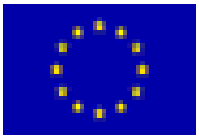
UNIVERSITA' DEGLI STUDI DI NAPOLI FEDERICO II



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ISOLATION OF SECONDARY METABOLITES FROM PLANTS AND THEIR USE AS LEAD COMPOUNDS FOR THE SYNTHESIS OF BIOLOGICALLY ACTIVE PRODUCTS

Tutore: Prof.ssa Marina Della Greca Candidata: Raffaella Purcaro

Relatore: Prof. Achille Panunzi

Coordinatore: Prof. Aldo Vitagliano

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Isolamento di metaboliti secondari di origine vegetale e loro utilizzo come modelli molecolari in nuove strategie sintetiche di sostanze bioattive.

I metaboliti secondari prodotti dalle piante sono quei composti non direttamente essenziali per i meccanismi fotosintetici e respiratori della pianta. La loro funzione è normalmente di natura ecologica in quanto sono usati o come meccanismi di difesa contro predatori o per la competizione interspecifica o per facilitare i processi riproduttivi.

L'ambiente con cui una specie vegetale si trova a comunicare per competizione, difesa o empatia è un ambiente complesso, caratterizzato da specie di natura diversa con le quali le piante instaurano una comunicazione di tipo chimico. Molti studi vengono condotti per comprendere l'origine, la funzione e il significato delle sostanze chimiche che mediano l'interazione tra organismi differenti. Meccanismi di interazione tra specie vegetali sono descritti con il termine allelopatia, che considera le interazioni positive o negative che una pianta esercita su di un'altra, mediante il rilascio di composti chimici nell'ambiente. Juglone, sulcotrione, scopoletina, 1,4-cineolo sono solo alcuni esempi di metaboliti fitotossici che, prodotti da una pianta le consentono il predominio vegetale del proprio habitat. Data l'elevata fitotossicità alcuni dei composti allelochimici isolati vengono impiegati in campo agronomico come erbicidi naturali (*Duke et al., 2002*).

Erbicidi di origine naturale potenzialmente possiedono caratteristiche di biocompatibilità difficilmente riscontrabili in un erbicida di sintesi. A ciò si aggiunge la maggiore specificità dei metaboliti naturali, perfezionati dall'evoluzione per agire in maniera altamente selettiva su target di interesse.

Inoltre, i prodotti di origine naturale rappresentano una notevole fonte di nuovi modelli molecolari con nuovi modi di agire che può essere utile per combattere la resistenza sviluppata da piante infestanti verso alcuni erbicidi di sintesi.

In questa ottica il gruppo di ricerca presso il quale ho svolto il mio lavoro di tesi, ha negli anni studiato i metaboliti secondari prodotti da piante infestanti dell'area mediterranea (*Cutillo et al., 2003*). In particolare nel corso del mio ciclo di dottorato ho condotto l'analisi sistematica dei metaboliti secondari delle specie *Aptenia cordifolia* e *Oxalis pes-caprae,* e intrapreso lo studio di *Phillyrea angustifolia.*

Per ciascuna delle piante è stata realizzata l'estrazione dei metaboliti mediante infusione, seguita da un processo di frazionamento degli estratti mediante tecniche cromatografiche (cromatografia su colonna di silice, cromatografia liquido-liquido in controcorrente, TLC e HPLC in fase diretta e inversa). L'impiego di tali tecniche ha consentito la purificazione delle

frazioni e l'isolamento dei metaboliti. La caratterizzazione strutturale delle molecole isolate e' stata realizzata con tecniche spettroscopiche, tra cui NMR mono e bidimensionale (^{1}H-^{1}H COSY, HMBC, HSQC, NOESY, ROESY), spettroscopia UV-VIS, dicroismo circolare e tecniche di spettrometria di massa (EI, ESI, MALDI).

Aptenia cordifolia, appartenente alla famiglia delle Aizoaceae, è una pianta erbacea perenne caratterizzata da fusti di piccole dimensioni legnosi solo alla base e da foglie succulenti verde scuro. Viene comunemente usata come pianta ornamentale, anche in ambienti ostili in quanto è in grado di sopravvivere in uno ampio range di salinità del terreno e riesce a crescere in ambienti secchi e molto esposti al sole. E' dotata di una notevole invasività ed in particolare quando ha a disposizione un'adeguata quantità di acqua riesce a sopraffare tutta la vegetazione circostante, crescendo su tutto ciò che incontra sul cammino.

La pianta fresca è stata posta in infusione prima in una soluzione idroalcolica (10% CH_3OH/H_2O), poi in CH_3OH ed, infine, AcOEt, a temperatura ambiente per sette giorni. Mediante i processi di purificazione sopra descritti dall'infuso idroalcolico di *A. cordifolia* sono stati isolati dodici composti con scheletro lignanico, di cui sei ossineolignani (*DellaGreca et al.,* 2005) e due lignanammidi, tre ammidi cinnamiche (*DellaGreca et al.,* 2006), cinque terpenoidi e diversi derivati fenolici (*DellaGreca et al., 2007*).

Oxalis pes-caprae è una pianta erbacea perenne di origine sudafricana ma largamente naturalizzata lungo le coste mediterranee e del basso Atlantico europeo. Ha proprietà rinfrescanti, astringenti, depurative e per via esterna ha potere antinfiammatori e decongestionante. Dato l'alto contenuto di acido ossalico le foglie risultano tossiche se ingerite in quantità eccessive.

Mediante i processi di estrazione e purificazione sopra descritti da *Oxalis pes-caprae* sono stati isolati sedici composti aromatici di natura esterea (*DellaGreca et al.,* in press) due lignani e quattro flavoni.

I metaboliti isolati sono stati sottoposti a saggi di fitotossicità sulla dicotiledone *Lactuca sativa,* specie test proposta come modello per le infestanti più comuni. I saggi sono stati condotti in accordo alla procedura ottimizzata da Macias et al.

E' stato valutato l'effetto che i metaboliti, a concentrazioni da 10^{-4} a 10^{-7} M, hanno sulla germinazione e sulla crescita dell'organismo vegetale testato.

Phillyrea angustifolia è una specie tipica della macchia mediterranea, si ritrova soprattutto lungo la costa tirrenica, con un fronte altimetrico che raggiunge i 600 metri. Colonizza terreni difficili e siccitosi e viene favorita in climi miti e caldi.

La fillirea, come la maggior parte delle specie della macchia, attua un "pirofitismo attivo" come strategia di ripresa dopo il fuoco, ossia ricresce rapidamente dopo un incendio a partire da polloni e rizomi e generalmente i cespugli superano il metro di altezza già dopo un anno dal fuoco, formando insieme ad altre specie, intricati grovigli difficili da penetrare (*Lopez-Soria et al. 1992*).

Dall'infuso idroalcolico della pianta sono stati finora isolati tre lignani e cinque secoiridoidi glucosilati, precedentemente già ritrovati in altre Oleaceae. Anche questi composti, insieme ad altri, saranno saggiati per valutarne una eventuale fitotossicità.

Inoltre, durante un periodo trascorso presso il centro di ricerca americano USDA-ARS, mi sono occupata dello studio di due piante, *Amyris madrensis* e *Swinglea glutinosa*. Entrambe appartengono alla famiglia delle Rutaceae che è fonte di molte classi di composti biologicamente attivi.

Amyris madrensis è una pianta sempreverde originaria dal Texas, molto resistente al caldo e alla siccità. Foglie secche della pianta sono state estratte per infusione con acetato di etile, una bioautografia dell'estratto mostrava la presenza di composti con attività fungicida. Ciò ci ha indotto alla successiva lavorazione dell'estratto mediante tecniche cromatografiche che hanno portato all'isolamento di diverse cumarine.

Swinglea glutinosa è una pianta tropicale, usata come pianta ornamentale o per costruire barriere naturali nei giardini. Dall'estratto organico delle radici della pianta sono stati isolati tre cumarine e nove acridoni alcaloidi. Alcuni dei composti isolati mostravano una buona attività inibente la crescita della specie *Oscillatoria peronata,* un cianobatterio dannoso per le colture di pesci.

L'ampio range di attività biologiche esibite dai lignani (*MacRae et al. 1984*) spiega l'enorme interesse verso l'isolamento e la sintesi di questi composti. Allo scopo di trovare un conveniente e blando metodo di preparazione di composti a struttura lignanica è stata studiato il comportamento dell'isoeugenolo e di altri propenil fenoli in reazioni fotoindotte (*Della Greca et al. in press)*. In particolar modo sono state prese in considerazione la fotoossigenazione sensibilizzata da coloranti e la fotoossidazione mediata da *t*-butil perossido, entrambe reazioni estremamente semplici e di basso impatto ambientale. Lignani ciclici e aciclici si sono ottenuti a partire dall'isoeugenolo mentre meno reattivi si sono mostrati gli altri substrati utilizzati nelle condizioni di reazione adoperate.

1. INTRODUCTION

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of the organisms. They are often restricted to a narrow set of species witin a phylogenetic group.

The function or importance of these compounds to the organism is usually of an ecological nature as they are used as defenses against predators, parasites and diseases, for interspecies competition, and to facilitate the reproductive processes (coloring agent, attractive smells).

Plants compete with other plants not only for space, light, water and nutrients, but also defend themselves against microorganisms, insects, and herbivores. It will not be surprising that plants use more than thorns, hairy surfaces, and though skins to discourage predators from taking a bite, rather they wield an arsenal of chemicals to deter enemies, fend off pathogens, make nutrients more available and protect against cold and other physical hazards of environments. These secondary metabolites, beneficial or deterrent to the growth of receptor organisms, could be regarded as an evolutionary expression of a quiet antagonism of plant to its enemies. One important aspect of this quiet antagonism is allelopathy. Allelopathy is defined as any direct or indirect effect (stimulatory or inhibitory) by one plant, including microorganisms, on another through production of chemical compounds released into the environment.[1]

Three criteria were proposed[2] to establish evidence for allelopathy:

1. The allegedly allelopathic plant must produce and release chemicals that modify the growth or function of other plants.
2. The amount of inhibiting substances produced must be sufficient to affect vital processes of the sensitive plants, like nutrient and water uptake by roots or energy fixation.
3. The observed field patterns of plant inhibition cannot be explained solely by physical or biotic factors.

Demonstrating that a plant has the described criteria is not an easy task. The finding of phytotoxins in the rhizosphere, density-dependent experiments to distinguish allelopathy and resource competition effects,[3] reduction of the negative power of one plant on the others by using substances with organic adsorptive capacity (activated carbon, gel filters)[4] to alter the rhizosphere chemistry, are just some of the more persuasive experiments that provide a deeper insight into allelopathy. Inhibition zones around the plant, wide spontaneous monoculture area, and root segregation may of course develop from other mechanisms of interference (resource competition, nutrient immobilization, indirect effects of herbivores), but such patterns establish a realistic ecological features in which to investigate allelopathy.[5]

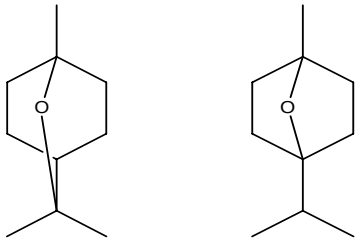
Allelochemical action is launched when the allelochemicals are released in the environment. This may happen through different processes, including foliar leaching, root exudation, residue decomposition, volatilisation and debris incorporation into soil.[6] Once released, phytotoxins diffuse in the soil transported by water. It has also been demonstrated that allelopathic interaction can occur between organisms through root to root contact.[6]

Allelochemicals so far identified belong to different classes of chemical compounds, as terpenoids, quinones, saponins, coumarins, tannins, flavonoids, cinnamic and benzoic acids, alkaloids.

Terpenoid class includes a wide range of mono-, di-, tri-, sesqui-, and tetraterpenoids. An impressive array of terpenoids from sunflower, responsible for inhibiting activity, have been described.[7]

Mechanisms of action have been studied for some chemicals having a wellproved allelopathic activity, but no unique way of interaction with cells has been found for structural related compounds.

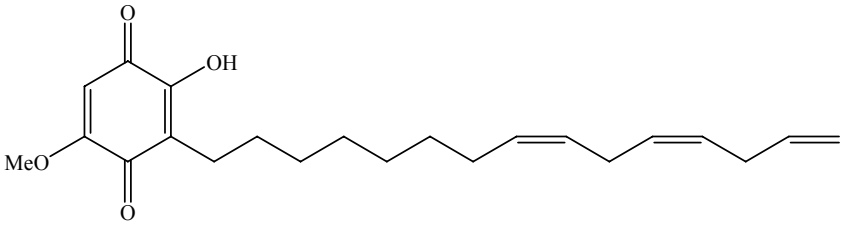
One of the first mechanisms of action described for allelochemicals[8] showed that the volatile 1,8-cineole and camphor reduce cell division causing a shorter root cells elongation and the generation of cells with irregular nuclear and large internal globules. It was successively reported that these monoterpenes cause structural modifications of small cellular organelles and the disruption of the nuclear membrane and other internal membranes.



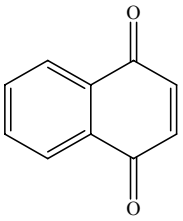
1,8-cineole 1,4-cineole

A natural analogue of 1,8-cineole is 1,4-cineole, which has been shown to be a potent inhibitor of asparigine synthetase, which is involved in nitrogen metabolism. The displacement of the oxygen bridge within the two monoterpenes described, deeply modifies the biological effects.

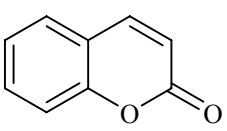
Two significant examples of bioactive quinones are juglone and sorgoleone.[9] They are described as having a role in *black walnut* and *Sorghum* allelopathy, respectively. They are both active at very low concentrations, several orders of magnitude lower than many allelochemicals from higher plants. It has been reported that both compounds affect plant energy metabolism, but it is still unclear what the target sites are.



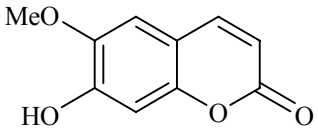
sorgoleone

juglone 

Many bioactive coumarins were isolated from plants and their action mechanisms are broadly distributed, including an internal role in seed dormancy by retarding cellular development.[10] Coumarin and scopoletin both decrease mitosis, and an inhibiting action on indoleacetic acid oxidation by scopoletin has been demonstrated. Roots absorbed scopoletin was proved to be transferred to shoots, where it decreased the photosyntesis rate, probably by stomatal closure.[11]

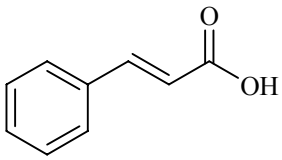


Coumarin

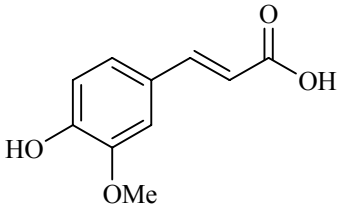


Scopoletin

Cinnamic and benzoic acids production has been reported for many allelopathic plants. In their mode of action these compounds do not have a specific molecular target. Effects have been reported on several phytormone interactions, ion uptake and content, photosynthesis, respiration, chlorophyll maintenance, membrane structures and ATPases transport, patterns of carbon flow, and a variety of enzymes. All these effects are said to be caused by ferulic acid, one of the better studied phenolic acids, in higher plants.[12]



Cinnamic acid



Ferulic acid

Secondary metabolites produced by plants and having biological activity have been broadly described in literature but only a small subset has been studied in connection with allelopahty, and their action mechanism is still far from being understood. Thus there is still a wide array of natural compounds to be investigated for a more complete view of allelopathy.

Allelochemicals produced by a plant may act as phytotoxins on the plant itself. Two possible mechanisms might prevent a plant from poisoning itself: a) resistance at the molecular target site; b) processes that avoid the allelochemical to interact with the molecular target site. While there is no evidence for the first mechanism, the second one is supported by several examples. Phytotoxins are

kept away from the molecular target through sequestration and chemical protection. Sequestration is realized by the compartmentalization of the toxic chemicals in cell areas which are not in contact with vital centres, as in vacuoles and trichomes. In some cases phytotoxins are modified by enzymatic processes that bond sugars or other moieties. In this way the modified phytotoxins can be

transported to a vacuole. The modified compounds are usually less phytotoxic, and their negative activity is even weaker when they are stored within vacuoles.

These compounds usually become phytotoxic only after they are released into the soil, where they are probably activated by the enzymatic removal of the function employed to ensure vacuole sequestration.

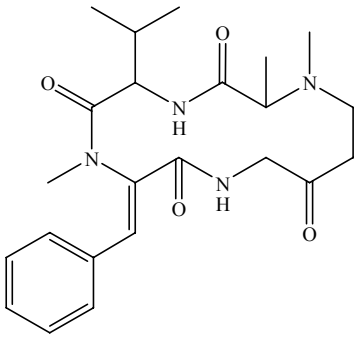
Chemical modification of allelochemicals is sometimes performed by plants, according to a species selected process, in order to lessen the phytotoxic effects of other species. Detoxification processes include oxidation, carbohydrate or other chemical conjugation, and segregation in depository cell compartments before excretion. These kinds of plant reactions are also observed in response to

herbicide treatments.

Allelochemicals have received special attention due to the agricultural potential of these compounds as natural herbicides,[13] both directly or after chemical modification that

implements physico-chemical properties. Widespread use of synthetic herbicides has resulted in herbiced-resistence weeds, and public concerns over the impact synthetic herbicides have on human health and the environment are increasing. These concerns are shifting attention to alternative weed control technologies based on natural products. Natural compounds often have a shorter environmental half life than synthetic compounds, thus reducing potential environmental impact. Herbicides designed by chemical synthesis have quite unspecific molecular target sites, usually aimed at undermining generic vital functions of the cell, causing drawbacks also to crops. Evolution, instead, selected a broad array of efficient and selective phytotoxins, which can be used as templates for new herbicides. Increasing the weeds resistance to common herbicides has aroused deep interest in the discovery of new molecular target sites for herbicides.

The obstacle for industrial application of these compounds sometimes is constituted by their structural complexity that makes their synthesis cost prohibitive. Structural simplification of the lead compounds often results in significantly lower biological activity. For example, tentoxin,[14] a cyclic tetrapeptide from the plant pathogen *Alternaria alternate*, has a really attractive soil behaviour. In fact, it shows high stability with good residual soil activity which is mainly exerted against an important spectrum of weed species. Several major crops are completely resistant to tentoxin toxicity. Unfortunately, much structure-activity research has not led to a simpler molecule with acceptable activity.[14]

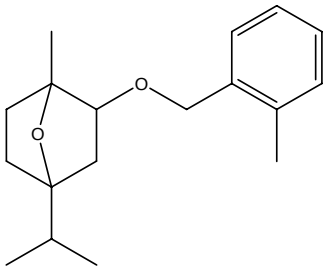


Tentoxin

Many natural products are phytotoxic, but few have both the correct physicochemical properties or level of biological activity to be good herbicides. For example, sorgoleone, which is a plant produced quinone, structurally related to plastoquinone. Together with many of its

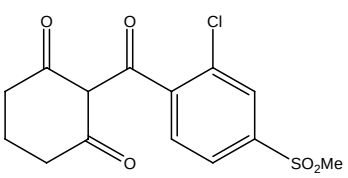
analogues, sorgoleone inhibits photosystem II of photosynthesis, apparently by preventing the binding of plastoquinone to the D-1 protein of PSII,[15] but it is a relatively weak herbicide.

Nevertheless there are many commercial herbicides deriving structurally from allelopathic compounds. An interesting example is provided by a herbicide widely used in Europe and Asia, cynmethylin. This herbicide is structurally similar to 1,4-cineole, a natural monoterpen, which acts on a target plant by inhibiting asparigine synthetase,[16] a previously unexploited molecular action site. The inhibition of this enzyme causes an interruption of plant growth. It seems that Cynmethylin is a pro-herbicide that requires metabolic bioactivation in soil by cleavage of the benzyl-ether side chain, giving a molecule with a 1,4-cineole structure, responsible for the allelopathic activity.[16]

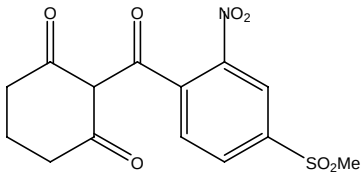


Cinmethylin

For agricultural purposes different kinds of triketone herbicides structurally derived from the natural compound leptospermone are widely used. Two interesting examples are sulcotrione and mesotrione.[17] These kinds of triketone herbicides act on the enzyme *phydroxyphenylpyruvate dioxygenase*, which is an enzyme involved in the biosynthesis of carotenoids and plastoquinone. The inhibition of its activity causes foliage bleaching in the treated plants, due to the loss of chlorophyll.



Sulcotrione



Mesotrione

The investigation of allelopathic compounds is just a small part of the studies of natural compounds. Nature still represents an infinite source of unknown molecules having new structures and functionalities that evolution refined for high specific biological roles. The functional-group diversity and architectural models of carbon skeletons designed by nature

continue to provide lessons for synthetic and medicinal chemists in their strategies for making biological active mimics. Biosynthetic pathways of natural compounds also provide interesting suggestions for monomer assembling and the tailoring of the growing product, which can be used to create new strategies for synthesising variants of natural products.[18]

My PhD project involved the study of two plants of the Mediterranean area, *Aptenia cordifolia* and *Oxalis pes-caprea*, in order to isolate and identify fitotoxic compounds.

These plants have been chosen because of their characteristics of persistency and diffusion, which are promising features for plant allelopathy.

The extraction of metabolites from these plants was realized by infusion in hydroalcoholic solution and then in methanol. Fractionation and purification of the extracts were realized by different chromatographic techniques (CC, HPLC, TLC), while pure compound identification was possible with spectroscopic techniques (^{1}H and ^{13}C NMR, UV-VIS, IR) and spectrometric techniques (ESI-MS, EI-MS, MALDI-MS). Characterized compounds have been tested on seeds of *Lactuca sativa* to detect their bioactivity, in accordance with a procedure defined by *Macias et al.*[19]

Furthermore, I have been concerned with the study of two Rutaceae species, *Amyris madrensis* and *Swinglea glutinosa*, at the USDA-ARS Natural Products Utilization Research Unit. In order to discover bioactive and environmentally safe compounds metabolites isolated from these plants have been evaluated for antifungal activity against the plant pathogen *Colletotrichum fragariae*, and for algicidal activity against *Oscillatoria perornata*, cianobacteria responsible for causing off-flavor in pond-cultured catfish.

The bioassays were performed in accord with the Wedge[20] and Schrader[21] methods, respectively.

Acetylcholinesterase inhibitory activity were also evaluated for these compounds.

Finally, in order to find convenient and mild approaches to the preparation of lignan-like compounds, light-promoted oxidations of propenyl phenols have been examineted under various conditions.

2. MATERIALS AND METHODS

2.1. General experimental procedures

Nuclear magnetic resonance (NMR) spectra have been recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a Varian 500 Fourier transform NMR spectrometer. Electronic Impact Mass Spectra (EI-MS) have been obtained with a HP 6890 spectrometer equipped with an MS 5973 N detector. Matrix Assisted Laser Desorption Ionization Mass Spectra (MALDI-MS) were recorded using a Voyager-DE MALDI-TOF mass spectrometer. High Resolution ElettroSpray Ionization Mass Spectra (HRESI-MS) were obtained using an Agilent 1100 HPLC coupled to a JOEL AccuTOF (JMS-T100LC). UV/Vis spectra were recorded in ethanol on a Perkin-Elmer Lambda 7 spectrophotometer. The analytical HPLC apparatus consists of an Agilent 1100 HPLC system equipped with a UV detector. The column is a 250 mm x 4.6 mm i.d., 5 μm Hibar LiChrosorb RP-18 (Merck). The preparative HPLC apparatus consists of a Shimadzu LC-10AD pump, a Shimadzu RID-10A refractive index detector, and a Shimadzu Chromatopac CR-6A recorder. Preparative HPLC was performed using a 250 mm x 10 mm i.d., reverse phase C-18 column (Phenomenex). Analytical TLC was made on Kieselgel 60 F254 or RP-18 F254 plates with 0.2 mm layer thickness (Merck). Spots were visualized by UV light or by spraying with H_2SO_4 - AcOH - H_2O (1:20:4). The plates were then heated for 5 min at 110 °C. Preparative TLC was performed on Kieselgel 60 F254 plates with 0.5 or 1 mm film thickness (Merck). Flash column chromatography was conducted on Kieselgel 60, 230-400 mesh (Merck), at medium pressure. Column chromatography was performed on Kieselgel 60, 70-240 mesh (Merck), or on Sephadex LH-20 (Pharmacia).

The methylene blue-sensitized photooxygenations were performed in a 50-ml Pyrex flask, by irradiation with an external 650-W halogen lamp (General Electric). A photoreactor (Helios Italquartz) equipped with a 500W high-pressure mercury lamp (through a Pyrex glass filter, λ >300 nm) was used for UV irradiation.

2.2. Extraction and isolation of compounds from *Aptenia cordifolia*

Plants of *Aptenia cordifolia* were collected in Campania, Italy, in August 2004, and identified by Prof. Antonino Pollio, Dipartimento di Biologia Vegetale, University Federico II, Naples,

Italy. A voucher specimen (HERBNAPY680) was deposited at the herbarium of the University Federico II.

Leaves extract fractionation.

Fresh leaves (12 kg) of *A. cordifolia* were powdered and extracted with H_2O - CH_3OH (9:1) at room temperature (25 °C) for 7 days. To an aqueous suspension (800 ml) of the hydroalcholic extract (450 g), cold $(CH_3)_2CO$ (1.0 l) was added, and the mixture was placed on a stirring plate overnight at -18 °C. The $(CH_3)_2CO$ addition led to heavy precipitation consisting mostly of proteinaceous material, which was removed by centrifugation. The solution was concentrated and successively chromatographed on Amberlite XAD-2, eluting with H_2O, CH_3OH, and $(CH_3)_2CO$.

Fraction eluted with H_2O was lyophilized (1.9 g), and the crude extract was subjected to silica gel flash column chromatography [CH_2Cl_2 - CH_3OH - H_2O (14:6:1)] to afford eight fractions. Fraction 1 was purified by reverse phase C-18 HPLC [CH_3OH - H_2O (9:1)] to give pure **35** (14 mg).

The fraction eluted with CH_3OH (45.0 g) was rechromatographed on silica gel column with CH_2Cl_2 and increasing CH_3OH. Fractions of 250 ml were collected and those with similar TLC profiles were combined to give thirteen fractions.

Fraction 4 (13.5 g), eluted with CH_2Cl_2 - CH_3OH (4:1), was purified by silica gel flash column chromatography (CH_2Cl_2 - CH_3OH gradient) to give fractions A-F. Fraction A (20 mg), eluted with CH_2Cl_2, was purified by reverse phase C-18 HPLC [CH_3OH - CH_3CN - H_2O (3:2:5)] to afford **19** (3 mg). Purification of fraction E (33 mg) by reverse phase C-18 HPLC [CH_3OH - CH_3CN - H_2O (3:1:6)] gave **6** (10 mg) and **7** (3 mg). Fraction F (103 mg) was identified as pure **26** (103 mg).

Fraction 5 (5.5 g), eluted with CH_2Cl_2 - CH_3OH (7:3), was rechromatographed on silica gel flash column eluting with CH_2Cl_2 - AcOEt - CH_3OH gradient to afford fractions A-I. Fraction A (91 mg) eluted with CH_2Cl_2 - AcOEt (4:1) was purified by preparative TLC [petrol ether - $(CH_3)_2CO$ (3:1)], to give **22** (8 mg). Fraction B (210 mg), eluted with AcOEt - CH_3OH (17:3), was purified by preparative TLC [CH_2Cl_2 - CH_3OH (9:1)] to afford **4** (20 mg); fraction F (52 mg), eluted with AcOEt - CH_3OH (4:1), was purified by preparative TLC [CH_2Cl_2 - CH_3OH (3:1)] to give **3** (6 mg).

Fraction 13 (5.5 g), eluted with CH_3OH, was repeatedly chromatographed on silica gel column with CH_2Cl_2 - CH_3OH gradient to give crude **18** which was purified by reverse phase C-18 HPLC [CH_3OH - CH_3CN - H_2O (3:2:5)] (4 mg).

Fraction eluted with $(CH_3)_2CO$ (50.0 g) was rechromatographed on silica gel column eluting with CH_2Cl_2 - $(CH_3)_2CO$ - CH_3OH gradient to give eleven fractions.

Fraction 1 (2.0 g), eluted with CH_2Cl_2, was purified by silica gel flash column chromatography [CH_2Cl_2 - $(CH_3)_2CO$ gradient)] to provide fractions A-M. Fraction M (45 mg), eluted with $(CH_3)_2CO$, was purified by preparative TLC [CH_2Cl_2 - $(CH_3)_2CO$ (4:1)] to yield **16** (2 mg) and **17** (3 mg).

Fraction 2 (1.5 g), eluted with CH_2Cl_2 - $(CH_3)_2CO$ (4:1), was purified by silica gel flash column chromatography [petrol ether - $(CH_3)_2CO$ gradient)] to give fractions A-O. Fraction D (103 mg), eluted with petrol ether - $(CH_3)_2CO$ (4:1), was purified by reverse phase C-18 HPLC [CH_3OH - CH_3CN - H_2O (2:2:3)] to give **30** (2 mg) and **31** (4 mg). Fraction G (103 mg), eluted with petrol ether- $(CH_3)_2CO$ (3:1) was purified by reverse phase C-18 HPLC [CH_3OH - CH_3CN - H_2O (1:1:3)] to give **29** (8 mg) and **34** (7 mg).

Fraction 3 (4.3 g), eluted with CH_2Cl_2 - $(CH_3)_2CO$ (3:2), was rechromatographed on silica gel flash column eluting with CH_2Cl_2 - $(CH_3)_2CO$ gradient to afford fractions A-I. Fraction D (51 mg), eluted with CH_2Cl_2 - $(CH_3)_2CO$ (9:1), was purified by preparative TLC [$CHCl_3$ - CH_3OH (9:1)] and reverse phase C-18 HPLC [CH_3OH - CH_3CN - H_2O (3:1:6)], to give **37** (8 mg). Fraction E (28 mg) eluted with CH_2Cl_2 - $(CH_3)_2CO$ (7:3) was purified by reverse phase C-18 HPLC [CH_3OH - CH_3CN - H_2O (6:3:11)], to give **36** (11 mg), **40** (2 mg), and **39** (3 mg) respectively. Fraction H (56 mg) was purified by preparative TLC [CH_2Cl_2 - $(CH_3)_2CO$ (4:1)] affording pure **9** (28 mg).

Fraction 6 (5.0 g) eluted with CH_2Cl_2 - CH_3OH (1:1) was rechromatographed on silica gel flash column eluting with CH_2Cl_2 - $(CH_3)_2CO$ - CH_3OH gradient to afford fractions A-Q. Fraction L (498 mg) eluted with CH_2Cl_2 - CH_3OH (4:1) was rechromatographed on silica gel flash column eluting with AcOEt - $(CH_3)_2CO$ - CH_3OH gradient to afford 11 fractions. Fraction eluted with AcOEt - $(CH_3)_2CO$ (9:1) was purified by reverse phase C-18 HPLC [H_2O - CH_3OH (1:1)], to give **25** (4 mg). Fraction O (2.3 g) eluted with $(CH_3)_2CO$ - CH_3OH (1:1) was rechromatographed on silica gel flash column, eluting with CH_2Cl_2 - $(CH_3)_2CO$ - CH_3OH gradient to afford 9 subfractions. The first subfraction (23 mg), eluted with CH_2Cl_2, was purified by reverse phase C-18 HPLC [CH_3OH - CH_3CN - H_2O - AcOH (1:5:4:0.1)] to afford **28** (10 mg). The second subfraction (13 mg), eluted with CH_2Cl_2 - $(CH_3)_2CO$ (9:1), contained **27** (13 mg). The third subfraction (46 mg) eluted with CH_2Cl_2 - $(CH_3)_2CO$ (4:1) was purified by preparative TLC [petrol ether - $(CH_3)_2CO$ (3:1)], to give **24** (4 mg) and **11 (**15 mg**)**. The forth subfraction (160 mg), eluted with CH_2Cl_2 - AcOEt (3:1), provided **10** (52 mg), **23** (9 mg), and **21** (11 mg) after purification by reverse phase C-18 HPLC [CH_3OH - CH_3CN - H_2O

(3:1:6)]. The fifth subfraction (250 mg), eluted with CH_2Cl_2 - AcOEt (1:1), gave **12** (10 mg), **13** (14 mg), and **14** (18 mg), after purification by reverse phase C-18 HPLC [CH_3OH - CH_3CN - H_2O (3:2:5)].

Fraction 8 (6.0 g) eluted with CH_3OH was rechromatographed on silica gel flash column eluting with $(CH_3)_2CO$ - CH_3OH gradient to afford fractions A-G. Fraction F (292 mg) eluted with $(CH_3)_2CO$ - CH_3OH (1:1) was rechromatographed on silica gel flash column eluting with $(CH_3)_2CO$ - CH_3OH (7:3) to afford fractions A-F. Fraction B (76 mg) was purified by preparative TLC [$(CH_3)_2CO$ - CH_3OH - H_2O (35:14:1)], to give **20** (8 mg), and finally fraction D (20 mg) was purified by reverse phase C-18 HPLC column [H_2O - CH_3OH (1:1)], to give **38** (5 mg).

Twigs extract fractionation.

Twigs of *A. cordifolia* (1.5 kg) were powdered and then extracted with H_2O - CH_3OH (9:1) at 25 °C for 7 days. To an aqueous suspension (100 ml) of the crude extract (45.0 g), cold $(CH_3)_2CO$ (100 ml) was added, and the mixture was placed on a stirring plate overnight at -18 °C. Addition of $(CH_3)_2CO$ led to the precipitation of mostly proteinaceous material, which was removed by centrifugation. The $(CH_3)_2CO$ was removed by evaporation, and the resulting clear aqueous extract was reduced to a volume of 150 ml, and then separated by Amberlite XAD-2 with H_2O, CH_3OH, and $(CH_3)_2CO$. The fraction eluted with CH_3OH (12.0 g) was purified by silica gel flash column chromatography to afford ten fractions. Fraction 1 (1.8 g), eluted with petrol ether - $(CH_3)_2CO$ (3:1), was purified by silica gel flash column chromatography using CH_2Cl_2 - $(CH_3)_2CO$ gradient to give fractions A-F. Fraction A (16 mg) was purified by reverse phase C-18 HPLC [CH_3OH - CH_3CN - H_2O (2:1:2)] to afford **32** (2 mg), **2** (1 mg), and **33** (2 mg). Fraction B (206 mg), eluted with CH_2Cl_2 - $(CH_3)_2CO$ (4:1), was purified by silica gel flash column chromatography eluting with CH_2Cl_2 - ethyl ether gradient to give 5 subfractions. The third subfraction (35 mg) was purified by preparative TLC [CH_2Cl_2 - ethyl ether (4:1)] to provide **1** (8 mg) and **8** (3 mg). Fraction D (110 mg), eluted with CH_2Cl_2 - $(CH_3)_2CO$ (7:3), was purified by silica gel flash column chromatography eluting with CH_2Cl_2 - CH_3OH (99:1) to afford six subfractions. The second subfraction (48 mg) was purified by preparative TLC [CH_2Cl_2 - CH_3OH (19:1)] to give **5** (8 mg) and **15** (9 mg).

2.3. Extraction and isolation of compounds from *Oxalis pes-caprae*

Leaves and twigs of *Oxalis pes-caprae* were collected in Bacoli-Naples, Italy, in April 2005, and identified by Prof. Antonino Pollio, Dipartimento di Biologia Vegetale of the University of Naples. A voucher specimen was deposited at the Botanical Gardens of the University Federico II of Naples, Italy.

Fresh leaves and twigs (20 kg) were infused with EtOAc and, successively, with CH_3OH for 7 days at room temperature (25 °C).

The EtOAc extract was concentrated by evaporation and fractionated into acidic and neutral fractions with aqueous NaOH 2M. The neutral fraction, washed with H_2O and concentrated under vacuum (70 g), was chromatographed on silica gel column to give twenty-eight fractions.

Fraction 3 (6.2 g), eluted with petrol ether - $(CH_3)_2CO$ (17:3), was rechromatographed by silica gel column. The fraction eluted with petrol ether - EtOAc (4:1) contained compound **45**, that was purified by silica gel flash column chromatography with petrol ether - $(CH_3)_2CO$ (9:1), (10 mg).

Fraction 12 (2.2 g), eluted with petrol ether - $(CH_3)_2CO$ (7:3), was rechromatographed on silica gel column. The fraction eluted with CH_2Cl_2 - $(CH_3)_2CO$ (97:3) contained compounds **46** and **54**, that were purified by preparative TLC [petrol ether - $(CH_3)_2CO$ (7:3), 11 and 4 mg].

Fraction 18 (7.3 g), eluted with CH_2Cl_2, was rechromatographed on silica gel column. The fraction eluted with CH_2Cl_2 - $(CH_3)_2CO$ (19:1) containing compounds **44**, **48**, and **49**, was purified by preparative TLC [CH_2Cl_2 - $(CH_3)_2CO$ (9:1)], to obtain fractions A-D. Fraction A (22 mg), contained compound **44**, that was purified by reverse phase C-18 HPLC [H_2O - CH_3OH - CH_3CN (3:1:1), 3 mg]. Fraction B (15 mg) contained compound **48** that was purified by reverse phase C-18 HPLC [H_2O - CH_3OH - CH_3CN (3:3:4), 2 mg]. Fraction C (15 mg) contained compound **49** that was purified by reverse phase C-18 HPLC [H_2O - CH_3OH - CH_3CN (4:3:3), 2 mg].

Fraction 20, eluted with CH_2Cl_2 - $(CH_3)_2CO$ (23:2) contained compounds **57** and **59** that were purified by preparative TLC [CH_2Cl_2 - $(CH_3)_2CO$ (23:2), 5 and 7 mg, respectively].

Fraction 22 (2.2 g), eluted with $(CH_3)_2CO$, was rechromatographed on silica gel column. The fraction eluted with CH_2Cl_2 - $(CH_3)_2CO$ (17:3) contained compounds **56** and **58**, that were purified by reverse phase C-18 HPLC [H_2O - CH_3OH - CH_3CN (4:3:3), 3 and 5 mg, respectively].

The MeOH extract was concentrated and partitioned between EtOAc and H_2O. The crude EtOAc residue (37 g) was chromatographed on silica gel eluting with petrol ether, ethyl ether, CH_2Cl_2, EtOAc, $(CH_3)_2CO$, CH_3OH, and H_2O to give thirty-five fractions.

Fraction 4 (480 mg), eluted with petrol ether - ethyl ether (19:1), was rechromatographed on silica gel eluting with petrol ether and $(CH_3)_2CO$ to give fractions A-E. Fraction B (40 mg), eluted with petrol ether - $(CH_3)_2CO$ (9:1), was subjected to TLC [petrol ether - $(CH_3)_2CO$ (4:1)] to give resorcinol (1,3-dihydroxybenzene) **60** (4.5 mg) and compound **53** (6.9 mg).

Fraction 10 (5.8 g), eluted with petrol ether - ethyl ether (9:1), was rechromatographed on silica gel eluting with petrol ether, ethyl ether, EtOAc, and CH_3OH to give fractions A-R. Fraction A (30 mg), eluted with petrol ether - ethyl ether (17:3), was subjected to TLC [petrol ether-$(CH_3)_2CO$, (4:1)] to give methyl 4-hydroxycinnamate **64** (18.5 mg) and compound **41** (5 mg).

Fraction 11 (850 mg), eluted with petrol ether - ethyl ether (4:1), was rechromatographed on silica gel eluting with petrol ether and ethyl ether to give fractions A-G. Fraction C (34 mg), eluted with petrol ether - ethyl ether (9:1), was subjected to TLC [petrol ether - $(CH_3)_2CO$, (3:1)] to give 4-hydroxybenzyl alcohol **61** (1.5 mg), **43** (4.1 mg), and **47** (3 mg).

Fraction 12 (2.2 g), eluted with petrol ether - $(CH_3)_2CO$ (17:3), was rechromatographed on silica gel eluting with petrol ether, ethyl ether, and $(CH_3)_2CO$ to give fractions A-S. Fraction C (80 mg), eluted with ethyl ether - $(CH_3)_2CO$ (4:1), was subjected to TLC [petrol ether - $(CH_3)_2CO$, (7:3)] to give 3-methoxy-4-hydroxybenzoic acid **62** (8.5 mg), 4-hydroxycinnamic acid **63** (4.5 mg), and compound **51** (4 mg).

Fraction 14 (134 mg), eluted with petrol ether - $(CH_3)_2CO$ (4:1), was rechromatographed on silica gel eluting with petrol ether, EtOAc, and $(CH_3)_2CO$ to give fractions A-F. Fraction B (65 mg), eluted with petrol ether - EtOAc (4:1), was subjected to Sep-Pak [CH_3CN - CH_3OH - H_2O, (2:1:2)] to give compound **42** (4.0 mg).

Fraction 20 (3.2 g), eluted with CH_2Cl_2 - EtOAc (4:1), was rechromatographed on silica gel eluting with CH_2Cl_2, $(CH_3)_2CO$, and CH_3OH to give fractions A-Q. Fraction B (50 mg), eluted with CH_2Cl_2 - $(CH_3)_2CO$ (19:1), was subjected to TLC [petrol ether - $(CH_3)_2CO$, (7:3)] to give sinapic acid **65** (28.5 mg) and compound **52** (11 mg). Fraction E (40 mg), eluted with CH_2Cl_2 - CH_3OH (9:1), was subjected to TLC [CH_2Cl_2 - CH_3OH (22:3)], to give caffeic acid **66** (16.5 mg), **50** (5 mg), and **55** (5 mg).

2.4. Extraction and isolation of compounds from *Swinlea glutinosa*

Dried roots (100 g) of *Swinlea glutinosa* were extracted at room temperature (25 °C) with EtOAc (1 l) twice for 12 h. After evaporation under reduced pressure at 40 ^{o}C, the extract was fractionated by silica gel flash column chromatography eluting with hexane and EtOAc starting from 10% of EtOAc up to 100%, and finally with EtOAc - CH_3OH (19:1). Fractions with similar TLC profiles were combined to yield sixteen fractions.

Fractions 1 e 2 were combined and purified by silica gel column chromatography with hexane - EtOAc (19:1) to afford fractions A-F. Fraction B was determined to be pure **69** (125 mg). Fraction D was further purified by HPLC [hexane - ethyl ether (4:1)] to give **68** (45 mg) and **67** (40 mg).

Fraction 4 was purified by crystallization from hexane and EtOAc to afford **77** (25 mg). Further purification of fraction 6, by silica gel column chromatography [$(CH_3)_2CO$ - hexane (1:9)] and TLC plates [CH_2Cl_2 - ethyl ether (19:1)], gave pure **78** (14 mg), **76** (10 mg), **73** (6 mg), **71** (17 mg), and **75** (21 mg).

Fraction 10 was rechromatographed on silica gel column eluting with CH_2Cl_2 - ethyl ether (97:3) affording **74** (7 mg) and **72** (20 mg).

Fraction 11 was rechromatographed on silica gel column eluting with CH_2Cl_2 - ethyl ether (19:1) to give 11 subfractions. The ninth subfraction was purified by TLC [CH_2Cl_2 - $(CH_3)_2CO$ (9:1)] to yield pure **70** (3 mg).

2.5. Extraction and isolation of compounds from *Amirys madrensis*

Dried leaves (400 g) of *A. madrensis* were ground in a blender. Ground plant material was extracted at room temperature with hexane (2 l) twice for 12 h. Following filtration through filter paper, the combined hexane extract was concentrated by evaporation under reduced pressure at 40^{o} C to afford 13.3 g of extract. Dried material was subsequently extracted with EtOAc and CH_3OH (4 l) providing 9.9 and 86 g of residue, respectively.

The EtOAc extract was subjected to silica gel flash column chromatography using hexane - EtOAc with increasing polarity. Fractions of 50 ml were collected and, on the basis of TLC similarities, were combined to produce twenty-seven fractions.

Fraction 6 (430 mg) was rechromatographed on silica gel column eluting with hexane - EtOAc (19:1), to give **86** (120 mg) and **82** (35 mg).

Fraction 15 was an off-white residue, and this fraction was dissolved in EtOAc and precipitated with hexane to obtain a white powder that was further purified by crystallization to afford white crystals of **80** (160 mg).

Fraction 19 (340 mg) was rechromatographed on silica gel column eluting with hexane - EtOAc 3:1 to afford fractions A-H. Fraction B was determined to be pure braylin **83** (35 mg). Fraction F (80 mg) was purified on silica gel column with CH_2Cl_2 - EtOAc (19:1) to provide **84** (15 mg).

Fraction 20 (610 mg) was rechromatographed on silica gel column eluting with hexane - $(CH_3)_2CO$ (9:1) affording **85** (14 mg).

Fraction 21 was dissolved in CH_3OH and precipitated with EtOAc to obtain a white powder that was further purified by crystallization to afford white crystals of **81** (230 mg). The mother liquor (250 mg) was rechromatographed on silica gel column eluting with hexane - $(CH_3)_2CO$ (9:1) to provide fractions A-G. Fractions E and F were combined (120 mg) and further purified using silica gel column chromatography with CH_2Cl_2 - EtOAc (9:1) to afford **79** (21 mg).

2.6. Bioassays

Phytotoxic activity

Pendimethalin, bioassayed as commercial mixture (Pendulum®) supplied by Ingegnoli Spa (Milano, Italy). Seed of *Lactuca sativa* L. (cv Cavolo di Napoli) were obtained from Ingegnoli Spa. All undersized or damaged seeds were discarded and the assay seeds were selected for uniformity. For the bioassays we used Petri dishes with one sheet of Whatman No. 1 filter paper as support. In four replicate experiments, germination and growth were conducted in aqueous solutions at controlled pH. Test solutions (10^{-4} M) were prepared using MES (2-[N-Morpholino]ethanesulfonic acid, 10 mM, pH 6) and the rest (10^{-5} - 10^{-7} M) were obtained by dilution. Parallel controls were performed. After adding 25 seeds and 2.5 ml of test solutions the Petri dishes were sealed with Parafilm® to ensure closed-system models. Seeds were placed in a KBW Binder 240 growth chamber at 25 °C in the dark. Germination percentage was determined daily for 5 days. After growth, the plants were frozen at -20 °C to avoid subsequent growth until the measurement process. Data are reported as percentage differences from control in the graphics. Thus, zero represents the control, positive values represent stimulation of the parameter studied and negative values represent inhibition.

Antifungal activity

Bioautography on silica gel TLC plates with *Colletotrichum fragariae* was used to identify the antifungal activity according to the previously published method.[20] Crude extracts and pure compounds from *A. madrensis* were applied to silica gel TLC plates and allowed to migrate in a suitable solvent. Each plate, dried, was subsequently sprayed with a spore suspension (10^5 spores/ml) of the fungus of interest and was incubated in a moisture chamber for 3 days at 26 °C with a 12 h photoperiod. The presence of antifungal constituents was indicated by clear zones of fungal growth inhibition on the TLC plates.

Algicide activity

Culture of the cyanobacterium *O. perornata* and of the green alga *S. capricornutum* were maintained separately in continuous, steady-state growth using the conditions outlined in Schrader et al.[21] to provide a source of cells growing at fairly constant rate. Stock solutions (2.0, 20.0, 200.0, and 2000.0 μM) of pure compounds were micropipeted to the empty wells (10 μM/well) of a 96-well microplate. The solvent was allowed to evaporate completely before adding continuous culture sample material (200 μM/well). Final concentrations for each pure compounds were 0.1, 1.0, 10.0, and 100.0 μM. Control did not include any test material, only culture material. Three replications were used for each concentration of pure compounds and the control. Microplates were placed in a growth chamber held at 29-30 °C and were illuminated continuously by fluorescent lights (40W, cool white) at a photon flux density of 21-27 μE m^{-2} s^{-1}. Absorbance measurements of each well were measured at 650 nm at 24 h intervals for 4 days using a Packard model SpectraCount microplate photometer. Mean values and standard deviations of absorbance measurements were calculated and graphed to determine the lowest observed effect concentration (LOEC) and the lowest complete inhibition concentration (LCIC).

Acetylcholinesterase inhibitory activity[22]

Acetylcholinesterase and galanthamine were purchased from Sigma, bovine serum albumin and 1-naphtyl acetate were obtained from Merk and Fast Blue B salt was from Fluka.

Acetylcholinesterase was dissolved in 150 ml of Tris-hydrochloric acid buffer at pH 7.8, bovine serum albumin (150 mg) was added and the solution was kept at 4 °C. After migration of the samples in a suitable solvent, the TLC plates were dried, sprayed with the enzyme stock solution and dried again. The plates were incubated at 37 °C for 20 min in a humid atmosphere. 10 ml of naphtyl acetate solution (prepared with 250 mg of naphtyl acetate in 100 ml of

ethanol) and 40 ml of the Fast Blue B salt solution (400 mg of Fast Blue B salt in 160 ml of distilled water) were mixed and sprayed onto the plates to give a purple coloration after 1-2 min.

2.7. Photooxidations

Methylene blue (MB)-sensitized photooxygenation of isoeugenol **87a**

Each solution of **87a** (100 mg, 0.61 mmol) was added of MB (22 mg, 0.06 mmol), and irradiated at 15 °C with a halogen lamp. During irradiation, dry oxygen was bubbled through the solution. Irradiation times, solvents, and concentrations are reported in Table 1. After solvent evaporation, chromatography of each residue on silica gel TLC [hexane - EtOAc (7:3)] followed by reverse phase C-18 HPLC column [H_2O - MeOH - MeCN (4:3:3)] led to compounds **88a**, **89**, **90**, **91**, and **96**, with the yields reported in Table 1. All products were spectroscopically characterized (IR, NMR, MS) and known compounds were identified by comparison of spectral data with those reported.

Table1. Methylene blue-sensitized photooxygenation of isoeugenol **87a**, at 15 °C under different conditions

Conc. (M)	Solvent	Time (h)	Isolated Yield (%)					
			87a	**88a**	**89**	**90**	**91**	**92**
0.36	CH_2Cl_2	30	3	30	15	-	3	trace
0.1	CH_2Cl_2	12	50	20	10	-	trace	trace
0.36	CH_3CN	30	30	20	10	-	trace	trace
0.1	CH_3CN	12	50	15	15	-	trace	-
0.1	H_2O	12	50	4	5	20	trace	2
0.1	H_2O-CH_3CN (2:1)	12	40	22	8	8	4	-

Irradiation of isoeugenol **87a** *with di-tert-butyl peroxide*

To a solution of **87a** (100 mg, 0.61 mmol) in CH_3CN dry (0.05 M) 0.23 ml of di-*tert*-butyl peroxide (two equivalents) were added. The sample was bubbled with a slow stream of argon for 30 min before the UV irradiation. After 14 h of irradiation the residue was analyzed by ^1H NMR. Chromatography purifications gave **88a** (~20%) and trace of compounds **89** and **91**.

Irradiation of isoeugenol **87a** *with chloranil*

24.5 mg of chloranil were added to a solution of **87a** (50 mg, 0.30 mmol) in CH_3CN dry (0.015 M). The sample was bubbled with a slow stream of argon for 30 min before the UV irradiation. After 8 h of irradiation the residue was analyzed by ^1H NMR. Chromatography purifications gave **88a** (~20%).

Methylene blue-sensitized photooxygenation of coniferyl alcohol **87b** *and ferulic acid* **87c**

Solutions of **87b** (100 mg, 0.56 mmol) and **87c** (100 mg, 0.52 mmol) in H_2O - CH_3CN (2:1) (0.1 M) were irradiated at 15 °C with a halogen lamp as above described for **87a**. During irradiation oxygen was bubbled through the solutions.

After 20 h ^1H NMR analysis of the residue from **87b** indicated the presence of compound **89** as the only product. The ^1H NMR spectrum of the residue from **87c** showed the presence of starting **87c** (80%) and its *cis*-isomer (20%).

Irradiation of coniferyl alcohol **87b** *and ferulic acid* **87c** *with di-tert-butyl peroxide*

To a solution of **87b** (50 mg, 0.28 mmol) in dry CH_3CN (0.05 M) 0.11 ml of di-*tert*-butyl peroxide were added. The sample was bubbled with a slow stream of argon for 30 min before the UV irradiation. After 14 h of irradiation the residue was analyzed by ^1H NMR. The mixture was purified by reverse phase C-18 HPLC column [H_2O – MeOH - MeCN (5:3:2)], to give compounds **88b** (30%) and **7** (10%).

Acid **87c** was treated similarly. After 14 h irradiation, the ^1H NMR spectrum of the residue showed the presence of starting **87c** (72%) and its *cis*-isomer (28%).

2.8. Spectroscopic data

Compound 16. (-)-*Pinoresinol* (2 mg). $[\alpha]_D^{25}$ = -10.0° (*c* 9.0, $CHCl_3$). MS: *m/z* 358 $[M]^+$. ^1H NMR ($CDCl_3$) δ: 6.82 (6H, m, H-6 and H-6', H-5 and H-5', H-2 and H-2'), 4.74 (2H, d, J=4.0 Hz, H-7 and H-7'), 4.25 (2H, dd, J=9.0 and 7.0 Hz, H-9a and H-9'a), 3.88 (2H, dd, J=9.0 and 4.0 Hz, H-9b and H-9'b), 3.10 (2H, m, H-8 and H-8'), 3.91 (6H, s, 3-OMe and 3'-OMe). ^{13}C NMR (CD_3OD) δ: 146.7 (C-3 and C-3'), 145.2 (C-4 and C-4'), 132.9 (C-1 and C-1'), 118.9 (C-6 and C-6'), 114.3 (C-5 and C-5'), 108.6 (C-2 and C-2'), 85.8 (C-7 and C-7'), 71.6 (C-9 and C-9'), 54.1 (C-8 and C-8'), 55.9 (3-OMe and 3'-OMe).

Compound 17. (±)-*Syringaresinol* (3 mg). $[\alpha]_D^{25}$ = 0° (*c* 3.5, $CHCl_3$). MS: *m/z* 418 $[M]^+$. ^1H NMR ($CDCl_3$) δ: 6.57 (4H, s, H-2, H-5, H-2' and H-5'), 4.72 (2H, d, J=4.0 Hz, H-7 and H-7'), 4.26 (2H, m, H-9a and H-9'a), 3.61 (2H, m, H-9b and H-9'b), 3.09 (2H, m, H-8 and H-8'), 3.89 (12H, s, 3-OMe, 3'-OMe, 5-OMe, 5'-OMe).

Compound 18. *Di*-erythro-*syringylglycerol*-β-O-*4,4'-syringaresinol ether* (4 mg). $[\alpha]_D^{25}$ = +3.1 (*c* = 0.05, CH_2Cl_2). MALDI-MS: *m/z* 893 $[M + Na]^+$. ^1H NMR ($CDCl_3$) δ: 6.65 (4H, s, H-2, H-6, H-2' and H-6'), 6.58 (4H, s, H-2'', H-6'', H-2''' and H-6'''), 4.99 (2H, d, J=4.0 Hz, H-7'' and H-7'''), 4.79 (2H, d, J=2.8 Hz, H-7 and H-7'), 4.36 (2H, dd, J=7.6 and 14.8 Hz, H-9a and H-9'a), 4.13 (2H, m, H-8'' and H-8'''), 3.99 (2H, m, H-9b and H-9'b), 3.88 (2H, m, H-9''a and H-9'''a), 3.50 (2H, dd, J=2.4 and 12.0 Hz, H-9''b and H-9'''b), 3.15 (2H, m, H-8 and H-8'), 3.90 (12H, s, 3-OMe, 3'-OMe, 5-OMe, 5'-OMe), 3.88 (12H, s, 3''-OMe, 3'''-OMe, 5''-OMe, 5'''-OMe). ^{13}C NMR ($CDCl_3$) δ: 153.5 (C-3, C-3', C-5 and C-5'), 147.1 (C-3'', C-3''', C-5'' and C-5'''), 137.6 (C-4 and C-4'), 136.0 (C-4'' and C-4'''), 134.2 (C-1 and C-1'), 130.4 (C-1'' and C-1'''), 102.9 (C-2, C-2', C-6, and C-6'), 102.6 (C-2'', C-2''', C-6'', and C-6'''), 87.1 (C-7'' and C-7'''), 85.8 (C-7 and C-7'), 72.7 (C-8'' and C-8'''), 72.0 (C-9 and C-9'), 60.6 (C-9'' and C-9'''), 54.5 (C-8 and C-8'), 56.4 and 56.3 (3-OMe, 3'-OMe, 3''-OMe, 3'''-OMe, 5-OMe, 5'-OMe, 5''-OMe, and 5'''-OMe).

Compound 19. *4-(4-Formylphenoxy)-3-methoxybenzaldehyde* (3 mg). MS: *m/z* 256 $[M]^+$. ^1H NMR (CD_3OD) δ: 9.75 (1H, s, H-7'), 9.72 (1H, s, H-7), 7.77 (2H, d, J=8.8 Hz, H-2' and H-6'), 7.43 (1H, s, H-2), 7.41 (1H, d, J=7.0 Hz, H-7), 6.91 (1H, d, J=7.0 Hz, H-5), 6.88 (2H, d, J=8.8 Hz, H-3' and H-5'), 3.92 (3H, s, 3-OMe). ^{13}C NMR (CD_3OD) δ: 193.3 (C-7 and C-7'), 164.6

(C-4'), 156.0 (C-4), 149.7 (C-3), 134.0 (C-2'), 131.1 (C-1'), 129.3 (C-1), 128.8 (C-6), 117.6 (C-3' and C-5'), 117.1 (C-5), 111.7 (C-2), 56.9 (3-OMe).

Compound 20. *4,4'-Oxyneolign-9,9'-dioic acid* (8 mg). UV λ_{max} (CH_3OH) nm: 278 (log ε 3.2). MALDI-MS: *m/z* 337 $[M+Na]^+$. ^1H NMR (CD_3OD) δ: 6.92 (4H, d, J=8.5 Hz, H-2 and H-2', H-6 and H-6'), 6.63 (4H, d, J=8.5 Hz, H-3 and H-3', H-5 and H-5'), 2.84 (4H, t, J=8.1 Hz, H-7 and H-7') 2.66 (4H, t, J=8.1 Hz, H-8 and H-8'). ^{13}C NMR (CD_3OD) δ: 180.6 (C-9 and C-9'), 157.4 (C-4 and C-4'), 130.7 (C-2, C-2', C-6, and C-6') 129.6 (C-1 and C-1'), 116.6 (C-3, C-3', C-5 and C-5'), 42.9 (C-8 and C-8'), 35.7 (C-7 and C-7').

Compound 21. *3-Methoxy-4,4'-oxyneolign-9,9'-dioic acid* (11 mg). UV λ_{max} (CH_3OH) nm: 280 (log ε 2.2). MALDI-MS: *m/z* 390 $[M+2Na]^+$. ^1H NMR (CD_3OD) δ: 7.05 (2H, d, J=8.5 Hz, H-2' and H-6'), 6.82 (1H, d, J=8.2 Hz, H-5), 6.74 (2H, d, J=8.5 Hz, H-3' and H-5'), 6.72 (1H, d, J=1.5 Hz, H-2), 6.70 (1H, dd, J=1.5 and 8.2 Hz, H-6) 2.89 (4H, t, J=8.0 Hz, H-7 and H-7') 2.66 (4H, t, J=8.0 Hz, H-8 and H-8'), 3.86 (3H, s, 3-OMe). ^{13}C NMR (CD_3OD) δ: 178.9 (C-9 and C-9'), 154.1 (C4'), 146.4 (C-3), 144.0 (C-4), 132.1 (C-1 and C-1'), 129.4 (C-2' and C-6'), 120.8 (C-6), 115.4 (C-3' and C-5'), 114.4 (C-5), 111.0 (C-2), 55.8 (3-OMe), 35.8 (C-8 and C-8'), 30.3 (C-7 and C-7').

Compound 22. *Dimethyl 3-methoxy-4,4'-oxyneolign-9,9'-dioate* (8 mg). UV λ_{max} (CH_3OH) nm: 280 (log ε 3.1). MALDI-MS: *m/z* 373 $[M+H]^+$. ^1H NMR ($CDCl_3$) δ: 7.05 (2H, d, J=9.0 Hz, H-2' and H-6') 6.83 (1H, d, J=8.0 Hz, H-5), 6.75 (2H, d, J=9.0 Hz, H-3' and H-5'), 6.70 (1H, d, J=1.5 Hz, H-2), 6.68 (1H, dd, J=1.5 and 8.0 Hz, H-6) 2.88 (4H, t, J=7.5 Hz, H-7 and H-7') 2.61 (4H, t, J=7.5 Hz, H-8 and H-8'), 3.87 (3H, s, 3-OMe), 3.67 (6H, s, 9-OMe and 9'-OMe). ^{13}C NMR ($CDCl_3$) δ: 173.8 (C-9 and C-9'), 154.4 (C4'), 146.7 (C-3), 144.2 (C-4), 132.7 (C-1 and C-1'), 129.6 (C-2' and C-6'), 121.0 (C-6), 115.5 (C-3' and C-5'), 114.6 (C-5), 110.2 (C-2), 56.1 (3-OMe), 51.9 (9-OMe and 9'-OMe), 36.3 (C-8 and C-8'), 30.9 (C-7 and C-7').

Compound 23. *3,3',5-Trimethoxy-4,4'-oxyneolign-9,9'-dioic acid* (9 mg). UV λ_{max} (CH_3OH) nm: 321 (log ε 0.9), 280 (2.6), 242 (2.5). MALDI-MS: *m/z* 427 $[M+Na]^+$. ^1H NMR ($CDCl_3$) δ: 6.82 (1H, d, J=8.5 Hz, H-5'), 6.70 (1H, d, J=8.5 Hz, H-6'), 6.44 (2H, s, H-2 and H-6), 2.90 (4H, t, J=7.5 Hz, H-7 and H-7') 2.67 (4H, t, J=7.5 Hz, H-8 and H-8'), 3.87 (9H, s, 3-OMe, 3'-OMe, and 5-OMe). ^{13}C NMR ($CDCl_3$) δ: 177.8 (C-9 and C-9'), 146.9 (C-3, C-3', and C-5),

144.0 (C-4'), 133.1 (C-1), 131.9 (C-4), 131.2 (C-1'), 120.7 (C-6'), 114.3 (C-5'), 110.8 (C-2'), 104.8 (C-2 and C-6), 55.7 (3'-OMe), 56.1 (3-OMe and 5-OMe), 35.7 (C-8 and C-8'), 30.7 (C-7 and C-7').

Compound 24. *3,3',5,5'-Tetramethoxy-4,4'-oxyneolign-9,9'-dioic acid* (4 mg). UV λ_{max} (CH_3OH) nm: 322 (log ε 1.5), 279 (3.0), 244 (2.8). MALDI-MS: *m/z* 457 $[M+Na]^+$. ^1H NMR ($CDCl_3$) δ: 6.42 (4H, s, H-2 and H-2', H-6 and H-6'), 2.88 (4H, t, J=8.0 Hz, H-7 and H-7'), 2.61 (4H, t, J=8.0 Hz, H-8 and H-8'), 3.88 (12H, s, 3-OMe and 3'-OMe, 5-OMe and 5'-OMe). ^{13}C NMR ($CDCl_3$) δ: 178.4 (C-9 and C-9'), 147.0 (C-3 and C-3', C-5 and C-5'), 145.2 (C-4 and C-4'), 132.0 (C-1 and C-1'), 104.9 (C-2 and C-2', C-6 and C-6'), 56.3 (3-OMe and 3'-OMe, 5-OMe and 5'-OMe), 36.1 (C-8 and C-8'), 31.2 (C-7 and C-7').

Compound 25. *3'-Methoxy-2,4'-oxyneolign-9,9'-dioic acid* (4 mg). UV λ_{max} (CH_3OH) nm: 280 (log ε 1.4). MALDI-MS: *m/z* 390 $[M+2Na]^+$. ^1H NMR (CD_3OD) δ: 7.23 (1H, m, H-4), 7.21 (2H, m, H-3 and H-6), 7.12 (1H, m, H-5), 6.82 (1H, d, J=2.0 Hz, H-2'), 6.67 (1H, d, J=8.0 Hz, H-5'), 6.63 (1H, dd, J=2.0 and 8.0 Hz, H-6'), 2.89 (2H, t, J=7.6 Hz, H-7), 2.82 (2H, t, J=7.8 Hz, H-7'), 2.44 (2H, t, J=7.6 Hz, H-8), 2.41 (2H, t, J=7.8 Hz, H-8'), 3.87 (3H, s, 3'-OMe). ^{13}C NMR (CD_3OD) δ: 182.2 (C-9 and C-9'), 148.6 (C-3), 145.8 (C-4), 144.2 (C-2') 135.7 (C-1) 129.2 (C-3', C-4', and C-6'), 127.1 (C-1'), 126.6 (C-5'), 122.0 (C-6), 116.4 (C-5), 113.5 (C-2), 56.5 (3-OMe), 41.9 (C-8), 41.6 (C-8'), 34.4 (C-7'), 34.0 (C-7).

Compound 26. *(Dimethylamino)-1-phenylethanol* (103 mg). $[\alpha]_D^{25}$ = +1.1 (*c* = 0.08, CH_2Cl_2). EI-MS: *m/z* 165 $[M]^+$, 150 $[M-Me]^+$. ^1H NMR ($CDCl_3$) δ: 7.40 (2H, d, J=7.0 Hz, H-2' and H-6'), 7.35 (2H, t, J=7.0 Hz, H-3' and C-5'), 7.28 (1H, t, J=7.0, H-4'), 4.86 (1H, dd, J=3.5 and 9.5 Hz, H-1), 2.81 (1H, dd, J=9.5 and 12.8 Hz, H-2a), 2.66 (1H, dd, J=3.5 and 12.8 Hz, H-2b), 2.35 (6H, s, 2×Me). ^{13}C NMR ($CDCl_3$) δ: 144.5 (C-1'), 130.0 (C-3' and C-5'), 129.3 (C-4'), 127.6 (C-2' and C-6'), 71.8 (C-1), 67.9 (C-2), 45.9 (2×Me).

Compound 34. (3R,9R)-*3,9-Dihydroxymegastigm-5-en-4-one* (7 mg). $[\alpha]_D^{25}$ = –76.0 (*c* = 0.1, CH_2Cl_2). EI-MS: *m/z* 226 $[M]^+$. ^1H NMR ($CDCl_3$) δ: 4.30 (1H, dd, J=5.8 and 14.0 Hz, H-3), 3.94-3.85 (1H, m, H-9), 2.54-2.45 (1H, m, H-7a), 2.34-226 (1H, m, H-7b), 2.15 (1H, dd, J=5.8 and 12.5 Hz, H-2a), 1.83 (3H, s, 13-Me), 1.77 (1H, br t, J=13.0 Hz, H-2b), 1.66-1.59 (2H, m, H-8), 1.29 (3H, s, 12-Me), 1.28 (3H, d, J=6.4Hz), 1.23 (3H, s, 11-Me). ^{13}C NMR ($CDCl_3$) δ:

200.2 (C-4), 166.1 (C-6), 127.9 (C-5), 69.1 (C-3), 68.2 (C-9), 45.3 (C-2), 37.7 (C-8), 37.5 (C-1), 29.8 (C-11), 27.1 (C-7), 25.6 (C-12), 23.8 (C-10), 11.9 (C-13).

Compound 35. *3*-O-*Methyl*-chiro-*inositol [(1*R,*2*R,*3*S,*4*R,*5*S,*6*R*)-6-Methoxycyclohexane-1,2,3,4,5-pentol]* (14 mg). $[\alpha]^{25}_{D}$ = +56 (*c* = 0.3, $CHCl_3$). ESI-MS: *m/z* 195 $[M+H]^+$. ^{1}H NMR ($CDCl_3$) δ: 3.91 (1H, dd, J=2.0 and 9.5 Hz, H-2), 3.89 (1H, d, J=2.0, H-6), 3.73 (1H, dd, J=2.0 and 9.5 Hz, H-1), 3.72 (1H, dd, J=2.0 and 9.5 Hz, H-5), 3.58 (1H, t, J=9.5 Hz, H-4), 3.35 (3H, s, 3-OMe), 3.25 (1H, d, J=9.0 and 9.5 Hz, H-3). ^{13}C NMR ($CDCl_3$) δ: 85.4 (C-3), 74.8 (C-4), 74.2 (C-1), 74.0 (C-6), 73.0 (C-5), 72.5 (C-2), 61.2 (Me).

Compound 36. (2S,E)-N-*[2-Hydroxy-2-(4-hydroxyphenyl)ethyl] ferulamide* (11 mg). Colourless oil. $[\alpha]^{25}_{D}$ = -3.0 (c 0.12, CH_3OH). IR ν_{max} (CH_2Cl_2) 3580, 3400, 2940, 1705, 1670, 1592, 1424, 1334, 1019 cm^{-1}. UV λ_{max} (CH_3OH) nm (log ε): 222 (3.9), 294 (2.5), 316 (2.4). EI-MS: *m/z* 329 $[M]^+$. ^{1}H NMR (CD_3OD) δ: 7.44 (1H, d, J=15.8 Hz, H-7), 7.22 (2H, d, J=8.9, H-2' and H-6'), 7.12 (1H, d, J=1.9 Hz, H-2), 7.03 (1H, dd, J=1.9 and 8.8 Hz, H-6), 6.79 (1H, d, J=8.8 Hz, H-5), 6.77 (2H, d, J=8.9 Hz, H-3' and H-5'), 6.45 (1H, d, J=15.8 Hz, H-8), 4.72 (1H, dd, J=4.9 and 7.8 Hz, H-7'), 3.87 (3H, s, 3-OMe), 3.53 (1H, dd, J=4.9 and 13.7 Hz, H-8'a), 3.44 (1H, dd, J=7.8 and 13.7 Hz, H-8'b). ^{13}C NMR (CD_3OD) δ: 169.6 (C-9), 158.1 (C-4'), 149.9 (C-3), 149.3 (C-4), 142.3(C-7), 134.5(C-1'), 128.4 (C-2' and C-6'), 123.3 (C-6), 118.7 (C-8), 116.5 (C-3' and C-5'), 116.1 (C-5), 111.7 (C-2), 73.5 (C-7'), 56.4 (3-OMe), 48.1 (C-8').

Compound 37. (E*)*-N-*[2-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)ethyl] ferulamide* (8 mg). Colourless oil. $[\alpha]^{25}_{D}$ = 0.0 (c 0.10, CH_3OH). IR ν_{max} (CH_2Cl_2) 3676, 3433, 3046, 2940, 1663, 1601, 1512, 1424, 1057 cm^{-1}. UV λ_{max} (CH_3OH) nm (log ε): 226 (2.9), 298 (1.2), 320 (1.1). EI-MS *m/z*: 359 $[M]^+$. ^{1}H NMR (CD_3OD) δ: 7.45 (1H, d, J=16.0 Hz, H-7), 7.13 (1H, d, J=1.5 Hz, H-2), 7.03 (1H, dd, J=1.5 and 8.5 Hz, H-6), 7.00 (1H, d, J=1.5 Hz, H-2'), 6.83 (1H, dd, J=1.5 and 8.6 Hz, H-6'), 6.80 (1H, d, J=8.5 Hz, H-5), 6.77 (1H, d, J=8.6 Hz, H-5'), 6.47 (1H, d, J=16.0 Hz, H-8), 4.73 (1H, dd, J=4.9 and 7.8 Hz, H-7'), 3.88 (3H, s, 3-OMe), 3.86 (3H, s, 3'-OMe), 3.54 (1H, dd, J=4.8 and 13.6 Hz, H-8'a), 3.44 (1H, dd, J=7.9 and 13.6 Hz, H-8'b); ^{13}C NMR (CD_3OD) δ: 170.0 (C-9), 150.5 (C-4'), 149.8 (C-3), 149.5 (C-4), 147.6 (C-3'), 142.8 (C-7), 128.7 (C-1), 123.8 (C-6), 120.5 (C-6'), 119.1 (C-8), 117.0 (C-5'), 116.5 (C-5), 112.0 (C-2), 111.3 (C-2'), 74.1 (C-7'), 56.9 (3 and 3'-OMe), 48.8 (C-8').

Compound 38. (E)-N-*[2-(4-Hydroxyphenyl)-2-propoxyethyl] ferulamide* (5 mg). Colourless oil. $[\alpha]^{25}_{D}$ = -2.0 (c 0.08, CH_3OH). IR ν_{max} ($CHCl_3$) 3625, 3522, 3400, 2943, 1705, 1673, 1594, 1512, 1425, 1334, 1019 cm^{-1}. UV λ_{max} (CH_3OH) nm (log ε): 280 (3.1). EI-MS: *m/z* 371 $[M]^+$. ^1H NMR (CD_3OD) δ: 7.46 (1H, d, J=19.5 Hz, H-7), 7.19 (2H, d, J=10.5, H-2' , H-6'), 7.15 (1H, d, J=2.5 Hz, H-2), 7.05 (1H, dd, J=2.5 and 9.5 Hz, H-6), 6.82 (2H, d, J=10.5 Hz, H-3' and H-5'), 6.81 (1H, d, J=9.5 Hz, H-5), 6.49 (1H, d, J=19.5 Hz, H-8), 4.37 (1H, dd, J=5.3 and 8.2 Hz, H-7'), 3.91 (3H, s, 3-OMe), 3.51 (1H, dd, J=5.3 and 14.0 Hz, H-8'a), 3.44 (1H, dd, J=8.2 and 14.0 Hz, H-8'b), 3.28 (2H, m, H-1″), 1.58 (2H, ses, J=7.6 Hz, H-2″), 0.92 (3H, t, J=7.6 Hz, H-3″). ^{13}C NMR (CD_3OD) δ: 169.7 (C-9), 158.8 (C-4'), 150.3 (C-3), 149.8 (C-4), 142.7 (C-7), 132.7 (C-1'), 129.6 (C-2' and C-6'), 128.8 (C-1), 123.8 (C-6), 119.2 (C-8), 117.0 (C-5), 116.8 (C-3' and C-5'), 112.2 (C-2), 81.5 (C-7'), 72.1 (C-1''), 56.9 (3-OMe), 47.7 (C-8'), 24.5 (C-2''), 11.5 (C-3'').

Compound 39. (E,E)-N,N-*Dityramin-4,4′-dihydroxy-3,5′-dimethoxy-β,3′-bicinnamamide* (3 mg).

Amorphous powder. IR ν_{max} ($CHCl_3$) 3684, 3595, 2927, 2857, 1740, 1598, 1509, 1455, 1057 cm^{-1}. UV λ_{max} (CH_3OH) nm (log ε): 336 (0.9), 315 (2.6), 246 (3.2). MALDI-MS *m/z*: 623 $[M-H]^-$. ^1H NMR (CD_3OD) δ: 7.59 (1H, s, H-7), 7.40 (1H, d, J=16.0 Hz, H-7′), 7.19 (1H, d, J=2.0, H-2'), 7.04 (2H, d, J=8.0 Hz, H-2″, H-6″), 6.91 (2H, d, J=8.0 Hz, H-2‴, H-6‴), 6.83 (1H, d, J=2.0 Hz, H-6'), 6.74 (1H, dd, J=2.0 and 8.0 Hz, H-6), 6.70 (2H, d, J=8.0 Hz, H-3″ and H-5″), 6.65 (1H, d, J=8.0 Hz, H-5), 6.62 (2H, d, J=8.0 Hz, H-3‴ and H-5‴), 6.52 (1H, d, J=2.0 Hz, H-2), 6.38 (1H, d, J=16.0, H-8'), 3.96 (3H, s, 3'-OMe), 3.47 (2H, t, J=6.8 Hz, H-8″), 3.43 (2H, t, J=6.8 Hz, H-8‴), 3.38 (3H, s, 3-OMe), 2.75 (2H, t, J=6.8 Hz, H-7″), 2.65 (2H, t, J=6.8 Hz, H-7‴). ^{13}C NMR (CD_3OD) δ: 170.4 (C-9), 169.5(C-9'), 157.3 (C-4'' and C-4‴), 150.6 (C-3), 149.9 (C-3'), 149.3 (C-4), 148.7 (C-4'), 141.9 (C-7'), 139.1 (C-7), 131.8 (C-1''), 131.6 (C-1‴), 131.2 (C-2'', C-2‴, C-6'', and C-6‴), 129.7(C-5'), 129.4 (C-8), 128.8 (C-1'), 127.0 (C-1), 125.1 (C-6'), 120.2 (C-8'), 116.8 (C-3'', C-3‴, C-5'', and C-5‴), 116.4 (C-5), 113.6 (C-2), 112.2 (C-2'), 57.2 (3'-OMe), 56.2 (3-OMe), 43.0 (C-8''), 36.2 (C-7''), 35.9 (C-7‴).

Compound 40. *7-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-N^2,N^3-bis(4-hydroxyphenethyl)-6-methoxy-1,2-dihydronaphthalene-2,3-dicarboxamide* (2 mg). Amorphous powder. $[\alpha]^{25}_{D}$ = 0.0 (c 0.20, CH_3OH). IR ν_{max} ($CHCl_3$) 3684, 3595, 3414, 3043, 2981, 1660, 1605, 1513, 1431, 1308, 945 cm^{-1}. UV λ_{max} (CH_3OH) nm (log ε): 223 (3.9), 284 (1.2), 333 (1.1). MALDI-MS

m/z: 623 $[M-H]^-$. ^1H NMR (CD_3OD) δ: 7.21 (1H, s, H-7), 6.97 (2H, d, J=8.5 Hz, H-2″ and H-6″), 6.88 (1H, s, H-2), 6.82 (2H, d, J=8.5 Hz, H-2‴ and H-6‴), 6.69 (1H, d, J=1.0 Hz, H-2′), 6.68 (2H, d, J=8.5 Hz, H-3″ and H-5″), 6.66 (1H, d, J=8.0 Hz, H-5′), 6.65 (2H, d, J=8.5 Hz, H-3‴ and H-5‴), 6.52 (1H, s, H-5), 6.41 (1H, dd, J=2.0 and 8.0 Hz, H-6′), 4.36 (1H, d, J=3.9 Hz, H-7′), 3.90 (3H, s, 3-OMe), 3.75 (3H, s, 3′-OMe), 3.70 (1H, d, J=3.9 Hz, H-8′), 3.42 (2H, t, J=6.8 Hz, H-8″), 3.23 (2H, t, J=6.8 Hz, H-8‴), 2.65 (2H, t, J=6.8 Hz, H-7″), 2.50 (2H, t, J=6.8 Hz, H-7‴). ^{13}C NMR (CD_3OD) δ: 175.0 (C-9'), 170.0 (C-9), 157.4 (C-4'' and C-4'''), 150.1 (C-3'), 148.7 (C-3), 147.2 (C-4), 146.0 (C-4'), 136.4 (C-1'), 135.1 (C-7), 133.0 (C-6), 131.9 (C-1''), 131.6 (C-1'''), 131.3 (C-2'' and C-6''), 131.2 (C-2''' and C-6'''), 128.0 (C-8), 125.3 (C-1), 121.9 (C-6'), 117.7 (C-5), 116.7 (C-3'', C-3''', C-5'', and C-5'''), 116.2 (C-5'), 113.7 (C-2), 113.0 (C-2'), 57.1 (3-OMe), 56.8 (3'-OMe), 50.3 (C-8'), 48.1 (C-7'), 43.0 (C-8''), 42.9 (C-8'''), 36.2 (C-7''), 35.9 (C-7''').

Compound 41. (E)-*3-methoxyphenyl 3,4,5-trimethoxycinnamate* (5 mg). Colourless oil. IR ν_{max} ($CHCl_3$) 2927, 1717, 1676, 1431 cm^{-1}. UV (CH_3OH) λ_{max} (log ε) nm: 241, 308. HREI-MS: *m/z* 344.1256 $[M]^+$. ^1H NMR ($CDCl_3$) δ: 7.77 (1H, d, J=15.9 Hz, H-7), 7.30 (1H, t, J=8.2 Hz, H-5'), 6.82 (2H, s, H-2 and H-6), 6.79 (1H, dd, J=2.0 and 8.0 Hz, H-4'), 6.77 (1H, dd, J=2.0 and 8.0 Hz, H-6'), 6.73 (1H, t, J=2.2 Hz, H-2'), 6.53 (1H, d, J=15.9 Hz, H-8), 3.91 (9H, s, 3,4, and 5-OMe), 3.82 (3H, s, 3'-OMe). ^{13}C NMR ($CDCl_3$) δ: 165.3 (C-9), 160.6 (C-3'), 153.6 (C-3 and C-5), 151.8 (C-1'), 146.6 (C-7), 146.3 (C-4), 129.9 (C-5'), 129.5 (C-1), 116.5 (C-8), 113.9 (C-6'), 111.7 (C-4'), 107.7 (C-2'), 105.6 (C-2 and C-6), 61.0 (4-OMe), 56.2 (3 and 5-OMe), 55.4 (3'- OMe).

Compound 42. (E)-*3-hydroxyphenyl 3,4,5-trimethoxycinnamate* (4 mg). Colourless oil. IR ν_{max} ($CHCl_3$) 3585, 1715, 1650, 1580, 1510 cm^{-1}. UV (CH_3OH) λ_{max} (log ε) nm: 219, 231, 290, 319. HREI-MS: *m/z* 330.1100 $[M]^+$. ^1H NMR ($CDCl_3$) δ: 7.78 (1H, d, J=16.0 Hz, H-7), 7.25 (1H, t, J=8.5 Hz, H-5'), 6.82 (2H, s, H-2 and H-6), 6.74 (1H, dd, J=2.0 and 8.5 Hz, H-4'), 6.72 (1H, dd, J=2.0 and 8.5 Hz, H-6'), 6.68 (1H, t, J=2.0 Hz, H-2'), 6.53 (1H, d, J=16.0 Hz, H-8), 3.91 (9H, s, 3, 4, and 5-OCH_3). ^{13}C-NMR ($CDCl_3$) δ: 165.4 (C-9), 156.6 (C-3'), 153.5 (C-3 and C-5), 152.1 (C-1'), 146.7 (C-7), 140.8 (C-4), 130.1 (C-5'), 129.6 (C-1), 116.3 (C-8), 113.8 (C-6'), 113.0 (C-4'), 109.3 (C-2'), 105.5 (C-2 and C-6), 61.0 (4-OMe), 56.2 (3 and 5-OMe),

Compound 43. (E)-*3-hydroxyphenyl sinapate* (4.1 mg). Colourless oil. IR ν_{max} ($CHCl_3$) 3330, 3047, 1726, 1682, 1515 cm^{-1}. UV (CH_3OH) λ_{max} (log ε) nm: 310. HREI-MS: *m/z* 316.0943 $[M]^+$. ^1H NMR ($CDCl_3$) δ: 7.77 (1H, d, J=16.2 Hz, H-7), 7.22 (1H, t, J=8.4 Hz, H-5'), 6.82 (2H, s, H-2 and H-6), 6.70 (2H, d, J=8.4 Hz, H-4' and H-6'), 6.66 (1H, d, J=2.1 Hz, H-2'), 6.47 (1H, d, J=16.2 Hz, H-8), 3.92 (6H, s, 3 and 5-OMe). ^{13}C NMR ($CDCl_3$) δ: 166.0 (C-9), 156.9 (C-3'), 151.6 (C-3 and C-5), 147.3 (C-7 and C-1'), 137.6 (C-4), 130.0 (C-1 and C-5'), 114.7 (C-8), 113.4 (C-6'), 113.2 (C-4'), 109.3 (C-2'), 105.4 (C-2 and C-6), 56.3 (3 and 5-OMe).

Compound 44. (E)-*3-methoxyphenyl sinapate* (3 mg). IR ν_{max} ($CHCl_3$) 2927, 1719, 1676, 1431 cm^{-1}. UV (CH_3OH) λ_{max} (log ε) nm: 241, 308. EI-MS: *m/z* 270 $[M]^+$ (8), 253 $[M\text{-}OH]^+$ (16), 147 $[M\text{-}C_7H_7O_2]^+$ (48), 123 $[M\text{-}C_9H_7O_2]^+$ (25). ^1H NMR ($CDCl_3$) δ: 7.79 (1H, d, J=15.9 Hz, H-7), 7.29 (1H, t, J=7.2 Hz, H-5'), 6.83 (2H, s, H-2 and H-6), 6.82 (1H, dd, J=2.2 and 7.2 Hz, H-6'), 6.68 (1H, dd, J=2.2 and 7.2 Hz, H-4'), 6.63 (1H, t, J=2.2 Hz, H-2'), 6.52 (1H, d, J=15.9 Hz, H-8), 3.92 (6H, s, 3 and 5-OMe), 3.91 (3H, s, 3'-OMe). ^{13}C-NMR ($CDCl_3$) δ: 167.6 (C-9), 162.0 (C-4), 161.7 (C-1'), 153.4 (C-3'), 148.3 (C-7), 131.5 (C-2 and C-6), 130.8 (C-5'), 127.0 (C-1), 116.9 (C-3 and C-5), 114.9 (C-8), 114.2 (C-4'), 113.9 (C-6'), 108.5 (C-2'), 56.3 (3 and 5-OMe), 56.0 (3'-OMe).

Compound 45. *2'-methoxyphenyl dihydrocinnamate* (10 mg). IR ν_{max} ($CHCl_3$) 3330, 3047, 1726, 1682, 1515 cm^{-1}. UV (CH_3OH) λ_{max} (log ε) nm: 310. EI-MS: *m/z* 256 $[M]^+$ (3), 225 $[M\text{-}OCH_3]^+$ (14), 207 $[M\text{-}C_6H_5O_2]^+$ (29), 109 $[M\text{-}C_{11}H_{11}O_4]^+$ (54). ^1H NMR ($CDCl_3$) δ: 7.29 (4H, m, H-2, H-3, H-5 e H-6), 7.20 (2H, m, H-4 and H-6'), 7.05 (1H, d, J=9,0 Hz, H-3'), 6.92 (2H, m, H-4' and H-5'), 3.76 (3H, s, OMe), 3.04 (2H, t, J=6,4 Hz, H-7), 2.92 (2H, t, J=6,4 Hz, H-8). ^{13}C NMR ($CDCl_3$) δ: 172.0 (C-9), 157.1 (C-2'), 148.8 (C-1'), 132.1 (C-1), 128.7 (C-3 and C-5), 127.7 (C-2 and C-6), 126.0 (C-4), 124.0 (C-4'), 122.0 (C-5'), 118.2 (C-6'), 115.5 (C-3'), 58.2 (2'-OMe), 36.0 (C-8), 31.0 (C-7).

Compound 46. (E)-*4'-acetylphenyl 4-hydroxycinnamate* (11 mg). Colourless oil. IR ν_{max} ($CHCl_3$) 3350, 3010, 1725, 1720, 1599 cm^{-1}. UV (CH_3OH) λ_{max} (log ε) nm: 312. EI-MS: *m/z* 282 $[M]^+$ (3), 265 $[M\text{-}OH]^+$ (14), 239 $[M\text{-}CH_3CO]^+$ (48), 147 $[M\text{-}C_8H_7O_2]^+$ (29), 135 $[M\text{-}C_9H_7O_2]^+$ (54). ^1H NMR ($CDCl_3$) δ: 8.03 (2H, d, J=9.0 Hz, H-3' and H-5'), 7.84 (1H, d, J=15.5 Hz, H-7), 7.51 (2H, d, J=8.5 Hz, H-2 and H-6), 7.28 (2H, d, J=9.0 Hz, H-2' and H-6'), 6.88 (2H, d, J=8.5 Hz, H-3 and H-5), 6.49 (1H, d, J=15.5 Hz, H-8), 2.62 (3H, s, Me). ^{13}C-NMR

($CDCl_3$) δ: 197.8 (C-7'), 165.5 (C-9), 158.0 (C-4), 155.0 (C-1'), 147.2 (C-7), 134.5 (C-4'), 130.7 (C-2 and C-6), 130.2 (C-3' and C-5'), 127.5 (C-1), 122.1 (C-2' and C-6'), 116.3 (C-3 and C-5), 114.4 (C-8), 26.8 (Me).

Compound 47. (E)-*3'-acethylphenyl 3,4,5-trimethoxycinnamate* (3 mg). Colourless oil. IR ν_{max} ($CHCl_3$) 3300, 1650, 1580, 1510 cm^{-1}. UV (CH_3OH) λ_{max} (log ε) nm: 219, 231, 290, 319. EI-MS: *m/z* 356 $[M]^+$ (5), 325 $[M\text{-}OCH_3]^+$ (15), 221 $[M\text{-}C_8H_7O_2]^+$ (51), 135 $[M\text{-}C_{12}H_{13}O_4]^+$ (20). ^{1}H-NMR ($CDCl_3$) δ: 7.84 (1H, d, J=7.5 Hz, H-4'), 7.80 (1H, d, J=16.0 Hz, H-7), 7.75 (1H, d, J=1.5 Hz, H-2'), 7.51(1H, t, J=7.5 Hz, H-5'), 7.38 (1H, dd, J=1.5 and 7.5 Hz, H-6'), 6.82 (2H, s, H-2 and H-6), 6.54 (1H, d, J=16.0 Hz, H-8), 3.90 (9H, s, 3, 4, and 5-OMe), 2.61 (3H, s, Me). ^{13}C NMR ($CDCl_3$) δ: 196.9 (C-7'), 165.0 (C-9), 153.5 (C-3 and C-5), 151.0 (C-1'), 147.0 (C-7), 140.6 (C-4), 138.5 (C-3'), 129.6 (C-5'), 129.4 (C-1), 126.4 (C-6'), 125.6 (C-4'), 121.4 (C-2'), 115.9 (C-8), 105.5 (C-2 and C-6), 60.9 (4-OMe), 56.1 (3 and 5-OMe), 26.6 (Me).

Compound 48. (E)-*4'-acethylphenyl sinapate* (2 mg). Colourless oil. IR ν_{max} ($CHCl_3$) 3330, 3047, 1726, 1682, 1515 cm^{-1}. UV (CH_3OH) λ_{max} (log ε) nm: 310. EI-MS: *m/z* 342 $[M]^+$ (3), 325 $[M\text{-}OH]^+$ (14), 311 $[M\text{-}OCH_3]^+$ (48), 207 $[M\text{-}C_8H_7O_2]^+$ (29), 135 $[M\text{-}C_{11}H_{11}O_4]^+$ (54). ^{1}H NMR ($CDCl_3$) δ: 8.02 (2H, d, J=8.8 Hz, H-3' and H-5'), 7.80 (1H, d, J=15.8 Hz, H-7), 7.27 (2H, d, J=8.8 Hz, H-2' and H-6'), 6.84 (2H, s, H-2 and H-6), 6.48 (1H, d, J=15.8 Hz, H-8), 3.94 (6H, s, 3 and 5-OMe), 2.61 (3H, s, Me). ^{13}C NMR ($CDCl_3$) δ: 195.8 (C-7'), 165.0 (C-9), 154.7 (C-3 and C-5), 154.6 (C-1'), 147.3 (C-4), 147.6 (C-7), 134.7 (C-4'), 134.6 (C-1), 130.0 (C-2' and C-6'), 121.8 (C-3' and C-5') 114.4 (C-8), 105.5 (C-2 and C-6), 56.4 (3 and 5-OMe), 26.6 (Me).

Compound 49. (E)-*4'-acethylphenyl 3,4,5-trimethoxycinnamate* (2 mg). Colourless oil. IR ν_{max} ($CHCl_3$) 2927, 1727, 1676, 1431 cm^{-1}. UV (CH_3OH) λ_{max} (log ε) nm: 245, 318. EI-MS: *m/z* 356 $[M]^+$ (3), 325 $[M\text{-}OCH_3]^+$ (16), 221 $[M\text{-}C_8H_7O_2]^+$ (48), 135 $[M\text{-}C_{12}H_{13}O_4]^+$ (25). ^{1}H-NMR ($CDCl_3$) δ: 8.01 (2H, d, J=8.0 Hz, H-3' and H-5'), 7.80 (1H, d, J=15.6 Hz, H-7), 7.26 (2H, d, J=8.0 Hz, H-2' and H-6'), 6.83 (2H, s, H-2 and H-6), 6.53 (1H, d, J=15.6 Hz, H-8), 3.89 (9H, s, 3, 4, and 5-OMe), 2.59 (3H, s, Me). ^{13}C-NMR ($CDCl_3$) δ: 196.0 (C-7'), 164.8 (C-9), 154.6 (C-1'), 153.5 (C-3 and C-5), 147.3 (C-7), 140.3 (C-4), 134.7 (C-4'), 130.0 (C-2' and C-6'), 129.4 (C-1), 121.8 (C-3' and C-5'), 115.9 (C-8), 105.6 (C-2 and C-6), 61.1 (4-OMe), 56.2 (3 and 5-OMe), 26.6 (Me).

Compound 50. (E)-*3'-(1-hydroxyethyl)-phenyl 3,4,5-trimethoxycinnamate* (5 mg). Colourless oil. IR ν_{max} ($CHCl_3$) 3250, 1580, 1510 cm^{-1}. UV (CH_3OH) λ_{max} (log ε) nm: 219, 319. EI-MS: *m/z* 358 $[M]^+$ (3), 341 $[M\text{-}OH]^+$ (14), 327 $[M\text{-}OCH_3]^+$ (48), 301 $[M\text{-}C_2H_5CO]^+$ (29), 221 $[M\text{-}C_8H_9O_2]^+$, 137 $[M\text{-}C_{12}H_{13}O_4]^+$ (54). ^1H NMR ($CDCl_3$) δ: 7.81 (1H, d, J=16.0 Hz, H-7), 7.37 (1H, t, J=7.6 Hz, H-5'), 7.25 (1H, d, J=7.6 Hz, H-4'), 7.18 (1H, t, J=2.0 Hz, H-2'), 7.03 (1H, dd, J=2.0 and 7.6 Hz, H-6'), 7.00 (2H, s, H-2 and H-6), 6.69 (1H, d, J=16.0 Hz, H-8), 4.85 (1H, d, J=6.4 Hz, H-7'), 3.88 (6H, s, 3 and 5-OMe), 3.81 (3H, s, 4-OMe), 1.44 (3H, d, J=6.4 Hz, Me). ^{13}C NMR ($CDCl_3$) δ: 167.7 (C-9), 155.4 (C-3 and C-5), 152.9 (C-1'), 148.6 (C-7), 142.0 (C-4), 131.9 (C-1), 131.8 (C-3'), 130.8 (C-5') 124.4 (C-4'), 121.8 (C-6'), 120.3 (C-2'), 118.0 (C-8), 107.6 (C-2 and C-6), 70.8 (C-7'), 61.7 (4-OMe), 57.3 (3 and 5-OMe), 26.1 (Me).

Compound 51. (E)-*2-hydroxyethyl 3,4,5-trimethoxycinnamate* (4 mg)**.** Colourless oil. IR ν_{max} ($CHCl_3$) 3350, 3010, 1725, 1599 cm^{-1}. UV (CH_3OH) λ_{max} (log ε) nm: 312. HREI-MS: *m/z* 282.1100 $[M]^+$. ^1H NMR ($CDCl_3$) δ: 7.63 (1H, d, J=16.0 Hz, H-7), 6.75 (2H, s, H-2 and H-6), 6.38 (1H, d, J=16.0 Hz, H-8), 4.35 (2H, m, H-1'), 3.90 (2H, m, H-2'), 3.88 (9H, s, 3, 4, and 5-OMe). ^{13}C NMR ($CDCl_3$) δ: 167.2 (C-9), 153.4 (C-3 and C-5), 145.4 (C-7), 141.8 (C-4), 129.7 (C-1), 116.8 (C-8), 105.3 (C-2 and C-6), 66.2 (C-1'), 61.4 (C-2'), 60.9 (4-OMe), 56.1 (3 and 5-OMe).

Compound 52. *2-hydroxyethyl senapate* (11 mg). Colourless oil. IR ν_{max} ($CHCl_3$) 3540, 1718, 1580, 1510 cm^{-1} . UV (CH_3OH) λ_{max} (log ε) nm: 219, 319. HREI-MS: *m/z* 268.0950 $[M]^+$. ^1H-NMR ($CDCl_3$) δ: 7.64 (1H, d, J=15.6 Hz, H-7), 6.78 (2H, s, H-2 and H-6), 6.35 (1H, d, J=15.6 Hz, H-8), 4.36 (3H, m, H-1'), 3.92 (6H, s, 3 and 5-OMe), 3.82 (3H, m, H-2'). ^{13}C NMR ($CDCl_3$) δ: 167.4 (C-9), 147.2 (C-3 and C-5), 145.7 (C-7), 138.8 (C-4), 129.7 (C-1), 115.3 (C-8), 105.1 (C-2 and C-6), 66.1 (C-1'), 61.5 (C-2'), 56.3 (3 and 5-OMe).

Compound 53. (E)-*2-acetoxyethy 3,4,5-trimethoxycinnamate* (7 mg)**.** Colourless oil. IR ν_{max} ($CHCl_3$) 3606, 3317, 1721, 1715, 1580, 1510 cm^{-1}. UV (CH_3OH) λ_{max} (log ε) nm: 219, 319. HREI-MS: *m/z* 324.1205 $[M]^+$. ^1H NMR ($CDCl_3$) δ: 7.64 (1H, d, J=16.0 Hz, H-7), 6.77 (2H, s, H-2 and H-6), 6.38 (1H, d, J=16.0 Hz, H-8), 4.42 (2H, m, H-1'), 4.36 (2H, m, H-2'), 3.90 (9H, s, 3,4, and 5-OMe), 2.11 (3H, s, COMe).^{13}C NMR ($CDCl_3$) δ: 170.8 (CO<u>Me</u>), 166.7 (C-9), 153.5 (C-3 and C-5), 145.5 (C-7), 142.0 (C-4), 129.8 (C-1), 116.7 (C-8), 105.4 (C-2 and C-6), 62.3 (C-1' and C-2'), 61.0 (4-OMe), 56.2 (3 and 5-OMe), 20.9 (2-CO<u>Me</u>).

Compound 54. *2-hydroxyethyl caffeate* (4 mg). Colourless oil. IR ν_{max} ($CHCl_3$) 3600, 3307, 1718, 1550, 1510 cm^{-1}. UV (CH_3OH) λ_{max} (log ε) nm: 219, 319. HREI-MS: *m/z* 224.0688 $[M]^+$. ^1H NMR ($CDCl_3$) δ: 7.58 (1H, d, J=16.5 Hz, H-7), 7.04 (1H, d, J= 2.0 H-2), 6.95 (1H, dd, J=2.0 and 7.5 Hz, H-6), 6.77 (1H, d, J=7.5 Hz, H-5), 6.29 (1H, d, J=16.5 Hz, H-8), 4.23 (2H, m, H-1'), 3.78 (2H, m, H-2'). ^{13}C NMR ($CDCl_3$) δ: 169.8 (C-9), 150.1 (C-3), 147.6 (C-7), 147.3 (C-4), 128.2 (C-1), 123.4 (C-6), 117.0 (C-8), 115.6 (C-5), 115.5 (C-2), 67.4 (C-1'), 61.7 (C-2').

Compound 55. (E)-*4-(4-(2-carboxyvinyl)phenoxy)benzoic acid* (5 mg). MALDI-MS: *m/z* 285 $[M+H]^+$. ^1H NMR (CD_3OD) δ: 8.05 (2H, d, J=8.4 Hz, H-2' and H-6'), 7.45 (1H, d, J=15.6 Hz, H-7), 7.24 (2H, d, J=8.1 Hz, H-2 and H-6), 7.12 (2H, d, J=8.4 Hz, H-3' and H-5'), 6.81 (2H, d, J=8.1 Hz, H-3 and H-5), 7.24 (1H, d, J=15.6 Hz, H-8). ^{13}C NMR (CD_3OD) δ: 171.3 (C-9), 170.6 (C-7'), 162.2 (C-4'), 156.2 (C-4) 148.0 (C-7), 130.0 (C-2' and C-6'), 128.3 (C-1), 126.1 (C-2 and C-6), 123.4 (C-1'), 117.4 (C-3, C-3', C-5, and C-5'), 115.6 (C-8).

Compound 56. *5,6,7,8,4'-pentamethoxyflavone* (3 mg). EI-MS: *m/z* 372 $[M]^+$ (34), 357 $[M-CH_3]^+$ (56), 341 $[M-OCH_3]^+$ (100). ^1H NMR ($CDCl_3$) δ: 7.88 (2H, d, J=9.0 Hz, H-2' and H-6'), 7.02 (2H, d, J=9.0 Hz, H-3' and H-5'), 6.60 (1H, s, H-3), 4.10 (3H, s, 5-OMe), 4.02 (3H, s, 8-OMe), 3.95 (6H, s, 6 and 7-OMe), 3.89 (3H, s, 4'-OMe). ^{13}C NMR ($CDCl_3$) δ: 177.3 (C-4), 162.2 (C-2), 161.1 (C-4'), 151.3 (C-7), 148.4 (C-8), 147.7 (C-9), 144.0 (C-5), 138.1 (C-6), 127.8 (C-2' and C-6'), 123.8 (C-1'), 114.8 (C-10), 114.4 (C-3' and C-5'), 106.7 (C-3), 62.3 (5-OMe), 62.1 (6-OMe), 61.9 (7-OMe), 61.7 (8-OMe), 55.5 (4'-OMe).

Compound 57. *5,6,7,8,3',4'-esamethoxyflavone* (5 mg). EI-MS: *m/z* 402 $[M]^+$ (26), 387 $[M-CH_3]^+$ (58), 371 $[M-OCH_3]^+$ (100). ^1H NMR ($CDCl_3$) δ: 7.57 (1H, dd, J=2.1 and 9.1 Hz, H-6'), 7.41 (1H, d, J=2.1 Hz, H-2'), 6.99 (1H, d, J=9,0 Hz, H-5'), 6.62 (1H, s, H-3), 4.11 (3H, s, 5-OMe), 4.03 (3H, s, 8-OMe), 3.98 (3H, s, 6-OMe), 3.97 (3H, s, 7-OMe), 3.96 (6H, s, 3' and 4'-OMe). ^{13}C NMR ($CDCl_3$) δ: 177.4 (C-4), 161.0 (C-2), 151.9 (C-4'), 151.4 (C-7), 149.3 (C-3'), 147.7 (C-9), 144.0 (C-5), 138.0 (C-6 and C-8), 124.0 (C-1'), 119.6 (C-6'), 114.8 (C-10), 111.0 (C-5'), 108.7 (C-2'), 106.7 (C-3), 62.3 (5-OMe), 62.0 (6-OMe), 61.8 (7-OMe), 61.7 (8-OMe), 56.1 (3'-OMe) 56.0 (4'-OMe).

Compound 58. *5-hydroxy-6,7,8,3', 4'-pentamethoxyflavone* (5 mg). EI-MS: *m/z* 388 $[M]^+$ (100), 373 $[M-CH_3]^+$ (96), 357 $[M-OCH_3]^+$ (55), 343 $[M-CH_3-OCH_3]^+$ (6,4). UV (CH_3OH)

λ_{max} (log ε) nm: 205, 251, 280, 337. ^{1}H NMR ($CDCl_3$) δ: 7.58 (1H, dd, J=2.1 and 9.0 Hz, H-6'), 7.42 (1H, d, J=2.1 Hz, H-2'), 6.99 (1H, d, J=9.0 Hz, H-5'), 6.61 (1H, s, H-3), 4.12 (3H, s, 8-OMe), 3.99 (6H, s, 3' and 4'-OMe), 3.97 (3H, s, 7-OMe), 3.96 (3H, s, 6-OMe). ^{13}C NMR ($CDCl_3$) δ: 179.3 (C-4), 158.8, (C-2), 152.9 (C-7), 149.1 (C-5), 149.0 (C-4'), 145.6 (C-3'), 142.9 (C-9), 136.2 (C-6), 132.9 (C-8), 123.7 (C-1'), 121.6 (C-6'), 114.6 (C-2'), 110.5 (C-5'), 107.5 (C-10), 106.5 (C-3), 62.1 (6-OMe), 61.7 (7-OMe), 61.2 (8-OMe), 56.1 (3'-OMe), 56.0 (4'-OMe).

Compound 59. *4'-hydroxy-5,6,7,8,3'-pentamethoxyflavone* (7 mg). EI-MS: *m/z* 388 $[M]^+$ (14), 373 $[M\text{-}CH_3]^+$ (100), 357 $[M\text{-}OCH_3]^+$ (10). UV (CH_3OH) λ_{max} (log ε) nm: 248, 268, 272, 340. ^{1}H NMR ($CDCl_3$) δ: 7.52 (1H, dd, J=1.5 and 9.0 Hz, H-6'), 7.39 (1H, d, J=1.5 Hz, H-2'), 7.04 (1H, d, J=9.0 Hz, H-5'), 6.60 (1H, s, H-3), 4.04 (3H, s, 8-OMe), 4.02 (3H, s, 5-OMe), 3.99 (3H, s, 3'-OMe), 3.96 (6H, s, 6 and 7-OMe). ^{13}C NMR ($CDCl_3$) δ: 177.4 (C-4), 161.0, (C-2), 151.9 (C-4'), 151.4 (C-7), 149.3 (C-3'), 147.7 (C-9), 144.0 (C-5), 106.7 (C-3), 138.0 (C-6 and C-8), 124.0 (C-1'), 119.6 (C-6'), 114.8 (C-10), 111.0 (C-5'), 108.7 (C-2'), 62.3 (5-OMe), 62.0 (6-OMe), 61.8(7-OMe), 61.7 (8-OMe), 56.1, (3'-OMe).

Compound 67. *Seselin* (40 mg). EI-MS: *m/z* 228 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 7.54 (1H, d, J=9.0 Hz, H-4), 7.15 (1H, d, J=8.4 Hz, H-5), 6.81 (1H, d, J=9.6 Hz, H-1'), 6.65 (1H, d, J=8.4 Hz, H-6), 6.15 (1H, d, J=9.6 Hz, H-3), 5.67 (1H, d, J=9.6 Hz, H-2'), 1.41 (6H, s, H-4' and H-5'). ^{13}C NMR ($CDCl_3$) δ: 161.1, (C-2), 156.5 (C-8a), 150.3 (C-7), 144.1 (C-4), 130.9 (C-2'), 128.0 (C-5), 115.1 (C-6 and C-4'), 113.7 (C-4a), 112.7 (C-3), 109.4 (C-8), 77.8 (C-3'), 28.3 (C-4' and C-5').

Compound 68. *5-methoxyseselin* (45 mg). EI-MS: *m/z* 258 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 7.91 (1H, d, J=9.4 Hz, H-4), 6.75 (1H, d, J=10.0 Hz, H-1'), 6.20 (1H, s, H-6), 6.09 (1H, d, J=9.4 Hz, H-3), 5.54 (1H, d, J=10.0 Hz, H-2'), 3.84 (3H, s, 5-OMe), 1.42 (6H, s, H-4' and H-5'). ^{13}C NMR ($CDCl_3$) δ: 161.5 (C-2), 157.6 (C-7), 156.7 (C-5), 151.2 (C-8a), 139.2 (C-4), 127.7 (C-2'), 115.1 (C-1'), 110.6 (C-3), 103.9 (C-4a), 102.8 (C-8), 95.5 (C-6), 78.2 (C-3'), 56.2 (5-OMe), 28.3 (C-4' and C-5').

Compound 69. *6-(3,3-dimethylallyl)seselin* (125 mg). HRESI-MS : *m/z* 297.1491 $[M+H]^+$. ^{1}H NMR ($CDCl_3$) δ: 7.54 (1H, d, J=9.6 Hz, H-4), 7.02 (1H, s, H-5), 6.84 (1H, d, J=10.2 Hz, H-1''), 6.16 (1H, d, J=9.6 Hz, H-3), 5.68 (1H, d, J=10.2 Hz, H-2''), 5.22 (1H, t, J=7.2 Hz, H-2'),

3.24 (2H, d, J=7.2 Hz, H-1'), 1.72 (3H, s, H-5'), 1.70 (3H, s, H-4'), 1.43 (6H, s, H-4'' and H-5''). ^{13}C NMR ($CDCl_3$) δ: 161.5 (C-2), 154.2 (C-7), 148.9 (C-8a), 144.3 (C-4), 133.2 (C-3'), 130.6 C-2''), 127.3 (C-5 and C-4a), 126.7 (C-6), 121.9 (C-2'), 115.6 (C-1''), 112.6 (C-3), 109.1 (C-8), 79.0 (C-3''), 28.3 (C-4'' and C-5''), 28.0 (C-1'), 26.0 (C-5'), 18.1 (C-4').

Compound 70. *4,9-dihydroxy-2-(2-hydroxypropan-2-yl)-11-methoxy-10-methyl-2,3-dihydrofuro[3,2-b]acridin-5(10H)-one.* (3 mg). HRESI-MS: m/z 370.1291 $[M-H]^-$. ^{1}H NMR (CD_6CO) δ: 7.79 (1H, dd, J=1.6 and 8.0 Hz, H-8), 7.27 (1H, dd, J=1.6 and 8.0 Hz, H-6), 7.15 (1H, t, J=8.0 Hz, H-7), 4.87 (1H, dd, J=8.0 and 9.2 Hz, H-2'), 3.85 (3H, s, 4-OMe), 3.82 (3H, s, N-Me), 3.20 (2H, m, H-1'a,b), 1.33 (3H, s, H-4'), 1.29 (3H, s, H-5'). ^{13}C NMR (CD_6CO) δ: 182.3 (C-9), 159.8 (C-3), 154.2 (C-1), 147.8 (C-5), 143.1 (C-4a), 137.3 (C-10a), 125.7 (C-4), 124.8 (C-8a), 122.9 (C-7), 119.7 (C-6), 116.8 (C-8), 107.5 (C-2), 107.3 (C-9a), 92.4 (C-2'), 72.0 (C-3'), 60.6 (4-OMe), 46.5 (N-Me), 27.5 (C-1'), 25.5 (C-5'), 24.2 (C-4').

Compound 71. *Glycocitrine IV* (17 mg). HRESI-MS: *m/z* 354.0598 $[M-H]^-$. ^{1}H NMR ($CDCl_3$) δ: 7.79 (1H, d, J=7.8 Hz, H-8), 7.06 (1H, d, J=7.8 Hz, H-6), 7.01 (1H, t, J=7.8 Hz, H-7), 5.29 (1H, t, J=7.2 Hz, H-2'), 3.76 (3H, s, 4-OMe), 3.75 (3H, s, N-Me), 3.39 (2H, d, J=7.2 Hz, H-1'), 1.78 (3H, s, H-5'), 1.66 (3H, s, H-4'). ^{13}C NMR ($CDCl_3$) δ: 182.9 (C-9), 157.8 (C-1), 155.2 (C-3), 147.7 (C-5), 139.4 (C-4a), 136.9 (C-10a), 132.3 (C-3'), 128.0 (C-4), 124.8 (C-8a), 122.8 (C-7), 122.3 (C-2'), 119.7 (C-6), 117.1 (C-8), 108.9 (C-2), 106.3 (C-9a), 60.2 (4-OMe), 45.1 (N-Me), 25.9 (C-5'), 21.9 (C-1'), 18.0 (C-4'),

Compound 72. *Citrusinine II* (20 mg). HRESI-MS: *m/z* 288.0335 $[M+H]^+$. ^{1}H NMR (CD_3OD) δ: 7.51 (1H, d, J=7.8 Hz, H-8), 6.93 (1H, d, J=7.8 Hz, H-6), 6.86 (1H, t, J=7.8 Hz, H-7), 6.01 (1H, s, H-2), 3.59 (3H, s, 4-OMe), 3.52 (3H, s, N-Me). ^{13}C NMR (CD_3OD) δ: 182.2 (C-9), 159.4 (C-1), 158.0 (C-3), 148.1 (C-5), 142.4 (C-4a), 137.1 (C-10a), 128.9 (C-4), 124.5 (C-8a), 122.9 (C-7), 119.7 (C-6), 116.1 (C-8), 106.3 (C-9a), 97.0 (C-2), 59.8 (4-OMe), 45.6 (N-Me).

Compound 73. *Citbrasine* (6 mg). HRESI-MS: *m/z* 300.0124 $[M-H]^-$. ^{1}H NMR (CD_3OD) δ: 7.87 (1H, d, J=7.8 Hz, H-8), 7.17 (1H, d, J=7.8 Hz, H-6), 7.10 (1H, t, J=7.8 Hz, H-7), 4.12 (3H, s, 2-OMe), 3.94 (3H, s, 3-OMe), 3.83 (3H, s, 4-OMe), 3.80 (3H, s, N-Me). ^{13}C NMR (CD_3OD) δ: 183.1 (C-9), 154.7 (C-1), 152.1 (C-2), 147.0 (C-5), 138.9 (C-3), 137.6 (C-10a),

134.5 (C-4), 124.6 (C-8a), 122.9 (C-7), 120.5 (C-6), 118.0 (C-8), 108.3 (C-9a), 61.9 (4-OMe), 61.3 (3-OMe), 61.0 (2-OMe), 46.6 (N-Me).

Compound 74. *Junosine* (7 mg). HRESI-MS: *m/z* 324.0471 $[M-H]^-$. ^1H NMR (CD_3OD) δ: 7.77 (1H, d, J=7.8 Hz, H-8), 7.09 (1H, d, J=7.8 Hz, H-6), 7.03 (1H, t, J=7.8 Hz, H-7), 6.32 (1H, s, H-4), 5.24 (1H, t, J=7.2 Hz, H-2'), 3.97 (3H, s, N-Me), 3.29 (2H, d, J=7.2 Hz, H-1'), 1.78 (3H, s, H-5'), 1.65 (3H, s, H-4'). ^{13}C NMR (CD_3OD) δ: 180.3 (C-9), 163.2 (C-1), 161.2 (C-3), 147.2 (C-5), 145.8 (C-10a), 133.8 (C-3'), 130.4 (C-4a), 124.8 (C-8a), 122.8 (C-7), 121.8 (C-2'), 119.2 (C-6), 116.3 (C-8), 108.4 (C-2), 104.5 (C-9a), 90.2 (C-4), 40.1 (N-Me), 24.8 (C-4'), 21.0 (C-1'), 16.7 (C-5').

Compound 75. *Oriciacridone* (21 mg). HRESI-MS: *m/z* 324.0219 $[M-H]^-$. ^1H NMR (CD_3OD) δ: 7.62 (1H, d, J=7.6 Hz, H-8), 7.08 (1H, d, J=7.6 Hz, H-6), 7.04 (1H, t, J=7.8 Hz, H-7), 6.17 (1H, s, H-2), 5.30 (1H, m, H-2'), 3.56 (3H, s, N-Me), 3.41 (2H, d, J=7.8 Hz, H-1'), 1.73 (3H, s, H-5'), 1.66 (3H, s, H-4'). ^{13}C NMR (CD_3OD) δ: 182.5 (C-9), 164.3 (C-3), 162.1 (C-1), 151.1 (C-4a), 148.7 (C-5), 138.2 (C-10a), 131.0 (C-3'), 124.6 (C-8a), 124.8 (C-2'), 122.7 (C-7), 119.2 (C-6), 115.7 (C-8), 107.9 (C-4), 106.7 (C-9a), 96.7 (C-2), 46.5 (N-Me), 25.9 (C-1'), 24.7 (C-5'), 17.0 (C-4').

Compound 76. *Citrusinine I* (10 mg). HRESI-MS: *m/z* 288.0122 $[M-H]^-$. ^1H NMR (CD_3OD) δ: 7.74 (1H, d, J=7.8 Hz, H-8), 7.06 (1H, d, J=7.8 Hz, H-6), 7.03 (1H, t, J=7.8 Hz, H-7), 6.29 (1H, s, H-2), 3.85 (3H, s, 4-OMe), 3.75 (3H, s, 3-OMe), 3.71 (3H, s, N-Me). ^{13}C NMR (CD_3OD) δ: 182.7 (C-9), 160.0 (C-1), 159.4 (C-3), 148.0 (C-5), 141.1 (C-4a), 137.6 (C-10a), 128.9 (C-4), 124.7 (C-8a), 122.9 (C-7), 120.1 (C-6), 116.7 (C-8), 106.5 (C-9a), 93.7 (C-2), 60.5 (4-OMe), 56.3 (3-OMe), 46.3 (N-Me).

Compound 77. *Atalaphyllidine* (25 mg). HRESI-MS: *m/z* 322.1025 $[M-H]^-$. ^1H NMR (CD_6CO) δ: 7.77 (1H, d, J=7.6 Hz, H-8), 7.33 (1H, d, J=7.6 Hz, H-6), 7.19 (1H, t, J=7.8 Hz, H-7), 6.75 (1H, d, J=9.6 Hz, H-1'), 6.11 (1H, s, H-2), 5.66 (1H, d, J=9.6 Hz, H-2'), 3.84 (3H, s, N-Me), 1.50 (6H, s, H-4' and H-5'). ^{13}C NMR (CD_6CO) δ: 182.2 (C-9), 164.4 (C-3), 161.3 (C-1), 148.9 (C-4a), 148.1 (C-5), 137.3 (C-10a), 125.2 (C-8a), 124.0 (C-2'), 123.6 (C-7), 121.1 (C-1'), 120.2 (C-6), 116.2 (C-8), 106.9 (C-4), 102.5 (C-9a), 97.4 (C-2), 76.8 (C-3'), 26.6 (C-4' and C-5'), 48.5 (N-Me).

Compound 78. *Pyranofoline* (14 mg). HRESI-MS: *m/z* 352.1142 $[M-H]^-$. ^1H NMR (CD_6CO) δ: 7.79 (1H, d, J=7.6 Hz, H-8), 7.31 (1H, d, J=7.6 Hz, H-6), 7.16 (1H, t, J=7.8 Hz, H-7), 6.72 (1H, d, J=9.6 Hz, H-1'), 5.71 (1H, d, J=9.6 Hz, H-2'), 1.52 (6H, s, H-4' and H-5'), 3.85 (3H, s, 4-OMe), 3.84 (3H, s, N-Me). ^{13}C NMR (CD_6CO) δ: 182.2 (C-9), 154.6 (C-3), 153.8 (C-1), 142.9 (C-4a), 148.1 (C-5), 137.2 (C-10a), 124.2 (C-8a), 124.0 (C-2'), 123.1 (C-7), 116.1 (C-1'), 120.2 (C-6), 116.2 (C-8), 129.3 (C-4), 106.5 (C-9a), 103.4 (C-2), 78.1 (C-3'), 27.8 (C-4' and C-5'), 60.1 (4-OMe), 46.5 (N-Me).

Compound 79. *8-hydroxy-2'-acetoxydihydroxanthyletin* (21 mg). HRESI-MS: *m/z* 305.1009 $[M+H]^+$. ^1H NMR ($CDCl_3$) δ: 7.59 (1H, d, J=9.6 Hz, H-4), 6.82 (1H, s, H-5), 6.20 (1H, d, J=9.6 Hz, H-3), 5.12 (1H, m, H-2'), 3.25 (2H, m, H-1'), 1.96 (3H, s, COMe), 1.57 (3H, s, H-4'), 1.52 (3H, s, H-5'). ^{13}C NMR ($CDCl_3$) δ: 170.5 (<u>CO</u>Me), 160.7 (C-2), 150.1 (C-7), 144.5 (C-4), 143.3 (C-8a), 128.1 (C-8), 125.3 (C-6), 114.5 (C-5), 113.4 (C-4a), 112.3 (C-3), 89.4 (C-2'), 82.3 (C-3'), 30.6 (C-1'), 22.5 (C-4'), 22.4 (C-5'), 21.3 (CO<u>Me</u>).

Compound 80. *Dehydrogeijerin* (160 mg). HRESI-MS: *m/z* 259.0937 $[M+H]^+$. ^1H NMR ($CDCl_3$) δ: 7.65 (1H, s, H-5), 7.60 (1H, d, J=9.4 Hz, H-4), 6.76 (1H, s, H-8), 6.54 (1H, s, H-2'), 6.21 (1H, d, J=9.4 Hz, H-3), 3.88 (3H, s, 7-OMe), 2.17 (3H, s, H-4'), 1.92 (3H, s, H-5'). ^{13}C NMR ($CDCl_3$) δ: 190.7 (C-1'), 160.6 (C-7), 157.5 (C-2), 156.9 (C-8a), 143.6 (C-4), 133.8 (C-3'), 130.6 (C-5), 128.4 (C-6), 125.0 (C-2'), 114.0 (C-3), 112.3 (C-4a), 99.8 (C-8), 56.5 (7-OMe), 28.3 (C-4'), 21.6 (C-5').

Compound 81. *Hopeyhopin* (230 mg). HRESI-MS: *m/z* 275.0915 $[M+H]^+$. ^1H NMR ($CDCl_3$) δ: 7.99 (1H, s, H-5), 7.68 (1H, d, J=9.5 Hz, H-4), 6.86 (1H, s, H-8), 6.27 (1H, d, J=9.5 Hz, H-3), 4.00 (3H, s, 7-OMe), 4.01 (1H, s, H-2'), 1.50 (3H, s, H-4'), 1.21 (3H, s, H-5'). ^{13}C NMR ($CDCl_3$) δ: 193.6 (C-1'), 162.3 (C-7), 160.1 (C-2), 158.9 (C-8a), 143.5 (C-4), 131.5 (C-5), 123.4 (C-6), 114.7 (C-3), 112.9 (C-4a), 100.1 (C-8), 68.1 (C-2'), 62.1 (C-3'), 57.0 (7-OMe), 24.5 (C-4'), 18.5 (C-5').

Compound 82. *2H-1-Benzopyran-2-one, 6-[(acetyloxy)(3,3-dimethyloxiranyl)methyl]-7-methoxy- (9CI)* (35 mg). HRESI-MS: *m/z* 319.0835 $[M+H]^+$. ^1H NMR ($CDCl_3$) δ: 7.62 (1H, d, J=9.5 Hz, H-4), 7.43 (1H, s, H-5), 6.77 (1H, s, H-8), 6.21 (1H, d, J=9.5 Hz, H-3), 5.92 (1H, d, J=8.8 Hz, H-1'), 3.87 (3H, s, 7-OMe), 3.07 (1H, d, J=8.8 Hz, H-2'), 2.07 (3H, s, COMe), 1.34 (3H, s, H-4'), 1.22 (3H, s, H-5'). ^{13}C NMR ($CDCl_3$) δ: 169.9 (<u>CO</u>Me), 160.9 (C-2), 159.9 (C-

7), 156.0 (C-8a), 143.5 (C-4), 127.3 (C-5), 123.8 (C-6), 113.8 (C-3), 112.6 (C-4a), 99.6 (C-8), 69.9 (C-2'), 65.1(C-2'), 59.9 (C-3'), 56.3 (7-OMe), 24.5 (C-4'), 21.3 (COMe), 19.8 (C-5').

Compound 83. *Braylin* (35 mg). HRESI-MS: *m/z* 259.1025 $[M+H]^+$. ^{1}H NMR ($CDCl_3$) δ: 7.54 (1H, d, J=9.4 Hz, H-4), 6.80 (1H, d, J=10.0 Hz, H-1'), 6.72 (1H, s, H-5), 6.20 (1H, d, J=9.4 Hz, H-3), 5.68 (1H, d, J=10.0 Hz, H-2'), 3.83 (3H, s, 5-OMe), 1.46 (6H, s, H-4' and H-5'). ^{13}C NMR ($CDCl_3$) δ: 161.3 (C-2), 146.0 (C-7), 145.8 (C-6), 144.5 (C-8a), 143.9 (C-4), 130.9 (C-2'), 115.2 (C-1'), 113.2 (C-3), 111.5 (C-4a), 110.3 (C-8), 108.7 (C-5), 77.2 (C-3'), 56.6 (6-OMe), 28.1 (C-4' and C-5').

Compound 84. *Heraclenin* (15 mg). HRESI-MS: *m/z* 287.0841 $[M+H]^+$. ^{1}H NMR ($CDCl_3$) δ: 7.76 (1H, d, J=9.6 Hz, H-4), 7.67 (1H, d, J=2.4 Hz, H-2'), 7.38 (1H, s, H-5), 6.80 (1H, d, J=2.4 Hz, H-1'), 6.35 (1H, d, J=9.6 Hz, H-3), 4.56 (2H, m, H-1''), 3.29 (2H, m, H-2''), 1.31 (3H, s, H-4'), 1.25 (3H, s, H-5'). ^{13}C NMR ($CDCl_3$) δ: 160.5 (C-2), 148.5 (C-7), 147.0 (C-2'), 144.5 (C-4), 143.8 (C-8a), 131.6 (C-8), 126.1 (C-6), 116.7 (C-4a), 114.9 (C-3), 114.0 (C-5), 107.0 (C-1'), 72.6 (C-1''), 61.5 (C-2''), 58.3 (C-3''), 24.7 (C-4''), 19.0 (C-1'').

Compound 85. *6,7,8-trimethoxycoumarin* (14 mg). EI-MS: *m/z* 214 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 7.59 (1H, d, J=9.6 Hz, H-4), 6.64 (1H, s, H-5), 6.24 (1H, d, J=9.6 Hz, H-3), 3.99 (3H, s, 6-OMe), 3.93 (3H, s, 8-OMe), 3.89 (3H, s, 7-OMe). ^{13}C NMR ($CDCl_3$) δ: 160.6 (C-2), 150.2 (C-6), 146.0 (C-8), 143.7 (C-4), 143.1 (C-7), 141.2 (C-8a), 115.2 (C-3), 114.5 (C-4a), 110.3, 103.8 (C-5), 61.9 (6-OMe), 61.6 (8-OMe), 56.4 (7-OMe).

Compound 86. *Methyleugenol* (120 mg). EI-MS: *m/z* 178 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 6.78 (1H, d, J=8.4 Hz, H-5), 6.71 (1H, d, J=1.6 Hz, H-2), 6.70 (1H, dd, J=1.6 and 8.4 Hz, H-6), 5.95 (1H, m, H-8), 5.05 (2H, m, H-9), 3.84 (3H, s, 3-OMe), 3.82 (3H, s, 4-OMe), 3.31 (2H, d, J=6.7 Hz, H-7). ^{13}C NMR ($CDCl_3$) δ: 148.8 (C-3), 147.3 (C-4), 137.6 (C-8), 132.5 (C-1), 120.5 (C-6), 115.5 (C-5), 111.8 (C-2), 111.2 (C-9), 55.7(3 and 4-OMe), 39.7 (C-7).

Compound 88a. (±)-*Dehydroisoeugenol.* EI-MS: m/z 326 $[M]^+$. IR ν_{max} cm^{-1}: 3370. ^{1}H NMR δ: 7.10-6.50 (5H, m, H-2, H-2', H-5, H-6, and H-6'), 6.36 (1H, d, J=15.7 Hz, H-7'), 6.10 (1H, dq, J=6.5 and 15.7 Hz, H-8'), 5.64 (1H, s, OH), 5.10 (1H, d, J=9.4 Hz, H-7), 3.89 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.45 (1H, m, H-8), 1.86 (3H, d, J=6.5 Hz, H-9'), 1.37 (3H, d, J=6.7 Hz, H-9). ^{13}C NMR δ: 146.7 (C-3'), 146.6 (C-3), 145.8 (C-4'), 144.1 (C-4), 133.3 (C-1),

132.2 (C-5'), 132.1 (C-1'), 130.9 (C-1), 123.4 (C-6), 119.4 (C-8'), 114.1 (C-2'), 113.3 (C-2), 109.3 (C-5), 108.9 (C-6'), 93.7 (C-7), 55.9 (2×OMe), 45.6 (C-8), 18.3 (C-9'), 17.6 (C-9).

Compound 88b. (±)-*Dehydrodiconiferyl alcohol*. EI-MS: *m/z* 358 $[M]^+$. ^{1}H NMR (CD_3OD) δ: 6.93 (2H, m, H-2' and H-6'), 6.82 (1H, dd, J=1.8 and 8.1 Hz, H-6), 6.79 (1H, d, J=1.8 Hz, H-2), 6.75 (1H, d, J=8.1 Hz, H-5), 6.55 (1H, d, J=15.8 Hz, H-7'), 6.18 (1H, dt, J=6.0 and 15.8 Hz, H-8'), 5.58 (1H, d, J=7.2 Hz, H-7), (2H, d, J=6.0 Hz, H-9'), 3.85 (6H, s, 3-OMe and 3'-OMe), 3.79 (2H, m, H-9), 3.50 (1H, m, H-8). ^{13}C NMR (CD_3OD) δ: 147.3 (C-3'), 149.3 (C-3), 141.4 (C-4'), 140.2 (C-4), 127.6 (C-7'), 126.5 (C-8'), 116.2 (C-6'), 132.4 (C-1), 127.2 (C-1'), 122.0 (C-6), 135.3 (C-5'), 116.8 (C-5), 115.3 (C-2), 109.6 (C-2'), 88.2 (C-7), 65.6 (C-9'), 63.2 (C-9), 56.3 (3-OMe and 3'-OMe), 50.4 (C-8).

Compound 90. (±)-Erythro-*guaiacylpropan-1,2-diol-β*-O-*4'-isoeugenylether*. ^{1}H NMR ($CDCl_3$) δ: 6.98-6.81 (6H, m, H-2, H-2', H-5, H-5', H-6, and H-6'), 6.38 (1H, d, J=15.7 Hz, H-7'), 6.17 (1H, dq, J=6.6 and 15.7 Hz, H-8'), 4.84 (1H, d, J=2.8 Hz, H-7), 4.31 (1H, m, H-8), 3.90 (6H, s, 2×OMe), 1.83 (3H, d, J=6.6 Hz, H-9'), 1.10 (3H, d, J=6.4, H-9). (±)-Threo-*guaiacylpropan-1,2-diol-β*-O-*4'-isoeugenylether*: ^{1}H-NMR ($CDCl_3$) δ: 6.98-6.91 (6H, m, H-2, H-2', H-5, H-5', H-6, and H-6'), 6.38 (1H, d, J=15.5 Hz, H-7'), 6.17 (1H, dq, J=6.6 and 15.5 Hz, H-8'), 4.74 (1H, d, J=8.8 Hz, H-7), 4.26 (1H, m, H-8), 3.88 (6H, s, 2×OMe), 1.83 (3H, d, J=6.6 Hz, H-9'), 1.04 (3H, d, J=6.3, H-9).

Compound 91. (±)-Threo-*guaiacylpropan-1,2-diol-α*-O-*4'-isoeugenylether*. HREI-MS: *m/z* 344.1620 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 6.75-6.95 (4H, m, H-2, H-6, H-2', H-5'), 6.66 (1H, d, J=7.0 Hz, H-5), 6.58 (1H, d, J=9.0 Hz, H-6'), 6.29 (1H, d, J=15.5 Hz, H-7'), 6.08 (1H, dt, J=6.5 and 15.5 Hz, H-8'), 4.43 (1H, d, J=7.8 Hz, H-7), 4.07 (1H, m, H-8), , 1.84 (3H, d, J=6.5 Hz, H-9'), 1.01 (3H, d, J=6.0 Hz, H-9), 3.90 (3H, s, 3-OMe), 3.89 (3H, s, 3'-OMe). ^{13}C NMR ($CDCl_3$) δ: 150.6 (C-3), 147.9 (C-3'), 147.1 (C-4), 145.1 (C-4'), 133.6 (C-1'), 131.0 (C-1), 130.2 (C-7'), 125.6 (C-8'), 120.9 (C-6), 119.1 (C-6'), 118.8 (C-5), 114.5 (C-5'), 109.8 (C-2'), 109.3 (C-2), 91.0 (C-7), 71.3 (C-8), 57.5 (3 and 3'-OMe), 18.9 (C-9'), 18.0 (C-9).

Compound 92. *4-formyl-2-methoxyphenyl 4-hydroxy-3-methoxybenzoate*. HREI-MS: *m/z* 302.0785 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 9.98 (1H, s, H-7'), 7.84 (1H, dd, J=2.0 and 8.4 Hz, H-6), 7.67 (1H, d, J=2.0 Hz, H-2), 7.54 (1H, d, J=1.6 Hz, H-2'), 7.52 (1H, dd, J=1.6 and 8.0 Hz, H-

6'), 7.33 (1H, d, J=8.0 Hz, H-5'), 7.02 (1H, d, J=8.4 Hz, H-5), 3.99 (3H, s, 3-OMe), 3.89 (3H, s, 3'-OMe). ^{13}C NMR ($CDCl_3$) δ: 192.2 (C-7'), 165.7 (C-7), 152.4 (C-3'), 151.2 (C-4), 146.7 (C-3), 146.1 (C-4'), 125.8 (C-6), 125.0 (C-5' and C-6'), 114.8 (C-5), 113.8 (C-2), 111.2 (C-2'), 57.0 (3 and 3'-OMe).

3. RESULTS AND DISCUSSION

Two spontaneous plants of the Mediterranean area, *Aptenia cordifolia* and *Oxalis pes-caprae,* besides two Rutaceous plants, *Swinglea glutinosa* and *Amyris madrensis,* have been investigated in order to study the metabolites they produce and to determine whether or not they possess biological activity.

All pure compounds were isolated by chromatographic techniques (CC, TLC, HPLC) and identified by using Mass Spectrometry (EI-MS, ESI-MS, MALDI-MS), NMR spectroscopy on ^{1}H and ^{13}C by one-dimensional and two-dimensional experiments, IR, and UV. For each plant the isolated compounds are described and the investigation modalities that allowed their structural elucidation are reported. Biological assays results are also illustrated.

3.1. *Aptenia cordifolia*

Aptenia cordifolia, belonging to the Aizoaceae family, is native of South Africa and now largely diffused in the United States (California, Florida, Oregon) and along the southern coast of Europe. It is a perennial herb, spreading over ground and neighbouring vegetation, with small, heart-shaped, dark green succulent leaves interspersed with small, purple flowers that open only in spring and summer. The most commonly grown plant, usually grown under the cultivar name of 'Red Apple', has brighter red flowers and is considered by some botanists to be actually a hybrid between *Aptenia cordifolia* and the closely related *Platythyra Aptenia haeckeliana*.[23]

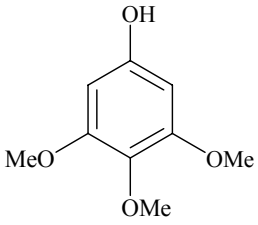
Fig. 1- *Aptenia cordifolia*

It is planted in parking lot planters, parkways, home gardens, in the urban interface near parks and preserves. It can tolerate some soil salinity and grows well in frost-free or almost frost free areas in full sun. When watered, red apple overwhelms all neighbouring vegetation, climbing over anything in its path, this invasiveness has recently become a serious problem. It has been used in landscaping adjacent to riparian areas within the urban interface, so it can easily spread into and dominate more natural riparian and wetland areas.

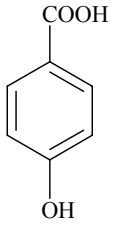
Fresh leaves and twigs of *A. cordifolia* were powdered and then extracted with H_2O - CH_3OH (9:1) at 25 °C for 7 days.

The hydroalcoholic extract of *A. cordifolia* leaves was reduced in volume and precipitated with $(CH_3)_2CO$. The $(CH_3)_2CO/H_2O$ soluble part was fractionated by Amberlite XAD-2 column chromatography and fractions were purified by silica gel chromatography and HPLC yielding compounds **3**, **4**, **6**, **7**, **9-14**, **16-31**, **34-40**.[24, 25, 26]

Repeated column chromatographies and preparative TLC of the hydroalcoholic extract of twigs of the plants, after acetone precipitation, afforded compounds **1**, **2**, **5**, **8**, **15**, **32**, **33**.[26] The structures of all the compounds were elucidated by their spectral data.



1



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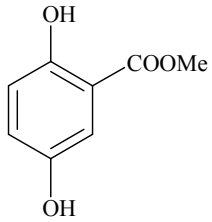

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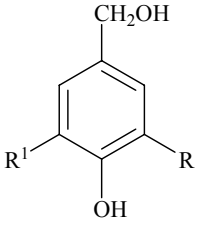
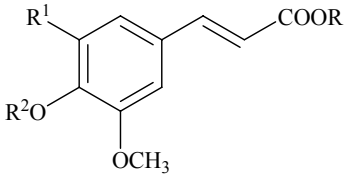
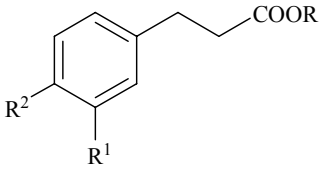


4 R = R^1 = H
5 R = R^1 = OMe



6 R = R^1 = R^2 = H
7 R = Me R^1 = R^2 = H
8 R = R^2 = H R^1 = OMe
9 R = H R^1 = OMe R^2 = Me



10 R = R^1 = R^2 = H
11 R = R^1 = H R^2 = OH
12 R = H R^1 = OMe R^2 = OH
13 R = H R^1 = R^2 = OMe
14 R = Me R^1 = R^2 = OMe
15 R = Et R^1 = R^2 = OMe

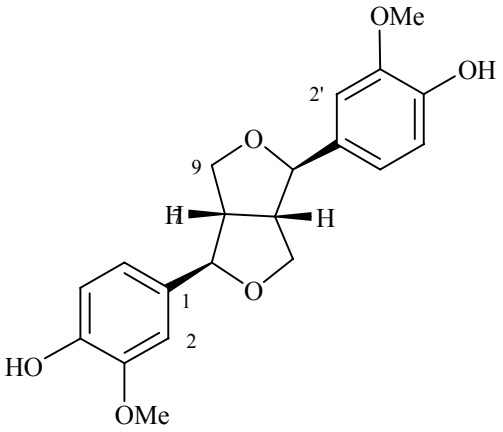
Phenols **1-5** were identified as 3,4,5-trimethoxyphenol, 4-hydroxybenzoic acid, methyl 2,5-dihydroxybenzoate, 4-(hydroxylmethyl)phenol, and 4-(hydroxymethyl)-2,6-dimethoxyphenol, respectively; metabolites **6-9** were identified as ferulic acid, methyl ferulate, sinapic acid, and 3,4,5-trimethoxycinnamic acid, respectively. Compounds **10-15** were identified as dihydrocinnamic acid, 4-hydroxy-dihydrocinnamic acid, dihydroferulic acid, 3,4-dimethoxy-dihydrocinnamic acid, and the corresponding Me and Et esters, respectively. All these compounds were identified by comparison of their spectroscopic data with those of authentic samples.

Compounds **16-25** were determined to have a lignan skeleton.

Lignans, widely distributed in the plant kingdom, are constituted by phenylpropanoid (C_6C_3) units linked by the central carbons of their side chains. Naturally occurring dimers that exhibit linkage other than this C8-C8' type linkage are known as neolignans, or oxyneolignans if the C_6C_3 units are linked by an oxygen atom.

Biogenetically they derive from the oxidative coupling of phenylpropanoid units, biosynthesized from the shikimic acid pathway, through radical processes.

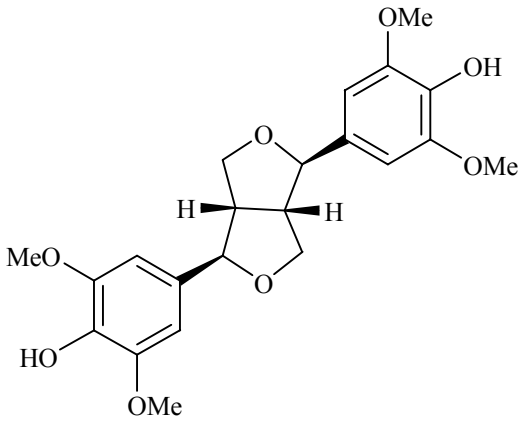
Repeating units of lignans generate lignins, which are involved in the lignification of plants cell walls to generate solid structures and watering conduction system in trees. Many lignans exhibit interesting antimicrobic, antiviral, herbicidal, or antifeedant activities that are thought to participate in plant defence mechanisms against biotic stresses.[27] Cancer protective effects of dietary lignans have been also demonstrated.[28]



16

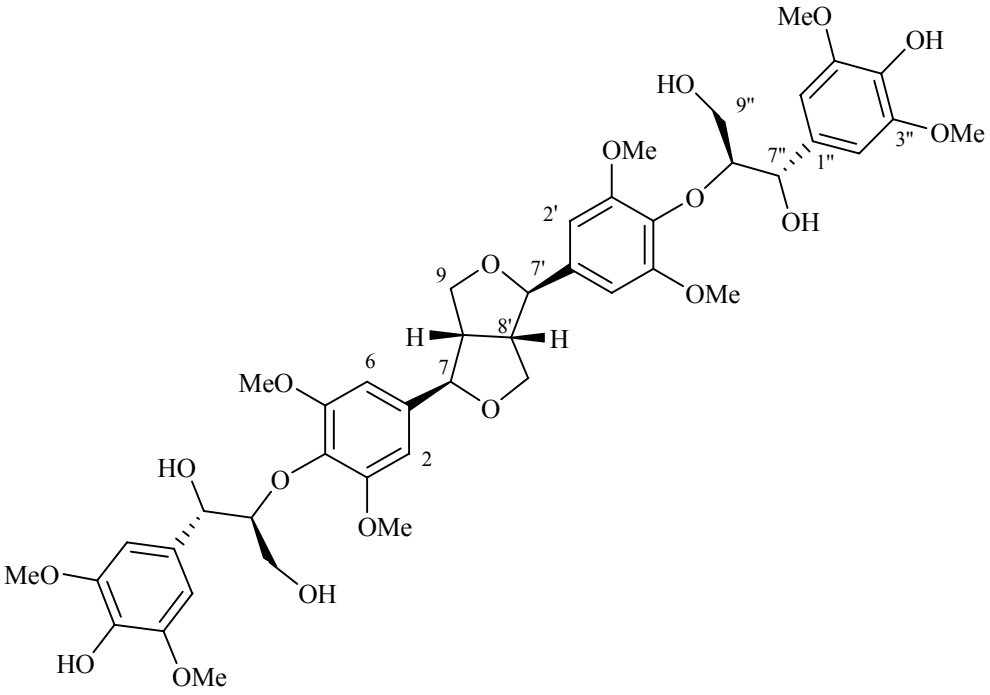
Compound **16** was identified as (-)-pinoresinol by analysing the spectroscopic data and comparing them to literature.[29] It has a molecular formula $C_{20}H_{22}O_6$ as deduced from the molecular ion peak $[M]^+$ *m/z* 358 in the EI mass spectrum and NMR data. The ^{13}C NMR

spectrum showed ten signals, that from a DEPT experiment were deduced to be three methines (δ 108.6, 114.3, and 118.9) and three quaternary carbons (δ 132.9, 145.2, and 146.7), in the aromatic region. At higher fields the signals of a methylenic carbon at δ 71.6, a benzylic methine at δ 85.8, an aliphatic methine carbon at δ 54.1, and a methylic carbon at δ 55.9 were present. These data were in agreement with a dioxabicyclic system, having two condensed furanic rings symmetrically substituted by phenylic rings. According to this hypothesis, ^{1}H NMR spectrum showed three aromatic protons of a 1,2,4-trisubstituted system, a benzylic proton bonded to an oxygen atom at 4.74 (d, J=4.0 Hz), two double doublets due to the two diastereotopic protons of the methylenic group at δ 4.25 (J=7.0 and 9.0 Hz), and δ 3.88 (J=4.0 and 9.0 Hz), a multiplet at δ 3.10 of a methinic proton, and the signal of two methoxyls at δ 3.91. Chemical shift values and couplings of the protons H-7 and H-9 defined the relative configuration of the compound. The absolute configuration was assigned by the measure of the optical rotation ($[\alpha]_D^{25}$= -10°) and comparing it to literature value.[29]



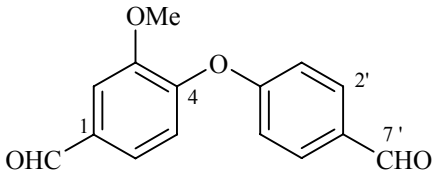
17

Compound **17** had the molecular ion peak $[M]^+$ at *m/z* 418 in the EI mass spectrum. The ^{1}H NMR spectrum showed the signals of a tetrasubstituted aromatic ring and of a C_3 side chain. In a ^{1}H-^{1}H COSY experiment a doublet at δ 4.72 coupled to a multiplet at δ 3.09, which, in turn, was correlated to two different multiplets at δ 4.26 and 3.61. These chemical shifts data are typical of a dioxabicyclic system. A singlet integrated for twelve protons appeared at δ 3.89. Comparison with literature[30] data univocally defined compound **17** as (±)-syringaresinol.



18

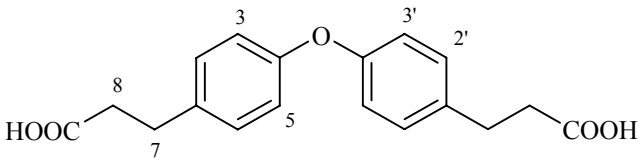
Spectral data suggested the molecular formula $C_{44}H_{54}O_{18}$ for the new compound **18**. It had a pseudo-molecular ion peak $[M+Na]^+$ at *m/z* 893 in the MALDI mass spectrum. In the ^{13}C NMR (DEPT) spectrum of **18** only sixteen signals were present, indicating a highly symmetric molecule. The ^{1}H NMR and ^{1}H-^{1}H COSY spectra revealed the connectivities of four H-atoms characteristic of one 3,7-dioxabicyclo[3.3.0]octane and two propane-1,2,3-triol moieties. The ^{1}H and ^{13}C NMR spectra of **18** showed the presence of four aromatic rings, each one with two H-atoms in *meta* position relative to each other. The COSY spectrum enabled us to define the two glycerol moieties as C-7", C-8", C-9" [δ(H): 4.99 (d, J=4.0 Hz); 4.13 (m), 3.88 (obscured), 3.50 (dd, J=2.4 and 12.0 Hz)]. The coupling constant of 4.0 Hz between H–7" and H–8" indicated an *erythro* relative configuration. The ^{13}C NMR and DEPT spectra of **18** showed two methyls, two methylenes, and five methines, as well as six quaternary carbons, which were connected by HMQC analysis. The functional groups were attached to the main skeleton with the aid of HMBC correlations. In the HMBC spectrum, H–7 correlated to C-2/C-6, C-8, and C-9. The doublet at δ 4.99 was attributed to H-7", with HMBC correlations with C-1", C-2"/C-6", and C-9". Finally, NOEs between the methyl groups at δ 3.90 and 3.88 with H-2/H-6 and H-2"/H-6", respectively, defined the positions of the methoxyl groups. From these data, the structure of the new dilignan **18** was elucidated as di-*erythro*-syringylglycerol-*β-O*-4,4'-syringaresinol ether.



19

Compound **19** showed the molecular ion peak $[M]^+$ at *m/z* 256 in the EI mass spectrum that, along with NMR data, suggested a molecular formula $C_{15}H_{12}O_4$. The UV spectrum revealed an absorption maximum at 276 nm, and the IR spectrum showed bands typical of carbonyl (1687 cm^{-1}) and phenyl (1604 cm^{-1}) groups.

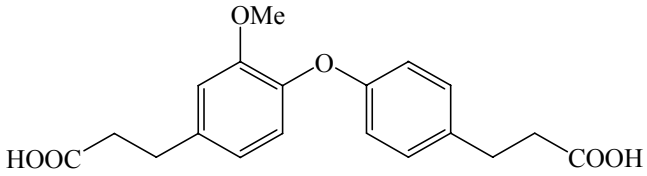
The ^1H NMR spectrum of **19** indicated the presence of four *ortho*-coupled H-atoms, two doublets, and one singlet in the aromatic region, as well a singlet integrated for three protons and two singlets due to formyl groups. In the ^{13}C NMR (DEPT) spectrum, twelve resonances were evident, including one methyl and seven aromatic methines. An HMQC experiment allowed the assignment of all H-atoms, with key HMBC cross-peaks of both H-2 and H-5 to C-3 and C-4, and of H-6 to C-1 and C-7. Furthermore H-2'/H-6' coupled with C-4' and C-7', H-7 showed a cross-peaks with C-1 and C-2/C-6, and H-7' interacted with C-1' and C-2'/C-6'. Finally, the methoxyl group was placed at C-3, based on an NOE between C-3 and H-2. From these data, the structure of **19** was derived as 4-(4-formylphenoxy)-3-methoxybenzaldehyde.



20

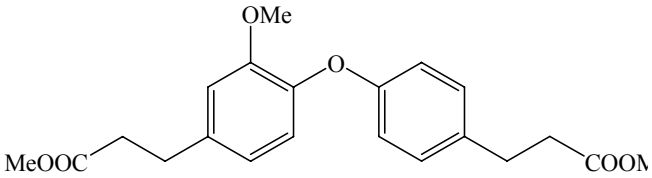
Compound **20** showed a pseudo-molecular ion peak $[M+Na]^+$ at *m/z* 337 in the MALDI spectrum. The UV spectrum revealed a band at 278 nm. The ^1H and ^{13}C NMR spectra indicated a highly symmetric molecule. In the ^1H NMR spectrum eight aromatic protons were present as two *ortho* coupled protons and eight methylene protons as two triplets in aliphatic region. The ^{13}C NMR spectrum showed only seven carbon signals that were assigned by a DEPT experiment as two methylenes and two methines. According to the structure in the HMBC spectrum both the H-7/H-7′ and H-8/H-8′ protons were correlated to the C-9/C-9′ at δ 180.6 and C-1/C-1′ at δ 129.6; furthermore the H-3/H-3′ protons were correlated to C-1/C-1′ and C-

4/C-4′ carbons at δ 157.4. From these data compound **20** was determined to be 4,4'-oxyneolign-9,9'-dioic acid.



21

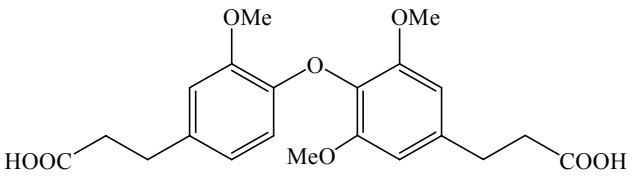
Compound **21** was identified as 3-methoxy-4,4'-oxyneolign-9,9'-dioic acid. The pseudo-molecular ion peak at *m/z* 390 $[M+2Na]^+$ in the MALDI spectrum, along with ^{13}C NMR spectrum, defined the molecular formula $C_{19}H_{20}O_6$. The ^1H NMR spectrum indicated the presence of four *ortho* coupled protons, two doublet protons, and one double doublet proton in the aromatic region; a methyl singlet of a methoxyl group and two triplets in the aliphatic region. In the ^{13}C NMR spectrum fourteen carbons were evident. The DEPT spectrum indicated the presence of a methyl, three methylenes, and five methines. The HMQC experiment allowed the assignment of the protons to the corresponding carbons. The HMBC spectrum showed cross-peaks of both the H-2 and H-5 protons with the C-3 and C-4 carbons and the H-6 with the C-1 and C-7. Furthermore the H-2′/H-6′ protons gave cross peaks with the C-4′ and C-7′ carbons, the H-7′ and the H-8′ protons gave cross peaks with the C-1′ and C-9′ carbons. These data led to the structure **21** as depicted. The assignment of the methoxyl at C-3 was confirmed by NOE between the methoxyl and the H-2 proton.



22

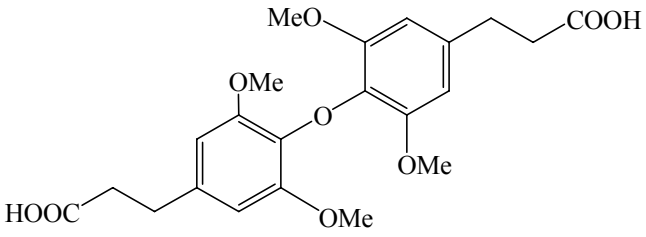
Compound **22** had a pseudo-molecular ion peak $[M+H]^+$ at *m/z* 373 in the MALDI mass spectrum. Its NMR spectrum closely resembled those of **21**, except for an additional signal at δ 3.67, due to two methoxyl groups. These data suggested that compound **22** is dimethyl 3-

methoxy-4,4'-oxyneolign-9,9'-dioate. The structure was also deduced from the NOE observed between the methoxyls and the H-8/H-8′ protons in the NOESY experiment. Compound **22** was also obtained by treatment of apteniol **21** with ethereal CH_2N_2.



23

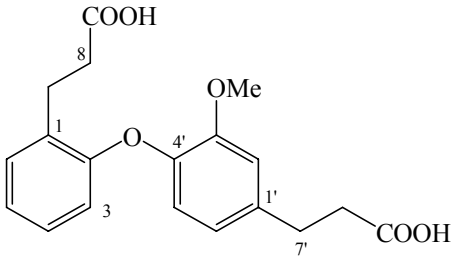
Compound **23** was deduced to be 3,3',5-trimethoxy-4,4'-oxyneolign-9,9'-dioic acid. It had the molecular formula $C_{21}H_{24}O_8$, as established by a pseudo-molecular peak at *m/z* 427 $[M+Na]^+$ in the MALDI mass spectrum and spectroscopic data. The ^1H NMR spectrum showed the presence of one aromatic ring with three coupled protons in a ABX system, an aromatic ring with two protons *meta* coupled, two methylenes as triplets and a methyl singlet of three methoxyls. In the ^{13}C NMR spectrum fifteen carbon signals were present, and the DEPT experiment evidenced two methyls, two methylenes, and four methines. The ^1H and ^{13}C resonances were assigned by combination of COSY, DEPT, HMQC, and HMBC experiments. The HMBC spectrum of compound **23** showed cross-peaks of both the H-2 and H-6 with C-4 and C-7, and both the H-2′ and the H-5′ protons with the C-1′ and C-4′ carbons. NOEs between the signal at δ 3.87 and the H-2, H-6 and H-2′ protons allowed to assign the methoxyls at C-3, C-5, and C-5′ positions.



24

Compound **24** was defined as 3,3',5,5'-tetramethoxy-4,4'-oxyneolign-9.9'-dioic. The UV spectrum revealed bands at 322, 279, and 244 nm. The ^1H NMR spectrum showed only one

singlet aromatic signal, two triplets in aliphatic region, and a methyl signal. In the ^{13}C NMR spectrum only eight carbon signals were present indicating a highly symmetric molecule. The ^{1}H and ^{13}C resonances of **24** were assigned by combination of COSY, DEPT, HMQC, and HMBC experiments. The DEPT spectrum showed one methyl, two methylenes, and one methine. According to the structure in the HMBC spectrum both the H-7/H-7′ and the H-8/H-8′ protons were correlated to the C-9/C-9′ at δ 178.4 and the C-1/C-1′ at δ 132.0; furthermore the H-2, H-6/H-2′, H-6′ protons were correlated to the C-1/C-1′ and C-4/C-4′ carbons. The NOE of the signal at δ 3.88 with H-2/H-6 and H-2′/H-6′ allowed assignment of the methoxyls at the C-3, C-3′, C-5, and C-5′ positions.



25

Compound **25** was determined to be 3’-methoxy-2,4’-oxyneolign-9,9’-dioic acid. The ^{1}H NMR spectrum showed the presence of one aromatic ring with three coupled protons in a ABX system, an aromatic ring with four adjacent protons, two methylenes as triplets and a methyl singlet of a methoxyl group. In the ^{13}C NMR spectrum sixteen carbon signals were present, and the DEPT experiment showed one methyl, four methylenes, and five methines. The HMQC experiment allowed the assignment of the protons to the corresponding carbons. The HMBC spectrum of compound **25** showed cross-peaks of H-7 with C-2, C-6, and C-9; H-8 with C-1, and both the H-2′ and the H-5′ protons with the C-1′ and C-4′ carbons. NOEs between the signal at δ 3.87 and H-2′, allowed assignment of the methoxyl at the C-3′ position.

Table 2. ^{1}H NMR Data of **20 - 25** (CD_3OD)[a] at 500 MHz

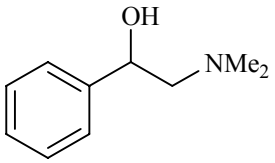
Position	**20**	**21**	**22**[b]	**23**[b]	**24**[b]	**25**
2	6.92 d (8.5)	6.72 d (1.5)	6.70 d (1.5)	6.44 s	6.42 s	
3	6.63 d (8.5)					7.21[c)]
4						7.23[c)]
5	6.63 d (8.5)	6.82 d (8.2)	6.83 d (8.0)			7.12 m
6	6.92 d (8.5)	6.70 dd (8.2, 1.5)	6.68 dd (8.0, 1.5)	6.44 s	6.42 s	7.21[c)]
7	2.84 t (8.1)	2.89 t (8.0)	2.88 t (7.5)	2.90 t (7.5)	2.88 t (8.0)	2.89 t (7.6)
8	2.66 t (8.1)	2.66 t (8.0)	2.59 t (7.5)	2.67 t (7.5)	2.61 t (8.0)	2.44 t (7.6)
2′	6.92 d (8.5)	7.05 d (8.5)	7.05 d (9.0)	6.72 s	6.42 s	6.82 d (2.0)
3′	6.63 d (8.5)	6.74 d (8.5)	6.75 d (9.0)			
5′	6.63 d (8.5)	6.74 d (8.5)	6.75 d (9.0)	6.82 d (8.5)		6.67 d (8.0)
6′	6.92 d (8.5)	7.05 d (8.5)	7.05 d (9.0)	6.70 d (8.5)	6.42 s	6.63 dd (8.0, 2.0)
7′	2.84 t (8.1)	2.89 t (8.0)	2.88 t (7.5)	2.90 t (7.5)	2.88 t (8.0)	2.82 t (7.8)
8′	2.66 t (8.1)	2.66 t (8.0)	2.61 t (7.5)	2.67 t (7.5)	2.61 t (8.0)	2.41 t (7.8)
3-OMe		3.86 s	3.87 s	3.87 s	3.88 s	
5-OMe				3.87 s	3.88 s	
3′-OMe				3.87 s	3.88 s	3.87 s
5′-OMe					3.88 s	
9-OMe			3.67 s			
9′-OMe			3.67 s			

[a] *J* values (in Hz) in parentheses; [b] recorded in $CDCl_3$500 MHz; [c] multiplicity was not determined due to overlapping.

Table 3. ^{13}C NMR Data of **20**-**25** (CD_3OD) at 125 MHz

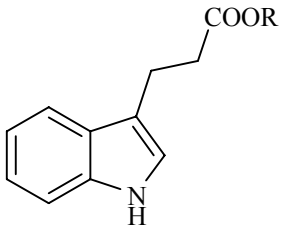
Position	**20**	**21**	**22**[a]	**23**[a]	**24**[a]	**25**
1	129.6	132.1	132.7	133.1	132.0	135.7
2	130.7	111.0	110.2	104.8	104.9	113.5
3	116.6	146.4	146.7	146.9	147.0	148.6
4	157.4	144.0	144.2	131.9	145.2	145.8
5	116.6	114.4	114.6	146.9	147.0	116.4
6	130.7	120.8	121.0	104.8	104.9	122.0
7	35.7	30.3	30.9	30.7	31.2	34.0
8	42.9	35.9	36.3	35.7	36.1	41.9
9	180.6	178.9	173.8	177.8	178.4	182.2
1′	129.6	132.1	130.0	131.2	132.0	127.1
2′	130.7	129.4	129.6	110.8	104.9	144.2
3′	116.6	115.4	115.5	146.9	147.0	129.2
4′	157.4	154.1	154.4	144.0	145.2	129.2
5′	116.6	115.4	115.5	114.3	147.0	126.6
6′	130.7	129.4	129.6	120.7	104.9	129.2
7′	35.7	30.3	30.9	30.2	31.2	34.4
8′	42.9	35.8	36.3	35.7	36.1	41.6
9′	180.6	178.9	173.8	177.8	178.4	182.2
3-OMe		55.8	56.1	56.1	56.3	56.5
5-OMe				56.1	56.3	
3′-OMe				55.7	56.3	
5′-OMe					56.3	
9-OMe			51.9			
9′-OMe			51.9			

[a] recorded in $CDCl_3$.



26

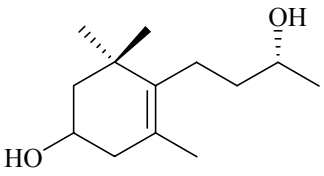
Compound **26** was identified as 2-(dimethylamino)-1-phenylethanol. The EI mass spectrum showed the molecular ion peak $[M]^+$ at *m*/*z* 165, with a prominent fragment $[M\text{-}Me]^+$ at *m*/*z* 150. The structure of **26** was established by means of ^1H and ^{13}C NMR (DEPT) as well as 2D NMR analyses, including COSY, HMQC, and HMBC experiments. The ^1H NMR spectrum indicated the presence of five aromatic protons at δ 7.40 (2H, d, J=7.0 Hz), 7.35 (2H, t, J=7.0 Hz), and 7.28 (1H, d, J=7.0 Hz); three double doublets at δ 4.86 (J=3.5 and 9.5 Hz), 2.81 (J=2.8 and 9.5 Hz), and 2.66 (J=3.5 and 12.8 Hz), and two methyl singlets at δ 2.35. In the ^{13}C NMR (DEPT) spectrum, seven resonances were evident due to one methyl, one methylene, and four methines. An HMQC experiment allowed the assignment of the protons to the corresponding carbons, and the HMBC spectrum showed cross-peaks of both H-1 and H-2 to C-1', and of H-2'/H-6' to C-1 and C-3'/C-5'. These data confirmed the structure of **26**, which had been isolated before from *Dolichothele uberiformis* as the corresponding hydrochloride[31], exhibiting an $[\alpha]_D^{25}$ value of -74°. Compound **26** isolated from *A. cordifolia* had $[\alpha]_D^{25}$ value of +1.1°.



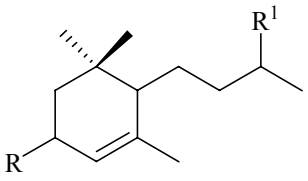
27 R = H
28 R = Me

Compounds **27** and **28** were identified as 3-(1H-indol-3-yl)propanoic acid and its Me ester, respectively.

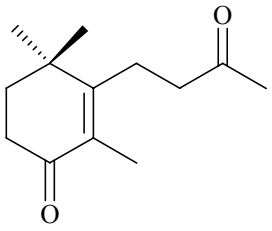
Compounds **29-34** were identified as C_{13} norisoprenoids. These molecules are generally found as aroma compounds in fruits and vegetables, but also in many leaf products such as tea or tobacco. C_{13} norisoprenoids are supposed to be structurally derived from higher molecular weight terpenoids such as carotenoids, through degradation processes.



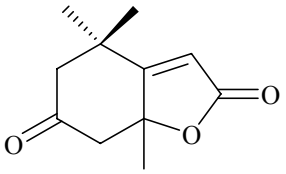
29



30 R = O R^1 = H, α OH
31 R = R^1 = O

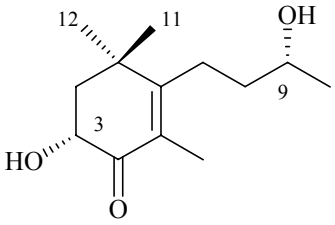


33



32

Compound **29** was determined to be 3-hydroxy-7,8-dihydro-*β*-ionone, and has been previously isolated from the fruits of *Prunus* species.[32] Compounds **30** and **31** had spectroscopic data corresponding to (9*R*)-9-hydroxymegastigm-4-ene-3-one and megastigm-4-ene-3,9-dione, in agreement with those isolated from *Chenopodium album.*[33] Compound **32** was identified as dehydrololiolide, previously isolated from *Vitis vinifera.*[34] Finally, the structure of 4-oxo-7,8-dihydro-*β*-ionone (**33**) was determined by spectroscopic comparison with a synthetic intermediate previously obtained during the preparation of canthaxanthin.[35]



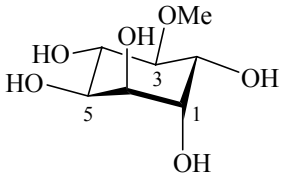
34

Compound **34** was identified as (3*R*,9*R*)-3,9-dihydroxymegastigm-5-en-4-one, previously identified in the metabolism of astaxanthin, a carotenoid non-provitamin A.[36] The molecular ion peak $[M]^+$ at *m/z* 226 in the EI mass spectrum and the ^{13}C NMR spectrum were in agreement with the molecular formula $C_{13}H_{22}O_3$. In the ^1H NMR spectrum of **34** there were three methyl singlets at δ 1.23, 1.29, and 1.83, one methyl doublet at δ 1.28, three methylenes groups at δ 2.15/1.77 (H-2), 2.50/2.30 (H-7), and 1.60 (H-8), a double doublet at δ 4.30 (H-3)

and a multiplet at δ 3.89 (H-9) due to two oxygenated methines. The ^{13}C NMR (DEPT) spectrum showed thirteen signals, including four methyls, three methylenes, two methines, and four quaternary carbons, which were assigned on the basis of HMQC experiments.

^{1}H-^{1}H COSY experiments with **34** showed a correlation series beginning with δ 3.89 (H-9), which was coupled to the methyl doublet at δ 1.28 (H-10) and to H-8 at δ 1.60. The latter, in turn, was correlated with H-7 at δ 2.50/2.30. The signal at δ 4.30 was correlated to the methylene at δ 2.15/1.77. The C=O group was placed in 4-position, and the OH groups were attached to C-3 and C-9, respectively, on the basis of HMBC experiments. HMBC correlations of H-2 and H-13 with C-4, of H-2 with C-3, and of H-8 and H-10 with C-9 confirmed the above assignments. In the NOESY spectrum of **34**, spatial interactions between the CH_3 groups at δ 1.23 and H-3, and between the CH_3 at δ 1.29 and H-2 were observed, which allowed us to differentiate between H-11 and H-12 methyls.

The absolute configuration of **34** was established by Mosher's method[37] upon conversion of **34** into the diastereoisomeric Mosher diesters. ^{1}H-^{1}H COSY experiments allowed us to identify the signals for H-2 and H-8 of the two diastereoisotopic diesters. Comparison of the chemical shifts of these protons in both the (*R*)- and the (*S*)-*Mosher* derivatives, and calculation of the corresponding shift differences, expressed as $\Delta\delta = \delta(R) - \delta(S)$, were in agreement with an (*R*)-configuration at both C-9 and C-3, as further confirmed by a positive Δδ value for CH_3-13, and negative ones for CH_3-11 and CH_3-12.[38]

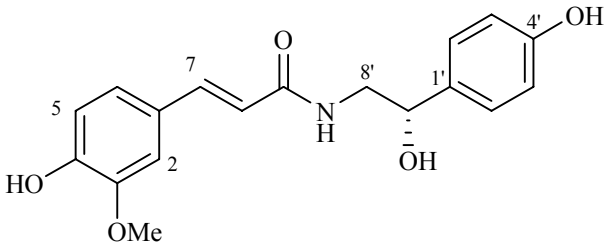


35

Compound **35** was identified as 3-*O*-methyl-*chiro*-inositol. The ESI mass spectrum showed the pseudo-molecular ion peak $[M+H]^+$ at *m/z* 195. The ^{1}H NMR spectrum showed a methoxyl group at δ 3.35, and six methines at δ 3.91, 3.89, 3.73, 3.62, 3.58, and 3.25. The ^{13}C NMR (DEPT) spectrum showed signals due to seven carbons, including one methyl and six methine groups. Analysis of the ^{1}H-^{1}H COSY and HSQC spectra of **35** suggested the presence of a sequence of six consecutive methine groups, and their ^{1}H NMR coupling constants were in good agreement with those previously reported.[39] The methoxyl group was positioned at C-3,

on the basis of HMBC correlations between C-3 and H-1, H-2, H-4, H-5, and the Me, respectively.

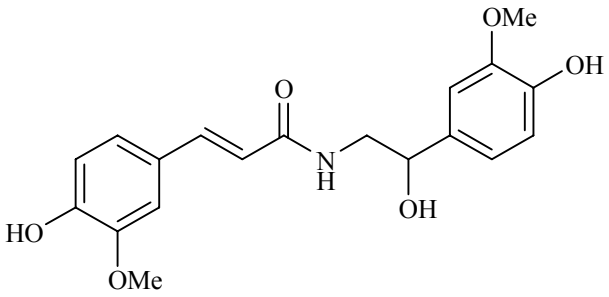
Furthermore, three cinnamic acid amides (**36-38**) and two lignanamides (**39-40**) were isolated from *A. cordifolia* leaves. Cinnamic acid amides are constituted of a cinnamic acid unit linked to a tyramine derivative. Hydrocinnamic acid amides have been observed in the leaves of virus-infected tobacco and the authors[40] suggested that they have antiviral effect. Several amides and lignanamides have been found in *Cannabis sativa* and *Procelia macrocarpa*,[41] some of them proved to have biological activities.



36

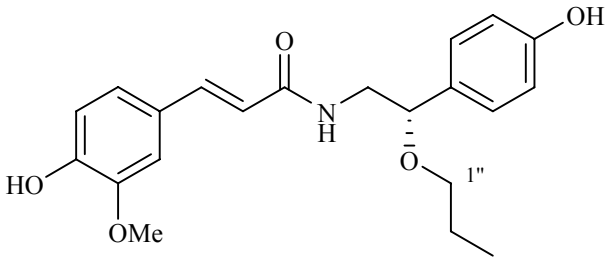
The molecular formula of compound **36** was deduced to be $C_{18}H_{19}NO_5$ as the EIMS spectrum showed the molecular ion peak $[M]^+$ at *m/z* 329. The UV spectrum revealed bands at 222, 294, and 316 nm. Signals at δ 7.44 (1H, d, J=15.8 Hz, H-7) and 6.45 (1H, d, J=15.8 Hz, H-8), in the ^{1}H NMR spectrum, indicated the presence of a *trans*-substituted double bond. Signals at δ 7.12 (1H, d, J=1.9 Hz, H-2), 6.79 (1H, d, J=8.8 Hz, H-5), and 7.03 (1H, dd, J=1.9 and 8.8 Hz, H-6) in the ^{1}H NMR spectrum suggested the presence of a 1,2,4-trisubstituted aromatic ring, as did signals at δ 111.7 (C-2), 116.1 (C-5), and 123.3 (C-6) in the ^{13}C NMR (DEPT) spectrum. In the HMBC spectrum, long-range correlations from the H-7 olefinic proton to the carbonyl carbon C-9 (δ 169.6) and the methine carbons C-2 and C-6 were observed, indicating the presence of a feruloyl group. Signals at δ 7.22 (2H, d, J=8.9 Hz, H-2', H-6') and 6.77 (2H, d, J=8.9 Hz, H-3', H-5') in the ^{1}H NMR spectrum, and signals at δ 128.4 (C-2' and C-6') and 116.5 (C-3' and C5'), in the ^{13}C NMR (DEPT) spectrum, suggested the presence of a *p*-substituted aromatic ring. In the ^{1}H NMR spectrum, the signals at δ 4.72 (1H, dd, J=4.9 and 7.8 Hz, H-7'), 3.53 (1H, dd, J= 4.9 and 13.7 Hz, H-8a'), 3.44 (1H, dd, J=7.8 and 13.7 Hz, H-8b'), as well as, signals at δ 73.5 (C-7'), and 48.1 (C-8') in the ^{13}C NMR (DEPT) spectrum indicated the presence of a 2-amino ethanoyl chain. In the HMBC spectrum, long-range correlations from the H-8' protons to the carbonyl carbon and the methine carbon (δ 73.5, C-7') were observed, indicating the

presence of a 2-hydroxy-2-(4-hydroxyphenyl) ethyl amine group (octopamine) linked to C-9 of the ferulic unit. The structure of compound **36** was *N*-[2-hydroxy-2-(4-hydroxyphenyl)ethyl] ferulamide. The optical rotation of **36** was found to be -3.0°, establishing the *S* (-) absolute configuration at C-7′ chiral center.[42] The *R* (+) isomer was previously isolated from root bark of *Lycium chinense*[43] and it showed antifungal activity. This substance was synthesized in a combinatorial library of a small molecule that selectively induces apoptosis in cancer cells.[44]



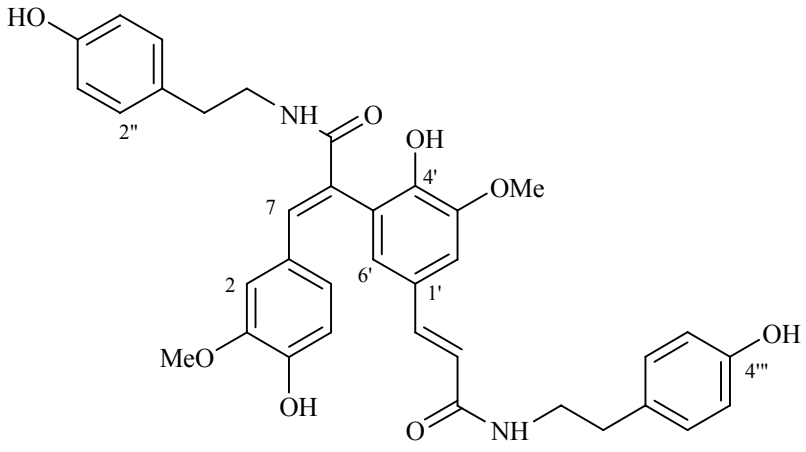
37

Data analysis of compound **37** has led to identify it as *N*-feruloyl normetanephrine. It had molecular formula $C_{19}H_{21}NO_6$ as deduced from the molecular ion peak $[M]^+$ at *m/z* 359 in the EIMS spectrum. The UV spectrum revealed bands at 226, 298, and 320 nm. The ^{13}C NMR spectrum showed the presence of seventeen signals. The DEPT experiment evidenced a methyl, a methylene, and nine methines. In the ^1H NMR spectra, signals corresponding to two 1,2,4-trisubstituted aromatic rings were present. The H-2, H-5, and H-6 of the ferulic moiety, in the ^1H NMR spectrum, were at δ 7.13, 6.80, and 7.03, as a narrow doublet, a doublet, and a double doublet, respectively. The H-2', H-5', and H-6' of the normetanephrine moiety were at δ 7.00, 6.77, and 6.83 as a narrow doublet, a doublet, and a double doublet, respectively. Furthermore, the spectrum showed the H-7 and H-8 *trans* olefinic protons at δ 7.45 and 6.47, the H-8' methylene as two double doublets at δ 3.54 and 3.44, and the H-7' as a double doublet at δ 4.73. In a NOE experiment the protons of the methoxyl group at δ 3.88 had relation with the proton doublet at δ 7.13 and the protons of the methoxyl at δ 3.86 had relation with the protons at δ 7.00. Finally, the HMBC experiment evidenced the following correlations: H-2' with C-4'; H-5' with C-1' and C-3'; H-6' with C-4' and C-7'; H-8' with C-9 and C-1'; H-2 with C-4; H-5 with C-1 and C-3; H-6 with C-4 and C-7; H-7 with C-9, and H-8 with C-1. This is the first report of *N*-feruloyl normetanephrine from plant extract. It was previously identified during the cloning and expression of a potato cDNA encoding hydroxycinnamoyl-CoA:tyramine N-(hydroxycinnamoyl) trasferase.[45]



38

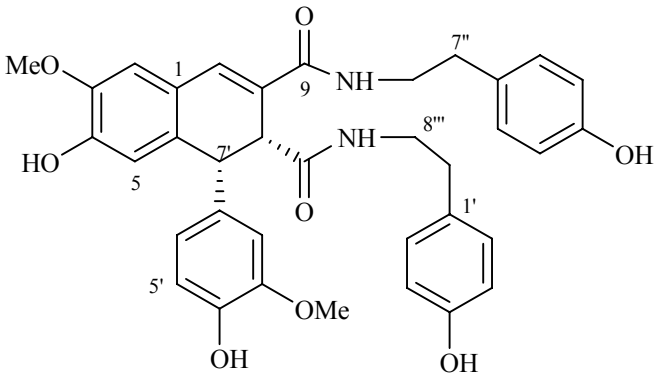
Compound **38** had never been isolated before. It had the molecular formula $C_{21}H_{25}NO_5$ as deduced from the molecular ion peak $[M]^+$ at *m/z* 371 in the EIMS spectrum. The general features of its MS and NMR spectra closely resembled those of **36**, except that for the presence of 42 mass unit more than **36**, and three signals in the ^1H NMR (δ 3.28, 1.58, and 0.92) and ^{13}C NMR (δ 72.1, 24.5, and 11.5) spectra attributed to an *n*-propyl group. The long-range correlations, in the HMBC spectrum, from the H-7' protons to the C-8' carbon and the methine carbon (δ 72.1, C-1") were observed, indicating that the *n*-propyl group was linked to the alcoholic hydroxyl.



39

Compound **39** showed the pseudo-molecular ion peak $[M-H]^-$ at *m/z* 623 in the MALDI mass spectrum. Its molecular formula was deduced to be $C_{36}H_{36}N_2O_8$ from NMR spectral data. The

UV spectrum revealed bands at 246, 315, and 336 nm. The ^{1}H and ^{13}C resonances of **39** were assigned by combination of COSY, DEPT, HMQC, and HMBC experiments. Two doublets at δ 7.40 and 6.38 (J=16.0 Hz), in the ^{1}H NMR spectrum, indicated the presence of a *trans*-substituted double bond. Furthermore, an olefinic proton singlet at δ 7.59 was evident. Signals at δ 6.52 (1H, d, J=2.0 Hz, H-2), 6.65 (1H, d, J=8.0 Hz, H-5), and 6.74 (1H, dd, J=2.0 and 8.0 Hz, H-6), in the ^{1}H NMR spectrum, suggested the presence of a 1,2,4-trisubstituted aromatic ring, as did correlations with the signals at δ 113.6 (C-2), 116.4 (C-5), and 116.8 (C-6) in the HSQC spectrum. The ^{1}H NMR spectrum showed two narrow doublets at δ 7.19 (1H, d, J=2.0 Hz, H-2′), 6.83 (1H, d, J=2.0 Hz, H-6′), of a 1,3,4,5-tetrasubstituted aromatic ring, correlated in the HSQC experiment at δ 112.2 and 125.1 carbon signals, respectively. Furthermore, eight *ortho*-coupled protons of two disubstituted aromatic rings were present as doublets at δ 7.04 (2H, d, J=8.0 Hz, H-2′′, H-6′′), 6.70 (2H, d, J=8.0 Hz, H-3′′, H-5′′), 6.91 (2H, d, J=8.0 Hz, H-2′′′, H-6′′′), and 6.62 (2H, d, J=8.0 Hz, H-3′′′, H-5′′′), correlated in the HSQC experiment to the carbon signals at δ 131.2 and 116.8. Signals at δ 3.47 (2H, t, J=6.8 Hz, H-8′′), 2.75 (2H, t, J=6.8 Hz, H-7"), 3.43 (2H, t, J=6.8 Hz, H-8"'), 2.65 (2H, t, J=6.8 Hz, H-7"'), correlated in the HSQC experiment to the carbons at δ 43.0 (C-8", C-8"'), 36.2 (C-7"), and 35.9 (C-7"'), indicated the presence of two 2-amino ethyl chains of a tyramine group. Long-range correlations between the H-7 olefinic proton and the carbonyl carbon (δ 170.4, C-9), the C-2 and C-6 methine carbons, and the C-1 and C-5′ quaternary carbons in the HMBC spectrum indicate the presence of a 8-linked feruloyl group. Long-range correlations from the H-7' proton to the carbonyl carbon (δ 169.5), the C-2′, C-6′, C-8′ methine carbons, and the C-1′ quaternary carbon, in the HMBC spectrum indicating the presence of a 5-linked feruloyl group. These correlations were consistent with a 8-5′ neolignan structure. The correlations from the H-8′′ protons to the C-9, and from the H-8′′′ protons to the C-9′ indicate the presence of two tyramine groups linked to C-9, and C-9′ carbons. The analysis of the NOESY spectrum evidenced NOEs between the H-7 proton with H-2 and H-6; the H-2 proton with 3-OMe; the H-2′ with H-8′ and 3′-OMe; the H-7′′ and H-8′′ with H-2′′ and H-6′′; the H-7′′′ and H-8′′′ with H-2′′′ and H-6′′′. These data confirmed the structure of compound **39** as depicted, it had never been isolated before.



40

Compound **40** showed the pseudo-molecular ion peak $[M-H]^-$ at *m*/*z* 623 in the MALDI mass spectrum. Its molecular formula was deduced to be $C_{36}H_{36}N_2O_8$. The ^1H and ^{13}C resonances of **40** were assigned by combination of COSY, DEPT, HMQC and HMBC experiments. The ^1H NMR spectrum of **40** showed the presence of two tyramine moieties, six aromatic and/or olefinic protons, two methoxy signals and two methine protons signals, which were coupled with each other. The ^1H and ^{13}C NMR data of **40** were almost coincident with those reported by Sakakibara *et al*.[46] for the dihydronaphthalene lignan, cannabisin D. The authors indicated a *trans* configuration of the phenyl group at C-7′ and the amide carbonyl at C-8' supported by the coupling constant between H-7′ and H-8′. Compound **40** showed, in the ^1H NMR spectrum, the relative protons as two doublet at 4.36 and 3.70 (J=3.9 Hz). The coupling constant between H-7′ and H-8′ indicates that the corresponding dihedral angle should be *ca* 45°. The minimized structure obtained by MM2 calculation[47] was used to generate dihedral angles and an angle of 52° was measured, which is compatible with a *cis* configuration (Fig. 2).[48] Correspondingly, the NOESY spectrum of **40** showed NOE between H-7′ with H-5 and H-2′, so the *quasi*-equatorial orientation of bond C-7′-H-7′ was supposed, and *quasi*-axial orientation of C-8′-H-8′.

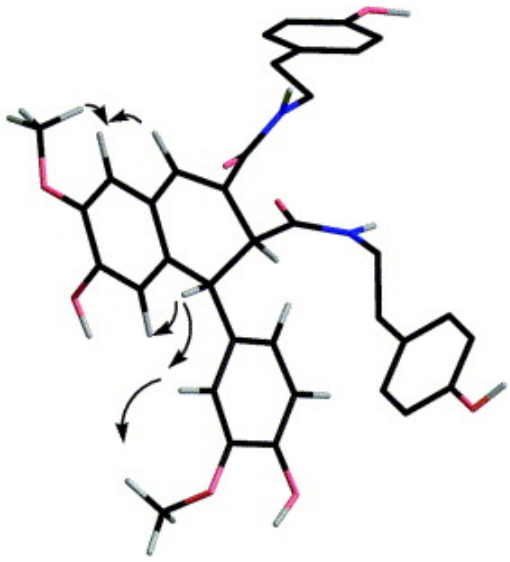


Fig. 2. Selected NOEs of minimized structure of compound **40**.

Table 4. NMR Data (CD_3OD, 500 MHz) for Compound **39**

Position	^{13}C (ppm)[a]	^{1}H δ (m, *J*/Hz)	NOESY	HMBC[b]
1	127.0			
2	113.6	6.52 (d, 2.0)	3-OMe	1, 3, 4, 7
3	150.6			
4	149.3			
5	116.4	6.65 (d, 8.0)		4
6	116.8	6.74 (dd, 8.0, 2.0)		2, 4, 7
7	139.1	7.59 (s)	2, 6	1, 2, 9, 5’
8	129.4			
9	170.4			
3-OMe	56.2	3.38 (s)	2	3
1’	128.8			
2’	112.2	7.19 (d, 2.0)	3’-OMe, 8’	3’, 6’, 7’
3’	149.9			
4’	148.7			
5’	129.7			1’, 4’
6’	125.1	6.83 (d, 2.0)	8’	1’, 2’, 4’, 7’
7’	141.9	7.40 (d, 16.0)		1’, 2’, 6’, 8’, 9’
8’	120.2	6.38 (d, 16.0)		1’, 9’
9’	169.5			
3’-OMe	57.2	3.96 (s)	2’	3’
1”	131.8			
2”	131.2	7.04 (d, 8.0)	7”, 8”	1”, 4”
3”	116.8	6.70 (d, 8.0)		1”, 2”, 4”, 6”
4”	157.3			
5”	116.8	6.70 (d, 8.0)		1”, 3”, 4”
6”	131.2	7.04 (d, 8.0)	7”, 8”	2”, 4”, 7”
7”	36.2	2.75 (t, 6.8)	2”, 6”	1”, 2”, 6”, 8”
8”	43.0	3.47 (t, 6.8)	2”, 6”	1”, 7”, 9
1”’	131.6			
2”’	131.2	6.91 (d, 8.0)	7”’, 8”’	1”’, 3”’, 4”
3”’	116.8	6.62 (d, 8.0)		1”’, 2”’, 4”’
4”’	157.3			
5”’	116.8	6.62 (d, 8.0)		1”’, 3”’, 4”’
6”’	131.2	6.91 (d, 8.0)	7”’, 8”’	2”’, 4”’, 7”’
7”’	35.9	2.65 (t, 6.8)	2”’, 6”’	8”’
8”’	43.0	3.43 (t, 6.8)	2”’, 6”’	9’, 1”’, 7”’

[a] ^{13}C NMR assignments are supported by a DEPT experiment.
[b] HMBC correlations from H to C.

Table 5. NMR Data (CD_3OD, 500 MHz) for Compound **40**

Position	^{13}C (ppm)[a]	^{1}H δ (m, *J*/Hz)	NOESY	HMBC[b]
1	125.3			
2	113.7	6.88 (s)	7, 3-OMe	3, 4, 6, 7
3	148.7			
4	147.2			
5	117.7	6.52 (s)	7’	1, 3, 7’
6	133.0			
7	135.1	7.21 (s)	2	2, 8, 9, 8’
8	128.0			
9	170.0			
3-OMe	57.1	3.90 (s)	2	3
1’	136.4			
2’	113.0	6.69 (d, 1.0)	3’-OMe, 7’	4’, 6’, 7’
3’	150.1			
4’	146.0			
5’	116.2	6.66 (d, 8.0)		1’, 4’
6’	121.9	6.41 (dd, 8.0, 2.0)		2’, 4’, 7’
7’	48.1	4.36 (d, 3.9)	5, 2’	1, 5, 6, 8, 1’, 2’, 6’, 6’, 9’
8’	50.3	3.70 (d, 3.9)		6, 7, 8, 9, 8’, 9’
9’	175.0			
3’-OMe	56.8	3.75 (s)	2’	3’
1”	131.9			
2”	131.3	6.97 (d, 8.5)	7”	1”, 3”, 4”, 7”
3”	116.7	6.68 (d, 8.5)		1”, 4”, 5”
4”	157.4			
5”	116.7	6.68 (d, 8.5)		1”, 3”, 4”
6”	131.3	6.97 (d, 8.5)	7”	1”, 4”, 5”, 7”
7”	36.2	2.65 (t, 6.8)	2”, 6”	2”, 6”, 8”
8”	43.0	3.42 (t, 6.8)		9, 1”, 7”
1”’	131.6			
2”’	131.2	6.82 (d, 8.5)	7”’	1”’, 4”’, 7”’
3”’	116.7	6.65 (d, 8.5)		1”’, 4”’, 5”’
4”’	157.4			
5”’	116.7	6.65 (d, 8.5)		1”’, 3”’, 4”’
6”’	131.2	6.82 (d, 8.5)	7”’	1”’, 4”’, 7”’
7”’	35.9	2.50 (t, 6.8)	2”’, 6”’	2”’, 6”’, 8”’
8”’	42.9	3.23 (t, 6.8)		9’, 1”’

[a] ^{13}C NMR assignments are supported by a DEPT experiment.
[b] HMBC correlations from H to C.

3.2. *Oxalis pes-caprae*

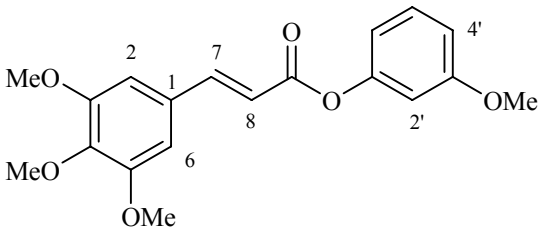
Oxalis pes-caprae, also known as 'Bermuda buttercup', is a native plant of South Africa, belonging to Oxalidaceae family. It is a perennial herb with a short, vertical stem that is mostly underground. It blooms in late winter or early spring with bright yellow flowers, its sour taste is due to oxalic acid present throughout the plant. It can form dense mats on the ground, outcompeting native plant species for light and space, and also works to inhibit the germination of native species. B. buttercup is most often associated with Mediterranean climates, but also occurs in subtropical and semi-arid regions. It is widely distributed in Italy and commonly found in cultivated lands. Additionally, it may cause oxalate poisoning in livestock if eaten in large quantities.

Fig. 3- *Oxalis pes-caprae*

Fresh leaves and twigs were infused with EtOAc and successively with CH_3OH for 7 days at room temperature.

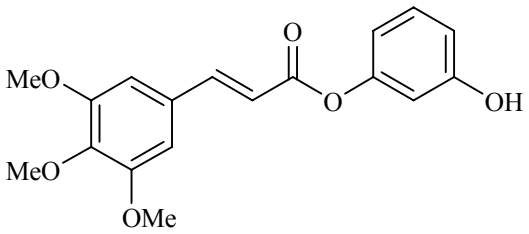
The EtOAc extract was concentrated by evaporation and fractionated into acidic and neutral fractions with aqueous NaOH 2M. The neutral fraction, washed with H_2O and concentrated under vacuum, was fractionated by silica gel column chromatography and the fractions were purified by preparative layer chromatography and HPLC yielding eight new cinnamic ester derivatives (**44-51**) and four flavons (**56-59**).

The MeOH extract was concentrated and partitioned between EtOAc and H_2O. The crude EtOAc residue was chromatographed on silica gel column and the fractions were purified by preparative layer chromatography and HPLC yielding six new cinnamic ester derivatives (**41-43**, **52-54**),[49] one lignan (**55**), and a few phenols and cinnamic acids (**60-66**).



41

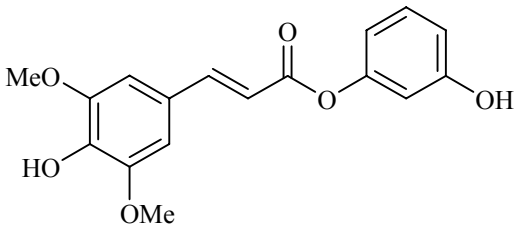
Compound **41** was determined to have the molecular formula $C_{19}H_{20}O_6$ by the molecular ion peak [M^+] at *m*/*z* 344 in the EIMS spectrum. The ^{1}H NMR and COSY spectra revealed two sets of 1,2,3,5-tetrasubstituted and 1,3-disubstituted benzene rings, a disubstituted *trans* double bond, and four methoxyls. The ^{13}C NMR spectrum showed fifteen carbon signals. The DEPT spectrum showed three methyls, and seven methines that were correlated to the corresponding protons by the HSQC experiment. In a NOESY experiment the protons of the methoxyl groups at δ 3.91 had relation with the proton singlet at δ 6.82 and the protons of the methoxyl at δ 3.86 had relation with the protons at δ 6.73 and 6.79. Finally, the HMBC experiment evidenced the following correlations: H-2/H-6 with C-3/C-5, C-4, and C-7; H-7 with C-2/C-6, C-8, and C-9; H-8 with C-1 and C-9; H-2' with C-3' and C-4'; H-4' and H-6' with C-1' and C-2'; H-5' with C-1' and C-3'. Thus, the structure of (*E*)-3'-methoxyphenyl 3,4,5-trimethoxycinnamate was established for compound **41**.



42

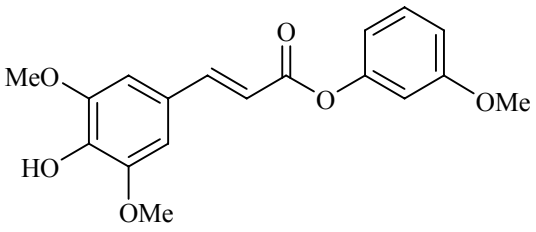
Compound **42** was identified as (*E*)-3'-hydroxyphenyl 3,4,5-trimethoxycinnamate. It had molecular formula $C_{18}H_{18}O_6$ as deduced from the molecular ion peak [$M]^+$ at *m*/*z* 330 in the EIMS spectrum. In the ^{1}H NMR spectrum, signals corresponding to two 1,2,3,5-tetrasubstituted and 1,3-disubstituted benzene rings were present. The H-2 and H-6 of the 3,4,5-trimethoxycinnamoyl moiety were at δ 6.82 as a singlet. In turn the H-5', H-4', H-6', and

H-2' of the 3-hydroxyphenyl moiety were at δ 7.25, 6.74, 6.72, and 6.68, respectively. Furthermore, the spectrum showed the H-7 and H-8 *trans* olefinic protons at δ 7.78 and 6.53, and three methoxyl groups at δ 3.91. In a NOESY experiment the protons of the methoxyl groups had relation with the proton singlet at δ 6.82. Finally, the HMBC experiment evidenced the following correlations: H-2/H-6 with C-3/C-5, C-4, and C-7; H-7 with C-1, C-2/C-6, and C-9; H-8 with C-1 and C-9; H-2' with C-3' and C-4'; H-4' and H-6' with C-1' and C-2'; H-5' with C-1' and C-3'.



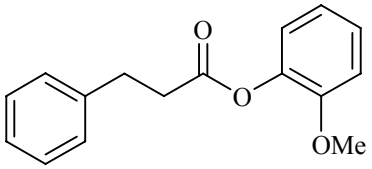
43

Spectra analysis of compound **43** defined it as 3'-hydroxyphenyl sinapate. It had molecular formula $C_{17}H_{16}O_6$ as deduced from the molecular ion peak $[M]^+$ at *m/z* 316.0943 in the HREIMS spectrum. The ^{13}C NMR spectrum showed the presence of thirteen signals. In the ^1H NMR spectrum the H-2 and H-6 signal of the sinapoyl moiety was at δ 6.82 as a singlet. The H-5', H-4'/H-6', and H-2', of the 3-hydroxyphenyl moiety were at δ 7.22, 6.70, and 6.66, respectively. Furthermore, the spectrum showed the H-7 and H-8 *trans* olefinic protons at δ 7.77 and 6.47, and two methoxyl groups at δ 3.92. In a NOESY experiment the protons of the methoxyl groups had relation with the proton singlet at δ 6.82. In a HMBC study, the correlations were comparable to those of **42**.



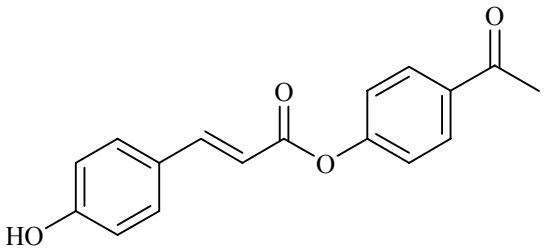
44

Compound **44** was determined to be (*E*)-3'-methoxyphenyl sinapate. The ^{13}C NMR spectrum showed fourteen carbon signals. The DEPT spectrum showed two methyls, eight methines, and five quaternary carbons. The ^{1}H NMR and ^{1}H-^{1}H COSY spectra revealed the presence of two 1,2,3,5-tetrasubstituted and 1,3-disubstituted benzene rings. The H-2/H-6 of the sinapoyl moiety were at δ 6.83, in turn the H-6', H-5', H-4', and H-2' of the 3-methoxyphenyl moiety were at δ 6.82, 7.29, 6.68, and 6.63, respectively. Furthermore, the spectrum showed the H-7 and H-8 trans olefinic protons at δ 7.79 and 6.52, and three methoxyl groups at δ 3.92 and 3.91. HMBC and NOESY experiments supported the hypothesized structure.



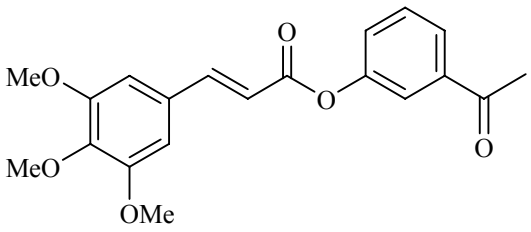
45

Compound **45** was identified as 2'-methoxyphenyl dihydrocinnamate. It had a molecular ion peak $[M]^{+}$ at *m/z* 256 in the EI mass spectrum that according to the ^{13}C NMR spectrum, suggested a molecular formula $C_{16}H_{16}O_3$. The ^{13}C NMR spectrum and a DEPT experiment indicated the presence of one methyl, two methylenes, seven methines, and four quaternary carbons. In the ^{1}H NMR spectrum H-2, H-3, H-5, H-6 appeared as a multiplet at δ 7.29, H-4 and H-6' as a multiplet at δ 7.20, H-3' as a doublet at δ 7.05, H-4' and H-5' as multiplet at δ 6.92. Furthermore, the spectrum showed a methoxyl group at δ 3.76 and two triplets at δ 3.04 and δ 2.92 mutually coupled of an ethylic chain. An HMBC showed correlations between the signal at δ 7.29 and C-1, C-4, C-7; H-3' and H-6' with C-1', C-2', C-4', and C-5'. In a NOESY experiment the protons of the methoxyl group at δ 3.76 had relation with the proton at δ 7.05.



46

Compound **46** was identified as (*E*)-4'-acetylphenyl 4-hydroxycinnamate. It had a molecular ion peak $[M]^+$ at *m/z* 282 in the EI mass spectrum that, according to the ^{13}C NMR spectrum, suggested a molecular formula $C_{17}H_{14}O_4$. The ^1H NMR and ^1H-^1H COSY spectra revealed two sets of 1,4-disubstituted benzene rings: H-2/H-6 and H-3/H-5 of the cinnamoyl moiety were at δ 7.51 and 6.88 as doublets, H-3'/H-5' and H-2'/H-6' of the acetylphenyl moiety were as doublets at δ 8.03 and 7.28, respectively. Furthermore, the ^1H NMR spectrum showed the H-7 and H-8 *trans* olefinic protons at δ 7.84 and 6.49 and one methyl group at δ 2.62 which had relation with H-3' and H-5' in a NOESY experiment. Finally, the HMBC experiment evidenced the following correlations: H-2/H-6 with C-3/C-5, C-4, C-1 and C-7; H-7 with C-1, C-2/C-6, and C-9; H-8 with C-1 and C-9; H3'/H5' with C-1', C-4', C-7'; H-8' with C-4'.

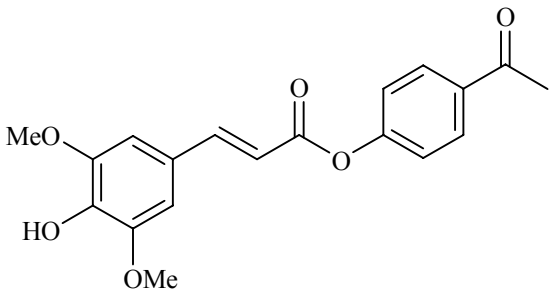


47

Spectral data of compound **47** and the molecular ion peak $[M]^+$ at *m/z* 356 in the EI mass spectrum suggested a molecular formula $C_{20}H_{20}O_6$. In the ^{13}C NMR spectrum seventeen carbon signals were present and the DEPT experiment evidenced three methyls and seven methines. Pattern signals in the ^1H NMR spectrum indicated the presence of two 1,2,3,5-tetrasubstituted and 1,3-disubstituted aromatic rings. Two olefinic protons appeared at δ 7.80 and 6.54, three methoxyl groups at δ 3.90 and one methyl at δ 2.61. The ^1H and ^{13}C resonances were assigned by combination of ^1H-^1H COSY, DEPT, HMQC, and HMBC experiments. The HMBC spectrum evidenced the following correlations: H-2/H-6 with C-3/C-5, C-4, C-1, and C-7; H-7

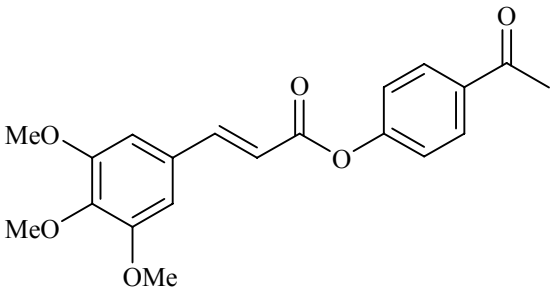
with C-1, C-2/C-6, C-8 e C-9; H-2' with C-1', C-3', C-4', C-6'; H-8' with C-3' and C-7'. According to the structure, in a NOESY experiment the protons of the methoxyl groups had relation with H-2/H-6 and the protons of the methyl group at δ 2.62 had relation with H-2' and H-4'.

Based on these findings, compound **47** was concluded to be (*E*)-3'-acetylphenyl 3,4,5-trimethoxycinnamate.



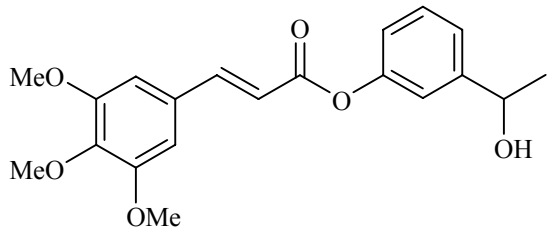
48

Compound **48** was determined to be (*E*)-4'-acetylphenyl sinapate. Spectral data and a molecular ion peak $[M]^+$ at *m/z* 342 in the EI mass spectrum indicated a molecular formula $C_{19}H_{18}O_6$. The ^{13}C NMR spectrum showed fourteen carbon signals, which have been attributed by a DEPT experiment to two methyls, five methines and seven quaternary carbons. In ^1H NMR spectrum two protons of a 1,2,3,5-tetrasubstituted aromatic ring resonated at δ 6.84 and protons of a 1,4 disubstituted aromatic ring resonated as two large doublets at δ 7.27 and 8.02. A singlet signal integrated for six protons appeared at δ 3.94 and a methyl group at δ 2.61. The connection of functional groups has been determined using HMBC correlations: H-2/H-6 gave cross peaks with C-3/C-5 and C-4; H-7 and H-8 with C-1 and C-9; H-2'/H-6' with C-1' and C-4'; H-3'/H5' and methyl protons with C-4' and the carbonyl carbon (δ 196.9). According to the structure in the NOESY experiment the methoxyl groups had relation with H-2/H-6 protons at δ 6.84.



49

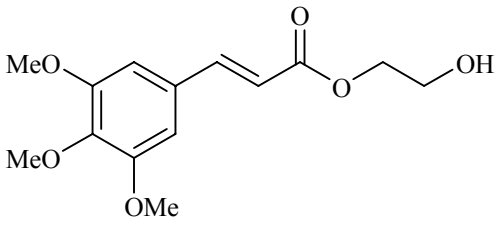
The molecular formula $C_{20}H_{20}O_6$ of compound **49** was suggested by the molecular ion peak $[M]^+$at *m/z* 356 in the EI mass spectrum and the ^{13}C NMR spectrum. The general features of its NMR spectra closely resembled those of **48**, except for the presence of nine methoxyl protons in the ^1H spectrum and a further methoxyl carbon in the ^{13}C NMR spectrum. Analysis of spectral data allowed to defined compound **49** as (*E*)-4'-acethylphenyl 3,4,5-trimethoxycinnamate.



50

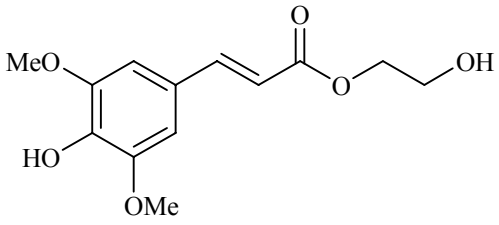
Compound **50** was identified as (*E*)-3'-(1-hydroxyethyl)-phenyl 3,4,5-trimethoxycinnamate. The molecular ion peak $[M]^+$ at *m/z* 358 in the EI mass spectrum and the ^{13}C NMR spectrum suggested the molecular formula $C_{20}H_{22}O_6$. The ^{13}C NMR (DEPT) spectra showed seventeen signals defined as three methyls, eight methines, six quaternary carbons. The ^1H NMR spectrum showed a singlet at δ 7.00 integrated for two protons attributed to H-2/H-6; a doublet at δ 7.25 and a double doublet at δ 7.03 attributed to H-4' and H-6' respectively, and two triplets at δ 7.37 and 7.18 relative to H-5' and H-2'. Furthermore, the spectrum showed H-7 and H-8 trans olefinic protons as two doublets at δ 7.81 and 6.69, a doublet and a quartet at δ 4.85 and δ 1.44 correlated in the ^1H-^1H COSY experiment and attributed to H-7' and H-8', respectively. A HMQC experiment allowed to assign the protons to the corresponding carbons.

In the HMBC spectrum protons H-2/H-6 were correlated to C-4 and C-7; H-2’ and H-5’ to C-1’, C-4’.



51

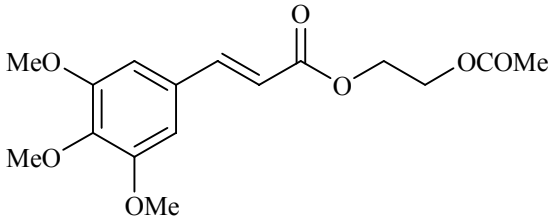
Compound **51** was identified as (*E*)-2-hydroxyethyl 3,4,5-trimethoxycinnamate. It had molecular formula $C_{14}H_{18}O_6$ as deduced from the molecular ion peak $[M]^+$ at *m*/*z* 282 in the EIMS spectrum. The ^{13}C NMR spectrum showed the presence of eleven signals. The DEPT experiment evidenced two methyls, two methylenes and three methines. In the ^1H NMR spectrum, signals corresponding to a 1,2,3,5-tetrasubstituted benzene ring were present. The H-2 and H-6 of the 3,4,5-trimethoxycinnamoyl moiety were at δ 6.75 as a singlet. The H-2' and H-3' of the hydroxyethyl chain were at δ 4.35, and 3.90, respectively. Furthermore, the H-7 and H-8 *trans* olefinic protons occurred at δ 7.63 and 6.38, and three methoxyl groups were observed at δ 3.88. In a NOESY experiment the protons of the methoxyl groups had relation with the proton singlet at δ 6.75. Finally, the HMBC experiment evidenced the following correlations: H-2/H-6 with C-3/C-5, C-4, and C-7; H-7 with C-1, C-2/C-6, and C-9; H-8 with C-1 and C-9; H-1' with C-9 and C-2'; H-2' with C-1'.



52

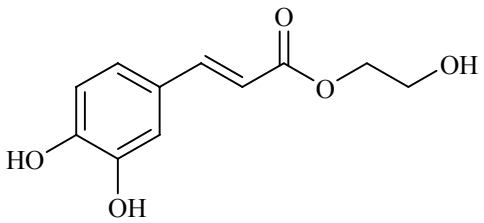
Compound **52** was defined to be 2-hydroxyethyl sinapate. It had molecular formula $C_{13}H_{16}O_6$ as deduced from the molecular ion peak $[M]^+$ at *m*/*z* 268 in the EIMS spectrum. The ^{13}C NMR

spectrum showed the presence of ten signals, assigned by DEPT experiment as a methyl, two methylenes, and three methines. In the ^{1}H NMR spectrum, signals corresponding to a 1,3,4,5-tetrasubstituted benzene ring were present. The H-2 and H-6 of the sinapoyl moiety, in the ^{1}H NMR spectrum, were at δ 6.77 as a singlet. The H-1' and H-2' of the 2-hydroxyethyl moiety were at δ 4.35 and 3.87, respectively. Furthermore, the spectrum showed the H-7 and H-8 *trans* olefinic protons at δ 7.64 and 6.38, and two methoxyl groups at δ 3.91. In a NOESY experiment the protons of the methoxyl groups had relation with the protons at δ 6.77. Finally, the HMBC experiment evidenced the following correlations: H-2/H-6 with C-3/C-5, C-4, and C-7; H-7 with C-1, C-2/C-6, and C-9; H-8 with C-1 and C-9; H-1' with C-9 and C-2'; H-2' with C-1'.



53

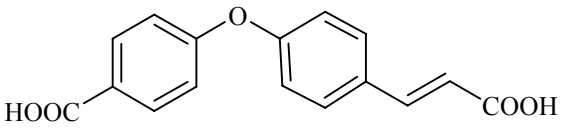
Compound **53** was identified as acetyl derivative of compound **44**. Its ^{1}H NMR spectrum showed the presence of a additional methyl at δ 2.11 and the ^{13}C NMR spectrum showed a methyl and carbonyl at δ 20.9 and 170.8, respectively. This hypothesis was confirmed by the EIMS spectrum that showed a molecular ion peak [M^{+}] at *m*/*z* 324 according to the molecular formula $C_{16}H_{20}O_{7}$.



54

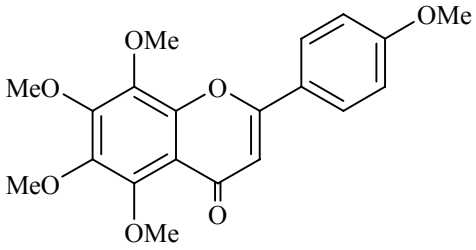
Compound **54** had molecular formula $C_{11}H_{12}O_{5}$ as deduced from the molecular ion peak $[M]^{+}$ at *m*/*z* 224 in the EIMS spectrum. The ^{13}C NMR spectrum showed the presence of eleven signals, assigned by DEPT experiment as two methylenes and five methines. In the ^{1}H NMR

spectrum, signals corresponding to a 1,2,5-trisubstituted benzene ring were present. The H-2, H-5, and H-6 of the caffeoyl moiety, in the ^{1}H NMR spectrum, were at δ 7.04, 6.77, and 6.95, as a narrow doublet, a doublet, and a double doublet, respectively. The H-1' and H-2' of the 2-hydroxyethyl moiety were at δ 4.24 and 3.79, respectively. Furthermore, the spectrum showed the H-7 and H-8 *trans* olefinic protons at δ 7.58 and 6.29. Finally, the HMBC experiment evidenced the following correlations: H-2 with C-3, C-4, and C-7; H-6 with C-2, C-4, and C-5; H-7 with C-1, C-2, C-6, and C-9; H-8 with C-1 and C-9; H-1' with C-9 and C-2'; H-2' with C-1'. All these features allowed to define compound **54** as 2-hydroxyethyl caffeate.



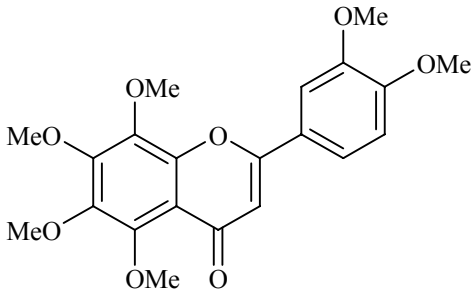
55

Compound **55** showed a pseudo-molecular ion peak $[M+H]^{+}$ in a MALDI mass spectrum at *m/z* 285 that, along with spectral data, indicated a molecular formula $C_{16}H_{12}O_5$. The ^{1}H NMR spectrum showed the presence of protons coupled in a AA'XX' system of two 1,4-disubstituted aromatic rings and two *trans* olefinic protons at δ 7.45 and 6.30. The ^{13}C NMR spectrum and a DEPT experiment showed five methines and six quaternary carbons, two of them at δ 170.6 and 171.3 relative to two carboxyl groups. The HMQC experiment allowed to attribute the protons to the corresponding carbons. The HMBC spectrum showed cross-peaks of H-7 and H-8 with C-9 and C-1; H-2/H-6 with C-4 and C-7; H-2'/H-6' with C-4' and C-7'. These data have led to identify compound **55** as *(E)*-4-[4-(2-carboxyvinyl)phenoxy] benzoic acid.



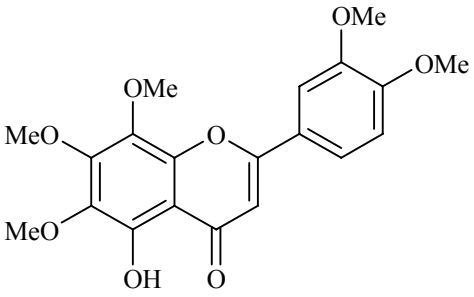
56

Compound **56** was determined to be 5,6,7,8,4'-pentamethoxyflavone. It had a molecular ion peak $[M]^+$ at *m/z* 372 in the EI mass spectrum that, along with the ^{13}C NMR spectrum, suggested a molecular formula $C_{20}H_{20}O_7$. ^{13}C NMR spectrum showed eighteen carbons defined by a DEPT experiment as five methoxyls, three methines, and seven quaternary carbons. ^1H NMR spectrum showed a pair of doublets at δ 7.88 and 7.02 attributed to H-2'/H-6' and H-3'/H-5', a singlet at δ 6.60 attributed to H-3, three singlets at δ 4.10, 4.02, and 3.89 each integrated for three protons, and a singlet at δ 3.95 integrated for six protons. These data were in agreement with those previously reported.[50]



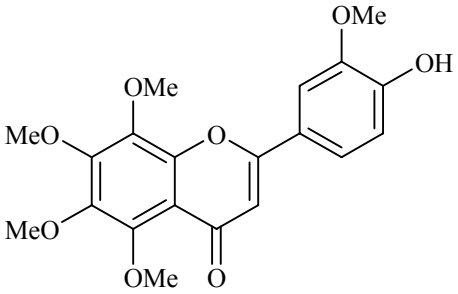
57

^1H NMR spectrum of compound **57** evidenced the presence of a 1,2,4-trisubstituted aromatic ring, a singlet at δ 6.62, and six methoxyl groups. ^{13}C NMR spectrum and a molecular ion peak $[M]^+$ at *m/z* 402 in the EI mass spectrum defined the molecular formula $C_{21}H_{22}O_8$. By comparing the spectral data with those previously reported, compound **57** was identified as 5,6,7,8,3',4'-esamethoxyflavone.[50]



58

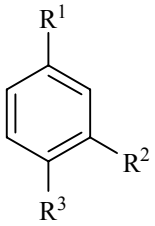
NMR data of compound **58** were similar to that of compound **57**. The ^{1}H NMR spectrum showed two doublets and a double doublet of a 1,2,4-trisubstituted aromatic ring, one singlet at δ 6.60, and five methoxyls. ^{13}C NMR spectrum showed twenty carbons defined as five methoxyls, four methines, and eleven quaternary carbons through a DEPT experiment. All spectroscopic data agreed with those reported for 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone.[50]

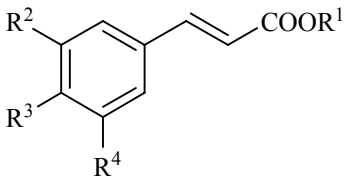


59

Compound **59** was identified as 4'-hydroxy-5,6,7,8,3'-pentamethoxyflavone on the basis of its spectroscopic data and by comparing them to data reported in the literature.[50] The ^{1}H NMR spectrum showed two doublets and a double doublet in the aromatic region, one singlet at δ 6.60, and five methoxyls. The ^{13}C NMR and DEPT spectrum showed the presence of five methoxyls, four methines and eleven quaternary carbons. The cross peak between a methoxyl group and H-2' in the NOESY experiment confirmed the hydroxyl position.

By comparison with authentic samples metabolites **60-66** were identified as 1,3-dihydroxybenzene, 4-hydroxybenzyl alcohol, 3-methoxy-4-hydroxybenzoic acid, 4-hydroxycinnamic acid, methyl 4-hydroxycinnamate, sinapic acid and caffeic acid, respectively.





60 R^1=R^2=OH R^3=H
61 R^1=CH_2OH R^2=H R^3=OH
62 R^1=COOH R^2=OMe R^3=OH

63 R^1=R^2=R^4=H R^3=OH
64 R^1=Me R^2=R^4=H R^3=OH
65 R^1=H R^2=R^4=OMe R^3=OH
66 R^1=R^2=H R^3=R^4=OH

3.3. *Swinglea glutinosa*

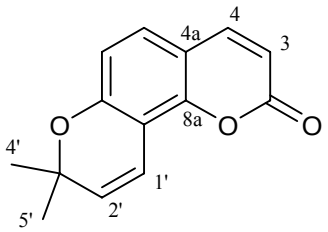
Swinglea glutinosa is a small tropical tree from the Rutaceae family. Brought to South America from South East Asia, it is used as ornamental plant in Colombia and as a natural barrier in rural area and gardens. *S. glutinosa* possesses fruits, which are not edible, but have an extraordinary pleasant smell. Essential oil from *S. glutinosa* fruit peel was obtained by microwave-assisted hydro-distillation. The complex odour bouquet exhibited by the essential oil depends upon its chemical composition, but not only on the presence of compounds at higher concentrations.

Extracts of the stem bark of this plant have been previously assessed for antiplasmodial activity showing promising activity in vitro against *Plasmodium falciparum.*[51]

We investigated the roots extract to define its metabolites composition.

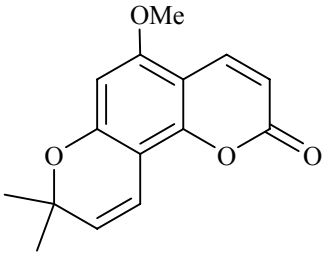
Fig. 4- *Swinglea glutinosa*

Dried root of *Swinlea glutinosa* was extracted at room temperature with EtOAc twice for 12 h. After evaporation under reduced pressure at 40 ^{o}C the extract was fractionated by silica gel flash column chromatography eluting with hexane and EtOAc. Fractions with similar TLC profiles were combined to yield 16 fractions, these were purified by column chromatography, TLC, and HPLC yielding three coumarins (**67**-**69**) and nine acridone alkaloids (**70**-**78**).
Coumarins constitute a class of widely distributed heterocyclic natural products exhibiting a broad pharmacological profile, including cytotoxic, antiviral or antibacterial activities.[52]



67

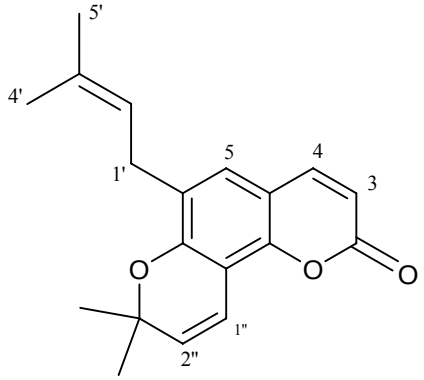
Compound **67** was isolated as colorless needles. The EI mass spectrum showed a molecular ion peak $[M]^{+}$ at *m/z* 228. The ^{1}H NMR spectrum showed two doublets at δ 7.54 and 6.15 (J=9.6 Hz) characteristic of H-4 and H-3 of a coumarin nucleus,[53] two doublets at δ 5.67 and 6.81 (J=9.6 Hz), and the singlet at 1.41 (6H) typical for the dimethylchromene ring. The remaining pair of doublets at δ 7.15 and 6.65 (J=8.4 Hz) was attributed to H-5 and H-6. These data were in agreement with those reported for seselin.[54] It has been reported to act as potential anticancer agent. [55]



68

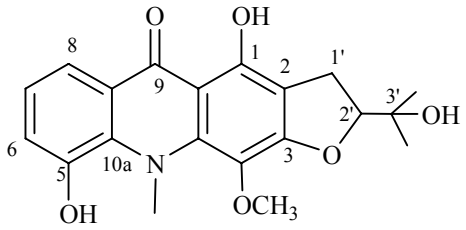
Spectral data of compound **68** identified it as 5-methoxyseselin. The ^{1}H NMR spectrum showed two doublets at δ 7.91 and 6.09 (J=9.4 Hz) attributed to the coumarin nucleus, the

characteristic signals of a dimethylchromene ring at δ 6.75 (1H, d, J=10.0 Hz), δ 5.54 (1H, d, J=10.0Hz), and δ 1.42 (6H, s), one singlet at δ 6.20, and one methoxyl group at δ 3.84. Comparison with literature data[56] confirmed the structure. Compound **68** has already been isolated from Rutaceae and it has been reported to have antibacterial activity. [57]



69

Compound **69** showed a pseudo-molecular ion peak $[M+H]^+$ at *m/z* 297.1491 in the HRESI mass spectrum suggesting the molecular formula $C_{19}H_{20}O_3$. The ^{13}C NMR and DEPT spectra indicated the presence of four methyls, one methylene, six methines, and seven quaternary carbons. The ^1H NMR spectrum showed two doublets at δ 7.54 and 6.16 (J=9.6 Hz) attributed to H-4 and H-3 of the coumarin nucleus, the characteristic signals of a dimethylchromene ring at δ 6.84 and δ 5.68 (d, J=10.2 Hz) and two methyls at δ 1.43 (6H, s). Furthermore, the spectrum showed a singlet at δ 7.02 and a prenyl group at δ 5.22 (1H, t, J=7.2 Hz), 3.24 (2H, d, J=7.2 Hz), 1.72 and 1.70 (each 3H). In a HMBC spectrum H-1'' had correlation with C-3'', and C-8a; H-2'' with C-1'' and C-8; H-5 with C-1', C-4, C-7, and C-8a. These data allowed to identified compound **69** as 6-(3,3-dimethylallyl) seselin. It has never been isolated before.



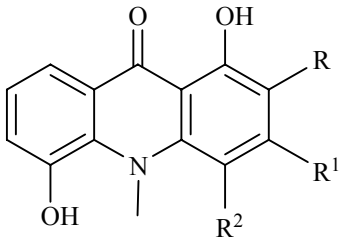
70

The molecular formula $C_{20}H_{21}NO_6$ of compound **70** was deduced on the basis of an HRESI mass experiment, which showed a pseudo-molecular ion peak $[M-H]^-$ at *m/z* 370.1291. The ^{13}C NMR spectrum showed twenty signals that were attributed by a DEPT experiment to four methyls, one methylenes, four methines, and eleven quaternary carbons. The presence of signal at δ 13.78, in the ^1H NMR spectrum, indicated a chelated phenolic hydroxyl group.

Signals relative to three aromatic protons in the ^1H NMR spectrum resonated in an ABX system in acetone-d_6 at δ 7.79 (1H, dd, J=1.6 and 8.0 Hz), δ 7.27 (1H, dd, J=1.6 and 8.0 Hz) and δ 7.15 (1H, t, J=8.0 Hz). They were attributed to H-8, H-6, and H-7, respectively, where the lower field proton at δ 7.79 was deshielded by the 9-carbonyl group. In the aliphatic region, protons at δ 3.20 (2H, m) and δ 4.87 (1H, dd, J=8.0 and 9.2 Hz), two methyl groups at δ 1.33 and δ 1.29, and a quaternary carbon, in the ^{13}C-NMR spectrum, at δ 72.0 were consistent with a hydroxyisopropyldihydrofuran moiety.

Two singlet signals at δ 3.87 and 3.70 (each 3H) correlated in the HSQC spectrum with ^{13}C signals at δ 60.6 and δ 46.5 were assigned to methoxyl and *N*-methyl groups, respectively.

Further support of the supposed structure was provided by an HMBC experiment. Proton at δ 7.79 (H-8) gave correlation with C-6, C-9, and C-10a; protons at δ 3.16 attributed to H-1'a,b gave heterocorrelation with C-1, C-3, and C-3'; protons at δ 1.26 and 1.40 relative to H-4' and H-5' methyls gave a cross peak with C-2' at δ 92.4. Based on literature[58] data, the structure of compound **70** was assigned as depicted.



71 R = $CH_2CH\text{-}C(CH_3)_2$ R^1 = OH R^2 = OMe
72 R = H R^1 = OH R^2 = OMe
73 R = R^1= R^2 = OMe
74 R = $CH_2CH\text{-}C(CH_3)_2$ R^1= OH R^2= H
75 R = H R^1 = OH R^2 = $CH_2CH\text{-}C(CH_3)_2$
76 R = H R^1 = R^2 = OMe

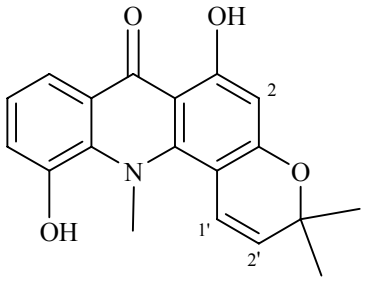
Compounds **71-78** contained a 1-hydroxy-9-acridone nucleus as common structural unit as suggested the ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum of compound **71** showed three aromatic protons in an ABX system, a methoxyl and a N-methyl at δ 3.79 and 3.78, and a prenyl moiety at δ 5.30 (1H, m), 3.40 (2H, d, J=7.3 Hz), 1.80 and 1.68 (each 3H, s). The substitution on acridone nucleus were deduced by C-H long range correlations observed in the HMBC spectrum. Comparison with literature data[59] allowed to define compound **71** as glycocitrine IV.

Compound **72** was identified as citrusinine II. Its ^1H NMR spectrum showed three ABC type protons and one singlet in the aromatic region, besides a methoxyl and a N-methyl groups. The combination of COSY, HMQC and HMBC spectra and comparison with data previously reported[60] confirmed the structure.

The ^1H NMR spectrum of compound **73** showed three protons coupled in an ABC system in the aromatic region, and four singlets each integrated for three protons at δ 4.12, 3.94, 3.83, and 3.80. Its spectral data were in agreement with those previously reported[61] for citrusinine I.

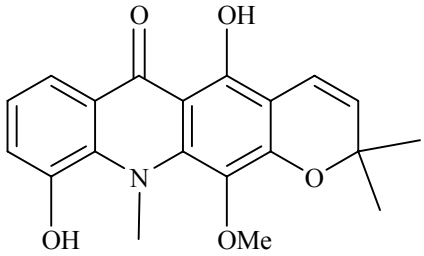
Compounds **74** and **75** were identified as junosine and oriciacridone. Their ^1H NMR spectra showed the presence of ABC-type aromatic protons, one isolated aromatic proton, a prenyl group, and a N-methyl group. HMQC and HMBC correlations allowed to locate the prenyl group at the C-2 position for compound **74** and at the C-4 position for **75**. Comparison with literature data[62] confirmed the structures.

Compound **76** had showed the same signal pattern as **72** but with two methoxyl groups. It was determined to be citrusinine I by comparing its data with those reported in the literature[61].



77

The ^{1}H NMR spectrum (acetone-d_6) of compound **77** showed the signals corresponding to four protons in the aromatic region. The ABX protons resonated at δ 7.78 (1H, d, J=7.6 Hz), 7.32 (1H, d, J=7.6 Hz), and 7.19 (1H, t, J=7.6 Hz) and were assigned to H-8, H-6 and H-7; one singlet at δ 6.11 was attributed to H-2 proton. The existence of a 2,2-dimethylpyran moiety was suggested by the coupled signals at δ 6.75 and 5.66 (2H, d, J=9.6 Hz), and two methyls at δ 1.50 (6H, s). N-methyl group was at δ 3.83. According to literature data[62] compound **77** was identified as atalaphyllidine.



78

Pattern signals in the ^{1}H NMR spectrum of **78** resembled that of **77** except for the presence of a methoxyl group at δ 3.85 instead of an aromatic proton at δ 6.11. H-1', H-2', and H-4'/H-5' of the dimethylpyran system were at δ 6.72, 5.71 and 1.52. Comparison with literature data[62] identified compound **78** as pyranofoline.

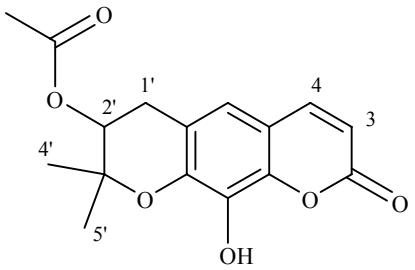
3.4. *Amyris madrensis*

Amyris madrensis, a densely-branched shrub, belongs to *Rutaceae* family. It is found only in Texas. The glossy evergreen leaves are dark green and leathery. The white flowers, inconspicuous but fragrant, appear during spring to fall, and the fruit is a small, attractive, reddish-brown drupe, greatly valued by birds. The aromatic leaves are used in making tea and as a tonic.

Only one publication is reported in literature about the chemical study of this plant.

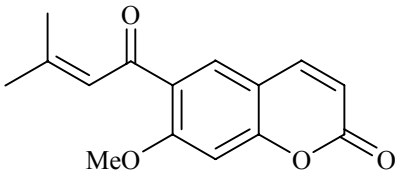
Fig. 5- *Amyris madrensis*

Dried leaves of *A. madrensis* were ground in a blender. Ground plant material was extracted at room temperature with hexane, EtOAc, and subsequently with CH_3OH. Preliminary bio-autography of the extracts on TLC plates revealed antifungal activity of the EtOAc extract. The occurrence of antifungal compounds was evidenced by the presence of clear zones on a dark background on the plate. That prompted us to investigate further. The EtOAc extract was fractionated by silica gel column chromatography and crystallization providing seven coumarins (**79-85**) and methyl eugenol (**86**).



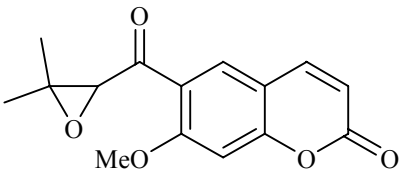
79

Compound **79** showed a pseudo-molecular ion peak $[M+H]^+$ at *m/z* 305.1009 in the HRESI mass spectrum suggesting the molecular formula $C_{16}H_{16}O_6$. The ^{13}C NMR spectrum showed sixteen carbons identified by a DEPT experiment as three methyls, one methylene, four methines, and eight quaternary carbons. In the aromatic region of the ^1H NMR spectrum two doublets characteristic for a coumarin nucleus were present at δ 7.59 and 6.20 and a singlet at δ 6.82, these signals were attributed to H-4, H-3, and H-7, respectively. Furthermore, the spectrum showed a triplet at δ 5.12, integrated for one proton (H-2'), correlated in a H-H COSY spectrum to a multiplet at δ 3.25 integrated for two protons (H-1'a,b). Three singlets, each integrated for three protons, were at δ 1.96, 1.57, and 1.52. The position of the substituents was determined by analyzing the HMBC spectrum, which revealed heteronuclear couplings of H-5 with C-8a, C-7, C-4, and C-1'; H-1' with C-7, C-6 and C-3'. These data defined the structure of compound **79** as depicted, it has been isolated for the first time.



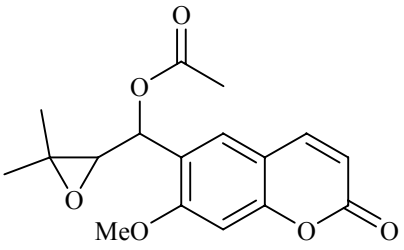
80

Compound **80** had a molecular formula $C_{15}H_{14}O_4$ suggested by the pseudo-molecular ion peak $[M+H]^+$ at *m/z* 259.0937 in the HRESI mass spectrum. ^1H and ^{13}C NMR spectra showed a pattern of signals typical of a coumarin nucleus and a 1-oxoprenyl chain with a carbonyl carbon at δ 190.7. Spectral data and comparison with literature data identified compound **80** as dehydrogeijerin.[63]



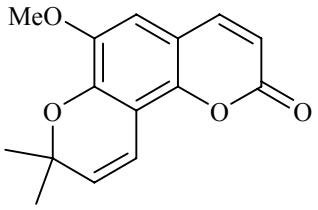
81

Compound **81** was determined to be hopeyhopin. The molecular formula $C_{15}H_{14}O_5$ was indicated by the pseudo-molecular ion peak $[M+H]^+$ at *m/z* 275.0915 in the HRESI mass spectrum. Signals at δ 4.01 in ^{1}H NMR spectrum (H-2') and δ 68.1 (C-2') and 62.1 (C-3') in the ^{13}C NMR spectrum suggested the presence of an oxirane ring. Literature data confirmed the assigned structure.[64]



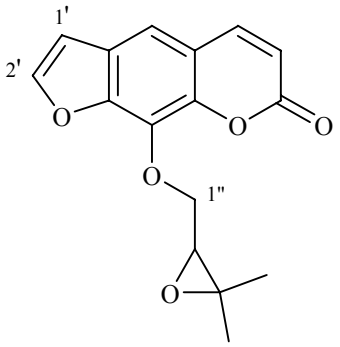
82

Compound **82** had molecular formula $C_{17}H_{18}O_6$ according to the pseudo molecular ion peak $[M+H]^+$ at *m/z* 319.0835 in the HRESI mass spectrum. The ^{1}H NMR spectrum showed two doublets at δ 7.62 and 6.21 (J=9.6 Hz) attributed to H-4 and H-3, two singlets a δ 7.43 and 6.77 relative at H-5 and H-8 respectively, and two doublets at δ 5.92 and 3.07 (J=8.8 Hz) attributed to H-1' and H-2'. Furthermore, the spectra showed a methoxyl group at δ 3.87 and three methyls at δ 2.07, 1.34, and 1.22. Comparison with literature data confirmed the structure.[65]



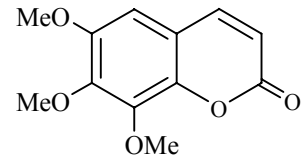
83

Compound **83** molecular formula $C_{15}H_{14}O_4$ according to the molecular ion peak $[M+H]^+$ at *m/z* 259.1025 in the HRESI mass spectrum.The ^1H NMR spectrum showed two doublets at δ 7.54 and 6.20 (J=9.4 Hz) attributed to the coumarin nucleus, the characteristic signals of a dimethylchromene ring at δ 6.80 (1H, d, J=10.0 Hz), δ 5.68 (1H, d, J=10.0 Hz), ad δ 1.46 (6H, s), one singlet at δ 6.72 and a methoxyl group at δ 3.84. These data were in agreement with those reported for braylin.[66]



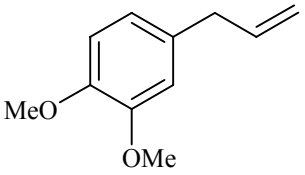
84

Compound **84** was determined to be heraclenin. The molecular formula $C_{16}H_{14}O_5$ was deduced by the molecular ion peak $[M+H]^+$ at *m/z* 287.0841 in the HRESI mass spectrum. In the ^1H NMR spectrum signals of the coumarin nucleus were at δ 7.76 (1H, d, J=9.6 Hz, H-4), 7.38 (1H, s, H-5), and 6.35 (1H, d, J=9.6 Hz, H-3); the furano protons appeared as doublets at δ 7.67 and 6.80 (J=2.4 Hz). Signals at δ 4.56 (2H, m), 3.29 (1H, m), 1.31 (3H, s) and 1.25 (3H, s) were attributed to H-1'', H-2'', H-4'', and H-5'', respectively. HMQC and HMBC experiments allowed to assigned the position of the substituents and the structure was confirmed by literature data.[67]



85

The EI mass spectrum of compound **85** showed a molecular ion peak $[M]^+$ at *m/z* 214 in the EI mass spectrum. The ^1H NMR spectrum accounted for three aromatic protons at δ 7.59 (d, J=9.6 Hz), 6.64 (s), and 6.24 (d, J=9.6 Hz), and three methoxyl groups at δ 3.99, 3.93, and 3.89. On the basis of this evidence, the compound was characterized as 6,7,8-trimethoxycoumarin and this was confirmed by literature data.[68]



86

The EI mass spectrum of compound **86** showed a molecular ion peak $[M]^+$ at *m/z* 178. The ^1H NMR spectrum showed signals of an ABX system attributed to a 1,2,4 trisubstituted aromatic ring, two multiplets at δ 5.95 and 5.05, and a doublet at δ 3.31 (J=6.7 Hz) of an allyl side chain. These data defined compound **86** as methyleugenol.

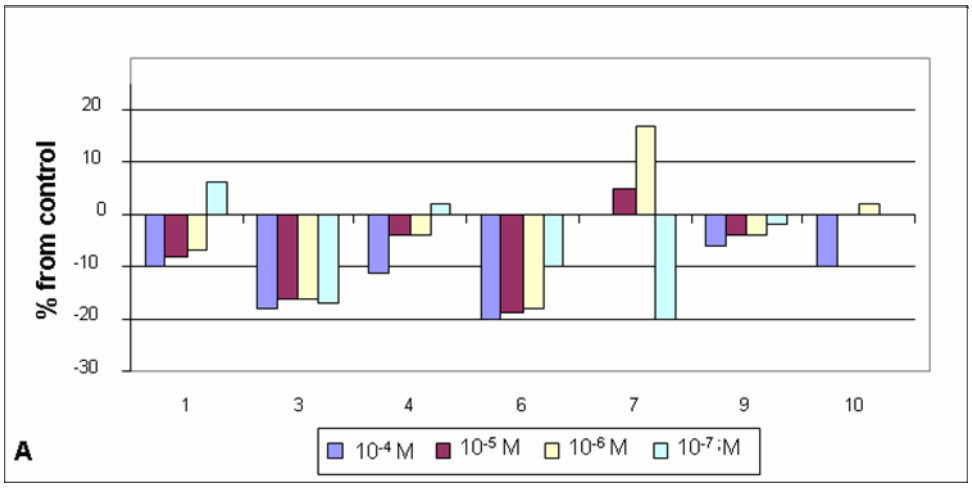
3.5. Bioassay

3.5.1 Phytotoxic activity of compounds isolated from A. cordifolia

The compounds isolated from *A. cordifolia* were tested for their phytotoxicity on the seeds of *Lactuca sativa*.[19] This species was selected as representative of main dicotyledon commercial crops.[19] It has been used extensively as a test organism because of its fast germination and high sensitivity, and allows comparison of bioassay results for many different compounds.[69] Aqueous solution of the isolated compounds, ranging between 10^{-4} and 10^{-7} M, were tested on germination, root length, and shoot length of treated lettuce seeds.

Figures 6A, B, C show the activity of the compounds **1**, **3**, **4**, **6**, **7, 9**, **10**, **13-15**, **17-19**, **26-29** and **32-35**. 3,4-Dimethoxy-dihydrocinnamic acid **13** and methyl 3-(1*H*-indol-3-yl) propanoate **28** were found to be the most active, reducing the germination by 90%, and the radical and shoot growth by 100% at 10^{-4} and 10^{-5} M, respectively. The other compounds showed slight inhibition of germination, with an activity of 10–30% at the highest concentration tested, with the exception of compounds **29** and **35**, which reduced the germination by 80 and 45% at 10^{-4} M, respectively. Exceptional was also compound **15**, which gave rise to a stimulation of germination at all concentrations tested (Fig. 6A). Further, the dilignan **18** showed a stimulation of root length at all concentrations tested, while compounds **29**, **32**, **33**, and **35** inhibited root growth of about 50% at the highest concentration tested (Fig. 6B). Dilignan **18** showed a stimulation of shoot length as well at all concentrations tested, compounds **32** and **33** reduced the shoot length by 60 and 80% at 10^{-4} M, respectively (Fig. 6C).

The activities of the above compounds isolated from *A. cordifolia* were compared with that of 4-hydroxybenzoic acid (HBA), a well-known and effective germination inhibitor.[70] The inhibition value for many compounds isolated from *A. cordifolia* were, at a concentration of 10^{-4} M, comparable to that of HBA. The bioactivities of the compounds tested showed a variable response on lettuce, and for some compounds, no dose-dependence effects were observed. The reason for this response could be due to differences in seed size, seed-coat permeability, differential uptake, and metabolism.[71]



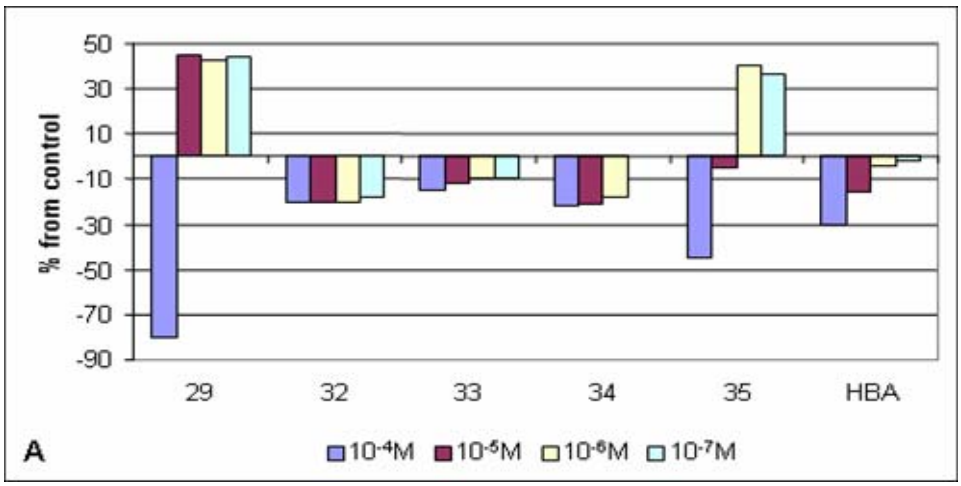
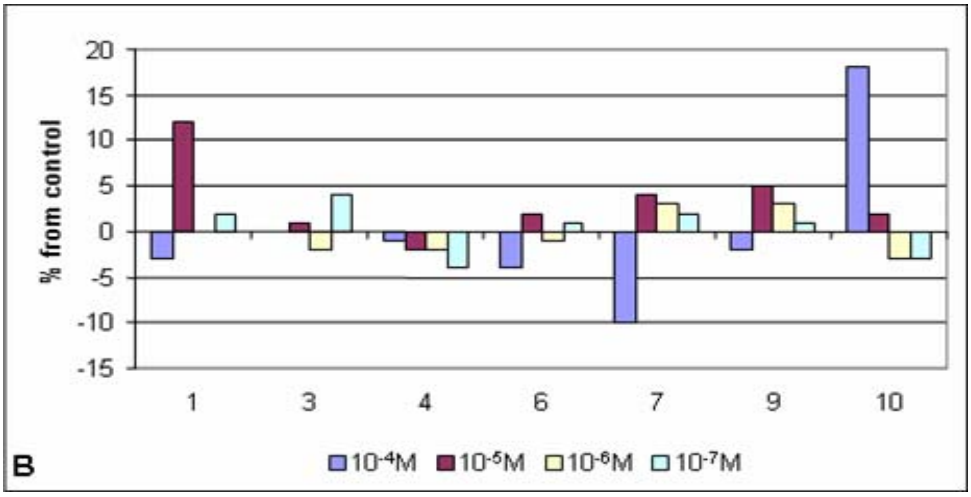
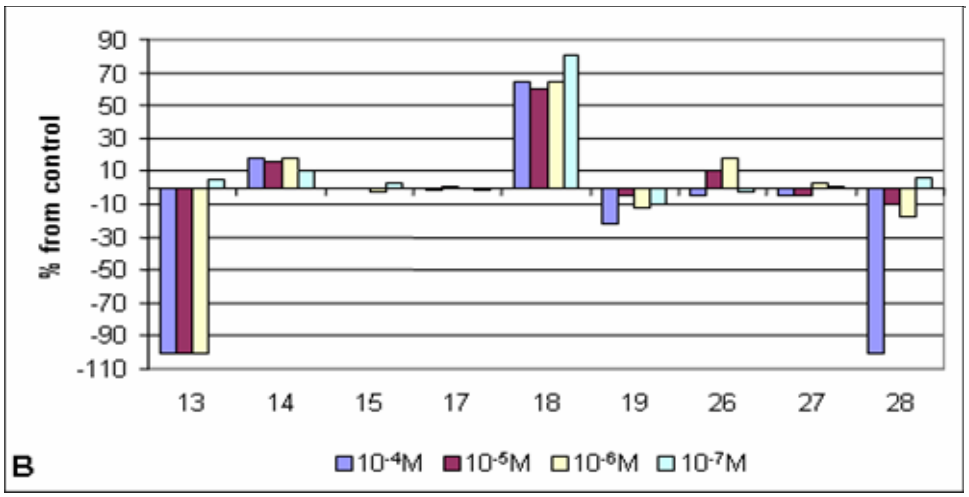


Fig. 6A- Effect on germination of *Lactuca sativa* L. Value presented as percentage differences from control.





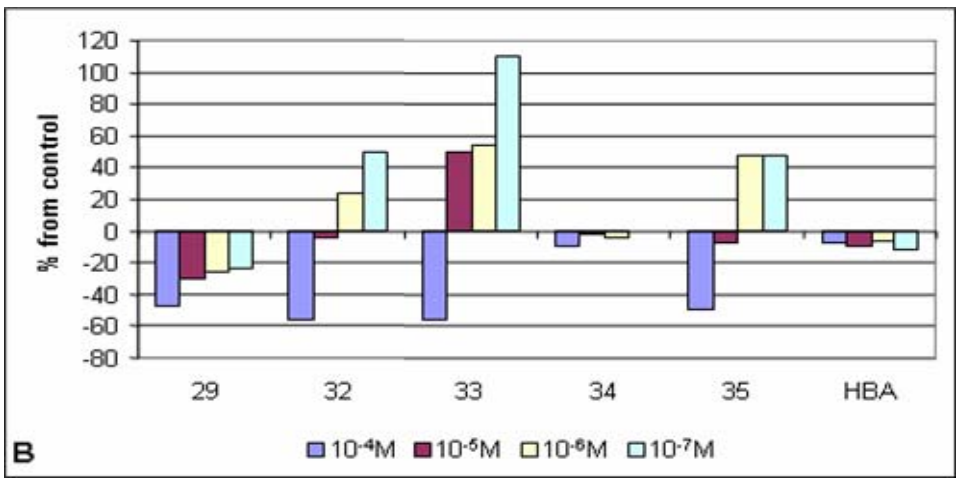
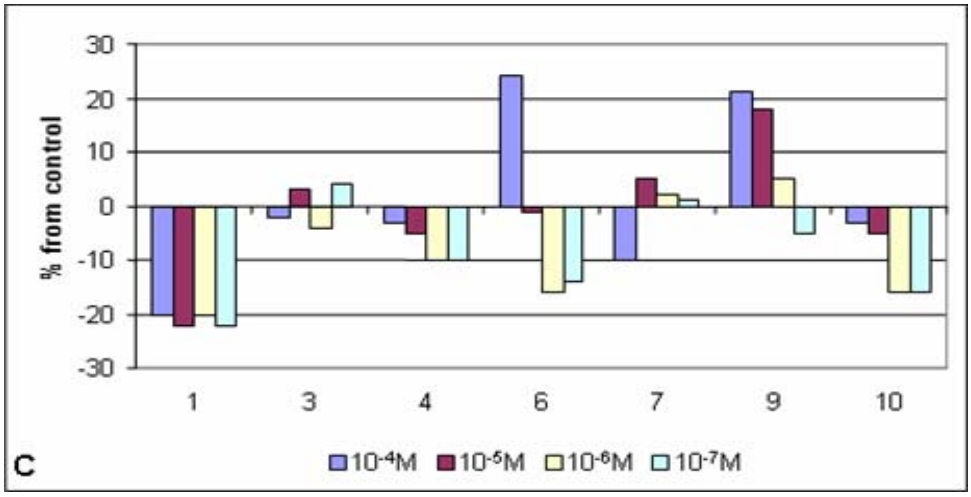
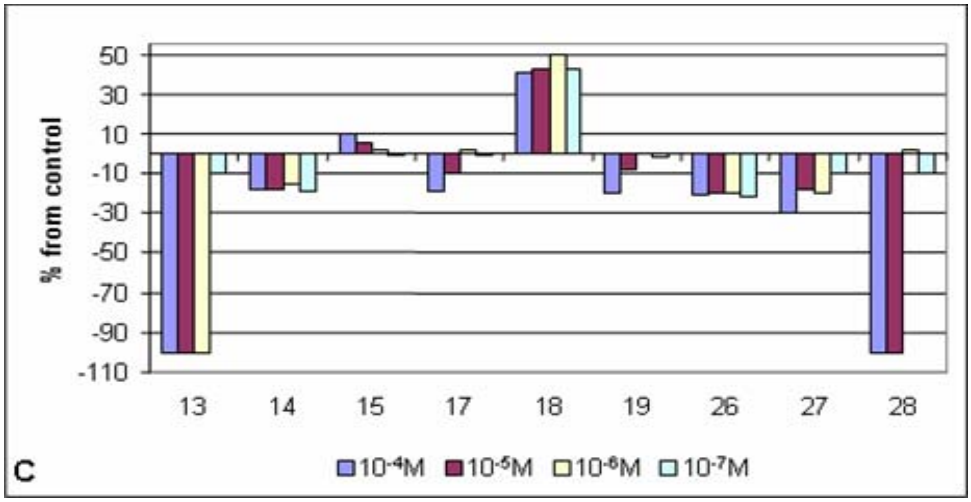


Fig. 6B- Effect on root length of *Lactuca sativa* L. Value presented as percentage differences from control





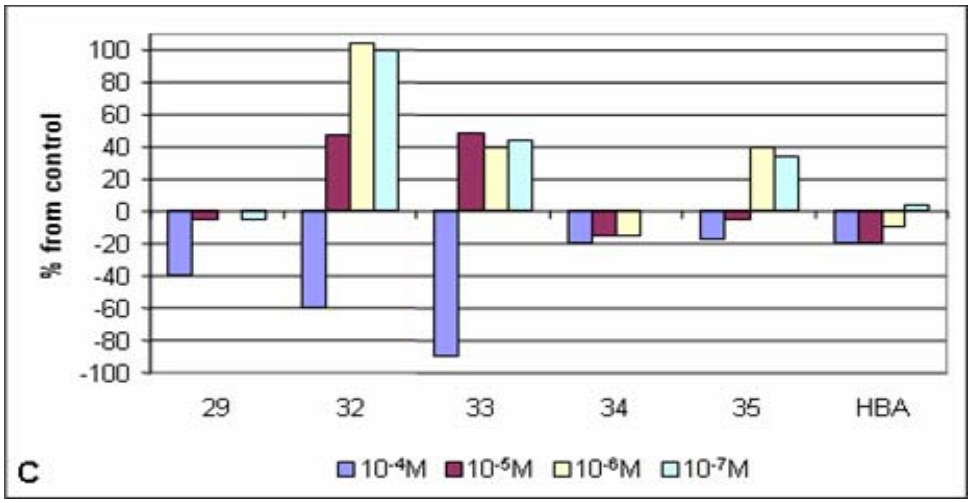
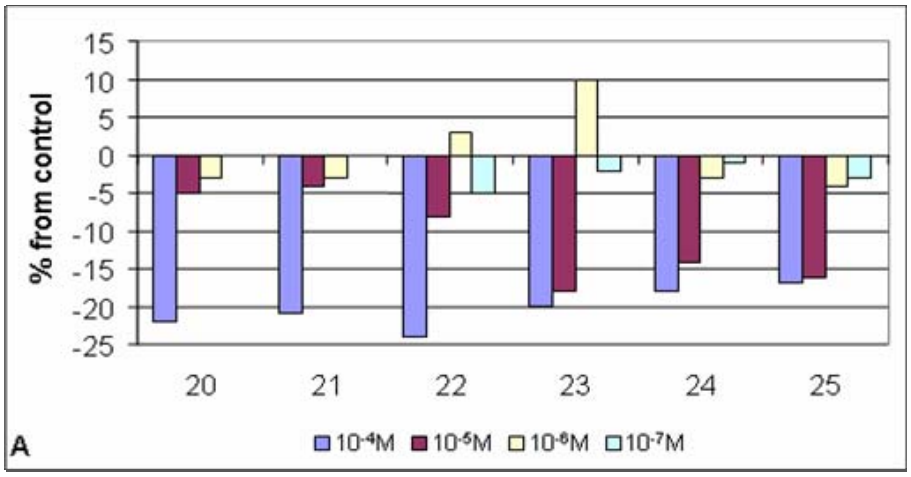
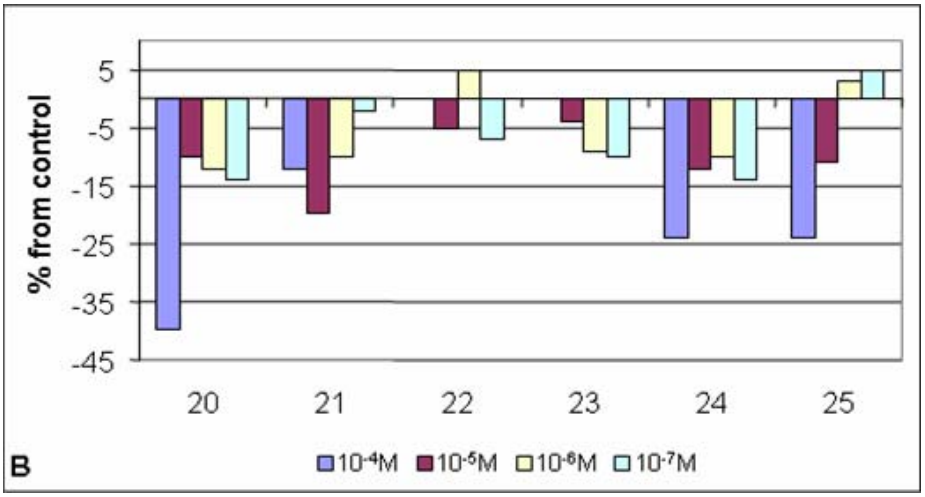


Fig. 6C- Effect on shoot lenght of *Lactuca sativa* L. Value presented as percentage differences from control

The activity of the oxyneolignans **20**-**25** are reported in Fig. 7. All compounds reduced the germination by 18-25% at 10^{-4} M, and dose dependence effects were observed. They reduced the root elongation by 20% at 10^{-4} M, but compound **20** was the most active (40%) when compared to the control and compound **22** was almost completely inactive. Amongst compounds **20**-**25**, only oxyneolignan **23** reduced shoot elongation by 15% at the highest concentration and no important effects were observed for compounds **20**, **22**, and **25**, while compounds **21** and **24** showed a stimulatory effects within 20-50%.





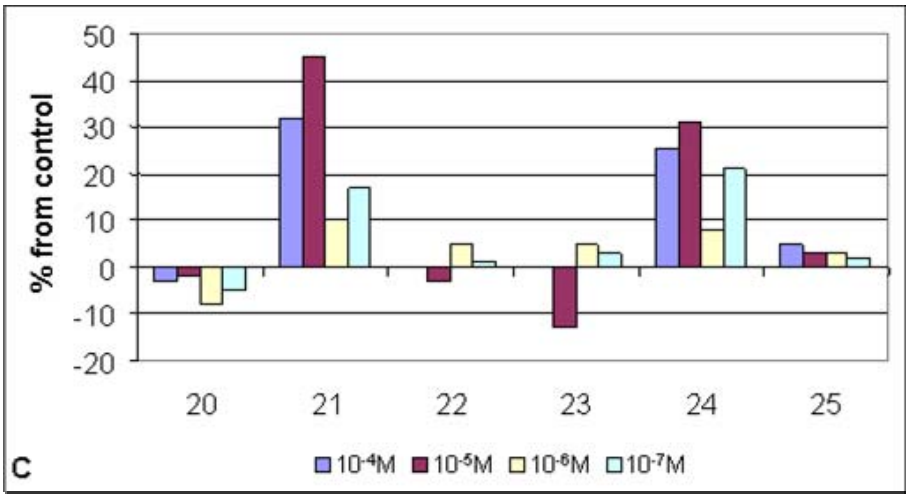
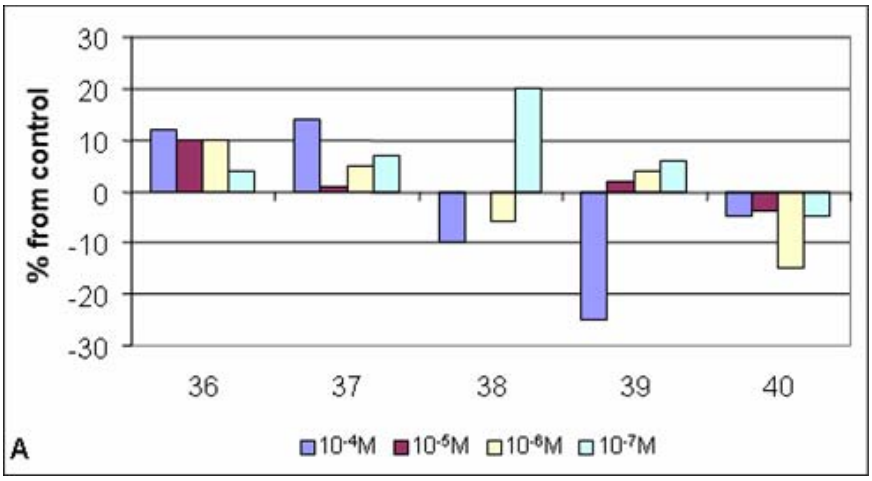
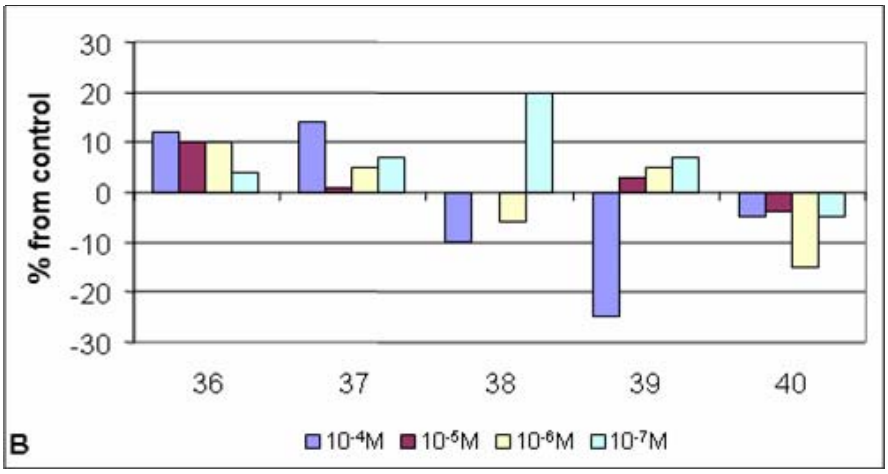


Fig. 7- Effect of compounds **20-25** on shoot lenght (A), root elongation (B), shoot elongation (C) of *Lactuca sativa* L.Value presented as percentage differences from control.

Cinnamic acid amides **37** and **38** (Fig. 8) reduced the germination by 20% compared to the control at 10^{-4} M, and dose dependence effects were observed. Compounds **36** and **39** were inactive, and compound **40** reduced the germination by 10 % at highest concentration tested. The root elongation of *L. sativa* was not affected by compounds tested, with exception of lignanamide **39** that showed 25% of inhibition at 10^{-4} M. Amongst compounds **36-40**, only amide **37** stimulated shoot elongation at all concentration tested and no important effects were observed for compounds **36**, **38**, **39,** and **40**.





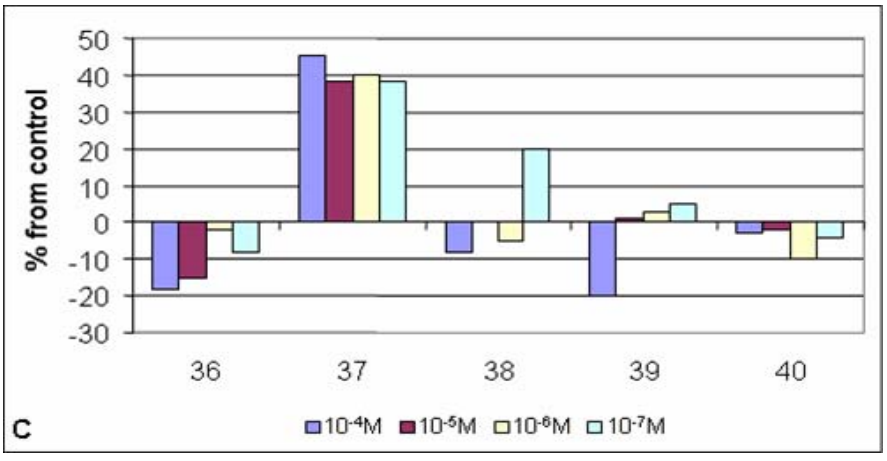
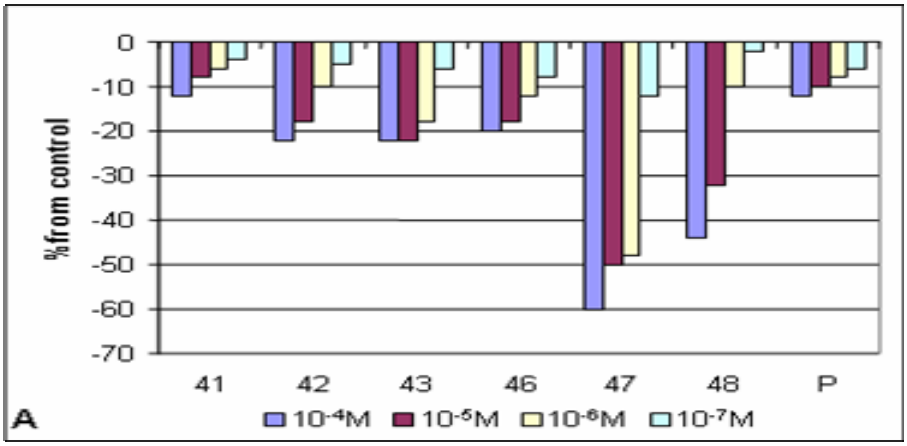


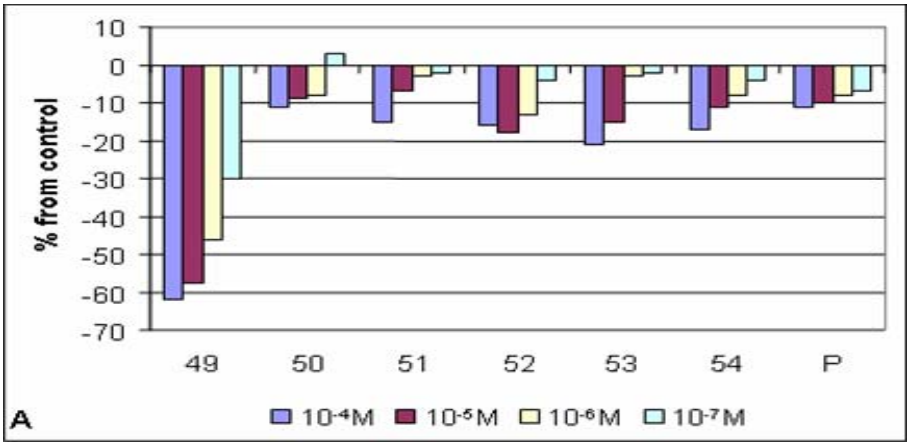
Fig. 8- Effect of compounds **36-40** on shoot lenght (A), root elongation (B), shoot elongation (C) of *Lactuca sativa* L.Value presented as percentage differences from control

3.5.2 Phytotoxic activity of compounds isolated from Oxalis pes-caprae

Some of the isolated compounds from *O. pes-caprae* were tested against on *Lactuca sativa* to evaluate the inhibitory or stimulatory effects on germination, root length, and shoot length of the tested seeds. The results are reported as percentage differences from the control in Fig. 9A, B, and C. The activities of the compounds were compared with that of pendimethalin (**P**), a commercial pre-emergence herbicide used widely in agriculture. Aqueous solutions of the compounds, ranging from 10^{-4} to 10^{-7} M, were investigated in accordance with the procedures optimized by Macias et al.[19]

The effects of the tested compounds on the inhibition of germination of *L. sativa* seeds (Fig. 9A) showed a variable behaviour within 10-20% at higher concentrations except for **47** and **49** that showed an inhibition of germination by 60-80% at the highest concentration tested.





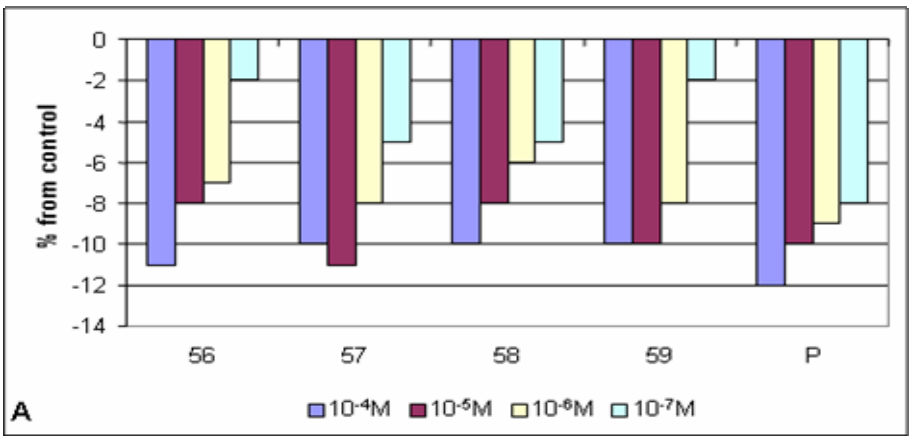
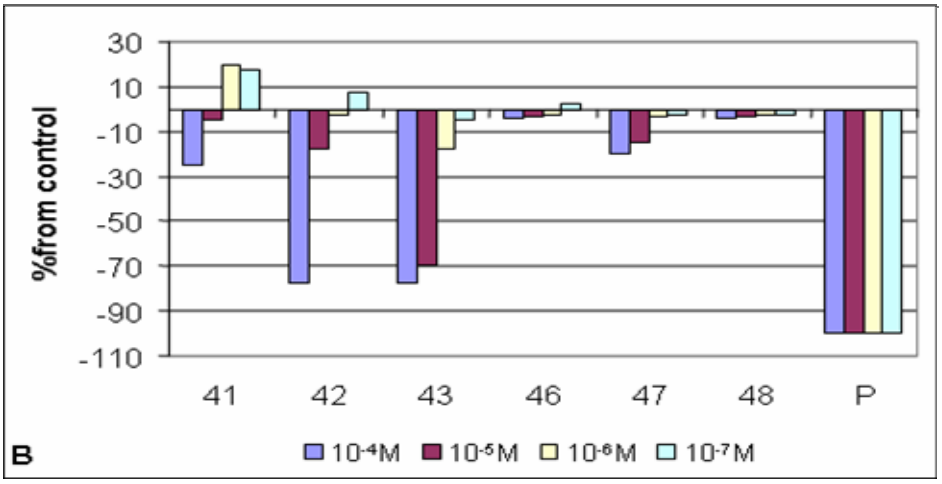


Fig. 9A- Effect on germination of *Lactuca sativa* L. Value presented as percentage differences from control.

The results reported in Fig. 9B show greater phytotoxic activities on lettuce root length by compounds **42**, **43**, **53**, and **54**, with respect to this standard herbicide. The tested compounds revealed ≥80% inhibition at 10^{-4} M concentration and compound **54** was found to be completely active up to 10^{-7} M. Compound **54** showed a radical and shoot inhibition of 100% at all concentration tested (Fig. 9B, C). This compound was tested at lower concentrations (10^{-8} and 10^{-9} M) and it showed no relevant effects on germination and shoot length, while slight stimulatory effects were observed on root length (results not shown).



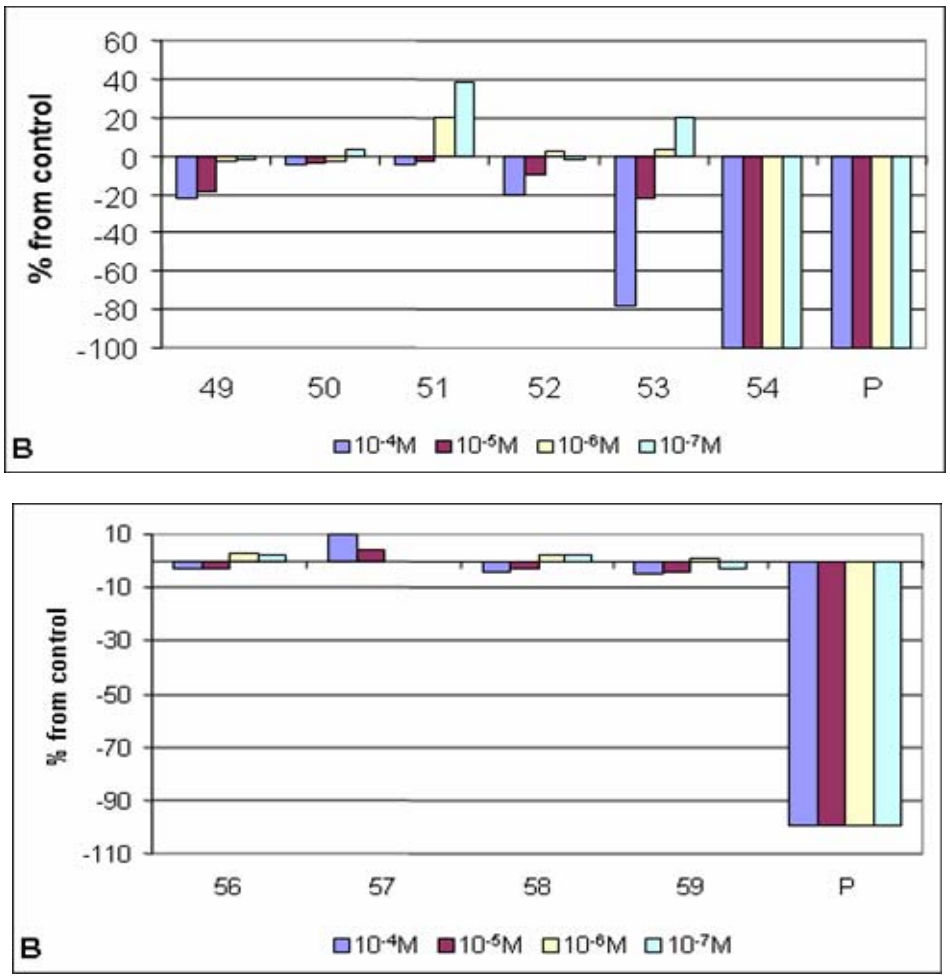


Fig. 9B- Effect on root length of *Lactuca sativa* L. Value presented as percentage differences from control.

Finally, compounds **41-43**, **53** reduced shoot length by 60-80% at the highest concentration tested (Fig. 9C). Compounds **47** and **49** had a good activity as well, they reduced the shoot elongation by 50% at 10^{-4} M; compounds **51** and **52** were moderately active only at higher concentration.

Flavons **56-59** affected lightly the germination and the shoot length at all concentration tested, while at lower concentration they showed a slight stimolatory effect on the root length.

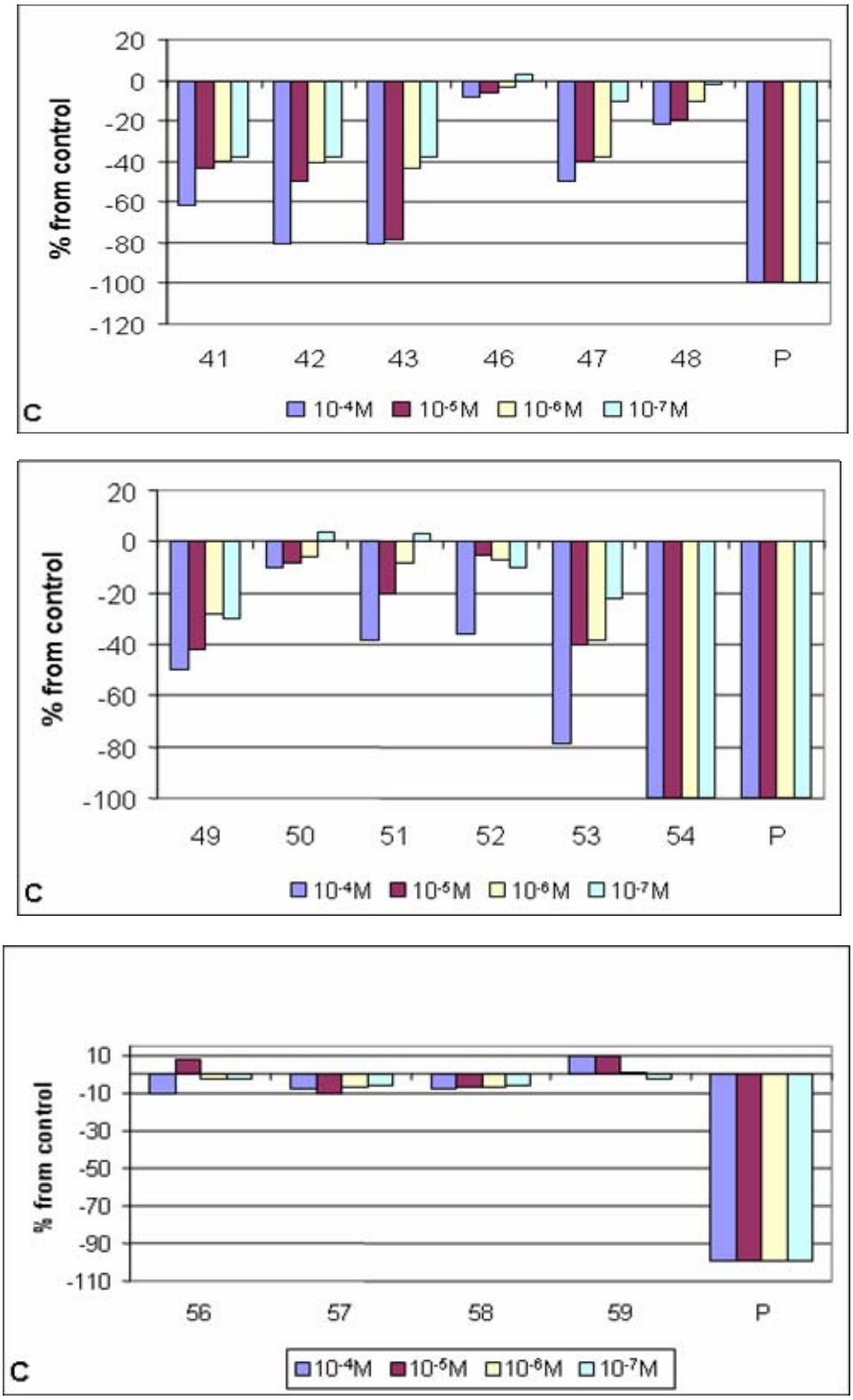


Fig. 9C- Effect on shoot lenght of *Lactuca sativa* L. Value presented as percentage differences from control.

3.5.3 Antialgal activity of compounds from Swinglea glutinosa *extract*

Pure compounds from *S. glutinosa* extract were evaluated for antialgal activity toward *Oscillatoria perornata* (Table 6). The most toxic of the isolated compounds was found to be compound **73** with a LCIC of 10 μM. It showed a selective toxicity toward *Oscillatoria perornata* when compared to *Selenastrum capricornutum*. Compounds **67**-**69**, **75** and **76** showed a moderate toxicity with a LCIC of 100 μM.

Table 6. Evaluation of pure compounds from *S. glutinosa* antialgal activity

	Oscillatoria perornata		***Selenastrum capricornutum***	
Compound	**LOEC** μM	**LCIC** μM	**LOEC** μM	**LCIC** μM
67	10	100	10	100
68	10	100	0.1	>100
69	10	100	>100	>100
71	100	>100	>100	>100
72	>100	>100	>100	>100
73	10	10	100	>100
74	>100	>100	>100	>100
75	100	100	>100	>100
76	100	100	100	>100
77	>100	>100	>100	>100

3.5.4 Antifungal activity of compounds isolated from Amyris madrensis

Bioutography of the ethyl acetate extract of *Amyris madrensis* leaves revealed the presence of active constituents against the crop pathogen *Colletotrichum fragariae*. Fractionation of the extract led to the isolation of compounds **79-86.** The antifungal activity of pure compounds was evaluated by bioautography according to the Wedge method[20] and evidenced by the presence of clear zones with a dark background on the TLC plate. The most active compounds that gave the most well-defined inhibitory zones are reported in Fig. 10.

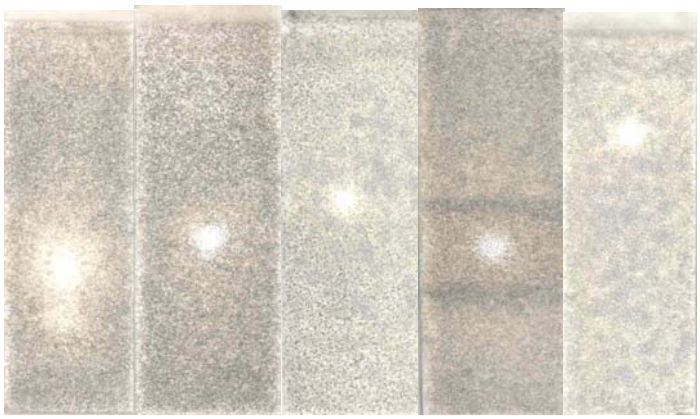


Fig. 10- Bioautography of pure compounds from *Amyris madrensis.* The plates were sprayed with *Colletotrichum fragariae.*

3.5.5 Acetylcholinesterase inhibitory activity

Alzheimer's diseas is the most common cause of senile dementia in later life. Inhibitors of acetylcholinesterase currently form the basis of the newest drugs available for the management of this disease. They function by correcting a deficiency of the neurotransimitter acetylcholine in the synapses of the cerebral cortex. Galanthamine, isolated from plants of the Amaryllidaceae family, is the most recent addition to drugs used for the treatmentof Alzhermer's disease.[22] In view of the potential of plants for the discovery of new acetylcholinesterase inhibitors, a TLC bioautography was used for the screening of pure compounds. This method is rapid and simple to use and gives quick access to information concerning the activity. The test relies on the cleavage by acetylcholinesterase of 1-naphthyl acetate to form 1-naphthol, wich in turn reacts with Fast Blue B salt to give a purple-coloured diazonium dye (Fig. 11). Regions of TLC plate which contain acetylcholinesterase inhibitors show up as white spots against the purple background. Pure compounds from *S. glutinosa* and galanthamine were applied onto the TLC plates and the bioautography was carried out.

Compounds that exhibited inhibitory activity are reported in Fig. 12. It shows areas of inhibition of the enzyme with white spots on the plate.

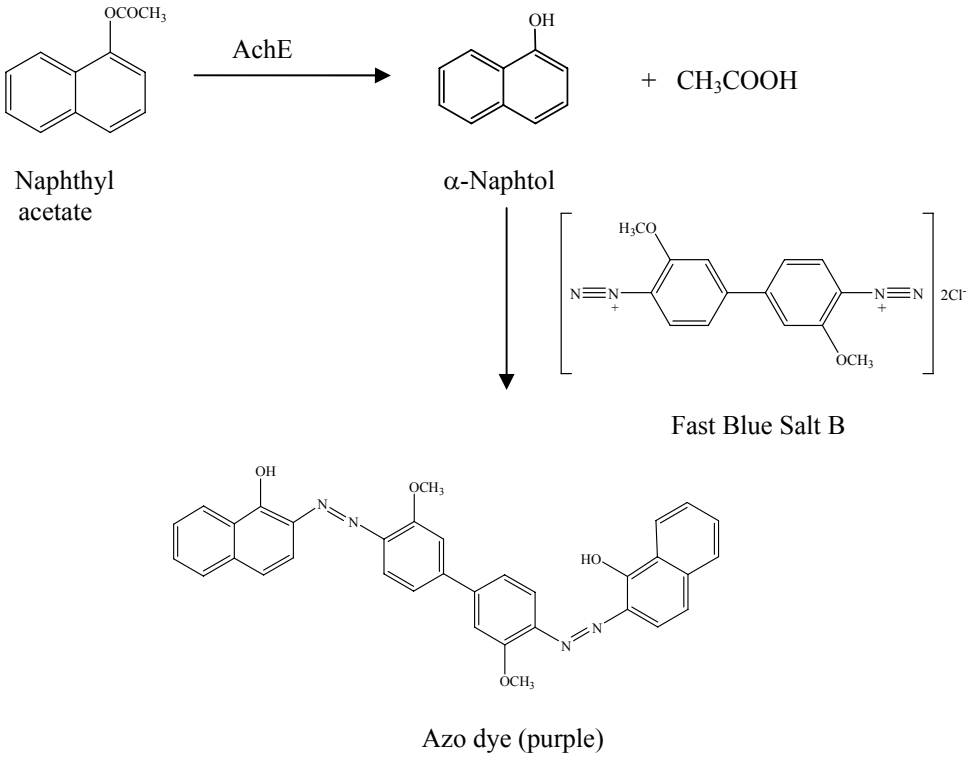


Fig. 11- Reaction of acetylcholinesterase with naphtyl acetate and the subsequent formation of the purple dye in the TLC bioassay

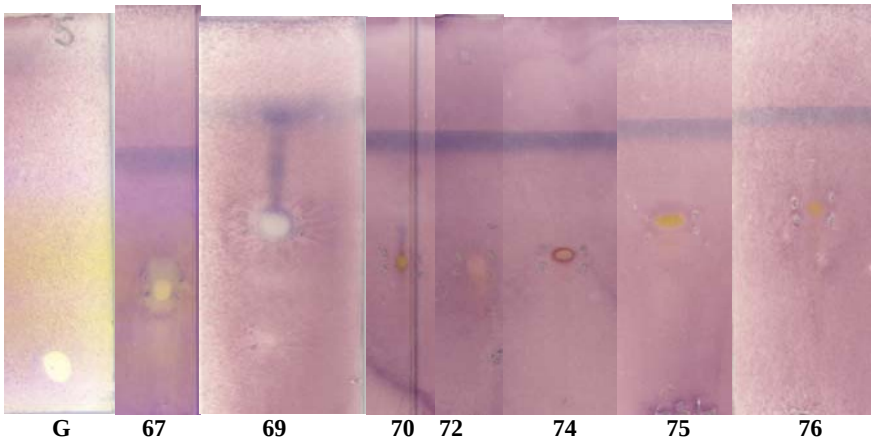
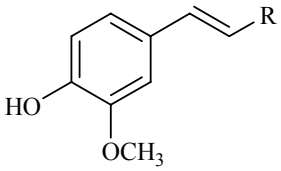


Fig. 12- Bioautograph showing the inhibition of AchE activity by galanthamine and compounds from *S. glutinosa*

3.6. Photo-oxidation of propenyl phenols

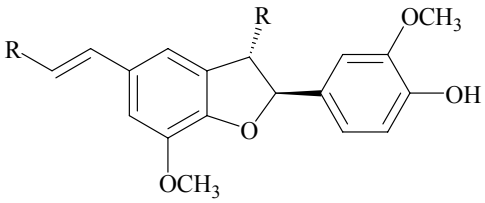
Isoeugenol **87a** was subjected to methylene blue–sensitized photooxygenation under various conditions (solvent, concentration).[72] More dilute solutions allowed reaction time to be reduced avoiding secondary oxygenation, mainly of the double bond. Moreover, the reaction mixture resulted less complex and product separation more easy. ^{1}H NMR analysis and preparative TLC and HPLC were used to identify and quantify the isolated products.



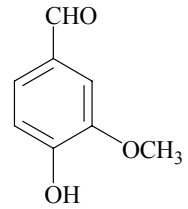
a R= CH_3
b R = CH_2OH
c R = COOH

87

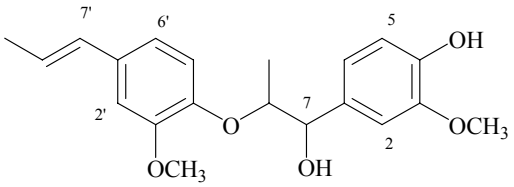
Compound **88a** was identified as dehydroisoeugenol by comparison with its spectral data with those reported in literature.[73] Compound **89** was identified as vanillin by comparison with commercial sample. Compound **90**, which was obtained as a 2:1 diastereoisomeric *erythro*/*threo* mixture, was identified as guaiacylpropan-1,2-diol-β-*O*-4'-isoeugenylether by comparison with literature spectroscopic data.[74]



88a R = CH_3
88b R= CH_2OH

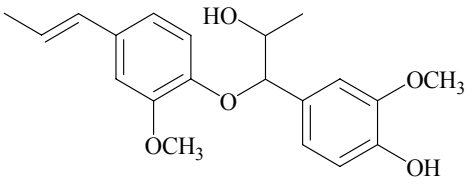


89



90 (*erythro and threo)*

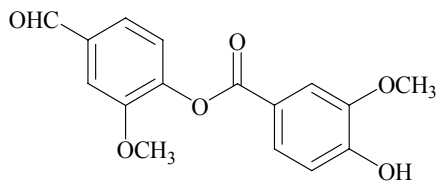
Compound **91** was a 9:1 diastereoisomeric *threo*/*erythro* mixture as observed in its ^{1}H NMR spectrum. The molecular formula $C_{20}H_{24}O_5$ was deduced by the HREIMS spectrum which showed the molecular ion peak $[M]^+$ at *m*/*z* 344.1620. Signals (major isomer) at δ 6.29 (1H, d, J=15.5 Hz, H-7'), and 6.08 (1H, dt, J=6.5 and 15.5Hz, H-8'), in the ^{1}H NMR spectrum, indicated the presence of a *trans*-substituted double bond. Six aromatic signals in δ 6.95-6.58 range in the ^{1}H NMR spectrum suggested the presence of two 1,2,4-trisubstituted aromatic rings, as did six methine signals at δ 109.3-120.9 range in the ^{13}C NMR (DEPT) spectrum. In the HMBC spectrum, long-range correlations from the H-7' olefinic proton to the methyl carbon C-9' at δ 18.9, the methine carbons C-2' at δ 109.8, and C-6' at δ 119.1 were observed. In the ^{1}H NMR spectrum, the signals at δ 4.43 (1H, d, J=7.8 Hz, H-7), 4.07 (1H, m, H-8), and 1.01 (3H, d, J=6.0 Hz, H-9), as well as signals at δ 91.0, 71.3, and 18.0 in the ^{13}C NMR (DEPT) spectrum attributed to C-7, C-8, C-9, respectively, indicated the presence of a propane 1,2-diol chain. In the HMBC spectrum, long-range correlations from the H-7 proton to the methyl (C-9), the methine carbons at δ 71.3, 109.3, 120.9, 131.0, and 145.1 assigned to C-8, C-2, C-6, C-1, and C-4', respectively were observed, indicating the presence of a 1-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol linked to C-4 of the isoeugenol unit that involve a 4'-*O*-7 linkage. Compound **91** was previously reported as an oxidatively dimerized isoeugenol. [75]



91 (*erythro and threo)*

Compound **92** was isolated from the irradiation mixture in water (2%). It had molecular formula $C_{16}H_{14}O_6$ as the HREIMS spectrum showed the molecular ion peak $[M]^+$ at *m*/*z* 302.0785. The ^{1}H NMR spectrum showed a singlet at δ 9.98 attributed to aldeidic group, as confirmed by a signal at δ 192.2 in the ^{13}C NMR spectrum. Six aromatic signals in δ 7.02-7.84 range, in the ^{1}H NMR spectrum and ^{1}H-^{1}H COSY suggested the presence of two 1,2,4-trisubstituted aromatic rings, as did six methine signals at δ 111.2-125.8 range in the

^{13}C NMR (DEPT) spectrum. These data, along with HSQC and HMBC spectra, confirmed the structure of compound **92** as depicted.

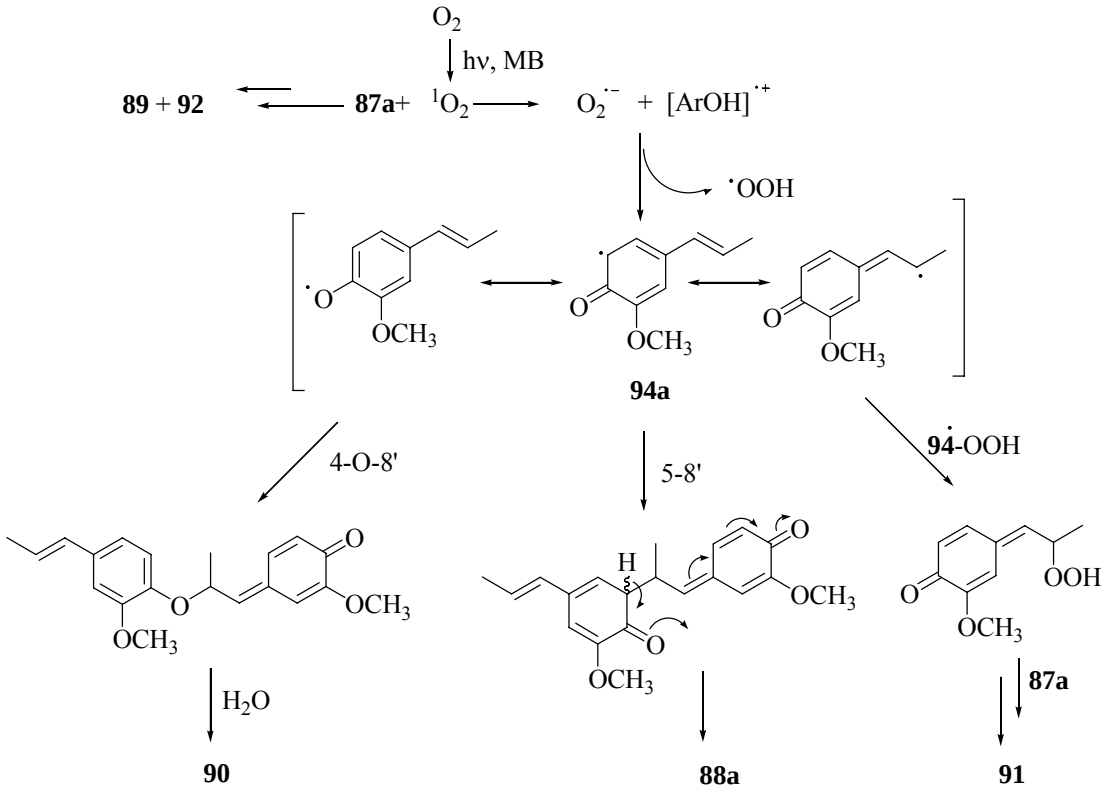


92

Appreciable amount of lignan **88a** was obtained both in organic solvents and in H_2O-CH_3CN. Compound **90** was found in 20 and 8% amount in H_2O and H_2O-CH_3CN as solvents. Compound **91** was present in small amount under all the conditions used.

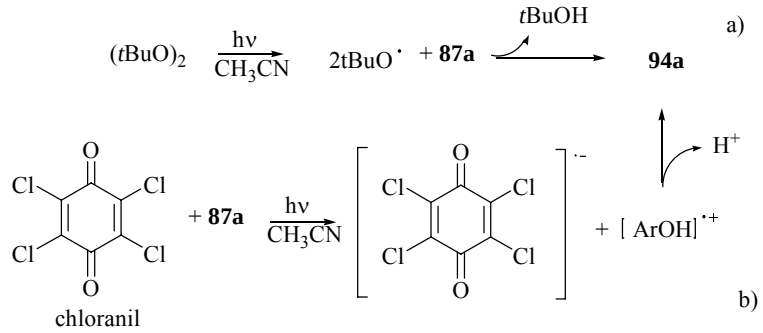
A rationalization of the results is reported in Scheme 1. In the formation of compounds **88a**, **90**, **91** the key intermediate should be radical **94a** deriving from electron-transfer reaction between **87a** and singlet oxygen followed by hydrogen abstraction.[76] Radical couplings and nucleophilic additions should lead to the final products **88a**, **90**, **91** (Scheme 1). As reported,[77] **88a** is formed by 5-8' coupling followed by an intramolecular cycloaddition 4-*O*-8'. Coupling and water addition to the resulting quinone methide appears to lead to compound **90**. The low yield can be explained on the basis of previous data,[4b] which evidenced the scarce competition of water addition respect to that of other nucleophiles such as methanol or ethanol, even in the presence of excess of water. Oxygenated species as OOH radical (or O_2)[75] may be involved in the formation of compound **91**, followed by the nucleophilic addition of **87a**.

Vanillin **89** is the cleavage product of the intermediate dioxetane deriving from addition of singlet oxygen to the double bond. According to Kuo,[77] it should be the precursor of compound **92** via abstraction of the phenolic hydrogen by oxygenated species and addition of the so formed radical to **89**.



Scheme 1

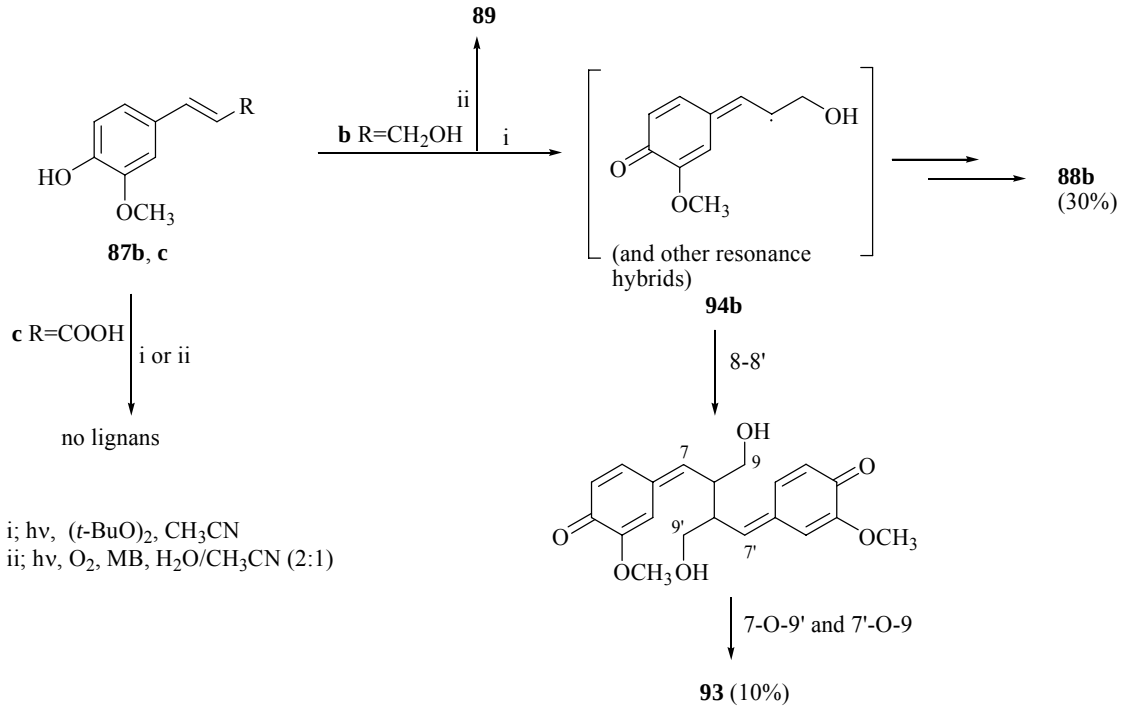
To minimize oxygen addition we used typical conditions for generating phenoxy radicals by different approaches (Scheme 2).[77,78] When isoeugenol **87a** was irradiated at $\lambda > 310$ nm in the presence of di-*tert*-butyl peroxide in dry CH_3CN in inert atmosphere,[77] the reaction mixture, analysed by ^1H NMR after 14 h, evidenced 60% conversion and the presence of compound **88a** (20%) in addition to trace amounts of **89** and **91**. Irradiation was also carried out in the presence of chloranil, a well-known electron-transfer sensitizer[77] able to abstract hydrogen,[78] in dry CH_3CN, in inert atmosphere. After 8 h, the reaction mixture, analysed by ^1H NMR, evidenced 40% conversion and the presence of only compound **88a** in 20% amount.



Scheme 2

The mild conditions (MB-sensitized photooxygenation in H_2O/CH_3CN and peroxide-promoted photo-oxidation) were also used for coniferyl alcohol **87b** and ferulic acid **87c**. The results are depicted in Scheme 3. The photooxygenation of **87b** in H_2O/CH_3CN and methylene blue as sensitizer led, after 20 h, only to vanillin **89**, evidently due to the activated double bond.[79] More satisfying was irradiation in the presence of the peroxide. The reaction mixture, analysed after 14 h by ^1H NMR, evidenced 90% conversion. HPLC separation gave compounds **88b** (30%) and **7** (10%), identified by comparison with literature spectroscopic data as dihydrodiconiferyl alcohol[80] and pinoresinol,[81] respectively. Radical **94b**, formed as above **94a**, partly gives the β-5 dimer **88b** and partly dimerizes to furofuran lignan **93** through initial 8-8' coupling followed by intramolecular 7-*O*-9' and 7'-*O*-9 cyclizations of alcoholic functions.

All attempts to oxidate ferulic acid **87c** under both conditions failed. No lignan derivatives were observed even after prolonged irradiation (30 h). *Cis-trans*-isomerization and formation of vanillin (trace) under MB-sensitized photooxygenation were the only events observed.


89
ii
b R=CH2OH
i
R
HO
OCH3
87b, c
c R=COOH
i or ii
no lignans
i; hν, (t-BuO)2, CH3CN
ii; hν, O2, MB, H2O/CH3CN (2:1)
OH
O
OCH3
(and other resonance hybrids)
88b
(30%)
94b
8-8'
OH
7
9
O
9'
7'
O
OCH3
OCH3 OH
7-O-9' and 7'-O-9
93 (10%)

Scheme 3

4. CONCLUSIONS

During the three years of my PhD studies four plants have been analysed, *Aptenia cordifolia*, *Oxalis pes-caprae* (two spontaneous plants of the Mediterranean area), *Swinglea glutinosa*, and *Amyris madrensis.* The plants have been treated by infusion procedure with an hydroalcoholic solution (10% MeOH-H2O) and/or organic solvent. The extracts obtained have been concentrated and fractionated by different chromatographic techniques (CC, TLC, HLPC), while the structural characterisation of pure compounds has been performed by spectroscopic and spectrometric techniques (^{1}H and ^{13}C NMR, UV, IR, EI MS, ESI MS, MALDI MS). The investigation of these plants has led to the identification of: three lignans (**16**, **17**, **19)**, one dilignan (**18**), six neolignans (**20-25, 55**), three cinnamic acid amides (**36-38**), two lignanamides (**39**, **40**), fourteen aromatic esters (**41-54**), fourteen cinnamic acid derivatives (**6-15**, **63-66**), five C-13 *nor*-terpenes (**29-34**), methylinositol (**35**), two tryptophan derivatives (**27-28**), eight phenol derivatives (**1-5, 60-62**), four flavons (**56-59**), ten coumarins (**67-69**, **79-85**), nine acridone alkaloids (**70-78**), and methyleugenol (**86**). The dilignan **18**, the lignan **19,** the six neolignans **20-25** and **55**, the cinnamic acid amide **38**, the lignanamide **39**, the aromatic esters **41-54**, and the two coumarins **69** and **79** have been isolated for the first time.

Some of the isolated compounds have been tested on seeds of a standard plant (*Lactuca sativa* L) to evaluate phytotoxic activity. The assays have been run in accordance with the procedures optimized by *Macias et al.*[19] The results reported have shown a strong inhibiting effect of many compounds like **13**, **28**, **42**, **43**, and in particular ester **54** which inhibits growth of the standard specie by 100% at all concentrations tested. Such a potent bioactivity for very diluted solution of compound **54** suggests further investigations of phytotoxic activity to fully understand the ecological role of this ester and to evaluate its potential use as a herbicide.

Some of the isolated compounds have been evaluated for antifungal activity against the plant pathogen *Colletotrichum fragariae*, and antialgal activity toward *Oscillatoria perornata.* The most active of the tested compounds were coumarins **80**, **81**, **82**, **84**, and **85** as showed clear zones of growth inhibition on the TL plates, while acridone alkaloid **73** showed a selective toxicity toward *Oscillatoria perornata* when compared to *Selenastrum capricornutum.*

Further studies with these compounds with *in vivo* systems are needed to further determine their potential as commercial fungicides or algicides.

The data presented enlighten the role covered by plant-originated compounds in the research of new eco-compatible agrochemicals. Natural substances still represent a fruitful field of investigation to detect unknown molecules having new structures and functionalities that

evolution has refined for highly specific biological roles. This thesis has also led to the identification and structural characterisation of some molecules having a new interesting carbon skeleton, that might contribute to the complicated process of discovery and definition of natural substances.

Finally, this work has showed the interesting possibility to obtain lignans using environmentally friendly photo-induced methods.

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