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Bioactive constituents from *Rollinia emarginata* (Annonaceae)

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From un Argentine collection of Ihe tropical tree *Rollinia emarginata* (Annonaceae), vomiíbliol, dehydrovomifoüol, blumenol C, loliolide, 7-epiloliolide, vanillin, dihydroactinolide, as well as other conimon plant constituents were obtained, and identified by theír NMR and MS reatures compared with authentic samples. Antifeedant and toxic actions were exerted to the polyphagous moth *Spodoplera frugiperda* by the ethanol extract of the plant, at 250 ppm in the larval diet. Additionally, in greeniíouse sludies, a 200 ppm aqueous solution of the exlract produced a post-emergency herbicida! elTect on the annual weed common lambsquarter *(Chenopodium álbum).*

Kcywords: Rollinia emarginata; Megastigmane derivatives: *Spodoplera frugiperda* antifeedant; *Chenopodium álbum* herbicide

1. Introduction

The pantropical family Annonaceae (custard apple family) comprises 130 genera and 2300 species. Most of them are trees of edible fruits distributed in África, Asia, Oceania, and America. Many species have been used in traditional medicine, in decoctions, as analgesics [1] and antiprotozoals [2], Extracts obtained from the lea ves of some species are used as domestic insecticides [3]. Phytochemical studies and, to a lesser extent, pharmacological studies on the species of Annonaceae have been intensified in the last 20 years. This is largely due to the discovery of the annonaceous acetogenins, a class of natural compounds with a wide variety of biológica! activities [4] that are found only in species of this family. The annonaceous acetogenins are the most powerful inhibitors of complex í (NADH: ubiquinone oxidoreducta.se) in mammalian and insect mitochondrial electrón transpon [5] systems. In addition, they are potent inhibitors of NADH oxidase of the plasma membranes of cáncer cells [6] leading to a decrease of the ATP production and consequent cell apoptosis. *Rollinia emarginata* Schlecht. (Annonaceae) is a 15-18m tall tree growing in Paraguay, Bolivia, Argentina, and Brazil. In Paraguay, this tree is called 'aratiku' or 'arachichu' by the Guaraní Indians

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meaning 'fruit of sky'. Stem bark of *R. emarginata* is mixed with yerba mate (/fox *paraguarensis.* Family Aquilfoliaceae) and consumad as a tea-like beverage to treat migraines and as a relaxant. A methanol extract from stem bark of a Paraguayan collection yielded four acetogenins [2], while the aerial parts of an Argentine collection [7] only gave three alkaloids.

As part of our search for bioactive compounds from South American plants, we evaluated the lethality to brine shrimps *(Artemia salina]* produced by ethanol extracts obtained from Argentine and Solivian collections of five species belonging to the Annonaceae family, *R. emarginata, R. occidentalis, R. intermedia, Annona cherimolia,* and *A. montana.* As the ethanol extract of *R. emarginata* was very toxic to brine shrimps (LD_{50} = 0.4ppm), it was submitted to acareful chemical investigation. Additionally, the ethanol extract of *R. emarginata* was tested in feeding experiments (choice and no choice tests) using second instar larvae of the polyphagous insect *Spodoptera frugiperda* Smith. The mortality and larval malformation produced by treated diets was also evaluated. Finally, the herbicidal action of the extract was also tested in a soybean greenhouse crop.

2. Results and discussion

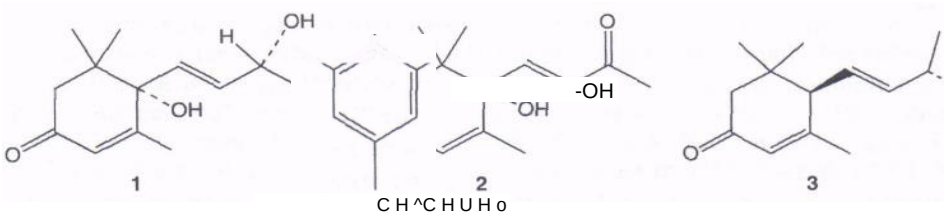
No annonaceous acetogenins were found in the ethanol extract of *R. emarginata.* However. this is the first report on the isolation of vomifoliol (1), dehydrovomifoliol (2), blumenol C (3), loliolide (4), 7-epiloliolide (5), vanillin (6), and isoelemicin (7) from a collection of the genus *Rollinia.* Compounds 1, 2, 3, 4, and 5, were identified by their NMR and MS features compared with authentic samples. GC-MS analysis indicated the presence of 6, 7, 8, stigmasterol, /J-sitosterol, /3-sitostenone, and stigmast-4-en-3-one as well as other volátiles, cornmon in higher plants. GC-MS of methylated polar fractions showed the presence of methyl jasmonate. Compounds **1-3** are bisnorsesqui-terpenes also known as megastigmane derivatives. Vomifoliol has been previously reported from *Vitis vinifera, Rauwolfia vomitoria, Crotón sparsiflorus, Podocarpus blumei, Phaseolus vulgar is* (kidney bean) and from *A. glabra* [8], a species of Annonaceae, among other higher plants. Compound 2 is a plant growth regulator in rice [9] and has been isolated from *Oryza sativa, Phaseolus vulgaris,* and *Cichorinm pumilum.* Blumenol C has been reported from *Podocarpus blumei* [10], while loliolide and its C-7 epimer have been found in several species of higher plants. Loliolide, which has also been isolated from algae, displays an inhibitory activity in lettuce seed germination [11] and has been reported to be a potent ant repellen! [12]. The aromatic compound vanillin is present in several species of the genus *Vanilla* and in other higher plants. Isoelemicin has been found in nutmeg oil [13] and other higher plant and is known to produce hypnotic effects.

Brine shrimp test. The brine shrimp lethality test (BST) was employed as a rapid and inexpensive bioassay to monitor the extraction of bioactive compounds [14]. Results correlate well with a series of pesticidal and cytotoxicity tests. Ethanol extracts from the aerial parts of *R. emarginata, R. occidentalis, R. intermedia, A. cherimolia,* and *A. montana* resulted toxic to some extent, giving LC_{50}s valúes of 0.4, 1.5, 147.6, 467.8, and 0.3ugmL~', respectively. The low LC_{50}s valúes found prompted us to test the insecticidal effects of the ethanol extract of *R. emarginata.*

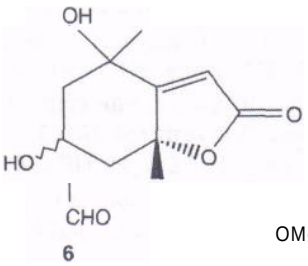
Antifeedant effect. The larval diet contained 250 ppm of extract. The two antifeedant tests (choice and no choice) yielded important informa tion. The choice test gave an FR_5fl = 0.35, vvhile an FR_5o = 0.73 was obtained in the no choice test. These results indícate that the extract displays a strong antifeedant effect at the concentration tested. However, it is important to point out that, in comparison with commercially available natural antifeedants containing the limonoid azadirachtin, the present extract is around 10 times milder.

Mortality. A diet treated with 250 ppm of the ethanol extract of *R. emarginata* kills 100% of the population of *S.frugiperda* at larval or pupal stages.

Herbicidal effects. The extract was evaluated as pre- and post-emergency herbicide resulting to be effective as a post-emergency herbicide in a soybean greenhouse crop affecting the annual weed common lambsquarter *(Chenopodium álbum* L.), when spraying with an aqueous 200 ppm solution of the extract.



C H ^C H U H o



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3. Materials and methods

3.1. *General experimental procedures*

TLC was carried out on silica gel precoated glass piales (Kiesel gel 60 F254, Merck). A saturated aqueous solution of ceric sulphate with 30% H_2SO_4 was employed as TLC detection reagent. Column chromatography was carried out on silica gel 60 (70 230 mesh, Merck) and on Sephadex LH-20 (CH_2C1_2 MeOH, 1:1). Preparative HPLC was perlbrmed by a Gilson pump system on reversed-phase Ultrasphere Beckman and Ultremex Phenomenex C8 and C18 columns (25 cm, 1 cm, 5um particle size) and mixtures of MeOH and H_2O as eluent. Mass spectra were recorded on a Hewlett-Packard 6890 Series GC System coupled to an HP 5973 Mass Selective

Detector. An HP-5MS (30mx0.25mm i.d. x0.25um) column with a temperatura programming from 50°C, then 50-280°C at 5^0Cmin~' and finally isothermal at 280°C for 15min, was used. 'H and ^{13}C-NMR were recorded in Varían Unity 300 and 400 MHz spectrometers in $CDC1_3$ as solvent.

3.2. ***Plañí material***

Aerial parts of *R. emarginata* Schldl. were collected at Colonia Benítez, Chaco, Argentina, in November 1998. A dried voucher specimen is on deposit at Herbario Fundación Miguel Lillo (L1L 604641) in Tucumán, Argentina.

3.3. ***Extraction and isolation***

Air-dried ground lea ves and twigs (512g) were extracted with 95% EtOH at room temperature. After solvent evaporation the crude extract named F001 (23.26g) was submitted to liquid liquid partition between a 1 : 1 mixture of H_2O and CHClj. The aqueous layer, after evaporation, gave 4.25 g of an extract named F002. The chloroform layer, after solvent evaporation, yiekled 9.97 g of extract F003. All insoluble portions were combined and named F004. F003 was again partitioned between 90% aqueous MeOH and «-hexane (1:1). The aqueous MeOH-soluble fraction (F005) gave 3g of extract after solvent evaporation and the n-hexane fraction (F006) weighted 6 g. F005 was chromatographed on silica gel and eluted with $CHC1_3$ and increasing amounts of EtOAc (0-100%) and finally MeOH to give 5 fractions. Fractions I-V and a methylated portion of fraction V were analyzed by GC-MS (table 1). HPLC of fraction III (Phenomenex Ultremex C18, CH_3CN-H_2O 17:3, 1 mLmirT^1) gave 9.3 mg of vanillin (Rt 12min) and 1.7mg of dihydroactinolide (Rt 14min) identified by comparison of their GC-MS features with those of authentic samples. HPLC (Phenomenex Ultremex C18. MeOH H_2O 2.5 : 1, 1 mLmin"^{1}) of fraction IV after a clean up with Sephadex LH 20 yielded 7-epiloliolide (2.7 mg), loliolide (4.7 mg), dehydrovomifoliol (2.1mg), blumenol C (3.2 mg), and vomifoliol (2.4 mg) identified by MS and $^!$H and

Tabie 1. Conslituents of *R. emt/rginuta* detected by GC-MS.

Compound	Kovat índex	Extract fraction	Compound	Kovát índex	Extract IVaction
a-Benzopyrone	-	F005 (I)	/8-Caryophyllene	1418	F006 (I)
Dihydroactinolide	-	F005 (I)	Humulene	1454	F006 (1)
írans-lsoelemicin	1573	F005 (I)	X-Cadinene	1461	F006 (I)
Spathulenol	1576	F005 (I)	/J-Selinene	1487	F006 (I)
Phytol	1949	F005 (1)	X-Selinene	1517	F006 (I)
Vanillin		F005 (III)	á-Cudinene	1524	F006 (I)
Dihydrovomilbliol		F005 (IV)	Limonene	1031	F006 (II)
Carvone	1242	F005 (IV)	1 0(1 4)- Aromadendrene	1439	F006 (III)
Blumenol C	-	F005 (IV)	Dihydroactinolide	-	F006 (III)
Vomifoliol	-	F005 (V)	Globulol	1583	F006 (III)
Methyl jasmonate		F005 (V) Methylated	Ergost-5-en-3-ol		F006 (III)
o-Copaene	1376	F006 (I)	Stigmasterol	-	F006 (III)
/5-Cubebene	1390	F006 (I)	/3-Sitoslerol		FOÜ6 (III)
a-Gurjunene	1409	F006 (I)	/3-Sitostenone	-	F006 (III)

^{13}C-NMR, in comparison with literature data of authentic samples. F006 vvas chromatographed on silica gel with n-hexane and increasing amouñts of EtOAc (O 100%) to give 3 fractions analyzed by GC-MS (table 1).

3.4. *Brine shrimp test*

The ethanol extracts and the chromatographic column fractions were tested in three concentra tions, 100, 10, and 1 ppm (three replica tes for each) in the brine shrimp test (BST) following the procedure previously described [14].

3.5. *Insects bioassays*

Larvae of *S. frugiperda* carne from a laboratory colony reared on artificial diet under controlled conditions as described previously [15].

3.5.1. Choice and no choice tests. The ethanol extract of *R. emarginata* (after complete solvent removal) vvas dissolved in acetone and added to the larval diet in a concentration of 250u,gg~' (ratio extract/diet). Treated and nontreated diets were employed in choice and no choice tests. Choice assays are employed to examine whether an insect will feed on a particular extract added to a test médium, or whether or not it prefers the control médium. If the latter is the case, the compound-extract acts as an antifeedant. If the converse is the case and the insects feed preferentially on the treated disc. trien the extract is said to act as a phagostimulant. These extracts differ from 'feeding deterrents' which suppress feeding on either control or treated media. In our experiments a control and a treated portion of diet were presented, in a test tube, to second instar larvae of the polyphagous herbivore *S. frugiperda* Smith. After a given period of time the diet vvas removed and weighed to obtain the amount of control (C) and treated *(T)* diet consumed. The diet is removed when no more than 50% of control diet has been eaten, that, in the present experiment, ranges from 24 to 48 h. Results of the choice test were then reported by the ratio *TIC* also known as FR_{50} [16] (feeding ratio when the control population has eaten half the diet). In addition to a choice test, another antifeedant assay was carried out, the no choice assay, in which each larvae was presented with either a control or a treated portion of diet. Insects were allowed to eat until 50% of the control diet has been consumed (24-48 h). The consumption is assessed by weight after the assay is terminated.

3.5.2. Toxicity bioassay. A portion of diet was impregnated with acetone and, after solvent removal, this portion was employed as control diet. Another portion was impregnated with an acetone solution of the ethanol extract of *R. emarginata* to leave 250ugg~' of diet. After evaporation of the solvent, control and treated diets were placed in test tubes. Two second instar larvae were placed in each tube and were kept at 27°C until emergency of the first-generation adults that occurs 18-22 days later. Mortality was registered by counting the emerged adults.

3.6. *Herbicide evaluation*

A 200 ppm aqueous solution of the ethanol extract of *R. emarginata* containing a surfactant was employed to spray three lOOm^2 plots of an experimental soybean field

containing lOcm-high plants of *Chenopodium álbum.* The herbicide solution was applied with a hand-held CO_2 sprayer. A no-herbicide weeded check was included for comparison.

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