ARGENTILACTONE FROM ANNONA AMBOTAY

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ARGENTILACTONE FROM *ANNONA AMBOTAY*

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RESUMEN

De corteza de tronco de *Annona ambotay*, árbol de la familia Annonaceae, y reportado en la Farmacopea Chacobo con el nombre Rononopa contra luxaduras, se aisló e identificó la argentilactona. Su estructura ha sido determinada por 1D-, 2D-RMN y EMIE. También, un nuevo ácido graso, acido 5-hidroxidodeca-*cis*-2,*trans*-6-dienoico fue detectado y estructuralmente propuesto sobre la base de EMIE y espectrometría IR, sin embargo se discute su presencia como un posible artefacto.

ABSTRACT

From the stem bark of *Annona ambotay*, a tree reported in the Chacobo pharmacopoeia under the name Rononopa and used for sprain health problems, argentilactone has been isolated. Its structure has been determined by 1D-, 2D-NMR and EIMS. Also, a new fatty acid, 5-hydroxydodeca-*cis*-2,*trans*-6-dienoic acid, has been found and structurally proposed on the basis of EIMS and IR spectrometry, the presence of the acid is however discussed as a possible artifact.

INTRODUCTION

In the course of our research program of new insecticide substances from bolivian plants, we have identified from *Annona ambotay* Aublet (Annonaceae), the compound argentilactone (**1**) and a new fatty acid, 5-hydroxydodeca-*cis*-2,*trans*-6-dienoic acid (**2**) which could be however regarded as an artifact of extraction procedures. The results of biological tests will be published elsewhere. This work constitutes a further contribution to the chemical knowledge of the species, reporting for the first time argentilactone as constituent in the species. *A. ambotay* is a small tree used by the ethnic group Chacobo in northeastern Bolivia (Beni department) for the treatment of sprains applying the stem-bark in poultice directly to the affected zone. The Annonaceae have been specially studied for nearly two hundred acetogenines that have been isolated from several species of *Annona* [1]. This genus comprises about hundred twenty species worldwide distributed in tropical and subtropical regions [2]. Previous work on the species *A. ambotay* from Brazil reports the presence of the sesquiterpenes β- and γ-elemene, β-caryophyllene, γ-muurolene and muurolol as the predominant volatile constituents of the essential oil from the bark [3]. From the trunk, the azaanthracene alkaloid named geovanine with liriodenine and *O*-methylmoschatoline, two oxoaporphine alkaloids together with flavonoids kaempferol, quercetin, (+)-dihydroquercetin, (+)-dihydrokaempferol, (±)-eriodictiol, (+)-catechin, taxifolin and steroids 5α-stigmastan-3,6-dione and sitosterol have been also reported [4].

RESULTS AND DISCUSSION

From the methylene chloride dry extract, coming up from a partition with the aqueous layer from a previously obtained hydroethanolic extract, an aliquot has been chromatographed in a vacuum liquid chromatographic silica gel system (VLC). The less polar fractions regrouped and treated in a preparative silica gel plate permitted isolation of **1** [5, 6]. The ^{13}C NMR spectrum from the DEPT experiment presented five methylene discrete signals at δ 31.3 (*t*, C-10), δ 29.9 (*t*, C-4), δ 29.0 (*t*, C-9), δ 27,7 (*t*, C-8) and δ 22.4 (*t*, C-11), all of very comparable intensity. The rest of signals corresponded to a methyl grouping at δ 13.9 (*q*, C-12), four vynilic carbons, namely δ 144.7 (*d*, C-3), δ 135.6 (*d*, C-7), δ 126.3 (*d*, C-6), δ 121.6 (*d*, C-2), an oxymethine carbon at δ 73.8 (*d*, C-5) and a carbonyl group at δ 164.1 (*s*, C-1). All carbon signals were correlated to protons in the ^{1}H NMR spectrum through cross-peaks in the XHCORR experiment.
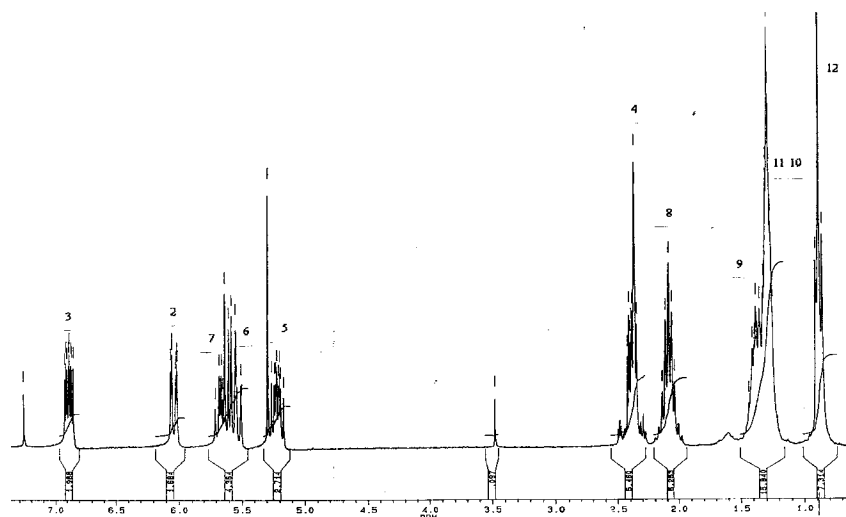
Compound 1, ^{1}H NMR spectrum (CDCl$_3$, 250 MHZ)
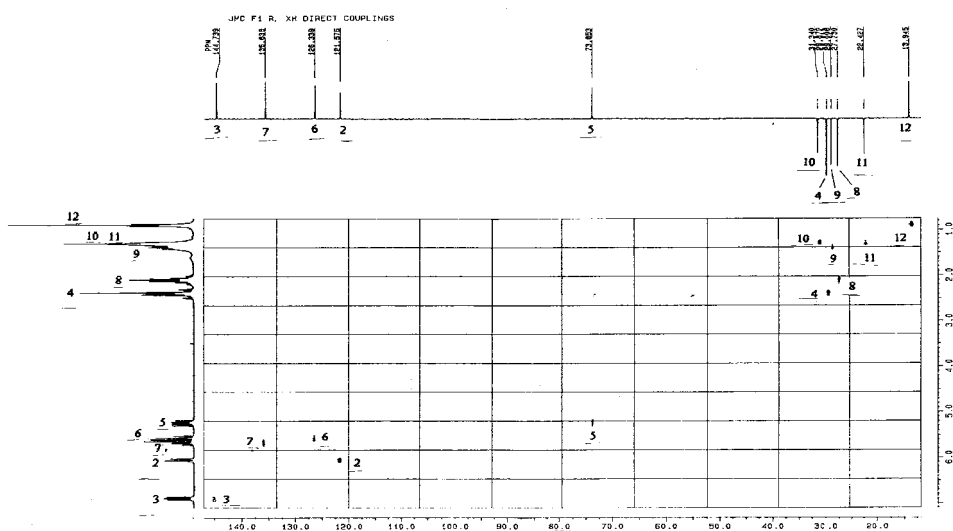
Compound 1, XHCORR direct heteronuclear correlations

The high field portion of the ^{1}H NMR spectrum showed a methyl signal at δ 0.89 (3H, *t*, $J_{12, 11}$ = 6.8 Hz, H-12) and a three-methylene bulk (6H) between δ 1.52 and δ 1.16. According to cross-peaks in the COSY spectrum the following proton chemical shifts could be attributed in the zone, δ 1.30 (2H, *m*, H-11), δ 1.26 (2H, *m*, H-10) and δ 1.37 (2H, *m*, H-9). At lower field, two allylic methylenes were found, the first at δ 2.14 (1H, *dq*, $J_{8, 8'}$ = 13.3 Hz, $J_{8, 9}$ = $J_{8, 9'}$ = $J_{8, 7}$ = 6.6 Hz, H-8) and δ 2.05 (1H, *dq*, $J_{8', 8}$ = 13.3 Hz, $J_{8', 9}$ = $J_{8', 9'}$ = $J_{8', 7}$ = 6.6 Hz, H-8') appearing as a whole amazingly first order nine-rays-composed-signal centered at δ 2.10. Each proton of methylene 8 occupies a space where 6 equidistant rays can be depicted. The more deshielded proton H-8 (centered at 2.14 ppm) presents a left extreme ray invisible and a right extreme ray very intense and overlapped with

the very intense left extreme ray of H-8' (centered at 2.05 ppm) which in turn has an invisible right extreme ray. In total, methylene 8 should exhibit an eleven-ray signal, showing in the spectrum only nine rays, the two extremes missing or invisible rays practically disappear because of the proximity of chemical shifts values for each proton 8.
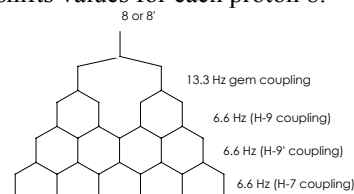
The second allylic methylene was centered at δ 2.39 (2H, *m*, H-4), being more deshielded because of direct bond to the vicinal oxymethine group. The COSY spectrum revealed a sequentially

autocorrelated eleven-proton spin system. Correlations departure, from H-12 δ 0.89 (3H, *t*, $J_{12,11}$ = 6.8 Hz), was followed through cross-peaks by the series H-11→H-10→H-9→H-8. From H-8 (δ 2.10) connectivities were established to the vynilic proton second order signals at δ 5.67 (1H, *m*, H-7) and δ 5.58 (1H, *m*, H-6) evidencing H-8→H-7 and the

allylic coupling H-8→H-6. Cross-peaks to both sides and much closer to the diagonal demonstrated the H-7→H-6 second order coupling. The COSY sequence continued, showing the series H-6→H-5→H-4→H-3→H-2.
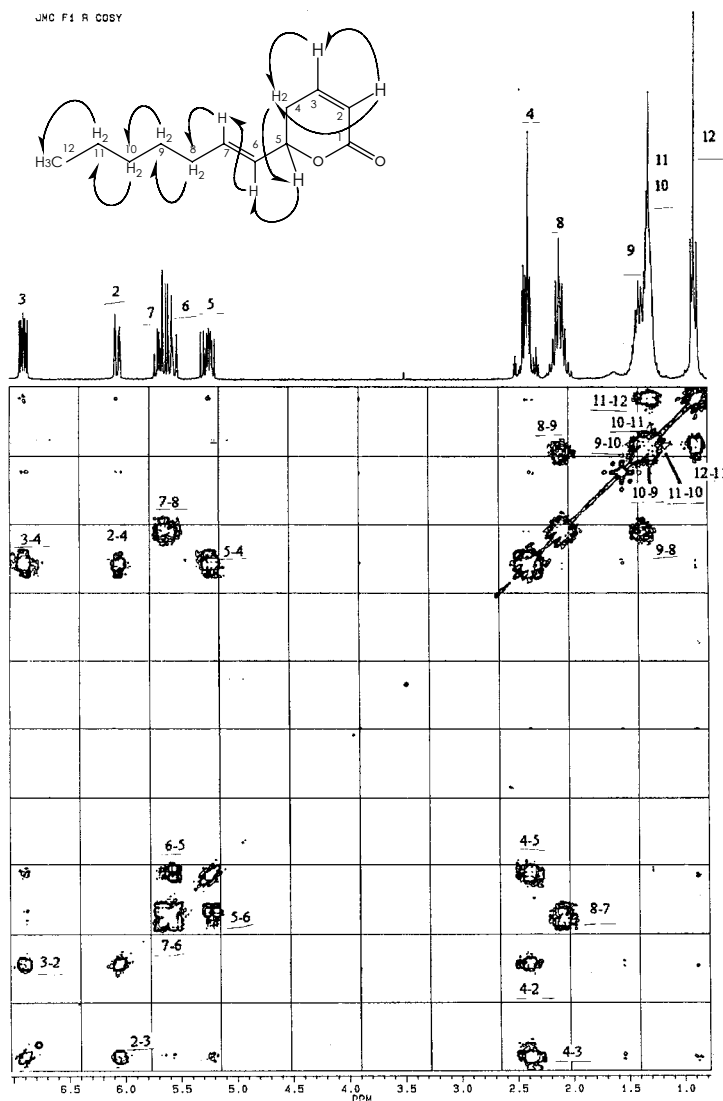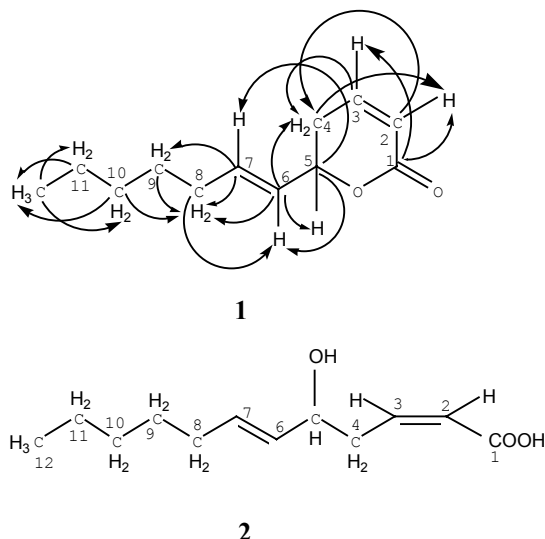

*Compound **1**, COSY homonuclear correlations pathway*

The sequence COLOC to detecte long range couplings, revealed remote connectivities as shown in the figure, clearly confirming the α,β-unsaturated carbonyl function through ^2J and ^3J bonds from δ 164.1 (*s*, C-1) to δ 6.90 (1H, *ddd*, $J_{3,2}$ = 9.8 Hz, $J_{3,4}$ = 5.3 Hz, $J_{3,4'}$ = 3.2 Hz, H-3) and to δ 6.05 (1H, *ddd*, $J_{2,3}$ = 9.8 Hz, $J_{2,4}$ = 2.1 Hz, $J_{2,4'}$ = 1.5 Hz, H-2). All atoms were correlated by heteronuclear remote couplings. The vicinal coupling constant value of 9.8

Hz between H-2 and H-3 led to establish a *cis* configuration for the first insaturation. The much low-field shifted chemical shift value of H-5 (δ 5.23) was justified by an oxy substitution on C-5 in a cyclic way, presenting in this manner a six membered α,β-unsaturated lactone ring. No cross-peak in the XHCORR spectrum to represent a remote connectivity between H-5 and carbonyl C-1 could be found in order to justify the lactonic structure. The

second insaturation belonging to the greasy moiety is proposed in a *trans* configuration according to published NMR data [6]. The EIMS spectrum of **1**

showed peaks for the molecular ion and other fragments that reconfirmed its identity as argentilactone [6].

1

2

*COLOC long-range correlations showed by argentilactone (**1**). 5-hydroxydodeca-cis-2,trans-6-dienoic acid (**2**)*
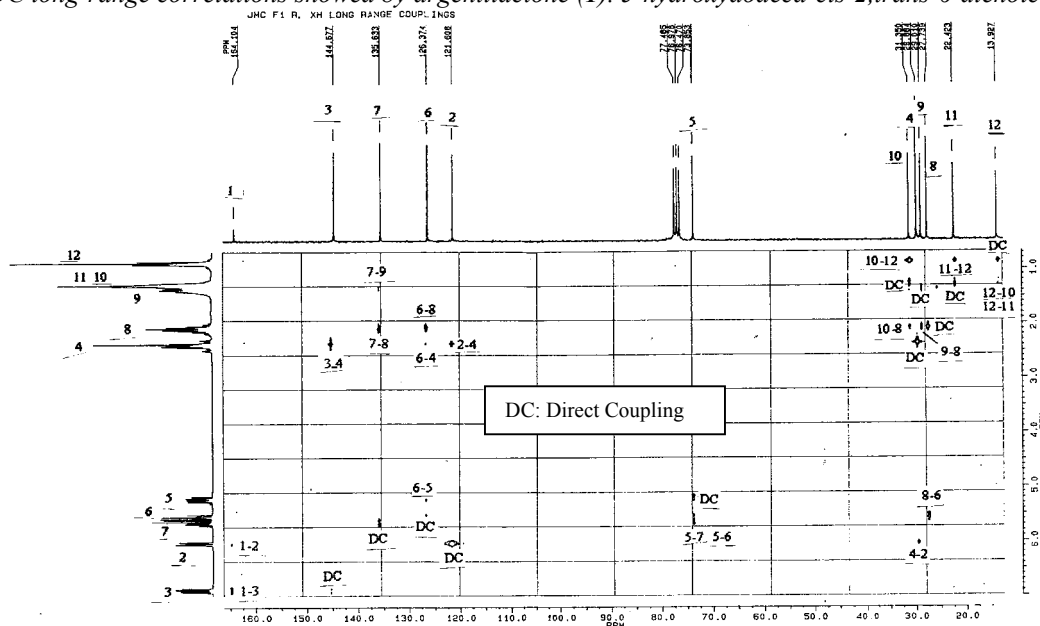

*Compound **1**, COLOC remote heteronuclear correlations*

In order to perform certain insecticide assays, another sample of **1** was obtained through a second extraction and separation from stem-bark of *A. ambotay*. The product obtained exhibited a similar R_f to that observed previously for **1** under similar conditions of elution. The product was submitted to EIMS and IR spectrometry to try to confirm its identity as argentilactone, however, the EIMS spectrum obtained did not fit the stepwise fragmentation pattern reported for argentilactone [6]. This fact and a close

analysis of the EIMS and IR spectra of the product obtained form the second isolation assay let us propose the existence of compound **2**. The IR spectrum features of **2** pointed out absorption bands for functions such as hydroxyl groups (3434 cm^{-1}), alkyl stretching vibrations (triplet between 2961 and 2861 cm^{-1}) and a carbonyl group at 1721 cm^{-1}, an absorption band at 968 cm^{-1} indicated a *trans* configuration for the double bond C-6 - C-7 [7]. This spectrum did not fit either the IR data of

argentilactone [6]. No molecular peak was present in the EIMS spectrum of **2** like most electron impact systems for long- and medium-chain acids, where molecular ions are either very small or cannot be detected [7]. From the supposed molecular peak value (m/z 212 for $C_{12}H_{20}O_3$), the loss of 17 mass units produced the base peak at m/z 195 ([M]$^+$-OH), and then m/z 177 ([M]$^+$-OH-H_2O) to arrive to peak m/z 149 after consecutive loss of the carbonyl group. At this point, two fragmentation pathways followed, peak at m/z 123 (149-C_2H_2), and the second way expressed by the following peaks: m/z 135, m/z 121 and m/z 107 representing sequential losses of three methylene groups from the fragment at m/z 149.
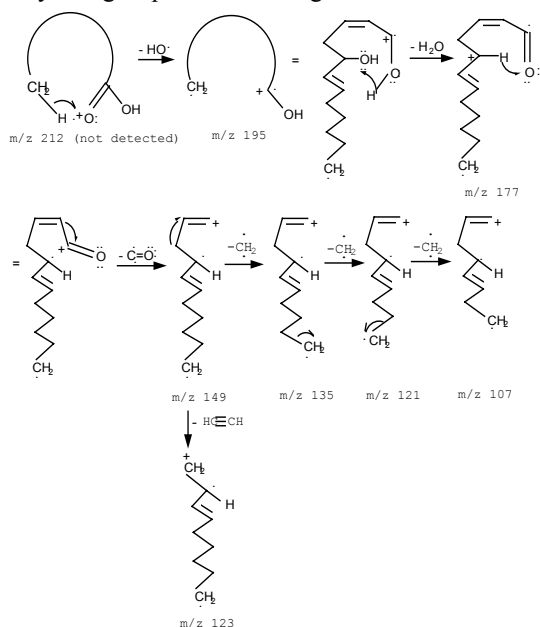
*Compound **2**, Stepwise fragmentation pattern*

Several longer-chain fatty acids like oleic, linoleic, myristic, palmitic, stearic, arachidic, palmitoleic and linoleic acids have been identified in *A. cherimolia*, *A. muricata*, *A. squamosa* as well as species from other annonaceae genus [2]. The possibility of formation of **2** from **1** as an artifact arises from the fact that during the second extraction, compound **1** that was supossed to be present in the methylene chloride layer was treated with anhydrous Na_2SO_4 to obtain a CH_2Cl_2 water-free solution. Considering sufficient water traces to form an oversaturated Na_2SO_4 aqueous solution to be in contact with **1**, the opening of the lactone ring and the addition of 18 mass units (H_2O) would be possible by a nucleophilic attack on the lactonic carbonyl by the alcali. Separately, to try to prove the possible artifact formation, an authentic sample of **1** suspended in methylene chloride was stirred at room conditions with a saturated Na_2SO_4 aqueous solution (slightly

basic) to try to obtain compound **2** with no sucsess, giving so, the possibility for **2** to be a new natural product.
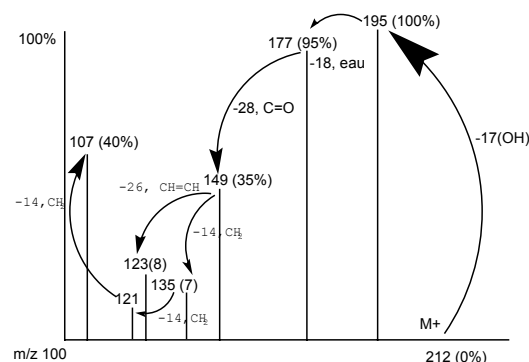
*Compound **2**, mass spectrum*

EXPERIMENTAL

General -. IR spectrum recorded in $CHCl_3$. ^1H and ^{13}C NMR spectra were recorded at 250 and 62.9 MHz in $CDCl_3$. Two dimensional NMR experiments were performed using standard Bruker microprograms.

Plant material - *A. ambotay* (Aublet) was collected and identified by Dr. Sylvie Bergeron in the Alto Ivon region of the Beni department (Bolivia) in November 1993. A voucher specimen is deposited at the National Herbarium of the San Andrés Major University of La Paz.

Extraction and isolation of compounds - First extraction. Dried, pulverized stem-bark (338 g) was extracted with 7 l of EtOH-H_2O (7:3) by maceration at room temp. for 24 hr. The remaining aq. layer from alcohol evaporation was partitioned into CH_2Cl_2 (200 mlX3) to yield a 12.3 g extract. 245 mg of the CH_2Cl_2 extract were chromatographed in silica gel 60 (70-230 mesh, 280 g) in a VLC system. Elution with pure CH_2Cl_2 gave rise to 85 mg of fr 2, which in turn was submitted to a silica gel prep. TLC (2 mm) and developed in CH_2Cl_2. Different bands were detected by irradiation under UV at λ 254 nm and by evaporation of iodine. Recovery of compound **1** from chromatography support was achieved by CH_2Cl_2-MeOH (9:1) to afford 53 mg of pure argentilactone (0.015%, R_f 0.42). The organic layer. Second extraction. A similar to the first extraction procedure was repeated employing (330) g of pulverized stem-bark of plant, 7 l of EtOH-H_2O (7:3), macerated for 24 hr. at room temp. The remaining aq. layer from alcohol evaporation was partitioned into CH_2Cl_2 (200 mlX3), the CH_2Cl_2 soluble layer was dried with Na_2SO_4 and conc. under red. press. to obtain 11.9 g of

extract. 250 mg of the CH_2Cl_2 extract were chromatographed in silica gel 60 (70-230 mesh,   g, VLC) to obtain   80   mg of fraction 2. This was submitted to a silica gel prep. TLC (2mm) to obtain 12 mg of **2**.

*Assay to prove the artifact **2** from **1*** - 4.5 mg of **1** were suspended in CH_2Cl_2 (3 ml) and stirred at room temp. for 50 hrs with a Na_2SO_4 aq. satd soln (5 ml, pH 8-9). The organic layer was separated from the aq. layer, dried at red press and the residue analysed by ^{1}H NMR to give **1**.

Argentilactone (**1**) - Pale yellowish oily liquid; $[\alpha]_D$ -11° (*c* 0.41 $CHCl_3$); EIMS *m/z* (rel. int.): 194 [M]$^+$ (7), 152 (4), 123 (8), 110 (8); ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.89 (3H, *t*, $J_{12, 11} = 6.8$ Hz, H-12), δ 1.26 (2H, *m*, H-10), δ 1.30 (2H, *m*, H-11), δ 1.37 (2H, *m*, H-9), δ 2.14 (1H, *dq*, $J_{8, 8'} = 13.3$ Hz, $J_{8, 9} = J_{8, 9'} = J_{8, 7} = 6.6$ Hz, H-8), δ 2.05 (1H, *dq*, $J_{8', 8} = 13.3$ Hz, $J_{8', 9} = J_{8', 9'} = J_{8', 7} = 6.6$ Hz, H-8'), δ 2.39 (2H, *m*, H-4), δ 5.23 (1H, *m*, H-5), δ 5.58 (1H, *m*, H-6), δ 5.67 (1H, *m*, H-7), δ 6.05 (1H, *ddd*, $J_{2, 3} = 9.8$ Hz, $J_{2, 4} = 2.1$ Hz, $J_{2, 4'} = 1.5$ Hz, H-2), δ 6.90 (1H, *ddd*, $J_{3, 2} = 9.8$ Hz, $J_{3, 4} = 5.3$ Hz, $J_{3, 4'} = 3.2$ Hz, H-3); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.9 (*q*, C-12), δ 22.4 (*t*, C-11), δ 27.7 (*t*, C-8), δ 29.0 (*t*, C-9), δ 29.9 (*t*, C-4), δ 31.3 (*t*, C-10), δ 73.8 (*d*, C-5), δ 121.6 (*d*, C-2), δ 126.3 (*d*, C-6), δ 135.6 (*d*, C-7), δ 144.7 (*d*, C-3), δ 164.1 (*s*, C-1).

5-hydroxydodeca-cis-2,trans-6-dienoic acid (**2**) - Oily liquid; IR vmax^{CHCl3} cm^{-1}: 3434, 2961, 2922, 2861, 1721, 1379, 1240, 1140, 1053, 1015, 968, 814; EIMS *m/z* (rel. int.): 209 (2), 197 (3), 196 (16), 195 (100), 177 (95), 152 (5), 151 (3), 150 (5), 149 (35), 147 (5), 135 (7), 123 (8), 121 (7), 110 (6), 109 (21), 107 (41).

ACKNOWLEDGMENTS

REFERENCES

1 - Hopp, D. C., Zeng, L., Gu, Z. M. and Mc Laughlin, J. L. (1996) *J. Nat. Prod.* **59**, 97.

2 - Leboéuf, M., Cavé, A., Bhaumik, B., Mukherjee, B. and Mukherjee, R. (1982) *Phytochemistry* **21**, 2783.

3 - Da Silva, M. L., Luz, A. I. R., Zoghbi, M. G. B., Ramos, L. S. and Maia, J. G. S. (1982) *Cienc. Cult.* **34**, 510.

4 - De Oliveira, A. B., De Oliveira, G. C., Carazza, F. and Maia, J. G. S. (1987) *Phytochemistry* **26**, 2650.

5 - Priestap, H., Bonafede, J. and Ruveda, E. (1977) *Phytochemistry* **16**, 1579.

6 - Fournet, A., Cavé, A. (1997) *Planta Medica* **63**, 433

7 - Gunstone, F. D. (1990) in *Methods in Plant Biochemistry* (Charlwood, B. V. and Banthorpe, D. V., eds), Vol. 4, p.1, Academic Press, London.