



BIOLOGICALLY ACTIVE FLAVONOIDS AND TERPENOIDS FROM *Egletes viscosa**

MARY ANNE S. LIMA, EDILBERTO R. SILVEIRA,† MARIA SILVÂNIA L. MARQUES, REGINA HELENA A. SANTOS‡ and MARIA TERESA P. GAMBARDELA‡

Curso de Pós-Graduação em Química Orgânica, Departamento de Química Orgânica e Inorgânica, Laboratório de Produtos Naturais, Universidade Federal do Ceará, Cx. Postal, 12.200, Fortaleza, CE, 60.021-970, Brazil; ‡Departamento de Química e Física Molecular, Instituto de Física e Química de São Carlos, C.P. 369, São Carlos, SP, 13560-250, Brazil

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Key Word Index—*Egletes viscosa*; Compositae; macela-da-terra; volatile constituents; neo-clerodane and open chain diterpenes; flavonoids; biological activity; X-rays; spectral data.

Abstract—The steam volatile components from the hexane extract of dried flower buds of *Egletes viscosa* were identified by gas chromatography-mass spectrometry as *trans*-carvyl acetate, *cis*-carvyl acetate, sabinyll acetate, verbenyl acetate, cyclopentaethyldiene, geranyl acetate and 5-methylfuranone, and *trans*-pinocarvyl acetate (major component). From the non-volatile residue, centipedic acid and a novel clerodane diterpene, 12-acetoxy-hawtriwaic acid lactone, were isolated. From the ethanol extract, ternatin (4',5-dihydroxy-3,3',7,8-tetramethoxyflavone), was isolated. Ternatin showed anti-inflammatory, hepatoprotection and gastroprotection properties, and, according to the NCI protocols, it showed moderate activity against HIV. The diterpenes showed antispasmodic activity. Structure determination of these secondary metabolites was accomplished by spectrometric methods, including 2D NMR, chemical interconversion and X-ray crystallographic analysis.

INTRODUCTION

In the northeast of Brazil, dried flower buds of *Egletes viscosa* Less. popularly known as 'macela-da-terra', are sold in herbal stores and are used in folk medicine for the treatment of digestive and intestinal problems. Braga reports its use in popular medicine as an emmenagogue and diaphoretic [1], whereas Correia mentions its stomachic and antidiarrhoea properties [2]. According to Simões *et al.*, the ethanol extract of dried flower buds of *E. viscosa* showed antidiarrhoea and antispasmodic activities [3]. Because of these properties, *E. viscosa* was selected for phytochemical analysis.

RESULTS AND DISCUSSION

Dried flower buds of *E. viscosa* were purchased from a herbal store in the 'Mercado São Sebastião', a popular 'flea market' in Fortaleza, Ceará, Brazil. Hexane extraction, at room temperature, yielded an oily brownish clear residue (EVF-H). Ethanol extraction of the resulting marc yielded a dark brown resin (EVF-E).

A report in the literature about the volatile constituents of *E. viscosa* [4] led us to perform a steam distillation on an aliquot of EVF-H. The steam distilled portion was analysed by GC-mass spectrometry, (MS) yielding the results presented in Table 1, which for the sake of comparison contains the reported data from the previous study [4].

Upon refrigeration of EVF-H, a solid material precipitated. Filtration and methanol-chloroform recrystallization yielded crystals of **1**, mp 150–152°, $[\alpha]_D^{25} - 123.9^\circ$ (*c* 5.0 in chloroform). High-resolution MS 372.1997 (calc. for $C_{22}H_{28}O_5$ 372.2014). Its ^{13}C NMR decoupled spectrum (Table 2) showed 22 lines in agreement with the molecular formula. APT and DEPT experiments allowed the determination of the hydrogenation pattern of each carbon revealing four unsaturated and two saturated non-hydrogenated carbons, four unsaturated and three saturated methines, six saturated methylenes and three methyl groups.

Proton NMR analysis (Table 2) revealed the characteristic signal pattern of a β -substituted furan ring i.e. signals at δ 7.41 (1H, s, H-16), 7.36 (1H, *m*, H-15), and 6.39 (1H, *m*, H-14). This was in agreement with the ^{13}C signals at δ 108.6 (CH, C-14), 126.1 (C, C-13), 138.5 (CH, C-15) and 143.3 (CH, C-16). The 1H NMR also showed the presence in **1** an angular methyl (δ 0.58, s, H-20), a tertiary methyl (δ 0.75, *d*, *J* = 6.8 Hz, H-17), an acetyl methyl (δ 2.00, s), an oxygenated methylene (δ 4.26, *dd*, H-19 α and

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†Author to whom correspondence should be addressed.

Table 1. GC-MS analysis of the volatile constituents of *E. viscosa*

Compound	KI*	Percentage	
		EVF-H/V	Ref. [4]
5-Methylfuranone	837	0.28	—
Ethylidenecyclopentane	876	0.63	—
α -Thujene	920	—	0.4
α -Pinene	927	—	2.0
β -Pinene	967	—	26.8
Mircene	981	—	0.7
δ -3-Carene	1000	—	2.7
α -Terpinene	1002	—	0.1
β -Phelandrene	1018	—	0.1
Linalool	1085	—	0.1
4-Terpineol	1100	—	0.2
Borneol	1111	—	0.1
<i>trans</i> -Pinocarveol	1116	1.52	1.1
Pinocarpone	1145	—	0.5
Verbenyl acetate	1247	3.29	—
<i>trans</i> -Pinocarvyl acetate	1313	51.69	49.0
<i>cis</i> -Carvyl acetate	1316	12.62	0.3
Mirtenyl acetate	1317	—	6.5
<i>trans</i> -Carvyl acetate	1326	20.40	—
Sabinyl acetate	1332	3.25	—
α -Terpenyl acetate	1362	0.84	1.1
Geranyl acetate	1372	0.54	1.1

*Kovat's Index [4].

Table 2. ^1H and ^{13}C NMR assignments for 1 and 12

C	1			12		
	^{13}C	HETCOR	COSY*	^{13}C	HETCOR	COSY*
1	20.2	1.99	←-] ←-] ←-]	25.6	1.69	
		1.10	←-] ←-] ←-]			
2	27.5	2.40	←-] ←-] ←-]	130.7	—	
		2.18	←-] ←-] ←-]			
3	140.0	6.76	←-] ←-] ←-]	123.5	5.11	←-] ←-]
4	135.6	—		27.9	2.12	←-] ←-]
5	45.6	—		34.5	2.27	←-] ←-]
6	34.2	1.86	←-] ←-] ←-]	132.2	—	
		1.20	←-] ←-] ←-]			
7	27.7	1.61	←-] ←-] ←-]	145.7	6.01	←-] ←-]
		1.44	←-] ←-] ←-]			
8	37.1	1.57	←-] ←-] ←-]	28.1	2.64	←-] ←-]
9	39.4	—		39.0	2.12	←-] ←-]
10	49.0	1.86		134.8	—	
11	41.9	2.15	←-] ←-] ←-]	124.5	5.20	←-] ←-]
		1.80	←-] ←-] ←-]			
12	64.8	5.93	←-] ←-] ←-]	28.4	2.27	←-] ←-]
13	126.2	—		25.0	2.46	←-] ←-]
14	108.6	6.39		124.9	—	
15	139.5	7.36		111.0	6.28	
16	143.3	7.41		142.5	7.34	
17	15.7	0.75	←-] ←-] ←-]	138.8	7.21	
18	169.1	—		15.9	1.60	
19	71.7	4.26	←-] ←-] ←-]	173.8	—	
		3.89	←-] ←-] ←-]			
20	17.5	0.59		17.7	1.60	
CH ₃ CO	21.5	2.00				
CH ₃ CO	170.0	—				

*Observed ^1H - ^1H correlations are connected by arrows (←- - - ->).

3.89, *dd*, H-19 β), an acylated carbinolic methine (δ 5.93, *dd*, $J = 4.5$ and 7.8 Hz, H-12) and an olefinic methine (δ 6.39, *dd*, $J = 2.3$ and 7.6 Hz, H-3).

In addition to the furan ring moiety, the ^{13}C NMR spectrum showed two carbonyl groups at δ 170.0 and 169.1, for an ester or lactone, a conjugated carbon-carbon double bond at δ 140.0 (CH, C-3) and 135.6 (C, C-4), a carbinolic methylene at δ 71.7 (CH₂, C-19), a carbinolic methine at δ 64.8 (CH, C-12), and 11 more saturated carbons. If two carbonyl groups, a furan ring and a carbon-carbon double bond account for six DBEs, from its molecular formula, **1** must be tricyclic. Because we had worked before with hardwickic acid (**2**) and derivatives from *Croton sonderianus* [5], it was very easy to relate all these features with a similar diterpene structure where either C-19 or C-20 has been oxidized. A search of the literature for clerodane model compounds revealed a lactone obtained after reaction of hawthornic acid (**3**) [6, 7], as a good model where, in addition, an acetoxy group had to be attached to a secondary carbon.

A comparison of the ^{13}C NMR data for **1** to those for 12-hydroxy-hardwickic acid (**4**) [8] revealed some similarities (Table 3). A closer relationship was observed after comparison of the ^1H and ^{13}C NMR data of 12-hydroxy-hawthornic acid (**5**), particularly when account was taken of the probable misassignments for C-3, C-4, C-13, C-15 or C-16, C-17 and C-20, reported for **5** [9].

In order to obtain the hydroxyl derivative of **1** for comparison with **5**, we attempted to hydrolyse it with

KOH-ethanol, but instead of **5** we obtained **6**. A similar alcohol addition to an α,β -unsaturated lactone moiety has already been observed for **7** under KOH-methanol conditions [10]. LiAlH_4 reduction of **1** yielded a mixture of products whose structures, **9-11**, were determined by spectroscopic analysis (Table 3). A similar hydride addition to the conjugated double bond has also been observed for **7** [7]. $^1\text{H}-^1\text{H}$ and $^1\text{H}-^{13}\text{C}$ COSY experiments were used to accomplish the assignments listed in Table 2.

The structure of **1** was finally solved by X-ray crystallographic analysis. Figure 1 shows a stereoscopic view of the relative stereochemistry of **1**. The absolute stereochemistry shown in Fig. 1 is arbitrary.

A search of the literature, including the review of clerodane diterpenes by Merrit and Ley [11], revealed no report of 12-acetoxy-hawthornic acid lactone. Thus, to the best of our knowledge, **1** is a novel secondary metabolite of the neo-clerodane diterpene class.

The mother liquors of **1** were chromatographed on silica gel to yield **12** as a clear oily fraction, homogeneous by TLC. Its IR spectrum showed a characteristic broad band centred at 3225 cm^{-1} . This, in conjunction with an intense absorption at 1680 cm^{-1} , indicated the conjugated carboxylic acid character of **12**. Its ^{13}C NMR spectrum (Table 2) showed 20 signals: 11 sp^2 (δ 173.8-111.0) and nine sp^3 carbons (δ 39.0-15.9). DEPT and APT techniques allowed the determination of the hydrogenation pattern of each carbon showing five sp^2

Table 3. ^{13}C NMR data for compounds **1**, **4**, **6** and **8-11**, and comparison with literature model **5** [9] [solvent, CDCl_3 ; except **5** (pyridine- d_5)]

C	1 (C)	4 (C)	5 [9] (C)	6 (P)	8 (C)	9 (C)	10 (C)	11 (C)
1	20.2	18.4	20.5	20.2	19.4	21.6	21.7	21.9
2	27.5	27.1	27.6	31.8	24.6	24.6	25.6	27.7
3	140.0	137.7	143.5	76.3	25.5	25.5	27.4	26.2
4	135.6	142.2	131.0	56.6	45.9	45.9	37.3	44.2
5	45.6	37.8	45.7	44.8	43.1	43.1	43.0	50.6
6	34.2	35.8	34.2	31.8	36.8	36.6	33.0	36.8
7	27.7	27.4	27.9	27.4	26.8	27.0	27.2	27.8
8	37.1	36.8	36.8	37.1	36.8	39.7	36.8	38.1
9	39.4	39.5	39.2	39.8	39.4	39.3	39.7	39.7
10	49.0	47.2	48.7	46.0	45.2	49.6	50.9	47.3
11	41.9	46.0	44.6	45.4	41.3	43.7	47.0	45.1
12	64.8	63.0	63.1	62.3	65.0	62.6	62.8	63.2
13	126.2	131.6	138.4	133.3	126.0	130.9	132.5	131.1
14	108.6	108.5	108.3	109.8	108.5	108.2	109.3	106.4
15	138.5	138.3	138.3	139.0	139.7	138.1	138.4	138.3
16	143.3	143.4	136.5	143.7	143.3	143.3	143.4	143.4
17	15.7	16.0	17.6	15.9	15.4	15.6	16.2	15.9
18	169.1	167.9	169.6	177.0	176.0	179.4	62.0	103.3
19	71.7	20.9	71.9	70.8	70.4	70.5	58.3	71.6
20	17.5	17.9	15.6	17.5	17.0	17.0	16.8	17.1
CH ₃ -C=O	170.0	—	—	—	170.0	—	—	—
CH ₃ -C=O	21.5	—	—	—	21.4	—	—	—
CH ₃ O	—	51.0	—	—	—	—	—	—
CH ₃ CH ₂ O	—	—	—	64.7	65.0	—	—	—
CH ₃ CH ₂ O	—	—	—	15.9	15.6	—	—	—

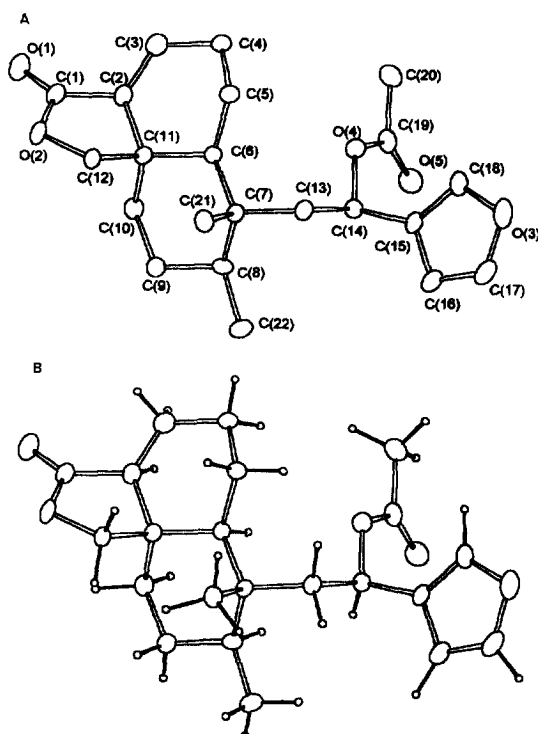
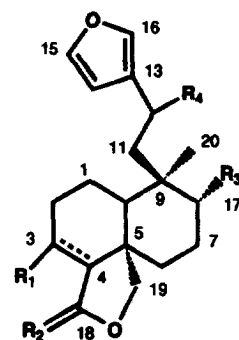


Fig. 1.(a) A computer generated perspective drawing of the final X-ray model of **1**. (b) Stereo view of **1** with hydrogens in idealized positions. For both (a) and (b), the absolute configuration shown is arbitrary.

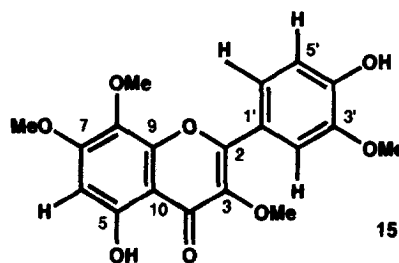
nonhydrogenated (δ 173.8, 134.8, 132.2, 130.6 and 124.9) and six monohydrogenated carbons (δ 145.7, 142.5, 138.8, 124.5, 123.4 and 111.0); three methyls (δ 25.6, 17.7 and 15.9) and six methylene carbons (δ 39.0, 34.5, 28.4, 28.1, 27.9 and 25.0). Its mass spectrum showed a molecular ion at m/z 316 and this, in agreement with the ^{13}C analysis, suggested the molecular formula $\text{C}_{20}\text{H}_{28}\text{O}_3$.

The ^1H NMR spectrum of **12** showed the presence of a β -substituted furan ring [δ 7.34 (1H, *m*, H-17), 7.21 (1H, *m*, H-16) and 6.28 (1H, *m*, H-15)], a vinyl proton in a conjugated double bond at [δ 6.02, 1H, *br t*, H-7], two other vinyl protons (δ 5.12, *m*, $J = 7.1$ and 1.2 Hz, H-3, and 5.21, *m*, $J = 6.9$ and 1.4 Hz, H-11), and two methylenes (δ 2.64, *q*, $J = 7.5$ Hz, H-8, and 2.47, distorted *t*, ($J = 7.2$ Hz, H-13). Multiplets were observed at δ 2.28 (4H, H-5 and H-12) and 2.14 (4H, H-4 and H-9), and singlets δ 1.70 (3H, H-1) and 1.60 (6H, H-18 and H-20).

The molecular formula, $\text{C}_{20}\text{H}_{28}\text{O}_3$, indicated seven DBEs. As a carbonyl, three carbon-carbon double bonds and a furan ring account for all DBEs, **12** must have an open chain. At this point two structures fitted the spectral data (**12** or **13**). ^1H - ^1H COSY, ^1H - ^{13}C direct correlation and ^1H - ^{13}C long-range correlation spectroscopy suggested structure **12** and allowed the assignments listed in Table 2. A comparison of the spectroscopic data (Table 4) for **12** and the methyl ester (**14**) obtained after treatment of **12** with an excess of diazomethane, with the published



	R ₁	R ₂	R ₃	R ₄	3-4
1	H	O	Me	OAc	double
5	H	O	Me	OH	double
6	βOEt	O	Me	OH	single
7	βOMe	O	CH_2OH	H	single
8	βOEt	O	Me	OAc	single
9	H	O	Me	OH	single
11	H	H, OH	Me	OH	single



data for centipedic acid and its methyl ester [12] secured their identity.

Silica gel column chromatography of EVF-E yielded an amorphous yellow solid, **15**, mp 216.2–216.8°, showing the characteristic positive microchemical reaction of flavonoids (HCl-Mg). Its ^1H NMR spectrum contained signals for four methoxy groups at δ 3.98, 3.95, 3.92 and 3.87, two exchangeable hydrogens (with D_2O) at δ 12.46 and 6.03, an aromatic ABX system at δ 7.06 (1H, *d*, $J = 8.1$ Hz, H-3'), 7.80 (1H, *dd*, $J = 2.1$ and 8.1 Hz, H-2'') and 7.81 (1H, *br s*, H-6'), and a singlet at δ 6.41 (1H, H-6). Its ^{13}C NMR spectrum (Table 4) showed two hindered methoxyl groups at δ 61.5 and 61.1, and two non-hindered ones at δ 56.3 and 56.0. It also showed 15 sp^2 carbons (δ 95.4–179.0). APT analysis indicated that the signals at δ 122.7, 114.7, 110.8, and 95.4 were due to hydrogenated carbons.

The absence of a hydrogenated carbon around δ 105.0 and the presence of a non-hydrogenated carbon at δ 138.6 indicated a flavonol moiety methylated at C-3. The UV spectrum showed a bathochromic shift after addition of

Table 4. ^{13}C NMR data for compounds **12**, **14** and **15** and comparison with literature data

C	12 *	14 *	14 [12]*	C	15 *	15 †	15 [14]†
1	25.6	25.6	25.7	2	155.7	155.6	155.6
2	130.7	131.4	131.8	3	138.6	137.7	137.4
3	123.5	123.4	123.7	4	179.0	178.3	178.0
4	28.1	27.9	28.1	5	157.4	156.4	156.2
5	34.5	34.7	34.8	6	95.4	95.7	95.6
6	132.2	132.0	132.0	7	158.4	157.6	147.5
7	145.7	142.0	141.8	8	128.8	128.3	128.2
8	27.9	27.8	28.0	9	148.4	148.2	158.0
9	39.0	39.1	19.3	10	105.3	104.4	104.4
10	134.8	134.9	135.1	1'	122.9	122.2	122.2
11	124.5	124.4	124.6	2'	110.8	111.7	111.1
12	25.0	24.9	25.1	3'	146.4	147.5	147.5
13	28.4	28.4	28.6	4'	148.3	150.1	150.6
14	124.9	124.8	125.0	5'	114.7	115.8	115.8
15	111.0	111.0	111.1	6'	122.7	120.8	120.1
16	138.8	142.5	142.6	OCH ₃	61.5	61.0	60.9
17	142.5	138.7	138.9	OCH ₃	60.1	60.1	59.6
18	15.9	15.8	15.9	OCH ₃	56.3	56.4	56.4
19	173.8	168.4	168.4	OCH ₃	56.0	55.9	55.5
20	17.7	17.7	17.7				
OCH ₃	—	51.0	51.0				

*CDCl₃.†DMSO-*d*₆.

sodium acetate, indicating on OH conjugated to a carbonyl. The steady complex formed after AlCl₃ addition, in agreement with the observation of a hydroxyl at δ 12.46 in the ^1H NMR spectrum, was good evidence for the positioning of a hydroxyl group at C-5. It was also indicative that the ABX system was in the 'C' ring, and that the 'A' ring must be pentasubstituted.

We discovered from the literature that the hydroxylated carbon of ring 'A' of a 5-hydroxy-6,7-dimethoxyflavone and a 5-hydroxy-7,8-dimethoxyflavone absorb at *ca* δ 91.0 and 96.0, respectively [13]. The value of δ 95.4 observed for **15**, indicated the 5, 7, 8 substitution pattern. Thus, **15** must be the 4',5-dihydroxy-3,3',7,8-tetramethoxyflavone, known as ternatin [13]. A comparison of the ^{13}C NMR data for **15** and ternatin (Table 4), in the same solvent DMSO-*d*₆, showed their common identity.

A 3-methyl-flavonol (3'-hydroxy-4',5',6,7-tetramethoxyflavone), a clerodane diterpene similar to hardwickic acid, and centipedic acid, have been isolated from *Centipeda orbiculares* (Compositae). According to Bohlmann and Mahanta [12], the *Centipeda* genus is botanically problematic. From our findings, *Centipeda* and *Egletes* should be placed in the same Tribus, the Astereae, in agreement with Bohlmann's suggestion [12].

Both diterpenes, **1** and **12**, and the flavonoid **15** from *E. viscosa* have been the subject of pharmacological assays in the Department of Pharmacology, Universidade Federal do Ceará, Brazil. The diterpenes showed an antispasmodic effect [3] whereas ternatin showed anti-inflammatory activity, hepatoprotection and gastroprotection properties [14–16]. The antiviral activity of

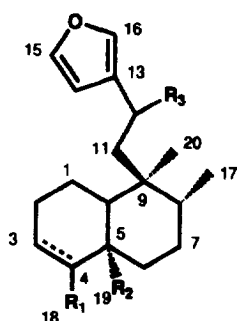
3-methoxyflavones is already known; according to the National Cancer Institute-(USA) protocols, ternatin showed moderate *in vitro* activity against HIV.

EXPERIMENTAL

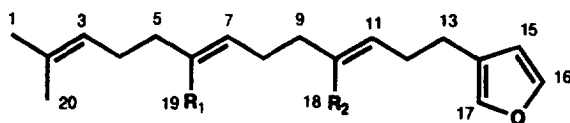
General experimental procedures. Mps: uncorr.; IR: KBr pellets (solids) or NaCl discs (oil films); ^1H (200 MHz) and ^{13}C (50 MHz) NMR: Bruker AC-200, CDCl₃, DMSO-*d*₆ or pyridine-*d*₅. δ 7.24, 2.49 and 7.19 for ^1H , and δ 77.0, 39.5 and 118.1 for ^{13}C as int. references; low resolution MS: Hewlett-Packard HP-5995-A (70 eV); HR MS: VG Analytical 7070 E-HF.

X-Ray analysis. A light white crystal was mounted on an Enraf-Nonius CAD-4 diffractometer equipped with graphite monochromated MoK α radiation. The lattice constants, determined by least-squares of 25 automatic centred reflections were: $a = 9.540(2)$, $b = 11.939(1)$, $c = 16.941(2)$ Å; $V = 1929.4(6)$ Å³, $Z = 4$; $D_{\text{calc}} = 1.289$ g cm⁻³ and the space group is P2₁2₁2₁. The intensity data were corrected for Lorentz and polarization effects, and the structure was solved by direct methods [17] and difference Fourier synthesis [18]. The H atoms were included in the final model at their calculated positions (C–H = 1.08 Å) with overall isotropic parameter $B = 6$ Å². The final full matrix least-squares refinement [18] with anisotropic thermal parameters for non-H atoms converged to $R = 0.049$ and $R_w = 0.055$ for 1099 observed reflections ($I \geq 3\sigma(I)$). The molecular illustrations shown in Fig. 1 was drawn with ORTEP [19].

The atomic parameters, tables of bond lengths and angles, and observed and calculated structure factors



	R ₁	R ₂	R ₃	3-4
2	CO ₂ H	Me	H	double
3	CO ₂ H	CH ₂ OH	H	double
4	CO ₂ Me	Me	OH	double
10	CH ₂ OH	CH ₂ OH	OH	single



	R ₁	R ₂
12	CO ₂ H	Me
13	Me	CO ₂ H
14	CO ₂ Me	Me

have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

Plant material. Dried flower buds of *E. viscosa* Less. were purchased at a herbal store in 'Mercado São Sebastião', Fortaleza, CE, Brazil, and were identified by Dr Afrânio G. Fernandes (Botanist, Dept. Biology, UFCE).

Extraction and isolation of constituents. Ground dried flower buds (1.84 kg) were exhaustively extracted with hexane at room temp. to yield 57.0 g clear viscous oily extract, designated EVF-H, after solvent evapn. Extraction of the marc with EtOH yielded 130.0 g brown resinous extract designated EVF-E. EVF-H was submitted to hydrodistillation to yield 0.8 ml volatile fraction designated EVF-H/V and a residue designated EVF-H/R. After *ca* 1 week at room temp., an amorphous ppt. started forming. A first crop of **1** was obtained by filtration. Crystallization from MeOH-HCl₃ yielded 2.62 g **1**, as orthorhombic crystals, mp 150–152°, [α]_D –123.9° (*c* 5.0 in CHCl₃). HR-EI-MS *m/z* 372.1997 (calc. for C₂₂H₂₈O₅, 372.2014); EI-MS *m/z* (rel. int.): 372

[M]⁺, (3.0), 330 [M – CH₂=C=O]⁺ (4.0), 312 [M – HOAc]⁺ (8.0), 218 [312 – 94]⁺ (30.0), 188 (26.0), 160 (20.0), 145 (25.0), 111 (22.0), 94 (CH₂=CH-furyl); IR $\nu_{\max}$ cm^{–1}: 3170, 3050, 2990, 1770, 1740, 1665, 1505, 1460, 1400, 1380, 1250, 1055, 1000, 980, 960, 880, 820, 790, 720; ¹H NMR (200 MHz, CDCl₃): Table 2; ¹³C NMR (50.0 MHz, CDCl₃): Table 2.

CC of EVF-H/R (52.0 g) on 200 g silica gel eluted with hexane, yielded 5.02 g of plates, mp 62.8–64.0° characterized as a long chain hydrocarbon and not further analysed, 1.21 g **1** and 1.61 g **12** as a pale yellow oil homogeneous by TLC. IR $\nu_{\max}$ cm^{–1}: 3250–2250, 1680, 1640, 1500, 1440, 1360, 1260, 1165, 1065, 1030, 870, 760; EI-MS *m/z* (rel. int.): 316 [M]⁺ (1.4), 301 [M – 15]⁺ (11.0), 248 (17.2), 175 (16.2), 150 (16.1), 149 (26.7), 136 (30.9), 135 (100.0), 134 (21.3), 133 (39.5), 131 (14.0), 122 (115.4), 121 (31.5), 119 (34.6), 95 (40.5), 94 (28.6), 93 (78.9), 91 (60.5), 81 (96.4); ¹H NMR (200 MHz, CDCl₃): Table 2; ¹³C NMR (50.0 MHz, CDCl₃): Table 2.

CC of EVF-E (90.0 g) adsorbed on 120 g silica gel yielded, on elution with CHCl₃, 12.0 g greenish resin designated EVFE-C. Rechromatography on silica gel yielded 830 mg yellow solid, **15**, after crystallization in CHCl₃–MeOH (1:1), mp 216.2–216.8°. IR $\nu_{\max}$ cm^{–1}: 3330, 2950, 1640, 1610, 1600, 1500, 1460, 1440, 1360, 1320, 1280, 1260, 1230, 1210, 1130, 1040, 1010, 920, 820; EI-MS *m/z* (rel. int.): 374 (52.0), 359 (100.0), 344 (70.0), 331 (6.0), 287 (8.0), 164 (5.0), 152 (12.5), 151 (14.2), 135 (6.5), 125 (7.0); ¹H NMR (200 MHz, CDCl₃): 12.46 (1H, s, OH), 7.81 (1H, *br s*, H-6'), 7.80 (*dd*, *J* = 2.1, 8.1 Hz, H-2'), 7.06 (1H, *d*, *J* = 8.1 Hz, H-3'), 6.41 (1H, s, H-6), 6.03 (1H, s, OH), 3.98 (3H, s, OMe), 3.95 (3H, s, OMe), 3.91 (3H, s, OMe), 3.88 (3H, s, OMe); ¹³C NMR (50 MHz, CDCl₃): Table 4.

Hydrolysis of 1. A soln of **1** (500 mg) in alcoholic 50% KOH (50 ml) was stirred at room temp. for 2 hr, until the starting material had completely reacted (monitored by TLC). Usual work-up yielded 310 mg of solid, **6**, mp 217.5–219.5°. EI-MS (rel. int.): 374 [M]⁺ (1.0), 358 (3.0), 330 (8.0), 280 (80.8), 234 (20.4), 173 (16.0), 133 (20.4), 57 (100.0) 41 (60.4); ¹H NMR (200 MHz, pyridine-*d*₅): δ 7.76 (1H, *br s*, H-16), 7.64 (1H, *br s*, H-15), 6.70 (1H, *br s*, H-14), 5.09 (1H, *br s*, H-12), 4.27 (2H, *q*, H-18), 3.64–3.46 (3H, *m*, CH₃CH₂O–, H-3), 1.13 (3H, *t*, *J* = 6.9 Hz, CH₃CH₂O–), 0.72 (3H, *d*, *J* = 6.7 Hz, H-17), 0.51 (3H, s, H-20); ¹³C NMR (50 MHz, pyridine-*d*₅): Table 3.

Acetylation of 6. Treatment of **6** (200 mg) with Ac₂O–pyridine (4:1) (2.0 ml), after usual work-up yielded a viscous oil, **8** (187 mg). ¹H NMR (200 MHz, CDCl₃): δ 7.38 (1H, *br s*, H-16), 7.34 (1H, *br s*, H-15), 6.35 (1H, *br s*, H-14), 5.90 (1H, *dd*, *J* = 3.3, 8.4 Hz, H-12), 4.13 (2H, s, H-18), 3.64–3.38 (3H, *m*, CH₃CH₂O– and H-3), 2.00 (3H, s, CH₃C=O), 1.20 (3H, *t*, 6.2 Hz, CH₃CH₂O–), 0.73 (3H, *d*, *J* = 6.6 Hz, H-17), 0.55 (3H, s, H-20); ¹³C NMR (50 MHz, CDCl₃): Table 3.

LiAlH₄ reduction of 1. Compound **1** (900 mg) was dissolved in Et₂O (25 ml) and mixed with 40 ml LiAlH₄ satd Et₂O soln. The reaction mixt. was stirred at room temp. for 42 hr, until the starting material had completely reacted (monitored by TLC). Excess of LiAlH₄ was

neutralized by adding hydrated Na_2SO_4 and filtered to yield 596 mg yellowish solid. CC on 12 g silica gel in a 60 ml cylindrical separatory funnel yielded **9** (47 mg), **10** (27 mg) and **11** (29 mg). Compound **9**, ^1H NMR (200 MHz, CDCl_3): δ 7.30 (2H, s, H-15 and H-16), 6.33 (1H, s, H-14), 4.75 (1H, dd, $J = 8.6, 2.7$ Hz, H-12), 4.26 (1H, d, $J = 9.1$ Hz, H-18a), 4.12 (1H, d, $J = 9.1$ Hz, H-18b), 2.30 (1H, br s, OH), 1.97–1.05 (15H, m), 0.70 (3H, d, $J = 6.7$ Hz, H-17), 0.50 (3H, s, H-20); ^{13}C NMR (50 MHz, CDCl_3): Table 3. Compound **10**, ^1H NMR (200 MHz, CDCl_3): δ 7.34 (2H, s, H-15, H-16), 6.38 (1H, s, H-14), 4.95 (1H, br d, H-19), 4.80 (1H, br, H-12), 3.97 (1H, d, $J = 8.4$ Hz, H-18a), 3.81 (1H, dd, $J = 3.0, 8.4$ Hz, H-18b), 3.45 (1H, br, OH), 2.43 (1H, dt, $J = 12.7, 2.6$ Hz, H-4), 1.98–1.02 (15H, m), 0.69 (3H, d, H-17), 0.49 (3H, s, H-20); ^{13}C NMR (50 MHz, CDCl_3): Table 3. Compound **11**, ^1H NMR (200 MHz, CDCl_3): δ 7.33 (1H, s, H-16), 7.31 (1H, s, H-15), 6.34 (1H, s, H-14), 4.72 (1H, m, H-12), 3.85 (1H, d, $J = 11.5$ Hz, H-18a), 3.31 (1H, dd, $J = 11.5, 3.5$ Hz, H-18b), 2.31–1.14 (18H, m), 0.72 (3H, d, H-17), 0.68 (3H, s, H-20); ^{13}C NMR (50 MHz, CDCl_3): Table 3.

Methylation of 12. Compound **12** (1 g) was dissolved in Et_2O (20 ml) and treated with excess of CH_2N_2 . Silica gel CC of the products yielded 541 mg of an oil, **14**, ^1H NMR (200 MHz, CDCl_3): δ 7.30 (1H, s, H-16), 7.18 (1H, s, H-17), 6.24 (1H, s, H-15), 5.81 (1H, br t, H-7), 5.17 (1H, br t, H-11), 5.07 (1H, br, H-3), 3.70 (3H, s, OCH_3), 2.50 (2H, dt, H-8), 2.43 (2H, t, H-13), 2.23 (2H, t, H-5), 2.21 (2H, t, H-12), 2.05 (4H, m, H-4, H-9), 1.65 (3H, s, H-20), 1.57 (3H, s, H-18); ^{13}C NMR (50 MHz, CDCl_3): Table 4.

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