Diterpene Glycosides from Egletes viscose

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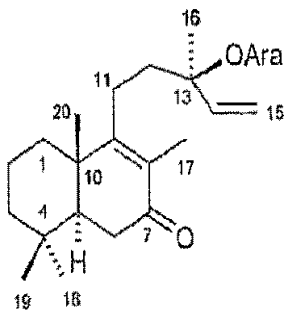
Abstract:

A phytochemical investigation of the $CHCl_3$-soluble extract of the entire plant of *Egletes viscosa* (Asteraceae), collected in Peru, afforded two new labdane glycosides, 13-hydroxy-7-oxolabda8,14-diene 13(R)-0-a-L-arabinopyranoside and 13–hydroxy-7-oxolabda-8,14-diene 13(R)-O-α-L-arabinopyranoside, along with four known compounds. The structures of the new compounds were elucidated by spectroscopic and chemical methods.

Article:

Egletes viscosa (L.) Less, (Asteraceae) is a small herb that is commonly known as "piro-sacha" and "macela-da-terra" in Peru and Brazil, respectively. It has been used in traditional medicine for the treatment of various diseases, including gastrointestinal disorders and as an emmenagogue [1], [2], [3]. Several biologically active flavonoids and terpenoids have been reported from this plant by a team of Brazilian researchers [1], [3], [4], although, to the best of our knowledge, it has not been examined for anticancer activity. Thus, as part of our collaborative program to search for new anticancer compounds from plants [5], the $CHCl_3$-soluble extract of the entire plant of *E. viscosa*, was examined. From this, two new labdane glycosides (1 and 2) and four known compounds, namely 5,7-dihydroxy-2',4'-dimethoxyflavonol [6], 8α,13-epoxylabdan-14,15-diol [7], 13-epi-sclareol [8], and tarapacol 14-O-α-L-arabinose [9], were isolated and identified; unfortunately, none of the compounds had significant anticancer activity.



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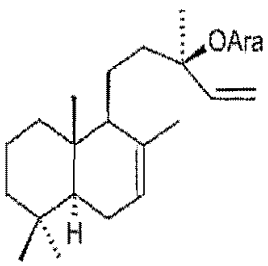

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Compound 1, $[\alpha]_D$: -49.3° (c 0.14, $CHC1_3$), was obtained as a colorless gum, The Na adduct of the molecular ion was determined as $C_{25}H_{40}O_6Na$ by HR-ESI-MS (m/z $[M\ Na]^+$ = 459.2717), indicative of an index of hydrogen deficiency of six. Three of these degrees of unsaturation were accounted for by an α,β-unsaturated ketone moiety, observed in the ^{13}C NMR spectrum (Table 1) at δ_c = 200.4 (C-7), 130.4 (C-8), and 168.1 (C-9), and a terminal double bond, which displayed classic cis/trans coupling of three vinylic protons in the ^1H-NMR spectrum (Table 1) at δ_H - 5.83 (1H, dd, J = 10.9, 17.6 Hz, H-14), 5.27 (1H, d, J = 17.6 Hz, H-15), and 5.31 (1H, d, J = 10.9 Hz, H-15). A fourth degree of unsaturation was attributed to the sugar moiety, which exhibited

characteristic proton signals at δ_H= 4.38 (1H, brt, J = 3.3 Hz, H-1') and 3.52 - 3.93 (5H, overlap, H-2'-H_2_5'), [9], [10]. Also apparent were five methyl proton signals δ_H = 1.4] (3H, s, H-16), 1.73 (3H, s, H-17), 0.88 (3H, s, H-18), 0.91 (3H, s, H-19), and 1.06 (3H, s, H-20)]. The labdane diterpenoid skeleton was deduced from the NMR data analysis, including COSY, HSQC, and HMBC experiments [11], [12], and this accounted for the final two degrees of unsaturanon. The presence of an L-arabinose unit was identified by comparison of the NMR data with those reported in the literature [9], [10], by TLC analysis after acid hydrolysis [13], and by measuring the α_D of the hydrolyzed sugar, which was positive as expected. The positions of the functional groups were determined unambiguously from an HMBC NMR experiment, and the relative stereochemistry was assigned based on ROESY correlations and literature comparisons [11], [12]; key correlations for these experiments are illustrated in Fig. 1. Moreover, the stereochemistry of C-13 is presumed to be R based on biogenetic similarities to 2, discussed below. Thus, the structure of this new labclane glycoside was elucidated as 13-hydroxy-7-oxolabda-8,14-diene 13(R)-O-α-L-arabinopyranoside (1).

Compound 2, $[\alpha]_D$ -19.5° (c 0.13, $CHC1_3$), gave the Na adduct of the molecular ion $[M + Na]^+$ at m/z = 445.2941 by HR-ESI-MS, consistent with an elemental formula of $C_{25}H_{42}O_5Na$, The ^{1}H-and ^{13}C-NIMR spectra of 2 (Table 1) were comparable to those of 1, except for the absence of conjugated ketone resonances and the presence of olefinic signals [δ_H = 5.37 (1H, brs, H-7), δ_c = 122.3 (C-7) and 135.1 (C-8)]. The structure of 2, including relative stereochemistry and identity of the sugar moiety, was determined in a manner comparable to that of 1. The aglycone core of 2 was identified as labda-7,14-dien-13(R)-ol by comparison of the NMR data and optical rotation (negative value) with the literature data [14], [15], [16]; this assignment is in agreement with all other known natural glycosides comprising the same aglycone. As a result, the new compound 2 was assigned as 13-hydro-xylabda-7,14-diene 13(R)-O-α-L-arabinopyranoside (2).

Table 1 NMR data of compounds **1** and **2** in $CDCl_3$

	1		2	
	δ_c	δ_H, *mult (J in Hz)*	δ_c	δ_H, *mult (J in Hz)*
1	36.2	1.32 dt (3.6, 12.8), 1.88 brd (12.4)	39.2	0.93 m, 1.79 brd (12.5)
2	18.9	1.59 m, 1.65 overlap	18.8	1.44 overlap, 1.54 overlap
3	41.5	1.20 dt (4.1, 13.4), 1.46 brd (13.2)	42.3	1.11 overlap, 1.38 overlap
4	33.4	–	32.9	–
5	50.5	1.66 overlap	50.2	1.47 overlap
6	35.4	2.34 (14.3, overlap), 2.49 dd (3.6, 17.5)	23.8	1.88 overlap, 1.93 overlap
7	200.4	–	122.3	5.37 brs
8	130.4	–	135.1	–
9	168.1	–	55.2	1.55 overlap
10	41.3	–	37.1	–
11	24.0	2.25 m	21.2	1.48 overlap, 1.71 overlap
12	40.6	1.69 overlap	44.3	1.53 overlap, 1.86 overlap
13	80.7	–	81.2	–
14	141.8	5.83 dd (10.9, 17.6)	142.0	5.78 dd (10.9, 17.5)
15	116.9	5.27 d (17.6), 5.31 d (10.9)	116.3	5.21 d (17.5), 5.27 d (10.9)
16	22.5	1.43 s	22.2	1.37 s
17	11.5	1.73 s	22.1	1.66 s
18	32.7	0.88 s	33.1	0.85 s
19	21.5	0.91 s	21.8	0.87 s
20	18.4	1.06 s	13.6	0.73 s
1′	98.1	4.38 brt (3.3)	97.6	4.37 brt (3.3)
2′	72.2	3.65 overlap	71.7	3.65 overlap
3′	73.2	3.65 overlap	72.8	3.65 overlap
4′	68.0	3.92 overlap	67.5	3.89 overlap
5′	65.2	3.52 d (11.5), 3.93 (3.2, overlap)	64.6	3.49 dd (2.9, 13.5), 3.91 (3.5, overlap)

All of the compounds isolated in this investigation were evaluated in the KB (human oral epidermoid carcinoma) cell cytotoxicity assay [17], but none of these displayed significant activity (EC_{50} values > 10 μg/mL). The typical average EC_{50} value for camptothecin in this assay, which was used as a positive control, is 0.01 μg/mL [17].

Materials and Methods

Optical rotations, IR spectra, and UV spectra were recorded on a Rudolph Research Autopol III automatic polarimeter, a Nicolet Avatar 360 FT-IR, and a Varian Cary 3 UV-Vis spectrophotometer, respectively. NMR experiments were performed on a Bruker AMX 500 spectrometer with TMS as an internal standard. EI-MS and ESI-MS were recorded on HP5989A and Finnigan LCQ instruments, respectively. HR-ESI-MS were performed with a 3-Tesla Finnigan FTMS-2000 Fourier Transform mass spectrometer. For this, the samples were prepared in 1:1 MeOH:THF + NaCl (to give the sodiated molecular ion), sprayed from a commercial Analytica electrospray ionization source, and focused into the FT-MS cell using a home-built set of ion optics. Preparative HPLC was carried out on a Varian Prostar 210 pump system (10 mL/min) using a YMC (Wilmington, NC) ODS-A (250 x 25 mm, i.d,, 5 μm) column.

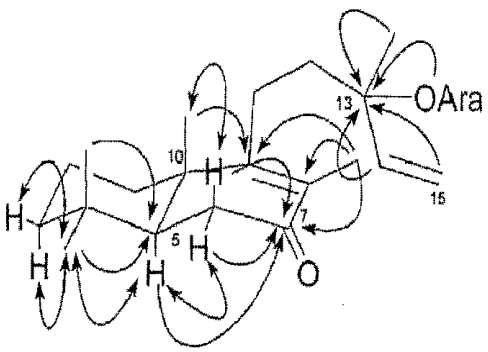

Fig. 1 Selected HMBC (→) and ROESY (←→) correlations of compound 1.

Egletes viscosa was collected in July, 1998 in the Province of Coronel Portillo, District of Yarinacocha, Peru. It was taxonomically identified by one of the authors (JGG), and a voucher specimen (A4652) has been deposited at the Field Museum of Natural History, Chicago, IL. We recognize that the above- and below-ground portions may have a slightly different profile of secondary metabolites. However, *E, viscosa* is a rather small herb, and as such, the entire plant was processed to supply adequate material for our programmatic studies [5].

The dried plant material (728 g) was extracted in a Soxhlet apparatus with hat MeOH overnight (2 x 3 L), and the MeOH extract concentrated under reduced pressure. This extract was partitioned between 10% aqueous MeOH and hexane, and the aqueous MeOH fraction was partitioned further between $CHCl_3$- MeOH (4 : 1) and water. The organic layer was washed with 1% saline until there was no further evidence of tannins [18]. Fractionation of the organic-soluble extract (4.7 g) was initiated by flash silica gel chromatography (250 g) using a $CHC1_3$-MeOH gradient (12 L. total) to afford eleven pooled fractions. Fractions 3 (0.2 g, eluted with 2.5% MeOH in $CHCl_3$), 4 (0.3 g, eluted with 3 % MeOH in $CHCl_3$), and 5 (0.4 g, eluted with 4% MeOH $CHCl_3$) were purified using HPLC (gradient of MeCN:H_2O, 70:30 to 85:15 over 45 min) to afford 5,7-dihydruxy-2',4'-dimethoxyfla-vonol [6], 8α,3-epoxylabdan-14,15-diol [7] {$[\alpha]_D$: -3.6° (c 0.11, $CHCl_3$)}, and 13-epi-sclareol [8], [19] {$[\alpha]_D$: +45° (c 0.11, $CHCl_3$)} from fraction 3, compound 2 (t_R = 36 min) from fraction 4, as well as compound 1 (t_R = 28 min) and tarapacol 14-O-α-L-arabi-nose [9] {$[\alpha]_D$: + 5.7° (c 0.23, $CHCl_3$)} from fraction 5.

13-Hydroxy-7-oxolabda-8,14-diene 13(R)-O-α-L-arobinopyranoside (1): Colorless gum, $[\alpha]_D$: -49,3° (c 0,14, $CHCl_3$); IR (CH_2Cl_2): ν_{max} = 3058, 1683 on^{-1}; UV (MeOH): λ_{max} (log ε)= 206 (3.65), 248 (3.67), 293 (3.54) nm; EI-MS: m/z (rel. int.) = 204 (100); HR-ESI-MS: m/z = 459.2717 $[M + Na]^+$ (calcd. for $C_{25}H_{40}O_6Na$: 459.2717); ^1H- and ^{13}C-NMR data: see Table 1.

13-Hydroxylabda-7,14-diene 13(R)-O-α-L-arabinopyranoside (2): Colorless gum, $[\alpha]_D$: -19.5° (c 013, $CHCl_3$); IR (CH_2C1_2): v_{max} = 3055 cm^{-1}; UV (Me0H): λ_{max} (log ε) = 204 (3,55) nm; El-MS: m/z (rel. int.) = 220 (100), 436 $[M]^+$; HD-ESI-MS: m/z = 445.2941 $[M + Na]^+$ (calcd. for $C_{25}H_{42}O_5Na$: 445.2924); ^1H- and ^{13}C-NMR data: see Table 1.

Compounds 1 and 2 and tarapacol 14-O-α-L-arabinose were applied to a silica gel TLC plate and were hydrolyzed with 1 N HCl for 1 h by heating. After removal of residual HCl under nitrogen, the plate was developed using $CHC1_3$-MeOH-H_2O (2:1:0.1). The air-dried plate was sprayed with 10% H_2SO_4 in MeOH-H_2O (5 :1) and heated at 120° C to identify spots that were attributed to arabinose by comparison to a reference standard of arabinose 0.3) [13]. Moreover, to determine if the sugar was L-arabinose or D-arabinose, aqueous solutions of 1 and 2 (5 mg, each) in 1 N NCl were heated at 110°C for 1 h under an N_2 atmosphere. After drying under N_2, the sugars and the aglycones were separated by liquid-liquid partitioning between $CHCl_3$ and H_2O. The sugar fractions were confirmed as L-arabinose (literature $[\alpha]_D$: + 104°) by comparison of their optical rotation values ($[\alpha]_D$: +95° and $[\alpha]_D$: + 88° for the sugars of 1 and 2, respectively).

All compounds were tested for cytotoxicity against human oral epidermoid carcinoma (KB) cells as described previously [17].

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References

1 Lima MAS, Silveira ER, Marques MSL, Santos RHA, Gambardela MTP. Biologically active flavonoids and terpenoids from Egletes viscosa. Phytochemistry 1996; 41: 217 -23

2 Rao VSN, Mendezes AMS, Gadelha MGT. Antifertility screening of some indigenous plants of Brazil, Fitoterapia 1988; 59: 17 -20

3 Rao VSN, Santos FA, Sobreira Tf, Souza MF, Melo CL, Silveira ER. Investigations on the gastroprotective and antidiarrhoeal properties of ternein, a tetramethoxyflavone from Egletes viscosa, Planta Med 1997; 63: 146-9

4 Guedes MM, Cunha AN, Silveira ER, Rao VSN. Antinociceptive and gastroprotective effects of diterpenes from the flower buds of Egletes viscosa. Planta Med 2002; 68: 1044- 6

5 Kinghorn AD, Farnsworth NR, Soejarto DD, Cordell GA, Swanson SM, Pezzuto JM et al. Novel strategies for the discovery of plant-derived anticancer agents. Pharm Biol 2003; 41 (supplement): 53 -67

6 Batterharn TJ, Highet RJ. Nuclear magnetic resonance spectra of flavanoids. Aust J Chem 1964; 17: 428-32

7 Rodriguez B, Valverde 5, Fraga BM, Gonzalez AG, Hernandez MG. De-termination of stereochemistry of C-14 in 8,13-epi-epoxy-14,15-dihy-droxylabdanes using proton NMR spectroscopy, An Quim 1976; 72: 850-4

8 Stierle DB, Stierle AA, Larsen RD. Terpenoid and flavone constituents of Pofemonium viscosum. Phytochemistry 1988; 27; 517-22

9 Torrenegra R, Robles J, Waibel R, Lovvel M. Achenbach H. Diterpenes and diterpene xylosides from Conyza trihecatactis. Phytochemistry 1994; 35: 195-9

10 Ahmed AA, Mahrnoud AA, Ahmed UM, El-Bassuony AA, Abd El-Razk MH, Pare PW et al. Manoyl oxide a-arabinopyranoside and grindelic acid diterpenoids from Grindelia integrifolia. J Nat Prod 2001; 64: 1365-7

11 Jakupovic J, Ellmauerer E, Bohlmann F, King RM, Robinson H, ent-Labdanes from Austrobrickeilia patens. Phytochemistry 1986; 25: 1927-9

12 Wu C-L, Lin H-R, Labdanoids and bis(bibenzyls) from Jungerinannin species. Phytochemistry 1997; 44: 101 -5

13 Kim N-C, Desjardins AE, Wu CD, Kinghorn AD. Activity of triterpenoid glycosides from the root bark of Mussaeacla macrophylla against two oral pathogens. J Nat Prod 1999; 62: 1379- 84
14 Uchio Y, Nagasaki M, Eguchi S, Matsuo A, Nakayama M, Hayashi S. Lab-dane diterpene glycosides with 6-deoxy-t-idose from Asterspathulifolius Maxim. Tetrahedron Lett 1980; 21: 3775-8
15 Khan MR, Gray AI, Waterman PG. Clerodane diterpenes from Zuelaaia guidonfa stem bark. Phytochemistry 1990; 29: 2939 -42
16 Choi SZ, Kvvon HC, Choi SU, Lee KR. Five new labdane diterpenes front Aster oharai. J Nat Prod 2002; 65: 1102 - 6
17 Gu J-Q, Graf TN, Lee D, Chai H-B, Mi Q, Kardono LDS et al. Cytotoxic and antimicrobial constituents of the bark of Diospyros maritima collected in two geographical locations in Indonesia, j Nat Prod 2004; 67: 1156 - 61
18 Wall ME, Wani MC, Brown DM, Fullas F, Oswald JB, Josephson FF et al, Effect of tannins on screening of plant extracts for enzyme inhibitory activity and techniques for their removal. Phytomeclicine 1996; 3: 281-5
19 Torrenegra R, Pedrozo J, Robles J, Waibel R, Achenbach H. Diterpenes from Gnaphalium pellitum and Gump/minim graveolens. Phytochemistry 1992; 31: 2415- 8