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SESQUITERPENE AND DITERPENE GLYCOSIDES FROM *XANTHIUM SPINOSUM*

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Key Word Index—*Xanthium spinosum*; Compositae; sesquiterpene lactones; kaurene glycosides.

Abstract—Two new sesquiterpene lactone glycosides and three new kaurene glycosides closely related to carboxyatractyloside and atractyloside, together with the 3′, 4′-didesulphated-carboxyatractyloside and 3′, 4′-didesulphated-atractyloside, were isolated from the aerial parts of *Xanthium spinosum*. Their structures were established by spectroscopic methods (^{1}H, ^{13}C, DEPT ^{13}C NMR and FAB mass spectr).

INTRODUCTION

The *Xanthium* genus (family Compositae, tribe Heliantheae) is represented by a relatively limited number of species distributed in nearly all parts of the world. The chemistry of this genus is quite homogeneous, sesquiterpene lactones being detected in all cases [1–4]. The occurrence of some toxic kaurene glycosides has been reported [5, 6]. These compounds inhibit mitochondrial ADP/ATP translocation [7] and produce nephrotoxic effects [8].

As a part of our continuing studies on the biologically active metabolites from South American Compositae, we report the isolation of two new sesquiterpene lactone glycosides (**1** and **2**), the new 2*β*-*O*-*β*-D-glucopyranosyl-15*α*-hydroxy-kaur-16-en-18,19-dicarboxylic acid (**3**), 4′-desulphated-carboxyatractyloside (**5**) and 4′-desulphated-atractyloside (**7**), together with the already known 3′,4′-didesulphated-carboxyatractyloside (**4**) and 3′,4′-didesulphated-atractyloside (**6**), from the aerial parts of *X. spinosum*.

RESULTS AND DISCUSSION

A chloroform–methanol extract of the aerial parts of *X. spinosum* afforded **1** and **2** purified by sequential Sephadex LH-20 column chromatography, and semi-preparative reversed-phase HPLC. The molecular formula of **1** $C_{21}H_{34}O_9$ was determined by ^{13}C NMR and DEPT ^{13}C NMR analysis and by FAB-mass spectrometry, in the negative ion mode, which gave a quasimolecular anion at *m/z* 429. Fragments at *m/z* 251 $[M - H - 178]^-$ and *m/z* 267 $[M - H - 162]^-$ (cleavage of a hexose unit with or without the glycosidic oxygen) were also evident.

The ^{13}C NMR spectrum of **1** (Table 1) exhibited 21 signals of which six were ascribable to a glucose unit and 15 to a sesquiterpene lactone. The aglycone signals were divided by ^{13}C NMR and DEPT ^{13}C NMR into three-CHOH groups (*δ* 74.2, 76.9 and 83.0), three >CH (*δ* 32.9, 40.7 and 42.2), three -CH_2 (*δ* 22.7, 37.4, 43.9), three CH_3 (*δ* 11.3, 21.3 and 22.4), one trisubstituted double bond CH=C (*δ*124.6 and 149.9) and one carboxylic group (*δ* 179.1). In the ^{1}H NMR spectrum of **1** (Table 1), in addition to three methyl doublets on saturated carbons (*δ*1.16, *J* = 7 Hz, Me-13; *δ*1.26, *J* = 7 Hz, Me-14; *δ*1.32, *J* = 7 Hz, Me-15), three signals ascribable to protons at oxygen-bearing carbons (*δ*3.96, *m*, *J* = 2.5, 7 and 10 Hz, H-4; *δ*4.22, *dd*, *J* = 3 and 10 Hz, H-2; *δ*4.79, *ddd*, *J* = 3.5, 7 and 11 Hz, H-8) and a double doublet for an olefinic proton (*δ*5.82, *J* = 5 and 9.5 Hz, H-5) were evident. The chemical shift of H-8 suggested that the hydroxyl group at C-8 was involved in a lactone cycle with the carboxylic function. Further features were signals corresponding to the anomeric proton (*δ*4.40, *dd*, *J* = 7.5 Hz) and to H_2-6 (*δ*3.68, *dd*, *J* = 5 and 12 Hz; *δ*3.88, *dd*, *J* = 3.5 and 12 Hz;) of a glucose unit. ^{1}H–^{1}H decoupling experiments in CD_3OD allowed the assignment of all protons of the aglycone portion, and the resulting sequence indicated the position of the lactone ring. The relative stereochemistry at C-7, C-8, C-10 and C-11 was determined by analysis of the coupling constants and by NOE difference spectroscopy. The chemical shift of H-8 (Table 1), downfield shifted if compared to that of the 8*β*-epimers, is reported to be diagnostic of an *α*-configuration [9]. A C-7/C-8 *cis*-fused lactone functional group in **1** was evident from the small coupling constant of 7 Hz observed between H-7 and H-8 [10]. Furthermore, irradiation of the H-8 signal enhanced the signals for H-7, H-10 and H-11, allowing the establishment of the *β*-stereochemistry of both the C-10 and C-11 methyl groups. In addition, in

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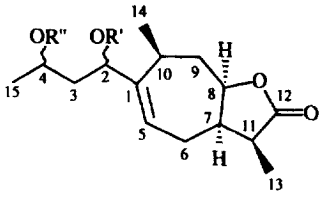
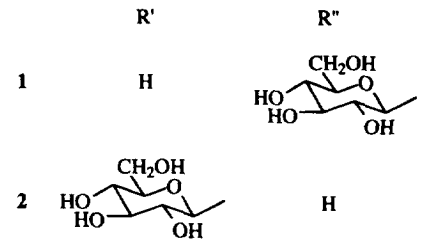
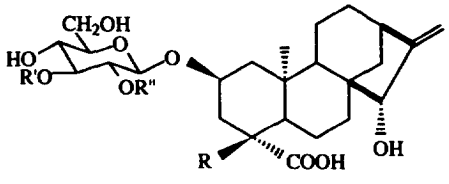




	R'	R"
1	H	
2		H



	R	R'	R"
3	COOH	H	H
4	COOH	H	-$COCH_2CH(Me)_2$
5	COOH	SO_3^-	-$COCH_2CH(Me)_2$
6	H	H	-$COCH_2CH(Me)_2$
7	H	SO_3^-	-$COCH_2CH(Me)_2$

the ^{13}C NMR spectrum of **1** (Table 1) reciprocal shielding γ-effects were observed due to the small dihedral angle between C-13 and C-6 [11], thus suggesting the β-methyl configuration of C-11.

Compound **2** showed a FAB-mass spectral fragmentation pattern superimposable on that of **1**; ^1H and ^{13}C NMR data suggested a close similarity between the two compounds; the main differences were the chemical shift of H-2 and H-4 and the resonances of C-2 and C-4 (Table 1). Comparison of the ^1H and ^{13}C NMR spectra of **1** and **2** prompted us to hypothesize that differences between the two terms should be confined to the position of the glucose unit. In particular the analysis of the observed shifts was in good agreement with the location of the sugar residue at C-4 in **1** and at C-2 in **2**. The location of the glucose unit at C-2 in **2** was also supported by the upfield shift exhibited by the resonance of the anomeric carbon if compared to that of the same carbon in **1** (δ100.0 in **2** versus δ104.2 in **1**), as expected for a glucose linked to an allylic alcoholic function [12]. Thus, **1** is the 4-*O*-β-D-glucopyranosyl-11α,13-dihydro-8-epi-desacetylxanthiuminol and **2** is the 2-*O*-β-D-glucopyranosyl-11α,13-dihydro-8-epi-desacetylxanthiuminol [13].

It is interesting to note that this is the first report of 8α-sesquiterpene lactones in *X. spinosum*, until now only xanthanolides with the opposite configuration at C-8 and a C-11/C-13 double bond had been isolated from this plant [4, 13]. Furthermore, the occurrence of sesquiterpene glucosides in a plant of the *Xanthium* genus is a very unusual finding.

Separation of the components of a methanolic extract of *X. spinosum*, by sequential Sephadex LH-20 column chromatography and semi-preparative reversed phase HPLC yielded **3**–**7**. NMR spectroscopic analysis indicated that **4** ($C_{31}H_{46}O_{12}$) was the 3',4'-didesulphated-carboxyatractyloside previously isolated from *X. pungens* [6] and **6** was the 3',4'-didesulphatedatractyloside isolated for the first time from *Coffea arabica* [14]. NMR data for **5** ($C_{31}H_{45}O_{15}S$) and **7** ($C_{30}H_{45}O_{13}S$) differed from those reported for **4** [6] and **6** [14] for the chemical shifts of H-1, H-2, H-3, C-1, C-2 and C-3 of the glucose unit (see Experimental). The observed resonances together with the FAB-mass spectra (see Experimental) suggested in **5** and **7** the occurrence of a sulphate group at C-3 of the glucose unit. Thus, **5** and **7** were defined, respectively, as the new 4'-desulphatedcarboxyatractyloside and 4'-desulphatedatractyloside.

Desulphation of **5** and **7** in dioxane and pyridine at 120° afforded **4** and **6**, respectively. The NMR spectral pattern of **3** ($C_{26}H_{38}O_{11}$), compared with that of **4** [6], showed the absence of the signals for the isopentanoyl moiety; thus, **3** was determined as the new 2β-*O*-β-D-glucopyranosyl-15α-hydroxy-kaur-16-en-18,19-dicarboxylic acid.

EXPERIMENTAL

The NMR spectra in CD_3OD were obtained at 250 and 500 MHz. The FAB-MS, in negative ion mode, and the DEPT experiments were performed as reported previously [15].

Plant material. Xanthium spinosum was collected at Cordillera del Condor (Ayabaca Province), Perù. A voucher sample is deposited at the Herbarium of the Museo de Historia Natural 'J. Prado' de la Universidad Nacional Mayor de San Marcos, Lima, Perù.

Isolation. The air-dried aerial parts of *X. spinosum* (570 g) were defatted with petrol and were successively extracted with $CHCl_3$ (28 g), $CHCl_3$–MeOH (9:1) (12.5 g) and MeOH (32 g). A portion of the $CHCl_3$–MeOH (9:1) residue (2.5 g) was submitted to CC on Sephadex LH-20 (80 × 2 cm). Frs (8 ml) were eluted with MeOH and checked by TLC on silica gel in $CHCl_3$–MeOH–H_2O (70:30:3). Frs 16–20 (80 mg) were further purified by HPLC on a C-18 μ-Bondapak column using MeOH–H_2O (2:3) as eluent to yield pure **1** (6 mg, R_t 22 min) and **2** (5 mg, R_t 24 min). Part of the MeOH residue (2.5 g) was submitted to Sephadex LH-20 CC using MeOH as the eluent. Frs 16–21 (A, 125 mg), 20–22 (B, 60 mg) and 23–30 (C, 95 mg), checked by TLC on silica gel in *n*-BuOH–HOAc–H_2O, were further fractionated by RP-HPLC using MeOH–H_2O (19:31, flow

Table 1. ^{1}H and ^{13}C NMR data for compounds **1** and **2** in CD_3OD

	1		**2**	
Position	δ_C	δ_H	δ_C	δ_H
1	149.9	—	147.6	—
2	74.2	4.22, 1H, *dd*	80.0	4.47, 1H, *dd*
3	43.9	1.62, 1H, *ddd*, H-3a	44.4	1.64, 1H, *ddd*, H-3a
		1.92, 1H *ddd*, H-3b		1.89, 1H, *ddd*, H-3b
4	76.9	3.96, *m*	66.6	3.88, *m*
5	124.6	5.82, 1H, *dd*	128.6	5.90, 1H, *dd*
6	22.7	2.32, 1H, *ddd*, H-6β	22.7	2.32, 1H, *ddd*, H-6β
		2.04, 1H, *ddd*, H-6α		2.00, 1H, *ddd*, H-6α
7	40.7	2.72, 1H, *m*	40.7	2.72, 1H, *m*
8	83.0	4.79, 1H, *ddd*	83.0	4.79, 1H, *ddd*
9	37.4	2.13, 1H, *ddd*, H-9α	37.3	2.14, 1H, *ddd*, H-9α
		2.07, 1H, *ddd*, H-9β		2.03, 1H, *ddd*, H-9β
10	32.9	2.60, 1H, *m*	33.1	2.54, 1H, *m*
11	42.2	2.93, 1H, *m*	42.5	2.94, 1H, *m*
12	179.1	—	179.1	—
13	11.3	1.16, 3H, *d*	10.7	1.16, 3H, *d*
14	21.3	126, 3H, *d*	20.9	1.23, 3H, *d*
15	22.4	1.32, 3H, *d*	22.6	1.28, 3H, *d*
1′	104.2	4.40, 1H, *d*	100.0	4.29, 1H, *d*
2′	75.2	*	75.2	*
3′	77.9	*	77.9	*
4′	71.6	*	71.3	*
5′	78.2	*	78.0	*
6′	62.8	3.68, *dd*, 1H, H-6′a	62.9	3.68, *dd*, 1H, H-6a
		3.88, *dd*, 1H, H-6′b		3.88, *dd*, 1H, H-6b

J (Hz): 2.3a = 3; 2,3b = 10; 3a,3b = 14.5; 3a,4 = 2.5; 3b,4 = 10; 4,15 = 7; 5,6β = 5; 5,6α = 9.5; 6α,6β = 14.5; 6α,7 = 3; 6β, 7 = 13; 7,8 = 7; 7,11 = 8; 8,9β = 11; 8,9α = 3.5; 9α,9β = 14; 9β,10 = 11; 9α,10 = 5; 4,15 = 7; 10,14 = 7; 11,13 = 7; 1′,2′ = 7.5; 5′, 6′a = 5; 5′,6′b = 3.5; 6′a,6′b = 12.

*Signals submerged by the solvent signal.

rate 2 ml min^{-1} for A; 3:7, flow rate 1.8 ml min^{-1} for B; 9:11, flow rate 2 ml min^{-1} for C) as eluent. Fr. A yielded pure **4** (13.6 mg, R_t = 16 min) and **5** (15.2 mg, R_t = 10 min). From fr. B, **3** (5.2 mg, R_t = 8 min) was obtained. Fr. C afforded **6** (4.8 mg, R_t = 28 min) and **7** (8.8 mg, R_t = 32 min).

Solvolysis of **5** *and* **7** *to give the desulphated analogues.* A soln of the compound (5 mg) in dioxane (0.2 ml) and pyridine (0.5 ml) was heated at 120° for 4 hr in a stoppered vial. After the soln had cooled, H_2O (2 ml) was added and the soln was extracted with *n*-BuOH. The *n*-BuOH extract was evapd under red. pres. to give **4** from **5** and **6** from **7**.

Compound **1**. $[\alpha]_D^{25}$ + 12.5 (MeOH; *c* 1); FAB-MS in negative ion mode: *m/z* 429 $[M-H]^-$, 267 $[M-H-162]^-$, 251 $[M-H-178]^-$. For ^{1}H and ^{13}C NMR: Table 1.

Compound **2**. $[\alpha]_D^{25}$ + 8.5 (MeOH; *c* 1); FAB-MS in negative ion mode: *m/z* 429 $[M-H]^-$, 267 $[M-H-162]^-$, 251 $[M-H-178]^-$. For ^{1}H and ^{13}C NMR: Table 1.

Compound **3**. $[\alpha]_D^{25}$ − 38.8 (MeOH; *c* 1); FAB-MS in negative ion mode: *m/z* 525 $[M-H]^-$, 347 $[M-H-178]^-$. ^{1}H NMR of the glucose unit: δ4.52 (*d*, *J* = 7.5 Hz, H-1), 3.20 (*dd*, *J* = 7.5, 9.5 Hz, H-2), 3.74 (*dd*, *J* = 5.0, 12.0 Hz, H-6a), 3.90 (*dd*, *J* = 3.5, 12.0 Hz, H-6b); the other signals were submerged by the solvent signal. ^{13}C NMR of the glucose unit: δ102.7 (C-1), 75.0 (C-2), 77.6 (C-3), 71.5 (C-4), 77.9 (C-5), 62.6 (C-6); for NMR data of the aglycone moiety see ref. [6].

Compound **5**. $[\alpha]_D^{25}$ − 43.2 (MeOH; *c* 1); FAB-MS in negative ion mode: *m/z* 688 $[M-H]^-$, 608 $[M-H-80]^-$, 603 $[M-H-85]^-$, 523 $[M-H-80-85]^-$. ^{1}H NMR of the glucose unit: δ4.83 (*d*, *J* = 7.5 Hz, H-1), δ4.86 (*dd*, *J* = 7.5, 9.5 Hz, H-2), δ4.43 (*dd*, *J* = 9.5, 9.5 Hz, H-3), δ3.65 (*dd*, *J* = 9.5, 9.5 Hz, H-4), δ3.72 (*dd*, *J* = 5.0, 12.0 Hz, H-6a), δ3.90 (*dd*, *J* = 3.5, 12.0 Hz, H-6b); the remaining signal was submerged by the sovent signal. ^{13}C NMR of the glucose unit: δ100.8 (C-1), 72.6 (C-2), 82.5 (C-3), 70.9 (C-4), 77.5 (C-5), 61.9 (C-6); for NMR data of the aglycone moiety and the isopentanoyl residue at C-2 of the glucose unit see ref. [6].

Compound **7**. $[\alpha]_D^{25}$ − 64.5 (MeOH; *c* 1); FAB-MS in negative ion mode: *m/z* 644 $[M-H]^-$, 564 $[M-H-80]^-$, 559 $[M-H-85]^-$. ^{1}H NMR of the glucose unit: δ 4.72 (*d*, *J* = 7.5 Hz, H-1), 4.82 (*dd*, *J* = 7.5, 9.5 Hz, H-2), 4.45 (*dd*, *J* = 9.5, 9.5 Hz, H-3),

3.65 (*dd*, J = 9.5, 9.5 Hz, H-4), 3.75 (*dd*, J = 5.0, 12.0 Hz, H-6a), 3.90 (*dd*, J = 3.5, 12.0 Hz, H-6b); the remaining signal was submerged by the solvent signal. ^{13}C NMR of the glucose unit: δ 100.8 (C-1), 72.2 (C-2), 82.4 (C-3), 70.5 (C-4), 77.0 (C-5), 62.3 (C-6); for NMR data of the aglycone moiety and the isopentanoyl residue at C-2 of the glucose unit see ref. [14].

Compounds **4** and **6** have been identified by experimentally derived ^{1}H and ^{13}C NMR spectra in comparison with lit. data [6, 14].

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