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TWO TOXIC KAURENE GLYCOSIDES FROM THE BURRS OF *XANTHIUM PUNGENS*

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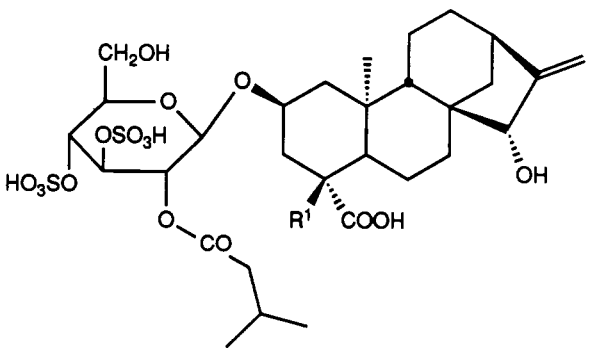
ABSTRACT.—Two H_2O-soluble toxic kaurene glycosides **3** and **4** responsible in part for the poisonous properties of the burr of *Xanthium pungens* have been isolated and identified. The structures of the compounds were elucidated using high resolution 2D nmr and mass spectral techniques.

The noogoora burr *Xanthium pungens* Widder (Compositae), known in North America as *Xanthium strumarium* L. or cocklebur plant, has become distributed in many parts of the world (1) and is especially noxious in South Africa and Australia. The toxic properties of the seeds and young seedlings that still contain the cotyledon have proven dangerous for pigs, cattle, and sheep grazing on these in the field. This problem becomes particularly evident in drought conditions where there has been enough rain to germinate the burrs, but not enough to maintain a useful supply of grazing feed. The major toxic principle of *Xanthium* species has been identified previously (2) as carboxyatractyloside, [**1**], a kaurene glycoside, first isolated from the Mediterranean thistle *Atractylis gummifera* (3).

We now report the isolation and identification of two H_2O-soluble analogues of carboxyatractyloside extracted from *X. pungens*. Structural elucidation was carried out using 2D nmr techniques combined with mass spectral analysis.

RESULTS AND DISCUSSION

Nmr spectroscopic analysis (Tables 1 and 2) indicated that the two compounds, **3** and **4**, isolated from *X. pungens* were closely related to carboxyatractyloside [**1**]. Both contained the same kaurene aglycone, as demonstrated by the diagnostic C-17 exocyclic methylene, the C-18, -19 *gem*-diacid, as well as the characteristic ^{13}C chemical shift of the C-15 carbon bearing the hydroxyl group. Through the use of various 2D nmr tech-



1 R^1=COOH
2 R^1=H

[1]Deceased.

TABLE 1. Selected ^{1}H-nmr Assignments for Compounds **3** and **4**.[a]

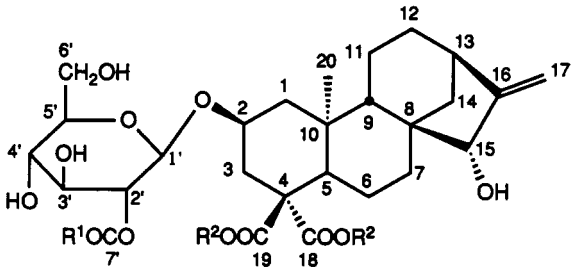
Proton	Compound	
	3	**4**
H-2	3.71 bm	3.83 bm
H-15	3.75 s	[b]
H-17	5.09 s, 5.16 s	4.86 s, 4.93 s
H-20	0.91 s	0.88 s
H-1′	4.64 d, J = 8.5 Hz	4.58 d, J = 8.0 Hz
H-2′	4.60 dd, J = 9.0, 9.0 Hz	[b]
H-3′	4.23 m	4.10 m
H-4′	3.51 m	[b]
H-5′	3.49 m	3.55 m
H-6′	3.70 bd, 3.84 bd	3.85–3.95 m
H-8′	2.18 d, J = 7.0 Hz	2.18 d, J = 7.0 Hz
H-10′	0.96 d, J = 6.5 Hz	1.33 m
H-11′	0.96 d, J = 6.5 Hz	0.69 d, J = 6.0 Hz
H-12′		0.94 d, J = 6.5 Hz

[a]In D_2O.
[b]Signal obscured by other resonances.

niques (^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR, and long-range analogues of both experiments), as well as mass spectral analysis, one could unambiguously assign the aglycone portions and most of the glycosidic portion of each extract.

The first of the two compounds, **3**, was found by microanalysis to contain no sulfur. The negative fab mass spectrum showed an $[M-H]^-$ ion at *m/z* 609. This was mass measured to give a formula of $C_{31}H_{45}O_{12}$, in agreement with the 31 carbon peaks observed in the ^{13}C nmr and the carbon multiplicities obtained from the APT (attached proton test) spectrum. This evidence together with the 2D nmr experiments suggested 3′,4′-didesulfated carboxyatractyloside as the structure for **3**.

The didesulfated derivative of atractyloside [**2**] has been previously identified as a constituent of *Coffea arabica* (4), while we have recently isolated **3** (in the form of its dimethyl ester **5**) from the burrs of *Xanthium spinosum*.[2] Confirmation of the above assignment was obtained from desulfation by acid hydrolysis of authentic carboxyatractyloside [**1**] to give compound **3** with nmr spectra identical to those of the naturally occurring compound from *X. pungens*.



3 R^1=$\overset{8'}{C}H_2\overset{9'}{C}H(\overset{10'}{C}H_3)_2$, R^2=H
4 R^1=$\overset{8'}{C}H_2\overset{9'}{C}H(\overset{12'}{C}H_3)\overset{10'}{C}H_2\overset{11'}{C}H_3$, R^2=H
5 R^1=$CH_2CH(Me)_2$, R^2=Me

[2]R.J. Capon, J.K. MacLeod, and P.B. Oelrichs, in preparation.

TABLE 2. ^{13}C-nmr Assignments for Compounds **1**, **3**, and **4**.

Carbon	Compound		
	1[a]	**3**	**4**
C-1	47.1	48.3	48.2
C-2	74.7	74.8	73.1
C-3	40.1	40.8	40.9
C-4	60.1	59.5	62.0
C-5	51.7	51.7	49.5
C-6	23.4	24.0	26.3
C-7	35.3	36.2	35.9
C-8	48.0	47.8	48.0
C-9	53.3	54.0	53.4
C-10	40.6	41.2	40.9
C-11	18.6	18.6	18.4
C-12	32.7	32.9	32.8
C-13	42.6	42.7	42.7
C-14	36.5	36.7	36.6
C-15	82.8	82.5	82.6
C-16	159.4	161.5	161.0
C-17	109.1	107.9	107.9
C-18	177.1[b]	176.7	178.0
C-19	177.6[b]	176.5	178.0
C-20	17.3	17.5	16.9
C-1′	99.3	100.8	100.7
C-2′	72.6	74.5	75.2
C-3′	79.3	74.8	76.3
C-4′	74.7	70.6	71.8
C-5′	75.0	76.7	78.5
C-6′	61.1	62.3	62.3
C-7′	175.4	172.5	172.5
C-8′	43.9	43.8	41.9
C-9′	25.8	25.8	32.2
C-10′	22.5[c]	22.6[b]	29.5
C-11′	22.6[c]	22.4[b]	11.5
C-12′	—	—	19.4

[a]Data for compound **1** are taken from Cole *et al.* (2).

[b,c]Assignments in the same column with the same superscript may be interchanged.

Microanalysis of the second compound also demonstrated that no sulfur was present. Its negative fab spectrum yielded an $[M-H]^-$ ion at *m/z* 623, which on accurate mass measurement corresponded to a formula of $C_{32}H_{47}O_{12}$, that is, a higher homologue of **3**. The ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR spectra again allowed unambiguous assignment of the structure of **4** which differed from **3** only in having a 3-methylpentanoyl ester moiety attached to C-2′ of the glycoside. Chemical confirmation of this side chain was carried out by basic hydrolysis of **4** followed by neutralization, extraction, and subsequent methylation using CH_2N_2. The gc-ms spectrum of this product was identical to that of the methyl ester of authentic 3-methylpentanoic acid.

The ^{13}C chemical shifts together with selected ^{1}H chemical shifts of carboxyatractyloside [**1**], **3**, and **4** are given in Tables 1 and 2. Both the positive and negative fab mass spectra of **3** and **4** showed the presence of dimer cluster ions. The positive fab spectrum of **3** exhibited a $[2M+Na]^+$ and a $[2M+K]^+$ ion at *m/z* 1243 and 1259, while its negative fab spectrum had a $[2M-H]^-$ ion at *m/z* 1219. For **4**, a $[2M+K]^+$ ion was present at *m/z* 1287 and the $[2M-H]^-$ ion at *m/z* 1247.

The stereochemistry of **3** and **4** could be assigned by correlation with carboxyatractyloside [**1**]. The excellent correlations of the nmr spectral data of **3** and **4** with **1** (Tables 1 and 2) preclude any alternative relative stereochemistries. Both **3** and **4** are portrayed with the same absolute stereochemistry assigned to carboxyatractyloside (3,5), because the optical rotations of **3** from hydrolysis of carboxyatractyloside and from *X. pungens* were identical. An attempt was also made to determine the chirality of the 3-methylpentanoyl ester in **4**. As compound **4** was available only in milligram quantities, a sufficient amount of the acid was not obtainable from hydrolysis of the toxin to measure its $[\alpha]_D$ value directly, even though the optical rotations for both *R* and *S* forms of **4** methylpentanoic acid are known (6). Alternative approaches, using nmr chiral shift reagents and capillary gc-ms after esterification of the acid with various chiral alcohols, were inconclusive.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Ir spectra were recorded on either a Shimadzu model IR-408 or a Perkin-Elmer model 683 instrument. Optical rotations were measured on a Perkin-Elmer model 241 spectropolarimeter at 21°. Fab mass spectra were obtained using a Finnigan MAT 90 spectrometer with glycerol as matrix. Exact mass measurements were carried out on the same instrument using CsI as reference. Low resolution ei mass spectra were obtained using a VG Micromass 7070F spectrometer operating at 70 eV. Gc-ms were run on a Hewlett Packard model 5970 system. ^{1}H- and ^{13}C-nmr spectra, including homo- and heteronuclear 2D experiments, were recorded on a Varian VXR 300S spectrometer. Chemical shift values are given in δ (ppm). Samples were run using D_2O or CD_3OD as solvent using either TMS or C_6H_6 as the internal reference (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). Both samples were freeze-dried from D_2O several times prior to use, to remove the exchangeable protons. Hplc was performed on a Waters Model 6000A instrument using a Whatman C-18 reversed-phase column.

PLANT MATERIAL.—The burrs of *X. pungens* were collected from plants growing in the Chinchilla region in southwestern Queensland where stock losses had been reported. A voucher specimen of the collection, identified by Dr. P.B. Oelrichs, is held at the Animal Research Institute, Brisbane.

TOXICITY TESTING.—This was carried out as previously reported (8). Both compounds **3** and **4** were toxic at the 3 mg/kg level. Comprehensive animal testing could not be carried out because of the small quantities of purified **3** and **4** that were available.

ISOLATION AND PURIFICATION OF **3** AND **4**.—The mixture containing **3** and **4** was isolated from the ground-up burrs of *X. pungens* (100 g) using essentially the same procedure described previously for the isolation of wedeloside from *Wedelia asperrima* (7), that is, a 50% aqueous MeOH extraction followed by polyamide column chromatography and hyflo super cel partition chromatography. Toxicity testing carried out at this stage on the crude mixture confirmed that the fraction containing **3** and **4** was active. Separation and purification of **3** and **4** was performed on a C-18 reversed-phase semipreparative hplc column using either MeCN-H_2O (30:70) or MeOH-H_2O (5:2) as solvent followed by chromatography on LH-20 using MeOH-H_2O (9:1) as eluent, giving purified compounds **3** (10 mg) and **4** (8 mg).

COMPOUND **3**.—Mp 175–178°; $[\alpha]_D$ −41.7 (c = 0.7, MeOH); fabms *m/z* $[M-H]^-$ 609.2905 (calcd for $C_{31}H_{45}O_{12}$, 609.2911); eims (70 eV) *m/z* (rel. int.) 563 (5), 447 (4), 445 (8), 355 (16), 289 (100), 217 (46), 204 (14), 156 (70); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

COMPOUND **4**.—Mp 218–220°; $[\alpha]_D$ −36.8 (c = 1.4, MeOH); ir (KBr) 3418, 2855, 1716, 1457; fabms *m/z* $[M-H]^-$ 623.3024 (calcd for $C_{32}H_{47}O_{12}$, 623.3068); eims (70 eV) *m/z* (rel. int.) 563 (3), 289 (100), 217 (32), 204 (18), 156 (57); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

IDENTIFICATION OF THE 2′-ESTER OF **4**.—Hydrolysis of **4** was carried out under alkaline conditions (20% KOH/at 90° for 7 h), followed by acidification, extraction with Et_2O, and treatment of the dried ($MgSO_4$) organic phase with ethereal CH_2N_2. Gc-ms of the Et_2O solution gave a peak corresponding in retention time and mass spectrum to an authentic sample of the methyl ester of 3-methylpentanoic acid: eims *m/z* (rel. int.) $[M]^+$ 130 (0.5), 115 (1), 101 (13), 99 (15), 74 (100), 59 (28), 43 (46).

DESULFATION OF CARBOXYATRACTYLOSIDE.—Carboxyatractyloside [**1**] (5 mg) was dissolved in MeOH containing 3% HCl (2 ml) and stirred at room temperature under N_2 for 48 h. After workup, the purified product showed ^{1}H- and ^{13}C-nmr spectra and optical rotation identical to those of compound **3**.

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