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BUCHANINOSIDE, A STEROIDAL GLYCOSIDE FROM *ELAEODENDRON BUCHANANII*

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Key Word Index—*Elaeodendron buchananii*; Celastraceae; unripe fruit; insect antifeedant; *Spodoptera exempta*; cardenolide; buchaninoside.

Abstract—A novel steroidal glycoside was isolated from the fruit of a tropical tree, *Elaeodendron buchananii*, as an antifeedant substance for *Spodoptera exempta*. The structure of the compound was determined to be a glycoside of 2α,3β-14-trihydroxy-16α-acetoxy-14β-carda-4,20 (22)-dienolide-7β,8β-epoxide.

INTRODUCTION

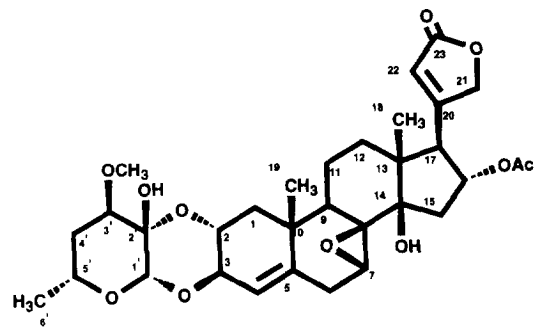
Elaeodendron buchananii, of the family Celastraceae, is a tropical tree that grows in east Africa and is poisonous to animal stock and human beings. Ingestion of its leaves, fruits or bark is said to cause sudden death. On the other hand, the roots can be dried and powdered for use in the treatment of wounds and in the primary symptoms of syphilis. Chewing of the plant has been said to cure diarrhoea [1].

Recently elabunin with moderate cytotoxicity was isolated from the roots of the plant [2].

The poisonous and medicinal properties of this plant have attracted our attention. We now report on the isolation and structural elucidation of a new steroidal glycoside from this plant named buchaninoside (**1**), which has antifeedant activity.

RESULTS AND DISCUSSION

The chloroform extract of the unripe fruit of *E. buchananii* was chromatographed on silica gel to give **1** as colourless crystals. At a dose of 100 μg, **1** showed 70% antifeeding activity against larvae of *Spodoptera exempta* (nut grass armyworm). The structural elucidation of **1** was undertaken using mass spectrometry and NMR spectroscopy. The high resolution electron impact mass spectrum of the compound ($M^+ + 1$: 603.2843) indicated its molecular formula to be $C_{32}H_{42}O_{11}$.



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The ^1H NMR spectrum (Table 1) showed the presence of a methoxyl group, an acetyl group, two singlet methyl and doublet methyl groups. Signals at 5.94 ppm (broad s), 4.84 ppm (*dd*, J = 18.0 and 1.7 Hz) and 4.91 ppm (*dd*, J = 18.0 and 1.7 Hz) suggested the presence of an α,β-unsaturated lactone. The ^1H NMR spectrum also showed the presence of many ether or hydroxyl groups in the molecule (Table 1).

The ^{13}C NMR spectrum (Table 2) of the compound showed 32 carbons: five methyl groups (including one methoxyl group), seven methylene groups (including one oxygenated methylene group), 11 methine carbons and nine quarternary carbons. Signals at 52.5 (CH) and 62.5 ppm (C) were considered to be due to an epoxide. Two characteristic signals at 90.3 and 95.9 ppm suggested the presence of acetals or hemiacetals.

According to the ^1H and ^{13}C NMR spectra including 2D measurements, and the molecular formula

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Table 1. ^{1}H Spectral data for buchaninoside (**1**)

	ppm	J(Hz)
1α	1.46	t ($J_{1\alpha,2\beta}$ = 12.0, $J_{1\alpha,1\beta}$ = 12.0 Hz)
1β	1.83	dd ($J_{1\beta,2\beta}$ = 3.5, $J_{1\beta,1\alpha}$ = 12.0 Hz)*
2β	4.14	ddd ($J_{2\beta,1\alpha}$ = 12.0, $J_{2\beta,1\beta}$ = 3.5, $J_{2\beta,3\alpha}$ = 9.0 Hz)
3α	4.54	ddd ($J_{3\alpha,2\beta}$ = 9.0, $J_{3\alpha,4}$ = 3.0, $J_{3\alpha,6\beta}$ = 2.5 Hz)*
4	5.28	dd ($J_{4,3\alpha}$ = 3.0, $J_{4,6\beta}$ = 2.0 Hz)*
5		
6α	2.50	dd ($J_{6\alpha,6\beta}$ = 17.5, $J_{6\alpha,7\alpha}$ = 5.5 Hz)
6β	2.80	ddd ($J_{6\beta,6\alpha}$ = 17.5, $J_{6\beta,3\alpha}$ = 2.5, $J_{6\beta,4}$ = 2.0 Hz)
7α	3.28	d ($J_{7\alpha,6\alpha}$ = 5.5 Hz)
8		
9α	1.55–1.95	m
10		
11α	1.55–1.95	m
11β	1.55–1.95	m
12α	1.55–1.95	m
12β	1.55–1.95	m
13		
14		
15α	2.32	ddd ($J_{15\alpha,15\beta}$ = 14.0, $J_{15\alpha,16\beta}$ = 8.0, $J_{15\alpha,14OH}$ = 2.5 Hz)
15β	2.21	dd ($J_{15\beta,15\alpha}$ = 14.0, $J_{15\beta,16\beta}$ = 8.0 Hz)
16β	5.28	ddd ($J_{16\beta,15\alpha}$ = 8.0, $J_{16\beta,15\beta}$ = 8.0, $J_{16\beta,17\alpha}$ = 4.0 Hz)*
17α	2.69	$br\,d$ ($J_{17\alpha,16\beta}$ = 4.0 Hz)
18	0.89	s
19	1.21	s
20		
21α	4.84	dd ($J_{21\alpha,21\beta}$ = 18.0, $J_{21\alpha,22}$ = 1.7 Hz)†
21β	4.91	dd ($J_{21\beta,21\alpha}$ = 18.0, $J_{21\beta,22}$ = 1.7 Hz)†
22	5.94	$br\,s$
23		
1′β	4.71	s
2′		
3′α	3.28	dd ($J_{3'\alpha,4'\alpha}$ = 3.0, $J_{3'\alpha,4'\beta}$ = 5.5 Hz)*
4′α	1.65	ddd ($J_{4'\alpha,3'\alpha}$ = 3.0, $J_{4'\alpha,4'\beta}$ = 14.0, $J_{4'\alpha,5'\beta}$ = 11.0 Hz)*
4′β	1.86	ddd ($J_{4'\beta,3'\alpha}$ = 5.5, $J_{4'\beta,4'\alpha}$ = 14.0, $J_{4'\beta,5'\beta}$ = 2.0 Hz)*
5′β	3.92	ddd ($J_{5'\beta,4'\alpha}$ = 11.0, $J_{5'\beta,4'\beta}$ = 2.0, $J_{5'\beta,6'}$ = 6.0 Hz)*
6′	1.24	d ($J_{6',5'\beta}$ = 6.0 Hz)
3′-OMe	3.41	s
16-OAc	2.04	s
14-OH	2.43	d ($J_{14OH,15}$ = 2.5 Hz)
2′-OH	3.70	s

*J values were obtained by ^{1}H decoupling or decoupling difference spectrum.
†Chemical shifts may be reversed.

($C_{32}H_{42}O_{11}$), **1** was assumed to have a structure similar to that of affinosides [3, 4], which are cardiac glycosides with sugar moieties doubly linked to the hydroxyl groups of the aglycones. Table 2 shows ^{13}C chemical shifts of affinoside A and those of **1**, which were established by an ^{1}H–^{13}C COSY experiment. By comparison with affinoside A data, **1** was deduced to have the same skeleton as that of affinoside A, but without a hydroxyl group at C-11 or carbonyl group at C-12, and with an acetyl group at C-16. The ^{13}C chemical shifts of **1** were very similar to those of affinoside A except for the C-11, C-12, C-16 and α positions of these carbons.

The H-3 signal of buchaninoside appeared as a doublet of double doublets at 4.54 ppm (J = 9.0, 3.0, 2.5 Hz). As the signal of H-6b, which appeared as a doublet of double doublets (J = 17.5, 2.5, 2.0 Hz), was transformed into a doublet of doublets (J = 17.5, 2.0 Hz) by irradiation of the H-3 signal, 2.5 Hz coupling was attributed to the long range coupling between H-3 and H-6. Such a coupling pattern was also observed in affinoside A [3].

Assignments of the two methyl groups (18 and 19), two methylenes (11 and 12), and two quarternary carbons (10 and 13) were undertaken by means of heteronuclear multiple bond connectivity (HMBC). The methyl protons at 0.89 ppm were assigned to H-18 due to a cross peak between C-17 and the protons. Assignments of C-13 and C-12 were also carried out using cross peaks between H-18 and carbon signal at 51.0 ppm (C-13), and H-18 and

Table 2. Comparison of the ^{13}C chemical shifts of buchaninoside (1) and affinoside A

	Buchaninoside		Affinoside A*	
1	41.8	CH_2	44.7	CH_2
2	68.3	CH	67.3	CH
3	69.9	CH	70.5	CH
4	121.0	CH	123.2	CH
5	139.8	C	140.0	C
6	30.0	CH_2	30.3	CH_2
7	52.5	CH	53.9	CH
8	62.5	C	64.6	C
9	45.4	CH	49.1	CH
10	40.2	C	41.3	C
11	20.3	CH_2	74.6	CH
12	40.3	CH_2	212.7	C
13	51.0	C	63.6	C
14	80.1	C	82.0	C
15	40.8	CH_2	36.5	CH_2
16	78.7	CH	28.6	CH_2
17	57.6	CH	42.4	CH
18	16.6	CH_3	18.5	CH_3
19	21.3	CH_3	21.3	CH_3
20	170.4	C†	173.1	C
21	73.5	CH_2	73.9	CH_2
22	118.5	CH	118.7	CH
23	173.8	C	174.1	C
1′	95.9	CH	96.2	CH
2′	90.3	C	92.0	C
3′	80.3	CH	81.4	CH
4′	32.8	CH_2	35.1	CH_2
5′	66.1	CH	66.4	CH
6′	20.8	CH_3	21.3	CH_3
3′-OMe	57.4	CH_3	58.3	CH_3
16-OAc	170.0	C†	—	
	20.9	CH_3	—	

*Abe and Yamauchi [3].
†Assignments may be reversed.

carbon signal at 40.3 ppm (C-12), respectively. A cross peak between C-3′ and the methoxyl signal at 3.41 ppm showed that the methoxyl group attaches at the 3′-position.

The stereochemistry of buchaninoside was assigned by means of J values, NOESY experiments and comparison with ^{13}C data for affinosides. The J values ($J5'$, $4'\alpha = 11.0$; $J4'\alpha$, $3' = 3.0$ Hz), and NOE cross peaks between 1′ and 5′, and 1′ and 2′-OH, suggested that the sugar moiety of **1** was identical to that of affinoside A. The orientation of OMe-3′ is β (axial).

The 2,3-*trans* configuration was confirmed by the J value between H-2 and H-3 ($J = 9.0$ Hz). As ^{13}C chemical shifts of C-2 to C-7 of **1** were in good agreement with those of affinoside A or other affinosides [3], **1**, was considered to have the same relative configuration as that of affinoside A at C-2 to C-7. The stereochemistry of the 7,8-epoxide was tentatively assigned as 7β, 8β, on the basis of the ^1H NMR comparison with the known 7β, 8β-epoxycardenolides [3, 5, 6].

Abe *et al.* reported that the signal due to H-17 in 16α-acetoxy cardenolide (e.g. 16α-acetoxycalotropagenin) was observed at 2.93 ppm (*d*, 4 Hz), whereas 16β-acetoxycardenolide (e.g. oleandrigenin) was at 3.37 ppm (*d*, 9 Hz) [7]. As H-17 in **1** is 2.69 ppm (*d*, 4 Hz), 16-acetoxy is deduced to be α-oriented.

The relative configuration of the aglycone of **1** was, therefore, assigned as 2α, 3β, 14-trihydroxy-16α-acetoxy-14β-carda-4, 20 (22)-dienolide-7β, 8β-epoxide.

EXPERIMENTAL

Plant material and purification. The unripe green fruits were hand picked in April 1986 at Chiromo Campus of the University of Nairobi. The plant was identified by comparison with the specimen kept at the Kenya National Museum.

The unripe fruits (5 kg) of *E. buchananii* were blended and soaked in $CHCl_3$ (5 l) for 2 weeks. It was filtered and the filtrate was evapd *in vacuo* to obtain 8.65 g of extract.

The extract (8 g) was loaded on to a silica gel column (200 g) and eluted with 3 l 30% EtOAc in petrol, 50% EtOAc in petrol (500 ml), followed by EtOAc (600 ml) and MeOH (300 ml). Compound **1** (15 mg) was obtained as crystals from the EtOAc fr., and recrystallized from mixture of EtOAc and hexane. The crystals were found to give a single peak on HPLC (30% H_2O in acetonitrile) and one spot on TLC (silica gel, 30% EtOAc in petrol).

Assay. Maize discs, 1 cm in diameter, were prepd from leaves of *Zea mais* by a cork borer. Test discs of each dose were treated with 100 μl Me_2CO soln containing 100, 50 and 10 $\mu g\ \mu l^{-1}$ **1**, while control discs were treated with 100 μl Me_2CO, using an Eppendorf pipette. The solvent was allowed to evaporate for 1 hr at room temp. (23°). Three treated discs of each dose were placed equidistantly along the perimeter of a glass petri dish (9 cm in diameter). Each of the control discs was then placed between the test discs. Four sixth instarlarvae of *S. exempta*, which had been starved for 2 hr, were then introduced into the dish. The dish was covered by a perforated aluminium lid and the larvae were allowed to feed for 2 hr. The remaining area of the discs was measured by a squire paper, and by difference, the area of the disc consumed were calcd. The percent antifeeding was obtained from the formula below:

$$\text{Antifeeding (\%)} = (A_c - A_t)/A_c \times 100$$

where A_c = area of control discs consumed, and A_t = area of test discs consumed.

It was repeated (×4) at each dose, and the average percent antifeeding was calcd.

Buchaninoside (**1**). Crystals, mp 259°, $[\alpha]_D - 44.6°$ ($CHCl_3$; *c* 0.56). IR ν_{max} cm^{-1}: 3499, 3430, 2930, 1782, 1753, 1238, 1092, 1052, 1030. ^1H and ^{13}C NMR: see Tables 1 and 2.

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