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NORCUCURBITACIN GENTIOBIOSIDES FROM *FEVILLEA TRILOBATA*

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ABSTRACT.—The new norcucurbitacin glycosides, andirobicin A gentiobioside [**2**], and andirobicin C gentiobioside [**1**], and the known fevicordin F gentiobioside [**3**], were isolated from the aqueous MeOH fraction of a liquid-liquid partition of the MeOH extract of the seeds of *Fevillea trilobata*. Their structures were determined by nmr and ms techniques.

Fevillea trilobata L. (Cucurbitaceae), a climbing plant that grows throughout Brazil and other countries of northern South America, has been used in folk medicine against various diseases (1). A preliminary bioassay of its seed oil has shown antimicrobial activity (2) and this oil was found to contain unsaturated conjugated acids (3). Recently, we described the isolation of andirobicin A and B glucosides, fevicordin A glucoside and cayaponosides B and D from the $CHCl_3$ fraction of a liquid-liquid partition of the MeOH extract of the seeds of *F. trilobata* (4). In the present study we report the isolation of andirobicin C and A gentiobiosides [**1** and **2**] and fevicordin F gentiobioside [**3**] as the major constituents of the aqueous MeOH fraction from the liquid-liquid partition of the MeOH extract.

The hot MeOH extract of the seeds of *F. trilobata* was partitioned between hexane and 80% aqueous MeOH, with H_2O added to the latter fraction to dilute it to 60% aqueous MeOH, and this fraction then being extracted with $CHCl_3$. The aqueous MeOH fraction was treated with MeOH and Me_2CO to yield a precipitate. The supernatant, after evaporation of the solvent, was further partitioned between *n*-BuOH and H_2O. The *n*-BuOH fraction was then chromatographed on a Si gel column and further purified by reversed-phase hplc to give three compounds as the major components.

Compounds **1**, **2**, and **3** had molecular formulas of $C_{41}H_{60}O_{16}$, $C_{42}H_{64}O_{17}$, and $C_{41}H_{62}O_{17}$, respectively, as determined by their hrfabms. The ^{1}H- and ^{13}C-nmr spectra of **1**–**3** all showed signals characteristic of a norcucurbitacin bearing a diglucoside moiety (4–7) (**1** δ_c 104.60, 105.41; **2** δ_c 104.61, 105.45; **3** δ_c 104.59, 105.39). The close resemblance of the ^{13}C-nmr chemical shifts of the carbons belonging to the norcucurbitacin skeleton with those of known norcucurbitacin glycosides (4–7) suggested that the attachment of the disaccharide in each case is at C-2. The ^{1}H- and ^{13}C-nmr spectra, analyzed with the aid of COSY, DEPT, and HETCOR techniques, while confirming the presence of the same disaccharide unit in all three compounds, helped to identify it as 6-*O*-β-D-glucopyranosyl-β-D-glucopyranose (gentiobiose). The ^{1}H- and ^{13}C-nmr data (Table 1) further suggested that **1**, **2**, and **3** differed only in their side-chains attached to C-17. Comparison of ^{1}H- and ^{13}C-nmr spectral data of **1**–**3** with those

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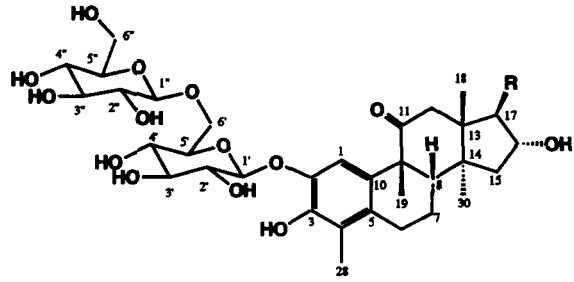

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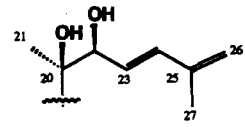

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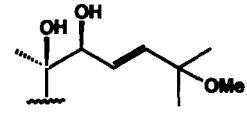

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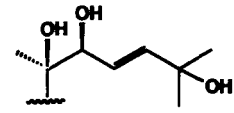

reported for known norcucurbitacin gentiobiosides suggested **3** to be identical with fevicordin F gentiobioside previously isolated from *Fevillea cordifolia* (7) and that **1** and **2** were hitherto unknown.

The ^{1}H-nmr spectrum of the side-chain of compound **1** showed the presence of four olefinic protons [δ 4.94 br s, 2H; 5.78 dd (15.6, 5.9)[2]; 6.36 d (15.6)], one vinyl methyl singlet at δ 1.82, one quaternary methyl group (δ 1.19 s) and one methine at δ 4.08 d (5.9) (δ_C 81.70). These data are compatible with the presence of an allylic hydroxy group at C-22 and a diene at C-23, -25. This was confirmed by further analysis utilizing COSY and HETCOR nmr spectra and by comparison with the ^{1}H- and ^{13}C-nmr data of cayaponoside B (4,6). Additional comparison of the ^{13}C-nmr chemical shifts of carbons 21 and 22 of compound **1** with those of the 20,22-*erythro*-dihydroxy series of cucurbitacins (8) indicated that compound **1** had a 20,22-dihydroxy system with the *erythro*-configuration at these carbons. Compound **1** was thus identified as the new norcucurbitacin gentiobioside 29-*nor*-1,2,3,4,5,10-dehydro-[2-(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyloxy)]-3,16α,20*R*,22*S*-tetrahydroxy-11-oxocucurbita-23*E*,25-diene.

The ^{1}H-nmr spectrum of the side-chain of compound **2** showed a methyl signal at δ 3.13 s (δ_C 50.81), two olefinic protons [δ 5.63 d (16.0); 5.71 dd (16.0, 4.7)] and a methine at δ 3.98 d (4.7) (δ_C 81.12). These spectral data indicated that compound **2** also had an allylic hydroxy group at C-22 and a methoxy group at C-25 in place of the C-25 (C-26) double bond in compound **1**. This conclusion was confirmed by complete analysis of the ^{1}H- and ^{13}C-nmr spectra by COSY and HETCOR techniques. Comparison with the ^{1}H- and ^{13}C-nmr data of andirobicin A glucoside (4) and of other compounds of the 20,22-*erythro*-dihydroxy series of cucurbitacins (8), showed that compound **2** is the new

[2]Coupling constants (Hz) are cited in parentheses.

TABLE 1. ^{13}C- (100.57 MHz) and ^{1}H- (400 MHz) Nmr Data of Compounds **1–3**.[a]

Position	δ_C			δ_H	
	1	**2**	**3**	**1**	**2**
1	113.29	113.34	113.29	6.61 s	6.57 s
2	144.84[b]	144.99[b]	144.79		
3	144.85[b]	144.83[b]	144.95		
4	124.98	125.01	124.99		
5	130.04[c]	130.05[c]	131.50		
6	24.82	24.81	24.82	β 2.56–2.70 m	β 2.56–2.67 m
				α 2.72–2.90 m	α 2.75–2.88 m
7	20.22	20.18	20.19	β 1.92–2.03 m	β 1.88–2.02 m
				α 2.22–2.34 m	α 2.18–2.32 m
8	43.98	43.97	43.98	2.14 br d (7.4)	2.11 br d (6.8)
9	52.02[d]	52.07[d]	52.05		
10	129.62[c]	130.02[c]	130.05		
11	217.52	217.41	217.58		
12	52.15	52.09	52.02	β 2.60 d (14.2)	β 2.56 d (13.9)
				α 2.77 d (14.2)	α 2.72 d (13.9)
13	ca.49[f]	52.00[d]	52.00		
14	51.94[d]	52.08[d]	52.08		
15	45.62	45.50	45.40	β 1.59 d (13.8)	β 1.55 d (13.6)
				α 1.92–2.03 m	α 1.88–2.02 m
16	72.20	72.23	72.23	4.61 br t (7.8)	4.58 br t (7.5)
17	56.97	56.80	56.56	2.35 d (6.8)	2.29 d (6.6)
18	19.86[e]	19.89[e]	19.89	0.97 s[b]	0.92 s[b]
19	29.14	29.15	29.13	1.30 s	1.26 s
20	77.18	77.03	77.05		
21	23.44	23.78	24.01	1.19 s	1.17 s
22	81.70	81.12	81.72	4.08 d (5.9)	3.98 d (4.6)
23	129.62	130.08	126.20	5.78 dd (15.6,5.9)	5.71 dd (16.0,4.8)
24	135.68	138.04	141.47	6.36 d (15.6)	5.63 d (16.0)
25	143.00	76.43	71.23		
26	116.72	26.16[g]	29.94	4.94 br s	1.19 s[c]
27	18.82	26.47[g]	30.06	1.82 br s	1.20 s[c]
28	11.54	11.54	11.50	2.08 s	2.05 s
30	20.22[e]	20.16[e]	20.16	0.99 s[b]	0.95 s[b]
1′.....	105.41	105.45	105.39	4.53 d (7.2)	4.49 d (7.3)
2′.....	74.74	74.72	74.72	3.42–3.54 m	3.37–3.52 m
3′.....	77.36	77.39	77.39	3.42–3.54 m	3.37–3.52 m
4′.....	71.01	71.01	71.01	3.42–3.54 m	3.37–3.52 m
5′.....	76.98	77.00	77.02	3.42–3.54 m	3.37–3.52 m
6′.....	69.44	69.43	69.39	3.82 dd (10.5,4.6)	3.79 dd (11.0,4.7)
				4.27 d (10.5)	4.24 d (11.0)
1″.....	104.60	104.61	104.59	4.35 d (7.8)	4.32 d (7.6)
2″.....	75.10	75.10	75.10	3.24–3.40 m	3.24–3.37 m
3″.....	77.86	77.87	77.86	3.24–3.40 m	3.24–3.37 m
4″.....	71.49	71.50	71.49	3.24–3.40 m	3.24–3.37 m
5″.....	77.98	77.99	77.98	3.24–3.40 m	3.24–3.37 m
6″.....	62.73	62.75	62.72	3.67 dd (12.4,4.7)	3.62 dd (12.8,5.0)
				3.86 d (12.4)	3.83 br d (12.8)
OCH_3		50.81			3.13 s

[a]Coupling constants (Hz) in parentheses; spectra recorded in CD_3OD.
[b–f]Signals interchangeable in the same column.
[g]Signal obscured by the solvent signal.

norcucurbitacin gentiobioside 29-*nor*-1,2,3,4,5,10-dehydro-25-methoxy-[2-(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyloxy)]-3,16α,20*R*,22*S*-tetrahydroxy-11-oxocucurbita-23*E*-ene.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra (δ ppm, *J* in Hz) were obtained on a Varian Unity 400 spectrometer (400 MHz) using CD_3OD as solvent (residual CHD_2OD as internal standard for ^1H nmr and $^{13}CD_3OD$ as internal standard for ^{13}C nmr). Cc employed Si gel 60 (230–400 mesh). Tlc analyses were performed by using precoated Si gel 60 F_{254} plates and detection was accomplished by uv_{254} irradiation and by spraying with 25% anisaldehyde in alcoholic H_2SO_4/HOAc followed by heating. Hplc separations were carried out on a Waters Nova-Pak C_{18} cartridge column with a Waters 990 Series photodiode array detector at 230 nm.

PLANT MATERIAL.—Seeds of *F. trilobata* were obtained as described previously (4).

EXTRACTION AND ISOLATION.—The extraction was performed as previously described (4). Part of the 60% aqueous MeOH fraction obtained (2.9 g) was treated with MeOH and Me_2CO, yielding a precipitate (214.4 mg). The oily supernatant was further partitioned between *n*-BuOH and H_2O. The *n*-BuOH fraction (267.0 mg) was chromatographed on a Si gel column [$CHCl_3$-MeOH (3:2) to MeOH] yielding 27 fractions. Fractions 8–13 (83.0 mg) were further purified by Si gel cc using a gradient of $CHCl_3$-MeOH (3.5:1) to MeOH as eluent, yielding cayaponoside D (19.3 mg) (4,6). Fractions 14–16 (18.7 mg) and 21–27 (10.6 mg) were submitted to hplc; compounds **1** (3.6 mg) and **2** (4.9 mg) were obtained from fractions 14–16 [MeOH-H_2O (1:1)], and compound **3** (2.6 mg) was obtained from fractions 21–27 [MeOH-H_2O (1:1)].

Andirobicin C gentiobioside [**1**].—Colorless amorphous powder. For ^1H- and ^{13}C-nmr spectral data, see Table 1; hrfabms *m/z* $[M+Na]^+$ 831.3779 ($C_{41}H_{60}O_{16}Na$ requires 831.3762).

Andirobicin A gentiobioside [**2**].—Colorless amorphous powder. For ^1H- and ^{13}C-nmr spectral data, see Table 1; hrfabms *m/z* $[M+Na]^+$ 863.4041 ($C_{42}H_{64}O_{17}Na$ requires 863.4023).

Fevicordin F gentiobioside [**3**].—Colorless amorphous powder. ^1H- and ^{13}C-nmr spectral data identical with those reported previously (7); hrfabms *m/z* $[M+Na]^+$ 849.3884 ($C_{41}H_{62}O_{17}Na$ requires 849.3867).

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