Journal of Natural Products
Vol. 58, No. 10, pp. 1600-1604, October 1995

OLEANANE TRITERPENE SAPONINS FROM THE CHINESE MEDICINAL HERB *CLINOPODIUM CHINENSIS*

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ABSTRACT.—Four oleanane triterpene saponins, 3β,16β,23-trihydroxy-12-keto-13β,28-epoxyolean-9(11)-en-3-yl-[β-D-glucopyranosyl(1→2)]-[β-D-glucopyranosyl(1→3)]-β-D-fucopyranoside [**1**] (clinopodiside D), 16β-propionyl-3β,23-dihydroxyoleana-11,21-dien-3-yl-[β-D-glucopyranosyl(1→2)]-[β-D-glucopyranosyl(1→3)]-β-D-fucopyranoside [**2**] (clinopodiside E), 3β,16β,21α,23,28-pentahydroxy-11-methoxyolean-12-en-3-yl-[β-D-glucopyranosyl(1→2)]-[β-D-glucopyranosyl(1→3)]-β-D-fucopyranoside [**3**] (clinopodiside F), and 3β,16β,21β,23,28-pentahydroxyoleana-11,13(18)-dien-3-yl-[β-D-glucopyranosyl(1→2)]-[β-D-glucopyranosyl(1→3)]-β-D-fucopyranoside [**4**] (clinopodiside G), were isolated and purified from the plant *Clinopodium chinensis*. Their structures were elucidated on the basis of interpretation of nmr and ms data and from chemical evidence. The results are of potential interest for medicinal reasons.

The whole plants of *Clinopodium chinensis* Benth. (Labiatae) are known to contain flavonoids (1), ursolic acid (2), and triterpenoid saponins (3). During our continuing phytochemical investigation of this plant, four new oleanane triterpene saponins, named clinopodisides D–G [**1**–**4**], were isolated from an *n*-

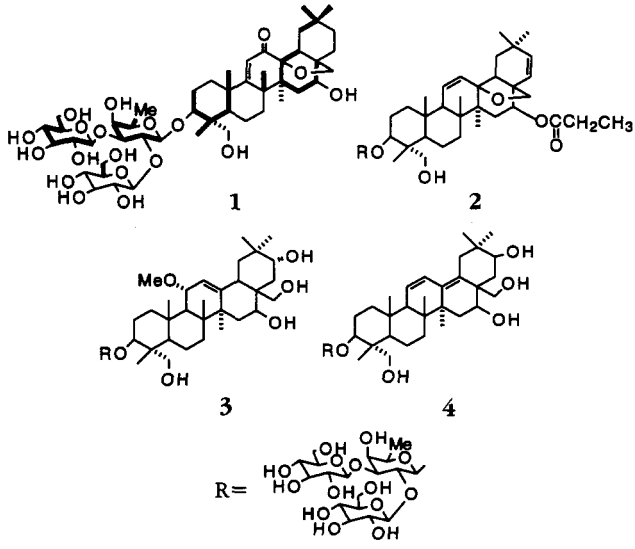

FIGURE 1. Structures of saponins **1**–**4**, with the bold lines showing observed C-C bonds by a computerized analysis of the 2D INADEQUATE nmr spectrum.

BuOH portion of the EtOH extract of *C. chinensis*. Brief details of the isolation and nmr spectra of the first two new compounds [**1** and **2**] were described in an earlier publication (4). In this paper we describe the isolation and structure elucidation of two other additional oleanane triterpene saponins, compounds **3** and **4**, and we present further nmr data to confirm the structure of compound **1**.

The structures of clinopodisides D [**1**] and E [**2**] were deduced mainly from ^{1}H- and ^{13}C-nmr spectra together with their fabms. Based on a comparison of spectral data with those in literature (5–7), their nmr assignments are given in Tables 1 and 2. In order to confirm the structure of **1**, a 2D INADEQUATE nmr experiment was carried out. With 110 mg of **1** our 2D INADEQUATE experiment took 156 h to complete and the computerized data analysis software 'XCCBond' (8,9) was used to detect the ^{13}C-^{13}C coupling signals from the low signal-to-noise spectrum recorded. Twenty-seven C-C bonds were unam-

TABLE 1. ^{13}C-Nmr Data for Compounds **1–4** in Pyridine-*d*$_5$ at 125 MHz.

Position	Compound				
	1 (mult.)[a]	**2** (mult.)[a]	**3** (mult.)[a]	**4** (mult.)[a]	C-C[b] for **1** ($J_{C\text{-}C}$, Hz)
1	37.2 (t)	38.9 (t)	40.3 (t)	38.5 (t)	
2	26.7 (t)	26.3 (t)	26.3 (t)	26.2 (t)	3 (37.5)
3	81.8 (d)	82.7 (d)	83.0 (d)	82.7 (d)	2 (37.5), 4 (37.7)
4	44.4 (s)	44.1 (s)	43.9 (s)	43.9 (s)	3 (37.7), 5 (34.5), 24 (36.2)
5	43.3 (d)	48.1 (d)	48.4 (d)	47.8 (d)	4 (34.5), 10 (33.3)
6	17.4 (t)	17.9 (t)	18.0 (t)	18.4 (t)	
7	34.6 (t)	31.9 (t)	33.4 (t)	32.5 (t)	
8	47.0 (s)	42.4 (s)	43.8 (s)	40.6 (s)	26 (33.5)
9	149.8[c] (s)	54.2 (d)	53.4 (d)	54.6 (d)	
10	40.2 (s)	36.5 (s)	38.3 (s)	38.5 (s)	5 (33.30), 25 (33.9)
11	122.5 (d)	132.5 (d)	76.2 (d)	127.1 (d)	
12	183.4 (s)	131.3 (d)	122.6 (d)	125.9 (d)	
13	86.1 (s)	85.1 (s)	147.8 (s)	136.8 (s)	14 (36.6), 18 (33.5)
14	46.7 (s)	45.4 (s)	44.2 (s)	44.3 (s)	13 (36.6), 15 (30.1), 27 (36.1)
15	37.0 (s)	36.5 (t)	35.0 (t)	35.1 (t)	14 (30.1), 16 (36.2)
16	63.0 (d)	64.8 (d)	67.6 (d)	76.5 (d)	15 (36.2), 17 (36.8)
17	46.5 (s)	46.8 (s)	43.7 (s)	45.0 (s)	16 (36.8)
18	47.3 (d)	53.4 (d)	52.4 (d)	133.3 (s)	13 (33.5)
19	37.6 (t)	35.3 (t)	37.2 (t)	38.8 (t)	20 (31.0)
20	31.9 (s)	46.1 (s)	31.8 (s)	36.6 (s)	19 (31.0), 29 (36.1), 30 (35.8)
21	34.6 (t)	129.4 (d)	72.8 (d)	73.3 (d)	
22	26.0 (t)	117.4 (d)	26.7 (t)	33.6 (t)	
23	64.5 (t)	64.9 (t)	64.2 (t)	64.7 (t)	
24	13.4 (q)	12.4 (q)	13.5 (q)	12.9 (q)	4 (36.2)
25	25.0 (q)	18.4 (q)	18.1 (q)	18.8 (q)	10 (33.9)
26	30.7 (q)	19.7 (q)	18.6 (q)	17.1 (q)	8 (33.5)
27	25.5 (q)	20.5 (q)	26.4 (q)	22.1 (q)	14 (36.1)
28	74.3 (t)	73.4 (t)	68.2 (t)	64.2 (t)	
29	33.7 (q)	28.8 (q)	30.7 (q)	20.9 (q)	20 (36.1)
30	24.4 (q)	26.0 (q)	18.1 (q)	30.1 (q)	20 (35.8)
Propionyl		180.0 (s)	54.3 (q OMe)		
		28.1 (t)			
		13.0 (q)			

[a]Determined from DEPT spectra.
[b]C-C bonds determined from 2D INADEQUATE data.
[c]Superimposed with solvent.

TABLE 2. ^{1}H-Nmr Data for Compounds **1–4** in Pyridine-d_5 at 500 MHz.

	Compound			
	1	**2**	**3**	**4**
Aglycone				
H-3	4.13 (m)	4.14 (m)	4.15 (m)	4.17 (m)
H-11		4.81 (9.5, 1.5)	3.79 (dd, 8, 3)	5.65 (d, 11)
H-12	6.15 (s)	5.94 (d, 10)	5.58 (d, 3)	6.60 (dd, 11, 2)
H-16	4.52 (m)	4.61 (m)	4.69 (m)	4.23 (m)
H-21		7.96 (d, 8.5)	4.16 (dd, 11, 4)	3.63 (t, 5)
H-22		7.34 (d, 8.5)	1.89/2.58	1.71/3.01
H-23	3.71/4.39	3.73/4.40	3.69/4.38	3.69/4.38
H-24	1.07 (s)	1.04 (s)	1.10 (s)	1.04 (s)
H-25	1.15 (s)	0.92 (s)	1.07 (s)	0.92 (s)
H-26	1.65 (s)	1.35 (s)	1.03 (s)	0.81 (s)
H-27	1.04 (s)	1.16 (s)	1.38 (s)	1.08 (s)
H-28	4.17/4.23	4.18/4.30	3.85/4.46	4.17/4.24
H-29	0.87 (s)	1.38 (s)	1.21 (s)	1.04 (s)
H-30	0.98 (s)	1.25 (s)	1.26 (s)	1.30 (s)
H-OMe			3.22 (s)	
Fuc				
H-1	4.88 (d, 8)	4.93 (d, 8)	4.92 (d, 8)	4.96 (d, 8)
H-2	4.66 (dd, 9, 5)	4.66 (dd, 9, 5)	4.67 (dd, 9.5)	4.67 (dd, 9, 5)
H-3	4.15 (m)	4.09 (m)	4.08 (m)	4.09 (m)
H-4	4.24 (m)	4.25 (m)	4.24 (m)	4.25 (m)
H-5	3.60 (m)	3.63 (m)	3.61 (m)	3.57 (m)
H-6	1.40 (d, 6)	1.38 (overlapped)	1.36 (d, 7)	1.41 (d, 7)
Glc at C-2 of fuc				
H-1	5.58 (d, 8)	5.57 (d, 8)	5.59 (d, 8)	5.57 (d, 8)
Glc at C-3 of fuc				
H-1	5.30 (d, 8)	5.29 (d, 7.5)	5.30 (d, 8)	5.25 (d, 8)
H-2	4.00 (t, 8)	3.99 (t, 8)	4.00 (t, 8)	3.99 (t, 8)
H-3	3.95 (br s)	3.93 (br s)	3.94 (br s)	3.93 (br s)
H-4	4.22 (m)	4.20 (m)	4.22 (m)	4.22 (m)
H-5	4.26 (m)	4.19 (m)	4.26 (m)	4.24 (m)
H-6	4.32/4.44	4.33/4.44	4.32/4.45	4.34/4.45

biguously detected as presented by bold lines in Figure 1 and are listed in Table 1 together with coupling constants. These results established part of the molecular skeleton of **1** and enabled the unambiguous assignment of most of the ^{13}C-nmr signals.

Saponin **3** has a formula of $C_{49}H_{82}O_{20}$ based on the fabms (*m/z* 1013 $[M+Na]^+$ and 997 $[M+Li]^+$) data and the ^{13}C-nmr spectrum, which showed 49 carbon signals including three anomeric carbons at δ 105.4, 104.3, and 104.3 (Table 1). On acid hydrolysis, **3** afforded glucose (glc) and fucose (fuc) as sugar components which were detected by gas chromatography after silylation. The positions of linkage of the sugars were determined by comparing the spectral data with those found in the literature (6) and were confirmed by HETCOR and ROESY nmr experiments. The two glucose units were found to be terminal and bound to the C-2 and C-3 positions of the fucose unit. Connectivities were established between H-1 of fucose at δ 4.92 and H-3 of the aglycone at δ 4.15, H-1 of glucose at δ 5.30 and H-3 of fucose at δ 4.08, and H-1 of glucose at δ 5.59 and H-2 of fucose at δ 4.67 in the ROESY spectrum. The ^{1}H-nmr spectrum showed six tertiary methyl groups characterized by singlets at δ 1.03, 1.07, 1.10, 1.21, 1.26, and 1.38, and one doublet at δ 5.58 attrib-

uted to the olefinic H-12. The ^{13}C-nmr and DEPT spectra exhibited signals for the C-12, C-13 double bond at δ 122.6 and 147.8, and for C-11 at δ 76.2, to which a methoxyl group (at δ 54.3) was attached. The β configuration of H-11 was determined on the basis of its nmr signal at δ 3.79 (dd, J=8 and 3 Hz) (6). The above analysis indicated that the structure of **3** appeared to be very similar to that of buddlejasaponin IVa (10). The significant 16-dalton difference in their mol wts (990 for **3** and 974 for buddlejasaponin IVa) indicated the presence of one more hydroxyl substituent in the aglycone of **3**. The ^{13}C-nmr chemical shifts of C-29 (−2.6 ppm) and C-30 (−6.0 ppm) when compared with those of buddlejasaponin IVa (6) strongly suggested that the additional hydroxyl group was located at C-21, since its location on C-22 would not have had effects of the same magnitude (11). The DQ-COSY spectrum showed connectivities between H-21 at δ 4.16 (overlapped) and H-22β at δ 1.89 (dd, J=13.5 and 11 Hz), and H-22β and H-22α at δ 2.58 (dd, J=13.5 and 4 Hz) (Table 2). Accordingly, the splitting pattern of H-21 should be a doublet of doublets with J=11 and 4 Hz, and the configuration of OH-21 was therefore α (12). Thus, the structure of **3** was concluded to be 3β,16β,21α,23,28-pentahydroxy-11-methoxyolean-12-en-3-yl-[β-D-glucopyranosyl(1→2)]-[β-D-glucopyranosyl(1→3)]-β-D-fucopyranoside, and this compound has been named clinopodiside F.

Saponin **4** gave a molecular formula of $C_{48}H_{78}O_{19}$ on the basis of fabms (*m/z* $[M+Na]^+$ 981 and $[M+Li]^+$ 965) and the ^{13}C-nmr spectrum, which showed a conjugated diene moiety at δ 127.1 (C-11), 125.9 (C-12), 136.8 (C-13), and 133.3 (C-18). Its structure was similar to that of buddlejasaponin IVb (6) but one more hydroxyl group was present in the molecule of **4** (Table 1). The presence of a hydroxyl group at C-21 was deduced from the upfield chemical shifts of C-29 (−4.0 ppm) and C-30 (−2.2 ppm). The signals of H-21 at δ 3.63 (t, J=5 Hz) and C-21 at δ 73.3 established the configuration of OH-21 to be β (13). The nmr assignments were performed by means of HETCOR and DQ-COSY methods. Consequently, **4** was identified as 3β,16β,21β,23,28-pentahydroxyoleana-11,13(18)-dien-3-yl-[β-D-glucopyranosyl(1→2)]-[β-D-glucopyranosyl(1→3)]-β-D-fucopyranoside, which has been given the trivial name clinopodiside G.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All nmr measurements were obtained on a Varian VXR 500 spectrometer equipped with a Sun 4/360 workstation. The fabms mass spectra were recorded on a Kratos triple-analyzer MS-50 instrument. Ir spectra were taken on a Perkin-Elmer 1600 instrument. Optical rotations were measured on a Perkin-Elmer 241 polarimeter using a Na lamp operating at 589 nm. Prep. and analytical hplc were carried out on a Gilson instrument with a column of Dynamax-60A (21.4 mm×25 cm) and of Dynamax-60A (4.6 mm×25 cm).

PLANT MATERIAL.—As described previously (3).

EXTRACTION AND ISOLATION.—For detailed procedures, see Liu *et al.* (3). The crude saponins were purified by hplc on a Dynamax-60A RP-18 column with MeOH-H_2O (linear gradient 2:3 to 4:1) and uv detector at 220 nm to afford saponins **1** (153 mg), **2** (38 mg), **3** (76 mg), and **4** (132 mg).

Clinopodiside D {*3β,16β,23-trihydroxy-12-keto-13β,28-epoxyolean-9(11)-en-3-yl-*[β-D-*glucopyranosyl(1→2)*]-[β-D-*glucopyranosyl(1→3)*]-β-D-*fucopyranoside*} [**1**].—$C_{48}H_{76}O_{19}$, white amorphous powder; $[\alpha]^{22}D$ +44.6° (*c*=0.26, MeOH); ^{13}C- and ^{1}H-nmr data, see Tables 1 and 2, respectively; fabms (Gly/NBA/TFA) *m/z* 979 $[M+Na]^+$, 963 $[M+Li]^+$, 957 $[M+H]^+$; aglycone eims *m/z* 486 $[M]^+$, 471, 453, 441, 277, 259, 245, 223, 201, 187.

Clinopodiside E {*16β-propionyl-3β,23-dihydroxyoleana-11,21-dien-3-yl-*[β-D-*glucopyranosyl(1→2)*]-[β-D-*glucopyranosyl(1→3)*]-β-D-*fucopyranoside*} [**2**].—$C_{51}H_{80}O_{19}$, white amorphous powder; $[\alpha]^{22}D$ +68.8° (*c*=0.08, MeOH); ^{13}C- and ^{1}H-nmr data, see Tables 1 and 2, respectively; fabms (Gly/NBA/TFA) *m/z* 996 $[M]^+$, 939 $[M-57]^+$, 834 $[M-glc]^+$, 777 $[M-57-glc]^+$, 527 [aglycone, $M+H]^+$, aglycone eims *m/z* 526 $[M]^+$, 469 $[M-57]^+$, 344, 281, 223, 203.

Clinopodiside F {*3β,16β,21α,23,28-pentahydroxy-11-methoxyolean-12-en-3-yl-*[β-D-*glucopyranosyl(1→2)*]-[β-D-*glucopyranosyl(1→3)*]-

β-D-*fucopyranoside*] [**3**].—$C_{49}H_{82}O_{20}$, amorphous powder; $[\alpha]^{22}D$ +17.5° (*c*=1.38, MeOH); ^{13}C- and ^{1}H-nmr data, see Tables 1 and 2, respectively; fabms (Gly/NBA/TFA) *m/z* 1013 $[M+Na]^+$, 997 $[M+Li]^+$, 851 $[M+Na-glc]^+$, 835 $[M+Li-glc]^+$.

Clinopodiside G [*3*β,*16*β,*21*β,*23,28-pentahydroxyoleana-11,13(18)-dien-3-yl*-[β-D-*glucopyranosyl*(*1*→*2*)]-[β-D-*glucopyranosyl*(*1*→*3*)]-β-D-*fucopyranoside*] [**4**].—$C_{48}H_{78}O_{19}$, white powder; $[\alpha]^{22}D$ +7.37° (*c*=1.14, MeOH); ^{13}C- and ^{1}H-nmr data, see Tables 1 and 2, respectively; fabms (Gly/NBA/TFA) *m/z* 981 $[M+Na]^+$, 965 $[M+Li]^+$, 820 $[M+Na+H-glc]^+$.

ACID HYDROLYSIS OF CLINOPODISIDES D–G [**1–4**].—A mixture containing each compound (5 mg), 1 ml MeOH-H_2O (1:1), and 0.5 ml 3 N HCl was refluxed in a sealed tube at 105° for 4 h. The solution was neutralized with 0.2 N NaOH, filtered, and then concentrated to dryness, to which was added 0.2 ml DMF and 0.2 ml BSTFA to undertake silylation at 75° for 15 min. The glucose and fucose were identified by gc: RTX-1 (30 m×0.32 mm i.d.); injection temperature; 230°; column temperature: 145° (1 min), 5°/min 178° (5 min), 1°/min 190°, 15°/min 275° (5.74 min); flow rate (splitless) He 30 ml/min; H_2 30 ml/min; air 300 ml/min; instrument HP-5890. The R_t values (min) are as follows: 8.49, 9.20, 10.04 (fucose), 15.85, 20.32 (glucose) for clinopodiside D [**1**]; 8.49, 9.20, 10.03 (fucose), 15.84, 20.30 (glucose) for clinopodiside E [**2**]; 8.50, 9.22, 10.05 (fucose), 15.88, 20.34 (glucose) for clinopodiside F [**3**]; 8.54, 9.26, 10.10 (fucose), 15.95, 20.43 (glucose) for clinopodiside G [**4**]; standards: 8.49, 9.27, 10.05 (fucose), 15.96, 20.35 (glucose).

ACKNOWLEDGMENTS

Mass spectral data were obtained at the Midwest Center of Mass Spectrometry, University of Nebraska at Lincoln. The authors are grateful for financial and other support from Brigham Young University. D.M.G. is supported by the NIH on grant GM 08521-34.

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Received 14 March 1995