Journal of Natural Products
Vol. 58, No. 2, pp. 184-188, February 1995

TRITERPENOID SAPONINS FROM *CLINOPODIUM CHINENSIS*

ZIMIN LIU,[1] ZHONGJIAN JIA,

State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, People's Republic of China

REX G. CATES,

Department of Botany and Range Science, Brigham Young University, Provo, Utah 84602

DU LI, and NOEL L. OWEN*

Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602

ABSTRACT.—Two new triterpenoid saponins, 3β,16β,23-trihydroxy-13,28-epoxyolean-11-en-3-yl-[β-D-glucopyranosyl(1→4)-β-D-glucopyranosyl(1→3)]-[β-D-glucopyranosyl(1→2)]-β-D-fucopyranoside [**1**] (clinopodiside B) and 28-*O*-β-D-glucopyranosyl-3β,23-dihydroxyoleana-12,21-dien-3-yl-β-D-glucopyranosyl-(1→3)-β-D-fucopyranoside [**3**] (clinopodiside C), together with three known saikosaponins, buddlejasaponins IV, IVa, and IVb, have been isolated from the plant, *Clinopodium chinensis*, and characterized using chemical evidence and spectroscopic methods, in particular 2D nmr spectroscopy.

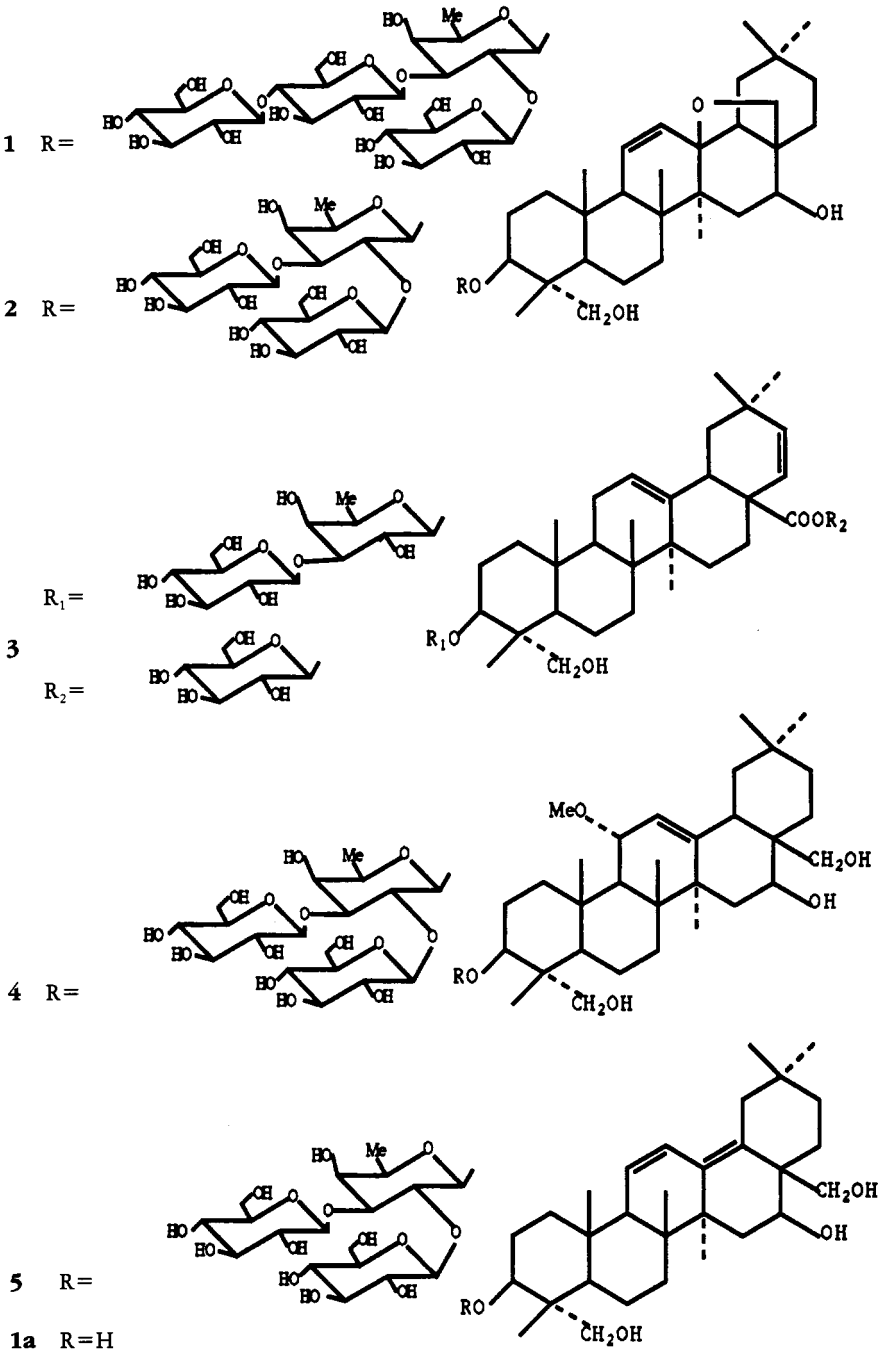
Clinopodium chinensis (Benth.) Kuntze (Labiatae) is a popular Chinese traditional medicinal herb, used as a salve for bruises and swelling, and is also purported to improve blood circulation (1). Chemical investigations of this plant were carried out by Dai *et al.* (2,3) in the early 1980s, and they reported the existence of flavonoids such as isosakuranelin, apigenin, didymin, hesperidin, and ursolic acid. In this paper, we describe some of the saponin components of *C. chinensis*, in particular the isolation and structure elucidation of two new triterpenoid saponins, named clinopodisides B [**1**] and C [**3**]. The related clinopodiside A has been reported by Xue *et al.* (4). Our analysis also confirmed the presence of didymin and ursolic acid.

RESULTS AND DISCUSSION

An *n*-BuOH extract of whole plants was passed through a Si gel (200–300 mesh) column to afford the crude saponins, which were further separated by hplc to obtain clinopodisides B [**1**] and C [**3**], and three known saponins, buddlejasaponins IV [**2**], IVa [**4**], and IVb [**5**], which were identified on the basis of nmr spectral data, ms and comparison with the literature (5,6).

The fabms of **1** showed pseudo-molecular ion peaks at *m/z* 1129 $[M+Na]^+$ and 1113 $[M+Li]^+$, which, together with the nmr results, enabled the molecular formula to be determined as $C_{54}H_{90}O_{23}$. Ir absorption bands at 3420 and 1653 cm^{-1} revealed the presence of hydroxyl groups and a double bond. After acid hydrolysis, the modified aglycone, saikogenin A [**1a**], was obtained as a crystalline solid, which had a molecular ion peak at *m/z* 472 $[M]^+$ (using eims) and a conjugated diene structure (from uv absorptions at 242, 250, and 260 nm) (7,8). On hydrolysis the sugar units of **1** were identified by paper chromatography. In the ^{1}H-nmr spectrum, the proton signals were assigned by means of COSY, and showed six singlet methyl proton signals at δ 0.73, 0.93, 0.95, 0.99, 1.04, and 1.27, two olefinic proton signals at δ 5.97 (1H, d, J=11 Hz, H-12) and 5.41 (1H, dd, J=11 and 3 Hz, H-11), and four sugar anomeric proton signals at δ 4.52 (d, J=7.3 Hz), 4.64 (d, J=7.4 Hz) and 4.89 (2H, d, J=7.7 Hz). Based on the

[1]Also of the Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602.



above results, it was concluded that saponin **1** has a similar structure to that of buddlejasaponin IV [**2**] (5,6,9), except that the former has one more glucose unit. When the ^{13}C-nmr spectrum (Table 1) was compared with literature values for **2** (9), glycosylation shifts were observed at C-3 (−1.6 ppm), C-4 (+6.1 ppm), and C-5 (−2.1 ppm) of the glucose affixed to C-3 of fucose. This showed that one more sugar (glucose) was present at C-4 of the glucose linked to the C-3 fucose position, when compared with **2**. Thus, the structure of **1** is 3β,16β,23-trihydroxy-13,28-epoxyolean-11-en-3-yl-[β-

TABLE 1. ^{13}C-Nmr Data of Clinopodisides B [**1**] and C [**3**].[a]

Carbon	Compound		Carbon	Compound	
	1	**3**		**1**	**3**
1	39.1	33.3	fuc at C-3 of aglycone		
2	26.0	24.9	1	104.7	101.3
3	84.5	80.5	2	76.1	71.1
4	44.3	40.9	3	85.3	82.5
5	47.7	45.1	4	72.3	71.3
6	18.0	19.3	5	70.9	69.7
7	32.2	33.3	6	16.8	17.8
8	42.8	39.2	glc at C-2 of fuc		
9	52.8	45.1	1	105.1	
10	36.6	35.9	2	76.1	
11	130.2	28.5[b]	3	77.8	
12	134.5	124.8	4	72.1	
13	85.8	144.5	5	77.7	
14	44.4	42.8	6	62.0	
15	35.7	29.4[b]	glc at C-3 of fuc		
16	65.4	29.5[b]	1	103.1	102.3
17	46.3	44.1	2	75.0	74.1
18	53.7	47.7	3	76.1	78.7
19	38.4	47.1[c]	4	78.2	72.0
20	31.7	30.7[c]	5	75.7	78.3
21	35.0	129.0	6	63.3	62.4
22	26.2	115.1	glc at C-4 of glc at C-3 of fuc/glc-28		
23	63.9	66.7	1	105.1	95.8
24	12.6	13.8	2	75.0	75.3
25	18.8	17.5	3	77.8	78.5
26	20.2	17.8	4	71.3	73.9
27	21.2	25.0	5	77.7	77.9
28	73.2	178.6	6	62.0	62.4
29	33.9	28.6[c]			
30	24.1	25.2[c]			

[a]Internal standard, TMS, solvent-MeOH-d_4, results expressed in δ values.
[b]Signals may be interchangeable.
[c]See text for explanation of values that are underlined.

D-glucopyranosyl(1→4)-β-D-glucopyranosyl(1→3)]-[β-D-glucopyranosyl(1→2)]-β-D-fucopyranoside, which has been accorded the trivial name clinopodiside B.

Saponin **3** was obtained as an amorphous white powder and gave positive colorations in the Liebermann-Burchard and Molish tests for triterpenoid saponins. After acid hydrolysis, it afforded a different aglycone but with the same type of sugar units as saponin **1**. The ^{1}H-nmr spectrum showed the existence of six tertiary methyl groups at δ 0.45, 0.49, 0.69, 0.70, 0.77, and 1.05. In the ^{13}C-nmr and DEPT spectra of **3** (Table 1), the carbonyl signal at δ_C 178.6 and the anomeric carbon signal of glucose at δ_C 95.8 clearly showed the presence of the glucosyl ester moiety at C-28 in the molecule. The double bond signals at δ_C 124.8 (CH) and 144.5 (C) were characteristic of an olean-12-en-28-oic ester and were definitely assigned to C-12,C-13, and the additional double bonds, at δ_C 115.1 (CH) and 129.0 (CH), were restricted to either C-21,C-22 or C-15,C-16 in the aglycone, since the doublets of these two additional olefinic protons occurred at δ 6.72 (d, J=9 Hz) and 7.19 (d, J=9 Hz) in the ^{1}H-nmr spectrum of **3**. The considerable shifts of C-30, C-29, C-20, and C-19 (underlined in Table 1) indicated that

the double bond was at C-21 rather than C-15. Meanwhile, the doublet of doublets of H-12 [δ 5.94 (1H, J=9 and 3 Hz)] revealed that no substituent group was present at C-11, and the downfield chemical shift of C-3 of fucose [δ_C 82.5 (CH)] showed that one glucose was linked to it. The nmr assignments of **3** were based on the DEPT spectrum and comparison with spectral data in the literature (10,11). Thus, the structure of **3** was concluded to be 28-*O*-β-D-glucopyranosyl-3β,23-dihydroxyoleana-12,21-dien-3-yl-β-D-glucopyranosyl-(1→3)-β-D-fucopyranoside, which has been named clinopodiside C.

In recent years, several papers have described phytochemical investigations of various species of *Clinopodium* and the presence of saikosaponin-type saponins were found in high yield (4,6,12). Saikosaponin-type saponins are also characteristic of the genus *Bupleurum* (Umbelliferae), which includes important medicinal plants in China. Thus, it is likely that some of the species in the genus *Clinopodium* could be useful for medicinal purposes.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All nmr measurements were performed on Varian VXR 500 and Bruker AM-400 NMR spectrometers. Samples were dissolved in 0.5 ml MeOH-d_4 or pyridine-d_5. The fabms and eims were recorded on ZAB-HS and Kratos triple analyzer MS-50 instruments. Ir spectra were taken on a 170SX instrument; uv spectra were recorded on a Shimadzu UV-240 spectrometer. Prep. and analytical hplc were carried out on a Gilson instrument with columns of Dynamax-60A (21.4 mm×25 cm) and Dynamax-60A (4.6 mm×25 cm).

PLANT MATERIAL.—Whole plants of *Clinopodium chinensis* Benth. were collected in July 1989, in Gansu Province, People's Republic of China. A voucher specimen was deposited in the Herbarium of Institute of Organic Chemistry, Lanzhou University, China.

EXTRACTION AND ISOLATION.—Air-dried whole plants (4.75 kg) were extracted twice with 95% EtOH at room temperature. The extract was concentrated under reduced pressure to obtain a residue (179 g), to which was added hot H_2O, and the resulting mixture was filtered to remove some pigments. The aqueous solution was extracted with petroleum ether (60–90°), EtOAc, and *n*-BuOH to yield the three corresponding residues (50 g, 46 g, and 20 g, respectively). The *n*-BuOH portion was chromatographed over Si gel to obtain the crude saponins, which were purified by hplc on a Dynamax-60A RP-18 column with MeOH-H_2O (linear gradient, 4:6 to 2:8) using a uv detector at 220 nm, to afford saponins **1** (30 mg), **2** (1103 mg), **3** (20 mg), **4** (806 mg), and **5** (400 mg).

Clinopodiside B [**1**].—Amorphous white powder, $C_{54}H_{90}O_{23}$; ^{1}H nmr (MeOH-d_4, TMS) δ 5.97 (1H, d, J=11 Hz, H-12), 5.41 (1H, dd, J=11 and 3 Hz, H-11), 4.89 (2H, d, J=7.7 Hz, H-1 of 2×glc), 4.64 (1H, d, J=7.4 Hz, H-1 of glc), 4.52 (1H, d, J=7.3 Hz, H-1 of fuc), 3.93–3.08 (m, H of sugars, H-3, 16, 23, and 28), 2.30 (1H, dd, J=13 and 5 Hz, H-18), 1.27 (3H, d, J=6.4 Hz, H-6 of fuc), 1.27, 1.04, 0.99, 0.95, 0.93, 0.73 (3H each, s, 6×Me); ^{13}C-nmr data, see Table 1; fabms (S-Gly) *m/z* 1129 $[M+Na]^+$, 1113 $[M+Li]^+$, 967 $[M+Na-glc]^+$, 805 $[M+Na-2\times glc]^+$, 643 $[M+Na-3\times glc]^+$, 607 $[M+Na-3\times glc-2H_2O]^+$.

Clinopodiside C [**3**].—Amorphous white powder, $C_{48}H_{76}O_{18}$; ^{1}H nmr (MeOH-d_4, TMS) δ 7.19 (d, J=9 Hz, H-21), 6.72 (d, J=9 Hz, H-22), 5.94 (dd, J=9 and 3 Hz, H-12), 5.12 (d, J=8 Hz, H-1 of glc), 5.08 (2H, m, H-1 of fuc and glc at C-3 of fuc), 3.63–2.92 (m, H of sugars, H-3, H-23), 1.04 (d, J=5 Hz, H-6 of fuc), 1.05, 0.77, 0.70, 0.69, 0.49, 0.45 (3H each, s, 6×Me); ^{13}C-nmr data, see Table 1; fabms (Gly/NBA/TFA) *m/z* 940 [M^+, not observed], 778 $[M-glc]^+$, 615 $[M-2\times glc-H]^+$; fabms (S-Gly) *m/z* 799 $[M+Na-glc-2H]^+$, 783 $[M+Li-2H]^+$, 641 $[M+Na-2glc+2H]^+$, 625 $[M+Li-2glc+2H]^+$.

Hydrolysis of clinopodisides B [**1**] *and C* [**1**].—A mixture containing each compound (5 mg), 1 ml MeOH-H_2O (1:1), and 0.5 ml 3 N HCl was refluxed using an oil bath for 3 h. The solution was neutralized with 0.2 N NaOH and then concentrated to dryness. The glucose and fucose were identified by paper chromatography using *n*-BuOH-HOAc-H_2O (4:1:5), upper phase (glucose, R_f=0.25; fucose, R_f=0.42).

Buddlejasaponin IV [**2**].—Amorphous white powder, $C_{48}H_{78}O_{18}$; fabms (S-Gly) *m/z* 965 $[M+Na]^+$, 949 $[M+Li]^+$, 803 $[M+Na-glc]^+$, 787 $[M+Li-glc]^+$, 641 $[M+Na-2glc]^+$, 625 $[M+Li-2glc]^+$. Its ^{13}C-nmr spectral data were assigned by the INADEQUATE nmr experiment, and our results are in agreement with those reported in the literature (5,6).

Buddlejasaponin IVa [**4**].—Amorphous white powder, $C_{49}H_{82}O_{19}$; fabms (S-Gly) *m/z* 997 $[M+Na]^+$, 981 $[M+Li]^+$, 819 $[M+Li-glc]^+$. Other spectral data as reported in the literature (5,6).

Buddlejasaponin IVb [**5**].—Amorphous white powder, $C_{48}H_{78}O_{18}$, with spectral data as reported in the literature (5,6).

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation of China and the Professional Development Fund from Brigham Young University. The support of Dr. David M. Grant is gratefully acknowledged. We are grateful to the Midwest Center for Mass Spectrometry at the University of Nebraska-Lincoln for their services (NSF, Biology Division, Grant No. DIR9017262). Thanks are due to Drs. Steven G. Wood and Jiping Zou in the Department of Botany and Range Science, Brigham Young University, for helpful advice and technical assistance.

LITERATURE CITED

1. "Flora of Beijing," Beijing Press, Beijing, 1987, vol. 2, p. 847.
2. J.-R. Dai, D.-W. Shi, and H.-Q. Zhang, *Zhongcaoyao,* **14**, 193 (1983).
3. D.-Y. Kong, J.-R. Dai, and D.-W. Shi, *Zhongcaoyao,* **16**, 518 (1985).
4. S.-R. Xue, J.Q. Liu, and G. Wang, *Phytochemistry,* **31**, 1049 (1992).
5. A. Yamamoto, T. Miyase, A. Ueno, and T. Maeda, *Chem. Pharm. Bull.,* **39**, 2764 (1991).
6. A. Yamamoto, T. Miyase, A. Ueno, and T. Maeda, *Chem. Pharm. Bull.,* **41**, 1270 (1993).
7. S. Shibata, I. Kitagawa, and H. Fujimoto, *Tetrahedron Lett.,* 3783 (1965).
8. S. Shibata, I. Kitagawa, and H. Fujimoto, *Chem. Pharm. Bull.,* **14**, 1023 (1966).
9. L. Pistelli, A.R. Bilia, and A. Marsili, *J. Nat. Prod.,* **56**, 240 (1993).
10. J. Bhattacharyya and E.V.L. Cunha, *Phytochemistry,* **31**, 2546 (1992).
11. F. Crespin, E. Olliver, C. Lavaud, A. Babadjamian, R. Faure, L. Debrauwer, G. Balansard, and G. Boudon, *Phytochemistry,* **33**, 657 (1993).
12. A. Yamamoto, H. Suzuki, T. Miyase, A. Ueno, and T. Maeda, *Phytochemistry,* **34**, 485 (1993).

Received 5 July 1994