Institute of Pharmacology and Toxicology[1], Academy of Military Medical Sciences, Beijing, and National Laboratory of Applied Organic Chemistry[2], Lanzhou University, Lanzhou, P.R. China

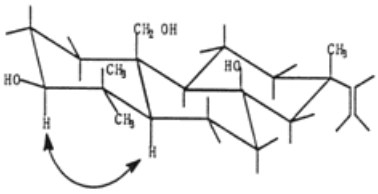
New isopimarane diterpene and new cineole type glucoside from *Nepeta prattii*

Zhen-Fu Hou[1], Yong-Qiang Tu[2] and Yu Li[2]

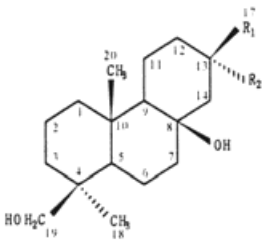
Together with sixteen known compounds, a new isopimarane diterpene (prattol) and a new cineole type glucoside were isolated from *Nepeta prattii*. Their structures were elucidated on the basis of spectral methods as isopimar-15-en-3β,8β,20-triol, and (1*R*, 2*R*, 4*S*)-1,8-epoxy-*p*-methan-2-O-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside.

1. Introduction

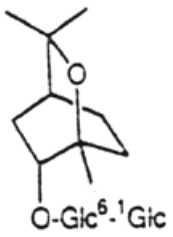
Some *Nepeta* species were used as Chinese folk medicine to resolve the exterior, dissipate cold, rectify the blood and resolve toxins. Efficacious drugs were made of these species for treatment of many diseases, e.g. headache, sore, scrofula and bleeding [1]. A great deal of literature exists on the chemical composition of the *Nepeta* genus. The types of compounds isolated belong to the classes of terpenoids and phenoloids. However, phytochemical study of the plant *Nepeta prattii* has not been reported so far. In the course of our search for biologically active substances from this plant, a new isopimarane diterpene, isopimar-15-en-3β,8β,20-triol, and a new cineole type glucoside, (1*R*,2*R*,4*S*)-1,8-epoxy-*p*-methan-2-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside along with sixteen known compounds were isolated. In this paper, we report the isolation and structural elucidation of these new compounds.



1



A R_1=CH_3, R_2= -CH=CH_2
B R_1= -CH=CH_2, R_2=CH_3



6

2. Investigations, results and discussion

From the methanol extract, we isolated two diterpenes, prattol (**1**), and ribenol (**2**) [2], four cineole type glucosides, (1*S*,2*S*,4*R*)-1,8-epoxy-*p*-methan-2-O-β-D-glucopyranoside (**3**), (1*R*,2*R*,4*S*)-1,8-epoxy-*p*-methan-2-O-β-D-glucopyranoside (**4**), (1*S*,2*S*,4*R*)-1,8-epoxy-*p*-methan-2-O-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside (**5**) [3], and (1*R*,2*R*,4*S*)-1,8-epoxy-*p*-methan-2-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (**6**), ten triterpenic acid, ursolic acid (**7**), 2α,3β-dihydroxyurs-12-en-28-oic acid (**8**), 2α,3α-dihydroxyurs-12-en-28-oic acid (**9**), 3β,13β-dihydroxyurs-11-en-28-oic acid (**10**) [4], 2α,3β,19-trihydroxyurs-12-en-28-oic acid (**11**), 2α,3α,23-trihydroxyurs-12-en-28-oic acid (**12**), 2α,3β,24-trihydroxyurs-12-en-28-oic acid (**13**), olealic acid (**14**), 2α,3β-dihydroxyolean-12-en-28-oic acid (**15**), and 2α,3α-dihydroxyolean-12-en-28-oic acid (**16**), and two steroids, β-sitosterol (**17**), and β-daucosterol (**18**). Among them, compounds **1, 4, 5, 6** are decribed for the first time as natural products, though **4, 5** had been isolated from a cell suspension culture of *Eucalyptus perriniana* following administration of 1,8-cineole [3], and compound **10** is isolated from the *Nepeta* genus for the first time. Compounds **8**, **9, 11, 12, 13, 15, 16** were all isolated in methyl ester form by previous treatment with CH_2N_2/Et_2O. The structures of the known compounds were identified by comparing their properties (m.p., MS, IR, ^{1}H NMR and ^{13}C NMR) with the reported values in the literature or by comparing with authentic samples. The structures of new compounds were deduced as follows.

Compound **1** was obtained as colorless cubic crystals from methanol, m.p. 222–224 °C. The EI-MS revealed the peak m/z 322, and the ^{13}C NMR and DEPT showed the presence of three CH_3, nine CH_2, four CH and four C. Then the molecular composition was deduced to be $C_{20}H_{34}O_3$. The ^{1}H NMR spectra indicated the presence of a $-CH{=}CH_2$ group, a $-CH_2OH$ unit and three methyl singlets. The ^{1}H-^{1}HCOSY and HMQC spectra revealed the following partial structures $-CH_2-CH_2-CHOH-$ and two $>CH-CH_2-CH_2-$. The C–C interconnectivity of all the fragments was established through HMBC experiment. The above information suggested compound **1** to be a pimarane or isopimarane diterpene, and three hydroxyl groups were located at C-3, C-8 and C-20. By further comparison of ^{13}C NMR data of **1** with those of the compound isopimar-15-en-8β,19-diol (**A**) [5] and pimar-15-en-8β,19-diol (**B**) [6], it was found that data at C-15, C-16 and C-17 of **1** were parallel to compound **A** but not compound **B**. Moreover, in the ^{1}H NMR spectra, the coupling constant values of 14-H (1.74, dd, J = 13.8, 1.7; 1.43, d, J = 13.8) indicated the presence of W-coupling of 17-CH_3 and 14α-H. The fact suggested 17-CH_3 and 14α-H could be axial. The 3β-configuration of the hydroxyl group was determined on the basis of the coupling constant values (3.53, dd, J = 9.7, 6.7) and the correlation between 3α-H and 5α-H in the NOESY spectra of **1**. Thus prattol (**1**) was elucidated to be isopimar-15-en-3β,8β,20-triol.

Compound **6** was obtained as amorphous solid. An $[M + Na]^+$ at m/z 517 in FAB-MS along with analysis of ^1H, ^{13}C NMR and DEPT spectra showed its molecular formula to be $C_{22}H_{38}O_{12}$. Two methine signals at δ 103.68, 100.36, two methene signals at δ 68.51, 61.10, and other eight methine signals at δ 70–77 in the ^{13}C NMR and DEPT spectra, and two anomeric proton signals at δ 4.72 (d, J = 7.6 Hz), 4.40 (d, J = 7.6 Hz) in the ^1H NMR spectrum implied the presence of a β-gentiobiose moiety. The remaining ten carbons signals were assigned to be a bicyclic monoterpene fragment based on the degree of unsaturation. By comparison of ^{13}C NMR spectral data of compound **6** with those of known compounds, (1*R*,2*R*,4*S*)-1,8-epoxy-*p*-menthan-2-O-β-D-glucopyranoside (**4**) and (1*S*,2*S*,4*R*)-1,8-epoxy-*p*-menthan-2-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (**5**) [3], it was found that ^{13}C NMR spectral data at C-1 to C-10, C-1′, and C-2′ of **6** were in accordance with compound **4**, and those at C-3′ to C-6′, and C-1″ to C-6″ of **6** were in agreement with compound **5**. Consequently, compound **6** was determined to be (1*R*,2*R*,4*S*)-1,8-epoxy-*p*-methan-2-O-β-D-glucopyranosyl-(1 → 6)-D-glucopyranoside.

3. Experimental

3.1. Equipment

Melting points were recorded on a Kofler apparatus and were uncorrected. IR spectra were run on a Nicolet 170 SX FT-IR instrument; EI-MS and FAB-MS were determined on a VG ZAB-HS mass spectrometer using 70 eV electron impact ionization; ^1H NMR (400.13 MHz), ^{13}C NMR (100.16 MHz) and 2D-NMR (^1H, ^1H-COSY, HMQC, HMBC, ^1H, ^1H-NOESY) spectra were recorded with a Bruker AM 400 FT-NMR spectrometer in $CDCl_3$ and DMSO-d_6 using TMS as internal standard; preparative HPLC was carried out on Gilson Model 303 Pump and Gilson UV Detector Model 116 with Wratman-partisil 10 ODS C_{18} column.

3.2. Plant material

Nepeta prattii was collected in August 1997, in Zhuanglang Gansu Province, P.R. China and identified by Professor Yong-Hong Zhang, Faculty of Pharmacy, Lanzhou Medicine College of P.R. China.

3.3. Extraction and isolation

The air-dried whole plants of *Nepeta prattii* (6.8 kg) were powdered and extracted four times (each three days) with MeOH (20 l) at room temperature. The extract was concentrated under reduced pressure. The residue (440 g) was suspended in H_2O and then extracted with pet. ether (10 l), EtOAc (10 l) and n-BuOH (8 l). The EtOAc extract (108 g) was subjected to CC over silica gel with pet. ether–Me_2CO (30 : 1–1 : 1) and MeOH gradient, when ten crude fractions were obtained (Fraction 1–10). Fraction 2 (pet. ether–Me_2CO; 20 : 1) on recrystallization with MeOH gave **17** (1.8 g). Fraction 3 (pet. ether–Me_2CO; 10 : 1) was further seperated by repeated over silica gel using C_6H_6–Et_2O (7 : 1), C_6H_6–Me_2CO (10 : 1) as eluants giving **2** and (65 mg) **10** (45 mg). Fraction 4 (pet. ether–Me_2CO; 7 : 1) on repeated chromatographic purification over a silica gel column and eluting with pet. ether–$CHCl_3$–Me_2CO (5 : 5 : 1), and by crystallization several times with heating MeOH gave pure compounds **7** (27 g) and **14** (0.4 g). Fractions 5, 6, 7 and 8 (pet. ether–Me_2CO; 5 : 1, 3 : 1, 2 : 1, 3 : 2) were seperated by CC over silic gel using C_6H_6–MeOH and $CHCl_3$–MeOH as eluants to get crude fractions. Each was methylated with CH_2N_2, then chromatographed on silia column and purified by recrystalliaztion. Compounds **1** (35 mg), **8a** (2.2 g) and **15a** (45 mg) were obtained from fraction 5; compounds **9a** (80 mg) and **16a** (20 mg) from fraction 6; compound **11a** (65 mg) from fraction 7; Compounds **12a** (30 mg) and **13a** (25 mg) from fraction 8. Fraction 9 (pet. ether–Me_2CO; 1 : 1) on recrystallization with MeOH gave compound **18** (2.9 g). The combination of fraction 10 (MeOH) and n-BuOH extract (57 g) was chromatographed on a column of Amberlite XAD-4 nonionic polystyrene resin eluting initially with H_2O followed by 95% EtOH. The latter (37 g) was chromatographed on a silica gel with $CHCl_3$–MeOH–H_2O (10 : 1 : 0.05–2 : 1 : 0.5), when six crude fractions were obtained (fraction 11–16). The mixture of **3** and **4** was isolated from fraction 12 ($CHCl_3$–MeOH–H_2O; 8 : 1 : 0.05); and the mixture of **5** and **6** was obtained from Fraction 14 ($CHCl_3$–MeOH–H_2O, 4 : 1 : 0.2). Two mixtures were further seperated by HPLC eluting with MeOH–H_2O (7 : 3 for 3, 4 and 1 : 1 for 5, 6) to give pure compounds **3** (40 mg), **4** (40 mg), **5** (35 mg) and **6** (35 mg).

3.4. Isopimar-15-en-3β,8β,20-triol (1)

Colorless cubic crystals, m.p. 222–224 °C (MeOH). IR (KBr, cm^{-1}): 3422, 2927, 1720, 1586, 1462, 1380, 1247, 1192, 1140, 1038. EI-MS (m/z, %): 322 (M^+, 0.1), 304 (M^+-H_2O, 6), 291 (82), 274 (30), 273 (19), 256 (33), 255 (41), 241 (25), 232 (74), 227 (26), 213 (39), 199 (26), 189 (100), 155 (39), 150 (39), 145 (54). ^1H, ^{13}C NMR: see Table 1.

3.5. Acetylation of 1

Compound **1** (20 mg) was added to 2 ml Ac_2O/pyridine (1 : 1). After allowing the mixture to sit at room temperature for 48 h, the mixture was evaporated to dryness in vacuo to give 3β,20-diacetoxy-isopimar-15-en-8β-ol (**1a**).

Table 1: ^1H, ^{13}C NMR spectral data of compound 1, A, and B (1, 400/100 MHz, C_5D_5N; A, B, 100/25 MHz, $CDCl_3$)

No.	^1H NMR	^{13}C NMR		
	1	**1**	**A**	**B**
1	1.64, 0.90	35.67	39.59	39.5
2	1.91	29.27	18.07	18.1
3	3.53 (dd, 9.7, 6.7)	78.35	35.74	35.6
4		39.71	38.68	38.7
5	1.05 (d, 12.3)	55.91	57.21	56.5
6	2.62 (m)	18.66	18.35	18.1
7	2.01, 1.43	43.99	43.99	42.3
8		70.27	72.49	72.5
9	0.93 (d, 11.7)	58.41	58.21	57.2
10		40.90	36.43	36.4
11	1.99	18.01	17.18	17.4
12	1.61, 1.38	39.09	38.13	36.1
13		37.20	37.20	37.1
14	1.74 (dd, 13.8, 1.7), 1.43 (d, 13.8)	50.79	51.57	53.4
15	5.88 (dd, 17.4, 10.7)	152.42	151.59	147.5
16	5.00 (d, 17.4), 4.91 (d, 10.7)	108.65	108.57	111.9
17	1.49 (s)	24.73	24.28	32.3
18	1.30 (s)	29.08	27.08	27.0
19	1.39 (s)	16.37	65.25	65.1
20	4.25 (d, 12.1), 3.79 (d, 12.1)	63.70	16.21	16.1

Table 2: ^{13}C NMR spectral data of compound 3, 4, 5 and 6 (100 MHz, DMSO-d_6)

No.	**3**	**4**	**5**	**6**	No.	**3**	**4**	**5**	**6**
1	71.56	71.03	71.48	70.59	1′	104.66	100.37	104.68	100.36
2	78.63	74.81	78.71	74.89	2′	73.73	73.39	73.55	73.31
3	33.35	31.12	33.53	31.31	3′	76.85	76.95	76.77	76.78
4	33.41	33.22	33.31	33.22	4′	72.25	70.15	70.26	70.18
5	21.81	21.60	21.82	21.60	5′	76.72	76.72	75.74	75.73
6	25.48	25.65	25.49	25.66	6′	61.24	61.14	68.52	68.51
7	24.21	24.53	24.13	24.45	1″			103.67	103.68
8	72.61	72.61	72.60	72.60	2″			73.56	73.55
9	28.87	28.84	29.05	29.02	3″			76.78	76.77
10	28.59	28.59	28.60	28.59	4″			70.26	70.26
					5″			76.84	76.85
					6″			61.12	61.10

3.6. 3β,20-Diacetoxy-isopimar-15-en-8β-ol (**1a**)

Colorless needle crystals, m.p. 166–168 °C (Me_2CO). ^1H NMR ($CDCl_3$): 5.69 (1 H, dd, J = 17.4, 10.7 Hz, H-15), 4.84 (1 H, d, J = 17.4 Hz, H-16a), 4.79 (1 H, d, J = 10.7 Hz, H-16b), 4.77 (1 H, d, J = 11.8 Hz, H-20a), 4.47 (1 H, dd, J = 11.5, 5.0 Hz, H-3), 4.40 (1 H, d, J = 11.8 Hz, H-20b), 2.05, 2.02 (each 3 H, s, CH_3COO-), 1.19 (3 H, s, Me-17), 0.90 (3 H, s, Me-19), 0.87 (3 H, s, Me-18). ^{13}C NMR ($CDCl_3$): 32.45 (C-1), 23.60 (C-2), 80.39 (C-3), 37.62 (C-4), 55.71 (C-5), 18.79 (C-6), 43.47 (C-7), 71.71 (C-8), 56.85 (C-9), 39.68 (C-10), 17.36 (C-11), 38.57 (C-12), 36.42 (C-13), 51.38 (C-14), 151.19 (C-15), 108.65 (C-16), 24.01 (C-17), 28.55 (C-18), 16.81 (C-19), 63.94 (C-20), 170.80, 170.62, 21.12, 21.12 ($2CH_3COO-$).

3.7. (1R, 2R, 4S)-1,8-Epoxy-p-menthan-2-O-β-D-glucopyranosyl-(1⟶6)-β-D-glucopyranoside (**6**)

Amorphous solid. FAB-MS (m/z): 517 $[M + Na]^+$. ^1H NMR (DMSO-d_6): 4.72 (1 H, d, J = 7.6 Hz, H-1″), 4.40 (1 H, d, J = 7.6 Hz, H-1′), 4.37 (1 H, br d, J = 11.6 Hz, H-6′b), 4.11 (1 H, br d, J = 11.6 Hz, H-6″b), 3.92 (1H, br d, J = 11.6 Hz, H-6′a), 3.87 (1 H, br d, J = 11.6 Hz, H-6″a), 2.8–3.3 (9 H, m, H-2exo, H-2′, H-2″, H-3′, H-3″, H-4′, H-4″, H-5′, H-5″), 2.56 (1 H, m, H-3exo), 1.09, 1.07, 0.97 (each 3 H, s, Me-7, Me-9, Me-10). ^{13}C NMR: see Table 2.

Acknowledgement: The authors are greatly indebted to Professor Yong-Hong Zhang (Faculty of Pharmacy Lanzhou medical college, P.R.China) for her help identification of the plant material, and the National Laboratory of Applied Organic Chemistry and Analysis Center, Lanzhou University, P.R.China for measuring 1D, 2D-NMR spectra and EI, FAB-MS, respectively.

References

1 Jiangsu College of New Medicine, The Encyclopedia of Traditional Chinese Medicine p. 1553. Shanghai Science and Technology Press, Shanghai 1986
2 Konishi, T.; Azuma, M.; Itoga, R.; Kiyosawa, S.; Fujiwara, Y.; Shimada, Y.: Chem. Pharm. Bull. **44**, 229 (1996)
3 Orihara, Y.; Furuya, T.: Phytochemistry **35**, 641 (1994)
4 Huang H., Sun H. D.; Zhao S. X.: Phytochemistry **42**, 1665 (1996)
5 Passannanti, S.; Paternostro, M.; Piozzi, F.: J. Nat. Prod. **47**, 885 (1984)
6 Matsuo, A.; Uto, S.; Nakayama, M.; Hayashi, S.: Tetrahedron Lett. **28**, 2451 (1976)

Received October 30, 2000
Accepted August 15, 2001

Prof. Yu Li
National Laboratory
of Applied Organic Chemistry
Lanzhou University
Lanzhou 730000
P.R. China