Phytochemistry, Vol. 29, No. 12, pp. 3956–3958, 1990
Printed in Great Britain.

0031–9422/90 $3.00 + 0.00

URSANE TRITERPENOIDS FROM *NEPETA ERIOSTACHIA*

S. P. S. BHANDARI, H. S. GARG, P. K. AGRAWAL and D. S. BHAKUNI

Central Drug Research Institute, Lucknow 226001, India

(*Received* 20 *February* 1990)

Key Word Index—*Nepeta eriostachiya*; Lamiaceae; trieterpenoids; nepetoic acid; 2α-methoxy-3β-hydroxyurs-12-en-28-oic acid; 2α-hydroxyursolic acid; 2α,3α-dihydroxyurs-12-en-28-oic acid; ursolic acid; sitosterol-β-D-glucoside.

Abstract—From the aerial parts of *Nepeta eriostachiya* two rare ursane triterpenoids, 2α,3α-dihydroxyurs-12-en-28-oic acid and 2α,3β-dihydroxyurs-12-en-28-oic acid, together with a new triterpene, nepetoic acid identified as 2α-methoxy-3β-hydroxyurs-12-en-28-oic acid were isolated as their methyl esters. Their structures were established by chemical and spectroscopic means.

INTRODUCTION

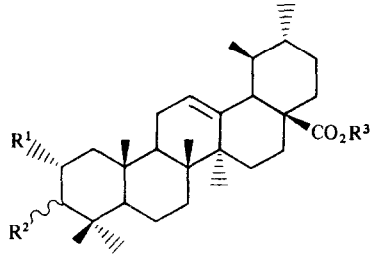
Various *Nepeta* species have been investigated for their triterpenoid contents [1–5]. The alcoholic extract of *Nepeta eriostachiya* collected from Ruttong Lahul, Himachal Pradesh, India showed mild diuretic activity, therefore, we carried out a detailed chemical investigation which led to the isolation of four ursane triterpenoids together with sitosterol-β-D-glucoside. The present paper reports the isolation and structure elucidation of these triterpenoids.

RESULTS AND DISCUSSION

The alcoholic extract after extraction with hexane and chloroform was chromatographed on a silica gel column using benzene with increasing amounts of ethyl acetate which led to a mixture of triterpene acids. The compounds ursolic acid (**1**) and 2α-hydroxy ursolic acid (**2**) were separated by fractional crystallization. The rest of the fractions were combined, then treated with diazomethane and rechromatographed on a silica gel column to afford methyl nepetoate (**3a**) and methyl-2α,3α-dihydroxyurs-12-en-28-oate (**4a**). The compounds **1**, **2** and **4** were characterized on the basis of their physical constants [6–8] and particularly by their ^{13}C NMR shielding data (Table 1).

Methyl nepetoate (**3a**) $C_{32}H_{52}O_4$, $[M]^+$ 500, exhibited IR absorption bands at 3340 cm^{-1} (hydroxyl) and 1725 cm^{-1} (ester). Its ^1H NMR spectrum showed signals for two secondary methyl groups (δ0.89 and 1.02) and five tertiary methyl singlets (δ0.72, 0.80, 0.96, 1.01 and 1.04), a trisubstituted olefinic proton (δ5.21) and a single proton doublet ($J = 10$ Hz) centred at δ2.22 characteristic of H-18 of an ursane type triterpenoid [9]. There were two methoxyl groups at δ3.34 and 3.57 assignable to a alkomethoxy and a carbomethoxy group respectively [10]. A methoxymethine was observed at δ3.20 whereas the presence of a doublet ($J = 10$ Hz) for a hydroxymethine at δ3.01 suggested the diequatorial substitution of methoxyl and hydroxyl groups at the C-2 and C-3 positions [11]. Abundant ions at *m/z* 262 and 203 resulted from the typical retro-Diels–Alder cleavage of ring C of urs-12-enes with a C-17 methoxy carbonyl and with no hydroxyl groups in rings D/E [12].

On acetylation **3a** gave a monoacetate, the ^1H NMR spectrum of which showed one acetoxy methyl signal at δ2.09, a carbinylic proton doublet ($J = 10$ Hz) at δ4.65, an alkomethoxy singlet at δ3.30, a carbomethoxy singlet at δ3.59 and a methoxy methine at δ3.36 together with an olefinic proton at δ5.26. All of these data led to the conclusion that **3a** must be a monomethyl ether of **2** having the methoxyl group at the C-2 position. The site of *O*-methylation was moreover established by the ^{13}C NMR spectral analysis of **3a** which exhibited 32 resonances. The nature of the resonances was determined by a DEPT spectrum which revealed the presence of 9 × Me, 8 × CH_2, 8 × CH and 7 × quaternary carbon signals. These were assigned by analogy with the reported values for the related triterpenoids [13, 14] and in view of methylation induced shifts [15, 16]. The presence of a methoxyl carbon signal at δ56.33, along with the appearance of C-2 at considerably low field position (δ78.72), clearly demonstrated C-2β as the site of methylation.



	R¹	R²	R³		R¹	R²	R³
1	H	β-OH	H	**2b**	OAc	β-OAc	Me
1a	H	β-OH	Me	**3a**	OMe	β-OH	Me
1b	H	β-OAc	Me	**3b**	OMe	β-OAc	Me
2	OH	β-OH	H	**4a**	OH	α-OH	Me
2a	OH	β-OH	Me	**4b**	OAc	α-OAc	Me

CDRI Communication No. 4734.

Moreover, the absence of a methine resonance at *ca* 88 ppm which would be expected in the case of 3-*O*-methylated triterpenoids [15] further confirmed the presence of a free 3β-hydroxy group. The appearance of the C-1 methylene signal at 4.09 ppm upfield in **3a** relative to **2a** was also consistent with the involvement of the C-2 hydroxyl group in *O*-methylation due to the methylation induced β-upfield shift [15–17].

Thus, all the spectroscopic data cited lead to the structure of methyl nepetoate as methyl-2α-methoxy-3β-hydroxyurs-12-en-28-oate and consequently nepetoic acid as 2α-methoxy-3β-hydroxyurs-12-en-28-oic acid. Moreover, to eliminate the possibility of **3** as being an artifact, methyl-2α-hydroxyursolate (**2a**) was treated repeatedly with diazomethane, but **3a** could not be detected.

A monomethyl ether of maslinic acid with an undetermined site of *O*-methylation which may be C-2 or C-3 has been recently characterized from the epicarp of olive drupe [18], whereas the oleanane analogue of nepetoic acid has been isolated from *Epilobium hirsutum* [4].

EXPERIMENTAL

Mps: uncorr. IR: KBr. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or pyridine-d_5 using 90 and/or 400 MHz spectrometers with TMS as int. standard. CC was on silica gel (60–120 mesh) (BDH); TLC was performed on silica gel G (BDH). The spots were visualized by spraying with 1% $Ce(SO_4)_2$ in 1 M H_2SO_4.

Extraction and isolation. The air-dried plant (12 kg) was powdered, extracted with 90% EtOH at room temp. and the extract concd. The total alcoholic extract concentrate (110 g) was fractionated with hexane and $CHCl_3$. The resulting residue was taken up in MeOH and filtered to remove inorganic salts. The MeOH extract (18 g) was chromatographed on a silica gel column using C_6H_6 with increasing amounts of EtOAc. The C_6H_6–EtOAc (7:1) and (5:1) eluates on crystallization with $CHCl_3$–MeOH and MeOH afforded ursolic acid (**1**) (5.13 g) [6] and 2α-hydroxyursolic acid (**2**) (1.02 g) [8], respectively. The mother liquor of these fractions, as well as the C_6H_6–EtOAc (4:1) eluates, were mixed to afford a mixt. of triterpene acids (8.24 g) (IR ν_{max}^{KBr} cm^{-1}: 1695) which could not be resolved by

Table 1. ^{13}C NMR chemical shifts of compounds **1a**, **1b**, **2a**, **2b**, **3a**, **4a** and **4b** and their acetyl derivatives (in $CDCl_3$ with TMS as int. ref.)

C	1a	1b	2a	2b	3a	4a	4b
1	38.82	38.33	46.50	44.23	42.41	41.98	39.13
2	27.37	23.51	68.97	70.04	78.72	66.47	68.26
3	78.81	80.83	83.95	80.74	81.62	78.90	77.31
4	38.83	37.60	39.17	39.37	38.84	38.32	38.47
5	55.41	55.33	55.36	54.99	55.13	48.12	49.75
6	18.34	18.19	18.34	18.24	18.15	18.03	17.88
7	33.01	32.92	32.91	32.84	32.91	32.80	32.78
8	39.60	39.54	39.40	39.37	39.62	39.74	39.80
9	47.51	47.51	47.58	47.57	47.59	47.33	47.51
10	37.03	36.86	38.26	38.12	38.01	38.23	38.17
11	16.91	16.96	23.38	23.41	23.58	23.30	23.36
12	125.53	125.43	125.38	125.16	125.28	125.41	125.34
13	138.08	138.18	138.28	138.37	138.28	138.29	138.36
14	42.02	41.98	42.10	42.12	42.11	42.16	42.20
15	28.21	28.02	28.01	28.02	27.99	28.01	28.08
16	24.35	24.21	24.24	24.24	24.21	24.25	24.28
17	48.11	48.05	48.11	48.11	48.08	48.12	48.14
18	52.82	52.90	52.91	52.93	52.90	52.92	52.96
19	39.13	39.01	39.06	39.06	39.02	39.06	39.13
20	38.82	38.85	38.89	38.96	38.84	38.90	38.94
21	30.71	30.62	30.66	30.66	30.65	30.66	30.69
22	36.73	36.58	36.62	36.60	36.61	36.64	36.64
23	28.21	28.02	28.62	28.48	28.70	28.47	27.15
24	15.59	16.86	16.76	17.65	16.67	21.86	21.53
25	15.73	15.40	16.70	16.48	16.36	16.41	16.29
26	16.91	16.96	16.96	16.95	16.98	16.93	16.97
27	23.33	23.26	23.61	23.55	23.58	23.75	23.75
28	177.72	177.76	178.22	177.81	177.97	178.01	177.88
29	23.61	23.51	16.99	16.95	16.97	16.98	17.09
30	21.22	21.06	21.12	21.08	21.11	21.11	21.12
CO_2Me	51.41	51.20	51.44	51.30	51.37	51.33	51.36
OMe					56.33		
OCOMe		170.63		170.59			170.48
OCOMe				170.30			170.26
OCOMe		21.06		20.97			20.98
OCOMe				20.75			20.87

chromatography. This was dissolved in Et_2O–MeOH (1:1), reacted × 5 with an ethereal soln of CH_2N_2 and worked-up as usual. The methylated products were chromatographed over silica gel using C_6H_6 and different combinations of C_6H_6–MeOH (99:1, 49:1, 97:3) to yield methyl ursolate (**1a**) (2.03 g) [6], methyl nepetoate (**3a**) (0.08 g), methyl-2α-hydroxyursolate (**2a**) (0.74 g) and methyl-2α,3α-dihydroxyurs-12-en-28-oate (**4a**) (0.18 g) [6–8], respectively.

Methyl nepetoate (**3a**). Needles (0.04 g), mp 172–174°, $[\alpha]_D$ +15° ($CHCl_3$), R_f 0.6 ($CHCl_3$–MeOH, 25:1). IR ν_{max}^{KBr} cm^{-1}: 3340, 2940, 2650, 1725, 1660, 1600, 1520, 1510, 1490, 1400, 1390, 1360, 1245, 1230, 1215, 1185, 1160, 1115, 1065, 965, 775, 690. ^1H NMR (90 MHz, $CDCl_3$): δ0.72, 0.80, 0.96, 1.01, 1.04, (3H each, *s*, 5 × Me), 0.89 and 1.02 (3H each, *d*, *J* = 6 Hz, 2 × Me), 2.22 (1H, *d*, *J* = 10 Hz, H-18), 3.01 (1H, *d*, *J* = 10 Hz, H-3), 3.20 (1H, *m*, H-2), 3.34 (3H, *s*, OMe), 3.57 (3H, *s*, CO_2Me), 5.21 (*t*, H-12). ^{13}C NMR: see Table 1. MS *m/z* (rel. int.): 500 $[M]^+$ (1), 499 (0.7), 468 $[M-MeOH]^+$ (0.6), 445 (0.6), 441 $[M-CO_2Me]^+$ (0.7), 409 (0.6), 331 (0.7), 262 (70), 238 (20), 203 (70), 189 (15), 133 (60), 44 (100). (Found: C, 76.06; H, 10.2 $C_{32}H_{52}O_4$ requires: C, 76.80; H, 10.4%).

Compound **3a** (34 mg) was treated with Ac_2O (1 ml) in pyridine (1 ml) at room temp. overnight and which on usual work-up afforded an amorphous solid **3b** (29 mg). IR ν_{max}^{KBr} cm^{-1}: OH absent, 2928, 2860, 2280, 2100, 1740, 1460, 1390, 1378, 1318, 1250, 1210, 1180, 1150, 1115, 1045, 960. ^1H NMR (80 MHz, $CDCl_3$): δ0.75, 0.83, 0.93, 0.99, 1.25, (3H each, *s*, 5 × Me), 0.87, 1.07 (6H, *d*, *J* = 6 Hz, 2 × Me), 2.09 (3H, *s*, OAc), 3.30 (3H, *s*, OMe), 3.36 (1H, *m*, H-2), 3.59 (3H, *s*, CO_2Me), 4.65 (1H, *d*, *J* = 10 Hz, H-3), 5.26 (*t* like, H-12). ^{13}C NMR: see Table 1. MS *m/z* (rel. int.): 542 $[M]^+$ (2), 540 (4.3), 510 (2), 482 (8.1), 481 (4.9), 480 (8.1), 468 (2.2), 467 (4.9), 452 (4.1), 451 (4), 450 (8.3), 437 (19), 435 (3.2), 408 (2), 389 (2.8), 366 (2.3), 339 (2.6), 314 (2.3), 313 (3.1), 262 (100), 203 (94), 189 (37), 133 (60), 44 (70). (Found: C, 75.02; H, 9.26, $C_{34}H_{54}O_5$, requires: C, 75.28; H, 9.96%).

Methyl-2α-hydroxyursolate (**2a**) (125 mg) was treated with an ethereal solution of CH_2N_2 (× 5) for 2 days and the reaction mixt. monitored by TLC which showed the absence of **3a**. It was further reacted with an ethereal solution of CH_2N_2 (× 10) for 5 days and worked-up as usual. The product was identified as unconverted starting material **2a**.

Acknowledgement—We are grateful to the staff of RSIC for spectra data.

REFERENCES

1. Lawrence, B. M., Hogg, J. W., Terhune, S. J., Morton, J. K. and Gill, L. S. (1972) *Phytochemistry* **11**, 2636.
2. Von Carstenn-Lichterfelde, C., Rodriguez, B. and Valverde, S. (1973) *Phytochemistry* **12**, 3002.
3. Popa, D. P., Salei, L. A. and Orgiyan, T. M. (1973) *Rastit Resur.* **12**, 247.
4. De Pascual Teresa, J., Corrales, B. and Grande, B. (1979) *An. Quim.* **75**, 135.
5. Ahmad, V. U. and Mohammad, F. V. (1986) *J. Nat. Prod.* **49**, 524.
6. Biessels, H. W. A., van der Kerk-van Hoof, A. C., Kettenes-van den Bosch, J. J. and Salemink, C. A. (1974) *Phytochemistry* **13**, 203.
7. Kojima, H. and Ogura, H. (1986) *Phytochemistry* **25**, 729.
8. Osman, A. M., Younes, M. E. and Sheta, A. E. (1974) *Phytochemistry* **13**, 2015.
9. Tori, K., Seo, S. and Tomita, Y. (1975) *Chem. Ind. (Lond.)* 434.
10. Higuchi, R., Kawasaki, T., Biswas, M., Pandey, V. B. and Das Gupta, B. (1982) *Phytochemistry* **21**, 907.
11. Kojima, H. and Ogura, H. (1989) *Phytochemistry* **28**, 1703.
12. Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1964) *Structure Elucidation of Natural Products by Mass Spectroscopy* Vol II, p. 122. Holden-Day, San Francisco.
13. Seo, S., Tomita, Y. and Tori, K. (1975) *Tetrahedron Letters* 7.
14. Ishii, H., Tori, K., Tozyo, T. and Yoshimura, Y. (1984) *J. Chem. Soc. Perkin Trans I* 661.
15. Blunt, J. W. and Munro, M. H. G. (1980) *Org. Magn. Reson.* **13**, 27.
16. Agrawal, P. K. and Thakur, R. S. (1985) *Magn. Reson. Chem.* **23**, 389.
17. Agrawal, P. K. and Bansal, M. C. (1989) in *Studies in Organic Chemistry* Vol. 39 (Agrawal, P. K., ed.), p. 450. Elsevier, Amsterdam.
18. Frega, N., Bonaga, G., Lercker, G. and Bortolomeazzi, R. (1989) *Riv. Ital. Sostanze Grasse* **66**, 107.