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TRITERPENES FROM THE LEAVES OF *PARSONSIA LAEVIGATA*

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Key Word Index *Parsonsia laevigata*; Apocynaceae; taraxerane-type triterpenoid; (4β)-D-friedoolean-14-ene-3β,24-diol.

Abstract –A novel taraxerane-type triterpene-diol, in addition to taraxerol and lupeol, was isolated from the leaves of *Parsonsia laevigata*. Its structure was shown to be (4β)-D-friedoolean-14-ene-3β,24-diol (taraxer-14-ene-3β,24-diol) by a combination of chemical and spectroscopic methods.

INTRODUCTION

Parsonsia laevigata Alston (Japanese name: Houraikagami) is a perennial liana and grows wild in the south-western islands of Japan. It is said that the larvae of *Idea leuconoe liukiuensis* ingest the leaves of this plant as a means of defence against attack by predators. In the process of investigations on the chemical constituents which are effective in the defence of the larvae, a novel taraxerane-type triterpene-diol, in addition to taraxerol and lupeol, was isolated from the leaves of the plant. We here wish to present the evidence which led to the establishment of the structure of the novel triterpene-diol.

RESULTS AND DISCUSSION

A chloroform-soluble fraction of an ethanol extract of the leaves was subjected to chromatography to give compound **1**, in addition to taraxerol and lupeol.

Compound **1** gave a positive Liebermann–Burchardt reaction and had a molecular formula of $C_{30}H_{50}O_2$ (HRMS *m/z* 442.3717). IR absorption bands at 3440 and 3330 cm^{-1} indicated the presence of hydroxyl groups. This was confirmed by the formation of a diacetate (**2**) which exhibited two doublet signals at δ4.37 and 4.16 due to methylene protons of a non-hindered CH_2OAc group and a double doublet signal at δ4.55 due to a methine proton of a $CH_2CH(OAc)$ grouping. In addition, it gave rise to a well-defined double doublet signal at δ5.53 due to an olefinic proton. This observation suggested the compound **1** was a taraxer-14-ene derivative [1].

Compound **1** was converted to a taraxerol (**3**), on partial tosylation with *p*-toluenesulphonyl chloride followed by reduction with lithium aluminium hydride. This proved that compound **1** was a taraxer-14-ene derivative in which one of the two hydroxyl groups was located on C-3 in the β-configuration. The location of the second hydroxyl group on either C-23 or C-24 was suggested by formation of an acetonide (**4**), on treatment of compound **1** with dry acetone and concentrated sulphuric acid. The chemical shifts of the methylene protons of the CH_2OAc group corresponding to this hydroxyl group were coincident with those of the axial CH_2OAc group of pentacyclic triterpenoids [2]. This indicated the location

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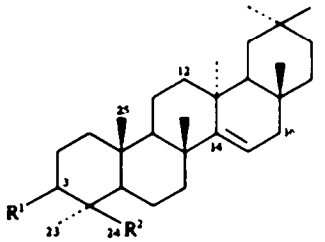
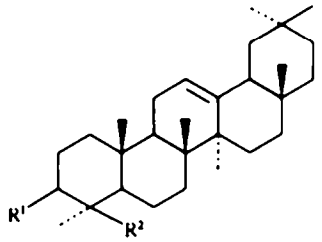


	R^1	R^2
1	OH	CH_2OH
2	OAc	CH_2OAc
3	OH	Me
4	O—C(Me)$_2$—O	CH_2
5	=O	Me



	R^1	R^2
6	OH	CH_2OH
7	OAc	CH_2OAc

of the acetoxyl group on C-24. This was supported by the shift of the ^{13}C NMR signal due to C-23 to a higher field than that due to the corresponding carbon of taraxeryl acetate [3] (Table 1). Thus, the second hydroxyl group in compound **1** was located on C-24 in the β-configuration. This finding was further confirmed by correlating the signals due to the methyl protons of the C-25 methyl group with those due to the methylene protons of the CH_2OAc group in the 2D NOE spectrum of compound **2**. Finally, the absolute configuration of compound **1** was determined on the basis of the agreement of the specific rotation and the CD curve of the compound **5** derived from compound **3** with those of taraxerone (**5**). Thus, compound **1** was shown to be taraxer-14-ene-3β,24-diol.

Finally, in the course of the above-mentioned structure elucidation, it was noted that compound **1** was isomerized readily to the corresponding olean-12-enes under mild acidic conditions. Thus it was isomerized to olean-12-ene-3β,24-diol (**6**) [4] on treatment with hydrochloric acid–ethanol, while both it and its diacetate (**2**) were converted to olean-12-ene-3β,24-diol diacetate (**7**) [4] on treatment with hydrochloric acid–acetic acid. This behaviour is in agreement with that generally observed for taraxer-14-enes [5].

Table 1. ^{13}C NMR data for compound **2** and taraxeryl acetate (100.5 MHz, $CDCl_3$, TMS as internal standard)

C	2	Taraxeryl acetate*
1	37.6	37.8
2	23.6	23.4
3	80.4	81.1
4	41.1	37.9
5	56.4	55.7
6	19.9	18.8
7	33.8	33.2
8	39.1	39.1
9	49.5	49.3
10	37.8†	37.6
11	17.8	17.6
12	33.2	36.7
13	37.9†	37.8
14	157.7	158.0
15	117.2	116.9
16	37.7	33.7
17	35.9†	35.8
18	48.9	48.9
19	41.7	41.3
20	28.9	28.8
21	35.2	35.2
22	36.8	37.5
23	22.6	28.0
24	65.5	16.5
25	15.4	15.5
26	25.8	26.0
27	30.0	29.9
28	29.9	29.9
29	33.4	33.4
30	21.4	21.3
$\underline{C}H_3COO$	21.3	21.3
	21.2	
$CH_3\underline{C}OO$	171.1	171.0
	170.6	

*Values are taken from ref. [3].

†Values may be interchanged although those given here are preferred.

EXPERIMENTAL

^1H NMR and ^{13}C NMR spectra, homonuclear (^1H) and heteronuclear (^1H ^{13}C) chemical shift correlated spectra and NOE correlated spectrum were taken in $CDCl_3$ with TMS as internal standard. EIMS spectra were taken at 70 eV using a direct insertion probe. Analytical and prep. TLC were carried out on Merck 60 GF_{254} silica gel plates (adsorbent thickness: 0.25 and 0.75 mm, respectively).

Extraction and isolation. Fresh leaves (13.5 kg) of *P. laevigata*, collected at Onna-son, Okinawa-prefecture in April, were immersed in EtOH at room temp. for 1 month. The EtOH soln was then taken to dryness and the concentrate obtained, after addition of H_2O, was shaken with $CHCl_3$. The $CHCl_3$ extract, after removal of the solvent, was chromatographed on a silica gel column (Waco-gel C-300) developed with $CHCl_3$ followed by $CHCl_3$–EtOAc mixtures with an increasing EtOAc content. The fraction eluted with $CHCl_3$ was re-chromatographed on a silica gel column to give taraxerol (250 mg, mp 283–285°) and lupeol (240 mg, mp 192°). These compounds were identified by comparison of their physical data and IR, NMR and MS data with those described in refs [6–8]. The fraction eluted with $CHCl_3$–EtOAc (9:1) gave **1** as colourless needles (190 mg).

Taraxer-14-ene-3β,24-diol (**1**). Mp > 320° (from $CHCl_3$, sub. 250°); IR ν_{max}^{KBr} cm^{-1}: 3440 and 3330 (OH), 3050, 1640 and 810 (>C=CH–); HRMS *m/z*: 442.3717, (M^+, calcd for $C_{30}H_{50}O_2$: 442.3810), 427.3604 $[M-Me]^+$, 318.2469 $[C_{21}H_{32}(OH)_2]^+$, 303.2440 $[C_{21}H_{32}(OH)_2-Me]^+$, 300.2721 $[C_{21}H_{32}(OH)_2-H_2O]^+$, 204.1850 $[C_{15}H_{24}]^+$, 189.1602 $[C_{15}H_{24}-Me]^+$. As all attempts to dissolve a sample of **1** in various solvents for measurements of NMR spectra and specific rotation failed, it was converted into its more soluble diacetate (**2**) by treatment with Ac_2O/C_5H_5N.

Diacetate (**2**). Mp 234–235° (from EtOH); $[\alpha]_D^{25}$ +10.1° ($CHCl_3$; *c* 0.99); IR ν_{max}^{KBr} cm^{-1}: 3050, 1630 and 810 (>C=CH–), 1710, 1705, 1280 and 1245 (–C(=O)–O–); ^1H NMR (400 MHz, $CDCl_3$): δ0.82 (3H, *s*, Me-28), 0.90 (3H, *s*, Me-30), 0.91 (3H, *s*, Me-29), 0.95 (3H, *s*, Me-27), 0.97 (3H, *s*, Me-25), 1.00 (3H, *s*, Me-23), 1.08 (3H, *s*, Me-26), 2.04 and 2.08 (each 3H, *s*, OAc), 4.16 and 4.37 (each 1H, *d*, J = 12 Hz, H-24), 4.55 (1H, *dd*, $J_{3\alpha,2\alpha}$ = 7 and $J_{3\alpha,2\beta}$ = 10 Hz, H-3), 5.53 (1H, *dd*, J = 4.3 and 7.5 Hz, H-15); ^{13}C NMR: see Table 1; EIMS *m/z* (rel. int.): 526 $[M]^+$ (17), 511 (6), 466 (41), 402 (31), 387 (7), 342 (9), 204 (100), 189 (17). The signals of ^1H and ^{13}C NMR spectra were assigned on the basis of ^1H ^1H and ^1H ^{13}C 2D correlated spectroscopy.

Conversion of compound **1** *to taraxerol* (**3**). *p*-Toluenesulphonyl chloride (39 mg) was added to a soln of **1** (25 mg) in dry C_5H_5N at 0°. The mixture was stirred for 1 hr at room temp. and then allowed to stand for 2 days at 60°. The reaction mixture was purified by prep. TLC with $CHCl_3$–EtOAc (9:1) to give a monotosylate (15 mg). $LiAlH_4$ (64 mg) was added to a soln of the monotosylate (15 mg) in dry THF and the mixture was refluxed for 1 hr. After decomposition of excess $LiAlH_4$ with MeOH and then H_2O, the mixture was extracted with $CHCl_3$. The $CHCl_3$ extract, after removal of the solvent, was subjected to prep. TLC with hexane–EtOAc (4:1) to give **3** (4 mg); mp 283–285° (from $CHCl_3$–MeOH); $[\alpha]_D^{28}$ +0.94° ($CHCl_3$; *c* 0.40); IR ν_{max}^{KBr} cm^{-1}: 3580 (OH), 3050, 1635 and 815 (>C=CH–); ^1H NMR (60 MHz, $CDCl_3$): δ0.81 (6H, *s*), 0.91 (6H, *s*), 0.95 (6H, *s*), 0.98 (3H, *s*), 1.10 (3H, *s*), 3.15 (1H, *m*, $W_{1/2}$ = 18 Hz), 5.53 (1H, *dd*, J = 4 and 7 Hz);

EIMS *m/z* (rel. int.): 426 [M]$^+$ (11), 411 (11), 393 (2), 302 (46), 287 (54), 204 (100), 189 (33). These physical and spectral data were in agreement with those of taraxerol (3), which was isolated from the same plant.

Acetonide (4). Concentrated H_2SO_4 (5 drops) was added to a soln of 1 (50 mg) in dry Me_2CO (20 ml) and dry Et_2O (100 ml). After standing at 5° for 2 days, the mixture was diluted with Et_2O and washed with 10% $NaHCO_3$. The Et_2O soln was taken to dryness and the concentrate obtained was subjected to chromatography on a silica gel column (Waco-gel C-300) with $CHCl_3$ to give the acetonide (4) (10 mg) as plates: mp 213° (from $CHCl_3$–MeOH); IR ν_{max}^{KBr} cm^{-1}: 3050, 1640 and 815 (>C=CH–), 1180, 1125 and 1090 (>C(–O–)–O–); ^{1}H NMR (60 MHz, $CDCl_3$): δ0.83 (s), 0.91 (s), 0.95 (s), 1.12 (s), 1.24 (s), 1.39 (s), 1.48 (s), 1.62 (s), 3.30 and 4.14 (each 1H, *d*, J = 12 Hz, H-24), and 5.55 (1H, *dd*, J = 4 and 7 Hz, H-15); EIMS *m/z* (rel. int.): 482 [M]$^+$ (32), 467 (11), 424 (12), 358 (20), 300 (22), 204 (100), 189 (22).

Oxidation of compound 3. Pyridinium chlorochromate (34 mg) was added to a soln of 3 (4.0 mg) in dry CH_2Cl_2. The mixture was stirred for 2 hr at room temp. The reaction mixture, after removal of the organic solvent, was subjected to prep. TLC with $CHCl_3$ to give 5 (2.4 mg): mp 245 247° ($CHCl_3$–MeOH), $[\alpha]_D^{30}$ +12.8° ($CHCl_3$; *c* 0.23); CD ($CHCl_3$; *c* 0.25): $[\theta]_{316}$ −165, $[\theta]_{308}$ 0, $[\theta]_{285}$ +924; IR ν_{max}^{KBr} cm^{-1}: 1705 (C=O); ^{1}H NMR (60 MHz, $CDCl_3$): δ0.83 (3H, s), 0.91 (6H, s), 0.95 (3H, s), 1.08 (9H, s), 1.14 (3H, s), 5.55 (1H, *dd*, J = 4 and 7 Hz); EIMS *m/z* (rel. int.): 424 [M]$^+$ (26), 409 (22), 300 (98), 285 (78), 204 (100). These physical and spectral data were the same as those of taraxerone (5) derived from taraxerol (3).

Isomerization of compound 1 *to olean-12-ene-3β,24-diol* (6). HCl (1 ml) was added dropwise at 78° to 1 (30 mg) suspended in EtOH (20 ml). The mixture was heated on a steam-bath for 15 min and then was rapidly evaporated to dryness under reduced pressure. The crude product was chromatographed on a silica gel column with C_6H_6 to give *olean-12-ene-3β,24-diol* (6) (25 mg) as needles: mp 248° (sub. 210° from $CHCl_3$–MeOH); $[\alpha]_D^{30}$ +77.6° ($CHCl_3$, *c* 0.79) (ref. [4] mp 248 250°, $[\alpha]_D$ +95°); HRMS *m/z*: 442.3825, (M$^+$, calcd for $C_{30}H_{50}O_2$: 442.3810); IR ν_{max}^{KBr} cm^{-1}: 3600 3100 (OH), 818 (>C=CH–); ^{1}H NMR (90 MHz, $CDCl_3$): δ0.83 (3H, s), 0.87 (9H, s), 0.94 (3H, s), 1.13 (3H, s), 1.25 (3H, s), 3.40 (1H, *m*, H-3), 3.32 and 4.22 (each 1H, *d*, J = 12 Hz, H-24), 5.18 (1H, *br t*, J = 3 Hz, H-12); EIMS *m/z* (rel. int.): 442 [M]$^+$ (5), 224 (5), 218 (100), 203 (25).

Conversion of compounds 1 *and* 2 *to olean-12-ene-3β,24-diol diacetate* (7). To 1 (30 mg) suspended in glacial HOAc (15 ml), conc. HCl (1 ml) was added dropwise at 95°. Usual work-up of the mixture gave *olean-12-ene-3β,24-diol diacetate* (7) (25 mg) as needles: mp 183 184° (from $CHCl_3$–MeOH); $[\alpha]_D^{30}$ +73.6° ($CHCl_3$, *c* 0.46) (ref. [4] mp 194–195°; $[\alpha]_D$ +80°; IR ν_{max}^{KBr} cm^{-1}: 1740, 1725, 1250 and 1235 (>C(=O)–O–), 815 (>C=CH–); ^{1}H NMR (100 MHz, $CDCl_3$): δ0.82 (3H, s), 0.86 (6H, s), 0.96 (6H, s), 1.01 (3H, s), 1.11 (3H, s), 2.02 and 2.05 (each 3H, s, OAc), 4.40 and 4.17 (each 1H, *d*, J = 12 Hz, H-24), 4.50 (1H, *dd*, $J_{3\alpha,2\alpha}$ = 7 and $J_{3\alpha,2\beta}$ = 10 Hz, H-3), 5.10 (1H, *m*, H-12); EIMS *m/z* (rel. int.): 526 [M]$^+$ (9), 511 (3), 466 (1), 307 (2), 218 (100), 203 (44). The same conversion was also observed on treatment of 2 with a mixture of glacial HOAc and HCl.

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REFERENCES

1. Hui, W. H. and Li, M. M. (1976) *J. Chem. Soc. Perkin Trans.* 1, 23.
2. Gaudemer, A., Polonsky, M. J. and Wenker, E. (1964) *Bull. Soc. Chim. Fr.* 407.
3. Katai, M. and Meguri, H. (1983) *Yukagaku* **32**, 311.
4. Meyer, A., Jeger, O. and Ruzicka, L. (1950) *Helv. Chim. Acta* **33**, 672, 687.
5. Beaton, J. M., Spring, F. S., Stevenson, R. and Stewart, J. L. (1955) *J. Chem. Soc.* 2131.
6. Corbett, R. E., Cumming, S. D. and Whitehead, E. V. (1972) *J. Chem. Soc. Perkin Trans.* 1, 2827.
7. Djerassi, C., Budzikiewics, H. and Wilson, J. M. (1962) *Tetrahedron Letters* 263.
8. Yoshihira, K., Tezuka, M. and Natori, S. (1971) *Chem. Pharm. Bull.* **19**, 2308.