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3-OXO-6β-HYDROXYOLEAN-18-EN-28-OIC ACID FROM *ORTHOPTERYGIUM HUANCUY*

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Key Word Index—*Orthopterygium huancuy*; Julianaceae; triterpenes; 6β-hydroxymoronic acid; 3-oxo-6β-hydroxy-olean-18-en-28-oic acid.

Abstract—A new triterpene, 3-oxo-6β-hydroxyolean-18-en-28-oic acid, was isolated from *Orthopterygium huancuy*. Betulonic, moronic and 3-oxo-6β-hydroxy-12-en-28-oic acids were also isolated.

Continuing our studies on the chemical components of South American plants, we present here the results obtained from a study of *Orthopterygium huancuy* (Gray) Hemsl. (Julianaceae), a species endemic of Peru.

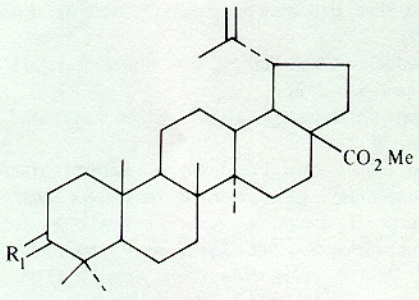
Chromatography of the alcoholic extract provided the triterpene betulonic acid. This compound was identified by its physical and spectroscopic constants and those of its methyl ester (**1**) [1]. Moronic acid was characterized in the methyl ester form (**2**) [2]. The reduction of **1** and **2** with sodium borohydride afforded the methyl esters of betulinic and morolic acids, **3** and **4**, respectively.

Two further triterpenoid acids were purified in the methyl ester form. The less polar was the methyl ester of 3-oxo-6β-hydroxyolean-12-en-28-oic acid (**3**). The other was the methyl ester of a new triterpene, the structure of which was assigned as 3-oxo-6β-hydroxyolean-18-en-28-oic acid, in accordance with the following considerations.

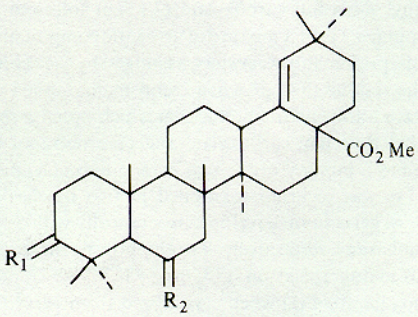
Its methyl ester (**5**) showed in the ^{1}HNMR signals of seven methyl groups, a proton geminal to an axial alcohol group and a vinylic hydrogen. The latter signal was a singlet, and the chemical shift was typical of an olean-18-ene with a free or esterified acid at C-28 [4]. Characteristic fragments of this skeleton were observed in the mass spectrum of **5** [5].

At a first approximation, the axial hydroxyl group was located at the 6β, 7α or 11β positions because, under normal acetylation conditions, the acetate was not formed. The chemical shift of the methyl groups at C-4, C-8 and C-10 pointed to 6β [6, 7]. The carbonyl group was located at C-3 by biogenetic considerations.

As the full assignment of carbon resonances in the olean-18-ene series has been made [2, 8], a ^{13}CNMR analysis of **5** was undertaken in order to confirm the structure. In Table 1 we give the carbon chemical shifts of



1 $R_1 = O$
3 $R_1 = \beta - OH$



2 $R_1 = O, R_2 = H_2$
4 $R_1 = \beta - OH, R_2 = H_2$
5 $R_1 = O, R_2 = \beta - OH$
6 $R_1 = R_2 = \beta - OH$
7 $R_1 = \alpha - OH, R_2 = \beta - OH$
8 $R_1 = \beta - OAc, R_2 = \beta - OH$
9 $R_1 = \beta - OAc, R_2 = O$

Table 1. Physical constants of isoadianone from *P. glauca* (two preparations) compared with reported data for adiantone (**2**) and isoadiantone (**1**)

	Mp	$[\alpha]_D$	^{1}H NMR methyl signals (δ) 4α	4β	8β	10β	14α	18α	22	ν_{max} (cm^{-1})	Ref.
Adiantone (**2**)	218°	+83°								1700‖	[5]
	222–224°	+81°									[7]
			0.84	0.82	0.98	0.80	0.94	0.59	2.10‡		[8]
Isoadiantone (**1**)	230–231.5°	−4°								1710‖	[5]
	232–234°	+2°									[7]
			0.84	0.81	0.96	0.79	0.96	0.70	2.13‡		[8]
*P. glauca**	227–228°	+1.5°	0.82	0.80	0.95	0.78	0.95	0.68	2.08‡	1705¶	—
P. glauca†	234–235°	+2.3°	0.85	0.83	0.98	0.80	0.96	0.70	2.13§	1702¶	—

*Akaline conditions.
†Neutral conditions.
‡At 60 MHz ($CDCl_3$).
§At 100 MHz ($CDCl_3$).
‖In CS_2.
¶In KBr.

Of the above compounds, only atranorin has previously been reported as a constituent of *P. glauca*.

EXPERIMENTAL

Isolation. P. glauca (from *Picea abies*; det. T. Tønsberg, Botany Dept., The University of Trondheim) (1.5 kg air-dried) was extracted with hexane (4 l.) in a Soxhlet extractor for 48 hr. Evaporation of the solvent furnished a residue (29.2 g, 1.9%) which separated on Si gel (750 g) into fractions eluted with hexane (0.18 g, 0.8%), toluene (13.65 g, 46.7%), Et_2O (6.9 g, 23.7%) and MeOH (8.46 g, 29%).

The hexane fraction, when purified on TLC (Si gel, hexane), gave a mixture of normal, satd hydrocarbons as determined by GC: *n*-C_{14} (3.0%), *n*-C_{15} (2.7%), *n*-C_{16} (3.2%), *n*-C_{17} (3.6%), *n*-C_{18} (4.6%), *n*-C_{19} (5.2%), *n*-C_{20} (4.6%), *n*-C_{21} (7.0%), *n*-C_{22} 3.9%), *n*-C_{23} (9.0%), *n*-C_{24} (2.7%), *n*-C_{25} (8.8%), *n*-C_{26} (2.9%), *n*-C_{27} (10.5%), *n*-C_{28} (3.6%), *n*-C_{29} (8.3%), *n*-C_{30} (3.0%), *n*-C_{31} (7.3%), *n*-C_{32} (2.3%) and *n*-C_{33} (3.8%).

The toluene fraction gave a crystalline mixture of atranorin and chloroatranorin (923 mg) upon concn. Further concn gave a crop of methyl β-orcinol carboxylate (2.157 g) followed by a voluminous ppt (*ca* 7 g) of a mixture of aromatic compounds and aliphatic lipids, presumably was esters, as judged by the ^{1}H NMR spectrum. The residue (5.47 g), when rechromatographed on Si gel (750 g), gave a toluene fraction (3.2 g) which ppted a second crop of methyl β-orcinol carboxylate and chloroatranol upon concn, as well as a mixture of the two compounds. The mother liquor (0.873 g) was rechromatographed in two batches on a Merck Lobar Si gel column developed isocratically with 5% and 2.5% Et_2O in hexane, respectively. Two homogeneous fractions which co-chromatographed on TLC and GC, gave colourless crystals of isoadiantone (**1**) when crystallized from Me_2CO.

30-*Nor*-21α-*hopan*-22-*one* (*isoadiantone*, **1**, 45 *mg*). Recrystallized from CH_2Cl_2–Me_2CO and had physical properties as reported in Table 1. IR ν_{max}^{KBr} cm^{-1}: 2945, 2865 (CH), 1702 (C=O), 1465, 1445, (CH_2), 1380, 1365 and 1350 (Me); EIMS (probe, 70 eV) *m/z* (rel. int.): 412.3696 $[M]^+$ (57), 397 $[M-15]^+$ (14), 369 $[M-43]^+$ (5), 206 (13), 191 (100), 177 (8), 149 (39) and 43 (64).

Chloroatranol (85 *mg*). Recrystallized from Me_2CO and had mp 139–140.5°; IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1644 (C=O), 1460, 1288, 1205, 1182, 1095, 825 and 750; ^{1}H NMR (90 MHz, $CDCl_3$): δ 2.31 (*s*, Me), 6.32 (*s*, Ar-H), 6.40 (*s*, OH), 10.20 (*s*, CHO) and 11.08 (*s*, OH); EIMS (probe, 70 eV) *m/z* (rel. int.): 188.0048 (30, calc. for $C_8H_7{}^{37}ClO_3$, 188.0053)/186.0076 (87, calc. for $C_8H_7{}^{35}ClO_3$, 186.0083) $[M]^+$, 187 (38)/185 (100) $[M-1]^+$, 168 (5), 140 (13) and 121 (3).

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5 and of the methyl ester of moronic acid (**2**) [2]. By comparison of both spectra it is seen that in the first the signal corresponding to C-6 has disappeared and that those of C-5 and C-7 were displaced downfield. It is also characteristic that the signal due to C-1 in **5** appeared at a lower field (2.5) than in **2**. This δ-effect is produced by the presence of an axial hydroxyl group at C-6. This shift, between cholestan-3β-ol and cholestan-3β,6β-diol is 1.4 [9], and between androstane and 6β-hydroxyandrostane 1.7 [10]. A similar δ-effect at C-7 in the olean-18-ene series is produced by the introduction of an 11β-hydroxyl group [8].

Table 1. ^{13}C NMR spectral data for compounds **2** and **5**

Carbon No.	2	5	Carbon No.	2	5
1	39.7	42.2	16	33.7	33.5
2	33.9	34.4	17	48.1	48.2
3	218.3	‡	18	136.7	137.1
4	47.1	48.9	19	132.4	132.9
5	54.8	56.5	20	31.9	32.1
6	19.6	69.5	21	33.4	33.5
7	33.9	42.2	22	33.4	33.5
8	40.4	39.8	23	20.9	23.6*
9	50.3	51.3	24	26.8	25.0*
10	36.8	36.8	25	15.8	17.3†
11	21.4	21.3	26	16.4	17.5†
12	25.9	26.0	27	14.8	15.1
13	41.3	40.5	28	176.8	177.1
14	42.4	42.7	29	30.3	30.4
15	29.3	29.5	30	29.1	29.1

*,†Values with the same sign may be interchanged.
‡Not recorded.

Finally, several reactions were carried out to relate our compound **5** with morolic acid methyl ester (**4**), but the route was not successful. By reduction of the methyl ester of 3-oxo-6β-hydroxyolean-18-en-28-oic acid (**5**) with sodium borohydride a mixture of two compounds was obtained. The less polar and major product was the 3β-hydroxyl derivative, **6**, and the other was identified as the 3α-compound, **7**. Acetylation of **6** in the usual way afforded the 3β-monoacetate, **8**. This compound was oxidized to give **9**. The reduction of this ketone by the Barton modification of the Wolff–Kishner reaction [11] and posterior methylation and acetylation did not afford the desired morolic acid methyl ester (**4**), only the initial ketone, **9**. Other pentacyclic triterpenoids with a 6β-hydroxyl group have been isolated from *Myroxylon balsamum* (Leguminosae) [3] and from *Pleurostylia opposita* (Celastraceae) [6].

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR were run on a 90 MHz and ^{13}C NMR on a 50.3 MHz instrument in $CDCl_3$ with TMS as int. reference. IR in $CHCl_3$ and column and dry chromatography were performed on Si gel.

Isolation of the products. The air-dried aerial part of the plant (2.7 kg) collected near Paira, Peru, were finely cut, extracted with EtOH and the extract concd *in vacuo*. The residue (96 g) was chromatographed by elution with petrol and petrol–EtOAc mixtures to give sitosterol (190 mg), betulonic acid and a triterpenoid acid mixture. This was treated with CH_2N_2 and chromatographed on dry column with petrol–EtOAc (9:1) giving the methyl esters of moronic acid (**2**) (580 mg), 3-oxo-6β-hydroxyolean-12-en-28-oic acid (35 mg) and 3-oxo-6β-hydroxyolean-18-en-28-oic acid (**5**) (80 mg).

Betulonic acid. Mp 236–238°, $[\alpha]_D$ + 30° ($CHCl_3$; *c* 0.32) (lit. [12] mp 253°, $[\alpha]_D$ + 31°). M^+ *m/z* 454.3457 (calcd for $C_{30}H_{46}O_3$, 454.3447). ^{1}H NMR: δ 0.95 (3H, *s*), 1.01 (6H, *s*), 1.03, 1.09 and 1,73 (each 3H, *s*), 3.03 (2H, *m*, H-2), 4.72 (2H, *d*, *J* = 12 Hz, H-29). MS *m/z*: 454 $[M]^+$, 439, 408, 393, 259, 248, 235, 219, 205, 203, 189, 185, 175, 147, 133. Methyl ester (**1**), mp 146–148°, $[\alpha]_D$ + 33° ($CHCl_3$; *c* 0.4) (lit. [12] mp 165°, $[\alpha]_D$ + 31.5°). IR ν_{max} cm^{-1}: 3020, 1700, 1640, 880. ^{1}H NMR 0.92, 0.96, 0.98, 1.02, 1.07 and 1.67 (each 3H, *s*), 3.68 (3H, *s*), 4.03 (2H, *d*, *J* = 12 Hz, H-29). MS *m/z*: 468 $[M]^+$, 453, 405, 393, 262, 249, 205, 203, 189 (100), 175, 149, 133.

Moronic acid methyl ester (**2**). Mp 146–148°, $[\alpha]_D$ + 59° ($CHCl_3$; *c* 0.38) (lit. mp 165°, $[\alpha]_D$ + 59°). M^+ *m/z* 468.3606 (calcd for $C_{31}H_{48}O_3$, 468.3603). IR and ^{1}H NMR spectra identical with those reported [13].

3-Oxo-6β-hydroxyolean-12-en-28-oic acid methyl ester. Mp 190–191°, $[\alpha]_D$ + 33° ($CHCl_3$; *c* 0.67) (lit. 190–191°, $[\alpha]_D$ + 35°). M^+ *m/z* 484.3530 (calcd for $C_{31}H_{48}O_4$ 484.3552). IR and ^{1}H NMR spectra identical to those reported [3].

3-Oxo-6β-hydroxyolean-18-en-28-oic acid methyl ester (**5**). Mp 204–205°, $[\alpha]_D$ − 7° ($CHCl_3$; *c* 0.32). M^+ *m/z* 484. 3558 (calcd for $C_{31}H_{48}O_4$ 484.3552). IR ν_{max} cm^{-1}: 3520, 1720, 1690, 820. ^{1}H NMR: δ 0.75, 0.97, 0.99, 1.16, 1.34, 1.42 and 1.46 (each 3H, *s*), 3.65 (3H, *s*), 4.45 (1H, *m*, H-6), 5.11 (1H, *s*, H-19). MS *m/z*: 484 $[M]^+$, 466, 425, 407, 302, 262, 249, 247, 203, 189, 187, 133.

Reduction of **1**. A soln of **1** (290 mg) in MeOH (2 ml) was stirred with $NaBH_4$ (600 mg) for 30 min. Usual work-up afforded betulinic acid methyl ester (**3**), mp 225–227°, $[\alpha]_D$ + 5° ($CHCl_3$; *c* 0.4) (lit. [14], mp 223–225°, $[\alpha]_D$ + 8°). ^{1}H NMR: δ 0.74, 0.80, 0.90 (each 3H, *s*), 0.94 (6H, *s*), 1.67 (3H, *br s*), 3.02 (1H, *t*, H-3), 3.65 (3H, *s*), 4.62 (2H, *d*, *J* = 12 Hz). MS *m/z* 470 $[M]^+$, 452, 410, 262, 220, 207, 203, 189 (100), 175, 135.

Reduction of **2**. Performed as above for **1**, affording morolic acid methyl ester (**4**), mp 230–233°, $[\alpha]_D$ + 28° ($CHCl_3$; *c* 0.2) (lit. [13], mp 228–229°, $[\alpha]_D$ + 26°. ^{1}H NMR: δ 0.75 (6H, *s*), 0.85 (3H, *s*), 0.94 (12H, *s*), 3.15 (1H, *t*, 3-H), 3.66 (3H, *s*), 5.14 (1H, *s*).

Reduction of **5**. A soln of **5** (59 mg) in dry MeOH (10 ml) was reduced as described above for **1**, yielding the alcohol **6** (40 mg), and **7** (15 mg). Compound **6** had mp 278–280°. ^{1}H NMR (pyridine-d_5): δ 0.91, 1.05, 1.08, 1.41, 1.53, 1.61 and 1.72 (each 3H, *s*), 3.46 (1H, *t*, H-3), 3.73 (3H, *s*), 4.83 (1H, *m*, H-6), 5.15 (1H, *s*, H-19). MS *m/z* 486 $[M]^+$, 468, 450, 427, 409, 391, 302, 287, 262, 249, 203, 189 (100), 187, 173, 133. Compound **7** had ^{1}H NMR: δ 0.74, 0.93, 0.97, 1.00 and 1.20 (each 3H, *s*), 1.23 (6H, *s*), 3.38 (1H, *t*, H-3), 3.67 (3H, *s*), 7.37 (1H, *m*, H-6), 5.12 (1H, *s*, H-19). MS *m/z* 485 $[M]^+$, 468, 450, 427, 409, 391, 287, 262, 249, 206, 203, 189 (100), 187, 173, 133.

Acetylation of **6**. A soln of **6** (38 mg) in pyridine was treated with Ac_2O overnight at room temp. Usual work-up gave **8** (40 mg), mp 320°. M^+ *m/z* 528.3806 (calcd for $C_{33}H_{52}O_5$ 528.3814). IR ν_{max} cm^{-1}: 3600, 1710, 1240, 840. ^{1}H NMR: δ 0.72, 0.93, 0.97, 0.99 and 1.22 (each 3H, *s*), 1.28 (6H, *s*), 2.07 (3H, *s*), 3.69 (3H, *s*), 4.45 (1H, *t*, H-3), 4.50 (1H, *m*, H-6), 5.13 (1H, *s*, H-19). MS *m/z* 528 $[M]^+$, 469, 450, 435, 391, 302, 262, 249, 203, 189 (100), 187, 173, 133.

Oxidation of **8**. The monoacetate **8** in dry CH_2Cl_2 (3 ml) was treated with pyridinium dichromate (5 mg) with stirring for 1 hr. After dilution with Et_2O and filtration through Celite, **9** was obtained; mp 228–230°, M^+ *m/z* 526.3655 (calcd for $C_{33}H_{50}O_5$, 526. 3658). IR ν_{max} cm^{-1}: 1715, 1705, 1240, 830. ^{1}H NMR: δ 0.91 (9H, *s*), 0.98 (9H, *s*), 1.26 (3H, *s*), 2.05 (3H, *s*), 3.63 (3H, *s*), 4.41 (1H,

t, H-3), 5.17 (1H, *s*, H-19). MS *m/z* 526 $[M]^+$, 466, 451, 407, 389, 249, 189 (100).

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CO-OCCURRENCE OF C-24 EPIMERIC 24-METHYL-$\Delta^{5,22}$-STEROLS IN THE SEEDS OF SOME *BRASSICA* AND *RAPHANUS* SPECIES OF CRUCIFERAE

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Key Word Index—*Brassica campestris*; *B. hirta*; *B. juncea*; *B. oleracea*; *B. napus*; *Raphanus sativus*; Cruciferae; seeds; sterol; brassicasterol; 22-dehydrocampesterol; reverse-phase HPLC.

Abstract—Reverse-phase HPLC has shown that the 24-methyl-$\Delta^{5,22}$-sterol fractions isolated from 27 seed and seed oil samples of *Brassica* and *Raphanus* species (Cruciferae) consist of *ca* 10–40% of the 24α-epimer, 22-dehydrocampesterol, in addition to the 24β-epimer, brassicasterol.

The seeds of some species of Cruciferae, e.g. *Brassica*, *Iberis* and *Raphanus*, can be characterized by the presence of appreciably large amounts (8–23%) of 24-methyl-$\Delta^{5,22}$-sterol in the 4-demethylsterol fraction of the unsaponifiable lipids [1]. After a 24β-methyl configuration was established, by chemical correlation with ergosterol (24β-methylcholesta-5,7,*trans*-22-trien-3β-ol), for the 24-methyl-$\Delta^{5,22}$-sterol (brassicasterol, **1**) isolated from the seeds of *B. rapa* [2], the 24-methyl-$\Delta^{5,22}$-sterol detected in the seeds of many Cruciferae plants was, to the best of the authors' knowledge, tentatively named 'brassicasterol' without proving the stereochemistry at C-24. Recently, we have examined by ^{13}C NMR spectroscopy the 24-methyl-$\Delta^{5,22}$-sterol fractions isolated from eight seed samples of *Brassica* sp. and demonstrated that all of the fractions were mixtures, epimeric at C-24, i.e. brassicasterol (24β-methylcholesta-5,*trans*-22-dien-3β-ol, **1**) and 22-dehydrocampesterol (24α-methylcholesta-5,*trans*-22-dien-3β-ol, **2**) [3]. More recently, the presence of **2** in the seeds of *B. juncea* was unambiguously proved by 400 MHz ^1H NMR spectroscopy after isolation by means of reverse-phase HPLC [4]. Expecting the widespread occurrence of **2**, accompanying **1** in the seeds of Cruciferae that contain 24-methyl-$\Delta^{5,22}$-sterols, we have undertaken a further investigation by HPLC of the 24-methyl-$\Delta^{5,22}$-sterol fractions isolated from three seed oil samples of *Brassica* sp. and 24 seed samples, including those previously examined by ^{13}C NMR [3], of the following Cruciferae: *Brassica campestris*, *B. hirta*, *B. juncea*, *B. oleracea*, *B. napus* and *Raphanus sativus*.

The acetylated 24-methyl-$\Delta^{5,22}$-sterol fractions, separated from the unsaponifiable lipids of the seed and seed oil samples of Cruciferae in the same manner as described previously [3], showed RR_t 1.10 (cholesteryl acetate, RR_t 1.00) on GLC (OV-1 glsss capillary column). This is consistent with the RR_t of the **1**- and **2**-acetates [4]. The steryl acetates were saponified and the resulting free sterol fractions were subjected to reverse-phase HPLC. The sterol fraction from each of the Cruciferae samples was separated into two well-resolved component peaks by HPLC with RR_t values of 0.86 and 0.92 (cholesterol, RR_t 1.00), which are in accord with those of authentic **2** and **1**, respectively. Thus, the two sterols isolated by HPLC could be identified as brassicasterol (**1**) and 22-dehydrocampesterol (**2**). Identification of these sterols was substantiated by their mass spectra, and further by the