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SATURATED HOPANE AND GAMMACERANE TRITERPENE-DIOLS FROM THE STEM BARK OF *ABIES VEITCHII*

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Key Word Index—*Abies veitchii*; Pinaceae; stem bark; triterpenoid; hopan-3α,22-diol; gammaceran-3β-21α-diol.

Abstract—Two saturated pentacyclic triterpene diols were isolated from the stem bark of *Abies veitchii* and their structures characterized as hopan-3α,22-diol and gammaceran-3β,21α-diol on the basis of chemical and spectral evidence.

INTRODUCTION

Previously, we have reported the isolation and structural determination of four 9β-lanostane triterpene lactones, **1**–**4**, and two lanostane type analogues, veitchiolide (**5**) and **6** from the stem bark of *Abies veitchii* Lindl. [1, 2]. Recently, we have also reported that several γ-keto acids, **1a** and **2a**, and their methyl esters, **1b** and **2b**, and the trisnor-α-hydroxy acid (**1c**) prepared from compounds **1** and **2** had a remarkable biological activity [3]. We have now isolated two new saturated pentacyclic triterpene diols, **7** and **8**, from the ether-soluble fraction of the methanol extract of the stem bark. The present paper deals with characterization of these compounds.

RESULTS AND DISCUSSION

Compound **7** was assigned the molecular formula $C_{30}H_{52}O_2$ (HRMS $[M]^+$ m/z 444.3964, requires 444.3967). It gave positive colour with Liebermann–Burchard reagent and showed an IR absorption band for a hydroxyl group at 3375, 1058 and 1025 cm^{-1}. Its ^1H and ^{13}C NMR spectra (Table 1) and EI mass spectrum (Scheme 1) suggested that the structure of **7** is not the known hopan-3β,22-diol [4] or 21α-*H*-hopan-3β,22-diol [5] but hopan-3α,22-diol. Oxidation of **7** with chromium trioxide in pyridine afforded a ketol (**7a**) having identical physical and spectral data with those of hydroxyhopanone [6, 7]. Consequently, the structure of **7** was established to be hopan-3α,22-diol. Compound **7** does not appear to have been previously isolated from natural sources, although it was biosynthesized by incubating a cell free system from *Acetobacter rancens*, *A. pasteurianum* or *Methylococcus capsulatus* with 2,3-epoxy-2,3-dihydrosqualene [8, 9].

Compound **8** has the molecular formula $C_{30}H_{52}O_2$ ($[M]^+$: m/z 444.3968; requires 444.3967) and showed positive colour with Liebermann–Burchard reagent. On acetylation it gave a diacetate (**8a**). In spite of the molecular formula, the ^1H and ^{13}C NMR spectra of **8** (Table 1) exhibited signals for only four methyl singlets and 15 carbons, respectively, indicative of a symmetric structure. Physical, IR, ^1H NMR and EI mass spectral data (Scheme 2) of **8** and **8a** were in good agreement with those of gammaceran-3β,21α-diol and its diacetate which have previously been synthesized from α-onocerin via γ-onocerin [10, 11] and also biosynthesized by the incubation of 2,3-epoxy-2,3-dihydro-squalene with a cell-free system from the protozoan *Tetrahymena pyriformis* [12], and thus the structure was unequivocally proved.

To the best of our knowledge, this is the first report for the isolation of **8** from plant sources, although tetrahymanol (**11**) was isolated from *T. pyriformis* [13] and gammacerane hydrocarbon and **11** have also been found in various crude petroleums, petroleum source rocks and geological sediments collected worldwide [14–16]. However, distribution of gammaceranes as chemical constituents in the plant kingdom are extremely rare and a literature survey revealed only the following studies dealing with the isolation of gammaceranoids from plant sources; (i) **11** from several polypodiaceous, pteridaceous, davallidaceous and aspidaceous ferns [17, 18], (ii) coriandrinonediol (**12**) (planar structure) from *Coriandrum sativum* (Umbelliferae) [19], (iii) monechmol (**13**) and its 3-*O*-β-D-glucoside (**14**) from *Monechma debile* (Acanthaceae) [20], (iv) gammacer-16-en-3α-ol (**15**), gammacer-16-en-3β-ol (**16**) and its acetate (**17**) along with two migrated gammaceranes, pichienyl acetate (**18**) and isopichienyl acetate (**19**), from *Picris hieracioides* subsp. *japonica* (Compositae) [21] and (v) compound **15** and three migrated gammaceranes, swertanone (**20**), swertanol (**21**) and episwertanol (**22**) from *Swertia chirata* (Gentianaceae), together with hop-22(29)-en-3β-ol (**23**) and a migrated hopane, chiratenol (**24**) [22–24].

It is worthy to note that **8** was isolated first together with **7** from *A. veitchii*, a Pinaceae tree having ancient morphological features, thought to have appeared between the Jurassic and Cretaceous periods in the East Eurasian Continent and growing now only in the subalpine belts of the Central Japan [25, 26], although it is well known that the origin of coniferous plants on the Earth was far later than that of ferns.

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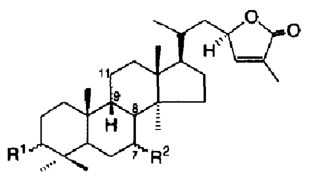
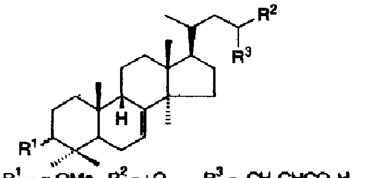
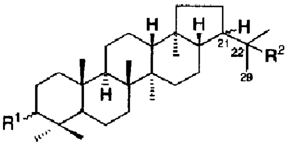


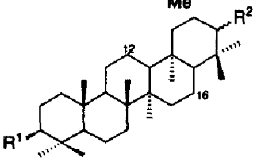
1 $R^1 = \alpha$-OMe, $R^2 = H$, Δ^7- ene
2 $R^1 = :O$, $R^2 = H$, Δ^7- ene
3 $R^1 = \alpha$-OH, $R^2 = H$, Δ^7- ene
4 $R^1 = \beta$-OH, $R^2 = H$, Δ^7- ene
5 $R^1 = \alpha$-OMe, $R^2 = \alpha$-OH, $\Delta^{9(11)}$- ene
6 $R^1 = \alpha$-OMe, $R^2 = H$, $\Delta^{7,9(11)}$- diene



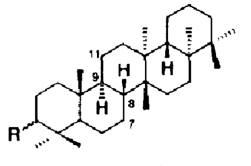
1a $R^1 = \alpha$-OMe, $R^2 = :O$, $R^3 = -CH_2\underset{Me}{C}HCO_2H$
1b $R^1 = \alpha$-OMe, $R^2 = :O$, $R^3 = -CH_2\underset{Me}{C}HCO_2Me$
1c $R^1 = \alpha$-OMe, $R^2 = \underset{H}{\overset{OH}{\cdot}}$, $R^3 = CO_2H$
2a $R^1 = R^2 = :O$, $R^3 = -CH_2\underset{Me}{C}HCO_2H$
2b $R^1 = R^2 = :O$, $R^3 = -CH_2\underset{Me}{C}HCO_2Me$



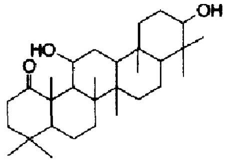
7 $R^1 = \alpha$-OH, $R^2 = OH$, 21β-H
7a $R^1 = :O$, $R^2 = OH$, 21β-H
7b $R^1 = H_2$, $R^2 = OH$, 21β-H
9 $R^1 = \beta$-OH, $R^2 = OH$, 21β-H
10 $R^1 = \beta$-OH, $R^2 = OH$, 21α-H
23 $R^1 = \beta$-OH, $R^2 = H$, $\Delta^{22(29)}$- ene



8 $R^1 = \beta$-OH, $R^2 = \alpha$-OH
8a $R^1 = \beta$-OAc, $R^2 = \alpha$-OAc
11 $R^1 = \beta$-OH, $R^2 = H_2$
13 $R^1 = \beta$-OH, $R^2 = H_2$, Δ^{12}- ene
14 $R^1 = \beta$-O-Glu, $R^2 = H_2$, Δ^{12}- ene
15 $R^1 = \alpha$-OH, $R^2 = H_2$, Δ^{16}- ene
16 $R^1 = \beta$-OH, $R^2 = H_2$, Δ^{16}- ene
17 $R^1 = \beta$-OAc, $R^2 = H_2$, Δ^{16}- ene



18 R = β-OAc, $\Delta^{9(11)}$- ene
19 R = β-OAc, Δ^8- ene
20 R = O, Δ^7- ene
21 R = β-OH, Δ^7- ene
22 R = α-OH, Δ^7- ene



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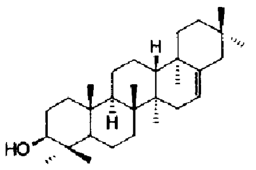

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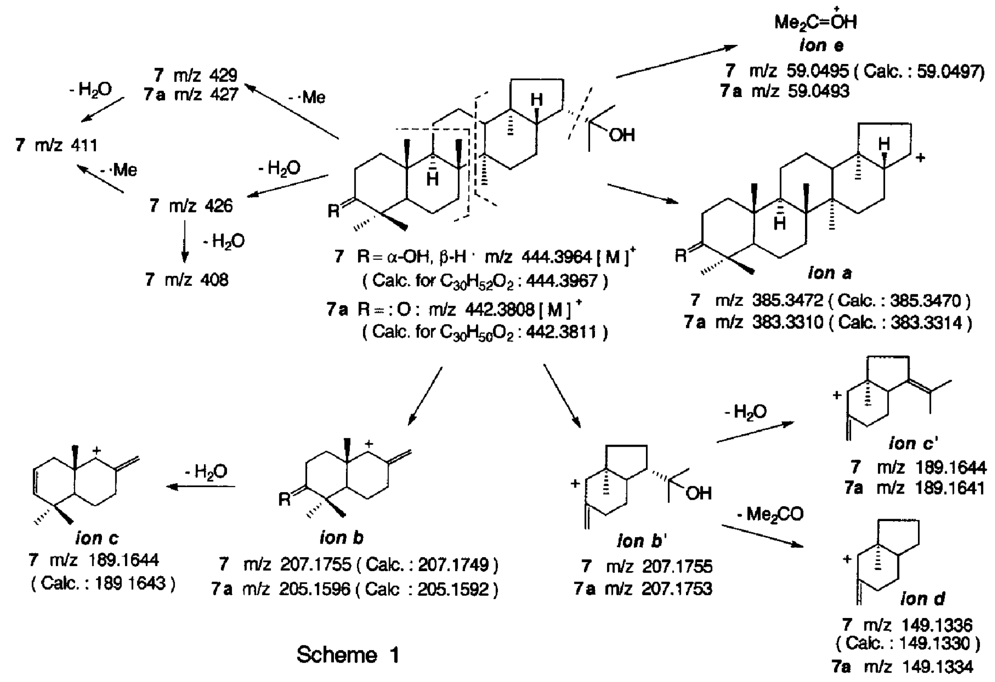
EXPERIMENTAL

General. Mps: uncorr. Optical rotations: $CHCl_3$; IR: KBr discs; ^1H (300 MHz) and ^{13}C (74.5 MHz) NMR: $CDCl_3$ with TMS as int. standard; EIMS (probe): 70 eV; CC silica gel 60 (70–230 mesh, Merck). TLC: silica gel HF_{254} and PF_{254} (Merck).

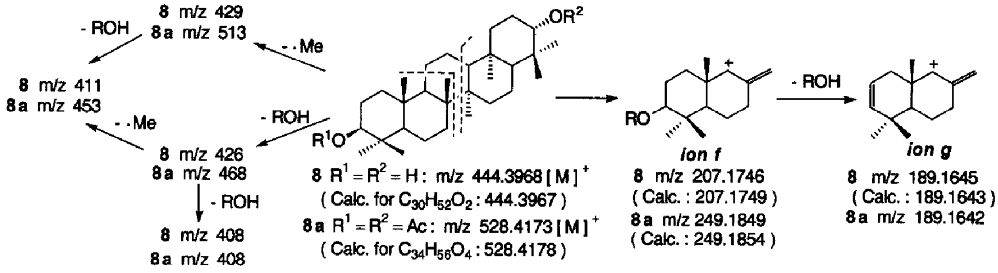
Extraction and isolation of compounds. Collection of the plant material, extraction, and separation of the Et_2O extract of the chopped and air-dried stem bark of *A. veitchii* (7.9 kg) on silica gel CC were as previously described [1, 2]. The stem bark was further extracted × 6 with boiling MeOH (20 l) for 5 hr. Removal of the solvent *in vacuo* gave a viscous residue, which was dissolved in Et_2O (2 l) and the resulting Et_2O soln was filtered to remove insoluble materials. Evapn of the Et_2O soln gave a brown residue (493.4 g), which was subjected to CC on silica gel (7 kg). Elution of the column with $CHCl_3$–EtOAc (20:1) (frs 117–141, each 1 l) afforded a crystalline solid (10.414 g). Two separations by CC of the solid with silica gel (300 and 100 g, respectively) furnished **7** (11 mg, 0.00013% of the dried bark) as a minor constituent from the fractions eluted with $CHCl_3$. Subsequent CC afforded **8** (271 mg, 0.0034%) from the frs eluted with a mixture of $CHCl_3$ and EtOAc (20:1).

Compound **7**. Leaflets, mp 278–280.5° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ +31° (*c* 0.18); IR ν_{max} cm^{-1}: 3375 (OH), 2923, 2850, 1455, 1385, 1358, 1139, 1058 and 1025; ^1H and ^{13}C NMR: see Table 1; EIMS: *m/z* (rel. int.) 444.3964 (9) $[M]^+$ (calc. for $C_{30}H_{52}O_2$: 444.3967), 429 (2) $[M-Me]^+$, 426 (7) $[M-H_2O]^+$, 411 (5) $[M-Me-H_2O]^+$, 385.3470 (5) $[M-C_3H_7O]^+$ (ion *a*), 207.1755 (79) $[C_{14}H_{23}O]^+$ (ions *b* and *b'*), 189.1644 (100) $[C_{14}H_{21}]^+$ (ions *c* and *c'*), 149.1336 (68) $[C_{11}H_{17}]^+$ (ion *d*) and 59.0495 (63) $[Me_2$: C=OH$]^+$ (ion *e*).

CrO_3 oxidation of compound **7**. To a soln of **7** (9 mg) in pyridine (1 ml) was added a soln of CrO_3 (9 mg) in pyridine (1.5 ml) with stirring at room temp. for 5 hr. Work-up as usual furnished a residue (9 mg), which was purified by prep. TLC (plate: 0.5 mm thick; C_6H_6–$CHCl_3$–EtOAc, 2:2:1) to give ketol **7a** as needles, mp 250–252° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ +62°, (lit.



Scheme 1



[4]: mp 250–252°, $[\alpha]_D$ +62° in $CHCl_3$); IR ν_{max} cm^{-1}: 3375, 2925, 2848, 1710 (C=O), 1460, 1385, 1358 and 1140; ^1H and ^{13}C NMR: see Table 1; EIMS: *m/z* (rel. int.) 442.3808 (10) $[M]^+$, 427 (3) $[M-Me]^+$, 424 (8) $[M-H_2O]^+$, 409 (11) $[M-H_2O-Me]^+$, 383.3314 (8) $[M-C_3H_7O]^+$ (ion *a*), 207.1753 (36) $[C_{14}H_{23}O]^+$ (ion *b'*), 205.1596 (30) $[C_{14}H_{21}O]^+$ (ion *b*), 189.1641 (82) $[C_{14}H_{21}]^+$ (ions *c* and *c'*), 149.1334 (100) $[C_{11}H_{17}]^+$ (ion *d*) and 59.0493 (53) [Me:C=OH]$^+$ (ion *e*). Physical and spectral data (mp, $[\alpha]_D$, IR, ^1H NMR and EIMS) of compound **7a** were identical with those of hydroxyhopanone already reported in the literature [4, 6, 7].

Compound **8**. Leaflets, mp above 310° (MeOH-$CHCl_3$), $[\alpha]_D^{23}$ +21° (*c* 0.36), (lit. [10]: mp 318–321°, $[\alpha]_D$ +23°), IR ν_{max} cm^{-1}: 3400 (OH), 2927, 2852, 1450, 1380, 1357, 1180, 1138, 1105, 1066 and 1022 (C–O); ^1H and ^{13}C NMR: see Table 1; EIMS: *m/z* (rel. int.) 444.3968 (16) $[M]^+$ (calc. for $C_{30}H_{52}O_2$: 444.3967), 429 (2) $[M-Me]^+$, 426 (3) $[M-H_2O]^+$, 411 (4) $[M-Me-H_2O]^+$, 408 (1) $[M-2H_2O]^+$, 207.1746 (100) $[C_{14}H_{23}O]^+$ (ion *f*) and 189.1646 (52) $[C_{14}H_{21}]^+$ (ion *g*).

Acetylation of compound **8**. Compound **8** (50 mg) was acetylated (2 ml Ac_2O–pyridine, 1:1) at room temp. for 24 hr. Usual work-up afforded a residue, which was subjected to prep. TLC (plate 2 mm thick, $CHCl_3$) to give a solid. Recrystallization of the solid from MeOH–$CHCl_3$ furnished diacetate **8a** (48 mg), as leaflets, mp above 310°, $[\alpha]_D^{23}$ +36° (*c* 0.42) (lit. [10]: mp above 355°, $[\alpha]_D$ +36°); IR ν_{max} cm^{-1}: 2945, 2875, 1724 (OAc), 1487, 1465, 1440, 1386, 1375, 1245 (OAc), 1142, 1068, 1020, 980, 943 and 900; ^1H and ^{13}C NMR: see Table 1; EIMS: *m/z* (rel. int.) 528.4173 (8) $[M]^+$ (calc. for $C_{34}H_{56}O_4$: 528.4178), 513 (3), 468.3951 (8) $[M-HOAc]^+$, 408.3749 (2) $[M-2\times HOAc]^+$, 249.1849 (21) $[C_{16}H_{25}O_2]^+$ (ion *f*) and 189.1643 (100) $[C_{14}H_{21}]^+$ (ion *g*). Physical and spectral data (mp, $[\alpha]_D$, IR, ^1H NMR and EIMS) of compounds **8** and **8a** were identical with those of gammacerane-3β,21α-diol and its diacetate, respectively, already reported in the literature [10–12].

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Table 1. ^{1}H (300 MHz) and ^{13}C NMR (74.5 MHz) spectral data of compounds **7**, **7a**, **8** and **8a** (in $CDCl_3$, TMS = 0)*

	^{1}H				^{13}C			
C	**7**	**7a**	**8**	**8a**	**7**	**7a**	**8**	**8a**
1					33.2	39.6	38.7	38.4
2					25.3	34.2	27.4	23.7
3	3.39 *t* J=3.1	—	3.20 *dd* J=11.6, 5.7	4.47 *dd* J=11.6, 5.7	76.2	218.2	79.0	81.0
4					37.5	47.4	38.8	37.8
5					48.8	54.9	55.1	55.2
6					18.3	19.7	18.4	18.2
7					33.2	32.6	33.1	33.0
8					41.8	41.9	41.7	41.7
9					50.0	50.0	50.2	50.1
10					37.2	36.8	37.0	36.9
11					20.9	21.5	21.2	21.1
12					24.1	24.1	21.2	21.1
13					49.9	49.6	50.2	50.1
14					41.9	41.6	41.7	41.7
15					34.7	34.4	33.1	33.0
16					21.9	21.9	18.4	18.2
17					53.9	53.9	55.1	55.2
18					44.1	44.1	37.0	36.9
19					41.3	41.3	38.7	38.4
20					26.6	26.6	27.4	23.7
21	—	—	3.20 *dd* J=11.6, 5.7	4.27 *dd* J=11.6, 5.7	51.1	51.1	79.0	81.0
22					73.9	73.9	38.8	37.8
23	0.76	1.08	0.97	0.84	28.3	26.6	28.0	28.0
24	0.94	1.02	0.76	0.84	22.1	21.1	15.4	16.4
25	0.83	0.93	0.82	0.85	15.7	15.7	16.0	16.0
26	0.96	1.00	0.96	0.95	17.0	16.4	16.5	16.5
27	0.96	0.96	0.96	0.95	17.1	16.9	16.5	16.5
28	0.76	0.77	0.82	0.85	16.2	16.2	16.0	16.0
29	1.18	1.18	0.76	0.84	28.7	28.7	15.4	16.4
30	1.21	1.21	0.97	0.84	30.9	30.9	28.0	28.0
OCO$\underline{\text{Me}}$	—	—	—	2.04	—	—	—	21.3
OCO$\underline{\text{Me}}$	—	—	—	2.04	—	—	—	21.3
O$\underline{\text{C}}$OMe	—	—	—	—	—	—	—	171.0
O$\underline{\text{C}}$OMe	—	—	—	—	—	—	—	171.0

*Assignments were made by 2D ^{1}H–^{1}H COSY, 2D ^{1}H–^{13}C COSY and 2D long range ^{1}H–^{13}C COSY experiments.

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