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TRITERPENES FROM *STAUNTONIA HEXAPHYLLA* CALLUS TISSUES

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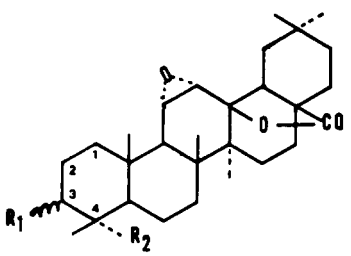
ABSTRACT.—A new triterpene **1** has been isolated from the extract of *Stauntonia hexaphylla* callus tissues. The structure was elucidated as 3α-hydroxy-11α,12α-epoxyoleanan-28,13β-olide [**1**] by ^{1}H-nmr, ^{13}C-nmr, and ms spectroscopic studies. Two known compounds, 28-norolean-12-ene-3β,17β-diol [**2**] and 11α-hydroxy-β-amyrin [**3**], were also isolated.

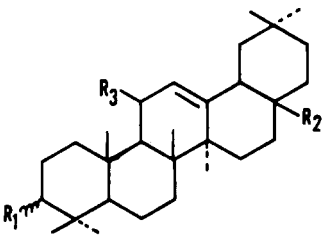
In an earlier paper, we reported the isolation and characterization of seventeen oleanane-type triterpenes including the 30-noroleanane type triterpenes and two phytosterols from callus tissues of *Stauntonia hexaphylla* Decne (Lardizabalaceae) (1). Eight of them were four pairs of 3-epimers. Recently, a new saponin, mubenoside A, was reported from the same callus tissues (2). Furthermore, a hypothetical biogenetic relationship between the 30-noroleanane triterpenes and the twelve other triterpenes isolated from the callus tissues of *St. hexaphylla* has been discussed (1). We now report the structural elucidation of three minor triterpenes, one of which is a new compound.

RESULTS AND DISCUSSION

Crude triterpene mixtures were obtained from the tissue culture as described in the Experimental section. The mixtures were chromatographed on hplc repeatedly, and compounds **1–3** were obtained. The ^{1}H-nmr spectrum (pyridine-d_5) of **1** showed the presence of seven characteristic tertiary methyl signals and two proton signals at δ 3.18 (m, 1H) and 3.26 (d, 1H, J = 3.7 Hz) (δ 3.01, s, 2H in $CDCl_3$) ascribable to epoxide protons C-11 and C-12. Moreover, the spectrum showed no other signal below 3.63 ppm, which suggested an α configuration for the hydroxy group at C-3. The ^{13}C-nmr spectrum of **1** further exhibited a signal at δ 75.1 (d) for C-3, which had a higher field chemical shift than that (δ 78.2 ppm) of the compounds with 3β-hydroxy configuration (Table 1). From the above-mentioned ^{1}H- and ^{13}C-nmr spectral data, the presence of an 3α-hydroxy group in **1** was presumed. Furthermore, the ^{13}C-nmr spectrum of **1** was compared with that of 3α-oleanolic acid [**7**] isolated from the same callus tissues (1), for the A-ring, and was identical. The ir spectrum of **1** showed an intense band at



1 R_1= ||||| OH, R_2=Me
4 R_1= ▬► ara, R_2=CH_2OH
8 R_1= ▬► OH, R_2=Me



2 R_1= ▬► OH, R_2=OH, R_3=H
3 R_1= ▬► OH, R_2=Me, R_3= ||||| OH
5 R_1= ▬► OH, R_2=COOH, R_3=H
6 R_1= ▬► OH, R_2=Me, R_3=H
7 R_1= ||||| OH, R_2=COOH, R_3=H

TABLE 1. ^{13}C-nmr Chemical Shifts of Compounds **1–7** (C_5D_5N, δ ppm).[a]

Carbon	Compound						
	1	**4**	**7**	**2**	**5**	**3**	**6**
C-1	33.0	38.5	33.6	39.0	39.0	39.5	38.5
C-2	27.5	25.7	26.3	28.1	28.1	27.4	27.0
C-3	75.1	81.6	75.2	78.2	78.2	78.7	78.9
C-4	37.0	43.6	37.6	39.4	39.4	39.0	38.7
C-5	48.6	47.1	49.3	55.9	55.9	55.1	55.1
C-6	17.3	17.4	18.7	18.9	18.9	18.4	18.3
C-7	31.5	31.2	33.3	33.5	33.3	32.9	32.6
C-8	40.9	40.9	39.9	40.0	39.8	43.3	39.7
C-9	49.8	49.8	47.9	48.3	48.2	49.7	47.6
C-10	37.0	36.4	37.9	37.5	37.4	37.9	37.0
C-11	52.9	52.8	23.7	24.0	23.8	81.7	23.4
C-12	57.4	57.3	122.6	122.7	122.6	121.2	121.7
C-13	88.0	87.7	144.8	145.7	144.9	153.2	145.0
C-14	41.9	41.7	42.3	42.1	42.2	41.8	41.7
C-15	27.0	27.1	28.3	28.1	28.4	26.4	26.2
C-16	21.7	21.6	23.8	26.4	23.8	27.4	27.3
C-17	44.1	44.1	46.5	71.0(s)	46.6	32.3	32.5
C-18	51.1	51.3	42.0	48.8	42.1	46.9	47.2
C-19	38.0	38.0	46.7	49.0	46.7	46.9	46.8
C-20	31.6	31.5	30.9	31.2	31.0	31.2	31.1
C-21	34.4	34.4	34.3	37.1	34.3	34.7	34.8
C-22	27.7	27.6	33.2	38.8	33.3	37.0	37.2
C-23	29.1	13.0	29.3	28.9	28.8	28.2	28.1
C-24	22.2	64.3	22.7	16.6	16.6	15.5	15.5
C-25	17.3	17.8	15.4	15.6	15.6	18.3	15.5
C-26	18.8	18.8	17.5	17.9	17.5	16.8	16.8
C-27	20.5	20.4	26.1	25.8	26.2	24.7	26.0
C-28	180.0	179.0	180.1	—	180.3	28.5	28.3
C-29	33.6	33.1	33.2	33.0	33.3	33.3	33.2
C-30	23.5	23.5	23.8	24.2	23.8	23.7	23.6

[a]Multiplicities were established by DEPT experiments. Compounds **3** and **6** were measured in $CDCl_3$.

1765 cm^{-1} indicating the presence of a γ-lactone. The ^{13}C nmr exhibited characteristic peaks at δ 52.9 (d) for C-11, 57.4 (d) for C-12, and 88.0 (s) for C-13, which agreed closely with those of the epoxy-γ-lactone moiety reported for the saponin **4** isolated from *Hedera rhombea* (3). Other chemical shifts of the ^{13}C nmr of **1** also coincided with those of the genin moiety of **4** except for predictable differences for the A ring (Table 1).

The mass spectrum of **1** exhibited the molecular ion at *m/z* 470 and showed characteristic fragments at *m/z* 207 and 189 comprising the A/B rings in **1**. Other fragments were similar to those of compound **8** (4,5). The combined spectral evidence led to the conclusion that compound **1** is 3α-hydroxy-11α,12α-epoxyoleanan-28,13β-olide, a 3-epimer of **8**. Compound **1** is a new compound.

Compound **2** showed seven methyl signals in the ^{1}H-nmr spectrum, and the ir spectrum of **2** showed bands for two hydroxy groups (ν 3685 and 3620 cm^{-1}). The ^{13}C-nmr spectrum of **2** showed 29 carbon signals, indicating a nortriterpenoid. The signals at δ 78.2 (d) and 71.0 (s) ppm indicated the presence of secondary and tertiary hydroxy groups, respectively. The signal of the carbonyl group usually observed in oleanolic acid [**5**] disappeared. However, the ^{13}C signals showed strikingly similar chemical shifts to those of **5** except for those of the D/E rings, and the signal assigned to C-17

showed a chemical shift at 71.0 ppm (s) which was ca. 25 ppm lower than that of **5** (Table 1). The mass spectrum of **2** showed the molecular ion at *m/z* 428 $[M]^+$ and a fragment corresponding to loss of H_2O at *m/z* 410 $[M-18]^+$.

Peaks due to retro-Diels-Alder cleavage (6) of the ring C/D were also observed at *m/z* 207, 202, and 189. The conformation of the D/E ring of **2** was determined as cis from observation of the rOe by a ROESY experiment between H-18 (β) and H-12. These physical data were identical with those reported for 28-norolean-12-ene-3β,17β-diol isolated from the pericarps of *Sapindus mukurossi* (Sapindaceae) (7). Compound **2** appears to be the second naturally occurring as 17-hydroxy nortriterpene compound.

The ^{1}H-nmr spectrum of compound **3** showed an olefinic proton at δ 5.44 (1H, d, $J=3.56$ Hz), protons of two hydroxymethine groups at δ 4.50 (1H, dd, $J=3.56$, 9.0 Hz) and 3.25 (1H, dd, $J=4.9$, 11.4 Hz), and eight methyl signals. The ^{1}H-nmr data of **3** were similar to those of 11α-hydroxy-β-amyrin reported from *Salvia glutinosa* (8). The structure of **3** was further supported by the ^{13}C-nmr data (Table 1) and by the mass spectrum. These three compounds have not been previously reported from *St. hexaphylla*. The callus tissues of *St. hexaphylla* produced 20 triterpenes, five of which were 3α derivatives. The occurrence of so many 3-epimers of oleanane triterpenes from the callus tissues of one plant is rare, and it is interesting from the standpoint of the metabolic ability of the callus tissues of *St. hexaphylla*.

EXPERIMENTAL

All mp's were uncorrected. Ir spectra were run in $CHCl_3$. ^{1}H nmr spectra were run at 200 MHz and 400 MHz and ^{13}C nmr spectra were recorded at 100.6 MHz at room temperature with $CDCl_3$ and pyridine-d_5 solutions with TMS as internal standard. Multiplicities for the ^{13}C-nmr spectra were determined by DEPT spectra at 90° and 135°. Mass spectra (70 eV) were taken with a direct probe.

PLANT MATERIAL.—*St. hexaphylla* was collected in August 1982 at the Medicinal Plant Garden of Tokyo College of Pharmacy. A voucher specimen (No. 7900074) is deposited in the plant garden.

DERIVATION AND CULTURE OF CALLUS TISSUES.—Callus tissues from the stalk were established in February 1983. Murashige and Skoog medium (minus glycine) (M&S) containing 2,4-D (1 mg/liter, 3 mg/liter) and kinetin (0.1 mg/liter) as plant growth regulators were used for induction of callus tissues. The callus tissues were subcultured every 5–6 weeks onto fresh M&S containing 2,4-D (1 mg/liter) and kinetin (0.1 mg/liter) at 26 ± 1° in the dark.

EXTRACTION AND ISOLATION.—The fresh callus tissues (820 g, fresh wt) were extracted with cold MeOH and EtOAc in a Waring blender. The solution was combined and concentrated under reduced pressure. The residue was partitioned between $CHCl_3$ and H_2O to obtain the organic-solvent-soluble fraction. The $CHCl_3$ solution was evaporated and chromatographed over a column of Si gel (Merck 9385), and gradient elution with $CHCl_3$ with increasing proportions of MeOH afforded the crude triterpene mixtures. The mixtures were purified repeatedly by cc on a Si gel column (CIG column system, Kusano) with hexane/EtOAc/MeCN, which afforded compounds **1–3** as minor constituents.

3α-Hydroxy-11α,12α-epoxyoleanan-28,13β-olide [**1**].—Mp 251–255° (dec); $CHCl_3$/MeOH colorless powder; $[\alpha]^{18}D$ +25 ($c=0.04$, $CHCl_3$); ir ν max ($CHCl_3$) 1765 cm^{-1}; ^{1}H nmr (C_5D_5N, δ, ppm) 0.78 (s, 3H), 0.87 (s, 3H), 0.89 (s, 3H), 1.01 (s, 3H), 1.10 (s, 3H), 1.20 (s, 3H), 1.26 (s, 3H), 2.52 (dd, 15.4, 3.6 Hz, 1H), 3.18 (m, 1H), 3.26 (d, 3.7 Hz), 3.63 (bs, 1H); eims *m/z* (%) $[M]^+$ 470 (50), 263 (33), 249 (15), 235 (50), 217 (28), 207 (24), 205 (43), 204 (42), 203 (63), 189 (100), 187 (32), 175 (49).

28-Norolean-12-ene-3β,17β-diol [**2**].—Mp 172–175°; colorless powder; $[\alpha]^{18}D$ + 54.4° ($c=0.062$, $CHCl_3$); ir ν max ($CHCl_3$) 3685, 3620 cm^{-1}; ^{1}H nmr (C_5D_5N, δ, ppm) 0.95 (s, 3H, Me-29), 0.99 (s, 3H, Me-25), 1.00 (s, 3H, Me-30), 1.08 (s, 3H, Me-24), 1.25 (s, 3H, Me-26), 1.28 (s, 3H, Me-27), 1.29 (s, 3H, Me-23), 2.56 (m, 2H, H-18, H-16), 3.48 (m, 1H, H-3), 5.34 (t, 1H, 4.0 Hz); sims *m/z* (%) $[M]^+$ 428 (1.5%), $[M-18]^+$ 410 (6.5) (a), 207 (7), 202 (100), 189 (7).

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