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A TRITERPENE FROM *AKEBIA QUINATA* CALLUS TISSUE

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Key Word Index—*Akebia quinata*; Lardizabalaceae; callus tissue; triterpene; 3α,24-dihydroxy-30-norolean-12,20(29)-dien oic-28-acid; quinatic acid.

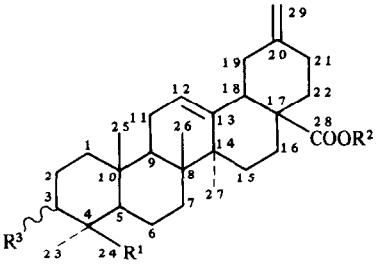
Abstract—A new triterpene was isolated from the methanol extracts of *Akebia quinata* tissue culture. The structure was determined by spectra and chemical transformations to be 3α,24-dihydroxy-30-norolean-12,20(29)-dien-28-oic acid.

INTRODUCTION

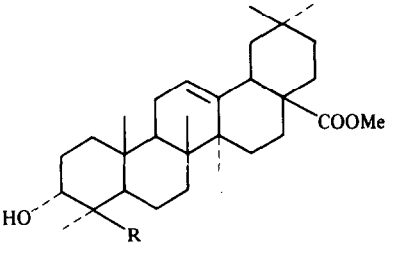
Recently we reported the isolation and structure determination of new triterpenes akebonic acid and its epimer together with two known triterpenes from the extract of the callus tissues of *Akebia quinata* (Lardizabalaceae, Japanese name Akebi [1]). In this paper, we report the isolation of a new triterpene.

RESULTS AND DISCUSSION

The crude triterpene mixture was obtained from the tissue culture as described in the Experimental. The mixture was chromatographed on HPLC repeatedly and compound **1** was obtained together with the compounds reported previously. The ^{1}H NMR spectrum (pyridine-d_5) of **1** showed four tertiary methyl signals, two proton signals at δ4.74(*s*) and 4.79(*s*) ascribable to the exomethylene protons and the hydroxymethylene protons at δ3.83 (*d*, $J = 10.8$ Hz) and 4.07 (*d*, $J = 10.8$ Hz) which were shifted downfield on acetylation **(1b)** to indicate the presence of an axial hydroxy methylene group attached to an asymmetric centre. Moreover, the ^{13}C NMR chemical shifts of the carbons of the C/D/E rings of **1** and akebonic acid **(2)** [1] are in good agreement with each other (Table 1). The mass spectrum of **1** showed $[M]^+$ at *m/z* 456 and exhibited a significant peak at *m/z* 232 and 187, which could be assigned to the fragments of the D/E rings derived by retro-Diels–Alder cleavage of the β-amyrin-Δ^{12} skeleton [2]. From the above mass spectral fragments, it is also evident that the two hydroxyl groups are present in the A/B ring portion. The axial (α)-orientation of the secondary hydroxyl groups was confirmed by the ^{1}H NMR data of **1** and **1b**. The ^{1}H NMR spectrum of **1** showed the presence of a triplet-like signal centred at δ3.70 and the signal shifted downfield to δ4.92 on acetylation. Furthermore, the ^{13}C NMR spectrum of **1** exhibited a signal at δ70.2 (*d*) for C-3 which was at higher field than that observed for related compounds with the 3β-hydroxy configuration [3]. From the above ^{1}H NMR and ^{13}C NMR spectral data a 3α-hydroxyl group was presumed to be present. Furthermore, the location of the second hydroxyl group at C-24 was suggested, because **1a** did not form the acetonide between the C-3α-hydroxyl and the 4β-CH_2OH group on treatment by the usual procedure [4]. Other evidence for the C-4 stereochemistry was obtained by the comparison of the average chemical shift value of the acetoxy-methylene protons of **1b** [$(a + b)1/2 = \delta 4.06$] with the ^{1}H NMR data reported for similar compounds [5, 6]. Also the ^{13}C NMR spectrum of the A/B ring of **1** similar to that of **3** [7] which was isolated from *Salvia nicolsoniana* or barbinervic acid which was isolated from leaves of *Clethra barbinervis* [8] (Table 1). The finding was further confirmed by correlating the signals due to the methyl protons of C-25 with those due to the methylene protons of the (C-24) group in the 2D NOE spectrum of compound **1a**. Thus, **1** was established as 3α,24-dihydroxy-30-norolean-12,20(29)-dien-28-oic acid **(1)** (quinatic acid). It is of interest that *A. quinata* callus produced the 30-noroleane type of triterpenoids.



	R^1	R^2	R^3
1	CH_2OH	H	---OH
1a	CH_2OH	Me	---OH
1b	CH_2OAc	H	---OAc
1c	CH_2OAc	H	---OH
2	Me	H	▬OH



3 R = CH_2OH

Table 1. ^{13}C NMR chemical shifts of compounds **1–3**

C	Chemical shift 1	2	3	C	Chemical shift 1	2	3
1	33.7	39.0	33.9	16	24.0	23.8	23.5
2	26.4	28.1	26.4	17	47.1	47.1	47.0
3	70.2	78.2	70.0	18	47.9	48.0	41.9
4	43.9	39.4	43.9	19	42.0	42.0	46.2
5	50.1	55.9	50.1	20	149.2	148.5	30.8
6	19.1	18.8	19.1	21	38.4	38.4	34.0
7	33.7	33.3	33.6	22	30.4	30.4	32.8
8	40.0	39.8	39.9	23	23.6	28.2	23.4
9	48.1	48.1	48.1	24	65.8	16.5	65.8
10	37.5	37.4	37.5	25	15.9	15.6	15.9
11	23.8	23.8	24.0	26	17.3	17.4	17.1
12	123.0	123.3	120.0	27	26.1	26.2	26.1
13	144.2	143.5	144.1	28	179.4	177.3	177.9
14	42.2	42.1	42.0	29	107.1	107.1	33.1
15	28.3	28.3	28.1	30			23.7
				COOMe			51.5

All signals were corroborated by DEPT techniques. The measurements were made in pyridine-d_5 with TMS as an internal standard.

EXPERIMENTAL

Mps: uncorr. The ^1H NMR spectra were recorded at 400 MHz and the ^{13}C NMR spectra were recorded at 100.6 MHz, at room temp. with $CDCl_3$ solns and pyridine-d_5 soln and TMS as int. standard. MS (70 eV) were taken with a direct probe.

Plant material. Akebia quinata Decne was collected in October, 1981 at the Medicinal Plant Garden of this college.

Derivation and culture of callus tissue. The callus tissue from the stalk was obtained in October, 1981. Murashige and Skoog's medium containing 2,4-D (1 mg/l; 3 mg/l) and kinetin (0.1 mg/l) as plant growth regulators were used for induction of callus tissue. The callus tissue was subcultured every 5 ~ 6 weeks onto fresh M & S medium (minus glycine) containing 2,4-D (1 mg/l) and kinetin (0.1 mg/l) at 26° ± 1 in the dark.

Extraction and isolation. The fresh callus tissue (1092 g, fr.wt, dry, wt 22.4 g) was extracted with cold MeOH and EtOAc in a Waring blender. The extracts were combined and concd under red. pres. to yield an extract which was partitioned between $CHCl_3$ and H_2O to obtain the organic solvent soluble fraction and the residual H_2O soln was further partitioned with *n*-BuOH saturated with H_2O. The BuOH soln was chromatographed over a column of silica gel (Merck 9385) and elution with $CHCl_3$ containing increasing proportions of MeOH afforded the crude triterpene mixture. The mixture was purified by repeated rechromatography on a silica gel column [HPLC, CIG column system (Kusano Scientific Co., Tokyo) with Iatrobeads as the stationary phase (60 silica gel, IATRON Co., Tokyo)] to afford compound **1.**

3α,24-*Dihydroxy*-30-*norolean*-12,20(29)-*dien*-28-*oic acid* **(1)** (*quinatic acid*). Mp 269–272°, colourless needles $[\alpha]_D^{18}$ + 66.6° (pyridine; *c* 0.375). ^1H NMR (pyridine-d_5): δ0.96 (3H, *s*), 1.01 (3H, *s*), 1.16 (3H, *s*), 1.62 (3H, *s*), 3.22 (1H, *dd*, *J* = 4, 12 Hz), 3.83 (1H, *d*, *J* = 10.8 Hz), 4.07 (1H, *d*, *J* = 10.8 Hz), 4.43 (1H, *br s*),4.74 (1H, *s*), 4.78 (1H, *s*), 5.51 (1H, *br s*). MS *m/z* rel. int.: 456 $[M]^+$ (2), 232 (100), 224 (21), 206 (47), 187 (97), 175 (52).

Methyl-3α,24-*dihydroxy*-30-*norolean*-12,20(29) *dien*-28-*oate* **(1a)** (*quinatic acid methylester*). Mp 233–236° (MeOH–$CHCl_3$), colourless needles, $[\alpha]_D^{18}$ + 110.8° ($CHCl_3$; *c* 0.318), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1710. ^1HNMR ($CDCl_3$): δ0.70 (*s*, 3H), 0.89 (*s*, 3H), 1.08 (*s*, 3H), 1.17 (*s*, 3H), 2.73 (*dd*, *J* = 4.0, 13 Hz), 3.48 (1H, *d*, *J* = 11 Hz), 3.67 (1H, *d*, *J* = 11 Hz), 3.60 (*s*, 3H), 3.82 (1H, *t*, *J* = 3 Hz), 4.61 (1H, *s*), 4.63 (1H, *s*), 5.34 (1H, *t*, *J* = 3 Hz) MS *m/z* (rel. int.); 470 $[M]^+$ (6), 452 (2), 411 (4), 393 (2), 246 (55), 206 (25), 187 (100),186 (65).

Acylation of **1**. Compound **1** was treated with Ac_2O and pyridine at room temp. over night. Ice was added to the reaction mixture, which yield a white ppt. The ppt. was filtered, dried and purified by HPLC. It afforded two colourless amorphous crystals (**1b**, diacetate) and (**1c**, monoacetate).

1b (*diacetate*); ^1H NMR ($CDCl_3$): δ0.73 (3H, *s*), 0.95 (6H, *s*), 1.22 (3H, *s*), 2.03 (3H, *s*), 2.09 (3H, *s*), 2.49 (1H, *t*, *J* = 13.5 Hz), 2.75 (1H, *dd*, *J* = 13.1, 4.5 Hz), 3.95 (1H, *d*, *J* = 11.4 Hz), 4.17 (1H, *d*, *J* = 11.4 Hz), 4.64 (2H, *s*),4.97 (1H, *t*-like), 5.35 (1H, *t*-like). MS *m/z* (rel. int.) 540 $[M]^+$ (5.2), 480 (7.9), 420 (5.7), 233 (17), 232 (84), 204 (17), 203 (7.6), 189 (25), 188 (59), 187 (100). **1c** (*monoacetate*): ^1H NMR ($CDCl_3$): δ0.75 (3H, *s*), 0.93 (3H, *s*), 1.06 (3H, *s*), 1.19 (3H, *s*), 2.04 (3H, *s*), 2.51 (1H, *t*, *J* = 13.8 Hz), 2.78 (1H, *dd*, *J* = 13.1, 4.7 Hz), 3.74 (1H, *t*-like),3.97 (1H, *d*, *J* = 11.4 Hz), 4.19 (1H, *d*, *J* = 11.4 Hz), 4.64 (2H, *s*), 5.36 (1H, *t*, *J* = 0.3 Hz). MS *m/z* (rel. int.): 498$[M]^+$ (2), 480 (3), 452 (2), 420 (2.4), 232 (78), 204 (13), 203 (20), 189 (22), 188 (65), 187 (100).

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