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TRITERPENOIDS OF *AKEBIA QUINATA* CALLUS TISSUE

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Key Word Index—*Akebia quinata;* Lardizabalaceae; callus tissue; triterpene; sterol.

Abstract—From tissue culture of *Akebia quinata*, three new triterpenes were isolated together with two known triterpenes, oleanolic acid and mesembryanthemoidgenic acid. The new triterpenes were characterized by spectroscopic means as 3β-hydroxy-30-norolean-12,20(29)-dien-28-oic acid, 3-epi-30-norolean-12,20(29)-dien-28-oic acid and 3β-hydroxy-29(or 30)-al-olean-12-en-28-oic acid.

INTRODUCTION

Akebia quinata Decne. is a creeping, woody vine which is widely distributed in Japan and East Asia. Its air dried stems are called Mokutsu in Japan, and it is used as a traditional oriental medicine in Japan and China, for example as an antiphlogistic, diuretic and analgesic.

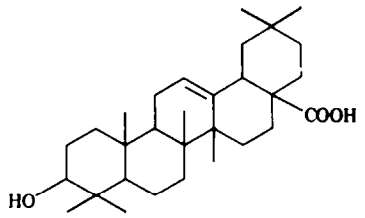
The stems [1–3], seed [4, 5] and pericarp [6, 7] have been reported to contain a number of triterpenoid saponins which on acid hydrolysis yield hederagenin, oleanolic acid and norarjunolic acid, respectively, as sapogenins. Also anthocyanins have been reported from the flowers [8]. We now report on the triterpene components of callus tissue of this plant.

RESULTS AND DISCUSSION

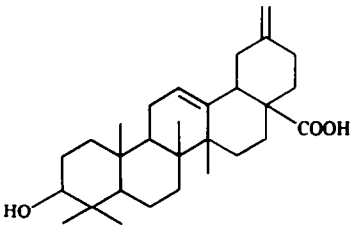
Callus tissue cultures were established from the stem of *A. quinata* (Akebi in Japanese) and they were cultured in the dark on Murashige and Skoog's medium containing 2,4-D (0.1 and 1.0 ppm) with kinetin (0.1 ppm).

The callus tissues were extracted successively with methanol and ethyl acetate and both extracts combined. The material was chromatographed on a silica gel column to give oleanolic acid (**1**), the new triterpenes **2**, **3** and **5**, mesenbryanthemoidigenic acid (**4**) and a mixture of sitosterol (**6**) and stigmasterol (**7**).

The structure of **1** was established by spectroscopic means and identification with an authentic sample (see



1



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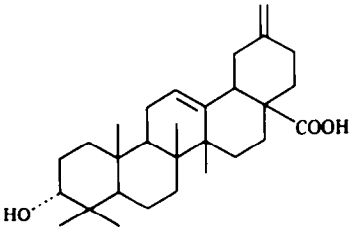

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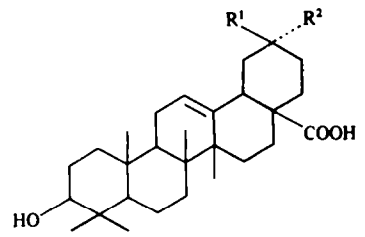
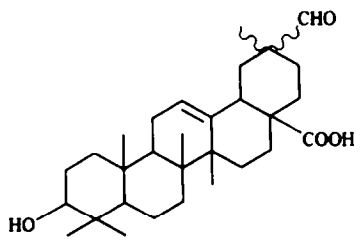


4 R^1 = Me, R^2 = CH_2OH
8 R^1 = CH_2OH, R^2 = Me



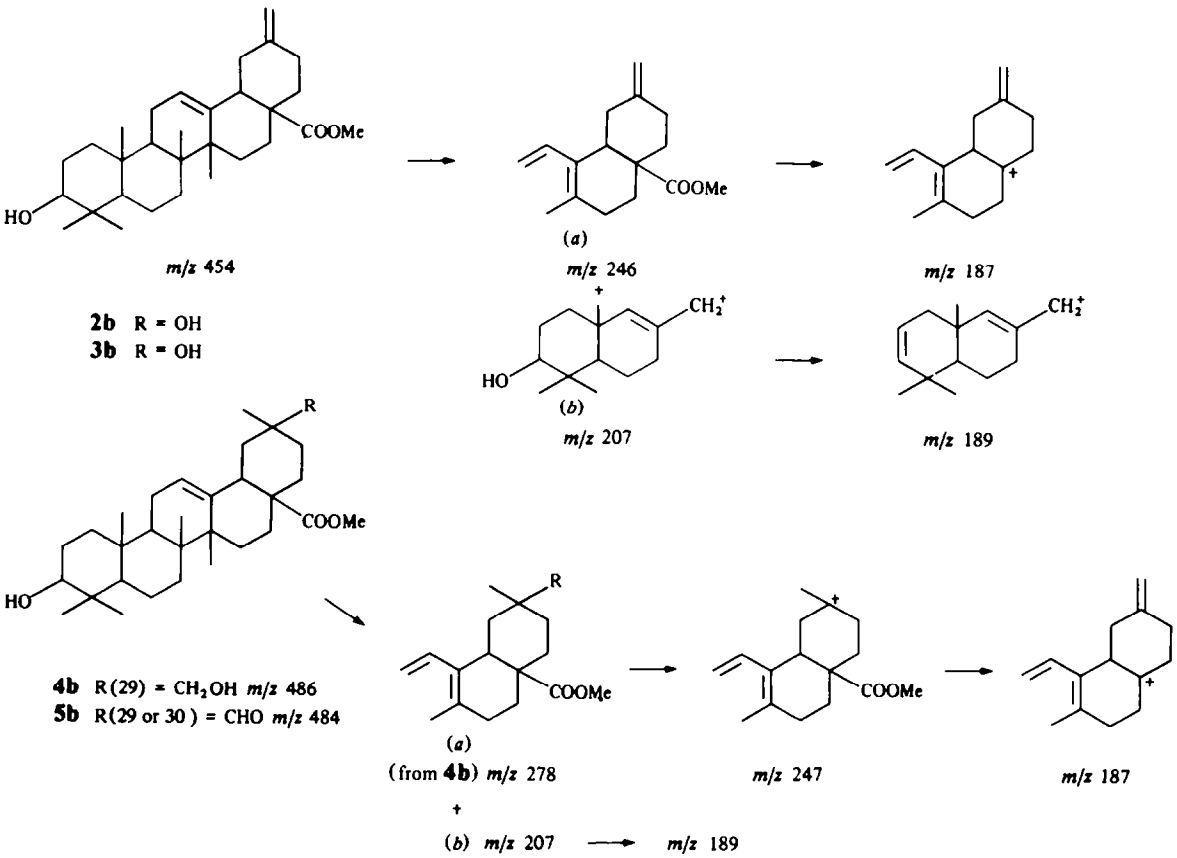
5

Experimental). Compound **2**, on treatment with diazomethane yielded a methyl ester (**2b**). The mass spectrum of compound **2b** showed $[M]^+$ at *m/z* 454 and it had the molecular formula $C_{30}H_{46}O_3$ as determined by high resolution mass spectrometry. The mass spectrum of **2b** exhibited the typical retro-Diels–Alder fragmentation of the C-ring of the β-amyrin Δ^{12}- skeleton [9]. The strong peaks at *m/z* 246 (*a*) and 187 [246 − COOMe] (59) were observed (Scheme 1). The mass number of the peak (*a*) is 16 mass units less than that of the peak due to the retro-Diels–Alder type fragmentation of oleanolic acid methyl ester (**1b**). The ^{1}H NMR spectrum of **2b** show five tertiary methyl signals, two protons at δ4.61 and 4.62 ascribable to the exo-methylene protons, and the H-18 proton at 2.79 (*dd*) [10]. Therefore, compound **2b** is presumed to be a 30-norolean-12-ene derivative carrying an exo-methylene group in ring D or E.

The ^{13}C NMR chemical shifts (Table 1) of **2b** were found to be similar to the authentic sample oleanolic acid methyl ester (**1b**) but with predictable differences. The low-field carbon signals at δ148.0 (*s*) and 106.8 (*t*) were assigned to the exo-methylene C-20 and C-29, respectively. The hydroxyl group at C-3 must have a β-equatorial configuration according to the signal at δ78.7 (*d*) [11] and also from the ^{1}H NMR chemical shift and the coupling pattern of the proton at C-3. The other assignments were supported by comparison of the ^{13}C NMR spectrum of **2b** with that of **1b**. Thus the structure of compound **2** was established as 3β-hydroxy-30-norolean-12,20(29)-dien-28-oic acid (akebonoic acid).

Compound **3**, on treatment with diazomethane, yielded a methyl ester (**3b**), which was found to be similar to **2b** but with predictable differences. The low-field ^{13}C NMR signal at δ76.2 (*d*) was assigned to C-3 with the α-configuration of the hydroxyl group. Other signals at δ33.0 (*t*), 25.2 (*t*), 37.4 (*s*) and 49.0 (*d*) were due to C-1, C-2, C-4 and C-5, respectively [12] (Table 1). These values for the chemical shifts also agreed with the α-configuration of the C-3 hydroxyl group, and this was also supported by the ^{1}H NMR chemical shift and the coupling pattern of the C-3 proton. The values of the chemical shifts for other carbons were similar to those of **2b**. The mass spectrum of **3b** exhibited the typical retro-Diels–Alder fragmentation and its fragments had the same *m/z* values to those of **2b** (Scheme 1). Thus, the structure of compound **3** was established as 3-epi-30-norolean-12,20(29)-dien-28-oic acid (3-epi-akebonoic acid). The compounds **2** and **3** are reported for the first time from natural sources. A noroleanane type triterpene, norarjunolic acid [7], has been isolated from the pericarp of *A. quinata* and other eupteleogenins have been reported from *Euptelea polandra* (Eupteleaceae) [13]. The 30-noroleanane type compounds are rare as natural products.

The mass spectrum of **4b** exhibited significant peaks at *m/z* 278 (*a*) and 247 [278 − CH_2OH], which could be assigned to fragments of the D and E rings derived by retro-Diels–Alder cleavage and *m/z* 207 (*b*) and 189 which could be unequivocally assigned to fragments of the A and B rings by a cleavage of the C ring and elimination of the hydroxyl groups (Scheme 1).



Scheme 1. Fragmentation of compounds **2**–**5**.

Table 1. ^{13}C NMR chemical shifts of compounds **1b–4b** and **8b**

Carbon	1b	2b	3b	4b	8b	Carbon	1b	2b	3b	4b	8b
1	38.5	38.5	33.0	38.5	38.7	16	23.4	23.5	23.4	23.5	23.4
2	27.1	27.2	25.2	27.2	27.0	17	46.6	47.0	47.1	47.0	46.8
3	78.7	79.2	76.2	79.1	78.9	18	41.3	41.4	41.4	40.5	40.3
4	38.7	38.8	37.4	38.8	38.7	19	45.8	47.2	47.2	40.1	40.3
5	55.2	55.2	49.0	55.3	55.3	20	30.6	148.0	148.2	35.8	35.2
6	18.3	18.3	18.3	18.4	18.5	21	33.8	30.0	30.0	31.6	32.8
7	32.6	32.7	32.7	32.7	32.8	22	32.3	37.5	37.5	32.7	32.1
8	39.4	39.4	39.5	39.3	39.3	23	28.1	28.1	28.3	28.1	28.1
9	47.6	47.7	47.5	47.7	47.7	24	15.6	15.6	22.3	15.6	15.7
10	37.0	37.1	37.1	37.1	37.2	25	15.3	15.3	15.3	15.3	15.4
11	23.1	23.3	23.3	23.1	23.4	26	16.8	16.8	16.9	16.9	16.9
12	122.1	123.2	123.0	122.8	122.8	27	26.0	26.0	26.2	25.9	26.0
13	143.4	143.1	143.2	143.2	143.2	28	177.9	177.5	177.5	178.1	178.5
14	41.6	41.6	41.8	41.6	41.7	29	33.1	106.8	107.0	74.4	28.9
15	27.7	27.7	27.7	27.7	27.7	30	23.6	—	—	19.0	65.8
						COOMe	51.3	51.6	51.6	51.6	50.6

All signals were corroborated from off-resonance decoupling experiments. ^{13}C NMR chemical shifts for compounds **1b–4b** and **8b** in $CDCl_3$ (ppm from TMS).

The ^{1}H NMR spectrum of **4b** showed six singlet methyl protons between δ0.70 and 1.12, carbomethoxy protons at 3.68 (*s*), hydroxymethylene protons at 3.30 (*s*, 2H) and an olefinic proton at 5.33 (*t*-like). The ^{13}C NMR spectrum (Table 1) of **4b** was very similar to that of **8b** (queretaroic acid) [11] except that the former exhibited signals δ74.4 (C-29) and 19.0 (C-30), the latter exhibited signals 28.9 (C-29) and 65.8 (C-30). This evidence supports the α (equatorial, i.e. C-29) rather than β (axial, i.e. C-30) orientation of the hydroxymethylene group at C-20 in the compound **4b**. Thus **4b** is represented as 3β,29-dihydroxyolean-12-en-28-oic acid (mesembryanthemoidigenic acid) [14]. This is the second report of compound **4** from a natural source.

Compound **5**, on treatment with diazomethane yielded a methyl ester (**5b**), which showed in its ^{1}H NMR spectrum signals attributable to an aldehyde group at δ9.39, a vinyl proton at 5.35 and six tertiary C-methyl groups at 0.75, 0.77, 0.91, 0.98, 1.13 and 1.16, suggesting it to have an aldehyde group at C-20 [15].

The mass spectrum of **5b** exhibited the $[M]^+$ ion at *m/z* 484 and its fragment ions (*a*) and (*b*) originating from the retro-Diels–Alder showed the analogues cleavage pattern (Scheme 1) to compounds with ions at **1–4** *m/z* 247 (*a*) [276 − CHO], 187 and 207 (*b*) and 189. Therefore compound **5** was determined as 3β-hydroxy-29(or 30)-aldolean-12-en-28-oic acid.

The compounds isolated were present in small amounts except for **1** and **2**, but it is very interesting from the biogenetic point of view that callus tissue of *A. quinata* produces the compounds **1–5**. Compounds **2**, **3**, **4** and **5** have not been reported from the original plant sources.

The ^{1}H NMR, ^{13}C NMR and mass spectra of substances **6** and **7** demonstrated the presence of a two sterol mixture, whose compositions were confirmed as equal amounts of sitosterol (**6**) and stigmasterol (**7**) by comparison with authentic samples using GLC.

EXPERIMENTAL

All mps are uncorr. IR spectra were recorded in $CHCl_3$. ^{1}H NMR spectra were run at 200 MHz (Varian) and 400 MHz (Bruker) and ^{13}C NMR spectra at 100.6 MHz at room temp. with $CDCl_3$ solns and TMS as internal standard. MS (70 eV) were taken with a direct probe.

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

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

Extraction and isolation. The fresh callus tissue was extracted with cold MeOH in a Waring blender and then with hot EtOAc. The extracts were combined and concd under red. pres. to yield an extract which was chromatographed over a column of silica gel. using hexane containing increasing proportions of EtOAc and MeOH. Compounds **1**, **6** and **2** were eluted with hexane–EtOAc (5:1); **2**, **3**, **4** and **5** were eluted with hexane–EtOAc (2:1). The compounds were finally purified by methylation with freshly prepared CH_2N_2 and then HPLC (silica gel Kusano) using the solvent system hexane–EtOAc–MeCN (2:1:0.5) and to give **1b**, **2b**, **3b**, **4b** and **5b**.

Oleanolic acid methyl ester (**1b**). Mp 196–198° ($CHCl_3$–MeOH), colourless needles, 90 mg, $[\alpha]_D^{24.5}$ + 64.6° (*c* 0.266; $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1705; ^{1}H NMR ($CDCl_3$): δ0.75 (*s*, 3H), 0.78 (*s*, 3H), 0.91 (*s*, 6H), 0.93 (*s*, 3H), 0.99 (*s*, 3H), 1.13 (*s*, 3H), 2.83 (*dd*, *J* = 4, 13 Hz, 1H), 3.22 (*m*, 1H), 5.28 (*t*, 1H). ^{13}C NMR ($CDCl_3$): δ15.3 (*q*), 15.6 (*q*), 16.8 (*q*), 18.3 (*t*), 23.1 (*d*), 23.4 (*t*), 23.6 (*q*), 26.0 (*q*), 27.1 (*t*), 27.7 (*t*), 28.1 (*q*), 30.6 (*s*), 32.3 (*t*), 32.6 (*t*), 33.1 (*q*), 33.8 (*t*), 37.0 (*s*), 38.5 (*t*), 38.7 (*s*), 39.3 (*s*), 41.3 (*d*), 41.6 (*s*), 45.8 (*t*), 46.6 (*s*), 47.6 (*d*), 51.3 (*q*), 55.2 (*d*), 78.7 (*d*), 122.1 (*d*), 143.4 (*s*), 177.9 (*s*). MS *m/z* (rel. int.): 470 $[M]^+$, (3), 262 (70), 207 (17), 203 (100), 189 (20).

Methyl 3β-hydroxy-30-norolean-12,20(29)-dien-28-oate (akebonoic acid methyl ester, **2b**). Mp 152–155° ($CHCl_3$–MeOH), colourless needles, 33 mg, $[\alpha]_D^{24}$ + 127.7° (*c* 0.658; $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1702, 1632; ^{1}H NMR ($CDCl_3$): δ0.72 (*s*, 3H), 0.78 (*s*, 3H), 0.91 (*s*, 3H), 0.98 (*s*, 3H), 1.16 (*s*, 3H), 2.79 (*dd*, *J* = 4.4, 13.5 Hz, 1H), 3.20 (*m*, 1H), 3.61 (*s*, 3H), 4.61, 4.62, (each *s*, 1H), 5.34 (*t*, 1H). ^{13}C NMR ($CDCl_3$): δ15.3 (*q*), 15.6 (*q*), 16.8 (*q*), 18.3 (*t*), 23.3 (*d*), 23.5 (*t*), 26.0 (*q*), 27.2 (*t*), 27.7 (*t*), 28.1 (*q*), 30.0 (*t*), 32.7 (*t*),

37.1 (*s*), 37.5 (*t*), 38.8 (*s*), 39.4 (*s*), 41.4 (*d*), 41.6 (*s*), 47.0 (*s*), 47.2 (*t*), 47.7 (*d*), 51.6 (*q*), 55.2 (*d*), 79.2 (*d*), 123.2 (*d*), 142.3 (*s*), 177.5 (*s*), 106.8 (*t*), 148.0 (*s*). MS *m/z* (rel. int.): 454.3485 (calc. for $C_{30}H_{46}O_3$, 454.3489) $[M]^+$ (10), 246 (45), 207 (23), 189 (11), 187 (100).

Methyl 3-epi-30-norolean-12,20(29)-dien-28-oate (3α *akebonic acid methyl ester*, **3b**). Mp 200–202° ($CHCl_3$–MeOH), colourless needles, 8.3 mg, $[\alpha]_D^{24} + 118.1°$ (*c* 0.166; $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1705, 1635. ^1H NMR ($CDCl_3$): δ0.72 (*s*, 3H), 0.84) (*s*, 3H), 0.92 (*s*, 3H), 0.95 (*s*, 3H), 1.16 (*s*, 3H), 2.79 (*dd*, *J* = 4.8, 13.4 Hz, 1H), 3.41 (*br s*, 1H), 3.60 (*s*, 3H) 4.61, 4.62 (each *s*, 1H), 5.35 (*t*, *J* = 4 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ15.1 (*q*), 16.9 (*q*), 18.3 (*t*), 22.3 (*q*), 23.3 (*t*), 23.4 (*t*), 26.2 (*q*), 25.2 (*t*), 27.7 (*t*), 28.3 (*q*), 30.0 (*t*), 32.7 (*t*), 37.1 (*s*), 37.5 (*t*), 33.0 (*t*), 37.4 (*s*), 39.5 (*s*), 41.4 (*d*), 41.8 (*s*), 47.1 (*s*), 47.2 (*t*), 47.5 (*d*), 51.6 (*q*), 49.0 (*d*), 76.2 (*d*), 123.0 (*d*), 143.0 (*s*), 177.5 (*s*), 107.0 (*t*), 148.2 (*s*). MS *m/z* (rel. int.): 454.3483 (calc. for $C_{30}H_{46}O_3$, 454.3486) $[M]^+$ (17), 246 (55), 207 (16), 189 (40), 187 (100).

Mesembryanthemoidigenic acid methyl ester (**4b**). Mp 200–203° ($CHCl_3$–MeOH), colourless crystal, 2.1 mg, $[\alpha]_D^{24} + 100°$ (*c* 0.022; $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710. ^1H NMR ($CDCl_3$): δ0.76 (*s*, 3H), 0.78 (*s*, 3H), 0.92 (*s*, 3H), 0.97 (*s*, 3H), 0.99 (*s*, 3H), 1.15 (*s*, 3H), 2.90 (*dd*, *J* = 4, 13 Hz, 1H), 3.25 (*m*, 1H), 3.30 (*s*, 2H), 5.32 (*t*, *J* = 4 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 15.3 (*q*), 15.6 (*q*), 16.9 (*q*), 18.4 (*t*), 19.0 (*q*), 23.1 (*t*), 23.5 (*t*), 26.0 (*q*), 27.2 (*t*), 27.7 (*t*), 28.1 (*q*), 31.6 (*t*), 32.7 (*t*), 35.8 (*s*), 37.1 (*s*), 38.5 (*t*), 38.8 (*s*), 39.3 (*s*), 40.1 (*t*), 40.5 (*d*), 41.6 (*s*), 47.0 (*s*), 47.7 (*d*), 55.3 (*d*), 51.6 (*q*), 74.4 (*t*), 79.1 (*d*), 122.8 (*d*), 143.2 (*s*), 178.1 (*s*). MS *m/z* (rel. int.): 486 $[M]^+$ (5), 278 (13), 247 (100), 207 (19), 189 (6), 187 (13).

Methyl 3β-hydroxy-29(or 30)-al-olean-12-en-28-oate (**5b**). Amorphous (0.6 mg) $[\alpha]_D^{24.5} + 21.2°$ (*c* 0.066; $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1700; ^1H NMR ($CDCl_3$): δ0.75 (*s*, 1H), 0.77 (*s*, 1H), 0.91 (*s*, 1H), 0.98 (*s*, 1H), 1.13 (*s*, 1H), 1.16 (*s*, 1H), 2.92 (*dd*, *J* = 4, 13 Hz, 1H), 3.25 (*m*, 1H), 5.35 (*t*, *J* = 4Hz, 1H), 9.39 (*s*, 1H). MS *m/z* (rel. int.): 484 $[M]^+$ (4.1), 247 (50), 207 (33), 189 (36), 187 (100).

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