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30-NOROLEANANE SAPONINS FROM CALLUS TISSUES OF *AKEBIA QUINATA*

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Key Word Index—*Akebia quinata*; Lardizabalaceae; callus tissues; 30-noroleanane saponins.

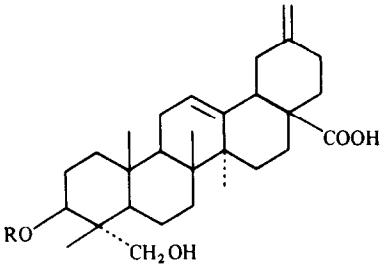
Abstract—Four new 30-nortriterpenoid saponins were isolated as major saponins from the callus tissues of *Akebia quinata*. The structures of these glycosides, tentatively named quinatoside A–D, were elucidated as 30-norhederagenin 3-*O*-α-L-arabinoside, 30-norhederagenin 3-*O*-β-D-glucopyranosyl (1→3)-α-L-arabinopyranoside, 30-norhederagenin 3-*O*-β-D-xylopyranosyl (1→2)-α-L-arabinoside and 30-noroleanolic acid 3-*O*-β-D-xylopyranosyl (1→2)-α-L-arabinopyranoside, respectively by means of ^{1}H NMR, ^{13}C NMR and SIMS. Furthermore, these saponins from the callus tissues were compared with the saponins isolated from the stem of *A. quinata*

INTRODUCTION

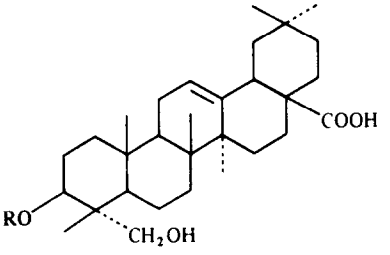
Previously, we reported the isolation and the structure determination of 30-noroleanane type triterpenoids such as akebonoic acid (**6**), its isomer and quinatic acid from the callus tissues of *Akebia quinata* (Lardizabalaceae) [1, 2]. In this paper, we report the structural elucidation of four new saponins (**1**–**4**) named quinatoside A–D, respectively, isolated from the methanol extract of the callus tissues of *A. quinata*. Furthermore, the saponins (**1**–**4**) from the callus tissues were compared with the saponins isolated from the stem of *A quinata*.

RESULTS AND DISCUSSION

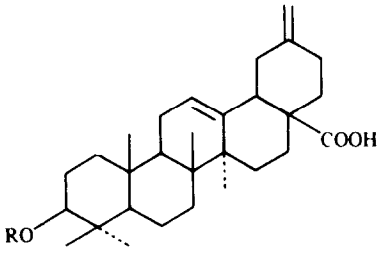
The crude saponin mixtures obtained from the methanolic extract of the callus tissues of *A. quinata* was



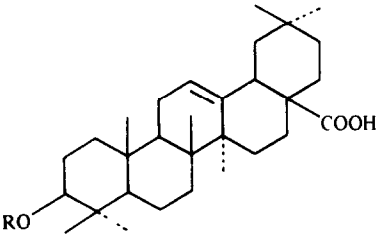
5 R = H
1 R = ara
2 R = ara$^{3-1}$glc
3 R = ara$^{2-1}$xyl



7 R = H
9 R = ara
10 R = ara$^{3-1}$glc
11 R = ara$^{2-1}$rha



6 R = H
4 R = ara$^{2-1}$xyl



8 R = H
12 R = ara$^{2-1}$rha

Table 1 ^{13}C NMR chemical shifts of aglycone moieties **1**-**4** (pyridine-d_5)

C	1	2	3	4	5	6	C	1	2	3	4	5	6
1	38 8	38 9	38 9	38 9	38 8	39 0	16	23 8	23 9	23 9	23 8	23 8	23 8
2	26 1	26 2	26 3	28 0	27 7	28 1	17	47 1	47 1	47 2	47 9	47 1	47 1
3	82 0	81 9	82 0	88 9	73 5	78 2	18	48 1	48 2	48 2	48 1	48 0	48 0
4	43 5	43 6	43 7	39 7	42 9	39 4	19	42 0	42 1	42 0	42 2	42 0	42 0
5	47 6	47 6	47 2	55 9	48 7	55 9	20	149 2	149 6	149 2	149 3	148 5	148 5
6	18 2	18 2	18 2	18 6	18 6	18 8	21	38 4	38 5	38 7	38 6	38 4	38 4
7	32 9	32 9	32 9	33 3	33 0	33 3	22	30 0	30 5	30 6	30 6	30 4	30 4
8	39 8	39 8	39 8	39 8	39 8	39 8	23	64 5	64 3	63 7	28 0	68 1	28 8
9	47 9	48 0	47 4	47 9	48 1	48 1	24	13 6	13 7	13 3	16 5	13 1	16 5
10	36 9	37 0	36 9	37 1	37 2	37 4	25	16 1	16 1	16 1	15 6	16 0	15 6
11	23 8	23 9	23 9	23 8	23 8	23 8	26	17 5	17 5	17 6	17 5	17 5	17 4
12	123 0	123 9	122 7	122 7	122 6	123 3	27	26 2	26 2	26 2	26 2	26 2	26 2
13	144 2	144 5	144 7	144 7	144 9	143 5	28	179 5	179 9	180 5	179 3	179 4	177 3
14	42 1	42 2	42 2	42 2	42 0	42 1	29	107 1	107 0	106 8	106 9	107 0	107 1
15	28 3	28 4	28 5	28 5	28 3	28 3	30						

The chemical shifts of **5** and **6** are cited from ref [3]

chromatographed on a silica gel and a reversed phase (RP-18) column and furnished four major saponins Saponin **1** exhibited in the ^{1}H NMR spectrum the presence of only four methyl groups and a characteristic exomethylene group at δ 4.75 (1H, *s*) and 4 80 (1H, *s*), and one anomeric proton at δ5 0 (1H, *d*, J=7 Hz) The SIMS of **1** exhibited a molecular peak at *m/z* 611 $[M+Na]^+$ indicating molecular weight of for the saponins 588 The ^{13}C NMR spectrum of **1** showed the presence of two olefinic bonds One of them was located between C-12 (δ 123 0) and C-13 (δ 144 2) and the other signals appeared at δ149 2 (*s*) and 107 1 (*t*) indicating the presence of an exomethylene group [1] Furthermore, the carbon signal of a hydroxymethylene group at C-23 was exhibited at δ64 5 (*t*) The aglycone moiety of **1** was presumed to be 30-norhederagenin (**5**) previously reported from the callus tissues of *Paeonia japonica* [3], by comparison of the ^{13}C NMR spectra (Table 1) On the other hand, the ^{13}C NMR spectrum of **1** showed the presence of only one anomeric carbon signal at δ106 7 and the five carbon signals of a sugar moiety, which were identical with those reported for an α-L-arabinosyl moiety [4] Furthermore, **1** afforded arabinose and a small amount of 30-norhederagenin on acid hydrolysis Therefore, the structure of quinatoside A was determined as 30-norhederagenin 3-*O*-α-L-arabinopyranoside (1)

Saponin **2** showed the presence of only the four methyl groups and the exomethylene protons at δ4 75 and 4 80 (each 1H, *s*) as well as **1** and furthermore, two anomeric protons at δ4 98 (1H, *d*, J=7 Hz) and 5 31 (1H, *d*, J=7 8 Hz) in the ^{1}H NMR spectrum The SIMS of **2** also showed a molecular peak at *m/z* 773$[M+Na]^+$ indicating a M_r for the saponin 750 The ^{13}C NMR spectrum of the aglycone moiety of **2** showed almost same chemical shifts with those of **1** (Table 1) Compound **2** also exhibited the presence of two anomeric carbon signals at δ106.6 and 106 4, respectively, and the presence of the other nine carbon signals due to the sugar moieties, which were identical with those published for a β-glucopyranosyl (1→3)-α-L-arabinosyl group [4] Therefore, the structure of quinatoside B was determined as 30-norhederagenin 3-*O*-β-glucopyranosyl (1→3)-α-L-arabinopyranoside (2)

Table 2 ^{13}C NMR chemical shifts of the sugar moieties **1**-**4** (in C_5D_5N)

C	1	2	3	4
ara 1′	106 7	106 6[a]	104 5	106 7
ara 2′	73 1	72 0	81 4	81 5
ara 3′	74 7	84 3	74 0	73 8
ara 4′	69 6	69 3	68 8	68 6
ara 5′	66 9	67 1	66 0	65 6
glc 1′		106 4[a]		
glc 2′		75 7		
glc 3′		78 4		
glc 4′		71 6		
glc 5′		78 8		
glc 6′		62 8		
xyl 1′			106 8	105 1
xyl 2′			76 2	76 1
xyl 3′			78 4	78 4
xyl 4′			71 0	71 1
xyl 5′			67 5	67 5

[a]The assignments may be interchanged in the column

Saponin **3** showed in the ^{1}H NMR spectrum the presence of four methyl groups and exomethylene protons as found for **1** and **2**, and two anomeric protons at δ5 08 (H, *d*, J=6 2 Hz) and 5 11 (1H, *d*, J=6 7 Hz), respectively Furthermore, the SIMS of **3** showed a molecular peak at *m/z* 721 $[M+H]^+$ The ^{13}C NMR spectrum of **3** showed almost the same chemical shifts as those of **1** and **2**. Furthermore, the spectrum also exhibited two anomeric carbon signals at δ106 8 and 104 5, and the other eight

carbon signals due to the sugar moieties, which were compared with the published data for a β-D-xylopyranosyl (1→2)-α-L-arabinopyranosyl group [5]. Therefore, the structure of quinatoside C were determined as 30-norhederagenin 3-*O*-β-D-xylopyranosyl (1→2)-α-L-arabinopyranoside (**3**)

Saponin **4** showed in the ^{1}H NMR spectrum the presence of five methyl groups, which was one more than present in compounds **1**–**3**. The characteristic exomethylene protons were exhibited at δ4 75 (1H, *s*) and 4.81 (1H, *s*) and signals for two anomeric protons were observed at δ4 89 (1H, *d*, *J*=6 2 Hz) and 5 08 (1H, *d*, *J*=6 9 Hz) The SIMS of **4** exhibited a molecular peak at *m/z* 727 [M +Na]$^+$ indicating a M_r 704 and the EI mass spectrum also exhibited a fragment ion corresponding to the aglycone moiety of **4** at *m/z* 440 [M−264]$^+$ The ^{13}C NMR spectrum of **4** showed the presence of two olefinic bonds (Table 1) and furthermore, the carbon signals of the aglycone moiety of **4** and of akebonoic acid (**6**), reported from the same extracts [1], were in good agreement with each other (Table 1).

The sugar moieties of **4** showed the same chemical shifts as those of **3** in the ^{13}C NMR spectrum (Table 1). Therefore, the structure of quinatoside D was determined as 30-noroleanolic acid 3-*O*-β-D-xylopyranosyl (1→2)-α-L-arabinopyranoside (4)

The saponins **1**–**4** have not been reported from the original plant, and this is the first report of these compounds from natural sources The aglycone portions of the saponins **1**–**4** were triterpenes such as the 30-noroleanane type which occurred as major triterpenoids components along with oleanolic acid in the chloroform extract of the callus tissues of *A quinata* [1]. Furthermore, these 30-noroleanane triterpenes have not been reported from the original plant except for norarjunolic acid from the pericarp [6] The stem of *A. quinata* was extracted with methanol to investigate the saponins and the saponin fractions showing the same R_f values on comparison with the saponins **1**–**4** on TLC (RP-18) were chromatographed on a reversed phase column (Rp-18) to yield the pure saponins (**9**–**12**) as the major components. The saponins were identified using spectral methods [7] and the aglycones of the saponins were hederagenin (**7**) and oleanolic acid (**8**) which are of common occurrence in plants However, 30-noroleanane type saponins are rare in natural sources except from the stem bark of *Guaiacum officinale* [8] and the leaves of *Acanthopanax senticosus* [9]

The above facts show the differences between the callus tissues and the original plants in their biosynthetic ability with regard to production of the secondary products. It is interesting that the biosynthesis of the 30-noroleanane type triterpenes in the Lardizabalaceous plants [10] and the speculative mode of biogenesis have been shown by using the callus tissues of *Stauntonia hexaphylla* (Lardizabalaceae) [11].

EXPERIMENTAL

Mps uncorr The ^{1}H NMR spectra were recorded at 400 MHz and the ^{13}C NMR spectra at 100 6 MHz, at room temp with pyridine-d_5 soln, and TMS as int. standard MS (70 eV) were taken with a direct probe Plant material and derivation and culture of callus tissues were described in ref [1]

Extraction and isolation. The fresh callus tissue (980 g, dry wt 35 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 satd with water The BuOH soln was chromatographed over a column of silica gel (Merck 9385) and eluted with $CHCl_3$ containing increasing proportions of MeOH to afford the crude saponin mixtures The mixtures were purified repeatedly by rechromatography over RP-18 (Fuji gel R 18-37) and afforded saponins **1**–**4**

30-*Norhederagenin* 3-O-α-L-*arabinopyranoside* (**1**) Mp 256–260° (decomp), colourless powder, $[\alpha]_D^{20}$ +78.3° (pyridine, *c* 0 166) ^{1}H NMR (C_5D_5N) δ0 92 (3H, *s*),0 93 (3H, *s*),1 00 (3H, *s*), 1 21 (3H, *s*), 2 60 (1H, *t*, *J*=13 Hz), 3 22 (1H, *dd*, *J*=4, 12 Hz), 4 75 (1H, *s*), 4 80 (1, H, *s*), 5 0 (1H, *d*, *J*=7 Hz), 5.49 (1H, *t*) SIMS *m/z* 611 [M+Na]$^+$, M.W 598 EIMS *m/z* (rel int) 456 [M−142]$^+$ (3), 410 (3), 232, 207 (23), 187 (100), 176 (25)

30-*Norhederagenin* 3-O-β-*glucopyranosyl*-(1→3)-α-L-*arabinopyranoside* (**2**) Mp 268–270°, colourless powder, $[\alpha]_D^{20}$ +106 3° (MeOH, *c* 0 222), ^{1}H NMR (C_5D_5N) δ0 93 (3H, *s*), 0 94 (3H, *s*), 1 01 (3H, *s*), 1 22 (3H, *s*), 2 62 (1H, *t*, *J*=12 Hz), 3 24 (1H, *dd*, *J* =4 4, 12 Hz), 4 75 (1H, *s*), 4 80 (1H, *s*), 4 98 (1H, *d*, *J*=7 4 Hz), 5 31 (1H, *d*, *J*=7 8 Hz), 5 49 (1H, *br s*) SIMS *m/z* 773 [M +Na]$^+$, M W 750, 456 [M−294]$^+$, 439[M−312]$^+$

30-*Norhederagenin* 3-O-β-D-*xylopyranosyl* (1→2)-α-L-*arabinopyranoside* (**3**) Mp 248–252°, colourless powder, $[\alpha]_D^{20}$ +86 2° (MeOH, *c* 0 163) ^{1}H NMR (C_5D_5N) δ0 96 (3H, *s*), 1.02 (3H, *s*), 1 06 (3H, *s*), 1 21 (3H, *s*), 2.81 (1H, *t*,*J*=13 2 Hz), 3.25 (1H, *m*), 4 74 (1H, *s*), 4 79 (1H, *s*), 5 08 (1H, *d*, *J*=6.2 Hz), 5.11 (1H, *d*, 6 7 Hz), 5 48 (1H, *br s*) SIMS *m/z*: 721 [M+H]$^+$

30-*Noroleanolic acid* 3-*O*-β-D-*xylopylanosyl* (1→2)-α-L-*arabinopyranoside* (**4**). Mp 290°>(decomp), $[\alpha]_D^{20}$ +43 7 (pyridine, *c* 0 274) ^{1}H NMR (C_5D_5N) δ0 88 (3H, *s*), 0 99 (3H, *s*), 1 08 3H, *s*), 1 28 (6H, *s*), 3 61 (1H, *m*), 4 75 (1H, *s*), 4 81 (1H, *s*), 4 89 (1H, *d*, *J* =6 2 Hz), 5 08 (1H, *d*, *J*=6 9 Hz), 5 49 (1H, *br*, *s*) SIMS *m/z* 727 [M+Na]$^+$, M_r 704 EIMS *m/z* (rel int) 440 [M−264]$^+$ (10), 232 (90), 207 (37), 187 (100)

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