Triterpenes and Triterpene Saponins from the Stems of *Akebia trifoliata*

Yoshihiro MIMAKI,* Minpei KURODA, Akihito YOKOSUKA, Hiroshi HARADA, Masato FUKUSHIMA, and Yutaka SASHIDA

Laboratory of Medicinal Plant Science, Tokyo University of Pharmacy and Life Science, School of Pharmacy; 1432–1, Horinouchi, Hachioji, Tokyo 192–0392, Japan. Received March 26, 2003; accepted May 14, 2003

To characterize the stems of *Akebia trifoliata* chemically, a detailed phytochemical examination was carried out on *A. trifoliata* stems, with particular attention to the triterpene and triterpene saponin constituents, and resulted in the isolation of three new triterpenes (1—3) and three new triterpene saponins (11—13), together with seven known triterpenes (4—10) and 12 known triterpene saponins (14—25). The structures of the new compounds were determined on the basis of spectroscopic analysis, including two-dimensional NMR spectroscopic data, and the results of hydrolysis. Four saponins (22—25), which were obtained in good yields and were not isolated from *Akebia quinata* stems, are concluded to be applicable as marker compounds in chemically distinguishing between *A. trifoliata* and *A. quinata* by conventional TLC examination. To the best our knowledge, the current work is the first chemical investigation of *A. trifoliata*.

Key words Akebiae Caulis; *Akebia trifoliata*; Lardizabalaceae; stem; triterpene; triterpene saponin

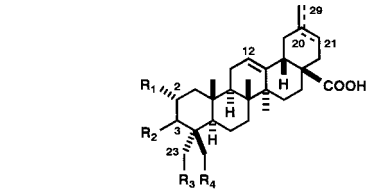
Akebiae Caulis (Japanese name: *moku-tsu*) is an important crude drug used in several Kampo prescriptions effective as a diuretic and an antiphlogistic. According to the *Pharmacopoeia of Japan*, Akebiae Caulis is prepared from the stems of *Akebia trifoliata* (THUNB.) KOIDZ. or *Akebia quinata* (HOUTT.) DECNE. (Lardizabalaceae). Although phytochemical analyses were extensively carried out on not only the stems[1—3] but also pericarps[4,5] and seeds[6,7] of *A. quinata* in 1970's, no chemical work appears to have been done on *A. trifoliata*, except for a study reporting the triterpene production of callus tissues derived from *A. trifoliata* stems.[8] Our preliminary TLC analysis of the MeOH extracts of *A. trifoliata* and *A. quinata* revealed that the chromatogram of *A. trifoliata*, which showed three prominent spots with *Rf* values ranging from around 0.5 to 0.7, was significantly different from that of *A. quinata*. This result prompted us to examine the triterpene and triterpene saponin constituents of the stems of *A. trifoliata*, which resulted in the isolation of three new triterpenes (**1**—**3**) and three new triterpene saponins (**11**—**13**), together with seven known triterpenes (**4**—**10**) and 12 known triterpene saponins (**14**—**25**). This paper reports the identification and structural determination of the isolated compounds on the basis of spectroscopic analysis, including two-dimensional NMR spectroscopic data, and the results of hydrolysis.

Results and Discussion

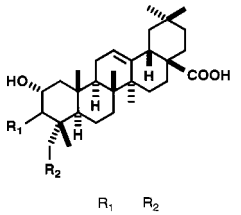
The *n*-BuOH-soluble fraction of the MeOH extract of *A. trifoliata* stems was chromatographed on porous-polymer polystyrene resin (Diaion HP-20), and the EtOH eluate portion was subjected to column chromatography on silica gel and octadecylsilanized (ODS) silica gel, and to reverse-phase preparative HPLC, giving compounds **1**—**25**. Compounds **4**—**10** and **14**—**25** were identified as 3α,24-dihydroxy-30-noroleana-12,20(29)-dien-28-oic acid (**4**),[9] 2α,3β,23-trihydroxy-30-noroleana-12,20(29)-dien-28-oic acid (**5**),[5] 2α,3β-dihydroxyolean-12-en-28-oic acid (**6**),[10] 2α,3α-dihydroxyolean-12-en-28-oic acid (**7**),[11] 2α,3β,23-trihydroxyolean-12-en-28-oic acid (**8**),[5] 2α,3β-dihydroxylup-20(29)-en-28-oic acid (**9**),[12] 2α,3α-dihydroxylup-20(29)-en-28-oic acid (**10**),[13] 3β-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid (**14**),[14] 3β-[(*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid (**15**),[15] 3β-hydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**16**),[16] 3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**17**),[4] 3β-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**18**),[14] 3β-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-[β-D-xylopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**19**),[17] 23-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**20**),[14] 3β-[(*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**21**),[18] 2α,3β,23-trihydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**22**),[19] 2α,3β,23-trihydroxyolean-12-en-28-oic acid *O*-β-D-xylopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**23**),[5] 2α,3β,23-trihydroxyurs-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**24**),[20] and 2α,3β,23-trihydroxy-30-norolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**25**),[5] respectively.

Compound **1** was obtained as an amorphous solid with the molecular formula $C_{29}H_{44}O_4$, as determined by the data of the positive-ion high-resolution electrospray ionization mass spectrum (HR-ESI-MS), showing an accurate $[M+Na]^+$ ion at *m*/*z* 479.3188, and ^{13}C-NMR spectrum (29 carbon signals). The ^{1}H-NMR spectrum of **1** showed signals for five tertiary methyl groups at δ 1.29, 1.24, 1.09, 1.01, and 0.98 (each s),

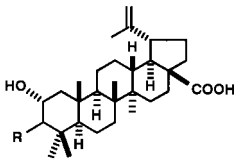
* To whom correspondence should be addressed. e-mail: mimakiy@ps.toyaku.ac.jp



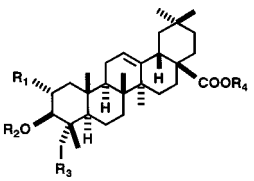
	R_1	R_2	R_3	R_4	
1	OH	β-OH	H	H	$\Delta^{20(29)}$
2	OH	α-OH	H	H	$\Delta^{20(29)}$
3	OH	β-OH	OH	H	$\Delta^{20(21)}$
4	H	α-OH	H	OH	$\Delta^{20(29)}$
5	OH	β-OH	OH	H	$\Delta^{20(29)}$



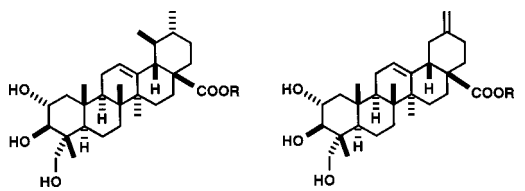
	R_1	R_2
6	β-OH	H
7	α-OH	H
8	β-OH	OH



	R
9	β-OH
10	α-OH



	R_1	R_2	R_3	R_4
11	OH	$^{+}Na^{-}O_3S$-	OH	Rha-(1→4)-Glc-(1→6)-Glc-
12	OH	$^{+}Na^{-}O_3S$-	OH	Glc-(1→6)-Glc-
13	H	GlcUA-(1→3)-[Rha-(1→2)]-Ara-	H	H
14	H	Glc-(1→3)-[Rha-(1→2)]-Ara-	H	H
15	H	Glc-(1→2)-[Glc-(1→3)]-Ara-	H	H
16	H	H	H	Rha-(1→4)-Glc-(1→6)-Glc-
17	H	Rha-(1→2)-Ara-	H	Rha-(1→4)-Glc-(1→6)-Glc-
18	H	Glc-(1→3)-[Rha-(1→2)]-Ara-	H	Rha-(1→4)-Glc-(1→6)-Glc-
19	H	Glc-(1→3)-[Xyl-(1→2)]-Ara-	H	Rha-(1→4)-Glc-(1→6)-Glc-
20	H	Rha-(1→2)-Ara-	OH	Rha-(1→4)-Glc-(1→6)-Glc-
21	H	Glc-(1→3)-Ara-	OH	Rha-(1→4)-Glc-(1→6)-Glc-
22	OH	H	OH	Rha-(1→4)-Glc-(1→6)-Glc-
23	OH	H	OH	Xyl-(1→3)-Rha-(1→4)-Glc-(1→6)-Glc-



	R
24	Rha-(1→4)-Glc-(1→6)-Glc-
25	Rha-(1→4)-Glc-(1→6)-Glc-

an exomethylene group at δ 4.81 and 4.76 (each br s), and an olefinic proton at δ 5.49 (t, J=3.5 Hz). The ^{1}H-NMR data and comparison of the ^{13}C-NMR spectrum of **1** with that of **5** showed their considerable structural similarity. The 20(29)-exomethylene group was ascertained by long-range correlations from the exomethylene proton signals to the methylene carbon signals at δ 41.9 and 30.4 assignable to C-19 and C-21, respectively, in the heteronuclear multiple-bond connectivity (HMBC) spectrum of **1**. Treatment of **1** with 2,2-dimethoxypropane resulted in the formation of an acetonide between the hydroxyl groups at C-2 and C-3, and the large proton spin-coupling constant of H-3, $^3J_{H\text{-}2,H\text{-}3}$=9.4 Hz, was consistent with the 2α and 3β configurations. The difference between **1** and **5** consisted only of a functional group attached to the C-4 quaternary carbon. The hydroxymethyl proton signals observed in the ^{1}H-NMR spectrum of **5** was displaced by the three-proton singlet signal at δ 1.29 (Me-23) in that of **1**, which, as well as the methyl singlet at δ 1.09 (Me-24) showed long-range correlations with δ 39.8 (C-4). The structure of **1** was formulated as 2α,3β-dihydroxy-30-noroleana-12,20(29)-dien-28-oic acid.

The ^{1}H-NMR spectrum of **2** ($C_{29}H_{44}O_4$) was very similar to that of **1**, showing signals for five tertiary methyl groups at δ 1.28, 1.15, 1.01, 0.96, and 0.91 (each s), an exomethylene group at δ 4.78 and 4.74 (each br s), and an olefinic proton at δ 5.48 (t, J=3.4 Hz). Although an acetonide was also formed between the hydroxyl groups at C-2 and C-3, the proton spin-coupling constants, $^3J_{H\text{-}2,H\text{-}3}$=2.6 Hz, $^3J_{H\text{-}1ax,H\text{-}2}$=11.6 Hz, and $^3J_{H\text{-}1eq,H\text{-}2}$=4.2 Hz, indicated that **2** was an isomer of **1** with regard to the C-3 hydroxyl group: the C-3 hydroxyl group was oriented to an α-axial while the C-2 hydroxyl group was oriented to an α-equatorial in **2**. This was further confirmed by nuclear Overhauser effect (NOE) correlations from H-2 to Me-24 (δ 0.91) and Me-25 (δ 0.96), and from H-3 to Me-23 (δ 1.28) and Me-24 in the phase-sensitive NOE correlation spectroscopy (NOESY) spectrum of **2**. Compound **2** was shown to be 2α,3α-dihydroxy-30-noroleana-12,20(29)-dien-28-oic acid.

The ^{1}H- and ^{13}C-NMR spectra of **3** ($C_{29}H_{44}O_5$) showed signals due to the C-2α hydroxyl group [δ_H 4.25 (ddd, J=11.5, 9.4, 4.2 Hz)], C-3β hydroxyl group [δ_H 4.20 (d, J=9.4 Hz)], C-12 olefinic group [δ_H 5.50 (t, J=3.2 Hz)/δ_C 122.6 (CH) and 144.4 (C)], C-23 hydroxymethyl group [δ_H 4.21 and 3.73 (each d, J=10.5 Hz)], and C-28 carboxyl group [δ_C 180.0)], suggesting that **3** was a 30-noroleanane derivative related to **5**. However, differences were recognized in the NMR signals arising from the E-ring part of the pentacyclic skeleton. Instead of the signals for an exomethylene group, signals assignable to a methyl group on a double bond [δ_H 1.65 (3H, s)] and a trisubstituted olefinic group [δ_H 5.34 (br s)/δ_C 132.7 (C) and 117.9 (CH)] were observed. In the HMBC spectrum, the methyl signal at δ 1.65 showed long-range correlations with a methylene carbon signal at δ 36.8, as well as the olefinic carbon signals at δ 132.7 and 117.9. The methylene carbon signal was associated with the one-bond coupled proton signals at δ 2.34 (dd, J=13.8, 10.6 Hz) and 1.94 (dd, J=13.8, 8.4 Hz) by the heteronuclear multiple quantum coherence (HMQC) spectrum, which showed proton spin-coupling correlations with the deshielded proton signal at δ 3.27 unequivocally attributable to H-18 in the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum and was assigned to H_2-19. On the other hand, the olefinic proton at δ 5.34 was shown to be coupled with the isolated methylene protons at δ 2.63 and 2.31 with J values of less than 0.5 Hz. One of the methylene proton signals at δ 2.31 showed HMBC correlations with C-17 (δ 44.8) and C-18 (δ 42.5), allowing the assignment of the δ 2.63 and 2.31 resonances to H_2-22. Thus the C-20(21) olefinic group bearing a methyl at C-20 was identified, and the structure of **5** was established as 2α,3β,23-trihydroxy-30-noroleana-12,20(21)-dien-28-oic acid.

Compound **11** was analyzed for $C_{48}H_{78}NaO_{22}S$ by the negative-ion HR-ESI-MS (*m/z* 1037.4629 $[M-H-Na]^-$) and positive-ion FAB-MS (*m/z* 1084 $[M+Na]^+$) data. The ^{1}H-NMR spectrum of **11** was typical of a triterpene triglycoside based upon olean-12-en-28-oic acid, showing six three-proton singlet signals at δ 1.13, 1.05, 0.97, 0.86×2, and 0.85, an olefinic proton signal at δ 5.34 (t, J=3.3 Hz), and three anomeric proton signals at δ 6.20 (d, J=8.1 Hz), 5.84 (br s), and 4.98 (d, J=7.9 Hz). Acid hydrolysis of **11** with 1.0 M HCl in dioxane–H_2O (1 : 1) gave 2α,3β,23-trihydroxyolean-12-en-28-oic acid (**8**) as the aglycon, and D-glucose and L-rhamnose as the carbohydrate moieties. The monosaccharides, including their absolute configurations, were identified by direct HPLC analysis of the hydrolysate, with detection carried out using an optical rotation (OR) detector. Furthermore, when 0.5 M $BaCl_2$ solution was added to the hydrolysate, it became cloudy, indicating the presence of sulfate ion. From the above NMR spectral and chemical data, together with the information from the positive FAB-MS and negative-ion ESI-MS-MS, the former showing a prominent fragment ion peak at *m/z* 981 $[M+Na-SO_3Na]^+$ and the latter at *m/z* 97 $[HSO_4]^-$, **11** was deduced to be a 2α,3β,23-trihydroxyolean-12-en-28-oic acid triglycoside with a sodium sulfate group. Analysis of the ^{1}H- and ^{13}C-NMR spectra of **11** allowed us to assume that the sugar sequence of the triglycoside moiety and its linkage position of **11** were the same as those of the concomitantly isolated saponins in this study (**16**—**22**, **24**, **25**). This was ascertained by HMBC correlations from H-1 of the terminal α-L-rhamnopyranosyl unit at δ 5.84 to C-4 of the inner β-D-glucopyranosyl unit (Glc′) at δ 78.2, H-1 of Glc′ at δ 4.98 to C-6 of the other inner β-D-glucopyranosyl unit (Glc) at δ 69.2, and from H-1 of Glc at δ 6.20 to C-28 of the aglycon at δ 176.6. The sulfate group was shown to be involved in a linkage at C-3 of the aglycon because the signals due to H-3 and C-3 were markedly displaced downfield at $\delta_{H\text{-}3}$ 4.96 (+0.77 ppm) and $\delta_{C\text{-}3}$ 84.1 (+5.9 ppm), respectively, when comparing the ^{1}H- and ^{13}C-NMR spectra of **11** with those of **22**. Accordingly, the structure of **11** was assigned as 2α,23-dihydroxy-3β-sulfoxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester sodium salt.

The molecular formula of **12** was determined by the negative-ion HR-ESI-MS and positive-ion FAB-MS to be $C_{42}H_{68}NaO_{18}S$, which was less than that of **11** by $C_6H_{10}O_4$, corresponding to the lack of one deoxyhexose. The ^{1}H-NMR spectrum of **12** exhibited signals for two anomeric protons at δ 6.21 (d, J=8.1 Hz) and 5.01 (d, J=7.8 Hz), as well as signals for six methyl groups at δ 1.14, 1.05, 0.96, 0.86×2, and 0.82, and an olefinic proton at δ 5.35 (t, J=3.3 Hz). The presence of a sodium sulfate group at C-3 of the aglycon in **12** as in **11** was ascertained by the ^{1}H-NMR [$\delta_{H\text{-}3}$ 4.95 (d, J=9.5 Hz)], ^{13}C-NMR ($\delta_{C\text{-}3}$ 84.2), FAB-MS (*m/z* 835 $[M+Na-SO_3Na]^+$), and ESI-MS-MS (*m/z* 97 $[HSO_3]^-$) data. When the ^{13}C-NMR spectrum of **12** was compared with that of **11**, the signals attributable to the terminal α-L-rhamnopyranosyl unit could not be detected, and the six signals corresponding to a terminal β-D-glucopyranosyl unit were observed at δ 105.1 (CH), 75.1 (CH), 78.4 (CH), 71.5 (CH), 78.3 (CH), and 62.5 (CH_2). Mild acid hydrolysis of **11** with 0.2 M HCl at 95 °C for 30 min gave **12** as one of the hydrolysates. These spectral and chemical data allowed the structural determination of **12** as 2α,23-dihydroxy-3β-sulfoxyolean-12-en-28-oic acid *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester sodium salt.

Compound **13** was deduced as $C_{47}H_{74}O_{17}$ from the positive-ion HR-ESI-MS (*m/z* 933.4911 $[M+Na]^+$+) and ^{13}C-NMR spectrum (47 carbon signals). The ^{1}H-NMR spectrum of **13** contained seven three-proton singlet signals at δ 1.30, 1.24, 1.13, 1.01, 0.99, 0.95, and 0.83, an olefinic proton signal at δ 5.46 (t, J=3.3 Hz), and three anomeric proton signals at δ 6.19 (br s), 5.22 (d, J=7.7 Hz), and 4.85 (d, J=5.9 Hz). Acid hydrolysis of **13** with 0.2 M HCl yielded 3β-hydroxyolean-12-en-28-oic acid (oleanolic acid), L-arabinose, D-glucuronic acid, and L-rhamnose, whereas enzymatic hydrolysis with β-D-glucuronidase gave a partial hydrolysate (**13a**) and D-glucuronic acid. Compound **13a** agreed with the product obtained by alkaline hydrolysis of **17** with 6% KOH

Table 1. ^{13}C-NMR Spectral Data for **1**—**3** and **11**—**13** in C_5D_5N

Position	**1**	**2**	**3**	**11**	**12**	**13**
1	47.8	42.8	47.7	48.1	47.9	38.9
2	68.6	66.1	68.8	67.4	67.5	26.6
3	83.8	79.3	78.2	84.1	84.2	88.1
4	39.8	38.8	43.7	44.4	44.4	39.6
5	55.9	48.7	47.9	47.3	47.2	56.0
6	18.8	18.4	18.5	18.3	18.3	18.5
7	33.2	33.2	32.5	32.6	32.5	33.2
8	39.8	40.0	40.0	39.9	39.9	39.7
9	48.1	47.9	48.2	48.0	47.9	48.0
10	38.5	38.7	38.4	37.8	37.8	37.0
11	23.9	23.8	23.9	23.9	23.9	23.7
12	122.9	123.0	122.6	122.7	122.6	122.5
13	144.2	144.2	144.4	144.2	144.2	144.8
14	42.1	42.2	42.1	42.1	42.1	42.1
15	28.3	28.2	28.7	28.2	28.2	28.3
16	23.8	23.8	25.0	23.8	23.8	23.7
17	47.0	47.0	44.8	47.0	47.0	46.6
18	47.9	47.9	42.5	41.6	41.6	41.9
19	41.9	41.9	36.8	46.1	46.1	46.4
20	149.1	149.1	132.7	30.7	30.7	30.9
21	30.4	30.4	117.9	33.9	33.9	34.2
22	38.3	38.4	37.5	32.6	32.4	33.2
23	29.3	29.5	66.5	64.7	64.6	28.0
24	17.7	22.3	14.3	14.1	14.1	17.0
25	16.8	16.6	17.3	17.5	17.5	15.6
26	17.4	17.4	17.4	17.5	17.4	17.3
27	26.1	26.1	27.2	26.1	26.1	26.2
28	179.4	179.4	180.0	176.6	176.6	180.2
29	107.1	107.1	23.3	33.0	33.1	33.2
30	—	—	—	23.7	23.6	23.7
1′				95.6	95.7	104.9
2′				73.8	73.8	74.9
3′				78.7	78.7	82.9
4′				70.8	70.9	68.6
5′				78.0	77.9	65.3
6′				69.2	69.4	—
1″				104.8	105.1	102.0
2″				75.3	75.1	72.5
3″				76.5	78.4	72.4
4″				78.2	71.5	73.9
5″				77.1	78.3	70.0
6″				61.2	62.5	18.6
1‴				102.6		105.1
2‴				72.5		74.7
3‴				72.6		77.6
4‴				74.0		73.3
5‴				70.3		77.6
6‴				18.5		172.7

in EtOH, through which the ester linkage of the triglycoside to C-28 of the aglycon was cleaved. The ^{1}H-NMR spectrum of **13** measured in C_5D_5N gave good spectral dispersion of the signals for the sugar moiety composed of L-arabinose, D-glucuronic acid, and L-rhamnose, and the ^{1}H- and ^{13}C-NMR assignments of each monosaccharide were established by interpretation of combined ^{1}H–^{1}H COSY and HMQC data. In the HMBC spectrum, the H-1 signal of the glucuronic acid moiety at δ 5.22 showed a long-range correlation with the carbon signal at δ 82.9 assigned to C-3 of the arabinosyl unit. Furthermore, HMBC correlations were observed between δ 6.19 (H-1 of rhamnosyl) and δ 74.9 (C-2 of arabinosyl), and between δ 4.85 (H-1 of arabinosyl) and δ 88.1 (C-3 of the aglycon). Thus the structure of **13** was defined as 3β-[(*O*-β-D-glucuronopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid.

Conclusions

The current work, to the best our knowledge, is the first chemical investigation of *A. trifoliata*. Compounds **1**—**3** are new 30-norolean-12-en-28-oic acid derivatives, and **11**—**13** are new triterpene saponins based upon olean-12-en-28-oic acid. Triterpene saponins are widely distributed in not only the plant kingdom but also in marine organisms, and more than 2600 saponins have been reported.[21] However, saponins with a sulfate group at C-3 of the oleanolic acid derivatives such as **11** and **12** have rarely been found in natural product research.[21]

The prominent spots A, B, and C detected on the reverse-phase TLC chromatogram were revealed to correspond to compounds **25**, **23**, and a mixture of **22** and **24**, respectively, which have not been isolated from *A. quinata* and could not be found by TLC analysis of the *A. quinata* extracts (Fig. 1). These compounds are concluded to be specific to *A. trifoliata* and applicable as marker compounds in chemically distinguishing between *A. trifoliata* and *A. quinata* by conventional TLC examination.

Experimental

Optical rotations were measured using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H-NMR, Karlsruhe, Germany) spectrometer using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard. FAB-MS were recorded on a Finnigan MAT TSQ-700 (San Jose, CA, U.S.A.) mass spectrometer using a dithiothreitol and dithioerythritol (3 : 1) matrix and ESI-MS on a Micromass LCT (Manchester, U.K.) mass spectrometer. Elemental analysis was carried out using an Elemental Vario EL elemental analyzer (Hanau, Germany). Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), silica gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed using a system comprised of a Tosoh CCPM pump (Tokyo, Japan), a Tosoh CCP PX-8010 controller, a Tosoh RI-8010, a Shodex OR-2 detector (Showa-Denko, Tokyo, Japan), and a Rheodyne injection port with a 2-ml sample loop for preparative HPLC and 20-μl sample loop for analytical HPLC. A Capcell Pak C_{18} UG80 column (10 mm i.d.×250 mm, ODS, 5 μm, Shiseido, Tokyo, Japan) was used for preparative HPLC, and a Capcell Pak NH_2 UG80 column (4.6 mm i.d.×250 mm, 5 μm, Shiseido) and an Aminex HPX-87H Ion Exclusion column (7.8 mm i.d.×250 mm, 9 μm, Bio-Rad Laboratories, Hercules, CA, U.S.A.) were employed for analytical HPLC.

Plant Materials The stems of *A. trifoliata* were collected in the fields of Hachioji ward, Tokyo, Japan, in October 1999. The plant was identified by one of the authors (Y.S.) and the plant specimen has been deposited in our laboratory (voucher no. AT-99-011). The plant materials used for TLC analysis were collected in the following places. *A. trifoliata*: Shiojiri, Nagano, in October 1999; *A. quinata*: Hachioji, Tokyo; and Shiojiri, Nagano, in October 1999.

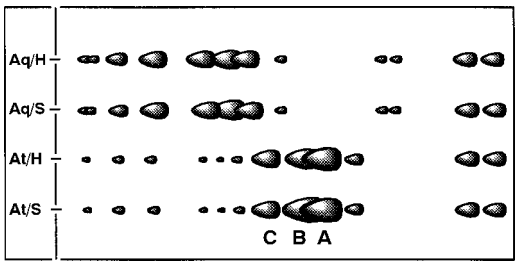


Fig. 1. Reverse-phase TLC Chromatogram of the MeOH Extracts of *A. quinara* and *A. trifoliata* Stems

Solvent: MeOH–H_2O (7 : 3). Aq/H: MeOH extract of *A. quinata* stems collected in Hachioji, Tokyo. Aq/S: MeOH extract of *A. quinata* stems collected in Shiojiri, Nagano. At/H: MeOH extract of *A. trifoliata* stems collected in Hachioji, Tokyo. At/S: MeOH extract of *A. trifoliata* stems collected in Shiojiri, Nagano.

Extraction and Isolation The plant material (fresh weight, 10.5 kg) was extracted with hot MeOH. The MeOH extract (995 g) was concentrated under reduced pressure and partitioned between H_2O and *n*-BuOH. The *n*-BuOH-soluble fraction (400 g) was passed through a Diaion HP-20 column eluted with 30% MeOH, EtOH, and EtOAc. Column chromatography of the EtOH eluate portion (220 g) on silica gel and elution with a stepwise gradient mixture of $CHCl_3$–MeOH (9 : 1; 4 : 1; 2 : 1; 1 : 1) and finally with MeOH alone gave six fractions (frs. I—VI). Fr. I was subjected to column chromatography on silica gel eluted with hexane–EtOAc (3 : 1), hexane–Me_2CO (3 : 1), $CHCl_3$–EtOAc (8 : 1; 7 : 1), and $CHCl_3$–MeOH (60 : 1) to give **7** (3.9 mg), **9** (14.5 mg), and **10** (11.7 mg). Fr. II was chromatographed on silica gel eluted with $CHCl_3$–MeOH (20 : 1) into two subfractions (frs. IIa and IIb). Fr. IIa was further separated by a silica gel column eluted with $CHCl_3$–MeOH (40 : 1; 30 : 1; 24 : 1) and an ODS silica gel column with MeOH–H_2O (4 : 1; 7 : 3) to give **1** (20.8 mg), **2** (25.5 mg), **4** (22.5 mg), and **6** (22.1 mg). Fr. IIb was subjected to column chromatography on silica gel eluted with $CHCl_3$–MeOH (30 : 1) and ODS silica gel with MeOH–H_2O (3 : 1), and to preparative HPLC using MeCN–MeOH–H_2O (1 : 1 : 1) to furnish **3** (8.8 mg), **5** (12.9 mg), and **8** (4.6 mg). Fr. III was subjected to a silica gel column eluted with $CHCl_3$–MeOH–H_2O (40 : 10 : 1) and divided into three subfractions (frs. IIIa, IIIb, and IIIc). Fr. IIIa was chromatographed on silica gel eluted with $CHCl_3$–MeOH–H_2O (30 : 10 : 1) and ODS silica gel with MeCN–H_2O (2 : 5) to yield **14** (280 mg) and **15** (106 mg). Fr. IIIb was chromatographed on silica gel eluted with $CHCl_3$–MeOH–H_2O (40 : 10 : 1) and ODS silica gel with MeCN–H_2O (1 : 3) to yield **13** (37.0 mg). Fr. IV was separated by column chromatography on silica gel eluted with $CHCl_3$–MeOH–H_2O (30 : 10 : 1; 20 : 10 : 1; 7 : 4 : 1) and ODS silica gel with MeCN–H_2O (2 : 5; 1 : 3; 1 : 4) to yield pure **23** (1.56 g) and **25** (2.19 g), **16** with a few impurities, and a mixture of **22** and **24**. Compound **16** (26.0 mg) was purified by preparative HPLC using MeOH–H_2O (7 : 3). The mixture of **22** and **24** was separated by subjecting it to preparative HPLC using MeOH–H_2O (4 : 3), giving **22** (512 mg) and **24** (460 mg). Fr. V was chromatographed on silica gel eluted with $CHCl_3$–MeOH–H_2O (20 : 10 : 1; 7 : 4 : 1) and ODS silica gel with MeCN–H_2O (1 : 3; 1 : 4; 2 : 9) to afford **11** (132 mg), **12** (20.8 mg), **20** (15.1 mg), and **21** (29.8 mg). Fr. VI was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH–H_2O (20 : 10 : 1; 7 : 4 : 1) and ODS silica gel column chromatography with MeOH–H_2O (4 : 3) and MeCN–H_2O (1 : 3) to give **17** (50.6 mg), **18** (156 mg), and **19** (16.9 mg).

Each (5 g) of the stems of *A. quinata* and *A. trifoliata* collected in Hachioji and Shiojiri was extracted with hot MeOH (5 ml). The MeOH extract was passed through a Diaion HP-20 column eluted with 30% MeOH, followed by EtOH. The EtOH eluate portion, in which the triterpene saponins were enriched, was used for TLC analysis.

Compound **1**: Amorphous solid. $[\alpha]_D^{27}$ +70.0° (c=0.10, MeOH). FAB-MS (negative mode): *m/z* 455 $[M-H]^-$. HR-ESI-MS (positive mode): *m/z* 479.3188 $[M+Na]^+$ (Calcd for $C_{29}H_{44}O_4Na$: 479.3137). *Anal.* Calcd for $C_{29}H_{44}O_4 \cdot 7/2H_2O$: C, 67.02; H, 9.86. Found: C, 66.93; H, 9.81. IR ν_{max} (film) cm^{-1}: 3333 (OH), 2924 and 2853 (CH), 1683 (C=O), 1053, 1031. ^{1}H-NMR (C_5D_5N) δ: 5.49 (1H, t, J=3.5 Hz, H-12), 4.81 and 4.76 (each 1H,

br s, H_2-29), 4.10 (1H, ddd, J=11.2, 9.4, 4.4 Hz, H-2), 3.41 (1H, d, J=9.4 Hz, H-3), 3.24 (1H, dd, J=13.5, 4.9 Hz, H-18), 1.29 (3H, s, Me-23), 1.24 (3H, s, Me-27), 1.09 (3H, s, Me-24), 1.01 (3H, s, Me-26), 0.98 (3H, s, Me-25). ^{13}C-NMR: see Table 1.

Preparation of Isopropylidene Derivative (1a) of 1 Compound **1** (10.6 mg) was dissolved in 2,2-dimethoxypropane (2 ml) containing a catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) (3 mg). After being allowed to stand for 12 h at room temperature, the reaction mixture was diluted with H_2O (5 ml) and extracted with Et_2O (5 ml×3). The Et_2O extract was washed successively with saturated $NaHCO_3$ (10 ml×2) and H_2O (10 ml×2), dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed on silica gel eluted with $CHCl_3$–Me_2CO (19 : 1) to give **1a** (5.2 mg). ^{1}H-NMR of **1a** (C_5D_5N) δ: 5.48 (1H, t, J=3.3 Hz, H-12), 4.81 and 4.76 (each 1H, br s, H_2-29), 3.83 (1H, ddd, J=11.9, 9.4, 3.8 Hz, H-2), 3.24 (1H, dd, J=13.3, 4.7 Hz, H-18), 3.18 (1H, d, J=9.4 Hz, H-3), 1.56 and 1.50 (each 3H, s, Me×2), 1.26, 1.15, 0.99, 0.94 and 0.90 (each 3H, s, Me-23—Me-27).

Compound **2**: Amorphous solid. $[\alpha]_D^{27}$ +142.0° (c=0.10, MeOH). FAB-MS (negative mode): m/z 455 $[M-H]^-$. *Anal.* Calcd for $C_{29}H_{44}O_4\cdot 3H_2O$: C, 68.20; H, 9.87. Found: C, 67.98; H, 9.62. IR ν_{max} (film) cm^{-1}: 3420 (OH), 2981, 2929 and 2858 (CH), 1686 (C=O), 1039. ^{1}H-NMR (C_5D_5N) δ: 5.48 (1H, t, J=3.4 Hz, H-12), 4.78 and 4.74 (each 1H, br s, H_2-29), 4.30 (1H, ddd, J=11.6, 4.2, 2.6 Hz, H-2), 3.77 (1H, d, J=2.6 Hz, H-3), 3.22 (1H, dd, J=13.5, 4.9 Hz, H-18), 1.28 (3H, s, Me-23), 1.15 (3H, s, Me-27), 1.01 (3H, s, Me-26), 0.96 (3H, s, Me-25), 0.91 (3H, s, Me-24). ^{13}C-NMR: see Table 1.

Preparation of Isopropylidene Derivative (2a) of 2 Compound **2** (10.0 mg) was treated with 2,2-dimethoxypropane as described for **1** to give **2a** (4.1 mg). ^{1}H-NMR of **2a** (C_5D_5N) δ: 5.51 (1H, t, J=3.3 Hz, H-12), 4.82 and 4.76 (each 1H, br s, H_2-29), 4.30 (1H, ddd, J=10.5, 5.9, 4.3 Hz, H-2), 3.83 (1H, d, J=4.3 Hz, H-3), 3.25 (1H, dd, J=13.7, 5.1 Hz, H-18), 1.61 and 1.43 (each 3H, s, Me×2), 1.25, 1.16, 0.97, 0.88 and 0.79 (each 3H, s, Me-23—Me-27).

Compound **3**: Amorphous solid. $[\alpha]_D^{27}$ +72.0° (c=0.10, MeOH). FAB-MS (negative mode): m/z 471 $[M-H]^-$. *Anal.* Calcd for $C_{29}H_{44}O_5\cdot 1/2H_2O$: C, 72.31; H, 9.42. Found: C, 71.97; H, 9.53. IR ν_{max} (film) cm^{-1}: 3361 (OH), 2932 (CH), 1689 (C=O), 1050. ^{1}H-NMR (C_5D_5N) δ: 5.50 (1H, t, J=3.2 Hz, H-12), 5.34 (1H, br s, H-21), 4.25 (1H, ddd, J=11.5, 9.4, 4.2 Hz, H-2), 4.21 and 3.73 (each 1H, d, J=10.5 Hz, H_2-23), 4.20 (1H, d, J=9.4 Hz, H-3), 3.27 (1H, dd, J=10.6, 8.4 Hz, H-18), 2.63 (1H, br d, J=16.8 Hz, H-22a), 2.34 (1H, dd, J=13.8, 10.6 Hz, H-19a), 2.31 (1H, br d, J=16.8 Hz, H-22b), 1.94 (1H, dd, J=13.8, 8.4 Hz, H-19b), 1.65 (3H, s, Me-29), 1.25 (3H, s, Me-27), 1.08 (3H, s, Me-25), 1.07 (3H, s, Me-24), 1.06 (3H, s, Me-26). ^{13}C-NMR: see Table 1.

Compound **11**: Amorphous solid. $[\alpha]_D^{27}$ −8.0° (c=0.10, MeOH). FAB-MS (positive mode): m/z 1084 $[M+Na]^+$, 981 $[M+Na-SO_3Na]^+$. FAB-MS (negative mode): m/z 1037 $[M-H-Na]^-$, 713 $[M-H-Na-$rhamnosyl$-$glucosyl$]^-$. HR-ESI-MS (negative mode): m/z 1037.4629 $[M-H-Na]^-$ (Calcd for $C_{48}H_{77}O_{22}S$: 1034.4627). ESI-MS-MS (negative mode): m/z 97 $[HSO_4]^-$. IR ν_{max} (film) cm^{-1}: 3387 (OH), 2941 (CH), 1733 (C=O), 1258, 1228, 1069, 1039. ^{1}H-NMR (C_5D_5N) δ: 6.20 (1H, d, J=8.1 Hz, H-1′), 5.84 (1H, br s, H-1‴), 5.34 (1H, t, J=3.3 Hz, H-12), 4.98 (1H, d, J=7.9 Hz, H-1″), 4.96 (1H, d, J=9.6 Hz, H-3), 4.35 (1H, ddd, J=11.2, 9.6, 3.7 Hz, H-2), 4.14 and 3.58 (each 1H, d, J=11.6 Hz, H_2-23), 3.14 (1H, dd, J=13.6, 4.0 Hz, H-18), 1.13 (3H, s, Me-27), 1.05 (3H, s, Me-25), 0.97 (3H, s, Me-26), 0.86 (3H×2, s, Me-29 and Me-30), 0.85 (3H, s, Me-24). ^{13}C-NMR: see Table 1.

Acid Hydrolysis of 11 A solution of **11** (30.5 mg) in 1.0 M HCl (dioxane–H_2O, 1 : 1, 4 ml) was heated at 95 °C for 1 h under an Ar atmosphere. After dilution with H_2O (6 ml), the reaction mixture was extracted with EtOAc (10 ml×3). The EtOAc extract was chromatographed on silica gel eluted with $CHCl_3$–MeOH (9 : 1) to afford **8** (12.4 mg). When a few drops of 0.5 M $BaCl_2$ solution was added to the H_2O residue (2 ml), it became cloudy (the qualitative test for sulfate ion). The H_2O residue (8 ml) was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and subjected to a Diaion HP-20 column eluted with 30% MeOH, followed by MeOH. The 30% MeOH eluate portion was passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA, U.S.A.), which was then analyzed by HPLC under the following conditions: column, Capcell Pak NH_2 UG80; solvent, MeCN–H_2O (17 : 3); flow rate, 0.9 ml/min; detection, RI and OR. Identification of L-rhamnose and D-glucose was carried out by comparison of their retention times and optical rotations with those of authentic samples. t_R (min): 8.07 (L-rhamnose, negative optical rotation), 16.66 (D-glucose, positive optical rotation).

Compound **12**: Amorphous solid. $[\alpha]_D^{27}$ +14.0° (c=0.10, MeOH). FAB-MS (positive mode): m/z 938 $[M+Na]^+$, 835 $[M+Na-SO_3Na]^+$. FAB-MS (negative mode): m/z 891 $[M-H-Na]^-$, 567 $[M-H-Na-$glucosyl×2$]^-$. HR-ESI-MS (negative mode): m/z 891.4056 $[M-H-Na]^-$ (Calcd for $C_{42}H_{67}O_{22}S$: 891.4048). ESI-MS-MS (negative mode): m/z 97 $[HSO_4]^-$. IR ν_{max} (film) cm^{-1}: 3388 (OH), 2931 (CH), 1739 (C=O), 1259, 1228, 1069. ^{1}H-NMR (C_5D_5N) δ: 6.21 (1H, d, J=8.1 Hz, H-1′), 5.35 (1H, t, J=3.3 Hz, H-12), 5.01 (1H, d, J=7.8 Hz, H-1″), 4.95 (1H, d, J=9.5 Hz, H-3), 4.33 (1H, overlapping, H-2), 4.13 and 3.59 (each 1H, d, J=11.7 Hz, H_2-23), 3.15 (1H, dd, J=13.5, 3.7 Hz, H-18), 1.14 (3H, s, Me-27), 1.05 (3H, s, Me-25), 0.96 (3H, s, Me-26), 0.86 (3H×2, s, Me-29 and Me-30), 0.82 (3H, s, Me-24). ^{13}C-NMR: see Table 1.

Partial Acid Hydrolysis of 11 A solution of **11** (30.1 mg) in 0.2 M HCl (dioxane–H_2O, 1 : 1, 4 ml) was heated at 95 °C for 30 min under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU column and subjected to a Diaion HP-20 column eluted with 30% MeOH, followed by MeOH. The MeOH eluate portion was chromatographed over silica gel eluted with $CHCl_3$–MeOH–H_2O (40 : 10 : 1) to give **12** (1.9 mg).

Compound **13**: Amorphous solid. $[\alpha]_D^{27}$ −4.0° (c=0.10, MeOH). FAB-MS (negative mode): m/z 909 $[M-H]^-$, 763 $[M-H-$rhamnosyl$]^-$, 733 $[M-H-$glucuronyl$]^-$. HR-ESI-MS (positive mode): m/z 933.4911 $[M+Na]^+$ (Calcd for $C_{47}H_{74}O_{17}Na$: 933.4824). *Anal.* Calcd for $C_{47}H_{74}O_{17}\cdot 11/2H_2O$: C, 55.88; H, 8.48. Found: C, 56.01; H, 8.24. IR ν_{max} (film) cm^{-1}: 3333 (OH), 2925 and 2856 (CH), 1689 and 1611 (C=O), 1055. ^{1}H-NMR (C_5D_5N) δ: 6.19 (1H, br s, H-1″), 5.46 (1H, t, J=3.3 Hz, H-12), 5.22 (1H, d, J=7.7 Hz, H-1‴), 4.85 (1H, d, J=5.9 Hz, H-1′), 3.32 (1H, dd, J=11.7, 3.9 Hz, H-3), 3.29 (1H, dd, J=15.4, 3.7 Hz, H-18), 1.30 (3H, s, Me-27), 1.24 (3H, s, Me-23), 1.13 (3H, s, Me-24), 1.01 (3H, s, Me-30), 0.99 (3H, s, Me-26), 0.95 (3H, s, Me-29), 0.83 (3H, s, Me-25). ^{13}C-NMR: see Table 1.

Acid Hydrolysis of 13 A solution of **13** (3.0 mg) in 0.2 M HCl (dioxane–H_2O, 1 : 1, 1 ml) was heated at 95 °C for 1 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by the addition of 0.2 M NaOH solution and subjected to a Diaion HP-20 column eluted with 40% MeOH, followed by EtOH–Me_2CO (1 : 1). The EtOH–Me_2CO (1 : 1) eluate portion was chromatographed over silica gel eluted with $CHCl_3$–EtOAc (19 : 1) to give oleanolic acid (1.2 mg). The 40% MeOH eluate portion was passed through a Sep-Pak C_{18} cartridge, which was then analyzed by HPLC under the following conditions: 1) column, Aminex HPX-87H Ion Exclusion; solvent, 0.005 M H_2SO_4 solution; flow rate, 0.7 ml/min; detection, RI and OR. Identification of D-glucuronic acid was carried out by comparison of its retention time and optical rotation with that of an authentic sample. t_R (min): 6.94 (D-glucuronic acid, positive optical rotation); 2) column, Capcell Pak NH_2 UG80; solvent, MeCN–H_2O (17 : 3); flow rate, 0.9 ml/min; detection, RI and OR. Identification of L-rhamnose and L-arabinose was carried out by comparison of their retention times and optical rotations with those of authentic samples. t_R (min): 9.23 (L-rhamnose, negative optical rotation), 11.49 (L-arabinose, positive optical rotation).

Enzymatic Hydrolysis of 13 Compound **13** (23.4 mg) was dissolved in an KH_2PO_4/Na_2HPO_4 buffer (pH 6.8, 2 ml) with β-D-glucuronidase (*Escherichia coli* in origin. Sigma, EC 3.2.1.31) (3.5 mg, 27.6×10^6 units) and incubated at room temperature for 168 h. The crude mixture was chromatographed on ODS silica gel eluted with MeOH–H_2O (4 : 1) to yield **13a** (1.6 mg) and D-glucuronic acid. D-Glucuronic acid was identified by direct TLC comparison with an authentic sample. *Rf* 0.17 ($CHCl_3$–MeOH–H_2O, 6 : 4 : 1).

Preparation of 13a from 17 Compound **17** (23.2 mg) was treated with 6% KOH in EtOH (5 ml) at room temperature for 3 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B (Organo, Tokyo, Japan) column and chromatographed on silica gel eluted with $CHCl_3$–MeOH–H_2O (60 : 10 : 1) to yield **13a** (11.3 mg).

References and Notes

1) Fujita M., Itokawa H., Kumekawa Y., *Yakugaku Zasshi*, **94**, 189—193 (1974).
2) Fujita M., Itokawa H., Kumekawa Y., *Yakugaku Zasshi*, **94**, 194—198 (1974).
3) Kumekawa Y., Itokawa H., Fujita M., *Chem. Pharm. Bull.*, **22**, 2294—2300 (1974).
4) Higuchi R., Kawasaki T., *Chem. Pharm. Bull.*, **24**, 1021—1032 (1976).
5) Higuchi R., Kawasaki T., *Chem. Pharm. Bull.*, **24**, 1314—1323 (1976).
6) Higuchi R., Miyahara K., Kawasaki T., *Chem. Pharm. Bull.*, **20**, 1935—1939 (1972).
7) Higuchi R., Kawasaki T., *Chem. Pharm. Bull.*, **20**, 2143—2149 (1972).
8) Ikuta A., *J. Nat. Prod.*, **58**, 1378—1383 (1995).
9) Ikuta A., Itokawa H., *Phytochemistry*, **27**, 3809—3810 (1988).

10) Singh B., Singh V. P., Pandey V. B., Rücker G., *Planta Med.*, **61**, 576—577 (1995).
11) Kumar N. S., Muthukuda P. M., Balasubramaniam S., *Phytochemistry*, **24**, 2454—2455 (1985).
12) Yagi A., Okumura N., Haraguchi Y., Noda K., Nishioka I., *Chem. Pharm. Bull.*, **26**, 1798—1802 (1978).
13) Kumar N. S., Muthukuda P. M., Wazeer M. I. M., *Phytochemistry*, **24**, 1337—1340 (1985).
14) Shao C.-J., Kasai R., Xu J.-D., Tanaka O., *Chem. Pharm. Bull.*, **37**, 311—314 (1989).
15) Miyase T., Melek F. R., El-Gindi O. D., Abdel-Khalik S. M., El-Gindi M. R., Haggag M. Y., Hilal S. H., *Phytochemistry*, **41**, 1175—1179 (1996).
16) Dubois M. A., Ilyas M., Wagner H., *Planta Med.*, **1986**, 80—83 (1986).
17) Miyase T., Sutoh N., Zhang D. M., Ueno A., *Phytochemistry*, **42**, 1123—1130 (1996).
18) Wegner C., Hamburger M., Kunert O., Haslinger E., *Helv. Chim. Acta*, **83**, 1454—1464 (2000).
19) Maeda C., Ohtani K., Kasai R., Yamasaki K., Duc N. M., Nham N. T., Cu N. K. Q., *Phytochemistry*, **37**, 1131—1137 (1994).
20) Mahato S. B., Sahu N. P., Luger P., Müller E., *J. Chem. Soc. Perkin Trans. II*, **1987**, 1509—1515 (1987).
21) Ahmad V. U., Basha A., "Spectroscopic Data of Saponins," Vols. 1—3, CRC Press, New York, 2000.