Phytochemistry, Vol. 26, No. 7, pp. 2023–2027, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

A TRITERPENE AND SAPONIN FROM ROOTS OF *ILEX PUBESCENS*

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(*Received* 21 *July* 1986)

Key Word Index—*Ilex pubescens*; Aquifoliaceae; triterpene saponin; pentacyclic triterpene; ilexsaponin A1; ilexgenin A.

Abstract—A new triterpene and its glycoside have been isolated from methanol extract of roots of *Ilex pubescens*. Their structures were established as 3β, 19α-dihydroxyurs-12-en-24,28-dioic acid and its 28-β-D-glucopyranosyl ester, based on chemical and spectral evidence.

INTRODUCTION

In China, 'mao-dong-qing' the root of *Ilex pubescens* Hook. et Arn. is widely used for the treatment of cardiovascular diseases and hypercholestaemia. Some phenolic compounds have been isolated from this plant [1]. We have investigated the constituents of the roots of this plant, and isolated a new triterpene and its glycoside. We have named them ilexgenin A and ilexsaponin A1, and established their structures as 1 and 2, respectively.

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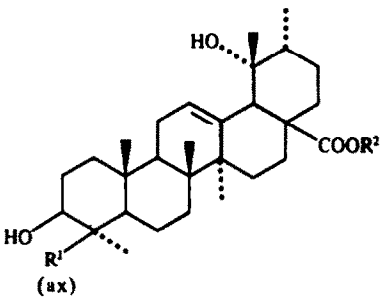
RESULTS AND DISCUSSION

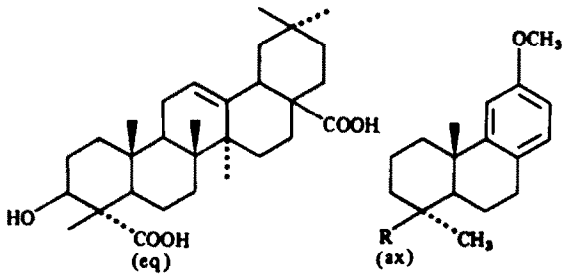
The dried roots of *Ilex pubescens* were extracted with benzene, followed by methanol. The methanol extract was suspended in water, then extracted successively with benzene and ethyl acetate. From the ethyl acetate fraction, two compounds, 1 and 2, were isolated by reversed phase and normal phase column chromatography.

Ilexgenin A (1), $C_{30}H_{46}O_6$, had mp > 300°. Its ^{13}C NMR spectrum revealed thirty carbon signals (CH_3– × 6, $-CH_2-$ × 9, >CH– × 4, >C< × 5, >CH–O × 1, >C–O × 1, >C=CH– × 1, CO × 2). Its IR spectrum showed bands at 3450 cm^{-1} for a hydroxyl group, at 1690 and 1685 cm^{-1} for a carboxyl group and at 1630 cm^{-1} for



	R^1	R^2
1	COOH	H
1a	$COOCH_3$	CH_3
1b	$COOCH_3$	H
2	COOH	Glc
2a	$COOCH_3$	Glc
3	CH_3	H
3a	CH_3	CH_3

Glc: β-D-glucopyranosyl



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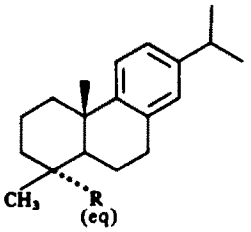
5 R = CH_3
6 R = $COOCH_3$



7 R = CH_3
8 R = $COOCH_3$

a double bond. The EI mass spectrum showed a peak at m/z 264, the characteristic retro-Diels–Alder cleavage peak of an olean-12-en or urs-12-en-28-oic acid derivative which possess a hydroxy group on either the D or E ring. The ^{1}H NMR spectrum showed the peak at δ3.08 (1H, *s*, H-18), which suggested the presence of the 19-*O*-substituted urs-12-ene skeleton [2]. The ^{13}C NMR spectrum of the methyl ester (**1a**) was compared with methyl pomolate (**3a**) (Table 1). The spectrum of **1a** showed one less methyl and one more carboxyl and methoxyl signals than that of **3a**. The rest of the spectrum was very similar to that of **3a**. In particular, carbon signals due to the D and E rings of **3a** appeared at the corresponding position in the spectrum of **1a**, while the signals of the A and B ring carbons were observed at somewhat different positions. In the EI mass spectrum of **1a**, retro-Diels–Alder cleavage peaks from the C and D rings appeared at m/z 278, 260 and 201 as well as in the spectrum of **3a**. However, a peak at m/z 208, arising by retro-Diels–Alder cleavage peak from the A and B rings of a pomolic acid (3)-type (non-substituted gem-dimethyl) triterpene, was not observed but a peak at m/z 252 was detected showing that one of the methyl groups at C-4 were replaced by $COOCH_3$ (Scheme 1). The ^{1}H NMR spectrum of **1a** showed the presence of a 3β-hydroxy by the signal at δ3.14 (1H, *dd*, J = 4, 11 Hz).

Table 1. ^{13}C NMR spectral data (δ) for **1**, **2** and related compounds

Carbon	3a*	1a	1	2	4†
Genin					
C-1	38.7	39.1	39.8	39.7	37.6
C-2	27.4	28.1	29.1	29.0	26.2
C-3	79.0	78.3	78.3	78.3	74.0
C-4	38.5	49.0	49.2	49.1	53.0
C-5	55.2	56.5	56.9	56.9	50.5
C-6	18.4	20.2	20.9	21.0	20.2
C-7	32.8	33.0	33.9	33.8	31.5
C-8	39.9	39.7	40.2	40.3	38.6
C-9	47.2	46.6	47.2	47.1	46.9
C-10	36.9	37.2	37.9	37.8	35.3
C-11	23.7	23.8	24.5	24.4	22.3
C-12	129.1	129.1	128.1	128.4	122.4
C-13	138.0	138.0	139.9	139.2	143.3
C-14	41.1	41.2	42.2	42.1	40.7
C-15	28.1	28.2	29.1	29.1	26.8
C-16	25.5	25.5	26.5	26.0	22.2
C-17	47.9	47.9	48.3	48.6	45.1
C-18	53.2	53.3	54.7	54.4	40.5
C-19	73.1	73.1	72.7	72.6	45.0
C-20	41.1	41.1	42.2	42.1	29.5
C-21	26.0	26.0	27.0	26.6	32.7
C-22	37.4	37.4	38.4	37.8	31.5
C-23	28.1	23.6	24.2	24.4	179.2
C-24	15.2	178.5	180.6	181.0	10.7
C-25	15.5	13.1	13.9	13.9	14.5
C-26	16.6	16.5	17.1	17.3	15.9
C-27	24.5	24.2	24.5	24.7	24.7
C-28	178.3	178.3	180.6	177.1	178.7
C-29	27.2	27.4	27.0	27.0	31.8
C-30	16.1	16.1	16.8	16.7	22.3
$COOH_3$	51.5	51.5			
		51.2			
Sugar					
G-1				95.7	
G-2				73.8	
G-3				78.7	
G-4				71.1	
G-5				79.1	
G-6				62.2	

*Cited from ref. [3].
†Cited from ref. [4].
3a, **1a**, in $CDCl_3$; **1**, **2**, **4**, in C_5D_5N.

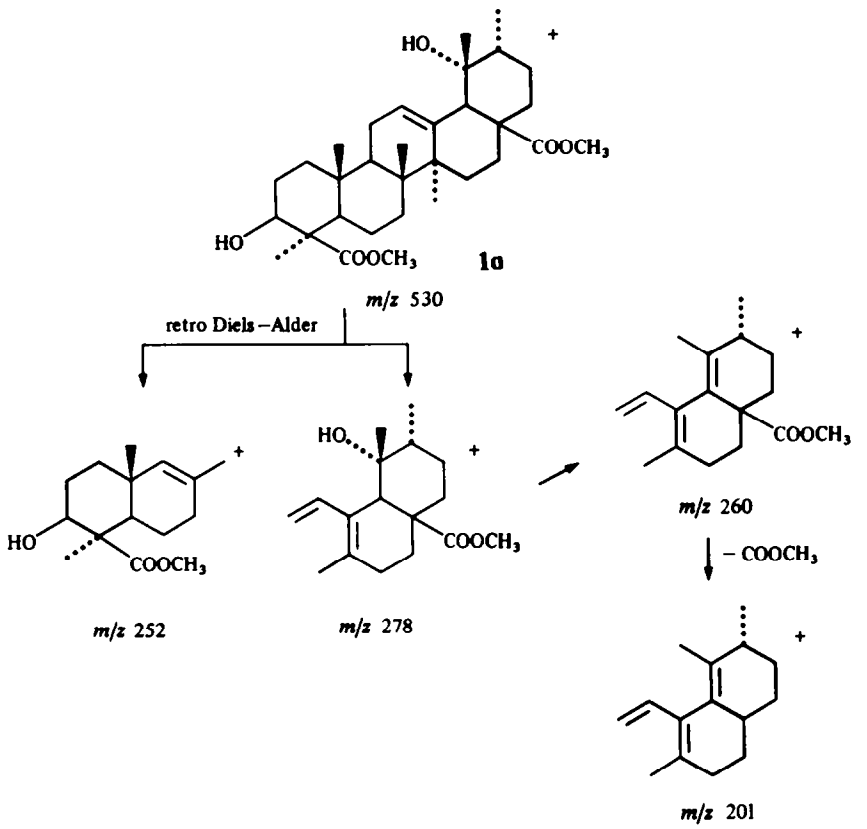
These facts indicate that **1a** is related to **3a**. The ^{13}C NMR spectrum of **1a** showed that the signal of the tertiary C-4 was shifted significantly downfield by 10.5 ppm, while the other tertiary carbons, C-8 and 10 were not. Therefore, the $COOCH_3$ group should be restricted to the C-23 or C-24 positions. The ^{13}C NMR spectrum of **1a** was compared with that of gypsogenic acid (**4**) [4] which has a C-23 equational –COOH group which results in significant differences in the chemical shifts of the A and B ring carbons. (Table 1). Since no ^{13}C NMR data for a triterpene with a C-24 carboxy methyl ester group were available, the data of several diterpenes (**5–8**) which have similar partial structures were compared [5]. Substitution ($CH_3 \rightarrow$ –COOMe) induced shift values in the ^{13}C NMR spectra for **5** and **6** corresponded to the values from **3a** and **1a**, while these for **7** and **8** was quite different (Table 2). These facts prove the location of the carbomethoxyl group in **3** is the C-24 (axial) position. Therefore, the structure of **1** is elucidated to be 3β,19α-dihydroxyurs-12-en-24,28-dioic acid.

A new saponin, ilexsaponin A1 (**2**), was obtained as an amorphous white powder. The IR spectrum of **2** exhibited hydroxyl (3400 cm^{-1}), carboxy (1685 cm^{-1}), and ester (1720 cm^{-1}) bands. The ^{13}C NMR spectrum of **2** showed characteristic signals of an ester-linked β-glucopyranose moiety [6]. The rest of the ^{13}C NMR signals were essentially the same as those of **1**, except one of the carbonyl signals, which was shifted downfield by 3.5 ppm. The aglycone of **2** was obtained by alkaline hydrolysis and identified as **1** by means of ^{13}C NMR spectroscopy. Acid hydrolysis of **2** afforded glucose, identified by GLC. The ^{1}H NMR spectrum of **2** supported the β-anomeric configuration (δ6.29, 1H, *d*, J = 6.8 Hz). To decide the location of the glucosyl ester, **2** was methylated by diazomethane followed by alkaline hydrolysis to afford **1b** [7]. The EI mass spectrum of **1b** showed fragment peaks at m/z 470, 452, 398, 264, 246, 252 which indicated that the methyl group was located on the C-24 carboxyl. On the contrary, peaks at m/z 456, 438, 384, 278, 260, 238, which would appear if there was a C-28 COOMe group, were not observed (Scheme 2). Therefore, the location of the glucosyl residue of **2** was deduced to be on the C-28 carboxyl group. It follows that **2** can be formulated as 3β,19α-dihydoroxyurs-12-en-24,28-dioic acid-28-β-D-glucopyranosylester.

EXPERIMENTAL

General procedure. NMR spectra were taken at 25° using TMS as internal standard; ^{13}C NMR at 25.15 MHz and ^{1}H NMR at 100 MHz. EIMS were taken at 75 eV. Mps were taken on a micro hot-stage and are uncorr.

Plant material. A sample of 'mao-dong-qing', the root of *Ilex pubescens*, was purchased in 1983 from Mikuni Co. Ltd (Osaka).

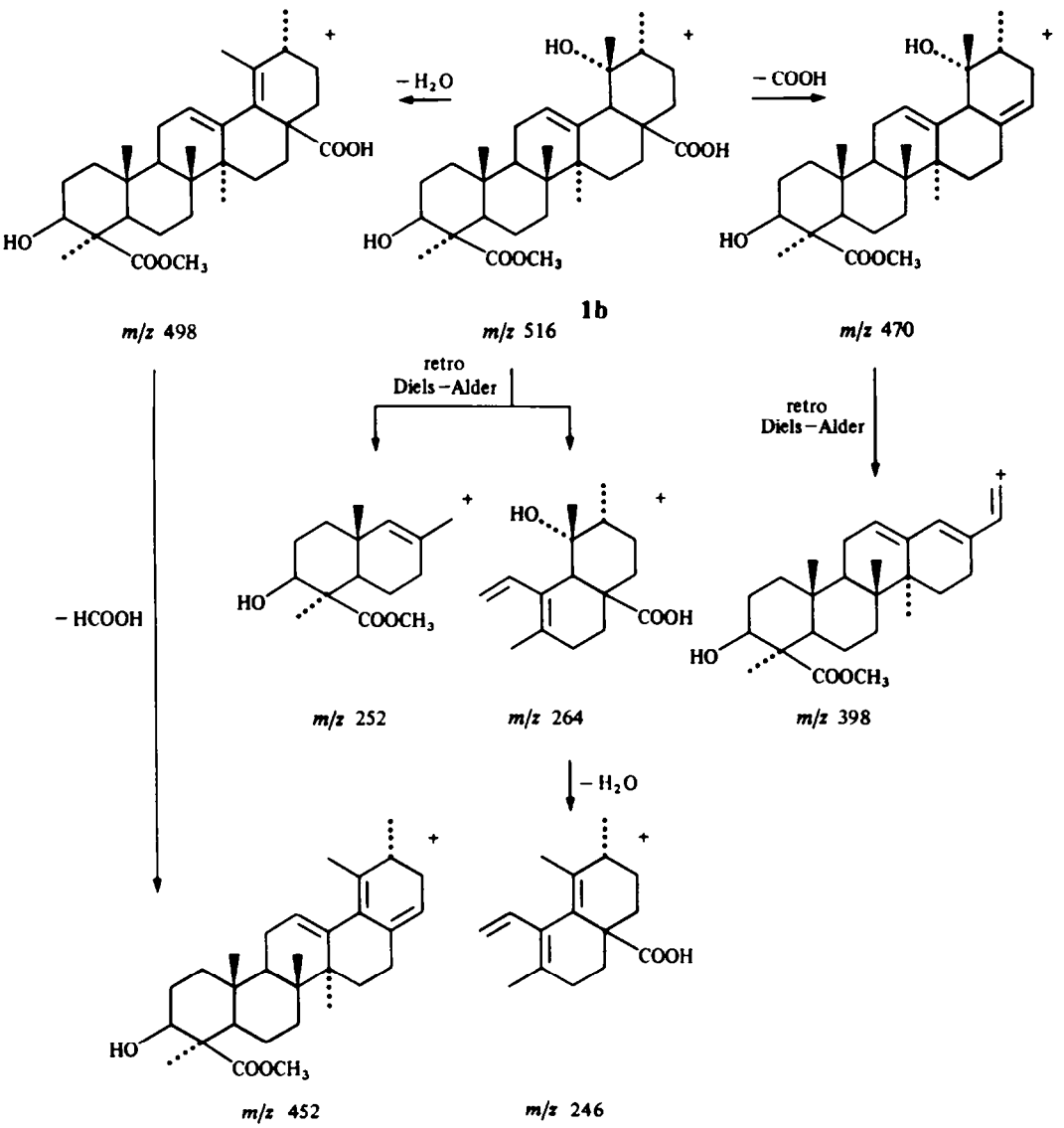


Scheme 1.

Table 2. ^{13}C NMR substitution induced shift ($\Delta\delta$) from **3a** to **1a**, and related compounds

Carbon	5	6	$\Delta\delta$ 6–5	7	8	$\Delta\delta$ 8–7		$\Delta\delta$ 1a–3a
C-1	38.9	39.4	+0.5	38.9	38.0	−0.9		+0.4
C-2	19.3	20.2	+0.9	19.3	18.6	−0.7		+0.7
C-3	41.7	37.6	−4.1	41.8	36.7	−5.1		−0.7
C-4	33.5	44.0	+10.5	33.4	47.7	+14.3		+10.5
C-5	50.3	52.8	+2.5	50.4	44.9	−5.6		+1.3
C-6	19.2	21.1	+1.9	19.1	21.7	+2.6		+1.8
C-7	29.6	31.2	+1.6	30.5	30.0	−0.5		+0.2
C-8	127.5	127.6	+0.1	134.8	134.7	−0.5		−0.2
C-9	151.4	149.3	−2.1	147.6	146.9	−0.7		−0.6
C-10	38.0	38.6	+0.6	37.5	37.0	−0.5		+0.3
C-11	110.1	111.2		124.2	124.1			
C-12	157.7	157.8		123.8	123.9			
C-13	110.7	111.2		145.3	145.7			
C-14	129.7	129.8		126.8	126.9			
C-15				33.5	33.5			
C-16				24.0	24.0			
C-17				24.0	24.0			
C-18	33.3	28.5	−4.8	33.3	179.1		(C-23)	−4.5
C-19	21.7	177.8		21.6	16.5	−5.1	(C-24)	
C-20	24.7	22.9	−1.8	24.9	25.1	+0.2	(C-25)	−2.4
$COOH_3$		51.2			51.9			

δValues of **5**–**8** were cited from ref. [5].



Scheme 2.

Extraction of triterpene and glycosides. Dried roots (4.7 kg) were crushed and extracted with C_6H_6 (20 l. × 2). The residue was re-extracted with MeOH (20 l. × 4) and the MeOH extract was taken to dryness (414 g). A suspension of the resulting extract in H_2O was washed with C_6H_6, then extracted with EtOAc to give an EtOAc extract (103 g). The EtOAc extract (100 g) was chromatographed on silica gel (Kieselgel 60, Merck, 3 kg) developing with $CHCl_3$–MeOH (10:1) and $CHCl_3$–MeOH–H_2O (15:2:0.1–15:10:2) mixture successively to yield 17 fractions.

From fraction 3 (eluate of $CHCl_3$–MeOH), compound **1** (370 mg) was obtained. A portion (1.6 g) of fraction 6 (26 g, eluate of $CHCl_3$–MeOH–H_2O, 15:2:0.1.) was subjected to chromatography on silica gel using EtOAc–$CHCl_3$–EtOH–H_2O, followed by reversed phase chromatography on ODS using MeOH–H_2O to afford compound **2** (585 mg). From a portion (1.6 g) of fraction 4 (11 g) eluted with $CHCl_3$–MeOH–H_2O (15:2:0.1), compound **2** (139 mg) was also obtained by a similar procedure to that above.

Compound **1**. White powder, $[\alpha]_D^{20}$ + 30.8° (pyridine: *c* 0.97). (found: C, 71.00; H, 9.33; $C_{30}H_{46}O_6$ requires: C, 71.68; H, 9.22%); EIMS *m/z* (rel. int.): 502 $[M]^+$ (4), 484 $[M-H_2O]^+$ (34), 456 $[M-HCOOH]^+$ (29), 438 $[M-HCOOH-H_2O]^+$ (15), 264 (8), 238 (21), 146 (100); HRMS *m/z*: 502.3295 $[M]^+$ (calc. for $C_{30}H_{46}O_6$: 502.3294); IR ν_{max}^{Nujol} cm^{-1}: 3450, 1690, 1685, 1630; ^1H NMR (pyridine-d_5): δ 1.16 (3H, *s*, Me), 1.18 (3H, *s*, Me), 1.48 (3H, *s*, Me), 1.74 (3H, *s*, Me), 1.78 (3H, *s*, Me), 1.14 (3H, *d*, *J* = 7.8 Hz, 30 Me), 3.08 (1H, *s*, H-18), 3.38 (1H, *dd*, *J* = 4.3, 11.6 Hz, H-3), 5.14 (1H, *br s*, 19-OH), 5.65 (1H, *br t*, H-12); ^{13}C NMR: Table 1.

Methylation of compound **1**. Compound **1** (75 mg) was treated with diazomethane followed by chromatography to give **1a** (di-Me ester): mp 199–203° (from MeOH): $[\alpha]_D^{20}$ + 78.2° (pyridine, *c* 1.01); (found: C, 72.27; H, 9.55; $C_{32}H_{50}O_6$ requires: C, 72.40; H, 9.50%); HRMS *m/z*: 530.3597 $[M]^+$ (calc. for $C_{32}H_{50}O_6$: 530.3607); EIMS *m/z* 530 $[M]^+$, 512, 470, 278, 252, 260, 201; ^1H NMR ($CDCl_3$): δ 0.70 (3H, *s*, Me), 0.76 (3H, *s*, Me), 1.21 (3H, *s*, Me), 1.26 (3H, *s*, Me), 1.40 (3H, *s*, Me), 0.94 (3H, *d*, *J* = 6.6Hz, 30-Me) 2.60 (1H, *s*, H-18), 3.14 (1H, *dd*, *J* = 4, 11 Hz, H-3), 3.60 (3H, *s*, OMe), 3.67 (3H, *s*, OMe), 5.35 (1H, *t*, *J* = 3.5 Hz, H-12); ^{13}C NMR: Table 1.

Compound **2**. White powder, $[\alpha]_D^{20} + 25.5°$ (pyridine; *c* 0.69). (found: C, 62.06; H, 8.43; $C_{36}H_{56}O_{11} \cdot 2H_2O$ requires: C, 61.69; H, 8.63%). EIMS (acetate) *m/z*: 331, $[Glc(Ac)_4]^+$; IR ν_{max}^{Nujol} cm^{-1}: 3400, 1720, 1685; ^1H NMR (pyridine-d_5): δ1.18 (3H, *s*, Me), 1.29 (3H, *s*, Me), 1.44 (3H, *s*, Me), 1.73 (6H, *s*, Me), 1.09 (3H, *d*, *J* = 6.1 Hz), 2.97 (1H, *s*, H-18), 3.30 (1H, *dd*, *J* = 4, 11 Hz, H-3), 5.21 (1H, *br s*, 19-OH), 5.61 (1H, *br t*, H-12), 6.29 (1H, *d*, *J* = 6.8 Hz, anomeric H). Mineral acid hydrolysis of **2** afforded D-glucose, identified by GC as its trimethylsilyl derivative.

Alkaline hydrolysis of compound **2**. A soln of **2** (25 mg) in 20% KOH (1 ml) was heated at 80° for 15 min. The soln was neutralized with 5% HCl, diluted with H_2O, and extracted with Et_2O. The Et_2O extract was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (15:1:0.1) to afford a white powder (19 mg), identified as **1** by ^1H NMR and ^{13}C NMR.

Methylation of compound **2** *followed by alkaline hydrolysis for MS*. A MeOH soln of **2** (30 mg) was treated with diazomethane and the resulting methyl ester was hydrolysed in KOH soln, yielding **1b** (8 mg).

Compound **1b**. White powder, HRMS *m/z*: 516.3482 $[M]^+$ (calc. for $C_{31}H_{48}O_6$: 516.3450); EIMS *m/z*: 516 $[M]^+$ 498, 470, 452, 398, 264, 252, 246, 234, 218, 201, 187, 175, 146. ^1H NMR (in pyridine-d_5): δ0.87, 1.14, 1.48, 1.59, 1.75 (each 3H, *s*, H-23, 25, 26, 27, 29) 1.14 (3H, *d*, *J* = 7.8 Hz, H-30); 3.10 (1H, *s*, H-18); 3.30 (1H, *dd*, *J* = 4, 11 Hz, H-3) 3.57 (3H, *s*, OMe); 5.11 (1H, *br s*, OH); 5.64 (1H, *t*, *J* = 3.5 Hz, H-12).

Acknowledgements—We are grateful to Dr. Masako Takani of Kanazawa University, and Professor Isao Kitagawa of Osaka University for gifts of authentic specimens of triterpenoids.

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