Triterpenoids and Phytosteroids from the Leaves of Cynanchum liukiuense

Bull. Fac. Sci., Univ. Ryukyus, No.70 : 83 – 92 (2000)

Triterpenoids and Phytosteroids from the Leaves of *Cynanchum liukiuense*

Kazuhito OGIHARA,* Saori NISHIZATO,* Masashi ISHIGAKI,* Matsutake HIGA,* and Seiichi YOGI*

*Department of Chemistry, Biology, and Marine Science, Faculty of Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

Abstract

Five triterpenoids were isolated from a chloroform-soluble fraction in a methanol extract of the leaves of *Cynanchum liukiuense* Werb, in addition to methyl stearate, phytosterols, and phytosterol glycosides. These triterpenoids were identified as 3β-acetyloxy-urs-12-ene (α-amyrin acetate), 3β-acetyloxy-olean-12-ene (β-amyrin acetate), (4β)-D-friedoolean-14-en-3β-ol (taraxerol), olean-12-en-3β-ol (β-amyrin), and lup-20(29)-en-3β-ol(lupeol), respectively. The phytosterols consisted of stigmastanol, stigmasterol, and campesterol by co-GC and GC-MS analyses and the phytosterol glycosides mainly did of the glucosides of stigmastanol, β-sitosterol, stigmasterol, and campesterol.

Introduction

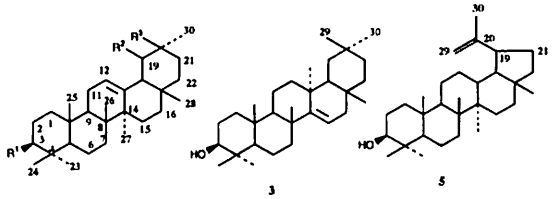
Cynanchum liukiuense is a perennial liana and grows in the Sakishima-Islands of Japan. This plant is known as food plant of danaid buttefly, *Salutura genutia*. In connection with a study on the triterpenoid constituents from food plants of some butterflies in Okinawa, we examined constituents in a chloroform-soluble fraction from a methanol extract of the leaves of *C. liukiuense* and isolated five triterpenoids, in addition to methyl stearate, phytosterols, and phytosterol glycosides. Herein, we describe the separation and identification of these constituents.

Results and Discussion

A chloroform-soluble fraction from a methanol extract of the leaves of *C. liukiuense* was subjected to column chromatography on silica gel to give **1**–**6**, in addition to **7** and **8** which were obtained as white prisms (**7**) and white powder (**8**), respectivery, after crystallization.

The compounds **1**–**5** gave a positive Liebermann-Burchard reaction (red to dark violet), which indicated that **1**–**5** were triterpenoids.

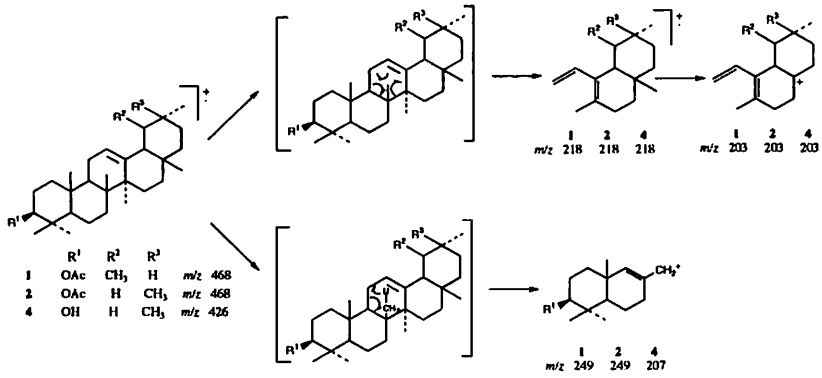
Compound **1** was suggested to have a molecular formula of $C_{32}H_{52}O_2$ by observations of 52 proton and 32 carbon signals in its ^1H and ^{13}C NMR spectra, respectively, and of a molecular ion peak at *m/z* 468 in its EIMS. The IR spectrum of **1** showed characteristic bands due to an ester of acetic acid at 1740 and 1240 cm^{-1}. The ^1H NMR spectrum of **1** showed a singlet at δ_H 2.04 due to methyl protons of an



	R^1	R^2	R^3
1	OAc	CH_3	H
2	OAc	H	CH_3
4	OH	H	CH_3

Received: June 30, 2000

acetyl group, in addition to signals due to six tertiary methyl groups at δ_H 0.80 (3H), 0.87 (12H), and 1.07 (3H), a doublet due to two methyl groups at δ_H 0.99 (6H), and a triplet due to a trisubstituted olefinic proton at δ_H 5.13 (1H, J= 3.6 Hz). The EIMS showed a base peak at *m/z* 218. These spectral data suggested that **1** was an urs-12-ene-type triterpene possessing one acetyloxy group. Moreover, the EIMS showed a fragment ion peak at *m/z* 249, which indicated that the acetyloxy group was located at A or B ring in the urs-12-ene skeleton (Scheme 1). Since the ^{1}H NMR spectrum showed a doublet of doublets at δ_H 4.50 (1H, *J*= 7 and 9 Hz) due to a methine proton joined at the carbon atom to which the acetyloxy group was bonded, the



Scheme 1. Mass fragmentation patterns of urs-12-ene and olean-12-ene compounds.

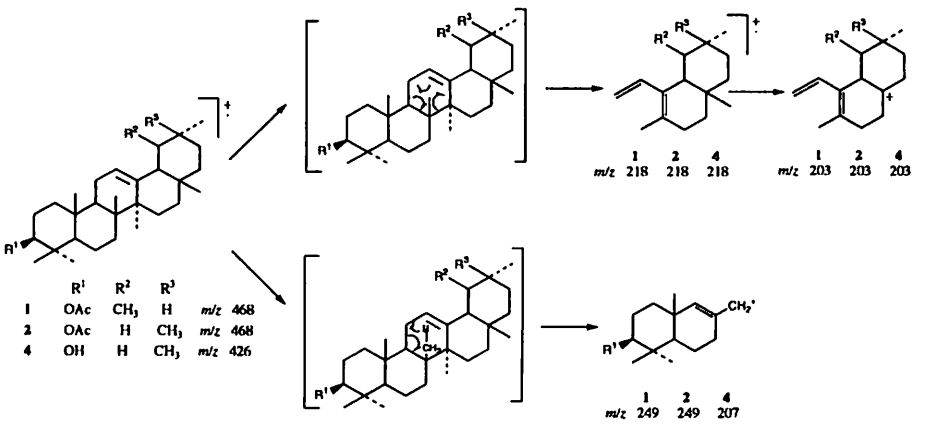
acetyloxy group was located on C-1, C-3, or C-7 in the β-configuration.

These results suggested that compound **1** was 3β-acetyloxy-urs-12-ene (α-amyrin acetate) which widely distributed in the natural sources. This suggestion was supported by comparison of the physical and spectral data of **1** with those described in references.[1-2]

Compound **2** was suggested to have a molecular formula of $C_{32}H_{52}O_2$ by observations of 52 proton and 32 carbon signals in its ^{1}H and ^{13}C NMR spectra, respectively, and of a molecular ion peak at *m/z* 468 in its EIMS. The IR spectrum of **2** showed characteristic bands due to an ester of acetic acid at 1733 and 1250 cm^{-1}. The ^{1}H NMR spectrum of **2** showed a singlet at δ_H 2.05 due to methyl protons of an acetyl group, in addition to signals due to eight tertiary methyl groups at δ_H 0.83 (3H), 0.87 (12H), 0.97 (6H), and 1.13(3H), and a triplet due to a trisubstituted olefinic proton at δ_H 5.18 (1H, *J*= 3.7 Hz). The EIMS showed a base peak at *m/z* 218. These spectral data suggested that **2** was an olean-12-ene-type triterpene possessing one acetyloxy group. Moreover, the EIMS showed a fragment ion peak at *m/z* 249, which indicated that the acetyloxy group was located at A or B ring in the olean-12-ene skeleton (Scheme 1). Since the ^{1}H NMR spectrum showed doublet of doublets at δ_H 4.50 (1H, *J*= 7 and 9 Hz) due to a methine proton joined at the carbon atom to which the acetyloxy group was bonded, the acetyloxy group was located on C-1, C-3, or C-7 in the β-configuration.

These results suggested that compound **2** was 3β-acetyloxy-olean-12-ene (β-amyrin acetate) which widely distributed in the natural sources. This suggestion was supported by comparison of the physical and spectral data of **2** with those described in references.[3-6]

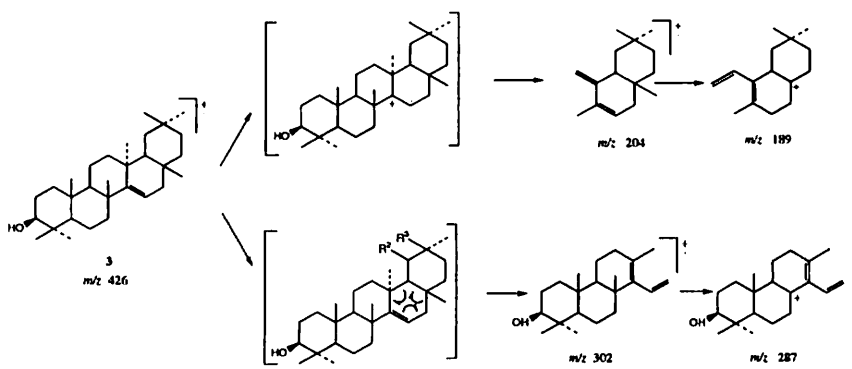
Compound **3** was suggested to have a molecular formula of $C_{30}H_{50}O$ by observations of 50 proton and 30 carbon signals in its ^{1}H and ^{13}C NMR spectra, respectively, and of a molecular ion peak at *m/z* 426 in its EIMS. The IR spectrum of **3** showed characteristic band due to hydroxy



Scheme 1. Mass fragmentation patterns of urs-12-ene and olean-12-ene compounds.

group at 3500 cm^{-1}. The ^1H NMR spectrum of **3** showed signals due to eight tertiary methyl groups at δ_H 0.80 (3H), 0.82 (3H), 0.91 (6H), 0.93 (3H), 0.95 (3H), 0.98 (3H), and 1.09 (3H), and a doublet of doublets due to a trisubstituted olefinic proton at δ_H 5.53 (1H, J= 3, 8 Hz). The EIMS showed a base peak at *m/z* 204. These spectral data suggested that **3** was an taraxer-14-ene-type triterpene possessing one hydroxy group. Moreover, the EIMS showed a fragment ion peak at *m/z* 302, which indicated that the hydroxy group was located at A, B, or C ring in the taraxer-14-ene skeleton (Scheme 2). Since the ^1H NMR spectrum showed a doublet of doublets at δ_H 3.20 (1H, J= 5 and 10 Hz) due to a methine proton joined at the carbon atom to which the hydroxy group was bonded, the hydroxy group was located on C-1, C-3, C-7, or C-11 in the β-configuration.

These results suggested that compound **3** was taraxer-14-en-3β-ol [(4β)-D-friedoolean-14-en-



Scheme 2. Mass fragmentation patterns of taraxerol

3β-ol: taraxerol] which widely distributed in the natural sources. The physical and spectral data of **3** coincided with those described in references.[7-9]

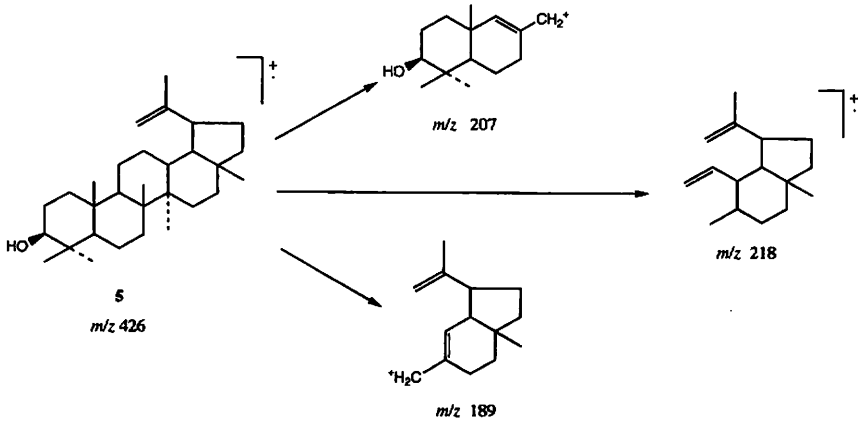
Compound **4** was suggested to have a molecular formula of $C_{30}H_{50}O$ by observations of 50 proton and 30 carbon signals in its ^1H and ^{13}C NMR spectra, respectively, and of a molecular ion

peak at *m/z* 426 in its EIMS. The IR spectrum of **4** showed characteristic band due to hydroxy group at 3500 cm^{-1}. The ^1H NMR spectrum of **4** showed signals due to eight tertiary methyl groups at δ_H 0.79 (3H), 0.83 (3H), 0.87 (6H), 0.94 (3H) 0.97 (6H), 0.99 (3H), and 1.13 (3H), and a triplet due to a trisubstituted olefinic proton at δ_H 5.31 (1H, J= 3.5 Hz). The EIMS showed a base peak at *m/z* 218. These spectral data suggested that **4** was an olean-12-ene-type triterpene possessing one hydroxy group. Moreover, the EIMS showed a fragment ion peak at *m/z* 207, which indicated that the hydroxy group was located at A or B ring in the olean-12-ene skeleton (Scheme 1). Since the ^1H NMR spectrum showed doublet of doublets at δ_H 3.31 (1H, J= 6 and 9 Hz) due to a methine proton joined at the carbon atom to which the hydroxy group was bonded, the hydroxy group was located on C-1, C-3, or C-7 in the β-configuration.

These results suggested that compound **4** was olean-12-en-3β-ol (β-amyrin) which widely distributed in the natural sources. The physical and spectral data of **4** coincided with those described in references.[2,10]

Compound **5** was suggested to have a molecular formula of $C_{30}H_{50}O$ by observations of 50 proton and 30 carbon signals in its ^1H and ^{13}C NMR spectra, respectively, and of a molecular ion peak at *m/z* 426 in its EIMS. The IR spectrum of **5** showed characteristic bands due to hydroxy group at 3500 cm^{-1} and due to terminal methylene group at 3070, 1635, and 890 cm^{-1}. The ^1H NMR spectrum of **5** showed signals due to six tertiary methyl groups at δ_H 0.78 (3H), 0.80 (3H), 0.85 (3H), 0.98 (3H), 1.06 (3H), and 1.28 (3H), a vinylic methyl group at δ_H 1.70 (3H), and a broad doublet due to a terminal methylene protons at δ_H 4.62 (1H, J= 11.61 Hz). The EIMS showed abundant fragment ion peaks at *m/z* 218, 207, and 189. These spectral data suggested that **5** was an lup-20(29)-ene-type triterpene possessing one hydroxy group. Moreover, the EIMS showed a fragment ion peak at *m/z* 207, which indicated that the hydroxy group was located at A or B ring in the lup-20(29)-ene skeleton (Scheme 3). Since the ^1H NMR spectrum showed a doublet of doublets at δ_H 3.21 (J= 6 and 9 Hz) due to a methine proton joined at the carbon atom to which the hydroxy group was bonded, the hydroxy group was located on C-1, C-3, or C-7 in the β-configuration.

These results suggested that compound **5** was lup-20(29)-en-3β-ol (lupeol) which widely distributed in the natural sources. The physical and spectral data of **5** coincided with those



Scheme 3. Mass fragmentation patterns of lupeol.

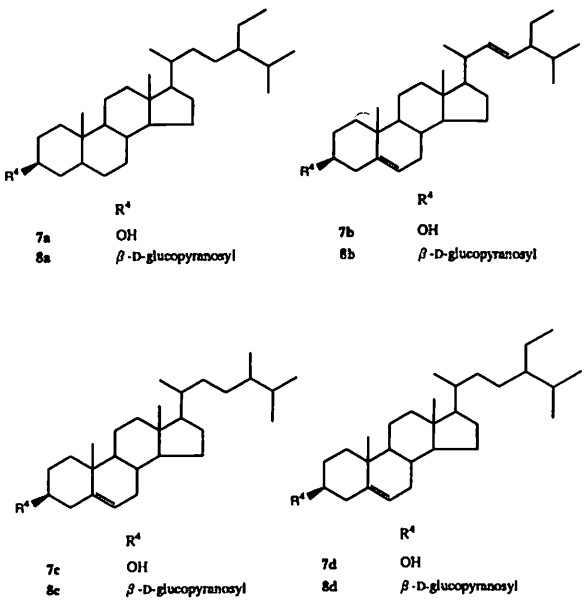
described in references.[11-12]

The IR spectrum of **6** showed characteristic bands due to ester at 1720 and 1250 cm^{-1}. The EIMS of **6** showed a molecular ion peak at *m/z* 298 and a fragment peak due to McLafferty rearrangement of fatty acid methyl ester at *m/z* 74.

These results suggested that **6** was methyl stearate which widely distributed in the natural sources. The physical data and IR and EIMS spectra of **6** coincided with those of authentic sample.

The white prism **7** and white powder **8** gave a positive Liebermann-Burchard reaction (green to dark green), which indicated that **7** and **8** were steroid.

The white prism **7** was estimated to be a single compound by indicating only one spot on analytical TLC. The EIMS showed a molecular ion peak at *m/z* 416, which suggested that **7** was a saturated sterol. But against this suggestion, the ^1H NMR spectrum showed signals due to some olefinic protons at δ_H 4.9−5.2, in addition to characteristic signals due to methyl protons of the steroid at high field region. This conflicting problem was settled by detailed analysis of the mass spectrum. The MS showed ion peaks at *m/z* 412 and 400 which were not able to be assigned to fragment ions besides the molecular ion peak at m/z 416, which indicated that **7** was a mixture of sterols. Stigmastanol (**7a**, 72.2 %), stigmasterol (**7b**, 19.1 %), and campesterol (**7c**, 19.1 %) were detected by co-GC and GC-MS analyses.



Thus, **7** was a mixture of phytosterols, stigmastanol, stigmasterol, and campesterol.

The white powder **8** was estimated to be a single compound by indicating only one spot on analytical TLC. The IR spectrum showed wide bands due to hydroxy groups at 3600-3200 cm^{-1} and due to C-O at 1200-1000 cm^{-1}, which suggested that **8** was glycoside. These results indicated **8** to be glycoside of sterol. Hydrolysis of **8** gave a kind of sugar and white prism corresponding to aglycon after chromatographic preparation and then recrystallization. The EIMS of the white prism showed a molecular ion peak at *m/z* 416, which suggested that the white prism was a saturated sterol. But against this suggestion, ^1H NMR spectrum of the white prism showed signals due to some olefinic protons at δ_H 4.9−5.2, in addition to characteristic signals due to methyl protons of the steroid at high field region. This conflicting problem was settled by detailed analysis of the mass spectrum. The MS showed ion peaks at *m/z* 414, 412, 400, and 394 which were not able to be assigned to fragment ions besides the molecular ion peak at *m/z* 416, which indicated that the white prism was a mixture of sterols. Stigmastanol (**7a**, 46.4 %), β-sitosterol (**7d**, 38.0 %), stigmasterol (**7b**, 3.5 %), campesterol (**7c**, 8.3 %), and two unidentified sterols (3.8 %) were detected by co-GC and GC-MS analyses. The sugar part was converted into alditol acetate. The alditol acetate was agreement with glucitol acetate derived

from D-glucose by co-GC and GC-MS analyses. Therefore, sugar moiety of **8** was identified as D-glucose. Since the ^{1}H NMR spectrum of **8** showed a doublet at δ_H 5.05 (J= 7.5 Hz) due to anomeric proton of sugar moiety, the D-gulcose was located on C-3 in the β-configuration as O-glucosyl linkage.

Thus, **8** was a mixture of stigmastanol-β-D-glucopyranoside (**8a**, 46.4 %), β-sitosterol-β-D-glucopyranoside (**8d**, 38.0 %), stigmasterol-β-D-glucopyranoside (**8b**, 3.5 %), campesterol-β-D-glucopyranoside (**8c**, 8.3 %), and β-D-glucopyranosides (3.8 %) of two unidentified sterols.

Experimental

Analytical TLC was carried out on Merck 60 F_{254} silica gel plate (thickness: 0.25 mm). GC and co-GC with authentic samples were performed on a Shimadzu gas-cheromatograph GC 14A instrument equipped with an FID and a glass capillary column (WCOT, 0.25 mm × 30 m; DB-1, 0.25 μ m) by programming the column temperature at 2°/min from 200 to 290° with He as a carrier gas. ^{1}H (90 and 270 MHz) and ^{13}C NMR (25 and 67.5 MHz) spectra were determined in $CDCl_3$ for **1**—**7** and in pyridine-d_6 for **8**, respectively with TMS as int. standard. EIMS spectra were obtained on a Hitachi M-2500 double focusing mass spectrometer at 70 eV. GC-MS analyses were performed on the same instrument as measurments of EIMS spectra conbined with a Hitachi GC-3000 instrument under following conditions: column, 0.25 mm × 30 m glass capillary column (WCOT) coated with OV-1; injector temperature 220° ; column temperature 2°/min from 200 to 290° ; Carrier gas He; splitless.

Extraction and isolation. Fresh leaves (5.9 kg) of *C. liukiuense*, collected at Hateruma Island, Okinawa-prefecture in April, were ground in a mixer and immersed in MeOH for 1 month. The MeOH soln was concd *in vacuo* and the obtained concentrate (201.6 g) was partitioned with $CHCl_3$ and H_2O. The $CHCl_3$ layer, after dried over anhydrous Na_2SO_4, was concd *in vacuo* and the obtained chloroform-soluble fraction (39.74 g) was subjected to column chromatography on silica gel (Wako-gel C-300) developed with $CHCl_3$ and then MeOH. The eluate with $CHCl_3$ was re-chromatographed on a silica gel column developed with n-hexane to give **3** (73mg), **6** (6 mg), and a mixture of **1** and **2**, and then with $CHCl_3$ to give **4** (30mg), **5** (28mg), and **7** (30mg). The mixture of **1** and **2** was re-crystallization with EtOH to give **1** (215 mg) and the filtrate, after **1** was filtered, was re-crestallized with hexane-EtOH (1:4) to give **2** (138mg). On the other hand, the eluate with MeOH was re-chromatographed on a silica gel column developed with $CHCl_3$-MeOH-H_2O (8:2:0.3) to give **8** (18 mg).

3β-*acetyloxy-urs-12-ene* (α-*amyrin acetate*: **1**). White needles, mp 213-215° (EtOH) [lit.[1]: mp 227° (petrol ether)]; IR ν_{max}^{KBr} cm^{-1}: 1740 and 1240 (O=C-O); ^{1}H (90 MHz) and ^{13}C NMR (67.5 MHz): see Table 1 and 2; EIMS *m/z* (rel. int.): 468 $[M]^+$ (21), 453 (5), 408 (3), 249 (10), 218 (100), 203 (20), and 189 (19). The physical and spectral data coincided with those described in references.[1-2]

3β-*acetyloxy-olean-12-ene* (β-*amyrin acetate*: **2**). Colorless prisms, mp 240-241° (EtOH) [lit.[3]: mp 238-241° (EtOH)]; IR ν_{max}^{KBr} cm^{-1}: 1740 and 1240 (O=C-O); ^{1}H (90 MHz) and ^{13}C NMR (22.5 MHz): see Table 1 and 2; EIMS *m/z* (rel. int.): 468 $[M]^+$ (10), 453 (2), 408 (1), 249 (10), 218 (100), 203 (22), and 189 (11). The physical and spectral data coincided with those described in references.[3-6]

(4β)-*D-friedoolean-14-en-3*β-*ol* (*taraxer-14-en-3*β-*ol*, *taraxerol*: **3**). Colorless plates, mp 287-288° (hexane) [lit.[7]: mp 282-285° (MeOH-$CHCl_3$)]; IR ν maxKBr cm-1: 3500 (OH); 1H (270 MHz) and 13C NMR (22.5 MHz): see Table 1 and 2; EIMS m/z (rel. int.): 426 $[M]^+$ (18), 411 (12), 302 (49), 287 (32), 204 (100), and 189 (11). These physical and spectral

data coincided with those described in references.[7-9]

Olean-12-en-3β-ol (*β-amyrin*: **4**). Colorless needles, mp 175-178° (EtOH-$CHCl_3$) [lit.[10]: mp 175-178° (EtOH-$CHCl_3$)]; IR ν_{max}^{KBr} cm^{-1}: 3500 (OH); ^1H (90 MHz) and ^{13}C NMR (22.5 MHz): see Table 1 and 2; EIMS *m/z* (rel. int.): 426 $[M]^+$ (18), 411 (4), 408 (5), 218 (100), 207 (12), 203 (20), and 189(18). These physical and spectral data coincided with those described in references.[2,10]

Lup-20(29)-en-3β-ol (*lupeol*: **5**). White needles, mp 191-192° (hexane) [lit.[11]: mp 192° (hexane)]; IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), and 3070, 1635, and 890 (=CH_2); ^1H (90 MHz) and ^{13}C NMR (22.5 MHz): see Table 1 and 2; EIMS *m/z* (rel. int.): 426 $[M]^+$ (100), 411 (22), 408 (6), 393 (6), 218 (67), 207 (89), and 189 (86). These physical and spectral data coincided with those described in references.[11-12]

Table 1. ^1H NMR data and coupling constants (Hz)* for triterpenoids 1-5

H	1	2	3	4	5
3	4.50 *dd* (7, 9)	4.50 *dd* (7, 9)	3.20 *dd* (5, 10)	3.31 *dd* (6, 9)	3.21 *dd* (6, 9)
12	5.13 *t* (3.6)	5.18 *t* (3.7)		5.31 *t* (3.5)	
15			5.53 *dd* (3, 8)		
23	0.87 *s*	0.87 *s*	0.98 *s*	0.99 *s*	0.85 *s*
24	0.87 *s*	0.87 *s*	0.80 *s*	0.79 *s*	0.78 *s*
25	0.87 *s*	0.97 *s*	0.93 *s*	0.94 *s*	0.98 *s*
26	0.87 *s*	0.97 *s*	1.09 *s*	0.97 *s*	1.06 *s*
27	1.07 *s*	1.13 *s*	0.91 *s*	1.13 *s*	1.28 *s*
28	0.80 *s*	0.83 *s*	0.82 *s*	0.83 *s*	0.80 *s*
29	0.99 *d* (2.8)	0.87 *s*	0.95 *s*	0.87 *s*	4.62 br.*d* (11.6)
30	0.99 *d* (2.8)	0.87 *s*	0.91 *s*	0.87 *s*	1.70 *s*
OAc	2.04 *s*	2.05 *s*			

* Couplimg constants are in parentheses.

Table 2. ^{13}C NMR data for triterpenoids 1-5

C	1	2	3	4	5
1	38.5	38.2	38.1	38.6	38.6
2	23.7	23.6	27.2	27.1	27.3
3	81.0	80.9	79.1	78.9	78.7
4	37.8	37.7	38.8	38.7	38.7
5	55.3	55.2	55.6	55.2	55.3
6	18.3	18.2	18.9	18.3	18.0
7	32.9	32.6	33.1	32.6	33.8
8	40.1	39.6	39.0	39.8	40.9
9	47.7	47.6	49.4	47.6	50.5
10	36.8	36.8	37.8	37.1	37.0
11	23.4	23.3	17.6	23.4	21.4
12	124.3	121.6	36.7	121.7	25.2
13	139.6	145.2	37.8	145.0	38.1
14	42.1	41.7	158.1	41.7	43.0
15	28.8	26.1	116.9	26.1	27.5
16	26.7	26.9	33.8	27.3	35.6
17	33.8	32.5	35.8	32.5	42.9
18	59.1	47.2	48.8	47.2	48.3
19	39.7	46.8	41.4	46.8	48.0
20	39.7	31.1	28.8	31.1	150.9
21	31.3	34.7	35.2	34.7	29.9
22	41.6	37.1	37.8	37.2	40.0
23	28.1	28.0	28.0	28.0	27.8
24	16.9	16.8	15.5	15.5	15.6
25	15.8	15.5	15.5	15.5	16.1
26	16.8	16.7	25.9	16.7	15.9
27	23.3	26.0	29.9	26.0	14.6
28	28.1	28.4	29.9	28.4	18.0
29	17.5	33.3	33.4	33.2	109.3
30	21.4	23.7	21.7	23.7	21.0
OAc	170.9	171.0			
	21.3	21.3			

Methyl stealate (**6**). White needles, mp 36.5-38.5° (EtOH); IR ν_{max}^{KBr} cm^{-1}: 1720 and 1250 (O=C-O); EIMS *m/z* (rel. int.): 298 $[M]^+$ (77), 267 (12), and 74 (100). These spectra coincided with those of the authentic sample.

Phytosterols (**7**). White prisms, mp 131-132° (EtOH); EIMS *m/z* (rel. int.): 416 (77), 412 (12), 400 (21). On the basis of co-GC and GC-MS analyses under conditions described above, **7** were composed of stigmastanol (**7a**, 72.2%), stigmasterol (**7b**, 19.1%), and campesterol (**7c**, 19.1%).

Phytosterol glucosides (**8**). White powder, mp 171-178° (EtOH); IR ν_{max}^{KBr} cm^{-1}: 3600-3200 (OH), and 1200-1000 (C-O); ^1H NMR (270 MHz) δ: 5.05 (d, J=7.5 Hz).

Hydrolysis of **8**. According to the method described in reference,[13-14] phytosterol glycoside was hydrolyzed. The white powder **8** (3 mg) dissolved in *n*-BuOH and 0.1M H_2SO_4 (1:1) were heated at 80° for 2 hr. After hydrolysis, reaction mixture was shaken with $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated to dryness and was analyzed by GC and GC-MS under conditions described above. Stigmastanol (**7a**, 46.4%), β-sitosterol (**7d**, 38.0%), stigmasterol (**7b**, 3.5%), campesterol (**7c**, 8.3%), and two unidentified sterols (3.8%) were detected by co-GC and GC-MS analyses. The H_2O layer was passed through an anion exchange resin column and the eluates obtained were concentrated to dryness. The residue was reduced with NaBH4 (6 mg) for 1 hr at room temp. The reaction mixture was passed through a cation exchange resin column and the eluate was concentrated to dryness. Boric acid was removed by codistillation with MeOH and the residue was acetylated with Ac_2O and pyridine (18 drops each) at room temp. overnight. The reagents were then evapd off *in vacuo*. The residue was peformed on GC and GC-MS analyses under conditions described above. From the glycoside, glucitol acetate was detected by GC. EIMS spectrum and GC retention time of the glucitol acetate obtained coincided with those of glucitol acetate derived from D-glucose.

Acknowledgements—The authors thank Professor Tatsuo Higa, College of Science, University of the Ryukyus, for the use of ^1H (270 MHz) and ^{13}C NMR (67.5 MHz) spectrometer and Professor Yosei Uehara, College of Science, University of the Ryukyus, for his helpful advice and useful comments on the manuscript.

References

1) Soldin, Marais, *J. Pharm. Soc.*, **55**, 452 (1966).
2) Kazuo Tohri, in *Tennenbutsukagaku '80A*, Mankoudou, Tokyo (1988).
3) J. M. Beaton, F. S. Spring, R. Stevenson, and J. L. Stewart, *J. Chem. Soc.*, **1955**, 2131.
4) B. Tursch, R. Savoir, R. Ottinger, and G. Chiurdoglu, *Tetrahedron Lett.*, **1967**, 539.
5) S. Ito, M. Kodama, M. Sunagawa, and T. Oba, *Tetrahedron Lett.*, **1969**, 2905.
6) K. Ogihara, R. Iraha, M. Higa, and S. Yogi, *Bull. Coll. Sci.,Univ. Ryukyus*, **64**, 53 (1997).
7) R. E. Corbet, S. D. Cumming, and E. V. Whitehead, *J. Chem. Soc. Perkin Trans.* 1, **1972**, 2827.
8) C. Djerassi, H. Budzikiewics, and J. M. Wilson, *Tetrahedron Letters*, **1962**, 263.
9) K. Ogihara, M. Higa, K. Hokama, and T. Suga, *Phytochemistry*, **26** (3), 783 (1987).
10) M Higa, K. Yokota, K. Ogihara, and S. Yogi, *Bull. Coll. Sci.,Univ. Ryukyus*, **62**, 45 (1996).

11) K. Yoshihira, M. Tezuka, and S. Natori, *Chem. Pharm. Bull.*, **19**, 2308 (1971).
12) E. Wenkert, G. V. Baddeley, and L. N. Moreno, *Org. Magn. Res.*, **11**, 337 (1978).
13) T. Warashina and T. Noro, *Chem. Pharm. Bull.*, **44** (2), 358 (1996).
14) T. Warashina and T. Noro, *Phytochemistry*, **44** (5), 9178 (1997).