Tetrahedron Vol 45, No 13, pp 4125 to 4134, 1989
Printed in Great Britain

0040-4020/89 $3.00+.00

TRITERPENOIDAL CONSTITUENTS OF UNCARIA FLORIDA VIDAL

Norio Aimi,[a)] Kittisak Likhitwitayawuid,[a,b)] Junko Goto,[a)]
Dhavadee Ponglux,[b)] Joju Haginiwa,[a)] and Shin-ichiro Sakai[a)]

a) Faculty of Pharmaceutical Sciences, Chiba University,
1-33 Yayoi-cho, Chiba 260, Japan
b) Faculty of Pharmaceutical Sciences, Chulalongkorn
University, Bangkok 10500, Thailand

(*Received in Japan* 30 *December* 1988)

Abstract- Chemical study on new triterpenoids of ursolic acid series in Uncaria florida Vidal (Rubaiceae) was carried out. A unique structural change accompanying diazomethane methylation was also studied.

Although the plants of the genus Uncaria (Rubiaceae) are well known for their rich content of alkaloids and tannins, much attention has also been focused on their triterpenoidal constituents.[1a-d)] In this paper we report isolation and structure determination of new type triterpenoids of a Formosan Uncaria sp., U. florida, and also chemical study on this structural class of triterpenoids. This work forms a part of our continuing study on triterpenoids of Rubiaceous plants.[2)]

The methanolic extract of Uncaria florida Vidal[3)] was partitioned between water and butanol. The butanol soluble fraction was then separated to a polar fraction containing acidic triterpenes and less polar oxindole alkaloid fraction on which we already reported our chemical study.[3)] Purification of the former fraction by use of SiO_2 column gave compound A. The remainder of this chromatography fractions were combined and the mixture was treated with diazomethane (24 hrs). Subsequent chromatographical purification of the obtained methyl ester mixture resulted in isolation of compounds B and C. On the other hand, a part of the carboxylic acid mixture was treated with diazomethane for a breaf period to give a different ester, a discussion on which will be made in the later part of this paper.

Compound A (1), mp 226-7°C, was found to have the molecular formula of $C_{30}H_{48}O_5$. ^{1}H-NMR spectrum showed seven methyl signals, among which six(23-H_3, 24-H_3, 25-H_3, 26-H_3, 27-H_3, and 29-H_3) were singlet and one (30-H_3) was doublet. The mass spectrum showed characteristic fragments at m/z 264 (a), 219(b), 201(c) and 370(d).[4] (Chart 1). These observations indicated that the basic skeleton of compound A was a urs-12-en-28-oic acid possessing one hydroxyl group at C-19.[4] From the comparison of the ^{13}C-NMR of **1** with the model compounds [5] the structure of compound A was clarified as 3β,6β, 19α-trihydroxy-urs-12-en-28-oic acid (1). Methylation of **1** with diazomethane gave the corresponding methyl ester (**4**), mp 220-1°C, $C_{31}H_{50}O_5$. Conventional acetylation of **4** afforded monoacetate (**5**), $C_{33}H_{52}O_6$, as an

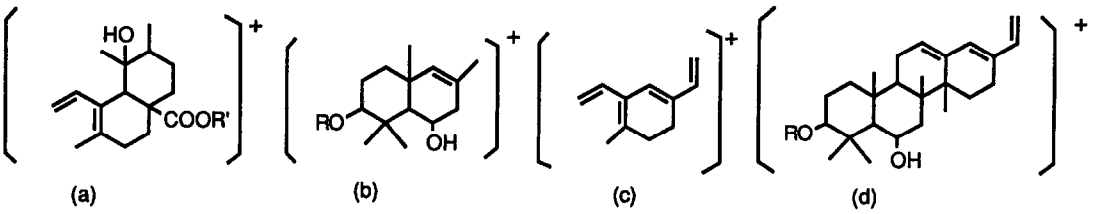
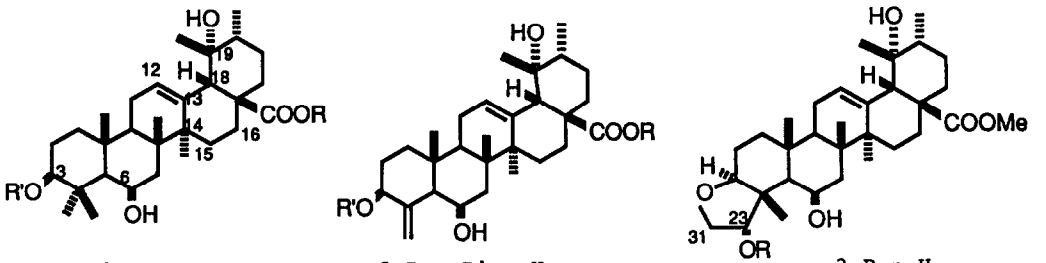


Chart 1.

amorphous powder. As was expected, the tertiary hydroxyl group at C_{19} and the highly hindered secondary hydroxyl group at C(6)β remained unacetylated.



1 R = R' = H
4 R = Me R' = H
5 R = Me R' = Ac

2 R = R' = H
6 R = Me R' = H
14 R = Me R' = Ac

3 R = H
7 R = Ac

Chart 2.

In the ^{1}H-NMR spectrum of **4** a 1H-signal was observed at δ 2.50 as a double-triplet with the coupling constants of J_1=J_2=13, J_3=5 Hz. When measured in pyridine-d_5 this signal moved further downward to δ 3.08. This characteristic signal was obviously assignable to 16α-H which was caused downfield shift by the anisotropic effect due to 19α-OH in an additive

manner to the same effect due to the Δ^{12} double bond. The same observation was made on the ^{1}H-NMR spectra of **1** and **5**, and also in those of the other compounds shown below.

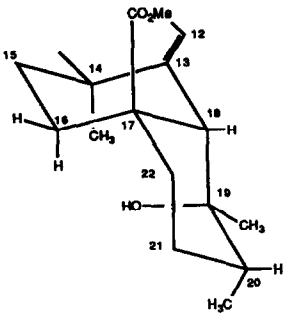


Fig. 1.

Compound A (**1**) is a prototype of the natural compounds of the structural class of ursolic acids possessing 3β,6β, and 19α-hydroxyl groups. Many of the more heavily functionalized natural products have been isolated from Rosaceae, Ericaceae, and other plant families,[5] but as far as we know this is the first finding of this fundamental compound from the nature.

Compound B (**6**), $C_{30}H_{46}O_5$, mp 146-8°C, was found to be a methyl ester of a nor-triterpene with the basic skeleton of carbon numbers of 29. As the result of the following investigation this compound was found to be a new nortriterpenoid, and to the corresponding acid (**2**) the name, floridic acid, was given. Compound B (**6**), therefore, was the methyl ester of floridic acid.

The ^{1}H-NMR spectrum indicated the structure of **6** as depicted above. (Chart 2). A particularly important observation was that a 2H-singlet signal was observed at δ 5.27 ppm, clearly indicating the presence of an exocyclic methylene grouping at C-4. Other expected signals were found for four singlet methyls (at δ 1.09, 1.09, 1.23, and 1.28), one doublet methyl signal at δ 0.95, signals due to 16α-H at δ 2.50, 18-H at δ 2.63, ester methyl at δ 3.61, 3α-H at δ 3.98, 6α-H at δ 4.45, and 12-H at δ 5.42. The ^{13}C-NMR spectrum also strongly denoted the structure. The olefinic carbons of the exocyclic methylene on the A ring were found at δ 150.9(C-4) and 104.4 (C-24). The neighboring carbons showed the expected changes of the chemical shifts from the other typical type of model compounds proving the proposed structure. Floridic acid (**2**) is the first norursane-type triterpene with exocyclic methylene at C-4 position.

Compound C (**3**) was the second methyl ester isolated from diazomethane-treated fraction. The molecular formula, $C_{32}H_{50}O_6$, indicated that further one extra carbon, in addition to the ester methyl group, was incorporated to a conventional triterpene framework. Spectral evidences, particularly from ^{13}C-NMR spectroscopy, indicated the structure depicted in Chart 2.

The structure of the tetrahydrofurane ring moiety fused to the A ring was evidenced by the ^{1}H-NMR signals at δ 4.09(23-H), δ 4.31 (31-H_a), and δ

3.57 (31-H_b). From conventional acetylation of compound C, monoacetate (**7**) was obtained. In the ^{13}C-NMR spectrum of **7** signals due to C-23 and C-31 were observed at δ 79.3 and δ73.7, respectively, showing the expected acylation shifts. The α-orientation of the hydroxyl group at C-23 was clarified by comparison of ^{13}C-NMR spectrum of **3** with those of closely related compounds derived from gypsogenin methyl ester by Komori et al.[6] The chemical shifts of carbons at 3, 4, 23, 24, and 31 of **3** are quite similar to the corresponding carbons of the derivative with 23α-hydroxyl group and not to 23β epimer. Komori et al. found that cleavage of glycosidic bond and concomitant formation of a fused tetrahydrofurane ring took place when they treated gypsogenin 3-O-glycoside and other saponins with diazomethane in methanol.[6] They applied this procedure to other saponins and found it to be a new method of cleavage of glycosidic bonds at 3β hydroxyl group of the natural products with 23-CHO group.

It was quite obvious that compound C (**3**) was formed as an artefact through the same type reaction on the ursane series of triterpenoid, and we turned our attention to isolation of the possible genuine constituent, e.g., **8**.

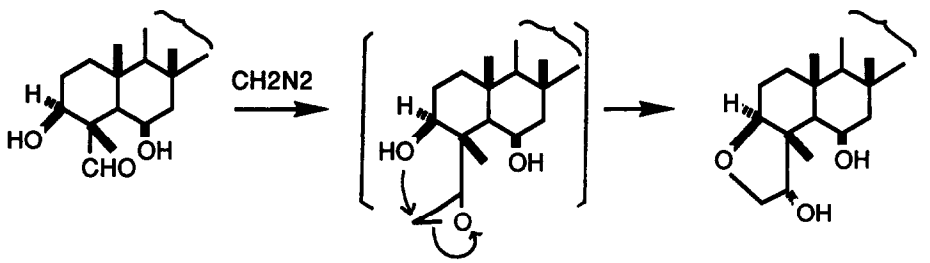
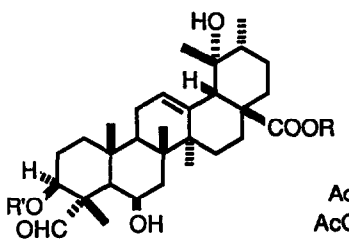


Chart 3.

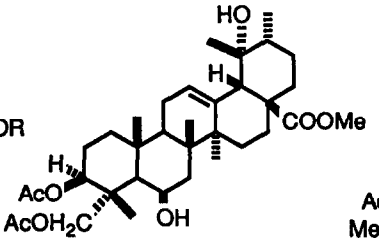
Trial was made to isolate the objective aldehydic carboxylic acid without using diazomethane treatment but the result was unsuccessful. Then the fraction was esterified with diazomethane for a short period of time, (15 min.), and the resulting material was acetylated to make the separation easy. Extensive chromatography afforded compounds D and E, both being new compounds.

Compound D (**10**), mp 197-199°C, was shown to have the molecular formula of $C_{33}H_{50}O_7$ by means of high resolution mass spectrometry. The ^{1}H-NMR spectrum was well consistent to the structure **10**, showing five singlet methyl signals (δ1.48, 1.37, 1.27, 1.23, and 1.00), one doublet methyl signal at δ 0.94 (J= 6.4 Hz). Other expected signals due to acetyl (δ1.98), ester methyl (δ3.60), 18-H (δ2.62), 16α-H(δ2.50, ddd, J= 13.2, 13.2, 5.1 Hz), and aldehyde (δ9.36) were also observed. The ^{13}C-NMR spectrum of

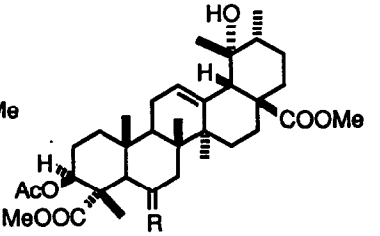
compound D strongly indicated correctness of the depicted structure. Through the comparison of the ^{13}C-NMR spectrum of **10** with that of **5** the stereochemistry at C-4 was proved; that is α-configuration of the carboxyaldehyde group. Thus the signals for C-3, C-5, and C-24 of **10** were observed at higher field than the corresponding signals in **5** by 5.7, 5.5,



8 R = R' = H
9 R = Me R' = H
10 R = Me R' = Ac



11



12 R = 

13 R = O

Chart 4.

and 5.9 ppm, respectively. These observations were in good accord to the given structure, in which γ-interactions to every of these carbons from the α-oriented aldehyde group were expected.

To obtain the final proof for the structure of **10** a chemical conversion to a compound with the known stereochemistry was carried out. Thus **10** was reduced with sodium borohydride and the resulting primary alcohol was acetylated to give **11**. Fully satisfactory identification of ^{1}H-NMR spectrum of **11** was made with that of methyl 3,23-diacetoxy-6β,19α-dihydroxyurs-12-en-28-olate derived from a natural compound isolated from Enkianthus campanulatus.[7] Thereafter the acetyl group of **10** was removed by use of sodium methoxide to give triol (**9**).

Now having the desired triterpene (**9**) in hand, its reaction with diazomethane for longer period of time was tested. To a solution of **9** in methanol, freshly prepared ethereal solution of diazomethane was added and the mixture was kept at room temperature for 48 hrs. The reaction product was found to be completely identical to **3**.

Compound E (**12**) was isolated as a colorless powder. The molecular formula, $C_{34}H_{52}O_8$, suggested this compound to be a dimethyl ester of a dioic acid. Examination of the ^{1}H-NMR spectrum gave the following observations; four tertiary methyl groups (δ0.98, 1.23, 1.24, 1.34, and 1.58), one

secondary methyl group (δ 0.94), two carbomethoxyl groups (δ3.62, δ3.67), one acetoxyl group (δ 1.99), and one olefinic proton (δ5.39). This spectrum also showed the characteristic signals of H-16α at δ 2.50 and H-18 at δ 2.62. These and other spectral properties clearly indicated that compound E was dimethyl 3β-acetoxy-6β,19α-dihydroxyurs-12-en-23,28-dioate (**12**). To confirm this structure more rigorously ^{13}C-NMR spectrum was measured. Shielding effects due to α-oriented C-23-methoxycarbonyl group were evidenced in the chemical shifts of C-3, C-5, and C-24. Furthermore the chemical shifts of the methyl carbons, C-24, C-25, C-26, and C-27, were compared with the calculated values basing on the increments proposed to this structure class of compounds.[8] As the result, completely satisfactory agreements were obtained to support the deduced structure. An attempt was made for preparation of compound E from compound D. But oxidation of **10** with chromic acid gave only corresponding 6-oxo compound (**13**) and oxidation of aldehyde function to carboxyl group was unsuccessful.

Experimental

General Procedure --- ^{1}H-NMR spectra were measured with JNM FX270 (270MHz) in $CDCl_3$ using TMS as an internal standard unless otherwise specified. ^{13}C-NMR spectra were taken in $CDCl_3$ using TMS as an internal standard on JNM FX270 (67.5 MHz) unless otherwise specified. For measurement of mass spectra, Hitachi M-60 or Hitachi 7M (for high resolution measurement) were used.

Plant Material --- The material plant was collected in Taiwan in 1969.[3] The botanical specimen has been deposited at the herbarium of Faculty of Pharmaceutical Sciences, Chiba University, Japan.

Extraction and separation of the triterpenoids from the plant --- The powdered plant material (1 kg) was extracted with MeOH at room temperature. The extract (154 g) was dissolved in water and extraction was made by use of n-BuOH. The organic layer (500 ml) was washed with 5 % Na_2CO_3 and then with water. Removal of the sovent gave 13.5 g of the residue, which was chromatographed over SiO_2 (650 g) with the developing solvent system of $CHCl_3$-MeOH-H_2O 9:2:0.3, and was separated coursely to two fractions. The less polar fraction (3.9 g) was found to be a mixture of oxindole alkaloids, on which we already reported our work.[3] The more polar fraction (2.62 g) was chromatographed over SiO_2 (78 g) and β-sitosteryl-β-D-glucoside (88.1 mg) was obtained. Further elution with $CHCl_3$-MeOH (2%) gave compound A (3β,6β,19α-trihydroxy-urs-12-en-28-oic acid)(**1**) (269 mg). The next fraction (681 mg) eluted with $CHCl_3$-MeOH (5%) was treated with excess amount of ethereal diazomethane in methanol for 24 hrs. After repeated purification with flash chromatography, compound B (methyl-3β,6β,19α-trihydroxy-23-norurs-4(24),12-dien-28-olate)(**6**)(111 mg), methyl-3β,6β,19α-trihydroxyurs-12-en-28-olate (**4**)(190 mg), and compound C (**3**) (68 mg) were obtained.

β-Sitosteryl-β-D-glucoside --- Colorless plates from MeOH. mp 269-272°C. [Found: C, 71.80; H, 10.21. $C_{35}H_{60}O_6$ 1/2H_2O requires C, 71.76; H, 10.49]. The ^{13}C-NMR spectrum in pyridine-d_5 was identical to the reported values.[8]

3β,6β,19α-Trihydroxy-urs-12-en-28-oic acid (1) --- Crystallized from MeOH-H_2O. mp. 226-227°C. [Found: C, 73.25; H, 9.78. $C_{30}H_{48}O_5$ requires C, 73.74; H, 9.90]. EI-MS m/z (%); 470(7)(M^+-H_2O), 442(7)(M^+-CO_2), 370 (5)(fragment d), 264(8)(fragment a), 246(31)(fragment a - H_2O), 224(6) (fragment b), 219(27)(fragment a -CO_2), 201(51)(fragment a -CO_2-H_2O), and 146(100)(fragment c). ^{1}H-NMR ($CDCl_3$-CD_3OD) δ: 1.06, 1.07, 1.18, 1.22, 1.27, 1.29 (each 3H, s, 23-H_3, 24-H_3, 25-H_3, 26-H_3, 27-H_3, 29-H_3), 0.95 (3H,d, J= 6.8 Hz, 30-H_3), 2.47 (1H, ddd, J= 14, 14, 4 Hz, 16α-H), 3.14 (1H, dd, J= 10, 4 Hz, 3α-H), 4.54 (1H, br.s, 6α-H), and 5.39 (1H, br.s., 12-H). ^{13}C-NMR (pyridine-d_5) δ ; 16.8(C-25), 16.8(C-30), 17.1(C-24), 18.0(C-26), 24.1(C-11), 24.7(C-27), 26.5(C-16), 27.0(C-21), 27.2(C-29), 28.4(C-2), 28.6 (C-24), 29.3(C-15), 37.1(C-10), 38.5(C-22), 39.7(C-4), 40.5(C-8), 41.3(C-7), 41.7(C-1), 42.4(C-20), 42.6(C-14), 48.3(C-9), 48.3(C-17), 54.7(C-18), 56.4(C-5), 67.8(C-6), 72.8(C-19), 78.8(C-3), 128.4(C-12), 139.3(C-13), and 180.7(C-28).

Methyl-3β,6β,19α-trihydroxy-urs-12-en-28-oate (4) -- Methyl ester **4** was obtained by mthylation of **1** with diazomethane. The sample was recrystallized from MeOH-H_2O. mp. 220-221°C. [Found: C, 74.18; H, 9.94. $C_{31}H_{50}O_5$ requires: C, 74.07; H, 10.03]. ν_{max}^{KBr} cm^{-1}: 3500, 1720. EI-MS m/z (%): 502(1)(M^+), 484(4)(M^+-H_2O), 278(2)(fragment a), 260(17)(fragment a -H_2O), 224(2)(fragment b), 206(36)(fragment b -H_2O). ^{1}H-NMR : 1.00, 1.08, 1.18, 1.22, 1.24, 1.30 (each 3H,s, 23-H_3, 24-H_3, 25-H_3, 26-H_3, 27-H_3, 29-H_3), 0.94 (3H,d, J=6.6 Hz, 30-H_3), 2.50(1H, ddd, J=13, 13, 5 Hz, 16α-H), 5.30(1H, t, J=3 Hz, 12-H). ^{1}H-NMR (pyridine-d_5) δ: 1.44, 1.44, 1.47, 1.67, 1.75 (each 3H, s, 23-H_3, 24-H_3, 25-H_3, 26-H_3), 1.13 (3H, d, J=6.6 Hz, 30-H_3), 2.90(1H, s, 18-H), 3.08(1H, ddd, J=13, 13, 5 Hz, 16α-H), 5.70(1H, br.s, H-12). ^{13}C-NMR (pyridine-d_5) δ; 16.7(C-30), 17.1(C-25), 18.1(C-24), 18.2(C-26), 24.0(C-11), 24.7(C-27), 26.1(C-16), 26.7(C-21), 27.0(C-29), 28.4(C-2), 28.7(C-23), 29.0(C-15), 37.1(C-10), 38.2(C-22), 39.7(C-4), 40.5(C-8), 41.3(C-1), 41.5(C-7), 42.2(C-20), 42.4(C-14), 48.3(C-9), 48.6(C-17), 51.5(OCH_3), 54.5(C-18), 56.6(C-5), 67.8(C-6), 72.6(C-19), 78.8(C-3), 128.7(C-12), 138.8(C-13), and 178.4(C-28).

Methyl-3β-acetoxy-6β,19α-dihydroxy-urs-12-en-28-oate (5) --- Acetylation of **4** (25 mg) with acetic anhydride (1.5 ml) in pyridine (1.5 ml) at room temperature afforded **5** (21.4 mg) as the sole product. Amorphous powder. ^{1}H-NMR δ: 0.96, 0.99, 1.23, 1.25, 1.33 (each 3H,s, 23-H_3, 24-H_3, 25-H_3, 26-H_3, 27-H_3), 0.96 (3H, d, J=6.6 Hz, 30-H_3), 2.06 (3H,s, OAc), 2.50 (1H, ddd, J=13, 13, 5 Hz, 16α-H), 4.55 (1H, br.s, 6-H), 5.39(1H, t, J=3 Hz, 12-H). ^{13}C-NMR δ; 15.1(C-30), 15.8(C-25), 16.8(C-24), 17.2(C-26), 20.3(Ac-Me), 22.7(C-2), 23.4(C-27), 24.4(C-11), 25.0(C-16), 26.4(C-29), 26.8(C-23), 27.1(C-21), 28.7(C-15), 35.4(C-10), 36.3(C-22), 37.5(C-4), 38.0(C-8), 39.2(C-1), 39.7(C-7), 40.1(C-20), 40.7(C-14), 46.4(C-9), 46.9(C-17), 50.6(OCH_3), 52.2(C-18), 54.7(C-5), 67.6(C-6), 72.2(C-19), 79.8(C-3), 128.2(C-12), 136.2(C-13), 170.0(OAc), and 177.2(C-28)

Methyl-3β,6β,19α-trihydroxy-23-nor-urs-4(24),12-dien-28-oate (6) --- Crystallization from acetone - water gave prisms, mp 146-148°C. [Found: C, 72.73; H, 9.53. $C_{30}H_{46}O_5$ $(CH_3)_2CO$ requires C, 72.75; H, 9.62]. ^{1}H-NMR δ: 1.09, 1.09, 1.23, 1.28 (each 3H,s, 25-H_3, 26-H_3, 27-H_3, 28-H_3), 0.95 (3H,d, J=6.6 Hz, 30-H_3), 2.50 (1H, ddd, J=13, 13, 5 Hz, 16α-H), 2.63 (1H, s, 18-H), 3.61 (3H, s, COOMe), 3.98 (1H, dd, J=11, 8 Hz, 3-H), 4.45 (1H, br.s, 6 -H), 5.27 (2H, s, 24-H_2), 5.42 (1H, t, J=3 Hz, 12-H). EI-MS m/z(%); 486(2)(M^+), 468(6)(M^+-H_2O), 450(6)(M^+-$2H_2O$), 426(23)(M^+-HCO_2CH_3), 354(33)(fragment d), 260(12)(fragment a -H_2O). ^{13}C-NMR δ; 16.1(C-30), 16.5(C-25), 18.5(C-26), 24.5(C-11), 24.6(C-27), 25.5(C-16), 26.0(C-21), 27.4(C-29), 28.1(C-15), 32.0(C-2), 37.5(C-22), 37.8(C-10), 39.0(C-1), 39.5(C-8), 40.5(C-7), 41.1(C-20), 42.2(C-14), 44.9(C-9), 48.0(C-17), 51.6(OMe), 53.6(C-6), 53.6(C-18), 69.9(C-6), 73.1(C-3), 73.1(C-19),

104.4(C-24), 129.6(C-12), 137.7(C-13), 150.9(C-4), and 178.3(C-28).

Compound C (3) --- Compound C (3) was obtained as an amorphous powder. [Found: 530.3581. $C_{32}H_{50}O_6$ requires 530.3604). ν (KBr)cm^{-1}: 3500, 1720. ^{1}H-NMR δ: 1.00, 1.07, 1.22, 1.26, 1.36 (each 3H, s, 24-H_3, 25-H_3, 26-H_3, 27-H_3, 29-H_3), 0.94 (3H, d, J=6.6 Hz, 30-H_3), 2.09 (1H, ddd, J=13, 13, 5 Hz, 16α-H), 2.62 (1H, s, 18-H), 3.39 (1H, dd, J=11, 4 Hz, 3-H), 3.57 (1H, d, J=11 Hz, 31-H_b), 3.61 (3H, s, COOMe), 4.09 (1H, d, 23-H), 4.31 (1H, dd, J=11, 5 Hz, 31-H_a), 4.45 (1H, br.s, 6α-H), 5.40 (1H, t, J=3 Hz, 12-H). ^{13}C-NMR ; 15.7(C-25), 16.1(C-30), 17.9(C-26), 18.2(C-24), 21.9(C-2),23.9(C-11), 24.8(C-27), 25.4(C-16), 26.0(C-21), 27.4(C-29), 28.2(C-15), 37.2(C-10), 37.4(C-22), 39.8(C-8), 40.5(C-1), 40.6(C-7), 41.1(C-20), 41.7(C-14), 47.5(C-5), 47.7(C-4), 47.9(C-17), 48.0(C-9), 51.7(OMe), 53.2(C-18), 71.0(C-6), 73.2(C-19), 76.1(C-31), 77.7(C-23), 83.1(C-3), 129.2(C-12), 137.3(C-13), and 178.5(C-28).

Compound C acetate (7) --- Compound C (3) (20 mg) was acetylated with Ac_2O (1.5 ml) in pyridine (1.5 ml) at room temperature. Usual work-up afforded amorphous 7 (21 mg) as the sole product. EI-MS m/z(%): 572(1)(M^+), 554(2)(M^+-H_2O), 512(11)(M^+-HCO_2CH_3), 278(5)(fragment a), 260(15)(fragment a -H_2O). ^{1}H-NMR δ: 0.99, 1.14, 1.23, 1.26, 1.37 (each 3H, s, 24-H_3, 25-H_3, 26-H_3, 27-H_3, 29-H_3), 0.94 (3H, d, J=6.5 Hz, 30-H), 2.05 (3H,s, OAc), 2.10 (1H, ddd, J=12, 12, 5 Hz, 16α-H), 2.63(1H, s, 18-H), 3.40 (1H, dd, J=12, 4.5 Hz, 3-H), 3.57 (1H, d, J=11 Hz, 31-H), 3.61 (3H, s, COOMe), 4.20 (1H, br.s, 6-H), 4.35 (1H, dd, J= 11, 4.5 Hz, 31-H), 5.00 (1H, d, J= 4.5 Hz, 23-H). ^{13}C-NMR ; 15.4(C-25), 16.1(C-30), 17.9(C-26), 18.1(C-24), 21.1(OAc), 21.9(C-2), 23.9(C-11), 24.6(C-27), 25.3(C-16), 26.0(C-21), 27.4(C-29), 28.1(C-15), 37.3(C-10), 37.3(C-22), 39.8(C-8), 40.6(C-1), 40.8(C-7), 41.1(C-20), 41.6(C-14), 46.6(C-4), 47.7(C-5), 47.9(C-17), 48.5(C-9), 51.6(OMe), 53.2(C-18), 70.7(C-6), 73.2(C-19), 73.7(C-31), 79.3(C-23), 84.3(C-3), 129.2(C-12), 137.2(C-13), 170.4(OAc), and 178.2(C-28).

Short time methylation of the acid mixture with diazomethane --- The triterpene carboxylic acid mixture (more polar fraction obtained in the separation steps of the procedure described above)(2.42 g) was treated with excess of diazomethane in ether for 15 min. The product was then acetylated and purification of the resulting material was carried out by using SiO_2 column and layer chromatography. As the result compound D (**10**)(123 mg), compound E (**12**)(53 mg), compound B acetate (**14**)(304 mg), and β-sitosteryl acetate (10 mg) were obtained.

Compound D (10) --- Compound D (**10**) was obtained as colorless prisms. mp 197-199 °C. [Found; 558.3521. $C_{33}H_{50}O_7$ requires 558.3558]. ν_{max}(KBr) cm^{-1}; 3520-3400, 1740-1700, 1370, 1240-1200, 1150. ^{1}H-NMR ; 0.94(3H, d, J=6.6 Hz, 30-H_3), 0.98(3H, s, 26-H_3), 1.00(3H, s, 26-H_3), 1.23(3H, s, 27-H_3), 1.27(3H, s, 29-H_3), 1.37(3H, s, 25-H_3), 1.48(3H, d, J=6.4 Hz, 24-H_3), 1.98(3H, s, OAc), 2.50(1H, ddd, J=13.2, 13.2, 5.1 Hz, 16α-H), 2.62(1H, s, 18-H), 3.60(3H, s, COOMe), 3.95(1H, br.s, 6-H), 4.96(1H, dd, J=10.3, 5.6 Hz, 3-H), 5.40(1H, t-like, 12-H), and 9.36(1H, s, 23-H). ^{13}C-NMR ; 10.9(C-24), 16.0(C-30), 16.9(C-25), 17.8(C-26), 21.0(OAc), 22.8(C-2), 23.5(C-11), 24.4(C-27), 25.3(C-16), 25.9(C-21), 27.4(C-29), 28.1(C-15), 35.5(C-10), 37.3(C-22), 34.5(C-8), 39.8(C-1), 40.8(C-7), 41.1(C-20), 41.8(C-14), 47.4(C-9), 47.9(C-17), 49.2(C-5), 51.6(OMe), 53.2(C-18), 54.9(C-4), 70.9(C-6), 73.1(C-19), 74.1(C-31), 170.3(OAc), and 205.1(C-23).

Compound E (12) --- Compound E (**12**) was obtained as a colorless powder. [Found; m/z 588.3616. $C_{34}H_{52}O_8$ requires 588.3597]. ν_{max}($CHCl_3$) cm^{-1}; 3600, 3500, 1740-1700, 1370, 1255, 1230-1200, 1150. ^{1}H-NMR δ; 0.94(3H, d, J=6.6 Hz, 30-H_3), 0.98(3H, s, 26-H_3), 1.23(3H, s, 27-H_3), 1.24(3H, s, 29-H_3), 1.34(3H, s, 25-H_3), 1.58(3H, s, 24-H_3), 1.99(3H, s, OAc), 2.50(1H,

ddd, J=12.9, 12.9, 4.6 Hz, 16α-H), 2.62(1H, s, 18-H), 3.62(3H, s, COOMe), 3.67(3H, s, COOMe), 3.97(1H, br.s, 6α-H), 5.14(1H, dd, J=10.2, 6.3 Hz, 3α-H), 5.39(1H, t, J=3.6 Hz, 12-H). ^{13}C-NMR δ; 13.5(C-24), 15.1(C-30), 16.9(C-25), 17.7(C-26), 21.1(OAc), 23.2(C-2), 23.5(C-11), 24.5(C-16), 26.0(C-21), 27.4(C-29), 28.1(C-15), 36.1(C-10), 37.4(C-22), 39.5(C-8), 39.8(C-1), 40.7(C-7), 41.2(C-20), 41.7(C-14), 47.5(C-9), 47.9(C-17), 51.6(OMe), 52.1(C-5), 52.2(OMe), 52.8(C-4), 53.2(C-18), 71.2(C-6), 73.2(C-19), 77.7(C-3), 128.9(C-12), 137.4(C-13), 170.1(OAc), 176.8(C-23).

Conversion of compound D (10) to 11 --- $NaBH_4$ (1 mg) was added to a solution of **10** (8 mg) in MeOH (3 ml). The reaction mixture was stirred at room temperature for 2.5 hr. Usual work-up gave a residue (8 mg) which was then treated with Ac_2O / pyridine (0.5 / 1 ml) overnight at room temperature. The product was purified with silica gel column to give **11** (9 mg). Amorphous powder. [Found; 602.3804. $C_{35}H_{54}O_8$ requires 602.3820]. EI-MS m/z (%); 602(3), 584(4), 542(17), 470(8), 306(10), 278(13), 265(10), 260(35), 250(26), 219(32), 218(28), 205(21), 201(73), 200(34), 179(100), 146(100), 133(38). ν_{max} ($CHCl_3$) cm^{-1}; 1740-1720, 1370, 1250, 1230-1200, 1150. ^{1}H-NMR δ; 0.95(3H, d, J=6.6 Hz, 30-H_3), 0.99(3H, s, 26-H_3), 1.23(9H, s, 24-H_3, 27-H_3, 29-H_3), 1.35(3H, s, 25-H_3), 2.03(3H, s, OAc), 2.05(3H, s, OAc), 2.51(1H, ddd, J=12.2, 12.2, 4.6 Hz, 16α-H), 2.63(1H, s, 18-H), 3.85(1H, d, J=12 Hz, 23-H_a), 3.98(1H, d, J=12 Hz, 23-H_b), 4.36(1H, br.s, 6-H), and 4.74(1H, dd, J=10.2, 6.3 Hz, 3-H).

Methyl 3β-acetoxy-19α-hydroxy-6-oxours-12-en-23-al-28-oate (13) --- A solution of CrO_3 (14 mg) in H_2O (0.5 ml) was added to 2 ml of pyridine which contained 9 mg of **11**. The mixture was kept at room temperature. The usual work-up gave 8 mg of **13** as a white mass. [Found 556.3407. $C_{33}H_{48}O_7$ requires 556.3401]. EI-MS m/z (%); 556(7), 496(23), 424(22), 278(8), 260(6), 250(4), 219(12), 218(8), 205(9), 201(25), 200(15), 179(88), 146(62), 133(23), and 43(100). ^{1}H-NMR δ; 0.76(3H, s, 26-H_3), 0.96(3H, d, J=6.4 Hz, 30-H_3), 1.00(3H, s, 25-H_3), 1.24 (3H, s, 29-H_3), 1.39(3H, s, 27-H_3), 1.62(3H, s, 24-H_3), 1.99(3H, s, OAc), 1.99(1H, d, J=15.1 Hz, 7α-H), 2.49(1H, d, J=15.1 Hz, 7β-H), 2.55(1H, ddd, J=13.8, 13.8, 4.7 Hz, 16α-H), 2.62(1H, s, 18-H), 2.67(1H, s, 5 -H), 3.59(3H, s, OMe), 4.75(1H, dd, J=11.1, 4.7 Hz, 3-H), 5.41(1H, t-like, 12-H), and 9.56(1H, s, 23-H).

ACKNOWLEDGEMENTS --- We thank Prof. Jinsaku Sakakibara, Nagoya City University, for identification of our compound (11) with the authentic specimen. This work was financially supported by a Grant-in-Aid for General Scientific Research from the Ministry of Education, Science, and Culture of Japan.

References and Notes

1) a) W. H. M. W. Herath, M. U. S. Sultanbawa, and G. P. Wannigama, Phytochem., **17**, 1979 (1978); b) F. R. Ahmed, A. S. Ng, and A. G. Fallis, Canad. J. Chem., **56**, 1020 (1978); c) W. H. M. W. Herath, M. U. S. Sultanbawa, G. P. Wanigama, and A. Cave, Phytochem., **18**, 1385 (1979); d) R. Cerri, R. Aquino, F. de Simone, and C. Pizza, J. Nat. Prod., **51**, 257 (1988).

2) N. Aimi, K. Yamaguchi, M. Takahashi, M. Iwata, S. Sakai, J. Haginiwa,

and Y. Iitaka, Tetrahedron, **37**, 983 (1981), and references cited therein.

3) J. Haginiwa, S. Sakai, K. Takahashi, M. Taguchi, and S. Seo, Yakugaku Zasshi, **91**, 575 (1971).

4) H. Budziekiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry", Vol. II, Holden-Day, Inc., San Fransisco, 1964, p. 122.

5) For examples; J. Sakakibara, T. Kaiya, H. Fukuda, and T. Ohki, Phytochem. **22**, 2553 (1983).

6) a); R. Higuchi, Y. Tokimitsu, N. Hamada, T. Komori, and T. Kawasaki, Liebig's Ann., **1985**, 1192.; b) R. Higuchi, Y. Tokimitsu, and T. Komori, Symposium Papers of 28th Symposium of Natural Products, October, 1986, Sendai, Japan, p. 224.

7) J. Sakakibara, T. Kaiya, and H. Fukuda, Phytochem., **23**, 627 (1984).

8) H. T. Cheung and D. G. Williamson, Tetrahedron, **25**, 119 (1969).

9) a) H. L. Holland, P. R. P. Diakow, and G. J. Taylor, Canad. J. Chem., **56**, 3121 (1978); b) I-M. Chang, H. S. Yun and K. Yamasaki, Kor. J. Pharmacog., **12**, 12 (1981)