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6β-HYDROXYURSOLIC ACID AND OTHER TRITERPENOIDS OF *ENKIANTHUS CERNUUS*

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Key Word Index—*Enkianthus cernuus*; Ericaceae; triterpene; steroid; 6β-hydroxyursolic acid; sitosteryl 6′-*O*-acyl-β-D-glucoside.

Abstract—From the leaves of *Enkianthus cernuus forma rubens*, a new triterpene, 6β-hydroxyursolic acid, was isolated together with several known compounds whose structures were established by chemical and spectroscopic means.

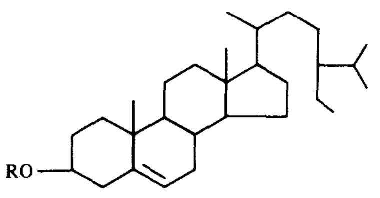
INTRODUCTION

In the course of our study on the constituents of the Ericaceae, we have now focused on *E. cernuus* Benth. et Hook. *forma rubens* Ohwi, a wild shrub distributed in the western part of Japan and used as a decorative plant. Only ursolic acid has been detected by TLC in it [1]. In this paper we report the presence of two new and several known compounds in the leaves of the plant.

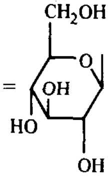
RESULTS AND DISCUSSION

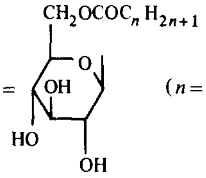
The leaves of the plant were extracted with benzene and methanol successively. The benzene extract was chromatographed on a silica gel column to give *n*-alkanes (**1**), wax alcohols (**2**), sitosterol (**3**), ursolic acid (**4**) and a new triterpene (**5**). The methanol extract was diluted with water and extracted with chloroform. From the chloroform extract 2α-hydroxyursolic acid (**6**), sitosteryl β-D-glucoside (**7**) and sitosteryl 6′-*O*-acyl-β-D-glucoside (**8**) were isolated.

The composition and relative quantities of compounds **1** and **2** were confirmed by GC/MS. The structures of the other known compounds were established by spectroscopic means and/or identification with authentic samples (see Experimental).

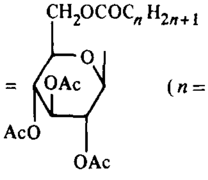


3 R = H

7 R =


8 R =


(n = 16 – 32)

8a R =


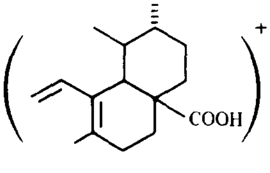
(n = 16 – 32)

n-C_nH_{2n+2} (n = 25 – 33)

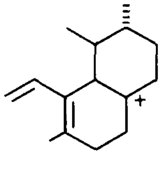
1

n-$C_nH_{2n+1}OH$ (n = 24 – 34)

2

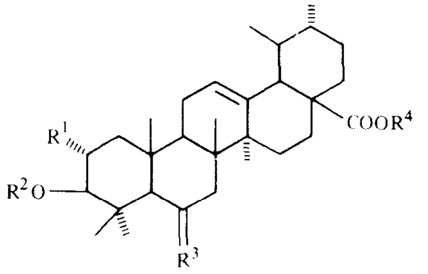


a: m/z 248



b: m/z 203

The IR spectrum of compound **5**, M^+ at m/z 472 ($C_{30}H_{48}O_4$), mp 230–235°, $[\alpha]_D$ + 36.7° (*c* 1.25; MeOH), showed the presence of hydroxyl and carboxyl groups (3400, 2910 and 1690 cm^{-1}). Its mass spectrum exhibited significant peaks at m/z 248 and 203 corresponding to ions a and b, suggesting that fragmentation was occurring in a manner typical of urs-12-enes or olean-12-enes with a C-17 carboxyl and no hydroxyl groups on rings D/E [2]. Acetylation of **1** gave a monoacetate (**5a**), which was esterified with diazomethane to give a methyl ester



	R^1	R^2	R^3	R^4
4	H	H	H	H
5	H	H	OH H	H
5a	H	Ac	OH H	H
5b	H	Ac	OH H	Me
5c	H	Ac	=O	Me
5d	H	H	OH H	Me
6	OH	H	H	H

monoacetate (**5b**). Compound **5b** was submitted to Jones' oxidation to afford a ketone (**5c**), mp 244–245°, $C_{33}H_{50}O_5$, whose IR spectrum showed no hydroxyl absorption. Therefore the four oxygens of compound **5** could be attributed to one carboxyl and two secondary hydroxyl groups. Alkaline hydrolysis of **5b** afforded a methyl ester (**5d**). The ^{13}C NMR spectrum of **5a** exhibited peaks at δ 126.0 (*d*) and 137.1 (*s*) due to C-12 and C-13, and its ^1H NMR spectrum showed a peak at δ 2.22 (*d*, *J* = 10 Hz) due to H-18. These facts revealed that **5** should have an ursane skeleton [3, 4]. Assuming that, of the two secondary hydroxyl groups, the one easily acylable was placed at C-3 and the position of the other was determined as follows. The ^1H NMR spectrum of **5c** showed a singlet at δ 2.24 due to H-5 and two doublets at δ 2.32 and 2.53 (each 1H, *J* = 12 Hz) due to H-7, suggesting that the second hydroxyl group should be attached to C-6. The C-3 hydroxyl group would have β-equatorial configuration according to the coupling pattern of H-3 (*dd*, *J* = 6 and 10 Hz). The configuration of the C-6 hydroxyl group was presumed to be β-equatorial because the peak at δ 4.50 (1H, *m*, $W_{h/2}$ = 8 Hz) in the ^1H NMR spectrum of **5d** could be assigned to H-6; this was in good agreement with the corresponding 6β-hydroxy derivative with an oleanane skeleton which showed the H-6 proton at δ 4.41 (1H, *m*, $W_{h/2}$ = 8 Hz) [5]. In addition, the calculated values of the tertiary methyl signals for methyl 6β-hydroxyursolate in the ^1H NMR spectrum agreed well with the experimental ones (Table 1) [6]. Thus the structure of **5** was established as 6β-hydroxyursolic acid.

Table 1. Methyl signals of **5d** (δ, in $CDCl_3$)

	24-Me	25-Me	26-Me	27-Me
Calc.*	1.18	1.30	1.08	1.02
Found	1.17	1.31	1.07	1.03

*The data are from ref. [6].

The ^1H NMR and ^{13}C NMR spectra of compound **8**, colourless oil, were very similar to those of sitosteryl β-D-glucoside (**7**) except that the former exhibited a large peak due to methylenes [δ 1.30, δ 30.1 (*t*)] and the presence of an ester carbonyl group (δ 173.6). On acetylation **8** gave an acetate (**8a**), whose ^1H NMR spectrum showed the presence of three acetoxyl groups (δ 1.98, 2.00, 2.02). Alkaline hydrolysis of **8** afforded **7** and a mixture of aliphatic carboxylic acids, whose composition was confirmed by GC/MS. The position of attachment of the carboxylic acids was revealed to be at C′-6 of the glucose moiety because of a downfield shift (1.9 ppm) of C′-6 and an upfield shift of C′-5 (3.6 ppm) in the ^{13}C NMR spectrum of **8**. Therefore the structure of **8** was confirmed as sitosteryl 6′-*O*-acyl-β-D-glucoside (alkyl: n-$C_{16}H_{33}$– to n-$C_{32}H_{65}$–). Aliphatic carboxylic acid esters of **7** have previously been found in nature [7, 8], but the length of the alkyl chains was limited to pentadecyl (n-$C_{15}H_{31}$–) or heptadecyl (n-$C_{17}H_{35}$–).

EXPERIMENTAL

All mps are uncorr. IR spectra were recorded on KBr discs. ^1H NMR spectra were run at 200 and 100 MHz, and ^{13}C NMR spectra at 50 and 25 MHz with TMS as internal standard. MS (70 eV) were taken with a direct inlet. Typical GC/MS operating conditions were as follows. The compounds were chromatographed on a glass column. The He carrier gas flow rate was 30 ml/min, the injection temp. was 300° and the jet separator and lines to the mass spectrometer were maintained at 200°. EIMS were obtained at an ionization voltage of 70 eV. The accelerating voltage was 3kV, the ion source temp. was 200° and the emission current was 300 μA.

Plant material. Leaves of *E. cernuus* Benth. et Hook. *forma rubens* Ohwi were collected in Tottori Prefecture, Japan, in 1981 by Chizu Agricultural Cooperative of Tottori and presented to us.

Extraction and isolation. The powdered, dried leaves (3.76 kg) were extracted under reflux with C_6H_6 and MeOH, successively. The MeOH extract was diluted with H_2O and extracted with $CHCl_3$ at room temp. The C_6H_6 extract was chromatographed over a silica gel column, eluting with hexane and then a mixture of C_6H_6–EtOAc. The hexane eluate afforded hydrocarbons (**1**). From the C_6H_6–EtOAc (19:1) eluate, **2** was obtained, from the C_6H_6–EtOAc (9:1) eluate **3**, and from the C_6H_6–EtOAc (3:7) eluate **4** and **5**, successively. The $CHCl_3$ extract was eluted with a mixture of $CHCl_3$–MeOH over a silica gel column. From the $CHCl_3$–MeOH (97:3) eluate **6** and **8** were separated, and from the $CHCl_3$ (19:1) eluate **7** successively.

Hydrocarbons (**1**). The GC operating conditions were: column 10% SE-30 on Chromosorb W (AW), GC over 200° programmed at 2°/min to 300°. The relative quantities obtained from the results of GC/MS were as follows. n-$C_{25}H_{52}$ 0.5%; n-$C_{26}H_{54}$ 0.2%; n-$C_{27}H_{56}$ 1.4%; n-$C_{28}H_{58}$ 0.6%; n-$C_{29}H_{60}$ 27.6%; n-$C_{30}H_{62}$ 1.3%; n-$C_{31}H_{64}$ 58.1%; n-$C_{32}H_{66}$ 0.7%; n-$C_{33}H_{68}$ 9.6%.

Alcohols (**2**). Compound **2** was acetylated and submitted to GC/MS. The GC operating conditions were the same as in the case of **1**. Relative quantities were: n-$C_{24}H_{49}OAc$ 0.7%; n-$C_{25}H_{51}OAc$ 0.2%; n-$C_{26}H_{53}OAc$ 12.9%; n-$C_{27}H_{55}OAc$ 0.7%; n-$C_{28}H_{57}OAc$ 15.9%; n-$C_{29}H_{59}OAc$ 0.7%; n-$C_{30}H_{61}OAc$ 38.5%; n-$C_{31}H_{63}OAc$ 0.4%; n-$C_{32}H_{65}OAc$ 22.5%; n-$C_{33}H_{67}OAc$ 0.6%; n-$C_{34}H_{69}OAc$ 6.9%.

Sitosterol (**3**). Purified as acetate (solvent C_6H_6–EtOAc, 9:1). MS m/z 456.3960 (M^+, calc. for $C_{31}H_{52}O_2$, 456.3967). The IR, ^1H NMR and ^{13}C NMR spectra were identical with those of the authentic sample.

Ursolic acid (**4**). Mp 289–292° (EtOH). Identical with the authentic sample in all respects.

6β-Hydroxyursolic acid (**5**). Purified by prep. TLC (solvent C_6H_6–EtOAc, 1:1). Mp 230–235° (MeOH). IR ν_{max} cm^{-1}: 3400, 2910, 1690. ^1H NMR (C_5D_5N): δ 0.98, 1.28, 1.44, 1.60, 1.72 (each 3H, *s*, Me), 3.02 (1H, *m*, H-3), 4.84 (1H, *m*, H-6), 5.46 (1H, *m*, H-12). MS: m/z 472.3517 (M^+, calc. for $C_{30}H_{48}O_4$, 472.3552), 248, 203.

Acetyl-6β-hydroxyursolic acid (**5a**). Purified by prep. TLC (solvent C_6H_6–EtOAc, 7:3). ^1H NMR ($CDCl_3$): δ 2.04 (3H, *s*, MeCO), 4.52 (2H, *m*, H-3 and H-6), 5.28 (1H, *m*, H-12). ^{13}C NMR ($CDCl_3$): δ 183.7 (C-28), 170.9 (Me$\underline{C}$O), 137.1 (C-13), 126.0 (C-12), 80.9 (C-3), 68.5 (C-6), 55.6 (C-5), 52.4 (C-18), 47.9 (C-17), 47.8 (C-9), 42.5 (C-14), 40.6 (C-8), 40.4 (C-20), 39.1 (C-19), 38.8 (C-1), 38.6 (C-4), 36.6 (C-22), 36.4 (C-10), 33.9 (C-7), 30.7 (C-21), 29.7 (C-15), 27.9 (C-23), 24.2 (C-16), 23.6 (C-2), 23.6 (C-27), 23.2 (C-11), 21.3 (C-30), 21.1 ($CO\underline{C}H_3$), 18.6 (C-24), 18.3 (C-29), 17.2 (C-26), 17.0 (C-25).

Methyl acetyl-6β-hydroxyursolate (**5b**). Purified by prep. TLC (solvent C_6H_6–EtOAc, 4:1). ^1H NMR ($CDCl_3$): δ 0.95, 0.95, 1.04, 1.25, 1.33 (each 3H, *s*, Me), 2.03 (3H, *s*, COMe), 2.22 (1H, *d*, $J = 10$ Hz, H-18), 3.58 (3H, *s*, OMe), 4.35 (1H, *m*, H-3), 4.50 (1H, *m*, $W_{h/2} = 8$ Hz, H-6), 5.24 (1H, *m*, H-12).

Methyl acetyl-6-oxo-ursolate (**5c**). Treatment of **5b** with Jones' reagent at room temp. The reaction mixture was poured into H_2O and extracted with Et_2O. The Et_2O extract was purified by prep. TLC (solvent C_6H_6–EtOAc, 9:1). Mp 244–245° (MeOH). IR ν_{max} cm^{-1}: 1722, 1703. ^1H NMR ($CDCl_3$): δ 0.80, 0.94, 0.97, 1.22, 1.30 (each 3H, *s*, Me), 2.07 (3H, *s*, COMe), 2.24 (1H, *s*, H-5), 2.31, 2.53 (each 1H, *d*, $J = 10$ Hz, H-7), 4.44 (1H, *m*, H-3), 5.34 (1H, *m*, H-12). ^{13}C NMR ($CDCl_3$): δ 211.4 (C-6), 177.7 (C-28), 170.9 (Me$\underline{C}$O), 137.9 (C-13), 125.0 (C-12), 80.0 (C-3).

Methyl 6β-hydroxyursolate (**5d**). Treatment of **5b** with dil. alkaline soln gave **5d**. ^1H NMR ($CDCl_3$): δ 1.03, 1.06, 1.07, 1.17, 1.31 (each 3H, *s*, Me), 3.15 (1H, *dd*, $J = 7$ and 9 Hz, H-3), 4.56 (1H, *m*, $W_{h/2} = 8$ Hz, H-6), 5.30 (1H, *m*, H-12).

2α-Hydroxyursolic acid (**6b**). Purified by prep. TLC (solvent C_6H_6–EtOAc, 4:1). Mp 210–212° (MeOH). Identified with the authentic sample.

Sitosteryl β-D-glucoside (**7**). Purified as acetate. Mp 160–163° (MeOH). Identified with the authentic sample. Complete assignment of the ^{13}C NMR signals of **7** was carried out with reference to those reported for sitosterol (**3**) [9] and methyl β-D-glucoside [10]. ^{13}C NMR (C_5D_5N): δ 140.9 (C-5), 121.8 (C-6), 102.5 (C-1′), 78.5 (C-3), 78.3, 78.2 (C-3′ and C-5′), 75.2 (C-2′), 71.7 (C-4′), 62.8 (C-6′), 56.9 (C-14), 56.3 (C-17), 50.3 (C-9), 46.1 (C-24), 42.5 (C-13), 40.0 (C-4), 39.3 (C-12), 37.5 (C-1), 36.9 (C-10), 36.4 (C-20), 34.3 (C-22), 32.1 (C-7 and C-8), 30.2 (C-2), 29.5 (C-25), 28.6 (C-16), 26.5 (C-23), 24.5 (C-15), 23.4 (C-28), 21.3 (C-11), 20.0 (C-27), 19.4 (C-19), 19.2 (C-26), 19.1 (C-21), 12.2 (C-29), 12.0 (C-18).

Sitosteryl 6′-O-acyl-β-D-glucoside (**8**). Purified by prep. TLC (solvent C_6H_6–EtOAc, 2:3). ^1HNMR (C_5D_5N): δ 0.70, 0.98 (each 3H, *s*, Me), 1.30 (many protons, *s*, $-CH_2-$), 3.62–5.20 (many protons, *m*), 5.40 (1H, *m*, H-6). ^{13}C NMR (C_5D_5N): δ 173.6 ($-\underline{C}OO-$), 141.0 (C-5), 123.9 (C-6), 102.4 (C-1′), 79.0 (C-3), 77.8 (C-3′), 74.7 (C-2′ and C-5′), 71.6 (C-4′), 64.7 (C-6′), 57.1 (C-14), 56.4 (C-17), 50.5 (C-9), 46.1 (C-24), 42.6 (C-13), 40.3 (C-4), 39.2 (C-12), 37.6 (C-1), 36.9 (C-10), 36.5 (C-20), 34.5 (C-8), 34.3 (C-22), 32.1 (C-7), 30.1 (large peak, C-2 and CH_2-), 29.6 (C-25), 28.6 (C-16), 26.6 (C-23), 25.4 (C-15), 23.4 (C-28), 23.0 ($-CH_2-$), 21.4 (C-11), 20.0 (C-27), 19.4 (C-19), 19.3 (C-26), 14.3 (Me), 12.2 (C-29), 12.1 (C-18).

Acetate of **8** (**8a**). Acetylation of **8** gave a triacetate which was purified by prep. TLC (solvent C_6H_6–EtOAc, 11:9). ^1H NMR ($CDCl_3$): δ 0.68, 0.97 (each 3H, *s*, Me), 1.26 (many protons, *s*, $-CH_2-$), 1.98, 2.00 2.02 (each 3H, *s*, MeCO). ^{13}C NMR ($CDCl_3$): δ 173.3, 170.2, 169.2 ($-\underline{C}OO-$), 140.3 (C-5), 122.1 (C-6), 99.6 (C-1′), 80.0 (C-3), 72.9 (C-3′).

Hydrolysis of **8**. Compound **8** in 5% $NaHCO_3$–MeOH soln was refluxed and extracted with Et_2O. From the extract **7** was obtained and was identified with the authentic sample. The mother liquor was acidified and extracted again with Et_2O. The extract was esterified with CH_2N_2 and submitted to GC/MS. The GC operating conditions were: column 3% OV-1 on Gas-Chrom Q, GC over 100° programmed at 4°/min to 300°. Relative quantities were: n-$C_{16}H_{33}COOMe$ 2.0%; n-$C_{17}H_{35}COOMe$ 0.8%; n-$C_{18}H_{37}COOMe$ 17.1%; n-$C_{19}H_{39}COOMe$ 1.0%; $2n$-$C_{20}H_{41}COOMe$ 2.0%; n-$C_{21}H_{43}COOMe$ 1.5%; n-$C_{22}H_{45}COOMe$ 15.2%; n-$C_{23}H_{47}COOMe$ 4.6%; n-$C_{24}H_{49}COOMe$ 6.7%; n-$C_{25}H_{51}COOMe$ 18.2%; n-$C_{26}H_{53}COOMe$ 4.6%; n-$C_{27}H_{55}COOMe$ 2.0%, n-$C_{28}H_{57}COOMe$ 14.5%; n-$C_{29}H_{59}COOMe$ 1.5%; n-$C_{30}H_{61}COOMe$ 6.1%; n-$C_{31}H_{63}COOMe$ 0.5%; n-$C_{32}H_{65}COOMe$ 1.8%.

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