# ONEKOTANOGENIN — A NEW TRITERPENE GENIN FROM THE HOLOTHURIAN *Psolus fabricii*

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The structure of onekotanogenin — a new triterpene genin from the holothurian *Psolus fabricii* — has been established with the aid of $^1H$ and $^{13}C$ NMR spectroscopy and a chemical correlation with the previously known cucumariogenin. Its structure has been determined as 20 (S)-acetoxy-3β,16-dihydroxylanost-7-en-8-oic acid 18,16-lactone. It has been shown that its 25,26-dihydro derivative is the native genin of psolusoside B — a minor glycoside from the holothurian *Psolus fabricii* Düben et Koren.

Continuing a chemical study of holothurians of the littoral of the island of Onekotan [1-3], we have established the structure of genin (I) obtained from psolusoside B (II) — a minor component of the glucosidic fraction from the holothurian *Psolus fabricii* Düben et Koren. Genin (I), differing substantially in its structure from all previously known aglycons of holothurian triterpene glycosides, we proposed to call onekotanogenin (from the site of collection of the animals).

Genin (I) was obtained by hydrolysis, with 2 N sulfuric acid in the presence of butanol, of the hydrogenated desulfated derivative (III). Derivative (III) was obtained, in its turn, from the glycoside (II) by solvolysis with a mixture of pyridine and dioxane followed by hydrogenation with Adams catalyst.

A comparison of the PMR spectrum of onekotanogenin (I) (Table 1) and derivative (IV) — product of the sodium tetrahydroborate reduction and subsequent pericyclization of the native genin from glycosides of the holothurian *Cucumaria japonica* [4] — showed agreement of the chemical shifts and the spin-spin coupling constants of the H-3α, H-7, H-9β, H-15α, H-15β, H-16, and $CH_3$-30, -31, and -32 signals. The assignment of the signals of the methyl groups in the spectrum was made with the use of the difference NOE procedure. Thus, when H-17 was irradiated, enhancement of the C-32 and $CH_3COO$ signals was obtained. When $CH_3$-32 was irradiated, the H-17 signal was enhanced. On the irradiation of H-9β and H-3α an enhancement of the $CH_3$-19 and $CH_3$-31 signals, respectively, was recorded. Irradiation of $CH_3$-21 enhanced the H-17 and H-16 signals. The downfield shifts of the $CH_3$-21 and H-17 signals by 0.16 and 0.47 ppm, respectively, in the PMR spectrum of (I) as compared with (IV) can be explained by the influence of the acetylation of the hydroxy group at C-20 [5, 6].

The acetylation of the hydroxyl at C-20 also explains the shift of the C-20 signal in the $^{13}C$ NMR spectrum of derivative (V) described in [4] from 72 ppm to 84.2 ppm for the genin (I), since according to information in the literature [7, 8], the α-effect of acetylation of

TABLE 1. PMR Spectrum of the Genin (I); Solvent $CDCl_3$ ($\delta_{TMS}$ = 0.

| Positions of the protons | δ, (J)* | Positions of the protons | δ, (J) | Positions of the protons | δ, (J) |
|---|---|---|---|---|---|
| H-3α | 3,20 m | H-16 | 4,81 d (2,0) | $CH_3$-30 | 0,86 s |
| H-7 | 5.55 m | H-17 | 2,97 s | $CH_3$-31 | 1,01 s |
| H-9β | 2,75 dm | $CH_3$-19 | 0,91 s | $CH_3$-32 | 1,37 s |
| H-15α | 1,97 Bdd (13, 4; 2, 0) | $CH_3$-21 | 1,40 s | $CH_3$-OAc | 2,00 s |
| H-15β | 2,09 Ad (13,4) | $CH_3$-26, 27 | 0,88 d (6,5) | | |

*The accuracy of measuring the constants was 0.25 Hz/point.

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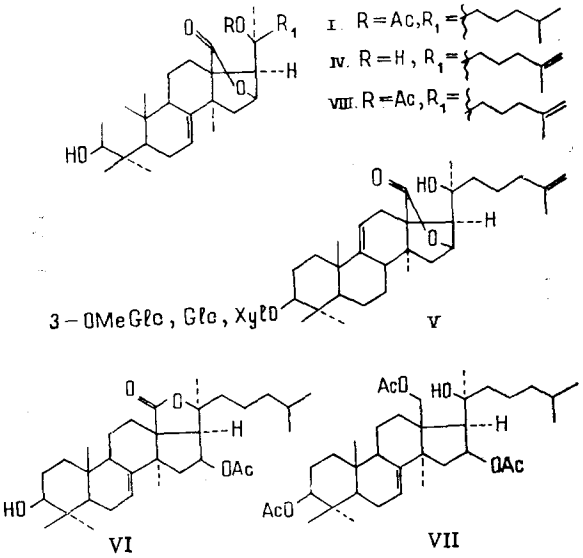


a tertiary hydroxyl is +11 to +12 ppm, with β-effect on the methyl carbon of about −1 ppm. Thus, the results of a comparison of the PMR spectra of onekotanogenin and the previously known derivative (IV) and also a comparison of the $^{13}C$ spectra of onekotanogenin with the spectrum of substance (V) described in the literature agree well with structure (I). For its definitive proof we performed a chemical correlation of onekotanogenin with the aglycon (VI) obtained by the hydrogenation of cucumarigenin from the glycosides of the holothurian *Cucumaria fraudatrix* [9], all the details of the structure of which have been confirmed by the results of x-ray structural analysis [10].

When genin (I) was reduced with lithium tetrahydroaluminate in dioxane, followed by acetylation, derivative (VII) (Table 3) was obtained. The reduction and subsequent acetylation of the aglycon (VI) led to an identical product (comparison of PMR spectra). Thus, the correctness of structure (I) has been confirmed by independent synthesis.

The $^{13}C$ NMR spectrum of genin (I) (Table 2) was close to that of the glycon moiety of glycoside (II). The C-3 signal in the spectrum of (II) (87.8 ppm) was shifted downfield in comparison with the corresponding signal at 79.1 ppm in aglycon (I) because of glycosylation at this position. Furthermore, the C-25 (144.8 ppm) and C-26 (110.4 ppm) signals indicated the presence of a 25(26)-double bond in the aglycon of glyoside (II). The presence of a 25(26)-double bond was also confirmed by the PMR spectrum of glycoside (II) taken in $C_5D_5N$. It contained: the signal of two protons of a double bond (4.82 ppm, 2H) and the signal of a methyl group at this double bond (1.71 ppm; difference decoupling). Thus, it follows unambiguously from a comparison of the $^{13}C$ and $^{1}H$ NMR spectra that the native genin of glycoside (II) is the 25(26)-dehydro derivative of onekotanogenin (VIII).

The overwhelming majority of the aglycons of holothurian tetrepene glycosides belong to the holostane series. The only exception is kuriligenin from the glycosides of *Duasmodactyla kurilensis* Levin [1]. Onekotanogenin, which contains a lactone group in the 18,16-position, differs substantially both from the holostane derivatives having an 18(20)-lactone group and from hirulogenin, having no lactone group at all.

## EXPERIMENTAL

Melting points were determined on a Boetius stage. Specific rotations were measured on a Perkin-Elmer 141 polarimeter at room temperature. $^{13}C$ and $^{1}H$ NMR spectra were obtained on a Brüker WM-250 spectrometer. The signals in the NMR spectra are given in the δ-scale, with TMS as standard. IR spectra were taken on Specord 75-IR instrument in chloroform, and mass spectra on a KLB-9000s mass spectrometer with direct introduction at an ionizing energy of 70 eV.

The animals were collected in the sea of Okhotsk in the littoral of the island of Onekotan (Kurila Islands) at a depth of 100 m by a scallop dredge during an expedition on the research ships "Mekhanik Zhukov" and "Dalarik" on August-September 1982. The holothurians were determined by V. S. Levin.

The psolusoside B(II) was isolated by the procedure described previously [2]. Mp 217-220°C with decomp., $[\alpha]_D^{20}$ −67° (c 0.1; pyridine).

Desolvation of Psolusoside B Followed by Hydrogenation. Glycoside (II) (250 mg) was boiled in 20 ml of pyridine–dioxane (1:1) for 1 h. The reaction mixture was evaporated to dryness, and the residue was chromatographed on silica gel in the chloroform–methanol (3:1) system. The purified product was dissolved in 50 ml of butanol–ethanol–water (10:5: 2.5), 40 mg of Adams catalyst that has been hydrogenated in 10 ml of ethanol was added, and the substance was hydrogenated at room temperature with vigorous stirring for two days. The reaction mixture was filtered, the spent catalyst was washed on the filter with chloroform–methanol (3:1), and the combined filtration was evaporated. This gave 240 mg of derivative (III), mp 215-219°C (decomp.), $[\alpha]_D^{20}$ −30° (c 0.2; pyridine).

Partial Acid Hydrolysis of the Hydrogenated Desulfated Derivative (III). With vigorous stirring, 240 mg of (III) was heated in the boiling water bath with 20 ml of 2 N $H_2SO_4$ and 10 ml of butanol for 30 min. The butanolic layer was separated off, the aqueous layer was washed twice with 5-ml portions of butanol, and the butanol layers were combined. Then the butanolic extract was washed with water (5 ml), with 1% $NaHCO_3$ solution (2 × 5 ml), and again with water (2 × 5 ml), and was evaporated to dryness. The dry residue was chromatographed on silica gel in the chloroform–methanol (6:1) system. This gave 205 mg of combined progenins and 14 mg of crude combined aglycons. By chromatographing this combined material on silica gel in the benzene–ethyl acetate (6:1) system, 7 mg of the aglycon (I) was obtained with mp 113-115°C, $[\alpha]_D^{20}$ −84.3° (c 0.3; chloroform), IR spectrum: 1768, 1730 $cm^{-1}$, Mass spectrum, m/z (%): 514 ($M^+$, 1.3), 484(1.3), 470(2.7), 488(3.3), 454(6), 409(6), 302(14.7), 284(1.3), 162(14.6), 145(10), 140(13.3), 122(53.3), 109(20), 96 (26.7), 95(26.7), 81(20), 69(33.3), 57(26.7), 55(26.7), 43(100), 41(26.7).

Reduction of Onekotanogenin (I) with $LiAlH_4$ Followed by Acetylation. A solution of 5 mg of the aglycon (I) in 5 ml of dioxane was treated with 100 mg of $LiAlH_4$ and the mixture was heated at the boil for 4 h. Then the excess of $LiAlH_4$ was neutralized with 2 ml of ethyl acetate and the reaction mixture was added to 5 ml of water and 5 ml of 6 N HCl. The resulting solution was extracted with hexane (3 × 5 ml), and the combined hexane layers were washed with a 1.5% solution of $NaHCO_3$ (5 ml) and with water (3 × 5 ml) and were then evaporated. The dry residue was covered with pyridine–acetic anhydride (1:1) and the mixture was left overnight. Then it was evaporated to dryness and the residue was chromatographed on silica gel in the benzene–ethyl acetate (6:1) system. This gave 1.5 mg of derivative (VII), amorphous, $[\alpha]_D^{20}$ +20° (c 0.04; chloroform).

TABLE 2. $^{13}C$ NMR Spectra of the Genin (I) and of the Aglycon Moiety of Glycoside (II). Solvent for (I) – $CDCl_3$; for (II) – $(CD_3)_2SO$ ($\delta_{TMS}$ = 0)

| Atom | I | II | Atom | I | II | Atom | I | II | Atom | I | II |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C-1 | 35,7 | 34,8 | C-9 | 45,9 | 45,1 | C-17 | 60,3 | 59,4 | C-25 | 28,1 | 144,8 |
| C-2 | 27,9 | 26,0 | C-10 | 36,0 | 34,7 | C-18 | 180,0 | 180,8 | C-26 | 22,5 | 110.4 |
| C-3 | 79.1[a] | 87.8 | C-11 | 22,0[b] | 20,9[b] | C-19 | 23,7 | 23,4 | C-27 | 22,5 | 21,7[a] |
| C-4 | 38,9 | 38,4 | C-12 | 20,2 | 19,2 | C-20 | 84,2 | 83,2 | C-30 | 16,2 | 16,5 |
| C-5 | 47.6 | 46,9 | C-13 | 53,8 | 53,8 | C-21 | 23,4 | 23,0[a] | C-31 | 28,9 | 28,0 |
| C-6 | 23,4 | 22,4 | C-14 | 45.7 | 44,9 | C-22 | 38,6 | 38,7 | C-32 | 34,5 | 33,7 |
| C-7 | 122,8 | 122,0 | C-15 | 44,5 | 43,6 | C-23 | 21,8[b] | 20.7[b] | OAc | 21,5 | 21,4[a] |
| C-8 | 146.7 | 146,8 | C-16 | 79,5[a] | 78,3 | C-24 | 39,3 | 37,0 | | 169,9 | 169,4 |

[a,b]Assignment of the signals ambiguous

TABLE 3. PMR Spectrum of Derivative (VII)*; Solvent $CDCl_3$ (δ, TMS = 0.

| Positions of the protons | δ (J) | Positions of the protons | δ (J) | Positions of the protons | δ (J) |
|---|---|---|---|---|---|
| H-7 | 5,58 m | H-15′ | 2,10 dd | $CH_3$-26, 27 | 0,87d (6,7) |
| H-16 | 5,52 m | $CH_3$-19 | 0,93 s | OAc | 2,04 s |
| H-3α | 4,47 m | $CH_3$-21 | 1,09 s | | 2,08 s |
| H-18 | 4,69 d | $CH_3$-30 | 0,90 s | | 2,11 s |
| H-18′ | 3,90 d (12,0) | $CH_3$-31 | 0,88 s | | |
| H-15 | 2,55 dd | $CH_3$-32 | 0,98 s | | |

*The interrelationship of the signals at 4.79 and 3.90 ppm and at 5.52, 2.55, and 2.10 ppm, respectively, have been confirmed by double resonance.

Reduction of the Aglycon (VI) with $LiAlH_4$ Followed by Acetylation. The aglycon (VI) (4 mg) was reduced by the procedure described above. This gave 1 mg of derivative (VII), amorphous, $[\alpha]_D^{20}$ +20°C (c 0.04; chloroform).

## CONCLUSIONS

From psolusodide B — a minor glycoside of *Psolus fabricii* — the genin onekotanogenin has been obtained. Its structure has been determined as 20(S)-acetoxy-3β,16-dihydroxylanost-7-en-18-oic acid 18,16-acetone . It has been shown that the native aglycon of psolusoside B is 20(S)-acetoxy-3β-16-dihydroxylanosta-3,25-dien-18-oic acid 18-16-lactone.

Aglycon (VI) from *Cucumaria fraudatrix* was made available to us by Sh. Sh. Afiyatullov.

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# PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS *Silene*

## XII. 5α-ECDYSTERONE 22-O-BENZOATE FROM *Silene scabrifolia*

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The new phytoecdysteroid 5α-ecdysterone 22-O-benzoate (I), $C_{34}H_{48}O_8$, mp 262-274°C (methanol—water) $[\alpha]_D^{20}$+45.8° (methanol), has been isolated from the epigeal organ of *Silene scarbiofolia* Kom. The alkaline hydrolysis of (I) led to 5α-ecdysterone (II) and benzoic acid. The isomerization of ecdysterone (0.6% $KHCO_3$ in $CH_3OH$) has yielded (II). Details of the IR, mass, and NMR spectra of compound (I) are given.

We have previously detected 2-deoxy-α-ecdysterone (I), ecdysterone (II), ecdysterone 22-O-benzoate (III) [1], and 2-deoxy-α-ecdysone 3-acetate (IV) in the plant *Silene scabrifolia* Kom. (family Caryophyllaceae) [2]. By rechromatographing the mother solution obtained on the isolation of ecdysterone 22-O-benzoate (III) on a column of silica gel we have isolated a new phytoecdysteroid (VI) with the composition $C_{34}H_{48}O_8$.

Bands in the IR spectrum at 1710, 1720, and 1280 $cm^{-1}$, in combination with the absorption characteristic of a benzene ring (1610, 1580, 725 $cm^{-1}$), and also strong peaks of ions with m/z 122 ($C_7H_6O_2$), 105 ($C_7H_5O$) and 77 ($C_6H_5$) in the mass spectrum indicated that the ecdysteroid (VI) contained a benzoic acid residue. The signals of five aromatic protons at 7.40 ppm (3 H) and 8.26 ppm (2 H) in the PMR spectrum showed the presence of a single benzoate group.

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