Y_1 is the amount of titrant consumed in the titration of a blank sample, ml;

P is the weight of the raw material, g; and

B is the loss in weight of the raw material on drying, %.

SUMMARY

1. A method has been developed for the quantitative determination of the aglycone of the combined patrinosides in the roots of *Patrinia intermedia*. The error of a single determination is ±5%.

2. The method can be used for the quantitative evaluation of a raw material.

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THE STRUCTURE OF THELENOTOSIDES A AND B FROM THE HOLOTHURIAN *Thelenota ananas*

V. A. Stonik, I. I. Mal'tsev, and G. B. Elyakov

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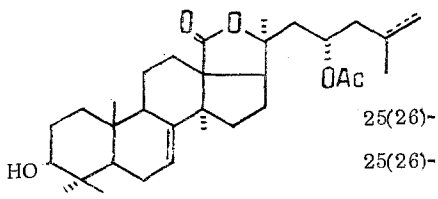
Two new triterpene tetraosides — thelenotosides A and B — have been obtained from the holothurian *Thelenota ananas*. Their complete structures have been determined as 23(S)-acetoxy-3β-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-O-β-D-quinovopyranosyl-(1→2)-β-D-xylopyranosyloxy]holost-7-ene and 23(S)-acetoxy-3β-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→2)-β-D-xylopyranosyloxy]holost-7-ene, respectively.

Continuing an investigation of the glycosides of holothurians of the family *Stichopodidae* [1, 2], we have studied the composition of the glycosidic fraction of the industrial Pacific Ocean holothurian *Thelenota ananas*. Two new physiologically active glycosides — thelenotosides A and B — have been obtained.

The native genins of the glycosides of *T. ananas* have a 7(8)- and not an 8(9)- double bond in the holostane nucleus, as was considered previously [3, 4] and are 23(S)-acetoxyholost-7-en-3β-ol (I) and 23(S)-acetoxyholosta-7,25-dien-3β-ol (II), respectively [5, 6].



25(26)-Dyhydro — I

25(26)-Dehydro — II

In order to determine the complete structures of the thelenotosides, we separated the total glycosides from *T. ananas* into a series of chromatographically individual fractions. Analysis of the ^{13}C NMR spectra of each of them showed that they were two-component mixtures

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with similar structures of the carbohydrate chains and having as their native aglycones compounds (I) and (II). In actual fact, the spectra contain signals both at 114.3 ppm (t) and at 141.6 ppm (s), which are characteristic for a Δ^{25}-bond and also at 23.1 and 23.4 ppm, showing saturation of the bond between atoms 25 and 26. To convert the mixtures isolated into individual compounds, the fractions were hydrogenated over Adams catalyst. After the hydrogenation of these substances, the signals at 114.3 and 141.6 ppm in the ^{13}C NMR spectra had disappeared.

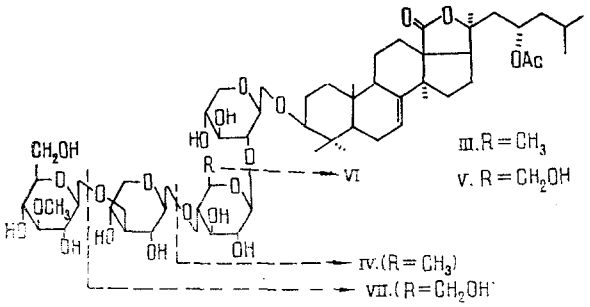
Thus, individual glycosides having only compound (I) as genin were obtained.

The least polar of the four glycosides that we obtained was shown to be identical with the previously known stichoposide A [7] from the holothurian *Stichopus chloronotus*, and the most polar was identical with stichoposide C [1] from the same holothurian. The other two glycosides, thelenotosides A and B have not been studied previously.

On acid cleavage, thelenotoside A (III) gave a mixture of D-quinovose, D-xylose, and 3-O-methyl-D-glucose in a ratio of 1:2:1. Methanolysis of a permethylate of (III) followed by acetylation of the methyl glycosides so formed gave the following monosaccharide derivatives, identified with the aid of GLC [8] and GLC-MS (in the order of increasing retention times): methyl 2,3,4,6-tetra-O-methyl-β-glucopyranoside, methyl 2,3,4,6-tetra-O-methyl-α-glucopyranoside, methyl 2-O-acetyl-3,4-di-O-methyl-α-xylopyranoside, methyl 4-O-acetyl-2,3-di-O-methyl-β-quinovopyranoside, methyl 4-O-acetyl-2,3-di-O-methyl-α-quinovopyranoside, methyl 3-O-acetyl-2,4-di-O-methyl-β-xylopyranoside, and methyl 3-O-acetyl-2,4-di-O-methyl-α-xylopyranoside.

The results of methylation showed that thelenotoside A has a linear carbohydrate chain with a 3-O-methyl-D-glucose residue present at its nonreducing end. Since the position of attachment followed unambiguously from the structure of the genin of thelenotoside A, to establish the complete structure of this glycoside it was necessary only to determine the sequence of attachment of the monosaccharide units in the carbohydrate chain and the configurations of the glycosidic bonds.

The enzymatic cleavage of (III) with a cellulase led to the formation of a progenin (IV) having two monosaccharide residues (xylose and quinovose) and identical with the known stichoposide A [7]. The formation of this progenin and the presence in the ^{13}C NMR spectrum of the signals of anomeric carbon atoms at 104.8 ppm (1 C) and 105.3 ppm (3 C) showing the β configurations of all the glycosidic bonds in thelenotoside A permitted its structure to be determined unambiguously as 23(S)-acetoxy-3β-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-O-β-D-quinovopyranosyl-(1→2)-β-D-xylopyranosyloxyl]holost-7-ene(III). An additional confirmation of formula (III) for this substance was obtained after its periodate oxidation followed by acid hydrolysis. In the hydrolysate obtained in this way only two monosaccharides were detected: D-xylose and 3-O-methyl-D-glucose (1:1).



Thelenotoside B (V) contained a carbohydrate chain of D-xylose, D-glucose, and 3-O-methyl-D-glucose residues in a ratio of 2:1:1. The methylation of (V) followed by methanolysis and acetylation gave methyl 2,3,4,6-tetra-O-methyl-α- and -β- glucopyranosides, methyl 2-O-acetyl-3,4-di-O-methyl-α-xylopyranoside, methyl 3-O-acetyl-2,4-di-O-methyl-α- and -β-xylopyranosides, and methyl 4-O-acetyl-2,3,6-tri-O-methyl-α- and -β-glucopyranosides. It followed from the results obtained that (V), like thelenotoside A, has a linear carbohydrate chain. The sequence of the linkage of the monosaccharide residues in (V) was determined with the aid of partial hydrolysis by 1 N oxalic acid. This gave two progenins: (VI) in minor amount and (VII) in major amount. The monoside (VI) gave xylose as the only sugar after acid hydrolysis. The hydrolysis of progenin (VII) yielded a mixture of D-xylose and D-glucose

(2:1). Consequently, the 3-O-methyl-D-glucose residue occupies the terminal position. The methylation of (VII) and methanolysis, with acetylation of the methyl glycosides obtained, gave a mixture of sugar derivatives in which methyl 2,3,4-tri-O-methyl-α-xylopyranoside, methyl 2-O-acetyl-3,4-di-O-methyl-α-xylopyranoside, and methyl 4-O-acetyl-2,3,6-tri-O-methyl-α- and -β-glucopyranosides were identified. These facts show that thelenotoside B contains the following sequence of monosaccharide residues (from the nonreducing end): 3-O-methyl-D-glucose→D-xylose→D-glucose→D-xylose, the xylose residue attached to the aglycone also being connected with a glucose residue by a 1→2 bond. The configurations of the glycosidic bonds in thelenotoside B as β followed from its ^{13}C NMR spectrum (C_1 signals in the carbohydrate chain at 104.8 ppm (1 C) and 105.2 ppm (3 C). On the basis of these results and those of the periodate oxidation of the glycoside, which led to the destruction of the glucose residue and one of xylose residues, we have ascribed to thelenotoside B the structure of 23(S)-acetoxy-3β-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→2)-β-D-xylopyranosyloxy]holost-7-ene (V).

The structures of the carbohydrate chains of thelenotoside A and B do not contradict the hypothesis that we have expressed on their biosynthesis from bioside blocks. In actual fact, each of these two glycosides has two such blocks, one of them (3-O-methylglucose—xylose) being found in both structures.

EXPERIMENTAL

All the spectral characteristics and physical constants were determined under the conditions given previously [1]. The animals were collected in the region of the Seychelles in 1979.

Complete acid hydrolysis and periodate oxidation, and also the methylation of the glycosides obtained, the isolation of the permethyl derivatives and their methanolysis and acetylation, were carried out under the conditions described previously [1].

Acetylation of the Glycosides. The glycosidic fraction from *T. ananas* was obtained by the method described previously [1]. The glycosides were separated by repeated column chromatography on silica gel L (40 × 100 μm) in the $CHCl_3$—MeOH—H_2O (75:25:1) system. A 200-mg sample of the mixture of glycosides under consideration was dissolved in 50 ml of ethanol, 5 mg of PtO_2 was added, and the mixture was stirred in an atmosphere of hydrogen at room temperature for 20 h. The solution was filtered and was concentrated to dryness in vacuum. From 2 g of glycosidic fractions we obtained 200 mg of stichoposide A, 700 mg of thelenotoside A, 500 mg of thelenotoside B, and 150 mg of stichoposide C.

Stichoposide A, mp 222-224°C (ethanol), $[\alpha]_D^{20}$ −40.0° (c 2.0; pyridine). According to the literature [7]: mp 220-223°C $[\alpha]_D^{20}$ −40.2°. It was shown to be identical with an authentic sample by a comparison of ^{13}C NMR spectra.

Stichoposide C, mp 260°C, $[\alpha]_D^{20}$ −41.0° (c 0.5; pyridine. According to the literature [1]: mp 260°C, $[\alpha]_D^{20}$−40.8°; a mixture with an authentic sample melted at 259-260°C. The ^{13}C NMR spectrum coincided completely with that of the standard sample.

Thelenotoside A, $C_{55}H_{88}O_{22}$, mp 241-242°C (ethanol), $[\alpha]_D^{20}$ −50.3° (c 2.7; pyridine).

Thelenotoside B, $C_{55}H_{88}O_{23}$, mp 208-210°C (ethanol), $[\alpha]_D^{20}$ −45.7° (c 1.4; pyridine).

Enzymatic Hydrolysis of Thelenotoside A. A mixture of 200 mg of the glycoside and 250 mg of cellulase was kept at 37°C for 22 days. Column chromatography of the mixture obtained yielded 9 mg of the progenin (IV) and 170 mg of unchanged glycoside.

Progenin (IV), mp 222-224°C (ethanol), $[\alpha]_D^{20}$ −40.0° (c 1.2; pyridine). A mixture with stichoposide A [7] melted at 220-222°C. The ^{13}C NMR spectrum of (IV) coincided completely with the corresponding spectrum of stichoposide A.

Partial Acid Hydrolysis of Thelenotoside B. A mixture of 100 mg of the glycoside and 5 ml of 1 N oxalic acid was stirred vigorously at 60°C for 36 h. The precipitate was filtered off, washed with water to neutrality, and dissolved on the filter in a 1:1 mixture of $CHCl_3$ and MeOH. The solution was evaporated to dryness and the residue (85 mg) was chromatographed on silica gel in the $CHCl_3$—MeOH—H_2O (75:25:1) system. In this way, 3 mg of progenin (VI), 14 mg of progenin (VII), and 50 mg of unchanged thelenotoside B were isolated.

Progenin (VI), mp 260-263°C [$CHCl_3$—MeOH (3:1)], $[\alpha]_D^{20}$ −39.4° (c. 3.0; pyridine).

Progenin (VII), mp 268-269°C (ethanol), $[\alpha]_D^{20}$ −42.1° (c 0.5; pyridine).

SUMMARY

The complete structures of thelenotosides A and B obtained from the holothurian *T. ananas* have been established as 23(S)-acetoxy-3β-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-O-β-D-quinovopyranosyl-(1→2)-β-D-xylopyranosyloxyl]holost-7-ene and 23(S)-acetoxy-3β-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→2)-β-D-xylopyranoxyloxy]holost-7-ene, respectively.

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^{13}C NMR SPECTRA OF STEROID GLYCOSIDES.

II. ACETATES OF PENNOGENIN GLYCOSIDES

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The ^{13}C NMR spectra of two acetates of pennogenin glycosides have been measured and an assignment has been made of the signals of the C atoms. The mutual influence of the aglycone and the component carbohydrate chains on the chemical shifts of the signals of the corresponding C atoms has been determined.

^{13}C NMR spectroscopy is being used successfully in structural studies of steroid glycosides [1-6]. The ^{13}C NMR spectra of acetates of the glycosides are being used for a similar purpose to a smaller degree [7, 8]. However, the study of the possibility of obtaining structural information from the spectra of the acetates is of interest, since natural steroid glycosides are frequently separated in the form of the corresponding acetates.

We have previously made an assignment of the signals in the ^{13}C NMR spectrum of pennogenin β-D-glucopyranoside acetate (I) [7]. In the present paper we give information on the ^{13}C NMR spectra of two other pennogenin glycoside acetates: the acetates of the O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside (II) and of the O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (III), which we have isolated previously [7]. We also give the glycosidation shifts for the aglycone ($\Delta\delta = \delta_C$ for the glycoside acetate − δ_C for the aglycone) and for the acetylated monosaccharides (II) and (III) ($\Delta\delta = \delta_C$ of the glycoside acetate − δ_C of the methyl glycoside acetate), illustrating the mutual influence of the aglycone and the component carbohydrate chains.

The assignments of the C-2' and C-5' signals (δ 71.6 and 71.75 ppm, respectively) in the spectrum of (I) [7] have been refined by the method of selective decoupling from protons. The signals of the C atoms of the aglycone moieties of (II) and (III), with the exception of the signals of ring A, are identical with those of pennogenin [9]. The values of the chemi-

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