STICHOPOSIDE E – A NEW TRITERPENE GLYCOSIDE FROM HOLOTHURIANS OF THE FAMILY STICHOPODIDAE

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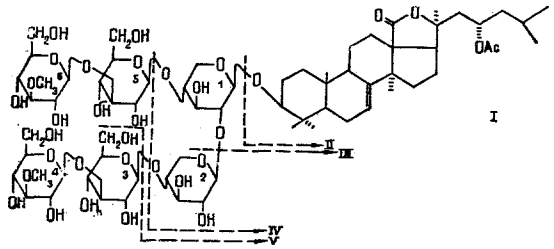
A new triterpene hexaoside – stichoposide E – has been isolated from an ethanolic extract of the holothurian Stichopus chloronotus by column chromatography on silica gel. Its complete structure has been determined as 23(S)-acetoxy-3β-{[O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→2)]-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-glucopyranosyl-(1→4)]-β-D-xylopyranosyloxy}holost-7-ene.

Continuing a study of the glycosides of holothurians of the family Stichopodidae [1-3], we have established the structure of stichoposide E (I) from Stichopus chloronotus (collected in the Australian littoral).

This compound, like other stichoposides from S. chloronotus had as its native aglycone 23(S)-acetoxyholost-7-en-3β-ol (II) [4]. In actual fact, a comparison of the ^{13}C NMR spectra of (I) with those of the known stichoposides C [1] and D [2] showed the complete coincidence of all the signals of the aglycone moieties of the glycoside.

The acid hydrolysis of (I) gave a mixture of artifactual genins [5] and D-xylose, D-glucose, and 3-O-methyl-D-glucose in a ratio of 2:2:2, these being identified by GLC and by GLC-MS in the form of the corresponding aldononitrile peracetates. The presence of six carbohydrate residues linked with one another by β-glycosidic bonds was confirmed for compound (I) by the ^{13}C NMR spectrum (Table 1), in which there were six signals of the C-1 atoms of monosaccharides in the 101.3-104.6 ppm region.

The Hakomori methylation [6] of stichoposide E followed by the methanolysis of the methylated derivative and the acetylation of the products obtained led to a mixture of sugar derivatives which was studied by GLC and GLC-MS. As a result we identified: methyl 2,3,4,6-tetra-O-methyl-α- and -β-glucopyranosides, methyl 4-O-acetyl-2,3-di-O-methyl-α- and -β-xylopyranosides, methyl 2,4-di-O-acetyl-3-O-methyl-α- and -β-xylopyranosides, and methyl 3-O-acetyl-2,4,6-tri-O-methyl-α- and -β-glucopyranosides.



I

The cleavage of (I) by Smith's method [7] gave the progenin (III), which, on a comparison of the constants, ^{13}C NMR spectra, and monosaccharide compositions, proved to be identical with a progenin obtained from stichoposides C [1] and D [2].

The enzymatic cleavage of stichoposide E under the action of cellulase on it led to the formation of the progenins (IV) and (V). Progenin (IV) proved to be a bioside (see Table 1) the carbohydrate chain of which included two xylose residues linked with one another by a β-(1→2) bond. On methylation and subsequent working up, (IV) yielded methyl 2,3,4-tri-O-methyl-α- and -β-xylopyranosides and methyl 2-O-acetyl-3,4-di-O-methyl-α-xylopyranoside.

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TABLE 1. ^{13}C NMR Spectra of the Glycoside (I) (DMSO, 333°K) and of the Progenins (IV) (C_5D_5N, 333°K) and (V) (C_5D_5N, 298°K)

Carbon atom	I	IV	V	Carbon atom	I	IV	V	Carbon atom	I	V
C_1	35,9	36,1	36,5	C_{26}	22,3	22,3	22,3	C_3^4	85,9	—
C_2	27,0	27,4	27,2	C_{27}	23,2	23,2	23,2	C_4^4	69,7	—
C_3	88,8	89,7	89,1	C_{30}	16,9	17,2	17,1	C_5^4	77,0	—
C_4	*	39,8	39,6	C_{31}	28,4	28,7	28,7	C_6^4	61,3	—
C_5	47,8	48,3	48,5	C_{32}	30,9	31,1	30,9	C_1^5	101,3[a]	103,1
C_6	22,5[a]	22,9[a]	23,0[a]	Ac	170,8	170,5	170,6	C_2^5	72,5	73,1
C_7	119,4	120,0	120,1		21,4	21,2	21,2	C_3^5	85,8	88,2
C_8	146,4	146,5	146,7	C_1^1	104,6	107,1	106,6	C_4^5	68,8	70,0
C_9	47,1	47,6	47,6	C_2^1	82,0	84,0	83,0	C_5^5	77,0	78,1
C_{10}	35,2	35,8	35,7	C_3^1	74,7	78,2	75,6	C_6^5	61,3	62,45
C_{11}	22,8[a]	23,0[a]	23,5[a]	C_4^1	76,6	71,0[a]	77,9	C_1^6	103,9	105,6
C_{12}	30,4	30,5	30,6	C_5^1	63,1	66,9	64,1	C_2^6	73,5	75,1
C_{13}	58,2	58,6	58,7	C_1^2	103,9	105,7	105,1	C_3^6	85 9	87,8
C_{14}	51,0	51,4	51,4	C_2^2	74,6	76,7	76,2	C_4^6	69,7	71,4
C_{15}	33,9	34,4	34,4	C_3^2	74,6	78,2	78,1	C_5^6	77,0	78,1
C_{16}	24,6	24,9	25,2	C_4^2	76,6	71,2[a]	71,0	C_6^6	61,3	62,45
C_{17}	54,3	54,4	54,4	C_5^2	63,6	67,6	67,2	OMe	60,0	60,5
C_{18}	180,0	179,4	179,6	C_1^3	101,6[a]					
C_{19}	23,9	24,2	24,0	C_2^3	72,5					
C_{20}	83,3	83,1	83,0	C_3^3	86					
C_{21}	26,7	27,1	27,2	C_4^3	68,8					
C_{22}	44,0	44,2	44,25	C_5^3	77,0					
C_{23}	68,1	68,2	68,7	C_6^3	61,3					
C_{24}	44,9	45,5	45,6	C_1^4	103,9					
C_{25}	24,3	24,7	24,8	C_2^4	73,5					

a – Assignment of the signals ambiguous; *signals masked by the signals of the DMSO.

After acid hydrolysis, progenin (V) gave D-xylose, D-glucose, and 3-O-methyl-D-glucose in a ratio of 2:1:1. Methylation followed by the treatment described above of the permethyl derivative obtained from (V) led to the formation of methyl 2,3,4-tri-O-methyl-α- and -β-xylopyranosides, methyl 2,3,4,6-tetra-O-methyl-α- and -β-glucopyranosides, methyl 2,4-di-O-acetyl-3-O-methyl-α- and -β-xylopyranosides, and methyl 3-O-acetyl-2,4,6-tri-O-methyl-α- and -β-glucopyranosides. In the ^{13}C NMR spectrum of compound (V), as compared with that of compound (IV), the C-4 signal in one of the xylose residues was shifted upfield by approximately 8 ppm as a result of glycosylation (see Table 1).

On the basis of these facts, formula (I) has been ascribed to stichoposide E.

The same glycoside has recently been identified, although after the completion of our investigation, by Japanese workers [8] in S. chloronotus collected in the littoral of Okinawa; they called it stichloroside A. Moreover, we have detected (I) in admixture with 25,26-dehydro derivatives, in an extract of another species, S. variegatus, collected in the Australian littoral. The hydrogenation of the mixture over PtO_2 at room temperature gave a product identical with (I) according to a comparison of constants and of ^{13}C NMR spectra, and a mixed melting point. The results obtained confirm our hypothesis of the absence of a relationship between the monosaccharide composition and the structure of the carbohydrate chains in the glycosides of these Stichopodidae, on the one hand, and ecological factors, especially the site of collection [2, 9], on the other hand, in contrast to the opinion of Japanese [10] and Belgian [11] workers.

EXPERIMENTAL

Melting points were determined on a Boëtius stage. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. ^{13}C NMR spectra were determined on a Bruker HX-90E spectrometer in DMSO and C_5D_5N. The signals in Table 1 are given in the δ system relative to TMS. GLC analysis was performed on a Tsvet-110 chromatograph using 150 × 0.3 cm glass columns containing 3% of F-1 on Chromaton HMDS with argon as the carrier gas (60 ml/min) at temperatures rising from 110 to 220°C at 5 deg/min. Chromato-mass spectrometric analysis was performed on a LKB-9000 S mass spectrometer using a 300 × 0.3 cm column containing 1.5% of QF-1 on Chromaton N-HMDS with helium as the carrier gas (30 ml/min). The analysis was performed under the following conditions: temperature of the evaporator 275°C, of the column 240°C, of the molecular separator 265°C, and of the ion source 255°C; ionizing voltage 70 V.

The animals were collected on the Great Barrier Reef (Australia) in January, 1980.

The glycosides were obtained by a method which we have described previously [1]. The amount of stichoposide E was 0.0003% of the crude weight of the holothurians.

Stichoposide E, mp 214-215°C (ethanol), $[\alpha]_D^{20}$ −48.8 (c 0.45; DMSO). For $C_{68}H_{110}O_{33}$, calculated, %; C 56.2; H 7.5; found, %; C 56.0; H 7.49. The results of the acid hydrolysis and the methylation of stichoposide E and its progenins are given in the text, and the ^{13}C NMR spectral characteristics for all the compounds are given in Table 1.

Hydrolysis of the Glycosides. Each of the glycosides (2 mg) was heated with 1 ml of 2 N HCl under reflux at 90-100°C for 2 h. Then the reaction mixture was extracted with chloroform. The aqueous layer was concentrated in vacuum to dryness. A solution of the residue in 1 ml of dry pyridine was treated with 5 mg of $NH_2OH \cdot HCl$ and the mixture was heated at 100°C for 1 h. Then 1 ml of acetic anhydride was added to it and it was heated at 100°C for another 1 h. The resulting solution was concentrated in vacuum to dryness. The residue was analyzed by the GLC method.

Smith Cleavage of Stichoposide E. A solution of 250 mg of the glycoside in 50 ml of water was treated with 100 mg of $NaIO_4$ and the mixture was left at 5°C for 12 h. Then it was deposited on a column of Teflon powder (Polikhrom-1) and was washed free from iodate and periodate ions with water. Oxidized glycosides were eluted with ethanol. The eluate (70 ml) was treated with 50 mg of $NaBH_4$ and the mixture was left at 20°C for 3 h. Then it was acidified to pH 5.0 with acetic acid and was concentrated in vacuum, and the boric acid was eliminated in the form of methyl borates by adding methanol to the residue in portions and driving off the solvent under vacuum. The final residue obtained was dissolved in 50 ml of water, and the solution was treated with 4 ml of concentrated HCl; after 20 min, the precipitate of progenin (III) that had precipitated was filtered off. The progenin was washed on the filter with water and was dissolved in 30 ml of a mixture of $CHCl_3$ and MeOH (1:1). The solution was concentrated in vacuum. The residue (140 mg) was purified on a column silica gel L (40-100 μ) in the $CHCl_3$–MeOH–H_2O (75:25:1) system. This gave 110 mg of the progenin (III).

The progenin (III), mp 248-249°C (ethanol), $[\alpha]_D^{20}$ −45.9° (c 1.2; pyridine). According to the literature [2]: mp 248-250°C, $[\alpha]_D^{20}$ −46.2. Melting point of a mixture, 248-249°C.

Enzymatic Cleavage of Stichoposide E. A solution of 200 mg of the glycoside in 50 ml of water was treated with 50 mg of cellulase. The mixture was thermostated at 38°C for seven days. The resulting solution was extracted with butanol (4 × 10 ml), and the butanolic extract was evaporated to dryness in vacuum. The resulting mixture was separated on a solumn of silica gel L (40-100 μ) in the $CHCl_3$–MeOH–H_2O (75:25:1) system. This gave 20 mg of the progenin (IV), 70 mg of the progenin (V), and 100 mg of unchanged stichoposide E.

The progenin (IV), mp 267-268°C (ethanol), $[\alpha]_D^{20}$ − 47.3° (c 0.45 pyridine).

The progenin (V), mp 261-263°C (ethanol), $[\alpha]_D^{20}$ − 41.6° (c 1.0, pyridine).

Methylation of Stichoposide E and of the Progenins (IV) and (V). A solution of the glycoside or of a progenin (10 mg) in 2 ml of methylsulfinyl anion (prepared from 350 mg of NaH and 15 ml of DMSO) was stirred at 50°C in an atmosphere of argon for 1 h. Then 1 ml of CH_3I was added to the reaction mixture and it was stirred at 20°C for another 1 h, after which it was diluted with water and extracted with $CHCl_3$ (3 × 2 ml). The extract was washed with 2 ml of saturated $Na_2S_2O_3$ solution and 2 ml of water and was concentrated in vacuum to dryness. The methylation product was purified by column chromatography on silica gel L (40-100 μ) in the hexane–ethyl acetate (3:1) system.

Methanolysis of the Methylated Products Obtained. The methylation product (3.3 mg) was boiled under reflux for 2 h with 1 ml of anhydrous methanol saturated with HCl. The solvent was evaporated off in vacuum, and the residue was treated with a mixture of pyridine and acetic anhydride (1:1) at 100°C for 1 h. The reaction mixture was concentrated in vacuum, and the residue was analyzed by GLC and by GLC-MS in order to identify the methyl glycosides.

SUMMARY

The complete structure of the new triterpene glycoside stichoposide E has been determined as 23(S)-acetoxy-3β-{[O-(3-O-methyl-B-D-glucopyranosyl)-(1 → 3)-O-β-D-glucopyranosyl-(1 → 4)-O-β-D-xylopyranosyl-(1 → 2)]-[O-(3-O-methyl-β-D-glucopyranosyl)-(1 → 3)-O-β-D-glucopyranosyl-(1 → 4)]-β-D-xylopyranosyloxy}holost-7-ene.

LITERATURE CITED

1. V. A. Stonik, I. I. Mal'tsev, A. I. Kalinovskii, K. Kondé, and C. B. Elyakov, Khim. Prir. Soedin., 194 (1982).
2. V. A. Stonik, I. I. Mal'tsev, A. I. Kalinovskii, and G. B. Elyakov, Khim. Prir. Soedin., 200 (1982).
3. V. A. Stonik, I. I. Mal'tsev, and G. B. Elyakov, Khim. Prir. Soedin., 62 (1982).
4. G. B. Elyakov, V. A. Stonik, et al., Dokl. Akad. Nauk SSSR, 259, 1367 (1981).
5. V. A. Stonik, V. F. Sharypov, A. I. Kalinovskii, and G. B. Elyakov, Dokl. Akad. Nauk SSSR, 245, 1133 (1979).
6. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
7. J. K. Hamilton and F. Smith, J. Am. Chem. Soc., 78, 5907 (1956).
8. I. Kitagawa, M. Kobayashi, T. Inamoto, T. Yasuzawa, and Y. Kyogoku, Chem. Pharm. Bull., 8, 2387 (1981).
9. V. A. Stonik, I. I. Maltsev, A. Kalinovsky, and G. B. Elyakov, First International Conference on the Chemistry and Biotechnology of Biologically Active Natural Products, Varna, Bulgaria, Vol. 3(2) (1981), p. 326.
10. I. Kitagawa, H. Yamanaka, M. Kobayashi, T. Nishino, I. Yosioka, and T. Sugawara, Chem. Pharm. Bull., 26, 3722 (1978).
11. A. Kelecom, B. Tursch, and M. Vanhaelen, Bull. Soc. Chim. Belg., 85, 277 (1976).