SUMMARY

1. cis-Abienol, epimanool, isocembrol, geranyllinalool, and labd-13E-en-8α,15-diol have been isolated from the oleoresin of the Yeddo spruce.

2. It has been established that, in contrast to species *Pinus* L., in the Yeddo spruce the biosynthesis of isocembrol is not accompanied by the formation of 4-epiisocembrol.

LITERATURE CITED

1. E. N. Shmidt, V. Beneshova, M. A. Chirkova, and V. A. Pentegova, Izv. Sibirsk. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk, Ser. No. 5, Issue No. 12, 116 (1969).
2. V. A. Raldugin, V. A. Khan, Zh. V. Dubovenko, and V. A. Pentegova, Khim. Prir. Soedin., 609 (1976).
3. V. A. Raldugin, and V. A. Pentegova, Khim. Prir. Soedin., 669 (1971).
4. V. A. Raldugin, V. A. Khan, Zh. V. Dubovenko, and V. A. Pentegova, Khim. Prir. Soedin., 299 (1976).
5. V. A. Raldugin and V. A. Pentegova, Khim. Prior. Soedin., 174 (1976).
6. P. F. Vlad, A. G. Russo, and Ch. K. Fan, Zh. Obshch. Khim., 39, 451 (1969).
7. A. H. Connor and J. W. Rowe, Phytochem., 15, 1949 (1976).
8. E. N. Shmidt and V. A. Pentegova, Khim. Prir. Soedin., 769 (1970).
9. N. V. Avdyukova, E. N. Shmidt, and V. A. Pentegova, Khim. Prir. Soedin., 653 (1977); B. Kimland and T. Norin, Acta Chem. Scand., 21, 825 (1967).
10 C. Asselineau, S. Bory, M. Fetizon, and P. Laszlo, Bull. Soc. Chim. Fr., 1429 (1961).
11. P. R. Jefferies and T. G. Payne, Aust. J. Chem., 18, 1441 (1965).
12. S. Chadrasekharan and T. Chakrabortty, J. Indian Chem. Soc., 45, 208 (1968).
13. P. Bernfeld, Biogenesis of Natural Compounds, 1st edn., Pergammon, Oxford (1963).
14. V. A. Babkin, Zh. V. Dubovenko, and V. A. Pentegova, Izv. Sibirsk. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk, Ser. No. 1, Issue No. 2, 168 (1970).

GLYCOSIDES OF MARINE INVERTEBRATES.

X. THE STRUCTURE OF STICHOPOSIDES A AND B FROM THE HOLOTHURIAN *Stichopus cloronotus*

V. F. Sharypov, A. D. Chumak,
V. A. Stonik, and G. B. Elyakov

UDC 547.996:593.96

The structures of two triterpene oligosides from the holothurian *Stichopus cloronotus* (Brandt) have been established; they are: 23ξ-acetoxy-3β-[O-β-D-quinovopyranosyl-(1→2)-β-D-xylopyranosyloxy]holost-7(8)-ene and 23ξ-acetoxy-3β-[O-β-D-glucopyranosyl-(1→2)-β-D-xylopyranosyloxy]holost-7(8)-ene.

The physiologically active glycosides of holothurians (class *Holothurioidea*) have now been investigated for more than two decades, but the complete structures of only four of them have been established [1-3]. The present paper gives the structures of two more such glycosides — stichoposides A (I) and B (II) from *Stichopus cloronotus* (Brandt) (Coetivy island, Seychelles archipelegro).

The glycosides of *S. cloronotus* after acid hydrolysis give the genin (III) [4], and differ from one another in their monosaccharide compositions. We have recently established that (III) is not the native genin but is formed from the 23-acetate of holost-7(8)-ene-3β,23ξ-diol (IV) as the result of 7(8)→8(9)→9(11) migration in the acid medium [5]. After this, the structural study of compounds (I) and (II) reduced in the main to determining the structures of their carbohydrate chains.

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 181-184, March-April, 1981. Original article submitted August 7, 1980.

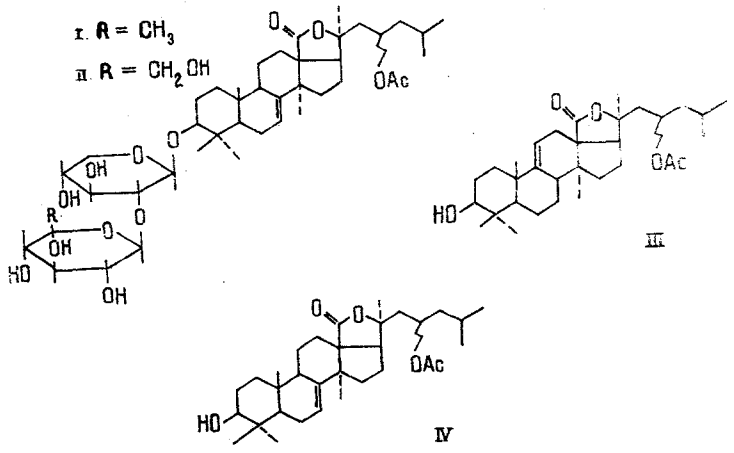
0009-3130/81/1702-0139$07.50

Analysis of the monosaccharide mixtures obtained from compound (I) and from compound (II), which was performed by the GLC-MS method for the peracetates of the aldonitrile derivative [6] in comparison with standard compounds, showed that the glycoside (I) contained D-xylose and D-quinovose residues, and glycoside (II) contained D-xylose and D-glucose residues. The assignment of the monosaccharides to the D series was made after a determination of the specific rotation of the mixtures obtained and, additionally, in the case of (II), by determining the specific rotation of each of the monosaccharides after their preliminary separation by preparative paper chromatography. The absence of sulfuric acid among the hydrolysis products and the results of elementary analysis showed that there were no sulfate residues in these glycosides, in contrast to holothurins A and B [2, 3].

The structures of the carbohydrate chains of the compounds investigated were established after their methylation by Hakomori's method [7], methanolysis of the permethanolates obtained, and acetylation of the products formed. After such treatment, by the GLC-MS method in comparison with standard samples we identified, for (I): methyl α- and β-2,3,4-tri-O-methylquinovopyranosides and methyl α- and β-2-O-acetyl-3,4-di-O-methylxylopyranosides, and for (II): methyl α- and β-2,3,4,6-tetra-O-methylglucopyranosides and methyl α- and β-2-O-acetyl-3,4-di-O-methylxylopyranosides. It followed from this that the carbohydrate chain of (I) has terminal quinovose and xylose, and that of (II) terminal glucose and xylose, the terminal monosaccharides in both cases being attached to the xylose by a 1→2 bond.

The β configurations of the glycosidic bonds were established by comparing the ^{13}C NMR spectra of (I) and (II) with the corresponding spectra of model methyl α- and β-xylo-, -quinovo-, and -glucopyranosides [8]. Each glycoside had two signals of anomeric carbon atoms in the 103-106 ppm region, as in the spectra of the model methyl β-glycosides.

The positions of attachment of the carbohydrate chains to the aglycone were also determined with the aid of ^{13}C NMR spectroscopy. A comparison of the spectra of I) and (II) with that of the genin (III) [9] showed a shift of the C-3 signal in the spectra of the glycosides to 90 ppm as compared with 78 ppm for the genin, which is connected with the glycosylation of the hydroxy group at C-3.



The results obtained permit stichoposide A (I) to be assigned the structure of 23ξ-acetoxy-3β-[O-β-D-quinovopyranosyl-(1→2)-β-D-xylopyranosyloxy]holost-7(8)-ene, and stichoposide B (II) that of 23ξ-acetoxy-3β-[O-β-D-glucopyranosyl-(1→2)-β-D-xylopyranosyloxy]holost-7(8)-ene.

EXPERIMENTAL

For chromatography we used type KSK silica gel, Silufol plates, and Whatman No. 3 paper. Glycosides were detected on the chromatograms with sulfuric acid, and monosaccharides with aniline phthalate with heating at 110°C for 5 min. The ^{13}C and ^{1}H NMR spectra were taken on a Bruker HX-90E instrument in deuterochloroform solutions with tetramethylsilane as internal standard. IR spectra were recorded on a Specord IR-75 instrument in chloroform, and UV spectra on a Specord UV-VIS instrument in ethanol. Angles of rotation were measured on a Perkin-Elmer 141 polarimeter in chloroform for glycosides and in water for sugars. The chromato-mass spectrometric (GLC-MS) study of the methanolysis products was carried out an LKB 9000S spectrometer with an ionizing voltage of 70 V using a 3 m × 3 mm column contain-

ing 3% of QF-1 on Chromosorb W. The temperature of analysis was 100-200°C, 2 deg/min. The carrier gas was helium at the rate of 60 ml/min. GLC analysis was performed on a Tsvet chromatograph using 2 m × 3 mm columns containing 5% of QF-1 on Chromaton N, 100-180°C, 2 deg/min. The carrier gas was argon at the rate of 60 ml/min.

Stichoposides A (I) and B (II). An aqueous alcoholic extract from the muscular sacs of the holothurians was concentrated in vacuum at 50°C. The aqueous layer was extracted three times with butanol, and the butanol layer was washed once with water and was evaporated to dryness. The residue was washed with benzene and was dissolved in chloroform, the insoluble part was separated off, the chloroform was evaporated, and the resulting mixture of glycosides was separated on silica gel in the chloroform–methanol (5:1) system. Stichoposide A: mp 220-223°C (methanol), $[\alpha]_D^{22}$ −40.2° (c 0.5). IR (cm^{-1}): 3450 (OH group), 740 (γ-lactone), 1725 (acetate). There was no absorption in the UV spectrum (above 210 nm). ^{13}C NMR, δ, ppm: 90.0 (C-3), 105.7 (C''_1), 106.2 (C'_1), 119.8 (C-7), 146.8 (C-8), 171.0 (acetate), 180.1 (C-18). Stichoposide B: mp 219-222°C (propanol), $[\alpha]_D^{22}$ −39.4° (c 0.9). IR (cm^{-1}): 3450 (OH group), 1738 (γ-lactone), 1723 (acetate). There was no absorption in the UV region of the spectrum. ^{13}C NMR, δ, ppm: 90.0 (C-3), 103.3 (C''_1), 104.2 (C'_1), 119.8 (C-7), 145.4 (C-8), 171.0 (acetate), 180.2 (C-18).

Acid Hydrolysis of (I). The hydrolysis of 10 mg of the glycoside was carried out with 5 ml of 2 N HCl at 100°C for 3 h. The precipitate of aglycone was extracted with chloroform, and the aqueous layer was evaporated. This gave 2.4 mg of combined sugars with $[\alpha]_D^{22}$ +28.5° (c 0.22). Calculated for the sum of D-xylose and D-quinovose (1:1), $[\alpha]_D$ +23.7° [10]. Xylose and quinovose were identified by chromatography on Silufol plates in the chloroform–methanol (2:1) system; this was confirmed by the GLC of the peracetates of the corresponding aldononitriles obtained from the combined monosaccharides by the method of Easterwood and Heeff [6].

Acid Hydrolysis of (II). The hydrolysis of 35 mg of the glycoside was carried out with 3 N sulfuric acid at 100°C for 3 h. The aglycones were extracted with chloroform, and the aqueous layer was neutralized with barium carbonate to a neutral pH, centrifuged, filtered, and evaporated. This gave 11 mg of combined sugars with $[\alpha]_D^{22}$ +22.4° (c 0.7). Calculated for the sum of D-xylose and D-glucose (1:1), $[\alpha]_D$ +36°. Xylose and glucose were identified by chromatography on Silufol and by the GLC of the acetates of the corresponding aldononitriles. By preparative paper chromatography of the combined monosaccharides in the butanol–pyridine–water (6:4:3) system, D-xylose and D-glucose were obtained and identified.

Methylation and Methanolysis of (I). The glycoside (20 mg) was stirred for 2 h in 2 ml of a mixture prepared from 60 mg of sodium hydride and 2.5 ml of absolute dimethyl sulfoxide in an atmosphere of nitrogen at 70°C for 2 h, and then 2 ml of methyl iodide was added and the reaction was continued for 2 h. The mixture was diluted with 5 ml of water and the methylation product was extracted with chloroform. The extract was washed with sodium thiosulfate solution and with water and was evaporated. Chromatography of the resulting product on silica gel in the hexane–acetone (5:1) system gave 6 mg of an amorphous substance with $[\alpha]_D^{22}$ −22° (c 0.4). IR (cm^{-1}): 1740, no hydroxy group absorption. UV spectrum: only terminal absorption. PMR, δ, ppm: 5.55 (m, H_7), 4.52 (d, J = 7 Hz, C_1-H) 4.22 (d, J = 6 Hz, C''_1-H). A mixture of 3 mg of the methylation product and 2 ml of anhydrous 10% methanolic hydrogen chloride was boiled for 3 h and evaporated, and the residue was acetylated and was analyzed by GLC-MS. Methyl α,β-2,3,4-tri-O-methylquinovopyranoside and methyl α,β-2-O-acetyl-3,4-di-O-methylxylopyranoside were identified.

Methylation and Methanolysis of (II). In the manner described above, 20 mg of glycoside yielded 8 mg of amorphous methylated product with $[\alpha]_D^{22}$ −32.5° (c 0.7). IR (cm^{-1}): 1746; hydroxy group absorption was absent. UV spectrum: terminal absorption. PMR, δ, ppm: 5.55 (m), 4.55 (d, J = 7 Hz), 4.24 (d, J = 6 Hz). After methanolysis of the product, methyl α,β-2,3,4,6-tetra-O-methylglucopyranoside and methyl α,β-2-O-acetyl-3,4-di-O-methylxylopyranoside were identified.

SUMMARY

The structures of two triterpene oligosides from the holothurian *Stichopus chloronotus* (Brandt) have been established.

LITERATURE CITED

1. I. Kitagawa, H. Yamanaka, M. Kobayashi, I. Yosioka, and T. Sugawara, Chem. Pharm. Bull., 26, 3722 (1978).
2. I. Kitagawa, T. Nishino, T. Matsuno, H. Akutsu, and Y. Kyogoku, Tetrahedron Lett., No. 11, 985 (1978).
3. I. Kitagawa, T. Nishino, and Y. Kyogoku, Tetrahedron Lett., No. 16, 1419 (1979).
4. I. Rothberg, B. M. Tursch, and C. Djerassi, J. Org. Chem., 38, 209 (1973).
5. V. A. Stonik, V. F. Sharypov, A. I. Kalinovskii, and G. B. Elyakov, Dokl. Akad. Nauk SSSR, 245, 1133 (1979).
6. V. M. Easterwood and B. S. L. Heeff, Svensk. Pappers., 23, 768 (1969).
7. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
8. P. A. Gorin and M. Mazurek, Can. J. Chem., 53, 1212 (1975).
9. A. I. Kalinovskii, V. F. Sharypov, V. A. Stonik, A. K. Dzizenko, and G. B. Elyakov, Bioorg. Khim., 6, 86 (1980).
10. P. Karrer and A. Boettcher, Helv. Chim. Acta, 36, 571 (1953).

TRANSFORMED STEROIDS.

121. SYNTHESIS OF THE δ-LACTONE OF 3β-ACETOXY-16α-HYDROXY-6-OXO-24-NOR-5α-CHOL-17(20)-EN-23-OIC ACID

A. V. Kamernitskii, V. A. Krivoruchko, I. G. Reshetova, and E. I. Chernoburova

UDC 542.91:547.92

The synthesis of the $\Delta^{17(20)}$-16α analog of natural chiogralactone is described. Attempts to introduce a 6-oxo group directly into the δ-lactone proved unsuccessful, since the first stage — saponification — took place with the formation of three products: the 3-hydroxy-δ-lactone, the 3-hydroxy-$\Delta^{20(22)}$-lactone, and the 15,17(20)-dienoic acid. The synthesis of the desired compound was effected from the ethyl ester of the 5,16-dienoic acid by the scheme 3-acetate→3-tosylate→6-hydroxy-3α,5α-cyclosteroid→6-oxo-3α,5α-cyclosteroid→6-oxo-5αH-δ-lactone. It has been shown that the cyclopropane ring in the 3α,5α-cyclosteroid δ-lactone is extremely stable under the conditions of acid treatments.

The aim of the present investigation was the synthesis of the 5αH-6-oxo-δ-lactone (VIII), the $\Delta^{17(20)}$-16α analog of natural chiogralactone [1]. An attempt to introduce a 6-oxo group directly into the lactone (I) via the 3α,5α-cyclosteroid showed that even in the first stage — saponification of the 3-acetoxy grouping in (I) the reaction did not take place unambiguously. Depending on the conditions (aqueous methanolic potash, sodium carbonate, 20°C, boiling), three products were formed: the $\Delta^{17(20)}$-3-hydroxylactone (IIa), the $\Delta^{20(22)}$-3-hydroxylactone (IIb), and the 15,17(20)-dienoic acid (IIIa). The structure of (IIa) was shown by its re-acetylation to (I); in the mass spectrum of the δ-lactone (IIb) there is an intense fragment (m/z = 111) which is characteristic for homoallyl cleavage at the C_{13-17}—C_{15-16} bonds. The acid (IIIa) was characterized in the form of the methyl ester (IIIb). It is formed as the result of the alkaline opening of the δ-lactone ring and the ready dehydration of the C_{16}-allyl alcohol on acid treatment. The positions of the double bonds in the ring and the side chain of (IIIb) were shown by comparing its UV spectrum (λ_{max} at 240 nm) with the spectra of the 16,20(22)-dienoic acids and their esters which we have obtained previously, in which λ_{max} is 270 nm, and also by the positions of the signals from the vinyl protons in the PMR spectrum (in a stronger field than those from the 16,20-(22)-dienes) [2, 3].

N. D. Zelinskii Institute of Organic Chemistry, Academy of Sciences of the USSR, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 184-187, March-April, 1981. Original article submitted August 20, 1980.

 0009-3130/81/1702-0142$07.50