Only a few sponges have Δ^7-sterols as their main component. At the same time, we detected such compounds in the deep-sea sample of H. panicea while stanols predominated in the other sample of the same species. Hitherto, Δ^5-sterols and, more rarely, Δ^0-sterols have been detected exclusively in sponges of the family Halichondriidae.

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CHEMICAL INVESTIGATION OF BIOMASS OF A CULTURE OF GINSENG CELLS.

II. 6-O-ACYL DERIVATIVES OF β-SITOSTEROL β-GLUCOSIDE

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The isolation of β-sitosterol, β-sitosterol β-D-glucoside, and a glycoside of oleanolic acid from the crude glycosidic fraction (CGF) of a methanolic extract of a culture of gingseng cells has been reported previously [1].

In the present paper we give the results of further investigations of a CGF of gingseng cells (strain BIO-2, Omutninsk Chemical Factory). The CGF was obtained by chromatographing a methanolic extract of the gingseng biomass on Polychrome-1 (water → 50% ethanol) [2] in order to free it from organic salts, free sugars, and amino acids [3]. The feebly polar part of the CGF was found to contain a feebly polar fraction, FPF, which, in TLC (SiO_2 L 5/40, Czechoslovakia) in system 1 (benzene—chloroform—methanol (15:15:3)) appeared in the form of a single spot with a R_f value of 0.5 and had the coloration characteristic for compounds of triterpene and steroid nature. The spot was revealed with 10% H_2SO_4 in ethanol followed by heating at 120-200°C. The FPF was isolated by preparative thin-layer chromatography in solvent system 1 and was recrystallized from acetone (7 mg was obtained — 0.017% calculated on the dry weight of the sample).

The IR spectrum of the FPF showed an absorption band at 1727 cm^{-7} characteristic of the stretching vibrations of an ester group.

The FPF was treated with a 5% solution of KOH in ethanol for 18-20 h at room temperature. Then the reaction mixture was diluted with water, neutralized with KU-2-8 ion-exchange resin (H^+ form), and extracted successively with pentane and chloroform. The chloroform extract contained a single compound, which was identified as β-sitosterol β-D-glucoside. The extractive substances that had passed into the pentane were treated with a saturated solution of diazomethane in ether for an hour, and the products were analyzed by GLC under the conditions given in [1]. This showed the presence of two compounds the RRTs of which were identical with the RRTs of authentic samples of methyl palmitate and linoleate in a ratio of 1:2:3 [sic], respectively. Consequently, the FPF is a mixture of β-sitosterol palmitoyl- and linoleoyl-β-D-glucosides.

A comparison of the chemical shifts in the ^1H NMR spectra of β-sitosterol β-D-glucoside and its acyl derivatives showed that in each case the acyl substitutent substituted the primary hydroxy group of the glucose residue [4].

The structures of the acyl derivatives of β-sitosterol β-D-glucose were confirmed by the performance of independent synthesis through the transesterification of methyl palmitate and linoleate with β-sitosterol β-D-glucoside in N,N-dimethylformamide in the presence of potassium carbonate [5].

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Thus, a cell culture of gingseng (strain BIO-2) produces, in addition to substances isolated previously [1], 6-O-acyl derivatives of β-sitosterol β-D-glucoside.

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AMINO ACID COMPOSITION OF COCOA SHELLS

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Cocoa shells form an industrial waste from the processing of cocoa beans, and make up 10-13% of the weight of the initial raw material. At the present time, investigations are being performed on the use of cocoa shells as a source of tanning substances, purine alkaloids, furfural, natural pigments, and aromatizers [1-3]. The fat of cocoa shells contains a considerable amount of vitamin D [4], and an extract has been proposed for use as an antioxidant for oils and fats [5].

The protein and polysaccharide composition of this material has been studied by enzymatic hydrolysis [6].

We have now studied the amino acid composition of cocoa shells. The samples analyzed were obtained from confectionery factories in Kiev and Erevan. For a preliminary qualitative characterization we used paper chromatography on type S Leningrad paper and a AAA-339 amino acid analyzer (Czechoslovakia). An aqueous ethanolic extract of cocoa shells was evaporated to small volume, and methanol was added to precipitate high-molecular-weight compounds. The mixture was centrifuged at 4000 rpm for 20 min, the deposit was separated off, and the resulting mother liquor was analyzed on paper in the BAW (4:1:2) solvent system. For a clearer separation of the compound, chromatography was carried out by the descending method. After the chromatograms had been treated with a 0.3% solution of ninhydrin, more than 11 spots were detected. Not all the amino acids could be identified completely by paper chromatography. Only six amino acids were identified.

For a deeper study of the qualitative and quantitative composition of the free amino acids of the cocoa shell extract, we used the amino acid analyzer. The raw material (50.0 g, accurately weighed) was exhaustively extracted with a mixture of chloroform and benzene (1:1) in a Soxhlet apparatus. After the solvents had been evaporated off, the crude product was transferred quantitatively into a round-bottomed flask, 500 ml of 40% aqueous ethanol was added, and the mixture was boiled under reflux for 1 h. The extracted meal was separated off by centrifugation. The combined extracts were evaporated to a syrupy state, and a fivefold amount of methanol was added to precipitate high-molecular-weight compounds. The precipitate was separated off, and the filtrate obtained was evaporated to dryness. The dry extract consisted of a light brown powder readily soluble in water. A solution of 12 mg of the powder in 2.2 ml of sodium citrate buffer solution was injected into the column of the amino acid analyzer. The qualitative composition of the amino acids in the sample was determined on the basis of retention times. A standard mixture consisting of 18 amino acids was used as internal standard. The amounts of amino acids found were recalculated to mg%. The peak areas were used as the parameters for calculation. The amino acid analyzer was calibrated on standard samples of amino acids in nanometers.

The results of the analysis are given below:

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