Viticosterone E 22-O-Benzoate 2,3-Diacetate (III). A solution of 50 mg of ecdysteroid (I) in 2 ml of pyridine was acetylated with 2 ml of acetic anhydride at room temperature for 24 h. The excess of reagents was eliminated in vacuum. The residue was chromatographed on a column of silica gel. Washing with system 2 yielded 35 mg of the acetate (III), $C_{40}H_{54}O_{11}$, mp 152-153°C (from methanol–water) $]\alpha]_D^{20}$ +65.5 ± 2° (c 0.31; methanol); ν_{max}^{KBr}, cm^{-1}: 3470 (OH), 1725, 1740, 1260 (ester group); 1630, 1590, 720 (benzene ring); 1670 (Δ^7-6-keto grouping).

Mass spectrum, m/z (%): 650 ($M^+ - CH_3COOH$, 0.4), 632(1), 614(1.6), 572(0.4), 551(1.6), 534(13), 528(10), 510(18), 500(13), 492(29), 468(4), 447(32), 429(45), 385(45), 384(31), 345(27), 334(79), 327(64), 311(43), 309(27), 283(27), 232(64), 231(42), 122(86), 105(100), 99(27), 81(61), 69(64).

CONCLUSIONS

From the epigeal part of Silene wallichiana Klotzsch (family Caryophyllaceae) has been isolated a new phytoecdysteroid, viticosterone E 22-O-benzoate.

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ANALYSIS OF OLIGOSPIROSTANOSIDES IN A SUSPENSION CULTURE OF Dioscorea deltoidea CELLS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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A method has been developed for the quantitative determination of oligospirostanosides in a culture of Dioscorea deltoidea cells with the aid of HPLC on a LiChrosorb RP-18 column. Elution was performed with acetonitrile–water (50-75%) with detection at 207 nm.

In recent years, several highly productive strains of a cell culture of Dioscorea deltoidea synthesizing steroid glycosides of both the furostanol and spirostanol series have been obtained in the Institute of Plant Physiology [IFR] of the Academy of Sciences of the USSR [1-3]. In a suspension culture of yam cells of the strain IFR DM-0.5, after hydrolysis with exogenous enzymes, deltonin and dioscin – spiro analogs of the deltoside and protodioscin formed in the cells in vitro [4] – were detected. We have previously developed a spectrophotometric method permitting the determination of the amounts of glycosides of the furostanol series in a cell culture [2, 3]. In the present paper we describe a method for analyzing oligospirostanosides. The method was developed with the use of a cell culture of Dioscorea deltoidea, strain IFR DM-0.5.

To convert the oligofurostanosides into oligospirostanosides the cell mass was first subjected to enzymatic hydrolysis with preparations of cellulytic and pectolytic enzymes. It must be mentioned that in this procedure an additional liberation of forms of the glycoside strongly bound to the cell walls is possible [5].

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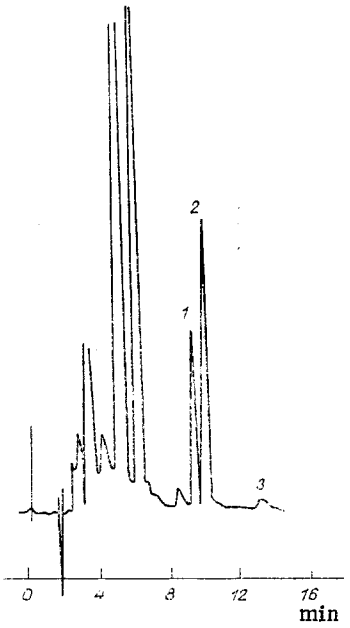

Fig. 1. Analysis of a methanolic extract of a cell culture of Dioscorea deltoidea by the HPLC method after enzymatic hydrolysis of the cells with "Pektinaza-500": 1) deltonin; 2) dioscin; 3) bioside.

A calibration graph was plotted with standard preparations of deltonin and dioscin. The difference in the correction factors of these glycosides is extremely small. Deltonin and dioscin were detected by the HPLC method in cells of the strain IFR DM-0.5 after fermentation with Pektinaza-500 (Fig. 1). A parallel analysis of three samples of a cell culture of the strain of yam by the same method showed good agreement of the results (% calculated on the dry weight of the cell mass):

Weight of cell culture, mg	Deltonin	Dioscin	Total amount of deltonin and dioscin
101	2.61	4.33	6.94
100	2.59	4.29	6.88
102	2.64	4.37	7.01

The mean concentration of oligospirostanosides was 6.94% on the dry weight. When the chromatogram obtained was analyzed, another small peak (3) was detected which was assigned to diosgenin rhamnoglucoside.

To elute the oligospirostanosides from a LiChrosorb RP-18 column we used mixtures of acetonitrile and water containing from 50 to 75% of acetonitrile. For the simultaneous determination of furostanol and spirostanol glycosides in one extract it would apparently be desirable to use a concentration gradient of acetonitrile in water.

EXPERIMENTAL

The HPLC Method. A LKB (Sweden) modular liquid chromatograph with an Ultra-Pak LiChrosorb RP-18 (7 μm) precolumn (4 × 30 mm) and an Ultra-Pak LiChrosorb RP-18 (5 μm) (4 × 250 mm) main column was used. Mixtures of acetonitrile with water (50-75%) were employed as the mobile phase. Detection was performed at 207 mn. The samples were injected in 70% methanol via a storage loop with a volume of 0.02 ml.

Calibration of Standard Samples. Absolute calibration was based on standard samples of deltonin and dioscin isolated from the rhizomes and leaves of Dioscorea deltoidea by a procedure described previously [6, 7]. Solutions of deltonin and dioscin were prepared in 70% of methanol with concentrations of from 0.01 to 0.05 μg/ml. The calculation was made from the areas of the peaks.

Separation by the TLC method was performed in the chloroform–methanol–water (65:35:10) system on Silufol plates (Czechoslovakia). The revealing agent was a 1% solution of vanillin in concentrated H_2SO_4.

Enzymatic Hydrolysis of a Cell Culture. Freeze-dried cells from a suspension culture of the yam (0.1 g) were hydrolyzed in the presence of the preparation Pektinaza-500 at pH 4.2 in 0.05 M acetate buffer at 55°C for 1 h.

The steroid glycosides were extracted with hot methanol. The extract was cooled and filtered. The total volume was made up to 25 ml. The completeness of the conversion of the oligofurostanol glycosides into oligospirostanol glycosides was checked by the TLC method.

CONCLUSIONS

A method has been developed for the quantitative determination of oligospirostanosides in a cell culture of Dioscorea deltoidea with the aid of HPLC on a LiChrosorb RP-18 column. Elution was performed with mixtures of acetonitrile and water (50-75%), and detection was carried out at 207 nm.

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STRUCTURE OF THE PRODUCTS OF OXIDATION OF VINDOLINE BY THE SARETT REAGENT

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The oxidation of vindoline with the Sarett reagent has given five lactams the structures of which have been established on the basis of the results of instrumental methods of analysis (the x-ray structural method, and the PMR, mass, and IR spectroscopy). Two of the vindoline derivatives – 4β-acetoxy-7,8-dihydroxy-16-methoxy-3α-methoxycarbonyl-3β,6β-epoxy-7,8,9,19-tetradehydrovincaminoreine 7,9-betaine and 4β-acetoxy-16-methoxy-3α-methoxycarbonyl-1-methyl-7,8-dioxo-3β,6β-epoxyaspidospermidine – have been obtained for the first time and are new compounds.

We have shown previously that vindoline (I) – an alkaloid of Catharanthus roseas (Madagascar periwinkle) is oxidized by chromic acid with the formation of various reaction products, depending on the pH of the medium [1-3]. The present work was devoted to determining the structures of the reaction products obtained on the oxidation of (I) with the Sarett reagent, which leads to the formation of a mixture of several substances. Four compounds (II, III, IV, and V) have been isolated by column chromatography on silica gel.

On the basis of IR, PMR, and mass spectra, (II) was shown to be identical with a lactam of the composition $C_{25}H_{30}N_2O_7$ that was obtained as an intermediate product in the synthesis of vindoline [4]. To confirm the structure of (II) and to establish its stereochemistry, we have made an x-ray structural study of a bromine derivative (IIa) of the lactam. Compound (II) was brominated in ether. Rhombic crystals of (IIa) were obtained from a mixture

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