The second sterol, with a molecular mass of 400, apparently consists of an ergostane derivative with one double bond. This is shown by the M^+, m/z 400, and $[M - CH_3]^+$, m/z 385, peaks. The last reaction in the chain in the biosynthesis of ergosterol – the final product of sterol synthesis of the yeast Saccharomyces cerevisiae – is the reaction of the C24(28) double bond in the ergosterol precursor ergosta-5,7,22,24(28)-tetraen-3β-ol. Apparently, because of the lower substrate specificity characteristic of the enzymes of secondary metabolism, the absence of two double bonds in the sterol molecule does not prevent C24(28)-reductase from performing this reaction, although it lowers the suitability of episterol in comparison with ergosta-5,7,22,24(28)-tetraen-3β-ol as its substrate. The reduction of the C24(28)-double bond in the side chain of episterol leads to the formation of an ergosta-7-en-3β-ol (fungisterol) molecule. Thus, in view of the characteristic features of the biosynthesis of ergosterol in yeast of this species, on the basis of the results described it may be stated with confidence that the mutants with respect to the NYS3 and NYS4 genes accumulate a mixture of episterol and fungisterol.

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STEROLS OF Salsola collina

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Continuing investigations begun previously [1] we have studied the composition of the sterols in an 80% ethanol extract of the epigeal part of Salsola collina Pall., gathered at the end of the vegetation period (in the stage of complete ripening of the seeds). From the fractions soluble in hexane and chloroform of an aqueous ethanolic extract we isolated sterol and sterol glycoside fractions. The total substances extracted by hexane were chromatographed on columns of silica gel with benzene–chloroform (1:0 and 1:1), and the free sterols were isolated. Elution of the column with chloroform–ethanol (1:0 and 19:1) gave a fraction of sterol glycosides. When the total substances extracted by chloroform were subjected to separation in silica gel, the free sterols were isolated by elution of the column with benzene–acetone (1:0 and 9:1) and the sterol glycosides with benzene–acetone (1:1).

In the products of the acid hydrolysis of the glycosidic fractions of the sterols we detected glucose and aglycons analogous in composition to the free-sterol fraction. In the ^{13}C NMR spectrum of the total glycosides the CSs of the carbon atoms of the β-D-glucose residue gave signals at 102.5, 78.8, 78.3, 75.4, 71.6, and 63.0 ppm. β-Sitosterol β-D-glucopyranoside with mp 296-298°C was isolated by the chromatographic separation of the glycoside fraction on silica gel and Molselekt G-10 using chloroform–methanol (19:1) as eluent. This substance was identified from its ^1H and ^{13}C NMR spectra [2, 3]. The acid hydrolysis and the β-sitosterol β-D-glucopyranoside gave D-glucose and β-sitosterol with mp 134-136°C [3].

The free sterols and the aglycon part of the products of the hydrolysis of the glycoside fractions were identified by chromato-mass spectrometry on an LKB-2091 instrument using a capillary column 25 m long with the deposited phase SE-30 in the isothermal regime at 280°C.

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The temperature of the separator was 300°C, the temperature of the ion source 310°C, and the energy of the ionizing electrons 70eV.

As a result, we identified the $C_{29}\Delta^5$ (M^+ 414) sterol β-sitosterol (I), the $C_{29}\Delta^{5,22}$ (M^+ 412) sterol stigmasterol (II), the $C_{28}\Delta^5$ (M^+ 400) sterol campesterol (III), and the C_{29} (M^+ 416) sterol 24-ethylcholestan-3-ol (IV). The sterol glycoside fraction consisted of a mixture of glycosides of sterols (I-IV). The isolation of sterols from a pentane extract of S. collina, including cholesterol and desmosterane has been reported previously [4]. Cholesterol and desmosterane were not detected in the material that we investigated.

The isolation of sterol glycosides from S. collina has not been reported previously.

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ENZYMATIC 5'-MONOPHOSPHORYLATION OF MODIFIED NUCLEOSIDES

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In the synthesis of nucleoside 5'-monophosphates frequent use is made of nucleoside phosphotransferases (NPTs), which catalyze the transfer of phosphate groups from molecules of organic monophosphate esters to the hydroxyls at the C-5' atoms of nucleosides. The sources of the enzymes are plants [1, 2] and bacteria [3, 4]. The latter source, in our view, is more promising because of the simplicity of production, since it is possible to use whole microbial cells as the enzyme preparation.

In order to expand the limits of use of NPTs in the production of modified nucleotides we have studied the possibility of synthesizing various 5'-mononucleotides modified in the carbohydrate moiety of the molecule with the aid of Erwinia herbicola 47/3 cells, which contain highly active NPTs. We selected the strain used earlier for the synthesis of AMP from adenosine (Ado) and p-nitrophenyl phosphate [5]. In this work we used Ado, 2'-deoxyadenosine (2'dAdo), guanosine (Guo), 2'-deoxyguanosine (2'dGuo), uridine (Urd), 2'-deoxyuridine (2'dUrd), cytidine (Cyd), 2'-deoxycytidine (2'dCyd), ribothymidine (Thd) and thymidine (2'dThd) from Fluka (Switzerland). The synthesis of 9-(β-D-arabinofuranosyl)adenine (ara-Ade) has been described previously [6]. 2',3'-Dideoxythymidine (2',3'ddThd), 2',3'-dideoxy-2',3'-didehydrothymidine (2',3'ddeThd); 3'-azido-2',3'-dideoxythymidine (3'N_3;2',3'ddThd); 1-(β-D-arabinofuranosyl)thymine (ara-Thy), 9-(β-D-xylopyranosyl)adenine (xylo-ade) and -guanine (xylo-Gua); 9-(β-D-arabinofuranosyl)guanine (ara-Gua); 3'-amino-2',3'-dideoxyadenosine (2'NH_2;2',3'ddAdo) and -thymidine (3'NH_2;2',3'ddThd); 3'-fluoro-2',3'-dideoxyadenosine (2'F;2',3'ddAdo) and its α-anomer (α-3'S;2',3'ddGuo) and -thymidine (3'F;2',3'ddThd) were supplied by E. I. Kvasyuk, G. V. Zaitsova, and N. E. Pupeiko (Institute of Bioorganic Chemistry of the Academy of Sciences of the Belorussian SSR, Minsk). 2'-Amino-2'-deoxyuridine (2'NH_2;2'dUrd), 2'-azido-2'-deoxyuridine (2'N_3;2'dUrd), and 3'-amino-3'-deoxycytidine (3'NH_2; 3'dCyd) were obtained from Professor A. A. Kraevskii (Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow).

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