and treated with a 1% dioxane solution of isatin hydrazone, after which they were heated at 100°C for 5-10 min.

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EPISTEROL AND FUNGISTEROL FROM MUTANT STRAINS OF THE YEAST *Saccharomyces cerevisiae*

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Mutant strains of the yeast Saccharomyces cerevisiae having blocks in the stages of the biosynthesis of ergosterol accumulate its intermediates. The use of nystatin-resistant mutants permits the sequence of stages in sterol synthesis to be established and strains to be obtained that accumulate sterols suitable for transformation into biologically active substances [1]. We have obtained a strain of the yeast Saccharomyces cerevisiae with mutations simultaneously in the two genes NYS3 and NYS4. Mutations in the NYS4 gene prevent the introduction of a double bond into the C22(23) position, and those in the NYS3 gene block dehydrogenation in the C5(6) position.

Gas-chromatographic analysis showed that the cells of the strains with mutations simultaneously in the two genes NYS3 and NYS4 accumulate two sterols the retention times of which exceed that of ergosterol. We succeeded in establishing the structures of these sterols by the method of mass spectrometry, which is widely used for these purposes [2]. The sterols were identified on the basis of the characteristics of fragmentary ions with values of m/z $\gg$ 200. It must be mentioned that many yeast sterols give identical fragmentary ions, and therefore for their identification the ratio of the intensities of the peaks must be taken into account. The sterols of the NYS3 and NYS4 strains have molecular ions with m/z 400 and 398. Their structures apparently differ by the number of double bonds. The peaks of the ions with m/z 385 and 383 correspond to the splitting out of a methyl group. The $[M - CH_3]^+$ ion is formed by the detachment of a methyl group both from C10 and from C13. The presence of a basic peak with m/z 271 in the spectrum indicates that double bonds are present both in the side chain and in the nucleus. The $[M - R]^+$ and $[M - R - 2H]^+$ ions are characteristic for Δ^5- and Δ^7-sterols with one double bond in the side chain [3]. The ratio of the intensities of the peaks with m/z 271 and 314 determines the position of the double bond in the nucleus. The m/z 271 peak is more intense than the m/z 314 peak if the sterol has a Δ^7-double bond [3]. High-intensity peaks of the $[M - R - 2H]^+$ and $[M - R - H_2O]^+$ ions are characteristic for Δ^5- and Δ^7-sterols [4]. However, the spectrum lacks the peaks characteristic for Δ^5- and $\Delta^{5,7}$-sterols that are connected with the breakdown of the tetracyclic nucleus [5]. The intensities of the peaks also permit the position of a double bond in the side chain to be determined. Intense peaks with m/z 257 and 255, and also a triplet of low-intensity peaks with m/z 299, 300, and 301 indicate that the double bond is present in the C24 position [3]. The fact that the intensity of the m/z 255 peak is greater than that of the m/z 213 peak indicates the presence of a Δ^{24}-bond in the side chain not suppressing fragmentation in ring D but leading to a predominance of dissociation with the formation of a m/z 255 ion [6]. Consequently, it may be concluded that the ergostane derivative with a molecular mass m/z 398 and two double bonds in the C7 and C24 positions is ergosta-7,24(28)-dien-3β-ol (episterol).

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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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