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METHYLATED STEROLS FROM POLYENE-RESISTANT STRAINS OF THE YEAST *Saccharomyces cerevisiae*

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Yeasts of the species *Saccharomyces cerevisiae* are rich in sterols and form a source of ergosterol, 22,23-dihydroergosterol, and zymosterol [1]. An increase in resistance to polyene antibiotics leads to an accumulation of new sterols by the yeast cells [2]. The composition of the sterols from strains of the yeast resistant to polyene antibiotics and having functional disturbances of the $\Delta^8 \rightarrow \Delta^7$ -isomerase or 22,23-dehydrogenase - enzymes regulating the biosynthesis of ergosterol - has been analyzed.

The sterol components were isolated from the unsaponifiable lipid fractions of yeast strains resistant to polyene antibiotics by thin-layer chromatography [Silufol plates impregnated with a 20% aqueous solution of AgNO_3 in the chloroform-acetone (95:5) system]. In nine strains the amount of compounds with R_f 0.73 detected was considerably greater than for other intermediates in the biosynthesis of ergosterol that we have identified previously [3]. The unknown sterols were identified by mass spectrometry on a Varian MAT instrument (temperature of the ion source 120°C; accelerating voltage 3.5 kV; ionization energy 70 eV).

Mass spectrum of compound (I) (direct introduction), m/z , %: 426 [$\text{C}_{30}\text{H}_{50}\text{O}$] $^+$, 87; 411 [$\text{C}_{29}\text{H}_{47}\text{O}$] $^+$, 100; 393 [$\text{C}_{29}\text{H}_{45}$] $^+$, 61; 383 [$\text{C}_{27}\text{H}_{43}\text{O}$] $^+$, 73; 327 [$\text{C}_{23}\text{H}_{35}\text{O}$] $^+$, 22; 245 [$\text{C}_{17}\text{H}_{25}\text{O}$] $^+$, 61.

Mass spectrum of compound (II) (direct introduction), m/z , %: 412 [$\text{C}_{29}\text{H}_{48}\text{O}$] $^+$, 35; 397 [$\text{C}_{28}\text{H}_{45}\text{O}$] $^+$, 100; 395 [$\text{C}_{28}\text{H}_{43}\text{O}$] $^+$, 30; 313 [$\text{C}_{22}\text{H}_{33}\text{O}$] $^+$, 12; 299 [$\text{C}_{21}\text{H}_{31}\text{O}$] $^+$, 25; 245 [$\text{C}_{17}\text{H}_{25}\text{O}$] $^+$, 49; 231 [$\text{C}_{16}\text{H}_{23}\text{O}$] $^+$, 72.

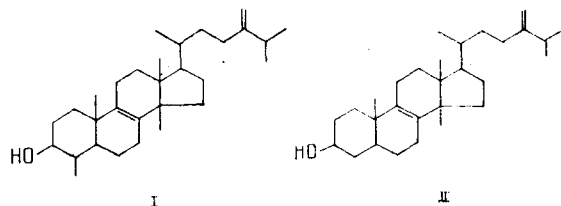
The presumed elementary compositions of the fragmentary ions formed on the breakdown of the molecules of compounds (I) and (II) were confirmed by the results of high-resolution mass spectrometry on a MKh-1320 instrument with direct introduction. The resolving capacity of the instrument was 10,000-12,000 at an accelerating voltage of 2.5 kV and an ionization energy of 70 eV.

Strong peaks of molecular ions with m/z 426 and 412, and also base peaks with m/z 411 and 397, respectively ($[M - 15]^+$) indicated that the compounds being analyzed were sterols methylated in positions 4 and 14 [4].

On comparing the mass spectra that we had obtained with the spectra given in [5] of 4,14-dimethylergosta-8,24(28)-dien-3 β -ol ($m/z \geq 200$) (%): 426 (39); 411 (100), 393 (10), 383 (7), 327 (14), 245 (21); and 14-methylergosta-8,24(28)-diene-3 β -ol ($m/z \geq 200$) (%): 412 (32), 397 (100), 379 (5), 369 (5), 313 (14), 299 (10), 245 (12.5), 231 (32); it was found that the spectra of compounds I and II contained the peaks of almost all the characteristic

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fragments of all these methylated sterols. This enabled us to identify compound (I) as 4,14-dimethylergosta-8,24(28)-diene-3-ol, and compound (II) as 14-methylergostadiene-3 β -ol.



These sterols have been described only in strains of yeast with a disturbed 14 α -demethylated function [6] and have not been identified in mutants belonging to other genetic classes.

Thus, methylated sterols have been identified for the first time in yeasts in which no disturbance of the function of the enzyme 14 α -demethylase has been detected.

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ALKALOIDS OF THE ROOTS OF *Vinca major* INTRODUCED INTO GEORGIA

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Continuing a chemical study of *Vinca major* L. introduced into Georgia, we have investigated the hypogeal organs of the plant for the presence of alkaloids [1, 2].

The roots (1.3 kg) of the plant collected in the phase of secondary autumn flowering (experimental field of the Institute of Pharmacochimistry of the Georgian Academy of Sciences, October, 1985) were extracted with acidified methanol. After the methanol had been distilled off, the acid extracts were made alkaline with a 25% solution of ammonia to pH 9-10, and the alkaloids were extracted with chloroform. The yield of purified alkaloids amounted to 0.385%, calculated on the air-dried raw material.

By separating the total material with respect to basicity with citrate-phosphate buffers having pH 7, 6, 5, 4, 3, and 2.2, four bases were isolated. The individual alkaloids were identified from the results of a comparison of their physicochemical constants and spectral characteristics with literature information and by determining mixed melting points with authentic samples of alkaloids.

Treatment with acetone of the fraction obtained from the buffer with pH 6 yielded a crystalline base (I) with mp 227-229°C (methanol), which was identified as vincamajoreine [4].

When subjected to column chromatography on alumina (neutral, activity grade II, 1:30) with elution by ethyl ether, the fraction from the buffer with pH 3 yielded (in fractions 1-3) the crystalline alkaloid (II) - vincamajine [3].

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