STEROLS FROM WINE YEAST BIOMASS

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In the processing of grapes a large amount of wastes accumulates. One of the possible methods for their utilization is the production of biologically active substances from them. In view of this, we have made a detailed study of the chemical compositions of extracts of wine yeast biomass. We give the results of an investigation of the (petroleum ether)-soluble fraction of an acetone extract of the yeast deposits from wine of the "Rkatsiteli" type. This material contains carbohydrates, alkyl ferulates, and esters of higher saturated alcohols and sterols the acyl moiety of which is represented by fatty acids. The bulk of the fraction under investigation consists of free sterols. It is known that in the intestine plant sterols form insoluble complexes with bile acids preventing the resorption of cholesterol and thus lowering its concentration in the blood by 20% [1]. On this is based the therapeutic use of, for example, β-sitosterol in the treatment of severe forms of hyperlipidemia and hypercholesteremia [1-4].

We have studied the composition of the sterols and their ester derivatives from wine yeast biomass. The petroleum ether fraction was analyzed by column chromatography on silica gel, by GLC, and by chromatomass spectrometry. The individual compounds isolated were identified from the results of the methods mentioned and by UV and IR spectroscopy, and also with the aid of their physicochemical constants. In column chromatography we used the hexane–ethyl acetate (98:2) system. The process was monitored by TLC on Silufol (solvent system for sterol esters: hexane–ethyl acetate (95:5), and for free sterols: hexane–ethyl acetate (90:10)).

The separated substances were detected by spraying with molybdophosphoric acid followed by heating. Cholesterol (1), stigmasterol (2), ergosterol (3), and β-sitosterol (4), first identified by TLC under these conditions, could not be isolated preparatively. Consequently, the cholesterol was separated from the phytosterols by using reversed-phase chromatography on silica gel impregnated with a 10% solution of undecane in petroleum ether (solvent system: acetic acid–water (90:10)). Such pairs of sterols as (4) and (2), and (3) and 5-dihydroergosterol (5) were difficult to separate. These compounds were separated in the form of acetates on silica gel impregnated with silver nitrate by means of chloroform–petroleum ether–acetic acid (25:75:0.5) [5].

Thus, in the sterol fraction (5% of the weight of the dry raw material) we detected nine sterols, of which six were identified — (1) (9.4%), campesterol (6) (7.0%), (2), (3.0%), (3) (32.0%), (4) (32.0%), and (5) (3.0%). Quantitative analysis was carried out by GLC using the method of internal normalization with respect to the areas of the peaks with β-sitosterol as standard. The determination of the structures of the unidentified sterols — (7) (5.0%), (8) (4.6%), and (9) (4.3%) — is continuing. The ester fraction consisted of compounds (4), (2), and (3) acylated with palmitic, stearic, and oleic acids. The palmitates of (2) and (4), the stearates of (2) and (4), and the oleate of (4) were isolated in the individual form.

GLC analysis was carried out with the use of the stationary phase OV-1 on Chromaton N-AW-HMDS. Chrom-4 chromatograph, column 300 × 0.3 mm, column temperature 260°C, carrier gas helium. Chromatomass spectrometric analysis was performed with the use of the phase SE-54 on a Varian MAT-220 instrument.

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APORPHINE ALKALOIDS OF *Thalictrum baicalense*

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We have previously reported on the isolation from the epigeal part of *Thalictrum baicalense* Turcz. of the new aporphine alkaloids baicaline [1] and baicalidine [2]. We now give the results of a further study of the alkaloid composition of *Th. baicalense*. The phenolic fraction of the combined bases [2] was chromatographed on a column of silica gel. The column was washed with chloroform and with chloroform–methanol.

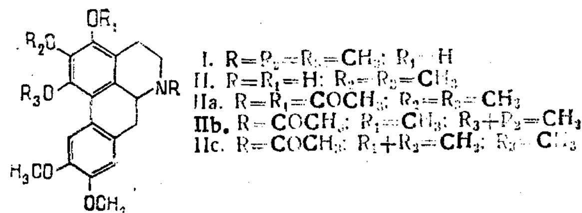
The fraction eluted by chloroform containing 1% of methanol yielded a base (I), and the fraction containing 3% of methanol a base (II). Base (II), $[\alpha]_D$ +61° (methanol). UV spectrum, λ_{max} (C_2H_5OH), nm: 220, 285, 303, 313; λ_{max} ($C_2H_5OH + OH^-$) 325 nm. PMR spectrum ($CDCl_3$, ppm, δ scale, 0 – HMDS): 3.63, 3.81, 3.83, and 3.88 (4 × OCH_3); 6.36 and 7.81 (2 s, 1 H each, 2 × ArH). The mass spectrum of (II) showed the peaks of ions with m/z 357 (M^+), 356, 342, 340, 328 $(M - 29)^+$, 327, 297.

The acetylation of (II) with acetic anhydride in the presence of pyridine yielded a O,N-diacetyl derivative (IIa). IR spectrum, λ_{max} (KBr), cm^{-1}: 1650 ($NCOCH_3$); 1760 ($OCOCH_3$). PMR spectrum, ppm: 3.64 (3 H) and 3.84 (9 H) (4 × OCH_3); 6.67 (s, 1 H) and 7.93 (s, 1 H) (2 × ArH); 2.30 (3 H, $OCOCH_3$); 2.09 and 2.13 (2 s, with a total intensity of 3 H; $NCOCH_3$). Mass spectrum: 441 (M^+), 382, 381, 340, 339, 327, 310, 293.

The facts given above showed that (II) was a new 1,2,3,9,10-pentasubstituted monophenolic noraporphine base. We have called it thalbaicaline. The position of the OH group was established from the results of a study of the mass spectra of (IIa), of N-acetylnorthalicmine (IIb), and N-acetylbaicaline (IIc). The mass spectra of (IIa) and (IIb) had peaks of medium intensity with m/z 381 $(M - 60)^+$ and 365 $(M - 32)^+$, respectively, while in the spectrum of (IIc), as was to be expected, these peaks were not visible. The results obtained indicated that the $OCOCH_3$ group in (IIa) is present at C-3 [3]. Consequently, thalbaicaline has the structure of 3-hydroxy-1,2,9,10-tetramethoxynoraporphine (II).

Base (I) had mp 191-193°C (ethyl acetate), $[\alpha]_D$ +73° (methanol). UV spectrum, λ_{max} (C_2H_5OH): 282, 305, 315 nm; λ_{max} ($C_2H_5OH + OH^-$) 326 nm; mass spectrum, m/z: 371 (M^+), 370, 356, 341, 328 $(M - 43)^+$. PMR spectrum, ppm: 2.46 (s, 3 H, NCH_3); 3.62 (3 H), 3.83 (6 H), and 3.88 (3 H) (4 × OCH_3); 6.65 and 7.77 (2 s, 1 H each, 2 × ArH).

Base (I) was identical with the N-methylthalbaicaline obtained from (II) (CH_2O/HCOOH) according to TLC, melting point, and mixed melting point. Thus, base (I) which we have called thalbaicalidine has the structure of 3-hydroxy-1,2,9,10-tetramethoxy-N-methylaporphine.



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