ISOLATION AND IDENTIFICATION OF STEROLS FROM YEASTS OF THE GENUS *Candida*

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It is known that yeasts of the genus *Candida* grown on media containing carbohydrates accumulate ergosterol (up to 0.2-0.5%) [1]. Since this source of ergosterol may be of practical interest, it appeared necessary to us to perform an analysis of the composition of the sterol fraction from the lipids of the yeast *Candida guilliermondii* grown on a medium containing carbohydrates. A similar analysis has been performed previously for the yeast *Candida utilis* [2].

The results of TLC showed that the unsaponifiable fraction of the yeast *C. guilliermondii* contains, in addition to sterols, considerable amounts of other components and, therefore, the usual isolation of pure sterols was difficult. After the preliminary precipitation of the sterols in the form of a mixture of digitonides and the decomposition of the latter, we obtained a mixture of sterols consisting mainly, according to TLC, of ergosterol.

From the mass spectrum of the mixture of sterols isolated it was possible to estimate that it contained substances with mol. wt. 396 (M^+ of ergosterol), 398, 400, 412, and 414 in a ratio of 96:1.2:1.6:0.5:0.6% (Fig. 1).

For the subsequent separation of the mixture of sterols, they were chromatographed repeatedly in a thin layer of nonfixed silica gel with 20% of Supercel. Five individual fractions were isolated, each of which gave a positive Liebermann–Burchard test. These fractions were repeatedly subjected to chromatographic separation.

From a fraction with R_f 0.5, after crystallization from methanol, we isolated substance (I) with mp 142-145°C, $[\alpha]_D^{20}$ −1° (c 1.0; chloroform). From its IR spectrum, this compound contained an OH group (3400 cm^{-1}), and it gave a positive Fieser test with SeO_2 [3], which may serve as a proof of the presence of a Δ^7 double bond in substance (I). This was confirmed by the NMR spectrum: δ 5.00 (>C=C<u>H</u>–, 1H) and 3.4 ppm (>C<u>H</u>–OH, 1H).

On the basis of the elementary analysis and mass spectrum (Table 1), substance (I) was ascribed the empirical formula $C_{28}H_{48}O$. Compound (I) formed an acetate and benzoate (Table 2).

As follows from Table 2, the constants of substance (I) and its derivatives are close to those of ergost-7-en-3β-ol (fungisterol). The identity of these compounds is also shown by the nature of the fragmentation of (I) under electron impact (presence of a C_9H_{19} side-chain, see Table 1), and also by the results of a direct comparison with an authentic sample.

From the fraction with R_f 0.55 after two crystallizations from ethanol we isolated ergosterol (II) with mp 158-159°C, likewise completely identical with an authentic sample. From the third fraction (R_f 0.6) after chromatography five times we obtained a further small amount of ergosterol, and also a sterol (III) with mp 135-137°C the molecule of which contained, according to its IR spectrum, an OH group (3418 cm^{-1}) and a double bond (1640, 830 cm^{-1}). The NMR spectrum showed the signal of an olefinic proton at 5.12 ppm. The mass spectrum of the sterol (III) agreed completely with the mass spectrum of β-sitosterol [2] (see Table 1). The acetate of sterol (III) had mp 118-120°C, which also corresponds to the

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TABLE 1. Fragmentation of Substances (I-V) under Electron Impact

Substance	M^+	$M^+ - CH_3$	$M^+ - H_2O$	$M^+ - C_2H_5$	$M^+ - CH_3 - H_2O$	$M^+ - C_2H_5 - H_2O$	M^+ − side chain (fragment A)	Fr. A − H_2O	Fr. A − C_2H_6	Fr. A − $C_2H_6 - H_2O$
5α-Ergost-7-en-3β-ol	400 (100)*	385 (31)	—	—	—	—	—	255 (43)	231 (48)	213 (41)
Ergosterol	396 (100)	381 (33)	378 (47)	—	363 (120)	—	271 (66)	253 (81)	229 (69)	211 (78)
β-Sitosterol	414 (100)	399 (37)	396 (31)	385 (40)	381 (27)	367 (38)	273 (67)	255 (77)	231 (69)	213 (77)
4α-Methylzymosterol	398 (100)	383 (62)	380 (38)	—	365 (52)	—	—	—	245 (113)	227 (116)
14α-Demethyllanosterol	412 (100)	397 (37)	—	—	379 (80)	—	301 (59)	—	259 (63)	241 (66)

*The relative intensities of the peaks (in %) are given in parentheses; the intensity of peak M^+ has been taken as 100%.

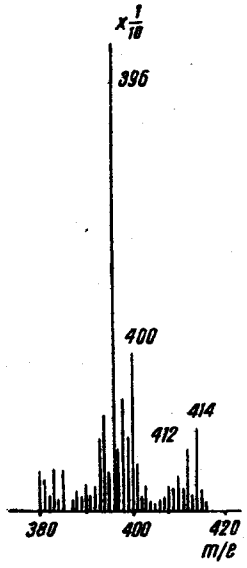


Fig. 1. Mass spectrum (region of the molecular peaks) of the mixture of sterols from the yeast *C. guilliermondii*.

acetate of β-sitosterol. Thus substance (III) was identified as β-sitosterol

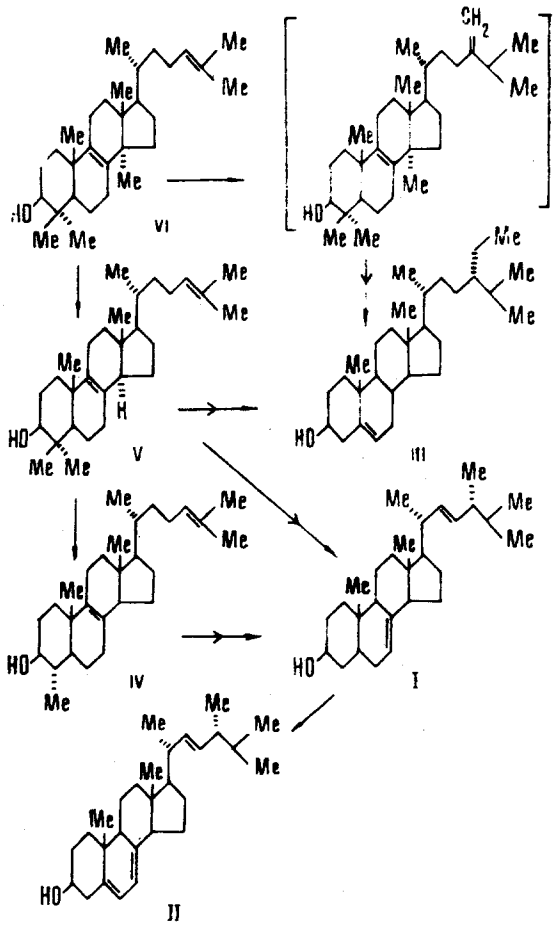
From the fourth fraction (R_f 0.75) by chromatographing it three times and crystallization from methanol we isolated a sterol (IV) with mp 126-128°C, $[\alpha]_D^{20}$ +28° (c 1.0; chloroform), in the IR spectrum of which absorption bands for an OH group (3410 cm^{-1}) and for a double bond (1650 and 1635 cm^{-1}) were observed.

On the basis of its elementary analysis and mass spectrum (see Table 1), this compound must be ascribed the empirical formula $C_{28}H_{46}O$. The acetate of the sterol (IV) had mp 135-139°C, $[\alpha]_D^{20}$ +45°. These facts coincide with the results obtained previously for 4α-methylzymosterol [2], and sterol (IV) is probably identical with the latter.

From the fifth fraction (R_f 0.8) we obtained a small amount of a sterol (V) with mp 135-138°C and $[\alpha]_D^{20}$ +35°. IR spectrum, cm^{-1}: 3480 (OH group), 1675 and 1630 (double bond). The NMR spectrum of compound (V) showed signals in the δ 1.53 ppm region (singlet, 3 H) and the 1.65 ppm region (singlet, 3 H), corresponding to two CH_3 groups on a double bond, and also the signal of an olefinic proton at 4.95 ppm (1 H). The acetate of the sterol (V) had mp 126-129°C, $[\alpha]_D^{20}$ +40°, these constants being close to those for 14α-demethyllanosterol isolated previously by Bloch et al. [5].

This structure for substance (V) was also confirmed by its mass spectrum (see Table 1), which is similar to that of lanosterol [6].

Taking literature information into account [7], an approximate scheme for the biosynthesis of ergosterol and the minor sterols from lanosterol (VI) in yeasts of the genus *Candida* can be represented in the following way (see scheme on p. 18):



EXPERIMENTAL

Chromatographic Separation of the Sterols. The fraction of unsaponifiable matter (50 g) from the lipids of the yeast *C. guilliermondii* was dissolved in 300 ml of ethanol, and a solution of 30 g of digitonin in 250 ml of 60% ethanol was added. The mixture was left at room temperature overnight. After centrifuging and washing with ethanol, the digitonide was purified by extraction with ether in a Soxhlet apparatus. This gave 25.2 g of a white powder which was decomposed with dimethyl sulfoxide as described by Issidorides et al. [8]. The mixture of sterols obtained (6.2 g) was chromatographed on a plate with a nonfixed layer of silica gel (100-150 mesh), containing 20% of Supercel (layer thickness 2 mm, 400 × 600 mm; 2 g of the mixture of sterols was deposited on each plate) in the benzene–ethyl acetate (8:2) system. Fractions were isolated with R_f 0.5, 0.55, 0.60, 0.75, and 0.8 (determined by burning the dried chromatogram with an incandescent Nichrome wire). The products were obtained by elution with chloroform. The starting zone was discarded. By thrice-repeated preparative chromatography, as described above, several fractions were isolated from these fractions.

Fraction with R_f 0.5 (90 mg). This fraction yielded 28 mg of ergost-7-en-3β-ol (I), $C_{28}H_{48}O$, mp 142-145°C (after two crystallizations from methanol), $[\alpha]_D^{20}$ –1° (c 1.0; chloroform). Literature data: mp 145-146°C, $[\alpha]_D^{20}$ 0° [4].

IR spectrum (tabled with KBr), cm^{-1}: 3400 (OH group), 1630 and 820 (double bond). NMR spectrum ($CDCl_3$): δ 3.4 (>C<u>H</u>–OH, 1 H) and 5.00 (>C=C<u>H</u>–, 1 H).

When the sterol was treated with an excess of acetic anhydride in pyridine (at room temperature for a day), ergost-7-en-3β-ol acetate was formed with a yield of 78%, mp 152-154°C (from methanol), $[\alpha]_D^{20}$ –2° (c 1.0; chloroform). Literature data: mp 157°C, $[\alpha]_D^{20}$ –5.3° [4].

TABLE 2. Constants of the Acetate and Benzoate of Substance (I)

Substance	mp, °C	$[\alpha]_D^{20}$, deg	Acetate		Benzoate	
			mp, °C	$[\alpha]_D^{20}$, deg	mp, °C	$[\alpha]_D^{20}$, deg
Substance I	143–6	–1	152–154	–2°	174–176	+1
Ergost-7-en-3β-ol*	145–6	0	157–158	–5,3	179	0

*The constants of the ergost-7-en-3β-ol and its derivatives were taken from the literature [4].

By the action of benzoyl chloride in pyridine (room temperature, 12 h) the ergost-7-en-3β-ol was converted into the corresponding benzoate. Yield 65%, mp 174-176°C (from acetone–hexane), $[\alpha]_D^{20}$ +1° (c 0.8; chloroform). Literature data: mp 179°C, $[\alpha]_D^{20}$ 0° [4].

Fraction with R_f 0.55 (4.35 g). From this chloroform eluted 3.2 g of ergosterol (II) with mp 159-159°C* (from ethanol). Ergosterol acetate, obtained with a yield of 81%, by treatment with acetic anhydride in pyridine had mp 177-178°C (from ethanol), $[\alpha]_D^{20}$ –85° (c 1.0; chloroform).

Fraction with R_f 0.60 (520 mg). The fraction was chromatographed five times on silica gel in the benzene–ethyl acetate (8:1) system as described above. Chloroform extracted 38 mg of β-sitosterol (III), $C_{29}H_{50}O$, mp 135-137°C, completely identical with an authentic sample.

IR spectrum, cm^{-1}: 3418 (OH group), 1630 and 830 (>C=CH–). β-Sitosterol acetate (obtained as described above): yield 83%, mp 118-120°C.

Fraction with R_f 0.75 (150 mg). Thrice-repeated thin-layer chromatography on silica gel in the benzene–ethyl acetate (8:2) system followed by two crystallizations from methanol yielded 35 mg of 4α-methylzymosterol (IV), $C_{28}H_{46}O$, mp 125-128°C, $[\alpha]_D^{20}$ +28° (c 1; chloroform). Literature data: mp 128-130°C, $[\alpha]_D^{22}$ +31° [5].

IR spectrum (tablet with KBr), cm^{-1}: 3410 (OH group), 1650 and 1635 (double bonds). NMR spectrum ($CDCl_3$), δ, ppm: 1.52 (CH_3), 1.60 (26,27–CH_3), 1.95 (4 H, allyl protons at C_7 and C_{11}), 2.89 (>CH–OH), and 4.9 (1 H, –CH=C<).

4α-Methylzymosterol acetate was obtained with a yield of 91% by treating (IV) with acetic anhydride in pyridine at 20°C for 12 h. It had mp 135-139°C (from ethanol), $[\alpha]_D^{20}$ +45° (c 1.0; chloroform). Literature data: mp 137-141°C, $[\alpha]_D^{24}$ +51.0° [5].

Fraction with R_f 0.8 (0.5 g). The fraction was chromatographed three times in a similar manner to what was done for the purification of the sterol (IV). This yielded 42 mg of 14α-demethyllanosterol (V), $C_{29}H_{48}O$, mp 135-138°C (from methanol), $[\alpha]_D^{20}$ +35° (c 1.0; chloroform). Literature data: mp 136-138.5°C, $[\alpha]_D^{23}$ + 40.4° [5].

IR spectrum (tablet with KBr), cm^{-1}: 3480 (OH group), 1675 and 1630 (double bonds).

NMR spectrum ($CDCl_3$), δ, ppm: 1.55 (CH_3, 3H), 1.65 (CH_3, 3 H), 1.95 (4 H, allyl protons at C_7 and C_{11}), 3.08 (>CH–OH, 1 H), 4.95 (–CH=C<, 1 H). Mass spectrum: see Table 1.

14-Demethyllanosterol acetate, obtained as described above with a yield of 70%, had mp 126-129°C (from ethanol), $[\alpha]_D^{20}$ +40° (c 1.0; chloroform). Literature data: mp 128-132°C, $[\alpha]_D^{24}$ + 41.8° [5].

Synthesis of 5α-Ergost-7-en-3β-ol (I). A solution of 3 g of ergosterol in 100 ml of ethyl acetate was hydrogenated at 40°C in the presence of 0.5 g of Raney nickel catalyst in an autoclave at a pressure of 10 atm of H_2 for a day. At a lower pressure the main product

*As in Russian original – Publisher.

was ergosta-7,22-dien-3β-ol, and the isolation of (I) became difficult. After the end of hydrogenation, the catalyst was separated by filtration. The filtrate was evaporated, and the crystalline residue was twice recrystallized from methanol. This gave 2.55 g (85%) of 5α-ergost-7-en-3β-ol (I) with mp 143-144.5°C (after drying, 5 mm Hg, 80°C), identical in all respects with the sample of (I) isolated above.

SUMMARY

By thin-layer chromatography on silica gel containing 20% of Supercel the following sterols have been isolated from a mixture of the sterols of the yeast *Candida guilliermondii* and identified: 5α-ergost-7-en-3β-ol, ergosterol, β-sitosterol, 4α-methylzymosterol, and 14α-demethyllanosterol. The amount of ergosterol in the mixture was 96%.

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