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A NEW TETRAETHENOID STEROL OF YEAST

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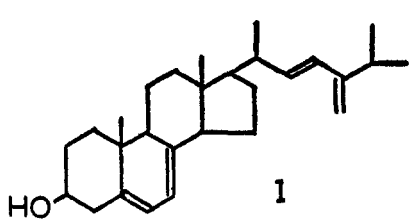
Ergosterol has long been known as the primary sterol of yeast (1) and several other yeast sterols have been reported. Smedley-MacLean (2) reported the presence of zymosterol, a dextrorotatory sterol. The structure of zymosterol was long in question until Barton (3) showed it to be 8,24-cholestadien-3β-ol. As a consequence of the establishment of the zymosterol structure and from the recent development of theories concerning optical rotation and structure, it would appear that two other dextrorotatory sterols of yeast reported by Wieland and others (4), namely ascosterol and fecosterol, are also unsaturated at the 8(9) position. Callow (5) reported the occurrence in yeast of "α-dihydroergosterol", since shown to be 5-dihydroergosterol (6). The neosterol described by Wieland (4) appears from the work of Barton (7) to be a mixture of 5-dihydroergosterol and ergosterol. Cerevisterol, described by Honeywell and Bills (8) has recently been shown (9) to be 7,22-ergostadiene-3β,5α,6β-triol. It therefore appears to be an oxidation product of ergosterol, but whether it is formed metabolically by yeast or whether it is formed from ergosterol by chemical oxidation during isolation of the sterols, is not clear. Wieland and Gough (10) have also described two other yeast sterols, episterol and hyposterol.

The new yeast sterol we wish to report is a tetraethenoid sterol having a ring structure identical with that of ergosterol, and having a conjugated double bond system in the side chain. Although the position of the conjugated double bond system in the side chain has not been positively established, the ultraviolet and infrared absorption characteristics lead us to believe that the new sterol has one of the structures (I or II) shown. The location of the ultraviolet maximum at 230 mμ and the infrared band at 11.3 μ lend credence to the presence of a vinylidene group (11). It may further be reasonable to assume that since the new sterol is a metabolic product of yeast, the enzyme systems which are involved in production of ergosterol and zymosterol would be more likely to place a second double bond in ergosterol at 24(28) than at 20(21). Hence we favor structure I and tentatively designate the new sterol as 24(28)-dehydroergosterol, or 5,7,22,24 (28)-ergostatetraen-3β-ol.



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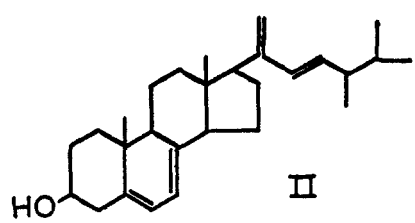

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This new yeast sterol is not a minor constituent. Under the usual conditions of yeast growth, such as that employed in the manufacture of bakers' yeast, the sterol produced by yeast is predominantly ergosterol (up to 80%). Although different strains of yeast vary in their tendency to produce 24(28)-dehydroergosterol, growth conditions can be attained under which any of a wide variety of yeast strains that we have examined is capable of making as much 24(28)-dehydroergosterol as ergosterol. Usually, growth conditions which favor sterol production will also favor production of 24(28)-dehydroergosterol.

The occurrence of 24(28)-dehydroergosterol was first noted through the appearance of an ultraviolet absorption band at 230 mμ. It was established that this band was not due to an α, β unsaturated ketone nor to a simple conjugated diene complexing with ergosterol, and that the material responsible for the 230 mμ maximum was a sterol which was precipitable by digitonin and also showed typical ergosterol absorption bands. Concentration of 24(28)-dehydroergosterol

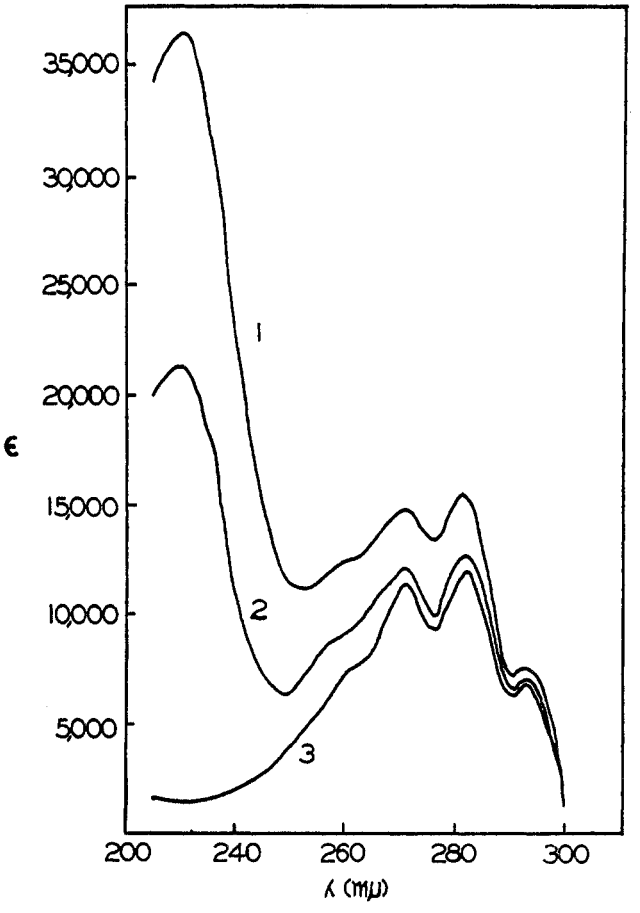

FIGURE 1. ULTRAVIOLET ABSORPTION SPECTRA IN ABSOLUTE ETHANOL OF: 1. Benzoate of 24(28)-dehydroergosterol. 2. 24(28)-Dehydroergosterol. 3. Ergosterol.

by fractional crystallization or chromatography of the free sterols or their esters proved to be extremely difficult. Isolation was finally accomplished by repeated recrystallization of the benzoate of a fraction containing a higher proportion of 24(28)-dehydroergosterol than of ergosterol. In this way there was obtained a benzoate having m.p. 149–151°, $[\alpha]_D^{25}$ −38° (1% in chloroform), and an ultraviolet absorption spectrum as shown in Figure 1. Its physical properties were not changed by six further recrystallizations. If the starting mixture of benzoates contained a higher proportion of ergosterol than of 24(28)-dehydroergosterol, ergosteryl benzoate was obtained upon repeated recrystallization, and if proportions were equal, no separation by fractional recrystallization could be obtained.

Saponification of the benzoate gave the sterol monohydrate, m.p. 118–120°, $[\alpha]_D^{25}$ −78° (1% in chloroform), and an absorption spectrum in absolute ethanol as shown in Figure 1 with three bands typical of ergosterol at 293 mμ (ε 6,900), at 281.5 mμ (ε 12,800), at 271 mμ (ε 12,200), and a fourth band at 230 mμ (ε 21,400). The acetate, m.p. 141–144°, $[\alpha]_D^{25}$ −42° (0.5% in chloroform), showed an ultraviolet absorption spectrum almost identical with that of the free sterol. The infrared absorption spectrum of 24(28)-dehydroergosterol with that of ergosterol is shown in Figure 2. The free sterol was very labile to oxidative deterioration, with deterioration beginning during 24 hours exposure to air, while the benzoate was more stable. The sterol was found to be about 5 times

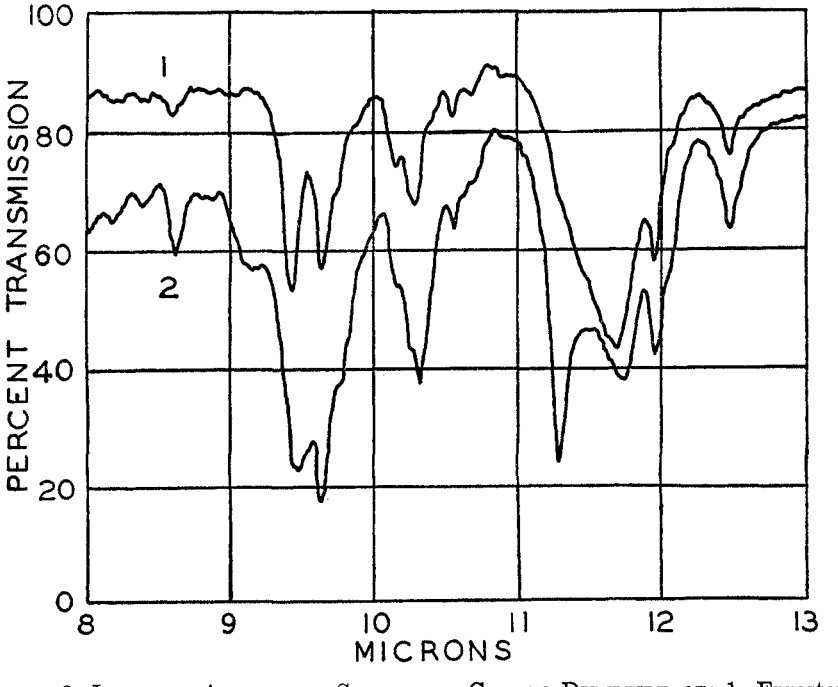

FIGURE 2. INFRARED ABSORPTION SPECTRA IN CARBON DISULFIDE OF: 1. Ergosterol. 2. 24(28)-Dehydroergosterol.

as soluble as ergosterol in a number of solvents. It reacted rapidly with maleic anhydride but did not give an easily crystallized adduct. Upon hydrogenation with Raney Nickel catalyst, 7-ergosten-3β-ol was obtained. Attempts to partially hydrogenate with the objective of obtaining 5-dihydroergosterol were unsuccessful. In the photocondensation or "sunshine" reaction, the sterol gave a bis compound as does ergosterol and this exhibited the 230 mμ absorption maximum (see Figure 3). Irradiation of an ethanol solution of a mixture of ergosterol and 24(28)-dehydroergosterol gave an irradiation product which showed the 230 mμ maximum in addition to the absorption in the 250–280 mμ region usually shown by the products of ergosterol irradiation. The vitamin D derived from 24(28)-dehydroergosterol, however, showed low activity in both rats and chicks.

In working with yeast sterols, we have also noted the presence of small amounts of a sterol other than 24(28)-dehydroergosterol which shows conjugated diene

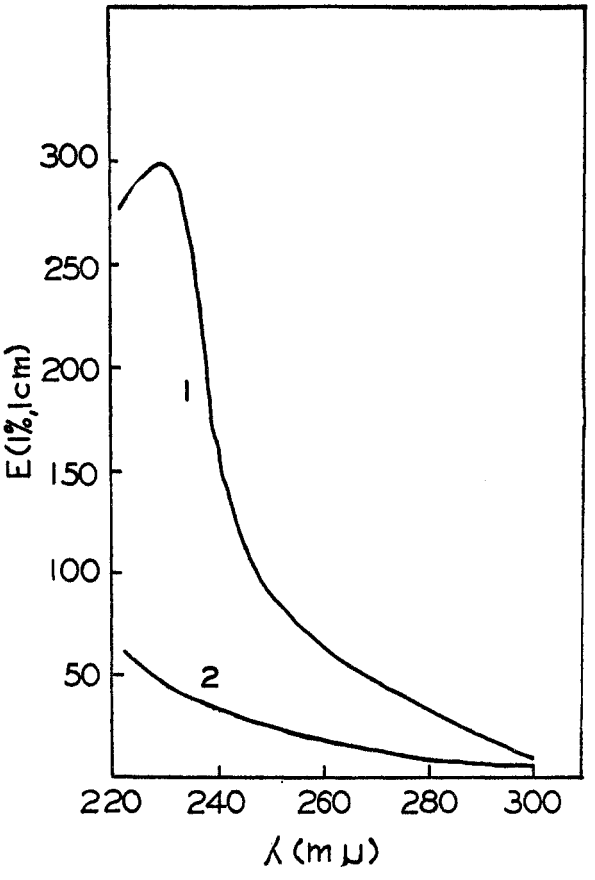

FIGURE 3. ULTRAVIOLET ABSORPTION SPECTRA IN ETHYL ETHER OF: 1. Photocondensation product from a mixture of 24(28)-dehydroergosterol and ergosterol. 2. Photocondensation product of ergosterol.

unsaturation in the side chain. We have not been successful in isolation of this sterol. Like 24(28)-dehydroergosterol, the material reacts rapidly with maleic anhydride and the unreacted material recovered after maleic anhydride treatment is much less dextrorotatory than the starting material. By this procedure, an optical rotation of about $[\alpha]_D$ +80° (chloroform) can be calculated for this material and it is likely that it is a sterol unsaturated at 8(9) and having conjugated diene unsaturation in the side chain.

EXPERIMENTAL

Melting points. All melting points are uncorrected.

Optical rotations. The sample (usually 500 mg.) was dissolved in chloroform in a volumetric flask to give a 1% solution and rotation determined in a 4-dm. tube using a sodium vapor lamp as the light source.

Absorption spectra. The ultraviolet spectra were determined in absolute ethanol using a Beckman quartz spectrophotometer (Model DU). The infrared spectra were determined on carbon disulfide solutions in a Baird Associates double beam spectrophotometer with the comparison cell empty.

Carbon and hydrogen determinations. These were carried out by Mr. Joseph F. Alicino, Metuchen, New Jersey.

Isolation of 24(28)-dehydroergosterol. A sterol fraction was obtained by crystallization from mother liquors of ergosterol production, whose spectral absorption at 281.5 mμ indicated the presence of 58% of sterols unsaturated at 5,7, and whose absorption at 230 mμ indicated 64% of sterols having conjugation in the side chain. To 200 g. of this material dissolved in 700 ml. of pyridine, 200 ml. of benzoyl chloride was added during 15 minutes with temperature rising to 80°. The mixture was allowed to stand overnight, becoming a nearly solid mass, was warmed on the water-bath to partly dissolve, and was poured into 3 l. of cold water. The benzoate was filtered, washed with water, and while still wet was dissolved in 600 ml. of hot ethyl acetate. Water was removed mechanically and by azeotropic distillation. Upon cooling the benzoate crystallized and was filtered, giving 150 g. of crude material. After 5 recrystallizations 35 g. of benzoate was obtained whose physical properties were unchanged in 6 additional recrystallizations, m.p. 149–151°, $[\alpha]_D^{25}$ −38° (1% in chloroform), E (1%, 1 cm.) at 281.5 mμ = 325 and at 230 mμ = 747 in absolute ethanol.

Anal. Calc'd for $C_{35}H_{46}O_2$ (498.72): C, 84.29; H, 9.30.

Found: C, 83.26; H, 9.15.

24(28)-Dehydroergosterol. 24(28)-Dehydroergosteryl benzoate (15 g.) was saponified by refluxing 2 hours with 7.5 g. of potassium hydroxide dissolved in 150 ml. of absolute ethanol. The free sterol was extracted with ethyl ether and crystallization of the 12.5 g. of ether residue gave 10 g. of lustrous white plates of the sterol monohydrate, m.p. 118–120°, $[\alpha]_D^{25}$ −78° (1% in chloroform), E (1%, 1 cm.) at 281.5 mμ = 308 and at 230 mμ = 518 in absolute ethanol. There was a weight loss of 4.14% upon drying in a vacuum oven for 5 hrs. at 70° (calculated for $C_{28}H_{42}O \cdot H_2O$, 4.37%).

Anal. Calc'd for $C_{28}H_{44}O_2$[412.63]: C, 81.50; H, 10.75.

Found: C, 82.41; H, 10.42 (sample was not dried).

24(28)-Dehydroergosteryl acetate. 24(28)-Dehydroergosterol (2.5 g.) was dissolved in 50 ml. of pyridine and 5.0 ml. of acetic anhydride was added. The mixture was allowed to stand for 1 hour, was heated 1 hour on the water-bath, and was poured into 500 ml. of ice water. The white precipitate was filtered, dissolved in 20 ml. of ethyl acetate, and upon cooling gave 2.0 g. of lustrous plates. The material obtained after one recrystallization from 95% ethanol, m.p. 141–144°, $[\alpha]_D^{25}$ −41.7° (0.5% in chloroform), had an ultraviolet absorption spectrum nearly identical with that of the free sterol.

Anal. Calc'd for $C_{30}H_{44}O_2$(436.65): C, 82.51; H, 10.16.

Found: C, 81.65; H, 9.93.

Hydrogenation of 24(28)-dehydroergosterol. To 5 g. of 24(28)-dehydroergosterol dissolved in 50 ml. of ethyl acetate was added 2 g. of Raney nickel suspended in ethyl acetate. The mixture then was hydrogenated in a Parr low pressure apparatus at 40–45 p.s.i. until hydrogen uptake ceased, requiring about 30 minutes. The catalyst then was filtered off, the solvent was distilled under reduced pressure, and the residue was crystallized from 150 ml. of 95% ethanol, giving 4.2 g. of 7-ergosten-3β-ol, m.p. 149–151°, $[\alpha]_D^{24}$ −3.0° (1% in chloroform), and no absorption in the 220–300 mμ region. No depression was obtained in a mixture melting point with a sample of 7-ergosten-3β-ol, m.p. 150–151°, $[\alpha]_D^{24}$ −3.5° (1% in chloroform), prepared by a method similar to that of Wieland and Benend (6), except that a 2:1 mixture of chloroform:absolute ethanol was used as solvent in hydrogenation of ergosteryl benzoate.

Photocondensation of a mixture of ergosterol and 24(28)-dehydroergosterol. A sterol fraction (18 g.) whose spectral absorption indicated the presence 82% of sterols unsaturated at 5,7 and 50% of sterols having diene conjugation in the side chain was placed in a square Pyrex bottle and dissolved in 1 liter of absolute ethanol containing 9 g. of eosin (yellowish). The mixture was boiled to remove air, stoppered while hot, and exposed to sunlight for 10 days. The fibrous crystals of the "pinacol" were filtered off and recrystallized from benzene, giving white crystals m.p. 180–189°, with the absorption spectrum shown in Figure 3. Similar treatment of ergosterol gave bisergostatrienol, m.p. 198–200°, with the absorption spectrum shown in Figure 3.

Irradiation of a mixture of ergosterol and 24(28)-dehydroergosterol. A sterol fraction (1 g.) whose spectral absorption indicated the presence of 59% of sterols unsaturated at 5,7 and 45% of sterols having diene conjugation in the side chain was dissolved in 400 ml. of 95% U.S.P. ethanol and irradiated for 90 minutes in a 7-cm. diameter quartz cell through which the alcohol solution was continuously circulated with a glass pump. The light source was a Hanovia Utility ultraviolet lamp, Type 16200, 125 watts. The irradiation mixture was concentrated to dryness by vacuum distillation, and the residue was taken up in a small volume of methanol. Upon chilling and filtering 0.44 g. of unchanged sterol was recovered. The methanolic filtrate was made to volume and an aliquot was submitted to a colorimetric vitamin D determination using the Nield, Russel, and Zimimerli (12) reagent with crystalline calciferol as reference material. Methanol was evaporated under a vacuum from another aliquot and the residue was dissolved in vegetable oil (Mazola). The oil solution was then assayed by the U.S.P. method (rats) and the A.O.A.C. method (chicks) for vitamin D potency. A similar irradiation was carried out on 1 g. of ergosterol, in which 0.44 g. of sterol was recovered and a sample of the methanol-soluble irradiation product was made up in vegetable oil for A.O.A.C. assay. Results were as follows:

	From ergosterol	From a mixture of ergosterol and 24(28)-dehydroergosterol
Sterol irradiated, g	1.0	1.0
Vitamin D formed (colorimetric det'n.), g	0.26	0.25
Vitamin D formed (U.S.P. assay), units	10,100,000	5,200,000
Vitamin D formed (A.O.A.C. assay), units	(not assayed)	162,000

The colorimetric measurement indicated that nearly equal quantities of vitamin D were formed in the two irradiations. Since the mixture of ergosterol and 24(28)-dehydroergosterol gave so much lower a response in the U.S.P. assay, it may be assumed the activity for rats of the vitamin D arising from 24(28)-dehydroergosterol is lower than that of ergosterol. The response in the A.O.A.C. assay given by the irradiation product of the sterol mixture is of such a low order that it may be assumed that the vitamin D arising from 24(28)-dehydroergosterol is not appreciably more active for chicks than that from ergosterol.

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SUMMARY

The isolation of a new tetraethenoid sterol from yeast is described. Its concentration in some yeasts approximates the ergosterol content. On the basis of chemical and spectroscopic evidence, its structure is proposed to be 5,7,22,24-(28)-ergostatetraen-3β-ol.

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