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ISOLATION OF ERGOSTA-5,7-DIENE-3β,23ξ-DIOL FROM YEAST

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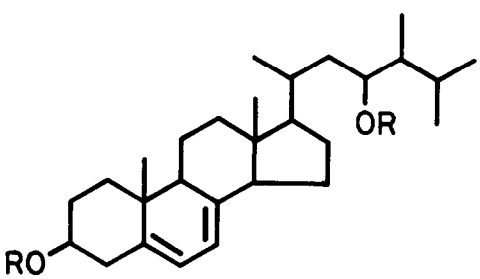
Abstract: A novel hydroxylated sterol was isolated from a Saccharomyces cerevisiae mutant defective in the Δ^{22}-desaturation of sterol side chain, and it was identified as ergosta-5,7-diene-3β,23ξ-diol.

In spite of the development of studies on sterols in yeast, scanty reports have been published on hydroxylated sterol(s) in it. In this communication, we report the isolation of a novel hydroxylated sterol from a Saccharomyces cerevisiae mutant N22,[1] which is defective in the Δ^{22}-desaturation of sterol side chain. The compound was identified as ergosta-5,7-diene-3β,23ξ-diol (1).



1, R = H
2, R = Ac
3, R = Me_3Si

From N22 cells grown on a synthetic medium without shaking, sterols were extracted and acetylated.[1b] One of the acetates which were detected in fraction I',[1b] on $AgNO_3$-TLC was shown to have a longer retention time than most sterols on GLC. The compound was suggested to be a diacetate ester (2)[2] of hydroxylated C_{28}-sterol by GC-MS. Compound 2 was further purified by TLC on silica gel plate (solvent, PhH-MeOH = 98 : 2; R_f, 0.51) and was hydrolyzed to obtain 1.[3] UV spectrum[3] of 1 showed absorption bands specific for $\Delta^{5,7}$-conjugated diene.[4] NMR spectrum[3] of 1 showed that 1 had 3β-hydroxyl group. Moreover, the incubation of N22 cells with [methyl-^{14}C]-L-methionine (1.83×10^7 dpm, 53 Ci/mol) as described in the previous paper[1b] enabled the incorporation of radioactivity into 1 (2.85×10^4 dpm; specific radioactivity, 1.0 Ci/mol). This indicates that 1 has C-28 carbon[5] which is known to originate from the methyl group of L-methionine. In addition, an intense peak[6] of m/z 173 corresponding presumably to fragment

$C_6H_{21}OSi$ formed by the cleavage of the bond between C-22 and C-23 was observed in MS of di-trimethylsilyl ether (3).[7] This indicates that 1 has a hydroxyl group at 23 position. Other analytical data were also consistent with the literature values[7] of synthetic (23RS)-ergosta-5,7-diene-3β,23-diol.

The cellular contents of 1 in strain M10, the parent strain of N22, and in a wild-type S. cerevisiae ATCC 12341, if present, were less than 2% of that found in N22 (about 70 μg/g dry cells). This suggests that the accumulation of 1 in N22 cells is due to the genetic characteristics of the mutant. Recently we reported the involvement of a cytochrome P-450 in the Δ^{22}-desaturation.[8] That paper seems to be the first one reporting the involvement of cytochrome P-450 in desaturation reaction. The Δ^{22}-desaturation is considered to proceed in two stages — hydroxylation of sterol accompanied by dehydration of the product. It seems that 1 accumulates owing to the lack of the enzyme involved in the dehydration.

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References and Notes

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2. Retention time of 2 relative to cholesteryl acetate on 3% SE-30 column was 2.6. m/z 498 (M^+), 438 (100%), 378, 363, 253.
3. Analysis of 1 by GLC on 3% SE-30 column showed that purity of 1 (retention time relative to cholesterol, 2.3) was about 90%. Analytical data of 1: λ_{max}^{EtOH} 271 (ε 13,800), 282 (15,000), 293 (8,700); m/z 414 (M^+), 381 (100%), 211; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (br m $C_3\alpha H$ and $C_{23}H$), 5.39 (m C_7H), 5.57 (m C_6H); ν_{max}(KBr) 3350 (OH), 1120 (C-O), 830 trisubstituted double bond), 800 (trisubstituted double bond).
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6. Retention time of 3 relative to cholesteryl trimethylsilyl ether on 3% SE-30 column was 2.4. m/z 558 (M^+), 453, 397, 307 (100%), 251, 173 (68%).
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