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SHORT COMMUNICATION

A SIMPLE METHOD FOR THE ISOLATION OF ZYMOSTEROL FROM A STEROL MUTANT OF *SACCHAROMYCES CEREVISIAE*

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Summary—A simple method is described for the direct isolation of zymosterol (5α-cholesta-8,24-dien-3β-ol) of high purity from a sterol mutant of *Saccharomyces cerevisiae*. This yeast strain, which is a double mutant of the *ERG6* (sterol transmethylase) and *ERG2* (C-8 sterol isomerase) genes, accumulates zymosterol as its major sterol component.

INTRODUCTION

Zymosterol is an intermediate sterol in the *Saccharomyces cerevisiae* ergosterol biosynthetic pathway. In wild type strains this sterol accumulates as a small percentage of the total sterol in comparison to ergosterol. The level of total zymosterol accumulation is strain- and growth-condition dependent and the total zymosterol accumulation may be as low as 2% [1] or as much as 20% [2] of total sterols. A yeast strain has previously been reported in which zymosterol is the predominant sterol accounting for >80% of the sterol dry weight [3, 4]. This strain is in actuality a double mutant of the *ERG6* gene (sterol transmethylase) and the *ERG2* gene (C-8 sterol isomerase). A strain defective in both of these enzyme activities accumulates principally zymosterol and grows poorly. This strain can revert back to either single mutants *erg6* or *erg2* and accumulate sterols consistent with these lesions. The *ERG6* and *ERG2* genes have been cloned [5, 6] and, using gene disruption techniques [7], strains deleted for these genes have been obtained. We have now reconstructed the *erg6/erg2* double mutant from the *erg6* and *erg2* single disrupted strains for the purpose of developing a strain of yeast capable of providing zymosterol of high purity without the need of extensive purification. This strain, which cannot revert due to the insertion of the wild type LEU2 gene into the coding sequence of the *ERG6* and *ERG2* genes as well as a 400 bp deletion in the coding region of *ERG6* [5, 6], accumulates zymosterol as its major sterol component.

In many instances the limited availability of zymosterol, which is not commercially available, is problematic for studies of the biosynthesis of cholesterol and related steroids. Moreover, it is known to decompose on storage [8, 9] which may necessitate obtaining new or additional samples. In the present communication, we have developed a simple method for the direct isolation of zymosterol from a mutant yeast strain. In addition, we have presented useful ^{1}H and ^{13}C NMR spectral assignments which will be of value in the identification of zymosterol.

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EXPERIMENTAL

The *erg6/2* double null mutant was isolated by genetic manipulations [10] and its genetic identity confirmed by yeast tetrad analysis and GLC. The strain was grown for 48 h in yeast complete medium (1% yeast extract, 2% peptone; and 2% glucose); cells were harvested and saponified in 25% alcoholic KOH for 2 h at reflux temperatures. Non-saponifiables were extracted into heptane and the sample dried under a stream of nitrogen. The residue was dissolved in ethanol and analyzed by TLC and GLC [6, 11, 12]. The dry weight of cells was determined by taking 10% of their wet wt and drying in a vacuum desiccator for 72 h prior to sample weighing.

Procedures for the recording of m.p., IR spectra, low resolution mass spectra (MS), proton and carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C NMR), and column chromatography have been reported previously [11, 12]. Details concerning distortionless enhancement polarization transfer (DEPT) experiments have been presented [11–13]. A sample of pure lanosterol was prepared by standard methods [14].

Analysis of the extract residues by TLC and GLC indicated the presence of two major sterol components (zymosterol, 80% and lanosterol, 15%). The residue was then subjected to column chromatography using a solvent gradient of diethyl ether (0 to 10%, v/v) in toluene. The purified zymosterol was recrystallized from acetone/water and characterized: m.p., 110–112°C. Values obtained for the m.p., IR and MS spectra were identical to those described previously [15]. The purified zymosterol showed a single component on GLC and TLC analysis.

RESULTS AND DISCUSSION

The purified zymosterol obtained from the *erg6/2* double mutant of *S. cerevisiae* represents the major sterol accumulated by this yeast strain. Analysis by GLC indicated that zymosterol constituted 0.875% (±0.063%) of the dry weight of harvested cells. A simple purification by silica gel column chromatography was able to separate zymosterol from its major impurity lanosterol (which was removed in a less polar fraction) and provided a purified product whose

Table 1. Carbon-13 chemical shifts for zymosterol (5α-cholesta-8,24-dien-3β-ol)[a]

Carbon atom	Chemical shift
C-1	35.19
C-2	31.73
C-3	71.25
C-4	38.41
C-5	40.83
C-6	25.54
C-7	27.23
C-8	128.31
C-9	135.05
C-10	35.76
C-11	22.84
C-12	36.99
C-13	42.18
C-14	51.95
C-15	23.81
C-16	28.78
C-17	54.85
C-18	11.25
C-19	17.85
C-20	36.10
C-21	18.69
C-22	36.06
C-23	24.84
C-24	125.24
C-25	130.92
C-26	17.64
C-27	25.72

[a]In ppm downfield from internal tetramethylsilane using $CDCl_3$ as solvent.

structure was confirmed by physical and instrumental methods. The ^{13}C NMR spectral assignments presented in Table 1 represent a more detailed and revised spectrum which was achieved using DEPT methods of spectral analysis [11–13]. A more detailed and revised ^{1}H and ^{13}C NMR spectral analysis of zymosterol is presented in Tables 1 and 2 [15]. The values in Table 1 were obtained using a DEPT-135 experiment which readily resolved the assignment of the chemical shift values for carbon atoms 20 and 22 and independently confirmed (with a greater degree of accuracy) the assignments attributed to the remaining carbon atoms. The two carbon signals for C-20 and C-22, which appear as a single line in the normal ^{13}C NMR spectrum, were resolved in the DEPT spectrum by virtue of the fact that one carbon is a methine carbon (C-20; 36.10 ppm) and the other is a methylene carbon (C-22; 36.06 ppm); thus they appeared as a positive and negative peak, respectively. In Table 2, a detailed analysis of the ^{1}H NMR is presented which includes a new assignment of the proton on C-21 and increased resolution of other salient protons.

Previous methods for the isolation of zymosterol have utilized wild strains of yeast which accumulate only a small percentage of this sterol in the total sterol fraction [1, 2, 15]. This has made the isolation of significant amounts of this sterol problematic and has required laborious procedures involving extensive and detailed chromatographic separation of the isolated sterol mixture, their ester derivatives, and finally a base catalyzed hydrolysis to remove the ester function and provide the final sterol product [15]. Using these and related procedures, the isolation of zymosterol has proved to be both inconvenient and difficult.

Table 2. Proton chemical shifts for zymosterol (5α-cholesta-8,24-dien-3β-ol)[a]

Proton	Chemical shift
H-3	3.616
H-18	0.609
H-19	0.950
H-21	0.945 (J = 6.6 Hz)
H-24	5.093 (J = 7.1, 1.4 Hz)
H-26[b]	1.603
H-27[b]	1.683

[a]In ppm downfield from internal tetramethylsilane using $CDCl_3$ as solvent.
[b]Assignments may be reversed.

We now report a simple and convenient method for the direct isolation of zymosterol, of high purity, from a mutant strain of yeast which accumulates this sterol as the major component of the total sterol fraction. Zymosterol is not commercially available and has not been prepared by chemical synthesis. For studies relating to the biosynthesis of cholesterol and related sterols and steroids, a sample of zymosterol is often required as a standard or starting material. The present communication offers a simple and convenient alternative to previous methods.

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