Sterol synthesis: a simple method for the isolation of zymosterol (5α-cholesta-8,24-dien-3β-ol) from yeast and spectral properties of zymosterol

Ulrike F. Taylor, Alemka Kisic, Robert A. Pascal, Jr., Akihiro Izumi, Mitsuhiro Tsuda, and George J. Schroepfer, Jr.[1]

Departments of Biochemistry and Chemistry, Rice University, Houston, TX 77001

Summary A relatively simple method is described for the isolation of zymosterol (5α-cholesta-8,24-dien-3β-ol) of high purity from baker's yeast. Also presented are detailed spectral properties, including ^{13}C NMR spectral analyses, of zymosterol and its acetate derivative.—**Taylor, U. F., A. Kisic, R. A. Pascal, Jr., A. Izumi, M. Tsuda, and G. J. Schroepfer, Jr.** Sterol synthesis: a simple method for the isolation of zymosterol (5α-cholesta-8,24-dien-3β-ol) from yeast and spectral properties of zymosterol. *J. Lipid Res.* 1981. **22:** 171–177.

Supplementary key words ^{13}C nuclear magnetic resonance · mass spectrometry · infrared spectrometry · medium pressure liquid chromatography

Zymosterol (5α-cholesta-8,24-dien-3β-ol) was first isolated from yeast by Smedley-Maclean in 1928 (1). In 1953, Ruzicka (2) speculated that this sterol might represent an intermediate in the biosynthesis of cholesterol from lanosterol. The results of subsequent

---

Abbreviations: UV, ultraviolet; IR, infrared; GLC, gas–liquid chromatography; GLC-MS, gas–liquid chromatography–mass spectral; TLC, thin-layer chromatography; MPLC, medium pressure liquid chromatography; PMR, proton magnetic resonance; NMR, nuclear magnetic resonance.

[1] To whom inquiries should be directed.

in vivo and in vitro studies have indicated that zymosterol does indeed serve as an efficient precursor of cholesterol in animal tissues (3–5). For studies of the biosynthesis of cholesterol we required quantities of this sterol, which is not commercially available and has not been prepared by chemical synthesis. Isolation from yeast, in which it occurs as one of the minor sterols, constitutes an alternative which is complicated by the necessity of separating this compound from a large number of other sterols. The purpose of this communication is to describe a relatively simple method for the isolation of zymosterol and to present the spectral properties of this sterol and its acetate derivative. A preliminary account of a portion of this work has been presented (6).

EXPERIMENTAL PROCEDURES AND RESULTS

General

Melting points were recorded in sealed, evacuated capillary tubes using a Thomas Hoover melting point apparatus. Ultraviolet (UV) spectra were measured on ethanol solutions of the sterols. Optical rotations were measured using a JASCO DIP-4 digital polarimeter with $CHCl_3$ solutions of the sterols. Infrared (IR) spectra were recorded on a Beckman IR-9 spectrometer using KBr pellets. Gas–liquid chromatographic (GLC) analyses were performed using a Hewlett-Packard Model 402 unit or a Model 5702A unit equipped with flame ionization detectors. The columns (1.8 m × 6 mm) were packed with 3% OV-17, 3% QF-1, 3% OV-22, or 3% OV-1 on Gas Chrom Q (100–120 mesh; Applied Science Laboratories, Inc., State College, PA). Mass spectral (MS) and gas–liquid chromatography–mass spectral (GLC–MS) analyses were made using an LKB 9000S spectrometer under operating conditions described previously (7–9). Trimethylsilyl ether derivatives were prepared as described previously (10). Thin-layer chromatographic (TLC) analyses were made on plates of silica gel G (Analtech, Newark, DE). Components on the plates were visualized after spraying with molybdic acid (11). Medium pressure liquid chromatography (MPLC) of steryl acetates on alumina-$AgNO_3$ columns was carried out as described previously (12, 13). Colorimetric assays of sterols and steryl acetates were made using a modified Liebermann-Burchard reagent (14). Proton magnetic resonance (PMR) spectra were recorded on $CDCl_3$ solutions of the sterols using a Varian EM-390 spectrometer operating at 90 MHz and using tetramethylsilane (TMS) as an internal standard. Peaks are reported as ppm (δ) downfield from the TMS standard. The ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-100-15 spectrometer operating at 25.2 MHz in the Fourier transform mode using $CDCl_3$ solutions of the sterols. The chemical shifts (δ) are expressed in ppm relative to TMS and are estimated to be accurate to ±0.05 ppm (($\delta TMS) = \delta(CDCl_3) + 76.9$ ppm)). The probe temperature was approximately 30°C. The ^{13}C NMR spectra (in $CDCl_3$) were first recorded in the proton noise-decoupling mode in order to measure the exact chemical shifts of all ^{13}C nuclei present. The degree of substitution of each carbon atom was determined by a second series of spectral measurements in the single-frequency, off-resonance decoupling (SFORD) mode.

Isolation of zymosteryl acetate

Baker's yeast (3.62 kg) was heated under reflux for 12 hr with 15% ethanolic (95% ethanol) KOH (9 liters). The resulting mixture was poured into cold water (6 liters) and extracted three times with petroleum ether (6-liter portions). The combined petroleum ether solutions were washed twice with cold water, dried over anhydrous $MgSO_4$, evaporated to dryness under reduced pressure, and further dried in a vacuum dessicator over P_2O_5 and KOH. The nonsaponifiable lipid (7.48 g) so obtained was dissolved in toluene (130 ml) with slight heating and the solution was filtered. The solvent was evaporated and a mixture of toluene and ether (95:5; 100 ml) was added and the resulting solution was subjected to chromatography on silicic acid (60–200 mesh; J. T. Baker Chemical Company, Phillipsburg, NJ) column (90 cm × 4 cm) using toluene–ether 95:5 as the eluting solvent. Fractions 20 ml in volume were collected and aliquots were taken for analysis by TLC (solvent system: toluene–ether 7:3). A total of 248 fractions were collected. A total of 7.26 g was recovered from the column (97% recovery of the material applied to the column). The contents (4.67 g) of fractions 121 through 248 were treated with a mixture of pyridine (55 ml) and acetic anhydride (50 ml) at room temperature for 3 hr and then heated at 60°C for 80 min. The mixture was poured into ice-water (600 ml) and extracted three times with petroleum ether (600-ml portions). The combined extracts were washed twice with 2 N HCl (100-ml portions) and three times with water (150-ml portions) and evaporated to dryness under reduced pressure. The resulting residue was dissolved in benzene (20 ml) and passed through a Unisil (100–200 mesh; Clarkson Chemical Company, Inc., Williamsport, PA) column (15 cm × 1 cm) using benzene (60 ml) as the eluting solvent. Evaporation of the solvent under reduced pressure yielded a white crystalline solid (4.77 g). The acetylated material was dissolved in hexane–toluene 80:20 and six portions (10–20 ml) of this solution were subjected to MPLC (60 psi) on an alumina-AgNO$_3$ column (100 cm × 1.5 cm) using the same solvent mixture as the eluting solvent at a flow rate of 5.1 ml per min. Fractions 20.4 ml in volume were collected. After 90–95 fractions had been collected, the eluting solvent was changed to toluene. The contents of the fractions were assayed by TLC (solvent system: toluene–ether 7:3), GLC (3% OV-17; 280°C), and colorimetric analysis (620 nm, 5 min after the addition of a modified Liebermann-Burchard color reagent). The latter analysis from one of the columns is presented in **Fig. 1.** The contents (163 mg) of fractions 24 through 40 (Fig. 1), corresponding to the mobility of pure zymosteryl acetate on TLC and GLC, were pooled. Fractions 41 through 81 (96 mg) contained zymosteryl acetate which was contaminated to a small extent by material with the properties (as judged by GLC and GLC–MS) of 3β-acetoxy-5α-cholesta-7,24-diene. The average total recovery of the material applied to the MPLC columns was 85%. The recovery of pure zymosteryl acetate from the six MPLC runs was 811 mg (17% yield from the crude acetylated material). An additional 513 mg was obtained from the six MPLC runs which appeared to be a mixture of zymosterol acetate and 3β-acetoxy-5α-cholesta-7,24-diene (see above). This material was subjected to MPLC as described above except that the eluting solvent used was hexane–toluene 85:15, yielding, in fractions 31 through 58, an additional 92 mg of pure zymosteryl acetate. The total recovery of the pure zymosteryl acetate was 902 mg (19% of the crude acetylated sterol fraction subjected to MPLC). Recrystallization from methanol gave needles melting at 106–107°C [literature: 104–106° (15), 103–104° (3), and 108° (16)]. The UV spectrum showed no specific absorbance due to a conjugated diene. The optical rotation ($[\alpha]_D^{26}$) was +35.2° (c, 0.92) [literature: +33.5° (15) and +35° (16)]. The infrared spectrum (**Fig. 2**) showed major absorbances at 2952, 2864, 1742, 1455, 1375, 1245, and 1038 cm^{-1}. The mass spectrum showed the following major ions in the high mass range: 426 (100%; M), 411 (45%; M-CH$_3$), 366 (19%; M-CH$_3$COOH), 351 (24%; M-CH$_3$-CH$_3$COOH), and 313 (16%; M-side chain-2H).[2]

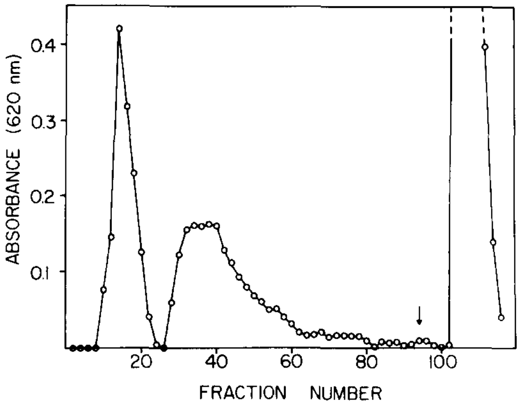

Fig. 1. Alumina-AgNO$_3$ MPLC (solvent, hexane–toluene 80:20) of steryl acetates after initial silicic acid column chromatography of unsaponifiable lipids of baker's yeast. The arrow indicates a change in eluting solvent to toluene. The contents of fractions 24 through 40, corresponding to the mobility of pure zymosteryl acetate on TLC and GLC, were pooled. Fractions 41 through 81 contained zymosteryl acetate which was contaminated to a small extent by material with the properties (GLC and GLC–MS) of 3β-acetoxy-5α-cholesta-7,24-diene. The contents of these fractions were further processed as indicated in the text.

The PMR spectrum showed the following peaks: 0.59 (s, 3H, C-18-CH$_3$), 0.93 (s, 3H, C-19-CH$_3$), 1.58 (s, 3H, C-26-CH$_3$ or C-27-CH$_3$), 1.66 (s, 3H, C-26-CH$_3$ or C-27-CH$_3$), 1.97 (s, 3H, CH$_3$ of acetate), 4.68 (m, 1H, C-3α-H), and 5.07 (t, 1H, C-24-H, J = 7 Hz). The ^{13}C NMR spectral data is summarized in **Table 1** along with previously published data on 3β-acetoxy-5α-cholest-8-ene (19). The compound showed a single component on GLC on four columns. Relative retention time (relative to 5α-cholestane) on the four columns were as follows: 3% OV-17 (270°C), 4.02; 3% QF-1 (240°C), 4.85; 3% OV-22 (270°C), 4.19; and 3% OV-1 (270°C), 2.86.

Zymosterol from zymosteryl acetate

Zymosteryl acetate (92 mg) in 1 N ethanolic (95% ethanol) KOH (20 ml) was heated to 40°C and then allowed to stand at room temperature for 12 hr. The mixture was poured into ice-water (100 ml) and extracted twice with petroleum ether (35-ml portions). The combined extracts were washed with water and evaporated to dryness under reduced pressure. The resulting residue was crystallized from methanol to give crystalline zymosterol (66 mg; 80% yield) melting at 110.5–112.0°C (literature; 108–109° (1, 3), 108–110° (15), 105–107° (20), 107–110° (21), 109° (16), 110° (22), and 110–113° (23)). The UV spectrum of the compound showed no specific absorbance due to a conjugated diene. The optical rotation ($[\alpha]_D^{25}$) was +52° (c, 0.45) [literature: +34.1° (ether) (1), +47.3° (15), +44° (20), +42° (21), +50° (16), +49° (22), and

---

[2] The mass spectrum of another Δ^{24}-unsaturated sterol, desmosterol (cholesta-5,24-dien-3β-ol) has been reported to have a very prominent ion at m/z 271 that has been shown to arise by a loss of the side chain and two hydrogens of the steroid nucleus (arising from hydrogen atoms attached to carbon atoms 12, 14, 16, and 17) (17, 18).

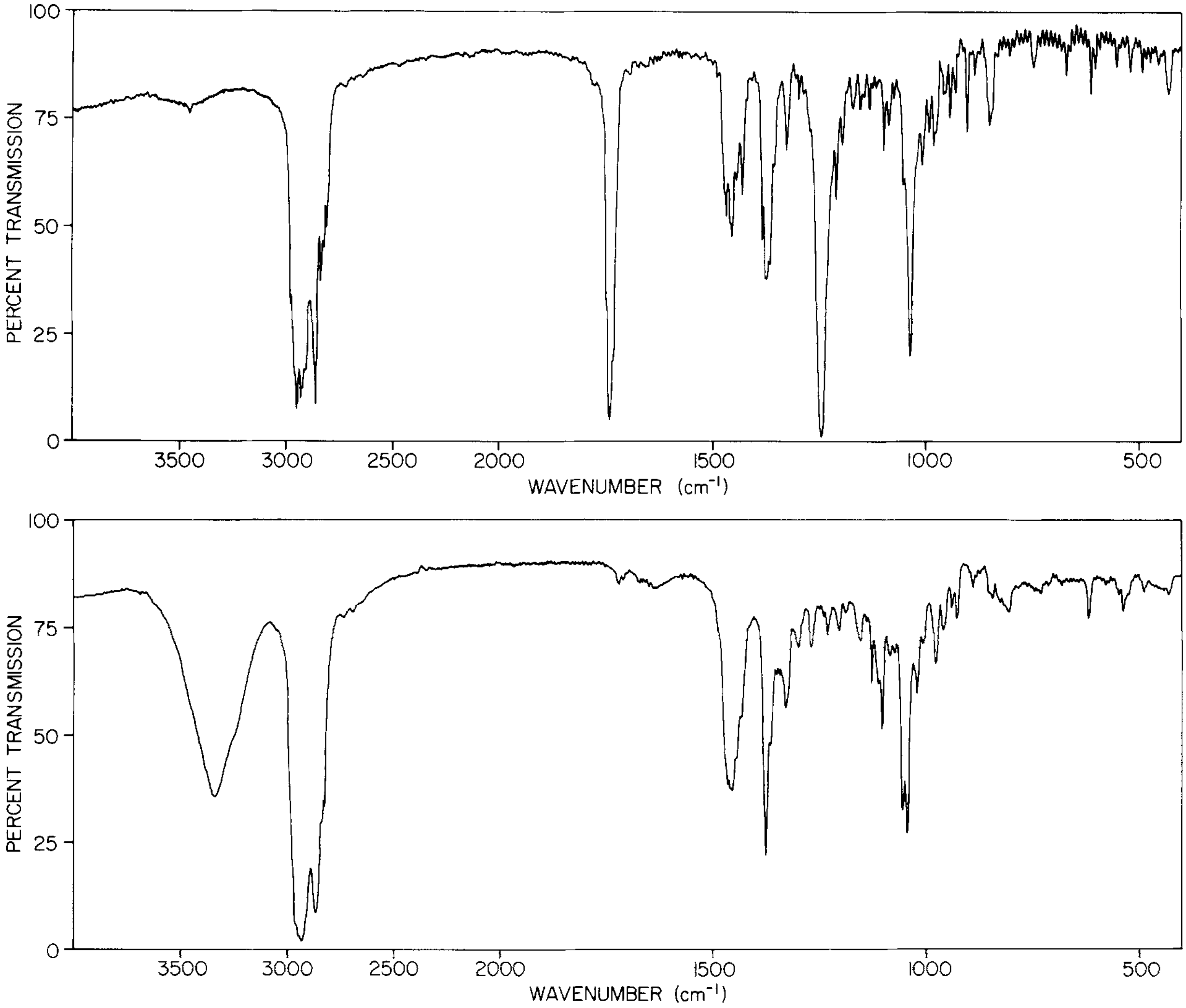

Fig. 2. Infrared spectra of zymosteryl acetate (above) and zymosterol (below).

+53.7° (23)]. The infrared spectrum (Fig. 2) showed major absorbances at 3340, 2930, 1454, 1377, and 1045 cm^{-1}. The mass spectrum showed the following major ions in the high mass range: 384 (100%; M), 382 (14%; M-2), 369 (55%; M-CH_3), 351 (11%; M-CH_3-H_2O) and 271 (18%; M-side chain-2H)[3]. The PMR spectrum showed the following peaks: 0.59 (s, 3H, C-18-CH_3), 0.93 (s, 3H, C-19-CH_3), 1.58 (s, 3H, C-26-CH_3 or C-27-CH_3), 1.66 (s, 3H, C-26-CH_3 or C-27-CH_3), 3.61 (m, 1H, C-3α-H), and 5.07 (t, 1H, C-24-H, J = 7 Hz). The ^{13}C NMR spectral data is summarized in Table 1 along with previously published data on 5α-cholest-8-en-3β-ol (19). The compound showed a single component on GLC on four columns. Relative retention time data for the trimethylsilyl ether derivative on four columns were as follows: 3% OV-17 (270°C), 2.43; 3% QF-1 (240°C), 2.09; 3% OV-22 (270°C), 2.37; and 3% OV-1 (270°C), 2.39.

^{13}C NMR spectra of zymosterol and zymosteryl acetate

The ^{13}C NMR spectral assignments for zymosterol and zymosteryl acetate are presented in Table 1. Peak assignments for the ring carbons and the angular methyl carbons of zymosterol and its acetate were based upon direct comparisons of the shielding values of these compounds with those of previously reported data (19) for the corresponding carbon atoms of 5α-cholest-8-en-3β-ol and its acetate derivative. The shift values of the ring carbons and the angular methyl carbons of the Δ^8-sterol (and its acetate) were within ±0.1 ppm of those of zymosterol (and its acetate) as shown in Table 1. The olefinic carbon peaks (C-24 and C-25) of zymosteryl acetate were easily identified by their characteristic shift values and by their splitting patterns present in the SFORD spectrum. For example, in the SFORD spectrum of zymosteryl acetate, the peak at 124.9 (C-24) occurred as a doublet while the peak at 130.4 ppm (C-25) occurred as a singlet. The peak assignments for C-26 and C-27 were based on the fact that C-26 should be strongly shielded (relative to C-27) due to the steric interaction between the C-23 methylene carbon and the C-26 methyl carbon in a cis-orientation to each other (24, 25). The shieldings for the remaining four side chain carbon atoms (C-20, C-21, C-22, and C-23) were only slightly affected by the introduction of the Δ^{24} double bond. The shielding values for C-20, C-21, and C-22 in zymosterol were almost identical (within ±0.2 ppm) to those values for the corresponding values for 5α-cholest-8-en-3β-ol. Assignments for these four carbon atoms were also confirmed by analyses of the SFORD spectra of zymosteryl acetate. In addition, the peak assignments for the side chain carbon atoms of zymosterol and its acetate were consistent with the results of peak assignments for the side chain of lanosterol (4α,4β,14α-trimethyl-5α-cholesta-8,24-dien-3β-ol) reported by Knight (26) and for the side chain of desmosterol (cholesta-5,24-dien-3β-ol) reported by Joseph-Nathan, Mejia, and Abramo-Bruno (27).[3]

TABLE 1. ^{13}C Chemical shifts of I, zymosterol (5α-cholesta-8,24-dien-3β-ol); II, cholest-8-en-3β-ol; III, zymosteryl acetate (3β-acetoxy-5α-cholesta-8,24-diene); and IV, 3β-acetoxy-5α-cholest-8-ene[a]

Carbon Atom	I	II[b]	III	IV[b]
1	35.1	35.1	34.7	34.8
2	31.5	31.5	27.4	27.5
3	70.9	70.9	73.2	73.3
4	38.2	38.2	34.0	34.1
5	40.7	40.7	40.4	40.5
6	25.5	25.4	25.2	25.3
7	27.1	27.1	26.9	27.0
8	128.0	128.0	128.0	128.1
9	134.8	134.8	134.3	134.4
10	35.6	35.6	35.5	35.6
11	22.8	22.7	22.6	22.7
12	36.9	36.9	36.7	36.8
13	42.0	42.0	41.9	42.0
14	51.8	51.8	51.6	51.7
15	23.7	23.9	23.6	23.9
16	28.7	28.7	28.6	28.7
17	54.7	54.8	54.6	54.8
18	11.2	11.2	11.1	11.2
19	17.8	17.8	17.5	17.6
20	36.0	36.2	35.9	36.2
21	18.6	18.7	18.5	18.7
22	36.0	36.1	35.9	36.1
23	24.7	23.7	24.6	23.7
24	125.0	39.4	124.9	39.4
25	130.6	27.9	130.4	27.9
26	17.6	22.4	17.5	22.5
27	25.7	22.7	25.5	22.7
C = 0			170.0	170.1
CH_3			21.2	21.3

[a] In ppm downfield from tetramethylsilane (Me_4Si); $\delta(Me_4Si) = \delta(CDCl_3) + 76.9$ ppm.

[b] Data from Tsuda and Schroepfer (19).

DISCUSSION

Prior to the introduction of chromatography, the isolation of zymosterol rested upon purification via repeated crystallization of the free sterol or an ester (from which the free sterol could be obtained by subsequent hydrolysis) (1, 15, 20). In view of our present knowledge of the complexity of the sterol composition of yeast and the published physical constants of the sterols isolated, one cannot but be impressed by the effort, patience, and skill at crystallization of these early investigators. However, from the limited data presented on these samples of zymosterol, it is clear that this approach did not lead to material of high purity. Treatment of the mixture of yeast sterols of *Saccharomyces cerevisiae* with maleic anhydride (to remove the bulk of ergosterol) combined with digitonin precipitation and recrystallization of the regenerated free sterol (21) provided a simple method for the isolation of zymosterol, but the material so obtained was obviously not of high purity. Similarly, early applications (4, 5) of alumina and silicic acid column chromatography (including a report by one of the authors of the present paper) could not, by our present knowledge, have yielded zymosterol of high purity. More recently, Barton, Kempe, and Widdowson (23) reported a detailed procedure that, while no GLC data were presented, gave zymosterol of apparent high purity. This procedure was quite lengthy. Sterol mixtures from *Saccharomyces cerevisiae*, from which the bulk of the ergosterol was stated to have been removed by the suppliers, were subjected to alumina column chromatography using stepwise gradient elution. The fifth main fraction was collected in ten subfractions. The first five subfractions were combined, benzoylated, and the resulting benzoates were, after fractional crystallization, subjected to TLC on $AgNO_3$-impregnated silica plates and crystallization. The purified benzoate was saponified to give the free sterol. Calculation of the yield of zymosterol from the yeast was not provided.

In the present study we have employed a more

---

[3] It is important to note that the numbering system for carbon atoms 26 and 27 utilized by the two laboratories differed. The nomenclature system used herein is that utilized by Knight (26), Eggert et al. (29) and by us (19, 30) previously, and differs from a system recently suggested by Popjak et al. (28) and used by Joseph-Nathan et al. (27).

simple approach which is applicable for larger-scale preparation of zymosterol of high purity. The non-saponifiable lipids of baker's yeast were isolated in a standard fashion and subjected to silicic acid column chromatography to remove non-polar materials (squalene, coenzyme Q, 2,3-epoxy-squalene, 4,4,14α-trimethyl-sterols, 4,4-dimethyl-sterols, and 4α-mono-methyl-sterols) and polar materials. The desired fraction obtained from the silicic acid column chromatography (as monitored by TLC) was acetylated and the resulting acetates were subjected to MPLC on alumina-$AgNO_3$ columns. This chromatographic system permits a facile separation of the desired zymosterol acetate from the acetates of yeast sterols containing the $\Delta^{5,7}$ conjugated diene system (ergosterol and related sterols). The separation of zymosteryl acetate from the acetates of other sterols which are more closely related structurally (i.e., 5α-cholesta-7,24-dien-3β-ol, 5α-cholesta-7,14-dien-3β-ol, and 5α-cholesta-8,14-dien-3β-ol) is more difficult and requires careful monitoring of the eluted materials not only by TLC and colorimetry but also by GLC. In spite of this difficulty, zymosteryl acetate of very high purity was obtained in good yield. The availability of pure zymosterol and its acetate allowed the recording of their infrared, nuclear magnetic resonance (^1H and ^{13}C), and mass spectral properties, all of which were previously unreported. Of special importance with regard to the assignment of structure for zymosterol (and its acetate derivative) were the results of ^{13}C NMR studies on these compounds which showed excellent agreement (allowing for the Δ^{24}-unsaturation) with previously recorded spectral data on 5α-cholest-8-en-3β-ol (and its acetate derivative) which had been prepared by an independent route by chemical synthesis.

This work was supported in part by grants (HL-15376 and HL-22532) from the National Institutes of Health and by a grant (C-583) from the Robert A. Welch Foundation.

Manuscript received 22 February 1980 and in revised form 16 June 1980.

REFERENCES

1. Smedley-Maclean, I. 1928. IV. The isolation of a second sterol from yeast-fat. *Biochem. J.* **22:** 22–26.
2. Ruzicka, L. 1953. The isoprene rule and the biogenesis of terpenic compounds. *Experientia.* **9:** 357–396.
3. Alexander, G. J., and E. Schwenk. 1957. Studies on biosynthesis of cholesterol. IX. Zymosterol as a precursor of cholesterol. *Arch. Biochem. Biophys.* **66:** 381–387.
4. Johnston, J. D., and K. Bloch. 1957. In vitro conversion of zymosterol and dihydrozymosterol to cholesterol. *J. Amer. Chem. Soc.* **79:** 1145–1149.
5. Schroepfer, G. J., Jr. 1961. Conversion of zymosterol-C^{14} and zymostenol-H^3 to cholesterol by rat liver homogenates and intact rats. *J. Biol. Chem.* **236:** 1668–1673.
6. Taylor, U. F., R. A. Pascal, Jr., M. Tsuda, and A. Kisic. 1980. A relatively simple method for the isolation of zymosterol from baker's yeast. *Federation Proc.* **39:** 1904.
7. Knapp, F. F., Jr., M. S. Wilson, and G. J. Schroepfer, Jr. 1976. Mass spectral fragmentation of 5α-hydroxysteroids. *Chem. Phys. Lipids.* **16:** 31–59.
8. Knapp, F. F., Jr., and G. J. Schroepfer, Jr. 1976. Mass spectroscopy of sterols. Electron ionization induced fragmentation of C-4 alkylated cholesterols. *Chem. Phys. Lipids.* **17:** 466–500.
9. Pascal, R. A., Jr., R. Shaw, and G. J. Schroepfer, Jr. 1979. Chemical synthesis of three 14α-hydroxymethyl cholestenols. *J. Lipid Res.* **20:** 570–578.
10. Schroepfer, G. J., Jr., R. A. Pascal, Jr., and A. A. Kandutsch. 1979. Inhibition of sterol synthesis in animal cells by 15-oxygenated sterols with the unnatural *cis*-C-D ring junction: 5α,14β-cholest-7-en-15β-ol-3-one and 5α,14β-cholest-7-en-15α-ol-3-one. *Biochem. Pharmacol.* **28:** 249–252.
11. Knapp, F. F., Jr., and G. J. Schroepfer, Jr. 1975. Chemical synthesis, spectral properties and chromatography of 4α-methyl and 4β-methyl isomers of (24*R*)-24-ethyl-5α-cholestan-3β-ol and (24*S*)-24-ethyl-cholesta-5,22-dien-3β-ol. *Steroids.* **26:** 339–357.
12. Pascal, R. A., Jr., C. Farris, and G. J. Schroepfer, Jr. 1979. Sterol biosynthesis. Medium pressure liquid chromatography of C_{27} sterol precursors of cholesterol on alumina-silver nitrate columns. *Federation Proc.* **38:** 633.
13. Pascal, R. A., Jr., C. L. Farris, and G. J. Schroepfer, Jr. 1980. Sterol synthesis. Medium pressure chromatography of C_{27} sterol precursors of cholesterol on alumina-silver nitrate columns. *Anal. Biochem.* **101:** 15–22.
14. Abell, L. L., B. B. Levy, B. B. Brodie, and E. E. Kendall. 1952. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.* **195:** 357–366.
15. Wieland, H., and M. Asano. 1929. Zur Kenntnis der Sterine der Hefe. *Annalen.* **473:** 300–313.
16. Bladon, P. 1958. Cholesterol. R. P. Cook, editor. Academic Press, Inc., New York. 93.
17. Wylie, S. G., and C. Djerassi. 1968. Mass spectrometry in structural and stereochemical problems. CXLVI. Mass spectrometric fragmentations typical of sterols with unsaturated side chains. *J. Org. Chem.* **33:** 305–313.
18. Massey, I. J., and C. Djerassi. 1979. Structural and stereochemical applications of mass spectrometry in the marine sterol field. Synthesis and electron impact mass spectral fragmentation of Δ^{24}- and $\Delta^{24(28)}$-3β-hydroxy-Δ^5-sterols. *J. Org. Chem.* **44:** 2448–2456.
19. Tsuda, M., and G. J. Schroepfer, Jr. 1979. Carbon-13 nuclear magnetic resonance studies of C_{27} sterol precursors of cholesterol. *J. Org. Chem.* **44:** 1290–1293.
20. Haussler, E. P., and E. Brauchli. 1929. Eine spezifische Farbenreaktion auf Ergosterin and seine Umwandlungsprodukte. *Helv. Chim. Acta.* **12:** 187–193.
21. Schwenk, E., G. J. Alexander, T. H. Stoudt, and C. A. Fish. 1955. Studies on the biosynthesis of cholesterol.

VII. Formation of cholesterol precursors by yeast. *Arch. Biochem. Biophys.* **55:** 274–285.

22. Fieser, L. F., and M. Fieser. 1959. Steroids. Reinhold Publishing Corporation, New York. 351.
23. Barton, D. H. R., U. M. Kempe, and D. A. Widdowson. 1972. Investigations on the biosynthesis of steroids and terpenoids. VI. The sterols of yeast. *J. Chem. Soc. (Perkin I).* **4:** 513–522.
24. Stothers, J. B. 1972. Carbon-13 NMR Spectroscopy. Academic Press, New York. 80–85.
25. de Haan, J. W., and L. J. M. van de Ven. 1973. Configurations and conformations in acyclic, unsaturated hydrocarbons. A ^{13}C NMR study. *Org. Magnetic Reson.* **5:** 147–153.
26. Knight, J. A. 1974. Carbon-13 NMR spectra of some tetra- and pentacyclic triterpenoids. *Org. Magnetic Reson.* **6:** 603–611.
27. Joseph-Nathan, P., G. Mejia, and D. Abramo-Bruno. 1979. ^{13}C NMR assignments of side-chain methyls of C_{27} steroids. *J. Am. Chem. Soc.* **101:** 1289–1291.
28. Popjak, G., J. Edmond, F. A. L. Anet, and N. R. Easton, Jr. 1977. Carbon-13 NMR studies on cholesterol biosynthesized from [^{13}C]mevalonates. *J. Am. Chem. Soc.* **99:** 931–935.
29. Eggert, H., C. L. VanAntwerp, N. S. Bhacca, and C. Djerassi. 1976. Carbon-13 nuclear magnetic resonance spectra of hydroxy sterols. *J. Org. Chem.* **41:** 71–78.
30. Tsuda, M., E. J. Parish, and G. J. Schroepfer, Jr. 1979. Carbon-13 nuclear magnetic resonance studies of allylic hydroxysterols. Assignments of structure to 5α-cholest-8(14)-ene-3β,7α,15α-triol, an inhibitor of sterol biosynthesis. *J. Org. Chem.* **44:** 1282–1289.