[Chem. Pharm. Bull. 32(3) 865—871 (1984)]

High Pressure Liquid Chromatography of Sterol Benzoates

MASUO MORISAKI and NOBUO IKEKAWA*

Department of Chemistry, Tokyo Institute of Technology, Meguro-ku, Tokyo 152, Japan

(Received June 18, 1983)

The high pressure liquid chromatographic behavior of forty sterol benzoates on a Zorbax ODS reverse phase column and on a normal phase column of Zorbax SIL is described.

Keywords——HPLC; sterol benzoate; cholestadienol; phytosterol

For our studies on sterol structure-biological function relationships,[1] a variety of cholestenols and cholestadienols have been prepared. Some of them are potential intermediates of cholesterol biosynthesis. During these experiments, we frequently observed the undesired formation of isomeric olefins. Although these by-products could be removed by repeated crystallization and/or extensive chromatography, analyses by high pressure liquid chromatography (HPLC) were very helpful for ascertaining the purity of the synthetic samples. In this paper, we describe the HPLC behavior of the benzoates of various cholestenols and cholestadienols, as well as several other C_{25-29} sterols. The techniques presently described utilize a single, relatively short column of Zorbax SIL (normal phase adsorption, 15 cm) or Zorbax ODS (reverse phase, 25 cm) with isocratic solvent systems, and gives satisfactory separation of various sterol benzoates in comparatively short (<1 h) elution times.

The benzoates of fully saturated stereoisomeric sterols, cholestanol,[2] coprostanol and 3-epicholestanol, were clearly separated from each other on reverse phase HPLC as shown in Fig. 1. Figure 1 also shows the separation of the benzoates of cholesterol, 3-epicholesterol and 20-isocholesterol. It is noted that the 3α-benzoates have shorter retention times than the 3β-isomers. The relations were reversed on a normal phase column (Table I). This behavior of sterol benzoates seems inconsistent with the fact that 3β-equatorial hydroxy steroids are

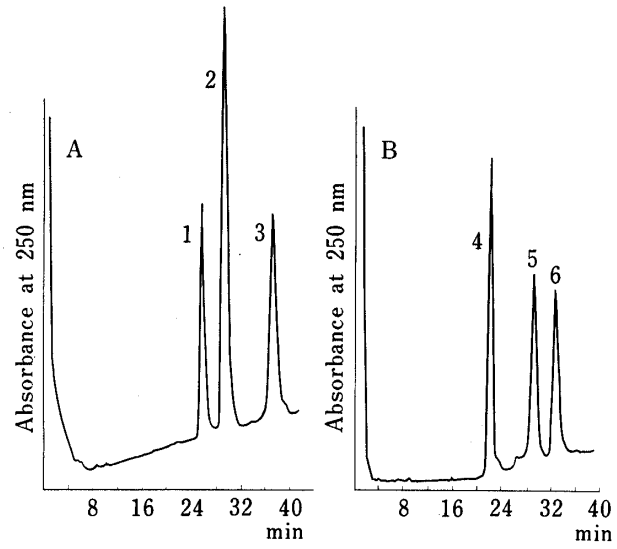

Fig. 1. Separation of Stereoisomeric Stanyl Benzoates (A) and Steryl Benzoates (B) on Zorbax ODS with Methanol (3.0 ml/min)

1, coprostanol; 2, 3-epicholestanol; 3, cholestanol; 4, 3-epicholesterol; 5, 20-isocholesterol; 6, cholesterol.

generally more polar than 3α-axial isomers.

Figure 2 indicates that the ODS reversed phase column was very effective for resolution of Δ^5 sterol benzoates differing in the number of carbon atoms (C_6, C_7, C_8, C_9 and C_{10}) in the side chain. Separation of some phytosterols is also shown in Fig. 2. Although cholesterol, campesterol and sitosterol were well separated, stigmasterol overlapped campesterol. Similar behavior of sterol acetates on μBondapak C_{18}[3] has been noted. Fucosterol and isofucosterol could not be resolved on Zorbax ODS, but they were easily separated on Zorbax SIL (Table I).

The C_{27} sterol benzoates with one double bond in various positions of the steroid nucleus[C-1, C-4, C-5, C-6, C-7, C-8 (9), C-8 (14), C-9 (11), C-14 and C-20 (22)] were fairly well resolved both on normal and reverse phase columns (Fig. 3). We failed to resolve the $\Delta^{8(14)}$ from Δ^{14} compounds, although they were reported[4,5] to be separated as the acetates on μPorasil.

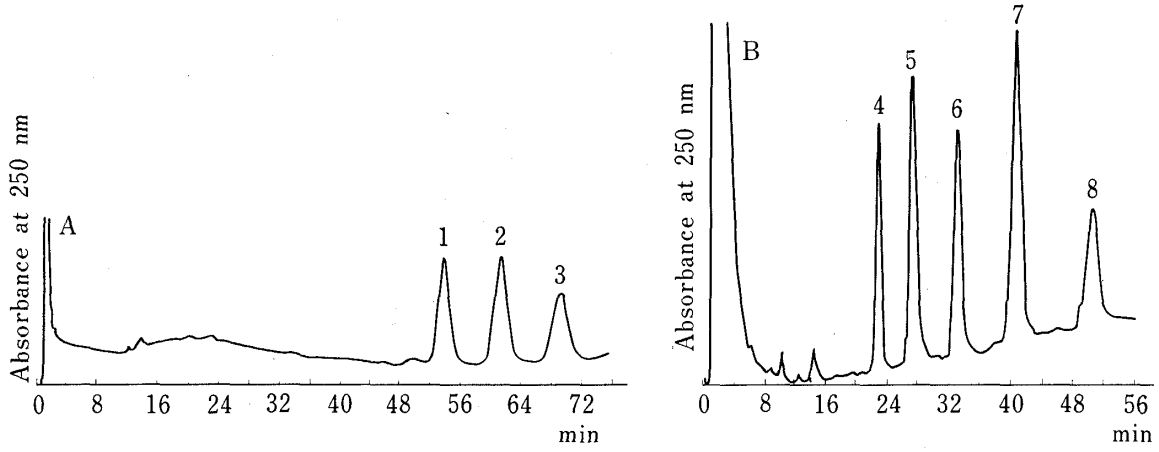

Fig. 2. Separation of Various Phytosterol Benzoates (A) and 20-Alkylated Pregnenol Benzoates (B) on Zorbax ODS with Methanol (A, 2.5 ml/min; B, 3.0 ml/min)

1, cholesterol, fucosterol and isofucosterol; 2, campesterol and stigmasterol; 3, sitosterol; 4, 20-isobutylpregnenol; 5, 20-isopentylpregnenol; 6, cholesterol; 7, 20-isoheptylpregnenol; 8, 20-isooctylpregnenol.

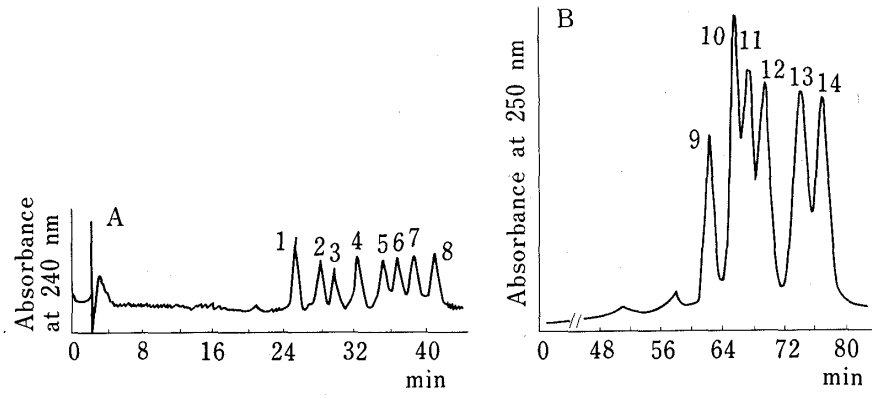

Fig. 3. Separation of Various Cholestenol Benzoates on (A) Zorbax SIL with Hexane–CH_2Cl_2 (80 : 1) at a Flow Rate of 1.0 ml/min, and on (B) Zorbax ODS with Methanol (1.1 ml/min)

1, Δ^1-cholestenol; 2, cholesterol; 3, Δ^4-cholestenol; 4, Δ^6-cholestenol; 5, $\Delta^{9(11)}$-cholestenol; 6, $\Delta^{20(E)}$-cholestenol; 7, $\Delta^{8(9)}$-, $\Delta^{8(14)}$- and Δ^{14}-cholestenol; 8, Δ^7-cholestenol; 9, Δ^4-cholestenol; 10, $\Delta^{9(11)}$-cholestenol; 11, $\Delta^{8(9)}$-cholestenol; 12, Δ^1-, $\Delta^{8(14)}$- and Δ^{14}-cholestenol; 13, Δ^6- and Δ^7-cholestenol; 14, cholesterol.

Separation of cholestadienol benzoates which have Δ^5 with an additional double bond at various positions in the side chain was more effectively accomplished by adsorption chromatography (Fig. 4) than by reverse phase HPLC. It can be seen that the isomeric pairs (*E* and *Z*) of the Δ^{20}, Δ^{22}, Δ^{23} compounds could be resolved from each other, but the 22*Z* and

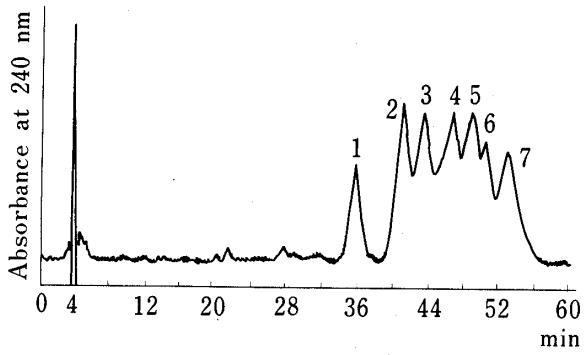

Fig. 4. Separation of Various Dehydrocholesterol Benzoates on Zorbax SIL with Hexane–CH_2Cl_2 (50 : 1) at a Flow Rate of 1.0 ml/min

1, cholesterol; 2, (20*Z*)-20-dehydrocholesterol; 3, (22*Z*)-22-dehydrocholesterol and (23*Z*)-23-dehydrocholesterol; 4, (22*E*)-22-dehydrocholesterol and (23*E*)-23-dehydrocholesterol; 5, 25-dehydrocholesterol; 6, 24-dehydrocholesterol; 7, (20*E*)-20-dehydrocholesterol.

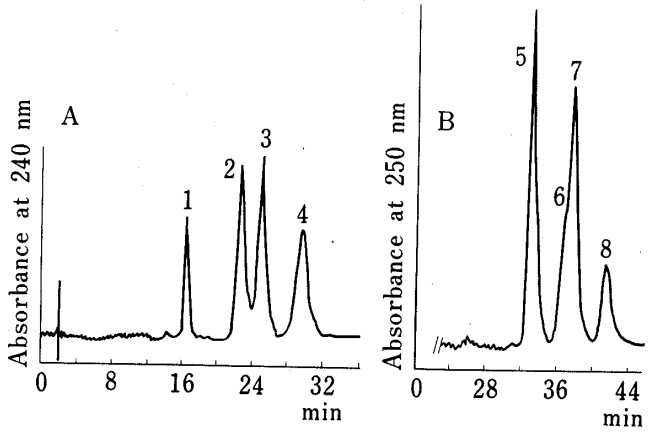

Fig. 5. Separation of Various Cholestadienol Benzoates on (A) Zorbax SIL with Hexane–CH_2Cl_2 (40 : 1) at a Flow Rate of 2.0 ml/min, or on (B) Zorbax ODS with methanol (1.5 ml/min)

1, $\Delta^{4,6}$-cholestadienol; 2, $\Delta^{5,7}$-cholestadienol; 3, $\Delta^{6,8}$- and $\Delta^{7,9(11)}$-cholestadienol; 4, $\Delta^{7,14}$- and $\Delta^{8,14}$-cholestadienol; 5, $\Delta^{7,14}$- and $\Delta^{8,14}$-cholestadienol; 6, $\Delta^{7,9(11)}$-cholestadienol; 7, $\Delta^{4,6}$- and $\Delta^{6,8}$-cholestadienol; 8, $\Delta^{5,7}$-cholestadienol.

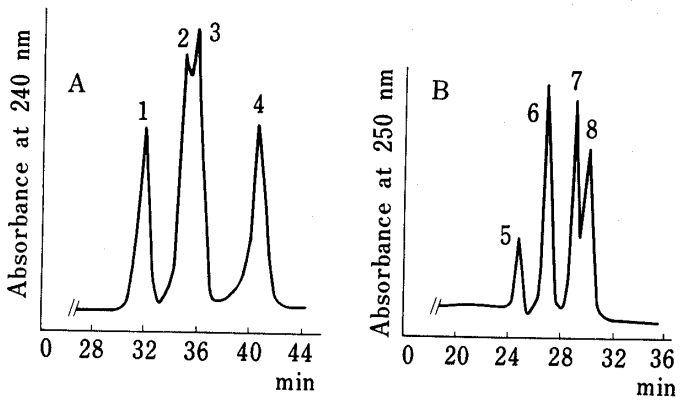

Fig. 6. Separation of Various Cholestadienol Acetates or Cholestadienol on Zorbax SIL: (A) Cholestadienol Acetates, Hexane–CH_2Cl_2 (20 : 1) at a Flow Rate of 1.5 ml/min; (B) Cholestadienols, Hexane–CH_2Cl_2 (2 : 1) at a Flow Rate of 1.0 ml/min

1, $\Delta^{5,7}$- and $\Delta^{4,6}$-cholestadienol; 2, $\Delta^{6,8}$-cholestadienol; 3, $\Delta^{7,9(11)}$-cholestadienol; 4, $\Delta^{7,14}$- and $\Delta^{8,14}$-cholestadienol; 5, $\Delta^{4,6}$-cholestadienol; 6, $\Delta^{5,7}$-cholestadienol; 7, $\Delta^{6,8}$- and $\Delta^{7,9(11)}$-cholestadienol; 8, $\Delta^{8(14)}$- and $\Delta^{7(14)}$-cholestadienol.

the 22*E* olefins overlapped with the 23*Z* and the 23*E* compounds, respectively. The 20*E* olefin was remarkably well separated from the 20*Z* isomer, and this may be due to a large difference in the conformations of their side chains.

Finally, the C_{27} sterols with a conjugated double bond at $\Delta^{4,6}$, $\Delta^{5,7}$, $\Delta^{6,8}$, $\Delta^{7,9(11)}$, $\Delta^{7,14}$ and $\Delta^{8,14}$ were chromatographed in the form of benzoates (Fig. 5), as well as the acetates or free alcohols (Fig. 6). Combination of the normal and reverse columns and the various derivatives made it possible to resolve five out of the six dienes, but separation of the 7,14-diene from the 8,14-diene could not be attained under our conditions.

HPLC of several sterols has been investigated with the free alcohols[6,7] or acetate

TABLE I. Relative Retention Times of Sterol Benzoates

Compound	Zorbax ODS Methanol	Zorbax SIL Hexane–CH_2Cl_2 (50 : 1)
Cholesterol benzoate	1.00	1.00
Cholestanol benzoate	1.12	0.95
Coprostanol benzoate	0.78	1.05
3-Epicholestanol benzoate	0.88	1.48
3-Epicholesterol benzoate	0.67	1.88
20-Isocholesterol benzoate	0.88	0.96
(20*R*)-20-Isobutyl-5-pregnen-3β-ol benzoate	0.69	1.07
(20*R*)-20-Isopentyl-5-pregnen-3β-ol benzoate	0.83	1.00
(20*R*)-20-Isoheptyl-5-pregnen-3β-ol benzoate	1.22	0.98
(20*R*)-20-Isooctyl-5-pregnen-3β-ol benzoate	1.53	0.95
Campesterol benzoate	1.14	1.00
24-Methylenecholesterol benzoate	0.82	1.54
Ergosterol benzoate	0.81	1.72
Sitosterol benzoate	1.29	1.00
Stigmasterol benzoate	1.14	1.04
Fucosterol benzoate	1.00	1.39
Isofucosterol benzoate	1.00	1.47
5α-Cholest-1-en-3β-ol benzoate	0.90	0.91
4-Cholesten-3β-ol benzoate	0.83	1.07
5α-Cholest-6-en-3β-ol benzoate	0.97	1.16
5α-Cholest-7-en-3β-ol benzoate	0.97	1.48
5α-Cholest-8(9)-en-3β-ol benzoate	0.93	1.39
5α-Cholest-8(14)-en-3β-ol benzoate	0.89	1.39
5α-Cholest-9(11)-en-3β-ol benzoate	0.86	1.27
5α-Cholest-14-en-3β-ol benzoate	0.95	1.39
(20*E*)-5α-Cholest-20(22)-en-3β-ol benzoate	0.81	1.34
(20*E*)-5,20(22)-Cholestadien-3β-ol benzoate	0.75	1.54
(20*Z*)-5,20(22)-Cholestadien-3β-ol benzoate	0.68	1.17
(22*E*)-5,22-Cholestadien-3β-ol benzoate	0.79	1.17
(22*Z*)-5,22-Cholestadien-3β-ol benzoate	0.71	1.32
(23*E*)-5,23-Cholestadien-3β-ol benzoate	0.75	1.29
(23*Z*)-5,23-Cholestadien-3β-ol benzoate	0.75	1.34
5,24-Cholestadien-3β-ol benzoate	0.78	1.45
5,25-Cholestadien-3β-ol benzoate	0.76	1.40
4,6-Cholestadien-3β-ol benzoate	0.75	1.10
5,7-Cholestadien-3β-ol benzoate	0.84	1.55
6,8(14)-Cholestadien-3β-ol benzoate	0.75	1.75
7,9(11)-Cholestadien-3β-ol benzoate	0.73	1.75
7,14-Cholestadien-3β-ol benzoate	0.66	2.34
8,14-Cholestadien-3β-ol benzoate	0.66	2.30

derivatives[3-5] using reverse phase columns of μBondapak C_{18}[3-7] or ODS-2,[6] and normal phase adsorption on μPorasil.[4,5] The resolution presently attained with sterol benzoates on Zorbax SIL or Zorbax ODS (Table I) was at least as good as those previously reported.[3-7] Analysis with benzoates has the important advantage of a much higher sensitivity of detection. The lower limit of detection of cholesterol benzoate with a UV detector set at 230 nm was of the order of 20 ng, as compared with 20 μg of cholesterol acetate by the use of a refractive index detector. Thus, the present method is extremely valuable for sterol detection, separation, isolation and/or identification.

Experimental

HPLC——HPLC analyses were performed at room temperature on a Shimadzu LC-3A instrument with a Zorbax SIL column (15 cm × 4.6 mm) using a ultraviolet (UV) detector (SPD-1), or on a Shimadzu LC-4A instrument with a Zorbax ODS column (25 cm × 4.6 mm) using a UV detector (SPD-2AS). The UV detectors were set at 240 or 250 nm. Other details of HPLC are given in the figure legends.

Sterol Benzoates——A solution of sterol (*ca.* 2 mg, *vide infra*) in pyridine (0.3 ml) containing benzoyl chloride (30 μl) was heated at 50 °C for 30 min, and then allowed to stand, after addition of H_2O (30 μl), at room temperature overnight. The mixture was partitioned between CH_2Cl_2 (3 ml) and 3% NaOH solution (3 ml). The lower layer was washed with 5% HCl and H_2O, dried over $MgSO_4$, and evaporated to dryness. The residue was chromatographed on a silica gel plate with benzene–*n*-hexane (1 : 1). The UV absorbing band at *Rf* 0.4 was scraped off and the sterol benzoate was eluted with CH_2Cl_2. The CH_2Cl_2 solution was concentrated to an appropriate volume for HPLC analysis.

Sterols——Cholesterol (Tokyo Kasei), campesterol (Research Plus Steroid Lab), ergosterol (Fluka) and stigmasterol (Merck) were commercial products. 5,7-Cholestadien-3β-ol, 5α-cholest-14-en-3β-ol and (20*Z*)-5,20(22)-cholestadien-3β-ol were gifts from Philips–Duphar, Prof. Y. Sato (Kyoritsu College of Pharmacy) and Dr. N. Koizumi (Teikoku Zoki Inc.), respectively. Sitosterol was purified from *Phellodendrom amurense*.[8] Fucosterol and 24-methylenecholesterol were purified from *Fucus evanescens*.[9] A mixture of cholestanol and coprostanol was prepared from cholesterol by catalytic hydrogenation on Pt[10] and they were resolved by flash chromatography[11] on silica gel with hexane–ethyl acetate (10 : 3). 3-Epicholesterol and 3-epicholestanol were prepared from cholesterol and cholestanol, respectively by the method of Bose *et al.*[12] Preparations of (20*E*)-5α-cholest-20(22)-en-3β-ol,[13] (20*E*)-5,20(22)-cholestadien-3β-ol,[13] (22*E*)-5,22-cholestadien-3β-ol,[14] 5,24-cholestadien-3β-ol,[15] 5,25-cholestadien-3β-ol,[16] (20*R*)-isobutyl-, isopentyl-, isoheptyl-, and isooctyl-5-pregnen-3β-ol,[17] 20-isocholesterol,[18] and a mixture of (23*E*)- and (23*Z*)-5,23-cholestadien-3β-ol[19] were previously described. The following compounds were prepared by the reported methods: 5α-cholest-1-en-3β-ol,[20] 4-cholesten-3β-ol,[21] 5α-cholest-6-en-3β-ol,[22] 5α-cholest-7-en-3β-ol,[23] 5α-cholest-8(9)-en-3β-ol,[24] 5α-cholest-8(14)-en-3β-ol,[25] 4,6-cholestadien-3β-ol,[26] 5α-cholesta-6,8(14)-dien-3β-ol,[27] 5α-cholesta-7,14-dien-3β-ol,[28] 5α-cholesta-7,9(11)-dien-3β-ol,[29] 5α-cholesta-8,14-dien-3β-ol,[30] isofucosterol,[31] (22*Z*)-5,22-cholestadien-3β-ol.[32]

5α-Cholest-9(11)-en-3β-ol——A mixture of 5α-cholest-9(11)-en-3α-ol[33] (211 mg, a gift from Dr. Y. Fujimoto of this laboratory), pyridinium chlorochromate (200 mg) and CH_2Cl_2 (7 ml) was stirred at room tempeature for 2 h. This was diluted with ethyl ether (7 ml) and filtered through a column of Florisil (5 g). Elution with 150 ml of CH_2Cl_2–ethyl ether (1 : 1) gave the crude 3-ketone (200 mg), which was treated with $LiAlH_4$ (50 mg) in tetrahydrofuran (7 ml) at room temperature for 10 min. Then ethyl acetate (0.5 ml) was added, followed by H_2O. Extraction with ethyl acetate, washing with dil. HCl and H_2O, drying over $MgSO_4$ and evaporation of the solvent gave a mixture of 3α- and 3β-ol (150 mg). This was chromatographed on silica gel with benzene–ethyl acetate (40 : 1) to give 5α-cholest-9(11)-en-3β-ol (120 mg), mp 122—123 °C (from methanol–ethyl ether). NMR δ: 0.60 (3H, s, 13-Me), 0.96 (3H, s, 10-Me), 3.5 (1H, m, 3α-H), 5.2 (1H, m, 11-H). MS *m/z*: 386 (M^+), 371 (M−Me), 368 (M−H_2O), 353, 273, 255.

(23*E*)-5,23-Cholestadien-3β-ol——This was prepared according to the scheme shown below, in which the key reaction is the reductive elimination of α-acetoxysulfone to produce predominantly or exclusively *trans*-olefin.[34] (20*R*)-20-(2-hydroxyethyl)-5-pregnen-3β-ol tetrahydropyranyl ether[35] (440 mg) was treated with *p*-toluenesulfonyl chloride (200 mg) in pyridine (3 ml) at room temperature overnight. Ice-water was added, then the mixture was extracted with ethyl acetate, washed with 1 N HCl and H_2O, and dried over $MgSO_4$. Evaporation of the solvent gave the 23-tosylate, which was refluxed with NaI (2 g) in acetone (35 ml) under stirring for 4 h. Most of the acetone was evaporated off and the residue was diluted with ethyl acetate. The mixture was washed with H_2O and dried over $MgSO_4$. Evaporation of the solvent gave a brown oil, which was chromatographed on silica gel with benzene to give the 23-iodide (390 mg), mp 123—125 °C (from *n*-hexane). A mixture of the 23-iodide (60 mg), benzenesulfinic acid sodium salt (100 mg, Aldrich) and dimethylformamide (3 ml) was stirred at 70—80 °C for 4 h. The reaction mixture was diluted with ethyl acetate, then washed with 2 N HCl and H_2O, and dried over $MgSO_4$. Evaporation of the solvent gave the crude sulfone (50 mg). NMR δ: 0.60 (3H, s, 13-Me), 3.0 (2H, m, 23-H_2), 7.5—8.0 (5H, m, phenyl). A

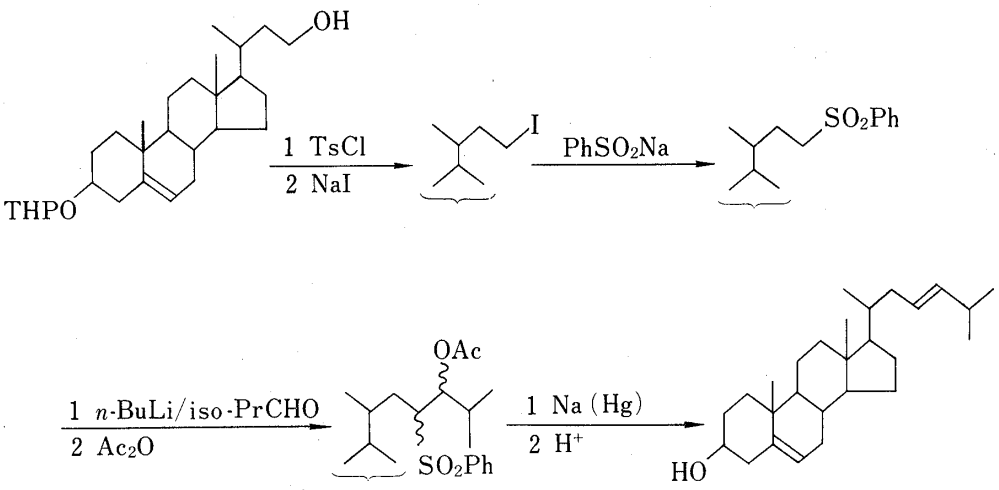

Chart 1

solution of this sulfone (50 mg) in tetrahydrofuran (1.5 ml) was treated with 1.2 M *n*-BuLi/*n*-hexane solution (0.1 ml) at −78 °C under argon. Then, 2-methylpropanal (50 μl) was added and the mixture was stirred at −78 °C for 1 h and then at room temperature for 1 h. Dilution with ethyl acetate, washing with H_2O, drying over $MgSO_4$ and evaporation of the solvent gave an oil (65 mg), which was treated with acetic anhydride/pyridine to give the crude 24-acetoxy-23-phenylsulfone (50 mg), after chromatography on silica gel with *n*-hexane–ethyl acetate (10 : 1). This product was stirred with 5% sodium amalgam (100 mg) in a mixture of ethyl acetate (0.5 ml) and methanol (1.0 ml) at −15 °C for 3 h and then at room temperature overnight. The mixture was extracted with ethyl acetate, washed with 1 N HCl and H_2O, and dried over $MgSO_4$. Evaporation of the solvent gave an oil, which was treated with conc. HCl (1 drop) in a mixture of CH_2Cl_2 (0.5 ml) and methanol (1 ml) at room temperature for 30 min. Extraction with ethyl acetate, washing with 5% $NaHCO_3$ and H_2O, drying over $MgSO_4$ and evaporation of the solvent gave an oil, which was subjected to silica gel TLC. Development with benzene–ethyl acetate (7 : 1) and elution with CH_2Cl_2 of the band at *Rf* 0.4 gave (23*E*)-5,23-cholestadien-3β-ol (5 mg), mp 136—137 °C (from *n*-hexane). NMR δ: 0.67 (3H, s, 13-Me), 1.0 (3H, s, 10-Me), 3.4 (1H, m, 3α-H), 5.3 (3H, m, 5,23,24-H), MS *m/z*: 384 (M^+), 366 ($M-H_2O$), 351 ($M-H_2O-Me$), 301 (cleavage at C-20,22), 283 ($301-H_2O$), 271. The spectroscopic and chromatographic properties were in accord with those of an authentic sample.[36]

References

1) Y. Isaka, M. Morisaki, and N. Ikekawa, *Steroids*, **38**, 417 (1981); H. Hagiwara, T. Nagasaki, Y. Saito, Y. Inada, T. Yasuda, H. Kojima, M. Morisaki, and N. Ikekawa, *Biochem. Inter.*, **5**, 329 (1982); S. Maruyama, M. Morisaki, and N. Ikekawa, *Steroids*, **40**, 341 (1982); S. Kawakami, M. Morisaki, and N. Ikekawa, *Chem. Pharm. Bull.*, in press. See also ref. 18.
2) Abbreviations: cholestanol, 5α-cholestan-3β-ol; coprostanol, 5β-cholestan-3β-ol; 3-epicholestanol, 5α-cholestan-3α-ol; cholesterol, 5-cholesten-3β-ol; 3-epicholesterol, 5-cholesten-3α-ol; 20-isocholesterol, (20*S*)-5-cholesten-3β-ol; campesterol, (24*R*)-24-methyl-5-cholesten-3β-ol; 24-methylenecholesterol, 5,24(28)-ergostadien-3β-ol; ergosterol, (22*E*)-5,7,22-ergostatrien-3β-ol; sitosterol, 5-stigmasten-3β-ol; stigmasterol, (22*E*)-5,22-stigmastadien-3β-ol; fucosterol, (24*E*)-5,24(28)-stigmastadien-3β-ol; isofucosterol, (24*Z*)-5,24(28)-stigmastadien-3β-ol.
3) H. Rees, P. L. Donnahey, and T. W. Goodwin, *J. Chromatogr.*, **116**, 281 (1976).
4) J. R. Thowsen and G. J. Schroepfer, Jr., *J. Lipid Res.*, **20**, 681 (1979).
5) E. Hansbury and T. J. Scallen, *J. Lipid Res.*, **21**, 921 (1980).
6) *Inter alia* L. N. Li, U. Sjostrand, and C. Djerassi, *J. Am. Chem. Soc.*, **103**, 115 (1981).
7) P. J. Trocha, S. J. Jasne, and D. B. Sprinson, *Biochemistry*, **16**, 472 (1979); J. M. DiBussolo and W. R. Nes, *J. Chromatogr. Sci.*, **20**, 193 (1982); R. J. Rodriguez and L. W. Parks, *Anal. Biochem.*, **119**, 200 (1982).
8) I. Nishioka, N. Ikekawa, A. Yagi, T. Kawasaki, and T. Tsukamoto, *Chem. Pharm. Bull.*, **13**, 379 (1965).
9) N. Ikekawa, M. Morisaki, and K. Hirayama, *Phytochemistry*, **11**, 2317, (1972).
10) E. B. Hershberg, E. Oliveto, M. Rubin, H. Staeudle, and L. Kuhlen, *J. Am. Chem. Soc.*, **73**, 1144 (1951).
11) W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
12) A. K. Bose, B. Lal, W. A. Hoffman III, and M. S. Manhas, *Tetrahedron Lett.*, **1973**, 1619.
13) K. Bannai, M. Morisaki, and N. Ikekawa, *J. Chem. Soc., Perkin Trans. 1*, 2116 (1976).
14) S. Takatsuto, B. Ying, M. Morisaki, and N. Ikekawa, *Chem. Pharm. Bull.*, **29**, 903 (1981).

15) H. Ohotaka, M. Morisaki, and N. Ikekawa, *J. Org. Chem.*, **38**, 1688 (1973).
16) M. Seki, J. Rubio-Lightbourn, M. Morisaki, and N. Ikekawa, *Chem. Pharm. Bull.*, **21**, 2783 (1973).
17) M. Morisaki, M. Shibata, C. Duque, N. Imamura, and N. Ikekawa, *Chem. Pharm. Bull.*, **28**, 606 (1980).
18) R. Goto, M. Morisaki, and N. Ikekawa, *Chem. Pharm. Bull.*, **31**, 3528 (1983).
19) M. Morisaki, C. Duque, N. Ikekawa, and M. Shikita, *J. Steroid Biochem.*, **13**, 545 (1980).
20) H. B. Henbest and R. A. L. Wilson, *J. Chem. Soc.*, **1956**, 3289.
21) Pl. A. Plattner, H. Heusser, and A. B. Kulkarni, *Helv. Chim. Acta*, **32**, 265 (1949).
22) D. R. James, R. W. Rees, and C. W. Shoppee, *J. Chem. Soc.*, **1955**, 1370; L. Caglioti, G. Cainelli, and G. Maina, *Tetrahedron*, **19**, 1057 (1963).
23) J. W. Cornforth, I. Y. Gore, and G. Popjak, *Biochem. J.*, **65**, 94 (1957).
24) P. J. Hylands, J. M. Midgley, C. Smith, A. F. A. Wallis, and W. B. Whalley, *J. Chem. Soc., Perkin Trans. 1*, **1977**, 817; M. Anastasia, A. Fiecchi, and G. Galli, *J. Org. Chem.*, **46**, 3421 (1981).
25) R. J. Chorvat and B. N. Desai, *J. Org. Chem.*, **44**, 3974 (1979).
26) V. A. Petrow, *J. Chem. Soc.*, **1940**, 66.
27) F. De. Simone, A. Dini, E. Finamore, L. Minale, C. Pizza, and R. Riccio, *J. Chem. Soc., Perkin Trans. 1*, **1981**, 1855.
28) E. J. Parish, T. E. Spike, and G. J. Schroepfer, Jr., *Chem. Phys. Lipids*, **18**, 233 (1977).
29) E. B. Hershberg, E. Oliveto, M. Rubin, H. Staeudle, and L. Kuhhen, *J. Am. Chem. Soc.*, **73**, 1144 (1951).
30) L. F. Fieser and G. Ourisson, *J. Am. Chem. Soc.*, **75**, 4404 (1953).
31) J. P. Dusza, *J. Org. Chem.*, **25**, 93 (1960).
32) R. F. N. Hutchins, M. J. Thompson, and J. A. Svoboda, *Steroids*, **15**, 113 (1970).
33) R. Breslow, R. J. Corcoran, B. B. Snider, R. J. Doll, P. L. Khanna, and R. Kaleya, *J. Am. Chem. Soc.*, **99**, 905 (1977).
34) P. J. Kocienski, B. Lythgoe, and S. Ruston, *J. Chem. Soc., Perkin Trans. 1*, **1978**, 829; P. J. Kocienski, B. Lythgoe, and I. Waterhouse, *ibid.*, 1045 (1980).
35) S. Maruyama, Y. Fujimoto, M. Morisaki, and N. Ikekawa, *Tetrahedron Lett.*, **23**, 1701 (1982).
36) Y. Fujimoto, M. Morisaki, and N. Ikekawa, *J. Chem. Soc., Perkin Trans. 1*, **1975**, 2302.