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Studies on Steroids. LXIII.[1] Synthesis of Cholesterol Analogs with a Modified Side Chain

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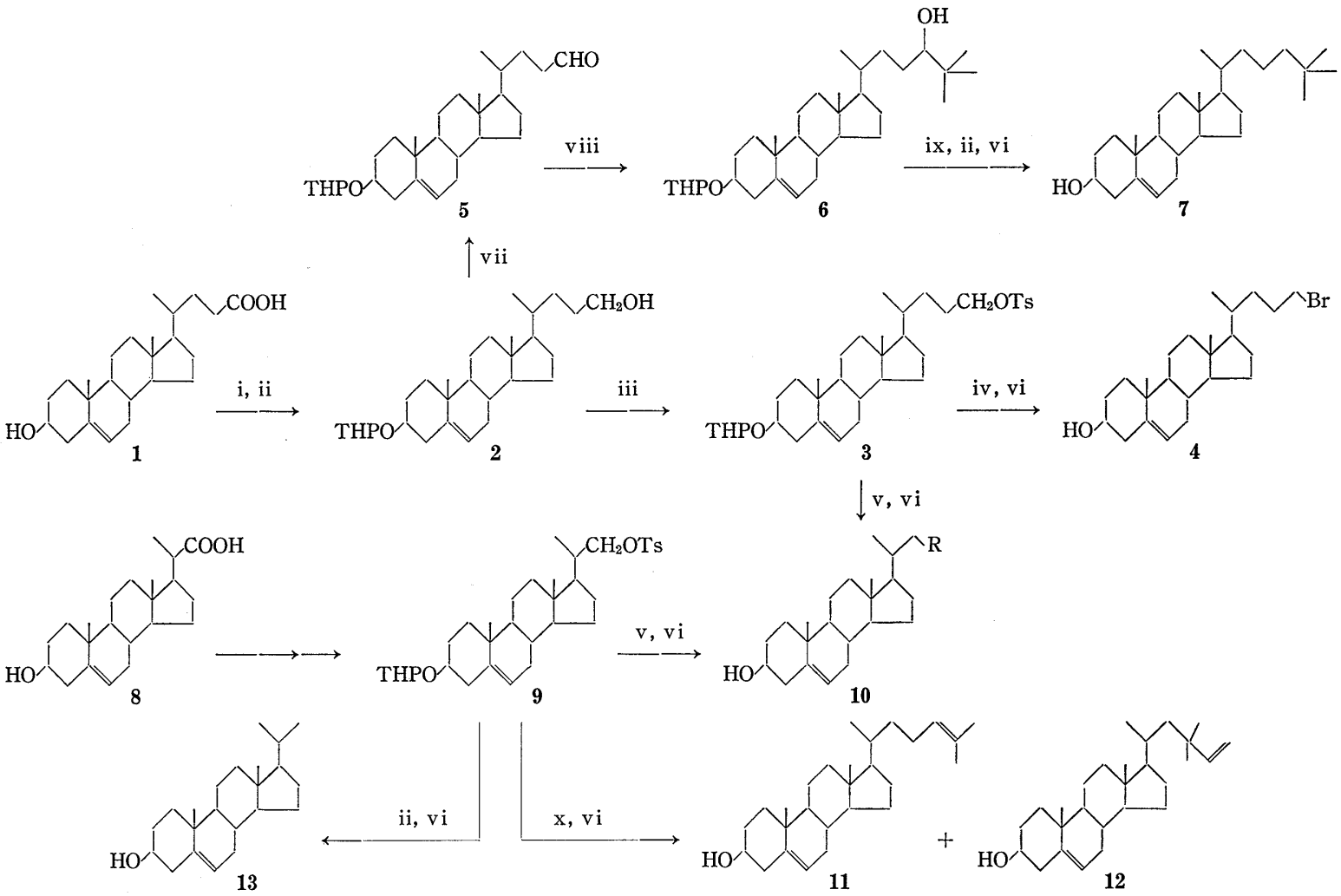
The Li_2CuCl_4-catalyzed Grignard reaction of the *p*-toluenesulfonates of 3β-tetrahydropyranyloxybisnorchol-5-en-22-ol (**9**) and 3β-tetrahydropyranyloxychol-5-en-24-ol (**3**) afforded several cholesterol analogs with a modified side chain (**10**). The reaction of **9** with 3-methyl-2-butenylmagnesium chloride gave a mixture of desmosterol (**11**) and its allylic isomer (**12**) in a ratio of 2:1. 25-Methylcholesterol (**7**) was prepared from the cholanal derivative (**5**) by reaction with *t*-butylmagnesium bromide followed by deoxygenation of the resulting 24-carbinol **6**.

Keywords——cholesterol analogs; sterol side chain; Li_2CuCl_4; 25-methylcholesterol; desmosterol

Cholesterol is not only a biogenetic precursor of steroid hormones but also functions as an important cell membrane constituent. However, those biological functions of cholesterol have not been fully rationalized in relation to its chemical structure. Bloch[3] has postulated that the planar structure of the rigid ring system of cholesterol has evolved in order to optimize its ability to condense the lipid phase of the membrane bilayer. We have focussed our attention on the biological significance of the sterol side chain and have now prepared several cholesterol analogs with a modified side chain for testing in various biological systems. These synthetic sterols may also be useful for the identification or structural determination of natural sterols with an unusually short or long side chain which are present in many marine invertebrates.[4] For the preparation of sterols lacking the oxygen function[5] in the side chain, cross coupling between the appropriate steroidal toluene sulfonate (tosylate) and Grignard reagents in the presence of Li_2CuCl_4[6] seemed most convenient. Using this method, Herz and Vàzquez[7] have recently prepared some modified sterols from 24-tosyloxychol-5-en-3β-ol methyl ether. In the present experiments, 17 cholesterol analogs **10** with a modified side chain were synthesized from the tetrahydropyranyl (THP) ether of 22-tosyloxybisnorchol-5-en-3β-ol (**9**) or 24-tosyloxychol-5-en-3β-ol (**3**).

The requisite 22-tosylate **9** was prepared from bisnorcholenic acid **8** as reported previously.[8] The other tosylate **3** was obtained by tosylation of the 24-alcohol **2**, which is easily available by THP etherification of commercial cholenic acid **1** followed by reduction with $LiAlH_4$.

1) Part LXII: C. Duque, M. Morisaki, N. Ikekawa, and M. Shikita, *Tetrahedron Lett.*, **1979**, 4479.
2) Location: *4259 Nagatsuta, Midori-ku, Yokohama 227, Japan.*
3) K. Bloch, "Reflections in Biochemistry," A. Kornberg, B.L. Horecher, L. Cornudella, and J. Oro Eds. Pergamon Press, Oxford, 1976, pp. 143—150.
4) R.M.K. Carlson, S. Popov, I. Massey, C. Delseth, E. Ayanoglu, T.H. Varkony, and C. Djerassi, *Bioorg. Chem.*, **7**, 453 (1978).
5) For the synthesis of sterols with various olefinic side chains by means of Wittig reaction, see Y.M. Sheikh and C. Djerassi, *Steroids*, **26**, 129 (1975).
6) *a*) M. Tamura and J. Kochi, *Synthesis*, **1971**, 303; *b*) Von G. Fouquet and M. Schlosser, *Angew. Chem.*, **86**, 50 (1974).
7) J.E. Herz and E. Vàzquez, *Steroids*, **27**, 133 (1976).
8) Y. Fujimoto, M. Morisaki, and N. Ikekawa, *J.C.S. Perkin I*, **1975**, 2302.

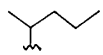
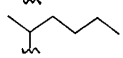
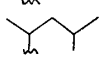
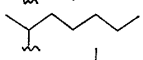
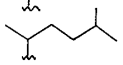
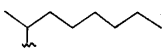
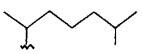
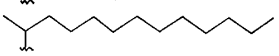
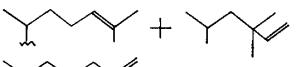
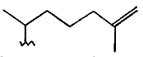
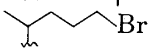
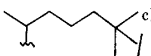
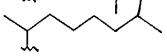
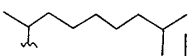
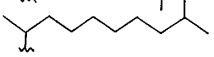


i, DHP; ii, LAH; iii, TsCl; iv, LiBr; v, RMgBr-Li_2CuCl_4; vi, H^+; vii, PCC; viii, ^{t}BuMgBr; ix, MsCl; x, $Me_2CH{=}CHCH_2MgCl$-Li_2CuCl_4

Chart 1

The copper-catalyzed coupling reaction was effected by treatment of the tosylates (**3** and **9**) with 30 mol equiv. of alkylmagnesium bromides in THF in the presence of 0.4 mol equiv. of Li_2CuCl_4. Acid hydrolysis of the crude product to remove the THP group afforded, after crystallization, the cholesterol analogs **10** in 50—80% yields. The structure and homogeneity of each of the reaction products were confirmed by gas chromatography–mass spectrometry (GC–MS) analysis of the trimethylsilyl ether; each gave a single peak on GC and showed the expected mass spectra (Table I).

Table I. Copper-catalyzed Coupling Reaction of the Tosylates (**3** and **9**)

Entry	Tosylate	Halide	Product **10**	Yield[a]	mp	RRT[b]	M+ of TMS ether
1	**9**	—	 [c]	—	135—136°	0.34	388
2	**9**	CH_3Br		83%	130—132°	0.40	402
3	**9**	C_2H_5Br		68	125—127°	0.50	416
4	**9**	*n*-C_3H_7Br		73	121—123°	0.66	430
5	**9**	iso-C_3H_7Br		54	144—146°	0.55	430
6	**9**	*n*-C_4H_9Br		67	133—136°	0.86	444
7	**9**	iso-C_4H_9Br		71	156—157°	0.74	444
8	**9**	*n*-$C_5H_{11}Br$		73	129—132°	1.14	458
9	**9**	iso-$C_5H_{11}Br$		55	145—147°	1.00	458
10	**9**	1-Br-decane		56	113—114°	5.04	528
11	**9**			2 : 1[d]	—	1.22 0.97	456 456
12	**3**			64	134—135°	1.17	456
13	**3**	*tert*-C_4H_9Br		54	118—120°	2.00	496 ; 494
14	**3**	—	 [c]	—	173—174°	1.14	472
15	**3**	iso-C_4H_9Br		58	127—129°	1.30	472
16	**3**	iso-$C_5H_{11}Br$		50	119—121°	1.69	486
17	**3**	iso-$C_5H_{13}Br$		75	114—116°	2.38	500

a) Isolation yield after crystallization.
b) GLC retention times are relative to cholesterol TMS ether on 1% OV-17 (1 m×4 mm i.d.) at 243°.
c) This was prepared by another method as described in the text.
d) Not isolated. The relative yields were estimated by GC analysis (Fig. 1B).

The shortest side chain analog (Entry 1), 20-methylpregnenolone (**13**) was prepared *via* hydrogenolysis of the 22-tosylate **9** with $LiAlH_4$. The vinyl Grignard reagent from 2-bromo-1-propene (Entry 12) afforded, on reaction with the 24-tosylate **3**, Δ^{25}-cholesterol[9] together with the 24-bromide **4** (10%). This bromide **4** seems to predominate when the reactivity of the Grignard reagent decreases. Thus, the attempted coupling (Entry 13) of *tert*-butylmagnesium bromide with **3** afforded only **4**, whose structure was ascertained by direct comparison with a sample prepared by treatment of **3** with LiBr.[10] The desired 25-methylcholesterol

9) M. Bergmann and J.P. Dusza, *J. Org. Chem.*, 23, 459 (1958).
10) *tert*-Butylmagnesium bromide is not intrinsically unreactive in the copper-catalyzed Grignard reaction. Thus, we reproduced the result of Fouguet and Schlosser[6b] that octyl tosylate coupled with ^{t}BuMgBr in the presence of Li_2CuCl_4 to provide 2,2-dimethyldecane in fair yield.

(**7**) (Entry 14) was therefore, prepared by an indirect method. Oxidation of the cholenol derivative **2** with pyridinium chlorochromate[11] gave the 24-al **5** in 71% yield, which, on reaction with *tert*-butylmagnesium bromide, furnished the *tert*-butyl carbinol **6** in 36% yield. The corresponding mesylate was reduced with $LiAlH_4$ and then hydrolyzed with acid to afford **7**.

When the allylic Grignard reagent from 3-methyl-2-butenyl chloride was reacted with the 24-tosylate **3** (Entry 11) in the presence of Li_2CuCl_4, a mixture of desmosterol (**11**)[12] and 23,23-dimethyl-26,27-bisnorcholesta-5,24-dien-3β-ol (**12**)[12g] was obtained in a ratio of *ca.* 2:1 as indicated by GC–MS analysis (Fig. 1A). When the same reaction was carried out without Li_2CuCl_4, the latter (**12**) was identified as the sole cross-coupled product, together with 20-hydroxymethylpregnenolone (Fig. 1B). Linstumelle *et al.*[13] recently reported that an allylic Grignard reagent catalyzed with CuI regioselectively coupled in the α position, affording the non-rearranged product. In accordance with these observations, the CuI-catalyzed Grignard reaction of **9** with 3-methyl-2-butenylmagnesium chloride afforded desmosterol (**11**) without accompanying allylic isomer (**12**). However, the material was contaminated with many other products, including 20-methylpregnenolone (**13**) (Fig. 1C).

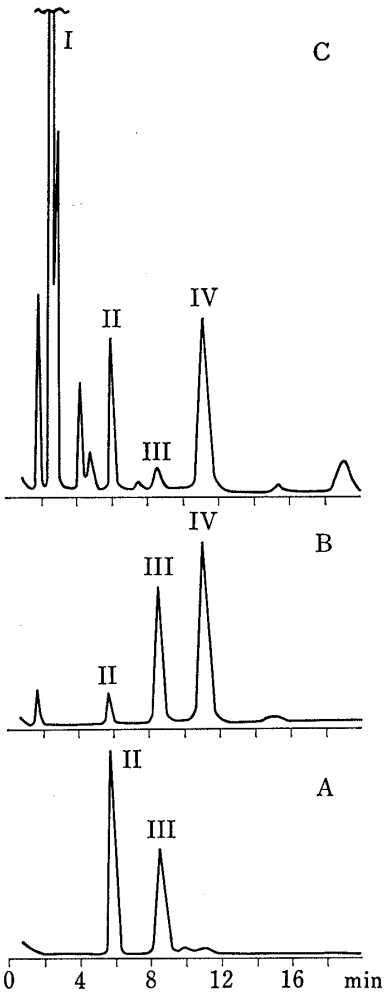


Fig. 1. Gas Chromatogram of the Grignard Reaction Products.

The 22-tosylate **9** was treated with 3-methyl-2-butenylmagnesium chloride without a catalyst (A), with Li_2CuCl_4 (B), or with CuI (C), and then hydrolyzed with acid. The TMS ethers of the crude products were analyzed by GC-MS using a 1% OV-17 column (1 m) at 243°. Peaks I (**13**), II (20-hydroxymethylpregnenolone), III (**12**) and IV (**11**) gave the following fragment ions (*m/e*):

I, 388 (M^+), 373 (M-Me), 298 (M-TMSOH), 283 (298-Me), 259 (M-129) and 129.
II, 476 (M^+), 461 (M-Me), 386 (M-TMSOH), 281 (M-2TMSOH-Me), 257 (M-TMSOH-129) and 129.
III, 456 (M^+), 441 (M-Me), 366 (M-TMSOH), 327 (M-129), 255, 253 and 129.
IV, 456 (M^+), 441 (M-Me), 366 (M-TMSOH), 351 (366-Me), 343 (M-side chain-2H), 327 (M-129), 253 and 129.

Experimental

Melting points were determined on a hot-stage microscope and are uncorrected. Proton magnetic resonance spectra were recorded on a Hitachi R-24A machine with $CDCl_3$ as a solvent and tetramethylsilane as an internal reference. Column chromatography was carried out with Merck Kieselgel 60 and thin-layer chromatography (TLC) was done on Merck precoated Kieselgel $60F_{254}$ plates (0.25 mm thick). Gas chromatography–mass spectrometry (GC–MS) was performed with a Shimadzu LKB 9000S machine. The following ab-

11) E.J. Corey and J.W. Suggs, *Tetrahedron Lett.*, **1975**, 2647.
12) For another synthesis of desmosterol see: *a*) U.M.H. Fagerlund and D.R. Idler, *J. Am. Chem. Soc.*, **79**, 6473 (1957); *b*) W. Bergmann and J.P. Dusza, *J. Org. Chem.*, **23**, 259 (1958); *c*) H. Ohtaka, M. Morisaki and N. Ikekawa, *J. Org. Chem.*, **38**, 1688 (1973); *d*) S.K. Dasgupta, D.R. Crump, and M. Gut, *J. Org. Chem.*, **39**, 1658 (1974); *e*) S.K. Dasgupta and M. Gut, *J. Org. Chem.*, **40**, 1475 (1975); *f*) T. Takeshita, S. Ishimoto, N. Ikekawa, *Chem. Pharm. Bull.*, **24**, 1928 (1976); *g*) N.A. Aptel, *J. Org. Chem.*, **43**, 2284 (1978).
13) F.D-Boumechal, R. Lorne, and G. Linstumelle, *Tetrahedron Lett.*, **1977**, 1181; G. Linstrumell, R. Lorne and H.P. Dang, *ibid.*, **1978**, 4069.

breviations are used: THF, tetrahydrofuran; THP, tetrahydropyranyl; TMS, trimethylsilyl; RRT, relative retention time.

Materials——Saturated alkyl bromides were products of Tokyo Kasei. 2-Bromo-1-propene and cholenic acid were obtained from Aldrich and Canada Packer, respectively. 3-Methyl-2-butenyl chloride was prepared by the method of Tanaka *et al.*[14]

Chol-5-ene-3β,24-diol 3-THP Ether (**2**)——A mixture of cholenic acid (**1**, 50 g), dihydropyran (44 ml), *p*-toluenesulfonic acid (30 mg) and benzene (1 l) was stirred at room temperature for 1 hr. THF (50 ml) and $LiAlH_4$ (11 g) were then added slowly at 0° and the mixture was stirred at room temperature overnight. Moist ether (300 ml) and H_2O (100 ml) were slowly added and the mixture was extracted with ethyl acetate, washed with brine, dried over $MgSO_4$ and concentrated to give the THP ether **2** (55 g), mp 131—132° (from acetone), δ 0.68 (3H, s, 13-Me), 1.0 (6H, m, 10- and 21-Me), 3.1—4.2 (3H, m, 3α-H and 6′-H_2 of THP), 4.7 (1H, m, 2′-H of THP), 5.35 (1H, m, 6-H). *Anal.* Calcd for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 77.73; H, 11.04.

24-Tosyloxychol-5-en-3β-ol THP Ether (**3**)——A solution of the 24-alcohol **2** (4.5 g) and *p*-toluenesulfonyl chloride (3 g) in pyridine (20 ml) was allowed to stand at room temperature overnight. Ice-water was added and the mixture was extracted with ethyl acetate, washed with 1 N HCl and H_2O, dried over $MgSO_4$ and evaporated to dryness. The residue was crystallized from acetone to give the 24-tosylate **3** (3.7 g), mp 100—105°, δ 0.67 (3H, s, 13-Me), 1.02 (3H, s, 10-Me), 2.45 (3H, s, aromatic Me), 3.1—4.2 (3H, m, 3α-H and 6′-H_2 of THP), 4.1 (2H, t, J=6 Hz), 4.75 (1H, m, 2′-H of THP), 5.4 (1H, m, 6-H), 7.5 and 8.0 (4H, a pair of d, J=9 Hz). *Anal.* Calcd for $C_{36}H_{52}O_5S$: C, 72.20; H, 9.09. Found: C, 71.74; H, 9.06.

General Procedures for the Copper-catalyzed Grignard Reaction (Table I)——Alkyl halide (5.3 mmol) was added to a suspension of Mg (128 mg, 5.3 mmol) in anhydrous THF (5 ml) through a syringe under argon. The mixture was stirred for 15 min at room temperature and then cooled to 0°. An aliquot (0.7 ml) of a solution of Li_2CuCl_4 (prepared by mixing 85 mg of LiCl, 135 mg of $CuCl_2$ and 10 ml of THF) was slowly added. Next, 2 ml of a THF solution of the tosylate (**3** or **9**) (0.175 mmol) was added to the stirred mixture during 1 min. After 2 hr at 0°, the reaction mixture was left at room temperature overnight and then 1 N HCl (10 ml) was added at 0°. After stirring for 15 min, the mixture was extracted with ether, washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and evaporated to dryness to give colorless crystals. The crude product was heated with a solution of conc.HCl (50 μl) in methanol (15 ml) for 10 min. Extraction with ether, washing with saturated $NaHCO_3$ solution and brine, drying over $MgSO_4$ and removal of the solvent gave colorless crystals which were recrystallized from methanol to give **10** (Table I). About 0.5 mg of **10** was treated with trimethylsilylimidazole (20 μl) at room temperature for 5 min to give the corresponding TMS ether, which was analyzed by GC–MS using 1% OV-17 (1 m×0.4 mm i.d.) at 243°.

Reaction of 9 with 3-Methyl-2-butenylmagnesium Chloride (See Fig. 1)——(a) Without Catalyst: The reaction was performed as described in "General procedures" except that Li_2CuCl_4 was omitted from the reaction mixture. The crude product was partitioned between *n*-hexane and 75% methanol. The upper fraction (**12**), mp 176—180° (from methanol) gave the following NMR data: δ 0.68 (3H, s, 13-Me), 1.0 (12H, m, 10-, 21- and 23-Me_2), 3.5 (1H, m, 3α-H), 4.87 (1H, q, J=10 and 2 Hz, 25-H_a), 4.90 (1H, q, J=17 and 2 Hz, 25-H_b), 5.3 (1H, m, 6-H), 5.85 (1H, q, J=10 and 17 Hz). The major component of the lower fraction had the same mobility on TLC as 20-hydroxymethylpregnenolone prepared by $LiAlH_4$ reduction of bisnorcholenic acid (**8**). The TMS ether of this authentic material was identical (RRT and mass spectrum) with peak II in Fig. 1.

(b) With Li_2CuCl_4: The reaction was carried out according to the "General procedures." Separation of desmosterol (**11**) from the crude product was effected in the form of the acetate by $AgNO_3$ (5%)-impregnated TLC, developing 3 times with hexane–CH_2Cl_2 (1: 1). *Rf* values of the **11**-acetate and **12**-acetate were 0.45 and 0.35, respectively.

(c) With CuI: Grignard reagent prepared from Mg (48 mg, 1.8 mmol), 3-methyl-2-butenyl chloride (185 mg, 1.8 mmol) and THF (5 ml) was treated at 0° with CuI (34 mg, 0.18 mmol) followed by a THF (1 ml) solution of the tosylate (**9**, 100 mg, 0.18 mmol). The subsequent procedure was as described in the "General procedures."

24-Bromochol-5-en-3β-ol (**4**)——(a) The reaction of the 24-tosylate **3** (1.0 g) with *tert*-butylmagnesium bromide according to the "General procedures" yielded the 24-bromide **4** (430 mg), mp 118—120° (from methanol), δ 0.68 (3H, s, 13-Me), 1.0 (6H, m, 10- and 21-Me), 3.3 (1H, t, J=6 Hz, 24-H_2) and 5.4 (1H, m, 6-H). *Anal.* Calcd for $C_{24}H_{39}OBr$: C, 68.07; H, 9.28. Found: C, 67.43; H, 9.54. The TMS ether derivative of this bromide showed *m/e* 496; 494 (M^+), 406; 404 (M-TMSOH), 391; 389 (M-TMSOH-Me), 367; 365 (M-129) and 129.

(b) The 24-tosylate **3** (750 mg) was refluxed with LiBr (1.0 g) in acetone (10 ml) for 1 hr. Cooling to 0° provided crystals of the 24-bromo-3-THP ether (540 mg) mp 113—115°; this material was treated with conc.HCl (20 μl) in methanol (5 ml) at 60° for 5 min. Extraction with CH_2Cl_2 and usual work-up afforded the 24-bromide **4** (420 mg); its spectral and chromatographic properties were identical with those of the sample prepared above (a).

14) J. Tanaka, T. Katagiri, and Y. Yamada, Yukikagobutsugoseiho No. 19. 1969, pp. 7—9.

3β-Tetrahydropyranyloxychol-5-en-24-al (5)——A solution of the 24-alcohol **2** (127 mg) in CH_2Cl_2 (5 ml) was added in one portion to a suspension of pyridinium chlorochromate[11] (97 mg) in CH_2Cl_2 (2 ml). After stirring for 2 hr at room temperature, the mixture was diluted with ether (50 ml) and filtered through a column of Florisil (3 g). The filtrate was concentrated and the residue was chromatographed on silica gel with benzene to give the 24-aldehyde **5** (90 mg), mp 135—137° (from hexane), δ: 0.70 (3H, s, 13-Me), 1.02 (3H, s, 10-Me), 3.1—4.2 (3H, m, 3α-H and 6′-H_2 of THP), 4.7 (1H, m, 2′-H of THP), 5.4 (1H, m, 6-H) and 9.7 (1H, t, J= 1.2 Hz). *Anal.* Calcd for $C_{29}H_{44}O_3$: C, 78.68; H, 10.47. Found: C, 78.97; H, 10.61.

25-Methylcholest-5-en-3β-ol (7)——A suspension of Mg (190 mg) in THF (5 ml) was treated with *tert*-butyl bromide (1.2 g) at room temperature. After stirring for 1 hr, the 24-aldehyde (**5**, 300 mg) in THF (4 ml) was added at 0° and stirring was continued at 0° for 1 hr and then at room temperature overnight. Cold NH_4Cl solution was added and the mixture was extracted with ether, washed with brine, dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel with benzene to give the *tert*-butyl carbinol **6** (122 mg), δ: 0.88 (9H, s, *t*-Bu). The bis-TMS ether derivative was prepared by acid hydrolysis followed by treatment with TMS-imidazole: *m/e* 560 (M^+), 545 (M-Me), 503 (M-Bu), 413 (M-Bu-TMSOH), 323 (M-Bu-2TMSOH). The carbinol **6** (120 mg) was treated with methanesulfonyl chloride (0.3 ml) in pyridine (2.0 ml) at 0° overnight. Usual work-up gave the mesylate (145 mg), δ: 0.97 (15H, ^{t}Bu, 20-Me and 10-Me), 2.98 (3H, s, Ms), which was then refluxed with $LiAlH_4$ (30 mg) in THF (5 ml) for 6 hr. Moist ether and NH_4Cl solution were added and the mixture was extracted with ether, washed with brine, dried over $MgSO_4$ and evaporated to dryness. The crude product was filtered through a short column of silica gel with benzene. The purified material was heated with conc HCl (20 μl) in a mixture of ether (2 ml) and methanol (4 ml) at 60° for 10 min. Work-up as usual and crystallization from methanol gave 25-methylcholesterol (**7**) (39 mg), mp 173—174°, δ: 0.68 (3H, s, 13-Me), 0.87 (9H, s, ^{t}Bu), 1.0 (6H, m, 10- and 20-Me), 3.5 (1H, m, 3α-H) and 5.35 (1H, m, 6-H). *Anal.* Calcd for $C_{28}H_{48}O$: C, 83.93; H, 12.08. Found: C, 83.42; H, 12.03.

20-Methylpregn-5-en-3β-ol (13)——The 22-tosylate **9** (100 mg) was refluxed with $LiAlH_4$ (50 mg) in THF (5 ml) for 30 min. Moist ether and 1 N HCl were added and the mixture was extracted with ether. Most of the ether was evaporated off, and the residue was washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel (5 g) with a mixture of benzene and ether (30: 1) to give, after crystallization from methanol, 20-methylpregnenolone (65 mg), mp 135—136°, δ: 0.68 (3H, s, 13-Me), 0.85 (3H, d, J=6 Hz, 20-Me_a), 0.94 (3H, d, J=6 Hz, 20-Me_b), 1.01 (3H, s, 10-Me), 3.5 (1H, m, 3α-H) and 5.3 (1H, m, 6-H).

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