(24) N. Harada and K. Nakanishi, *J. Amer. Chem. Soc.*, **91**, 3989 (1969); M. Koreeda, N. Harada, and K. Nakanishi, *Chem. Commun.*, 548 (1969).
(25) Melting points are uncorrected. Rotations were run in chloroform unless otherwise stated, uv spectra in 95% ethanol on a Cary Model 14 recording spectrophotometer, ir spectra as KBr pellets on a Perkin-Elmer Model 257 grating spectrometer, CD curves in absolute methanol on a Jasco Model ORD/UV recording spectrometer, mass spectra on a high-resolution MS-902 mass spectrometer at 70 meV, and nmr spectra on Varian A-60, Bruker HFX-90, and HFX-270 nmr spectrometers. Analyses were performed by Dr. F. Pascher, Bonn, Germany.
(26) W. Herz and G. Högenauer, *J. Org. Chem.*, **27**, 905 (1962).
(27) By spin decoupling.

Synthesis of 5α-Cholesta-7,24-dien-3β-ol and Cholesta-5,7,24-trien-3β-ol[1]

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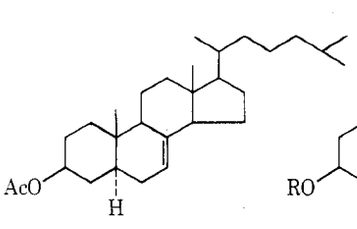
The title compounds were synthesized and were utilized for the identification of products of the *in vitro* incubation of mevalonic acid with yeast homogenates.

In the course of studies of the biosynthesis of sterols from (3*RS*,2*R*)-[2-^{14}C,2-^{3}H]mevalonic acid (MVA) and (3*RS*,2*S*)-[2-^{14}C,2-^{3}H]MVA in yeast homogenates, an unknown metabolite was obtained in a significant radioactive yield.[3] Frequently the metabolite contained *ca.* 20% of the total ^{14}C radioactivity of the nonsaponifiable residue. The acetate of the unknown on hydrogenation over nickel sponge in ethyl acetate[4] gave 5α-cholest-7-en-3β-ol acetate (**1**), thus revealing a C_{27} structure.[3] Analysis of the tritium content of the 7-en-3β-ols (**1**) derived from the *R* and the *S* metabolites indicated the incorporation in each case of four isotopic hydrogens. On theoretical grounds the presence of a tritium atom at C-26 of the metabolite and in **1** was assumed *a priori*. We have determined[3] the distribution of the isotopic hydrogens at C-1 and C-7 of **1** and have also deduced the distribution of ^{3}H at C-15. Based on our data it became clear that the metabolite retained both the 2-pro *R* and 2-pro *S* hydrogens of MVA at C-22. This establishes that the unknown does not have a C-22 double bond.[5] In view of the fact that the biosynthetic product had a C_{27} and *not* a C_{28} framework, it seemed reasonable to assume that it still retained the C-24 unsaturation required for the introduction of the 24-alkyl moiety.[6] The body of the available evidence suggested therefore either 5α-cholesta-7,24-dien-3β-ol (**2a**) and/or cholesta-5,7,24-trien-3β-ol (**3a**) as the likely structure for the metabolite.



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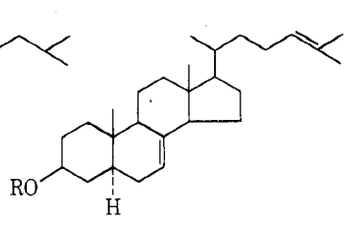
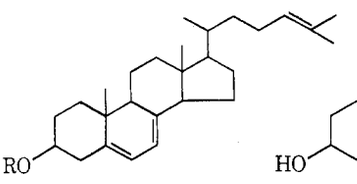
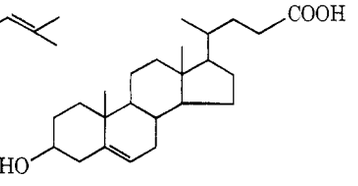


2a, R = H
b, R = CH_3CO



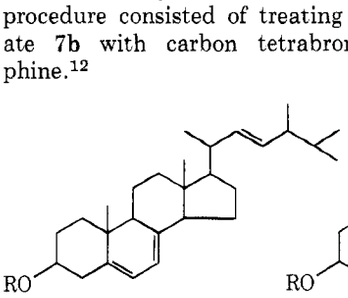
3a, R = H
b, R = CH_3CO



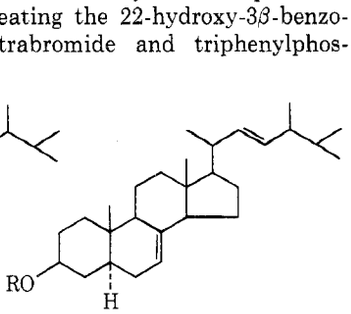
4

Cholesta-5,7,24-trien-3β-ol (**3a**) was previously prepared by Scallen.[7] Selective hydrogenation of the 5(6) double bond of **3a** afforded[8] 5α-cholesta-7,24-dien-3β-ol (**2a**). Since we required somewhat larger amounts of the diene **2a** and the triene **3a**, we undertook the preparation of these compounds and concentrated first on the synthesis of 5α-cholesta-7,24-dien-3β-ol (**2a**). We projected several approaches (*e.g.*, using **4** as starting material); however, the availability of ergosterol (**5a**) influenced our decision on a route *via* **7a** which we planned to couple with $(CH_3)_2C{=}CHCH_2X$.

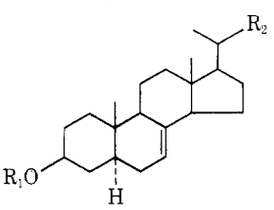
With this in mind, a benzene solution of ergosteryl benzoate (**5b**) was hydrogenated in the presence of tris(triphenylphosphine)rhodium chloride catalyst[9] to give 5α-ergosta-7,22-dien-3β-ol benzoate (**6**) in nearly quantitative yield. The diene **6** was dissolved in methylene chloride–pyridine[10] and ozonized at −78°. Following a reductive work-up, the aldehyde **7a** was isolated and subsequently reduced with sodium borohydride to the alcohol **7b**. The alcohol **7b** was converted to the bromide **7c** by two methods. The less convenient, two-step procedure involved the preparation first of the 22-tosyl ester 3β-benzoate **7d**. Displacement of the tosyl moiety was then carried out by warming a mixture of **7d**, lithium bromide, and dimethyl sulfoxide[11] to yield **7c** in *ca.* 70–75% yield. The preferred procedure consisted of treating the 22-hydroxy-3β-benzoate **7b** with carbon tetrabromide and triphenylphosphine.[12]



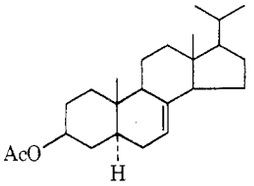
5a, R = H
b, R = C_6H_5CO
c, R = CH_3CO
d, R = p-$CH_3C_6H_4SO_2$



6, R = C_6H_5CO



7a, R_1 = C_6H_5CO; R_2 = CHO
b, R_1 = C_6H_5CO; R_2 = CH_2OH
c, R_1 = C_6H_5CO; R_2 = CH_2Br
d, R_1 = C_6H_5CO; R_2 = $CH_3C_6H_4SO_2$



8

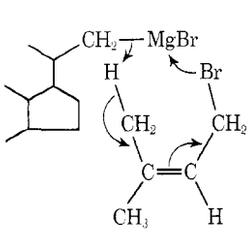
The coupling of the bromide **7c** with γ,γ-dimethylallyl bromide [$(CH_3)_2C{=}CHCH_2Br$] was carried out in the presence of magnesium.[13] In general the reaction posed some problems mainly due to self-condensation of dimethylallyl bromide. Also, under certain conditions, the C-3 benzoate moiety seemed to react preferentially with dimethylallylmagnesium bromide. When the reaction was carried out as described in the Experimental Section, two major steroidal products were formed, and these were resolved by argentation layer chromatography of their acetates.

The more polar product was **2b**, which after saponification gave the required **2a**, mp 96–98° (reported[8] mp 99–102°). The product **2a** gave a single peak on glc. Its mass spectrum had a peak for the molecular ion (*m/e* 384, M^+) and the fragmentation pattern was consistent with the assigned structure.[14] More important, however, was the nmr spectrum, which showed a multiplet at 5.25 ppm corresponding to the two vinylic hydrogens at C-7 and C-24. The crucially important signals for the vinylic 26- and 27-methyls were located at 1.60 (3 H) and 1.68 (3 H) ppm. Finally the chemical shifts of 0.54 ppm of the 18-methyl and 0.8 ppm of the 19-methyl are in good agreement with the calculated[15] and reported[26] values of 0.55 and 0.80 ppm, respectively. The presented evidence fully supports structure **2a**.

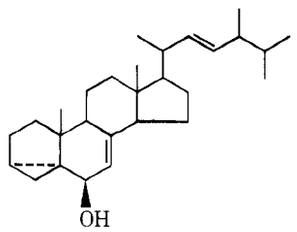
The less polar major product of the coupling reaction was identified as 20-methyl-5α-pregn-7-en-3β-ol acetate (**8**), mp 122–123°. The mass spectrum of **8** had a peak at *m/e* 358 for the molecular ion which corresponds to $C_{24}H_{38}O_2$. The nmr spectrum in $CDCl_3$ had a signal at 5.15 ppm for the C-7 vinylic proton and a pair of doublets at 0.86 (J = 6 Hz, 3 H) and 0.95 ppm (J = 6 Hz, 3 H) for the 21 and 22 secondary methyls. Finally, the chemical shifts of the 18- and 19-methyls are also in agreement with the proposed structure.[15]

The formation of the 20-methylpregnene is mechanistically interesting, since it seems to involve the reductive elimination of the 22-bromide from **7c**. A possible rationalization of the results is presented in **9**.

For the synthesis of cholesta-5,7,24-trien-3β-ol (**3a**) initially we again considered ergosterol as the starting material. We planned to protect the sensitive 5,7-diene moiety by converting ergosterol (**5a**) to isoergosterol[16] (**10**). However, ozonization[10] of **10** and work-up of the ozonide with zinc and acetic acid gave mainly the dehydrated cyclodiene **11**. In view of this difficulty and the anticipated difficulties of coupling the steroidal C_{22} bromide with dimethylallyl bromide, we abandoned this approach.



9



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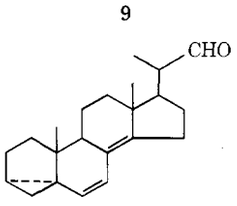

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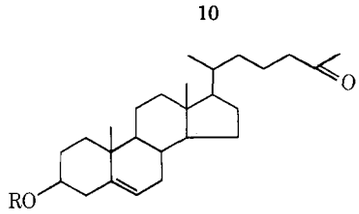
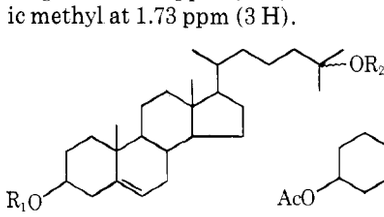


12a, R = H
b, R = CH_3CO
c, R = tetrahydropyranyl

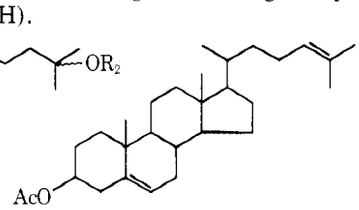
An alternative route from 26-norcholest-5-en-25-on-3β-ol (**12a**) was considered and explored. We planned to introduce the C-7 double bond first, then carry out a Grignard reaction[17] with CH_3MgI on the 25-ketone and finally dehydrate the C-25 hydroxyl.

Prior to embarking on this route it was necessary to devise a procedure for the selective dehydration of the 25-hydroxyl without disturbing the homoannular diene system of ring B. From the outset we omitted mineral acids from our considerations, since these are known to cause isomerization of 5,7-dienes.[18] The dehydration procedures with acetic anhydride–acetic acid[19] or with methyl(carboxysulfamoyl)triethylammonium hydroxide inner salt[20,21] (**20**) seemed more promising. Therefore the stability of cholesta-5,7-dien-3β-ol acetate (**21**) toward these reagents was tested, and the reaction was followed by uv and argentation tlc. In the course of the prolonged boiling with acetic anhydride–acetic acid[19] required for the removal of a 25-hydroxyl, the 5,7-diene **21** rearranged, as evidenced by the disappearance of the characteristic uv absorption. In contrast, treatment of **21** with reagent **20** did not cause rearrangement and the starting material was recovered in good yield.

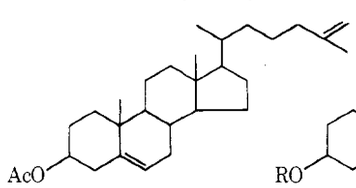
However, the question of the relative yields of Δ^{24} and Δ^{25} isomers still remained. For this purpose the 3β-acetoxy 25-ketone **12a** was treated with methylmagnesium iodide[17] and the resulting 3β,25-diol **13a** was acetylated with pyridine and acetic anhydride. In addition to the expected 3β-monoacetate **13b**, about 10% of the 3β,25-diacetate **13c** was also formed. The monoacetate **13b** was then dehydrated by the method of Burgess, *et al.*,[20] to yield a mixture of olefinic products. The mixture was resolved by argentation layer chromatography and two diene acetates were isolated. The more mobile diene (43%) proved to be desmosterol acetate (**14**), mp 96–98°, *m/e* 366 (M^+ − acetate). The less mobile product (46%) was the 5,25-diene acetate **15**, mp 108–109°, *m/e* 366 (M^+ − acetate). The nmr spectrum of **15** had a signal for the 26-methylene hydrogens at 4.75 ppm (2 H) and a singlet for a single vinylic methyl at 1.73 ppm (3 H).



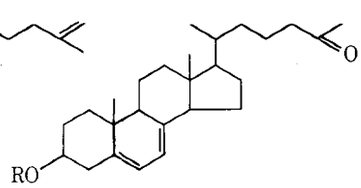
13a, $R_1 = R_2 = H$
b, $R_1 = CH_3CO$; $R_2 = H$
c, $R_1 = R_2 = CH_3CO$



14



15



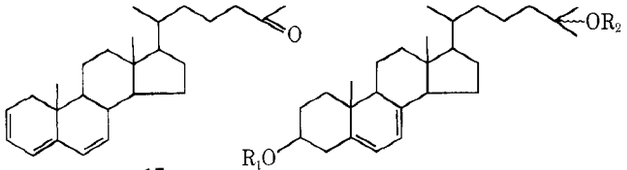
16a, R = H
b, R = CH_3CO

With this information at hand we proceeded with the synthesis of the 5,7,24-triene **3b** using the 3β-tetrahydropyranyl ether **12c** as a protective group for the 3-hydroxyl. Hence the ether **12c** was treated with *N*-bromosuccinimide in the presence of pyridine. The recovered mixture of bromides was dehydrobrominated with collidine in boiling xylene.[22] The uv spectrum of the crude dehydrohalogenation residue showed absorption maxima at 250, 272, and 283 nm. The absorption at 272 and 283 nm was interpreted as indicative of the presence of the required 5,7-diene

group. Fractionation of the crude residue by argentation layer chromatography showed a single product, which was recovered and identified as the triene **17**. The triene **17**, mp 97–98°, had uv absorption maxima at 294, 307, and 320 nm. The mass spectrum had a peak at *m/e* 366 for the molecular ion, revealing the elimination of the tetrahydropyranoxy moiety. The absence of this moiety was confirmed by the nmr spectrum, which showed the presence of five vinylic protons as expected for **17**. A similar, but minor reaction was observed in the course of *N*-bromosuccinimide bromination and dehydrobromination of cholesteryl acetate.[23] Apparently, in the present case, exposure of the product to argentation tlc ($AgNO_3$–SiO_2) promoted the elimination and resulted in the formation of the triene as the main product.

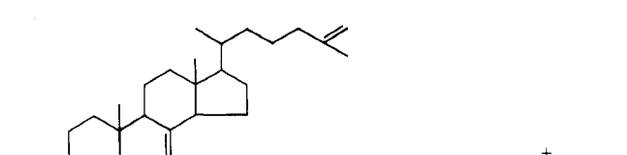
Under the circumstances we abandoned the use of the tetrahydropyranyl moiety as a protective group and prepared 26-norcholesta-5,7-dien-25-on-3β-ol acetate (**16b**) from acetate **12b** by *N*-bromosuccinimide bromination followed by dehydrobromination. The obtained 25-keto-5,7-diene **16b** was treated in the dark under nitrogen with methylmagnesium iodide.[17] The resulting diol **18a** was immediately reacetylated with pyridine and acetic anhydride to yield the 3-monoacetate **18b** as well as about 10% of the diacetate **18c**.

The 3-monoacetate-25-ol **18b** was treated with methyl(carboxysulfamoyl)triethylammonium hydroxide inner salt (**20**) and again the dehydration proceeded toward C-24 and C-25. The two trienes (**3b** and **19**) were resolved by argentation layer chromatography. The less polar fraction (35%) was the required cholesta-5,7,24-trien-3β-ol acetate (**3b**), mp 78–81°. The product showed ultraviolet absorption maxima at 265, 272, and 283 nm as expected for the 5,7-diene. The nmr spectrum of **3b** had signals for the three vinylic hydrogens at C-6, C-7, and C-24, and for the 26 and 27 vinylic methyls.[26] Saponification of **3b** provided[7] **3a**. The more polar compound was cholesta-5,7,25-trien-3β-ol acetate (**19**, 31%), mp 91–96°. The ir, nmr, and mass spectra fully support structure **19**.



17

18a, $R_1 = R_2 = H$
b, $R_1 = CH_3CO$; $R_2 = H$
c, $R_1 = R_2 = CH_3CO$



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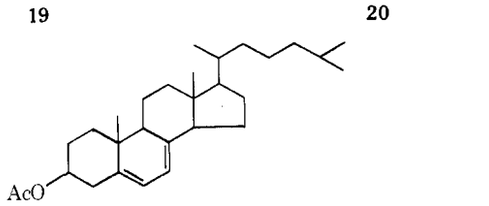

21

It is worthy of note that **18b** can be conveniently used in the synthesis of vitamin D metabolites and its analogs.

Finally we wish to report that the major unknown yeast metabolite was identified as the triene **3a**. In addition a small amount of the metabolite diene **2a** was also isolated. The results of the biosynthetic studies will be reported elsewhere.

Experimental Section

Physical Measurements. Melting points were taken on a hot-stage apparatus and are corrected. Infrared (ir) spectra were recorded on a Perkin-Elmer Model 237 spectrophotometer as KBr wafers.[24] Absorption frequencies are quoted in reciprocal centimeters. Ultraviolet (uv) spectra were recorded on a Perkin-Elmer Model 202 spectrophotometer in methanol solutions. Nuclear magnetic resonance (nmr) spectra were recorded in $CDCl_3$ on a Varian DA-60 or an EM 360 spectrometer at 60 MHz. Chemical shifts are quoted in parts per million downfield from tetramethylsilane as internal standard (s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet).

Mass spectra were recorded on a Du Pont 21-491 or a Varian M-66 instrument using the direct probe insertion system with a temperature of source of 210° and an ionization voltage of 70 eV. The masses of eliminated fragments are given in brackets after the molecular ion.

Chromatography. Analytical thin layer chromatography (tlc) was carried out on precoated silica I B-F Baker Flex plates in the indicated solvent systems. The products were detected under ultraviolet light and by spraying with an ethanolic solution of phosphomolybdic acid or aqueous sulfuric acid. Preparative layer chromatography was carried out on plates coated with silica gel (Merck $HF_{254-366}$).

The purity of steroidal samples was tested by gas–liquid chromatography (glc) on a Hewlett-Packard 7620A instrument using a 6-ft glass column (o.d. 6 mm, i.d. 2 mm) packed with 3% OV-101 on Gas Chrom Q (80–100 mesh) support or 1% SE-30 on Gas Chrom Q (80–100 mesh) support. The temperature was set isothermally at 230° and the helium flow was 30 ml/min.

5α,6-Dihydroergosteryl Benzoate (6). Ergosteryl benzoate (**5b**, 25 g) in benzene (750 ml) was hydrogenated in the presence of tris(triphenylphosphine)rhodium chloride (3.0 g) at room temperature and atmospheric pressure. One equivalent of hydrogen was absorbed in 16 hr. The solution was then evaporated to dryness, and the residue was slurried with ether. The resultant suspension was filtered through a column of 1 kg of alumina. The product was eluted with ether (10 l.). Removal of the solvent gave a nearly quantitative yield of 5α,6-dihydroergosteryl benzoate (**6**). The uv spectrum of the product indicated the absence of absorption at 272 and 284 nm.

23,24-Dinor-5α-chol-7-en-22-al-3β-ol Benzoate (7a). 5α,6-Dihydroergosteryl benzoate (**6**, 31.27 g) in dichloromethane (10 l.) containing pyridine (5.9 ml) was cooled to −78°. Ozone (1.5 equiv) was passed through the solution at a rate of 42 mg/min (determined by iodometry) for 110 min. The excess of ozone was removed by bubbling nitrogen for 10 min and then the solution was treated for 3 hr with dimethyl sulfide (20 ml) and methanol (20 ml). During this period the temperature was progressively increased to 22°. The solution was evaporated to dryness, and the resulting residue was dissolved in chloroform (600 ml)–methanol (300 ml) and shaken with saturated sodium bisulfate (400 ml) for 10 min. The viscous mixture was extracted with ether (4 × 1000 ml) and each time centrifuged for 5 min at 500 rpm to break the resultant emulsion. The ether extract was dried and evaporated, giving unreacted 5α,6-dihydroergosteryl benzoate (**6**, 10 g). The aqueous phase was neutralized with sodium hydroxide and brought to pH 8 with saturated sodium bicarbonate. The solution was then extracted with 3 × 1000 ml of chloroform and again centrifuged. The organic extract was washed once with water, dried over sodium sulfate, and evaporated to leave a crude material which after preparative tlc (hexane–ethyl acetate, 9:1) yielded 8.5 g of purified aldehyde **7a**. Finally crystallization from chloroform–methanol gave a homogeneous product: mp 200–202°; ir 2700, 1730, 1720 cm^{-1}; nmr δ 0.6 (s, 3 H, 18-CH_3), 0.88 (s, 3 H, 17-CH_3), 1.15 (d, J = 6 Hz, 3 H, 21-CH_3), 4.86 (m, 1 H, 3α-H), 5.15 (m, 1 H, 7-H vinylic), 9.65 (m, J = 3 Hz, 1 H, 22-CHO); mass spectrum *m/e* 434 (M^+) (−15, −28, −58, −122).

23,24-Dinor-5α-chol-7-ene-3β,22-diol 3-Benzoate (7b). To a solution of the 22-aldehyde **7a** (8.4 g) in chloroform (200 ml) and methanol (150 ml) at room temperature, was added sodium borohydride (750 mg). After 30 min a second portion of sodium borohydride (750 mg) was added. The reaction was terminated (after a total of 60 min) with a solution of ammonium chloride (5 g) in water (200 ml). Chloroform (200 ml) was added and the organic phase was washed with water, dried over sodium sulfate, and evaporated, giving the crude alcohol **7b** (7.5 g). This material was

further purified by preparative tlc (hexane–ethyl acetate, 4:1) and crystallized from a methanol–chloroform mixture (needles): mp 207–209°; ir 3640, 3480, 1715 cm^{-1}; nmr δ 0.575 (s, 3 H, 18-CH_3), 0.86 (s, 3 H, 19-CH_3), 1.06 (d, J = 6 Hz, 3 H, 21-CH_3), 1.6 (s, 1 H, 22-OH, D_2O exchangeable), 3.58 (m, 2 H, 22-CH_2), 4.9 (m, 1 H, 3α-H), 5.2 (m, 1 H, 7-H vinylic); mass spectrum m/e 436 (M^-) (−15, −59, −122, −137, −181).

23,24-Dinor-5α-chol-7-ene-22-bromide-3β-ol Benzoate (7c). A. A mixture of the 22-alcohol **7b** (7.3 g), carbon tetrabromide (17.8 g), and triphenylphosphine (13.2 g) in ether (5 l.) was stirred at room temperature for 1 hr, and then the solution was evaporated. The product was first filtered through a column of silica gel (800 g) from which it was eluted with chloroform, and was then purified by preparative tlc (hexane–ethyl acetate, 25:1). The obtained 22-bromide **7c** (3.0 g) was crystallized twice from a methanol–chloroform mixture to yield 2.7 g (white needles): mp 199–201°; ir 1715, 765 cm^{-1}; nmr δ 0.58 (s, 3 H, 18-CH_3), 0.86 (s, 3 H, 19-CH_3), 1.12 (d, J = 6 Hz, 3 H, 21-CH_3), 3.6 (m, 2 H, 22-CH_2), 4.96 (m, 1 H, 3α-H), 5.2 (m, 1 H, 7-H vinylic); mass spectrum m/e 500 (M^+), 498 (−123, −122, −137, −245, −287, −395).

B. The 22-alcohol **7b** (180 mg) and *p*-toluenesulfonyl chloride (500 mg) in 10 ml of dry pyridine were kept at 4° for 12 hr. After the usual work-up the tosylate **7d** (200 mg) was crystallized from methanol–chloroform (needles): mp 148–150°; ir 1715, 960 cm^{-1}.

The tosylate **7d** (200 mg) in dry dimethyl sulfoxide (10 ml) was treated with lithium bromide (39 mg) at 70° for 6 hr. The mixture was poured into water and extracted with ether, and the extract was washed with water (4 × 20 ml), then dried and evaporated. The product was separated by preparative tlc (hexane–ethyl acetate, 9:1), giving the bromide **7c** (160 mg) and recovered tosylate **7d** (88.6 mg). Bromide **7c** was crystallized from methanol–chloroform and its physical constants were as above.

20-Methyl-5α-pregn-7-en-3β-ol Acetate (8). To magnesium turnings (10 g) in dry ether (20 ml) and dry tetrahydrofuran (freshly distilled from lithium aluminum hydride) was added γ,γ-dimethylallyl bromide (2 ml). The reaction started immediately and the temperature was lowered to 4° by immersion in an ice–water bath. After 15 min, 22-bromide **7c** (561 mg) in ether (25 ml) and tetrahydrofuran (25 ml) were added all at once, along with γ,γ-dimethylallyl bromide (4 ml). The reaction mixture was stirred at room temperature and additional γ,γ-dimethylallyl bromide (6 ml) in ether (25 ml) was added dropwise during 8 hr. Stirring was continued for 12 hr, and the reaction was stopped by addition of aqueous ammonium chloride. The product was extracted with chloroform, and the extract was washed with water (3 × 70 ml), dried (sodium sulfate), and evaporated. A sterol fraction (261 mg) slightly less mobile than cholesterol was isolated by preparative tlc (hexane–ethyl acetate, 5:1).

This fraction was treated with acetic anhydride (7 ml) and pyridine (3.5 ml) at 50° for 1 hr. The volatile components were removed under reduced pressure, and the residue was fractionated by argentation tlc (silica gel–20% silver nitrate; chloroform–petroleum ether (bp 60–90°)–acetic acid, 25:75:0.5; developed three times). The mixture was resolved into the less polar **8** and the more polar **2b**.

The less polar 20-methyl-5α-pregn-7-en-3β-ol acetate (**8**, 172 mg) was crystallized from methanol–chloroform (white plates): mp 122–123°; ir 1733 cm^{-1}; nmr δ 0.53 (s, 3 H, 19-CH_3), 0.80 (s, 3 H, 18-CH_3), 0.86 (d, J = 6 Hz, 3 H, 21-CH_3), 0.95 (d, J = 6 Hz, 22-CH_3), 2.01 (s, 3 H, 3β-acetoxy), 4.66 (m, 1 H, 3α-H), 5.15 (m, 1 H, 7-H vinylic) [in benzene solution peaks appeared at 0.55 (s, 3 H, 18-CH_3), 0.72 (s, 3 H, 19-CH_3), 0.88 (d, J = 6 Hz, 3 H, 21-CH_3), 0.975 (d, J = 6 Hz, 3 H, 22-CH_3), 1.75 (s, 3 H, 3β-acetoxy), 4.83 (m, 1 H, 3α-H), 5.18 (m, 1 H, 7-H vinylic)]; mass spectrum m/e 358 (M^+) (−15, −33, −60, −103).

5α-Cholesta-7,24-dien-3β-ol (2a). The more polar **2b** (93.4 mg), recovered from the above coupling experiment, was dissolved in tetrahydrofuran (10 ml) and treated with potassium hydroxide in methanol (5%, 10 ml). The mixture was stirred overnight, then acidified and diluted with water. The product was extracted with chloroform (100 ml) and after the usual work-up **2a** was obtained. Crystallization from methanol provided 5α-cholesta-7,24-dien-3β-ol (**2a**, 55 mg) (needles); mp 96–98° (lit.[8] mp 99–102°); ir 3350 cm^{-1}; nmr δ 0.54 (s, 3 H, 18-CH_3), 0.8 (s, 3 H, 19-CH_3), 0.95 (d, J = 6 Hz, 3 H, 21-CH_3), 1.60 and 1.68 (s, 6 H, 26-CH_3 and 27-CH_3), 1.68 (s, 1 H, 3β-OH, D_2O exchangeable), 3.56 (m, 1 H, 3α-H), 5.25 (m, 2 H, 7, 24-H vinylics) [reported[26] δ 0.554 (s, 3 H, 18-CH_3), 0.813 (s, 3 H, 19-CH_3)]; mass spectrum m/e 384 (M^+) (−18, −68, −113, −129).

Ergosteryl Tosylate (5d). Ergosterol (**5a**, 5 g) and *p*-toluenesulfonyl chloride (8.1 g, freshly crystallized from hexane) in pyridine (80 ml, distilled over sodium hydroxide) were stirred for 12 hr at 4° in the dark. The mixture was then poured into a cold solution (400 ml) of sodium bicarbonate (4%). After 15 min the precipitate was quickly filtered, washed with cold water, and dried in a stream of air for a short time. The product was dissolved in chloroform (200 ml) and filtered through a mixture of sodium sulfate–magnesium sulfate, and removal of the solvent gave impure ergosteryl tosylate (**5d**).

The tosylate is unstable and decomposes during tlc. Repetitive crystallizations from acetone yielded 1.25 g of ergosteryl tosylate (**5d**), mp 90–100°.

3,5α-Cycloergosta-7,22-dien-6β-ol (Isoergosterol, 10). To a refluxing mixture of sodium bicarbonate (400 mg), water (50 ml), and acetone (200 ml), ergosteryl tosylate (**5d**, 800 mg) was added in one portion. The boiling was continued for 5 min; then the condenser was removed and 100 ml of acetone was distilled under reduced pressure.

After cooling, water (100 ml) was added and the product was collected by filtration. The solid was washed with water (2 × 50 ml) and dried for 3 hr at 80°. Crystallization from hexane gave isoergosterol (**10**, 600 mg): mp 129–130° (lit.[16] mp 129–130°); ir 3480 cm^{-1}; nmr δ 0.65 (s, 3 H, 18-CH_3), 0.83 (d, J = 6 Hz, 3 H, 21-CH_3), 1.08 (s, 3 H, 19-CH_3), 151 (s, 1 H, 6β-OH, D_2O exchangeable), 3.4 (m, 1 H, 6α-H), 5.2 (m, 2 H, 22-, 23-H vinylics), 5.45 (m, 1 H, 7-H vinylic); mass spectrum m/e 396 (M^-) (−18, −33, −59, −143, −197).

23,24-Dinor-3,5α-cyclocholesta-6,8(14)-dien-22-al (11). Ozone was admitted for 100 sec to a solution of isoergosterol (**10**, 213.3 mg) in dichloromethane (100 ml) cooled to −78° at a rate of 19.4 mg/min (determined by iodometry) (1.25 equiv). The excess of ozone was removed by bubbling nitrogen; then zinc (500 mg) and acetic acid (1.5 ml) were added. The mixture was stirred under nitrogen for 10 min at −78°, then for 1 hr at 0°. The zinc was filtered and the filtrate was washed with saturated sodium bicarbonate and water and dried (sodium sulfate). Removal of the solvent gave a residue which was purified by preparative tlc (hexane–ethyl acetate, 3:4).

From the more mobile zone (R_f 0.61) **11** was isolated. Crystallization from methanol yielded 23,24-dinor-3,5α-cyclocholesta-6,8(14)-dien-22-al (**11**, 65 mg): mp 105°; uv λ_{max} 260 nm (ε 21,200); ir 2680, 1730 cm^{-1}; nmr δ 0.78 (s, 3 H, 18-CH_3), 1.11 (s, 3 H, 19-CH_3), 1.15 (d, J = 6 Hz, 3 H, 21-CH_3), 5.23 and 6.15 (d, J = 10 Hz, 2 H, 6-, 7-H vinylics), 9.53 (d, J = 4 Hz, 1 H, 22-H aldehyde); mass spectrum m/e 310 (M^+) (−15, −57, −67).

26-Norcholest-5-en-25-on-3β-ol Tetrahydropyranyl Ether (12c). 26-Norcholest-5-en-25-on-3β-ol (**12a**, 12.0 g) was added to a benzene solution (250 ml) containing dihydropyran (15 g, distilled over sodium hydroxide) and *p*-toluenesulfonic acid monohydrate (200 mg). After 6 hr the resultant solution was poured into ice-cold aqueous sodium bicarbonate and the product was extracted with ether (500 ml). After the usual work-up, crystallization from ethanol–ether yielded 9.22 g of **12c**: mp 108–109°; ir 2910, 1705 cm^{-1}; nmr δ 0.66 (s, 3 H, 18-CH_3), 1.0 (s, 3 H, 19-CH_3), 3.53 (m, 1 H, 3α-H), 4.66 (m, 1 H, pyran C**H**—O), 5.31 (m, 1 H, 6-H vinylic).

26-Norcholesta-2,4,6-trien-25-one (17). A mixture of 26-norcholest-5-en-25-on-3β-ol tetrahydropyranyl ether (**12c**, 206 mg), petroleum ether (10 ml, bp 64–67°, distilled over sulfuric acid),[22] pyridine (0.67 ml), *N*-bromosuccinimide (300 mg, freshly crystallized from water), and bromine (1 drop) was refluxed under nitrogen. The mixture was irradiated with a sunlamp (250 W) for 20 min and then cooled (10°) and diluted with petroleum ether (30 ml). The solution was washed with water (3 × 25 ml) and dried over sodium sulfate. The solvent was distilled (below 10°) under reduced pressure.

The resulting oily residue was rapidly added to a boiling mixture of xylene (10 ml) and collidine (0.4 ml) and refluxing was continued for 30 min under nitrogen. After cooling, ether (100 ml) was added and the organic extract was washed with dilute hydrochloric acid (50 ml, 0.01 *N*), then with water (3 × 50 ml), and dried. Removal of the solvent gave an oil which showed maximal uv absorption at 250, 272, and 283 nm.

This oil was fractionated by tlc (silica gel–15% silver nitrate, hexane–ethyl acetate, 10:3; developed twice). The plates indicated a single product, which was eluted and crystallized from MeOH to yield 26-norcholesta-2,4,6-trien-25-one (**17**, 122 mg): mp 97–98°; uv λ_{max} 294 nm, 307 (ε 15,300), 320; nmr δ 0.71 (s, 3 H, 18-CH_3), 0.95 (s, 3 H, 19-CH_3), 0.95 (d, J = 6 Hz, 3 H, 21-CH_3), 2.15 (s, 3 H, 27-CH_3), 5.5–6.13 (m, large, 5 H, 2-, 3-, 4-, 6-, 7-H vinylic); mass spectrum m/e 366 (M^+) (−15, −113, −128).

26-Norcholesta-5,7-dien-25-on-3β-ol Acetate (16b). A mixture

of 26-norcholesta-5-en-25-on-3β-ol acetate (**12b**, 1 g), petroleum ether (100 ml), and pyridine (1.5 ml) was treated with *N*-bromosuccinimide (1.5 g) as described for **17**. The obtained brominated product was dissolved in xylene (50 ml) containing collidine (2 ml) and refluxed for 30 min under nitrogen in the dark. The dehydrobrominated product was purified by argentation tlc (silica gel–15% silver nitrate, hexane–ethyl acetate, 4:1; developed three times). Two bands with R_f 0.62 and 0.52, respectively, were detected.

Elution of the band with R_f 0.62 gave an oily residue (132 mg). The nmr spectrum indicated that the product still contained some impurity. However, judging from the nmr (chemical shifts of 18- and 19-methyls, the number of vinylic protons), the uv, and the mass spectra, the major component (*ca.* 70–80%) of the mixture was tentatively identified as 26-norcholesta-4,6-dien-25-on-3β-ol acetate.

The product from the slower band was crystallized from methanol to yield **16b** (238 mg): mp 135–136°; uv λ_{max} 263 nm (ε 7590), 272 (11,200), 283 (11,620), 294 (6810); ir 1730, 1710 cm^{-1}; nmr δ 0.63 (s, 3 H, 18-CH_3), 0.96 (s, 3 H, 19-CH_3), 2.05 (s, 3 H, 3β-acetate), 2.15 (s, 3 H, 27-CH_3), 4.66 (m, 1 H, 3α-H), 5.35 and 5.55 (d, J = 6 Hz, 2 H, 6-, 7-H vinylic); mass spectrum m/e 426 (M^+) (−60, −75, −173).

Cholesta-5,7-diene-3β,25-diol (18a). A solution of methyl iodide (3 g, 21.2 mmol) in dry ether (50 ml) was slowly added to a stirred suspension of magnesium turnings (520 mg, 21.2 mmol) in dry ether (50 ml) under nitrogen. Addition of a crystal of iodine initiated a vigorous reaction. The solution was maintained at reflux until the magnesium was consumed (30 min). Then 26-norcholesta-5,7-dien-25-on-3β-ol acetate (**16b**, 750 mg, 1.77 mmol) in dry ether (75 ml) was added dropwise and the reaction mixture was refluxed for 3 hr in the dark under nitrogen. The stirring was continued for 7 hr at room temperature and the reaction was terminated with a saturated solution of ammonium chloride (200 ml). The product was extracted with ether to yield, after workup, a crude residue (524 mg) which was stored at −10° under vacuum in the dark.

A portion of this material (24 mg) was resolved by tlc (hexane–ethyl acetate, 3:2) into two bands. The minor zone (R_f 0.66) contained the saponified starting material (**16a**).

The second zone (R_f 0.38) was identified as cholesta-5,7-diene-3β,25-diol (**18a**): mp 165–166°; uv λ_{max} 263 nm (ε 7590), 273 (11,310), 283 (11,820), 295 (6905); ir 3350 cm^{-1}; nmr δ 0.68 (s, 3 H, 18-CH_3), 0.99 (d, J = 6 Hz, 3 H, 21-CH_3), 1.01 (s, 3 H, 21-CH_3), 1.23 (s, 6 H, 26-, 27-CH_3), 3.53 (m, 1 H, 3α-H), 5.36 and 5.6 (d, J = 6 Hz, 2 H, 6-, 7-H vinylic); mass spectrum m/e 400 (M^+) (−18, −33, −36, −51, −147).

Cholesta-5,7-diene-3β,25-diol 3-Acetate (18b). A mixture of the crude **18a** (500 mg), acetic anhydride (12 ml), and pyridine (6 ml) was stored in the dark under nitrogen for 16 hr. The reagents were distilled *in vacuo* and the residue was then purified by preparative tlc (hexane–ethyl acetate, 4:1, developed twice). Two fractions (with R_f 0.73 and 0.44) were isolated.

The residue of the less polar zone (R_f 0.73) was crystallized from methanol to yield cholesta-5,7-diene-3β,25-diol diacetate (**18c**, 50 mg): uv λ_{max} 263, 274, 283, and 295 nm; ir 1730 cm^{-1}; nmr δ 0.61 (s, 3 H, 18-CH_3), 0.96 (s, 3 H, 19-CH_3), 1.43 (s, 6 H, 26-, 27-CH_3), 1.96 (s, 3 H, 25-acetate), 2.05 (s, 3 H, 3β-acetate), 4.66 (m, 1 H, 3α-H), 5.36 and 5.6 (d, J = 6 Hz, 2 H, 6-, 7-H vinylic); mass spectrum m/e 424 (M^+ − acetate) (−60, −75, −171).

The residue of the second zone (R_f 0.44) was crystallized from methanol–chloroform to yield cholesta-5,7-dien-3β,25-diol 3-acetate (**18b**, 401 mg); mp 108–110°; uv λ_{max} 265 nm (ε 7700), 273 (11,370), 283 (11,510), 295 (6810); ir 3440, 1730 cm^{-1}; nmr δ 0.68 (s, 3 H, 18-CH_3), 0.95 (s, 3 H, 19-CH_3), 1.21 (s, 6 H, 26-, 27-CH_3), 2.03 (s, 3 H, 3β-acetate), 4.66 (m, 1 H, 3α-H), 5.35 and 5.6 (d, J = 6 Hz, 2 H, 6-, 7-H vinylic); mass spectrum m/e 424 (M^+ − water) (−60, −78, −93, −171).

Cholest-5-ene-3β,25-diol 3β-Acetate (13b). Treatment of cholest-5-en-25-on-3β-ol acetate (**12b**) with methylmagnesium iodide as described above gave the diol **13a**, mp 172–174° (lit.[17] mp 172–174°). The diol **13a** was then acetylated to yield as the main product **13b**: mp 134–136° (lit.[17] mp 138–140°); ir 3440, 1730 cm^{-1}; nmr δ 0.66 (s, 3 H, 18-CH_3), 1.01 (s, 3 H, 19-CH_3), 1.20 (s, 6 H, 26- and 27-CH_3), 1.8 (s, 3 H, 3β-acetate), 4.56 (m, 1 H, 3α-H), 5.36 (m, 1 H, 6-H vinylic); mass spectrum m/e 384 (M^+ − acetate) (−15, −18, −33, −129, −131, −139, −171).

Cholesta-5,24-dien-3β-ol Acetate (14). A mixture of cholest-5-en-3β,25-diol 3β-acetate (**13b**, 150 mg) and methyl(carboxysulfamoyl)trimethylammonium hydroxide inner salt[20] (**20**, 500 mg) in benzene (40 ml) was refluxed under dry nitrogen. After 10 min the starting material was consumed as evidenced by tlc (hexane–ethyl acetate, 4:1). The reaction was stopped by addition of water (10 ml). The organic phase was washed with saturated sodium chloride (2 × 20 ml) and water (2 × 20 ml), dried, and concentrated. The residue was fractionated by tlc (silica gel–18% silver nitrate, hexane–ethyl acetate, 4:1, developed twice). Two major bands with R_f 0.62 and 0.50 were observed.

The product from the R_f 0.62 zone was crystallized from methanol–chloroform to yield cholesta-5,24-dien-3β-ol acetate (**14**, 65 mg): mp 96–98° (lit.[25] mp 99–100°); ir 1730 cm^{-1}; nmr δ 0.7 (s, 3 H, 18-CH_3), 0.96 (d, J = 6 Hz, 3 H, 21-CH_3), 1.03 (s, 3 H, 19-CH_3), 1.63 and 1.7 (s, 6 H, 26- and 27-CH_3). 2.03 (s, 3 H, 3β-acetate), 4.62 (m, 1 H, 3α-H), 4.75 (t, J = 6 Hz, 1 H, 24-H vinylic), 5.36 (m, 1 H, 6-H vinylic); mass spectrum m/e 366 (M^+ − acetate) (−15, −68, −85, −113, −121, −138, −153).

The product from the second zone (R_f 0.50) was crystallized from methanol to yield cholesta-5,25-dien-3β-ol acetate (**15**, 70 mg): mp 108° (lit.[25] mp 112°); ir 1730 cm^{-1}; nmr δ 0.7 (s, 3 H, 18-CH_3), 0.95 (d, J = 6 Hz, 3 H, 21-CH_3), 1.03 (s, 3 H, 19-CH_3), 1.73 (s, 3 H, 27-CH_3) 2.03 (s, 3 H, 3β-acetate), 4.62 (m, 1 H, 3α-H), 4.65 (s, 2 H, 25-CH_2), 5.36 (m, 1 H, 6-H vinylic); mass spectrum m/e 366 (M^+ − acetate) (−15, −42, −71, −85, −111, −113, −121, −138, −153).

Treatment of Cholesta-5,7-dien-3β-ol Acetate (21) with Methyl(carboxysulfamoyl)triethylammonium Hydroxide Inner Salt (20). A mixture of cholesta-5,7-dien-3β-ol acetate (10 mg) and **20** (100 mg) in benzene (2 ml) was refluxed under nitrogen for 40 min. Aliquots were removed at 5-min intervals and tested by tlc (hexane–ethyl acetate, 5:1); only the starting material was detected. After the termination of the reaction the starting material was recovered.

Cholesta-5,7,24-trien-3β-ol Acetate (3b). A mixture of cholesta-5,7-diene-3β,25-diol 3-acetate (**18b**, 250 mg) and **20** (250 mg) in dry benzene (20 ml) was refluxed under nitrogen. After 15 min a sample of the reaction mixture was removed and tlc (hexane–ethyl acetate, 4:1) revealed the absence of starting material. The reaction was stopped and the mixture was worked up as described above. The crude product was purified by tlc (silica gel–18% silver nitrate, hexane–ethyl acetate, 4:1, developed twice). Two major bands were observed and eluted.

The residue of the less polar zone was crystallized from methanol to give cholesta-5,7,24-trien-3β-ol acetate (**3b**, 85 mg): mp 78–81°; uv λ_{max} 265 nm, 272 (ε 10,980), 283 (11,400), 294 (6650); ir 1730 cm^{-1}; nmr δ 0.63 (s, 3 H, 18-CH_3), 0.95 (s, 3 H, 19-CH_3), 0.95 (d, J = 6 Hz, 21-CH_3), 1.61 (s, 3 H, 26-CH_3), 1.68 (s, 3 H, 27-CH_3), 2.03 (s, 3 H, 3β-acetate), 4.66 (m, 1 H, 3α-H), 5.1 (t, J = 6 Hz, 1 H, 24-H vinylic), 5.36 and 5.57 (d, J = 6 Hz, 2 H, 6-, 7-H vinylic); mass spectrum m/e 424 (M^+) (−60, −75, −101, −145, −171).

The residue of the more polar fraction was crystallized from methanol and yielded cholesta-5,7,25-trien-3β-ol acetate (**19**, 78 mg): mp 91–96°; ir 1730 cm^{-1}; nmr δ 0.63 (s, 3 H, 18-CH_3), 0.95 (s, 3 H, 19-CH_3), 0.95 (d, J = 6 Hz, 3 H, 21-CH_3), 1.7 (s, 3 H, 27-CH_3), 2.03 (s, 3 H, 3β-acetate), 4.66 (m, 1 H, 3α-H), 4.66 (s, 2 H, 26-CH_2), 5.36 and 5.56 (d, J = 6 Hz, 2 H, 6-, 7-H vinylic); mass spectrum m/e 424 (M^+) (−60, −75, −101, −145, −171).

Cholesta-5,7,24-trien-3β-ol (3a). A mixture of cholesta-5,7,24-trien-3β-ol acetate (**3b**, 20 mg), tetrahydrofuran (5 ml), and lithium aluminum hydride (100 mg) was refluxed in an atmosphere of nitrogen in the dark for 2 hr. To the cold reaction mixture was added saturated sodium sulfate (25 ml) and the obtained residue was removed by filtration. The filtrate was washed with water (2 × 25 ml) and dried and the solvent was removed to yield a powder (15.2 mg). The residue was crystallized from methanol to yield **3a** (needles): mp 98–99° (lit.[7] mp 102–102.5°); ir 3400 cm^{-1}; nmr δ 0.62 (s, 3 H, 18-CH_3), 0.96 (s, 3 H, 19-CH_3), 1.01 (d, J = 6 Hz, 3 H, 21-CH_3), 1.62 (s, 3 H, 26-CH_3), 1.7 (s, 3 H, 27-CH_3), 3.66 (m, 1 H, 3α-H), 5.1 (t, J = 6 Hz, 1 H, 24-H vinylic), 5.36 and 5.46 (d, J = 6 Hz, 2 H, 6-, 7-H vinylic) [reported[7,26] δ 0.62 (s, 3 H, 18-CH_3), 0.955 (s, 3 H, 19-CH_3), 1.63 (s, 3 H, 26-CH_3), 1.69 (s, 3 H, 27-CH_3)]; mass spectrum m/e 382 (M^+) (−15, −18, −33, −59, −129, −131, −171).

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Registry No.—**2a**, 651-54-7; **2b**, 51373-26-3; **3a**, 1715-86-2; **3b**, 17137-77-8; **5a**, 57-87-4; **5b**, 5035-30-3; **5d**, 51373-27-4; **6**, 4356-17-6; **7a**, 51373-28-5; **7b**, 32308-36-4; **7c**, 51373-29-6; **7d**, 51373-30-9; **8**, 51373-31-0; **10**, 2774-59-6; **11**, 32308-45-5; **12a**, 7494-34-0; **12b**,

7548-94-9; **12c**, 51464-63-2; **13a**, 2140-46-7; **13b**, 10525-22-1; **14**, 2665-04-5; **15**, 10525-24-3; **16b**, 24281-79-6; **17**, 51373-32-1; **18a**, 22145-68-2; **18b**, 24281-78-5; **18c**, 34679-19-1; **19**, 51373-33-2.

References and Notes

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Pyrazolopyrimidine Nucleosides. V. Methylation of the C-Nucleoside Antibiotic Formycin and Structural Elucidation of Products by Magnetic Circular Dichroism Spectroscopy[1]

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The direct methylation of formycin (**9**) has furnished the two monomethyl derivatives, 7-amino-1-methyl-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (**10**) and 7-amino-2-methyl-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (**12**). An unequivocal assignment of the above structures was made by a comparison of the magnetic circular dichroism (MCD) curves obtained for the model compounds 7-amino-2,3-dimethylpyrazolo[4,3-*d*]pyrimidine (**6**) and 7-amino-1,3-dimethylpyrazolo[4,3-*d*]pyrimidine (**7**) with the MCD spectra of **10** and **12**. The unequivocal synthesis of **6** and **7** was accomplished by ring annulation of the appropriately substituted pyrazole precursors. The synthesis of 1,3-dimethylpyrazolo[4,3-*d*]pyrimidin-7-one (**8**) and 2-methyl-3-(β-D-ribofuranosyl)-pyrazolo[4,3-*d*]pyrimidin-7-one (**11**) was accomplished by an unusual displacement of the exocyclic amino group in 1 *N* sodium hydroxide.

The antibiotics formycin and formycin B were isolated[3] from *Norcardia interforma* and found[4-6] to be C-nucleosides which were isomeric with the naturally occurring nucleosides adenosine and inosine, respectively. These antibiotics are of considerable interest since they are C-nucleosides and belong to the same class of compounds as showdomycin,[7] pseudouridine,[8] and pyrazomycin.[9] Formycin has demonstrated inhibition of Ehrlich carcinoma, mouse leukemia L-1210, Yoshida rat sarcoma, HeLa cells, and *Xanthomonas oryzae* as well as some antiviral activity.[3,12] Formycin 5′-triphosphate acts as a source of biological energy[13] and ribopolynucleotides with formycin replacing adenosine, at the binding site of t-RNA to ribosomes, have shown[14] no mistranslation of the messenger. In fact, formycin has shown the ability to act as a substrate for a number of enzymes specific for adenosine, including adenosine kinase[11] and, unfortunately, adenosine deaminase.[10] The resemblance of formycin to adenosine is thus apparent in many biological systems. Since formycin is such an excellent substrate for adenosine deaminase, this would suggest that although formycin hydrobromide has been found to exist in the syn conformation, there must be a population of formycin in the anti conformation in solution and *in vivo*. In fact, a recent X-ray study[15] has revealed that formycin, *per se*, exists on the average somewhere between the classical syn and anti forms (amphi form[16]) in the solid state. A recent study has established that adenosine derivatives in the syn conformation are not substrates for adenosine deaminase and this prompted us to initiate a study[17] designed to restrict rotation around the glycosyl (carbon–carbon) bond of formycin and increase the per cent of nucleoside in the syn conformation.

The isomeric purine nucleosides, when alkylated on an imidazole nitrogen, form salts with a positively charged heterocyclic ring[18] which can then undergo a facile ring opening.[19] However, formycin presents a unique opportunity to alkylate a ring nitrogen of a bicyclic nucleoside without the usual quaternization. These alkylated derivatives of formycin should be chemically very similar to formycin and yet the 2-alkyl derivative should exhibit