(Z)-17(20)-Dehydrocholesterol. A New Sterol with C-21 and C-22 Spatially Fixed

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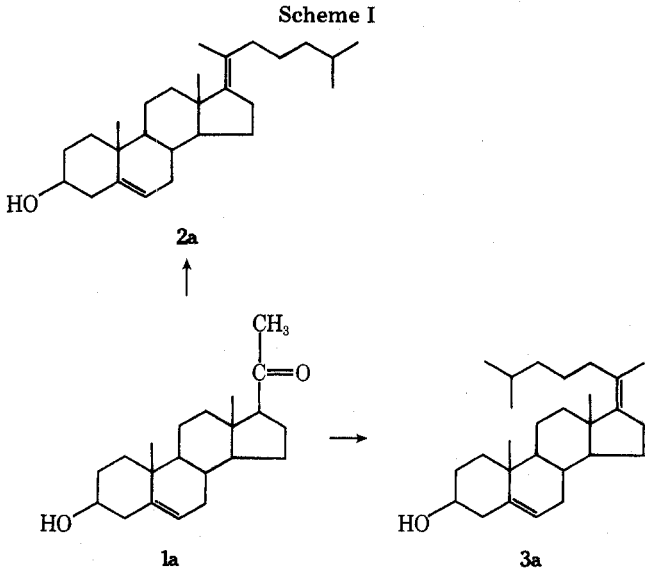
(Z)-3β-Hydroxycholesta-5,17(20)-diene, in which the side chain lies on the same side of the 17(20) bond as the sterol nucleus (C-22 cis oriented with respect to C-13), was prepared by two independent routes from pregnenolone. In one case the compound arose directly along with the previously described E isomer and E-$\Delta^{20(22)}$ analogue by the acid-catalyzed dehydration of the Grignard addition products, 20α- and 20β-hydroxycholesterol. The product composition was the same when either of the latter epimers was used and is consistent with a reaction path through an equilibrium mixture of rotationally isomeric carbonium ions bearing a positive charge on C-20 with C-22 toward or away from C-13. In the second route the cyanohydrin of pregnenolone was dehydrated with $POCl_3$ in pyridine. The (E)-$\Delta^{5,17(20)}$-nitrile produced was equilibrated in base to give the thermodynamically more stable Z isomer with the nitrile group toward C-13. After Grignard addition, reduction, and removal of the resultant 22-hydroxyl group, the title sterol was obtained. The presence and geometry of the $\Delta^{17(20)}$ bond were demonstrated by proton magnetic resonance and mass spectrometry and by conversion of the diene, through addition of osmium tetroxide followed by reduction, to the known 3β,17α,20α-trihydroxycholest-5-ene, as well as to 3β,20α-dihydroxycholest-5-ene by hydroboration. The physical and chemical evidence indicates that sterols with the natural configuration at C-20 assume a preferential conformation about the 17(20) bond such that C-21 and C-22 lie pseudoequatorially to the rear of the 17(20) bond with the third substituent on C-20 pseudoaxially oriented to the front opposing C-18.

$\Delta^{17(20)}$-Sterols are of interest owing to the fact that the side chain is fixed in space about the 17(20) bond and can be used to study the effect of spatial orientation on biological behavior, e.g., the ability to support the correct architectural characteristics of membranes or to fit into the active sites of enzymes. Furthermore, $\Delta^{17(20)}$-sterols occur naturally,[1,2] and a knowledge of their stereochemistry is of significance in understanding the mechanism of cyclization of squalene oxide.[2] In previous work[3] by one of our groups pregnenolone (**1a**) led to the $\Delta^{17(20)}$ derivative (**2a**) of cholesterol in which the side chain lies to the right (*E*) (Scheme I). More recently one of us

Scheme I

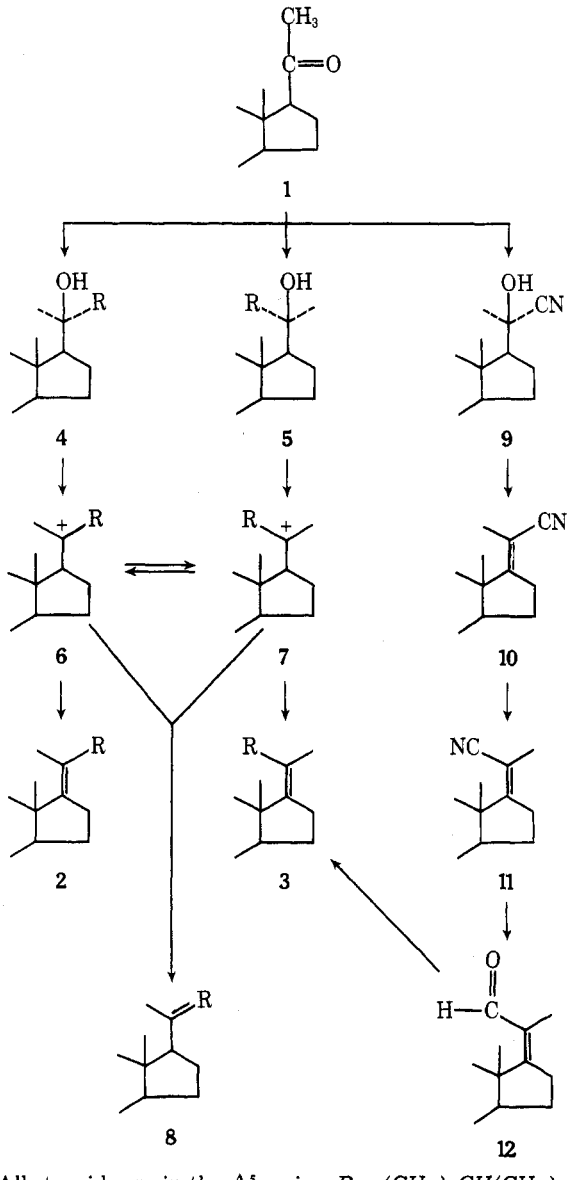


discovered a new route from pregnenolone to the *E* isomer, and both of us independently obtained the *Z* isomer (**3a**) from the same starting material but by different routes. Upon realization of this overlap, we decided to publish the work jointly. In the meantime a third route (by remote oxidation and double bond migration) to Z-$\Delta^{17(20)}$ steroids has appeared.[4] It differs among other ways from those reported here in that use of a 3β-hydroxy steroid as starting material necessarily requires inversion at C-3. In addition to obtaining the (Z)-3β-hydroxy-$\Delta^{5,17(20)}$-sterol (**3a**) and proving its structure by physical and chemical means, we have been able to shed further light on the question of rotation about a single bond between C-17 and C-20.

The most direct route (Scheme II) to the $\Delta^{17(20)}$-sterols entailed acid-catalyzed dehydration of the epimeric mixture of 20-hydroxycholesterols (**4** and **5**) derived from Grignard addition of the isohexyl group to pregnenolone (**1**, 3β-hydroxypregn-5-en-20-one). This yielded a ternary mixture in high yield of the *Z* and *E* isomers of 17(20)-dehydrocholesterol (**3** and **2**, respectively) and their E-$\Delta^{20(22)}$ analogue[5] (**8**). Each moved differently in gas–liquid chromatography, and each was obtained pure by preparative adsorption chromatography. The (Z)-$\Delta^{17(20)}$-sterol (**3**) moved faster than the other two sterols on a column of deactivated Al_2O_3, while the *E* isomer (**2**) was separable from the $\Delta^{20(22)}$ analogue (**8**) by argentation chromatography. Separation was also achievable on a column of lipophilic Sephadex.

The same mixture of dienes was obtained when either 20α-(**4**) or 20β-hydroxycholesterol (**5**) was used as starting material in the dehydration. The ratio of products [(Z)-$\Delta^{17(20)}$:(E)-$\Delta^{17(20)}$:(E)-$\Delta^{20(22)}$] was 1:1:3 in both cases. Since equilibration did not occur after dehydration, the following interpretation of the mechanism is suggested (Scheme II). The proton magnetic resonance spectra of the epimeric 20-hydroxycholesterols (Table I) indicate that in their preferred conformation C-22 lies to the right in the α isomer (**4**) and to the left in the β isomer (**5**) with the 20-hydroxyl group antiparallel to the 17α hydrogen atom (opposing C-18) in both cases.[6] While such an arrangement would fulfill the requirements for a concerted elimination of water, only one of the isomeric pair of dienes (**2** and **3**) should have been obtained in each case [the *Z* isomer (**3**) from the β epimer (**5**) and the *E* isomer (**2**) from the α epimer (**4**)]. Since both isomers were obtained from each epimer, a concerted mechanism apparently does not operate. However, the alternative, a two-step process with the intermediacy of a racemized, planar carbonium ion at C-20 (**6** and **7**) fulfills the stereochemical requirements. When C-20 is in the planar state, examination of molecular models reveals little difference between the nonbonded interactions of C-21 and C-22 with

Scheme II[a]

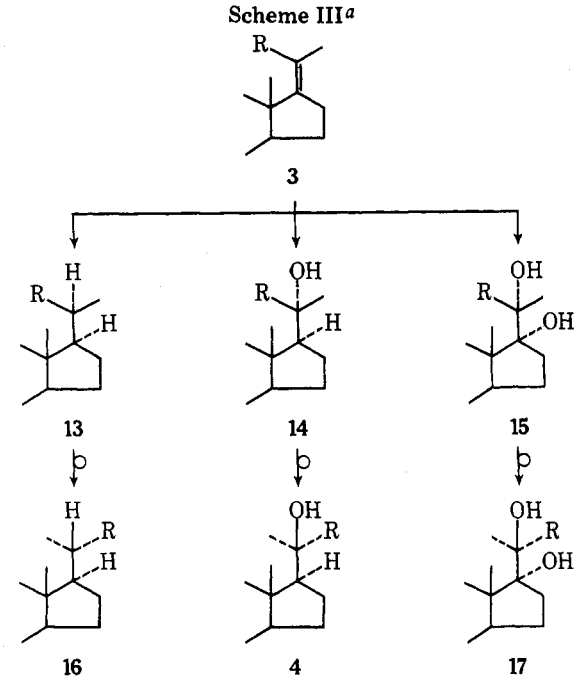


[a] All steroids are in the Δ^5 series: R = $(CH_2)_3CH(CH_3)_2$ or $CH(CH_2)_2CH(CH_3)_2$; a, free alcohol; b, acetate; c, tetrahydropyranyl ether; d, 6β-methoxy-3,5-cyclo derivative.

the nucleus (C-12, C-18, and C-16) in the *Z* and *E* conformers. On the reasonable assumption that the tertiary carbonium ion has a half life long enough to permit rotation, approximately equal amounts of the two conformers (**6** and **7**) should exist at equilibrium which in turn would lead to approximately equal amounts of the *Z* and *E* dienes from either of the 20-hydroxy epimers (**2** from **6** and **3** from **7**) in agreement with observation.

The analysis in the preceding paragraph also leads to an explanation for the preferred conformations of the epimeric 20-hydroxycholesterols (**4** and **5**).[6] When C-20 is tetrahedral in the conformer with C-22 to the right (trans oriented to C-13), C-21 and C-22 both lie toward the rear of the 17(20) bond and therefore away from C-18 and C-16. Conversely, rotation of 180° places C-21 and C-22 in the front with substantially more interaction with C-18 and C-16. This would require, as is found, that in both epimers the 20-hydroxyl group should lie pseudoaxially in front (opposing C-18) with C-21 and C-22 pseudoequatorially to the rear.[7] As will be seen in the subsequent discussion, the same analysis explains the data known for the 17α-hydroxy derivatives of the 20-hydroxycholesterols. An important extrapolation of the argument is that cholesterol itself should also exist preferentially in the conformer with C-22 to the right (**16**, Scheme III), while in 20-isocholesterol C-22 should lie to the left.

Scheme III[a]



↻ indicates rotation of 180° about the 17(20) bond

[a] Compounds **13** and **16** are 6β-methoxy-3,5-cyclo derivatives and all other steroids are in the Δ^5 series: in all cases R is $(CH_2)_3CH(CH_3)_2$; a, free alcohol; b, acetate..

In the second route (Scheme II) to (*Z*)-17(20)-dehydrocholesterol (**3**) the cyanohydrin (**9**) derived from pregnenolone (**1**) was dehydrated as previously described[8] to the (*E*)-$\Delta^{17(20)}$-nitrile (**10**) which in turn was isomerized to the more stable *Z* isomer (**11**). After protection of the 3β-hydroxyl group by conversion to its tetrahydropyranyl ether the cyano group was reduced with diisobutylaluminum hydride to the imine which without isolation was hydrolyzed to the aldehyde. Reaction of the latter with isopentylmagnesium bromide yielded the 22-hydroxy derivative (**12**) of (*Z*)-17(20)-dehydrocholesterol (**3**) which was isolated as its 22-methyl ether. The allylic methoxy group was reductively removed by treatment with lithium in ethylamine, and after hydrolysis of the tetrahydropyranyloxy group (*Z*)-17(20)-dehydrocholesterol (**3**) was obtained. The physical properties, including the proton magnetic resonance spectra (Table I), were identical with those of the sample derived from the first route.

The assignment of the *Z* geometry to the $\Delta^{5,17(20)}$-dienic product (**3**) follows from previous assignments of geometry to the (*E*)- and (*Z*)-$\Delta^{5,17(20)}$-nitriles.[7] Among other things which led to an assignment of structure, the protons of C-21 in the (*Z*)-$\Delta^{17(20)}$-nitrile (**11**) (bearing the C-21 methyl group away from C-13) appeared further upfield than in the *E* isomer (**10**) and no significant homoallylic coupling occurred in the *Z* isomer (**11**) with the C-16 protons as expected from other work,[9] while in the *E* isomer (**10**) the C-21 protons appeared as a triplet having J = 1.6 Hz. The same phenomenon was found with the isomeric 17(20)-dehydrocholesterols. The C-21 protons in the *Z* isomer (**3**) derived from the *Z* nitrile (**11**) appeared as an unresolved triplet at 1.55 ppm but as a distinct triplet (J = 1.7 Hz) at 1.68 ppm in the *E* isomer (**2**) derived from the *E* nitrile (**10**). The upfield shift in the signal from the C-21 protons of the *Z* isomer compared to the *E* isomer was also in agreement with expectation.[3,4,7,9]

In addition to the physical evidence chemical proof of structure was obtained (Scheme III). The (*Z*)-17(20)-dehy-

Table I. Singlet ^1H NMR Signals from Sterols in Parts per Million (δ)

Sterol	C-18	C-21	Origin of sterol
Cholesterol (16)	0.68		Natural product
17α-Hydroxycholesterol	0.70		From 16-dehydrocholesterol by epoxidation and reduction
17α-Hydroxycholesterol	0.70		From (Z)-17(20)-dehydrocholesterol by hydroboration
20α-Hydroxycholesterol (4)	0.87	1.28	From pregnenolone by Grignard reaction
20α-Hydroxycholesterol (4)	0.87	1.28	From (Z)-17(20)-dehydrocholesterol by hydroboration
20β-Hydroxycholesterol (5)	0.87	1.13	From 21-norcholest-5-en-3-one by Grignard reaction
17α,20α-Dihydroxycholesterol (18)	0.85	1.30	From 17α-hydroxy-21-norcholest-5-en-3-one by Grignard reaction
17α,20α-Dihydroxycholesterol (18)	0.85	1.30	From (Z)-17(20)-dehydrocholesterol by osmylation
17α,20β-Dihydroxycholesterol	0.90	1.23	From 17α-hydroxycholesterol by Grignard reaction
(E)-17(20)-Dehydrocholesterol (2)	0.86	1.68	From (E)-3β-hydroxy-20-cyanopregna-5,17(20)-diene
(E)-17(20)-Dehydrocholesterol (2)	0.86	1.68	From dehydration of 20α-hydroxycholesterol
(E)-17(20)-Dehydrocholesterol (2)	0.86	1.68	From dehydration of 20β-hydroxycholesterol
(Z)-17(20)-Dehydrocholesterol (3)	0.87	1.53	From (Z)-3β-hydroxy-20-cyanopregna-5,17(20)-diene
(Z)-17(20)-Dehydrocholesterol (3)	0.87	1.55	From dehydration of 20α-hydroxycholesterol
(Z)-17(20)-Dehydrocholesterol (3)	0.87	1.55	From dehydration of 20β-hydroxycholesterol

drocholesterol (3) was converted through the 3,5-cyclo derivative to 17α,20α-dihydroxycholesterol (15) by addition of osmium tetroxide and subsequent reductive cleavage of the Os–O bond and retro-3,5-cyclo rearrangement. Similarly, hydroboration led to 20α-hydroxycholesterol (14) (and 17α-hydroxycholesterol), and reduction of the 3,5-cyclo derivative without retro rearrangement led to 6β-methoxy-3,5-cyclo-5α-cholestane (13) which was identical with the product derived by rearrangement of cholesterol.

The proton magnetic resonance spectra (Table I) of the 20α-hydroxy- and 17α,20α-dihydroxycholesterol (4 and 17) derived from the Z-$\Delta^{5,17(20)}$-diene (3) were identical with samples prepared by the appropriate Grignard reactions with pregnenolone and with 17α-hydroxy-21-norcholest-5-en-20-one, respectively. The downfield shifts in the NMR signals (Table I) from the C-18 protons of 4 and 17 indicated that the 20-hydroxy groups were pseudoaxially in front opposing C-18 in both cases. If the 20-hydroxyl groups were not so oriented, the signal from the C-18 protons should have been near the value for cholesterol and its 17α-hydroxy derivative.[7] Since attack on the $\Delta^{17(20)}$ bond must have occurred from the rear giving 14 and 15, rotation about the 17(20) bond must have taken place afterwards leading to 4 and 17, respectively. This adds further weight to the conclusion that the most stable conformation about the 17(20) bond is the one with C-21 and C-22 pseudoequatorially oriented to the rear as shown by 4, 5, 16, and 17.

Experimental Section

General. Melting points were determined on a Kofler hot stage. NMR spectra were obtained in $CDCl_3$ at ambient temperature on a 220 or a 60 MHz instrument (Varian Associates) with an internal standard of tetramethylsilane. Chemical shifts are reported in parts per million. Mass spectra were determined on a Varian Associates M-66 spectrometer. Gas–liquid chromatography was performed in a 6-ft U-tube of 1% nitrile silicone gum (XE-60) on Chromosorb W with helium as the carrier gas at 232 °C on an F and M Model 400 instrument. Retention times are given as values relative to the retention time of cholesterol. Adsorption chromatography, unless otherwise described, was carried out on a column of alumina deactivated with 10% of water. The solvent system was ether graded into hexane. Argentation chromatography was performed with benzene graded into hexane on silicic acid impregnated with 20% of silver nitrate. Lipophilic Sephadex used for some of the separations was Lipidex-5000 supplied by Packard Instrument Co., Inc., and the solvent system was 5% hexane in methanol. The microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y. Some of the mass and NMR spectra were performed by Morgan-Schaffer Corp., Montreal 252, Quebec, Canada.

(Z)-3β-Hydroxycholesta-5,17(20)-diene (3a), (E)-3β-Hydroxycholesta-5,17(20)-diene (2a), and (E)-3β-Hydroxycholesta-5,20(22)-diene (8a) from Dehydration of 3β,20α- and 3β,20β-Dihydroxycholest-5-ene (4a and 5a). The acetate (4b) of the 20α-hydroxy steroid (4) was prepared as previously described from pregnenolone acetate (1a) by the Grignard reaction.[6,10,11] Purification was achieved by several crystallizations. While it was accompanied by the 20β epimer (5b)[6] the latter was more readily obtained pure from 3β-hydroxy-21-norcholest-5-en-20-one by Grignard addition with methylmagnesium iodide.[11] Dehydration was accomplished with 1.55 g of either of the acetates at 32 °C in 220 ml of methanol to which 4.4 ml of concentrated hydrochloric acid was added. The course of the reaction was followed by GLC, and dehydration was found to be complete during 4.0 h. Partial hydrolysis of the acetoxy group also occurred. The solvents and HCl were removed on a rotary evaporator at a temperature (ca. 50 °C) above that of the reaction which resulted in completion of the hydrolytic process. The resulting mixture showed three peaks on GLC with relative retention times of 0.88, 0.94, and 1.01 corresponding respectively to (Z)-3β-hydroxycholesta-5,17(20)-diene (3a), (E)-3β-hydroxycholesta-5,17(20)-diene (2a), and (E)-3β-hydroxycholesta-5,20(22)-diene (8a). The peaks heights were in the ratio of 1:1:3, respectively. The mixtures were essentially identical when either starting material (4a or 5a) was used, and no difference in the rates of dehydration was observed.

The (Z)-3β-hydroxycholesta-5,17(20)-diene (3a) was separated from the other two dienols by chromatography on deactivated alumina. It (3a) moved faster than the other two sterols (2a and 8a). Combination of appropriate fractions yielded 0.11 g of 3a as an oil which solidified on standing and moved in GLC with a relative retention time of 0.88. Rechromatography of fractions in which 3a was mixed with the other sterols gave an additional 0.06 g. After crystallization from acetone it (3a) melted at 119–121 °C: NMR δ 0.87 (s, 3 H, C-18), 1.02 (s, 3 H, C-19), 1.55 [s (broad), 3 H, C-21], 0.88 (d, J = 6 Hz, 6 H, C-26 and C-27); MS m/e (rel intensity) 384 (M^+, 47), 369 ($M^+ - CH_3$, 21), 351 (369 − H_2O, 24), 299 ($M^+ - C_6H_{13}$, 78), 281 (299 − H_2O, 25), 271 (299 − C_2H_4, 100), and 253 (271 − H_2O, 30). The benzoate of 3a melted at 93–94 °C: NMR δ 0.88 (s, 3 H, C-18), 1.08 (s, 3 H, C-19), 1.56 [s (broad), 3 H, C-21], and 0.88 (d, J = 6 Hz, 6 H, C-26 and C-27); MS m/e (rel intensity) 488 (M^+), 473 ($M^+ - CH_3$), 403 ($M^+ - C_6H_{13}$), 366 ($M^+ - C_6H_5COOH$, 63), 351 (366 − CH_3, 24), 281 (366 − C_6H_{13}, 100), 253 (281 − C_2H_4, 18), 228 (253 − C_2H, 17), and 212 (228 − CH_2 − 2 H, 24).

Material (0.78 g) from the alumina chromatography which moved slower than the (Z)-$\Delta^{5,17(20)}$-dienol (3a) and displayed only GLC peaks for 2a and 8a was crystallized from methanol. The precipitate (0.32 g) was composed principally of 8a. The sterol in the filtrate was acetylated (pyridine/acetic anhydride) giving 0.45 g which was chromatographed by the argentation method. The first steryl acetate to be eluted was (E)-3β-acetoxycholesta-5,17(20)-diene (2b). Combination of fractions showing a GLC peak only for 2b amounted to 0.09 g: mp 76–79 °C (methanol); NMR δ 0.86 (s, 3 H, C-18), 1.03 (s, 3 H, C-19), 1.68 (t, J = 1.7 Hz, 3 H, C-21), and 0.87 (d, J = 6, 6 H, C-26 and C-27); MS m/e (rel intensity) 366 ($M^+ - CH_3COOH$, 40), 351 (366 − CH_3, 30), 281 (366 − C_6H_{13}, 100), and 253 (281 − C_2H_4, 18). The free alcohol (2a) melted at 108–110 °C and showed a GLC peak at 0.94. The benzoate, which melted at 145–147 °C, had essentially the same mass spectrum as did the benzoate of the Z isomer except that the fragment with m/e 228 was missing. The properties of 2a and its benzoate agreed with the literature.[3]

(E)-3β-Acetoxycholesta-5,20(22)-diene (8b) appeared in fractions following those containing the (E)-$\Delta^{5,17(20)}$-dienyl acetate (2b). It (8b, 0.20 g) melted at 123–124 °C (methanol): NMR δ 0.55 (s, 3 H, C-18),

1.02 (s, 3 H, C-19), 1.63 (s, 3 H, C-21), 0.89 (d, J = 6.5 Hz, 6 H, C-26,27), 2.04 (s, 3 H, CH_3 of acetoxy group); MS m/e (rel intensity) 366 ($M^+ - CH_3COOH$, 100), 351 (366 − CH_3, 11), 281 (366 − C_6H_{13}, 4), 255 (281 − C_2H_2, 3), 254 (281 − C_2H_3, 7), 253 (281 − C_2H_4, 11), 228 (253 − C_2H, 41), 213 (228 − CH_3, 25), 211 (228 − CH_3 − 2 H, 21). The free alcohol (**8a**) melted at 136–138 °C (methanol) and showed a GLC peak with a relative retention time of 1.02: NMR δ 0.55 (s, 3 H, C-18), 1.01 (s, 3 H, C-19), 1.62 (s, 3 H, C-21), 0.88 (d, J = 6 Hz, 6 H, C-26,27). The NMR and mass spectra of the $\Delta^{5,20(22)}$-dienyl acetate (**8b**) derived from **4b** were identical with those of **8b** derived from **5b.** The properties of the (*E*)-$\Delta^{5,20(22)}$-dienol (**8a**) and its acetate (**8b**) agreed with the literature.[3,12]

When a mixture of the three dienols was chromatographed on Sephadex, **3a** was eluted first followed in order by **2a** and **8a.**

After 15 mg of (*E*)-3β-hydroxycholesta-5,20(22)-diene (**8a**) was dissolved in 10.0 ml of methanol and 0.20 ml of concentrated acid was added at 32 °C, samples were withdrawn at intervals beginning with 5.0 min and extending to 4 h. There were neither quantitative nor qualitative changes in the GLC peak, and no other peaks appeared.

(*Z*)-3β-Tetrahydropyranyloxy-20-cyanopregna-5,17(20)-diene (11c) from 10b. To a solution of 20 g of (*E*)-3β-acetoxy-20-cyanopregna-5,17(20)-diene (**10**)[8] in 250 ml of dry (freshly distilled from CaH_2) dimethyl sulfoxide 20 g of potassium *tert*-butoxide was added and the solution was stirred in a nitrogen atmosphere for 24 h. Then the solution was cooled in an ice bath and acetic acid was slowly added until the solution was neutral. After much water and ice were added the mixture was extracted with ethyl acetate and the extract was washed with water, dried with sodium sulfate, and evaporated. Thereby 12.3 g of crude **11a** was obtained which was, without further purification, dissolved in 100 ml of tetrahydrofuran containing 200 mg of *p*-toluenesulfonic acid and 8 ml of dihydropyran. This solution was kept at 20 °C for 18 h and then poured into a saturated ice-cold sodium bicarbonate solution. The product was extracted with ether, washed with water, and dried over sodium sulfate and the solvent evaporated. Purification by chromatography over Alcoa alumina gave, after recrystallization from ether, 10.30 g of pure **11c:** mp 167–171 °C; ir 2200 (conjugated–CN), 1030 and 970 cm^{-1} (ether); NMR δ 0.95 (s, 3 H, 18-CH_3), 1.33 (s, 3 H, 19-CH_3), and 1.83 (s, 3 H, 21-CH_3).

Anal. Calcd for $C_{27}H_{39}NO_2$: C, 79.17; H, 9.60. Found: C, 78.91; H, 9.45.

(*Z*)-3β-Tetrahydropyranyloxypregna-5,17(20)-diene 20-Carbaldehyde (12c) from 11c. The solution of 10.0 g of the nitrile **11c** in 400 ml of dry toluene was cooled to −70 °C, under a nitrogen atmosphere, while stirring. Then 27.0 ml of a 20% solution of diisobutylaluminum hydride in hexane was added. The solution was kept at −70 °C for an additional 30 min and then kept at room temperature overnight. Then 800 ml of a saturated ammonium chloride solution was added and the mixture was stirred vigorously for 1 h. The product was extracted with ethyl acetate, and the extract washed with water, dried, and concentrated. Purification by preparative TLC (benzene–ethyl acetate, 9:1) gave, after recrystallization from methanol, 7.0 g of pure **12c:** mp 179–181 °C; ir 1655 (–CHO), 1610 (C=C), 1030 and 960 cm^{-1} (ether); NMR δ 1.03 (s, 3 H, 18-CH_3), 1.10 (s, 3 H, 19-CH_3), 1.70 (s, 3 H, 21-CH_3), 5.35 (m, 1 H, 6-CH), and 10.27 (–CHO).

Anal. Calcd for $C_{27}H_{40}O_3$: C, 78.59; H, 9.77. Found: C, 78.43; H, 9.56.

The acetate (**12b**) was obtained in a similar manner from **11b.** A solution of 5.70 g of the *Z* nitrile (**11b**) in 200 ml of toluene was reduced with diisobutylaluminum hydride as described for its tetrahydropyranyl ether (**11c**). The residue containing the crude reduction product was acetylated and the acetate isolated in the usual fashion. The crude residue was purified by filtration of its benzene solution through a Florisil column (200 g) and the eluates were crystallized from methanol to give 3.90 g of aldehyde (**12b**): mp 140–143 °C; ir 1720 (OAc), 1635 (conjugated –CHO), and 1250 cm^{-1} (OAc); NMR δ 1.06 (s, 3 H, 18-CH_3), 1.10 (s, 3 H, 19-CH_3), 1.71 (s, 3 H, 21-CH_3, $W_{1/2}$ = 4 Hz), and 10.17 (s, 1 H, CHO).

Anal. Calcd for $C_{24}H_{34}O_3$: C, 77.80; H, 9.25. Found: C, 78.09; H, 9.18.

(*Z*)-3β-Hydroxycholesta-5,17(20)-diene (3a) from 12c. A stirred Grignard reagent solution, prepared from 1.20 g of magnesium turnings of 8.5 ml of 1-bromo-3-methylbutane in 75 ml of ether, was diluted with 50 ml of dry benzene and then the solution of 5.20 g of **3** in 50 ml of benzene was added dropwise and the solution stirred overnight. The reaction mixture was hydrolyzed with an ice-cold saturated solution of ammonium chloride. The organic material was extracted with ethyl acetate and the extract was washed with water, dried over sodium sulfate, and concentrated. The unstable crude alcohol was methylated at once by dissolving it in 25 ml of dry tetrahydrofuran and adding this solution to a solution of sodium methylsulfinylmethide in dimethyl sulfoxide (50 ml of Me_2SO and 1.0 g of NaH 50%). The solution was stirred for 3 h under nitrogen at room temperature, then 15 ml of methyl iodide was added and the solution was stirred overnight. Addition of ice and cold water followed by ether extraction gave a crude product which was purified by chromatography over 300 g of Florisil. The allylic ether was obtained pure by elution with ether–hexane (1:9) yielding 5.0 g of (*Z*)-3β-tetrahydropyranyloxy-22-methoxycholesta-5,17(20)-diene (**22**—isomeric mixture) as a mobile liquid: ir 1030 and 960 cm^{-1} (ether); NMR δ 0.83 (s, 3 H, 18-CH_3), 0.93 (s, 3 H, 19-CH_3), 0.94 (d, J = 6 Hz, 6 H, 26- and 27-CH_3), 3.17 and 3.18 (22-OCH_3) and 5.32 (m, 1 H, 6-CH).

Anal. Calcd for $C_{33}H_{54}O_3$: C, 79.46; H, 10.92. Found: C, 79.46; H, 10.86.

To a solution of 4.9 g of the allylic ether in 100 ml of anhydrous ethylamine was added rapidly 1.4 g of lithium, cut into small pieces, and the mixture was stirred until a blue color persisted for 20 min. Then the mixture was slowly poured into a cold saturated ammonium chloride solution, the product extracted with ether, and the extract washed with water, dried over sodium sulfate, and concentrated to give 4.5 g of a gummy product. To the solution of 2.10 g of the crude $\Delta^{5,17(20)}$-dienyltetrahydropyranyl ether (**3c**) in 20 ml of a tetrahydrofuran–methanol (1:1) solution was added 5 drops of concentrated hydrochloric acid and the solution heated on a steam bath for 2 h. The 3β-hydroxy product was extracted with ether, and the extract washed with water, dried, and concentrated to give, after recrystallization from acetone, 1.30 of (*Z*)-3β-hydroxycholesta-5,17(20)-diene (**3a**): mp 120–123 °C; ir 3200 and 1050 cm^{-1} (–OH); NMR δ 0.85 (s, 3 H, 18-CH_3), 0.87 (d, J = 6 Hz, 6 H, 26,27-CH_3), 1.53 (s, 3 H, 21-CH_3), 1.57 (OH), 5.35 (m, 1 H, 6-CH).

Anal. Calcd for $C_{27}H_{44}O$: C, 84.31; H, 11.53. Found: C, 84.47; H, 11.72.

(*Z*)-3α,5-Cyclo-6β-methoxy-5α-cholest-17(20)-ene (3d) from 3a. To a solution of 800 mg of the alcohol **3a** in 10 ml of pyridine was added 600 mg of *p*-toluenesulfonyl chloride and the solution kept at room temperature overnight. Then it was poured into ice, the product was extracted with ether, and the extract was washed with ice-cold 2 N hydrochloric acid and water, dried over anhydrous sodium sulfate, and concentrated to give crude tosylate. A solution of 2.50 g of the tosylate in 50 ml of methanol and 10 ml of pyridine was heated under reflux for 3 h and then most of the solvents were removed in vacuo. The residue was diluted with water and extracted with ether, dried, and concentrated. Purification on a Florisil column (100 g) gave, with 2% ether in hexane, eluates containing 1.3 g of syrupy **3d** which failed to crystallize after 2 years standing: ir 1090 cm^{-1} (–OCH_3); NMR δ 0.88 (d, 26,27-CH_3), 0.92 (18-CH_3), 1.03 (19-CH_3), and 3.33 (–OCH_3). The corresponding acetate, (*Z*)-3α,5-cyclo-6β-acetoxy-5α-cholest-17(20)-ene, was similarly prepared from the intermediate tosylate of **3a** by heating its solution in 20 ml of acetone and 5 ml of water containing 700 mg of potassium acetate under reflux for 20 h. The solvents were removed under vacuum, the residue extracted with pentane, the solution dried and concentrated, and the crude product was purified by preparative TLC to give 400 mg of syrup which was acetylated with acetic anhydride and pyridine at room temperature for 18 h. The usual workup, followed by purification or preparative TLC, gave, after recrystallization from methanol, 517 mg of the 6-acetoxy derivative: mp 114–116 °C; ir 1720 and 1230 cm^{-1} (acetate); NMR δ 0.88 (d, J = 6 Hz, 6 H, 26,27-CH_3), 0.92 (s, 3 H, 18-CH_3), 1.01 (s, 3 H, 19-CH_3), 1.55 (s, 3 H, 21-CH_3), 2.05 (s, 3 H, Ac), and 4.54 (m, 1 H, 6-CH).

Anal. Calcd for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87. Found: C, 81.72; H, 11.07.

3α,5-Cyclo-5α-cholestan-6β-yl Methyl Ether (16d) from 3d. The solution of 100 mg of the syrupy olefin **3d** in 10 ml of ethyl acetate was hydrogenated (100 mg of 10% Pd/C, H_2 at 45 psi) for 40 h. The product was purified by TLC and crystallized from methanol to give 85 mg of **16d,** mp 69–71 °C,[13] ir and NMR identical with those of an authentic sample prepared from cholesterol.

(20*S*)-20-Hydroxycholesterol (4a) from 3d. To an ice-cold solution of 250 mg of the 3,5-cyclo derivative (**3d**) in 7 ml of tetrahydrofuran was added 2.5 ml of diborane–tetrahydrofuran complex and the solution stirred for 1 h at 0 °C and for an additional 1 h at 22 °C. Then 4 ml of a 2 N sodium hydroxide solution was added dropwise, the mixture was cooled to 0 °C and 4 ml of a 30% hydrogen peroxide solution was added, while stirring, and the mixture kept at 0 °C for 1 h. The product was extracted with ethyl acetate, the extract washed with a 10% sodium bicarbonate solution and water and dried, and the solvent evaporated. To the solution of the residue in 35 ml of dimethyl sulfoxide and 5 ml of water at 0 °C was added, dropwise, 2 ml of 7% perchloric acid and then 10 ml of tetrahydrofuran was added to give

a homogenous solution. The solution was kept at room temperature for 3 days, then it was diluted with water and extracted with ethyl acetate. The product was prepurified on TLC and gave 76 mg of solids. Purification on a Celite partition column (isooctane–methanol, 1:9) gave 21 mg of (20S)-20-hydroxycholesterol (**4a**), mp 130–132 °C (MeOH), ir and NMR identical with those of an authentic sample (Table I). In addition there was obtained 43 mg of 17α-hydroxycholesterol, mp 175–177 °C (MeOH); the ir and NMR spectra were indistinguishable from those of an authentic sample[14] (Table I).

(20S)-3β,17α,20-Trihydroxycholest-5-ene (17a) from 3d. To a solution of 200 mg of the 3,5-cyclo derivative (**3d**) was added 250 mg of osmium tetroxide and the mixture was kept for 5 days in the dark. Then it was poured into a solution of 500 mg of lithium aluminum hydride in 140 ml of ether and the mixture was heated under reflux for 3 h. The excess hydride was decomposed with a saturated aqueous solution of sodium sulfate and the crude product isolated in the usual fashion. The total residue was hydrolyzed with perchloric acid in the same fashion as indicated (above) for the alcohol **4a**. The product, crystallized from methylene chloride, gave 25.0 mg with mp 160–162 °C;[15] the ir and NMR spectra were superimposable on those obtained from authentic material (Table I).

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Registry No.—**2a**, 21903-19-5; **2b**, 56312-72-2; **3a**, 41083-88-9; **3d**, 59873-54-0; **4a**, 516-72-3; **4b**, 7484-20-0; **5a**, 7484-22-2; **5b**, 7429-99-4; **8a**, 59905-87-2; **8b**, 54548-85-5; **10b**, 3092-00-0; **11b**, 5143-83-9; **11c**, 58449-04-0; **12b**, 59873-55-1; **12c**, 59873-56-2; **16d**, 2867-93-8; **17a**, 382-78-5; dihydropyran, 25512-65-6; 1-bromo-3-methylbutane, 107-82-4; (Z)-3β-tetrahydropyranyloxy-22(R)-methoxycholesta-5,17(20)-diene, 59873-57-3; (Z)-3β-tetrahydropyranyloxy-22(S)-methoxycholesta-5,17(20)-diene, 59873-58-4; (Z)-3α,5-cyclo-6β-acetoxy-5α-cholest-17(20)-ene, 59873-59-5.

References and Notes

(1) Y. M. Sheikh, B. Tursch, and C. Djerassi, *Tetrahedron Lett.*, 3721 (1972).
(2) R. C. Ebersole, W. O. Godtfredsen, S. Vangedal, and E. Caspi, *J. Am. Chem. Soc.*, **95**, 8133 (1973).
(3) N. K. Chaudhuri, R. Nickolson, J. G. Williams, and M. Gut, *J. Org. Chem.*, **34**, 3767 (1969).
(4) B. B. Snider, R. J. Corcoran, and R. Breslow, *J. Am. Chem. Soc.*, **97**, 6580 (1975).
(5) Evidence for the configuration (*E*) of the 20(22)-dehydrocholesterol melting at 124 °C as the acetate has been presented by J. P. Schmit, M. Piraux, and J. F. Pilette, *J. Org. Chem.*, **40**, 1586 (1975), who obtained it by the Wittig reaction. This is the isomer obtained from the dehydration of the 20-hydroxycholesterols as well as of 22α-hydroxycholesterol [K. Tsuda and R. Hayatsu, *J. Am. Chem. Soc.*, **81**, 5987 (1959)]. The *Z* isomer has been described by W. G. Anderson, C. Y. Byon, M. Gut, and F. H. Bissett, *Tetrahedron Lett.*, 3193 (1976).
(6) This has been discussed at greater length by W. R. Nes and T. E. Varkey, *J. Org. Chem.*, **41**, 1652 (1976).
(7) From an analysis of the NMR signals from the C-18 protons of pregnanes substituted at C-20, e.g., 20α- and 20β-hydroxyprogesterone, C. H. Robinson and P. Hofer, *Chem. Ind. (London)*, 377 (1966), have concluded similarly that the preferred conformation about the 17(20) bond is the one in which the two large groups project to the rear and the H atom to the front. The NMR data provided by Robinson and Hofer together with that given in the present paper (Table I) also consistently show a downfield shift in the signal from C-21 in the cases where C-21 lies to the left compared to the value for the conformer or the $\Delta^{17(20)}$ isomer with C-21 to the right.
(8) N. K. Chaudhuri and M. Gut, *J. Am. Chem. Soc.*, **87**, 3737 (1965).
(9) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry", Holden-Day, San Francisco, Calif., 1964, p 110, and references cited therein; M. Tanabe and R. H. Peters, *J. Org. Chem.*, **36**, 2403 (1971).
(10) V. Petrow and I. A. Stuart-Webb, *J. Chem. Soc.*, 4675 (1956).
(11) A. Mijares, D. I. Cargill, J. A. Glasel, and S. Lieberman, *J. Org. Chem.*, **32**, 810 (1967).
(12) K. Tsuda and R. Hayatsu, *J. Am. Chem. Soc.*, **81**, 5987 (1959).
(13) E. G. Ford and E. S. Wallis, *J. Am. Chem. Soc.*, **59**, 1415 (1937), gives mp 78–78.5 °C.
(14) N. K. Chaudhuri, R. C. Nickolson, and M. Gut, *Steroids*, **16**, 495 (1970), gives 168–170 °C.
(15) N. K. Chaudhuri, J. G. Williams, R. C. Nickolson, and M. Gut, *J. Org. Chem.*, **34**, 3759 (1969).

Approaches to Analogues of Dehydrogliotoxin. 6.[1] An Efficient Synthesis of a Gliotoxin Analogue with Anti-Reverse Transcriptase Activity[2]

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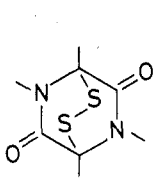
The addition of α-ketoacyl chlorides **4** to indolenine-2-carboxamides **3**, followed by spontaneous, diastereoselective ring closure to 3,6-disubstituted dioxopiperazines (**5** → **6**), provides an efficient, new synthesis of gliotoxin analogues. Compound **6** was converted into the mercaptoalkene **11** by treatment with H_2S. Regiospecific and diastereoselective addition of H_2S to the exo methylene group gave cis dithiol **12**. This zinc ion catalyzed reaction is believed to proceed via the chelate intermediate **19a**. Several oxidation procedures were studied for the conversion of **12** into disulfide **13**. The tri- and tetrasulfides **21** and **22** were obtained from **12** by reaction with SCl_2 and S_2Cl_2, respectively; the monosulfide **20** was obtained from **13** by treatment with $(C_6H_5)_3P$. Analogies between this synthesis and what is known about the biosynthesis of gliotoxin are discussed. Compound **13** thus obtained (81% overall yield) was found to inhibit the enzyme reverse transcriptase, while having no effect on transcriptase; its activity is comparable to that of gliotoxin.

The epidithiodioxopiperazine system **1**, common to a number of fungal metabolites, including dehydrogliotoxin (**2**), the sporidesmins, aranotins,[5] and others,[6] appears to be the site of the potent antiviral, antibacterial, or antifungal activities of this group of compounds. Several syntheses of simple derivatives of **1** have appeared[7] and Kishi and coworkers have recently reported a 12-step synthesis of (±)-dehydrogliotoxin (**2**).[8] We wish to report the development of



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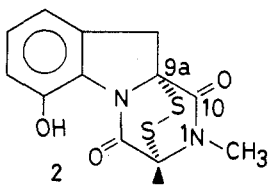

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