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STEREOCHEMICALLY CONTROLLED SYNTHESES OF 20,22-EPOXYCHOLESTEROLS

Masato Koreeda*, Naoyuki Koizumi, and Beverly A. Teicher

Department of Chemistry, The Johns Hopkins University, Baltimore, Maryland 21218

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Although the side-chain cleavage reaction of cholesterol to pregnenolone in mammalian adrenal cortex has been extensively studied, the mechanism of this reaction has not yet been definitively proven.[1] Evidence for a sequential hydroxylation pathway involving (20S)-20- or (22R)-22-hydroxy- and (20R,22R)-20,22-dihydroxycholesterols has been well established.[2] While each of the proposed intermediates is enzymatically converted to pregnenolone, detailed kinetic studies reveal a significant discrepancy between the amount of pregnenolone formed from cholesterol in the incubation system, and the amount formed from the proposed sequential hydroxylation mechanism.[3] This finding stimulated the proposal of different mechanisms for the side-chain cleavage. In a series of communications, Kraaipoel et al.[4] described the biosynthetic conversion of 20,22-dehydrocholesterol (unspecified stereochemistry) to pregnenolone in bovine adrenal mitochondria and suggested a mechanism involving 20,22-olefin and 20,22-epoxycholesterol intermediates.

A more rigorous study of this problem requires developing synthetic routes which will unambiguously provide stereochemically pure epoxides. This communication presents highly stereochemically controlled syntheses of each of the four 20,22-epoxycholesterols. A proficient means of constructing oxygenated cholesterol side-chains utilizing the 1,3-dithiane anion[5] is also described.

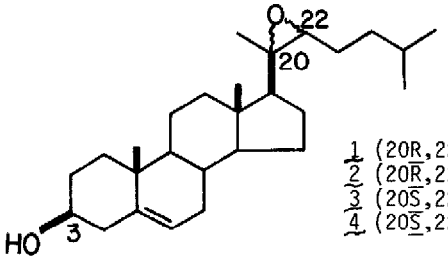
Three of the four possible 20,22-epoxides were obtained by treating their corresponding 20,22-α-glycol 22-mesylates with base, which gives inversion of configuration at C-22 during epoxide formation; and the fourth by direct epoxidation of the 20,22-olefin. Since (20R,22R)-20,22-dihydroxy-5α-cholestanol (5,6-dihydro-8a) has been prepared from 5,6-dihydro-7,[6] a convenient synthesis of 7 was sought. Addition of 2-lithio-1,3-dithiane to pregnenolone tetrahydropyranyl (THP) ether 5 (dry THF, 0°, 6 hrs, argon) followed by hydrolysis of the resulting adduct 6 with $HgCl_2/CaCO_3$ in THF/acetonitrile/water (reflux, 50 hrs) and acetylation, gave the desired aldehyde 7 in 80% yield from 5 (mp. 182-184°; NMR: ^1H (δ ppm) 1.33 (s, 3H, 22-H) and 9.57 (s, 1H, 21-H), ^{13}C (δ ppm) 79.5 (20-C) and 203.5 (21-C)).[#] The aldehyde was converted into the stereochemically

[#] NMR spectra were taken with a Jeol MH-100 (^1H) and a Varian CFT-20 (^{13}C) in $CDCl_3$.

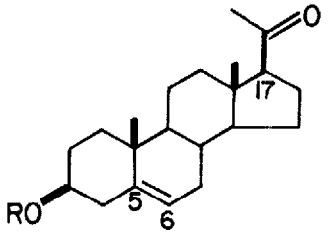
pure 20R,22R-triol 8a (mp. 177-179°) with an excess of isoamylmagnesium bromide in benzene/ether in 95% yield. The 22-mesylate of the triol 3-pivaloyl ester 8b, which was selectively prepared from 8a in 88% yield, when treated with KOH in methanol/THF at reflux for 11 hrs afforded the 20R,22S-epoxide 2 in 84% yield from 8a (amorphus powder; NMR: ^1H (δ ppm) 0.80 (s, 3H, 13-Me), 1.28 (s, 3H, 20-Me), and 2.64 (m, 1H, 22-H), ^{13}C (δ ppm) 14.7 (18-C), 61.1 (20-C), and 66.5 (22-C); mass spec.: M^+ at m/e 400).

An effective use of the 1,3-dithiane anion system is illustrated by the following synthesis of the α-hydroxyketone 10a. Addition of 2-lithio-2-isoamyl-1,3-dithiane to 5 yielded adduct 9 in 70% yield (-25°, THF, 7hrs, argon). Hydrolysis with $HgCl_2/CaCO_3$ of 9 gave the 20R-α-hydroxyketone 10a in 51% yield (mp. 178-180°; CD (dioxane) $\Delta\varepsilon_{281}$ -3.33; NMR: ^1H (δ ppm) 0.91 (s, 3H, 13-Me) and 1.43 (s, 3H, 20-Me), ^{13}C (δ ppm) 80.1 (20-C) and 214.2 (22-C)). This constitutes a very convenient synthetic route to 10a; an earlier reported synthesis[7] of 10a required five synthetic steps from pregnenolone acetate. Sodium borohydride reduction of the α-hydroxyketone 3-benzoate 10b in methanol/THF afforded predominantly the 20R,22S-diol 11 in 81% yield (mp. 186-188°).[8] The minor product, the 22R-isomer, was formed in 12% and was easily separated from the major product by fractional recrystallization using a chloroform/methanol solvent system. Conversion of 11 into the 20R,22R-epoxide 1 was carried out via the 22-mesylate as described above in 91% yield (mp. 131-133°; NMR: ^1H (δ ppm) 0.80 (s, 3H, 13-Me), 1.29 (s, 3H, 20-Me), and 2.65 (m, 1H, 22-H), ^{13}C (δ ppm) 13.2 (18-C), 60.1 (20-C), and 60.3 (22-C)).

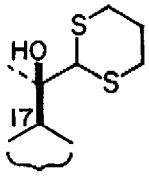
The two epoxides with a 20S-configuration were prepared via E-20(22)-dehydrocholesterol 3-benzoate 12. A Wittig reaction on pregnenolone,[9] followed by benzoylation provided 12 in 75% yield. Treatment of the olefin 12 with osmium tetroxide in ether, followed by $NaHSO_3$ reduction gave the α-glycols 13 (72%, mp. 186-188°) and 8c (5%), which were purified by fractional recrystallization from acetone. The major product 13 was assigned the 20S,22S-configuration based on the CD spectrum of the α-hydroxyketone 14 (mp. 191-192°; CD (dioxane) $\Delta\varepsilon_{281}$ +1.38; NMR: ^1H (δ ppm) 0.72 (s, 3H, 13-Me) and 1.31 (s, 3H, 20-Me), ^{13}C (δ ppm) 79.8 (20-C) and 215.3 (22-C)) as opposed to the CD spectrum of 20R-α-hydroxyketone 10a. The α-hydroxyketone 14 was obtained by treatment of α-glycol 13 with N-chlorosuccinimide/dimethyl sulfide in toluene under argon, followed by addition of triethylamine.[10] This oxidation provided the α-hydroxyketone 14 in 96% yield from the α-glycol 13. This α-glycol was converted to the 20S,22R-epoxide 3 through base treatment of the 22-mesylate in 83% yield (mp. 133-134°; NMR: ^1H (δ ppm) 0.90 (s, 3H, 13-Me), 1.30 (s, 3H, 20-Me), and 2.44 (m, 1H, 22-H), ^{13}C (δ ppm) 14.4 (18-C), 61.1 (20-C), and 65.1



1 (20R,22R)
2 (20R̄,22S̄)
3 (20S̄,22R̄)
4 (20S̄,22S̄)



5 R = THP



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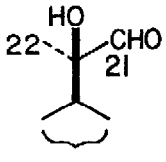
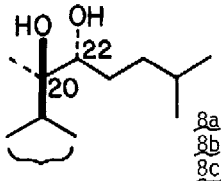
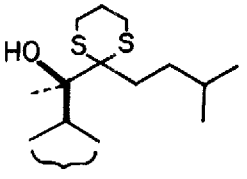


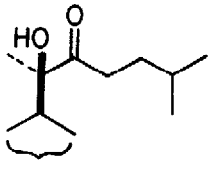
7 = Ac



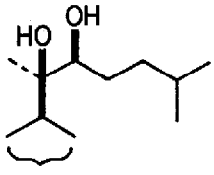
8a R = H
8b R = t-Bu-CO-
8c R = Bz



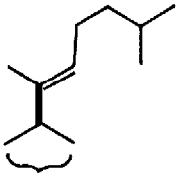
9 R = THP



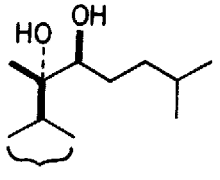
10a R = H
10b R = Bz



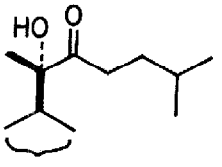
11 R = Bz



12 R = Bz



13 R = Bz



14 R = Bz

(22-C)).

The 20S,22S-epoxide 4 was obtained in a less stereochemically controlled manner. Direct epoxidation of E-20(22)-dehydrocholesterol 3-benzoate 12 with 1.2 mol. equiv. of m-chloroperbenzoic acid (MCPBA) provided regiospecifically 20,22-epoxides. However, the stereoselectivity of this reaction was not remarkable under the various conditions examined. Greatest stereoselectivity was obtained using 1.2 mol. equiv. of MCPBA in methylene chloride at 0^{o} for 2 hrs. These conditions produced the 20S,22S-epoxide 4 3-benzoate in 45% yield and 20R,22R-epoxide 1 3-benzoate in 26% yield. Base hydrolysis of 20S,22S-epoxide 4 3-benzoate in KOH/methanol/THF gave pure 20S,22S-epoxide in 95% yield (mp. 132.5-133.5^{o}; NMR: ^{1}H (δ ppm) 0.68 (s, 3H, 13-Me), 1.30 (s, 3H, 20-Me), and 2.92 (m, 1H, 22-H), ^{13}C (δ ppm) 13.6 (18-C), 59.6 (22-C), and 60.5 (20-C)).

Two oxidation reactions on E-20(22)-dehydrocholesterol have been described above. Both of these show a preference for the 20-re,22-si face[11] entry of the reagent at the 20,22-double bond; this contrasts markedly with catalytic hydrogenation data obtained on the same olefin.[9]

Recently, Morisaki, et al.[12] published results indicating that E-20(22)-dehydrocholesterol and the 20,22-epoxycholesterols are not good intermediates in the side-chain cleavage reaction in their incubation system. Burstein, et al.,[13] using (20S)-[20-^{18}O]-20-hydroxycholesterol and (22R)-[22-^{18}O]-22-hydroxycholesterol, showed retention of ^{18}O in the (20R,22R)-20,22-dihydroxycholesterol formed in the incubation system, thus providing evidence against the possibility of dehydration of a hydroxycholesterol to form a 20,22-olefin intermediate. They tested E- and Z-20(22)-dehydrocholesterols and two of the 20,22-epoxides in their incubation system and found little conversion of these compounds to pregnenolone.

We are independently investigating the mechanism of the side-chain cleavage reaction.[14]

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