Hypocholesterolemic Agents. 9.[1] C-20 Epimeric 22,25-Diazacholesterols†

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The potent hypocholesterolemic properties of 22,25-diazacholesterol (7) prompted the synthesis of the 20β epimer (2) in order to determine the effect of side-chain stereochemistry on biological activity. Both epimeric diazasteroids were prepared by SN2 displacement of the appropriate C-20 tosylate. The desired products were accompanied by large amounts of olefinic and *D*-homo rearranged by-products. Only the epimer (20α) whose stereochemistry coincided with that of cholesterol showed appreciable hypocholesterolemic activity.

One successful approach to the development of hypocholesterolemic agents has been the preparation of compounds which will in some manner interfere with the endogenous synthesis of cholesterol. Previous papers in this series have described the synthesis and potent hypocholesterolemic properties of certain aza- and diazacholesterols.[2-4] Not only were these compounds found to inhibit cholesterol synthesis in laboratory animals[5-7] and man,[8] but their ability to interfere with cholesterogenesis in insects has also been demonstrated.[9] Moreover, the pronounced avian antifertility action of 20,25-diazacholesterol (Ornitrol)[10] suggests that these compounds may also affect other phases of cholesterol metabolism.

The pronounced hypocholesterolemic properties of 22,-25-diazacholesterol have prompted other groups to investigate similar isosteres of cholesterol. Irmscher, *et al.*,[11] reported the synthesis of 22-oxa-25-azacholesterol and found that 25-azacholesterols were potent hypocholesterolemics regardless of whether C-22 was present as a carbon atom or replaced by oxygen or nitrogen. The 20β epimer of 22-oxa-25-azacholesterol, which has the opposite stereochemistry to cholesterol at C-20, was prepared by Cross, *et al.*,[12] but unfortunately no biological activity was reported. As previously noted in this series,[13] alterations of the stereochemistry of 20,25-diazacholesterol at C-17 caused a marked decrease in hypocholesterolemic activity. Our continued interest in the relation of stereochemistry to biological action for these compounds prompted the present study dealing with the synthesis and biological evaluation of the 20β epimer of 22,25-diazacholesterol.

The most direct route to the 20β epimer (2) appeared to be aminolysis of a 20α-tosyloxypregnane derivative with the appropriate amine. Several laboratories have studied the solvolysis of both 20α- and 20β-tosyloxypregnanes and have indicated that the nature of the products depends largely on reaction conditions. For example, treatment of 5α-pregnan-20α-ol tosylate with NaN₃ in hexamethylphosphotriamide solution led to the 20β-azide (52%) and the trans elimination product, 5α-pregn-17(20)-ene (48%).[14] On the other hand, only elimination products were obtained when the tosylate was refluxed with pyridine for 2 hr. Similar treatment of the 20β-tosylate gave a mixture of the cis and trans Δ¹⁷ olefins along with the *D*-homo rearranged product, 17α-methyl-5α-*D*-homoandrostan-17aβ-ol tosylate. *D*-Homoannulation is a common finding in the solvolysis of 20β-tosylates in the pregnane series.[15,16]

In our studies, heating pregn-5-ene-3β,20α-diol 20-tosylate (1b) in 2-dimethylaminoethylamine afforded the desired 20β-diazacholesterol (2) in low yield. Acetylation of the neutral fraction followed by chromatography on silicic acid treated with AgNO₃ yielded two isomeric 3β-acetoxypregna-

dienes (3 and 4) and a rearranged *D*-homoacetate (5). The olefinic by-products (3, 4) were readily identified by nmr. Not only were the chemical shifts for 3 in agreement with those reported for similar Δ¹⁷-*trans*-pregnanes,[14] but also the melting point for 3 agreed with that previously reported.[17] The more polar 3β-acetoxypregnadiene analyzed for C₂₃H₃₄O₂ and the infrared spectrum showed strong bands at 6.11, 9.98, and 10.92 μ, indicative of a vinylidene group. The nmr spectrum (100 MHz)‡ confirmed the presence of four vinyl protons and the C-20 olefin 4 was assigned to this product. Lee and Wolff[16] deduced on the basis of nmr data that a C-20 olefin was present after acetolysis of a 20β-tosylated pregnane but were unable to isolate the olefin in pure form.

The last product eluted from the column was assigned structure 5 on the basis of its nmr spectrum and independent synthesis. The C-18 CH₃ and C-17 CH₃ resonances at δ 0.88 and 0.80 (d, *J* = 6 Hz), respectively, were in agreement with those reported by Lee and Wolff.[16] Furthermore, uranediol rearrangement of pregn-5-ene-3β,20α-diol 3-acetate 20-tosylate (1b) with formic acid, followed by hydrolysis of the 20-formate and subsequent acetylation, gave a product identical in all respects with that obtained by aminolysis of 1b and subsequent acetylation.
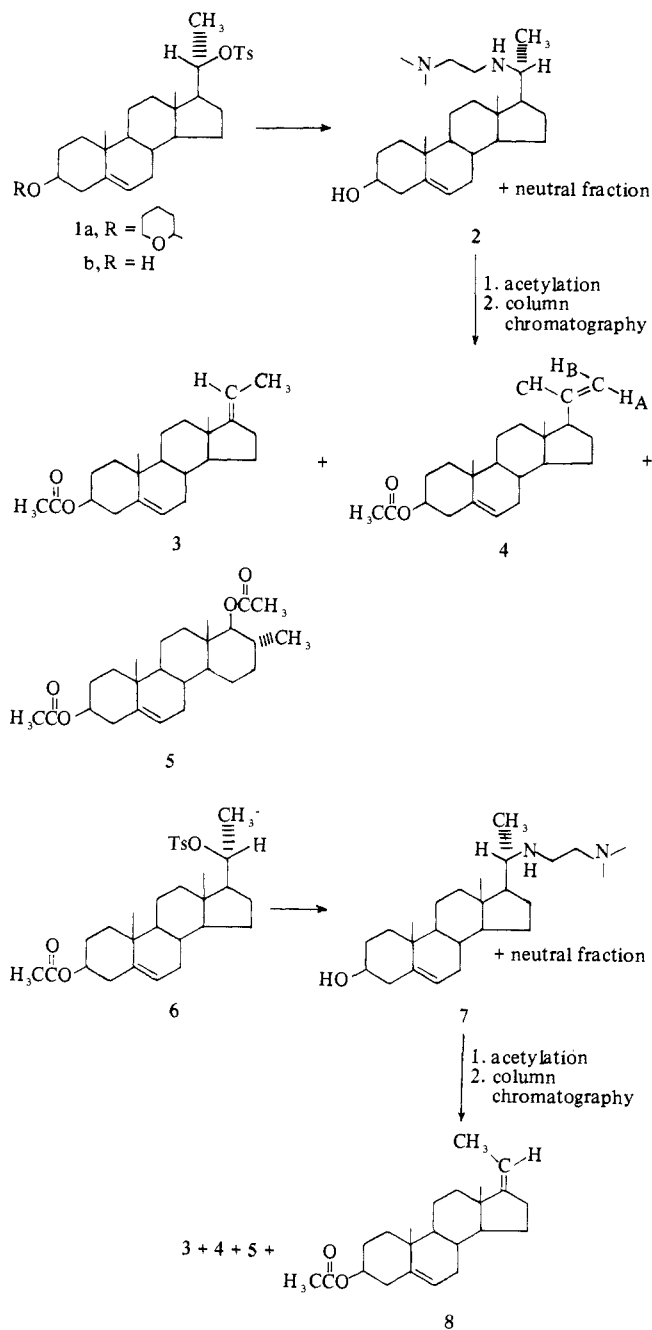
For comparison purposes, the 20β-tosyloxypregnane derivative 6 was also subjected to the same reaction conditions. Heating 6 with 2-dimethylaminoethylamine afforded a low yield of 22,25-diazacholesterol (7) previously prepared by an alternate route.[3] Acetylation of the neutral fraction followed by chromatography on silicic acid impregnated with AgNO₃ afforded the three isomeric 3β-acetoxypregnadienes 8, 3, and 4 as well as the rearranged *D*-homoacetate 5. The known cis olefin 8 was readily identified by its nmr spectrum. The melting point was also in agreement with that previously reported for this compound.[18]

The formation of the same *D*-homoacetate 5 by aminolysis of both the 20α- and 20β-tosylates was surprising and argues against a concerted mechanism involving a nonclassical 17,20-bridged transition state as previously proposed for these rearrangements.[19] Our results are in agreement with the recent findings of Hirschmann, *et al.*,[20] who obtained the same *D*-homoandrostane derivative upon formolysis of epimeric 20-tosyloxypregnanes. These workers cite evidence to support distinct reaction paths for the two tosylates.

The configuration of 20β-22,25-diazacholesterol was confirmed by nmr spectroscopy. Robinson and Hofer[21] have noted that for epimeric 20-amino steroids the C-21 methyl resonance for the 20α epimer is downfield, relative to that for the 20β compound, by 0.07–0.11 ppm. In our case, the C-21 methyl resonance for the β epimer 2 was 0.97 ppm

1a, R = (tetrahydropyranyl)

b, R = H

2 + neutral fraction

1. acetylation
2. column chromatography

3 + 4 + +

5

6 → 7 + neutral fraction

1. acetylation
2. column chromatography

3 + 4 + 5 + 8

(J = 5.5 Hz), whereas the one for the α epimer 7 was 1.08 ppm (J = 6.5 Hz) and in complete accord with the above findings. Moreover, a downfield shift of 4–4.5 Hz for the C-18 methyl resonance was noted for the β epimer but not the α epimer when the spectra were taken in pyridine. On the basis of previous studies[22] dealing with pyridine-induced solvent shifts, solvent interaction and subsequent deshielding of the C-18 protons would only be expected for the 20β epimer.

Both 2 and 7 were evaluated for hypocholesterolemic activity as their dihydrochloride salts. Both compounds were administered orally to male rats made hypercholesterolemic with 6-propylthiouracil according to the method of Ranney and Cook.[7] In the assay, 7 was found to cause a 35% reduction in serum cholesterol at a dose of 1 mg/kg. On the other hand, the 20β epimer 2, which possesses the unnatural stereochemistry at C-20, was found to have approximately 10% the hypocholesterolemic activity of 7.[§] This result is

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consistent with our previous studies in this series which have shown that any structural departure from the cholesterol molecule usually leads to a marked reduction in hypocholesterolemic activity.

Experimental Section[#]

Pregn-5-ene-3β,20α-diol 3-Tetrahydropyranyl Ether 20-p-Toluenesulfonate (1a). p-Toluenesulfonyl chloride (0.3 g) was added to a soln of pregn-5-ene-3β,20α-diol 3-tetrahydropyranyl ether[7] (0.3 g) in dry pyridine (5 ml), and the mixture was stirred at room temp for 6 hr. The reaction mixture was then poured onto ice H_2O, and the ppt was filtd, washed well with H_2O, and recrystd from aqueous Me_2CO to give 1a (0.45 g, 81%): mp 128–130°; [α]D –37°; nmr ($CDCl_3$) δ 0.62 (C-18 CH_3), 0.74 (C-19 CH_3), 1.26 (d, J = 6.5 Hz, C-21 CH_3).** Anal. ($C_{33}H_{48}O_5S$) C, H.

Pregn-5-ene-3β,20α-diol 20-p-Toluenesulfonate (1b). Ethanolic HCl soln (0.2 N, 5 ml) was added to a soln of 1a (100 mg) in THF (5 ml), and the mixture was stirred for 1 hr at room temp. The reaction mixture was then decompd with H_2O and extd with $CHCl_3$. The organic layer was sepd, dried (Na_2SO_4), and evapd, and the residue recrystd from aqueous Me_2CO to give 1b (65 mg, 69%): mp 136–138°; [α]D –47°; nmr ($CDCl_3$) δ 0.65 (C-18 CH_3), 0.98 (C-19 CH_3), 1.30 (d, J = 6 Hz, C-21 CH_3). Anal. ($C_{28}H_{40}O_4S$) C, H.

20β-(2-Dimethylaminoethyl)aminopregn-5-en-3β-ol (2). A soln of pregn-5-ene-3β,20α-diol 20-toluenesulfonate (1b, 1.5 g) in freshly distd 2-dimethylaminoethylamine (20 ml) was heated at gentle reflux with stirring under N_2 for 2 days. The solvent was removed completely *in vacuo*, and the residue extd with 10% HCl and Et_2O. The organic and aqueous phases were sepd, and the latter was made alkaline with 10% NaOH soln. The ppt was filtered, washed well with H_2O, and recrystd from EtOAc to give 2 (150 mg, 12%): mp 122–124°; [α]D –60°; nmr ($CDCl_3$) δ 0.72 (C-18 CH_3), 1.00 (C-19 CH_3), 0.97 (d, J = 5.5 Hz, C-21 CH_3), 2.2 (–N(CH_3)$_2$). Anal. ($C_{25}H_{44}N_2O$) C, H.

The ether layer from the original extn was washed with dil $NaHCO_3$ soln and H_2O and dried (Na_2SO_4). The solvent was removed *in vacuo*, and the residue acetylated with Ac_2O in pyridine. The usual work-up afforded a mixture of products (1 g) which was chromatographed on silicic acid (Mallinckrodt) impregnated with 5% $AgNO_3$. Elution with hexane–C_6H_6 (9:1, 300 ml) gave *trans*-3β-acetoxypregna-5,17(20)-diene (3, 200 mg): mp 142–143° (from MeOH, lit.[17] mp 139–140°); [α]D –71°; nmr ($CDCl_3$) δ 0.73 (C-18 CH_3), 1.56 (C-21 CH_3, dd, $J_{H21,H20}$ = 7 Hz, $J_{H20,H16}$ = 1.5 Hz), and 2.30 (C-16 methylene protons). Further elution with the same solvent system (500 ml) gave a mixture of 3 and 4 (200 mg). This was followed by a fraction (200 ml) contg only 3β-acetoxypregna-5,20(21)-diene (4, 110 mg): mp 133–134° (from MeOH); [α]D –77.8°; nmr ($CDCl_3$, 100 MHz) δ 0.60 (C-18 CH_3), 4.97 (dd, trans "A" proton at C-21, J_{AC} = 16 Hz, J_{AB} = 2.1 Hz), 5.00 (dd, cis "B" proton at C-21, J_{AB} = 2.1 Hz, J_{BC} = 8 Hz), 5.58 (C-6 vinyl proton), and 5.77 (a pair of triplets, C-20 "C" proton, J_{AC} = 16 Hz, J_{BC} = 8 Hz, $J_{C,H17}$ = 6.5 Hz). Anal. ($C_{23}H_{34}O_2$) C, H. Elution with hexane–C_6H_6 (7:3) (300 ml) gave a mixture of 4 and 5 (150 mg). This was followed by a fraction contg only 17α-methyl-D-homoandrost-5-ene-3β,17aβ-diol diacetate (5, 90 mg): mp 214–216° (from MeOH); [α]D –112.4° (lit.[11] mp 210–211°, [α]D –112°); nmr ($CDCl_3$) δ 0.80 (C-17 CH_3), 0.88 (C-18 CH_3). Anal. ($C_{25}H_{38}O_4$) C, H.

17α-Methyl-D-homoandrost-5-ene-3β,17aβ-diol Diacetate (5). A soln of pregn-5-ene-3β,20β-diol 3-acetate 20-p-toluenesulfonate[18] (1 g) (6) in formic acid (20 ml) was refluxed for 30 min, cooled, and poured into H_2O. The ppt was filtered, washed, and dried, and the formate group was hydrolyzed with methanolic KOH at room temp overnight. After the usual work-up, the residue was acetylated with Ac_2O in pyridine to give 5 (0.5 g, 64%), mp 215–216°, identical in all respects with that obtained by the aminolysis of 1b or 6, followed by acetylation and chromatography.

Aminolysis of Pregn-5-ene-3β,20β-diol 3-Acetate 20-p-Toluenesulfonate. A soln of 6 (2 g) in freshly distd 2-dimethylaminoethylamine (20 ml) was heated to gentle reflux for 2 days and worked up

**Characteristic nmr signals reported here are in agreement with those reported earlier for several C-20 functional pregnanes by Leboeuf, *et al.*[14]

as described above. The basic fraction afforded 22,25-diazacholes-terol (7, 120 mg): mp 110–112° (lit.[3] mp 112–114°); nmr (CDCl₃) δ 0.70 (C-18 CH₃), 1.00 (C-19 CH₃), 1.08 (d, *J* = 6.5 Hz, C-21 CH₃), 2.17 (–N(CH₃)₂). The neutral fraction (1 g) was acetylated and chro-matographed on silicic acid impregnated with 5% AgNO₃. Elution with hexane–C₆H₆ (9 : 1, 200 ml) gave *cis*-3β-acetoxy-5,20(21)-preg-nadiene (8, 200 mg, 16%): mp 87–89° (from MeOH); [α]D –65° (lit.[18] mp 89°; [α]D –70°). Further elution with the same solvent gave a mixture of 8 and 3 (200 mg). This was followed by a fraction (200 ml) contg pure 3 (100 mg, 8%). The next fraction eluted from the column contained a mixture of 3 and 4 (150 mg). This was fol-lowed by a fraction contg only 4 (110 mg). Further elution with hexane–C₆H₆ (7 : 3) gave the *D*-homoacetate 5 (100 mg).

References

(1) M. C. Lu, F. Kohen, and R. E. Counsell, *J. Med. Chem.*, 14, 136 (1971) (paper 8).

(2) R. E. Counsell, P. D. Klimstra, L. N. Nysted, and R. E. Ranney, *ibid.*, 8, 45 (1965).

(3) R. E. Counsell, P. D. Klimstra, R. E. Ranney, and D. L. Cook, *J. Med. Pharm. Chem.*, 5, 720 (1962).

(4) R. E. Counsell, P. D. Klimstra, and R. E. Ranney, *ibid.*, 5, 1224 (1962).

(5) R. E. Ranney and R. E. Counsell, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 21, 96 (1962); *Proc. Soc. Exp. Biol. Med.*, 109, 820 (1962);

(6) R. E. Ranney, D. L. Cook, W. E. Hambourger, and R. E. Coun-sell, *J. Pharmacol. Exp. Ther.*, 142, 132 (1963);

(7) R. E. Ranney and D. L. Cook, *Arch. Int. Pharmacodyn.*, 154, 51 (1965).

(8) J. M. Martt and C. R. Talbert, *Circulation*, 28, 763 (1963); J. M. Martt, C. R. Talbert, and G. E. Lee, *Ann. Intern. Med.*, 61, 870 (1964); B. A. Sachs and L. Wolfman, *Arch. Intern. Med.*, 116, 336 (1965).

(9) J. A. Svoboda and W. E. Robbins, *Science*, 156, 1637 (1967).

(10) J. E. Wooford and W. H. Elder, *J. Wildl. Manage.*, 31, 507 (1967).

(11) K. Irmscher, J. M. Kraemer, and H. Halpaap, *Steroids*, 7, 557 (1966).

(12) A. D. Cross, E. Denot, R. Acevedo, and P. Crabbé, *Steroids*, 5, 585 (1965).

(13) V. V. Ranade, F. Kohen, and R. E. Counsell, *J. Med. Chem.*, 14, 38 (1971).

(14) M. Leboeuf, A. Cavé, and R. Goutarel, *Bull. Soc. Chim. Fr.*, 1619, 1624, 1628 (1969).

(15) H. Hirschmann, F. B. Hirschmann, and A. P. Zala, *J. Org. Chem.*, 31, 375 (1966).

(16) H. Lee and M. E. Wolff, *ibid.*, 32, 192 (1967).

(17) R. Fischer, G. Lardelli, and O. Jeger, *Helv. Chim. Acta*, 33 1335 (1952).

(18) R. Goutarel, C. Conreur, L. Djakouré, M. Leboeuf, and A. Cavé, *Tetrahedron*, 24, 7013 (1968).

(19) "Steroid Reaction Mechanisms," D. N. Kirk and M. P. Hart-short, Ed., Elsevier, New York, N. Y., 1968, p 302.

(20) F. B. Hirschmann, D. M. Kautz, S. S. Deshmane, and H. Hirschmann, *Tetrahedron*, 27, 2041 (1971).

(21) C. H. Robinson and P. Hofer, *Chem. Ind. (London)*, 377 (1966).

(22) P. V. Demarco, E. Farkas, D. Doddrell, B. L. Mylari, and E. Wenkert, *J. Amer. Chem. Soc.*, 90, 5480 (1968).

3(2H)-Isoquinolones. 1. 3-Oxygenated Analogs of Papaverine as Peripheral Vasodilators†

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Two types of papaverine analogs, *N*-substituted-6,7-dialkoxy-1-(3,4-dialkoxybenzyl)-3(2H)-isoquinolone hydrochlorides (5, 10) and 3-alkoxypapaverines (6) were prepared, and the cardiovascular effects of rep-resentative compounds were studied in comparison with papaverine and ethaverine. The 3(2H)-iso-quinolone derivatives elicit a rapid lowering of blood pressure when administered iv or id to anesthetized dogs. The *N*-methyl-3(2H)-isoquinolone derivative (5a) shows relatively less positive inotropic effect than papaverine hydrochloride, both iv and id, and produces a longer vasodilator response. Compounds 6b and 10 exhibit greater vascular selectivity than 5a upon rapid iv administration in dogs, although 6b is much less effective when given id in the rat. Although no obvious structure–activity relationship is evi-dent among the N-substituents in the isoquinolones studied, it is apparent that the *N*-substituted-3(2H)-isoquinolone variation of papaverine allows enhanced selectivity and/or duration of peripheral vasodilator activity in the dog.

Clinically interesting 1-benzylisoquinolines include such compounds as papaverine (1),[1] dioxyline (2),[2] and etha-verine (3).[3] Although papaverine enjoys considerable use as an antispasmodic and peripheral vasodilator, its utility is hampered by untoward cardiac effects and short dura-tion of action.

We have investigated the synthesis and vasodilator prop-erties of isoquinoline derivatives, structurally related to papaverine, but having oxygen functions in the 3 position.

Chemical Synthesis. The reaction of 2-[(3,4-dimethoxy-phenyl)acetyl]-4,5-dimethoxyphenyl acetic acid (7) with NH₄OAc was reported to give the 3(4H)-isoquinolone (4a).[4,5] However, our spectral data support the existence of 4 under most conditions as the 3(2H)-isoquinolone, 4b,
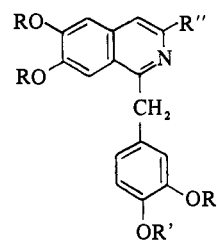
1, R,R' = CH₃; R" = H
2, R,R" = CH₃; R' = C₂H₅
3, R,R' = C₂H₅; R" = H

in agreement with a recent communication.[6] Other workers[7,8] have demonstrated an equilibrium between 3(2H)-isoquinolones and 3-isoquinolinols, in which polar solvents (H₂O, EtOH) favor the lactam form and nonpolar solvents (Et₂O, dioxane) allow the lactim to predominate.