Sterol Metabolism X: Epimeric 23-Hydroxycholesterols

JOHAN E. VAN LIER and LELAND L. SMITH

Abstract □ Synthesis of the epimeric 23-hydroxycholesterols has been achieved by sodium borohydride reduction of 3β-hydroxycholest-5-en-23-one. Tentative configurational assignments were made based on specific rotation data. The configurations of the epimeric 24-hydroxycholesterols are discussed, and a reconsideration of their configurational assignment is suggested.

Keyphrases □ 23-Hydroxycholesterols, epimeric—synthesis □ Absolute configuration—23-hydroxycholesterols, epimeric □ IR spectrophotometry—identification □ Adsorption chromatography—identification

Although naturally occurring 20-, 22-, 24-, 25-, and 26-hydroxysterols have been studied extensively, 23-hydroxysterols have received relatively little attention. 23-Hydroxysterols have limited representation in nature in the fungal metabolite 23*S*-hydroxylanosterol (lanosta-8,24-dien-3β,23*S*-diol)(1), the chiograsterols (2), the plant hormone antheridiol (3), and possibly a 23-hydroxycholesterol (cholest-5-ene-3β,23-diol) sulfate in human infant meconium (4). Other 23-hydroxylated steroids include several bile acids (5), the jervine and veratramine classes of steroidal alkaloids (6), and a variety of triterpenoid compounds (7–18). In view of a continuing interest in the autoxidation of cholesterol in the side chain (19, 20) and in the occurrence of side-chain-oxidized cholesterol derivatives in human tissues (21–23), the authors sought to prepare the as yet undescribed epimeric 23-hydroxycholesterols for record purposes.

THEORETICAL

Sodium borohydride reduction of 3β-hydroxycholest-5-en-23-one (24) gave a mixture of epimeric 3β,23-diols separable by chromatography. Twice the yield of the lower melting, chromatographically more mobile, more levorotatory epimer was obtained compared to the higher melting, more polar, more dextrorotatory epimer. Although the epimeric 3β,23-diols were resolved from one another by adsorption chromatography, they were unresolved on gas chromatography; their corresponding 3β,23-diacetates also were not resolved.

The 23-hydroxycholesterol epimers could be distinguished from one another by means of their IR absorption spectra, but except for minor differences in ion intensity the epimers were not distinguishable by their mass spectra, either as the free 3β,23-diols or as their 3β,23-diacetates. Mass spectra of the 3β,23-diols were characterized by a strong molecular ion (*m/e* 402) and by ions at *m/e* 384 $(M-18)^+$ and *m/e* 369 $(M-33)^+$, representing loss of water and of a methyl radical and water, respectively. Mass spectra of the 3β,23-diacetates exhibited no molecular ion; the highest mass observed, also the base peak, was *m/e* 426 $(M-60)^+$, representing loss of the elements of acetic acid. The next highest mass at *m/e* 366 $(M-120)^+$ corresponds to loss of two molecules of acetic acid.

Assignment of absolute configuration to the epimers may be made by analogy to arguments advanced in assignment of absolute configuration to the epimeric 23-lanosterols (25). Since *S*(+)-4-methylpent-3-en-2-ol and *S*(+)-4-methylpentan-2-ol derived therefrom have the same absolute configuration and sign of specific rotation (26, 27), these two secondary alcohols serving as model compounds for assignment of the respective 23-hydroxylanosterol and 23-hydroxycholesterol derivatives imply that the more dextrorotatory epimers in both sterol series have the same absolute configuration. The absolute configuration of 23-hydroxysterols corresponding to that of *S*(+)-4-methylpentan-2-ol and *S*(+)-4-methylpent-3-en-2-ol is the $23\beta_F$-configuration by Plattner's convention (28). The $23\beta_F$-configuration corresponds to the 23*S*-configuration by the Cahn-Ingold-Prelog sequence rule nomenclature for the more dextrorotatory 23-hydroxylanosterol (25). Because of a change in priority of the C_{22}-steroid residue relative to the terminal isobutyl group of 23-hydroxycholesterol, the more dextrorotatory $23\beta_F$-hydroxycholesterol and the lanost-8-ene-3β,$23\beta_F$-diol(25) derived by catalytic reduction of 23*S*-hydroxycholesterol are of the 23R-configuration.

Other than the as yet undescribed cholest-5-ene-3β,21-diol and (25*S*)-cholest-5-ene-3β,26-diol, all possible cholesterol derivatives hydroxylated in the side chain have now been prepared and absolute configurations assigned. However, previously assigned absolute configurations (29, 30) of the epimeric 22-hydroxycholesterols have been recently reversed, the naturally occurring epimer now being termed cholest-5-ene-3β,$22\beta_F$(22R)-diol (31–33). Furthermore, it appears that revision of the previously accepted absolute configurations of the epimeric 24-hydroxycholesterols is in order. The prior assignment of the $24\beta_F$(24*S*)-configuration (29) to the naturally occurring, more levorotatory 24-hydroxycholesterol cerebrosterol (cholest-5-ene-3β,24-diol) (34–36) was made in reference to molecular rotations of model levorotatory *n*-alkylisopropylcarbinols, in the same manner as used by Entwistle and Pratt (25) and by the authors with methylisobutenylcarbinol and methylisobutylcarbinol for assignment of configuration to the 3β,23-diols. The model levorotatory alkylisopropylcarbinols used for this purpose (29) were erroneously given the *S*-configuration[1] which led to a $24\beta_F$-assignment for the levorotatory 24-hydroxycholesterol epimer. However, when proper recognition is made that the levorotatory model compounds used for this assignment are of the R-configuration, a revision of absolute configuration obtains, and the more levorotatory, naturally occurring 24-hydroxycholesterol cerebrosterol becomes cholest-5-ene-3β,$24\alpha_F$-(24R)-diol.[2]

In view of the revised absolute configurational assignments of the 22-hydroxycholesterols (31-33), the presently suggested revised absolute configurational assignments of the 24-hydroxycholesterols, and the absolute configurations of the 23-hydroxycholesterols assigned herein, it is now apparent that the three hydroxycholesterols of β_F-configuration: $22\beta_F$(22R)-hydroxycholesterol, $23\beta_F$(23R)-hydroxycholesterol, and $24\beta_F$(24*S*)-hydroxycholesterol, are the more dextrorotatory of their respective epimeric pairs. This deduction follows from recognition that the dextrorotatory enantiomers of the appropriate model secondary alcohols *S*(+)-6-methylheptan-2-ol (38), *S*(+)-4-methylpentan-2-ol (26, 27), and *S*(+)-3-methylbutan-2-ol (29) have the same *S*-configuration.

[1] Model *n*-alkylisopropylcarbinol Compounds XXX (p. 1987) of Klyne and Stokes (29) used for assignment of absolute configuration to the 24-hydroxycholesterol epimers appear to have the *S*-configuration but are stated to be levorotatory, whereas model Compound XXI [*S*(+)-3-methylbutan-2-ol] is dextrorotatory and its enantiomeric model Compound XXII [R(−)-3-methylbutan-2-ol] is levorotatory (p. 1986). From the printed figures, both Models XXX and XXI have the *S*-configuration, but XXX should have the R-configuration to be levorotatory (37). This point is repeated by Fieser and Fieser (6).

[2] Prior reports from this laboratory (21–23) have used without revision the absolute configuration of cerebrosterol as assigned by Klyne and Stokes (29) but converted to the Cahn-Ingold-Prelog sequence rule nomenclature, thus 24*S*-hydroxycholesterol (cholest-5-ene-3β,24*S*-diol). These prior usages should now be revised. On the same basis the more levorotatory epimer of another 24-hydroxysterol epimeric pair previously assigned $24\beta_F$(24*S*)-configuration (38) should also be revised.

EXPERIMENTAL[3]

3β-Hydroxycholest-5-en-23-one—A solution of 40 mg. of 3β-hydroxynorchol-5-en-23-ic acid, m.p. 188–190°, $\tilde{\nu}_{max.}^{KBr}$ 3300, 2650, 1680, 1270, and 1040 cm.$^{-1}$, in 5 ml. of dry pyridine–acetic anhydride (2:1) was held overnight at room temperature. The mixture then was diluted with 50 ml. of distilled water. The precipitated acetate was filtered, washed with water, dried under vacuum ($\tilde{\nu}_{max.}^{KBr}$ 2650, 1720, 1680, 1250, and 1040 cm.$^{-1}$), and dissolved in 50 ml. of absolute diethyl ether. One drop of dry pyridine and 0.7 ml. of thionyl chloride were added, and after 3 hr. at room temperature the ether was evaporated under vacuum. Crystallization from diethyl ether–hexane give 318 mg. of the acid chloride acetate, m.p. 149–154°, $\tilde{\nu}_{max.}^{KBr}$ 1800, 1720, 1250, 1030, and 760 cm.$^{-1}$ [lit. m.p. 155–156° (24)]. To 240 mg. of magnesium turnings was added under nitrogen 1.45 g. of freshly distilled isobutyl bromide in 30 ml. of absolute diethyl ether. The mixture was stirred vigorously until the reaction started, and gentle stirring was continued until all magnesium dissolved. The Grignard solution was cooled to −5° and 1 g. of cadmium chloride was added. After stirring for 10 min., 30 ml. of dry benzene was added, and after 15 min. the acid chloride acetate (318 mg.) in 10 ml. of dry benzene was added. The stirred mixture was allowed to come to room temperature and was held overnight. The mixture was cooled in an ice bath and the complex destroyed by cautious addition of 10 ml. of 2 *N* sulfuric acid and then 100 ml. of water. The reaction mixture was extracted three times with 100-ml. portions of diethyl ether; the combined ether extracts were washed with sodium bicarbonate solution, with water, and with saturated sodium chloride solution, and were dried over anhydrous sodium sulfate. The dried solution was evaporated under vacuum, redissolved in 50 ml. of methanol containing 50 mg. of sodium methoxide, and refluxed for 45 min. After cooling, 100 ml. of water was added, and the mixture was neutralized with 2 *N* sulfuric acid and extracted three times with 100-ml. portions of diethyl ether. The ether extract was washed with sodium bicarbonate solution, with water, and with saturated sodium chloride solution before drying over anhydrous sodium sulfate and evaporated under vacuum. Gas chromatographic analysis of the product indicated the presence of four components. The reaction products were dissolved in a small amount of methylene chloride and chromatographed on a 2.5 × 60-cm. column packed with synthetic polysaccharide (Sephadex LH-20) prepared with methylene chloride. Elution with neat methylene chloride (13.5-ml. fractions taken automatically) gave several fractions which were analyzed individually by TLC. From Fraction No. 13 there was obtained on evaporation of the solvent 145 mg. of the desired 23-ketone, m.p. 136–140° [lit. m.p. 141–143° (24)]; $\tilde{\nu}_{max.}^{KBr}$ 3400, 1700, and 1050 cm.$^{-1}$; R_C 0.93 in benzene–ethyl acetate (3:2).

An unidentified steroidal ketone was recovered from Fraction No. 14, obtained from the polysaccharide column from which the 23-ketone had just been eluted. Evaporation of the methylene chloride gave 70 mg. of material; upon recrystallization from hexane–diethyl ether, there was obtained 18 mg. of white crystals, m.p. 119–125°; $\tilde{\nu}_{max.}^{KBr}$ 3520, 3450, 1730, 1720, 1060, and 1020 cm.$^{-1}$; $\tilde{\nu}_{max.}^{CCl4}$ 3630, 1740, 1155, 1055 cm.$^{-1}$; R_C 0.89 in benzene–ethyl acetate (3:2); magenta color with 50% sulfuric acid.

Anal.—Calcd. for $C_{24}H_{36}O_3$: C, 77.34; H, 9.74. Found: C, 77.08; H, 9.95.

Cholest-5-ene-3β,23α$_F$(23*S*)-diol—A solution of 150 mg. of 3β-hydroxycholest-5-en-23-one dissolved in 20 ml. of methanol was treated overnight at room temperature with an excess of sodium borohydride. The solvent was evaporated under vacuum and the residue was dissolved in 100 ml. of diethyl ether and washed with 2 *N* sulfuric acid, with water, with sodium bicarbonate solution, with water, and with saturated sodium chloride solution. The ether solution was dried over anhydrous sodium sulfate and evaporated under vacuum. The solid residue was found by TLC to be a mixture of epimeric 23-hydroxycholesterols. The mixed epimers were chromatographed on a 2.5 × 60-cm. column of silica gel prepared and irrigated with hexane–diethyl ether (3:1) with 15-ml. fractions being collected automatically. TLC examination of each fraction established that the more mobile epimer was present in Fractions 36–46, and the more polar epimer in Fractions 48–60. Fractions 46–48 contained both epimers. The mixed fraction was rechromatographed on a 20 × 20-cm. chromatoplate of silica gel HF_{254} 1 mm. thick, irrigated with benzene–ethyl acetate (3:2). Recovery of both resolved 3β,23-diols from the chromatoplate afforded additional material which was added to the appropriate initial fraction. Crystallization from hexane–ethyl acetate of the fractions containing the more mobile epimer gave 91 mg. (67%) of the 3β,23α$_F$(23*S*)-diol. Recrystallization from diisopropyl ether–diethyl ether gave the analytical sample, m.p. 136–137°; [α]$_D$ −30°; $\tilde{\nu}_{max.}^{KBr}$ 3400 and 1050 cm.$^{-1}$; R_C 0.84 in benzene–ethyl acetate (3:2); blue-purple color with 50% sulfuric acid; r_T 2.25 (3% QF-1), 1.66 (3% SE-30); mass spectrum: *m/e* 402 (100%), 384 (32%); 369 (13%), 351 (11%), 300 (14%), 291 (16%), 273 (23%), 255 (17%), *etc.*

Anal.—Calcd. for $C_{27}H_{46}O_2$: C, 80.53; H, 11.52. Found: C, 80.25; H, 11.82.

Cholest-5-ene-3β,23α$_F$(23*S*)-diol 3β,23-Diacetate—A solution of 55 mg. of the more mobile 3β,23α$_F$-diol in 5 ml. of dry pyridine–acetic anhydride (2:1) was held overnight at room temperature and worked up by precipitation with 10 ml. of water. The diacetate was centrifuged down (15 min. at 10,000 × *g*) and the supernatant was discarded. After washing the derivative several times with distilled water, the sample was dried under vacuum and recrystallized from methanol, yielding 50 mg. of 3β,23*S*-diacetate, m.p. 143–146°; [α]$_D$ −35°; $\tilde{\nu}_{max.}^{KBr}$ 1730, 1240, and 1030 cm.$^{-1}$; R_C 1.43 in benzene–ethyl acetate (3:2); blue-purple color with 50% sulfuric acid; r_T 4.62 (3% QF-1), 2.69 (3% SE-30); mass spectrum: *m/e* 426 (100%), 366 (10%), 351 (7.5%), 326 (5.7%), 282 (11%), *etc.*

Anal.—Calcd. for $C_{31}H_{50}O_4$: C, 76.49; H, 10.36. Found: C, 76.26; H, 10.45.

Cholest-5-ene-3β,23β$_F$(23R)-diol—Crystallization from hexane–ethyl acetate of the appropriate fractions containing the more polar epimer afforded 44 mg. (33%) of the 3β,23β$_F$-diol. Recrystallization from diisopropyl ether–diethyl ether gave the analytical sample, m.p. 175–176°; [α]$_D$ −22°; $\tilde{\nu}_{max.}^{KBr}$ 3400 and 1050 cm.$^{-1}$, different from the spectrum of the 23*S*-epimer; R_C 0.74; blue-purple color with 50% sulfuric acid; r_T 2.25 (3% QF-1), 1.66 (3% SE-30), unseparable by gas chromatography from the 23*S*-epimer; mass spectrum: essentially the same as for the 23*S*-epimer.

Anal.—Calcd. for $C_{27}H_{46}O_2$: C, 80.53; H, 11.52. Found: C, 80.35; H, 11.56.

Cholest-5-ene-3β,23β$_F$(23R)-diol 3β,23-Diacetate—Acetylation of 17 mg. of the 3β,23β$_F$-diol in the same manner used for the 3β,23α$_F$-diol gave 11 mg. of the 3β,23β$_F$-diacetate, crystallized from methanol, m.p. 111–114°; [α]$_D$ −9°; $\tilde{\nu}_{max.}^{KBr}$ 1730, 1240, and 1030 cm.$^{-1}$, different from the spectrum of the 23-epimeric diacetate; R_C 1.43; blue-purple color with 50% sulfuric acid; r_T 4.62 (3% QF-1), 2.69 (3% SE-30), inseparable by gas chromatography from the 23*S*-epimer.

Anal.—Calcd. for $C_{31}H_{50}O_4$: C, 76.49; H, 10.36. Found: C, 76.74; H, 10.48.

[3] Melting points were taken on a calibrated Kofler block under microscopic magnification. Optical rotations were obtained in 1.0% solutions in chloroform using a 2-dm. tube. IR absorption spectra were recorded over the range 4000–400 cm.$^{-1}$ with a Perkin-Elmer model 337 spectrophotometer equipped with a beam condenser. Adsorption TLC was conducted using silica gel HF_{254} (E. Merck GmbH., Darmstadt, Germany) and the solvent system benzene–ethyl acetate (3:2). Thin-layer chromatographic mobilities (R_C) are expressed in terms of cholesterol as unit mobility. Gas chromatography was conducted on a Hewlett-Packard F and M model 402 gas chromatograph using 3% QF-1 and 3% SE-30 columns as described previously in detail (22). Retention times (r_T) are given in terms of cholesterol as unit time.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 17, 1969, from the *Department of Biochemistry, University of Texas Medical Branch, Galveston, TX 77550*

Accepted for publication December 11, 1969.

The authors acknowledge gifts of 3β-hydroxychol-5-enic acid from Dr. M. J. Thompson, U. S. Department of Agriculture, Beltsville, Md., and Dr. H. L. Herzog, Schering Corp., Bloomfield, N. J. Financial support for these studies was by U. S. Public Health Service Research Grant HE-10160.

Paper IX of the series: L. L. Smith and J. E. van Lier, *J. Atheroscler. Res.*, to be published.

New Compounds: Mannich Bases from 1,2-Diphenylindolizine—*N*-Substituted Cyclohexylaminomethyl Derivatives

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Abstract □ Seven new Mannich bases, involving *N*-substituted cyclohexylamines and 1,2-diphenylindolizine, have been synthesized as potential biologically active compounds.

Keyphrases □ Mannich bases—synthesis □ *N*-Substituted cyclohexylamines—synthesis from 1,2-diphenylindolizine □ 1,2-Diphenylindolizine—Mannich base synthesis

In previously reported work, it has been shown that certain Mannich bases derived from indolizines exhibited CNS-depressant activity (1–3). As part of a continuing exploration of indolizines with potential biological activity, a series of Mannich bases involving *N*-substituted cyclohexylamines was synthesized from 1,2-diphenylindolizine (4) (Table I).

EXPERIMENTAL[1]

The appropriate secondary amine (0.045 mole) was combined with 30 ml. of 1,4-dioxane and 2.25 ml. of 40% aqueous formaldehyde (0.030 mole). The mixture was placed in the refrigerator and allowed to stand for 48 hr. To the mixture was then added 4.1 g. of 1,2-diphenylindolizine (0.015 mole); the resulting clear solution was stirred at room temperature for 72 hr., during which

[1] Melting points were taken on a Thomas-Hoover melting-point apparatus and are uncorrected. Elemental analyses were obtained from Strauss Microanalytical Laboratories, Oxford, England.