STEROL METABOLISM. XXXI. SYNTHESIS OF THE EPIMERIC [24-^{3}H] CHOLEST-5-ENE-3β,24-DIOLS

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SUMMARY

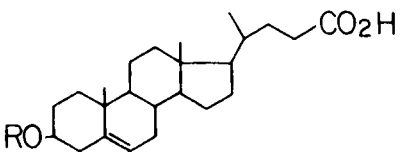
A synthesis of [24-^{3}H] (24S)-cholest-5-ene-3β,24-diol (cerebrosterol) in 12.1% radiochemical yield and of its 24-epimer has been achieved by sodium borotritide reduction of 3β-hydroxycholest-5-en-24-one derived from 3β-hydroxychol-5-enic acid by an improved procedure. The epimeric 3β,24 diols were separated as the dibenzoate esters, for which circular dichroism data are presented in support of the assigned absolute stereochemistry.

INTRODUCTION

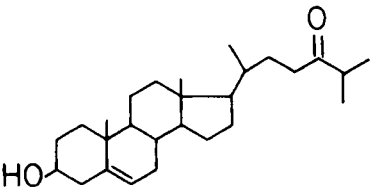
For planned studies of the metabolism in mammalian brain of the sterol (24S)-cholest-5-ene-3β,24-diol (cerebrosterol) (IIIa) a need arose for the isotopically labeled sterol of high specific activity. Radioactive IIIa from incubations of brain tissue with [4-^{14}C] or [1,2-^{3}H] cholest-5-en-3β-ol (cholesterol) (1,2) was not of the required high specific activity, and another means of synthesis of IIIa was sought.

Previously reported chemical syntheses of the epimeric cholest-5-ene-3β,24-diols IIIa and IVa involved reduction of 3β-hydroxycholest-5-en-24-one (II) and resolution of the product epimeric 3β,24-diols as the dibenzoate esters IIIb and IVb by crystallization (3,4) or by chromatography (5). Synthesis of the epimeric [24-^{3}H] 3β,24-diols IIIa and IVa is thus a matter of sodium borotritide reduction of the 24-ketone II. However, chemical synthesis of II by the action of diisopropyl cadmium on

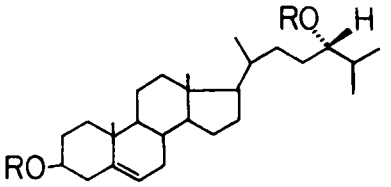
the acid chloride of 3β-acetoxychol-5-enic acid (Ib) (6) in our hands and in those of others (7) was of diminished success. Furthermore, the alternative preparation of II from fucosterol (24-ethylcholesta-5,E-24(28)-dien-3β-ol) is limited by the availability of fucosterol from seaweed (7-9). Our present radiochemical synthesis of the epimeric [24-^{3}H] 3β,24-diols IIIa and IVa derives from these prior findings but includes an improved synthesis of the intermediate 24-ketone II formed by the action of isopropyl lithium (10) on the cholenic acid Ia.



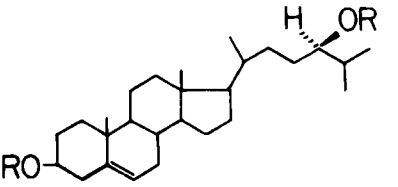
Ia. R=H
Ib. R=COCH3



II.



IIIa. R=H
IIIb. R=COC6H5



IVa. R=H
IVb. R=COC6H5

EXPERIMENTAL

Melting points were taken on a calibrated Kofler block under microscopic magnification. Infrared absorption spectra were obtained on 1.5 mm potassium bromide

disks incorporating the sample, using a Perkin Elmer Model 337 spectrophotometer equipped with a beam condensing lens. Thin-layer chromatography was conducted on 20 x 20 and 20 x 40 cm chromatoplates of Silica Gel HF_{254} (E. Merck GmbH., Darmstadt), 0.25 mm thick for analytical purposes. Mobility data given in R_c values with cholesterol as unit mobility. Gas chromatography was conducted on 1.83 m long 4 mm diameter silanized glass U-tubes packed with 3% SP-2401 on 100-200 mesh Supelcoport (Supelco Inc., Bellefonte, Pa.). Column temperature was 230° C; injection temperature was 250° C; detector temperature was 265° C. Relative retention times (t_R) are given in terms of cholesterol as unit retention time.

3β-Hydroxycholest-5-en-24-one (II).

To a vigorously stirred suspension of 112 mg (0.33 mmole) of 3β-hydroxychol-5-enic acid (Steraloids Inc., Pawling, N. Y.) in 10 ml of anhydrous benzene at 0° under nitrogen was added 600 μl (1.14 mmole) of isopropyl lithium in pentane (Alfa Products, Ventron Corp., Beverly, Mass.). The mixture was stirred at room temperature for 4 hr and then poured into vigorously stirred ice water. The slurry was extracted three times with ethyl acetate, and the organic layer was separated, dried over anhydrous sodium sulfate and evaporated under vacuum. The solids were chromatographed on a 20 x 40 cm 2 mm thick chromatoplate of Silica Gel HF_{254} with benzene-ethyl acetate (1:1, v/v). The 24-ketone II zone was eluted with ethyl acetate, and the product was recrystallized from hexane, yielding 60 mg of II, m.p. 136-138° C (lit. m.p. 137-138.5° C (6), 137-138° C (8)); ν_{max}^{KBr} 3400, 1705, 1060 cm^{-1}; R_c 0.98 in benzene-ethyl acetate (1:1, v/v); greenish color with 50% sulfuric acid spray; t_R 1.75; identical in these properties with those of an authentic reference sample of II.

The zone containing unaltered starting material I was similarly eluted and recrystallized, giving 35 mg of I, identified as such by comparison of its physical properties with those of the authentic reference sample.

[24-^{3}H] Cholest-5-ene-3β,24-diols (IIIa and IVa).

To a suspension of 200 mg (0.5 mmole) of II in 4 ml of absolute ethanol at 0° C was added 7 mg (0.185 mmole) of sodium borotritide (25 mCi) (New England Nuclear,

Boston, Mass.) in 1 ml of absolute ethanol. The mixture was stirred at room temperature for 2 hr, and 2 ml of 10% aqueous acetic acid was added. The mixture was concentrated under vacuum to half-volume and extracted with ethyl acetate. The ethyl acetate extracts were evaporated, and the residue was crystallized from hexane-diethyl ether to yield 161 mg of epimeric 3β,24-diols IIIa and IVa identified by thin-layer chromatography.

[24-^{3}H] Cholest-5-ene-3β,24-diols 3β,24-Dibenzoates (IIIb and IVb).

The mixture of 3β,24-diol epimers IIIa and IVa (160 mg) dissolved in 2 ml of anhydrous pyridine was stirred overnight at 4°C with 1 ml of benzoyl chloride. Water (3 ml) was added, and after stirring for 4 hr the mixture was extracted with diethyl ether. The ether layer was washed successively with water, 2N HCl, 1N NaOH, and then evaporated under vacuum. The oily product was chromatographed on 20 x 40 cm chromatoplates 0.25 mm thick irrigated with benzene-ethyl acetate (5:3, v/v) to remove remaining benzoic acid. The mixed sterol dibenzoates IIIb and IVb were eluted from the chromatoplate and rechromatographed on four 20 x 40 cm chromatoplates 0.25 mm thick developed six times with benzene-hexane (1:1, v/v). The less mobile ultraviolet light absorbing zone was extracted with diethyl ether and crystallized from methanol to give 66 mg of [24-^{3}H] (24S)-cholest-5-ene-3β,24-diol (cerebrosterol) dibenzoate (IIIb), m.p. 179-181°C (lit. m.p. 179-181°C (3,4)); $\tilde{\nu}_{max}^{KBr}$ 1710, 1280, 1110, 710 cm^{-1}; homogeneous on thin-layer and gas chromatography; identical in these properties with those of an authentic reference sample of IIIb.

The more mobile zone similarly eluted and crystallized from methanol gave 83 mg of [24-^{3}H] (24R)-cholest-5-ene-3β,24-diol dibenzoate (IVb), m.p. 142-143°C (lit. m.p. 141-142°C (3,4)); $\tilde{\nu}_{max}^{KBr}$ 1710, 1280, 710 cm^{-1} (different from IIIb in the region 900-1010 cm^{-1}); homogeneous on thin-layer and gas chromatography; identical in these properties with those of an authentic reference of sample IVb.

[24-^{3}H] (24S)-Cholest-5-ene-3β,24 diol (Cerebrosterol) (IIIa).

A solution of 66 mg of IIIb in 3 ml of methanol containing 15 mg of sodium meth-

oxide was maintained at room temperature for 2 hr. The reaction mixture was evaporated under vacuum, the residue extracted with ethyl acetate, and the extract was washed with 2N HCl, dried over anhydrous sodium sulfate, and evaporated under vacuum. The residue was crystallized from hexane-diethyl ether to give 19 mg of [24-^{3}H] (24S)-cholest-5-ene-3β,24-diol (cerebrosterol) (IIIa), m.p. 172-173°C (lit. m.p. 175-176°C (3,4)); $\tilde{\nu}_{max}^{KBr}$ 3400, 1060 cm^{-1}; R_c 0.60 in benzene-ethyl acetate (4:1, v/v); pink color with 50% sulfuric acid used as spray; t_R 2.47; identical in these properties with those of an authentic reference sample of IIIa. The sample was homogeneous as regards radioactivity on thin-layer chromatography and had a specific activity of 74.4 μCi/mg.

[24-^{3}H] (24R)-Cholest-5-ene-3β,24-diol (IVa).

A solution of 83 mg of IVb in methanol containing 20 mg of sodium methoxide was maintained at room temperature for 2 hr, and then processed in the manner described for hydrolysis of IIIb. The yield thus was 20 mg of [24-^{3}H] (24R)-cholest-5-ene-3β,24-diol (IVa), m.p. 181-182°C (lit. m.p. 182-183°C (3,4)); $\tilde{\nu}_{max}^{KBr}$ 3400, 1060 cm^{-1}; R_c 0.60 in benzene-ethyl acetate (4:1, v/v); pink color with 50% sulfuric acid used as spray; t_R 2.47; identical in these properties with those of authentic reference sample of IVa. The sample was homogeneous as regards radioactivity on thin-layer chromatography and had a specific activity of 80.6 μCi/mg.

Circular Dichroism Studies

Circular dichroism spectra were recorded on 0.015 mg/ml solutions of cholesterol 3β-benzoate, IIIb, and IVb in methanol-dioxan (9:1, v/v) using a JASCO ORD-CD spectrophotometer. Circular dichroism data (all at 230 nm) are: for cholesterol 3β-benzoate, $\Delta\epsilon$ +6.36; for IIIb, $\Delta\epsilon$ +2.31; for IVb, $\Delta\epsilon$ +8.73.

DISCUSSION

The present synthesis affords both epimeric cholest-5-ene-3β,24-diols IIIa and IIIb labeled with tritium at the C-24 position. By the nature of the sodium borotritide reduction conditions all of the isotope introduced into the sterols may be considered to be located in the assigned C-24 positions. Because of the ease of syn-

thesis and purification procedures, relatively large amounts of the [24-^{3}H] sterols may be prepared. The route is particularly accessible in view of the improved procedure for synthesis of the intermediate 24-ketone II, which was prepared from 3β-hydroxychol-5-enic acid (Ia) in 50% yields. Although yields were not high, the reaction was quite easily worked up, and starting material was readily recovered. The low yield of II and high recovery of Ia from the reaction was due in large part to the poor solubility of Ia in benzene. Use of more polar solvents such as tetrahydrofuran or diethyl ether was unsuccessful because of the instability of isopropyl lithium in those solvents.

As an adjunct to the present synthesis we have reconsidered the matter of the absolute configuration of the C-24 carbon atom in these 3β,24-diols. The absolute stereochemistry of the naturally occurring 3β,24-diol (cerebrosterol) (IIIa) has been assigned the (24S)-configuration (11), which is equivalent to the 24β$_F$-configuration in other nomenclature (12). In confusion over the sign of rotation of proper model compounds used for the assignment we had questioned the matter (13). On reexamination, it is now clear that a change of sign of rotation obtains in the homologous series of key model compounds, the model compound (2S)-3-methylbutan-2-ol being dextrorotatory but (3S)-2-methylpentan-3-ol, (3S)-2-methylhexan-3-ol, and (3S)-2-methylheptan-3-ol being levorotatory (14). By confining the comparison of molecular rotation of the more levorotatory 3β,24-diol cerebrosterol (IIIa) to the levorotatory higher homologs of (3S)-configuration, the correct assignment of the (24S)-stereochemistry to IIIa is made (11).

In support of this matter we have measured the circular dichroism spectra of the 3β,24-dibenzoate esters IIIb and IVb in comparison with cholesterol 3β-benzoate. The incremental contributions Δ ($\Delta\epsilon$) to the circular dichroism of the sterol ester molecule by the individual 24-benzoyloxy ester groups are -4.05 for IIIb and +2.37 for IVb, thus of the same respective signs found for molecular rotational differences $\Delta [M]_D$ used previously (11). The benzoate sector rule developed for assignment of absolute configuration of cyclic secondary alcohols (15) cannot be used with assurance

in the present case, for the flexibility of the sterol side-chain compromises the matter. Nonetheless, on the assumption that the C_8-side-chains of IIIb and IVb adopt extended conformations such that Newman projections for all vicinal carbon atoms are staggered, then the circular dichroism should receive a contribution to the negative region from the C-22/C-23 bond in IIIb and a contribution to the positive region from the C-22/C-23 bond in IVb. This formulation is thus entirely in accord with the (24S)-configuration for IIIa and (24R)-configuration for IVa.

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