COMMUNICATIONS TO THE EDITOR

THE CONSTITUTION OF CEREBROSTEROL, A HYDROXYCHOLESTEROL ISOLATED FROM HORSE BRAIN

Sir:

We recently reported[1] isolation from horse brain of a substance, m.p. 175–176°, α_D −48.3° Chf, diacetate, m.p. 96–97°, α_D −27.6° Chf, isomeric with all known cholestenediols and hence designated cerebrosterol[2]; in later work the sterol was also isolated from human brain. The steroidal nature of the substance was established by hydrogenation to the saturated diol, cerebrostanediol, oxidation of this to cerebrostandione, and Wolff–Kishner reduction to cholestane.

Observation that cerebrosterol is precipitated by digitonin, gives a positive Liebermann–Burchard test, is levorotatory, is converted on Oppenauer oxidation to an α,β-unsaturated diketone, and forms a diacetate indicated that it is a secondary alcoholic derivative of cholesterol. Some of the possible locations for the extra secondary hydroxyl group can be eliminated: C_2, C_4, C_7 (known alcohols or ketones); C_{11} (cerebrostenedione forms a disemicarbazone); C_1 (cerebrostenedione undergoes Wolff–Kishner reduction without alkaline cleavage and hence is not a 1,3-diketone); C_{15} or C_{16} (absence of a band at 5.77μ in the infrared spectra of cerebrostanedione and cerebrostenedione). Of the remaining positions: C_{12}, C_{22}, C_{23}, and C_{24}, the location C_{24} at first seemed excluded since our cerebrostenedione melts at 118–119°, whereas Hey, Honeyman and Peal[3] report the m.p. 90–91° for Δ^4-cholestene-3,24-dione obtained by ozonization of fucostadienone. However, in an Oppenauer oxidation of 24-ketocholesterol[4] we obtained Δ^4-cholestene-3,24-dione melting at 118–119° (λ^{EtOH} 242 mμ, log E = 4.22, α_D + 83.5° Chf, λ^{Chf} 5.86, 6.01, 6.18μ; calcd.: C, 81.35; H, 10.62; found: C, 81.32; H, 10.64) and identical with cerebrostenedione (mixed m.p., infrared spectrum).

Previous workers[4,5] have reported that 24-ketocholesterol on reduction according to Meerwein–Ponndorf or with lithium aluminum hydride affords a single diol, m.p. 166–169°; diacetate, m.p. 93–95°. We repeated the Meerwein–Ponndorf reduction and on fractional crystallization as dibenzoate isolated two products, m.p. 179–181°, α_D −15.5° Chf (calcd.: C, 80.61; H, 8.91; found: C, 80.65; H, 8.83), and m.p. 141–142°, α_D −11.8° Chf (found: C, 80.68; H, 8.90). Hydrolysis of the first dibenzoate yielded a product identical with cerebrosterol, which is thus assigned the constitution of Δ^5-cholestene-3β,24ξ^1-diol. Saponification of the second dibenzoate gave the 24ξ^2-epimer, m.p. 182–183°, α_D −26.8° (calcd.: C, 80.54; H, 11.52; found: C, 80.52; H, 11.57); diacetate, m.p. 100–102°, α_D −37.2° Chf.

On mild Oppenauer oxidation the synthetic 24ξ^1- and 24ξ^2-diols afforded, along with Δ^4-cholestene-3,24-dione, Δ^4-cholestene-24ξ^1-ol-3-one, m.p. 138°, α_D +79.5° Chf, λ^{EtOH} 242 mμ (log E 4.22), λ^{Chf} 2.8, 6.01, 6.18μ (calcd.: C, 80.94; H, 11.07; found: C, 80.98; H, 11.00), acetate, m.p. 97°, α_D + 86.5° (calcd.: C, 78.68; H, 10.47; found: C, 78.70; H, 10.38), and Δ^4-cholestene-24ξ^2-ol-3-one, m.p. 143°, α_D + 93.5° (found: C, 80.90; H, 11.02), acetate, m.p. 91°, α_D + 70° (found: 78.70, H, 10.45). Both products on chromic acid oxidation afforded Δ^4-cholestene-3,24-dione.

Catalytic hydrogenation of Δ^5-cholestene-3β,24ξ^1-diol gave cholestane-3β,24ξ^1-diol, m.p. 202–203°, α_D + 24° Di (calcd.: C, 80.14; H, 11.96; found: C, 80.09; H, 11.92), diacetate, m.p. 119°, α_D +22.5° Chf (calcd.: C, 76.18; H, 10.72; found: C, 76.19; H, 10.62). These substances were not depressed in m.p. on admixture with cerebrosterol and its diacetate, respectively. Chromic acid oxidation of cholestane-3β,24ξ^1-diol afforded a dione, m.p. 122°, α_D +40° Chf, λ^{Chf} 5.87μ (calcd.: C, 80.94; H, 11.07; found: C, 80.80; H, 11.03) that showed no depression in m.p. on admixture with cerebrostanedione.

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VISTER RESEARCH LABORATORIES
CASATENOVO (COMO), ITALY
ALBERTO ERCOLI
PIETRO DE RUGGIERI

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(1) A. Ercoli, S. Di Frisco and P. de Ruggieri, *Gazz. Chim. Ital.*, **83**, 78 (1953).

(2) Previously[1] named cerebrostenediol.

(3) D. H. Hey, J. Honeyman and W. J. Peal, *J. Chem. Soc.*, 2881 (1950).

(4) B. Riegel and I. A. Kaye, THIS JOURNAL, **66**, 723 (1944).

(5) D. H. Hey, J. Honeyman and W. J. Peal, *J. Chem. Soc.*, 4836 (1952).

XYLOSE ISOMERASE[1]

Sir:

Cohen[2] has recently described an enzyme in *E. coli* which catalyzes the equilibrium reaction: D-ribulose ⇄ D-arabinose and named it "pentose isomerase."

We now wish to report the finding of an enzyme which catalyzes the reaction: D-xylose ⇄ D-xylulose. This enzyme obtained from *Pseudomonas hydrophila* is specific for xylose and does not catalyze the formation of ketopentoses from D-ribose, D- or L-arabinose, D-lyxose or L-rhamnose. It will therefore be referred to as "xylose isomerase." A similar enzyme from *Lactobacillus pentosus* has been briefly reported by Lampen.[3]

The enzyme preparation used was an acetone-dried powder obtained, as previously described,[4] from a cell-free extract of *P. hydrophila* grown on D-xylose, except that the cells were ruptured by sonic vibration (10 kc., 1.0 amp., 10 min.).

The xylose isomerase was found to be a soluble

(1) Issued as N.R.C. No. 3011.

(2) S. S. Cohen, *J. Biol. Chem.*, **201**, 71 (1953).

(3) J. O. Lampen, "Symposium on Phosphorus Metabolism," Vol. II, Johns Hopkins Press, Baltimore, Md., 1952, p. 363.

(4) R. M. Hochster and R. W. Watson, *Nature* (*Lond.*), **170**, 357 (1952).