

## COMMUNICATIONS TO THE EDITOR

## THE CONSTITUTION OF CEREBROSTEROL, A HYDROXYCHOLESTEROL ISOLATED FROM HORSE BRAIN

Sir:

We recently reported<sup>1</sup> isolation from horse brain of a substance, m.p. 175–176°,  $\alpha_D -48.3^\circ$  Chf, diacetate, m.p. 96–97°,  $\alpha_D -27.6^\circ$  Chf, isomeric with all known cholestenediols and hence designated cerebrosterol<sup>2</sup>; 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  $\alpha,\beta$ -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<sub>2</sub>, C<sub>4</sub>, C<sub>7</sub> (known alcohols or ketones); C<sub>11</sub> (cerebrostenedione forms a disemicarbazone); C<sub>1</sub> (cerebrostenedione undergoes Wolff–Kishner reduction without alkaline cleavage and hence is not a 1,3-diketone); C<sub>15</sub> or C<sub>16</sub> (absence of a band at 5.77 $\mu$  in the infrared spectra of cerebrostanediol and cerebrostenedione). Of the remaining positions: C<sub>12</sub>, C<sub>22</sub>, C<sub>23</sub>, and C<sub>24</sub>, the location C<sub>24</sub> at first seemed excluded since our cerebrostenedione melts at 118–119°, whereas Hey, Honeyman and Peal<sup>3</sup> report the m.p. 90–91° for  $\Delta^4$ -cholestene-3,24-dione obtained by ozonization of fucostadienone. However, in an Oppenauer oxidation of 24-ketocholesterol<sup>4</sup> we obtained  $\Delta^4$ -cholestene-3,24-dione melting at 118–119° ( $\lambda^{EtOH}$  242  $\mu$ ,  $\log E = 4.22$ ,  $\alpha_D + 83.5^\circ$  Chf,  $\lambda^{Chf}$  5.86, 6.01, 6.18 $\mu$ ; 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<sup>4,5</sup> 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°,  $\alpha_D -15.5^\circ$  Chf (calcd.: C, 80.61; H, 8.91; found: C, 80.65; H, 8.83), and m.p. 141–142°,  $\alpha_D -11.8^\circ$  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  $\Delta^3$ -cholestene-3 $\beta$ ,24 $\xi^1$ -diol. Saponification of the second dibenzoate gave the 24 $\xi^2$ -epimer, m.p. 182–183°,  $\alpha_D -26.8^\circ$  (calcd.: C, 80.54;

H, 11.52; found: C, 80.52; H, 11.57); diacetate, m.p. 100–102°,  $\alpha_D -37.2^\circ$  Chf.

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

Catalytic hydrogenation of  $\Delta^5$ -cholestene-3 $\beta$ ,24 $\xi^1$ -diol gave cholestane-3 $\beta$ ,24 $\xi^1$ -diol, m.p. 202–203°,  $\alpha_D + 24^\circ$  Di (calcd.: C, 80.14; H, 11.96; found: C, 80.09; H, 11.92), diacetate, m.p. 119°,  $\alpha_D + 22.5^\circ$  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-3 $\beta$ ,24 $\xi^1$ -diol afforded a dione, m.p. 122°,  $\alpha_D + 40^\circ$  Chf,  $\lambda^{Chf}$  5.87 $\mu$  (calcd.: C, 80.94; H, 11.07; found: C, 80.80; H, 11.03) that showed no depression in m.p. on admixture with cerebrostanediol.

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XYLOSE ISOMERASE<sup>1</sup>

Sir:

Cohen<sup>2</sup> has recently described an enzyme in *E. coli* which catalyzes the equilibrium reaction: D-ribulose  $\rightleftharpoons$  D-arabinose and named it "pentose isomerase."

We now wish to report the finding of an enzyme which catalyzes the reaction: D-xylose  $\rightleftharpoons$  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.<sup>3</sup>

The enzyme preparation used was an acetone-dried powder obtained, as previously described,<sup>4</sup> 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

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(2) Previously<sup>1</sup> named cerebrostenediol.

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

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