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.

We are indebted to Professor L. F. Fieser for his helpful advice and for the infrared absorption spectra.

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(2) Previously[1] named cerebrostenediol.

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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.

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enzyme, heat sensitive and stable for more than one year when stored as the acetone powder at 4° over calcium chloride. Up to 80% enzyme inactivation was obtained by prolonged dialysis at 0–4°. Addition of 0.01 *M* Mn^{++} or Mg^{++} restored 92 and 70% of the lost activity, respectively. The optimum *p*H was 7.5.

Ketopentose was determined by the cysteine–carbazole method,[5] by the use of $FeCl_3$–orcinol spectra, and by paper chromatography in several solvent systems. An equilibrium of 16% D-xylulose and 84% D-xylose was reached in 3 hr. at 27° under the conditions used. However, this equilibrium was shifted to 81.5% D-xylulose and 18.5% D-xylose by the addition of borate (0.062 *M*) to trap the ketopentose. The isomerase reaction did not require phosphate and could be carried out aerobically or anaerobically. The enzyme was not inhibited by iodoacetate, fluoride, azide or β-methyl-D-xyloside. A typical example of the isomerase reaction showing the rate of ketopentose formation anaerobically (without added borate) is given in Table I.

TABLE I

RATE OF CONVERSION OF D-XYLOSE TO D-XYLULOSE BY XYLOSE ISOMERASE

Reaction components per 3-ml. aliquot were: acetone powder 30 mg., D-xylose 80 mg., NaF 0.02 *M*, $MgCl_2$ 0.0033 *M*, $NaHCO_3$ 0.02 *M* in 95% N_2 + 5% CO_2 at 27°. Proteins and nucleic acids were precipitated with trichloroacetic acid and protamine sulfate. Supernatant containing the reaction product was treated with cysteine-carbazole and after 1 hr. at room temperature the resulting colors were read spectrophotometrically.

Enzymatic reaction time, min.	% D-xylose converted to D-xylulose
0	0.00
10	4.25
30	9.00
60	12.00
90	14.85
120	15.50
180	16.25
240	16.00

Paper chromatograms run with phenol–water (4:1),[6] benzyl alcohol–acetic acid–water (3:1:3)[7] and toluene–dioxane–water (4.17:12.5:1) all resulted in good separations of control D-xylulose,[8] D-ribulose,[9] D-xylose and D-ribose. With each solvent system, the product of the enzymatic reaction, freed from D-xylose by bromine oxidation followed by removal of the resultant xylonic acid with Amberlite IRA 400 resin, had an R_f value identical with authentic D-xylulose. The reaction product gave no ribulose spot and did not exhibit the characteristic spectrophotometric peak for ribulose at 540 mμ in the $FeCl_3$–orcinol reaction.

Positive identification of the product of the isomerase reaction as D-xylulose was effected as follows: the reaction product was freed of protein,[10] adjusted to *p*H 7.5, concentrated *in vacuo* (at 30–40°)[11] to a small volume which was then transferred to a cellulose column[12,13] and the components separated using water-saturated butanol as the mobile phase. A modified cysteine–carbazole spot test was used to identify the effluent ketopentose. When combined, the ketopentose fractions consisted of pure sirupy D-xylulose having $[\alpha]^{25}D$ −32.2° (*c* 2.7% in H_2O)[14] and gave a crystalline *p*-bromophenylhydrazone, melting at 128–129° (uncor.) which was unchanged on admixture with an authentic sample.[14]

In view of our previous report[4] on the enzymatic phosphorylation of D-xylose, the existence of xylose isomerase raises the question whether the substrate in the phosphorylation is D-xylose, D-xylulose or both sugars. Conclusive evidence is not yet available and this phase of the problem is under investigation.

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ON THE ENZYMATIC MECHANISM OF COENZYME A ACETYLATION WITH ADENOSINE TRIPHOSPHATE AND ACETATE[1]

Sir:

The activation of acetate was recently partially clarified when it was found that this reaction involved a pyrophosphate split of ATP, indicating the over-all reaction

$$ATP + HS\text{·}CoA + acetate \rightleftharpoons AMP + PP + acetyl\sim S\text{·}CoA \quad (1)$$

As intermediary, an S-phosphoryl derivative had been suggested as initial product of a reaction ATP and CoA.[2,3] More recent work in our laboratories, however, made such a mechanism more and more unlikely, suggesting, rather, mechanisms involving enzyme-bound intermediaries.[4,5] A considerable clarification of a mechanism of this type appeared to be possible by the use of isotopes. Through testing for exchange with labelled pyrophosphate as well as labelled acetate, it appeared possible to obtain a rather precise information on the intermediate course of the reaction.

Radioactive pyrophosphate was prepared by

(1) The following abbreviations are used in this communication: ATP = adenosine triphosphate; HS·CoA = sulfhydryl-coenzyme A; CoA = coenzyme A; AMP = adenosine monophosphate; PP = pyrophosphate; ex = enzyme, whereby "x" may represent the group which takes part in the chemical reactions; and Ad = adenosine. This investigation was supported in part by research grants from the U. S. Public Health Service, from the Rockefeller Foundation, and from the Atomic Energy Commission.
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