

solid which on systematic recrystallization from butanone, alcohol and ethyl acetate furnished 434 mg. of cholesteryl acetate; m.p. 112.7–114.7°, no depression on mixing with an authentic sample.

***i*-Cholestane (II).**^{3,5}—A mixture of 3 g. of sodium, 60 cc. of diethylene glycol, 5.00 g. (13.0 mmoles) of *i*-cholestan-6-one¹¹ and 10 cc. of 85% hydrazine hydrate was heated under reflux for two hours.⁴ The condenser was removed and the boiling solution was evaporated until the pot temperature had reached 205°. Boiling of the two-phase mixture was then continued for four hours under reflux. The reaction mixture was diluted with water and extracted with ligroin (Skellysolve B, b.p. 60–70°). The ligroin solution after washing and drying was chromatographed on a column of 20 g. of alumina (Fisher adsorption alumina, 80–200 mesh). Elution with ligroin furnished 3.80 g. of *i*-cholestane, m.p. 76–78°, upon removal of the solvent. Further elution of the column with benzene gave 879 mg. of the azine (see below) representing 17.7% of the starting material, m.p. 220–239°.

The *i*-cholestane was crystallized from acetone to give 3.48 g. (72.4%); m.p. 77.4–79.1°. Two more crystallizations from acetone gave the purified hydrocarbon as plate-like crystals: m.p. 78.4–79.1° (cor.); $[\alpha]_D^{20} +78.5^\circ$ (20.0 mg. of hydrocarbon made up to 2 cc. with chloroform, $\alpha_D^{20} +1.57^\circ$, *l*, 2 dm.).

Schmid and Kagi³ give m.p. 80–80.5° and $[\alpha]_D^{20} +79.6^\circ$.

The azine fraction was crystallized five times from butanone to give the purified product as needles: m.p. 239.8–243.5° (dec.) after softening at 220°; $[\alpha]_D^{20} +121^\circ$ (42.3 mg. made up to 5 cc. with benzene, $\alpha_D^{20} +2.05^\circ$, *l*, 2 dm.).

Anal. Calcd. for C₂₇H₄₈N₂ (hydrazone): C, 81.34; H, 11.63; N, 7.03. Calcd. for C₂₄H₃₈N₂ (azine): C, 84.75; H, 11.59; N, 3.66. Found: C, 84.89; H, 11.55; N, 3.65.

Rearrangement of *i*-Cholestane (II) to Compound III.⁸—*i*-Cholestane (0.49 g., 1.32 mmoles) was heated under reflux for eight hours with 0.5 cc. of 48% hydrobromic acid in 15 cc. of acetone. This mixture was diluted with 2–3 cc. of water and allowed to crystallize at 5° as heavy blades: 0.45 g.; m.p. 51–61°. One recrystallization from acetone gave 0.32 g.; m.p. 61.5–64.5°; $[\alpha]_D^{20} +61.2^\circ$ (50.9 mg. of hydrocarbon was dissolved up to 4.94 cc. with chloroform, $\alpha_D^{20} +1.26^\circ$, *l*, 2 dm.).

Schmid and Kagi³ give m.p. 64.5–65° and $[\alpha]_D^{19} +57.9^\circ$ for this compound. Chromatography of this hydrocarbon effected no change in properties but the resulting product was more stable on storage. Hydroiodic acid in acetone was an effective rearrangement catalyst. A ligroin (b.p. 40–42°) solution of *i*-cholestane was shaken with concentrated sulfuric acid at 0–10° to effect this rearrangement. Sulfuric acid and acetic acid at 100°¹³ also effected the reaction though in reduced yield. Hydrochloric or sulfuric acids in acetone catalyzed the reaction slowly. The rearrangement failed to occur with hydrobromic acid in ethanol.

The oxide of III was prepared using perbenzoic acid in chloroform on III. After chromatography and crystallization from ethyl acetate the purified oxide was obtained: m.p. 96.5–97.5°; $[\alpha]_D^{20} +43^\circ$ (33.4 mg. dissolved up to 1.96 cc. with chloroform, $\alpha_D^{20} +0.73^\circ$, *l*, 1 dm.). The literature³ reports m.p. 97.5–98.5°; $[\alpha]_D^{19} +40.8^\circ$.

Bromination of Compound III.—Compound III (154 mg., 0.416 mmole, m.p. 62.5–64°) was dissolved in 2 cc. of ether and treated with 3 cc. of acetic acid containing 67 mg. of bromine.⁷ The brown color was discharged immediately and on cooling the solution in ice and salt there was obtained 90 mg. (48%) of a white crystalline compound: m.p. 106–109°; $[\alpha]_D^{20} -101^\circ$ (16.2 mg. made up to 1.96 cc. in chloroform, $\alpha_D^{20} -0.82^\circ$, *l*, 1 dm.).

Anal. Calcd. for C₂₇H₄₆Br: C, 72.13; H, 10.09; Br, 17.78. Calcd. for C₂₇H₄₆Br₂: C, 61.13; H, 8.74; Br, 30.13. Found: C, 72.63, 72.93; H, 10.41, 10.37.

In cold alcoholic silver nitrate this bromide reacts rapidly to form a white precipitate, suggesting an allylic bromide structure. No way was found to purify the compound.

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(13) E. Kaiser and J. J. Svarz, *THIS JOURNAL*, **71**, 517 (1949).

A Substance with Rh Activity. A Correction

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In a previous communication,² the isolation of a crystalline substance, m.p. 157°, from blood lipids was reported. Many attempts to duplicate this work have been unsuccessful. Further investigation of the remaining 40 mg. of the material previously isolated has established its identity as Amytal (5-ethyl-5-isoamylbarbituric acid).³ It was evidently incorporated in some of the early blood liquid samples by accidental contamination.

Reexamination of the original material by sodium fusion, contrary to previous work, showed the presence of nitrogen. Combustion gave analytical figures in excellent agreement for Amytal.

Anal. Calcd. for C₁₁H₁₈O₃N₂: C, 58.39; H, 8.02; N, 12.38. Found: C, 58.50; H, 7.95; N, 12.15.

The melting point of the lipid material, an authentic sample of amytal and a mixture of the two, were all 156–157°.

A methylation product, m.p. 87–88.5°, obtained by Read⁴ by treatment with alkali and methyl sulfate, is evidently N,N'-dimethyl ethylisoamylmalonamide.

Anal. Calcd. for C₁₂H₂₄O₂N₂: C, 63.12; H, 10.6. Found: C, 63.35; H, 10.9.

Veronal is reported to react in this way on alkaline methylation to yield N,N'-dimethyl diethylmalonamide.⁵

(1) Eli Lilly and Company Fellow, 1951–1952.

(2) C. C. Price, D. H. Read, T. J. Bardos and C. Chen, *THIS JOURNAL*, **70**, 3527 (1948).

(3) H. A. Shonle, *ibid.*, **45**, 243 (1923); M. M. Tiffeneau, *Bull. soc. chim.*, **33**, 183 (1923).

(4) D. H. Read, Ph.D. Dissertation, University of Notre Dame, 1949.

(5) R. Cohn, *Pharm. Z.*, **53**, 29 (1912).

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Enzymatic Dephosphorylation of Casein

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Phosphoproteins such as casein have thus far been considered to be resistant toward the action of purified phosphatases from mammalian tissues.^{1–3} Since casein, however, is a mixture of at least two proteins which differ in solubility, phosphorus content^{4,5} and electrophoretic behavior^{5,6} it seemed possible that differences also may exist in the action of phosphatases on the various fractions. It has now been found that one of these fractions, α -casein, is readily dephosphorylated in the pH range of 5.6 to 6.6 by prostate phosphatase with liberation of about 42% of the phosphorus. The enzyme has no effect on β -casein, whereas on prolonged exposure, to the enzyme, of "unfractionated" casein about 12% of phosphorus is set free.

The casein preparations used in these experiments are similar to those described by Warner⁵

(1) Rimington and Kay, *Biochem. J.*, **20**, 777 (1926).

(2) Schmidt and Thannhauser, *J. Biol. Chem.*, **149**, 369 (1943).

(3) Anagnostopoulos, Pacht, Bourland and Grabar, *Bull. soc. chim. Biol.*, **33**, 699 (1951).

(4) Linderström-Lang, *Compt. rend. Lab. Carlsberg*, **17**, No. 9 (1929).

(5) Warner, *THIS JOURNAL*, **66**, 725 (1944).

(6) Mellander, *Biochem. Z.*, **300**, 240 (1939).