Anal. Calcd. for $C_{13}H_{18}O_4$: C, 65.53; H, 7.61. Found: C, 65.38; H, 7.59.

Hydrolysis with ethanolic sodium hydroxide yielded 3,4-diethoxybenzoic acid which, upon recrystallization from water, was obtained as fluffy white needles melting at 162–163°.

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.74; H, 6.71.

Herzig[11] treated the tetraethyl ether of quercitin with alcoholic potassium hydroxide and obtained 3,4-diethoxybenzoic acid melting at 165–166°. He prepared the ethyl ester and recorded a melting point of 56–57°.

The other two chromatographic bands were combined and eluted. The acetone eluate, upon removal of solvent, yielded crystals and oil. The crystals were removed and recrystallized from petroleum ether (b.p. 65–110°) to give ethyl 4-ethoxy-3-hydroxybenzoate as slightly yellow crystals melting at 77–78° and not depressing the melting point of a mixture with ethyl 4-ethoxy-3-hydroxybenzoate prepared from authentic 4-ethoxy-3-hydroxybenzoic acid.[12]

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.89; H, 6.79.

The oil removed from the crystals of ethyl 4-ethoxy-3-hydroxybenzoate was boiled with dilute sodium hydroxide solution, cooled and acidified with dilute sulfuric acid. The solid obtained was filtered, washed with water, and recrystallized from dilute methanol to yield 3-ethoxy-4-hydroxybenzoic acid as white needles melting at 164–165° and not depressing a mixed melting point with authentic 3-ethoxy-4-hydroxybenzoic acid.

The approximate yields obtained in this experiment were: ethyl 4-ethoxy-3-hydroxybenzoate, 15%; ethyl 3,4-diethoxybenzoate, 20%; and 3-ethoxy-4-hydroxybenzoic acid, 30%.

Acknowledgment.—The authors wish to thank Mr. Donald McDonnell for the analyses and Mr. John Carlson for the microbiological data reported in this paper.

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APPLETON, WIS.

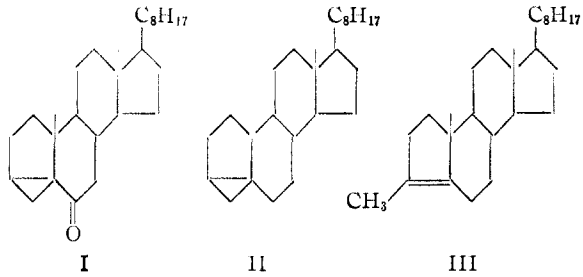
Reductions of *i*-Cholestan-6-one[1]

BY FRANKLIN S. PROUT AND BYRON RIEGEL

RECEIVED JANUARY 17, 1952

We have confirmed the preparation of *i*-cholestane (II) by the Wolff–Kishner reduction of *i*-cholestan-6-one (I)[2] recently reported by Schmid and Kagi,[3] although we have used the Huang-Minlon[4] modification of this reaction. The properties of this hydrocarbon agree with those of *i*-cholestane reported by Schmid and Kagi[3] and by Schmid and Karrer who prepared this hydrocarbon by action of lithium aluminum hydride on cholesteryl *p*-toluenesulfonate.[5]

i-Cholestane (II) failed to add hydrogen under atmospheric pressure using Raney nickel (W-4)[6] in dioxane or prereduced platinum oxide in glacial acetic acid. In both cases *i*-cholestane was recovered in 88% yield by direct crystallization of the hydrocarbon. In contrast hydrogenation of *i*-cholestane in the presence of platinum oxide and acetic acid by Schmid and Kagi[3] was followed by



I II III

treatment of the hydrocarbon with cold concentrated sulfuric acid (−10°) to give a hydrocarbon having a m.p. 43.5–44.5°, $[\alpha]^{15}D$ + 54.4° (chloroform). This compound actually appears to be an impure rearrangement product of *i*-cholestane, since *i*-cholestane was converted to compound III by shaking with concentrated sulfuric acid at 0–10°.

i-Cholestane (II) when treated with bromine according to the directions of Hauptmann[7] absorbed some bromine. The absorption was apparently random and incomplete since the only product obtained was a small amount (36%) of starting material.

Using hydrobromic acid in acetone,[8] we have confirmed the acid-catalyzed rearrangement of *i*-cholestane (II) described by Schmid and Kagi.[3] The resulting hydrocarbon (III), brilliantly characterized by these workers,[3] has been further characterized by its reaction with bromine.[7] Here the addition of bromine was accompanied by liberation of hydrogen bromide and furnished an allylic monobromide not obtained by Schmid and Kagi.[3]

In another sequence *i*-cholestan-6-one was reduced quantitatively with aluminum isopropoxide to give the *i*-cholestan-6-ol as an oil.[9] This oil was converted to cholesteryl acetate in boiling acetic acid with zinc acetate in an over-all yield of 94%.

Acknowledgment.—The authors wish to express their appreciation for a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council for the support of part of this work.

Experimental[10]

The Conversion of *i*-Cholestan-6-one (I) to Cholesteryl Acetate.—One gram (2.62 mmoles) of *i*-cholestan-6-one[11] was converted quantitatively to *i*-cholestan-6-ol by reduction with aluminum isopropoxide and isopropyl alcohol.[9] Half of this crude oil (1.27 mmoles) was heated under reflux in a mixture of 25 cc. of acetic acid, 1 g. of zinc acetate dihydrate and 1 cc. of acetic anhydride for six hours.[12] The mixture was diluted with water to give 511 mg. (94%) of

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(7) *Cf.* H. Hauptmann, *ibid.*, **69**, 562 (1947).

(8) *Cf.* B. Riegel, G. P. Hager and B. L. Zenitz, *ibid.*, **68**, 2562 (1946).

(9) I. M. Heilbron, J. Hodges and F. S. Spring, *J. Chem. Soc.*, 759 (1938).

(10) All melting points are uncorrected, except for one. The analyses were performed by Misses Margaret Hines and Virginia Gibbs of Northwestern University and by Micro-Tech Laboratories, Skokie, Illinois. Analyses for carbon and hydrogen content of *i*-cholestane (II), Compound III and the oxide of III were determined but are not reported.

(11) The *i*-cholestan-6-one (m.p. 97–98°) was prepared for us by Drs. Frank A. Vingiello and William L. Hartop according to the procedure of Windaus and Dalmer (Ref. 2).

(12) *Cf.* J. H. Beynon, I. M. Heilbron and F. S. Spring, *J. Chem. Soc.*, 406 (1937).

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-one[11] 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) respresenting 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.); $[\alpha]^{26}D$ +78.5° (20.0 mg. of hydrocarbon made up to 2 cc. with chloroform, $\alpha^{26}D$ +1.57°, *l*, 2 dm.).

Schmid and Kagi[3] give m.p. 80–80.5° and $[\alpha]^{20}D$ +79.6°.

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°; $[\alpha]^{26}D$ +121° (42.3 mg. made up to 5 cc. with benzene, $\alpha^{25}D$ +2.05°, *l*, 2 dm.).

Anal. Calcd. for $C_{27}H_{46}N_2$ (hydrazone): C, 81.34; H, 11.63; N, 7.03. Calcd. for $C_{54}H_{88}N_2$ (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.[3]—*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°; $[\alpha]^{20}D$ +61.2° (50.9 mg. of hydrocarbon was dissolved up to 4.94 cc. with chloroform, $\alpha^{20}D$ +1.26°, *l*, 2 dm.).

Schmid and Kagi[3] give m.p. 64.5–65° and $[\alpha]^{19}D$ +57.9° 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°; $[\alpha]^{26}D$ +43° (33.4 mg. dissolved up to 1.96 cc. with chloroform, $\alpha^{26}D$ +0.73°, *l*, 1 dm.). The literature[3] reports m.p. 97.5–98.5°; $[\alpha]^{19}D$ +40.8°.

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°; $[\alpha]^{27}D$ −101° (16.2 mg. made up to 1.96 cc. in chloroform, $\alpha^{27}D$ −0.82°, *l*, 1 dm.).

Anal. Calcd. for $C_{27}H_{45}Br$: C, 72.13; H, 10.09; Br, 17.78. Calcd. for $C_{27}H_{46}Br_2$: 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.

DEPARTMENTS OF CHEMISTRY OF
NORTHWESTERN AND DE PAUL UNIVERSITIES
EVANSTON, ILLINOIS
CHICAGO 14, ILLINOIS

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A Substance with Rh Activity. A Correction

BY CHARLES C. PRICE AND GIANCARLO BERTI[1]

RECEIVED APRIL 24, 1952

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.

Reëxamination 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 $C_{11}H_{18}O_3N_2$: 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 Read[4] by treatment with alkali and methyl sulfate, is evidently N,N'-dimethyl ethylisoamylmalonamide.

Anal. Calcd. for $C_{12}H_{24}O_2N_2$: 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]

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DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NOTRE DAME
NOTRE DAME, INDIANA

Enzymatic Dephosphorylation of Casein

BY GERTRUDE E. PERLMANN

RECEIVED MARCH 26, 1952

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 *p*H 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 Warner[5]

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