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.

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

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]

(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

BY GERTRUDE E. PERLMANN

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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 *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]

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

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

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

and were kindly provided by Dr. Thomas L. McMeekin of the Eastern Regional Laboratory. One-ml. samples containing 5 mg. of protein in veronal acetate buffer of *p*H 6.4 were incubated with 0.05 mg. of prostate phosphatase for 6 and 24 hours, respectively. Preliminary to the estimation of the inorganic phosphate that is released by the enzyme, one ml. of 20% trichloroacetic acid was added and the protein precipitate removed by centrifugation. The results with the three preparations are summarized in Table I.

TABLE I

ACTION OF PROSTATE PHOSPHATASE ON CASEIN FRACTIONS

Protein	Phosphorus content, %	Time of incubation at 37° in hours	Phosphorus released by enzyme, % of total phosphorus
"Unfractionated"	0.8	6	0
casein		24	12.5
α-Casein	1.0	6	24.0
		24	42.0
β-Casein	0.6	6	0
		24	0

During the dephosphorylation of α-casein the solubility of the protein decreases. Simultaneously several new components appear in the electrophoretic pattern, as is shown in Fig. 1. Here the full curve is the tracing of the pattern of α-casein whereas the dashed line is that of the protein after 20% of the phosphorus had been liberated.

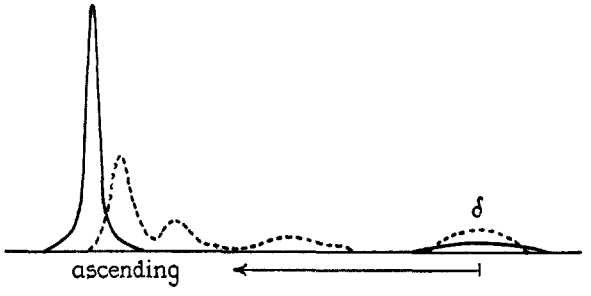

Fig. 1.—Superimposed tracings of electrophoretic patterns of α-casein, ——, and partially dephosphorylated α-casein, - - - - -. Patterns recorded after electrophoresis of 0.5% protein solutions in 0.1 ionic strength sodium phosphate buffer of *p*H 6.8 at a potential gradient of 6 volts per cm. for 8200 seconds.

In experiments in which α-casein and β-casein are remixed in different proportions, if the total concentration of the β-component exceeds 30%, the enzyme reaction is partially inhibited, the degree of inhibition being proportional to the concentration of the β-casein. From these results it emerges that the failure of previous investigators to dephosphorylate crude casein without a preceding transformation to phosphopeptone may be due to the inhibiting action of β-casein on the dephosphorylation of the α-form.

I wish to express my sincere thanks to Dr. Gerhard Schmidt of the Boston Dispensary for a generous sample of prostate phosphatase.

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Reduction of N-Nitrosodiphenylamine to *unsym*-Diphenylhydrazine by Lithium Aluminum Hydride[1]

BY R. H. POIRIER AND F. BENINGTON

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The reduction of nitrosodimethylamine to *unsym*-dimethylhydrazine by adding the nitrosamine to an excess of lithium aluminum hydride has recently been described by Schueler and Hanna.[2] Their attempt to apply this procedure to the preparation of *unsym*-diphenylhydrazine by the reduction of N-nitrosodiphenylamine yielded only diphenylamine. We have found, however, that by using equimolar quantities of the reactants, *unsym*-diphenylhydrazine is obtained in 73% yield, along with approximately 20% of diphenylamine. Moreover, the yield of hydrazine is increased to more than 90% by an "inverse" order of addition, that is, by adding a solution of lithium aluminum hydride to N-nitrosodiphenylamine. The course of reaction is best expressed by the equation

$$2Ph_2NNO + 2LiAlH_4 \longrightarrow (Ph_2NN)_2AlLi + LiAlO_2 + 2H_2$$
$$\downarrow 2H_2O$$
$$2Ph_2NNH_2 + LiAlO_2$$

Experimental

To 9.9 g. (0.05 mole) of N-nitrosodiphenylamine[3] in 50 ml. of dry ether at 10° was slowly added 57 ml. of a 0.97 molar solution of lithium aluminum hydride (0.055 mole) in ether. A precipitate, presumably $LiAlO_2$, appeared during the addition of the hydride to the nitrosamine. After standing at 10° for one hour, excess hydride and the product complex were decomposed by adding 25 ml. of wet ether followed by 100 ml. of a 30% solution of potassium sodium tartrate. The aqueous phase was separated and extracted with four 100-ml. portions of ether. Upon treating the combined ether solutions successively with water, brine solution, and ether previously equilibrated with concentrated hydrochloric acid, 10.85 g. of crude *unsym*-diphenylhydrazine hydrochloride precipitated. The crude product decomposed at 140–145°. Recrystallization from absolute ethanol gave 9.9 g. (90%) of silvery gray needles which began to decompose at 140°.[4]

Anal. Calcd. for $C_{12}H_{13}N_2Cl$: N, 12.7. Found: N, 12.2.

This product gave a mono-acetyl derivative which, after recrystallization from ethanol, melted at 188.5°,[5] and did not depress the melting point of an authentic sample.

Anal. Calcd. for $C_{14}H_{14}N_2O$: N, 12.4. Found: N, 11.9.

(1) This is a part of the research supported by the United States Air Force under Contract AF 33(038)-12656.

(2) F. W. Schueler and C. Hanna, THIS JOURNAL, **73**, 4996 (1951).

(3) S. Wexman, *Farm. Chilena*, **20**, 299 (1946).

(4) All decomposition and melting points are uncorrected.

(5) D. Vorländer and G. Bittins, *Ber.*, **68B**, 2269 (1935), reported 186° as the melting point for N-monoacetyldiphenylhydrazine, and 125° for N,N-diacetyldiphenylhydrazine.

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Infrared Spectrum of Cyclobutene. A Correction

BY JOHN D. ROBERTS AND C. W. SAUER

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Reëxamination of the infrared spectra reported for cyclobutene[1] has revealed that the samples were heavily contaminated with carbon dioxide (strong absorption at 2350 $cm.^{-1}$). The infrared spectrum of carbon dioxide-free cyclobutene pre-

(1) J. D. Roberts and C. W. Sauer, THIS JOURNAL, **71**, 3925 (1949).