

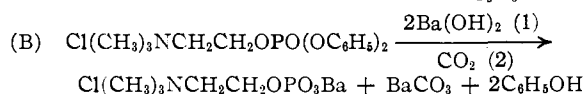
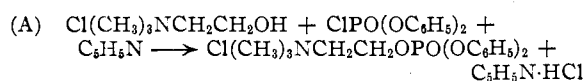
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, BANTING INSTITUTE, UNIVERSITY OF TORONTO]

Phosphorylcholine

BY ERICH BAER

In 1944 Baer and McArthur¹ reported the synthesis of phosphorylcholine utilizing diphenylphosphoryl chloride as phosphorylating agent. Although the method gives the cholinephosphate in excellent yields and with a high degree of purity it suffers from the disadvantage that it requires considerable amounts of gold salt for the isolation and platinum salt for the catalytic cleavage of the diphenylphosphorylcholine. Moreover, the method involves the use of special equipment for the catalytic hydrogenolysis. The preparation of larger amounts of phosphorylcholine according to this procedure is expensive and cumbersome.

A more convenient method of preparation of phosphorylcholine is as follows: Choline chloride is phosphorylated in pyridine by means of diphenylphosphoryl chloride (equation A). The excess of pyridine is distilled off *in vacuo* and the mixture of diphenylphosphorylcholine chloride and pyridine hydrochloride boiled with an aqueous solution of baryta (equation B). After removing the excess of barium hydroxide with carbon dioxide and extracting the phenol with ether, the barium salt of phosphorylcholine chloride is precipitated with ethanol and dried. The crude barium salt is obtained in a yield of 60 to 63% of the theoretical amount and is sufficiently pure (96 to 97%) to be used directly for most biological purposes. It can be purified further by reprecipitation from water with ethanol. The properties of the barium salt so prepared are identical with those of the product obtained by the costly and laborious procedure previously reported.¹



During the course of the investigation the reineckate and sulfate of diphenylphosphorylcholine were prepared. Since they might be useful under special circumstances, their preparation and their properties are described in the experimental part.

Experimental

Phosphorylation of Choline Chloride.—In a wide-necked and thick-walled flask (500 ml.) equipped with a rubber stopper carrying a mercury-sealed stirrer were placed 150 ml. of glass beads² (6 mm. diameter), 150 ml. of dry pyridine,³ 28.8 g. of pure diphenylphosphoryl chlo-

ride,⁴ and 13.95 g. of choline chloride.⁵ The mixture was stirred for two days at room temperature. At the end of this time the crystalline sludge was taken up in 150 ml. of water, filtered into a distilling flask and evaporated *in vacuo* to a thick sirup (bath temperature 45–50°).

Phosphorylcholine Chloride Barium Salt.—The residue from the vacuum distillation, containing diphenylphosphorylcholine chloride and pyridine hydrochloride, was dissolved in 430 ml. of water and, after the addition of 140 g. of barium hydroxide (octahydrate), 0.5 ml. of octyl alcohol and a few pumice stones, the mixture was refluxed (soda-lime tube) for seventy-five minutes (timed from the beginning of boiling). At the end of this period a vigorous stream of carbon dioxide was passed through the still hot solution until it had become neutral to phenolphthalein. The mixture was filtered with suction through a pre-coated filter (Hyflo Super-Cel) and the solid washed on the filter three times with 50 ml. portions of distilled water. The combined filtrates were extracted with 400 ml. of ether, to remove the phenol, and the aqueous solution was evaporated to dryness under reduced pressure at a bath temperature of 45 to 50°. The residue was taken up in 150 ml. of water, the solution diluted with an equal volume of 99% ethanol and set aside for one hour. The voluminous precipitate, containing most of the impurities, was separated as completely as possible by centrifuging at high speed and washed in the centrifuge tube several times with small portions of 50% ethanol (total volume 100 ml.). To the combined mother liquor and washings were added very gradually in small portions 600 ml. of 99% ethanol.⁶ The barium salt of phosphorylcholine chloride, which precipitated in the form of glistening leaflets, was centrifuged off⁷ and by means of 80% ethanol (approximately 75 ml.) transferred to a Büchner funnel, on which it was washed in succession with 99% ethanol and dry ether. The air-dried barium salt, weighing 24–25 g., was freed from its water of crystallization by keeping the finely powdered substance in a vacuum of 0.05 mm. at 100° over phosphorus pentoxide for a period of sixteen hours. The anhydrous barium salt of phosphorylcholine chloride weighed from 21.5 g. to 22.5 g. (60 to 63%) and was found to be sufficiently pure (97%) for most biochemical studies.

For analytical purposes a portion of the barium salt was re-precipitated from water with ethanol and dried *in vacuo* as described above. The recovery was 87%.

Anal. Calcd. for $\text{C}_9\text{H}_{15}\text{O}_4\text{NCIPBa}$ (355): C, 16.90; H, 3.69; N, 3.94; Cl, 9.99; P, 8.74; Ba, 38.67. Found (crude barium salt): N, 3.83; Cl, 9.67; P, 8.53; Ba, 38.83; (recrystallized barium salt): C, 16.75; H, 3.99; N, 3.78; Cl, 9.67; P, 8.67; Ba, 38.71, 38.67.

Diphenylphosphorylcholine Reineckate.—Choline chloride (13.95 g.) was phosphorylated as described above and the reaction mixture evaporated *in vacuo* to a thick sirup. The residue was dissolved in 500 ml. of water containing 40 g. of potassium carbonate⁸ and a small amount of

(4) P. Brigl and H. Müller, *Ber.*, **72**, 2121 (1939). B. p. (8 mm.) 192–194°. Older preparations should be redistilled before use.

(5) The choline chloride was thoroughly dried *in vacuo* over phosphorus pentoxide in a drying pistol treated with boiling acetone vapors.

(6) The addition of a larger volume of ethanol caused the contamination of the barium salt of phosphorylcholine chloride with barium chloride.

(7) Filtration of the mixture by suction at this stage is not advisable, since the filter is very quickly clogged with an almost impenetrable mat of the barium salt.

(8) The weakly alkaline reaction prevents the simultaneous precipitation of pyridine reineckate.

(1) E. Baer and C. S. McArthur, *J. Biol. Chem.*, **154**, 451 (1944).

(2) The glass beads have the dual purpose of bringing the choline chloride into a fine state of dispersion and preventing the formation of a sticky gum enclosing unreacted starting material.

(3) The pyridine was boiled, under reflux with barium oxide, distilled under anhydrous conditions and kept over Drierite.

Hyflo Super-Cel. The solution was filtered and the solid on the filter washed with 500 ml. of water. The combined filtrates were run into a freshly prepared and filtered solution of 48 g. of ammonium reineckate⁹ in 2000 ml. of water. The precipitate was filtered with suction, washed on the filter thoroughly with water, methanol and finally with ether and dried over calcium chloride *in vacuo* to constant weight. The yield of crude reineckate was 60.8 g. (93%). For further purification it was dissolved in 1000 ml. of dry acetone and the solution filtered through a pre-coated filter (Hyflo Super-Cel). The clear filtrate¹⁰ was diluted with 3000 ml. of distilled water, of which the first 1000 ml. was added very slowly. The reineckate was filtered with suction, washed with water, methanol and ether, and dried *in vacuo*. The yield of pure diphenylphosphorylcholine reineckate was 53.5 g. (81.7%); m. p. 162–164°. *Anal.* Calcd. for $C_{21}H_{21}O_4N_7PS_4Cr$ (654.5): N, 14.95; P, 4.75. Found: N, 15.01, 15.05; P, 4.62, 4.75, 4.68.

Diphenylphosphorylcholine Sulfate.—A solution of 12.7 g. of silver sulfate in 1700 ml. of warm (50°) water was added, together with a small amount of Hyflo Super-Cel, to the clear solution of 53.5 g. of diphenylphosphorylcholine reineckate in 1300 ml. of acetone. The mixture was filtered with suction through a pre-coated filter, the residue washed with water and the combined filtrates concentrated under diminished pressure (bath 45–50°) to a volume of 150 ml. Traces of diphenylphosphorylcholine reineckate, if present, were decomposed by the dropwise addition of a dilute solution of silver sulfate, avoiding an excess of silver sulfate. The silver reineckate

(9) The commercial ammonium reineckate is often not sufficiently pure. It was found more economical to prepare the ammonium salt as described in "Organic Syntheses," Coll. Vol. II, p. 555.

(10) The acetone solution can be used directly for the preparation of the diphenylphosphorylcholine sulfate.

was removed, the aqueous solution taken to dryness under reduced pressure and the residue triturated with 250–300 ml. of dry acetone. After standing for several hours on ice, the crystals were filtered with suction, washed on the filter several times with small quantities of ice-cold acetone and dried for twenty-four hours at 0.05 mm. over calcium chloride at room temperature. The diphenylphosphorylcholine sulfate was obtained in almost quantitative yield (31.2 g.). For analytical purposes the sulfate was purified by dissolving it, at room temperature, in a mixture of 96 parts (by volume) of dry acetone and 4 parts of methanol and removing the insoluble material. The filtrate was evaporated to dryness under reduced pressure and the residue triturated with dry acetone. The sulfate was filtered with suction and dried *in vacuo* at room temperature over calcium chloride.

Anal. Calcd. for $(C_{17}H_{23}O_4NP)_2SO_4 + 2H_2O$ (804.5): N, 3.48; P, 7.70; SO_4 , 11.92. Found: N, 3.53; P, 7.68; SO_4 , 12.19.

The sulfate, when being kept for several days at room temperature over phosphorus pentoxide at a pressure of 0.001 mm., loses its water of crystallization and forms a viscous mass (P, 7.96%). On exposing the anhydrous sulfate to moist air, it regains its water of crystallization and solidifies (P, 7.58).

The diphenylphosphorylcholine sulfate (hydrate) is readily soluble in water, methanol, glacial acetic acid, tetrachloroethane and insoluble in dry acetone, ethyl acetate and benzene.

Summary

A convenient procedure for the preparation of phosphorylcholine is reported.

TORONTO, CANADA

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Studies on the Reduction of Aromatic Ketones by the Clemmensen Method

By H. LEON BRADLOW¹ AND CALVIN A. VANDERWERF

The fact that reduction of *p,p'*-difluorobenzophenone by a modification of the Clemmensen procedure has been found to yield almost 80% of the theoretical amount of the pinacolone 1,2,2,2-tetra-*p*-fluorophenylethanone,² prompted this study of the applicability of the Clemmensen method to the reduction of aromatic ketones. Although a few examples of the successful reduction of aromatic ketones by the Clemmensen method have been reported, the reduction of benzophenone and many related compounds is generally considered to be unsatisfactory because of the formation of resinous products.³

Experimental⁴

Materials.—Amalgamated zinc was prepared according to Martin⁵ with two modifications: (1) the zinc was first washed with dilute hydrochloric acid to remove any oxide coating, and (2) the amalgamated zinc was washed with

distilled water. Of the ketones submitted to reduction, numbers I, VII, VIII, XIV and XV were obtained from commercial sources, whereas the remainder were prepared according to standard procedures.

General Procedures.—Three somewhat varied procedures were used for the reduction of ketones, as follows:

PROCEDURE A.—About 0.05 mole of the ketone was refluxed vigorously for forty-eight hours with 12 g. of amalgamated zinc, 18 cc. of water, 8 cc. of concentrated hydrochloric acid and 15 cc. of toluene. Concentrated hydrochloric acid (5 cc.) was added every eight hours.

PROCEDURE B.—Same as A, excepting that 20 g. of amalgamated zinc, 40 cc. of concentrated hydrochloric acid, 15 cc. of water and 20 cc. of toluene were used, and that 10 cc. of concentrated hydrochloric acid was added every eight hours.

PROCEDURE C.—About 0.05 mole of the ketone was refluxed for from twenty-four to thirty-six hours with 20 g. of amalgamated zinc, 20 cc. of ethanol and 50 cc. of concentrated hydrochloric acid. Additional acid (5 cc.) was added after eight and after sixteen hours.

Isolation of the reduction products was effected in two general ways. Where the formation of dimeric products⁶ was indicated by the appearance of crystalline deposits on the zinc, the reaction mixtures were exhaustively steam distilled in order to remove any monomeric reduction prod-

(1) H. P. Cady Fellow, 1946.

(2) Bradlow and VanderWerf, *THIS JOURNAL*, **69**, 662 (1947).

(3) See Martin, "Organic Reactions," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1942, p. 159.

(4) All melting points corrected; boiling points uncorrected.

(5) Martin, *THIS JOURNAL*, **58**, 1438 (1936).

(6) The exact nature of these varied; in some cases pinacols were formed, in others pinacolones, and occasionally mixtures of both.