[CONTRIBUTION FROM THE RESEARCH LABORATORY OF MERCK & CO., INC.]

Some Improvements in the Synthesis of Cocarboxylase Chloride

BY JOHN WEIJLARD

The previously published method[1] for the synthesis of cocarboxylase has been simplified, especially by the reversal of the purification steps and the elimination of the silver precipitation stage which gave erratic results on larger scale experiments. Tests are reported which show that cocarboxylase can be stored for more than a year without detectable hydrolysis.

Experimental Part

One hundred cc. of 85% phosphoric acid was heated in a 400 cc. Pyrex beaker over a free flame until fumes appeared and a slight though definite crystalline deposit was noted. Twenty-four grams of anhydrous sodium pyrophosphate was added and the mixture stirred until all was in solution. The hot acid was cooled to 135°, the beaker was placed in an oil-bath, preheated to 155°, and 40 g. of vitamin B_1 was added all at once and mixed well. It was kept for ten minutes in the 155° oil-bath with almost continuous hand stirring, then cooled to room temperature and dissolved in 2 liters of water; 1280 cc. 2.5 *N* hydrochloric acid was added and the volume made up to 4 liters with water.

Crude cocarboxylase phosphotungstate was precipitated by adding an excess of 25% phosphotungstic acid with stirring (750 cc.). The mixture was centrifuged and the supernatant liquid discarded.

The crude phosphotungstate was dissolved in 16 liters of acetone. As the solid disintegrated, crude cocarboxylase separated as a sticky substance. It was kept at 0–2° overnight; the supernant liquid was then decanted and discarded. The sticky residue was dissolved in 600 cc. of 0.1 *N* hydrochloric acid; the acid solution was centrifuged and the clear liquid was decanted. The residue was agitated with 200 cc. of tenth normal hydrochloric acid and then centrifuged. Ten volumes of acetone (8 liters) was added to the combined acid solutions and the mixture chilled overnight at 0–2°. The supernatant liquid was discarded and the residue (a soft glassy mass) dissolved in 200 cc. water. The aqueous solution was cooled in ice and precipitated with 860 cc. of concentrated barium hydroxide solution, a slight excess. The precipitated barium phosphate was centrifuged off at 5° and the clear supernatant fluid was decanted, acidified with hydrochloric acid to congo red, and cooled immediately in ice. The precipitate was agitated with 200 cc. of water, then centrifuged, and the supernatant washing added to the main solution.

To the cooled solution, 10% sulfuric acid was added to precipitate the excess of Ba^{++}, adjusting so that not more than a slight trace of sulfuric acid was added in excess. The barium sulfate was separated by centrifuging and washed once in the centrifuge with 30 cc. of water. The united solutions and washings (1260 cc.) were mixed with 12.6 liters of acetone and kept at 0–2° overnight.

The supernatant liquid was siphoned off and discarded. The residue, a glassy mass, was dissolved in 150 cc. of tenth normal hydrochloric acid, 800 cc. of absolute alcohol was added and the mixture allowed to stand at 0° for four days.

The supernatant liquid was discarded; the solid precipitate which had formed was washed first with absolute alcohol, then with ether, by decantation. The still soft mass (10.4 g.) was dissolved in 70 cc. of 0.1 *N* hydrochloric acid, treated with 0.5 g. of carboraffin and filtered after ten minutes contact at room temperature. The solution was made up to 200 cc. with 0.1 *N* hydrochloric acid. Three volumes (600 cc.) of absolute alcohol was added and the mixture cooled in the ice box three hours; then 7 volumes (1400 cc.) of acetone was added and the mixture refrigerated at 6–7° for forty-eight hours with frequent scratching. The slightly turbid solution was decanted, the crystals were collected on a filter and washed with alcohol, then ether; yield 6.3 g. main crop of semi-crude cocarboxylase.

The 6.3 g. of semi-crude was dissolved in 120 cc. of 0.1 *N* hydrochloric acid, and absolute alcohol added to a permanent cloudiness, 350 cc. being required. After a few hours of cooling, 10 cc. more of alcohol was added and the mixture was allowed to crystallize in the refrigerator for four days with occasional scratching. The crystals were collected and washed with absolute alcohol, then ether, and dried *in vacuo* over sulfuric acid for a few hours; yield 3.96 g. main crop of pure cocarboxylase; m. p. 238–240°. *Anal.* Calcd. for $C_{12}H_{21}O_8N_4ClSP_2 \cdot {}^1/_2H_2O$: C, 30.66; H, 4.30; N, 11.91; H_2O, 1.91. Found: C, 30.40; H, 4.18; N, 11.71; H_2O, 1.95.

The mother liquors and alcoholic washings from the semi-crude as well as from the pure cocarboxylase were allowed to stand for a week in the ice box, and additional small crops of semi-crude material were obtained. These were recrystallized as outlined above; yield 0.87 g., m. p. 238–240°. Calcd. for $C_{12}H_{21}O_8N_4ClSP_2 \cdot {}^1/_2H_2O$: C, 30.66; H, 4.30; N, 11.91; H_2O, 1.91. Found: C, 30.52; H, 4.36; N, 11.53; H_2O, 1.80.

The total yield of cocarboxylase chloride was 4.83 g.

Stability of Cocarboxylase.—Cocarboxylase chloride having been prepared by this method and stored at room temperature in the dark for more than a year was again analyzed. Calcd. for $C_{12}H_{21}O_8N_4ClSP_2 \cdot {}^1/_3H_2O$: C, 30.89; H, 4.24; N, 12.00; P, 13.29; H_2O, 1.27. Found: C, 30.72; H, 4.19; N, 11.66; P, 13.64; H_2O, 1.15. Apparently there was a slight loss of water of crystallization.

It was tested for ionic phosphate by dissolving 0.005 g. of cocarboxylase in 10 cc. of water, adding 2 g. of ammonium nitrate, followed by 10 cc. of ammonium molybdate solution U. S. P. X. No turbidity developed in one hour. This test is so sensitive that 0.000010 g. of phosphorus as ionic phosphate produces a yellow turbidity in ten minutes. From this it is obvious that no hydrolysis has taken place on storing. The melting point remained unchanged, 238–240°.

Since this paper was prepared H. Weil-Malherbe's[2] ex-

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[1] Weijlard and Tauber, THIS JOURNAL, **60**, 2263 (1938).

[2] H. Weil-Malherbe, *Biochem. J.*, **34**, 980 (1940).

cellent paper on cocarboxylase has appeared. In that paper it was suggested that the use of silver might be avoided.

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Summary

An improved process for the synthesis of cocarboxylase chloride has been presented as well as evidence indicating that cocarboxylase is stable under normal conditions except slight loss of water of crystallization.

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NOTES

Polymorphic Forms of Substituted Phenols

BY RICHARD T. ARNOLD, HAROLD KLUG, JOSEPH SPRUNG AND HAROLD ZAUGG

In connection with the research on another problem, we prepared 6-hydroxytetralin (β-tetralol) by fusion of the sodium sulfonate with alkali according to Schroeter.[1] This sample of tetralol melted at 53–54° in contrast to the reported value of 62°. It formed transparent, huskily built crystals when precipitated from low-boiling ligroin.

Another sample of β-tetralol from the decomposition of 6-tetralin diazonium chloride melted at 62° and formed opaque needles from low-boiling ligroin.

If a large crystal of the form melting at 54° was brought into contact with one fine needle of the 62° modification, it turned opaque at the point of contact and this opaque boundary passed across the crystal so that in about five minutes the transformation was complete. The resulting melting point of the big crystal was then 62°.

Using a Buerger[2] precision powder camera of 57.3 mm. radius, powder photographs were taken at room temperature using Fe K_α radiation. The powder samples were extruded rods of powder crystals prepared in the device described by Lukesh[3] using LePage's glue as a binder. The following photographs were taken: (a) 54° form, (b) 62°—from 54° by contact with 62° form, (c) 62° form. Both (b) and (c) were identical and different from (a).

In a similar manner 4-hydroxyhydrindene has been obtained in two crystalline forms. The first, m. p. 39.5–40°, was produced by alkali fusion of the sodium sulfonate and precipitated from ligroin in needles.

From the 4-hydrindenediazonium chloride we obtained 4-hydrindenol melting at 49–50° as described by Linder.[4]

Powder photographs of these two forms showed them to be entirely different. The melting point of the low-melting form (39.5–40°) was raised to 49–50° by contact with the 49–50° modification.

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[1] Schroeter, *Ann.*, **426**, 120 (1922).

[2] Buerger, *Am. Mineral.*, **21**, 11–17 (1936).

[3] Lukesh, *Rev. Sci. Instruments*, **11**, 200 (1940).

[4] Linder, *Monatsh.*, **72**, 219 (1939).

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p-(*p*-Aminophenyl)-benzenesulfonamide

BY CONARD K. DONNELL, JAMES H. DIETZ AND WILLIAM T. CALDWELL

Recently Van Meter, Bianculli and Lowy[1] reported the preparation of *p*-(*p*-aminophenyl)-benzenesulfonamide from *p*-aminobiphenyl. We, too, had prepared this compound in the same way with results essentially like theirs; however, we determined the orientation of the sulfonamide group differently by means of an independent synthesis from *p*-nitrobiphenyl, a synthesis which is, of course, equally applicable to the preparation of other N′-substituted sulfanilamides. The structure of the requisite intermediate *p*-(*p*-nitrophenyl)-benzenesulfonic acid was proved by Gabriel and Dambergis,[2] by showing that the same product is obtained upon sulfonation of *p*-nitrobiphenyl as upon nitration of *p*-phenyl-

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[1] Van Meter, Bianculli and Lowy, THIS JOURNAL, **62**, 3146 (1940).

[2] Gabriel and Dambergis, *Ber.*, **13**, 1410 (1880).