A summary of the weights and yields of the products of this reaction is given in Table I.

Reaction of Phenylmagnesium Bromide with Ethyl α-Benzoyl-α-bromopropionate.—This reaction was carried out in the same manner as described above for the acetate, except that 50 g. of bromo ester and an equivalent quantity of Grignard reagent were used. At the completion of the reaction most of the ether was removed by distillation and the magnesium enolate precipitated by the addition of dry petroleum ether. This salt was filtered off, decomposed with cold dilute hydrochloric acid, and the keto ester extracted with ether. After drying and removal of the ether, distillation of the residue gave 30.4 g. (85%) of ethyl α-benzoylpropionate, b. p. 127–128° (1 mm.). The petroleum ether was distilled from the filtrate through a 30-cm. Vigreux column and the residue fractionated at atmospheric pressure. A yield of 22.0 g. (81%) of bromobenzene, b. p. 154–155°, was obtained. The above products were the only substances which could be isolated from the reaction mixture.

Summary

The rates of reaction of ethyl benzoylbromoacetate and ethyl α-benzoyl-α-bromopropionate with potassium iodide and hydrazine and the course of the reaction of each of these bromo esters with phenylmagnesium bromide have been determined. The results which were obtained, together with the values found for the molecular refractions of these bromo esters, indicate that they exist to a certain extent in a hypobromite structure.

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Crystalline Antineuritic Vitamin (B_1) Obtained with the Aid of Picrolonic Acid

BY ATHERTON SEIDELL AND MAURICE I. SMITH

It was shown several years ago[1] that by means of benzoylation at one stage of the process an acetone precipitated concentrate of high antineuritic activity could be prepared. Since then such concentrates have been subjected to a great variety of fractionation procedures. Until recently no notable advance in their purification has been attained.

Although the preparation of a picrolonate of the antineuritic vitamin has been reported by several workers,[2] in each case some other compound, usually the hydrochloride, was first isolated and, for purposes of confirmatory evidence, this was converted to the picrolonate or other well characterized salt. The procedures by which crystalline compounds of the

(1) Seidell, *J. Biol. Chem.*, **82**, 633–640 (1929); Seidell and Smith, *U. S. Pub. Health Reports*, **45**, 3194–3200 (1930); Seidell and Birckner, THIS JOURNAL, **53**, 2288–2295 (1931).

(2) Jansen and Donath, *Med. Dienst. Volkogezondheid Ned-Indie*, Part I (1927); Windaus, Tschesche, *et al.*, *Nachr. Ges. Wiss. Goettingen*, 207 (1931); same paper, *Z. physiol. Chem.*, **204**, 123–128 (1932); Ohdake, *Proc. Imp. Acad. (Japan)*, **7**, 102–105 (1913); **8**, 179–182 (1932); *Bull. Agr. Chem. Soc. (Japan)*, **8**, 11–46, 111–119 (1932); Kinnersley, O'Brien and Peters, *J. Physiol.*, **76**, 17 P (1932).

antineuritic vitamin have, so far, been obtained are extremely tedious and very wasteful of the active material. The yields have apparently not yet been sufficient to permit adequate studies of the chemical identity of the active compound.

A more direct and efficient procedure for obtaining a crystalline antineuritic compound has been the object of the experiments described in this and in the preceding papers from this Laboratory.

Of the many reagents which have been tried picrolonic acid applied to our acetone precipitated concentrates[1] of an activity of about 0.1 mg. as determined by the rat method previously described[3] has given the best results. The explanation of this appears to be that a large proportion of the constituents of our vitamin concentrate do not combine with picrolonic acid and remain in the solution from which those compounds that do form picrolonates either precipitate directly or separate upon slow evaporation of the solvent. Of these picrolonates the least soluble, which consequently precipitate first, contain much less of the active compound than those which deposit later. These latter upon crystallization from alcohol yield a definitely crystalline material of high physiologic antineuritic activity.

The many factors which determine the separation of the active from the inactive picrolonates have, so far, not been learned with sufficient precision to ensure that a constant yield of vitamin picrolonate can be regularly obtained. Although the crystalline product has been isolated repeatedly the amount obtained has frequently been only a few per cent. of that corresponding to the total vitamin present. The highest recovery of vitamin as crystalline picrolonate so far obtained from acetone precipitated concentrate has been approximately 25%.

As the result of numerous experiments the following procedure has been found most successful. One-half gram of acetone precipitated vitamin concentrate is dissolved in 25 cc. of water and to this a warm 3.3% solution of recrystallized picrolonic acid in 80% methyl alcohol is added in successive quantities of 1.0 to 2.0 cc. The yellow precipitate formed is thrown down by centrifugation after each addition of the picrolonic acid reagent. When 5.0 to 6.0 cc. of reagent has been added and precipitation is still not complete the supernatant cloudy liquid is decanted and allowed to stand in the cold room (+5°) until next day. The clear supernatant solution is now decanted and upon evaporation in a partially evacuated desiccator containing sulfuric acid, gradually yields a partially crystalline deposit. When the volume of liquid has been reduced to between 5.0 and 10.0 cc. it is withdrawn and the deposit washed by flowing several small portions of water over it and withdrawing each with a fine pipet.

The deposit obtained as just described may consist of pale yellowish aggregates of needles, rods or clusters of prisms together with some amorphous material which appears to be sufficiently lighter to permit its elutriation with methyl alcohol.

The partially crystalline deposit, which usually melts at about 180°, is dissolved in 90% methyl alcohol by warming, followed by centrifuging and decanting the clear solution. This upon evaporation may yield a fluffy amorphous precipitate which should be removed by centrifugation. The final approximately 10 cc. of clear alcoholic solution

(3) Smith, *U. S. Pub. Health Reports*, **45**, 116-129 (1930).

is evaporated slowly in a desiccator. A crystalline ring soon forms at the surface of the liquid. This is dislodged and on continuing the evaporation, a deposit of well-formed rods or prisms, usually in star-shaped clusters, collects at the bottom of the liquid. These when dried on hardened filter paper and later in a desiccator melt with effervescence at approximately 227°.

In the most successful experiment made as above, 18 mg. of crystalline picrolonate was obtained from 500 mg. of acetone precipitated vitamin concentrate. In this case the curative dose for polyneuritic rats of the vitamin concentrate was 0.1 mg. and that of the crystalline picrolonate was 0.015 mg. (see Table I). Calculating from these results there were 5000 curative doses in the crude concentrate and 1200 doses in the crystals. This corresponds to a recovery of approximately 25% of the vitamin in the crystalline form.

The physiological tests were made as described previously[3] by injecting the samples into a tail vein of rats brought to the polyneuritic state by a diet deficient in the antineuritic vitamin. Due to their slight solubility in water, warming is necessary to effect solution of the picrolonate samples. Physiological salt solution is used for dissolving and diluting the samples to such extent that the desired dose is contained in about one-half to one cubic centimeter used for the injection.

TABLE I

PHYSIOLOGICAL TESTS ON POLYNEURITIC RATS OF THE ACTIVITY OF VITAMIN CONCENTRATE AND OF CRYSTALLINE PICROLONATE

Rats used	Dose administered, mg.	Results R = Recovery P = Paralysis not relieved	Duration of cure, days
	I. Vitamin Concentrate No. 33-C		
2	0.150	R R	10–15
5	.100	R R R R R	5–10
4	.075	R R P P	0–9
	II. Crystalline Picrolonate No. 33.91		
3	0.030	R R R	9–17
3	.020	R R R	4–11
3	.015	R R R	6–8
2	.012	P P	0

We have tested by the same method three samples of vitamin hydrochloride crystals kindly sent us by Professors Jansen, Windaus and Peters. The minimum curative dose for these varied between 0.008 and 0.012 mg. Analyses of the picrolonate made from the hydrochloride by Ohdake and from the gold salt by Windaus, Tschesche and co-workers[2] indicate that the compound is made up of two molecules of picrolonic acid to one of vitamin. This corresponds to a content of approximately 35% of the vitamin base. Analyses of the hydrochloride show that it contains approximately 80% of the base. Hence, the curative dose of the picrolonate should be about twice that of the hydrochloride. Since the curative dose of our picrolonate is 0.015 mg. as compared with 0.008 mg. as the minimum curative dose of the best of the three hydrochlorides submitted to us, it is evident that the crystals obtained directly by means of picrolonic acid as herein described are equally as pure as those obtained by the transformation of other vitamin salts into the picrolonate.

Summary

Vitamin concentrate prepared from brewers' yeast by adsorption on fuller's earth followed by extraction, benzoylation and acetone precipitation as previously described, when dissolved in water and treated with an alcoholic solution of picrolonic acid, yields initial precipitates which are relatively inactive. The filtrate from these when evaporated yields a semi-crystalline deposit rich in the antineuritic vitamin. This picrolonate deposit when purified by recrystallization from methyl alcohol is converted to characteristic rods or prisms which are curative for polyneuritic rats in doses of 0.015 milligram.

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The Preparation of Certain Cryptophenols

BY LARKIN HUNDLEY FARINHOLT, WILTON C. HARDEN AND DANIEL TWISS

It has been observed that many phenols when passed through the body become conjugated. This conjugation represents a detoxification, usually accompanied by a corresponding decrease in germicidal activity. A similar loss of germicidal activity may be produced *in vitro*, when the phenol is in contact with organic material. In this case, however, the effect is due to precipitation of the phenol by chemical combination with albumins. It has also been shown in the case of strongly germicidal phenols that the margin between the effective concentration for bacteria and the harmful dose for the host is very small, and that such phenols are in general too toxic and too corrosive to be taken in effective quantities. In view of these facts, it seemed of interest to attack the problem of producing effectively germicidal phenols for internal use from a new standpoint. On the basis of the following considerations, a number of cryptophenols have been prepared and submitted to pharmacological investigation, the results of which will be reported elsewhere.

Cryptophenols are those phenols in which the acidity has been reduced to such an extent that they do not dissolve in aqueous sodium hydroxide, but only in Claisen solution. It might be expected that this change in properties would be accompanied by a simultaneous decrease in corrosive action and susceptibility to conjugation. On the other hand, it seems reasonable to assume, in the light of present knowledge, that such weakly acid phenols would also, in general, be less germicidal than those which are strongly acid, although there is not necessarily a direct proportionality between acidity and germicidal action. If it be found, however, that