was added as before, and the mixture allowed to stand for several hours in the cold. The resultant precipitate was combined with Precipitate (I) and the whole recrystallized two more times in the manner just described. We thus obtained 540 mg. of colorless crystals[18] which melted with decomposition at 255° (corr.). All the filtrates obtained during the recrystallization process were combined and reworked to yield a further crop of crystals amounting to 15 mg. The total yield (555 mg.) represents a recovery of 9.9% of the vitamin originally present in the yeast.

Anal. Calcd. for $C_{12}H_{18}ON_4SCl_2$: C, 42.71; H, 5.38; N, 16.62; S, 9.51; Cl, 21.06. Found: C, 42.83, 42.70; H, 5.40, 5.39; N, 16.31, 16.18; S, 9.59, 9.39, 9.42; Cl, 20.87, 20.70; ash, 0.17.

(18) A photograph of the crystals may be found in *Ind. Eng. Chem., Anal. Ed.*, **9**, 290 (1937).

Biological Assay.—The antineuritic potency of the crystals was tested on mice by a method to be described elsewhere. It was found to be identical with that of the vitamin B_1 hydrochloride obtained from rice polishings in this Laboratory.

Summary

A method is described for isolating vitamin B_1 hydrochloride from brewers' yeast by the use of a synthetic zeolite.

NEW YORK, N. Y. RECEIVED JUNE 24, 1937

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, FORDHAM UNIVERSITY]

The Use of Synthetic Zeolites in the Isolation of Vitamin B_1. III. Experiments with Wheat Germ[1]

BY LEOPOLD R. CERECEDO AND JOHN J. THORNTON

In previous papers,[2,3] the isolation of vitamin B_1 from rice polishings and from brewers' yeast has been described. In the present communication we wish to report the isolation of the vitamin from wheat germ. So far as we are aware, only one attempt at the isolation of the vitamin from wheat germ has been reported in the literature, namely, by Guha and Drummond.[4] They prepared a concentrate of vitamin B_1 from wheat embryo, the pigeon-curative day-dose of which was found to be 0.005 mg., and which promoted good growth in rats in daily doses of 0.015 mg. Their observations led them to believe that vitamin B_1 consisted of more than one factor.

In our experiments with wheat germ, we were able to draw upon the experience gained in working out the methods for the isolation of the vitamin from rice polishings and from yeast. As in the case of yeast, we found that a precipitation of the wheat germ extract with lead acetate previous to the treatment with the zeolite was an essential step. In addition, we found it expedient to fractionate the silicotungstate of the vitamin with lead acetate before attempting the final purification. This procedure has enabled us to isolate vitamin B_1 from wheat germ for the first time. The substance has been found to be identical with the compound isolated from rice polishings and from brewers' yeast.

(1) Presented at the Chapel Hill meeting of the American Chemical Society, April, 1937.

(2) Cerecedo and Hennessy, THIS JOURNAL, **59**, 1617 (1937).

(3) Cerecedo and Kaszuba, *ibid.*, **59**, 1619 (1937).

(4) Guha and Drummond, *Biochem. J.*, **23**, 880 (1929).

Experimental Part

1. Extraction.—Eighteen kilograms of wheat germ was placed into each of 6 wooden casks of 52-gallon (197-liter) capacity. As a preliminary to the extraction proper, each batch of the wheat germ was thoroughly mixed with 20 gallons (77 liters) of 15% ethyl alcohol, to which enough sulfuric acid was added to give a *p*H of 4.0–4.5. The contents of each cask were extracted four times with 40 gallons (153 liters) of 15% alcohol at *p*H 4–4.5. In the first cask the 40 gallons (153 liters) was made up of the 20 gallons (77 liters) used to wet down the wheat germ and 20 gallons of fresh extracting liquid. In all the other casks the 40 gallons was made up of the 20 gallons already present and the 20 gallons which was decanted from the preceding casks after standing overnight. Efficient extraction was ensured by intermittent stirring. The *p*H was kept at 4–4.5 throughout the extraction process.

The four portions of the extract were transferred successively from cask No. 6 to the collecting cask No. 7 and, after addition of 2 gallons (7.7 liters) of 95% ethyl alcohol, thoroughly mixed. To the mixture solid barium hydroxide was added slowly with constant stirring until a *p*H of 7.0 had been reached. The resultant precipitate was allowed to settle overnight. The following day the supernatant liquid was decanted and treated with a saturated solution of lead acetate at a *p*H of 6.8–7.0 until precipitation was complete. After standing overnight the supernatant liquid was pumped off and treated with dilute sulfuric acid so as to remove the excess of lead and barium. The precipitate settled in about twenty-four hours, giving a clear supernatant liquid, which was separated from the precipitate and treated with barium hydroxide until the *p*H was 4.5. When the mixture had stood overnight the precipitate settled well. The supernatant liquid was siphoned off and was now ready for the next step. The final volume of extract was approximately 125 liters and contained 90% of the vitamin originally present in the wheat germ.

2. Treatment of the Extract with the Zeolite.—The preparation of the zeolite and the treatment of the

extract for removal of the vitamin have been described by Cerecedo and Hennessy[2].

3. Removal of the Vitamin from the Zeolite.—Fifty-five liters of a normal ammonium nitrate solution was heated to 80° and allowed to percolate through the charged zeolite at the rate of 500 cc. per minute. This was followed by 4 gallons (15 liters) of hot water so as to wash out any liquid adhering to the zeolite. The filtrate was cooled and then adjusted to *p*H 7.5 with dilute sodium hydroxide solution. After a few hours the resulting precipitate had settled sufficiently to allow the supernatant liquid (approximately 70 liters) to be siphoned off.

4. Precipitation of the Silver Salt of the Vitamin.—To the liquid obtained from step 3 we added dilute nitric acid with stirring until the *p*H was 2.0, then freshly prepared silver oxide to a *p*H of 4.0. The resultant precipitate was allowed to settle and, after standing for five hours, the supernatant fluid (I) decanted and set aside for further treatment. The precipitate was centrifuged off, suspended in 200 cc. of water and the *p*H of the suspension adjusted to 2.0 with dilute nitric acid. On warming the mixture to 60° with stirring, the precipitate dissolved. The solution was cooled and then the *p*H adjusted to 4.0 by adding gradually with constant stirring fresh silver oxide. After standing for thirty minutes, the mixture was centrifuged, and the supernatant fluid combined with that set aside (I). More silver oxide was added with stirring to the combined liquids until the *p*H was 7.5. The mixture was covered to keep out the light and then allowed to stand in the ice box until the following day, when the supernatant fluid was siphoned off. The silver precipitate, obtained between *p*H 4.0 and 7.5, was collected and treated with concentrated hydrochloric acid until the *p*H was 2.0. The mixture was stirred, diluted to 200 cc. with water and then centrifuged. The centrifugate (A) was set aside, and the precipitate treated once more with hydrochloric acid. After dilution to 50 cc. with water and stirring, the mixture was centrifuged and the centrifugate combined with (A). Dilute sodium hydroxide solution was added with stirring to the combined liquids until the *p*H was 7.0 and the resultant precipitate removed. Thus, a solution (255 cc.) was obtained which contained 30% of the activity originally present in the wheat germ.

5. Preparation of the Silicotungstate of the Vitamin.—The solution obtained in step 4 was diluted to 400 cc. with water and made 0.6 *M* with sulfuric acid. A 10% solution of silicotungstic acid, prepared according to Scroggie,[5] was added until no more precipitate was produced. After standing overnight in the ice box, the precipitate was centrifuged off, washed with 0.6 *M* sulfuric acid and suspended in 200 cc. of water. Dilute sodium hydroxide solution was then added to the suspension with rapid stirring until the *p*H was 7.0. This treatment dissolved the precipitate. To this solution, the *p*H of which was kept between 6.5 and 7.0 with dilute sodium hydroxide, a saturated solution of lead acetate was added until, on centrifuging a sample, the supernatant liquid was found to be perfectly clear. The mixture was centrifuged and the centrifugate (I) set aside for further treatment. The precipitate was again suspended in 200 cc. of water and subjected twice to the treatment just described. Centrifugates (II) and (III), obtained from these treatments, were combined with (I). Dilute sulfuric acid was added to the combined liquids until the *p*H was 2.0, whereupon the resultant precipitate was centrifuged off and discarded. Ten cc. of 10% silicotungstic acid was added to the filtrate and the mixture allowed to stand in the ice box overnight. After testing with a few drops of silicotungstic acid solution for complete precipitation, it was centrifuged. The precipitate was collected and washed with a small volume of 0.6 *M* sulfuric acid. It was then decomposed by adding 15 cc. of a saturated solution of barium hydroxide and stirring thoroughly for two minutes at 0°. The precipitate was centrifuged off immediately and set aside for further treatment (Precipitate I). To the supernatant liquid dilute sulfuric acid was added to a *p*H of 8.0. This treatment yielded a precipitate of barium sulfate which was collected and reworked with barium hydroxide in the manner just described. The filtrates resulting from these two treatments were combined and treated with dilute hydrochloric acid until the *p*H was 2.0 (Solution A). Precipitate (I) was subjected to two further treatments with barium hydroxide at 0°. The filtrates from the second and third treatments were added to the filtrate from the first treatment (Solution A) after adjusting the *p*H to 2.0 with hydrochloric acid. The combined filtrates were taken to dryness *in vacuo*.

6. Crystallization of the Vitamin.—The dry semicrystalline residue obtained in step 5 was extracted three times with 5 cc. of hot 95% alcohol. The three extracts were combined and the mixture allowed to stand in the ice box until thoroughly chilled, whereupon it was centrifuged, and the precipitate discarded. The solution was cautiously evaporated to a small volume (1–2 cc.) on the steam-bath, and then placed in the ice box. The next morning colored crude crystals had formed, which were separated from the mother liquor. They were triturated with a small amount of absolute ethyl alcohol (about 3 cc.). This treatment removed most of the color from the precipitate. After decantation of the mother liquor, the crystals were treated with 1 cc. of water, 3 cc. of 95% alcohol, and then with dioxane until a faint cloudiness just persisted. After standing overnight in the cold, colorless crystals of vitamin B_1 hydrochloride, resembling those isolated from rice polishings and from brewers' yeast, were obtained. They melted at 253° (corr.) with decomposition. The yield was 40 mg., representing a recovery of 1.8% of the material originally present in the wheat germ.

Anal. Calcd. for $C_{12}H_{18}ON_4SCl_2$: C, 42.71; H, 5.38; N, 16.62; S, 9.51; Cl, 21.03. Found: C, 42.98, 42.86; H, 5.84, 5.79; N, 16.46; Cl, 20.61, 20.84; ash, 0.75.

Biological Assay.—The antineuritic potency of the crystals was tested on mice by a method to be described elsewhere. It was found to be identical with that of the vitamin B_1 hydrochloride isolated in this Laboratory from rice polishings and from brewers' yeast.

Summary

The isolation of vitamin B_1 from wheat germ has been described.

(5) Scroggie, THIS JOURNAL, **51**, 1057 (1929).

NEW YORK, N. Y. RECEIVED JUNE 24, 1937