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 a commercial sample of natural vitamin B_1 hydrochloride (Merck).

Summary

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

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, FORDHAM UNIVERSITY]

The Use of Synthetic Zeolites in the Isolation of Vitamin B_1. II. Experiments with Brewers' Yeast[1,2]

BY LEOPOLD R. CERECEDO AND FRANK J. KASZUBA

In a recent paper Cerecedo and Hennessy[3] reported the isolation of vitamin B_1 from rice polishings by means of a new method using a synthetic zeolite. In the present communication we wish to describe our experiments with brewers' yeast. Several workers[4] have reported the isolation of the vitamin from yeast, but the methods used have been laborious and costly. Furthermore, the yields obtained have been poor. Inasmuch as our preliminary experiments with rice polishings had shown that the synthetic zeolite, "Decalso" seemed to have a selective action on the vitamin, it occurred to us that also in the case of yeast the procedure of isolation might be simplified by the use of this material. However, several changes in the process described in the previous paper had to be made before we succeeded in obtaining the pure substance. These changes were: first, a precipitation of the extract with neutral lead acetate before the treatment with the zeolite. The idea which guided these experiments was based upon the findings of Rosedale[5] and of Chick and Roscoe[6] to the effect that lead acetate in neutral or slightly alkaline solution precipitates nearly all vitamin B_2, whereas a large part of vitamin B_1 is left in solution. We have found that an extract of brewers' yeast, after precipitation with barium hydroxide and lead acetate, still contains more than 90% of the vitamin B_1 present in the original yeast. The second change consisted in precipitating the vitamin by means of silver oxide. The use of this reagent for the precipitation of nitrogenous bases has been recommended by Kiesel.[7] When the procedure had been thus modified, it enabled us to isolate the vitamin from brewers' yeast as the hydrochloride in a very pure form and in a yield of almost 10%.

Experimental Part

1. Extraction.[8]—Five hundred pounds (228 kg.) of pressed brewers' yeast[9] was placed in batches of 100 lb. (45.4 kg.) each into 5 wooden casks of 52 gallon (196-liter) capacity. To each cask 15 gallons (76 liters) of 15% ethyl alcohol was added. After adjusting the *p*H to 4–4.5 with sulfuric acid, added while the mixture was being thoroughly stirred, the contents of each cask were heated by means of a steam coil to 80°, and kept at this temperature for one-half hour. Twenty-three gallons (87 liters) of 15% ethyl alcohol was then added to one of the casks (cask No. 1), the *p*H being kept at 4–4.5. The mixture was stirred for one hour and allowed to stand overnight. The following day the supernatant liquid (about 30 gallons (113 liters)) was transferred from cask No. 1 into cask No. 2, and 30 gallons (113 liters) of fresh 15% alcohol was added to cask No. 1. The contents of cask No. 1 were stirred for one-half hour, those of cask No. 2 for two hours, the *p*H being maintained at 4–4.5. Thus, the continuous extraction, as described in the previous paper, was begun. The extract so obtained was treated with solid barium hydroxide to a *p*H of 7.0 while stirring vigorously. After standing overnight, the supernatant liquid was decanted into an empty tank and treated with a saturated solution of neutral lead acetate at a *p*H of 6.8,[10] until precipitation was complete. The next morning the supernatant liquid was pumped into another tank and treated with sulfuric acid to a *p*H of 4.5. The final extract, a liquid of pale lemon-yellow color, was decanted, after standing overnight, from the lead sulfate precipitate. Thus, each portion of

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

(2) This investigation was begun in the latter part of 1932.

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

(4) Windaus, Tschesche, Ruhkopf, Laquer and Schultz, *Z. physiol. Chem.*, **204**, 123 (1932); Kinnersley, O'Brien and Peters, *Biochem. J.*, **27**, 232 (1933); Jansen, Wibaut, Hubers and Wiardi, *Rec. trav. chim.*, **52**, 366 (1933); Ohdake, *Bull. Agr. Chem. Soc. Japan*, **10**, 71 (1934).

(5) Rosedale, *Biochem. J.*, **21**, 1266 (1927).

(6) Chick and Roscoe, *ibid.*, **23**, 498 (1929).

(7) Kiesel, *Z. physiol. Chem.*, **161**, 147 (1926).

(8) The method of continuous extraction had to be used in this investigation as well as in the previous one on account of the lack of adequate filtering devices. With the proper equipment the process can be shortened considerably.

(9) The yeast was generously supplied by the Jacob Ruppert Brewery, New York.

(10) It is essential that this *p*H be maintained during the addition of the lead acetate. Failure to do this results in a final product which is not crystalline but gummy, and from which the yield of crystals is unsatisfactory.

the extract as it came from cask No. 5 was first treated with barium hydroxide and then with lead acetate. The volume of the combined extracts, showing a 90–95% recovery of the activity of the yeast, was about 90 gallons (340 liters).

2. **Treatment of the Extract with the Zeolite.**—The zeolite was made ready for use as follows: 40 lb. (18 kg.) of Decalso[11] was suspended in water and, while the suspension was being thoroughly stirred, concentrated sulfuric acid gradually added to a *p*H of 4.5. This *p*H was maintained, by further addition of sulfuric acid, for fifteen minutes. The liquid was removed and the acid treatment repeated three times. The zeolite was then thoroughly washed with water, placed in a tower[12] to a depth of 12–15 inches (30.5–38 cm.), and preheated to 75° by allowing hot water to pass through the tower. Forty-five gallons (171 liters) of the extract (*p*H 4.5) was heated to 75° and allowed to percolate through the zeolite layer at a rate of 1 liter per minute. The zeolite was then washed with four 10-gallon (38-liter) portions of hot water. This treatment was found to remove practically all the vitamin from the extract.

3. **Removal of the Vitamin from the Zeolite.**—Nine gallons (34 liters) of a molar ammonium nitrate solution was heated to 75° and allowed to pass through the charged zeolite at a rate of 500 cc. per minute. This was followed by 5 gallons (19 liters) of hot water so as to wash out any liquid remaining in the zeolite layer.

The remaining 45 gallons (171 liters) of extract was treated in the same manner. The combined effluent liquids were cooled, neutralized with 6 *N* sodium hydroxide, and the resultant precipitate removed.

4. **Preparation of the Silver Salt of the Vitamin.**—The water clear solution obtained in the previous step was adjusted to a *p*H of approximately 3.0 with 6 *N* nitric acid. Freshly prepared alkali-free silver oxide was then added slowly with stirring to a *p*H of 4.0. The resultant precipitate was immediately filtered off and the filtrate treated with silver oxide and concentrated ammonium hydroxide to a *p*H of 7.0. A small amount of silver nitrate solution was added at this point to ensure complete precipitation. The mixture was allowed to stand until the next day, when most of the supernatant liquid was decanted and the silver salt collected by centrifuging. It was then washed with water, triturated with a minimum amount of concentrated hydrochloric acid, and finally treated with water in excess to precipitate the silver chloride, which was removed. The resultant clear liquid was neutralized with 6 *N* sodium hydroxide and the precipitate removed by centrifuging.

5. **Preparation of the Silicotungstate of the Vitamin.**—The clear, colorless liquid obtained from step 4 was immediately acidified with dilute sulfuric acid to a *p*H of 4.0 and treated with a 10% solution of silicotungstic acid, prepared according to Scroggie,[13] until precipitation was complete. After standing for several hours in the cold, the precipitate was collected, washed[14] three times and suspended in about 100 cc. of distilled water. A 6 *N* solution of sodium hydroxide was added slowly, with vigorous stirring, to a *p*H of 7–7.5. Insoluble matter was centrifuged off and the centrifugate adjusted to *p*H 4.0 with dilute sulfuric acid. After addition of a few drops of silicotungstic acid solution, the mixture was allowed to stand in the cold for several hours. The precipitate was collected by centrifuging and washed three times. It was decomposed with solid barium hydroxide[15] in the presence of crushed ice,[16] the amount of baryta used being about equal to the amount of the precipitate. The mixture was stirred vigorously for three minutes, centrifuged, and filtered into 3–5 cc. of 6 *N* sulfuric acid. The residue was then repeatedly stirred with more ice and barium hydroxide if necessary until the filtrates were practically colorless. The combined filtrates[17] were adjusted to *p*H 7.5–8.0 with saturated baryta solution and immediately acidified with a few drops of concentrated hydrochloric acid to a *p*H of 5.0. After warming on the steam-bath, the mixture was centrifuged. The clear, pale yellow centrifugate (A) was set aside momentarily, while the precipitate was added to the silicotungstate residue and reworked with baryta. The final liquid thus obtained was combined with the previous centrifugate (A).

6. **Crystallization of the Vitamin.**—The liquid (*p*H 5.0) obtained in the previous step was concentrated *in vacuo* at 40° to a small volume. Twenty-five cc. of warm 95% alcohol was added to the residue and, after cooling, the insoluble matter removed. The filtrate was then taken to dryness in a vacuum desiccator over sulfuric acid. A slight brown coloration in the semicrystalline material was removed almost completely by absolute alcohol. The purification of the residue so obtained (Residue A) will be described below. From the absolute alcohol solution we recovered a small amount of the crystalline vitamin B_1 hydrochloride in the following manner. The gummy substance obtained after removal of the absolute alcohol by evaporation was dissolved in 0.2 cc. of hot water and 10 cc. of absolute ethyl alcohol. After cooling, 6 cc. of petroleum ether was added and the mixture shaken thoroughly. A gummy precipitate formed which adhered to the walls of the container and permitted the decantation of an almost clear liquid. This liquid, after standing for forty-eight hours in the cold, deposited crystals which were collected and recrystallized from 95% alcohol (Precipitate I).

The main yield of the vitamin was obtained as follows: to the semicrystalline residue obtained after treatment of the crude product with absolute alcohol (Residue A) a drop of concentrated hydrochloric acid and 4 cc. of distilled water were added. The mixture was warmed on the steam-bath and treated with 8 cc. of hot 95% alcohol. After cooling and removing the insoluble matter, dioxane was added to the clear solution until the first permanent opalescence was obtained. On standing overnight in the cold, clusters of long needles were deposited. They were collected, dissolved in a minimum amount of water and treated with a volume of 95% alcohol which was slightly larger than the volume of water used. Again dioxane

(11) The Decalso was generously supplied by The Permutit Company, New York.

(12) The tower has been described by Cerecedo and Hennessy.[3]

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

(14) The wash liquid was a normal solution of sulfuric acid containing a few drops of silicotungstic acid.

(15) Recrystallized twice from water.

(16) A quantity of ice was used which on melting yielded about 15 cc. of water.

(17) The combined filtrates were kept just on the acid side by adding more sulfuric acid when the *p*H reached 6.5.

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