Ethylcreatinine Sulfate.—Several residues left after crystallization of the creatinine ethyl sulfate were collected and after standing several days a crystalline mass appeared. This was recrystallized from absolute alcohol and proved to be ethylcreatinine sulfate, m. p. 168°. It was converted to the picrate, m. p. 96°, hydrochloride, m. p. 264°, aurichloride, m. p. 153°, chloroplatinate, m. p. 190–217°, dec., corresponding to data given in the literature.

Anal. Calcd. for $C_6H_{12}ON_3 \cdot H_2SO_4$: N, 17.57; SO_4, 39.98. Found: N, 16.68, 17.00; SO_4, 40.41.

N^2-Methyl-5-benzalcreatinine.—Eight grams of methyl creatinine sulfate was dissolved in the least amount of water. Five grams of sodium carbonate was added and the mixture evaporated to dryness on the water-bath. The methylcreatinine was taken up in hot absolute alcohol and the solution evaporated to a sirup. Five cc. of redistilled benzaldehyde was added and the mixture heated in an oil-bath at 140–150° for three hours. The resulting product was treated with dilute hydrochloric acid, which caused the gummy mass to become hard. It was then washed with ether and suspended in sodium bicarbonate solution to destroy any acidity and filtered immediately, washed with cold water, and recrystallized from hot water. Pale flat needle-shaped yellow crystals were formed. These were filtered, washed with cold water and dried in a vacuum desiccator, m. p. 126°; mixed melting point with N^2-methyl-5-benzalcreatinine prepared by methylating benzalcreatinine, 126°.

N^2-Methyl-5-furfuralcreatinine.—Three grams of furfuralcreatinine was added to 15 cc. of methyl sulfate on the steam-bath and allowed to remain for one hour. The product was washed with ether, recrystallized from hot water and air dried, m. p. 132°; mixed melting point with N^2-methyl-5-furfuralcreatinine prepared by condensing furfural with methylcreatinine, 132°.

Summary

1. Creatinine has been methylated in good yield by using methyl sulfate. The use of ethyl sulfate gave poor results in the ethylation of creatinine, creatinine ethyl sulfate being the main product.

2. Further proof is presented for the structure of methylcreatinine.

3. Methylcreatinine sulfate, ethylcreatinine sulfate and creatinine ethyl sulfate have been described for the first time.

NIAGARA FALLS, N. Y. RECEIVED JUNE 21, 1937

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, FORDHAM UNIVERSITY]

The Use of Synthetic Zeolites in the Isolation of Vitamin B_1. I. Experiments with Rice Polishings[1,2]

BY LEOPOLD R. CERECEDO AND DOUGLAS J. HENNESSY

Organic bases of moderate strength have been shown by Whitehorn[3] to be removed from solution when passed through a bed of permutit, the process being that of base exchange. Whitehorn further showed that the reaction could be reversed by passing a solution of a salt such as potassium chloride through the permutit layer, whereupon the organic base is removed and is found in the filtrate.

As far as we are aware only naturally occurring silicates, such as acid clays,[4] and fuller's earth,[5] have been used heretofore in the isolation of vitamin B_1. The findings of Whitehorn suggested the idea of using permutit or other zeolites of a similar composition. Certain advantages in using synthetic. zeolites offered themselves, the most important being the use of neutral salts for the recovery of the vitamin from them, provided the base exchange principle were found to be applicable. Other factors were: the known composition of the material, the means of controlling the size of the particles, the possibility of modifying the composition of the zeolites if necessary, and the possibility of regenerating the material following its use.

A systematic investigation was carried out to determine the best conditions for the removal of the vitamin from extracts by means of the zeolites and the most suitable way of recovering the substance from the zeolites. Once these were established, it was found that a single silver precipitation, followed by a precipitation with silicotungstic acid, yielded highly potent concentrates, from which on recrystallization crystals of pure vitamin hydrochloride could be obtained.

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

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

(3) Whitehorn, *J. Biol. Chem.*, **56**, 751 (1923).

(4) Jansen and Donath, *Mededeel. Dienst Volksgezondheit Nederland Indië*, pt. 1, 186 (1926); Ohdake, *Proc. Imp. Acad. Tokyo*, **7**, 102 (1931); Van Veen, *Rec. trav. chim.*, **50**, 610 (1931).

(5) Windaus, Tschesche, Ruhkopf, Laquer and Schultz, *Z. physiol. Chem.*, **204**, 123 (1932); Seidell and Smith, THIS JOURNAL, **55**, 3380 (1933); Williams, Waterman and Keresztesy, *ibid.*, **56**, 1187 (1934).

Experimental Part

1. Extraction.—Thirty kilograms of rice polishings are placed in each of five wooden casks of 52-gallon (200-liter) capacity. As a preliminary to the extraction proper, each batch of the material is mixed thoroughly with 22 gallons (84 liters) of 15% alcohol to which enough sulfuric acid is added to give a *p*H of 4–4.5. The contents of each cask are extracted four times with 44 gallons of 15% alcohol at *p*H 4–4.5. In the first cask the 44 gallons are made up of the 22 gallons used to wet down the polishings and 22 gallons of fresh extracting liquid. In all the other casks the 44 gallons are made up of the 22 gallons already present and the 22 gallons which are decanted from the preceding casks after standing overnight. Efficient extraction is ensured by intermittent stirring. The *p*H is kept at 4–4.5 throughout the extraction process.

As each 22-gallon portion of the extract is decanted from cask No. 5, it is brought to *p*H 7–7.5 by stirring in a hot saturated solution of barium hydroxide. To the mixture 1.8 liters of 95% alcohol is added to prevent fermentation. The following day the supernatant liquid is decanted and adjusted to *p*H 4.5–5.0. Each of the three succeeding portions of extract, decanted from cask No. 5, is combined with the residue from the previous barium hydroxide treatment and treated with barium hydroxide, etc. The combined extracts, which show a 80–85% recovery of the activity of the rice polishings, total 70–75 gallons, and are ready for the next step.

2. Treatment of the Extract with the Zeolite.—Preliminary experiments showed that various zeolites may be used for removing the vitamin from solution. Recently we have used Decalso[6] exclusively. The zeolite was made ready for use by stirring it several times with water and then with sulfuric acid at *p*H 4.0. The removal of the vitamin from the extract was carried out in a tower made of lead clad metal, which was 60 inches (1.5 meters) high and 11 inches (28 cm.) in diameter. The tower contained a perforated bottom, over which a cloth was placed. It was filled to a height of 18 inches (46 cm.) with the acid-washed zeolite, the ratio in weight of zeolite to rice polish being 1:10. The zeolite was pre-heated to 75° by allowing hot water to pass through the tower. The heating was done by means of a steam coil in the upper part of the tower. The extract (*p*H 4.5) was also heated to 75° and run through the zeolite layer at a rate of 1 liter per minute, the outflow being regulated by an outlet valve at the bottom of the tower. When all the extract had passed through, the zeolite was washed with four 10-gallon (38-liter) portions of hot water.

3. Removal of the Vitamin from the Zeolite.—Twenty gallons (76 liters) of a molar ammonium nitrate solution was heated to 75° and passed 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 resulting filtrate was cooled and then adjusted to *p*H 7.5 with ammonium hydroxide solution.

4. Precipitation of the Silver Salt of the Vitamin.—One liter of 10% silver nitrate solution was added to the liquid resulting from step 3, keeping the *p*H at 7.5. After standing overnight, the precipitate was collected by centrifuging, then suspended in 1 liter of water and, keeping the *p*H at 3.0 with nitric acid, heated at 50°, with stirring, for fifteen minutes. This treatment dissolved part of the precipitate. After cooling, the mixture was brought to *p*H 5.0 with ammonium hydroxide. The resulting precipitate was discarded. The filtrate, after addition of a small amount of silver nitrate solution, was brought to *p*H 7.0 with ammonium hydroxide. The precipitate, formed after standing overnight in the cold, was collected, washed with a small quantity of water and decomposed with hydrochloric acid. After removal of the silver chloride, the filtrate was neutralized with sodium hydroxide and the ensuing precipitate discarded.

5. Precipitation of the Silicotungstate of the Vitamin.—The use of silicotungstic acid for the precipitation of the vitamin has been recommended by Jansen.[7] In our experiments we used a sample of silicotungstic acid prepared according to Scroggie.[8] The solution resulting from step 4 was diluted to 500 cc., enough sulfuric acid added to make it 0.6 *N*, and precipitated with a 10% solution of silicotungstic acid. The precipitate was collected and washed with 100 cc. of 0.6 *N* sulfuric acid containing a few drops of silicotungstic acid solution. It was then mixed with 20 cc. of a cold suspension of twice recrystallized barium hydroxide. The mixture was stirred well for three minutes and centrifuged immediately. The supernatant liquid was decanted and acidified at once with sulfuric acid. The precipitate resulting from the barium hydroxide treatment was subjected to two further treatments with barium hydroxide in the manner above described. The filtrates from the second and third treatments were added to the filtrate of the first treatment and the *p*H adjusted to 8.0 with barium hydroxide and sulfuric acid. Three cc. of concentrated hydrochloric acid was added, and the resultant precipitate was removed by centrifuging.

6. Crystallization of the Vitamin.—The filtrate was evaporated to dryness *in vacuo* at 40° and the residue extracted three times with 8 cc. of boiling 95% alcohol. The combined liquids were cooled and centrifuged. Evaporation of the filtrate to a small volume yielded on standing a crystalline precipitate. After removal of the mother liquor, this was washed twice with 2 cc. of boiling absolute alcohol. The residue was taken up in 2 cc. of water and 5 cc. of 95% alcohol. To the solution, dioxane was added until, on thorough mixing, a faint cloudiness persisted. On standing in the cold, colorless crystals were obtained, which were collected and rinsed with cold absolute alcohol. The melting point of the crystals varied with the rate of heating. The usual value obtained is 255° (corr.).

In accordance with the observations of previous workers, we find that the yields of the vitamin hydrochloride vary. They depend to a great extent on the quality of rice polishings. From 100 kg. of polish we have obtained between 240 and 260 mg. of crystalline material melting at 246°. The yield of the pure substance (m. p. 255°, corr.) is between 130 and 140 mg.

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, 42.26, 42.39; H, 5.13, 5.45; N, 16.49; S, 9.46; Cl, 20.84; ash, 0.7.

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

(7) Jansen, *Rec. trav. chim.*, **48**, 984 (1929).

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

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.

NEW YORK, N. Y. RECEIVED MAY 21, 1937

[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.