liquid at −22°. The melting points of the unrecrystallized specimens of the original debromination acid have ranged in this Laboratory from −12.8 to −14.5°. McCutcheon has recently reported a value of −16.25 to −17°. We are convinced that these variations in melting points are due to the presence of varying amounts of the contaminating isomer.

Discussion

In the preceding work we have shown that debromination linoleic and linolenic acids, prepared by reduction of the corresponding bromo-acids in neutral methyl alcohol, contain about 12 and 15%, respectively, of isomeric acids which give nearly theoretical iodine numbers for C_{18} two and three bond acids but lower melting points and much lower tetrabromide and hexabromide numbers than the original debromination acids. It does not seem likely that the isomeric acids have conjugated bonds. Attempts to further isolate the contaminating isomer in the case of linolenic acid have been so far unsuccessful, because the differences in solubility are too small. It seems logical to conclude that the contaminating isomers are of the *cis–trans* type, which yield no tetrabromides or hexabromides insoluble in petroleum ether or ether, respectively. The origin of these isomeric acids is not certain. The high melting points of our bromides make it improbable that they contain appreciable amounts of isomeric bromides, which might give rise to other acids. We believe that the contaminating isomeric acids arise from isomerization during the debromination procedure. It has been our experience in this Laboratory that linoleic and linolenic acids, prepared in a neutral alcoholic reduction medium, such as we have employed above, do not differ from those prepared in a strongly acid medium in such properties as melting point, iodine number, and yield of insoluble bromides. Therefore, it seems likely though not certain that preparations of debromination acids, previously reported in the literature, have been more or less contaminated with these isomeric acids and that the final crystal fractions described above are the purest acids so far prepared. The crystallization procedure is the only method at present available for removing these isomeric acids.

Summary

Linoleic and linolenic acids, prepared by the debromination procedure, have been shown by repeated low temperature crystallizations to contain 12 to 15%, respectively, of isomeric acids of low melting points and low or zero tetra- and hexabromide numbers. The constants of the highly purified acids are described. The origin of the isomeric acids is discussed.

COLUMBUS, OHIO RECEIVED DECEMBER 7, 1940

[CONTRIBUTION FROM THE ORGANIC RESEARCH LABORATORY, NATIONAL OIL PRODUCTS CO.]

An Amino Analog of Vitamin B_1

BY DONALD PRICE AND FRANK D. PICKEL

Bergel and Todd[1] synthesized a number of analogs of vitamin B_1, none of which showed any measurable vitamin activity in animal tests. They, moreover, stated that any significant alteration in the structure of the vitamin would cause almost complete loss of activity, and their statement has been largely borne out by later work.

Finkelstein and Elderfield[2] reported two pyridine analogs of the vitamin which were inactive toward polyneuritic rats at levels of 100 γ per rat. Schmelkes[3] announced the preparation of 2-methyl-3-β-hydroxyethyl-N-((2-methyl-6-amino-pyrimidyl-(5))-methyl)-pyridinium bromide hydrobromide, the exact pyridine analog of vitamin B_1 and stated that it showed activity. He later published[4] his synthesis of this substance but gave no further data concerning its activity. The same substance prepared by a different synthesis was also reported by Baumgarten and Dornow,[5] who stated that a 26-fold quantity was required for activity equal to that of vitamin B_1. In a later paper the same authors[6] showed that their previously published structure as well as that of Schmelkes was in error due to the Clemmensen reduction having taken an unforeseen course, in each case the hydroxyl group in the pyridine side-

(1) F. Bergel and A. R. Todd, *J. Chem. Soc.*, 1504 (1937).

(2) J. Finkelstein and R. C. Elderfield, *J. Org. Chem.*, **4**, 365 (1939).

(3) F. C. Schmelkes, *Science*, **90**, 113 (1939).

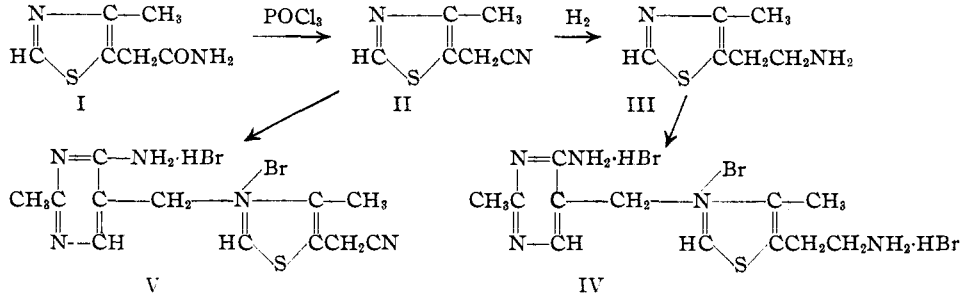
(4) F. C. Schmelkes and R. R. Joiner, THIS JOURNAL, **61**, 2562 (1939).

(5) P. Baumgarten and A. Dornow, *Ber.*, **73**, 44 (1940).

(6) *Ibid.*, **73**, 353 (1940).

chain occupying the α rather than the β position. More recently, however, Tracy and Elderfield[7] have announced the synthesis of the true pyridine analog of vitamin B_1, but have not yet reported upon its biological activity.

There is some evidence in the literature[8] to support the possibility that amines may be directly converted to the corresponding hydroxy compounds in the animal body. In view of this it was felt that the preparation and biological assay of an analog of vitamin B_1 in which the hydroxy group in the side-chain of the thiazole nucleus was replaced by an amino group would not be without interest. The synthesis was carried out as indicated herewith.



4-Methylthiazole-5-acetamide (I) was prepared by a modification of the method of Cerecedo and Tolpin[9] by treatment of the corresponding ethyl ester with ammonia. Dehydration with phosphorus oxychloride gave 4-methyl-5-cyanomethylthiazole (II) smoothly and in good yield. The latter was reduced to 4-methyl-5-β-aminoethylthiazole (III)[10] catalytically in the presence of either Raney nickel or palladium. The readiness with which this reduction proceeded is somewhat surprising in view of the well-known tendency of sulfur compounds to poison hydrogenation catalysts.

The thiazole amine was then condensed with 2-methyl-5-bromo-methyl-6-amino pyrimidine to give the amino analog of vitamin B_1 (IV). Proof that the condensation product actually has the structure (IV), and that the condensation did not take place through the amino group of the thiazole side-chain is furnished by the fact that it gives a positive thiochrome test.[11] This is hardly reconcilable with a structure resulting from condensation through the β-amino group of the thiazole. Furthermore, the compound gives a positive reaction with diazotized sulfanilic acid.[12] As has been pointed out by Buchman[13] this color test, which is given by the vitamin itself is a property of quaternary salts of the hydroxythiazole. It also gives a positive test with diazotized *p*-aminoacetophenone.[14]

The vitamin analog (IV) was assayed according to the official U. S. P. Curative method[15] for one-tenth the vitamin content of the U. S. P. crystalline B_1 reference standard and was found to be inactive at this level.

4-Methyl-5-cyanomethylthiazole (II) also condenses quite readily with 2-methyl-5-bromomethyl-6-aminopyrimidine to yield a cyano analog of vitamin B_1 (V).

Experimental

4-Methylthiazole-5-acetamide (I).[16]—To 108 g. of crude ethyl 4-methylthiazole-5-acetate hydrobromide was added 75 cc. of concentrated ammonium hydroxide and the liberated base immediately separated from the aqueous layer. The latter was extracted with two 25-cc. portions of ether and after removal of the ether the residue was added to the ester-base above. The whole was then stirred with concentrated ammonium hydroxide until a clear, homogeneous solution was obtained (seventy-five to ninety minutes). On standing for about three hours 33 g. of quite pure amide (m. p. 134.5–135.5°) separated and was filtered off. Sixteen grams more was obtained by evaporation of the filtrate to dryness and crystallization from dioxane. The crystallized material melted at 136°, the melting point reported by Cerecedo and Tolpin.[9]

4-Methyl-5-cyanomethylthiazole. (II).—A mixture of 8.8 g. of 4-methylthiazole-5-acetamide with 50 cc. of phos-

(7) Ann H. Tracy and R. C. Elderfield, *Science*, **92**, 180 (1940).

(8) Guggenheim and Löffler, *Biochem. Z.*, **72**, 325 (1916); Ewins and Laidlaw, *J. Physiol.*, **41**, 78 (1910); P. Mayer, *Z. physiol. Chem.*, **42**, 59 (1904); *cf.* also S. Frankel, "Die Arzneimittel-Synthese," Berlin, 1919, p. 174.

(9) L. R. Cerecedo and J. G. Tolpin, THIS JOURNAL, **59**, 1661 (1937).

(10) This compound is described in British Patent 456,751 where it is prepared by a Hoffmann or Curtius degradation of 4-methyl-5-thiazolyl propionamide. *Cf.* also C. R. Harington and R. C. G. Moggridge, *J. Chem. Soc.*, 446 (1939).

(11) G. Barger, F. Berger and A. R. Todd, *Nature*, **136**, 259 (1935); *Ber.*, **68**, 2375 (1935).

(12) Johnson and Clapp, *J. Biol. Chem.*, **5**, 163 (1908); *cf.* also Pauly, *Z. physiol. Chem.*, **42**, 516 (1904).

(13) E. R. Buchman, THIS JOURNAL, **58**, 1803 (1936).

(14) D. Melnick and H. Field, Jr., *J. Biol. Chem.*, **127**, 505 (1939).

(15) U. S. Pharmacopoeia XI, 1939 Supplement, p. 129.

(16) The authors are indebted to Dr. Everette L. May of this Laboratory for this improved procedure as well as for most of the microanalyses reported in the present paper.

phorus oxychloride was heated at 115–120° for half an hour. The excess reagent was then removed *in vacuo* at 40° and the viscous red residue poured into ice water and sodium carbonate added until the solution was distinctly alkaline, whereupon the nitrile separated as an oil. The mixture was extracted with ether, the ether extract dried over anhydrous sodium sulfate, and after removal of the solvent the residual oil (6.3 g.) distilled under reduced pressure. The fraction distilling at 92–93° (2 mm.) was collected; yield about 81% of the theoretical.

Anal. Calcd. for $C_6H_6N_2S$: C, 52.14; H, 4.38; N, 20.29. Found: C, 51.81; H, 4.38; N, 20.05.

In alcoholic solution 4-methyl-5-cyanomethylthiazole forms a picrate which melts at 171°.

Anal. Calcd. for $C_{12}H_9O_7N_5S$: N, 19.07. Found: N, 18.92.

4-Methyl-5-β-aminoethylthiazole (III).[10]—A solution of 5.9 g. of 4-methyl-5-cyanomethylthiazole in 150 cc. of absolute alcohol was added to the nickel obtained from 23 g. of Raney nickel alloy by the usual digestion and drying procedure. The reduction was then carried out under three atmospheres of hydrogen, the reaction being stopped at the end of one and one-half hours, and the alcohol solution decanted from the catalyst. After removal of the alcohol *in vacuo* the residue was distilled and the fraction boiling at 82–85° (2 mm.) collected. The weight of the distillate was 2 g. or about 33% yield. The compound forms a picrate of m. p. 227°.

Anal. (picrate). Calcd. for $C_{18}H_{16}O_{14}N_8S$: C, 36.00; H, 2.69; N, 18.68. Found: C, 36.15; H, 2.95; N, 18.54.

The reduction can also be carried out in the presence of palladium on zirconium oxide if the nitrile is dissolved in glacial acetic acid containing a little hydrochloric acid.

4 - Methyl - 5 - β - aminoethyl - N - ((2 - methyl - 6 amino)-5-pyrimidyl-methyl)-thiazolium Bromide Dihydrobromide (IV).—To a solution of 1.4 g. of 4-methyl-5-β-aminoethylthiazole in 4 cc. of *n*-butanol, 3.6 g. of 2-methyl-5-bromomethyl-6-aminopyrimidine di-hydrobromide was added and the mixture heated at 120–125° for half an hour. After filtration the residue was extracted with two 5-cc. portions of absolute ethyl alcohol, after which it was dissolved in aqueous ethanol and reprecipitated with dioxane. The bromide-dihydrobromide appears as rosets of very fine needles; m. p. 250–251°. The compound forms a picrate m. p. 204–206° (from water) and gives a positive thiochrome test[11] as well as a positive Pauly reaction[12]; yield 28% of the theoretical.

Anal. Calcd. for $C_{12}H_{20}N_5SBr_3$: C, 28.46; H, 3.98; N, 13.84. Found: C, 28.37; H, 4.03; N, 13.95.[17]

4 - Methyl - 5 - cyanomethyl - N - ((2 - methyl - 6 - amino)-5-pyrimidyl-methyl)-thiazolium Bromide Hydrobromide (V).—One hundred fifty milligrams of 4-methyl-5-cyanomethylthiazole was dissolved in 0.2 cc. of butanol and 150 mg. of 2-methyl-5-bromomethyl-6-aminopyrimidine di-hydrobromide added and the mixture heated to 120° for fifteen minutes. Rosets of white needles appeared as the pyrimidine dissolved; 2 cc. of absolute alcohol was then added and the solution filtered. The reaction product was then purified by dissolving it in 95% ethyl alcohol and reprecipitating with dioxane, m. p. 231–232°. It forms a picrate which melts at 199–200° and it gives a positive Pauly reaction[12] but fails to give the thiochrome test. This is probably due to rapid hydrolysis of the cyano group in the thiazole side-chain, whereby extraction of the thiochrome derivative from the alkaline solution by the butanol is prevented, thus giving an apparently negative test.

Anal. Calcd. for $C_{12}H_{15}N_5SBr_2 \cdot H_2O$: C, 32.83; H, 3.91; N, 15.95. Found: C, 32.91; H, 4.16; N, 16.02.

Summary

Two analogs of vitamin B_1, one having a cyano and the other an amino group in the side chain of the thiazole nucleus have been prepared. The latter was assayed and found to be inactive.

HARRISON, NEW JERSEY RECEIVED JANUARY 3, 1941

(17) The nitrogen determinations on this and the succeeding compound were carried out by the micro-Kjeldahl method, using red phosphorus and hydriodic acid, described in Pregl–Roth, "Die quantitative organische Mikroanalyse," Berlin, 1935, p. 111. In agreement with the experience of Wintersteiner, Williams and Ruehle (THIS JOURNAL, **57**, 517 (1935)), both the ordinary Pregl micro-Kjeldahl and the Dumas methods appeared to give quite low results.