was considered to be arachidic acid. The composition of the saturated acids is given in Table III.

Summary

A study has been made of the composition of the glycerides of watermelon seed oil (Cuban Queen Variety). The fatty acids consist of 8.84% palmitic acid, 5.61% of stearic acid, 0.72% of arachidic acid, 13.03% of oleic acid and 68.38% of linoleic acid. The unsaponifiable matter amounts to 1.19%.

The presence of arachidic acid in watermelon seed oil has not been mentioned by previous investigators.

WINTER HAVEN, FLA. RECEIVED FEBRUARY 6, 1939

[CONTRIBUTION NO. 691 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

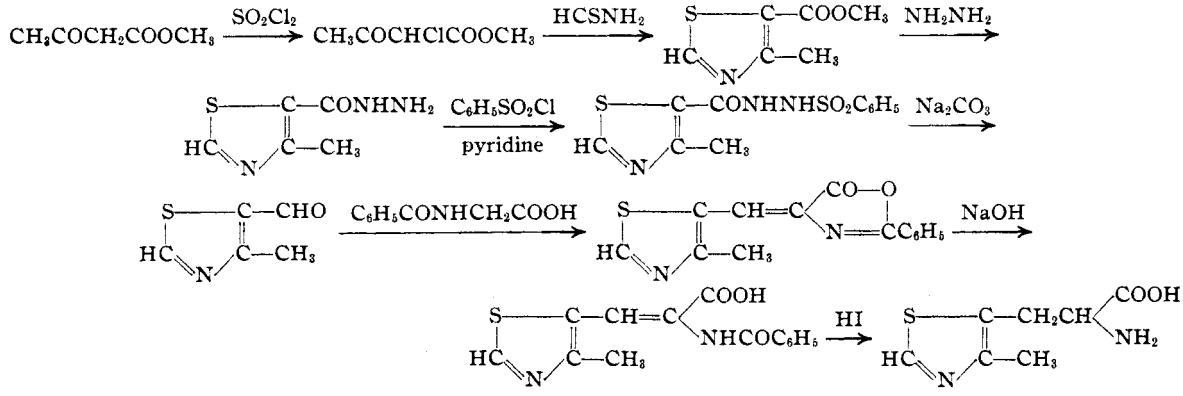
Thiamin Analogs. I.[1] β-(4-Methylthiazolyl-5)-alanine[2]

BY EDWIN R. BUCHMAN AND EDWIN M. RICHARDSON

The thiamin (vitamin B_1) molecule comprises two parts which may be referred to as the pyrimidine half and the thiazole half, the vitamin itself being a quaternary salt of its thiazole half. Not only may the synthesis of the vitamin be accomplished *in vitro*[3] by a coupling of the two halves but also the same transformation may be effected enzymatically[4] in certain organisms, so that it is reasonable to assume that this reaction represents the last step in the natural biogenesis of the substance.

The thiazole half, with its $-CH_2CH_2OH$ group, bears a certain resemblance to β-phenylethyl alcohol, tyrosol, and tryptophol, substances which may be derived from the amino acids, phenylalanine, tyrosine, and tryptophan by the action of fermenting yeast[5] and whose β-hydroxyethyl side chain results from the degradation of the alanine grouping. We have seen in this similarity a clue to a possible precursor of the vitamin thiazole and accordingly have synthesized the appropriate amino acid, β-(4-methylthiazolyl-5)-alanine, in order to investigate its biological significance.[6]

The synthesis was accomplished in a straightforward manner as indicated below



The transformation of the thiazole ester to the aldehyde represents an extension to the thiazole series of the recently published[7] method of Stevens for converting aromatic acids to the corresponding aldehydes. The over-all yield of amino acid from acetoacetic ester is about 6% or a yield of 9 g. from 100 g. of the starting material.

The evidence accumulated to date does not permit definite conclusions regarding the biological role of the amino acid. It is of interest that

(1) Paper 18 in the R. R. Williams series.

(2) Presented before the Pacific Division of the American Association for the Advancement of Science at the San Diego meeting, June, 1938.

(3) R. R. Williams and J. K. Cline, THIS JOURNAL, **58**, 1504 (1936); J. K. Cline, R. R. Williams and J. Finkelstein, *ibid.*, **59**, 1052 (1937).

(4) J. Bonner and E. R. Buchman, (a) *Proc. Natl. Acad. Sci.*, **24**, 431 (1938); (b) *ibid.*, in press.

(5) F. Ehrlich, *Ber.*, **40**, 1047 (1907); **44**, 139 (1911); **45**, 883 (1912).

(6) Professor C. R. Harington in a communication to one of us (E. R. B.) has disclosed that, independently motivated by considerations similar to those which have influenced us, he also has synthesized this amino acid and investigated some of its biological properties. We have been privileged to view Professor Harington's manuscript prior to publication and take this opportunity again to thank him for the extended courtesy.

(7) J. S. McFayden and T. S. Stevens, *J. Chem. Soc.*, 584 (1936).

pea roots[4a] are able to convert the substance to the vitamin thiazole, whereas *Phycomyces Blakesleeanus*[8] and *Staphylococcus aureus*[9] do not bring about this transformation to an appreciable extent. While such findings are consistent with the view[10] that we are dealing with a progenitor of the vitamin, the final proof rests on its actual isolation from natural sources.[11]

Experimental

4-Methyl-5-carbomethoxythiazole.—286 grams (89% yield) of methyl α-chloroacetoacetate, b. p. 83–86° at 21 mm. was obtained[12] by the action of 270 g. of sulfuryl chloride on 232 g. of methyl acetoacetate (Eastman practical); 300 g. of crude thioformamide was added[13] with shaking to a solution, kept at 0°, of 384 g. of chloro ester, prepared as above, in 50 cc. of alcohol. Cooling was continued intermittently during the next hour to avoid a rise in temperature above 25° and the mixture was allowed to stand for three days at room temperature. Dilute hydrochloric acid was then added to the reaction product and non-basic material removed by washing with ether. The aqueous phase was cautiously made alkaline with potassium carbonate and the thiazole ester taken up in chloroform and distilled. The yield was 236 g. (58% of the theoretical); b. p. 115–116° at 23 mm.; m. p. (recrystallized from alcohol) 76°.[13,14]

4-Methylthiazole-5-aldehyde.—157 grams of the thiazole ester was refluxed from a water-bath with 250 g. of hydrazine hydrate (42% solution) and 200 cc. of alcohol for two and one-half hours. The hydrazide formed was allowed to crystallize at room temperature, was filtered off and washed with 50% alcohol. The product (113 g.) had m. p. 166° which was unchanged on subsequent recrystallization from alcohol. The mother liquors gave an additional 12.5 g. of hydrazide bringing the total yield to 80%.

Anal. Calcd. for $C_5H_7N_3SO$: C, 38.25; H, 4.49; N, 26.75. Found: C, 38.07; H, 4.61; N, 26.71.

To a suspension of 126 g. of the hydrazide in 2300 cc. of pyridine (good commercial grade) was added with stirring 200 g. of benzenesulfonchloride over a period of one hour keeping the temperature at about 20° by means of a cooling bath. The resulting clear, slightly colored solution was stirred for an additional three hours and the pyridine distilled off directly from a water-bath under water pump vacuum. The residue was poured into water, the precipitate filtered, and crystallized from alcohol, giving 195 g. of product, m. p. 165–167° (81% yield). After a second recrystallization from alcohol the benzenesulfonacylhydrazide melted constantly at 170° (mixed m. p. with hydrazide, below 145°).

Anal. Calcd. for $C_{11}H_{11}N_3S_2O_3$: C, 44.4; H, 3.73; N, 14.13. Found: C, 44.81; H, 3.41; N, 14.00.

Fifty grams of the sulfonhydrazide in 250 g. of ethylene glycol (Eastman practical) was treated at 160° bath temperature with 44 g. of anhydrous sodium carbonate. The addition of the alkali was made in one portion and resulted in a brisk evolution of gas (care must be taken that the reaction vessel is large enough to accommodate the foam produced). After two to three minutes, when the effervescence had subsided, the mixture was removed from the bath, hot water was added and the solution cooled and saturated with potassium carbonate at room temperature. Several such batches as above were combined and the thiazole aldehyde was recovered by repeated extraction with chloroform and subsequent distillation. A 40% yield of crystalline product, b. p. 112–118° at 21 mm., was obtained, which was contaminated only by a small amount of ethylene glycol and could be used directly for the next step in the synthesis. For analysis a portion was recrystallized from isopropyl alcohol, prisms, m. p. 75°.

Anal. Calcd. for C_5H_5NSO: C, 47.25; H, 3.97; N, 11.02. Found: C, 47.17; H, 4.13; N, 10.85.

The aldehyde is soluble in water and the usual organic solvents, very slightly soluble in petroleum ether; phenylhydrazone, m. p. 158–159°; dinitrophenylhydrazone not melted at 235°; no picrate was precipitated on mixing the components in ether. The yield of pure aldehyde from the thiazole ester was 23% of the theoretical.

Azlactone of α-Benzoylamino-β-(4-methylthiazolyl-5)-acrylic Acid.—A mixture of 5 g. of the unrecrystallized thiazole aldehyde, 7.88 g. of hippuric acid, 3.25 g. of freshly fused sodium acetate and 12.5 g. of acetic anhydride was heated for a few seconds over a free flame until the mass became liquid and then for two hours in a bath at 100°. The reaction product was washed thoroughly with hot water and with a small amount of cold alcohol. Recrystallization from benzene gave 7.5 g. (70% yield) of the azlactone, fine yellow needles, m. p. 199–200°.

Anal. Calcd. for $C_{14}H_{10}N_2SO_2$: C, 62.2; H, 3.73; N, 10.37. Found: C, 62.18; H, 4.07; N, 10.43.

β-(4-Methylthiazolyl-5)-alanine.—It was found possible to convert the azlactone directly into the amino acid by treatment with red phosphorus, hydriodic acid and acetic anhydride but the yield was not as high as in the two-step transformation described below which involves a preliminary opening of the lactone ring with alkali. Three grams of the azlactone was stirred in a bath at 100° with a solution of 0.5 g. of sodium hydroxide in 250 cc. of water for forty-five minutes. After cooling, the solution was filtered, the filtrate carefully acidified with hydrochloric acid and the precipitate recrystallized from 95% alcohol; 2.8 g. (87% yield) of acid was obtained, crystals with a faint yellow tinge, m. p. 217°.

Anal. Calcd. for $C_{14}H_{12}N_2SO_3$: N, 9.72. Found: N, 9.95.

(8) J. Bonner, private communication.

(9) B. C. J. G. Knight, private communication.

(10) For an alternative theory regarding the formation of the thiazole half, see reference 4a; a relationship to the thiomethylpentose from yeast [P. A. Levene and H. Sobotka, *J. Biol. Chem.*, **65**, 551 (1925)] is also conceivable.

(11) It may be noted that with respect to sulfur lability, the new amino acid exhibits properties resembling those of sulfur constituents of proteins, which properties have been ascribed [D. Blumenthal and H. T. Clarke, *J. Biol. Chem.*, **110**, 343 (1935)] to as yet undiscovered amino acids.

(12) Compare F. Allihn, *Ber.*, **11**, 569 (1878); B. B. Dey, *J. Chem. Soc.*, **107**, 1646 (1915).

(13) Compare H. T. Clarke and S. Gurin, THIS JOURNAL, **57**, 1879 (1935).

(14) A. Windaus, R. Tschesche and R. Grewe, *Z. physiol. Chem.*, **228**, 27 (1934); E. R. Buchman, R. R. Williams and J. C. Keresztesy, THIS JOURNAL, **57**, 1849 (1935).

2.95 g. of the acrylic acid[15] was mixed with 2.25 g. of red phosphorus and 14 cc. of acetic anhydride and 14 cc. of hydriodic acid (57%) was added dropwise with shaking. The containing flask was connected to a reflux condenser and heated for three hours in a bath at 120°. After cooling, the phosphorus was filtered off and washed with acetic acid. The filtrate was evaporated to dryness, taken up in water and then extracted with ether. Concentration of the aqueous phase gave a sirup, to which excess ammonium hydroxide was added and the resulting clear solution again evaporated to dryness. The residue was extracted with hot absolute alcohol and the alcohol insoluble portion crystallized from hot water yielding 1.40 g. (70% of the calcd.) of the amino acid, stout white prisms, containing one-half molecule of water which was not lost on drying at room temperature.

Anal. Calcd. for $C_7H_{10}N_2SO_2 \cdot {}^1/_2H_2O$: C, 43.08; H, 5.69; N, 14.36. Found: C, 43.02; H, 5.76; N, 14.17.

The amino acid is readily soluble in hot water; practically insoluble in absolute alcohol. When heated in a capillary, it did not melt but decomposed with evolution of gas at about 237°; an almost colorless liquid remained in the tube. By heating an aqueous solution of the acid with cupric carbonate it was found possible to prepare the blue copper salt which crystallized from the concentrated solution and from which, on treatment with hydrogen sulfide, the original material could be regenerated. A determination[16] of the percentage of sulfur labilized[11] by plumbite, by bromine and by fuming nitric acid gave respectively the figures 51, 75 and 70.

(15) An attempt was made to accomplish the reduction of the acid using Adams platinum catalyst. Although the recrystallized thiazole did not "poison" the catalyst, as evinced by undiminished subsequent activity of the same catalyst using benzaldehyde as a test object, only unchanged starting material could be recovered.

Acknowledgment.—Grateful acknowledgment is made to Dr. R. T. Major of Merck and Co., Inc., for providing chemicals and microanalyses and to the Research Corporation for a grant of funds, defraying the costs of the investigation.

Summary

There has been described the synthesis of an amino acid which is a theoretically possible precursor of the thiazole half of vitamin B_1.

(16) We are indebted to Mr. Harold W. Strickler for these analyses.

PASADENA, CALIF. RECEIVED FEBRUARY 2, 1939

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND THE DEPARTMENT OF ZOÖLOGY OF THE UNIVERSITY OF WISCONSIN]

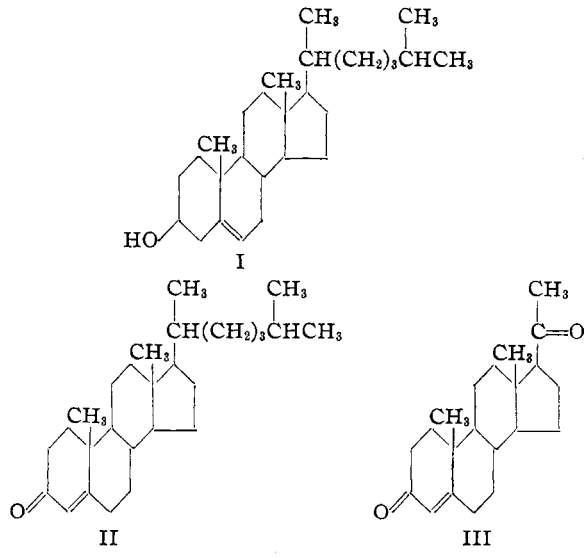
The Preparation of Progesterone from Cholesterol

BY M. A. SPIELMAN AND R. K. MEYER

Although the corpus luteum hormone, progesterone (III), is very much in demand, there is no really satisfactory way of obtaining it. Corpus luteum tissue is expensive and the extraction laborious, while stigmasterol, from which it is made artificially,[1] is virtually unavailable in America.

Cholesterol (I) is a cheap sterol and therefore a desirable starting material. However, it is obvious that a very small yield would be expected in the direct removal of most of the cholesterol side chain by oxidation, and to offset this, the preparative method would have to be both simple and inexpensive. This paper reports the details of such a method.

Two ways of preparing progesterone from cholesterol have been published, and in each, cholestenone (II) is an intermediate. Tavastsherna[2] converted cholestenone to the dibromide and oxidized it in benzene solution with aqueous sulfuric acid and potassium permanganate. The yield claimed is 40–60 mg. from 10 g. of cholestenone dibromide,[3] but several attempts made in this Laboratory have produced no hormone what-



(1) Butenandt, Westphal and Cobler, *Ber.*, **67**, 1611 (1934); Butenandt and Westphal, *ibid.*, **67**, 2085 (1934).

(2) Tavastsherna, *Arch. sci. biol.* (*U. S. S. R.*), **40**, 141 (1936); *C. A.*, **31**, 6670 (1937).

(3) The 15% yield given in the German summary and quoted in *Chemical Abstracts* is not in keeping with the Russian text.