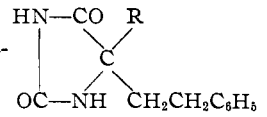
same procedure, the 5-isopropyl and 5-isobutyl-5-styrylhydantoins were hydrogenated to the corresponding 5-(2-phenylethyl) derivatives. Certain data for these hydantoins are collected in Table II.

TABLE II

5-ALKYL-5-(2-PHENYLETHYL-HYDANTOINS



R	M. p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
$-CH_2CH_3$[a]	196–198				
$-CH(CH_3)_2$	231–235	68.28	68.08	7.32	7.34
$-CH_2CH(CH_3)_2$	177–181	69.21	68.31	7.69	7.42

[a] Herbst and Johnson, ref. 8, reported m. p. 198–199°.

Summary

1 By interaction with potassium cyanide and ammonium carbonate, five-alkyl 2-anilino-2-phenylethyl ketones were converted into 5-alkyl-5-styrylhydantoins with elimination of aniline.

2. Two new 5-branched alkyl-5-phenylethylhydantoins have been prepared by catalytic hydrogenation of the corresponding styryl compounds.

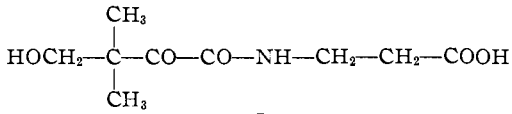
AUSTIN, TEXAS RECEIVED FEBRUARY 4, 1949

[FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

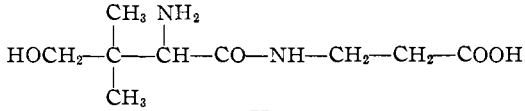
Synthesis of Compounds Related to Pantothenic Acid[1]

BY S. H. LIPTON[2] AND F. M. STRONG

Among the numerous structural analogs of pantothenic acid which have been described, only a few possess appreciable vitamin activity (hydroxypantothenic acid,[3] pantothenol[4]), while several others are metabolic inhibitors of pantothenic acid (pantoyltaurine[5,6,7] phenylpantothenone[8]). In view of known biochemical interrelationships between structurally similar hydroxy, keto and amino compounds, it seemed of interest to study the biological properties of the keto (I) and amino (II) analogs of pantothenic acid.



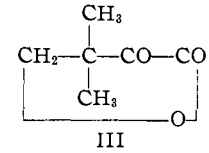
I



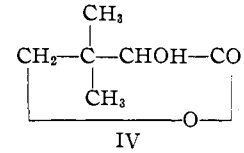
II

Efforts to prepare the keto analog (I) are described in the present paper.[9]

The proposed synthesis of the keto analog (I) involved condensation of β-alanine with the corresponding keto lactone (III), or derivatives thereof.



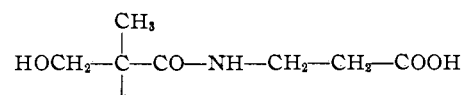
III



IV

Since the keto lactone (III) prepared from dimethylpyruvic acid and formaldehyde according to the method of Kuhn and Wieland[10] melted appreciably higher than their product and considerable difficulty was encountered in attempting to condense it with β-alanine, some doubt arose as to its identity. Therefore, it was also prepared by an alternative method, namely, the oxidation of *d,l*-pantolactone (IV) by lead tetraacetate. The two products were identical, and on reduction consumed one mole of hydrogen with the formation of *d,l*-pantolactone (IV) which yielded microbiologically active[11] *d,l*-pantothenic acid when condensed with β-alanine.

Condensation of the keto lactone (III) with β-alanine gave mixtures from which no "ketopantothenic" acid (I) could be isolated, although catalytic reduction of the crude products followed by microbiological estimation of pantothenic acid activity indicated that 13–15% of the keto analog was formed under certain conditions. However, two other nitrogenous, acidic compounds were obtained from this condensation. Analysis indicated one of these corresponded to I minus a molecule of carbon monoxide, and since in addition it had no carbonyl function, it was formulated as N-(α,α-dimethyl-β-hydroxypropionyl)-β-alanine (V).



V

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Committee.

(2) From the Ph.D. dissertation of S. H. Lipton, 1948; present address: Department of Obstetrics and Gyuecology, University of Chicago.

(3) Mitchell, Snell and Williams, THIS JOURNAL, **62**, 1791 (1940).

(4) Pfaltz, *Z. Vitaminforsch.*, **13**, 236 (1943).

(5) Snell, *J. Biol. Chem.*, **139**, 975 (1941); **141**, 121 (1941).

(6) Barnett and Robinson, *Biochem. J.*, **36**, 357, 364 (1942).

(7) Kuhn, Wieland and Moeller, *Ber.*, **74**, 1605 (1941).

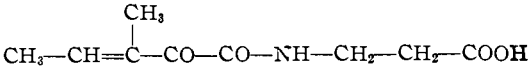
(8) Woolley and Collyer, *J. Biol. Chem.*, **159**, 263 (1945).

(9) The synthesis and biological inactivity of the amino analog (II) have very recently been reported by Folkers, *et al.*, THIS JOURNAL, **70**, 3088 (1948).

(10) Kuhn and Wieland, *Ber.*, **75**, 121 (1942).

(11) Dann and Satterfield, *Biol. Symposia*, **12**, 273 (1947).

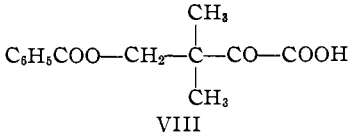
The second product (VI) readily formed carbonyl derivatives but alcohol derivatives could not be obtained. The composition of VI and its derivatives corresponded to I minus a molecule of water. The β-hydroxy ketone structure of I would undoubtedly favor ready dehydration, but since I is also a neopentyl alcohol, dehydration would be accompanied by rearrangement, probably to the unsaturated β-alanide (VII).



VII

However, the product (VI) gave negative tests for unsaturation and on hydrogenation consumed only one mole of hydrogen. The substance was recovered unchanged after boiling in 9*N* hydrochloric acid overnight, but was cleaved by alcoholic alkali to yield β-alanine and an unidentified, sirupy, ether-soluble product. The structure of VI, therefore, is still in doubt. Neither V or VI shows any appreciable pantothenic acid activity toward *L. arabinosus*,[11] nor did they act as metabolic antagonists of pantothenic acid.

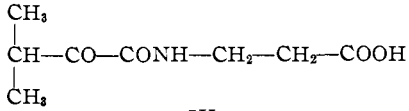
An attempt to oxidize pantothenic acid directly to the keto analog (I) with lead tetraacetate resulted in destruction of most of the vitamin but no I could be detected among the products. Condensation of the oxime and phenylhydrazone of III with β-alanine did not lead to I. It was thought that condensation with β-alanine esters might be effected smoothly with the acid chloride corresponding to III and for this purpose efforts were made to prepare the benzoate, VIII, and convert it into the acid chloride.



VIII

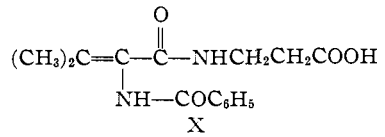
This approach to I also failed, since instead of VIII only unchanged III was obtained after benzoylation.

Another approach which was investigated was concerned with reactions leading to dimethylpyruvyl-β-alanine IX, which it was hoped might be condensed with formaldehyde to yield I.

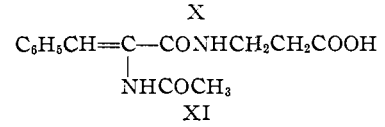


IX

For the preparation of IX the coupling of an appropriate azlactone with sodium β-alanate was contemplated. As model reactions, the azlactones of α-benzamido-β,β-dimethylacrylic acid[12] and of α-acetamidocinnamic acid[13] were coupled with sodium β-alanate and the corresponding β-alanides (X and XI) were obtained in approximately 90% yields. Selective hydrolysis



X



XI

of the latter compounds by acetic acid–hydrochloric acid mixtures, as employed previously,[14] did not lead to any of the α-keto-N-acyl-β-alanides. In the hydrolysis of X, 94% of α-benzamido-β,β-dimethylacrylic acid[12] was isolated. Hydrolysis of XI led to the isolation of an unknown non-acidic solid, m. p. 189–190°, but none of the desired phenylpyruvyl-β-alanine or derivatives could be isolated. Evidence that some cleavage of the acetamido group had occurred was afforded by isolation of a small amount of ammonium chloride from the hydrolysis mixture. It would appear, therefore, that the acyl-β-alanyl amide linkage is much more labile to acid hydrolysis than are corresponding amides of α-amino acids, and that the selective hydrolysis of acyldehydropeptides[14] as a route to α-keto "peptides" is not applicable to β-alanides.

Experimental

α-Keto-β,β-dimethyl-γ-butyrolactone, III.—The method of preparing III reported by Kuhn and Wieland[10] was confirmed in every respect except that the pure product (from benzene) melted at 68–70° instead of 60°. It was also prepared by the following, more convenient, method.

A mixture of 64 g. of freshly distilled *dl*-pantolactone[15] and 228 g. of lead tetraacetate[16] was refluxed in benzene suspension for eight and one-half hours, then allowed to stand overnight, filtered, and the lead diacetate washed with benzene. The solvent was removed and the partly crystalline residue stirred up with 1:1 benzene–petroleum ether. Filtration and washing with the same solvent mixture gave 18.2 g. of white, crystalline product, and 3.0 g. (32.6% total yield) more was obtained by concentrating the filtrate. The pure product (from benzene), m. p. 68–70°, gave no depression with that from dimethylpyruvic acid.[10]

Anal. Calcd. for $C_6H_8O_3$: C, 56.3; H, 6.30, sapon. equiv., 128.1; hydrogen uptake (Pt), 1.00 mole. Found: C, 56.1; H, 6.39; sapon. equiv., 129.2; hydrogen uptake, 0.99 mole.

When a four-fold excess of anhydrous hydrazine was mixed with III at room temperature, heat was evolved and the solid went into solution. After one hour the mixture was dissolved in alcohol, concentrated to a sirup at reduced pressure, and the sirup extracted with hot benzene. The residue from evaporating the benzene extract was crystallized from alcohol, and gave colorless needles of the hydrazone, m. p. 200–202°.

Anal. Calcd. for $C_6H_{10}O_2N_2$: C, 50.7; H, 7.08. Found: C, 51.1; H, 6.95.

The phenylhydrazone of III, obtained in 88% yield,

(12) Ramage and Simonsen, *J. Chem. Soc.*, 532 (1935).

(13) "Org. Synth.," Coll. Vol. II, p. 1 (1944).

(14) Fruton and Bergmann, *J. Biol. Chem.*, **166**, 449 (1946).

(15) The *dl*-pantolactone was kindly furnished by Merck and Company, Inc., Rahway, New Jersey.

(16) Oesper and Deasy, THIS JOURNAL, **61**, 972 (1939).

consisted of colorless needles from chloroform, m. p. 169–171°.

Anal. Calcd. for $C_{12}H_{14}O_2N_2$: C, 66.1; H, 6.46. Found: C, 66.2; H, 6.59.

A sample of this phenylhydrazone which had been twice recrystallized from chloroform was allowed to stand overnight at 5° in contact with the chloroform mother liquor. The next day the colorless crystals had redissolved to form a yellow solution which on concentration deposited yellow crystals, m. p. 66–67°. Recrystallization from petroleum ether did not change the m. p. This yellow product appeared to be an isomer of the original phenylhydrazone of III, probably the corresponding azo compound.

Anal. Calcd. for $C_{12}H_{14}O_2N_2$: C, 66.1; H, 6.46; N, 12.86. Found: C, 66.4; H, 6.56; N, 12.94.

The 2,4-dinitrophenyl hydrazone of III was obtained in nearly quantitative yield as yellow cubical crystals, which after recrystallization from glacial acetic acid, melted at 242–243.5°.

Anal. Calcd. for $C_{12}H_{12}O_6N_4$: C, 46.8; H, 3.90. Found: C, 46.7; H, 4.00.

The oxime of III was obtained in 74% yield as white crystals from water, m. p. 167–170°.

Anal. Calcd. for $C_6H_9O_3N$: C, 50.4; H, 6.34. Found: C, 50.8; H, 6.29.

The identity of III was further confirmed by reducing a sample over Pt and converting the reduced product to the 3,5-dinitrobenzoate which melted at 163–164°, alone and when mixed with an authentic specimen of *dl*-pantolactone 3,5-dinitrobenzoate.[17] The reduced product was also condensed with sodium β-alanate by the method of Parke and Lawson[18] and found by microbiological assay[11] to yield the same amount of pantothenic acid activity as *d,l*-pantolactone under identical conditions.

Condensation of α-Keto-β,β-dimethyl-γ-butyrolactone with Sodium β-Alanate.—A mixture of 1.0 g. of III and 0.87 g. of dry sodium β-alanate was heated under reflux in 6 cc. of absolute isopropyl alcohol for three hours. After removing the solvent the residual viscous oil was dissolved in 20 cc. of water, adjusted to *p*H 1.9 by addition of hydrochloric acid, and extracted with ether for sixteen hours in a continuous extraction apparatus. The oily residue obtained by evaporation of the ether extract was then extracted several times by stirring it thoroughly with hot benzene.

The benzene-insoluble oil was dissolved in about 5 volumes of ethanol and on standing a crystalline solid (V) separated. After three recrystallizations from water–alcohol mixtures, pure V, m. p. 213–215°, was obtained in a yield of 10%. V was a nitrogenous acid, but failed to give a test for the ketone group.

Anal. Calcd. for $C_8H_{15}O_4N$: C, 50.8; H, 8.00; neut. equiv., 189.2. Found: C, 50.7; H, 7.99; neut. equiv., 190.0, 189.2.

Compound V was assayed microbiologically at levels up to 400 μg. per tube, but showed less than 0.05% the activity of pantothenic acid. Likewise at levels of 300 μg. per tube it failed to inhibit the growth promoting effect of 0.1 μg. of pantothenic acid.

The mother liquor from V was concentrated under reduced pressure, and the residue dissolved in a small volume of chloroform. A crystalline solid, VI, separated in about 30% yield from this solution. After several recrystallizations from chloroform the m. p. was 131–133°. Qualitative tests indicated that VI was a nitrogenous keto acid. It gave no evidence of unsaturation either with bromine in carbon tetrachloride or with aqueous permanganate solution.

Anal. Calcd. for $C_9H_{13}O_4N$: C, 54.3; H, 6.56; N, 7.03; neut. equiv., 199.2. Found: C, 54.1, 54.0; H, 6.50, 6.57; N, 6.93, 6.95; neut. equiv., 199.2; hydrogen uptake, 1.04 moles.

(17) Stiller, Keresztesy and Finkelstein, THIS JOURNAL **62**, 1779 (1940).

(18) Parke and Lawson, *ibid.*, **63**, 2869 (1941).

Neither VI nor its reduction product had activity as pantothenic acid when tested microbiologically. Likewise, VI failed to inhibit the growth-promoting action of 0.12 μg. of pantothenic acid when tested in amounts of 50 and 200 μg.

The 2,4-dinitrophenylhydrazone of VI was obtained in the usual way in 89% yield as pale yellow crystals from glacial acetic acid, m. p. 257–259°.

Anal. Calcd. for $C_{15}H_{17}O_7N_5$: C, 47.4; H, 4.52. Found: C, 47.3; H, 4.70.

The phenylhydrazone of VI was obtained as fine plates from alcohol, m. p. 169–170°.

Anal. Calcd. for $C_{15}H_{19}O_3N_3$: C, 62.3; H, 6.62. Found: C, 62.6; H, 6.40.

The semicarbazone of VI was obtained in 63% yield as colorless crystals from alcohol, m. p. 171.5–173.5°.

Anal. Calcd. for $C_{10}H_{16}O_4N_4$: C, 46.9; H, 6.30. Found: C, 47.4; H, 6.27.

In order to effect the hydrolysis of VI, a solution of 1 g. in 20 cc. of 1 *N* alcoholic sodium hydroxide was refluxed three hours. After neutralizing, the solvent was removed from the solution and the residue was extracted with hot ethanol. Additions of absolute ether to the ethanol caused a fine white solid to separate. This solid melted at 198–200°, following recrystallization from alcohol–water, and gave no melting point depression when mixed with an authentic sample of β-alanine. The other product of the cleavage, which was obtained as a sirup by concentrating the alcohol mother liquor, was not identified.

Condensation Experiments.—In order to determine conditions for the condensation of III with β-alanine which gave the highest yield of I, a number of different condensation conditions, both dry and in solvent, were employed. The extent of formation of I was estimated by hydrogenating small amounts of the crude condensates, and determining microbiologically the increase in pantothenic acid content caused by the reduction. Condensations in the dry state were done by fusing a 250-mg. amount of III with an equivalent amount of sodium β-alanate. It was found that the highest formation of I (13–15%) occurred when III was fused with sodium β-alanate in the dry state at 100° for five minutes (Table I).

Oxidation of Sodium *dl*-Pantothenate by Lead Tetraacetate.—Oxidation of sodium *dl*-pantothenate was carried out using dioxane and also glacial acetic acid as the solvents. The suspensions of sodium *dl*-pantothenate were refluxed for a four-hour period with an equivalent amount of lead tetraacetate. The oxidation mixtures were then concentrated under reduced pressure to thick oils which were assayed microbiologically both before and after hydrogenation.

Oxidation in dioxane as the solvent reduced the amount of pantothenic acid to approximately one-fourth its original value, and in glacial acetic acid almost all the pantothenic acid was destroyed. However, in each case hydrogenation of these oxidation mixtures did not restore any of the activity. Thus, no formation of I in these oxidations was indicated.

Attempted Preparation of α-Keto-β,β-dimethyl-γ-benzoxybutyric Acid, VIII.—To 0.5 g. of III dissolved in 2.5 times the equivalent amount of a 10% sodium hydroxide solution at 0° was added 0.55 g. of benzoyl chloride. The solution was shaken vigorously until the odor of benzoyl chloride disappeared; then it was acidified to *p*H 5.6 with hydrochloric acid. The white precipitate, collected and recrystallized from benzene, melted at 120–121° alone and at 119–121° when mixed with an authentic sample of benzoic acid. No VIII was obtained. By evaporation of the aqueous layer and extraction into ether, unchanged III, m. p. 68–70° was recovered.

N-(α-Benzamido-β,β-dimethylacrylyl)-β-alanine, X.—To 10.06 g. (0.05 mole) of the azlactone of α-benzamido-β,β-dimethylacrylic acid[12] dissolved in 75 cc. of acetone was added 4.45 g. (0.05 mole) of β-alanine dissolved in 50 cc. of 1 *N* sodium hydroxide. The reaction occurred with evolution of heat. After stirring the solution for ten

minutes at room temperature, 50 cc. of 1 *N* hydrochloric acid was added and the solution was concentrated under reduced pressure to *ca.* 100 cc. The condensation product (X) was collected on a Buchner funnel and washed with cold water. The total yield was 12.83 g. (88.5%). After recrystallization from 4:1 alcohol–acetone, the product melted at 178–179°.

Anal. Calcd. for $C_{15}H_{18}O_4N_2$: neut. equiv., 290.1. Found: neut. equiv., 284.6

Hydrolysis of X.—An amount of 7.25 g. (0.025 mole) of **X** was refluxed on the steam-bath for one hour with a mixture of 25 cc. of 1 *N* hydrochloric acid and 15 cc. of glacial acetic acid. The white crystalline solid which separated was collected and found to weigh 5.15 g. When recrystallized from hot ethyl alcohol, it melted at 218–219° (dec.), which agrees with the previously reported value for α-benzamido-β,β-dimethylacrylic acid.[12]

Anal. Calcd. for $C_{12}H_{13}O_3N$: neut. equiv., 219.2. Found: neut. equiv., 219.8, 220.6.

TABLE I

EFFECT OF CONDENSATION CONDITIONS UPON FORMATION OF THE KETO ANALOG OF PANTOTHENIC ACID

Conditions of condensation			Pantothenic acid activity,[a] %	
Time in hours	Temp., °C.	Solvent	Unreduced	Reduced[b]
3	80	Isopropyl alc.	0.063	2.7
1	95–100	None	0	7.3–8.3
1	180	None	0.001	0
6.5	95–105	None	0.049	0.39
5 (min.)	100	None	0	13–15

[a] Determined microbiologically with *L. arabinosus*.[11] [b] Observed activity doubled to allow for the formation of *d,l*-pantothenic acid on reduction.

By evaporating the mother liquor to dryness β-alanine hydrochloride was obtained as white crystals which melted at 122° after recrystallization from hot alcohol.

N-(α-Acetamidocinnamoyl)-β-alanine, XI.—This compound was prepared by coupling the azlactone of α-acetamidocinnamic acid[13] with β-alanine according to the procedure used for the preparation of **X**. The yield of XI, pale yellow crystals, m. p. 154–155.5° from alcohol, was 92.4%.

Anal. Calcd. for $C_{14}H_{16}O_4N_2$: C, 60.8; H, 5.84; N, 10.1; neut. equiv., 276.3. Found: C, 59.7; H, 5.70; N, 10.0; neut. equiv., 276.5.

For hydrolysis of **XI**, 11.0 g. was refluxed two hours on the steam-bath with a mixture of 20 cc. of 1.0 *N* hydrochloric acid and 20 cc. of glacial acetic acid. The solution was then diluted with 3 volumes of water and cooled in ice. The precipitate (4.4 g.) was recrystallized from hot ethanol, from which it was obtained as long needles, m. p. 189–190°. It was a non-acidic compound of unknown structure. In earlier attempts, hydrolysis times of fifteen minutes to one hour were used. In these studies much of the XI was isolated unchanged. Some oily material was obtained from the hydrolysis which gave a positive qualitative ketone test but neither it nor an oxime derivative could be obtained pure. In one case the water-soluble fraction after hydrolysis was taken to dryness *in vacuo*, and the residue taken up in hot alcohol. Upon concentrating and cooling the alcohol solution, a colorless, inorganic, water-soluble, crystalline solid was deposited, which sublimed on heating and gave positive qualitative tests for chloride and ammonia. The yield of the ammonium chloride was about one-fourth of the theoretical.

Discussion

Although the desired keto analog of pantothenic acid (I) was not obtained in pure form, the appearance of considerable pantothenic acid activity after reduction of crude preparations of I, together with the inactivity of the same preparations before reduction, show quite definitely that I was formed, and that it cannot replace pantothenic acid, at least for *L. arabinosus*. In view of the similar inactivity of the amino analog (II),[9] it would appear that the metabolic action of pantothenic acid does not involve such transformations as dehydrogenation or transamination at the α-hydroxyl position.

Summary

The synthesis of α-keto-β,β,dimethyl-γ-butyrolactone (III) by two different methods gave in each case the identical product.

The condensation of this keto lactone (III) with sodium β-alanate was found to yield under certain conditions 13–15% of the keto analog (I) of pantothenic acid. This product was not isolated but was inactive for *Lactobacillus arabinosus*. Two crystalline products of the condensation of III with sodium β-alanate were isolated. One of these (V) was tentatively identified as α,α-dimethyl-β-hydroxypropionyl-β-alanine. The other (VI) was a β-alanide, but its structure was not definitely established. Attempts to produce I by several other methods were unsuccessful.

MADISON, WISCONSIN RECEIVED JANUARY 10, 1949