Synthesis of α-Ketopalmitic Acid Methyl Ester.—α-Hydroxypalmitic acid (m. p. 86°) was prepared from α-bromopalmitic acid as described by Le Sueur.[4] On treating 5 g. of silver salt of α-hydroxypalmitic acid with 4 g. of methyl iodide in benzene solution on a water-bath for five hours, 3.5 g. of α-hydroxypalmitic acid methyl ester was obtained which melted at 58–59°. The ester dissolved less in cold petroleum ether or benzene in comparison with lanopalmic acid methyl ester. The oxidation of α-hydroxypalmitic acid methyl ester to α-ketopalmitic acid methyl ester was carried out in a manner similar to the preparation of lanopalminonic acid methyl ester, and the resulting product was purified by recrystallization from aqueous methanol and then from low boiling ligroin; glistening laminas melting at 47°.

Anal. Calcd. for $C_{14}H_{29}COCO_2CH_3$: C, 71.76; H, 11.36. Found: C, 71.54; H, 11.10.

Cleavage of the Oxime of III.—For the oximation of III, 3 g. of the substance was added to 10 cc. of aqueous-alcoholic solution containing 0.74 g. of hydroxylamine hydrochloride and 1.1 g. of sodium carbonate, and allowed to stand for three days at room temperature. Dilution with water precipitated the oxime, which was filtered, washed and dried. It weighed 3.1 g. and showed a m. p. of 92° after recrystallization from petroleum ether.

Anal. Calcd. for $C_{17}H_{32}O_2NOH$: N, 4.68. Found: N, 4.62.

In order to carry out a Beckmann rearrangement, 2 g. of the oxime was treated with excess of phosphorus pentachloride in ethereal solution at −5°. The acid amide purified by the crystallization from petroleum ether melted at 111–112°.

Anal. Calcd. for $C_{17}H_{33}O_3N$: N, 4.68. Found: N, 4.41.

One gram of the acid amide obtained above was hydrolyzed at 150–160° for six hours in a sealed tube containing 20 cc. of concentrated hydrochloric acid yielding 0.6 g. of pentadecylic acid which melted at 52–52.5° and showed no depression of m. p. on addition of *n*-pentadecylic acid. The amide of the acid melted at 102°, the m. p. of *n*-pentadecylic acid amide.

Anal. Calcd. for $C_{15}H_{30}O_2$: C, 74.31; H, 12.48; mol. wt., 242. Found: C, 74.52; H, 12.60; mol. wt. (from neutralization no.), 243. Calcd. for acid amide ($C_{15}H_{31}ON$): N, 5.81. Found: N, 5.87.

Oxidation of Lanopalminonic Acid with Hydrogen Peroxide.—Lanopalminonic acid obtained by the saponification of III with dilute alcoholic potash is flat needles from ligroin melting at 69°. On mixing the keto acid (1.6 g.) with 30% hydrogen peroxide solution (10 cc.), the evolution of gas was observed. After the stirring was continued as long as any gas was evolved, the oxidized acid (1.4 g.) was collected on filter paper, washed and crystallized from methanol; laminas of silky luster, m. p. 52°. The acid did not depress the m. p. of *n*-pentadecylic acid.

Oxidation of Lanopalmic Acid with Lead Peroxide.—A mixture of 0.5 g. of lanopalmic acid and 5 g. of lead peroxide was distilled in a current of steam. An oily substance distilling over and having an odor of higher aldehyde is treated with hydroxylamine and the oxime was recrystallized, after removing acid substances, from aqueous methanol; yield 1.3 g. The oxime obtained was small needles and melted at 83–84°.

Anal. Calcd. for $C_{15}H_{30}NOH$: N, 5.81. Found: N, 5.88.

Acknowledgment.—It is a pleasant duty of the author to thank Prof. Y. Tanaka for kind advice and encouragement.

Summary

Lanopalmic acid has been isolated from the mixed acids of Merino sheep wool wax, and the acid has been proved to be a stereoisomer of α-hydroxypalmitic acid.

TOKYO, JAPAN RECEIVED NOVEMBER 15, 1937

(4) Le Sueur, *J. Chem. Soc.*, **87**, 1895 (1905).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL AND COLLOIDAL CHEMISTRY, THE HEBREW UNIVERSITY]

The Behavior of Peptides when Heated in β-Naphthol

BY N. LICHTENSTEIN

When **dipeptides** are heated in β-naphthol at a temperature of 135–150°, they dissolve and form diketopiperazines which may be separated in good yield on cooling by removing the β-naphthol with ether. By this method the respective anhydrides of the following dipeptides were obtained: *d,l*-leucyl-*d,l*-leucine, *d,l*-leucylglycine, glycyl-*d,l*-leucine, *d,l*-valylglycine, *d,l*-alanyl-*d,l*-leucine, glycyl-*d,l*-phenylalanine.

Benzoylated dipeptides, such as benzoyl-*d,l*-leucylglycine, benzoylglycylglycine and benzoylglycyl-*d,l*-phenylalanine dissolve, but suffer no ring closure and may be recovered unchanged. Glycylglycine which is insoluble in hot β-naphthol also remains unchanged.

If **tripeptides** are treated in the same manner, the terminal amino acid in the free form and the diketopiperazine corresponding to the first amino acid, which contains the free amino group, and to the second adjacent amino acid of the peptide, are obtained.

In this fashion glycine and *d,l*-alanyl-*d,l*-leucine

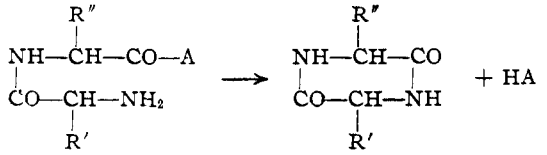
anhydride may be obtained from *d,l*-alanyl-*d,l*-leucylglycine; glycine and *d,l*-alanine anhydride from *d,l*-alanyl-*d,l*-alanylglycine; *d,l*-leucine and *d,l*-alanine anhydride from *d,l*-alanyl-*d,l*-alanyl-*d,l*-leucine; *d,l*-phenylalanine and *d,l*-alanylglycine anhydride from *d,l*-alanylglycyl-*d,l*-phenylalanine; *d,l*-leucylglycine anhydride and *d,l*-phenylalanine from *d,l*-leucylglycyl-*d,l*-phenylalanine, glycine and *d,l*-leucylglycine anhydride from *d,l*-leucylglycylglycine.

The separation of the anhydride from the free amino acid may be accomplished easily by treating the carefully dried mixture with hot absolute alcohol in which, as is known, diketopiperazines but no free amino acids are soluble.

Benzoyl-*d,l*-alanyl-*d,l*-alanylglycine dissolved in hot β-naphthol, as did the unbenzoylated tripeptide, but as in the corresponding case of the benzoyl dipeptides, underwent no change.

A **tetrapeptide,** *d,l*-leucylglycylglycyl-*d,l*-leucine, treated in the same fashion with β-naphthol, yielded only *d,l*-leucylglycine anhydride.

The transformation undergone by peptides in hot β-naphthol seems to consist of a ring closure by the combination of the free amino group in the first acid, with the CO group in the second acid. In this process, a hydrogen atom of the free amino group, and the residue attached to the CO group in the second acid are jointly displaced as in the scheme



If residue A is a carboxylic OH group, as is the case in dipeptides, ring closure with the liberation of water occurs. If residue A is an amino acid, as is the case in tripeptides, then together with a diketopiperazine the free amino acid is obtained. If residue A is a dipeptide, as is the case in the tetrapeptide mentioned, then on displacement this residue itself forms an anhydride.

The above findings suggest an easy method for the preparation of diketopiperazines from the corresponding dipeptides, particularly in cases where a direct anhydration by heating is not practicable. As several preliminary experiments suggest, it may not be necessary first to isolate the dipeptide in pure form; the mixture obtained on amination of the halogen acyl body may directly be heated with β-naphthol, and on removing the naphthol with ether the diketopiperazine may be obtained by extraction with hot chloroform.

In the case of tripeptides, the method of heating with β-naphthol constitutes a comparatively simple method of identifying the terminal amino acid bearing the free carboxyl group.

If under the same conditions the proteins ovalbumin or edestin are heated with β-naphthol, they dissolve, and after removal of the β-naphthol, beautifully crystalline products predominantly insoluble in hot alcohol are obtained.[1]

It may be recalled that A. Fodor and collaborators[2] treated a number of proteins with non-hydrolyzing media such as hot water-free glycerol, hot resorcinol and hot β-naphthol and on pouring the solutions thus obtained into absolute alcohol separated decomposition products whose weight constituted 70% of the original protein, and which on the basis of their investigations were concluded to be cyclic polypeptide chains of a much higher molecular weight than simple diketopiperazines.[3]

Though the crystalline products obtained by us from edestin and ovalbumin on treatment with β-naphthol have not yet been investigated more closely, it seems right to conclude on the basis of the insolubility of the predominant part of these products in hot alcohol that they are not mixtures of simple diketopiperazines. It remains to be shown whether our finding with lower peptides, that these form diketopiperazines or diketopiperazines plus an amino acid when heated in β-naphthol, is generally applicable to higher peptides. If this proves to be the case, a comparative study of the behavior of proteins and polypeptides in hot β-naphthol may furnish new insight into the structure of proteins.

Experimental

WITH COLLABORATION OF S. HESTRIN, E. DIMANT AND H. BRZOZA

The Peptides Used.—The peptides were prepared in general according to the methods indicated in Abderhalden's "Biochemisches Handlexikon," Vols. IV and XII.

The benzoyl *d,l*-alanyl-*d,l*-alanylglycine was obtained by benzoylation of the tripeptide with benzoyl chloride in aqueous solution with addition of sodium bicarbonate.

(1) Similar findings in this institute with other proteins are reported elsewhere.

(2) A. Fodor and S. Kuk, *Kolloid-Z.*, **74**, 66 (1936); A. Fodor and N. Lichtenstein, *Enzymologia*, **1**, 311 (1936); **4**, 36 (1937).

(3) When a diketopiperazine, *d,l*-leucine anhydride, was subjected by us to the treatment applied in Fodor's experiments, the material was recovered unchanged. A secondary association of diketopiperazines to form higher molecular structures is therefore out of the question under the conditions of Fodor's experiments.

The coupling product obtained on acidification was freed of benzoic acid by extraction with petroleum ether and then recrystallized twice in water. The m. p. of the substance obtained was 214° (uncorr.); 94.9 mg. in aqueous solution, using phenolphthalein as indicator, required 2.98 cc. of 0.1 *N* sodium hydroxide, calcd. 2.96 cc.; Kjeldahl nitrogen determination, subs. 107.1 mg. required 9.92 cc. of 0.1 *N* hydrochloric acid, calcd. 13.08, found 12.98% N.

The Treatment with β-Naphthol.—The peptides were always carefully mixed with five times their weight of β-naphthol and heated in a round flask on the oil-bath at a bath temperature of 135–150°. The heating was continued for three hours, though in a few experiments it was shown that the transformation obtained on solution, which occurred at different rates with different peptides, was already complete long before this time, *e. g.*, in the case of *d,l*-leucyl-*d,l*-leucine anhydrization was effected within one-half hour. It is preferable on completion of the reaction to pour the still hot solution into a porcelain dish from which the mass of substance may be removed easily on cooling. This is then ground in a mortar and stirred repeatedly with ether. The suspension is then filtered under reduced pressure and the residue is washed with ether. The anhydrides obtained from dipeptides were recrystallized directly from hot alcohol to which *carbo animalis* had been added. The products obtained from tripeptides must, before treatment with absolute alcohol, be dried carefully in a vacuum desiccator over sulfuric acid. It is best to continue the drying for several days. The amino acid remaining after boiling the mixture with alcohol may, if necessary, be purified by taking up in a little water and precipitating with absolute alcohol. The diketopiperazines which separate out on the concentration of the alcoholic extracts, may be recrystallized from alcohol or ethyl acetate.

It may be pointed out that on treating the cooled β-naphthol solution with ether, benzoyl dipeptides at first go into solution but separate out again on standing. Their separation may be accelerated and rendered more complete by the addition of petroleum ether. The substances obtained are almost pure and may be recrystallized from hot water.

The Reaction Products.—The results obtained with dipeptides are summarized in Table I, those with tripeptides in Table II.

The product obtained on treatment of the tetrapeptide *d,l*-leucylglycylglycyl-*d,l*-leucine was dried after the treatment with ether in a vacuum over sulfuric acid. The product gave no ninhydrin and no copper salt reaction. On recrystallizing from alcohol it could be identified as consisting only of *d,l*-leucylglycine anhydride by the m. p., the mixed m. p. (239°), the nitrogen content (28.7 mg. required in the Kjeldahl determination 3.36 cc. of 0.1 *N* hydrochloric acid. Calcd. 16.47%, found 16.40% N) and the molecular weight (0.2554 g. dissolved in 22.0709 g. glacial acetic acid

TABLE I

PROPERTIES AND ANALYSES OF PRODUCTS

Starting material	End-product, * anhydride	M. p., °C. *mixed m. p. also	Ninhydrin and Cu salt reacn.	Titration, cc. of 0.1 *N* soln. for mg. of sample	Kjeldahl nitrogen, % or cc. soln. Calcd.	Found
d,l-Leucylglycine	*d,l*-Leucylglycine*	239*	Negative		16.47	16.40
Glycyl-*d,l*-leucine	*d,l*-Leucylglycine*	239*	Negative		16.47	16.50
d,l-Leucyl-*d,l*-leucine	*d,l*-Leucine*	273*	Negative		12.39	12.35
d,l-Valylglycine	*d,l*-Valylglycine*	245	Negative		17.94	17.61
d,l-Alanyl-*d,l*-leucine	*d,l*-Alanyl-*d,l*-leucine*	244	Negative		15.22	15.07
Glycyl-*d,l*-phenylalanine	Glycyl-*d,l*-phenylalanine*	273	Negative		13.73	13.46
Glycylglycine[a]	Glycylglycine		Positive	Linderstrøm-Lang, alc. HCl, 73.6	5.57	5.60
Benzoyl-*d,l*-leucylglycine	Benzoyl-*d,l*-leucylglycine	163*		NaOH, 126.5	4.33	4.30
Benzoylglycylglycine	Benzoylglycylglycine			Raw product, NaOH, 108.4	4.50	4.40
Benzoylglycyl-*d,l*-phenylalanine	Benzoylglycyl-*d,l*-phenylalanine	172*		NaOH, 43.1	1.32	1.36

[a] Insoluble in naphthol.

TABLE II

PROPERTIES AND ANALYSES OF PRODUCTS

Starting material	End-products * for anhydride	M. p., °C. * mixed m. p. also	Ninhydrin and Cu salt reaction	Titrations, cc. of 0.1 *N* HCl for mg. of samples	Kjeldahl nitrogen analysis % or cc. soln. Calcd.	Found
d,l-Alanyl-*d,l*-leucylglycine	*d,l*-Alanyl-*d,l*-leucine*	246	Negative		15.22	15.11
	Glycine[a]	179*[b]	Positive		18.66	18.69
d,l-Alanyl-*d,l*-alanylglycine	*d,l*-Alanine*	274	Negative		19.71	19.65
	Glycine[a]		Positive	Linderstrøm-Lang, alc., 47.4	6.32	6.28
d,l-Alanyl-*d,l*-alanyl-*d,l*-leucine	*d,l*-Alanine*	273.5	Negative		19.71	19.45
	d,l-Leucine		Positive		10.69	10.73
				Linderstrøm-Lang, alc., 32.6	2.49	2.55
d,l-Alanylglycyl-*d,l*-phenylalanine	*d,l*-Alanylglycine*	240	Negative		21.89	21.60
	d,l-Phenylalanine[a]		Positive	Linderstrøm-Lang, alc., 30.8	1.86	1.92
d,l-Leucylglycyl-*d,l*-phenylalanine	*d,l*-Leucylglycine*	239*	Negative		16.47	16.49
	d,l-Phenylalanine[a]		Positive	Linderstrøm-Lang, alc., 93.7	5.67	5.65
d,l-Leucylglycylglycine	*d,l*-Leucylglycine*	239*	Negative		16.47	16.69
	Glycine[a]	179*[b]	Positive			
Benzoyl-*d,l*-alanyl-*d,l*-alanylglycine	Benzoyl-*d,l*-alanyl-*d,l*-alanylglycine	213–214*	Negative	Aqueous soln., NaOH, 85.8	2.67	2.65

[a] Sweet taste. [b] 3,5-Dinitrobenzoyl derivative.

gave a Δ of 0.25° corresponding to a molecular weight of 180.5. Calcd. 170.1).

The amino acids glycine, *d,l*-leucine, *d,l*-alanine and *d,l*-phenylalanine do not dissolve in β-naphthol and remain unchanged.

The Treatment of Proteins with β-Naphthol.—Edestin and ovalbumin mixed with respectively 5 and 10 times their weight of β-naphthol were treated in the same manner as were the peptides. The products obtained after washing with ether were seen under the microscope, to consist of crystals. On repeated boiling with absolute alcohol, over 80% of the substance remains undissolved. On evaporation of the first alcoholic extract, a brown mass which in part dissolved in ether but was not investigated further, was obtained. The part insoluble in absolute alcohol, on reëxamination under the microscope, proved beautifully crystalline as was the mother material.

Summary

On heating with β-naphthol at a temperature of 135–150°, dipeptides enter into solution and yield diketopiperazines; this circumstance gives an easy method for the preparation of the latter.

Tripeptides treated in a corresponding manner with β-naphthol are decomposed so that they yield the terminal amino acid in a free form and a diketopiperazine consisting of the first amino acid which carries the free amino group and the second adjacent amino acid. This property provides a method for the identification of the amino acid bearing the free carboxyl group in tripeptides.

A tetrapeptide treated under the same conditions yielded a diketopiperazine corresponding to the two amino acid pairs.

Benzoylated dipeptides and a benzoylated tripeptide, though soluble in β-naphthol, underwent no change. Free amino acids and glycylglycine do not dissolve in β-naphthol and remain unchanged.

Edestine and ovalbumin dissolve in hot β-naphthol and yield beautifully crystalline products which are largely insoluble in hot alcohol and therefore are not simple diketopiperazines.

The significance of the above findings for our knowledge of protein structure is discussed.

JERUSALEM, PALESTINE RECEIVED SEPTEMBER 27, 1937

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

α-Ethyl-*l*-sorbopyranoside and its Tetraacetate

BY ROY L. WHISTLER AND R. M. HIXON

During a study of Fischer's method[1] for glycoside formation with relation to *l*-sorbose it was observed that a solution of this sugar in ethanol underwent an optical rotatory change analogous to the change occurring during the formation of α-methyl-*l*-sorboside in methanol (Fig. 1). The close similarity in the optical changes indicated a reaction between sorbose and the ethanol solution with formation of an ethyl sorboside, which like the methyl sorboside would be expected to be of the *alpha* configuration. The reaction is essentially complete in four hours at room temperature.

A small quantity of *l*-sorbose was subjected to the action of a 1% solution of hydrogen chloride in dry ethanol with recovery, at the end of four hours, of fine colorless needles having a melting point of 116° and an optical rotation[2] of −73.9°. The strong negative rotation as compared to *l*-sorbose (−43.4°) indicates an α-glycoside. On solution in dilute hydrochloric acid the hydrolysis curve followed closely that found for α-methyl-*l*-sorboside, taking ten days for complete hydrolysis in 0.1 *N* acid and approximately thirty days in 0.015 *N* acid (Fig 1). The true end-point in the latter solution was indefinite due to mold growth which became noticeable after twenty-eight days.

Acetylation of the α-ethyl-*l*-sorboside produced an α-ethyl-*l*-sorboside tetraacetate which was identical with that obtained through the ethylation of sorbose tetraacetate. Thus, the ring structure of α-ethyl-*l*-sorboside must be the same as that in sorbose tetraacetate. The structure of sorbose tetraacetate-[3,4] as 1,3,4,5-tetraacetylsorbose can be established through the fact that acetylation of α-methyl-*l*-sorbopyranoside whose configuration has been proved[5] yields an α-methyl-*l*-sorboside tetraacetate[6] identical with the compound obtained through the methylation[5] of sor-

(1) Fischer, *Ber.*, **28**, 1145 (1895).

(2) All rotations are specific rotations taken with the D-line of sodium at 26°.

(3) Arragon, *Compt. rend.*, **196**, 1133 (1933).

(4) Arragon, *ibid.*, **198**, 1508 (1937).

(5) Whistler and Hixon, THIS JOURNAL, **59**, 2047 (1937).

(6) Arragon, *Bull. soc. chim. biol.*, **17**, 831 (1935).