precipitate of the acid was filtered off after standing for three days at room temperature. It was purified through its sodium salt, and recrystallized from aqueous alcohol. The yield was 2.3 g. (23%).

Monoperphthalic Acid Oxidation.—The quinone (4.74 g.) was added to an excess of monoperphthalic acid in ether solution and the mixture was allowed to stand at room temperature for thirty hours. The precipitate of acid was filtered and the acid purified as before. The yield was 2.08 g. (31.2%).

The pure dicarboxylic acid melted at 186–190° (dec.) when the temperature of the block was raised 4° per minute.

Anal. Calcd. for $C_{11}H_{10}O_5$: C, 59.46; H, 4.54; neut. equiv., 111.3. Found: C, 59.32; H, 4.80; neut. equiv., 111.1.

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A Peptide Derivative Related to Gramicidin

BY JOSEPH S. FRUTON

The studies of Hotchkiss[1] and of Gordon, *et al.*,[2] have shown that gramicidin is a cyclopeptide which is characterized by an unusually high content in D-leucine and L-tryptophan, these two components accounting for approximately one half of the total amino acids found after complete hydrolysis of gramicidin. In the course of our studies on the effect of peptides and peptide derivatives on bacterial growth, the substance D-leucyl-L-tryptophan diketopiperazine was synthesized. The synthesis involved the reaction of carbobenzoxy-D-leucyl azide with L-tryptophan methyl ester, followed by the catalytic hydrogenation of the coupling product. Treatment of the resulting dipeptide ester with ammonia gave the diketopiperazine.

If the antibacterial action of gramicidin were due solely to the presence of D-leucine or L-tryptophan residues, the synthetic diketopiperazine might have been expected to exhibit some inhibition of the growth of organisms affected by gramicidin. It has been found, however, that the diketopiperazine, when tested at concentration levels of 1 to 10 μg. per ml. of culture medium, shows no appreciable action on *Escherichia coli*, *Staphylococcus aureus*, *Clostridium welchii* or *Brucella abortus*, and only a slight antibacterial effect was noted with *Streptococcus hemolyticus*. Control experiments with gramicidin, at 1 and 5 μg. per ml., showed complete inhibition of the growth (in 12 hours) of *S. hemolyticus*. Further experiments on the antibacterial activity of peptide derivatives related to gramicidin and tyrocidine are in progress.

Experimental

N-Carbobenzoxy-D-leucyl-L-tryptophan Methyl Ester.—Three grams of carbobenzoxy-D-leucinhydrazide[3] was dissolved in a mixture of 25 ml. of water, 10 ml. of glacial acetic acid and 5 ml. of concentrated hydrochloric acid. The solution was chilled to 0° and, with shaking, there was added, in small portions, a solution of 0.7 g. of sodium nitrite in 10 ml. of water. The azide separated as an oil and was extracted with ether. The ethereal solution was washed successively with cold water, cold aqueous bicarbonate solution, and again with cold water. The ethereal layer (60 ml.) was dried briefly over sodium sulfate and added to a solution of 2.5 g. of L-tryptophan methyl ester[4] in 60 ml. of ether. The reaction mixture was left at room temperature for eighteen hours, and then washed successively with dilute hydrochloric acid, water, aqueous bicarbonate solution, and water. After being dried over sodium sulfate, the solution was concentrated to a small volume under reduced pressure. The careful addition of petroleum ether (30–60°) gave a sirup which crystallized readily. After recrystallization from ethyl acetate–petroleum ether, the substance (2.7 g.) melted at 125–127°.

Anal. Calcd. for $C_{26}H_{31}O_5N_3$: N, 9.0. Found: N, 9.2.

D-Leucyl-L-tryptophan Diketopiperazine.—One gram of the above carbobenzoxydipeptide ester was dissolved in a mixture of 15 ml. of methanol and 0.2 ml. of glacial acetic acid and was hydrogenated at atmospheric pressure in the presence of palladium black. The hydrogenation required two hours, after which time the catalyst was removed by filtration. The filtrate was added to 30 ml. of methanol which had previously been saturated with dry ammonia at 0°. The mixture was left at room temperature for two days, then concentrated under reduced pressure, and the resulting crystalline product was dissolved in 10 ml. of hot absolute alcohol. On chilling the alcoholic solution, 0.56 g. of the diketopiperazine crystallized; m. p. 218–219° (dec.).

Anal. Calcd. for $C_{17}H_{21}O_2N_3$: C, 68.2; H, 7.1; N, 14.0. Found: C, 67.9; H, 7.1; N, 14.0.

(1) Hotchkiss, *J. Biol. Chem.*, **141**, 171 (1941).
(2) Gordon, Martin and Synge, *Biochem. J.*, **37**, 86 (1943).
(3) This compound was prepared in the manner described for the L-form by Bergmann, *et al.*, *J. Biol. Chem.*, **109**, 325 (1935).
(4) Abderhalden and Kempe, *Z. physiol. Chem.*, **52**, 207 (1907).

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Indole from Formyl-toluidine

BY ALEXANDER GALAT AND HARRIS L. FRIEDMAN

Of the numerous methods of preparation of indole described in the literature, ring closure of *o*-formyltoluidine is the most direct and convenient. Tyson[1] has shown that yields up to 79% may be obtained with potassium alkoxides, whereas sodium alkoxides give little or no product. This is a peculiarity of the formyl group, for the higher acyl derivatives are readily dehydrated by sodium alkoxides.[2]

The use of potassium metal in the dehydration of *o*-formyltoluidine adds both expense and an element of danger to large scale preparations. It occurred to us that if the potassium *ion* had a catalytic effect in the reaction, it would be possible to use an inexpensive potassium salt with sodium alkoxide.

This possibility was tested as follows: sodium (4.6 g.) was dissolved in 100 ml. of anhydrous methanol and 27 g. of *o*-formyltoluidine was added. Complete solution resulted on warming.

(1) Tyson, THIS JOURNAL, **63**, 2024 (1941).
(2) Madelung, *Ber.*, **45**, 1130 (1912).