solutions decomposed to a dark red solution in a few hours after contact with air. The solid (0.7 g.) on quick crystallization from acetone (200 ml.) under nitrogen atmosphere gave colorless long needles which melted at 141–142°.

Anal. Calcd. for $C_{42}H_{60}O_5N_2$: C, 74.9; H, 9.0; N, 4.2. Found: C, 75.0, 75.2, 74.3; H, 9.1, 9.3, 9.0; N, 4.3, 4.0.

Hydrolysis of compound VI. The crystalline compound (0.2 g.) was dissolved in acetone (25 ml.) by warming, and 50% sulfuric acid (5 ml.) was added to it. This was gently heated under reflux for about 5 min. The acetone was removed under reduced pressure, the resulting solid dissolved in benzene and extracted with 5% sodium hydroxide. The benzene layer on drying and concentration gave (0.077 g.) of the impure quinone. Steam distillation gave 34 mg. of pure 2,6-di-*tert*-butylbenzoquinone.

The alkaline layer was acidified and the precipitate collected (0.12 g.). Crystallization from benzene gave shining yellow flakes, m.p. 220°, which were identical with the quinone oxime (IV), in their melting point, ultraviolet, and infrared spectra.

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A Study with C^{14} of the Hydrolysis of Unsymmetrical 2,5-Piperazinedione into Dipeptides

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The hydrolysis of 2,5-piperazinedione has been extensively studied.[1–4] With unsymmetrical 2,5-piperazinediones it should in theory give a mixture of two dipeptides. Previous work was done at that time when the modern techniques of chromatography and electrophoresis were unknown. The analytical methods then used were based on the titration of free carboxyl or amino group liberated during the hydrolysis. The emphasis was therefore more on the study of the ideal conditions and of the rate of the hydrolysis rather than on the relative proportions and identities of the products formed.

As no definite rules have been laid out for the opening of unsymmetrical 2,5-piperazinediones, this method of synthesis of dipeptides, first proposed by Fischer and Shrauth,[5] has been neglected.

In a recent publication[6] it has been shown that the partial hydrolysis of 1,4-diazaspiro[4.5]decane-2,5-dione (V) by 1*N* hydrochloric acid gives two dipeptides: glycylamino-1 cyclopentanecarboxylic acid (IV) and amino-1-cyclopentanecarboxylglycine (VI), these two peptides having been identified by paper chromatography. Connors and Ross[7] in a recent work have obtained by the opening of the same piperazinedione only one peptide, VI.

Theoretically it became interesting to study quantitatively the yield of the two peptides expected after the hydrolysis. One could expect that assymetry of the molecule will favor the opening of one peptide bond over the other. However, there are practical difficulties in this study because of the similarity in the chemical properties of the two peptides and their abnormal reaction with ninhydrin.[6] To overcome this, we incorporated in this substituted 2,5-piperazinedione a radioactive carbon. Starting from glycine-1-C^{14} we have prepared IV by the carbodiimide method. This peptide was then cyclized into the corresponding piperazinedione (V), which after hydrolysis gave the two radioactive peptides. These were separated by paper chromatography and the radioactivity of each was determined. The relative proportion of the two peptides was 58% for IV and 42% for VI. In this case, the difference in the respective yields is not sufficient to allow for theoretical considerations. However, this new method using C^{14} incorporated into the piperazinedione appears the ideal one for similar studies with other unsymmetrical piperazinediones.

EXPERIMENTAL

N-Carbobenzoxyglycine-1-C^{14} (I). A solution containing 5.05 mg. of glycine-1-C^{14} (total activity, 0.1 mc.) in 10 ml. of water was made. An aliquot of 4 ml. was taken and 2 g. of glycine were dissolved in it. Then sodium hydroxide and benzyl chloroformate were added and I was isolated as usual. Yield: 4.4 g. (79%) m.p. 119–120°.[8] Specific activity: 3.3×10^3 c.p.m./mg.

Anal. Calcd. for $C_{10}H_{11}NO_4$: N, 6.70. Found: N, 6.75.

Benzylamino-1-cyclopentanecarboxylate (II). This compound was prepared by a method recently described.[6]

Benzyl N-carbobenzoxyglycyl-1-C^{14}-amino-1-cyclopentanecarboxylate (III). To a solution of 4.0 g. of I, and 4.4 g. of II in 40 ml. of tetrahydrofuran was added 4.2 g. of *N,N'*-dicyclohexylcarbodiimide, and III was isolated according to Sheehan and Hess[8] by recrystallizing with ethyl acetate and petroleum ether. This yielded 6.8 g. (86%) of III, m.p. 107–108°. Specific activity: 1.6×10^3 c.p.m./mg.

Anal. Calcd. for $C_{23}H_{26}N_2O_5$: N, 6.83. Found: N, 6.94.

Glycyl-1-C^{14}-amino-1-cyclopentanecarboxylic acid (IV). A solution of 4.1 g. of III in 30 ml. of ethanol containing a little acetic acid was hydrogenated for 6 hr. over 0.1 g. of palladium 10% on carbon. The catalyst was filtered, and the solution evaporated to dryness, washed with acetone, and filtered. The insoluble peptide was recrystallized from water and acetone. Yield: 1.3 g. (70%), m.p. 277°.[6] Specific activity: 2.8×10^3 c.p.m./mg.

Anal. Calcd. for $C_8H_{14}N_2O_3$: N, 15.05. Found: N, 15.08.

1,4-Diazospiro[4.5]decane-2,5-dione-5-C^{14} (V). A mixture of 1 g. of IV and 6 g. of β-naphthol[9] was heated for 3 hr. at 145° in an oil bath, with occasional stirring. After cooling, the yellowish residue was thoroughly extracted two or three times with ether to remove the β-naphthol. After dissolving

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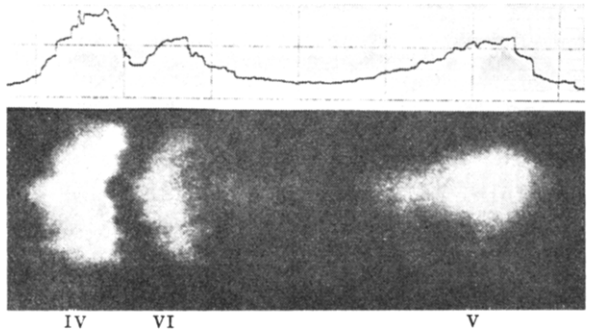
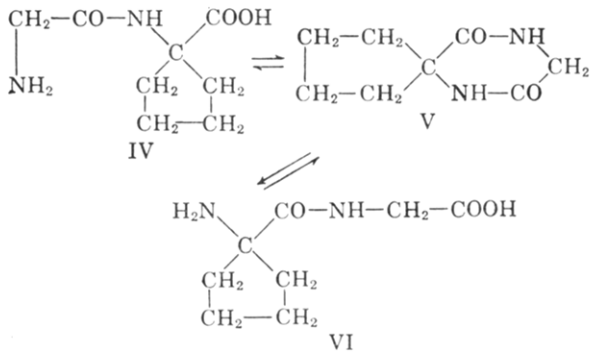


Fig. 1. Radiochromatogram after partial hydrolysis of V

in a little boiling water, the residue was purified with charcoal and crystallized out upon cooling. Yield: 0.63 g. (70%), m.p. 276°. Specific activity: 2.2×10^3 c.p.m./mg.

Anal. Calcd. for $C_8H_{12}N_2O_2$: N, 16.67. Found: 16.63.

Partial hydrolysis of 1,4-diazaspiro[4.5]decane-2,5-dione-5-C[14]. The piperazinedione (0.2 g.) was hydrolyzed by dissolving in 10 ml. of 1*N* hydrochloric acid and by boiling during 5–6 minutes. The solution was cooled and the volume was exactly completed to 10 ml. An aliquot of 0.1 ml. was submitted to paper chromatography in 2,4,6-trimethylpyridine (1 part), 2,4-lutidine (85%) (1 part), and water (2 parts), in order to separate the two peptides and the nonhydrolyzed piperazinedione.



Measure of the radioactivity (*a*) *By elution from the paper.* The paper band was sectioned and the radioactive spots of the two peptides and of the nonhydrolyzed piperazinedione were eluted with water. This water was collected on planchets which were dried under an infrared light. The radioactivity was determined using a Nuclear Chicago detector D 47. The mean actual counts for fifteen determinations were 59×10^3 c.p.m. for IV, 43×10^3 c.p.m. for VI, and 60×10^3 c.p.m. for the nonhydrolyzed V. This gave a proportion of 58% in favor of IV and 42% in favor of VI.

(*b*) *By direct measure on the paper.* The paper band was passed directly into a Nuclear Chicago Actigraph. The areas of the two first curves obtained (Fig. 1) were determined and found to be in a proportion of 58% for IV and 42% for VI.

The paper band was also placed on a Kodak X-Ray Royal Blue Film during seven days, after which time the film was developed (Fig. 1). Three spots were found and their relative intensities were of the same order as the ones found by the actigraph and the elution method.

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Diacetylorthanilamide

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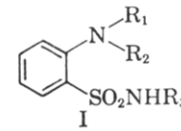
Ekbom[1] heated orthanilamide (I. $R_1 = R_2 = R_3 = H$) with acetic anhydride and obtained a product m.p. 191.5–192.5° to which he assigned the structure I ($R_1 = R_2 = COCH_3$, $R_3 = H$). Parke and



I

Williams[2] stated that acetic anhydride and orthanilamide gave mainly diacetylorthanilamide and that acetylation with pyridine and acetic anhydride yielded the same compound almost quantitatively. The latter authors reported a melting point of 190° and named this product in the Experimental section *o*-diacetylaminobenzenesulfonamide, thus assigning the same structure as Ekbom. Recently, Yale, Losee, and Bernstein[3] in referring to the work of Parke and Williams showed the structure of the acetylation product as I ($R_1 = R_2 = COCH_3$, $R_3 = H$).

We have prepared diacetylorthanilamide, m.p. 196°, using pyridine and acetic anhydride according to the procedure of Parke and Williams.[2] However, a consideration of infrared spectral[4] and pK_a[5] data shows that the compound is 2-acetylsulfamylacetanilide (I. $R_1 = H$, $R_2 = R_3 = COCH_3$). Two bands of medium to strong intensity attributable to —C=O vibrational modes are present in the infrared spectrum of diacetylorthanilamide at 5.78 μ and 6.00 μ. The band at 6.00 μ is also found in 2-sulfamylacetanilide[1] (I. $R_1 = R_3 = H$, $R_2 = COCH_3$) and clearly results from the presence of the acetamido substituent. The band at 5.78 μ corresponds well with the expected lower wave length absorption of $-C=O$ present in the group $-SO_2NHC(=O)-CH_3$ and correlates with the absorption at 5.80 μ shown by 2-acetylsulfamyl-*N*-methylacetanilide[6] (I. $R_1 = CH_3$, $R_2 = R_3 = COCH_3$). Further evidence is obtained by a consideration of absorptions due to N—H stretching vibrations. Diacetylorthanilamide has a sharp band in its infrared spectrum at 2.95 μ corresponding to the N–H absorption

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