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N-Phenylacetyl-L-cysteinyl-D-valine

BY FREDERICK W. HOLLY, ELIZABETH W. PEEL, EILEEN L. LUZ AND KARL FOLKERS

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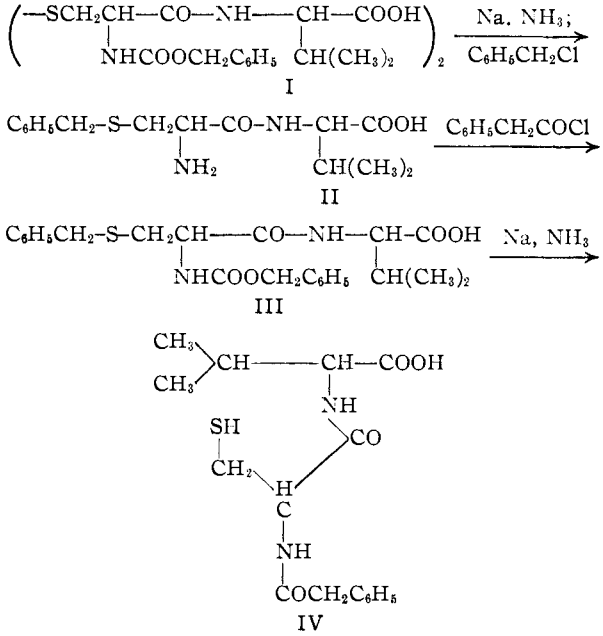
N-Phenylacetyl-L-cysteinyl-D-valine has been synthesized. Against *B. megatherium* this compound exhibited a low order of antibiotic activity and a low degree of synergistic action when combined with benzylpenicillin.

The dipeptide, N-phenylacetyl-L-cysteinyl-D-valine (IV), because of its structural relation to the penicillins, has been synthesized in order to test it for antibiotic activity. Similarly, attention has been called to the similarity in structure between the penicillins[1,2] and glutathione, and more recently, to the related dipeptides, the N-(N'-phenylacetylseryl)-D-penicillamines, which have been synthesized and been shown to be inactive as biological precursors of benzyl penicillin.[3] Dr. H. B. Woodruff of the Microbiology Department has assayed N-phenylacetyl-L-cysteinyl-D-valine for antibiotic activity. By a cup assay test using *B. megatherium* this dipeptide had antibiotic activity equivalent to 12 penicillin units per milligram at a concentration of 1 mg./ml. The sample also showed a low degree of synergistic action when combined with benzylpenicillin; when 1 mg. per ml. was added to a solution of penicillin containing 94 units per ml., the mixture assayed 164 units per ml.

A reaction of N,N'-dicarbobenzoxy-L-cystinyl chloride with D-valine formed N,N'-dicarbobenzoxy-L-cystinyl-D-valine (I). Reduction and benzylation of this product (I) yielded S-benzyl-L-cysteinyl-D-valine (II) which was converted into N-phenylacetyl-S-benzyl-L-cysteinyl-D-valine (III). Removal of the benzyl group from the intermediate III gave N-phenylacetyl-L-cysteinyl-D-valine (IV).



(1) E. Fischer, *Science*, **106**, 146 (1947).
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(3) W. Baker and W. D. Ollis, *J. Chem. Soc.*, 556 (1951).

Experimental[4]

N,N'-Dicarbobenzoxy-L-cystine.—L-Cystine was converted to the dicarbobenzoxy derivative[5] by treatment with carbobenzoxy chloride[6] in aqueous sodium hydroxide. In one typical preparation 100 g. of L-cystine yielded 176 g. of N,N'-dicarbobenzoxy-L-cystine, m.p. 112–124°, with preliminary softening at 80° (micro-block). Three recrystallizations of the material from chloroform did not improve the melting point; solubility analysis[7] in chloroform showed the product to be about 80% pure. This product was used in subsequent reactions.

N,N'-Dicarbobenzoxy-L-cystinyl Chloride.[5]—Fifteen grams of N,N'-dicarbobenzoxy-L-cystine of about 80% purity was suspended in 300 ml. of anhydrous ether, and the mixture was cooled in an ice–salt-bath. To the mixture 18 g. of powdered phosphorus pentachloride was added in one portion; the cooling bath was removed, and the mixture was shaken for 30 minutes. During this period partial solution and reprecipitation occurred, giving a white, fluffy product. A sample of this material, washed with chloroform on a porous plate, melted at 68–69°. The main fraction was collected on a filter, dissolved immediately in hot chloroform, and precipitated with petroleum ether. The crystalline product was collected on a filter and used immediately in a synthesis of N,N'-dicarbobenzoxy-L-cystinyl-D-valine.

Although the above procedure was satisfactory for preparing small quantities (0.03 mole) of the acid chloride, it was not consistently applicable for the preparation of larger quantities (0.2 mole). Use of other solvents and changing the order of addition of reagents did not improve the synthesis, and accordingly the acid chloride was prepared in small amounts and was used immediately.

N,N'-Dicarbobenzoxy-L-cystinyl-D-valine (I).—To a solution of 5.0 g. of D-valine[8] in 75 ml. of water containing 2.8 g. of sodium hydroxide at −5° was added N,N'-dicarbobenzoxy-L-cystinyl chloride (prepared from 10 g. of the acid) during a five-minute period with stirring. The mixture was stirred at −5° for 40 minutes and warmed to 20° during a 15-minute period. Concentrated hydrochloric acid was added until the solution was about *p*H 1. The white crystalline solid that formed was collected on a filter and washed with water. The crude product was dissolved in warm dioxane and was reprecipitated with water giving 11 g. of impure dipeptide, m.p. 115–130°. Two recrystallizations from ethyl acetate yielded 4 g. of N,N'-dicarbobenzoxy-L-cystinyl-D-valine (I), m.p. 165–172° (micro-block); $[\alpha]^{23}D$ −86.8° (*c* 0.908 in dimethylformamide).

Anal. Calcd. for $C_{32}H_{42}N_4O_{10}S_2$: C, 54.37; H, 5.99; N, 7.93. Found: C, 54.07; H, 6.32; N, 7.20.

S-Benzyl-L-cysteinyl-D-valine (II).—Two grams of N,N'-dicarbobenzoxy-L-cystinyl-D-valine was dissolved in 50 ml. of liquid ammonia. To the solution was added 0.8 g. of sodium in portions until a deep blue color persisted for several minutes. Then 0.72 ml. of benzyl chloride was added to the solution, and the ammonia was allowed to evaporate.

The white residue was dissolved in water and the cloudy solution was extracted twice with ether; the aqueous solution was acidified with dilute hydrochloric acid to *p*H 5–6. The crystalline precipitate that formed was collected on a filter, washed with water, and dried *in vacuo* to give 1.8 g. of crude product. Two recrystallizations from ethanol gave

(4) We are indebted to Mr. Richard Boos and his associates for the microanalyses.
(5) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).
(6) Carter, Frank and Johnson, *Org. Syntheses*, **23**, 13 (1943).
(7) We are indebted to Mr. F. A. Bacher for the solubility analysis data.
(8) Obtained by resolution of DL-valine; E. Fischer, *Ber.*, **39**, 2320 (1906).

1 g. of S-benzyl-L-cysteinyl-D-valine (II), m.p. 204–209°, transition at 185–195° (micro-block); $[\alpha]^{23}D$ +23.5° (*c* 1.63 in 2.5 *N* hydrochloric acid).

Anal. Calcd. for $C_{15}H_{22}N_2O_3S$: C, 58.04; H, 7.15; N, 9.03. Found: C, 58.31; H, 7.10; N, 8.79.

N-Phenylacetyl-S-benzyl-L-cysteinyl-D-valine Triethylamine Salt.—A solution of 3.6 g. of S-benzyl-L-cysteinyl-D-valine in 11.5 ml. of 1.01 *N* sodium hydroxide was cooled until it was partially frozen and 12.7 ml. of 1.01 *N* sodium hydroxide was added. With stirring, 1.7 ml. of phenylacetyl chloride was added to the partially frozen solution. The mixture was stirred at 0° for 30 minutes and an additional 30 minutes with no external cooling. The clear solution was acidified with dilute hydrochloric acid to *p*H 1 and was extracted twice with ether. After the ether extract was dried over sodium sulfate, 2 ml. of triethylamine was added to the clear ether solution. A crystalline product formed that was recrystallized from a mixture of acetone and ether to give 4.3 g. of the triethylamine salt of N-phenylacetyl-S-benzyl-L-cysteinyl-D-valine, m.p. 111–121°, which was used without further purification in the preparation of the dipeptide (IV).

A sample was also prepared by dissolving crude N-phenylacetyl-S-benzyl-L-cysteinyl-D-valine in acetone, adding triethylamine (20% excess), and then adding ether until crystallization started. The solution was cooled and the crystalline product was removed, washed with acetone–ether, and with ether, and dried to give N-phenylacetyl-S-benzyl-L-cysteinyl-D-valine triethylamine salt, m.p. 127–130°; $[\alpha]^{23}D$ −15.1° (*c* 1.52 in chloroform).

Anal. Calcd. for $C_{29}H_{43}N_3O_4S$: C, 65.75; H, 8.18; N, 7.93. Found: C, 65.65; H, 7.88; N, 7.56.

N-Phenylacetyl-L-cysteinyl-D-valine (IV).—Four grams of N-phenylacetyl-S-benzyl-L-cysteinyl-D-valine triethylamine salt was dissolved in 50 ml. of liquid ammonia; 0.9 g. of sodium was added in portions until a permanent blue color formed. To the solution, 5 g. of ammonium sulfate was added and the ammonia was allowed to evaporate. The residue was dissolved in water, the solution was extracted with ether, and the aqueous solution was acidified with 2 *N* sulfuric acid to *p*H 1. The crystalline product that separated was collected on a filter, washed with water, and dried *in vacuo* to give 2.7 g. of N-phenylacetyl-L-cysteinyl-D-valine (IV), m.p. 178–188°, softening at 173° (micro-block). After five recrystallizations from water–methanol the product melted at 174–193°, $[\alpha]^{23}D$ −45.3° (*c* 0.852 in 0.5 *N* ammonium hydroxide).

Anal. Calcd. for $C_{18}H_{22}N_2O_4S$: C, 56.78; H, 6.55; N, 8.28. Found: C, 56.99; H, 6.68; N, 8.08.

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[CONTRIBUTION FROM THE NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, FEDERAL SECURITY AGENCY]

Analogs of the Carcinogen 2-Acetylaminofluorene: The Isomeric 4-Acetylaminofluorene[1]

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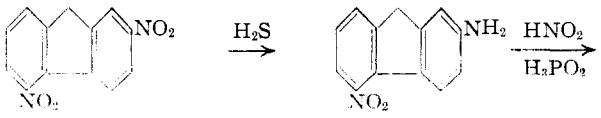
A direct synthesis of 4-acetylaminofluorene, an isomer of the carcinogen 2-acetylaminofluorene, is described. The ultraviolet absorption spectra of both compounds in relation to their structures are discussed. Preliminary results indicate that the 4-isomer is not an active carcinogen.

Since the discovery of the carcinogenic effect of 2-acetylaminofluorene[3] a number of related compounds have been prepared and tested for the purpose of studying the effect of chemical structure on biological activity.[4-9]

Of the possible acetylaminofluorenes the 4-isomer has so far been unknown. It is the purpose of this paper to describe a convenient synthesis of this compound achieved according to the reaction scheme



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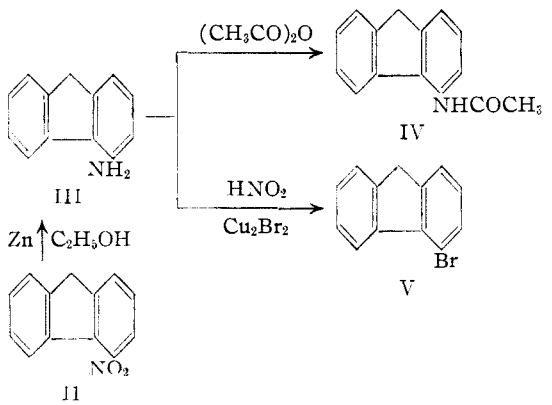

The dinitration of fluorene and separation of 2,7- and 2,5-dinitrofluorene was carried out according to Courtot.[10]

Monoreduction of 2,5-dinitrofluorene with hydrogen sulfide in ammoniacal ethanol gave 2-amino-5-nitrofluorene as a red gummy material, which did not crystallize satisfactorily from a variety of solvents. The addition of dilute sulfuric acid to a hot acetic acid solution of the product, however, resulted in the deposition of the crystalline acid sulfate salt. After this treatment the free base obtained from the salt crystallized readily.

(1) Presented at the Meeting of the XIIth International Congress of Pure and Applied Chemistry, New York, September, 1951.

(2) Public Health Service Research Fellow of the National Cancer Institute, 1949–1951.

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(4) C. Hoch-Logeti, *Brit. J. Cancer*, **1**, 391 (1947).

(5) E. C. Miller, J. A. Miller, R. B. Sandin and R. K. Brown, *Cancer Research*, **9**, 504 (1949).

(6) H. P. Morris, C. S. Dubnik and J. M. Johnson, *J. Natl. Cancer Inst.*, **10**, 1201 (1950).

(7) H. P. Morris and C. S. Dubnik, *Cancer Research*, **10**, 233 (1950).

(8) E. K. Weisburger, THIS JOURNAL, **72**, 1758 (1950).

(9) E. K. Weisburger, J. H. Weisburger and F. E. Ray, *J. Org. Chem.*, **16**, 1697 (1951).

(10) Ch. Courtot and J. Moreaux, *Compt. rend.*, **217**, 453 (1943).