of water and the precipitated material was extracted with 200 ml. of ether in three portions. The combined ether extracts were dried over magnesium sulfate and the solvent was evaporated on the steam-bath; crude yield 2.80 g., m.p. 156–170° dec. This material was shown to be 74% hydroperoxide, using iodometric assay in ethanol,[10] or to represent a yield of 59%.

Two recrystallizations of this crude material gave 2.10 g. of white leaflets from ethanol–water, m.p. 190–191° dec.

Fifty mg. of the material from alcohol–water was dissolved in benzene and put on a 14-cm. alumina column and eluted with one liter of benzene. At this point, a small band which was visible only under ultraviolet light and which had the purple fluorescence characteristic of authentic tri-(*p*-nitrophenyl)-carbinol had moved about 5 cm. A very minute yellow band remained at the very top of the column. Chloroform was then passed through the column and the carbinol band was removed to give *ca.* 5 mg. of material melting at 157–160°. Following this material there appeared the hydroperoxide which was invisible under both ultraviolet and white light and which melted at 186.5–187.5 dec. The small yellow band remained at the top of the column. *Anal.* Calcd.: C, 55.55; H, 3.19. Found: C, 55.80; H, 3.28.

The material melting at 190–191° gave infrared absorption peaks characteristic of O–H stretching (2.95 μ) and O–O stretching[11] (shoulder at 11.7 μ) in a Nujol mull. No carbonyl absorption was indicated.

C. Hydrogen Iodide Cleavage.—Excess sodium iodide was added to 179.7 mg. of hydroperoxide dissolved in 20 ml. of absolute ethanol and the solution heated to incipient boiling, cooled, excess thiosulfate added and the solution flooded with water. The solution was warmed and allowed to cool slowly giving 150.7 mg. (87%) of tri-(*p*-nitrophenyl)-carbinol, m.p. 190–191°.[12] The infrared spectrum of a Nujol mull showed absorption peaks characteristic of O–H stretching (2.90 μ) and tertiary carbon–oxygen stretching (7.10 μ) were observed. No absorption was observed at the carbonyl and peroxide wave lengths.

Oxidation of this material with excess chromic acid in boiling acetic acid gave a 10% yield of long white needles which melted at 189–190°,[13] and depressed the melting point of the starting material, indicating that this product is 4,4'-dinitrobenzobenzophenone.

The Reaction of Tri-(*p*-nitrophenyl)-methide Ion with Tri-(nitrophenyl)-methyl Hydroperoxide.—In a one-liter flask closed with a stopper bearing a stopcock was placed 249 mg. of hydroperoxide, 218 mg. of hydrocarbon and 1.0 g. of potassium hydroxide. The flask was flushed with nitrogen, 100 ml. of absolute ethanol was added and the flask stoppered. The flask outlet was opened to a vacuum line and the solvent briskly boiled to expel residual oxygen and the stopcock was then closed. The blue solution then was shaken occasionally and allowed to stand at room temperature for ten hours. Crystals were noted on the bottom and sides of the flask. These were separated by decantation, washed with alcohol and the mother liquor and washings combined and quickly flooded with water. The crystals were triturated with alcohol–water and removed by filtration. The crystals were bright green and decolorized rapidly in air to give a yellow solution. The crystals appeared to be dark brown, but when scratched appeared green. Solution of the crystals in ethyl acetate resulted in rapid decolorization.[14]

Evaporation of the ethyl acetate followed by the addition of pentane precipitated long yellow needles melting at 155–157°. Continued recrystallization lowered and broadened the melting point range. The crude yield of this unidentified material was estimated at 50 mg.

The original mother liquor was extracted with ethyl acetate, separated, dried and the solvent removed in an air jet. The yield of crude material was 201 mg. This material was recrystallized three times from ethanol–water to give 110 mg. of material melting at 165–170°. The mother liquor from the first recrystallization deposited leaflets of hydroperoxide. The material melting at 165–170° was chromatographed on a small alumina column using benzene to develop the chromatogram. Three bands were observed: a small yellow band at the top of the column, a fast purple-red band (hydrocarbon) and a slower moving band invisible in white light but purple under ultraviolet radiation. This band was collected separately by elution with chloroform and recrystallized from ethanol water to give *ca.* 100 mg. of short white needles melting at 189–189.5°, and showing no depression when mixed with carbinol obtained from cleavage of the hydroperoxide.

The Reaction of Tri-(*p*-nitrophenyl)-methyl Hydroperoxide with Ethanolic Hydroxide Ion.—A solution of 118.9 mg. of tri-(*p*-nitrophenyl) hydroperoxide melting at 190–191° was made up of 25.00 ml. at 43.2 ± 0.1° in 0.0870 *N* potassium hydroxide in absolute ethanol. The rate of appearance of *p*-nitrophenol was followed by periodically observing the optical density of the solution at 460 mμ with a Beckman model DU spectrophotometer. It was found that a maximum of 12% reaction had occurred after 11 hours at 43.2°. After standing at this temperature for three weeks 15 ml. of the solution was flooded with water and extracted with ether. Recrystallization of the residue obtained on evaporation of the ether gave 35 mg. of material melting at 180–191° without decomposition and giving no hydroperoxide test. One more recrystallization from ethanol–water raised the melting point to 192°. A mixed melting point with hydroperoxide was depressed at 175° and the compound gave an infrared absorption spectrum in a Nujol mull identical with authentic carbinol.

A control experiment which contained no base was carried out simultaneously. After three weeks the presence of hydroperoxide was detected easily with potassium iodide. Treatment of a small sample with solid potassium carbonate produced an immediate yellow coloration which was discharged on the addition of acid.

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(14) K. Ziegler and E. Boye (ref. 5a) report an identical description of the tri-(*p*-nitrophenyl)-methyl radical.

Galactosyl Glycerol from *Gelidium pristoides* and *Gracilaria confervoides*

BY JOHN R. NUNN AND MAVIS M. VON HOLDT

RECEIVED OCTOBER 13, 1954

The recently reported work of Putman and Hassid[1] on the structure of α-D-galactopyranosyl-2-glycerol from the red alga, *Irideae laminaroides*, has prompted us briefly to record similar work which was in progress in this Laboratory at the time of the appearance of the above report.

Methanolic extraction of *Gelidium pristoides* and subsequent deionization (using Amberlite IR 120 and IR 4B resins) of the product yielded a sirup, which was chromatographed on a charcoal–Celite column.[2] The main fraction (1% of dried seaweed) crystallized in colorless needles, m.p. 127–127.5°, $[\alpha]^{19}D$ 172° (*c* 0.70, water) (Found: C, 42.8; H, 7.2. Calcd. for $C_9H_{18}O_8$: C, 42.5; H, 7.2). It was nonreducing to Fehling solution. This substance was hydrolyzed (*N* sulfuric acid for 4 hours), and the products, which were separated by paper chromatography, were shown to be glycerol (tri-*p*-nitrobenzoate, mixed m.p. 191–193°), and galactose (mucic acid, mixed m.p. 215°). A quantitative estima-

(1) E. W. Putman and W. Z. Hassid, THIS JOURNAL, **76**, 2221 (1954).

(2) R. L. Whistler and D. F. Durso, *ibid.*, **72**, 677 (1950).

tion of glycerol and galactose in the hydrolysate revealed a molar ratio of 1.0:0.95. When the substance was oxidized in 0.02 *M* sodium metaperiodate solution in the usual way, formic acid (0.94 mole per mole of substance) was liberated, and 1.99 moles periodate per mole of substance was consumed. This, together with the fact that formaldehyde could not be detected in the reaction mixture, indicated that the galactose was linked through its reducing group to C2 in the glycerol; thus showing that the substance was galactosyl-2-glycerol, and corroborating the work of Putman and Hassid.[1] The glycoside was obtained from *Gracilaria confervoides* in 0.8% yield.

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NATIONAL CHEMICAL RESEARCH LABORATORY
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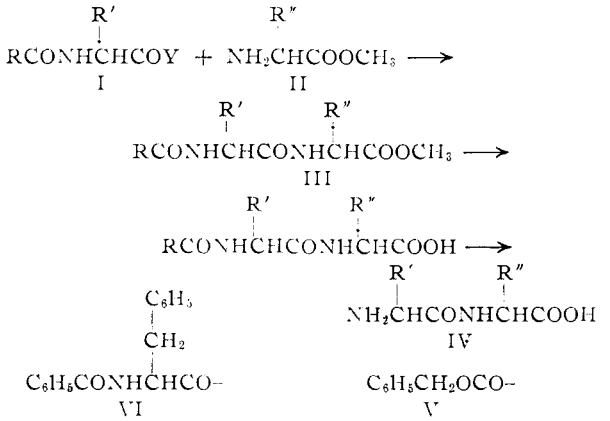
Enzymatic Removal of the Protecting Group in Peptide Synthesis[1]

BY ROBERT W. HOLLEY

RECEIVED DECEMBER 28, 1954

Peptide synthesis, in the laboratory, usually involves the reaction of an N-acylamino acid derivative I with an amino acid ester, II. The product III is saponified, and the acyl protecting group is removed to give the peptide IV. The classical example of an N-acyl protecting group is the carbobenzoxy group V.



In the synthesis of large peptides, particularly those containing amino acids with reactive side chains, a variety of protecting groups is needed in order to permit the selective removal of the protecting group from one reactive group in the molecule, allowing reaction with this group while other reactive groups remain protected. With this need in mind, we have been searching for new protecting groups which might be removed under highly selective conditions, and have investigated the possibility of using a protective group which can be removed by a hydrolytic enzyme. In the initial experiments, chymotrypsin was chosen as the enzyme and the benzoyl-L-phenylalanyl group VI was chosen as the protecting group. It is known that chymotrypsin catalyses the hydrolysis of benzoyl-L-phenylalanine amides.[2]

L-Leucyl-L-leucine (IV, R′ = R″ = $(CH_3)_2$-$CHCH_2$—) has been prepared in this way. Benzoyl-L-phenylalanine ethyl ester was converted to the hydrazide, and to the azide, and the azide was allowed to react with L-leucine methyl ester. The product, benzoyl-L-phenylalanyl-L-leucine methyl ester, was converted to the hydrazide, and the corresponding azide was reacted with L-leucine methyl ester. The benzoyl-L-phenylalanyl-L-leucyl-L-leucine methyl ester, which was obtained, was saponified, and the benzoyl-L-phenylalanyl group was removed by hydrolysis catalyzed by chymotrypsin. Yields throughout the synthesis were good. An 80% yield of L-leucyl-L-leucine hydrate was obtained in the final, enzyme-catalyzed step. As would be anticipated from the known specificity of chymotrypsin, the only products were benzoyl-L-phenylalanine and L-leucyl-L-leucine.

To the best of our knowledge, the enzymatic removal of a protecting group has not been used before in the synthesis of peptides. The present work establishes that the general approach can be used successfully. However, work with various hydrolytic enzymes and various protecting groups will be required to establish the scope of the procedure. One limitation is clear. If a proteolytic enzyme such as chymotrypsin is used, the peptide which is to be synthesized must not contain a linkage susceptible of hydrolysis by the enzyme. Also, in some instances the separation of the enzyme from the peptide which has been synthesized may cause difficulty.

Although it is not anticipated that enzymatic removal of a protecting group, as illustrated here with the benzoyl-L-phenylalanyl group, will compete with the use of the carbobenzoxy group in the synthesis of simple peptides, it is possible that this type of approach may be of value in special problems of peptide synthesis.

Experimental[3]

Benzoyl-L-phenylalanine Hydrazide.—A mixture of 2.2 g. (7.4 mmoles) of benzoyl-L-phenylalanine ethyl ester,[4] 8 ml. of absolute ethanol and 0.80 ml. of hydrazine hydrate was heated at 70–80° for 4 hr. The mixture was cooled and filtered, and the crystalline hydrazide was washed with cold absolute ethanol. It was recrystallized from 34 ml. of 95% ethanol; 1.6 g. (76%); m.p. 196–199°. It was recrystallized for analysis.

Anal. Calcd. for $C_{16}H_{17}N_3O_2$: N, 14.8. Found: N, 14.8.

Benzoyl-L-phenylalanyl-L-leucine Methyl Ester.—A mixture of 1.1 g. (6.0 mmoles) of L-leucine methyl ester hydrochloride and 25 ml. of ether was cooled in an ice-bath and 12.5 ml. of cold 50% (w./v.) potassium carbonate solution was added. The mixture was shaken and the ethereal solution was separated. The aqueous layer was washed with 5 ml. more ether and the ethereal solutions were combined and dried over anhydrous sodium sulfate at 0°, during preparation of the azide described below.

(1) Journal Paper No. 994, New York State Agricultural Experiment Station. This investigation was supported in part by research grants, G-3435 and G-3435(C), from the National Institutes of Health, Public Health Service.

(2) H. Neurath and G. W. Schwert, *Chem. Revs.*, **46**, 69 (1950).

(3) All melting points were determined on a microscope hot-stage and are corrected. Analyses were performed by Dr. G. Weiler and Dr. F. B. Strauss, Oxford, England.

(4) S. Kaufmann and H. Neurath, *Arch. Biochem.*, **21**, 437 (1949).