Drs. R. G. Hayter and R. D. Feltham for discussions and their interest in this work.

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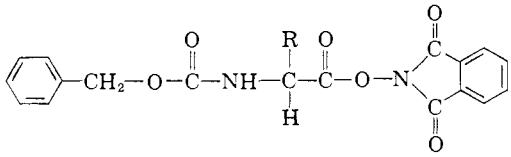
RECEIVED JANUARY 9, 1961

A NOVEL ACTIVATED ESTER IN PEPTIDE SYNTHESES

Sir:

Bodanszky and du Vigneaud[1,2] have introduced the *p*-nitrophenylesters of N-carbobenzoxyamino acids for the synthesis of oligopeptides. The advantage of this method is obvious and has been discussed thoroughly by these authors. In extending this conception it seemed of interest to us to study activated esters of the general type (I) for their properties as N-acylating agents.



I, R = -H

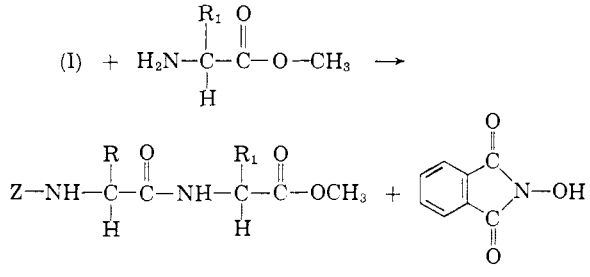
= $-CH_2-CH(CH_3)_2$ (L)

= $-CH_2-C_6H_5$ (D, L and L)

= $-CH_2-S-CH_2-C_6H_5$ (L)

and Z-L-prolyl-hydroxyphthalimide ester

These activated esters (I) are synthesized readily from N-carbobenzoxyamino acids and N-hydroxyphthalimide[5,6] in the presence of dicyclohexylcarbodiimide.[3] They crystallize with ease from ethanol or carbon tetrachloride and have been obtained in yields of 40–80% of the theory depending on the amino acid employed. In the presence of another amino acid ester, the active esters (I) react within seconds, at 0° and in quantitative fashion with formation of a protected dipeptide according to the equation



The N-hydroxyphthalimide can be removed completely by shaking the reaction mixture with an aqueous solution of sodium bicarbonate in which the anion of the former is readily soluble with formation of a brilliant red coloration. With this method Z-L-leucyl-L-leucine-methyl ester and Z-glycyl-D,L-phenylalanine methyl ester have been synthesized in excellent yield and purity.

(1) M. Bodanszky, *Nature*, **183**, 1324 (1959).
(2) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
(3) D. F. Elliot and D. W. Russell, *Biochem. J.*, **66**, 49P (1957).

The tripeptide Z-glycyl-L-phenylalanylglycine methyl ester, synthesized by stepwise addition of glycine-ethyl ester to Z-L-phenylalanylhydroxyphthalimide ester, then decarbobenzoxylation and a new addition of Z-glycylhydroxyphthalimide ester, showed no trace of racemization in the test according to Anderson and Callahan.[4]

For the preparation of N-hydroxyphthalimide 1 mole of hydroxylamine hydrochloride and 2 moles of triethylamine are heated in 500 ml. of absolute ethanol until complete dissolution has occurred. To the hot solution 1 mole of N-carboethoxyphthalimide[5,6] is added at once with mechanical stirring. The solution changes to a deep red, owing to the formation of the triethylammonium salt of N-hydroxyphthalimide.

After immediate cooling to room temperature the solution is poured into 3 l. of acidified water. The product crystallizes spontaneously in form of fine, nearly colorless needles. After filtration, washing with water and drying over P_2O_5 *in vacuo* the product is suitable for the preparation of activated esters; m.p. 230°; yield 70% (of theoretical).

The substance otherwise is identical with the previously described compound.[7,8] Details of this procedure will be published shortly.

Acknowledgment.—We are indebted to Professor F. Zilliken for valuable discussions.

(4) G. W. Anderson and F. M. Callahan, *J. Am. Chem. Soc.*, **80**, 2902 (1958).
(5) G. H. L. Nefkens, *Nature*, **185**, 309 (1960).
(6) G. H. L. Nefkens, G. I. Tesser and R. J. F. Nivard, *Rec. trav. chim.*, **79**, 688 (1960).
(7) L. Cohn, *Ann.*, **205**, 295 (1880).
(8) N. I. Putokhin, *J. Russ. Phys. Chem. Soc.*, **62**, 2203 (1930).

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RECEIVED JANUARY 6, 1961

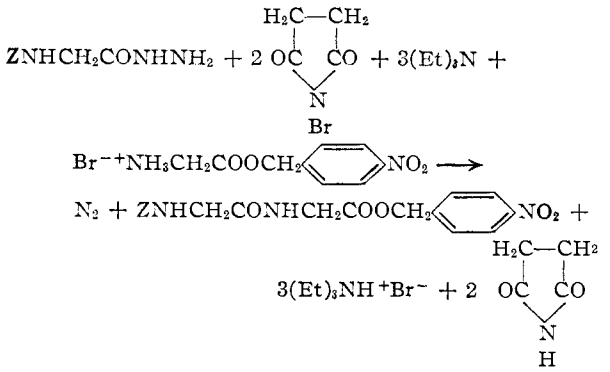
PEPTIDE SYNTHESIS VIA OXIDATION OF HYDRAZIDES

Sir:

Recently it was observed[1] that γ-glutamylhydrazide reacts with two equivalents of N-bromosuccinimide (NBS) to give pyrrolidonecarboxylic acid and nitrogen in quantitative yield. Accordingly, it appeared that this procedure under proper conditions might serve as a new method for peptide synthesis.

Z.Gly.$NHNH_2$ and Gly.$OBz(NO_2)$.HBr[2] were coupled instantaneously upon addition of two equivalents of NBS. The reaction was carried out in an ice-bath in the presence of three equivalents of triethylamine (TEA) using tetrahydrofuran (THF) as solvent. Z.Gly-Gly.$OBz(NO_2)$ was isolated after two minutes from the reaction mixture by addition of about 5 volumes of water, and recrystallized from ethanol–water, m.p. 99°, yield 86% (*Anal.* Calcd. for $C_{18}H_{18}O_7N_3$: C, 56.85; H, 4.77; N, 10.47. Found: C, 57.07; H, 4.95; N, 10.51). The over-all reaction may be summarized as shown

(1) P. M. Gallop, S. Seifter and C. Franzblau, unpublished results.
(2) Z, benzyloxycarbonyl; $OBz(NO_2)$, *p*-nitrobenzyl ester.



Employing dioxane, dimethylacetamide, and acetonitrile as solvents for the above reaction, yields ranging from 82 to 86% were obtained. Dimethylformamide gave a yield of 40% which may be consistent with the observation that this solvent is oxidized by NBS.

These dipeptides were prepared as above in THF: Z.D.Ala-Gly.OBz(NO_2), m.p. 116–117° (crystallized from ethanol-water), yield 75% (*anal.* Calcd. for $C_{20}H_{21}N_3O_7$: C, 57.83; H, 5.10; N, 10.11. Found: C, 58.09; H, 5.29; N, 9.81), Z_2.Lys-Gly.OBz(NO_2), m.p. 94° (crystallized from ethanol–water), yield 83% (*anal.* calcd. for $C_{31}H_{34}N_4O_9$: C, 61.37; H, 5.65; N, 9.24. Found: C, 61.73; H, 5.82; N, 9.19), Z_2Lys-Glu.OBz-OEt (NO_2), m.p. 105–106° (crystallized from ethanol-water), yield 82% (*anal.* Calcd. for $C_{36}H_{42}N_4O_{11}$: C, 61.18; H, 5.99; N, 7.93. Found: C, 61.44; H, 6.12; N, 8.26), Z.Gly-Phe.OEt oil[3] yield 76%.

Racemization was tested using the procedure of Anderson and Callahan.[4] Z.Gly-Phe.OEt was converted to the hydrazide m.p. 142°[3] in 94% yield and treated with Gly.OEt and NBS in THF. After crystallization of the product from 2% solution in ethanol, 1.1% of the D,L form (m.p. 118–131°) came out first and the L form (m.p. 116–117°) came out later in 63% yield.

Since carboxyl activation may occur in the presence of a free amino group under the above conditions, the method appeared suitable for the polymerization of tripeptide hydrazides. By treating Pro-Gly-Gly.$NHNH_2$[5] in dimethylacetamide with NBS and isolating the material left after dialysis against water, a polymer $(PGG)_n$ was obtained of molecular weight of 1400 as determined by dinitrophenylation with dinitrofluorobenzene.

The method is simple, rapid and should have application to syntheses in which side chains which are sensitive to NBS oxidation are absent.

This work was supported by grant A-3083 of the National Institutes of Health, U.S.A. Public Health Service, and by the National Science Foundation grant NSF-G-13957 (to P. M. G.).

(3) G. W. Kenner and R. J. Stedman, *J. Chem. Soc.*, 2069 (1952).

(4) G. W. Anderson and F. M. Callahan, *J. Am. Chem. Soc.*, **80**, 2902 (1958).

(5) F. F. King, J. W. Clark-Lewis, D. A. A. Kidd and G. R. Smith, *J. Chem. Soc.*, 1039 (1954).

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RECEIVED DECEMBER 30, 1960

BOOK REVIEWS

Amino Acids, Proteins and Cancer Biochemistry. Papers presented at the Jesse P. Greenstein Memorial Symposium, Division of Biological Chemistry, American Chemical Society, September 16, 1959. Edited by John T. Edsall. Academic Press Inc., 111 Fifth Avenue, New York 3, N. Y. 1960. ix + 244 pp. 16 × 23.5 cm. Price, $7.00.

This symposium was designed to portray some of the major current developments in the two fields of Greenstein's greatest interest, those of amino acid and protein chemistry, and of the biochemistry of cancer. The first chapter is an account of Greenstein's life and work, by Edsall and Meister, and the book ends with a bibliography of his writings. The body of the book consists of ten reviews. All are well written, and should be easily comprehensible to chemists in other specialities. As might be expected from a symposium, each paper reflects the point of view of its authors, although all are well documented.

Six papers deal with amino acids and proteins. Winitz, *et al.*, describe the use of amino acids in chemically defined diets. The value of such diets in metabolic studies is illustrated by experiments on glycine formation in the rat. Meister discusses amino acid activation and peptide bond biosynthesis; his studies on the specificity of the tryptophan-activating enzyme are particularly interesting. Scheraga, *et al.*, give physico-chemical evidence for the existence of internal hydrogen bonds in ribonuclease, and propose a three-dimensional molecular model for the enzyme. Neurath, *et al.*, review their elegant studies on the structure and function of pancreatic carboxypeptidase A, with particular reference to the role of metals. Sober and Peterson discuss the evaluation of protein mixtures by column chromatography.

A transition to the second subject of the book is effected by the paper of Roberts and Simonsen, on free amino acids and related substances in normal and neoplastic tissues, with special reference to glutamine. Kit discusses the nucleic acids of normal tissues and tumors, and summarizes the evidence (as of 1959) on the nuclear synthesis of RNA. Weinhouse provides a critical review of enzyme activities and tumor progression. He discusses (1), Greenstein's conclusion that tumors, in their biochemical characteristics, resemble one another more closely than they resemble their tissues of origin; (2) the deletion hypothesis of carcinogenesis proposed by the Millers; and (3) his (Weinhouse's) disagreement with the Warburg hypothesis that the cancer process is initiated by the deletion of a crucial stage in respiration, so that fermentation, rather than oxidation of glucose, supplies the energy required for growth. Racker, Wu and Alpers also discuss carbohydrate metabolism in tumor cells. They are in partial agreement with both the Warburg and Weinhouse schools, but make the interesting suggestion that in intact cells glycolysis is limited by the availability of inorganic phosphate; since the transport of phosphate is slow, its availability is limited by its regeneration from ATP; hence rapidly growing cells that convert ATP to ADP in the course of many synthetic processes can express their glycolytic capacity more effectively than other cells. A similar idea,