

error, with the sum of the three peaks lying downfield, indicating that the doublet at 2.92 ppm is half of a symmetrical AB system ($J_{H\alpha\beta}/\Delta\nu = 0.13$) associated with protons H_α and H_β , one variety *cis* and the other *trans* to a BCl_2 group. The postulated overlap in the assigned H_α peak is verified by the intensity ratio of the resolved doublet at 2.92 ppm (calcd, 1.27; found 1.28).⁴

In the above discussion it has been assumed that both adducts are single, crystalline isomers, resulting from *cis* addition of B_2Cl_4 to the carbon-carbon π bonds of a conjugated system. That such an interpretation is correct is implied by the simplicity of the proton resonance spectra of these adducts. Further, in the case of adduct I, the relatively narrow melting range (46.5–47.5°) supports this view. (This latter criterion could not be applied to adduct II which, as mentioned above, decomposes before its melting point.)

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Martel Zeldin, Thomas Wartik
Department of Chemistry
The Pennsylvania State University
University Park, Pennsylvania
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Racemization Control in the Synthesis of Peptides by the Mixed Carbonic-Carboxylic Anhydride Method

Sir:

Of presently known methods for forming the peptide bond, only the azide procedure is considered to be safe to use to avoid racemization in sensitive cases.¹ Consequently, the synthesis of peptides by the stepwise addition of amino acids to the amino end of the growing peptide chain has become the most common procedure, because racemization is unlikely when commonly used amino-protecting groups and methods for forming the peptide bond are used. Since the azide procedure has limitations,² reliable methods of coupling suitably protected peptides are badly needed to avoid the inherent disadvantages of the stepwise approach, particularly when long-chain peptides are desired. Our reinvestigation of the mixed carbonic-carboxylic anhydride procedure,³ briefly reported here, indicates that it might fill the need when reaction conditions are suitably controlled.

We have recently reported⁴ that racemization is proportional to the time allowed for mixed anhydride formation (activation time), using our test system of forming the mixed anhydride between Z-Gly-L-Phe-OH and ethyl carbonate by the reaction of ethyl chloroformate with the dipeptide derivative in the presence of triethylamine, adding H-Gly-OEt, isolating Z-Gly-Phe-Gly-OEt, and separating the racemate by fractional crystallization from ethanol. Since yields are a

(1) E. Schröder and K. Lübke, "The Peptides," Vol. 1, Academic Press Inc., New York, N. Y., 1965, p 326. Abbreviations used here are standard, and are described in this book.

(2) Reference 1, p 82.

(3) T. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1951); R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951); J. R. Vaughan, Jr., and R. L. Osato, *J. Am. Chem. Soc.*, **73**, 3547 (1951).

(4) G. W. Anderson, F. M. Callahan, and J. E. Zimmerman, *Acta Chim. Hung.*, **44**, 51 (1965).

little better if isobutyl chloroformate is the reagent,⁵ we have subsequently used this compound routinely. Although several other solvents have given good results, tetrahydrofuran was used in the experiments reported here.

The most important factor has been found to be the nature of the tertiary amine used. Most of our studies have been done at a reaction temperature of -15° . Yieldwise, optimum activation times vary with the tertiary amine used; with 1 equiv of amine, a 4-min activation time is more than adequate for any useful amine. We have routinely allowed 12 min before adding the ethyl glycinate in order to exaggerate racemization when it occurs. Workup of reaction mixtures was the customary removal of solvent under vacuum, solution of the product in ethyl acetate, and washing of this with water, bicarbonate solution, water, 1 *N* hydrochloric acid, and water. After drying over sodium sulfate, the ethyl acetate solution was concentrated under vacuum and the crystalline product was fractionally crystallized from a 2% solution in ethanol.

Under the above conditions, racemization could not be avoided completely when triethylamine was used. With trimethylamine, using exactly 1 equiv or a 5% excess of isobutyl chloroformate to ensure no excess of the amine, a 90% yield of pure Z-Gly-L-Phe-Gly-OEt was obtained and no racemate; in contrast, with 2 equiv, only a trace of L isomer was obtained, but 68% of DL isomer was isolated.

Another test was the synthesis of For-Phe-Gly-OEt (L) in 82% yield with no racemization by the use of 1 equiv of $(CH_3)_3N$ with 10% excess of isobutyl chloroformate, and a 1.5-min activation time at -6° ; Sheehan and Yang⁶ reported a 50% yield of racemate by a conventional mixed anhydride procedure, and they were unable to avoid racemization by changing reaction conditions. Likewise, Z-Val-Tyr-Ileu-OMe was obtained by us in 84% yield without racemization, whereas Schwarz and Bumpus⁷ reported considerable racemization.

Of numerous aliphatic tertiary amines, those containing at least one methyl group were generally useful in giving good yields and no racemate when not used in excess, and those not containing a methyl group were not, indicating the importance of steric factors. It was now of interest to test inductive effects. One amine tested, N-methylmorpholine, was found to give 94% yields of pure L peptide and no DL when either 1 or 2 equiv was used. Several other promising amines are being investigated.

Williams and Young⁸ have found that benzoyl-L-leucine is more readily racemized than Z-Gly-L-Phe-OH and have developed as a test system the activation of this compound and subsequent reaction with H-Gly-OEt. The amount of racemate present in the resulting Bz-Leu-Gly-OEt is estimated by comparing the optical rotation with that of the pure material and by saponifying and fractionally crystallizing the resulting Bz-Leu-Gly-OH. We have found that when small amounts of racemate are present in the ester, these may be detected by ether extraction and fractionally crystallizing the

(5) J. R. Vaughan, Jr., and R. L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).

(6) J. C. Sheehan and D.-D. H. Yang, *ibid.*, **80**, 1154 (1958).

(7) H. Schwarz and F. M. Bumpus, *ibid.*, **81**, 890 (1959).

(8) M. W. Williams and G. T. Young, *J. Chem. Soc.*, 881 (1963).

extracted material from alcohol-water. Thus, when 10 mg of DL-Bz-Leu-Gly-OEt was added to 1.00 g of the L isomer, 30% of the DL form was readily recovered by this procedure.

Application of this technique to the reaction product of 1 equiv of L-Bz-Leu-OH and 1 equiv of N-methylmorpholine with 5% excess isobutyl chloroformate in THF at -15° for 4 min followed by 5% excess H-Gly-OEt showed that no racemate was formed. A 93% yield of pure L-Bz-Leu-Gly-OEt, mp $157-158^{\circ}$, was found. (From several experiments, we have found $[\alpha]^{25D} -32.5 \pm 0.5^{\circ}$ (c 3, EtOH) for this product; further recrystallization did not give the -34° reported by Williams and Young. A differential thermal analysis gave a single endotherm at 159° .) A similar experiment with 2 equiv of N-methylmorpholine gave a 16% yield of racemate and a 50% yield of L isomer. By older procedures, Williams and Young found crude yields of 85-88% with estimated L isomer 18-22%.

On the basis of our present evidence, steric factors appear to be particularly important for the complete formation of the mixed anhydride. Racemization *via* oxazolones seems likely.

Although N-methylmorpholine is the safer amine to use in the mixed anhydride procedure, the fact that good results were obtained with proper use of the strong racemizer trimethylamine is made even more striking by the following experiment. Z-Gly-Phe-N₃ (L) was made from the corresponding hydrazide (20 mmoles) at -5° in ethyl acetate-1 *N* hydrochloric acid by treatment with sodium nitrite. The ethyl acetate solution was washed with aqueous potassium bicarbonate to remove excess acid, dried over sodium

sulfate a few minutes while chilling, then divided into two equal portions. A solution of 10 mmoles of trimethylamine in THF was added to one, and both were kept for 12 min in an ice-salt bath. Then 10 mmoles of ethyl glycinate was added to each. After refrigeration (-3°) overnight, each was worked up to yield 3.14 g of product, mp $\sim 83-92^{\circ}$. Thin layer chromatography (silica gel, chloroform-methanol 2:1) showed spots at R_f 0.8 (Z-Gly-Phe-Gly-OEt) and 0.65 (Z-Gly-Phe-NH₂). Fractional crystallizations from 2% solutions in ethanol gave a 1.6% yield of DL tripeptide from the reaction done with trimethylamine and none from the control. Yields of L tripeptide were 19 and 24%, respectively. Concentration of the alcohol solutions remaining and recrystallization of products from ethyl acetate yielded 18% of pure Z-Gly-Phe-NH₂, mp $143-144^{\circ}$, $[\alpha]^{25D} +9.74^{\circ}$ (c 2, EtOH), from the trimethylamine reaction and 9%, mp $143-144^{\circ}$, $[\alpha]^{25D} +10.8^{\circ}$ (c 2, EtOH) from the control. Authentic Z-Gly-Phe-NH₂ (L), made by reaction of NH₃ with the mixed anhydride from Z-Gly-Phe-OH (L) and isobutyl chloroformate, had $[\alpha]^{25D} +9.5^{\circ}$ (c 2, EtOH). *Anal.* Calcd for C₁₉H₂₁N₃O₄: C, 64.21; H, 5.96; N, 11.83. Found: C, 64.66; H, 6.21; N, 11.88 for the product from the control peptide synthesis. The isolation of the DL tripeptide provides the first example of racemization by the azide method, to our knowledge.

George W. Anderson, Joan E. Zimmerman
Francis M. Callahan

Organic Chemical Research Section, Lederle Laboratories
American Cyanamid Co., Pearl River, New York

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Book Reviews

Oxidation and Combustion Reviews. Volume 1. Edited by C. F. H. TIPPER, Ph.D., D.Sc., Senior Lecturer in the Department of Inorganic, Physical, and Industrial Chemistry, University of Liverpool. American Elsevier Publishing Co., Inc., 52 Vanderbilt Ave., New York, N. Y. 1965. 344 pp. 16 × 23 cm. \$14.50.

The study of combustion and oxidation must have started before recorded history, and because of the complexity of the subject it is likely to be studied for a similar period of time in the future. The titles of the papers given at the 1964 International Combustion Symposium (over 1400 pages in small print) reveal a tremendous range of topics varying from experimental fires to reaction kinetics. Dr. Tipper envisages that the series of "Oxidation and Combustion Reviews," of which the first volume has recently been published, should cover a similarly wide field including, in later volumes, the more physical and the engineering aspects of the subject.

Judging by the first volume, it is intended that each volume should contain a wide range of topics. The first volume concentrates on the chemistry of the oxidation process in both gaseous and solid oxidations. There are six chapters, each written by different authors in differing styles with differing degrees of success. Therefore it is best to review each chapter separately.

The first chapter by Ben-Ain and Lucquin deals with the branched-chain process in slow combustion and in cool flames. Judging by the dates of the references given, this subject has not progressed

much recently and the authors may be criticized for a rather complacent acceptance of some unsubstantiated theories. Thus they accept that consumption of reactants competing with chain branching causes a slow combustion reaction to reach a maximum rate.

The chapter on oxidation reactions induced by ionizing radiation is little more than a very useful list of references with a brief summary of the results obtained in this field.

Gas-phase photooxidation reactions are described by McMillan and Calvert in a well-argued account which begins by detailing some effects (*e.g.*, quenching) associated with electronically excited molecules. This is followed by a good account of the reactions of alkyl, acyl, peracyl, and alkoxy radicals. The reactions are each considered separately in preference to a separate treatment of the photooxidation of each parent compound.

The chapter by J. H. Thomas on oxidation reactions involving nitrogen dioxide deals, by contrast, in considerable detail with the kinetics of the reactions between nitrogen dioxide and (a) hydrogen, (b) hydrocarbons, (c) halogen-containing compounds, and (d) formaldehyde and formic acid. This is a good review.

Oxidative degradation of high polymers is ably dealt with by W. L. Hawkins in a more descriptive account of the environmental factors which in practice are responsible for the oxidation of polymers in general use. A description of the natural degradation is followed by an account of laboratory experiments designed to give some idea of the reaction mechanisms involved and the products obtained.