if sulfur were participating in electron-pair acceptor type conjugation.

TABLE II

QUALITATIVE RELATIONSHIP BETWEEN $\bar{\sigma}_R$ VALUES FOR $-\overset{\cdot\cdot}{\underset{\cdot\cdot}{S}}\!\!\diagdown\!\!X$ AND $\bar{\sigma}_R$ VALUES FOR X

$-\overset{\cdot\cdot}{\underset{\cdot\cdot}{S}}\!\!\diagdown\!\!X$ Group	σ_p for X[a]	$\bar{\sigma}_R$ for $-\overset{\cdot\cdot}{\underset{\cdot\cdot}{S}}\!\!\diagdown\!\!X$[b] (ionization of benzoic acids)	$\bar{\sigma}_R$ for $-\overset{\cdot\cdot}{\underset{\cdot\cdot}{S}}\!\!\diagdown\!\!X$[b] (ionization of phenols)
$-SCH_3$	−0.17	−0.24	−0.03
$-SCOCH_3$	+0.52	+0.10	+0.12
$-SCN$	+0.63	+0.06	+0.14

[a] H. H. Jaffe, *Chem. Revs.*, **53**, 191 (1953). [b] The $\bar{\sigma}_R$ values for $-\overset{\cdot\cdot}{\underset{\cdot\cdot}{S}}\!\!\diagdown\!\!X$ were calculated from the data of Bordwell and Boutan (2) using the ρ_I values given in Table II of reference (3). The aliphatic σ_I values of Taft and Lewis were used (see (*a*), Table I) except for the thiolacetoxy ($-SCOCH_3$) and thiocyanate groups. The σ_I value for the thiolacetoxy group was obtained from the data of Bordwell and Boutan[2] using equations (1) and (6) of reference (3) and the ρ_I and α values given in Table II of reference (3). The σ_I value of the thiocyanate group has not been experimentally determined, and was estimated from the equation:

$$\sigma_{I_{-AX}} = \sigma_{I_{-X}}\left(\frac{1}{2.8}\right) + \sigma_{I_{-AH}}$$

where 1/2.8 is a fall-off factor. This equation comes from unpublished work of R. W. Taft, Jr., and I. C. Lewis which is patterned after earlier work of G. E. K. Branch and M. Calvin, *The Theory of Organic Chemistry*, Prentice-Hall, Inc., New York, N. Y., 1941, pp. 201–225. In general, the method gives good agreement with experimental results.

Although the observed electron-pair acceptor participation by sulfur is small, the $(p\text{-}d)\pi$ conjugation need not necessarily be small since the observed effect could be the net result of $(p\text{-}d)\pi$ conjugation, such as shown in resonance form (VI), acting against a somewhat smaller magnitude of ordinary $(p\text{-}p)\pi$ conjugation of the type shown in resonance forms (IV) and (I).

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Phosphorus Pentoxide as a Reagent in Peptide Synthesis

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At the time Schramm and Wissman[1] reported on the use of phosphorus pentoxide for the synthesis of peptides, an essentially identical procedure was being developed in this laboratory. The purpose of this communication is, first of all, to report that, contrary to the findings of the above authors, racemization can occur when acylated peptides are employed as intermediates and, secondly, to supply additional information about the potential usefulness of this synthetic procedure.

The general procedure was as follows: A diethyl phosphite solution containing one mole of acylated amino acid, one mole of amino acid (or peptide) ester hydrochloride, and two moles of tri-*n*-butylamine, was added to a solution of phosphorus pentoxide in diethylphosphite. After being heated on a steam bath for forty minutes, the reaction mixture was poured into an aqueous sodium bicarbonate solution. Crystallization frequently occurred at this point. The reaction was carried out in a hood, since phosphine appeared as a by-product.

A number of the compounds not hitherto synthesized by this method are listed in Table I and include peptides containing the following amino acid residues: glycine, D-alanine, L-phenylalanine, L-tyrosine, L-tryptophan, ε-*N*-carbobenzoxy-L-lysine, and L-glutamic acid dibenzyl ester. The protecting groups include *N*-benzoyl, *N*-carbobenzoxy,

TABLE I

PEPTIDE DERIVATIVES

Compound[a]	Yield,[b] %	M.P., found	M.P., lit.
Z·Gly-Phe·OH(DL)	70[c]	160	160[d]
Z·Gly-Phe·OH(L)	64[c]	125–126	125–126[e]
Z·Ala-Gly·OBz(L)	85	112	111[f]
Z·Ala-Ala·OBz(D-D)	75	138	138[g]
Z·Phe-Gly·OC_2H_5(L)	77	109	109–110[h]
B·Gly-Z·Lys·OCH_3(L)[i]	75	145	145[j]
Tr·Gly-Gly·OC_2H_5	65	161	163[k]
Z·Ala-Tyr·OC_2H_5(L-L)	50	136	137[l]
Z·Try-Gly·OH(L)	32[c]	156	156[m]
Z·Gly-Try·OH(L)	50[c]	142	142[m]
Z·Ala-Glu·$(OBz)_2$(L-L)	65	103	104–105[n]

[a] The abbreviations are those of E. Brand and B. F. Erlanger, *J. Am. Chem. Soc.*, **73**, 3508 (1951). Tr = trityl = triphenylmethyl; B = benzoyl; Z = carbobenzoxy; Bz = benzyl. [b] Yield of purified products. [c] Over-all yield of coupling reaction and subsequent saponification of methyl ester. [d] J. Vaughan, Jr., and R. L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952). [e] K. Hofmann and M. Bergmann, *J. Biol. Chem.*, **134**, 225 (1940) report $[\alpha]_D^{25}$ +38.5 (5.0% in ethanol); we find $[\alpha]_D^{25}$ +37.8 (0.5% in ethanol). [f] B. F. Erlanger and E. Brand, *J. Am. Chem. Soc.*, **73**, 3508 (1951). [g] This is m.p. of L-L isomer as reported in ref. *f*. [h] G. W. Anderson and R. W. Young, *J. Am. Chem. Soc.*, **74**, 5308 (1952). They also report $[\alpha]_D^{25}$ −16.0 (2% in ethanol); we find $[\alpha]_D^{25}$ −17.3 (2% in ethanol). [i] This compound was saponified and converted, by hydrogenolysis, to hippuryl L-lysine: $[\alpha]_D^{25}$ −5.2 (2.5% in water); ref. *e* reports the same rotation. [j] M. Bergmann, L. Zervas, and F. Ross, *J. Biol. Chem.*, **111**, 245 (1935). [k] G. Amiard, R. Heymes, and L. Velluz, *Bull. Soc. Chim. France*, 191 (1955). [l] M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **145**, 247 (1942). [m] E. L. Smith, *J. Biol. Chem.*, **175**, 39 (1948). [n] H. Sachs and E. Brand, *J. Am. Chem. Soc.*, **75**, 4608 (1953). They also report $[\alpha]_D^{25}$ −16.6 (2% in glacial acetic acid); we find $[\alpha]_D^{25}$ −16.8 (2% in glacial acetic acid).

(1) G. Schramm and A. Wissman, *Ber.*, **91**, 1073 (1958).

and *N*-trityl. Except for the peptide containing tyrosine and one containing tryptophan, the yields are good. The yield of tyrosine peptides could probably be increased by protecting the phenolic hydroxyl group.[2]

In order to ascertain whether racemization would occur when an acylated dipeptide was used as an intermediate, the sensitive test of Anderson and co-workers[3] was applied. Carbobenzoxyglycyl L-phenylalanine was condensed with glycine ethyl ester hydrochloride in the presence of tri-*n*-butylamine and the products were examined. Approximately 40% of the total product was found to be carbobenzoxyglycyl DL-phenylalanylglycine ethyl ester. The experimental conditions were modified in a number of ways without effecting a decrease in the extent of racemization (see Experimental section).

Schramm and Wissman[1] reported that no racemization occurred when carbobenzoxyglycyl L-leucylglycine ethyl ester was synthesized by the condensation of carbobenzoxyglycyl L-leucine with glycine ethyl ester hydrochloride in the presence of tri-*n*-butylamine and phosphorus pentoxide. Since their procedure was quite similar to the one used in our laboratory, the degree of racemization must be influenced by the nature of peptide being synthesized. Our experiments demonstrate, however, that racemization is possible when acylated peptides are employed as intermediates in this reaction.

It would appear from our work, as well as from that of Schramm and Wissman,[1] that phosphorus pentoxide is a valuable reagent for the synthesis of simple peptide derivatives of amino acids which lack reactive groups in their side chains or whose reactive groups are suitably protected. It is especially adaptable to the synthesis of large quantities of peptides in small reaction volumes, as illustrated in the Experimental section by the preparation of 90 g. carbobenzoxyglycyl L-phenylalanine. It has also been found to be a useful reagent for the preparation of hydroxamic acids and anilides directly from carboxylic acids. The synthesis of hexanohydroxamic acid from the acid and hydroxylamine hydrochloride is described in another paper,[4] as is the synthesis of a new substrate for trypsin, benzoyl DL-arginine-*p*-nitroanilide hydrochloride.[5]

EXPERIMENTAL

The following procedure was used to prepare all compounds listed in Table I. In crystallizing these compounds, the solvents used were the same as those described in the literature for the particular peptide derivative (see Table I).

Standard procedure: Carbobenzoxyglycyl L-phenylalanine. A suspension of 85.5 g. (0.397 mole) of L-phenylalanine methyl ester hydrochloride (Mann Research Laboratories), 83.2 g. (0.397 mole) of carbobenzoxyglycine,[6] and 148 g. (190 ml., 0.795 mole) of tri-*n*-butylamine in 125 ml. of diethylphosphite was added to a solution of 56 g. (0.397 mole) of phosphorus pentoxide (Mallinckrodt, A.R.) in 175 ml. of diethylphosphite (Victor Chemical Works) (warming is necessary to effect solution of the phosphorus pentoxide). The reaction mixture was heated on a steam bath for 40 min., poured into 700 ml. of water containing an excess of sodium bicarbonate, and the resulting oil extracted into ethyl acetate. After washing with dilute hydrochloric acid and water, the ethyl acetate extract was dried over anhydrous sodium sulfate and concentrated *in vacuo* to yield 145 g. of an oil. The above procedure should be carried out in a hood, since a considerable quantity of phosphine appears as a by-product.

The oily ester was saponified by allowing it to stand in a solution of 236 ml. 2*N* of sodium hydroxide in 500 ml. of methanol for 90 min. at room temperature. It was then acidified with concentrated hydrochloric acid, concentrated in volume *in vacuo* and poured into 200 ml. of water. The resulting oil was extracted into ethyl acetate, washed with water, and dried over anhydrous sodium sulfate. Removal of the solvent *in vacuo* gave a solid mass which could be crystallized from ethyl acetate–ether. The yield was 45 g. The mother liquor was found to contain unsaponified ester which was allowed to react for an additional hour at room temperature with a solution of 150 ml. 2*N* of sodium hydroxide in 200 ml. of methanol. The reaction mixture was treated as above and yielded 51 g. of material, which was combined with the 45 g. obtained previously and recrystallized from ethyl acetate–ether. The yield was 90 g. (64% over-all yield for both steps); m.p. 125–127°; $[\alpha]_D^{24}$ +37.5 (0.5% in ethanol); reported,[7] m.p. 125–126°, $[\alpha]_D^{24}$ +38.5 (5% in ethanol).

Racemization studies: Carbobenzoxyglycyl-L-phenylalanylglycine. Carbobenzoxyglycyl-L-phenylalanine (356 mg., 1 mmole), glycine ethyl ester hydrochloride (140 mg., 1 mmole), and 0.48 ml. (2 mmoles) of tri-*n*-butylamine were suspended in 5.0 ml. of diethylphosphite. This mixture was added to a solution of 142 mg. (1 mmole) of phosphorus pentoxide in 5.0 ml. of diethylphosphite and the whole reaction mixture heated on a steam bath for 20 min. After this heating period, it was poured into 15 ml. of water, extracted into ethyl acetate, and the ethyl acetate extract was washed successively with water, dilute hydrochloric acid, and dilute sodium bicarbonate. After drying over anhydrous sodium sulfate, the ethyl acetate was removed *in vacuo*, the residue was dissolved in absolute alcohol and allowed to crystallize in the refrigerator; yield, 140 mg. (32%), m.p. 130°, $[\alpha]_D^{25}$ 0 (*c*, 2, ethanol). According to Anderson and Callahan,[3] this is the racemized product, *i.e.*, carbobenzoxyglycyl-DL-phenylalanylglycine ethyl ester.

The mother liquor was taken to dryness *in vacuo* and the residue crystallized from ethyl acetate-petroleum ether; yield, 140 mg., m.p. 118–120°, $[\alpha]_D^{25}$ −12.8 (*c*, 2, ethanol). Literature[3] reports m.p. 120–120.5° and $[\alpha]_D^{25}$ −13.2 (*c*, 2, ethanol) for carbobenzoxyglycyl L-phenylalanylglycine ethyl ester.

Essentially the same results were obtained when experimental conditions were changed as follows: (a) decreasing the reaction time to 10 min.; (b) using free glycine ethyl ester in place of the hydrochloride and 1 mole of tri-*n*-butylamine; (*c*) heating the glycine ethyl ester with phosphorus pentoxide for 10 min. prior to addition of other rea-

(2) *Cf.* K. Blau and S. G. Waley, *Biochem. J.*, **57**, 538 (1954); S. G. Waley and J. Watson, *Biochem. J.*, **57**, 529 (1954); E. Wunsch, G. Fries, and A. Zwick, *Ber.*, **91**, 542 (1958).

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

(4) W. Cohen and B. F. Erlanger, *J. Am. Chem. Soc.*, **82**, 3928 (1960).

(5) B. F. Erlanger, N. Kokowsky, and W. Cohen, in preparation.

(6) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(7) K. Hofmann and M. Bergmann, *J. Biol. Chem.*, **134**, 225 (1940).

gents; (d) decreasing the volume in order to discourage intramolecular reactions; (e) carrying out the reaction at room temperature for 45 min. (the yield was decreased considerably but the same degree of racemization occurred); and (f) adding only 1 mole of tri-*n*-butylamine to the reaction mixture.

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Nitration with Uranium Nitrate–Nitrogen Tetroxide–Water Complex in the Presence of Acetic Anhydride[1]

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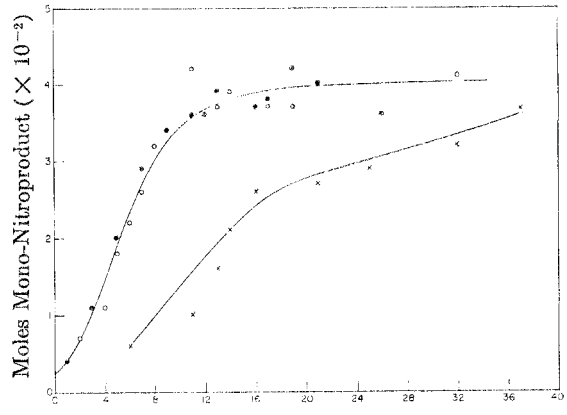
The type of influence which acetic anhydride has on the nitrating ability of nitric acid is not definitely understood. Menke[2] examined the nitrating abilities of inorganic nitrates with acetic anhydride. He found that nitrates of metals such as Fe^{+3}, Cu, Ni, Co, Al, Ce, and UO_2^{++} acted as very strong nitrating agents in acetic anhydride. Menke suggested possibly that acetyl nitrate is formed in the nascent state and this is then decomposed as the temperature rises. Also, he found that the velocity of nitration could be modified by a change in temperature, the choice of inorganic nitrate, and by using mixtures of acetic anhydride and acetic acid in various proportions. Putokhin[3] nitrated thiopene with cupric nitrate–trihydrate in acetic anhydride and obtained good results. He found the reaction quieter with 80% acetic acid in place of acetic anhydride and almost nil with 60% acetic acid. Dewar and Maitlis[4] nitrated quinoline using lithium nitrate with a little copper nitrate in acetic anhydride at 100°. Traverso[5] examined the nitration of tetralin with copper and aluminum nitrates in acetic acid and acetic anhydride. Borewell and Garbisch[6] in a recent study of the behavior of acetic anhydride and 70% nitric acid feel that the exothermic reaction between these compounds forming acetyl nitrate is essential for successful nitrations, and the acetyl nitrate so formed is the nitrating agent.



Moles Acetic Anhydride (× 10^{-2})
Nitrations with I. $UO_2(NO_3)_2 \cdot N_2O_4 \cdot H_2O$. II. $UO_2(NO_3)_2 \cdot 6H_2O$
○ Nitrobenzene I
× Nitrobenzene II
● Nitro-o-xylenes I

We initially intended to nitrate aromatic compounds with uranium nitrate–nitrogen tetroxide–water complex[7] in acetic anhydride. It was noted that as the quantity of acetic anhydride was increased the reaction became increasingly vigorous. We therefore, investigated the influence of increasing amounts of acetic anhydride on the amount of mononitration products of benzene and *o*-oxylene. An excess of aromatic compound was used as a solvent. Nitration with uranyl nitrate hexahydrate was also investigated for comparison to the above uranyl nitrate complex.

EXPERIMENTAL

Benzene, 25 cc. and varying quantities of acetic anhydride (from 0.02 to 0.40 mole) were mixed in a 125 cc. Erlenmeyer flask immersed in an ice bath with a magnetic stirrer. Uranium nitrate–nitrogen tetraoxide-water complex (0.02 mole) was slowly added to this mixture with constant stirring. Approximately 20 min. was taken for addition. A slight exothermic reaction occurred during addition of complex while the reaction flask was in the ice bath. When all the complex had been added and the solution was well stirred, it was removed from the ice bath and stirring was continued at room temperature. A very vigorous exothermic reaction occurred, and a yellow precipitate settled out of the solution. The temperature was kept below 60° until the reaction had subsided. The yellow precipitate has been identified as uranyl acetate. The entire reaction mixture was then washed twice with ice water and once with 5% sodium carbonate solution. The resulting organic layer was vacuum distilled. The nitrobenzene obtained was weighed and the amount in moles was recorded in Fig. 1 as a function of the moles of acetic anhydride used.

Benzene nitrations were also carried out using uranyl nitrate hexahydrate (0.02 mole) in the same manner as above with the exception that addition of the solid nitrate was at room temperature, as the reaction was much less vigorous than that with the complex.

(1) This work was supported by a grant from the Chemistry Branch of the Division of Research, United States Atomic Energy Commission.
(2) J. B. Menke, *Rec. Trav. Chim.*, **44**, 141 (1925).
(3) N. I. Putokhin, *Sbornik Nauch. Trudov Kuibyshev, Ind. Inst. im. V. V. Kuibyshiva*, No. 5, 271 (1955).
(4) M. J. S. Dewar, P. M. Maitlis, *Chem. & Ind.*, **48**, 685 (1955).
(5) Georgio Traverso, *Ann. Chim.*, **45**, 706 (1955).
(6) F. G. Bordwell and C. W. Garbisch, Jr., *J. Am. Chem. Soc.*, **82**, 3588 (1960).
(7) J. R. Lacher, Keith Ensley, Anne Tenge, and J. D. Park, *J. Org. Chem.*, **24**, 1347 (1959).