J. Adler N. J. Greenfield, and G. D. Fasman, *Methods Enzymol.*, **27**, 675 (1974).
(26) Y.-H. Chen, J. T. Yang, and K. H. Chau, *Biochemistry*, **13**, 3350 (1974).
(27) C. C. Baker and I. Isenberg, *Biochemistry*, **15**, 629 (1976).
(28) (a) M. Goodman, F. Naider, and R. Rapp, *Bioorg. Chem.*, **1**, 310 (1971); (b) see also: P. Marche, J.-L. Morgat and P. Fromageot, *Eur. J. Biochem.*, **40**, 513 (1973).
(29) J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis", W. H. Freeman, San Francisco, Calif., 1969.
(30) P. Edman and G. Begg, *Eur. J. Biochem.*, **1**, 80 (1967).
(31) H. D. Niall, *Methods Enzymol.*, **27**, 942 (1973).
(32) W. R. Gray, *Methods Enzymol.*, **25**, 121 (1972).
(33) A. Margin, *Tetrahedron Lett.*, **33**, 3145 (1971).
(34) F. C. Westall, J. Scotchler, and A. D. Robinson, *J. Org. Chem.*, **37**, 3363 (1972).
(35) D. Yamashiro and C. H. Li, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 4945 (1974).
(36) These cleavages were performed by Mr. Jim Geever of the Armour Pharmaceutical Co.
(37) N. H. Tan and E. T. Kaiser, *J. Org. Chem.*, **41**, 2787 (1976).

Examples of Amino Acid Transaminations with *o*-Formylbenzoic Acid

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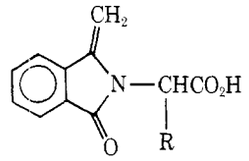
The present paper describes an unusual example of a nonenzymatic transamination of two amino acids by a nonpyridoxal-type carbonyl compound. During the course of some recent research on the interaction of certain *o*-carbonylbenzoic acids with amino acids, *o*-formylbenzoic acid was found to undergo transamination reactions with L-glutamic acid and L-alanine in the presence of acetic acid and toluene. The products of this novel reaction are an α-ketocarboxylic acid (**4**) and *N*-(3-phthalidyl)phthalimidine (**3**). Structural proof of the products was based on derivative formation, spectral and elemental analyses, and synthesis. α-Amino-*o*-toluic acid (**6**) was a probable intermediate in the transformation. Apparently, *o*-formylbenzoic acid condensed with **6** or with phthalimidine (**5**) to form product **3**. With L-glutamic acid, an additional product (the anhydride of **2**, where R = $CH_2CH_2CO_2H$) was obtained which resulted from a simple condensation of starting materials.

In a recent publication,[2] *o*-acetylbenzoic acid was reported to condense readily with various amino acids and produce 3-methylenephthalidylamino acids (**1**).



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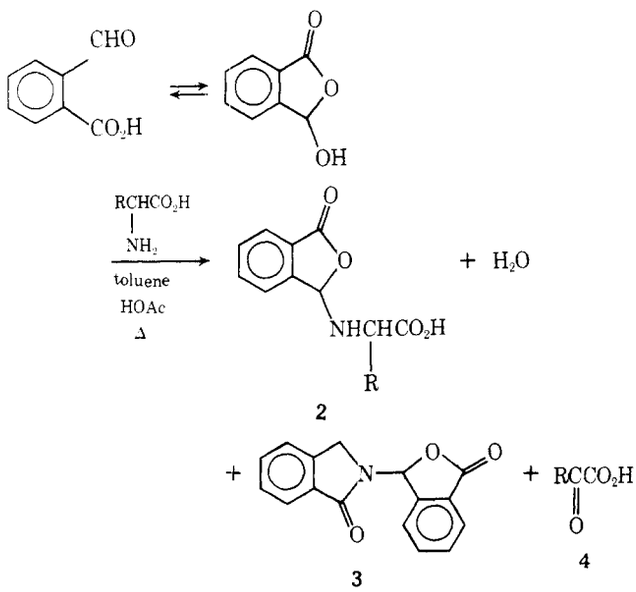
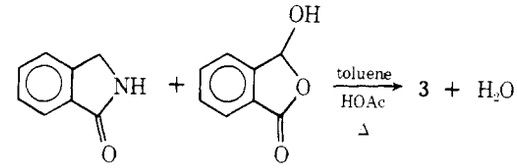
When *o*-formylbenzoic acid was treated with L-glutamic acid, the expected product, **2** (R = $CH_2CH_2CO_2H$), was formed, but only in 19% yield. However, other products were obtained which indicated that transamination had occurred: **3** in 19% yield and **4** (R = $CH_2CH_2CO_2H$) in 17% yield as the semicarbazone derivative. The yield of product **3** was increased to 46% when dioxane was used in place of toluene. Transamination was apparently the only reaction when L-alanine and L-phenylalanine were each treated with *o*-formylbenzoic acid. None of the condensation product, **2**, was obtained with these amino acids. In the reaction of L-alanine with *o*-formylbenzoic acid, product **3** was formed in 66% yield and a crystalline phenylhydrazone derivative of pyruvic acid (**4**, R = CH_3) was isolated in 19% yield.



2 **3** **4**

The structure of the base-insoluble product (**3**) was established by an unambiguous synthesis from phthalimidine[3] (**5**) and *o*-formylbenzoic acid under conditions identical to those used with the latter compound and an amino acid. The product from this experiment was isolated in 50% yield and was identical to that obtained from the transamination reaction with respect to the thin-layer chromatogram R_f value, infrared spectrum, and melting point. Similar reactions of amides with *o*-formylbenzoic acid are in the literature.[4]



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The formation of α-amino-*o*-toluic acid (**6**) and the keto acid (**4**) may be rationalized by analogy with the well-established mechanism for the transamination with pyridoxal phosphate. Experiment has shown that no reaction occurs in the absence of acetic acid. In fact, a large excess of the reagent was found to afford optimum yields of products. Apparently, α-amino-*o*-toluic acid (**6**) reacts with *o*-formylbenzoic acid or its tautomer as soon as it is formed and two molecules of water are eliminated. Two equally plausible routes are possible for this transformation. In the first, **6** cyclizes to phthalimidine (**5**) which then condenses with *o*-formylbenzoic acid, a reaction which has already been demonstrated (see above). The

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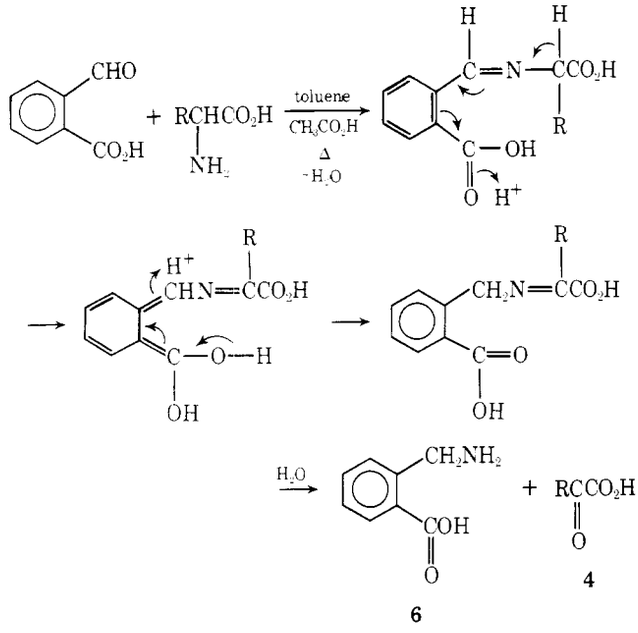
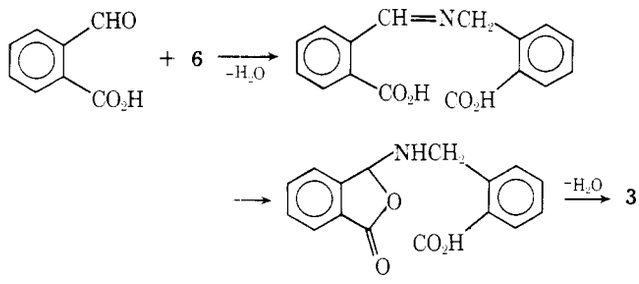
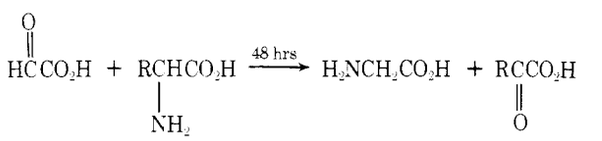


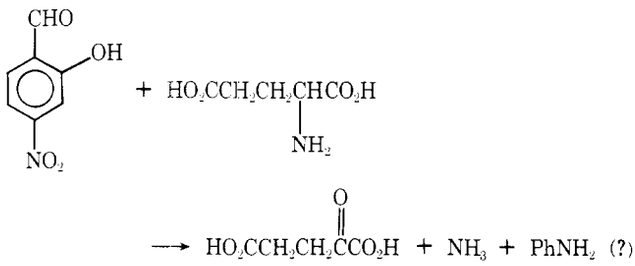
second alternative could produce **3** by the following transformation.



The chief difference between the transamination reaction reported herein and those catalyzed by pyridoxal phosphate is that the former shows no evidence for decarboxylation. The present reaction, then, is an unusual example of a nonenzymatic transamination of amino acids by a non-pyridoxal-type



carbonyl compound that is not accompanied by decarboxylation. As far as we are aware, only two analogous reactions have been reported. The first involved the reaction of glyoxylic acid with various amino acids under physiological conditions to produce glycine and an α-keto acid.[5] The second was the conversion of L-glutamic acid into α-ketoglutaric acid by 4-



or 6-nitrosalicylaldehydes.[6] The authors of that report described the latter reaction as an oxidative deamination rather than a transamination, since ammonia was produced and an "aromatic amine". Apparently, the nitro moiety was the oxidant. They suggested that pyridoxal-like activity in benzenoid compounds requires the presence of a phenolic hydroxyl group (since *p*-nitrobenzaldehyde failed to form significant amounts of keto acids) and the presence of a powerful electron-attracting group in the 4 or 6 position of salicylaldehyde (as shown by the failure of salicylaldehyde itself and 4-carboxysalicyaldehyde to bring about the reaction). Although the carboxyl group is meta directing, it was considered not to be a sufficiently powerful electron-attracting group to effect the deamination.

Experimental Section

IR spectra were recorded on a Perkin-Elmer Model 521 or a Beckman Acculab 3 spectrophotometer. Mass spectra were obtained using a duPont 21-472 instrument. ^{1}H-NMR spectra were run on a Perkin-Elmer R-24B instrument and ^{13}C-NMR spectra on a JEOL FX60Q. Melting points are corrected. Microanalyses were performed by the Analytical Department, Bristol Laboratories, Syracuse, N.Y.

The Transamination of L-Alanine. A mixutre of 3.0 g (20 mmol) of *o*-formylbenzoic acid, 0.89 g (10 mmol) of L-alanine, 3.66 mL (3.84 g, 64 mmol) of HOAc, and 30 mL of toluene was heated to the reflux temperature until no more water was collected in a Dean and Stark trap (about 16 h). The reaction mixture was allowed to cool to 25 °C and diluted with an equal volume of water; the pH was adjusted from 3.0 to 8.5 with aqueous NaOH. An insoluble white solid, *N*-(3-phthalidyl)phthalimidine (**3**), was collected by filtration and recrystallized from HOAc: wt 1.74 g (66%); mp 241.0–241.5 °C; IR (Nujol) 1765 (γ-lactone carbonyl), 1700 cm^{-1} (γ-lactam carbonyl), no absorption in the NH or OH region except for that of Nujol; ^{1}H NMR (Me_2SO-d_6) 7.5 (multiplet, ~8 aromatic H's), 7.05 (singlet, 1 benzyl H on phthalidyl ring), 3.95 and 3.75 (two singlets, 2 benzyl H's on phthalimidine ring); ^{13}C NMR ($CDCl_3/Me_4Si$ = 0, 20 MHz) 44.7 (1 C), 81.4 (1 C), 123.1–144.6 ppm (10 C); mass spectrum *m/e* 265 (100, M$^+$), 237 (30), 221 (55), 133 (33), 132 (20), 44 (15), 28 (15). Anal. Calcd for $C_{16}H_{11}NO_3$ (**3**): C, 72.45; H, 4.18; N, 5.28; mol wt, 265.27. Found: C, 72.19; H, 4.26; N, 5.20.

The pH 8.5 aqueous filtrate from above was adjusted to pH 2.0 with 6 N HCl and then extracted twice with *n*-BuOH. The organic extracts were washed with water and distilled under reduced pressure until all of the easily volatilized material was removed. The residual oil weighed 1.47 g and was dissolved in CH_2Cl_2. Petroleum ether (bp 30–60 °C) was added, and a small quantity of crystals (**3**) separated. The CH_2Cl_2–petroleum ether solvents were removed from the filtrate and replaced with EtOH. To this solution was added 1.1 g (10 mmol) of phenylhydrazine and two drops of HOAc. This mixture was diluted with approximately an equal volume of water, and the resultant solution was heated to the boiling point and then allowed to cool. Yellow crystals of pyruvic acid phenylhydrazone were collected and dried: wt 0.25 g; mp 191.5–194.0 °C dec with gas evolution (lit. mp 192 °C); IR (Nujol) 3280 (NH stretching), 2600–2800 (bonded carboxyl OH), 1675 (α,β-unsaturated carboxyl carbonyl), 1660 (imine), 1450 and 1250 (CO stretching), 700–750 cm^{-1} (aromatic CH stretching). A second fraction of crystals was obtained: wt 87 mg; mp 189.5–194.0 °C dec. Total yield of pyruvic acid phenylhydrazone was 19%.

The Transamination of L-Glutamic Acid. A similar mixture containing 1.47 g (10 mmol) of L-glutamic acid was heated to the reflux temperature for 20 h. About 0.45 mL (25 mmol) of water was collected in a Dean and Stark trap during this period. The reaction mixture was allowed to cool and 30 mL of water was added. The pH was adjusted from 3.0 to 8.7 with aqueous NaOH and the crystals (**3**) were collected by filtration and dried: wt 0.21 g; mp 229.5–234.5 °C. Another fraction of **3** was obtained by concentration of the toluene portion of the filtrate, wt 0.31 g. Both of these fractions were identical with authentic **3** by IR and TLC and the total yield of **3** was 19.4%. In another experiment run exactly as above, except that dioxane was used in place of toluene, the yield of **3** was 1.21 g (45.8%).

The pH 8.7 aqueous layer that remained after the separation of the *N*-(3-phthalidyl)phthalimidine (**3**) was acidified to pH 2.0 with 6 N HCl and then extracted twice with *n*-BuOH. The organic layer was washed with water and distilled under reduced pressure until all of the easily volatilized material was removed. The oily residue was triturated with 15 mL of acetone to afford a white crystalline solid (the anhydride of **2** where R = $CH_2CH_2CO_2H$) which was recrystallized from methanol: wt 0.49 g (18.6%); mp 225.5–228.0 °C dec with gas evolution; IR (Nujol) 1770 (γ-lactone carbonyl), 1790 and 1740 (cyclic anhydride), 1050 and 950 cm^{-1} (COC stretching); ^{1}H NMR (Me_2SO-d_6) 7.7 (multiplet, 4 aromatic H's), 7.05 (singlet, 1 benzyl H

on phthalidyl ring), 3.8 (multiplet, 1 methine H on glutaric anhydride ring), 2.2 ppm (multiplet, 4 methylene H's on glutaric anhydride ring). Anal. Calcd for $C_{13}H_{11}NO_5$ (the anhydride of **2**, where R = $CH_2CH_2CO_2H$): C, 59.77; H, 4.24; N, 5.36. Found: C, 59.68, 59.78; H, 4.58, 4.43; N, 5.34, 5.89.

After separation of the above product, the remaining base-soluble, BuOH-extracted, reaction mixture was chomatographed on a silica gel column with a mixture of C_6H_6 and EtOAc (70:30) in order to obtain a fraction that corresponded to 2-ketoglutaric acid (**4**, R = $CH_2CH_2CO_2H$), wt 1.54 g. This was treated with an equal weight of semicarbazide hydrochloride and 2.3 g of NaOAc in hot aqueous EtOH. Crystals of 2-ketoglutaric acid semicarbazone separated on cooling: wt 180 mg; mp 219.5–220.5 °C dec with gas evolution (lit. mp for 2-ketoglutaric acid semicarbazone is 220 °C); IR (Nujol) 3440 (NH stretching), 2500–2700 (bonded carboxyl OH), 1640–1700 (several bands for carboxyl carbonyl, imine, and urea moieties), 1440 and 1255 cm^{-1} (CO stretching). Another fraction of 2-ketoglutaric acid semicarbazone was obtained by treatment of the pH 2.0 BuOH-extracted aqueous solution with 1.0 g of semicarbazide hydrochloride and 1.5 g of NaOAc: wt 62.4 mg; mp 211.5–214.0 °C dec with gas evolution. The total yield of this product was 243 mg (16.6%).

***N*-(3-Phthalidyl)phthalimidine (3) from Phthalimidine and *o*-Formylbenzoic Acid.** A solution of 0.80 g (6 mmol) of phthalimidine,[3] 0.90 g (6 mmol) of *o*-formylbenzoic acid, 1.1 mL (1.15 g, 19.2 mmol) of HOAc, and 10 mL of toluene was heated to reflux for 6 h. A trap was used to collect the water of reaction. A white solid was separated by filtrating: wt approximately 2 g: mp 238.6–242.0 °C. This was suspended in MeOH for a few minutes and the insoluble fraction was collected and dried: wt of **3** was 0.79 g (49.7%); mp 241.5–242.5 °C; IR was identical to that of the product isolated from the transamination experiments.

Registry No.—**2** (R = $CH_2CH_2CO_2H$) anhydride, 65898-29-5; **3**, 65898-30-8; **4** (R = $CH_2CH_2CO_2H$), 328-50-7; **H** (R = $CH_2CH_2CO_2H$) semicarbazone, 2704-31-6; *o*-formylbenzoic acid, 119-67-5; L-alanine, 56-41-7; pyruvic acid phenylhydrazone, 5330-70-1; L-glutamic acid, 56-86-0; phthalimidine, 480-91-1.

References and Notes

(1) Department of Chemistry, Clemson University, Clemson, S.C. 29631.
(2) C. A. Panetta and A. L. Miller, *Synthesis*, 43 (1977).
(3) S. Danishefsky, T. A. Bryson, and J. Putenpurayil, *J. Org. Chem.*, **40**, 796 (1975).
(4) D. D. Wheeler, D. C. Young, and D. S. Erley, *J. Org. Chem.*, **22**, 547 (1957).
(5) H. I. Nakada and S. Weinhouse, *J. Biol. Chem.*, **204**, 831 (1953).
(6) M. Ikawa and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 653 (1954).

Iminium Salts from α-Amino Acid Decarbonylation. Application to the Synthesis of Berbines[1]

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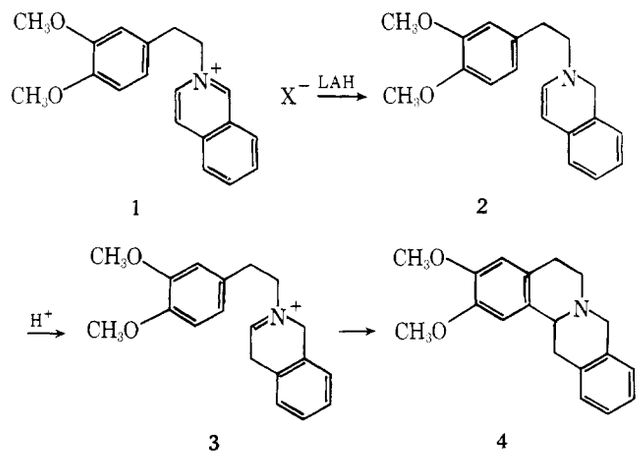
Berbines are synthesized from α-(tertiary amino) acids in high yields through decarbonylation to regiospecific iminium salts followed by an acid-catalyzed cyclization reaction. Syntheses of the α-(tertiary amino) acids from various phenylalanines which involve as the key step alkylation of a 1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid with a 2-phenylethyl bromide are described. The synthesis of isopropyl 1,2,3,4-tetrahydro-7,8-dimethoxy-3-isoquinolinecarboxylate (**15**), obligatory to the synthesis of 9,10-dimethoxyberbines by the above method, is described. It utilizes a metalation to align four contiguous substituents on the aromatic nucleus followed by a difficult selective reduction of an amide α to an ester.

A general, high-yield, regiospecific method for generating iminium salts should have broad applicability to the preparation of nitrogen-containing fused ring systems. One such system is the berbines, a class of naturally occurring and synthetic bases of the isoquinoline alkaloid group. Compounds of this type, such as 2,3-dimethoxyberbine (**4**),[2] have been synthesized via an iminium salt **3** derived from lithium aluminum hydride (LAH) reduction of an isoquinolinium salt **1**, followed by acid treatment of the dihydroisoquinoline **2** (Scheme I).

The overall yields of berbines reported for this process vary from low to moderate (18–66%).[2-4] Three factors may detract from the efficacy of generating the iminium salt by this reductive process. First, though reduction of the isoquinolinium salt with LAH produces the dihydroisoquinoline, this may be slowly reduced itself by LAH to the 1,2,3,4-tetrahydroisoquinoline.[5] Second, the resulting dihydroisoquinoline is subject to dimerization on acid treatment.[6,7] Third, dihydroisoquinolines are reported to disproportionate to a 1,2,3,4-tetrahydroisoquinoline and isoquinoline especially when a C-4 substituent is present.[8,9] In addition this classical process has significant limitations in potential substitution patterns.

We reported recently that iminium salts could be isolated from α-tertiaryamino acids by mild treatment (room temperature or warming in $POCl_3$) in high yields (>90%) and

Scheme I. Synthesis of Berbines via Partial Reduction of Isoquinolinium Salts



importantly regiospecifically by command of the position by the carboxyl substituent.[10] This decarbonylation of an α-tertiaryamino acid would avoid the pitfalls of the previous method. As a test of its effectiveness, we have applied our decarbonylative iminium salt procedure to the synthesis of a variety of berbines. Our process consists in every case of

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