magnetic stirring with an air space between the flask and driving unit; the temperature fluctuated slightly above room temperature, and at the finish was 26.5°. During the stirring the preparation was observed to enter a period of solution followed by a period of precipitation. The crystalline precipitate was centrifuged, washed with hexane, and dried; yield, 0.14 g. The preparation was extracted with water at room temperature, the extracts combined, and recrystallization effected by refrigeration, yield, 0.07 g. This was identified as glycine anhydride by microscopic examination of its optical characteristics.

Conversion of glycylvaline complex to glycylvaline anhydride. A mixture of 0.50 g. of glycylvaline and 12.5 ml. of butyl titanate in 37.5 ml. of ethyl alcohol was heated until the glycylvaline dissolved (a few minutes). The solution remained clear for several weeks at room temperature and was unchanged by overnight storage at −12°. Thereafter the solution gradually deposited long slender needles. The crop was permitted to grow for a month and was then centrifuged. An attempt to wash the precipitate with ethyl alcohol, to avoid possible precipitation of complex with hexane, caused the precipitate to dissolve. The precipitate was recovered by adding water to hydrolyze residual butyl titanate, drying, and extracting the residue with ethyl alcohol. The ethyl alcohol extract was dried and extracted with water to clarify it, and the water extract dried; yield, 0.12 g. The product contained no ash, gave negative ninhydrin and biuret tests, a positive diketopiperazine test, and melted at 240–242° with sublimation and slight decomposition (reported[10] m.p. of glycylvaline anhydride, 245°). Glycylvaline melted with decomposition at 228–233°; m.p. of the mixture was 228–240° with partial decomposition and some sublimation.

Anal. Calcd. for $C_7H_{12}N_2O_2$: C, 53.8; H, 7.75; N, 17.9. Found: C, 53.9; H, 7.57; N, 17.8.

Acknowledgment. The authors wish to thank Dr. F. T. Jones for microscopic identification and photomicrographs, Dr. K. J. Palmer for X-ray analysis, Dr. J. E. Moore for paper chromatography, Dr. K. P. Dimick for gas chromatography, and L. M. White and G. E. Secor for microanalytical carbon, hydrogen, and nitrogen determinations.

ALBANY, CALIF.

(10) P. Bergell, *Hoppe-Seyler's Z. physiol. Chem.*, 97, 300 (1916).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Preparation of Benzyl and *p*-Nitrobenzyl Esters of Amino Acids[1]

JAMES E. SHIELDS,[2a] WILLIAM H. McGREGOR[2b] AND FREDERICK H. CARPENTER

Received September 8, 1960

The use of an azeotropic procedure to prepare the benzyl and *p*-nitrobenzyl esters of several amino acids is described. Included are the *p*-nitrobenzyl esters of N^{ϵ}-tosyl-L-lysine, N^{ϵ}-(*p*-nitrobenzyloxycarbonyl)-L-lysine and L-histidine, compounds of difficult accessibility utilizing previously published methods.

The synthesis of peptides frequently involves the coupling of an *N*-carbobenzoxyamino acid or peptide with an amino acid or peptide ester. When methyl or ethyl esters are used, a saponification step is required to uncover the carboxyl group of the coupled product.[3] It is often convenient and sometimes mandatory to avoid this saponification step. In such cases the benzyl ester has been used to cover the carboxyl group.[4] The benzyl ester can be removed concomitantly with the carbobenzoxy group by hydrogenolysis[4] or with less facility by the use of hydrogen bromide in acetic acid.[5] Improved methods of preparation have made the benzyl esters of amino acids more generally available for peptide synthesis.[6] Recently Maclaren, Savige and Swan[6f] employed an azeotropic distillation method to prepare *S*-benzyl-L-cysteine benzyl ester. Carbon tetrachloride was used to form the azeotrope, and *p*-toluenesulfonic acid was used to catalyze the esterification. We are reporting our experience in the preparation of several benzyl esters of amino acids in which a similar procedure was used with the exception that benzenesulfonic acid[6a] instead of *p*-toluenesulfonic acid was used as the catalyst. The condensed azeotrope was passed through a bed of silica gel or anhydrous calcium sulfate to remove the water before being returned to the pot. The use of commercial preparations of

(1) This work was supported in part by grant A-608 of the National Institute for Arthritis and Metabolic Diseases of the National Institutes of Health.

(2)(a) National Science Foundation predoctoral fellow, 1956–1959. (b) Present address: Union Carbide Research Institute, 32 Depot Plaza, White Plains, N. Y.

(3) For a review of the subject see: J. S. Fruton, *Advances in Protein Chem.*, 5, 1 (1949); M. Goodman and G. W. Kenner, *Advances in Protein Chem.*, 12, 466 (1957).

(4) M. Bergmann, L. Zervas, and L. Salzmann, *Ber.* 66, 1288 (1933); M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, 117, 189 (1937).

(5)(a) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, 17, 1564 (1952). (b) G. W. Anderson, J. Blodinger, and A. D. Welcher, *J. Am. Chem. Soc.*, 74, 5309 (1952); N. F. Albertson and F. C. McKay, *J. Am. Chem. Soc.*, 75, 5323 (1953). (c) D. Ben-Ishai, *J. Org. Chem.*, 19, 62 (1954).

(6)(a) H. K. Miller and H. Waelsch, *J. Am. Chem. Soc.*, 74, 1092 (1952); (b) H. Sachs and E. Brand, *J. Am. Chem. Soc.*, 75, 4610 (1953); (c) J. D. Cipera and R. V. V. Nichols, *Chemistry and Industry*, 16 (1955); (d) B. F. Erlanger and R. M. Hall, *J. Am. Chem. Soc.*, 76, 5781 (1954); (e) K. C. Hooper, H. N. Rydon, J. A. Schofield, and G. S. Heaton, *J. Chem. Soc.*, 3148 (1956); N. Izumiya and S. Makisumi, *J. Chem. Soc. Japan*, 78, 662 (1957) and *Chem. Abstr.*, 53, 5148 (1959); K. Hofmann, M. E. Woolner, G. Spühler, and E. T. Schwartz, *J. Am. Chem. Soc.*, 80, 1486 (1958); (f) J. A. Maclaren, W. E. Savige, and J. M. Swan, *Australian J. Chem.*, 11, 345 (1958).

these drying agents containing an indicator, aided in following the course of the reaction and in estimating the amount of drying agent needed for a run. Preparation of the benzyl esters of the amino acids as their benzenesulfonic acid salts rather than *p*-toluenesulfonic acid salts has some advantages in subsequent synthetic procedures involving the generation of a free amino ester from the salt. In the procedure of Miller and Waelsch[6a] for preparation of the free base by solution of the salt in chloroform with an equivalent of triethylamine followed by precipitation of the triethylammonium salt with ether, we found that the benzenesulfonic acid gave an insoluble, crystalline salt which was easily removed, whereas *p*-toluenesulfonic acid gave an oil. The formation of this oil interferes with the isolation of the ester.

One potential disadvantage in the use of benzyl esters in peptide synthesis is their lability to anhydrous hydrogen bromide. It is frequently desirable to uncover the amino group of an intermediate peptide while retaining an ester on the carboxyl group. If anhydrous hydrogen bromide in acetic acid is used to cleave the carbobenzoxy group, partial or complete cleavage of the benzyl ester may result.[5a,c] To avoid this acid cleavage, Schwyzer and Sieber[7] have suggested the use of *p*-nitrobenzyl esters to obtain improved acid stability with a group which can subsequently be removed by hydrogenolysis.[8] The only published reports of the synthesis of *p*-nitrobenzyl esters of amino acids have followed the classical methods for the preparation of esters of this alcohol.[9] The *N*-acylamino acid or peptide has been treated with *p*-nitrobenzyl bromide in the presence of a tertiary amine.[7,10] This route to the ester requires that the amino group of the amino acid or peptide, as well as any basic groups of the side chains, be protected against alkylation by the *p*-nitrobenzyl halide. Since the amino acid must be acylated before and de-acylated after esterification, this route adds two extra steps to the synthesis of the ester where a free amino acid is to be used for the starting material. Moreover, in the particular case of histidine, esterification by the alkylation method was not possible.[10b]

The successful use in this laboratory of the carbon tetrachloride azeotropic method for preparing benzyl esters of amino acids prompted an attempt to use this method for the preparation of amino acid esters of *p*-nitrobenzyl alcohol directly from the free amino acids and the alcohol. The azeotropic method proved successful in the preparation of the *p*-nitrobenzyl esters of L-alanine, L-phenylalanine, L-histidine, N^{ϵ}-tosyl-L-lysine and N^{ϵ}-(*p*-nitrobenzyloxycarbonyl)-L-lysine. Preparation of the *p*-nitrobenzyl esters by the alkylation method would involve undesirable complexities for the latter three compounds.

Free amino acids are not appreciably soluble in carbon tetrachloride, nor is benzenesulfonic acid. As a result, in the preparation of *p*-nitrobenzyl esters by the azeotropic method, a two-phase system is obtained. On heating, the benzenesulfonic acid melts and forms the second liquid phase. This is lighter than the carbon tetrachloride solution and remains on the surface. In many instances this upper phase solidified as the reaction progressed. The reaction was usually stopped at this point. Some esterifications did not yield a solid upper phase till the solution was allowed to cool.

In the case of N^{ϵ}-(*p*-nitrobenzyloxycarbonyl)-L-lysine, it was found that using an excess of *p*-nitrobenzyl alcohol in the reaction mixture improved the yields. When the ratio of alcohol to amino acid was increased from 1.5 to 3.5, the yield increased from 50 to 90%. In the esterification of L-alanine, no improvement was found (55 to 60% in either case).

It was of some interest to determine whether or not the *p*-nitrobenzyloxycarbonyl group could be cleaved from an amino acid *p*-nitrobenzyl ester with hydrogen bromide-acetic acid without concomitant splitting of the ester bond. The *p*-nitrobenzyl ester of *p*-nitrobenzyloxycarbonyl-L-alanine was made by the alkylation method. The compound was treated with hydrogen bromide in acetic acid at 60° for 45 min., to give a 62% yield of L-alanine *p*-nitrobenzyl ester hydrobromide. This experiment demonstrates not only the marked stability of the *p*-nitrobenzyl ester to acid cleavage, but the differential stability of carboxylate esters as compared to carbamate esters.

EXPERIMENTAL[11]

Amino acid benzyl ester benzenesulfonates. These were prepared in a manner similar to that of Maclaren, Savige and Swan.[6f] Twenty mmoles of amino acid, 21 mmoles of benzenesulfonic acid monohydrate, 14 ml. of benzyl alcohol, and 50 ml. of carbon tetrachloride were refluxed for 15 hr. The reflux condensate was passed through a continuous extraction apparatus[12] containing silica gel to remove water before the solvent was returned to the pot. While the carbon tetrachloride solution was still warm, ether or carbon tetrachloride was added, and the mixture was placed in the refrigerator overnight. The product was recrystallized from alcohol and ether.

(7) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **42**, 972 (1959).

(8) F. H. Carpenter and D. T. Gish, *J. Am. Chem. Soc.*, **74**, 3818 (1952).

(9) E. E. Reid, *J. Am. Chem. Soc.*, **39**, 124 (1917); J. A. Lyman and E. E. Reid, *J. Am. Chem. Soc.*, **39**, 701 (1917).

(10)(a) R. Schwyzer, B. Iselin, and M. Feurer, *Helv. Chim. Acta*, **38**, 69 (1955). (b) H. Schwarz and K. Arakawa, *J. Am. Chem. Soc.*, **81**, 5691 (1959).

(11) The analyses were performed by the Microchemical Laboratory, Department of Chemistry, University of California, Berkeley. Melting points are capillary melting points and are uncorrected.

(12) Similar in design to a "Crank case dilution distilling receiver" (Kimble Laboratory Glassware catalogue number 22009), modified to hold a larger amount of drying agent.

L-Valine benzyl ester benzenesulfonate and hydrochloride. L-Valine (2.34 g.) and 3.7 g. of benzenesulfonic acid monohydrate were allowed to react according to the procedure described above to give 6.8 g., 93%, of material which was recrystallized from methanol and ether and dried over phosphorus pentoxide *in vacuo* for analysis; m.p. 177–180°, $[\alpha]_D^{24}$ +14° (*c*, 3.78 pyridine).

Anal. Calcd. for $C_{18}H_{23}O_5NS$: N, 3.83; S, 8.77. Found: N, 4.13; S, 9.15.

Conversion to the hydrochloride was accomplished by stirring 2 g. of ester with 9 ml. of chloroform and 0.77 ml. of triethylamine until solution occurred. Ether was added to precipitate the triethylammonium benzenesulfonate, which was filtered off; and hydrogen chloride was bubbled through the filtrate. The crystalline product was dried over phosphorus pentoxide *in vacuo* at room temperature; wt. 1.16 g., 87%, m.p. 138.5–140°. This material did not lower the melting point of a sample prepared by the polyphosphoric acid method of Erlanger and Hall[6d] from 5.0 g., 4.3 mmoles, of L-valine, 50 ml. of benzyl alcohol, and 10 g. of polyphosphoric acid. This product was crystallized from hot ethyl acetate; wt. 3.15 g., 30%, m.p. 138–139°, $[\alpha]_D^{24}$ +9.1° (*c*, 3 pyridine).

Anal. Calcd. for $C_{12}H_{18}O_2NCl$: C, 59.14; H, 7.44; N, 5.75; Cl, 14.55. Found: C, 59.20; H, 7.40; N, 5.59; Cl, 14.59.

L-Glutamic acid dibenzyl ester benzenesulfonate and hydrochloride. L-Glutamic acid (2.94 g.) and 3.7 g. of benzensulfonic acid monohydrate were treated as above; yield 9.0 g., 90%. The crude product was recrystallized from methanol and ether and dried over phosphorus pentoxide *in vacuo* at room temperature; m.p. 115–117°, $[\alpha]_D^{24}$ +18° (*c*, 3.11 pyridine).

Anal. Calcd. for $C_{25}H_{27}O_7NS$: N, 2.88; S, 6.60. Found: N, 3.09; S, 6.60.

A sample was converted to the hydrochloride by stirring 2 g. in 9 ml. of chloroform and 0.58 ml. of triethylamine and working up as above. The product was crystallized from methanol and ether; wt. 1.35 g., 90%, m.p. 100–102° (*cf.* lit.[6b] m.p. 100–102°). A mixed melting point with a sample of L-glutamic acid dibenzyl ester hydrochloride prepared by the method of Sachs and Brand[6b] showed no depression.

L-Alanine benzyl ester benzenesulfonate. L-Alanine (1.78 g.) and 3.7 g. of benzenesulfonic acid monohydrate were allowed to react as described. The product was recrystallized from ethanol and ether; wt. 6.1 g., 91%, m.p. 111.5–113.5°. The product was dried over phosphorus pentoxide *in vacuo* at room temperature for analysis, $[\alpha]_D^{24}$ +7.5° (*c*, 4.5 pyridine).

Anal. Calcd. for $C_{16}H_{19}O_5NS$: N, 4.15; S, 9.50. Found: N, 4.29; S, 9.63.

L-Phenylalanine benzyl ester benzenesulfonate and hydrochloride. L-Phenylalanine (3.3 g.) and 3.7 g. of benzenesulfonic acid monohydrate were reacted according to the procedure outlined above; yield 8.3 g., 100%, m.p. 162–164°. The product was dried over phosphorus pentoxide *in vacuo* at room temperature for analysis, $[\alpha]_D^{24}$ +19° (*c*, 3.29 pyridine).

Anal. Calcd. for $C_{22}H_{23}O_5NS$: N, 3.39; S, 7.75. Found: N, 3.57; S, 7.82.

The product was converted to the hydrochloride as previously described, using 2 g. of the ester benzenesulfonate, 10 ml. of chloroform, and 0.68 ml. of triethylamine; yield 1.2 g., 85%. After recrystallization from ethyl acetate and petroleum ether, the product showed m.p. 203–205° (*cf.* lit. m.p. 203° reported by Erlanger and Hall[6d]).

N^{ϵ}-Tosyl-L-lysine p-nitrobenzyl ester benzenesulfonate. A suspension of N^{ϵ}-tosyl-L-lysine[13] (3.00 g., 0.010 mole), *p*-nitrobenzyl alcohol (2.31 g., 0.015 mole), and benzenesulfonic acid monohydrate (2.4 g., 0.014 mole) in 200 ml. of carbon tetrachloride was refluxed. The condensate was passed through a bed of silica gel in a continuous extractor[12] to remove water. The mixture was refluxed for 1 day or more, the reaction being judged complete when the insoluble oil on the surface of the carbon tetrachloride had entirely solidified. This solid was then crystallized from 95% aqueous acetone by the slow addition of ether. The resulting product was recrystallized from hot water to give a white crystalline solid, wt. 4.08 g., 69%, m.p. 170–172°, $[\alpha]_D^{24}$ +3.2° (*c*, 4 dimethylformamide).

Anal. Calcd. for $C_{26}H_{31}O_9N_3S_2$: C, 52.60; H, 5.26; N, 7.08. Found: C, 51.90; H, 5.15; N, 6.84.

N^{ϵ}-(p-Nitrobenzyloxycarbonyl)-L-lysine. The copper chelate was prepared according to the procedure of Roeske, Stewart, Stedman, and du Vigneaud[13a] and was acylated with *p*-nitrobenzyl chloroformate according to Gish and Carpenter.[14] To 200 ml. of boiling water were added L-lysine monohydrochloride (18.3 g., 0.010 mole) and, in portions, basic copper carbonate (*ca.* 12 g.). The hot suspension was filtered to remove excess copper carbonate, and the residue on the filter was washed with 50 ml. of hot water. To the combined filtrates were added 50 ml. of 2*N* potassium bicarbonate and 150 ml. of purified dioxane.[15] The solution was cooled in an ice bath. *p*-Nitrobenzyl chloroformate (27 g., 0.0125 mole) was made up as a 2*N* solution in dioxane (total volume, 64 ml.). This solution and 4*N* potassium hydroxide (32 ml.) were added in portions to the lysine solution with mechanical stirring and continuous cooling in ice. The stirring was continued for about 0.5 hr. after all the reactants had been added. Then the blue solid was washed by decantation with water, alcohol and ether; wt. 30 g., 70%. The blue copper complex was suspended in 200 ml. of *N* hydrochloric acid and treated with hydrogen sulfide for 0.5 hr. while being stirred magnetically. The precipitated copper sulfide was filtered off and washed with water. The combined filtrates were neutralized with ammonium hydroxide to *p*H 6. The white precipitate was filtered and recrystallized from 600 ml. of boiling water. The crystalline product was dried *in vacuo* over phosphorus pentoxide; wt. 21.5 g., 66%, m.p. 228° dec.

A sample was treated with decolorizing carbon and recrystallized from boiling water; m.p. 240–241° dec., $[\alpha]_D^{24}$ +14.2° (*c*, 4 6*N* hydrochloric acid).

Anal. Calcd. for $C_{14}H_{19}O_6N_3$: C, 51.69; H, 5.89; N, 12.92. Found: C, 51.31; H, 5.75; N, 12.42.

N^{ϵ}-(p-Nitrobenzyloxycarbonyl)-L-lysine p-nitrobenzyl ester benzenesulfonate. A suspension of N^{ϵ}-(*p*-nitrobenzyloxycarbonyl)-L-lysine (6.50 g., 0.020 mole), benzenesulfonic acid monohydrate (4 g., 0.022 mole) and *p*-nitrobenzyl alcohol (10 g., 0.07 mole) in carbon tetrachloride (250 ml.) was refluxed and the condensate passed through a bed of anhydrous calcium sulfate (indicating Drierite) for 1 day or more. The solid material from the reaction mixture was crystallized from 700–800 ml. of boiling water. The mass of white, feathery crystals was dried *in vacuo* over phosphorus pentoxide; wt. 11.13 g., 90%, m.p. 155–157° with sintering at 150°.

A sample (0.50 g.) was treated with decolorizing charcoal and recrystallized from 30 ml. of boiling water; wt. 0.35 g., 70% recovery, m.p. 168–169°, $[\alpha]_D^{24}$ +3.7° (*c*, 3 dimethylformamide).

---

(13)(a) R. Roeske, F. H. C. Stewart, R. J. Stedman, and V. du Vigneaud, *J. Am. Chem. Soc.*, **78**, 5883 (1956). (b) The compound melted at 237–238° dec., $[\alpha]_D^{24}$ +14.5° (*c*, 3 5.9*N* hydrochloric acid) prepared by the method of Roeske, *et al.*, whereas they reported m.p. 237–238° dec., $[\alpha]_D^{25}$ +13.6° (*c*, 3: 2*N* hydrochloric acid). B. F. Erlanger, W. V. Curran and N. Kokowsky *J. Am. Chem. Soc.*, 81, 3051 (1959) reported m.p. 234–237° dec., $[\alpha]_D^{24}$ +16.4° (*c*, 2: 6*N* hydrochloric acid) for the same material.

(14) D. T. Gish and F. H. Carpenter, *J. Am. Chem. Soc.*, **75**, 950 (1953).

(15) Purified according to E. Eigenberger in *Organic Solvents*, A. Weissberger, E. S. Proskauer, J. A. Riddick, and E. E. Toops, Jr., eds., Interscience, New York, 1955, p. 372.

Anal. Calcd. for $C_{27}H_{30}O_{11}N_4S$: C, 52.42; H, 4.89; N, 9.06. Found: C, 52.11; H, 5.03; N, 8.52.

L-*Phenylalanine p-nitrobenzyl ester benzenesulfonate.* A suspension of L-phenylalanine (1.65 g., 0.010 mole), benzenesulfonic acid monohydrate (2 g., 0.011 mole), and *p*-nitrobenzyl alcohol (2.1 g., 0.014 mole) in carbon tetrachloride (130 ml.) was refluxed and the condensate passed through a bed of silica gel for 1 day. The solution was decanted, and the remaining solid was dissolved in 95% aqueous acetone. Crystallization was brought about by the slow addition of ether, giving a tan solid. This was recrystallized from boiling water with treatment with decolorizing charcoal. The product was dried *in vacuo* over phosphorus pentoxide; wt. 2.93 g., 64%, m.p. 190–191°, $[\alpha]_D^{24}$ +11.0° (*c*, 4 dimethylformamide).

Anal. Calcd. for $C_{22}H_{22}O_7N_2S$: C, 57.64; H, 4.84; N, 6.11. Found: C, 58.01; H, 4.84; N, 5.91.

Although the benzenesulfonic acid salt of free L-phenylalanine melted at 189–190°, admixture of the above ester salt with the free amino acid salt gave m.p. 148–153°.

L-*Alanine p-nitrobenzyl ester benzenesulfonate.* L-Alanine (0.89 g., 0.010 mole), *p*-nitrobenzyl alcohol (2.0 g., 0.013 mole), and benzenesulfonic acid monohydrate (2.3 g., 0.013 mole) were suspended in carbon tetrachloride (250 ml.) and refluxed for 2 days. The condensate was passed through a bed of indicating silica gel. The solution was decanted after cooling, and the remaining solid was crystallized from 95% aqueous acetone by the slow addition of ether. The resulting tan solid (wt. 3.13 g., 82%) was treated with decolorizing charcoal and recrystallized from boiling water. The product was dried *in vacuo* over phosphorus pentoxide; wt. 1.66 g., 44%, m.p. 158–160°, $[\alpha]_D^{24}$ +7.1° (*c*, 7.5 pyridine).

A second crop, wt. 0.47 g., 12%, was obtained from the mother liquors.

Anal. Calcd. for $C_{16}H_{18}O_7N_2S$: C, 50.26; H, 4.74; N, 7.33. Found: C, 50.81; H, 4.64; N, 7.18.

*p-Nitrobenzyloxycarbonyl-*L*-alanine p-nitrobenzyl ester.* *p*-Nitrobenzyloxycarbonyl-L-alanine was prepared according to the procedure of Gish and Carpenter[14] for the DL-compound to give a colorless oil. A solution of this oil (2.4 g., 0.009 mole) in 35 ml. of ethyl acetate was treated with *p*-nitrobenzyl bromide (2.4 g., 0.011 mole) and triethylamine (1.4 ml., 0.010 mole). The reaction mixture was allowed to stand at room temperature for 3 days. The crystallized triethylammoniun bromide was filtered off and washed with fresh ethyl acetate. The combined filtrates were washed with *N* hydrochloric acid, water, ice-cold freshly prepared *N* sodium bicarbonate, and cold water. The ethyl acetate solution was dried with anhydrous magnesium sulfate, filtered, and evaporated *in vacuo*. The solid residue, wt. 1.97 g., m.p. 119–122°, was triturated with ether, cooled to 0°, and filtered; wt. 1.39 g., 38%, m.p. 131–134° with sintering at 125°. This was recrystallized from benzene-pentane; wt. 1.18 g., 33%, m.p. 132.5–135° with sintering at 125°, $[\alpha]_D^{24}$ −1.3° (*c*, 4 chloroform).

Anal. Calcd. for $C_{18}H_{17}O_8N_3$: C, 54.05; H, 4.25; N, 10.42. Found: C, 55.42; H, 4.34; N, 10.52.

L-*Alanine p-nitrobenzyl ester hydrobromide.* *p*-Nitrobenzyloxycarbonyl-L-alanine *p*-nitrobenzyl ester (3.00 g., 0.0074 mole) was dissolved in 50 ml. of 35% (by wt.) hydrogen bromide in glacial acetic acid. The reaction mixture was heated at 60° for 45 min. and then poured into 400 ml. of ether. The white solid was dissolved in methanol and crystallized by the addition of ether; wt. 1.39 g., 62%, m.p. 177–178.5°, $[\alpha]_D^{25}$ −3.0° (*c*, 5 dimethylformamide).

Anal. Calcd. for $C_{10}H_{13}O_4N_2Br$: C, 39.37; H, 4.20; N, 9.19; Br, 26.20. Found: C, 39.33; H, 4.40; N, 9.18; Br, 26.14.

DL-*Histidine p-nitrobenzyl ester dibenzenesulfonate.* DL-Histidine monohydrochloride dihydrate (2.98 g., 0.013 mole), benzenesulfonic acid monohydrate (5 g., 0.03 mole), and *p*-nitrobenzyl alcohol (5 g., 0.03 mole) were suspended in 50 ml. of carbon tetrachloride. The reaction mixture was refluxed 2 days. The azeotrope was dried with anhydrous calcium sulfate before returning to the pot. The solid residue was washed with ether and dissolved in 4 ml. of water. Acetone (100 ml.) was added to the aqueous solution to precipitate unesterified histidine as the salt; however, no precipitate formed, so the solution was evaporated to dryness *in vacuo*. The residual oil was dissolved in 5 ml. of 95% ethanol. The product crystallized on standing at room temperature overnight; wt. 4.83 g., 60%, m.p. 215–217°.

Anal. Calcd. for $C_{26}H_{26}N_4O_{10}S_2$: C, 49.50; H, 4.32; N, 9.24. Found: C, 49.87; H, 4.16; N, 9.03.

L-*Histidine p-nitrobenzyl ester dibenzenesulfonate.* A suspension of L-histidine (1.55 g., 0.010 mole), *p*-nitrobenzyl alcohol (4.5 g., 0.030 mole), and benzenesulfonic acid monohydrate (4 g., 0.025 mole) in 50 ml. of carbon tetrachloride was refluxed, and the condensate was passed through a bed of anhydrous calcium sulfate for 2 days. The solution was decanted from the solid, which was taken up in 5 ml. of *ca.* 90% ethanol. Ether was added slowly with scratching to crystallize the product as the monohydrate; wt. 5.61 g., 90%, m.p. 92–95°, $[\alpha]_D^{29}$ −4.95° (*c*, 3 pyridine).

Anal. Calcd. for $C_{26}H_{26}N_4O_{10}S_2.H_2O$: C, 48.07; H, 4.52; N, 8.97. Found: C, 48.38; H, 4.54; N, 8.90.

A sample was dried *in vacuo* at 78° for 12 hr. The loss in weight was 2.71%. Theoretical loss in weight for 1 mole of water of hydration per mole of ester, 2.88%.

BERKELEY 4, CALIF.