



heated at refluxing temperature for an hour or until complete solution had been effected. An excess of ammonia was added to the clear solution whereupon a white solid precipitated at once; m. p. 187–189°. The product was collected, dried and recrystallized from 250 ml. of Cello-solve; yield 37.8 g. (84% yield) of desired product; m. p. 191–192°. Further recrystallization did not change the melting point.

Anal. Calcd. for $C_{17}H_{19}N_3O_2S_2$: N, 11.63. Found: N, 11.71.

The presence of a free primary aromatic amino group was confirmed by diazotization and coupling with 2-naphthol to produce a red dye.

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Crystalline Carbobenzyloxy Derivatives of DL-Valine and DL-Leucine¹

The carbobenzyloxy derivatives of DL-leucine² and of L-leucine³ have been reported as oils. The corresponding L-valine derivative has been reported as an oil which crystallized after weeks of storage.⁴ In studies of peptide synthesis⁵ and in enzyme-substrate investigations⁶ crystalline, preferably pure, carbobenzyloxy derivatives of DL-valine and DL-leucine were sought. The procedure given below has served to yield these derivatives readily in a few days in an analytically pure, crystalline condition. Seeds of the carbobenzyloxy-DL-valine thus obtained have been used to induce crystallization in an oily mass in another laboratory.⁷

Carbobenzyloxy-DL-valine.—Eleven and seven-tenths gram (0.10 mole) of DL-valine (Dow) was dissolved in 25 ml. of 4 *N* sodium hydroxide. With cooling in an ice-bath, there were added simultaneously with stirring, over a period of forty-five minutes, 25 ml. of 4 *N* sodium hydroxide and 23 ml. of carbobenzyloxy chloride solution⁸ (from 200 g. of 20% phosgene in toluene and 38 ml. of benzyl alcohol, allowed to react and then concentrated under reduced pressure to 80 g.). The solution was acidified with hydrochloric acid, which deposited an oil. The mass was crystallized by dissolving in dilute sodium hydroxide, reprecipitating with hydrochloric acid and permitting it to stand overnight in the refrigerator; yield, 21 g. (84%) with a m. p. of 75–77° (uncor.). In later preparations, ether or ethyl acetate was used to extract the initial oil obtained upon acidification, and the extracts were dried and the solvent removed by evaporation. The residue was crystallized by dissolving in benzene, precipitating with hexane, and seeding with material from the first preparation; yields, 16 g. (65%) and 20.5 g. (82%), m. p. 76–78° (uncor.). *Anal.* Calcd. for $C_{12}H_{17}O_4N$: N, 5.58. Found: N, 5.59. The m. p. reported for the L isomer was 64–65°.⁴

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(2) Bergmann and Fraenkel-Conrat, *J. Biol. Chem.*, **119**, 707 (1937).

(3) Bergmann, Zervas and Fruton, *ibid.*, **115**, 593 (1936).

(4) Synge, *Biochem. J.*, **42**, 101 (1948).

(5) Fling and Fox, unpublished experiments.

(6) Fox, Pettinga, Halverson, and Wax, *Arch. Biochem.*, **25**, 21 (1950).

(7) Private communication from Dr. J. W. Hinman.

(8) Bergmann and Zervas, *Ber.*, **65**, 1192 (1932).

Carbobenzyloxy-DL-leucine.—Twenty-six and two-tenths grams (0.20 mole) of DL-leucine (Dow) was treated with carbobenzyloxy chloride as above. Acidification of the reaction mixture with concentrated hydrochloric acid yielded an oil which was taken up with three 75-ml. portions of ether. The combined ethereal extracts were washed with 25 ml. of water and re-extracted carefully with three portions (75, 50 and 50 ml.) of saturated sodium bicarbonate solution. The combined bicarbonate extracts were then acidified with concentrated hydrochloric acid to congo red. The oil which separated was taken up with ether. The washed ethereal extract was dried over sodium sulfate.

The dry ethereal solution was then concentrated to a sirupy consistency by distilling off the solvent under reduced pressure, and the material was set into the freezer (–12°). There was some initial evidence of crystallization overnight. Fifty ml. of benzene was added and the mixture was heated to 50°. The sirup did not dissolve. Two hundred ml. of hexane was added and the mixture placed in the freezer. It was stirred periodically with a stirring rod which had a few crystals adhering to it. Crystallization commenced after one day and a white amorphous mass was obtained.

Since it gave evidence of melting at room temperature, the solid was filtered off in a cold room, washed with cold hexane and dried in a vacuum desiccator. Forty grams of product melting at 44–52° was obtained. This material was dissolved in a minimum amount of warm benzene and filtered. Sufficient hexane was added to the cold filtrate to cause some oiling, and the mixture was seeded. The material crystallized slowly, going through a gummy stage, but eventually it yielded a smooth, creamy paste. This was filtered off in the cold, dried by suction and then in a vacuum desiccator. The yield was 35.5 g. (67%), and the material melted at 45–48° (uncor.).

Anal. Calcd. for $C_{14}H_{19}O_4N$: N, 5.28. Found: N, 5.17, 5.14.

In later runs, the two derivatives were crystallized more readily by washing the oils obtained in syntheses from one-half mole of amino acid, with three 500-ml. portions of water and rubbing under renewed portions of hexane until crystallization ensued. The possibility of atmospheric seeding in repetitions cannot be excluded. The yield of carbobenzyloxy-DL-valine by this last procedure was 113 g. (90%) with m. p. 74–76° (cor.) and 125 g. (94%) of carbobenzyloxy-DL-leucine, m. p. 46–49° (cor.). N contents of the derivatives were, respectively, 5.56 and 5.24%.

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1-Benzyl-5-*p*-chlorophenylbiguanide Monohydrochloride

A mixture of 5.8 g. of *p*-chlorophenyldicyandiamide¹ and 5.4 g. of benzylamine hydrochloride was ground well in a mortar, and then by means of an oil-bath heated in a flask for a few moments at 190°. Heating was discontinued as soon as the melt turned dark and smoke began to form. The product was leached with several portions of boiling water. Upon cooling 7 g. (64% yield) of white crystalline 1-benzyl-5-*p*-chlorophenylbiguanide monohydrochloride was obtained; m. p. 220°. Recrystallization from dilute hydrochloric acid did not raise the melting point.

Anal. Calcd. for $C_{11}H_{16}ClN_5 \cdot HCl$: N, 20.71. Found: N, 20.74.

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(1) Walther and Grieshammer, *J. prakt. Chem.*, [2] **92**, 251 (1915); Curd and Rose, *J. Chem. Soc.*, 729 (1948).