ethereal layer was washed with water, sodium carbonate solution, and again with water. The base in the ether layer was dried and converted to the hydrochloride which melts at 200°. The base was liberated from an aqueous solution of the hydrochloride and allowed to react with an excess of methyl iodide in ether. The quaternary salt melted at 181–182° as first obtained and at 182–185° after one crystallization from alcohol-ether mixture.

Anal. Calcd. for $C_{21}H_{29}IN_2O_2$: C, 55.8; H, 6.4. Found: C, 55.5; H, 6.6.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY,
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Preparation of DL-*beta*-(2-Fluorenyl)alanine[1]

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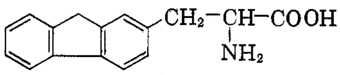
An amino acid derived from fluorene, DL-β-(2-fluorenyl)alanine, was synthesized from fluorene-2-carboxaldehyde or ethyl fluorene-2-carboxylate as starting materials. The physical and chemical properties were determined and the amino acid was characterized as its hydrochloride salt and as the *N*-benzenesulfonyl derivative.

The preparation of DL-β-(2-fluorenyl)alanine was undertaken for work involving cancer chemotherapy with 2-substituted fluorenes containing biologically important polar side chains. The compound was thought to be of interest due to the known carcinogenic activity of 2-fluorenylamines, and because of the desirability of obtaining amino acids derived from the fluorene ring system for growth and antagonism studies. The compound (I) may also be considered as an indenophenyl alanine.



I

The amino acid was synthesized from fluorene-2-carboxaldehyde or from ethyl fluorene-2-carboxylate as starting materials. These substances were reduced with lithium aluminum hydride to the corresponding carbinol, which was transformed to the bromide with phosphorus tribromide. The carbinol and bromide have been previously prepared by another method,[2] but the hydride reduction seemed to be a simpler process. The bromide was used to alkylate the sodium derivative of diethyl acetamidomalonate, and the product was hydrolyzed and decarboxylated with difficulty by hydrochloric acid to the amino acid hydrochloride. The latter gave the desired compound when treated with ammonium hydroxide.

The amino acid is a high melting, very insoluble substance. The physical properties showed some resemblance to those of the corresponding 5-acenaphthenyl alanine,[3] but it was somewhat more stable than the latter, especially in hot alkaline media. The compound was characterized as the hydrochloride and as the *N*-benzenesulfonyl derivative.

EXPERIMENTAL

Melting points are uncorrected and were taken on a Fisher-Johns block.

2-Fluorenemethanol. A. From ethyl fluorene-2-carboxylate. The ethyl ester[4] of fluorene-2-carboxylic acid was employed due to its much greater solubility in solvents compared to the free acid.

A mixture of 8 g. of lithium aluminum hydride and 200 ml. of ether was refluxed for 1 hr. and cooled. To this was added slowly with stirring, a solution of 6.75 g. of the ethyl ester in 50 ml. of benzene. The liquid was then refluxed for 0.5 hr. and excess hydride decomposed with ethanol and dilute hydrochloric acid. The organic solvents were evaporated at room temperature and the aqueous-acid suspension filtered to yield the solid carbinol. Extraction of the latter with ether, and filtration, removed inorganic residues. The filtrate was diluted with petroleum ether and decolorized with Norit, and then filtered and evaporated. After recrystallization from dilute aqueous acetone, the material weighed 5.04 g. or 90.6%. On heating, it softened at about 120° and melted at 125–142° (lit. 131[2]). When the compound was recrystallized twice from ether-petroleum ether, it had m.p. 138–142°, and after 5 more recrystallizations, m.p. 140.5–142.5°. The melting point of the substance is sensitive to impurities but preparative methods and analysis of subsequent products would seem to confirm its identity.

B. From fluorene-2-aldehyde. The aldehyde was prepared by the method of Ayling, Hinkel, and Beynon.[5] Inorganic salts were removed from the product by extraction of the aldehyde with ether, filtration, and evaporation of the solvent.

A solution of 8 g. of lithium aluminum hydride in ether, prepared as above, was treated gradually with a solution of 6.9 g. of the aldehyde in 100 ml. of ether-benzene. A yellow-green color formed together with a light colored precipitate. The solution was refluxed 10 min. and then decomposed by ethanol and dilute hydrochloric acid. The organic solvents were removed at room temperature and the dilute acid suspension of the product was filtered. After washing and drying, the crude aldehyde weighed 6.87 g. or 98.5%. Without further purification, the cream-white solid had m.p. 126–130°. Both preparations of the carbinol gave the same bromide and subsequent derivatives.

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[1] The work described in this paper was carried out under a research grant (No. C-327 and CY-2915) to Prof. D. M. Greenberg, from the National Cancer Institute, United States Public Health Service.

[2] J. von Braun and H. Engel, *Ber.*, **57**, 191 (1924).

[3] D. C. Morrison, *J. Org. Chem.*, **23**, 33 (1958).

[4] D. C. Morrison, *J. Org. Chem.*, **23**, 1772 (1958).

[5] L. E. Hinkel, E. E. Ayling, and J. H. Beynon, *J. Chem. Soc.*, 339 (1936).

2-Fluorenylmethyl bromide. The carbinol (2.17 g., 0.011 mole) was added gradually to a solution of 3 ml. of phosphorus tribromide in 20 ml. of benzene. The mixture was shaken until the solid dissolved and then left several days at room temperature. Probably this long reaction time is not necessary. The benzene solution was poured slowly with stirring into a mixture of ether and water. The organic phase was extracted 3 times with water, filtered, and dried over anhydrous sodium sulfate. It was then evaporated to dryness under vacuum. A yield of 2.6 g. (90.7%) of cream colored solid was obtained. This material had m.p. 94–97° (lit.[2] 95°). When stored sealed at 0° for 1 week, the m.p. was lowered to 90–96°. The best preparation obtained showed m.p. 95–100° and all recrystallizations lowered this value, especially if done from aqueous solvents.

Diethyl (2-fluorenylmethyl)acetamidomalonate. A solution containing 0.24 g. of sodium (0.0104 mole) and 2.22 g. of diethyl acetamidomalonate (0.0104 mole) in 150 ml. of anhydrous ethanol (distilled from sodium and diethyl phthalate) was prepared. This was treated with a solution of 2.59 g. of the bromide (0.01 mole) in benzene-ethanol. Little or no heat was evolved and the mixture was refluxed for 16 hr. Acetic acid (5 ml.) was added and the mixture was distilled to a small volume under vacuum. It was then steam distilled for 0.5 hr. An orange oil was left in aqueous suspension and this crystallized on cooling and standing. This product was recrystallized once from aqueous acetone and dried. It weighed 3.5 g. or 88.6%. For purification, the ester was recrystallized from aqueous acetone and from ether-petroleum ether, m.p. 149.5–150.5°.

Anal. Calcd. for $C_{23}H_{25}NO_5$: C, 69.87; H, 6.33. Found: C, 69.64; H, 6.12.

DL-*β-(2-Fluorenyl)alanine hydrochloride.* Hydrolysis and decarboxylation of the ester required long refluxing with hydrochloric acid, or some ester or intermediate product was recovered. The best procedure found is described. A solution of 8.64 g. of the ester in 150 ml. of glacial acetic acid was heated to boiling under reflux and 125 ml. of concentrated hydrochloric acid was slowly added while maintaining reflux. The mixture was refluxed for 6 days and then evaporated to dryness under vacuum. The residue was repeatedly extracted with boiling 3*N* hydrochloric acid and the extracts filtered while above 90°. Storage of the combined filtrates at 0° overnight produced a crystalline deposit which was filtered, washed, and dried. The filtrates were boiled to a small volume and further crops taken. The combined yield of hydrochloride was 5.35 g. or 84.5%. The salt was decolorized in ethanol-benzene solution with Norit and then recrystallized from this solvent 5 times. Recrystallization from boiling dilute hydrochloric acid was less effective in purification. On heating, the hydrochloride sintered and discolored above 230° and melted 240–258° with decomposition, forming an orange-brown melt.

Anal. Calcd. for $C_{16}H_{16}NO_2Cl$: C, 66.32; H, 5.53. Found: C, 66.41; H, 5.35.

DL-*β-(2-Fluorenyl)alanine.* The amino acid hydrochloride was digested with a mixture of equal volumes of concentrated ammonium hydroxide and water on the steam bath until solution was obtained. The hot solution was filtered and the filtrate boiled down to concentrate the solution and to eliminate ammonia. Crystallization of the free amino acid began in the heated solution and was completed on cooling overnight on ice. It was then filtered, washed with water, and dried. From 1.37 g. of hydrochloride, 1.13 g. of amino acid were obtained or 94.3%. The amino acid formed a white powder which could be recrystallized by repeating the solution in hot dilute ammonia and concentrating. The analytical sample was vacuum dried for analysis.

Anal. Calcd. for $C_{16}H_{15}NO_2$: C, 75.89; H, 5.93. Found: C, 76.28; H, 6.06.

On heating, the amino acid begins to discolor (light orange-brown) at 210–215° and melts at 225–233° to a brown liquid with decomposition. On further recrystallization of the analytical sample, the melting point was lowered. This amino acid was nearly insoluble in boiling water, but dissolved in hot, dilute acids and alkalies, more readily in the latter. When boiled in suspension in dilute aqueous acetic acid with ninhydrin, the insoluble particles became purple and the liquid light brown, while with excess amino acid the liquid became greenish-brown. No change in appearance of the compound was observed during storage for 2 years.

*N-Benzenesulfonyl-*DL-*β-(2-fluorenyl)alanine.* This derivative was made by acylation of the fluorenylalanine dissolved in an excess of *N* potassium hydroxide, by stirring vigorously with an ether solution of benzenesulfonyl chloride. After several hours stirring, the mixture was filtered and the insoluble potassium salt washed with ether and dried. It was dissolved in hot water and acidified with an excess of dilute hydrochloric acid. The suspension of product was cooled in ice for some time and then filtered, washed with water, and dried. It was recrystallized a number of times from ether, m.p. 184–186°.

Anal. Calcd. for $C_{22}H_{19}NSO_4$: C, 67.18; H, 4.83. Found: C, 67.64; H, 5.14.

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[COMMUNICATION NO. 1969 FROM THE KODAK RESEARCH LABORATORIES]

Dithiocarbamates. I. Quaternary Ammonium Dithiocarbamates

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A series of dithiocarbamates containing tertiary amine groups has been alkylated to give quaternary ammonium or pyridinium dithiocarbamates. Proof of the structure of these products is based largely on their ultraviolet absorption spectra. The ultraviolet spectra of various thiono and dithiocarbamates are compared.

A number of dithiocarbamates have been prepared which contain a tertiary amine group; these have been quaternized to give water-soluble quaternary ammonium or pyridinium dithiocarbamates. The synthesis of the intermediate bases was carried out by an adaptation of the well known metathetical reaction between the alkali metal salt of a dithiocarbamate and an alkyl halide,[1] either or both moieties containing the tertiary

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(1) M. Kulka, *Can. J. Chem.*, **34**, 1093 (1956); G. Nachmias, *Ann. Chim.* (Paris), **7**, 584 (1952).