128. *Ferrocene Derivatives. Part VI.*[1] DL-*Ferrocenylalanine.*

By J. M. Osgerby and P. L. Pauson.

Syntheses of DL-ferrocenylalanine from ferrocenecarboxyaldehyde by the azlactone method, and, more efficiently, from (ferrocenylmethyl)trimethylammonium iodide *via* (ferrocenylmethyl)formamidomalonic ester are described. The latter starting material has also been converted into ferrocenyl-acetic and -propionic acid.

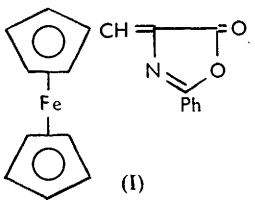
The availability of ferrocenecarboxyaldehyde [1] suggested several obvious routes to amino-acids containing the ferrocene nucleus. The biological properties of ferrocenylalanine appeared to be of potential interest and its preparation by the azlactone method was therefore chosen for initial study.

Condensation with hippuric acid converted the aldehyde smoothly into the desired azlactone (I) and this was readily hydrolysed to α-benzamido-β-ferrocenylacrylic acid. Reduction with sodium and ethanol converted both this acid and its azlactone precursor (I) into β-ferrocenyl-α-*cyclo*hexylformamidopropionic acid (II). This acyl derivative (II) of ferrocenylalanine (III) was unaffected by carboxypeptidase and gave only a trace of ninhydrin-positive material on alkaline hydrolysis. Acid hydrolysis afforded the desired amino-acid (III) but only in minute yield. Numerous variations of the conditions failed to improve this or to reduce the extensive decomposition to tar which accompanied hydrolysis. As amides of formic acid are hydrolysable under much milder conditions, attention was turned to the formamidomalonic ester method. This ester is usually condensed in the form of its sodio-derivative with a reactive halide. The necessary ferrocenylmethyl chloride or bromide was, however, then unknown and, although Dr. K.

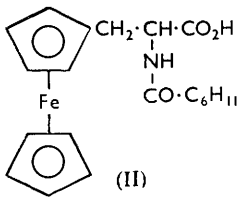
[1] Part V, preceding paper.

Schlögl [2] kindly informed us that he was attempting its preparation for the same purpose, we decided to explore the use of the much more readily available quaternary salt (IV).

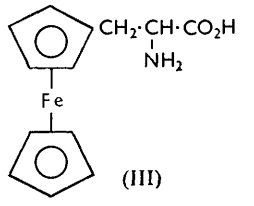
The preparation of (ferrocenylmethyl)trimethylammonium iodide (IV) from ferrocene by the action of methylenebisdimethylamine in glacial acetic acid [3] was improved by the addition of phosphoric acid to the reaction mixture. Under these conditions the salt (IV) was isolated in quantitative yield without purification of the intermediate tertiary



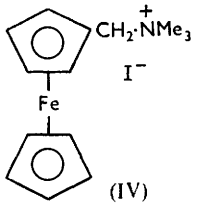
(I)



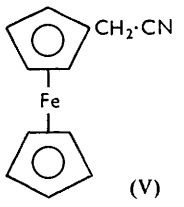
(II)



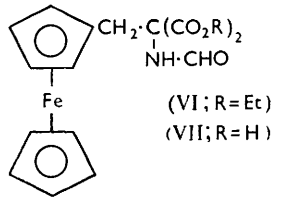
(III)



(IV)



(V)



(VI; R = Et)
(VII; R = H)

amine. The same conditions have been applied to the preparation of *N*-(ferrocenylmethyl)piperidine in 70% yield.

To demonstrate the reactivity of the salt (IV) in condensations of the type required for its conversion into (ferrocenylmethyl)formamidomalonic ester (VI) its reactions with potassium cyanide and with diethyl sodiomalonate were first studied. Cyanide reacts smoothly to yield ferrocenylacetonitrile (V) which has been identified by hydrolysis to the corresponding acid. We had already obtained the latter by the Willgerodt reaction * on acetylferrocene followed by alkaline hydrolysis of the resultant thiomorpholide.

Diethyl malonate reacted with (IV) in the presence of sodium ethoxide to give an oily ester which was hydrolysed and decarboxylated to ferrocenylpropionic acid, also obtained by catalytic hydrogenation of ferrocenylacrylic acid [1] and identical with an authentic specimen [5] kindly supplied by Dr. E. Csendes. Similar condensation of the quaternary ammonium salt (IV) with formamidomalonic ester afforded the desired product (VI). Hydrolysis of this by 5N-hydrochloric acid yields the amino-acid (III) directly. Alternatively, alkaline hydrolysis converts the ester (VI) into the corresponding free acid (VII) which is then further hydrolysed and decarboxylated to (III) by brief treatment with acid.

Experimental

Azlactone (I).—Ferrocenecarboxyaldehyde (1 g.; 4·7 mmoles), hippuric acid (1 g.; 5·6 mmoles), and anhydrous sodium acetate (0·4 g.; 5 mmoles) were dissolved in acetic anhydride (4 ml.) by refluxing for 5 min. in a stream of nitrogen. The solution was then heated for 1·5 hr. on a steam-bath, cooled, diluted with methanol (6 ml.), and refrigerated overnight. Filtration followed by recrystallisation from benzene–ligroin (b. p. 60—80°) afforded the *azlactone* (I)

* The use of this method was briefly reported by one of us [4] and has recently been extended and mproved by Rinehart *et al.*[5] and by Graham *et al.*[5a]

[2] Schlögl, personal communication, and *Monatsh.*, 1957, **88**, 601.
[3] Hauser and Lindsay, *J. Org. Chem.*, 1956, **21**, 382; 1957, **22**, 355.
[4] Pauson, *Quart. Reviews*, 1955, **9**, 391.
[5] Rinehart, Curby, and Sokol, *J. Amer. Chem. Soc.*, 1957, **79**, 3420.
[5a] Graham, Lindsey, Parshall, Peterson, and Whitman, *ibid.*, p. 3416.

(0·964 g.; 58%) as deep purple crystals, m. p. 188° (Found: C, 66·5, 67·7, 67·4; H, 4·55, 4·3, 4·5; N, 3·8, 4·1. $C_{20}H_{15}O_2NFe$ requires C, 67·2; H, 4·2; N, 3·9%).

No crystalline azlactone could be obtained when acetylglycine was employed in place of hippuric acid.

α-Benzamido-β-ferrocenylacrylic Acid.—The above azlactone (I) (2 g.; 5·6 mmoles) was refluxed for 15 min. with a solution of sodium hydroxide (0·6 g.) in 50% aqueous ethanol (25 ml.). After cooling and acidifying with 2N-hydrochloric acid, the red precipitate was collected and recrystallised from glacial acetic acid. *α-Benzamido-β-ferrocenylacrylic acid* (2 g.; 95%) was obtained as red rods, m. p. 218—219° (vac.) (Found: C, 63·7; H, 4·65; N, 3·9. $C_{20}H_{17}O_3NFe$ requires C, 64·0; H, 4·5; N, 3·7%).

β-Ferrocenyl-α-(cyclohexylformamido)propionic Acid (II).—To a solution of the above acrylic acid (1 g.; 2·7 mmoles) in absolute ethanol (40 ml.), sodium (4 g.) was added, and the mixture refluxed for 1 hr. After cooling, the solution was poured into water (100 ml.) and filtered, and the filtrate acidified with 2N-hydrochloric acid. The product was collected after refrigeration, to ensure complete precipitation, and recrystallised from 50% aqueous ethanol. The *acid* (II) was obtained as orange-yellow plates, m. p. 222—223° (decomp.; vac.) (Found: C, 62·5; H, 6·5; N, 3·9. $C_{20}H_{25}O_3NFe$ requires C, 62·7; H, 6·6; N, 3·7%).

The same product was obtained in lower overall yield (38%) when the azlactone (I) (1 g.) was similarly reduced (by using 5·3 g. of sodium).

(Ferrocenylmethyl)trimethylammonium Iodide (IV).—Ferrocene (9 g.; 0·05 mole) was added to a mixture of methylenebisdimethylamine (8 g.; 0·08 mole), phosphoric acid (8 g.; *d* 1·75), and glacial acetic acid (80 ml.) in a three-necked flask. The mixture was heated on a boiling-water bath for 10 hr. with continuous stirring and passage of a slow stream of nitrogen. After cooling, the solution was poured into water (approx. 100 ml.) and extracted with ether to remove any trace of unchanged ferrocene. The aqueous layer was then made alkaline with sodium hydroxide and extracted with ether, and the extract dried and evaporated (finally *in vacuo*). The residue (dimethylaminomethylferrocene) was taken up in benzene and excess of methyl iodide was carefully added to precipitate the methiodide [3] as a yellow solid, m. p. 220° (decomp.).

Piperidinomethylferrocene.—Ferrocene (0·9 g.; 0·005 mole) was added to a mixture of methylenebispiperidine (1·8 g.; 0·01 mole), phosphoric acid (1 g.; *d* 1·75), and glacial acetic acid (10 ml.). The resulting solution was refluxed under nitrogen for 8 hr., cooled, diluted with an equal volume of water, and extracted with ether. Neutralisation of the aqueous layer precipitated *piperidinomethylferrocene* (0·72 g.), and a further quantity (0·22 g.) was extracted from the mother-liquors with ether. The product crystallised in yellow leaflets from aqueous ethanol and after further crystallisation from ligroin (b. p. 40—60°) had m. p. 84—85·5° (Found: C, 68·1; H, 7·25; N, 4·8. $C_{16}H_{21}NFe$ requires C, 67·9; H, 7·5; N, 4·95%).

Ferrocenylacetonitrile (V).—The quaternary salt (IV) (3·9 g.; 0·01 mole) was added to a solution of potassium cyanide (1·0 g.; 0·015 mole) in absolute ethanol (10 ml.), and the mixture refluxed for 2 hr. under nitrogen. After cooling, ether was added to precipitate unchanged quaternary salt (0·92 g.), and the mixture filtered. The filtrate was diluted with water, and the aqueous layer extracted with more ether. The combined ether extracts were dried and evaporated, and the residue crystallised from aqueous ethanol, giving yellow leaflets (0·83 g.; 48%), m. p. 81°. After further purification by chromatography on alumina and crystallisation from ligroin (b. p. 40—60°) *ferrocenylacetonitrile* had m. p. 85° (Found: C, 64·0; H, 5·2; N, 6·1. $C_{12}H_{11}NFe$ requires C, 64·0; H, 4·9; N, 6·2%).

Ferrocenylacetic Acid.—(a) *From the nitrile.* The nitrile (V) (0·105 g.) was refluxed with a solution of sodium hydroxide (1 g.) in a mixture of ethanol (9 ml.) and water (7 ml.) for 1·5 hr. The cooled solution was diluted with more water and extracted with ether, and the aqueous layer acidified with dilute hydrochloric acid. The precipitated *ferrocenylacetic acid* was extracted with ether and crystallised from *cyclo*hexane, from which it formed pale yellow platelets (0·07 g.; 60%) which shrank to a semisolid mass at *ca.* 140° and finally melted at 161° (vac.) (Found: C, 59·4; H, 5·5. $C_{12}H_{12}O_2Fe$ requires C, 59·05; H, 5·0%).

(b) *From acetylferrocene.* A mixture of acetylferrocene (1 g.), sulphur (0·2 g.), and morpholine (0·6 g.) was heated on a steam-bath for 1·5 hr. and then at 130° ± 10° for 1 hr. After cooling, extraction of the black mass with ligroin (b. p. 40—60°) yielded *ferrocenylacet-thiomorpholide*, m. p. 125—126°. Recrystallisation from ligroin (b. p. 60—80°) gave orange-yellow needles (0·2 g.), m. p. 127—128° (Found: C, 58·8; H, 6·0. $C_{16}H_{19}ONSFe$ requires

C, 58·4; H, 5·8%). Reduction of reaction time or temperature afforded mixtures containing much unchanged ketone.

The thiomorpholide (0·1 g.) was refluxed for 3 hr. with a solution of potassium hydroxide (1 g.) in water (2 ml.) and ethanol (8 ml.). After cooling, acidification caused liberation of hydrogen sulphide and precipitation of the acid, which was taken up in ether, extracted with sodium hydrogen carbonate solution, and reprecipitated with dilute hydrochloric acid. Yield: 55 mg. (74%) of ferrocenylacetic acid, identical with the above product in m. p. and infrared spectrum.

β-*Ferrocenylpropionic Acid*.—(a) *From ferrocenylacrylic acid*. A solution of β-ferrocenylacrylic acid [1] (0·1 g.) over 10% palladium on charcoal (0·03 g.) absorbed 9·5 ml. of hydrogen during 5 min. Evaporation of the filtered solution left β-ferrocenylpropionic acid (0·1 g.), m. p. 115—117·5°, raised by recrystallisation from *cyclo*hexane to 117—118° (lit.: [5, 6] 115—116°) (Found: C, 60·8; H, 5·6. Calc. for $C_{13}H_{14}O_2Fe$: C, 60·5; H, 5·5%).

(b) *From the quaternary salt* (IV). To a solution of sodium (0·3 g.; 0·013 mole) in absolute ethanol (50 ml.), diethyl malonate (5·5 g.; 0·035 mole) was added, followed by the quaternary salt (IV) (5 g.; 0·013 mole). The mixture was refluxed in a current of nitrogen for 5 hr. on a steam-bath. Most of the solvent was evaporated off under reduced pressure, water was then added, and the mixture extracted with ether. Evaporation of the dried ether solution left diethyl (ferrocenylmethyl)malonate as an oil which failed to crystallise and was therefore hydrolysed directly by heating on a steam-bath with 10N-sodium hydroxide. After all the ester had disappeared, the alkaline solution was cooled, neutralised, and extracted with ether. The ether solution was washed with water, dried, and evaporated, and the residue crystallised once from water. (Ferrocenylmethyl)malonic acid (0·7 g.; 18%) so obtained formed yellow leaflets, m. p. 144—145°, which decomposed on keeping and were not purified further. It was sublimed at 155°/0·3 mm. and then crystallised from ether at −78° to yield β-ferrocenylpropionic acid, m. p. 115°.

Similar results were obtained in another experiment when the reaction between diethyl sodiomalonate and the quaternary salt (IV) was carried out in *n*-butyl ether at 160° (8 hr.).[7] The m. p.s of both the above samples of ferrocenylpropionic acid were undepressed by admixture with an authentic specimen.[6]

Diethyl (Ferrocenylmethyl)formamidomalonate (VI).—The quaternary salt (IV) (5·01 g.; 0·013 mole) was added to a solution of diethyl sodioformamidomalonate, prepared by dissolving first sodium (0·6 g.; 0·026 mole) and then diethyl formamidomalonate (5·28 g.; 0·026 mole) in anhydrous ethanol (100 ml.). The mixture was refluxed and stirred for 19 hr. in a slow current of nitrogen. It was then filtered, and the solvent removed under reduced pressure. After addition of water to the residue, the mixture was extracted thrice with ether, and the ether solution dried and evaporated to leave an oil (5·17 g.) which solidified on cooling to −78°. Recrystallisation from aqueous ethanol gave yellow needles of *diethyl (ferrocenylmethyl)formamidomalonate* (3·81 g.; 73%), m. p. 90—91° (Found: C, 57·15; H, 6·2; N, 3·4, 3·65. $C_{19}H_{23}O_5NFe$ requires C, 56·9; H, 5·8; N, 3·5%).

(Ferrocenylmethyl)formamidomalonic Acid (VII).—The above ester (VI) (1 g.) was refluxed for 1·5 hr. with a solution of sodium hydroxide (1 g.) in water (7 ml.) and ethanol (9 ml.). The cooled reaction mixture was acidified with dilute hydrochloric acid and filtered, and the residue washed well with water. Recrystallisation from acetone–ligroin (b. p. 60—80°) afforded pale yellow crystals of *(ferrocenylmethyl)formamidomalonic acid* (0·43 g.; 50%), m. p. 160—161° (Found: C, 52·2; H, 4·7; N, 3·6. $C_{15}H_{15}O_5NFe$ requires C, 52·2; H, 4·4; N, 4·1%).

DL-*Ferrocenylalanine* (III).—(a) *From* (VI). The ester (VI) (0·44 g.) was refluxed with 6N-hydrochloric acid (12 ml.) under nitrogen for 45 min. (oil-bath temp. 125°). Evaporation of the cooled solution under reduced pressure yielded the crude hydrochloride (0·26 g.; 76%). This was dissolved in water and titrated to pH *ca.* 7 with 0·1N-sodium hydroxide. The precipitated DL-*ferrocenylalanine* (DL-α-amino-β-ferrocenylpropionic acid) (III) recrystallised from water as a yellow monohydrate, m. p. 290° (decomp.) (Found: C, 53·4; H, 5·9; N, 5·1. $C_{13}H_{15}O_2NFe,H_2O$ requires C, 53·6; H, 5·9; N, 4·8%). The same product was obtained in lower overall yield by the two-step hydrolysis of (VI) to (VII) as described above, followed by refluxing (VII) with 2N-hydrochloric acid (2 hr.).

(b) *From* (II). No hydrolysis took place when the *cyclo*hexylformamido-derivative (II) of

[6] Woodward and Csendes, personal communication; cf. refs. 4 and 5.
[7] Snyder, Smith, and Stewart, *J. Amer. Chem. Soc.*, 1944, **66**, 200.

the amino-acid was dissolved in 0·1N-lithium hydroxide, brought to pH 8·1 with 0·1N-phosphate buffer, and incubated with carboxypeptidase for up to 72 hr. When refluxed (8 hr.) with 50% sodium hydroxide, 60% of (II) was recovered unchanged and only a trace of unidentified ninhydrin-positive product formed. Complete destruction of all but 5% of (II) occurred on heating with water at 140—160° for 6 hr. Refluxing with 2N-hydrochloric acid appeared to have no effect on (II), but when this acid (0·3 g.) was heated (17 hr.) in a sealed tube with 6N-hydrochloric acid (10 ml.) and *n*-butanol (10 ml.) and the aqueous layer which separated on cooling was neutralised with ammonia, it yielded DL-ferrocenylalanine (4 mg.) after evaporation to dryness and extraction with *n*-butanol.

The authors thank Dr. K. Schlögl for keeping them informed of his parallel investigations.[2] They are indebted to the Minnesota Mining and Manufacturing Company for a grant, and to E.I. du Pont de Nemours & Co. for gifts of ferrocene.

THE UNIVERSITY, SHEFFIELD 10. [*Received, July 19th,* 1957.]