THE OPTICALLY ACTIVE 3-FERROCENYLALANINE AND ITS APPLICATION IN PEPTIDE CHEMISTRY*

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Racemic 3-ferrocenylalanine was resolved in enantiomers using brucine. The absolute configuration was estimated by ozonolytic degradation of the N-trifluoroacetyl derivative of the (—)-enantiomer yielding D-aspartic acid. Diastereoisomeric cyclo(D-3-ferrocenylalanyl-L-prolyl) and cyclo-(L-3-ferrocenylalanyl-L-prolyl) were synthesized using conventional methods of peptide synthesis. Circular dichroism spectra of these cyclodipeptides are discussed and compared with spectra of the corresponding diastereoisomeric cyclodipeptides containing phenylalanine.

Non-protein amino acids have been used for examination of the side-chain effects on physical, chemical and biological properties of peptides. The appropriate design induces the enhancement of steric and/or electronic factors when compared with protein amino acids. The extreme values of these factors can be reached and, in certain degree, also their separation. The information attained with these model structures can be utilized in stereochemical studies on biologically active peptides. In the preceeding papers[1-3], we examined from this standpoint tert-leucine (2-amino-3,3-dimethylbutanoic acid) in which the extreme bulkiness of the tert-butyl side chain is the most decisive structural feature. In this contribution we describe the synthesis and absolute configuration of another uncommon amino acid — 3-ferrocenylalanine (2-amino-3-ferrocenylpropanoic acid, Fal**, *I*). In this compound the side chain bears an aromatic substituent which can be compared with aromatic rings of common amino acids (*e.g.* with phenyl in phenylalanine). However, the side chain in 3-ferrocenylalanine is much bulkier. We checked the possibility to synthesize peptides containing 3-ferrocenylalanine (*I*) by application of conventional methods of peptide synthesis.

DL-3-Ferrocenylalanine (*I*) was prepared according to the literature[5]. The resolution was carried out using brucine salts of the N-formyl derivatives. After resolution, both enantiomers exhibited comparable CD parameters. Also after deformylation

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** Nomenclature and symbols follow the published conventions[4].

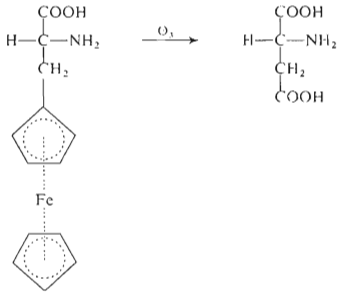
both enantiomers exhibited comparable chiroptical characteristics. Therefore, reasonable optical purity may be assumed for the resolved material. The absolute configuration was estimated by direct chemical correlation. Laevorotatory ($[\alpha]_D$ in methanol) amino acid was treated with trifluoroacetanhydride in trifluoroacetic acid to yield the N-trifluoroacetyl derivative and this was ozonolyzed, without purification, in 5% aqueous trifluoroacetic acid. The trifluoroacetyl protecting group, which was very convenient in the degradation step, was not stable during the isolation of degradation products. Finally, we isolated fully deprotected aspartic acid which was identified to be the D-enantiomer by comparison with an authentic sample.

The ferrocene system in *I* is rather sensitive to oxidation and to acids. Serious difficulties might be encountered in syntheses of peptides containing 3-ferrocenylalanine (*I*). Therefore, we decided to synthesize a very simple peptide to reach the fundamental information about the stability of the ferrocene moiety under conditions characteristic for conventional methods of peptide chemistry. The pair of diastereoisomeric cyclodipeptides – cyclo(L-3-ferrocenylalanyl-L-prolyl) (*II*) and cyclo(D-ferrocenyl-L-prolyl) (*III*) was selected for this purpose because the final products would be also interesting for further stereochemical examination. Both the enantiomeric 3-ferrocenylalanines were transformed in N-benzyloxycarbonyl derivatives (isolated as dicyclohexylammonium salts) which were coupled to the L-proline methyl ester using N,N′-dicyclohexylcarbodiimide. We were unable to obtain the resulting (chromatographically pure) protected dipeptides in crystalline state. Therefore, we used them directly for further syntheses. The N-deprotection was effected by hydrogenolysis and the cyclization by treatment with methanolic ammonia. The cyclodipeptides *II* and *III*, resulting in satisfactory yields, were crystalline and well defined compounds. All operations involved in these syntheses were performed in a standard manner. Application of the nitrogen atmosphere in long-term operations was the only special caution. Taking in account the greenish colour of all reaction mixtures (but not of the isolated products) we may guess that the ferrocene system was destroyed to a small extent in all steps. However, neither the yields nor the quality of isolated peptides were significantly deteriorated by this process.

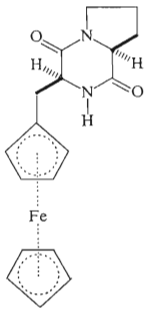
In the further step, we measured the CD spectra of cyclodipeptides *II* and *III* in acetonitrile and in 2,2,2-trifluoroethanol (Figs 1 and 2). In the long wavelength region, two very weak bands at 456 and 330 nm and one stronger at 260 nm are present the location of which corresponds to bands observed[6] in the ultraviolet and magnetic circular dichroic spectra of ferrocene and acetylferrocene. The signs of these bands depend on the absolute configuration of the ferrocenylalanine residue and the bands may be ascribed to the $d-d$ electronic transitions in the ferrocene moiety. In the short wavelength region, the bands belonging to the amide groups transitions govern the CD curve shape; one positive $n-\pi^*$ band centered at 220 to 230 nm and a couple of $\pi-\pi^*$ bands (positive with maximum at 200–215 nm and negative with maximum at 185–195 nm). The short wavelength bands originating

in ferrocene electronic transitions are superposed on the amide bands. However, these bands are not well developed and are hardly discernible (probably, one of them is located in 2,2,2-trifluoroethanol at about 210 nm).

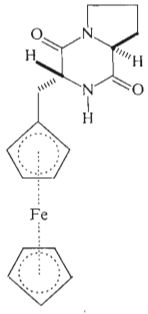
The CD spectrum of the *trans*-disubstituted cyclodipeptide *III* is in the short wavelength region significantly different from that of *cis*-disubstituted isomer *II*. The mutual relation of CD curves of both diastereoisomers is very similar to the situation observed for analogous cyclodipeptides containing phenylalanine instead of 3-ferrocenylalanine (Fig. 2). This relationship of CD curves seems to be of general



(−)-*I*



II



III

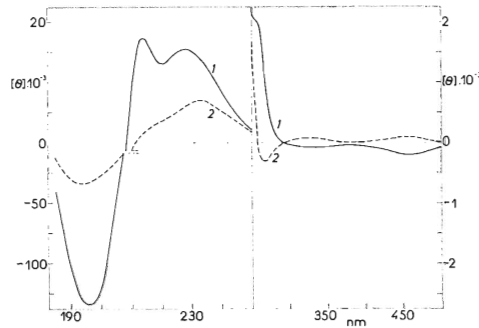


FIG. 1

Circular Dichroism Spectra of Cyclo(D-ferrocenylalanyl-L-prolyl) *1* and Cyclo(L-ferrocenylalanyl-L-prolyl) *2* in Acetonitrile

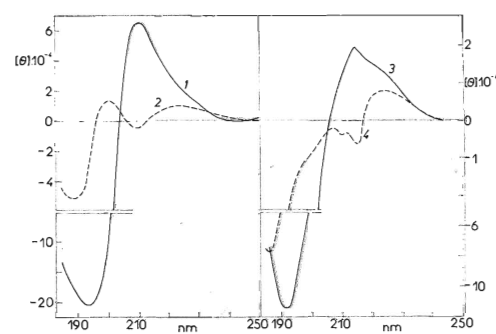


FIG. 2

Circular Dichroism Spectra of Cyclo(D-ferrocenylalanyl-L-prolyl) *1*, Cyclo(L-ferrocenyl-L-prolyl) *2*, Cyclo(D-phenylalanyl-L-prolyl) *3* and Cyclo(L-phenylalanyl-L-prolyl) *4* in 2,2,2-Trifluoroethanol

significance for cyclodipeptides cyclo(Pro-X) in which X is an amino acid with bulky side chain (*e.g.* valine). The characteristic differences in CD spectra of diastereoisomers can be summarized as follows: With *cis*-disubstituted isomers the positive $\pi-\pi^*$ band exhibits always lower intensity; the maximum of this band is described in some cases by a negative ellipticity value, in other cases this band coalesces with the more intense $n-\pi^*$ band and is not clearly discernible. Also the positive $n-\pi^*$ band and the negative $\pi-\pi^*$ band exhibit lower intensities in the CD spectra of *cis*-isomers, *cf.*[7,8]. The observed regularity corroborates the absolute configuration of 3-ferrocenylalanine enantiomers determined chemically (see above) which is, of course, unequivocal. However, the observation could be exploit for determination of the absolute configuration of amino acids on which the chemical correlation or other known methods are not applicable.

EXPERIMENTAL

Melting points were determined on a Kofler block. Optical rotations were measured on the Perkin–Elmer 141 MCA polarimeter. Samples for elemental analysis were dried at room temperature over phosphorus pentoxide at 130 Pa. The purity of compounds was checked using thin-layer chromatography on silica gel plates (Kieselgel G., Merck) in the solvent systems 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5) and 2-butanol–90% formic acid–water (75 : 13·5 : 11·5), detection by ninhydrin and/or chlorination. Solutions were dried with $MgSO_4$ and taken down on a rotatory evaporator (water-pump vacuum, bath temperature 40°C).

Resolution of the N-Formyl-DL-3-ferrocenylalanine

N-Formyl-DL-3-ferrocenylalanine (4·45 g) was dissolved in boiling ethanol (100 ml) and the hot ethanolic solution of brucine (6·89 g in 60 ml) was added. The mixture was left standing at 0°C for 2 days and the crystals which separated were filtered off (portion A, 6·58 g). The filtrate was taken to dryness yielding portion B (4·45 g). The portion A was dissolved in water (50 ml), filtered and 24 ml of 1M-NaOH were added under mixing. The precipitated brucine was filtered off and washed with water. The combined filtrates were extracted with chloroform (three times) and the remnants of chloroform in the aqueous layer were evaporated *in vacuo*. Diluted hydrochloric acid (1 : 1) was dropped to the solution and N-formyl derivative which separated after cooling was filtered off, 2·5 g. The portion B was worked up in the same way yielding 1·31 g of the enantiomeric N-formyl derivative. CD spectra of both N-formyl derivatives were recorded and the whole resolution procedure was repeated with both portions till the satisfactory reproducibility of CD curves in two subsequent resolution steps was reached. The original portion A of the brucine salt yielded 1·52 g of the N-formyl-D-ferrocenylalanine, $[\alpha]_D$ —59·8° (*c* 0·42, methanol), the CD curve exhibits a maximum at 448 mn, $[\Theta]$ +71·5° (deg cm^2 dmol^{-1}, ethanol). For $C_{14}H_{15}FeNO_3$ (301·1) calculated: 55·84% C, 5·02% H, 4·65% N; found: 55·85% C, 5·12% H, 4·49% N. The original portion B of the brucine salt yielded 1·03 g of the L-enantiomer, $[\alpha]_D$ +59·8° (*c* 0·79, methanol), the CD curves exhibit a maximum at 447 nm, $[\Theta]$ —72·6° (deg cm^2 dmol^{-1}). Found: 55·70% C, 5·17% H, 4·62% N.

L(+)-3-Ferrocenylalanine (L-*I*)

N-Formyl-L-3-ferrocenylalanine (1·0 g) was suspended in acetic acid (5 ml), concentrated hydrochloric acid was added and the mixture was refluxed for 25 min and evaporated *in vacuo*. The residue was dissolved in water, the remnants of the solid material were separated by filtration and the solution was neutralized with aqueous ammonia. The precipitate was filtered off, washed with water and dried over KOH. Yield 0·79 g (87%) of a compound which does not melt below 300°C; $[\alpha]_D$ +53·2° (*c* 0·29, acetic acid). For $C_{13}H_{15}FeNO_2 + 1\ H_2O$ (291·1) calculated: 53·63% C, 5·89% H, 4·81% N; found: 53·10% C, 5·85% H, 4·81% N.

N-Formyl-D-3-ferrocenylalanine (1·5 g) yielded in the same way as given for the L-enantiomer the amino acid D-*I* (1·25 g, 86%), m.p. above 300°C, $[\alpha]_D$ —48·3° (*c* 0·26, acetic acid). Found: 53·92% C, 5·64% H, 4·88% N.

Ozonolysis of the D(—)-3-Ferrocenylalanine (*D-I*)

Trifluoroacetic anhydride (2·7 ml) was added to the solution of amino acid D-*I* (0·50 g) in trifluoroacetic acid (10 ml). The mixture was stirred for 30 min in nitrogen atmosphere and evaporated and the residue was dissolved in 5% aqueous trifluoroacetic acid. Ozone was bubbled through the solution for 5·5 h. Then, 30% hydrogen peroxide (20 ml) was added, the mixture was stirred for 2 h at 60°C, the catalyst (Pd black) was added and the mixture again stirred at 60°C for two days. The catalyst was filtered off, the solution was evaporated, the residue triturated successively with light petroleum, diethyl ether and ethyl acetate and dissolved in 1M-NaOH. The solution was filtered, acidified with 1M-HCl and evaporated. The residue was dissolved in a small volume of water and chromatographed on a Dowex-50 column. At first, the acidic material was eluted with water. Aqueous ammonia (5%) eluted the amino-acid fraction, after evaporation 13·2 mg. The material was identical with an authentic sample of D-aspartic acid according to the thin-layer chromatography, paper electrophoresis and amino-acid analysis. The CD curve (in water and in 0·1M-HCl) of both compounds exhibited the same wavelengths for extrema. However, with the ozonolysis product, the dichroic absorption was reduced to 25%. In the reaction mixture after ozonolysis, only glycine was detected besides aspartic acid (amino-acid analyzer, relation Asp : Gly ca 6 : 1). The decrease in dichroic absorption of the D-aspartic acid is due to racemization during the isolation steps.

N-Benzyloxycarbonyl-L-3-ferrocenylalanine

Benzyloxycarbonyl chloride (0·7 ml) was added to the solution of L-3-ferrocenylalanine (0·50 g) in aqueous sodium hydroxide (0·6 g in 10 ml). The mixture was stirred in a nitrogen atmosphere for 2 h at room temperature and extracted with diethyl ether. The aqueous layer was acidified (diluted hydrochloric acid 1 : 1) and extracted with ethyl acetate. The extract was dried and evaporated. The oily residue yielded the dicyclohexylammonium salt, 0·71 g (70%), m.p. 158—160°C (decomposition); $[\alpha]_D$ +61·9° (methanol). For $C_{33}H_{46}FeN_2O_4$ (590·6) calculated: 67·11% C, 7·85% H, 4·74% N; found: 67·59% C, 7·72% H, 4·35% N.

The D-enantiomer was prepared in the same manner in a yield of 73%, m.p. 160—161°C (decomposition); $[\alpha]_D$ —62·9° (*c* 0·41, methanol). Found: 67·42% C, 7·67% H, 4·63% N.

Cyclo(L-3-ferrocenylalanyl-L-prolyl) (*II*)

The salt of proline methyl ester with bis(*p*-toluenesulphonyl)imide (0·42 g) was added to the solution of the dicyclohexylammonium salt of N-benzyloxycarbonyl-L-3-ferrocenylalanine (0·50 g)

in dichloromethane (20 ml), the mixture was cooled to —20°C, N,N′-dicyclohexylcarbodiimide (0·2 g) was added, and mixed for 15 min at —20°C. After 2 days standing at 0°C, the separated N,N′-dicyclohexylurea was filtered off, the filtrate was washed with 1M-HCl, water, 0·5M aqueous $NaHCO_3$ solution, and water, dried and evaporated. The oily residue was chromatographically homogeneous; yield 0·44 g of benzyloxycarbonyl-L-3-ferrocenylalanyl-L-proline methyl ester.

The mixture of this protected dipeptide (0·43 g) with methanol (20 ml) and 1M-HCl (1 ml) was hydrogenated in the presence of Pd/C catalyst. The residue after filtration and evaporation of the reaction mixture was dissolved in methanolic ammonia, the solution left for 2 days at room temperature, evaporated and the residue chromatographed on a silica gel column. Elution with dichloromethane containing 10% of ethanol yielded 0·15 g (51%, related to the fully protected dipeptide) of *II* melting at 178—179°C. After crystallization from ethanol–diethyl ether m.p. 182—183°C. CD curves see Figs 1 and 2. For $C_{18}H_{20}FeN_2O_2$ (352·2) calculated: 61·38% C, 5·72% H, 7·95% N; found: 61·84% C, 5·84% H, 8·25% N.

Cyclo(D-3-ferrocenylalanyl-L-prolyl) (*III*)

The synthesis was performed in a similar manner as for *II*. The oily benzyloxycarbonyl-D-3-ferrocenylalanyl-L-proline methyl ester was prepared in a yield of 0·42 g (starting from 0·68 g of the D dicyclohexylammonium salt) and was chromatographically homogeneous. This dipeptide (0·36 g) yielded 0·10 g (40%) of *III*, m.p. 164—166°C; the sample for analysis m.p. 165—166°C (dichloromethane–light petroleum). For $C_{18}H_{20}FeN_2O_2$ (352·2) calculated: 61·38% C, 5·72% H, 7·95% N; found: 61·23% C, 5·73% H, 7·75% N. For CD curves see Figs 1 and 2.

CD Measurements

The spectra were recorded on a Roussel Jouan Dichrograph CD 185 model II in 0·01—1·0 cm cells at 22—25°C. The concentration of solutions was about 0·5—1·5 mg per 1 ml with cyclodipeptides and about 5 mg per 1 ml with N-formyl-3-ferrocenylalanines. The spectral data are given in molar ellipticities, $[\Theta]$ (deg cm^2 dmol^{-1}) and are not corrected for the solvent refractivity index.

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REFERENCES

1. Pospíšek J., Bláha K.: *Peptides 1976. Proc. 14th European Peptide Symp. Wépion 1976*, (A. Loffet, Ed.), p. 95. Editions de l'Université de Bruxelles, Brussels 1976.
2. Pospíšek J., Bláha K.: This Journal *42*, 1069 (1977).
3. Maloň P., Pančoška P., Budějínský M., Hlaváček J., Pospíšek J., Frič I., Bláha K.: This Journal, in press.
4. IUPAC-IUB Commission on Biochemical Nomenclature. Symbols for Amino Acid Derivatives and Peptides. Recommendations 1971. Biochemistry *11*, 1726 (1972).
5. Schlögl K.: Monatsh. Chem. *88*, 601 (1957).
6. Nielson D., Farmer M., Eyring H.: J. Phys. Chem. *80*, 717 (1976).
7. Madison V., Young P. E., Blout E. R.: J. Amer. Chem. Soc. *98*, 5358 (1976).
8. Pančoška P., Frič I., Bláha K.: This Journal *44*, 1296 (1979).