189. Synthesis of Optically Active, Ring-Substituted N-Benzyloxycarbonylphenylalanines *via* 2-Benzyloxycarbonylamino-2-arylalkylmalonates

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Dedicated to Professor *F. Leuthardt* on the occasion of his 70th birthday

(10. V. 73)

Summary. Diethyl or dimethyl benzyloxycarbonylaminomalonate was reacted with ring-substituted benzyl halides and the resulting arylalkyl derivatives (**3** to **6**) half-saponified to the DL-monoacid-monoesters (**7** to **10**). Decarboxylation by refluxing in dioxane afforded the N-benzyloxycarbonyl-DL-amino acid esters (**11** to **14**), which were resolved into their optical antipodes by enzymic hydrolysis of the ester group with Subtilisin, type *Carlsberg*. Enzymic hydrolysis led to the N-benzyloxycarbonyl-L-amino acids (**15** to **18**) and to the corresponding D-amino acid esters. The latter were converted to the N-benzyloxycarbonyl-D-amino acids (**19** and **20**) by alkaline hydrolysis of the ester groups. These derivatives could be used directly for further peptide synthesis. The following compounds were prepared: N-benzyloxycarbonyl derivatives of *p*-methyl-L-phenylalanine (**15**), *p*-methyl-D-phenylalanine (**19**), *p*-fluoro-L-phenylalanine (**16**), *m*-fluoro-L-phenylalanine (**17**), *m*-fluoro-D-phenylalanine (**20**) and penta-fluoro-L-phenylalanine (**18**). The free amino acids were obtained by removal of the benzyloxycarbonyl group with HBr in acetic acid.

Introduction. – For investigations of the binding properties of the active site in various proteolytic enzymes we were in need of a number of L and D amino acids with 'unnatural' side chains, as well as of the peptides containing these new amino acids. For this purpose we developed a simplified method to synthesize optically active amino acids and their N^{α}-derivatives *via* acylamino malonates [1]. The principle of this method is depicted in the scheme:

$$R'NHCR(COOR'')_2 \xrightarrow[1\ equ.]{OH^-} R'NHCR\begin{matrix}COOR'' \\ COOH\end{matrix} \quad (DL)$$

$$\xrightarrow[100°C]{\Delta} R'NHCHRCOOR'' \ (DL) \xrightarrow{protease}$$

$$R'NHCHRCOOH \ (L) + R'NHCHRCOOR'' \ (D)$$

The usual synthetic routes to optically active amino acids proceed to the free racemic amino acids which then have to be resolved. This is often achieved by an enzymic reaction which needs appropriate derivation of the free DL-amino acids, *e.g.* acetylation or esterification[3]). The pathway shown in the scheme leads directly to N-protected DL-amino acid esters which are already substrates for the enzymic resolution by proteolytic enzymes. The protecting group of the amino function (R'

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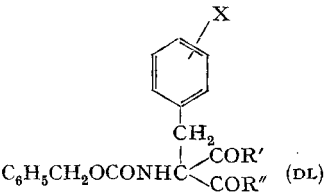
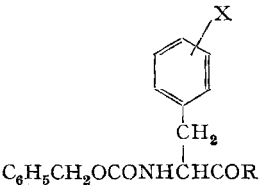
in the above scheme) is introduced in the first step and is never removed until the stage of the optically active amino acid is reached. By choosing a suitable amino protection it is therefore possible to end up directly with a N-protected, optically active amino acid which can be used for further peptide synthesis. Such a short-cut is desirable since usually the *de novo* synthesis of amino acids and their derivatives provides a bottle-neck in the preparation of peptides containing 'unnatural' amino acids.

In the present paper we report the synthesis by this new route of optically active N-benzyloxycarbonyl derivatives of three fluorophenylalanines and of *p*-methylphenylalanine. Fluorophenylalanines have been obtained by stereospecific enzymic synthesis of their phenylhydrazides by means of papain [3]. N-Chloroacetyl-*p*-methyl-DL-phenylalanine has been resolved by carboxypeptidase [4], and N-trifluoroacetyl-pentafluoro-DL-phenylalanine was hydrolyzed stereospecifically by the action of acylase [5]. Resolution of ring-substituted, racemic phenylalanine esters by α-chymotrypsin has also been reported recently [6].

Results and Discussion. – Diethyl or dimethyl benzyloxycarbonylaminomalonate (**1** or **2**) was reacted in the usual way with aryl-substituted benzyl halides to form the 2-arylalkyl-2-benzyloxycarbonylaminomalonates **3** to **6**. Mild alkaline hydrolysis gave the monoacid-monoesters **7** to **10**, which were easily crystallized and characterized in the form of their dicyclohexylamine salts. The reason for the smooth hydrolysis of only one ester group is the electrostatic repulsion between the ionized monoester produced in the first step of saponification and the hydroxyl ion catalyzing further hydrolysis.





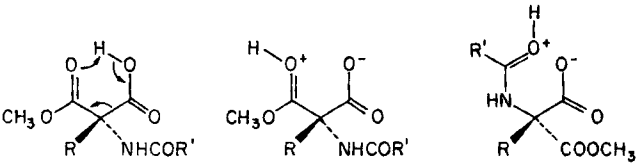
Nr.	X	R′	R″
3	*p*-CH_3	$-OCH_2CH_3$	$-OCH_2CH_3$
4	*p*-F	$-OCH_2CH_3$	$-OCH_2CH_3$
5	*m*-F	$-OCH_3$	$-OCH_3$
6	penta-F	$-OCH_3$	$-OCH_3$
7	*p*-CH_3	$-OCH_2CH_3$	$-O^-NH_2^+(C_6H_{11})_2$
8	*p*-F	$-OCH_2CH_3$	$-O^-NH_2^+(C_6H_{11})_2$
9	*m*-F	$-OCH_3$	$-O^-NH_2^+(C_6H_{11})_2$
10	penta-F	$-OCH_3$	$-O^-NH_2^+(C_6H_{11})_2$

Nr.	X	R	
11	*p*-CH_3	$-OCH_2CH_3$	(DL)
12	*p*-F	$-OCH_2CH_3$	(DL)
13	*m*-F	$-OCH_3$	(DL)
14	penta-F	$-OCH_3$	(DL)
15	*p*-CH_3	$-OH$	(L)
16	*p*-F	$-OH$	(L)
17	*m*-F	$-OH$	(L)
18	penta-F	$-OH$	(L)
19	*p*-CH_3	$-OH$	(D)
20	*m*-F	$-OH$	(D)

The monoacid-monoesters **7** to **10** were freed from their dicyclohexylamine salts and decarboxylated by boiling in dioxane for 24 h. In the case of monoethyl 2-acetamido-2-arylalkylmalonates decarboxylation obeyed first order kinetics with a half-time of about 10 min [1]. Decarboxylation of compounds **7** to **10** was also of first order but with a half-time of 4 to 5 h. This lower reaction rate seems to be a general

behaviour of benzyloxycarbonylaminomalonates since it was observed regardless of the structure of the R-side chain (see scheme).

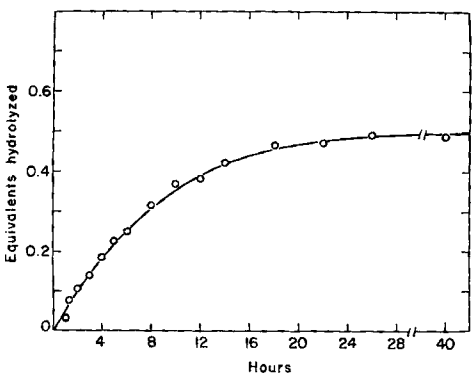
Two different reaction mechanisms, characterized by either a concerted step or by a zwitterionic intermediate, have been considered for decarboxylation reactions [7]. Structure I



I II III

formulates a concerted pathway for the malonates in question, II and III are possible zwitterionic intermediates which subsequently decarboxylate. In II and III an 'electron sink' is formed by protonating carbonyl oxygens which facilitates the decarboxylation step, although the low basicity of a carbonyl group provides a high energy barrier to this pathway. Assuming that structure III contributes to catalysis, the difference in the decarboxylation rate between acetamido- and benzyloxycarbonylaminomalonates is readily explained by the lower basicity of the carbonyl oxygen in the benzyloxycarbonyl group (R' = benzyl in III). This interpretation is unsatisfactory, however, if the reaction proceeds exclusively through the concerted pathway I.

The benzyloxycarbonyl-DL-amino acid esters **11** to **14** were resolved enzymically by Subtilisin, type *Carlsberg*. In essence, the procedure was as described previously [1]. The sparingly water-soluble substrates were suspended or emulsified in water or water-acetonitrile 9:1, and hydrolysis was achieved by adding the enzyme at pH 8. The uptake of alkali at constant pH was monitored by means of an automatic titration device providing an easy control of the course of hydrolysis. The figure shows the time



The course of the hydrolysis of N-benzyloxycarbonyl-*p*-methyl-DL-phenylalanine *ethyl ester* **11** (4 g, 11.7 mmol) by Subtilisin *Carlsberg* (20 mg) in 50 ml of 0.01M KCl at pH 8.0 and 25°

course of the resolution of ester **11**. The reaction stopped after hydrolysis of 0.5 equivalents of substrate as was expected for a stereospecific reaction. In case of esters **12** and **14**, reaction stopped after hydrolysis of 0.41 and 0.37 equivalents, respectively[4]).

[4]) An attempt to increase yield of hydrolysis by addition of more enzyme after 24 h up to an approximate enzyme/substrate ratio of 1:30 failed.

The remaining unhydrolyzed D-esters were therefore of low optical purity. The reason for this incomplete reaction is unknown. Product inhibition can be ruled out since the benzyloxycarbonyl-L-amino acids **16** and **18** have similarly low inhibition constants towards Subtilisin *Carlsberg* of about 0.05 to 0.1 mol/l at pH 8, as have the acids **15** and **17** [8].

The products of the enzymic reaction, being amino protected L-amino acids (**15** to **18**), were soluble in the aqueous medium at pH 8 to 9, whereas the hydrolysis-resistant esters of the D-isomers could be removed by filtration and/or extraction with ethyl acetate. The benzyloxycarbonyl L-amino acids were recovered by acidifying and extracting the aqueous hydrolysis mixture with ethyl acetate.

It was of interest to try to introduce various N-blocking groups into the aminomalonates. Preliminary experiments, however, showed that so far only the benzyloxycarbonyl group proved to be stable throughout all reaction steps involved. The *t*-butoxycarbonyl and the *o*-nitrophenylsulfenyl groups are partly removed during the decarboxylation in boiling dioxane which precludes the synthesis of *t*-butoxycarbonyl- and *o*-nitrophenylsulfenyl-amino acids *via* this route.

Another limitation to the method lies in the specificity requirements of the subtilisins as well as of other proteolytic enzymes. This is exemplified by the failure of the subtilisins and of chymotrypsin to resolve N-benzyloxycarbonyl-*p*-phenyl-DL-phenylalanine ethyl ester [8]. From literature data [6] [9] and from our experience (this paper and [1]) we conclude, however, that benzene derivatives with substituents of the size of halogens, methyl or hydroxy groups in any position of the phenyl ring allow the use of proteolytic enzymes for the resolution of the DL-amino acid esters.

Experimental Part

M.p. were uncorrected. Optical rotations were measured with a *Perkin-Elmer* automatic polarimeter (1 dm cell). Thin layer chromatography was performed on Silicagel coated aluminium sheets (*Riedel-DeHaën*, Hannover) in the systems: (1) ethyl acetate/light petroleum ether 1:1; (2) methanol/ethyl acetate 1:1; (3) diisopropyl ether/chloroform/acetone/2-propanol/water/formic acid 10:10:10:2:1:2; (4) diisopropyl ether/chloroform/dioxane/methanol/water/triethylamine 10:10:10:2:1:3 and (5) dioxane/methanol/dimethylformamide/triethylamine 10:1:1:1. Spots were detected by charring.

Non aqueous titrations of acidic groups were performed in dimethylformamide using sodium methoxide in methanol/benzene as titrant and thymolblue as indicator [10], or using potassium *t*-butoxide in *t*-butylalcohol-benzene as titrant and 2-nitrodiphenylamine as indicator [11]. Basic groups were titrated in acetic acid with $HClO_4$ in acetic acid as titrant and methyl violet as indicator [12]. Dimethyl and diethyl aminomalonate hydrochlorides were obtained from *Fluka* AG, Buchs. Subtilisin, type *Carlsberg*, lot 90910, was from *Novo-Industri* A/S, Copenhagen.

Diethyl-(N-benzyloxycarbonyl)aminomalonate (**1**). To diethylaminomalonate (42.4 g, 0.2 mol) in 200 ml of water, benzyl chloroformate (34 g, 0.2 mol) was added in small portions over a period of about 20 minutes. The reaction mixture was vigorously stirred and cooled in an ice bath whilst the pH was kept between 9 and 10 by dropwise addition of 4N NaOH. When the uptake of alkali had ceased, the mixture was brought to pH 7 with 1N HCl, and the precipitated product was collected by filtration and dried *in vacuo* over KOH. Recrystallization from light petroleum ether yielded 43.2 g (70%) of colorless material, m.p. 32–33°. Titration equivalent: calc. 309.3, found 315[5]).

$C_{15}H_{19}NO_6$ (309.3) Calc. C 58.24 H 6.19 N 4.53% Found C 58.40 H 6.28 N 4.40%

[5]) The proton at the second carbon atom is weakly acidic and can be titrated with potassium *t*-butoxide under nonaqueous conditions [11].

Dimethyl-(N-benzyloxycarbonyl)aminomalonate (**2**) has been prepared similarly, as reported earlier [1].

Diethyl 2-(4-methylbenzyl)-2-benzyloxycarbonylaminomalonate (**3**). α-Bromo-*p*-xylol (9.3 g, 50 mmol) was added with stirring into a solution of malonate **1** (15.5 g, 50 mmol) in 70 ml of 0.71M ethanolic sodium ethoxide. The mixture was kept at reflux temperature for 2 h, cooled and filtered. The filtrate was evaporated, and the oily residue taken up in ethyl acetate (300 ml). The organic layer was washed with water, 0.1N HCl, 5% $NaHCO_3$, water and saturated NaCl-solution, dried ($MgSO_4$), boiled with activated charcoal and evaporated to give diester **3** as a yellowish oil which was homogenous in TLC. (R_f(3) 0.6; R_f(1) 0.45) and which was used without further purification for the subsequent step.

Monoethyl 2-(4-methylbenzyl)-2-benzyloxycarbonylaminomalonate, dicyclohexylamine salt (**7**). For partial hydrolysis, 14.9 g (36 mmol) of the above oil in 210 ml of 0.28N KOH in ethanol (prepared by mixing appropriate amounts of 6.1N aqueous KOH and ethanol) was allowed to stand at room temperature for one hour. The reaction was followed by titrating the excess base in aliquots withdrawn at different time intervals. After about 45 min., base consumption levelled off at a base strength of the reaction mixture of 0.1N, indicating the hydrolysis of only one ester group. The excess base was neutralized with 1N HCl, most of the ethanol was evaporated, and the residual aqueous solution was acidified to pH 2–3 with 1N HCl. The product was extracted with 3 × 100 ml of ethyl acetate, the organic extract was washed with water and saturated NaCl-solution, dried ($MgSO_4$) and evaporated to give the monoacid-monoester as a colorless oil (R_f(2) 0.7). The oil was dissolved in 2-propanol (100 ml), and dicyclohexylamine (9.05 g, 50 mmol) was added. The dicyclohexylamine salt **7** precipitated upon addition of a few ml of diethyl ether and was recrystallized once from 2 propanol/methanol 5:1, m.p. 159°, yield 65%.

$C_{33}H_{46}O_6N_2$ (566.7) Calc. C 69.94 H 8.18 N 4.94% Found C 70.00 H 8.14 N 4.87%

The following compounds were prepared accordingly.

Diethyl 2-(4-fluorobenzyl)-2-benzyloxycarbonylaminomalonate (**4**), colorless oil R_f(3) 0.72, R_f(1) 0.59; yield 82%.

$C_{24}H_{24}FNO_6$	Calc.	C 65.30	H 5.48	F 4.30%	N 3.17
(441.5)	Found	,, 65.61	,, 5.32	,, 4.50%	,, 2.95

Monoethyl 2-(4-fluorobenzyl)-2-benzyloxycarbonylaminomalonate, dicyclohexylamine salt (**8**), m.p. 134° (recrystallized from 2-propanol), yield 78%.

Titration equivalent: calc. 570.70, found 582.0.

$C_{32}H_{43}FN_2O_6$	Calc.	C 67.35	H 7.59	F 3.33%	N 4.90
(570.7)	Found	,, 67.34	,, 7.70	,, 3.12%	,, 5.05

Dimethyl 2-(3-fluorobenzyl)-2-benzyloxycarbonylaminomalonate (**5**), oil, R_f(1) 0.81, R_f(3) 0.9; yield 79%.

$C_{22}H_{20}FNO_6$ (413.4) Calc. C 63.92 H 4.88% Found C 64.35 H 4.95%

Monomethyl 2-(3-fluorobenzyl)-2-benzyloxycarbonylaminomalonate, dicyclohexylamine salt (**9**), m.p. 136° (recrystallized from 2-propanol/ethanol), yield 91%.

Titration equivalent: calc. 556.7, found 548.0.

$C_{31}H_{41}FN_2O_6$ (556.7) Calc. C 66.89 H 7.42 N 5.03% Found C 67.05 H 7.57 N 4.82%

Dimethyl 2-(pentafluorobenzyl)-2-benzyloxycarbonylaminomalonate (**6**), m.p. 95–96° (recrystallized from diisopropyl ether/hexane), yield 78.2%.

$C_{20}H_{16}F_5NO_6$	Calc.	C 52.07	H 3.50	F 20.57%	N 3.04
(461.34)	Found	,, 52.12	,, 3.60	,, 20.74%	,, 3.00

Monomethyl 2-(pentafluorobenzyl)-2-benzyloxycarbonylaminomalonate, dicyclohexylamine salt (**10**), m.p. 125° (recrystallized from 2-propanol), yield 87%.

$C_{31}H_{37}F_5N_2O_6$	Calc.	C 59.23	H 5.93	F 15.11%	N 4.46
(628.7)	Found	,, 59.40	,, 6.10	,, 15.30%	,, 4.51

*N-Benzyloxycarbonyl-*p*-methyl-*DL*-phenylalanine ethylester* (**11**). The monoacid-monoester **7** was freed from its dicyclohexylamine salt as follows: The salt (10.3 g, 18.2 mmol) was suspended in

250 ml of 1N H_2SO_4. The aqueous suspension was extracted with 4 × 150 ml of ethyl acetate, and the organic extract was dried ($MgSO_4$) and evaporated to give the free acid of **7** as a colorless oil. This oil was dissolved in 100 ml of dioxane, and the solution was kept at reflux temperature for 24 h. The rate of decarboxylation could be followed by titrating the free acid content of samples withdrawn at different time intervals. After 24 h no more free acid could be detected in the reaction mixture. The solvent was evaporated and the oily residue recrystallized from ether/light petroleum ether, m.p. 46–47°, yield 71%.

$C_{20}H_{23}NO_4$ (341.4) Calc. C 70.36 H 6.79 N 4.10% Found C 71.12 H 6.62 N 4.32%

The following racemic N-benzyloxycarbonylamino acid esters were obtained by decarboxylating the corresponding monoacid-monoesters as outlined above.

N-Benzyloxycarbonyl-p-*fluoro*-DL-*phenylalanine ethylester* (**12**): m.p. 87–88° (recrystallized from cyclohexane), yield 89%.

$C_{19}H_{20}FNO_4$ (345.4) Calc. C 66.02 H 5.84 N 4.06% Found C 65.53 H 5.74 N 3.81%

N-Benzyloxycarbonyl-m-*fluoro*-DL-*phenylalanine methyl ester* (**13**): yellowish oil, $R_f(2)$ 0.75, yield 92%.

N-Benzyloxycarbonyl-pentafluoro-DL-*phenylalanine methylester* (**14**): colorless oil after chromatography on silicagel with ethylacetate/methanol/chloroform 10:1:3 as eluent. Yield 57%, $R_f(4)$ 0.72, $R_f(5)$ 0.8.

$C_{18}H_{14}F_5NO_4$ (403.3) Calc. C 53.61 F 23.55% Found C 53.79 F 22.95%

N-Benzyloxycarbonyl-p-*methyl*-L-*phenylalanine* (**15**). Powdered racemic ester **11** (4 g, 11.7 mmol) was suspended in 50 ml of 0.01M KCl (10^{-4}M in KH_2PO_4 to prevent pH-jumping on addition of alkali). After adjusting the pH to 8, hydrolysis was initiated by adding 20 mg of Subtilisin *Carlsberg* to the vigorously stirred mixture. The mixture was held at 25° and the pH kept constant by means of a pH-stat assembly (0.5N KOH as titrant). Alkali uptake (11.4 ml, 5.7 mmol) ceased after about 24 h (see Fig.). The heterogenous reaction mixture was diluted with one volume of ice-cold 5% $NaHCO_3$-solution, and the crystalline ester of the D-isomer **19** was collected by filtration. The filtrate was extracted with 100 ml of ethyl acetate, the organic extract was dried ($MgSO_4$) and evaporated to yield some additional ester of **19**. The aqueous fraction was acidified with 2N HCl to about pH 2, and the free acid was extracted into 3 × 150 ml ethyl acetate. The organic extract was washed with H_2O and saturated NaCl-solution, dried ($MgSO_4$) and evaporated. The remaining solid material was twice recrystallized from diisopropylether/light petroleum ether to yield 1.32 g (35.9%, theory 50%) of N-benzyloxycarbonyl-*p*-methyl-L-phenylalanine (**15**): m.p. 102–103°, $[\alpha]_D^{18} = +26°$ ($c = 3$, dioxane).

$C_{18}H_{19}NO_4$ (313.6) Calc. C 68.99 H 6.11 N 4.47% Found C 68.75 H 6.06 N 4.40%

p-*Methyl*-L-*phenylalanine* was prepared by removing the benzyloxycarbonyl group from **15** with HBr in acetic acid [13]. The hydrobromide, obtained by ether precipitation, was dissolved in 1 ml of water, and the free *p*-methyl-L-phenylalanine was precipitated by adjusting the pH to 6.5 and adding 1 volume of ethyl alcohol. Yield 62% after one recrystallization from water/ethanol, m.p. 211–218° (lit. m.p. 196–206° [4]), $[\alpha]_D^{18} = -10.3°$ ($c = 1.3$ in 0.5N HCl), (lit. $[\alpha]_D^{25} = -6.5°$ ($c = 0.1$ in 0.5N HCl) [4]).

N-Benzyloxycarbonyl-p-*methyl*-D-*phenylalanine* (**19**). The ethyl ester of **19** obtained by filtration and extraction of the reaction mixture after enzymic hydrolysis (see above) was dissolved in 20 ml of ethanol and 15 ml of 0.4N NaOH. After 3 h at room temperature the solution was acidified to about pH 2 and the free acid **19** extracted into 2 × 100 ml of ethyl acetate. Washing (H_2O, satd. NaCl-solution), drying ($MgSO_4$) and evaporating the organic extract gave a colorless oil which solidified on standing for several hours. Recrystallization from diisopropylether/light petroleum ether gave **19** in 28% yield (theory 50%), m.p. 104–105°, $[\alpha]_D^{18} = -22°$ ($c = 2$, dioxane).

$C_{18}H_{19}NO_4$ (313.6) Calc. C 68.99 H 6.11 N 4.47% Found C 69.21 H 6.10 N 4.21%

p-*Methyl*-D-*phenylalanine* was prepared as outlined for the L-isomer. Yield 68%, m.p. 192–194°, $[\alpha]_D^{18} = +9.8°$ ($c = 1.3$ in 0.5N HCl).

The following N-benzyloxycarbonyl-L-amino acids and their D-isomers were prepared by enzymic hydrolysis of the corresponding DL-esters according to the general prescription given for **15**.

N-Benzyloxycarbonyl-p-*fluoro*-L-*phenylalanine* (**16**). 8.2 g (23.8 mmol) of the racemic ester **12** was hydrolyzed in 80 ml of 0.01M KCl. Subtilisin *Carlsberg* was added in two portions of 25 mg each at the beginning of the reaction and after 12 h. Hydrolysis stopped after about 18 h when only 0.41 of the theoretically 0.5 equ. of the DL-ester were hydrolyzed. **16** was obtained in 38% yield (50% theoretically) after one recrystallization from diisopropyl ether/light petroleum ether, m.p. 101–104° (lit. m.p. 102–104° [14]), $[\alpha]_D^{19} = -5.8°$ ($c = 2$, methanol), (lit. $[\alpha]_D^{23} = -6.8°$ ($c = 2$, methanol) [14]).

$C_{17}H_{16}FNO_4$	Calc.	C 64.35	H 5.08	F 5.99%	N 4.41
(317.3)	Found	,, 64.35	,, 5.38	,, 6.03%	,, 4.63

The unhydrolyzed ester of the D-isomer was not purified further since it was expected to contain some L-isomer due to the incomplete enzymic hydrolysis.

p-*Fluoro*-L-*phenylalanine* was obtained by removing the blocking group from **16** and isoelectric precipitation at pH 6.8 in water/ethanol 1:1 followed by recrystallization from water/ethanol; m.p. 240–243° (dec.) (lit. 250–255° [3]), $[\alpha]_D^{20} = -25.1°$ ($c = 1$, H_2O), (lit. $[\alpha]_D^{25} = -23°$ ($c = 2$, H_2O) [3]).

N-Benzyloxycarbonyl-m-*fluoro*-L-*phenylalanine* (**17**). The racemic ester **13** (4.2 g, 12.2 mmol) was hydrolyzed with 20 mg of Subtilisin *Carlsberg* in 50 ml of 0.01M KCl containing 10% (*v/v*) of acetonitrile. Vigorous stirring kept the substrate emulsified in the reaction mixture. Additional enzyme (5 mg each time) was added after 5 and 18 h. Hydrolysis was complete after 32 h; yield 39% (theory 50%), m.p. 124° (from diisopropyl ether), $[\alpha]_{20}^{D} = -6.2°$ ($c = 1$, methanol).

$C_{17}H_{16}FNO_4$	Calc.	C 64.35	H 5.08	F 5.99%	N 4.41
(317.3)	Found	,, 64.13	,, 5.17	,, 5.67%	,, 4.43

m-*Fluoro*-L-*phenylalanine*, prepared as the *p*-fluoro-isomer, had m.p. 235–239° (dec.) and $[\alpha]_D^{18} = -25.2°$ ($c = 1$, H_2O), (lit. $[\alpha]_D^{25} = -24°$) ($c = 2$, H_2O) [3]).

N-Benzyloxycarbonyl-m-*fluoro*-D-*phenylalanine* (**20**) was obtained in 29% yield (theory 50%) by alkaline hydrolysis of the D-ester, m.p. 126–127°, $[\alpha]_D^{18} = +7.35°$ ($c = 1$, methanol).

N-Benzyloxycarbonyl-pentafluoro-L-*phenylalanine* (**18**). The racemic ester **14** (5.2 g, 12.9 mmol) was emulsified by vigorous stirring in 50 ml of 0.01M KCl containing 10% (*v/v*) of acetonitrile. Hydrolysis was accomplished with 50 mg of Subtilisin *Carlsberg* added in 2 equal portions at the beginning of the reaction and after 5 h. The reaction stopped after about 11 h when only 0.37 of the theoretically 0.5 equ. of the DL-ester were hydrolyzed. **18** was obtained in 21% yield (theory 50%) after recrystallization from diisopropyl ether/hexane, m.p. 118–119°, $[\alpha]_D^{18} = +1.34°$ ($c = 1.5$, dioxane).

$C_{17}H_{12}F_5NO_4$	Calc.	C 52.45	H 3.11	F 24.40%	N 3.60
(389.3)	Found	,, 52.41	,, 3.30	,, 24.81%	,, 3.51

Pentafluoro-L-*phenylalanine*, obtained by removal of the blocking group from **18** and isoelectric precipitation at pH 6.5 in water/ethanol 5:1, had m.p. 250–255° (dec.) (lit. m.p. 261° [5]) and $[\alpha]_D^{18} = 19.3°$ ($c = 1$, H_2O), (lit. $[\alpha]_D^{25} = +22.4°$ ($c = 1$, H_2O) [5]).

The skillful aid by Mr. *I. Jacobson* is gratefully acknowledged. We thank Mr. *A. Deshe* for measuring the optical rotations.

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190. Conformational Study by ESR. of Some Alkyl Substituted 6a-Thiathiophthene Radical Anions

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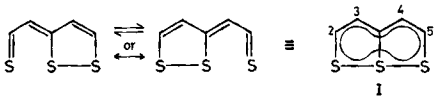
(17. V. 73)

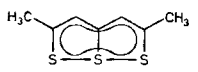
Summary. ESR. data are reported for the radical anions ($II^{\ominus}$ to $VI^{\ominus}$) of five alkyl substituted *6a-thiathiophthenes*. Rates and activation parameters for the inversion of the 3,4-trimethylene chain in $IV^{\ominus}$, $V^{\ominus}$ and $VI^{\ominus}$ have been obtained by means of an iterative least squares computer program ESRCEX. Preferential conformations of the alkyl substituents are discussed in terms of the $\langle\cos^2\theta\rangle$ dependence of the β-proton coupling constants and with the aid of molecular models. Experimental evidence strongly suggests that the partial rotation of the ethyl and isopropyl groups in $V^{\ominus}$ and $VI^{\ominus}$ is correlated with the inversion of the 3,4-trimethylene chain.

A few years ago, the ESR. data were reported [1] for the radical anions of several symmetrically substituted derivatives of *6a-thiathiophthene* (I) [2]. In the temperature range of investigation (−60 to +20°), the radical anions were found to retain the mirror plane passing through the C–S bond.

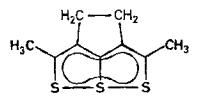


I

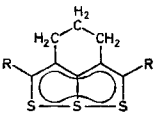
In the present paper, we deal in more detail with two of the radical anions considered in the previous paper ($II^{\ominus}$ and $V^{\ominus}$) [1] and extend the study to three other alkyl substituted derivatives ($III^{\ominus}$, $IV^{\ominus}$ and $VI^{\ominus}$). The ESR. spectra of $IV^{\ominus}$, $V^{\ominus}$ and $VI^{\ominus}$ display marked temperature dependence due to inversion of the 3,4-trimethylene chain and restricted rotation ofthe 2,5-alkyl groups ($V^{\ominus}$ and $VI^{\ominus}$).



II



III



IV R=CH_3
V R=CH_2CH_3
VI R=$CH(CH_3)_2$

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