[CONTRIBUTION NO. 192 FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, FORDHAM UNIVERSITY]

STUDIES ON THE CHEMISTRY OF HETEROCYCLICS. VIII. ENZYMATIC RESOLUTION OF RACEMIC β-2-THIENYLALANINE AND PREPARATION OF SOME SUBSTITUTED β-2-THIENYLALANINES[1]

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Previous studies in this laboratory have demonstrated the applicability of the N-methylformanilide (1) and rhodanine (2) syntheses to reactions in the thiophene series. Among the compounds made available through these investigations was the amino acid analog of phenylalanine, *viz.*, β-2-thienylalanine, which has proved to be of interest in growth inhibition experiments both on microorganisms (3) and on the rat (4). Since different effects were noted in these metabolism studies with the racemic amino acid and its optical isomers, it was decided to use an enzymatic method (4a) for its resolution thus obviating the more tedious chemical resolution previously applied (3). For this purpose a carboxypeptidase preparation (5) was utilized because of its effectiveness in asymmetrically hydrolyzing chloroacetylphenylalanine (6). This investigation indicated some major differences between thienyl- and phenyl-alanine. It was found that while the enzyme did not attack chloroacetyl-D-thienylalanine, it completely hydrolyzed the L-isomer but only in the presence of a buffer. However no buffer was required for the enzymatic hydrolysis of chloroacetyl-L-phenylalanine (6). It was also noted that the chloroacetylated thienyl enantiomorphs were susceptible to racemization when hydrolyzed with 2 *N* HCl, followed by three successive evaporations to dryness. Substitution of 1 *N* HBr for 2 *N* HCl with one evaporation produced optically-pure amino acids. The results from the two methods are compared in more detail in the experimental section.

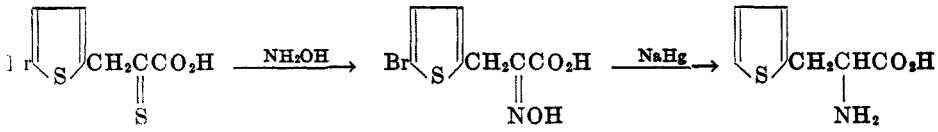
In view of the biological importance of β-2-thienylalanine the alkyl- and halogen-substituted thienylthiopyruvic acids, reported previously (2), were further reacted to obtain their corresponding thienyl amino acids. The preparation of the alkyl-substituted amino acids proceeded readily like that of the first member of the series, but the halogen-substituted acids presented some unforeseen results. When the 5-bromothienyloximino acid was reduced with 2% sodium amalgam, hydrogenolysis occurred yielding β-2-thienylalanine instead of the expected β-(5-bromo-2-thienyl)alanine (Chart I). This was proved conclusively by the analysis and melting point of the amino acid isolated and from the melting point of its phenylurea derivative. Prior investigations have demonstrated the removal of bromine from the thiophene ring with hydrogen in the presence of palladium on charcoal (7, 8).

[1] For Papers VI and VII of this series see *J. Org. Chem.*, **15**, 81, 89 (1950). This investigation was carried out under the auspices of the Office of Naval Research. For a preliminary communication see *Arch. Biochem.*, **25**, 460 (1950). The analyses were carried out by Dr. F. Bühler and A. A. Sirotenko of this Department.

[2] Abridged from a part of the dissertation presented to the Graduate School of Fordham University, 1950, in partial fulfillment of the requirements of the degree of Doctor of Philosophy.

The preparation of β-(5-chloro-2-thienyl)alanine was achieved in lower yields than the alkyl-substituted products and under anomalous conditions. In the other cases cited above, the free amino acid precipitated from solution after the reaction mixture had been cooled. The chloro compound failed to precipitate even after standing for one week in a refrigerator. However when the solution was diluted with water to twice the original volume and chilled, the amino acid

CHART I



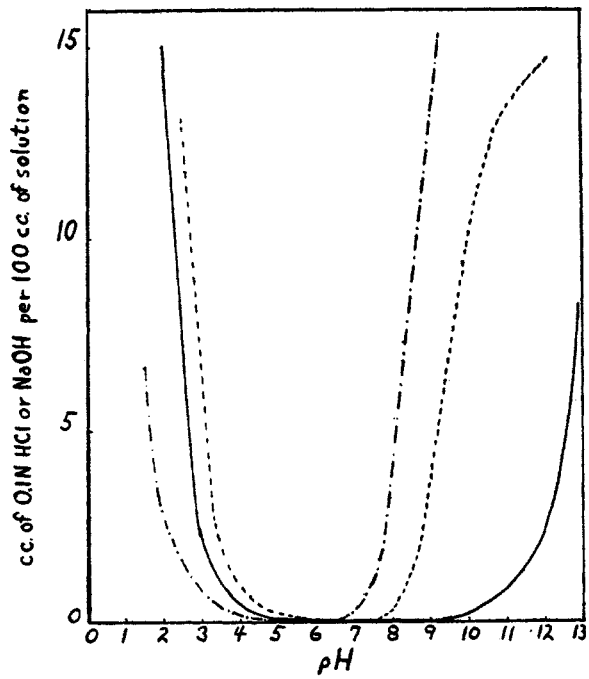


FIGURE 1. TITRATION CURVES OF β-2-THIENYLALANINE AND β-(5-CHLORO-2-THIENYL)-ALANINE. Water blank ————; β-2-Thienylalanine - - - -; β-(5-Chloro-2-thienyl)-alanine —·—·—·.

appeared. It may be noted that since small additions of lactic acid were used to keep the reduction media acidic, the *p*H of the solution at the end of the reaction was about 5.7, while the *p*H of the solution after dilution was 4.9, A marked difference between β-2-thienylalanine and β-(5-chloro-2-thienyl)alanine was shown by titrating them in an aqueous HCl solution with NaOH. These results are presented in Figure 1. These observations indicate that the presence of the chlorine atom has an appreciable effect on the properties of the resultant amino acid.

EXPERIMENTAL[3]

*Chloroacetyl-β-2-thienyl-*DL*-alanine.* Applying the method described for the chloroacetylation of L-phenylalanine (9), 9.5 g. (0.055 mole) of β-2-thienyl-DL-alanine was dissolved in 56 cc. of 1 *N* NaOH and placed in an ice-bath. To this solution was added, with shaking and in small portions, 83 cc. of 1 *N* NaOH and a solution of 8.8 g. of chloroacetyl chloride in ether. Dry hydrogen chloride gas was then passed into the alkaline mixture for 30 minutes, which precipitated the chloroacetylated compound as an oil. After standing overnight cold, the oil solidified giving a yield of 11.35 g. (82%). Recrystallization from hot water (Norit) gave 9.75 g. of white crystals (71%).

Carboxypeptidase preparation. The enzyme was obtained from 10 kilos of fresh-frozen beef pancreas (5), and stored with 480 cc. of water at −14°. One-eighth of this suspension was used for each enzymatic digestion. The amount of chloroacetylated compound used as substrate varied between 9.5 g. and 30 g.

TABLE I

SPECIFIC ROTATIONS OF OPTICAL ISOMERS OF β-2-THIENYLALANINE

COMPOUND	$[\alpha]_D^{21}$ OBSERVED		$[\alpha]_D^{21}$ LITERATURE	
	L-form	D-form	L-form	D-form
β-2-Thienylalanine	−31.4[a]	+31.4[b]	−31.7 (12)	+31.6 (12)
Chloroacetyl-β-2-thienylalanine	+46.5[c]	−47.2[d]	—	—

[a] 1.0834 g. in 50 cc. of water. [b] 0.2624 g. in 25 cc. of water. [c] 0.8468 g. in 50 cc. of absolute alcohol. [d] 0.9487 g. in 50 cc. of absolute alcohol.

Resolution procedure. Chloroacetyl-β-2-thienyl-DL-alanine (9.5 g., 0.038 mole) was suspended in water and dissolved by the cautious addition of 6 *N* LiOH with vigorous stirring. The enzyme was brought into solution by adjusting the suspension to *p*H 7.6 with 0.2 M LiOH. After the insoluble globulins were removed by filtration, 75 cc. of MacIlvaine buffer (*p*H 7.6) was added and the solution was mixed with the substrate, forming a total volume of about 300 cc. The digestion was carried out at 37° for 48 hours. The mixture was then acidified with glacial acetic acid to *p*H 5.0, and evaporated *in vacuo* to half of its original volume. The L-amino acid filtered off at this point amounted to 1.2 g. (36%) after washing with cold water and absolute alcohol. The filtrate was concentrated further *in vacuo*, layered with ethyl acetate, acidified to *p*H 2.0 with conc'd HCl, and extracted five times with ethyl acetate. The extract was dried with sodium sulfate and evaporated *in vacuo* to an oil which crystallized after washing with petroleum ether. Upon filtration there was obtained 3.2 g. (67%) of chloroacetyl-β-2-thienyl-D-alanine. This product, after recrystallization from hot water, had a specific rotation of $[\alpha]_D^{21}$ −47.2°, which is in agreement with its L-antipode obtained by chloroacetylation of the L-amino acid.

Chloroacetyl-β-2-thienyl-D-alanine (1.35 g., 0.055 mole) was refluxed for 70 minutes with 1 *N* HBr and evaporated to dryness *in vacuo*. The white residue was dissolved in absolute alcohol and adjusted to *p*H 5.2 with 4*N* LiOH whereupon 0.65 g. (69%) of the D-amino acid precipitated.

In a previous experiment no buffer was used during the enzymatic digestion and while some optically-pure L-amino acid was obtained, the chloroacetylated D-compound was evidently contaminated with unhydrolyzed chloroacetylated L-amino acid because it had the specific rotation $[\alpha]_D^{21}$ −31.1°. This mixture when hydrolyzed with 2 *N* HCl yielded a free amino acid with no optical activity.

[3] The authors wish to acknowledge the cooperation of Dr. J. V. Fiore and S. N. Timasheff.

Data relative to the four optically-active compounds described are recorded in Table I.

To verify the identity of the chloroacetylated racemic acid, used as the starting material in the resolution, it was converted to the unsaturated azlactone by treatment with acetic anhydride and pyridine (10). This product showed no depression in melting point when

TABLE II

ANALYTICAL AND MELTING-POINT DATA OF OPTICAL ISOMERS

COMPOUND	NITROGEN		M.P., °C.	
	Calc'd	Found	Observed	Literature
β-2-Thienyl-DL-alanine	8.18	8.27	273–275[c] 238–244[d]	274–275[c] (13) 243–245[d] (3)
β-2-Thienyl-D-alanine	8.18	8.38	273–276[c] 239–246[d]	239–244[d] (12)
β-2-Thienyl-L-alanine	8.18	8.25	272–274[c] 238–244[d]	239–244 (12)
Chloroacetyl-β-2-thienyl-DL-alanine	5.65	5.85	127–128	
Chloroacetyl-β-2-thienyl-D-alanine	5.65	5.90	119.5–120	
Chloroacetyl-β-2-thienyl-L-alanine	5.65	5.65	120.5–121.5	
2-Methyl-4-(2-thenal)-5-oxazolone[a]	7.26	7.34	131–132.5	
2-Methyl-4-(2-thenal)-5-oxazolone[b]	7.26	7.27	131.5–132.5	

[a] From chloroacetyl-β-2-thienyl-DL-alanine. [b] From 2-thenaldehyde. [c] Preheated oil-bath to 270°. [d] Oil-bath heated at rate of 8°/minute.

TABLE III

5-THIENYL-SUBSTITUTED 3-(2-THIENYL)-2-OXIMINOPROPIONIC ACIDS DERIVED THROUGH THE RHODANINE SYNTHESIS

5-THIENYL SUBSTITUENT	M.P.[a, f], °C.	YIELD[b], %	ANALYSES			
			Calc'd		Found	
			C	H	C	H
5-Methyl[c]	155–156	94	48.24	4.52	48.05	4.44
5-Ethyl[c]	152.5–153.5	94	50.70	5.16	50.90	5.23
5-Propyl[d]	143–144	93	52.90	5.72	53.20	5.33
5-Chloro[e]	153.5–154.5	95	38.26	2.73	38.29	2.73
5-Bromo[e]	156–157	90	31.81	2.27	31.78	2.44

[a] Oil-bath heated rapidly to within 7–8° of the recorded melting point. [b] Yields represent per cent conversion from the corresponding 2-thienylthiopyruvic acids. [c] Recrystallized from toluene. [d] Recrystallized from benzene. [e] Recrystallized from chloroform. [f] All melting points indicate decomposition.

mixed with an authentic sample prepared from 2-thenaldehyde by means of the Erlenmeyer azlactone synthesis (11).

The analyses and melting points of the isomeric amino acids and their chloroacetylated compounds are listed in Table II together with those of the azlactone.

Preparation of oximino propionic acids. The procedure employed was the same for all, as exemplified by the preparation of the 5-methyl compound. Benzene and chloroform were

TABLE IV

β-2-Thienylalanines Prepared by the Rhodanine Synthesis

Starting material	Amino acids									Phenylurea derivatives				
	Product	Yield, %	m.p.,[g] °C.	Analyses						M.p.,[g] °C.	Analyses			
				Calc'd			Found				Calc'd		Found	
				C	H	N	C	H	N		C	H	C	H
5-Methyl-2-thenaldehyde	β-(5-Methyl-2-thienyl)alanine	65	253[a]–255	51.89	5.94	7.56	52.10	6.14	7.70	162.5–163	59.21	5.26	59.49	4.99
5-Ethyl-2-thenaldehyde	β-(5-Ethyl-2-thienyl)alanine	62	235[a]–238	54.27	6.53	7.03	54.56	6.24	7.19	165.5–166.5	60.37	5.66	60.48	5.41
5-Propyl-2-thenaldehyde	β-(5-Propyl-2-thienyl)alanine	55	217[a]–220	56.34	7.04	6.57	56.74	6.93	6.72	158 –159	61.44	6.02	61.76	6.08
5-Chloro-2-thenaldehyde	β-(5-Chloro-2-thienyl)alanine	47	226[a]–228	40.87	3.89	6.81	40.97	3.85	6.82	163.5–164	51.77	4.00	51.58	4.12
5-Bromo-2-thenaldehyde	β-2-Thienylalanine	50	273[b,c]–275	33.60	3.20	5.60	49.00[d]	5.27[d]	8.22[d]	175[e,f]–176	—	—	—	—

[a] Oil-bath was heated at a rate of 6° per minute. [b] β-2-Thienylalanine has m.p. 274–275° (13). [c] Mixed m.p. with an authentic sample of β-2-thienylalanine showed no depression. [d] Calculated for β-2-thienylalanine: C, 49.12; H, 5.26; N, 8.18. [e] Phenylurea derivative of β-2-thienylalanine has m.p. 175–176° when the oil-bath is preheated to 165° (2). [f] Mixed m.p. with an authentic sample of the phenylurea derivative of β-2-thienylalanine showed no depression. [g] All melting points indicated decomposition.

substituted for toluene in the recrystallization of some of these products. These variations are indicated in Table III as well as other pertinent data relative to the compounds thus synthesized.

3-(5-*Methyl-2-thienyl*)-*2-oximinopropionic acid.* To a solution of 1.7 g. of sodium in 49 cc. of ethanol was added 5.16 g. of hydroxylamine hydrochloride. This solution after filtration was added to 4.6 g. (0.023 mole) of 5-methyl-2-thienylthiopyruvic acid and heated on a steam-bath for 30 minutes. After removal of the alcohol under a vacuum the residue was dissolved in 12 cc. of 5% NaOH and filtered. The cooled filtrate was carefully acidified under ether with 11 cc. of 10% HCl and the precipitated acid filtered off and dried *in vacuo* over potassium hydroxide. The water layer was removed in a separatory-funnel and extracted three times with ether. The combined ether extracts were dried over Drierite and evaporated to dryness. Total yield, 4.31 g. (94%).

Preparation of amino acids and their ureides. The preparation of β-(5-methyl-2-thienyl)-alanine described below was followed in all instances with the exception that the 5-chloro compound was precipitated by diluting the final reaction mixture with water to twice the original volume. All the amino acids prepared exhibited a positive ninhydrin reaction. The phenylurea derivatives were prepared in the usual manner with phenyl isocyanate. In Table IV are listed the compounds so obtained.

β-(5-*Methyl-2-thienyl*)*alanine.* 3-(5-Methyl-2-thienyl)-2-oximinopropionic acid (4.41 g., 0.022 mole) was dissolved in 60 cc. of absolute alcohol and 200 g. of 2% sodium amalgam was added in small portions with heating on a steam-bath. Small additions of lactic acid were made during the reduction to keep the solution acidic to litmus. When addition of the the amalgam was complete, the solution was decanted and left in the refrigerator overnight. Filtration afforded 2.85 g. of amino acid. By evaporation of the solution to a syrup and treatment with absolute alcohol a further 0.2 g. was obtained; total yield, 3.05 g. (74%).

The aqueous solutions of β-2-thienylalanine and β-(5-chloro-2-thienyl)alanine used for titration contained 0.2424 g. and 0.4443 g. of amino acid per 100 ml. of solution, respectively. A Fisher titrimeter was employed to obtain the data presented in Figure 1.

SUMMARY

Racemic β-2-thienylalanine was resolved into its optical isomers through the asymmetric hydrolysis of its chloroacetylated derivative using a purified beef pancreas carboxypeptidase preparation.

Four thienyl amino acids were prepared from 5-methyl-2-thenaldehyde, 5-ethyl-2-thenaldehyde, 5-propyl-2-thenaldehyde, and 5-chloro-2-thenaldehyde.

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