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TETRAZOLE ANALOGUES OF AMINO ACIDS AND PEPTIDES IV[1]

RESOLUTION OF RACEMIC TETRAZOLE ANALOGUES OF N-BENZYLOXYCARBONYL AMINO ACIDS BY MEANS OF HYDRAZIDE OF L-TYROSINE

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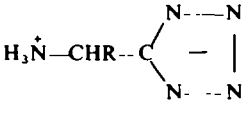
Abstract—Racemic tetrazole analogues of N-benzyloxycarbonyl derivatives of alanine, S-benzyl-cysteine, leucine, phenylalanine and valine were resolved by means of L-tyrosine hydrazide. The resolution of tetrazole analogue of N-benzyloxycarbonyl-DL-phenylalanine provided the L-enantiomer. From the other fractionation experiments tetrazole analogues of N-benzyloxycarbonyl-D-alanine, -S-benzyl-D-cysteine, -D-leucine, and -D-valine were obtained.

5-SUBSTITUTED tetrazoles behave as acids comparable in character to carboxylic acids.[2] In the first paper of this series the synthesis of a number of tetrazole analogues of amino acids **1** was described.[3] The present paper deals with the optical resolution of these compounds. The resolution was achieved by fractional crystallization of diastereomeric salts formed from racemic tetrazolic acid **2** and an optically active resolving base. This is the first report of employing of the acidic properties of 5-tetrazolyl residue for resolution of racemic compounds.



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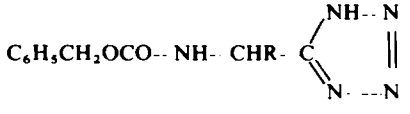

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Tetrazole analogues of amino acids were in the form of their benzyloxycarbonyl derivatives **2**. The hydrazide of L-tyrosine served as the optically active base. This compound introduced in 1966 to peptide chemistry by Vogler and Lanz[4] is an excellent resolving agent especially for the preparation of enantiomers of N-benzyloxycarbonyl amino acids.[4–8]

The fractionation experiments were carried out in MeOH. The difference in solubility of diastereomeric salts followed the pattern observed for N-benzyloxycarbonyl amino acids. This means that the precipitating material was enriched in the diastereomeric salt formed from the D-enantiomer of the tetrazole analogue of the amino acid (derivatives of alanine, S-benzyl-cysteine, leucine and valine). Only in the case of the tetrazole analogue of phenylalanine did the precipitating material contain the L–L diastereomeric salt. It should be pointed out that attempts to resolve N-benzyloxycarbonyl-DL-phenylalanine by means of L-tyrosine hydrazide resulted in separation of a material enriched in the D–L-diastereomeric salt.[4]

TABLE 1. RESULTS OF FRACTIONATION OF DIASTEREOMERIC SALTS FORMED FROM RACEMIC TETRAZOLE ANALOGUES OF N-BENZYLOXYCARBONYL AMINO ACIDS AND HYDRAZIDE OF L-TYROSINE

No	Racemic tetrazolic acid[a]	MeOH[b] ml	Time of crystallization hr	Precipitating material (salt "A")						Material recovered from mother liquor (crude salt "B")		
				Crude salt "A"		Sterically homogeneous salt "A"						
				Yield %	M.p. °C	Number of recrystallizations	Yield %	M.p. °C	Configuration	Yield %	M.p. °C	Configuration
1	Z-AlaT	2	13	100	154 159	4	41	166–168	D–L	96	161–164	L–L
2	Z-CysT/BZL/	8	16	102	169–171	2	89	171–172	D–L	93	143–149	L–L
3	Z-LeuT	1·25	20	95	166–169	3	59	174–176	D–L	99	147–150	L–L
4	Z-PheT	6	16	101	148–153	3	60	153–154	L–L	97	156–159	D–L
5	Z-Valt	1·5	18	103	171–175	2	72	183–184	D–L	90	80–89	L–L

[a] Hydrazide of L-tyrosine was used in equimolar amount; proposal of nomenclature and symbolism of tetrazole analogues of amino acids is given in ref. 10; Z—benzyloxycarbonyl; AlaT, CysT(BZL), LeuT, PheT, ValT—tetrazole analogues of alanine, S-benzyl-cysteine, leucine, phenylalanine and valine, respectively.

[b] The given volume of MeOH was used for 1 mmole quantities of tetrazolic acid and resolving base.

The steric purity of the crude precipitating salts "A" were in the range 45 to 70 per cent. The differences in solubility were, however, so pronounced that after several crystallizations from MeOH sterically homogeneous diastereomeric salts were obtained. From the mother liquor after evaporation of the solvent the material enriched in the other diastereomeric salt "B" was obtained. Attempts to purify these materials by recrystallization were, however, unsuccessful. The results of fractionation of diastereomeric salts are summarized in Table 1.

From the purified sterically homogeneous diastereomeric salts "A" the respective

TABLE 2. ENANTIOMERS OF TETRAZOLE ANALOGUES OF N-BENZYLOXYCARBONYL AMINO ACIDS PREPARED FROM STERICALLY HOMOGENEOUS SALTS "A"

No	Compound	Yield %	M.p. °C	$[\alpha]_D^{20}$ c = 1	Lit. data of reference L-form M.p. °C	$[\alpha]_D^{20}$ c = 1	Ref.
1	Z-D-AlaT	89	139–141	+34·5[a]	140–141	−36[a]	3
2	Z-D-CysT(BZL)	92	138–139	+35·5[a]	138–140	−36[a]	3
3	Z-D-LeuT	91	100–102	+39·5[a]	101–102	−40[a]	3
4	Z-L-PheT	96	182–183	−47[b]	181–182	−48[b]	9
5	Z-D-ValT	93	156–158	+33[a]	156–158	−33[a]	This paper

[a] In MeOH [b] In DMF

TABLE 3. TETRAZOLE ANALOGUES OF N-BENZYLOXYCARBONYL AMINO ACIDS OBTAINED FROM CRUDE SALTS "A" AND "B"

No	Compound	Material obtained from crude salt "A" Yield %	M.p. °C	$[\alpha]_D^{20}$ c = 1	Enriched in form	Material obtained from crude salt "B" Yield %	M.p. °C	$[\alpha]_D^{20}$ c = 1	Enriched in form
1	Z-AlaT	85	124–128	+16·5[a]	D	87	118–124	−11[a]	L
2	Z-CysT(BZL)	93	108–115	+24[a]	D	94	105–108	−25[a]	L
3	Z-LeuT	92	93–96	+22·5[a]	D	98	95–105	−13[a]	L
4	Z-PheT	96	167–171	−27·5[b]	L	96	166–171	+19[b]	D
5	Z-ValT	90	143–146	+20[a]	D	95	135–142	−18[a]	L

[a] In MeOH [b] In DMF

TABLE 4

No	Compound	Yield %	M.p. °C	Formula	N% calc.	found
1	Z-DL-Cys(BZL)-NH_2[a]	93	131–131·5	$C_{18}H_{20}N_2O_3S$	8·13	7·90
2	Z-DL-Leu-NH_2	74	129–131	$C_{14}H_{20}N_2O_3$	10·60	10·51
3	Z-DL-Val-NH_2	86	187–189	$C_{13}H_{18}N_{22}O_3$	11·19	11·01
4	Z-L-Val-NH_2	78	210–211	$C_{13}H_{18}N_2O_3$	11·19	11·23

[a] Prepared from S-benzyl-DL-cysteine obtained according to Liberek and cow.[12]

TABLE 5

No	Compound		Yield %	M.p. °C
1	Z-NH-CH(CH_2SBZL)-CN	DL	73	oil
2	Z-NH-CH($CH_2CH(CH_3)_2$)-CN	DL	81	53–54[a]
3	Z-NH-CH($CH(CH_3)_2$)-CN	DL	72	oil
4	Z-NH-CH($CH(CH_3)_2$)-CN	L	70	oil

[a] Found: 11·10% N. Calc. for $C_{14}H_{18}N_2O_2$: N, 11·38%

TABLE 6

No	Compound	Yield %	M.p. °C	Formula	N% calc.	found
1	Z-DL-CysT(BZL)	65	120–122	$C_{18}H_{19}N_5O_2S$	18·94	18·82
2	Z-DL-LeuT	86	110–112	$C_{14}H_{19}N_5O_2$	24·21	24·33
3	Z-DL-ValT	92	153–155	$C_{13}H_{17}N_5O_2$	25·44	24·97
4	Z-L-ValT[a]	94	156–158	$C_{13}H_{17}N_5O_2$	25·44	25·09

[a] $[\alpha]_D^{20}$ −33° (c = 1, MeOH)

enantiomers of 5-substituted tetrazoles were obtained by acidification with HCl. The optical rotations and other physical constants of these products were measured and compared with those of reference compounds of unquestionable L-configuration, prepared previously from L-amino acids.[3, 9] The results of preparations of enantiomers from sterically homogeneous diastereomeric salts "A" are presented in Table 2. For comparison tetrazole analogues of N-benzyloxycarbonyl amino acids were also obtained from crude salts "A" and "B". The optical rotations of these products are recorded in Table 3.

Attempts to resolve racemic tetrazole analogue of N-benzyloxycarbonyl phenylalanine by means of ephedrine[11] were unsuccessful.

EXPERIMENTAL

M.ps are uncorrected. The polarimeter was a Hilger and Watts model capable of being read to 0·01° and was used in conjunction with a sodium lamp.

Synthesis of tetrazole analogues of N-benzyloxycarbonyl amino acids

The starting materials for resolution were prepared from racemic N-benzyloxycarbonyl amino acids by generally applicable method described in Part I of this series.[3] The procedure involves preparation of the amides of N-benzyloxycarbonyl amino acids, dehydration of the intermediate amides to the corresponding

nitriles, and finally formation of the 5-tetrazolyl moiety from the cyano group by the action of sodium azide and ammonium chloride. The reference tetrazole analogues of N-benzyloxycarbonyl-L-valine was obtained from N-benzyloxycarbonyl-L-valine according to the outlined sequence of reactions.[3] The synthesis of tetrazole analogues of N-benzyloxycarbonyl-DL-alanine and -DL-phenylalanine was already described in Part I.[3]

Amides of N-*benzyloxycarbonyl amino acids.* The compounds were prepared by the mixed anhydride procedure with carbonic acid ethyl ester.[3] The results are summarized in Table 4.

Nitriles of N-*benzyloxycarbonyl amino acids.* The prepared amides were dehydrated by the action of phosphoryl chloride in pyridine.[3,13] The results are summarized in Table 5.

Tetrazole analogues of N-*benzyloxycarbonyl amino acids.* A suspension of N-benzyloxycarbonyl amino nitrile (10 mmoles), NaN_3 (0·72 g–10·4 mmoles), and NH_4Cl (0·59 g–11 mmoles) in DMF (6 ml) was heated to 95–110° for 16–24 hr, and the solvent was evaporated under reduced pressure. To the residue 1 N HCl aq (15–20 ml) was added (caution: HN_3 evolved) and the solid was then collected, washed with water, and recrystallized from MeOH—H_2O. The results are summarized in Table 6.

The syrupy crude tetrazoles were extracted three times with 15 ml of AcOEt. The AcOEt solution was washed with H_2O, dried over $MgSO_4$ and evaporated under reduced pressure.

Hydrazide of L-*tyrosine.* This compound was prepared according to Curtius[14] by hydrazinolysis of methyl ester of L-tyrosine. The material used as resolving base was recrystallized twice from MeOH: m.p. 195–196°; $[\alpha]_D^{20}$ +78° (c = 1, AcOH). Lit. m.p. 195·5°.[14]

Resolution of tetrazole analogues of N-*benzyloxycarbonyl-*DL-*amino acids*

Separation of diastereomeric salts. Tetrazole analogue of N-benzyloxycarbonyl-DL-amino acid (1 mmole) and hydrazide of L-tyrosine (1 mmole) in MeOH (for volume of solvent see Table 1) were heated to reflux temperature and some undissolved material was filtered off. The filtrate was set aside at −5° in a refrigerator for 24 hr. The separated material (crude salt "A") was collected and recrystallized several times from a minimal amount of MeOH.

The crude salt "B" was obtained from the mother liquor after evaporation of MeOH.

Enantiomers of tetrazole analogues of N-*benzyloxycarbonylamino acids.* A suspension of finally ground diastereomeric salt "A" in 1 N HCl aq (5 ml) was stirred for 15 min and then left for 2–4 hr in a refrigerator. The product was filtered and washed several times with cold water. Results are summarized in Table 2.

The same procedure was applied to crude salts "A" and "B". The results are presented in Table 3.

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