for *p*-iodophenol and *p-tert.*-butylphenol, ΔS is small and positive. This must be due to desolvation of the phenol and gel.

Again, *p-tert.*-butylphenol is an exception. The decrease in free energy is entirely associated with an increase in entropy, which suggests that the adsorption of *p-tert.*-butylphenol is controlled by some steric process.

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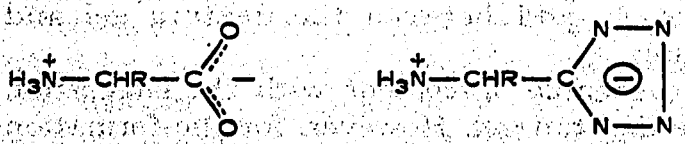
Tetrazole analogues of amino acids and peptides

II. Paper and thin-layer chromatography of tetrazole analogues of amino acids

Like amino acids (I), tetrazole analogues of amino acids (II)[1–3]—the compounds in which the carboxyl group is replaced by a 5-tetrazolyl group—exist in a zwitterion form.



I II

A number of tetrazole analogues of amino acids were prepared and characterised in the first paper[3] of this series. As a further part of the study the chromatographic behaviour of these compounds was investigated, and the sensitivity of the reaction with ninhydrin was determined.

The physical and chemical properties of tetrazole analogues of amino acids are very similar to the properties of amino acids. They are soluble in water, alkalis, aqueous acids, and sparingly soluble or insoluble in organic solvents. All tetrazole analogues of amino acids have high melting points, and all decompose at the melting temperatures. The dissociation constants of the tetrazole analogues of the amino acids are comparable to the respective constants for the amino acids.

We propose that the tetrazole analogues of amino acids, as a group, should be

called aminoalkyltetrazoles and abbreviated to AT. In order to emphasise the analogy between a particular aminoalkyltetrazole and its corresponding amino acid we suggest that the common name of the amino acid followed by the word tetrazole is used. For example, the tetrazole analogue of alanine would be called "alaninetetrazole", and for shorthand notation written as AlaT.

Experimental

Three adsorbents were used: (A) Whatman paper No. 1; (B) pre-coated cellulose TLC plates (Merck DC Fertigplatten Cellulose F); and (C) silica gel TLC plates (Merck Kieselgel G).

Silica gel TLC plates were prepared by the following method: 6 g Silica Gel G were shaken vigorously in an erlenmeyer flask with 12 ml of distilled water for about 2 min. The slurry was spread evenly on a dry plate (20 × 20 cm) and then the plate was dried at room temperature for about 24 h.

The solvent systems used were: S_1, methanol–water (7:3)[4]; S_2, *n*-propanol–water (4:1)[5]; S_3, *n*-butanol–acetic acid–water (4:1:1)[6,7]; S_4, pyridine–isoamyl alcohol–

TABLE I[a]

CHROMATOGRAPHIC DATA FOR AMINO ACIDS AND THEIR TETRAZOLE ANALOGUES

Solvents: S_1, methanol–water (7 : 3); S_2, *n*-propanol–water (4 : 1); S_3, *n*-butanol–acetic acid–water (4 : 1 S_4, pyridine–isoamyl alcohol–water (7 : 7 : 6); S_5, phenol–water (3 : 1, w/w). Adsorbents: A, Whatman paper N B, cellulose TLC plates (Merck DC – Fertigplatten Cellulose F); C, silica gel TLC plates (Merck Kieselge Technique: ascending; length of development: 24 cm (Whatman paper) or 10 cm (TLC plates).

No.	*Amino acid*	*Tetrazole analogue of amino acid*	*R_F values in solvent systems*													
			S_1			S_2			S_3			S_4			S_5	
			Adsorbent			*Adsorbent*			*Adsorbent*			*Adsorbent*			*Adsor*	
			A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	
1	Gly		0.55	0.52	0.46	0.11	0.15	0.25	0.09	0.11	0.22	0.16	0.13	0.20	0.38	
2		GlyT	0.57	0.54	0.56	0.18	0.23	0.47	0.14	0.13	0.39	0.27	0.25	0.36	0.40	
3	β-Ala		0.64	0.62	0.38	0.16	0.20	0.17	0.19	0.22	0.31	0.16	0.14	0.21	0.64	
4		β-AlaT	0.64	0.61	0.50	0.25	0.29	0.38	0.19	0.19	0.40	0.27	0.26	0.34	0.60	
5	Ala		0.71	0.71	0.50	0.21	0.27	0.32	0.20	0.22	0.30	0.21	0.19	0.28	0.55	
6		AlaT	0.72	0.73	0.58	0.30	0.38	0.53	0.27	0.28	0.47	0.34	0.33	0.47	0.57	
7	Pro		0.72	0.71	0.38	0.27	0.30	0.17	0.25	0.26	0.19	0.23	0.22	0.17	0.88	
8		ProT	0.73	0.71	0.49	0.34	0.40	0.33	0.31	0.31	0.33	0.36	0.35	0.29	0.87	
9	Abut		0.76	0.78	0.52	0.31	0.37	0.39	0.32	0.32	0.35	0.26	0.26	0.33	0.67	
10		AbutT	0.78	0.80	0.59	0.44	0.54	0.62	0.45	0.42	0.55	0.44	0.43	0.50	0.69	
11	Val		0.78	0.80	0.53	0.42	0.46	0.40	0.46	0.44	0.42	0.34	0.32	0.34	0.73	
12		ValT	0.80	0.81	0.59	0.54	0.62	0.63	0.55	0.54	0.63	0.52	0.47	0.58	0.74	
13	Nva		0.78	0.81	0.54	0.46	0.51	0.40	0.49	0.51	0.48	0.36	0.37	0.36	0.77	
14		NvaT	0.79	0.83	0.59	0.61	0.66	0.62	0.59	0.63	0.60	0.54	0.52	0.58	0.76	
15	Ile		0.80	0.85	0.55	0.55	0.60	0.43	0.58	0.61	0.50	0.43	0.43	0.48	0.80	
16		IleT	0.81	0.86	0.60	0.66	0.72	0.66	0.65	0.70	0.65	0.60	0.59	0.60	0.79	
17	Phe		0.72	0.72	0.58	0.49	0.54	0.55	0.53	0.51	0.59	0.49	0.47	0.52	0.87	
18		PheT	0.74	0.75	0.63	0.61	0.68	0.71	0.62	0.62	0.70	0.64	0.63	0.58	0.81	
19	Leu		0.81	0.82	0.55	0.55	0.63	0.54	0.61	0.60	0.61	0.48	0.44	0.49	0.82	
20		LeuT	0.83	0.83	0.60	0.67	0.75	0.75	0.71	0.69	0.72	0.65	0.60	0.66	0.80	
21	Cys/BZL/		0.71	0.71	0.59	0.61	0.70	0.63	0.58	0.66	0.62	0.65	0.63	0.62	0.86	
22		CysT/BZL/	0.73	0.73	0.70	0.69	0.77	0.72	0.66	0.73	0.72	0.73	0.72	0.68	0.83	

[a] Symbols in this paper are according to SCHWYZER *et al.*[11].

water (7:7:6)[8]; S_5, phenol–water (3:1, g/g)[6,7,9]; and were prepared according to the literature.

1% solutions of the amino acids (AA) and their tetrazole analogues (AT)[3] in 0.1 *N* hydrochloric acid were used for spotting. Sample spots were applied in amounts of 10–30 μg (Whatman paper) or 1–10 μg (TLC plates). A 0.25% solution of ninhydrin in acetone was used for the detection of the AA and AT on the chromatograms.

Results and discussion

R_F values of AA and AT from comparative runs in five solvents are given in Table I.

The highest R_F values for AT were observed in solvents S_1 and S_5. Both these systems gave approximately the same R_F values for each pair involving the amino acid and the corresponding tetrazole analogue of the amino acid. In the other solvent systems studied (S_2, S_3, S_4), the observed differences of R_F values for such corresponding pairs were in range 0.05 to 0.24, the R_F value of the AT being, in general, higher than that of the AA.

Lower AA and AT (glycine, alanine, β-alanine, α-aminobutyric acid and their tetrazole analogues) developed with S_2, S_3 or S_4 on silica gel TLC plates usually gave higher R_F values than those obtained for the other two adsorbents.

S_4 (pyridine–isoamyl alcohol–water) appeared to be the best system for the

TABLE II

SENSITIVITY OF THE TETRAZOLE ANALOGUES OF AMINO ACIDS TO THE NINHYDRIN REACTION ON WHATMAN PAPER NO. 1

No.	*Amino acid*	*Tetrazole analogue of amino acid*	*Sensitivity (μg)*		*Colour of spot*[a]
1	Gly		0.1	(0.2)[b]	violet-brown
2		GlyT	0.2		brown-violet
3	β-Ala			(0.4)[b]	violet
4		β-AlaT	0.4		grey-blue
5	Ala		0.2	(0.2)[b]	violet
6		AlaT	0.3		violet
7	Pro			(0.5)[b]	yellow-brown
8		ProT	3.0		yellow-orange
9	Abut			(0.1)[b]	violet
10		AbutT	0.2		violet
11	Val		0.2	(0.1)[b]	violet
12		ValT	0.4		violet
13	Nva				violet
14		NvaT	0.4		violet
15	Ile			(0.1)[b]	violet
16		IleT	0.5		violet
17	Phe		0.5	(0.7)[b]	violet
18		PheT	1.0		violet
19	Leu			(0.1)[b]	violet
20		LeuT	0.4		violet
21	Cys/BZL/				violet
22		CysT/BZL/	0.5		violet

[a] After 18 h.
[b] Reported by SAIFER AND ORESKES[10].

separation of aminoalkyltetrazoles not only because of differences in R_F values but also because the spots after spraying with ninhydrin were dense and intensely coloured. Development of AT by phenol–water solvent system (S_5) resulted in a low sensitivity for the ninhydrin reaction. In spite of thorough removal of phenol from the chromatograms it was difficult to calculate the R_F values on Whatman paper and cellulose TLC plates and very difficult on silica gel TLC plates, though the spots of amino acids could be easily detected with ninhydrin on these plates.

After spraying the chromatograms with ninhydrin the tetrazole analogues of amino acids gave a yellow colour. The spots changed to grey-brown and finally to violet.

The reactivity and sensitivity of the various aminoalkyltetrazoles with ninhydrin was determined by the SAIFER AND ORESKES method[10]. The limits of the sensitivity of the tetrazole analogues of amino acids (Table II) are comparable with the sensitivity values of the respective amino acids.

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