ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN—POLONIA
VOL. LII/LIII,12 SECTIO AA 1997/1998

Medical Academy in Lublin

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The synthesis of thiohydantoin derivatives and their elution behavior in TLC system

Synteza i analiza chromatograficzna tiohydantoinowych pochodnych metodą TLC

1. INTRUDUCTION

Among analytical methods applied in the amino acid studies, the chromatographic techniques were most valuable. The separation and identification of free amino acids, their derivatives and the qualitative analysis of the amino acid composition of the proteins became possible owing to the constantly improved chromatographic methods.

Numerous syntheses of the reagents reacting with amino acids were worked out. With the amino groups blocked, the derivatives obtained show the decreased polarity. Moreover, the derivation favourably affects the sensitivity and range of delection.

The most frequently used reagents reacting with amino acids are: phenylisothiocyanate /PTC/ /1/, chloride of 4-N,N-dimethylaminoazobenzene-4'-sulphonic acid /DABS-Cl/ /2/, 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate /DABITC/ /3/. In the chromatographic analysis of amino acids Iskierko et al. /4/ applied 4-N,N-di-n-butylaminoazobenzene-4'-isothiocyanate /DBABITC/.

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The aim of the present paper was to analyze the effects of the structure on the chromatographic separation of the selected protein amino acids in the form of their thiohydantoin derivatives and the selectivity of the chromatographic separation with regard to free amino acids. Thus, a new reagent synthesized in our Department i.e. 4-N,N-dibenzylaminoazobenzene-4'-isothiocyanate /DPMABITC/ was used. The reagent preparation was based on the synthesis methods of other azobenzene derivatives /3,5,6/ with some modifications. The compound structure was determined by means of the elemental analysis, the infrared and ^{1}H NMR spectra.

2. EXPERIMENTAL

MATERIALS AND METHODS

The following 15 α-amino acids were used in the studies: alanine /Ala/, arginine /Arg/, phenylalanine /Phe/, glycine /Gly/, histidine hydrochloride /His/, asparginic acid /Asp/, glutamic acid /Glu/, leucine /Leu/, lysine hydrochloride /Lys/, methionine /Met/, proline /Pro/, threonine /Thr/, tryptophan /Try/, tyrosine /Tyr/ and valine /Val/ – all from Merck.

DPMABITC was synthesized in the Department of Fundamental Chemistry, Medical School in Lublin.

Preparation of DPMABITC of the amino acids studied

The course of the amino acid derivation using DPMABITC is presented in Figure 1. The microsynthesis of the DPMABITC-derivatives of the individual amino acids was carried out using DPMABITC in the molar ratio of reagent: amino acid – 1:1. Several experimental assays of the synthesis proved that those molar ratios of DPMABITC–AMK were the optimal proportions for obtaining the thiohydantoin derivatives. The Ala, Arg, Gly, Leu, Met, Phe, Pro, Val and Try amino acids in the amounts of 50 mole each were dissolved in 1cm^3 of buffer, pH 10.4/0.4cm^3 of triethylamine–TEA + 5cm^3 of 2.0 mole acetic acid + 50cm^3 of acetone + 50cm^3 of water. The Asp, Glu, His, Lys, Thre and Tyr amino acids were dissolved in the buffer of pH 10.65/0.5cm^3 of TEA+ 5cm^{3}of 2.0 mole acetic acid + 50 cm^{3}acetone + 50cm^3 of water/. The 50μmole of DPMABITC solution dissolved in 4cm^3 of acetone were added . The reaction was carried out in the 10cm^3 flasks in the nitrogen atmosphere heating the reaction mixtures for 72min. at the temperature of 55–58 °C. The contents of the

flasks were evaporated to dryness with nitrogen at the temperature of 35–40 °C and vacuum dried over phosphorus pentoxide. Then, 0.6cm^3 of TEA was added to each sample and heated in the nitrogen atmosphere for 40 minutes at the temperature of 50–55 °C. The reaction mixtures were dried with nitrogen stream and in the vacuum exsiccator over potassium hydroxide and phosphorus pentoxide.

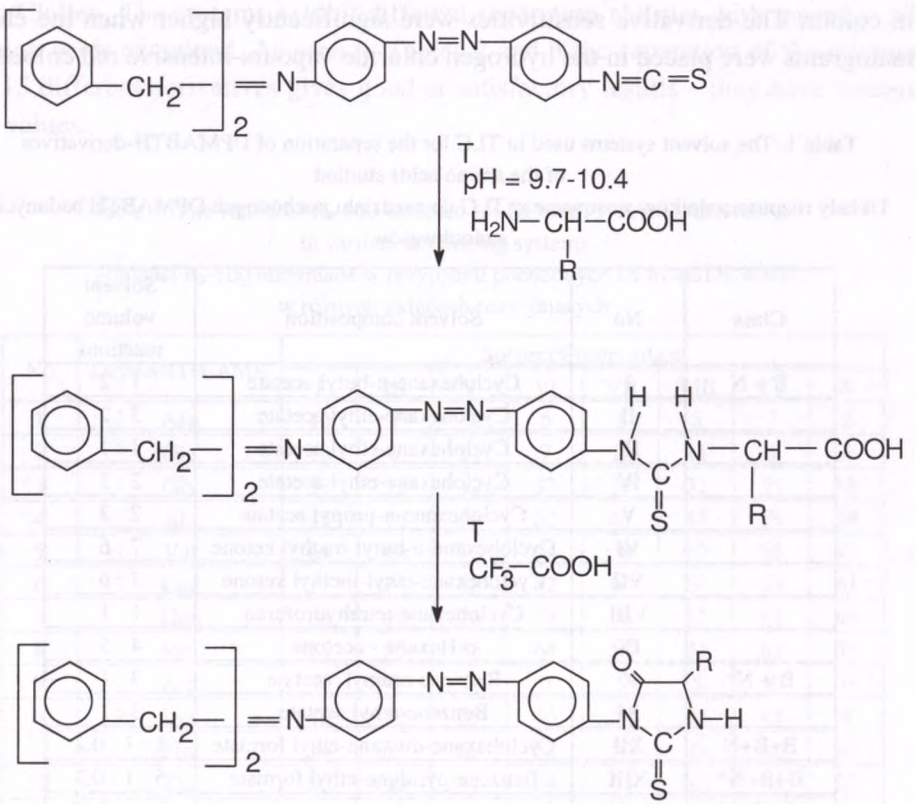

Fig. 1. Synthesis of DPMABITC- and DPMABTH-derivatives of amino acids (AMK)
Synteza pochodnych DPMABITC i DPMABTH aminokwasów (AMK)

Chromatographic separation of DPMABTH-AMK

The DPMABTH-AMK obtained were dissolved in 0.5cm^3 of acetone /analytically pure/ and chromatographically analyzed by TLC in the DS II hori-

zontal chromatographic chambers /7/. The Merck-DC-Plastikfolien Kieselgel 80 adsorbents were used. The chromatograms were developed by means of the ascending chromatography at the distance of 16cm at a constant temperature. The separation was performed using 18 eluents of various capacities for forming the hydrogen bonds with the absorbent active surface. The chromatograms were dried with warm air stream. The DPMABTH-AMK were visible in the form of red spots. In the eluent systems containing acetic acid the spots became purple in colour. The derivative sensitivities were significantly higher when the chromatograms were placed in the hydrogen chloride vapours-intensive red colour.

Table 1. The solvent systems used in TLC for the separation of DPMABTH-derivatives of the amino acids studied

Układy rozpuszczalników stosowane w TLC do rozdziału pochodnych DPMABTH badanych aminokwasów

Class	No	Solvent composition	Solvent volume reactions
B + N	I	Cyclohexane-n-butyl acetate	1 : 2
	II	Cyclohexane-ethyl acetate	3 : 2
	III	Cyclohexane-ethyl acetate	1 : 1
	IV	Cyclohexane-ethyl acetate	2 : 3
	V	Cyclohexane-n-propyl acetate	2 : 3
	VI	Cyclohexane-n-butyl-methyl ketone	7 : 6
	VII	Cyclohexane-ethyl-methyl ketone	7 : 6
	VIII	Cyclohexane-tetrahydrofuran	1 : 1
	IX	n-Hexane - acetone	4 : 5
B + N*	X	Benzene-n-amyl acetate	3 : 1
	XI	Benzene-ethyl acetate	3 : 1
B+B+N	XII	Cyclohexane-dioxane-ethyl formate	1 : 1 : 0.2
B+B+N*	XIII	Benzene-pyridyne-ethyl formate	5 : 1 : 0.2
AB+B+N*	XIV	Benzene-n-amyl acetate-acetic acid	2 : 1 : 0.25
	XV	Benzene-n-butyl acetate-acetic acid	2 : 1 : 0.25
	XVI	Benzene-pyridine-acetic acid	5 : 1 : 0.25
	XVII	Benzene-ethyl acetate – acetic acid	4 : 1 : 0.25
AB+ B+ N	XVIII	Cyclohexane-dioxane-acetic acid	2 : 1 : 0.4

3. RESULTS, DISCUSSION AND CONCLUSIONS

The Tables 2 and 3 present the separation of 15 amino acids 4-N,N-dibenzylaminoazobenzene-4'-thiohydantoin derivatives obtained by using DPMABITC /4,5/. The separation was performed with 18 eluent systems selected out of 52 various adsorption solvents classified according to Pimentel and Mc Clellen. The systems exhibit different separation abilities with regard to all amino acids examined. As seen in Tables 2 and 3 the separation of the mixture of 15 different derivatives gives good or satisfactory results – they have various R_F values.

Table 2. The values of $R_F \cdot 100$ obtained for DPMABTH-AMK derivatives in various developing systems
Wartości $R_F \cdot 100$ otrzymane w przypadku pochodnych DPMABTH-AMK w różnych układach rozwijających

No	DPMABTH-AMK	Solvent Composition							
		I	III	V	VI	VII	VIII	IX	X
1	Asp	13	7	9	6	8	8	7	2
2	Glu	18	9	11	7	10	11	9	5
3	Gly	37	33	42	37	37	32	51	18
4	Ala	45	46	56	52	47	44	59	24
5	Val	64	66	75	74	58	59	66	43
6	Leu	71	74	83	82	63	64	69	61
7	Thre	66	67	76	78	56	55	63	49
8	Met	55	55	65	64	52	51	61	31
9	Arg	0	0	0	0	5	5	8	0
10	Lys	49	29	39	40	38	35	45	8
11	Phe	58	53	69	62	50	49	60	35
12	Tyr	50	40	61	54	41	40	49	20
13	Try	53	44	63	56	44	42	55	26
14	Pro	47	50	60	60	54	46	64	46
15	His	4	5	7	4	5	3	6	0

It was experimentally proved that R_F of only some derivatives were similar or almost identical. For example, in system I this concerns the derivatives of methionine and tryptophan, valine and threonine; in system III-methonine and phenylalanine, valine and threonine; in system VI-tyrosine and trypthophan.

However, this seems insignificant as using the mixture of two or three solvent systems listed in the Tables 2 and 3 one can separate and identify the

DPMABTH-AMK derivatives except for arginine and histidine which do not move or just slightly migrate /system VII, IX, XII, XIII, XVI/ from the starting line. The behaviour of histamine and arginine results from the ability of nitrogens of those amino acids to form hydrogen bonds with polar solvents which bind with the silica gel active surface. The most selective separation of DPMABTH-AMK was achieved in systems VII, XIII, XVI. Table 4 illustrated the effects of the percentage of the polar ethyl acetate solvent in the non-polar cyclohexane solvent on R_F of each thiohydantoin derivative. As the data show the increased polar eluent content results in the significant increase of R_F. This relation is of some practical significance for the DPMABTH-AMK separation since within certain limits of the eluent concentration difference it enables to regulate R_F of the thiohydantoin derivatives.

Table 3. The values of $R_F \cdot 100$ obtained for DPMABTH-AMK derivatives in various developing systems
Wartości $R_F \cdot 100$ otrzymane w przypadku pochodnych DPMABTH-AMK w różnych układach rozwijających

No	DPMABTH-AMK	Solvent Composition							
		XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
1	Asp	5	27	11	20	18	13	11	34
2	Glu	8	30	13	29	27	24	15	40
3	Gly	19	34	33	40	36	35	27	43
4	Ala	28	45	41	53	43	44	35	54
5	Val	40	61	55	67	57	58	47	63
6	Leu	50	66	62	80	68	65	60	71
7	Thre	43	54	51	69	60	50	52	60
8	Met	36	50	46	61	53	47	56	58
9	Arg	0	5	4	0	0	4	0	6
10	Lys	16	48	22	3	32	20	20	31
11	Phe	38	37	49	64	51	53	41	67
12	Tyr	22	40	38	50	41	40	25	47
13	Try	31	43	35	57	46	38	31	51
14	Pro	47	57	58	60	55	60	50	65
15	His	0	4	6	4	4	6	0	9

The DPMABITC introduction to the amino acid molecules caused the decrease of their polarity and the increase of their molar mass, which in turn, facilitated the detection, improved the sensitivity and selectivity of the chromatographic separation of the thiohydantoin derivatives of the amino acids studied. The colour change of DPMABTH-AMK in hydrochloride may have some prac-

tical application in distinguishing α-amino acids from their β- and γ-isomers. The results confirm the abilities and the beneficial effects of DPMABITC on the TLC separation of α-amino acids.

Table 4. The effect of the percentage of polar solvent on the value of $R_F \cdot 100$ for DPMABTH-AMK derivatives

Wpływ procentowej zawartości polarnego rozpuszczalnika na wartości $R_F \cdot 100$ pochodnych DPMABTH-AMK

No	DPMABTH-AMK	Solvent Composition		
		II	III	IV
1	Asp	5	7	9
2	Glu	6	9	12
3	Gly	20	33	41
4	Ala	29	46	61
5	Val	45	66	72
6	Leu	58	74	79
7	Thre	50	67	70
8	Met	36	55	63
9	Arg	0	0	3
10	Lys	14	29	38
11	Phe	39	53	66
12	Tyr	24	40	53
13	Try	28	44	56
14	Pro	34	50	59
15	His	0	5	7

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STRESZCZENIE

Zastosowano 4-N,N-dibenzyloaminoazobenzeno-4'-izotiocyjanian (DPMABITC) do otrzymywania tiohydantoinowych pochodnych 15 α-aminokwasów (DPMABTH-AMK). Barwne pochodne rozdzielano metodą chromatografii cienkowarstwowej (TLC) na żelu krzemionkowym przy zastosowaniu układów rozpuszczalników różnych klas.