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THIN-LAYER CHROMATOGRAPHIC MOBILITY OF DIASTEREOISOMERS

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SUMMARY

Conditions were determined for the chromatographic separation of diastereoisomeric compounds, *viz.* derivatives of α-hydroxy-β-(indolyl-3)-butyric acids and β-methyltryptophans, in a thin sorbent layer. For the majority of the investigated compounds, the mobility of the A diastereoisomer (αRβS/αSβR configuration) is higher than that of the B diastereoisomer (αRβR/αSβS configuration). Sorption isotherms were studied for certain pairs of diastereoisomers and sorption forces were shown to be a decisive factor in the process of separating isomers. Possible conformations of the diastereoisomers are considered and a supposition is put forward that a greater sorptivity of one isomer compared with the other is determined by the greater steric accessibility of polar groups in this isomer in the preferable conformation in the adsorbed state. A suggestion is made concerning the conformation of molecules in the adsorbed state on the basis of the data derived for the comparative chromatographic mobility of the diastereoisomers.

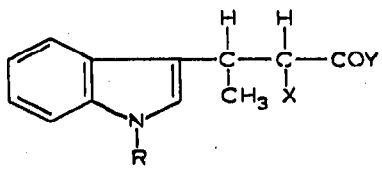
INTRODUCTION

The study of the chromatographic behaviour of substances belonging to different stereochemical series is both of theoretical interest and of practical importance. As a rule, only one of the diastereoisomers has valuable biological activity (thus, for instance, the *erythro* analogue of levomycetin has no valuable medicinal properties; the properties of ephedrine and ψ-ephedrine are very different). The study of the influence of the configurations of compounds on their chromatographic mobility carried out for several pairs of diastereoisomers having the same molecular fragments could allow the elucidation of the mechanism of the chromatographic process and provide information on the conformations of these compounds. Data have been reported on the chromatographic mobility of diastereoisomers with a relatively rigidly fixed conformation (steroids, alkaloids, carbohydrates). For such substances, differences in the chromatographic mobilities of the diastereoisomers were explained by differences in the steric accessibilities of polar groups in the molecules, for instance, the axial or equatorial position of the polar substituent[1–3]. The study of the chromato-

graphic mobility of diastereoisomers which contain asymmetric carbon atoms in the open chain proved to be a much more complicated problem.

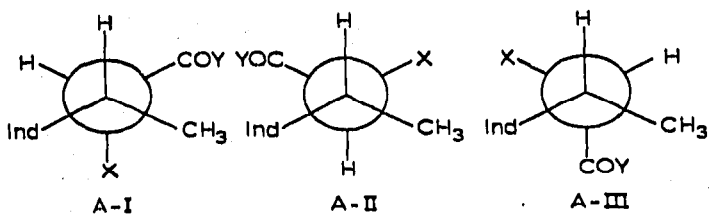
We studied the chromatographic behaviour of fourteen pairs of diastereoisomeric compounds which were derivatives of α-substituted β-(indolyl-3)-butyric acids. The configuration of the diastereoisomers which were the subject of our studies had been established earlier by chemical and nuclear magnetic resonance (NMR) spectroscopic methods[4,5].

In the present paper, we call compounds that have the configuration of indolmycin "A series compounds" and those with the configuration of isoindolmycin "B series compounds". An α-hydroxy- or an α-amino-β-(indolyl-3)-butyric acid of the A series has the αRβS/αSβR configuration, and the corresponding acid of the B series has the αRβR/αSβS configuration. Possible conformations with respect to the C_α–C_β bond for both diastereoisomers of the general formula

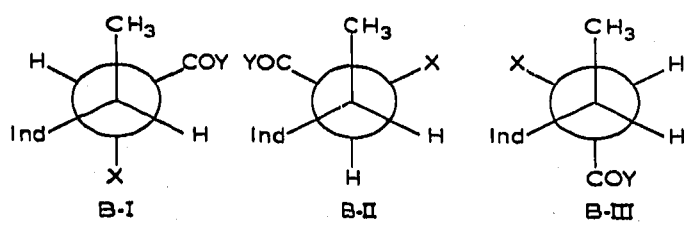


are given below.

A series



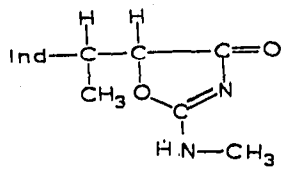
B series

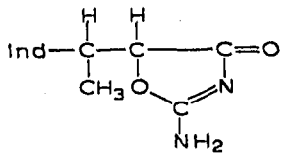


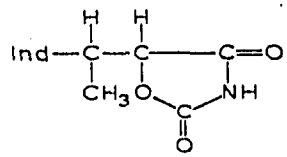
Compounds studied

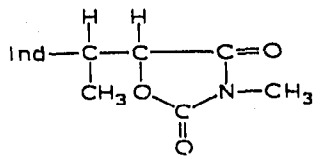
IA, IB	Ind—CH(CH_3)—CH(OH)—COOH	α-Hydroxy-β-(indolyl-3)-butyric acids
IIA, IIB	Ind—CH(CH_3)—CH(OH)—$COOCH_3$	Methyl α-hydroxy-β-(indolyl-3)-butyrates
IIIA, IIIB	Ind'—CH(CH_3)—CH(OCH_3)—COOH	α-Methoxy-β-(1-methyl-indolyl-3)-butyric acids
IVA, IVB	Ind'—CH(CH_3)—CH(OCH_3)—$COOCH_3$	Methyl α-methoxy-β-(1-methylindolyl-3)-butyrates

VA, VB Ind—CH(CH_3)—CH(OH)—$CONHCH_3$ α-Hydroxy-β-(indolyl-3)-butyricmethylamides

VIA, VIB  2-Methylamino-5-α-(indolyl-3')-ethyl-2-oxazolin-4-ones (racemic indolmycin and isoindolmycin)

VIIA, VIIB  2-Amino-5-α-(indolyl-3')-ethyl-2-oxazolin-4-ones

VIIIA, VIIIB  5-α-(Indolyl-3')-ethyl-oxazolidin-2,4-diones

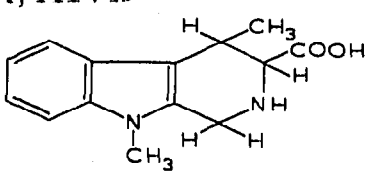
IXA, IXB  3-Methyl-5-α-(indolyl-3')-ethyloxazolidin-2,4-diones

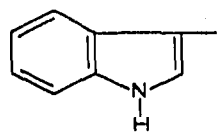
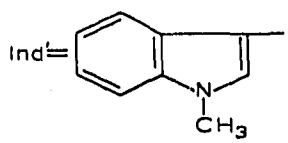
XA, XB Ind—CH(CH_3)—CH(NH_2)—COOH β-Methyltryptophans

XIA, XIB Ind—CH(CH_3)—CH($NHCOCH_3$)—COOH N-Acetyl-β-methyltryptophans

XIIA, XIIB Ind'—CH(CH_3)—CH(NH_2)—COOH 1,β-Dimethyltryptophans

XIIIA, XIIIB Ind—CH(CH_3)—CH($CHCOCH_3$)—$COOCH_3$ Methyl esters of N-acetyl-β-methyltryptophans

XIVA, XIVB  1,6-Dimethyl-5-carboxy-3,4,5,6-tetrahydro-β-carboline

Ind =  and Ind' = 

Materials

Adsorbents used were silica gel, prepared according to Stahl (A); silica gel, unfixed layer (B); aqueous silicic acid (C); and aluminium oxide (D).

The solvents were used without additional purification. For detection, use was made of a 1% solution of *p*-dimethylaminobenzaldehyde in a 1:1 solution of 25%

isomers to be adsorbed) are determined by differences in the steric accessibility of polar groups of the diastereoisomers in the preferable conformations. It should be noted that the conformation of molecules in the adsorbed state may not coincide with their conformation in solution. In this case, the differences in the chromatographic mobility are determined by the conformations of adsorbed molecules.

By NMR techniques, it has been shown that for α-hydroxy-β-(indolyl-3)-butyric acids and their derivatives, conformations with gauche-oriented C–H bonds are preferable (conformations A-I and A-III for compounds of the A series and conformations B-II and B-III for compounds of the B series)[4]. For amino acids, the contribution of conformations with the transoidal arrangement of the C_α-H and C_β-H bonds is essential[5,6]. In our case, however, for both the hydroxy and amino derivatives of indolylbutyric acids, the same regularities were observed in the adsorption of diastereoisomers. This evidently signifies that the conformation in the adsorbed state does not correspond to the preferable conformation of diastereoisomers in solution.

It appears that for all the compounds studied there exist common regularities in the choice of the preferable conformation in the adsorbed state, and differences in the chromatographic mobility of the diastereoisomers are determined by these conformations.

We shall now consider regularities in the choice of preferable conformations in the adsorbed state for diastereoisomers and the accessibility of polar groups of diastereoisomers in these conformations.

The choice of preferable conformations of molecules of compounds belonging to the A and B series is determined by the tendency of the most bulky groups (which have the greatest dipole moments) to be spaced a maximum distance apart. In this case, for an isomer of the A series, an A-I conformer will be predominant, where the indole ring and COY group (COOH, $CONHCH_3$ or $COOCH_3$) are in the transoidal position, while for an isomer of the B series, a B-I conformer will be predominant. Amino acids are known to be sorbed by enzymes in such a conformation (thus, phenylalanine is adsorbed in a conformation when the phenyl nucleus and the COX group are also in the transoidal position; the same regularities have been observed for tryptophan)[7].

From the comparison of the chromatographic mobilities of *cis*- and *trans*-β-arylacrylic acids (including isomeric Ind—CH=CH—COOH), a conclusion can be drawn that isomers with aryl and COOH groups in the *cis* position are more difficult

		ΔR_F	R_F		ΔR_F	R_F		ΔR_F	R_F		ΔR_F
A	*XIIIB*		*XIVA*	*XIVB*		*XVA*	*XVB*		*XIA*	*XIB*	
	0.68	−0.07	0.10	0.00	−0.10	0.27	0.22	−0.05	0.49	0.55	+0.06
	–	–	–	–	–	–	–	–	0.65	0.75	+0.10
	0.73	−0.10	0.46	0.36	−0.10	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	0.64	0.58	−0.06	–	–	–

to sorb than the corresponding *trans*-isomers[8]. Steric accessibilities of the indole ring in the A-I conformer and in the B-I conformer are approximately the same; the same holds for the carboxyl group. The X group (*e.g.* hydroxy, amino or methoxy), is screened to a considerably greater extent in the A-I conformer than in the B-I conformer. Accordingly, the B conformer can be expected to be held stronger by the sorbent than the A-I conformer. Hence, if A-I and B-I conformations are predominant for the diastereoisomers, differences in the abilities of the diastereoisomers to be adsorbed are determined by the differences in the steric accessibilities of the X group, which is more accessible in the B isomer than in the A isomer. In such a case, it can be expected that the B isomer will be held by the sorbent more strongly than the A isomer, that is, $\Delta R_F = R_F(B) - R_F(A) < 0$.

If the choice of preferable conformations of the adsorbed state is determined by the dipole–dipole attracting interactions of the indole ring and the COY group (COOH, $COOCH_3$, etc.) and dipole–dipole repelling interactions of the indole ring and the X group, then for the A isomer the A-II conformation is predominant, and for the B isomer the B-II conformation is predominant. In these conformations, the indole group and the X group have approximately the same steric accessibilities, and the COY group in A-II is more accessible than the COY group in the B-II conformer. In such a case, greater absorptivity of the A series compound could be expected compared with that of the B isomer, but no such phenomenon is actually observed.

For tetra-substituted ethanes, conformers with the gauche arrangement of the C_α-H and C_β-H bonds often prove to be more advantageous than transoidal conformers[8,9]. In such a case, the A-III and B-III conformers may prove to be most advantageous, since all their bulky substituents are brought closer together. In the A-III and B-III conformers, the accessibilities of the indole ring are almost the same, the X group is more accessible in A-III and the COY group is more accessible in B-III. Consequently, for those compounds in which the COY group precedes the X group (COOH, OH) in the adsorption series, the B-III conformer will be sorbed more strongly than A-III, and for the compounds with the reverse relationship (COY = $COOCH_3$, X—OH), the A-III conformer will be sorbed more strongly than B-III. In our case, however, no reversal of the chromatographic mobility of pairs of diastereoisomeric acids is observed, compared with that of a pair of diastereoisomeric esters, that is, for both IA and IB on the one hand and IIA and IIB on the other hand, $\Delta R_F = R_F(B) - R_F(A) < 0$. Therefore, the supposition that the preferable conformations of diastereoisomers in the sorbed state are A-III and B-III can be rejected.

For other diastereoisomeric compounds, namely derivatives of β-substituted α-aminobutyric acids, the conformations of the adsorbed state are such that the most polar substituent at C_β and the COOH group are in the transoidal position. It can be expected that stronger sorption (and hence lower mobility in thin-layer chromatography) will be observed with the diastereoisomer which, in this conformation, has a more accessible NH_2 group (see chromatographic mobilities of threonine (XVB) and allothreonine (XVA) in Table III).

The chromatography of diastereoisomers VIIIA and VIIIB, IXA and IXB and XIA and XIB in different systems of solvents on different sorbents, in all the cases when the separation of the diastereoisomers was attained, revealed another regularity of the chromatographic mobility (see Tables III and IV). The sorption isotherms obtained for VIIIA and VIIIB (Fig. 2) showed the isomer of the A series to be sorbed to

TABLE IV

SYSTEMS OF SOLVENTS AND SORBENTS USED FOR SEPARATION AND R_F AND ΔR_F VALUES FOR PAIRS VIII AND IX OF DIASTEREOISOMERS

No. of system[a]	*Mobile phase*	R_F		ΔR_F	R_F		ΔR_F
		VIIIA	*VIIIB*		*IXA*	*IXB*	
1A	Acetone–chloroform (7:3)	0.36	0.53	+0.17	–	–	–
2A	Acetone–benzene (7:1)	0.50	0.70	+0.20	–	–	–
3A	Ether	0.27	0.43	+0.16	–	–	–
4B	Ether	0.29	0.46	+0.17	–	–	–
5A	Ether–carbon tetrachloride (120:5)	0.25	0.42	+0.17	–	–	–
6A	Ethyl acetate	0.26	0.47	+0.21	–	–	–
7B	Ethyl acetate	0.33	0.50	+0.17	–	–	–
8A	Ether–petroleum ether (10:1)	–	–	–	0.54	0.71	+0.17

[a] Letters indicate the sorbent used (see *Materials* section).

a greater extent than that of the B series. Evidently, for IX and XI compounds, the chromatography is also of the adsorption type. To explain the reversal of the chromatographic mobility for the three pairs of compounds, we shall now consider those differences which exist in the structures of the compounds belonging to this group and of the compounds of the main group of substances discussed. In all the compounds discussed earlier, the dipole moment of the X groups (OH, NH_2, OCH_3, etc.) is less than that of the COOH group (or $COOCH_3$ or $CONHCH_3$). For N-acetyl-β-methyltryptophans, this relationship is reversed (the dipole moment of the $NHCOCH_3$ group is much greater than that of the COOH group). For adsorption in this case, the predominance will lie not with the A-I and B-I conformations that have groups which are completely remote from one another (indole ring and COOH), but with the A-II and B-II conformations, where in the transoidal position are found the indole ring and the most polar of all the groups positioned at C_α, *viz.* the $NHCOCH_3$ group. As pointed out above, for the A-II and B-II conformers, in view of the differences in the steric accessibilities of the COOH group, the A-II conformer will be held more strongly than the B-II conformer by the sorbent, which explains the greater absorptivity and lower

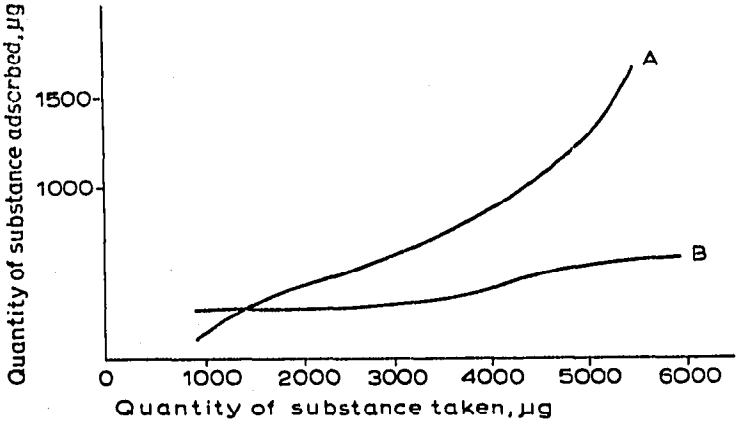


Fig. 2. Sorption isotherms. VIIIA and VIIIB, sorbent A, ethyl acetate as mobile phase.

mobility observed for XIA than for XIB. Evidently, a shift of the conformation equilibrium can be observed for derivatives of oxazolidinediones (VIII, IX) as well, but in this case all is not clear. The question remains open why the relationship $\Delta R_F = R_F(B) - R_F(A) > 0$ does not hold for methyl esters of the diastereoisomeric N-acetyl-β-methyltryptophans.

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