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THIN-LAYER CHROMATOGRAPHY ON SILICA GEL AS A METHOD FOR ASSIGNING THE RELATIVE CONFIGURATIONS TO SOME ALIPHATIC DIASTEREOMERIC COMPOUNDS

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SUMMARY

Separation of 37 diastereomeric pairs of compounds from the group Ar–CH(X)–CH(Y)–Ar′ (X and Y are polar groups; Ar and Ar′ are phenyl or *m*-, *p*-alkoxy substituted phenyl groups) is achieved by thin-layer chromatography on silica gel. In all cases the erythro-isomers have higher R_F values than do the corresponding threo-isomers, irrespective of the polarity of the developing solvents as well as of the formation of an intramolecular hydrogen bond between X and Y. The phenomena are explained on the basis of the preferred conformations of the diastereomers. Thin-layer chromatography on silica gel is proposed as a method for assigning the relative configurations of diastereomeric pairs of compounds of the above type.

INTRODUCTION

Separation of aliphatic diastereomeric compounds with two asymmetric carbon atoms has been achieved by thin-layer chromatography (TLC) on silica gel[1,2], boric acid-impregnated silica gel[3,4], Fasertonerde (alumina with fibre structure)[5] and cellulose[2]. Only in two of the papers quoted above do the authors correlate the chromatographic behaviour of the diastereomeric compounds with their relative configurations. On examining the separation of the methyl esters of diastereomeric fatty acids of higher molecular weights with two adjacent hydroxyl groups by TLC on boric acid-impregnated silica gel, MORRIS[3] has found that the threo-isomers have higher R_F values than do the erythro-isomers. He has assumed that the threo-compounds complex much more readily and, since the complexes are less polar than the original diols, these migrate much faster than the erythro-isomers. DREFAHL *et al.*[4] have separated diastereomeric aliphatic amino alcohols by TLC on silica gel, impregnated with boric acid, and have established that the diastereomer occurring, hydrogen bonding intramolecularly to a lesser degree, has a higher R_F value. This

has been explained by these authors with the assumption that the solvent in the developing system, itself capable of intermolecular hydrogen bonding, interacts more strongly with the isomer having less intramolecular hydrogen bonding. Furthermore, the difference in the extent of complex formation between boric acid and the diastereomeric amino alcohols has been pointed out as a contributing factor. Thus, the above two explanations for the difference in TLC behaviour of diastereomeric aliphatic compounds are based only on studies of compounds with intramolecular hydrogen bonding from the type HO...HO and NH_2...HO and with boric acid-impregnated silica gel as adsorbent.

In recent years many diastereomeric pairs of compounds, derivatives of 1,2-diarylethanes with two asymmetrical carbon atoms, were synthesised in our laboratory for various purposes. Their relative configurations were assigned which permitted us to investigate the separation by TLC on silica gel of a substantial number of diastereomeric pairs of compounds belonging to the group of Ar–CH(X)–CH(Y)–Ar' (X and Y are polar groups; Ar and Ar' are phenyl groups or *m*-, *p*-alkoxylated ones), as well as to establish whether a correlation exists between chromatographic behaviour and relative configurations of the compounds studied.

EXPERIMENTAL

The adsorbents used for thin-layer plates were Silica Gel DG (Riedel de Haen, 30 g and 80 ml of distilled water) and cellulose powder (Schuchardt, 20 g, 1 g of gypsum and 85 ml of water). The slurry was spread with an apparatus according to Stahl with a coating thickness of approximately 250 μ. The coated plates were air dried and kept in a dust-free chamber. The samples were applied 1.5 cm from the edge of the plate with a 2-cm distance between them. The length of run was 10 cm. The chromatograms were developed at room temperature in a glass chamber saturated with the solvent system. The cases of multiple development are indicated in Table I. Both Dragendorff's reagent followed by spraying with ether–iodine (for the basic compounds) and sulphuric acid were used for detection. The solvent systems used were A = ether–heptane (2:1); B = heptane–ethyl acetate–methanol–ammonia (12:10:1.5:1, the upper layer); C = ether–petroleum ether (2:1), D = ether–heptane (1:5); E = benzene–ethyl acetate–ethanol–ammonia (50:40:5:5, the upper layer); F = ether–heptane (1:1); G = ether; H = ether–petroleum ether (1:1); I = ether–acetone (3:2); J = ether–acetone (4:1); K = ether–acetone (9:1); L = ether–acetone (7:3); M = ether–methanol (17:3); N = ether–methanol (1:1); O = ethyl acetate–methanol–acetic acid (16:4:2); P = benzene–methanol–acetic acid (60:6:1); and Q = benzene–methanol–acetic acid (30:1:1). Whatman No. 1 paper was used for paper chromatography (PC) with ascending development of the chromatograms.

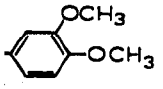
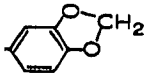
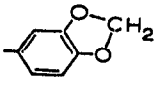
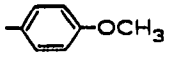
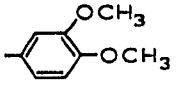
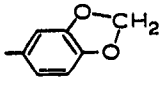
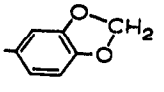
Methyl esters of threo- and erythro-3-hydroxy-2,3-diphenylpropionic acids (23 and 24, Table I) were prepared by methylation with diazomethane of the hydroxy acids 73 and 74, respectively[6].

RESULTS AND DISCUSSION

The results from the separation by TLC on silica gel of the diastereomeric pairs of compounds of the type mentioned above prepared by us and other authors

ABLE I

R_F VALUES OF THE DIASTEREOMERIC PAIRS OF Ar–CH(X)–CH(Y)–Ar'

r	Ar'	X	Y	Configuration	Reference	Compound No.	R_F	Solvent system
h	Ph	NH_2	$COOCH_3$	threo	7,8	1	0.30	A developed twice
				erythro		2	0.39	
		NH_2	$COOCH_3$	threo	9	3	0.48	B developed twice
				erythro		4	0.55	
		NH_2	$COOCH_3$	threo	9	5	0.27	A
				erythro		6	0.41	
h		NH_2	$COOCH_3$	threo	9	7	0.69	B developed twice
				erythro		8	0.76	
h	Ph	$NHCH_3$	$COOCH_3$	threo	10	9	0.39	A
				erythro		10	0.55	
		$NHCH_3$	$COOCH_3$	threo	10	11	0.48	A–B (1:1)
				erythro		12	0.60	
		$NHCH_3$	$COOCH_3$	threo	10	13	0.27	A–B (1:1)
				erythro		14	0.39	
		$NHCH_3$	$COOCH_3$	threo	10	15	0.31	A
				erythro		16	0.51	
Ph	Ph	$N(CH_3)_2$	$COOCH_3$	threo	10	17	0.34	C
				erythro		18	0.42	
Ph	Ph	NHPh	$COOCH_3$	threo	11	19	0.27	D[a] developed twice
				erythro		20	0.34	
Ph	Ph	$NHCONH_2$	$COOCH_3$	threo	12	21	0.25	E developed twice
				erythro		22	0.30	
Ph	Ph	OH	$COOCH_3$	threo	—	23	0.51	F
				erythro		24	0.59	
Ph		OH	$COOCH_3$	threo	13	25	0.38	B–H (1:5)
				erythro		26	0.47	

[a] Resolved by M. MLADENOVA.

(continued on p. 386)

TABLE I *(continued)*

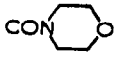
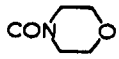
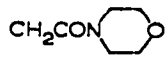
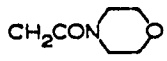
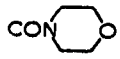
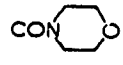
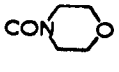
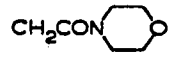
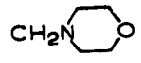
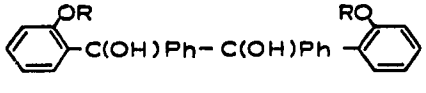
Ar	*Ar'*	*X*	*Y*	*Configuration*	*Reference*	*Compound No.*	R_F	*Solvent system*
Ph	Ph	$CON(CH_3)_2$	CH_2COOCH_3	threo	14	27	0.52	G
				erythro		28	0.68	
Ph	Ph	$CON(C_2H_5)_2$	CH_2COOCH_3	threo	14	29	0.32	H
				erythro		30	0.39	
Ph	Ph		CH_2COOCH_3	threo	14	31	0.43	G
				erythro		32	0.52	
Ph	Ph	$CON(CH_3)_2$	$CH_2COOC_2H_5$	threo	14	33	0.60	G
				erythro		34	0.70	
Ph	Ph	$CON(C_2H_5)_2$	$CH_2COOC_2H_5$	threo	14	35	0.40	H
				erythro		36	0.46	
Ph	Ph		$CH_2COOC_2H_5$	threo	14	37	0.52	G
				erythro		38	0.58	
Ph	Ph	$CON(CH_3)_2$	$CH_2CON(CH_3)_2$	threo	15	39	0.61	I
				erythro		40	0.70	
Ph	Ph	$CON(CH_3)_2$	$CH_2CON(C_2H_5)_2$	threo	15	41	0.49	I
				erythro		42	0.66	
Ph	Ph	$CON(CH_3)_2$		threo	15	43	0.45	I
				erythro		44	0.60	
Ph	Ph	$CON(C_2H_5)_2$	$CH_2CON(CH_3)_2$	threo	15	45	0.52	J
				erythro		46	0.58	
Ph	Ph	$CON(C_2H_5)_2$	$CH_2CON(C_2H_5)_2$	threo	15	47	0.31	G
				erythro		48	0.43	
Ph	Ph	$CON(C_2H_5)_2$		threo	15	49	0.32	K
				erythro		50	0.43	
Ph	Ph		$CH_2CON(CH_3)_2$	threo	15	51	0.32	J
				erythro		52	0.44	
Ph	Ph		$CH_2CON(C_2H_5)_2$	threo	15	53	0.59	J
				erythro		54	0.70	
Ph	Ph			threo	15	55	0.38	L
				erythro		56	0.54	

TABLE I *(continued)*

Ar	*Ar'*	*X*	*Y*	*Configuration*	*Reference*	*Compound No.*	R_F	*Solvent system*
Ph	Ph	NH_2	CH_2OH	threo	7	57	0.44	M
				erythro		58	0.54	
Ph	Ph	$NHCH_3$	CH_2OH	threo	16	59	0.45	M
				erythro		60	0.57	
Ph	Ph	$CH_2N(CH_3)_2$	CH_2CH_2OH	threo	17	61	0.38	N
				erythro		62	0.53	
Ph	Ph	$CH_2N(C_2H_5)_2$	CH_2CH_2OH	threo	17	63	0.35	N
				erythro		64	0.52	
Ph	Ph		CH_2CH_2OH	threo	17	65	0.15	G
				erythro		66	0.46	
Ph	Ph	CH_2OH	CH_2CH_2OH	threo	17	67	0.64	G
				erythro		68	0.85	
Ph	Ph	$NHCH_3$	COOH	threo	10	69	0.46	O developed twice
				erythro		70	0.52	
Ph	Ph	$NHCONH_2$	COOH	threo	12	71	0.15	P developed three times
				erythro		72	0.21	
Ph	Ph	OH	COOH	threo	18	73	0.22	Q
				erythro		74	0.31	

are given in Table I. The relative configurations of the compounds studied are known (see the references).

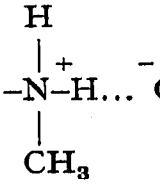
The most obvious conclusion drawn from the data (Table I) is that in all cases of separation of the 37 diastereomeric pairs of compounds studied, the erythro-isomers possess higher R_F values than do the corresponding threo-isomers, irrespective of whether intramolecular hydrogen bonding between X and Y exists and of the polarity of the solvent system used (see systems D and O). It is worth noting that TLC on silica gel of 6 diastereomeric pairs of compounds (from the group



R is an alkoxyl group) has shown higher R_F values for the *ms*-forms than for the

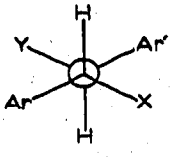
corresponding D,L-isomers[1].

Intramolecular hydrogen bonding is observed only in some of the diastereomeric pairs of compounds investigated—in compounds 57–66 of the type N...HO, in 67, 68 of the type HO...HO. A zwitterion structure and intramolecular hydrogen bonding

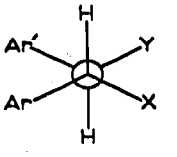


of the type $-\overset{H}{\underset{CH_3}{N}}-H\ldots\ ^{-}OOC-$ is assumed for the diastereomeric pair of 69, 70 (compare with ref. 2). Obviously a generally valid explanation, similar to that given by the authors cited above[3,4], in our case is impossible.

Although the variety of X and Y in the compounds studies is rather large, and therefore we are dealing with different classes of organic compounds, some common relationships even if only qualitative have been established for the behaviour of the individual compounds of the two steric series. For instance, in the cases in which N...HO intramolecular hydrogen bonding leading to the formation of a six-membered ring (57–60) is possible, it is predominant in the threo-isomers[7,16]; on formation of an eight-membered ring (61–66) the hydrogen bond is stronger again in the threo-isomers[17]. In the compounds with $COOCH_3$ and $CON(CH_3)_2$ groups, the signals for the methyl protons of these groups appear in the erythro-isomers at a higher field[10,13–15,19]. A simple relationship has been found between absolute configuration and sign of rotation in the optically active compounds[20]. Characteristic differences have also been established in the chemical behaviour of such compounds. For example, some cases of *cis*-cyclisation have been investigated. When the ring closure occurs by including a unit between X and Y, the threo-isomers show higher reactivity[7,12,21,22]. Further, if the open-chained compounds are optically active, then a larger change of the molecular rotation due to the ring closure has been observed in the erythro-series[23–25]. When a ring is closed by including a unit between X and Ar', the erythro-isomers show higher stereospecificity[26]. All these differences in behaviour are readily explained by the preferred conformations of the compounds studied. Investigation of the NMR spectra[10,13,19,27] of the latter has shown that it is a general tendency for the preferred conformations of the compounds from the erythro- and threo-series to be of the type A and B, respectively. The antiperiplanar arrangement of the methine hydrogen atoms is characteristic for A and B. Alternatively, X and Y as well as Ar and Ar' in the erythro-series are antiperiplanar,



A(erythro)



B(threo)

while in the threo-series they are synclinal. Thus the two steric series in this particular case appear as *series of conformational similarity*.

Detailed investigation both of the adsorption of each of the compounds studied on silica gel and of its solvation by the solvent systems used would be rather a

labour-consuming problem. However, the variety of X and Y (strongly polar in some cases and weakly polar in others) as well as the variety of the developing solvents on the one hand, when one and the same result is obtained ($R_{F\,(\text{erythro})} > R_{F\,(\text{threo})}$) on the other hand, indicate that definite factors are predominant. Here again we consider that the preferred conformations of the compounds are decisive.

Apparently the interaction between the most polar groups and the silica gel adsorbent will be the strongest. This is supported by the fact that the more polar X and Y are, the more polar the developing solvent must be. Hence, the threo-isomers should be more strongly adsorbed than the erythro-isomers for the following reasons. When the two most polar substituents are in steric proximity they can *both* react (incl. specifically[28]) with the surface of the adsorbent. These groups are close to each other in the preferred conformations of the threo-isomers. Actually, conformations with close X and Y groups are possible and also are present in the erythro-series. However, these conformations, because of additional steric hindrance, are less stable and hence their adsorbates should also be less stable. Therefore, it is reasonable to expect that in all cases the threo-isomers should exhibit a higher tendency of adsorption and respectively a lower R_F value. The influence of the interaction between the substance adsorbed and the developing solvent should also be in the same direction. Depending on the solvents used, either X and Y or aryl groups will solvate more strongly. Solvation of the groups increases their effective volume. Therefore, when the two strongest solvating groups are synclinal, they will mutually hinder their solvation. Hence, conformation A preferred in the erythro-series, is more favourable here. *Vice versa*, such a conformation is less favourable in the threo-series. Certainly this is a case of no great differences since with acyclic compounds the energy differences between the conformers are not great but nevertheless great enough for realising the resolution of 37 diastereomeric pairs of compounds on silica gel. We consider the importance of the adjacent aryl groups mainly as a factor causing the preferred conformations indicated above of the compounds studied and not as groups favourably interacting with the surface of the adsorbent.

The compounds with intramolecular hydrogen bonding of the type N...HO (57–66) and HO...HO (67, 68) show the same order of retention of the diastereomeric compounds by TLC on silica gel as it is in the compounds lacking such a bond. This fact can be used as a proof of our assumption that these bonds are cleaved by the action of the silica gel. Therefore, in these cases as in the ones discussed above, the threo-isomers will adsorb more strongly and solvate more weakly. This assumption is entirely reasonable considering that the silica gel hydroxyl groups are more acidic than the alcohol hydroxyl groups[29]. The results found by FISCHER AND KOCH[5] on chromatographic separation of aliphatic diastereomeric diols by TLC on Fasertonerde and by PC may also be used to support the same assumption. These authors have established in the case of TLC on Fasertonerde that the erythro- respective *ms*-isomer has a higher R_F value than the threo- respective D,L-isomer, while in the case of PC of the same compounds the order of retention of the diastereomeric compounds in the chromatograms is reversed. We consider that on Fasertonerde (a strong polar adsobent) cleavage of the intramolecular hydrogen bond of the type HO...HO takes place whereas on cellulose such a cleavage cannot occur. That is why the order of retention of the diastereomeric compounds is different in both cases. We cannot refer to our attempts to separate 59 from 60 and 67 from 68 by

TLC on cellulose and by PC, since on developing the chromatograms even with the least polar solvents (benzene, petroleum ether, hexane, heptane and others) the substances merely migrate to the front without any detectable separation of the diastereomeric compounds.

Our investigations on assignment of the relative configurations of other acyclic as well as cyclic diastereomeric compounds on the basis of their chromatographic behaviour will continue in the future.

CONCLUSION

We propose that TLC on silica gel be used as a method for assignment of the relative configurations of diastereomeric pairs of compounds of the type Ar–CH(X)–CH(Y)–Ar', where X and Y are polar groups and Ar and Ar' are phenyl or *m*-, *p*-alkoxylated phenyl groups. Erythro configuration should be assigned to the diastereomer with a higher R_F value while threo-configuration to that with a lower R_F value, irrespective of whether an intramolecular hydrogen bond of the type stated above between X and Y is formed.

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