Examination of Molecular Associations *via* Thin-Layer Electrophoresis II: Interactions of Amphetamine Isomers with Optically Active and Racemic Acids

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Abstract □ The different degree of migration of *d*- and *l*-amphetamine isomers on alumina, cellulose, and silica gel layers, in the presence of optically active and racemic mandelic and tartaric acids and the inactive salicylic acid, was examined using thin-layer electrophoresis. The observed differences were interpreted in terms of molecular associations.

Keyphrases □ Amphetamine isomers—interactions with optically active and racemic mandelic and tartaric acids, thin-layer electrophoresis, molecular associations □ Molecular associations—interactions of amphetamine isomers with optically active and racemic mandelic and tartaric acids, thin-layer electrophoresis □ Electrophoresis, thin layer—interactions of amphetamine isomers with optically active and racemic mandelic and tartaric acids and inactive salicylic acid, molecular associations

A number of chromatographic methods have been used successfully for the resolution of racemic compounds. In 1951, Kotake *et al.* (1) first reported resolution of some racemic amino acids using paper chromatography. In 1952, Curti and Colombo (2) succeeded in the resolution of racemic compounds by modifying the surface of inactive adsorbents, such as a silica gel column, by treatment with an active compound, namely (+)-camphorsulfonic acid. Dalgliesh (3, 4) also resolved a number of amino acids on cellulose paper. The different degree of adsorption of the isomers on the flat cellulose area was thought to be the principal reason leading to the resolution. When L-isomers were faster moving, stronger adsorption forces operated on the D-isomers and vice versa.

The solvent effect has also been discussed, but evidence showed that the resolution is independent of the optical character of the solvent, since inactive solvents also give resolution of the amino acids (1, 5). Sakan *et al.* (6) suggested that studies of the effect of paper rather than the solvent system should be made, and Fujisawa (7) later found that resolution is due to the asymmetric adsorption on cellulose.

Studies of the molecular associations of optically active isomers, when in solution or in solid phase or in a solution–solid system, have been successfully conducted using IR, melting-point, X-ray crystallography, conductimetry, and paper and thin-layer chromatographic techniques (8–11).

The purpose of the present investigation is to establish a molecular association pattern using thin-layer electrophoresis.

Table I—Distances Traveled by *d*- and *l*-Amphetamine Isomers (in Centimeters) on Cellulose, Alumina, and Silica Gel Plates in the Presence of Optically Active and Racemic Mandelic and Tartaric Acids and Inactive Salicylic Acid

Acids	pH	Amines: *l*-Amphetamine	Amines: *d*-Amphetamine	Current, mamp.
Cellulose Layer				
d-Mandelic	2.6	6.3	7.2	7.5
l-Mandelic	2.6	7.2	6.3	7.5
dl-Mandelic	2.6	7.2	7.2	7.5
d-Tartaric	2.3	6.2	7.5	4.0
l-Tartaric	2.3	7.5	6.2	4.0
dl-Tartaric	2.3	7.4	7.4	4.0
Salicylic	2.7	7.0	7.0	4.0
Alumina Layer				
d-Mandelic	2.6	6.5	6.5	11.0
l-Mandelic	2.6	6.5	6.5	11.0
dl-Mandelic	2.6	6.5	6.5	11.0
Silica Gel Layer				
d-Mandelic	2.6	5.0	5.0	11.0
l-Mandelic	2.6	5.0	5.0	11.0
dl-Mandelic	2.6	5.0	5.0	11.0

EXPERIMENTAL

Materials—The following were used: *d*-mandelic acid[1], m.p. 133.5° [lit. (12) m.p. 133.8°] and specific rotation +156° [lit. (12) +156° in water]; *l*-mandelic acid[1], m.p. 132.5° [lit. (12) m.p. 132.8°] and specific rotation −156.2° [lit. (12) −156.2° in water]; and *dl*-mandelic acid[2], m.p. 120.2° [lit. (12) m.p. 120.5°]. Mandelic acids were recrystallized from ethyl alcohol, filtered through sintered-glass funnels, and dried before melting points and optical rotations were taken. The tartaric acids used were: *d*-tartaric acid[3], m.p. 169.5° [lit. (13) m.p. 168–170°] and specific rotation +12° [lit. (13) +12° in water]; *l*-tartaric acid[3], m.p. 169.5° [lit. (13) m.p. 168–170°] and specific rotation −12° [lit. (13) −12° in water]; and *dl*-tartaric acid[3], m.p. 206° [lit. (13) m.p. 206°]. Tartaric acids were recrystallized from absolute alcohol, filtered, and dried in a desiccator before melting points and optical rotations were taken. Also used were: salicylic acid USP[4], m.p. 210° [lit. (13) m.p. about 211°]; *d*-amphetamine, puriss.[3], b.p. 204° [lit. (12) b.p. 203–204°] and n_D^{20} = 1.517 [lit. (12) 1.517]; and *l*-amphetamine, puriss.[3], b.p. 204° [lit. (4) b.p. 203–204°] and n_D^{20} = 1.5172 [lit. (12) 1.517].

Apparatus—A thin-layer electrophoresis cell[5] with 500-v. power supply unit was used.

Plates—Precoated alumina, cellulose, and silica gel plates[6] (20 × 10 cm.), 250-nm. thickness, were employed.

General Procedure—Aqueous solutions of the various acids and alcoholic solutions of the amphetamines, at exactly 0.1 *M* concentrations, were prepared. The acid solutions were also used as buffers. The precoated plates were sprayed with acid buffer solutions and joined to the cells by buffer-soaked wicks[7]. The *d*- and *l*-amphetamine samples were applied to the plates, 3 cm. from the anode, and equilibrated with the buffer solvent for a few minutes;

[1] Eastman Organic Chemicals.
[2] National Biochemical Co.
[3] Aldrich.
[4] Fisher.
[5] Camag Chemie A. G.
[6] Analtech, Inc.
[7] MN-paper for electrophoresis, Macherey, Nagel and Co.

then the system was switched on and adjusted to 350 v. To eliminate any temperature increase during the experiment, water for cooling was circulated through the system. After 75 min. the plates were removed, air dried, and developed in iodine fumes. The distances traveled by the spots were measured and recorded.

RESULTS AND DISCUSSION

The different rates of migration observed between *d*- and *l*-amphetamine isomers using optically active and racemic mandelic and tartaric acids, and the optically inactive salicylic acid as buffer solvents on alumina, cellulose, and silica gel plates, are depicted in Table I. The pH values of the acids, as well as the current (in milliamperes), are also indicated. The experimental field strength was 17.5 v./cm. (350 v. for 20 cm.).

The results indicate that two optically active isomers of the amine moved differently on cellulose layers when the optically active isomers of the acids were used as buffers. Specifically, the isomers possessing the opposite sign of rotation to that of the acid used as the supporting phase were always slower than the ones with the same sign of rotation. When the racemic acids were employed as buffers, both amine isomers migrated equal distances; a similar result was obtained when a nonoptically active acid (namely, salicylic) was used.

The results using thin layers of silica gel and the same buffer system were different from that of the cellulose thin layer in that both amine isomers traveled the same distance. When using a thin layer of alumina, the amine spots were again unaffected by the presence of the optically active acids.

In an electric field, the rate of migration of an ion is the sum of the electric potential and a number of other resisting forces. The direction of migration also depends on the charges. The amphetamines, possessing an amino group, are protonated at the low pH of the experiment, and they move toward the cathode. It is during this movement that a number of resisting forces would be encountered. Adsorption, for example, should not affect the solute ions in an ideal stabilizing medium. In actuality, however, adsorption should affect the separation of different types of ions in a solution by selectivity, and this kind of separation may be impossible in the absence of these adsorptive forces. However, plain adsorptivity is not the case here, since *d*- and *l*-amphetamine ions are similar, differing only in the sign of rotation.

On the other hand, if only adsorption was influencing the migration, the results could have been reproduced when alumina or silica gel plates were employed.

With regard to the difference in migratory power between *d*- and *l*-isomers of the amine, in the presence of optically active acids, it is indicated that an association of the acid and base is present and that this association involves interaction of the hydrocarbon moieties as well as electrostatic attraction of the cationic and anionic heads of the molecules involved (8–11). The results indicate that a *d*-amine isomer moves slower on an *l*-acid buffer-buffered support phase than the *l*-amine isomer. Similarly, *l*-amine travels slower on *d*-acid than *d*-amine on *d*-acid. The association, therefore, of *d*/*l*-diastereoisomer is stronger than that of *l*/*l*-diastereoisomer (similarly for *l*/*d*- and *d*/*d*-diastereoisomers) and, in fact, this follows the pattern of racemic resolution. This stronger association of oppositely charged diastereoisomers will be the retaining factor during the movement of the ions, compared to the weaker associated diastereoisomers, when cellulose layers are used.

When racemic acids replaced the optically active acids, no selective preference of the amine isomer toward the support phase could be found; both isomers migrated an equal distance. A similar result was found when the optically inactive salicylic acid was used.

The absence of preferential migration between *d*- and *l*-isomers on alumina and silica gel plates, as compared to cellulose plates, can be looked upon as the result of the effect of the carboxyl groups present in prepared cellulose. The importance of the carboxyl groups (present in cellulose) also appeared in earlier studies of some racemic amino acids (8). Since racemic mixtures of certain α-amino acids were resolved on cellulose thin layers, using a nonasymmetric solvent system, asymmetric adsorption forces in cellulose must be involved. The carboxyl groups present in cellulose were found to be implicated, since treatment of the layers with diazomethane (and, therefore, methylation of the carboxyl groups) suppressed resolution of the racemic amino acids. Different association of the isomers with the cellulose surface (probably with the carboxyl groups) during the development of the electropherograms was one of the main factors leading to the different migration. The difference in association between the isomers and cellulose surface can be concluded from the observed difference in migration of the two isomers on cellulose. Slower migration indicates stronger interaction between the isomers and the cellulose. However, the involvement of the carboxyl group can only be supplementary to the effect of the optical activity of the acids used as support phases, since the nonoptically active salicylic acid did not produce any preferential difference in the migration of the amine isomers even on cellulose.

The reported differences in traveling distance between the isomers (Table I), 0.7–0.9 cm., can be considered sufficient for establishing the explanation for the observed association phenomena if one considers that according to theory (14), two samples of an initial diameter (at the point of application) of 0.5 cm. and with relative mobilities differing by 1% will require approximately 100 cm. migration to produce a separation of only 0.5 cm. According to this reference, therefore, the observed molecular association produces a 20% difference in mobility between *d*- and *l*-amphetamine, which indicates a rather strong molecular association.

This study of interaction of asymmetric molecules with asymmetric groups at a surface becomes important because of the potential implications in the design of models of drug–receptor interactions. Moreover, the wide use of thin-layer electrophoresis over other methods for the examination of biological products will facilitate these studies.

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