

Separation of *cis* and *trans* isomers of α,β -unsaturated acids by thin-layer chromatography

Chemical constituents of plants often occur as esters of different α,β -unsaturated acids like angelic acid, tiglic acid, β,β -dimethylacrylic acid etc. In such compounds, the acid part is usually identified by isolation of the acid in a pure state after saponification of the corresponding esters. In many instances the process of isolation is further complicated by the conversion of the α,β -unsaturated acid from a labile *cis* form to a more stable *trans* configuration during saponification.

In the course of chemical investigations on some plants we encountered a number of angelic acid and β,β -dimethylacrylic acid esters of some triterpenes and it was found necessary to identify extremely small amounts of these α,β -unsaturated acids, and in particular to differentiate the *cis* and *trans* isomers (*i.e.* angelic acid and tiglic acid) by TLC. Lack of any convenient method for the resolution of these compounds by TLC prompted us to develop a suitable method.

At the outset, attempts were made to separate angelic acid, tiglic acid and β,β -dimethylacrylic acid by conventional TLC on Silica Gel G using a number of different solvent systems, but without any success. All the three acids migrated to an equal extent on the thin-layer chromatogram.

Certain triglyceride mixtures have been resolved into classes according to their degree of unsaturation and within these classes, certain isomeric unsaturated triglycerides have been resolved by TLC over Silica Gel G impregnated with silver nitrate¹. SUKH DEV *et al.*² have also separated certain isomeric olefinic sesquiterpenoids by TLC over Silica Gel G impregnated with silver nitrate. Following their methods, we tried to separate the aforesaid α,β -unsaturated acids. However, all the three acids again gave rise to long streaking spots which could not be employed for identification purposes. The following solvent systems were tested:

- (1) Chloroform-methanol (95:5, v/v)
- (2) Chloroform-methanol (9:1, v/v)
- (3) Benzene-methanol-acetic acid (95:3:2, v/v).

It has been reported recently³ that silica gel plates impregnated with ammoniacal silver nitrate, give much better resolution than silica gel plates impregnated with silver nitrate itself. Following this method, we have been able to separate the above mentioned acids. Three different solvent systems were employed:

- (A) Chloroform-methanol (95:5, v/v)
- (B) Chloroform-methanol (9:1, v/v)
- (C) Chloroform-methanol (2:1, v/v).

The R_F values of angelic acid, tiglic acid and β,β -dimethylacrylic acid in the three solvent systems mentioned above are tabulated below.

Experimental

β,β -Dimethylacrylic acid and tiglic acid were obtained commercially and angelic acid was prepared from tiglic acid according to the method of Mock *et al.*⁴ A slurry of Silica Gel G (E. Merck, 3.0 g) in ammoniacal silver nitrate solution (3.60 ml, made by adding liquor ammonia to a 5% aq. silver nitrate solution drop by drop till the precipitate first formed completely dissolved) was applied to the glass plates (10 × 20 cm) with an improvised spreader (layer thickness 0.35 mm). The plates were air-

TABLE I

Solvent system	<i>R_F</i> values		
	<i>Angelica acid</i>	<i>Tiglic acid</i>	<i>β,β-Dimethylacrylic acid</i>
A	0.50	0.14	0.03
B	0.63	0.38	0.21
C	0.78	0.41	0.30

dried for 15 min and then activated by heating in an air oven at 110° for 30–40 min. The acids in chloroform solution (100–200 μg) were spotted to the plates in the usual way.

The solvent was allowed to run up to a height of 14–15 cm from the base line. The plates were then air-dried and sprayed with an ethanolic solution (0.1 %) of 2,7-dichlorofluorescein and then exposed to U.V. light. Greenish-yellow fluorescent spots were observed in case of all the three acids. All the operations were carried out in an air conditioned room at a temperature of 22–24°.

Discussion

The strong co-ordination complex formed by the electrophilic complex amino ion $\text{Ag}(\text{NH}_3)_2^+$, present in the ammoniacal silver nitrate solution, with the nucleophilic π -bonds of the olefinic compounds probably explains the better separation of the α,β -unsaturated acids by this method. Of the three solvent systems employed, best results were achieved with solvent system C, as will be seen from Table I.

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