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Note

Separation and identification of some hydroxyacetophenones and their derivatives by thin-layer chromatography

H. M. CHAWLA, INDU GAMBHIR and LILY KATHURIA
Research Laboratories, Rajdhani College, New Delhi-110015 (India)
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Polyhydroxyacetophenones are the most important precursors for the laboratory and biogenetic synthesis of naturally occurring polyphenols. Being almost identical in molecular weight and ring size, isomeric acetophenones are difficult to separate by the usual chromatographic and chemical methods.

Recently, we have been investigating the separation of natural products by thin-layer chromatography (TLC) by impregnating silica gel plates with nitrobenzene prior to the development of the chromatograms[1–4]. This impregnation, however, did not give satisfactory results with polysubstituted acetophenones, but when chlorobenzene was used in place of nitrobenzene all of the hydroxyacetophenones were well resolved on a single chromatogram. The initial results are reported in this paper, and quantitative investigations on the reasons for the improved separations are still in progress.

EXPERIMENTAL

Materials

Glass plates (20 × 5 × 0.3 cm) were coated with silica gel G (Glaxo Labs., Bombay, India) and activated at 130° for 30 min before use. The plates were allowed to stand in a chromatographic tank containing chlorobenzene for 12 h, then removed and dried in a desiccating cabinet for 24 h. The hydroxyacetophenones to be separated were recrystallized and a solution in benzene or ethyl acetate was used for chromatography.

Solvent system

Benzene–acetone (24:1) plus 0.4% of glacial acetic acid was used as the solvent and the plates were either (i) sprayed with concentrated sulphuric acid followed by heating at 110° for 15 min or (ii) by keeping the plates overnight at room temperature, or (iii) exposed to iodine vapour to reveal the spots. Unlike the reaction with many polyphenols, exposure of the chromatograms to ammonia vapour gave no colour and hence was unsuitable for detection.

Procedure

By means of a fine glass capillary, concentrated solutions of the compounds

in benzene (1 mg/ml), except compound 3 (which was dissolved in ethyl acetate), together with a solution of their mixture, were applied at the baseline of the chromatographic plate at a distance of 2 cm from the lower end. The plates were developed in the above solvent system for 1 h in a tank previously saturated with solvent vapour. Chromatography was also carried out by using plates that had not been impregnated with chlorobenzene prior to the development. This gave a poor separation of the compounds.

RESULTS AND DISCUSSION

Several solvent systems recommended for phenols and ketones were tried but the above system gave the best results with polysubstituted acetophenones and their derivatives (Table I). Using pure benzene, only compounds 5, 7 and 9 moved; with benzene–ethyl formate (9:1), the carbonyl compounds moved with the solvent front; (c) benzene–acetone (24:1) resolved many components of the mixture but compounds 5, 6 and 9 merged and could not be separated clearly. The addition of 0.4% of glacial acetic acid to the last solvent system separated these unresolved components, but larger amounts of acid resulted in overlapping of other components.

TABLE I

R_F VALUES OF HYDROXYACETOPHENONES

No.	*Compound*	R_F *X 100*	*Colour with conc.* H_2SO_4	*Colour with iodine vapor*
1	2,4,5-Trihydroxy-6-methoxyacetophenone	13	Reddish brown	Brown
2	2,4,6-Trimethoxybenzaldehyde	26	Purple	Dark yellow
3	2-(Veratroyloxy)-4,6-dimethoxyacetophenone	45	Orange-brown	Light yellow
4	2,5-Dihydroxy-4-methoxy-6-benzyloxy-acetophenone	59	Orange	Brown-red
5	2-Hydroxy-4,5-dibenzyloxy-6-methoxy-acetophenone	74	Dark brown	Dark yellow
6	2-Benzoyloxy-3,4,6-trimethoxyacetophenone	75	Brown	Light yellow
7	2-(Anisoyloxy)-4,5-dibenzyloxy-6-methoxy-acetophenone	78	No colour	Light yellow
8	2-Hydroxy-4-benzyloxy-6-methoxy-acetophenone	84	Orange yellow	Light yellow
9	α-Anisoyloxy-2-hydroxy-4,5-benzyloxy-6-methoxyacetophenone	89	Dark yellow	Light yellow

The greater separation on addition of acetic acid is probably due to protonation of the acetophenone carbonyl group, which deactivates the benzene ring of the polysubstituted acetophenones. This supports our earlier hypothesis that there is a loose π-type complexation between the adsorbed organic liquid on the silica gel plates and the substrates[1,2,4,5]. Detailed investigations are currently being carried out by effecting a large number of separations of closely related compounds and impregnating the plates with organic liquids with different dielectric constants.

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