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Note

Thin-layer chromatographic analysis of oxo and thio compounds

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When aliphathic alcohols are oxidized with silver compounds and bromine^{1–4}, the products are ketones and tetrahydrofurans. Our present study has indicated that significant quantities of other products such as aldehydes and acids may result from oxidation of cyclic alcohols. Gas-liquid chromatography (GLC) is an ideal technique for the determination of ketones and some aldehydes. Acids, however, are very difficult to analyze by GLC unless they are derivatized first^{5–8}. In general, in the published thin-layer chromatographic (TLC) methods^{9–13} ketones are derivatized by a reaction with 2,4-dinitrophenylhydrazine before or during development.

The aim of this study was to develop a TLC procedure that would be sensitive enough to detect ketones and possibly acids and aldehydes without prior derivatization. After unsuccessfully testing 22 different chromogenic reagents that had been reported previously for conjugated and specific ketones, we turned our attention to 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INPTC) as a possible chromogenic reagent. This compound is sensitive to ketones, aldehydes and acids as indicated by tests performed on 26 compounds containing carbonyl or thio-carbonyl groups.

EXPERIMENTAL

Materials

The solvents used were purchased from either Burdick & Jackson Labs., (Muskegon, Mich., U.S.A.) or Fisher Scientific (Silver Spring, Md., U.S.A.). The ketonic pesticides and metabolites were obtained from the Pesticide Reference Standard Section, Registration Division, Environmental Protection Agency (Washington, D.C., U.S.A.). All of the remaining ketones, acids and aldehydes were obtained from commercial sources.

The TLC plates (aluminum oxide and silica gel) with a thickness of 100 μ m and dimensions 20 × 20 cm were obtained from J. T. Baker (Phillipsburg, N.J., U.S.A.).

The chromogenic reagent was prepared by dissolving 0.5 g INPTC in 100 ml of methanol. The basic spraying solution was prepared by dissolving 5 g of potassium hydroxide in 10 ml of water in a 100 ml volumetric flask and then diluting to volume with methanol.

Thin-layer chromatography

About 0.1 to 1.0 g of each ketone, acid or aldehyde was placed in separate 10-ml volumetric flasks and then diluted to volume. Water was used for the dilution of ethyleneurea and hydantoin, methanol for kepone and ethylenethiourea, and methylene chloride for 4-methylcyclohexanone, ordram, cycloheximide and 4,4'-bis(dimethylamine) benzophenone. All of the remaining compounds were diluted with hexane. Using a template, the compounds were applied as spots at 2 cm intervals on an imaginary line 1 in. from the bottom of the TLC plate. Another line was drawn across the plate 12 cm above this line to mark the solvent front for propanol-2-isooctane (1:3). A line at 10 cm was used for the other solvent systems.

A TLC chamber (4.5 × 23 × 23 cm) was lined with blotting paper and saturated with the developing solvent mixtures. About 25 ml of the developing solvent were poured into a trough at the bottom of the tank. The plates were then developed until the solvent front reached the drawn line (about 30 min). The plate was air dried for 5 min in a hood, and then sprayed with INPTC. The plate was then allowed to stand in the hood for 5 min and then heated for 5 min at 75° in an oven. Characteristic colored spots were observed as indicated in Tables I and II. When the plate was sprayed with a basic solution, the spots changed colors as indicated in Tables I and II. Fig. 1 represents several ketones including some with ketonic impurities also detected by the procedure.



Fig. 1. TLC on silica gel. Solvent system, acetonitrile-water (9:1). A = 0.5 mg pentanone-2; B = 0.5 mg pentanone-3; C = 0.5 mg cyclobutanone; D = 0.5 mg cyclopentanone; E = 0.25 mg cyclohexanone; F = 0.25 mg 4-methylcyclohexanone; G = 0.25 mg 3-methylcyclohexanone; H = 0.25 mg octanone-2. Note: commercial products contained impurities.

RESULTS AND DISCUSSION

There are three major problems in the TLC of many low-molecular-weight compounds: volatility, unsatisfactory color formation and solubility. Low-molecular-weight samples are generally more soluble than high-molecular-weight samples. We felt that there is enough affinity between the low-molecular-weight compounds and aluminum oxide and silica gel adsorbents to make TLC analyses possible, provided that a suitable mobile phase is selected. For example, when the propanol-2-isooctane (1:3) mixture was used as the mobile phase for 3-pentanone, the ketone could not be detected. With hexane-isooctane (1:1), a streak was observed extending from the origin to about 60 mm. However, when the polar solvent mixture acetonitrile-water (9:1) was used, a compact spot with a R_F value of 0.66 was observed.

To find a sensitive and practical chromogenic reagent for low-molecular-weight non-conjugated aliphatic and cyclic ketones has always been a problem. In fact, we tested 22 different chromogenic reagents, including many which had been reported as

TABLE I

TLC OF ALIPHATIC AND CYCLIC KETONES, ETHYLENETHIOUREA AND SOME ACIDS AND ALDEHYDES ON ALUMINA SHEETS

Solvent systems: A, propanol-2-isooctane (1:3); B, acetonitrile-water (9:1). Distance developed: A = 12 cm, B = 10 cm. ND = Not detected; NA = not analyzed.

Compound	Amount (μg) [*]	R_F		Color observed	
		A	B	INPTC	KOH
Pentanone-2	400	ND	0.84	pink	blue
Pentanone-3	400	origin	0.69	pink	blue
Cyclopentanone	400	ND	0.76	pink	blue
4-Methylcyclohexanone	200	0.67	0.83	pink	greenish blue
Octanone-2	200	0.54	0.77	pink	yellowish green
Menthone	200	0.65	0.82	yellow	yellowish orange
3-Methylcyclohexanone	200	p.63	0.81	pink	blue
5-Methyloctanone-2	200	0.69	0.77	pink	blue
Cyclooctanone	100	0.68	0.80	yellow	green
Cyclohexanone	100	0.57	0.82	pink	blue
Cholestan-6-one	60	0.64	NA	pink	blue
4-Androsten-17 β - ol-3-one-17-acetate	40	0.59	0.79	pink	blue
Decachlorooctahydro-1,3,4- metheno-2H-cyclobuta(cd)- pentalen-2-one	20	origin	NA	pink	blue
4,4'-bis(dimethylamine) benzophenone	100	origin to 40 mm	origin to 80 mm	pink	blue
Cyclobutanone	500	NA	0.76	pink	blue
2-Ethylhexanoic acid	150	NA	origin to 25 mm	pink	blue
<i>n</i> -Heptanoic acid	150	NA	origin to 10 mm	pink	blue
<i>n</i> -Heptaldehyde	150	NA	0.80	pink	blue
S-Ethylhexahydro- 1H-azepine-1-carbothioate	60	NA	0.78	pink	blue
Hydantoin	20	origin	NA	pink	blue
Ethylene thiuram monosulfide	2	NA	0.63	pink	blue

* Amount easily detectable.

useful for conjugated ketones. In most cases, large sample sizes were needed and in some instances even with large samples, certain ketones would not yield a color reaction.

However a color reaction was possible with the chromogenic reagent INPTC and as little as 20 μg could be detected as indicated in Tables I and II. For spot testing the amounts can be about ten times lower. For example 10 μg of cyclohexanone is detectable by spot testing.

To gather more information on the specificity of the procedure, several cycloalcohols were included in some of the TLC experiments. The alcohols were not detected by the INPTC spray as illustrated in Fig. 2 (areas F and G).

The characteristic color for each compound after spraying with INPTC is shown in the tables. For cyclooctanone, a yellow spot was observed after the INPTC spraying, whereas for cyclohexanone, a pink spot is observed. The results have triggered our interest in the nature of the product formed on TLC plates by INPTC and

TABLE II

TLC OF ALIPHATIC AND CYCLIC KETONES, ETHYLENETHIOUREA AND SOME ACIDS AND ALDEHYDES ON SILICA GEL SHEETS

Solvent systems: A, propanol-2–isooctane (1:3); B, acetonitrile–water (9:1); C, hexane–isooctane (1:1). Distance developed: A = 12 cm, B = 10 cm, C = 10 cm. ND = Not detected; NA = not analyzed.

Compound	Amount (μg)	R_F			Color observed	
		A	B	C	INPTC	KOH
Pentanone-2	200–500	ND	0.68	ND	pink	blue
Pentanone-3	200–500	ND	0.66	origin to 60 mm	pink	blue
Cyclopentanone	100–500	ND	0.58	0.63	pink	blue
4-Methylcyclohexanone	100–500	0.50	0.58	0.09	pink	blue
Octanone-2	200–250	0.40	0.52	0.23	pink	blue
Menthone	100–300	0.18	0.84	0.30	pink	blue
3-Methylcyclohexanone	100–250	ND*	0.58	0.50	pink	blue
5-Methyloctanone-2	250	NA	0.48	0.22	pink	blue
Cyclooctanone	100–150	0.46	0.86	0.48	greenish yellow	yellow
Cyclohexanone	100–250	0.52	0.65	0.55	pink	blue
Cholestan-6-one	60–100	0.48	origin to 80 mm	0.55	pink	green
4-Androsten-17 β -ol-3-one-17-acetate	40–60	0.41	0.83	0.25	dark pink	reddish brown
Decachlorooctahydro-1,3,4-metheno-2H-cyclobuta(cd)-pentalen-2-one	20–40	origin	0.79	NA	no color	pink
4,4'-bis(dimethylamine) benzophenone	100–150	NA	origin to 85 mm	origin	pink	blue
Cyclobutanone	500	NA	0.15	ND	pink	blue
2-Ethylhexanoic acid	200	NA	0.71	0.38	no color	blue
<i>n</i> -Heptanoic acid	200	NA	0.69	0.28	no color	pink
<i>n</i> -Heptaldehyde	200	NA	0.81	0.52	pink	orange
S-Ethylhexahydro-1H-azepine-1-carbothioate	50–60	NA	0.84	0.40	pink	dark pink
Ethyleneurea	40	NA	0.43	NA	pink	purple
Ethylenethiourea	40	NA	0.67	NA	pink	purple
Endrin ketone	40	NA	0.79	NA	no color	pink
Cycloheximide	40	NA	0.70	NA	pink	brown
<i>n</i> -Butyraldehyde	80	NA	0.85	NA	pink	orange

* None detected at low level.

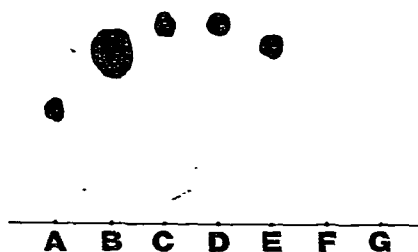


Fig. 2. TLC on silica gel. Solvent system, acetonitrile–water (9:1). A = 40 μg ethyleneurea; B = 40 μg ethylenethiourea; C = 40 μg endrin ketone; D = 40 μg kepone; E = 40 μg cycloheximide; F = 0.3 mg cyclohexanol; and G = 0.3 mg 3-methylcyclohexanol.

the carbonyl compound. Initial studies indicate that the solid adsorbent is a necessary component of the reaction and that different types of product are formed in the various cases. Preliminary evidence suggests that the highly colored compounds are 1:1 adducts, whereas the carbonyl compounds forming yellow products are of an entirely different nature. Studies are currently underway to determine the structure of these products.

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