Journal of Chromatography, 130 (1977) 392–395

CHROM. 9641

Note

Fluorescence method for detecting aldehydes at picomole level on thin-layer chromatographic plates*

J. CHRISTOPHER YOUNG

Chemistry and Biology Research Institute, Agriculture Canada, Ottawa, Ontario K1A OC6 (Canada)

(Received July 2nd, 1976)

Aldehydes have been detected on thin-layer chromatographic (TLC) plates after they had been sprayed with solutions of 4-amino-5-hydrazino-1,2,4-triazole-3-thiol[1], biphenyl-2-amine[2], 2,4-dinitrophenylhydrazine (DNPH)[3], malachite green[4], phosphomolybdic acid (PMA)[5], combined DNPH–PMA[6], and others[3]. Alternatively, derivatives have been prepared *in situ* (*e.g.*, DNPH[7]) or externally and applied to the plate (*e.g.* various hydrazones[8–14], semicarbazones[15], and oximes[20]), and then developed. Detection limits for these methods generally exceed 1 μg.

While the author was investigating the chemistry of the photolytic decomposition of N-nitrosamines on TLC plates, a method for the detection of aldehydes at levels less than 1 μg was required. Observations from concurrent studies on the photochemistry of imines (possible reaction intermediates derived from N-nitrosamines) gave rise to the fluorescence method reported in this paper.

EXPERIMENTAL

Materials

Aldehydes [numbers in brackets] were purchased from Aldrich (Milwaukee, Wisc., U.S.A.) [2, 7, 8, 11, 12, 15, 18, 22, 25, 26], BDH (Toronto, Ont., Canada) [17], Eastman-Kodak (Rochester, N.Y., U.S.A.) [4, 6, 10, 13, 16, 23], Fisher Scientific (Pittsburgh, Pa., U.S.A.) [1], K & K (Plainview, N.Y., U.S.A.) [5], Matheson, Coleman and Bell (Norwood, Ohio, U.S.A.) [3, 19, 20, 24], and Polaks Frutal Works (Middletown, N.Y., U.S.A.) [21]. Standard solutions of the aldehydes in methylene chloride or diethyl ether were freshly prepared before each determination. Acetophenone was purchased from Anachemia Chemicals (Montreal, Canada); aniline and 1-naphthylamine from BDH; fluorescamine from Fisher Scientific; phosphomolybdic acid from Merck (Montreal, Canada). N-benzylideneaniline was synthesized from benzaldehyde and aniline[16]. All other reagents came from Eastman-Kodak. Solvents were of reagent grade and were used as received from commercial sources. Eastman Chromagram sheets (No. 13181), 0.1 mm silica gel with fluorescent indicator, were stored in a dry atmosphere and activated by heating at 105° for 1 h prior to use.

* Contribution No. 928

Spray reagents

Solutions of 1% (v/v) aniline in methylene chloride, 0.5% (w/v) DNPH in 2 *N* hydrochloric acid, 0.1 mg/ml fluorescamine in acetone, 10% (w/v) PMA in 95% ethanol, and 10% (v/v) triethanolamine in methylene chloride were used as spray reagents.

Thin-layer chromatography and detection of aldehydes

Standard solutions containing 1 μg or less of aldehydes and ketones were spotted and then overspotted with 5 μg of aniline. After 10 min the chromatograms were developed in hexane–diethyl ether–methylene chloride (10:3:2), irradiated for 5 min with UV light using the apparatus previously described[19], immediately thereafter sprayed sequentially with fluorescamine and triethanolamine, and viewed under long-wave UV light. Instead of aniline, 5 μg each of some other aromatic amines were spotted over 1 μg of benzaldehyde, and the plates were then developed and treated as before. Alternatively, the aldehydes (*ca.*, 10 μg each) were spotted, developed, and sprayed with aniline or DNPH.

Photolysis of N-benzylideneaniline

On each of four plates 10 μg of N-benzylideneaniline was spotted, irradiated, and developed. One plate was sprayed with DNPH–PMA[6] and another with PMA–DNPH[6]. On each of the remaining plates, 5 μg of benzaldehyde was spotted at R_F 0.42 and 5 μg of aniline at R_F 0.58. After 10 min these plates were developed in a second direction and sprayed with DNPH–PMA or PMA–DNPH.

Photolysis of 4-hydroxy- and 4-dimethylaminobenzylideneanilines prepared in situ

Solutions containing 2 μg of the 4-hydroxy- and 4-dimethylamino aromatic aldehydes were spotted and then overspotted with 5 μg of aniline. After 10 min the chromatograms were developed, irradiated for 5 min, immediately developed in a second direction, sprayed with fluorescamine and triethanolamine, and viewed under long-wave UV light.

RESULTS AND DISCUSSION

The basis for the method described herein followed from the observations that on TLC plates a mixture of aniline and benzaldehyde spontaneously formed the corresponding imine; upon UV irradiation the imine regenerated aniline; and a characteristic yellow fluorescence resulted after treatment with fluorescamine[17] and viewing under long-wave UV light.

$$R\text{–}CHO + H_2N\text{–}C_6H_5 \underset{\text{TLC}}{\overset{\text{UV}}{\rightleftharpoons}} R\text{–}CH{=}N\text{–}C_6H_5 + H_2O$$

The identity of aniline and benzaldehyde as products from the photolysis of N-benzylideneaniline on silica gel was confirmed by comparison of R_F values and colour reactions with those of authentic material. The imine, aniline, and benzaldehyde gave yellow, no colour, and yellow spots, respectively, with DNPH–PMA and brown,

brown, and yellow spots, respectively, with PMA–DNPH. Furthermore, overspotting the presumed aniline and benzaldehyde with benzaldehyde and aniline, respectively, afforded the imine. Furey and Kan[18] reported photochemical hydrolysis of N-benzylideneaniline in solution to aniline and benzaldehyde. To determine the analytical scope of this new detection technique, a variety of other aldehydes were then screened. Table I summarizes the results.

The majority of the aromatic aldehydes examined afforded imines and gave detection limits of 20–30 ng (*ca.*, 200 pmole). However, those aldehydes having 4-

TABLE I

THIN-LAYER CHROMATOGRAPHIC FLUORESCENCE DETECTION LIMITS OF SOME ALDEHYDES AND R_F VALUES OF THEIR CORRESPONDING ANILINEIMINES

Detection limits determined on activated silica gel by spotting aldehyde, overspotting with 5 μg aniline, after 10 min developing in hexane–diethyl ether–methylene chloride (10:3:2), irradiating with UV light, immediately thereafter spraying with fluorescamine reagent, and viewing under long-wave UV light. R_F values of aldehydes were determined by spraying the developed plate with 2,4-dinitriphenylhydrazine. Abbreviations: ni = no imine detected; nf = no fluorescence observed from UV-irradiated and fluorescamine-treated imine; r = red spot; y = yellow spot. R_F aniline = 0.42.

No.	*Aldehyde*	R_F		*Detection limit*	
		Aldehyde	*Imine*	*ng*	*pmoles*
	Aromatic				
1	Benzaldehyde	0.58	0.65	20	200
2	4-Chlorobenzaldehyde	0.57	0.67	20	150
3	Cinnamaldehyde	0.52	0.58	25	200
4	2,3-Dimethoxybenzaldehyde	0.50	0.55	25	150
5	3,5-Dimethoxybenzaldehyde	0.52	0.60	30	175
6	2-Hydroxybenzaldehyde	0.56	0.65	25	175
7	3-Hydroxybenzaldehyde	0.19	0.25	30	225
8	5-Hydroxy-2-nitrobenzaldehyde	0.05	ni	—	—
9	Indole-3-aldehyde	0.12	ni	—	—
10	4-Methoxybenzaldehyde	0.46	0.56	30	225
11	4-Methylbenzaldehyde	0.60	0.68	30	250
12	3-Nitrobenzaldehyde	0.39	0.55	30	200
	Aromatic 4-hydroxy				
13	2,4-Dihydroxybenzaldehyde	0.15	0.19y		nf
14	3,5-Dimethoxy-4-hydroxybenzaldehyde	0.11	0.12y		nf
15	4-Hydroxybenzaldehyde	0.17	0.23y		nf
16	4-Hydroxy-3-methoxybenzaldehyde	0.17	0.22y		nf
	Aromatic 4-dimethylamino				
17	4-Dimethylaminobenzaldehyde	0.38	0.53y		nf
18	4-Dimethylaminocinnamaldehyde	0.36	0.39r		nf
	Aliphatic				
19	Citral	0.52	0.51, 0.59	125	800
20	Citronellal	0.64	ni	—	—
21	Eicosanal	0.57	ni	—	—
22	Hexanal	0.55	0.72	100	1000
23	Hydrocinnamaldehyde	0.46	0.69	70	500
24	Nonanal	0.63	0.74	150	1000
25	Pentanal	0.48	0.68	200	2500
26	Phenylacetaldehyde	0.37	0.64	50	450

hydroxy or 4-dimethylamino functionalities yielded coloured imines, which ultimately did not give observable fluorescence. Two-dimensional development of these imines demonstrated that UV irradiation liberated some aniline, which for the most part recombined with the aldehyde to afford the imine.

Some aliphatic aldehydes could also be detected although the detection limits were higher than for aromatic aldehydes. Aniline did not react to form appreciable amounts of imines with ketones such as acetophenone, benzophenone, and cyclohexanone, nor with 1,4-benzoquinone, 1,4-naphthoquinone, and anthraquinone.

Other amines were tested with benzaldehyde as substitutes for aniline. They included 2-chloro-, 3-chloro-, 4-chloro-, 4-hydroxy-, 4-nitro-, and 2-phenylaniline, benzylamine, cyclohexylamine, 1-naphthylamine, and 1,3-diaminobenzene. In all cases the yield of fluorophor, if formed at all, was less than from aniline.

The method described in this paper allows the detection of certain aldehydes on TLC plates at levels lower than hitherto reported. Interfering primary amines, which form fluorophors with fluorescamine[17], could be removed prior to analysis. The presence of other primary amines might not be a problem since the yellow fluorophor from aniline (emission maximum 484 nm) can easily be distinguished from the blue-white fluorophors from aliphatic amines (emission maximum 460–465 nm)[19].

The 4-hydroxy- and 4-dimethylamino aromatic aldehydes, which formed coloured imines, could be detected directly by using a solution of aniline as the spray reagent. About 0.1 μg each was clearly observable.

REFERENCES

1 C. H. Rahn and H. Schlenk, *Lipids*, 8 (1973) 612.
2 T. Nakai, T. Ohta and M. Takayama, *Agr. Biol. Chem.*, 38 (1974) 1209.
3 G. Zweig and J. Sherma (Editors), *Handbook of Chromatography*, Vol. II, CRC Press, Cleveland, Ohio, 1972, p. 153.
4 R. J. Teichman, G. H. Takei and J. M. Cummins, *J. Chromatogr.*, 88 (1974) 425.
5 R. Marcuse, *J. Chromatogr.*, 7 (1962) 407.
6 J. C. Young, *J. Chromatogr.*, 124 (1976) 115.
7 P. Froment and A. Robert, *Chromatographia*, 4 (1971) 173.
8 R. Chayen, R. Dvir, S. Gould and A. Harell, *Anal. Biochem.*, 42 (1971) 283.
9 M. H. Hashmi and M. A. Shahid, *Mikrochim. Acta*, (1968) 1045.
10 F. C. Hunt, *J. Chromatogr.*, 35 (1968) 111.
11 F. C. Hunt, *J. Chromatogr.*, 40 (1969) 465.
12 D. C. Johnson and E. G. Hammond, *J. Amer. Oil Soc.*, 48 (1971) 653.
13 J. S. Kirchner, *Technique of Organic Chemistry, Vol. XII, Thin-Layer Chromatography*, Interscience, New York, 1967, p. 378.
14 G. Zweig and J. Sherma (Editors), *Handbook of Chromatography*, Vol. I, CRC Press, Cleveland, Ohio, 1972, pp. 513–515, 626–629.
15 B. J. Camp and F. O'Brien, *J. Chromatogr.*, 20 (1965) 178.
16 L. A. Bigelow and H. Eatough, *Org. Synth. Collect. Vol., I* (1941) 80.
17 S. Udenfriend, S. Stein, P. Bohlen, W. Dairman, W. Leimgruber and M. Weigele, *Science*, 178 (1972) 871.
18 R. L. Furey and R. O. Kan, *Tetrahedron*, 24 (1968) 3085.
19 J. C. Young, *J. Chromatogr.*, 124 (1976) 17.
20 J. S. Kirchner, *Technique of Organic Chemistry, Vol. XII, Thin-Layer Chromatography*, Interscience, New York, 1967, p. 389.