

Notes

A new method for the detection of some aromatic acids

The classical indicator methods for the detection of aromatic acids on paper chromatograms are relatively insensitive and yield transient colours. A recent method¹ involving the detection of the sodium salts of acids with chloranilic acid is sensitive to 1 μg but its use is limited to chromatograms developed with unbuffered and non-acidic solvent systems. This paper describes a new sensitive method based on the formation of fluorescent hydroxy derivatives by means of hydroxyl radicals photochemically generated in dilute aqueous hydrogen peroxide.

Experimental

All compounds, except terephthalic acid (for which pyridine was the solvent) were dissolved in ethanol and 5 μl aliquots (5 μg) were applied to Whatman No. 1 paper for descending chromatography. The chromatograms after development and drying were lightly sprayed with an aqueous 0.3% solution of hydrogen peroxide. The wet chromatograms were placed on a glass plate and were illuminated with a Mineralight U.V. lamp (Model V-41) equipped with a filter to absorb most of the emitted visible radiation above 400 $\text{m}\mu$. Within a few minutes blue fluorescent spots became visible.

TABLE I
R_F VALUES AND SENSITIVITIES OF AROMATIC ACIDS TO THE
U.V.-HYDROGEN PEROXIDE DETECTION METHOD

Compound	<i>R_F</i> × 100			Sensitivity (μg)
	Solvent A	Solvent B	Solvent C	
Terephthalic acid	0	24*	40*	0.2
Isophthalic acid	0	31*	52	1
<i>o</i> -Phthalic acid	0	23*	31	2
Benzoic acid	47	not detected	not detected	2
Trimesic acid	0	1	10	0.5
Phenylacetic acid	not detected	not detected	not detected	(>>20)**
<i>o</i> -Toluic acid	not detected	not detected	not detected	(>>20)**
<i>m</i> -Toluic acid	59	not detected	90	2
<i>p</i> -Toluic acid	57	95	90	2
<i>o</i> -Chlorobenzoic acid	not detected	not detected	not detected	(>>20)**
<i>m</i> -Chlorobenzoic acid	69	91	89	3
<i>p</i> -Chlorobenzoic acid	68	91	89	3
2,4-Dichlorobenzoic acid	74	not detected	88	4
3,4-Dichlorobenzoic acid	75	90	88	3
3,4,5-Trimethoxybenzoic acid	44		84	1

Solvent A: *n*-butanol saturated with 5 *N* NH_4OH^3 ; Solvent B: solvent "B" of $\text{RE}10^3$; Solvent C: benzene-acetic acid-water (2:2:1)⁴.

* Streaky spot.

** 20 μg gives no detectable fluorescence.

Results and discussion

R_F values and sensitivities for the aromatic acids investigated are shown in Table I. The sensitivity is the minimum weight of compound found to yield an easily seen fluorescence in a spot approximately 1 cm² in area when viewed in a darkened room. No solvent development was done in those experiments in which sensitivity was measured.

The maximum fluorescence from terephthalic acid, isophthalic acid, trimesic acid and 3,4,5-trimethoxybenzoic acid was usually attained after illumination for about 1/2 min. For the other compounds yielding a positive reaction illumination for at least 3 min was required for maximum fluorescence. Prolonged illumination of terephthalic acid spots changed the colour of their fluorescence from blue to white.

The failure to detect benzoic acid, *m*-toluic acid, and 2,4-dichlorobenzoic acid in some solvent systems may be due to interference from the fluorescent solvent front.

Since the 6-methylsalicylic acid at least is known to be fluorescent³, the apparent absence of reaction with *o*-toluic acid must be attributed to preferential radical attack on the methyl group to produce non-fluorescent products. It is noteworthy that BATES AND URI⁵ found no evidence for hydroxylation of *o*-toluic acid by radicals photochemically generated from the ferric ion-pair complex Fe³⁺+OH⁻. Also in the case of phenylacetic acid, it is probable that radicals attack the side-chain in preference to the ring.

Attempts were made to produce fluorescent reaction products by photochemical decomposition of the compounds in the absence of hydrogen peroxide. For this purpose unfiltered radiation from the Mineralight lamp was used to illuminate wet (sprayed lightly with water) samples of the acids. In only one compound 3,4-dichlorobenzoic acid was an intense fluorescence (white) detected. Very faint fluorescence was detected for *p*-chlorobenzoic acid, 2,4-dichlorobenzoic acid, trimesic acid, and terephthalic acid.

Although the method is by no means general for aromatic acids, a number of acids can be detected because many hydroxy acids fluoresce. Even if specific isomers are non-fluorescent, the mixture of isomeric hydroxy acids produced may well fluoresce. Prolonged illumination and respraying with peroxide to produce dihydroxy acids might prove useful in cases where the monohydroxy acids do not fluoresce. The hydroxylation technique under carefully controlled conditions followed by subsequent rechromatography might yield unique reproducible product patterns useful for the identification of complex aromatic molecules.

Defence Research Chemical Laboratories,
Ottawa, Ontario (Canada)

DOUGLAS W. GRANT

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⁵ H. G. C. BATES AND N. URI, *J. Am. Chem. Soc.*, 75 (1953) 2754.

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