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Colour reactions of some aromatic ethers found in essential oils*

The higher boiling constituents of many essential oils contain a group of compounds loosely termed "aromatic ethers". These compounds are usually alkoxy-substituted allyl- or propenylbenzenes. Although there have been a number of reports dealing with gas chromatographic studies on this group of substances there have been relatively few publications so far which describe the thin-layer chromatography (TLC) of these compounds. FISHBEIN *et al.* have reviewed the chromatography of a number of methylenedioxyphenyl compounds, including such aromatic ethers as safrole, myristicin and apiole¹.

The aromatic ethers usually occur in essential oils together with large amounts of various terpene constituents, consequently the use of some chromogenic reagents useful for detecting terpenes, such as 2,2-diphenyl-1-picrylhydrazyl², has been investigated. This communication reports the results of a survey of the colour reactions given by eight "aromatic ethers" of this general type with ten different chromogenic reagents.

Experimental

Aromatic ethers. Safrole, eugenol and isoeugenol were obtained from the Aldrich Chemical Co. Eugenol methyl ether and isoeugenol methyl ether were gifts from Fritzsche, Dodge and Olcott Inc. Myristicin and elemicin were gifts from Dr. A. THOMAS and Mr. R. CHAPPAZ of Firmenich et Cie. (Geneva). Apiole was obtained from parsley seed oil (Fritzsche) by preparative gas-liquid chromatography (GLC).

Where necessary the compounds were purified by preparative TLC and/or preparative GLC methods.

Chromogenic reagents. The following reagents were used:

(i) CNTNF — A solution of 9-dicyanomethylene-2,4,7-trinitrofluorene (2 g) in acetone (100 ml) prepared immediately before use (*cf.* FISHBEIN³).

(ii) TNB — A solution of 1,3,5-trinitrobenzene (2 g) in acetone (100 ml) prepared immediately before use (*cf.* HUTZINGER⁴).

(iii) DPPH — A solution of 2,2-diphenyl-1-picrylhydrazyl (15 mg) in chloroform (25 ml). The plates were heated at 110° for 5–10 min immediately after spraying (*cf.* BERGSTRÖM AND LAGERCRANTZ²).

(iv) Gibbs' reagent — A solution of 2,6-dibromoquinone chlorimide (0.4 g) in methanol (100 ml). The plates were exposed to ammonia fumes directly after spraying.

(v) Vanillin-sulphuric acid — A solution of vanillin (1 g) in conc. sulphuric acid (100 ml).

(vi) Conc. sulphuric acid — The plates were lightly sprayed with reagent grade conc. sulphuric acid.

(vii) Furfural-sulphuric acid — A 2% solution of furfural in 62.5% sulphuric acid. The plates were heated for 15–30 min at 110–120° after spraying (*cf.* BEROZA⁵).

(viii) Chromotropic acid reagent — Conc. sulphuric acid (15 ml) was slowly added to a solution of chromotropic acid (4,5-dihydroxy-2,7-naphthalenedisulphonic

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TABLE I

COLOUR REACTIONS OF SOME AROMATIC ETHERS ON SILICA GEL PLATES

The colours reported are those observed by viewing the developed chromatograms (after spraying) in daylight. The initial colour produced by the particular reagent is reported together with subsequent major colour changes. Abbreviations for colours: Y = yellow; Gr = green; O = orange; Br = brown; P = purple; Pr = pink; R = red; Gy = grey; V = violer; I = violer; V = grey; Y = yellow; d = dark; t = trace; — = no colour. R_F values on silica plates in an *n*-hexane-chloroform (3:2) developing solvent: myristicin, 0.71; safrole, 0.86; eugenol, 0.31; isoeugenol, 0.27; eugenol, 0.42; isoeugenol methyl ether, 0.42; elemicin, 0.27; apiole, 0.41.

Chromogenic reagents ^a	(i) C _{NTNF} ₃	(ii) TNB ^a	(iii) DPPH ^{a,b}	(iv) Gibbs' reagent	(v) Vanillin- H ₂ SO ₄	(vi) Conc. H ₂ SO ₄	(vii) Furfural- H ₂ SO ₄	(viii) Chromo- tropic acid	(ix) SbCl ₃ ⁻ SbCl ₄ ⁻ (1:1)
Myristicin	Br	Y	Y	Br → Gy	tr	Y → —	Br → Gy	P	Br → tr
Safrole	Br → —	Y	Y	Gy-Br → Gy	— → tr	IP → —	Gy-Br → Gy	IP → P	—
Eugenol	Br	Y	Y	Br	P → Y	R → tr	Gy-Br → Gy	Gy	dBr
Isoeugenol	Gr	Y	Y	Y	R-P → P	O → tr	R	Gy	P

Eugenol methyl ester	Br	Y	Y	$\kappa\text{-DI} \rightarrow \text{uY}$	$\text{r} \rightarrow \text{I}$	$\kappa \rightarrow -$	$\text{uY-DI} \rightarrow \text{uY}$	$\text{uY} \rightarrow \text{u}$	$\text{uY} \rightarrow \text{u}$
Isoeugenol methyl ether	$\text{Gy} \rightarrow \text{Br}$	O-Y	Y	$\text{P} \rightarrow \text{Br}$	$\text{R-Pk} \rightarrow \text{IPk}$	$\text{Pk} \rightarrow -$	P	$\text{Gy-P} \rightarrow \text{P}$	IV
Elemicin	tr	Y	Y	$\text{Y} \rightarrow \text{tr}$	$\text{---} \rightarrow \text{tr}$	—	Br	$\text{Y-Br} \rightarrow \text{tr}$	Gy-Br
Apiole	Br	Y	Y	$\text{Br} \rightarrow \text{Gy}$	tr	—	Br	$\text{P} \rightarrow \text{P-Br}$	Br

^a CNTNF = 9-dicyanomethylene-2,4,7-trinitrofluorene; TNB = 1,3,5-trinitrobenzene; DPPH = 2,2-diphenyl-1-picrylhydrazyl; NBDF = p-nitrobenzenediazonium fluoroborate.

^b All colours on a purple background.

acid) (1 g) in water (15 ml). The plates were heated for 15–20 min at 110–120° after spraying (cf. BEROZA⁵, GUNNER AND HAND⁶).

(ix) Antimony trichloride–antimony pentachloride reagent — A mixture of equal volumes of solutions of antimony trichloride in chloroform (1:3, w/w) and antimony pentachloride in chloroform (1:4, v/v) (cf. STAHL AND JORK⁷).

(x) NBDF — A solution of *p*-nitrobenzenediazonium fluoroborate (1 g) in acetone (100 ml). After initial spraying with this reagent the plates were oversprayed with 0.1 *N* methanolic potassium hydroxide.

Colour development. The aromatic ethers (5–10 µg) were applied, in chloroform solution to the origin of Brinkmann Silica Gel F₂₅₄ pre-coated TLC plates (thickness 0.25 mm). The plates were then developed in the solvent system *n*-hexane–chloroform (3:2). After drying in air each developed chromatoplate was sprayed with one of the various chromogenic reagents mentioned above. The initial colour development and subsequent major changes in the colour of the spots and the backgrounds were carefully noted.

Mass spectroscopy. A sample (20 µg) of a typical aromatic ether (myristicin) was chromatographed, on a silica gel plate, in the manner described above. The plate was sprayed with the CNTNF reagent and the silica carrying the myristicin–CNTNF complex was scraped off and introduced directly into the mass spectrometer (a DuPont/CEC 21-110B instrument) using a standard probe for direct introduction into the ion source. The sample temperature was carefully raised and in this manner the mass spectra of both compounds forming the complex could be obtained due to the differential sublimation of the two components.

Results and discussion

The colours obtained from the aromatic ethers by the action of several different chromogenic reagents after chromatography on Silica Gel G using *n*-hexane–chloroform (3:2) as developing solvent are shown in Table I.

In general the aromatic ethers are not particularly reactive chemically and their detection on chromatograms presents certain problems. The double bond in the side-chain can be demonstrated by reagents used to detect unsaturation, however such reagents are relatively non-specific. The stable free-radical reagent DPPH falls into this category and all the compounds tested gave pale yellow spots on a violet background. The possible use of a number of electron-acceptor reagents for the detection of the aromatic ethers on developed chromatograms was reinvestigated; the electron-acceptors used included some of those previously reported by FISHBEIN³ in 1966. The two electron-acceptor reagents mentioned in Table I, *viz.* 9-dicyanomethylene-2,4,7-trinitrofluorene (CNTNF) and 1,3,5-trinitrobenzene (TNB), appeared to give the most satisfactory results and gave definite colours with the majority of the compounds tested, although in the case of TNB the colours given by the different aromatic ethers were not well differentiated. Gibbs' reagent (*i.e.* 2,6-dibromo-quinone chlorimide) gave good results with reasonably well differentiated colours. The chromogenic reagents v, vi, vii and viii gave quite strong distinguishable colours with the aromatic ethers, the chromotropic acid reagent being the best. However, they are all very corrosive reagents involving the use of concentrated sulphuric acid and heat and consequently cannot be used when the chromatography has been carried out on paper or plastic-backed thin layers. The antimony trichloride–antimony

pentachloride reagent described by STAHL AND JORK⁷ gave good results with most of the compounds tested. The possible use of the stabilized diazonium salt reagent (*p*-nitrobenzenediazonium fluoroborate, NBDF) was investigated, but it gave only relatively weak, relatively non-specific colours, with the aromatic ethers investigated.

The main advantage of the electron-acceptor reagents, *e.g.* reagents i and ii, is the non-destructive nature of their mode of action, in contrast to several of the other corrosive reagents used in this investigation. Differential sublimation of the separate components of these complexes occurs in the mass spectrometer and this enables a more positive identification of microgram quantities of the aromatic ether to be made. This has been demonstrated in the case of the myristicin-CNTNF complex and the spectra obtained are shown in Fig. 1. The mass spectrum obtained for myristicin directly from the addition complex on silica is essentially the same as that reported earlier for myristicin by WILLHALM *et al.*⁸.

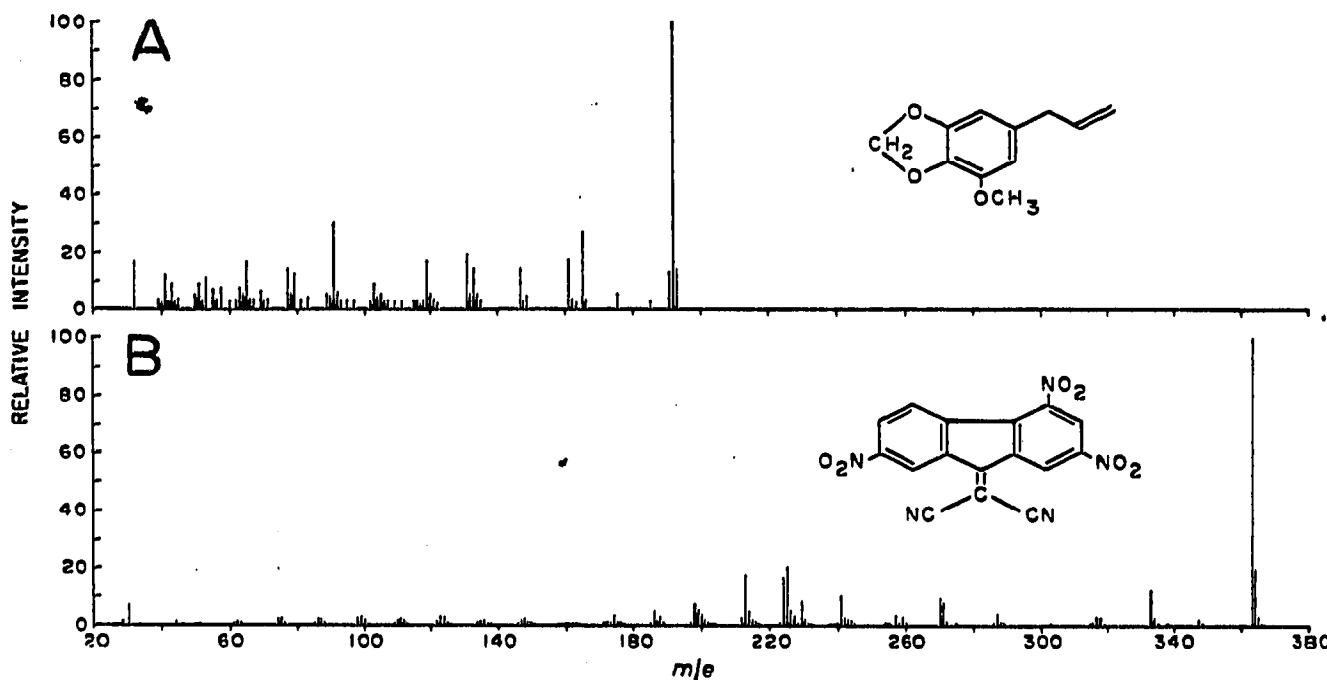


Fig. 1. The 70-volt mass spectra of the myristicin-CNTNF complex at different sample temperatures: (A) spectrum at 80° (myristicin) and (B) spectrum at 215° (9-dicyanomethylene-2,4,7-trinitrofluorene).

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