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Note

CHROM. 6334

The use of electron-acceptor reagents for the non-destructive detection of some phenothiazine derivatives on thin layers*

During the past quarter of a century phenothiazines have become increasingly important in medicine due to their antihistaminic and psychotherapeutic activity[1]. Numerous methods for the qualitative and quantitative analysis of these compounds, which have been described in the literature, have involved the use of colorimetry[2–4] and many of these methods have been adapted for use in thin-layer and paper chromatography.

The phenothiazines can be detected on developed thin-layer chromatograms by virtue of their absorption in UV light and by the use of numerous chromogenic reagents including the classical alkaloid reagents such as Dragendorff's reagent[5] and the iodoplatinate reagent[6,7]. Other reagents that have been used are those containing strong acids such as the FPN reagent[4,6,8,9] (ferric chloride, perchloric acid and nitric acid), aqueous or ethanolic sulfuric acid[10–12], a phosphomolybdic acid, ferric chloride hydrochloric acid mixture[13], potassium perchlorate in phosphoric acid[14], Folin–Ciocalteu[8], followed by 50 % sulfuric acid[15]. Further examples of the use of this type of chromogenic reagent for the detection of phenothiazines can be found in some review articles (*cf.* refs. 16–19). Other chromogenic reagents which have been used for the phenothiazines include bromine vapor[20,21] and a bromine–aniline[21] reagent.

Phenothiazines, being strong electron donors, have been known for some time to form highly colored charge-transfer complexes with electron-acceptor reagents[22–26]. MEUNIER has utilized the electron-acceptor *p*-benzoquinone for the detection of phenothiazines, on thin layers after chromatography, by using a chromogenic reagent system consisting of *p*-benzoquinone in the presence of hydrochloric acid gas[18,27]. The colors formed were attributed to the formation of cation radial ions[18,27]. The ability of phenothiazines to form charge-transfer complexes with iodine has also been made use of by GASCO AND BODRATO, who reported that such complexes run as distinct spots on silica layers using a solution of iodine in chloroform as solvent[28].

Recent work carried out in these laboratories on the utilization of electron-acceptor reagents for the chromatographic detection of indoles[29–31] has led to an evaluation being made of their possible use as chromogenic reagents for other compounds of pharmacological interest. This communication reports the results obtained by studying the interaction of nine different electron-acceptor reagents with thirteen common phenothiazine derivatives, several of which are widely used clinically.

Experimental

Phenothiazines. Chlorpromazine base, promethazine base, methotrimeprazine base, prochloroperazine base, methiomeprazine base and trimeprazine tartrate were

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gifts from Poulenc Ltd. Promazine hydrochloride was a gift from John Wyeth and Brother (Canada) Ltd. Triflupromazine hydrochloride (Vesprin) was a gift from E. R. Squibb and Sons Ltd., and Stelazine was a gift from Smith, Kline and French (Canada) Ltd. Phenothiazine base, 2-chlorophenothiazine base, 2-methoxyphenothiazine base and 2-trifluoromethylphenothiazine base were obtained from the Aldrich Chemical Co.

Where necessary the phenothiazine free base was obtained by decomposing the salt with 20% aqueous ammonia solution and extracting the liberated free base with ether.

Chromogenic reagents. The following reagents were used, all freshly prepared directly before use:

TCNE — A solution of tetracyanoethylene (1 g) in acetonitrile (100 ml).

TNF — A solution of 2,4,7-trinitro-9-fluorenone (1 g) in acetonitrile (100 ml).

CNTNF — A solution of 9-dicyanomethylene-2,4,7-trinitrofluorene (1 g) in acetonitrile (100 ml).

TetNF — A solution of 2,4,5,7-tetranitro-9-fluorenone (1 g) in acetonitrile (100 ml).

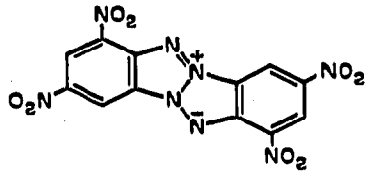
Chloranil — A solution of chloranil (1 g) in acetonitrile (100 ml).

TCNQ — A solution of 7,7,8,8-tetracyanoquinodimethane (1 g) in acetonitrile (100 ml).

TNB — A solution of 1,3,5-trinitrobenzene (1 g) in acetonitrile (100 ml).

HNS — A saturated solution of 2,2',4,4',6,6'-hexanitrostilbene (a gift from the Ministry of Aviation, U. K. Government) in acetonitrile.

TACOT — A solution of tetranitrodibenzo-1,3a,4,6a-tetraazapentalene (0.01 g) in acetonitrile (100 ml). The tetranitro compound was prepared by nitration of dibenzo-1,3a,4,6a-tetraazapentalene (a gift from E. I. du Pont de Nemours and Co. Ltd.) by the method of CARBONI *et al.*[32].



TACOT

Color development. The phenothiazines were applied in 0.1, 0.5, 1.0, 3.0, 5.0 and 10.0-μg quantities from benzene solution (10 mg free base in 10 ml benzene) to Eastman-Kodak "Chromagram" non-fluorescent silica gel (6061) sheets. The plates were sprayed with one of the various chromogenic reagents mentioned above. The initial color development and any subsequent changes in the color of the spots or any background color were noted. The detection limits were also determined.

Mass spectrometry. Mass spectra were determined using a DuPont/C.E.C. 21-110B instrument.

Results and discussion

The colors obtained from the phenothiazines on Silica Gel G by the action of several different electron-acceptor reagents are shown in Table I. Although the colors produced were not very well differentiated from compound to compound (*e.g.* the TNF reagent, with one exception, gave grey colors with all the phenothiazines

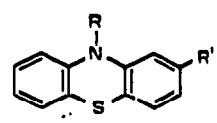
TABLE I

COLOR REACTIONS OF SOME PHENOTHIAZINES ON SILICA GEL PLATES

The colors reported are those observed by viewing the developed chromatograms (after spraying) in daylight. The initial color produced by the particular reagent is reported together with subsequent major color changes. Abbreviations for colors: Gr = green; Gy = grey; Y = yellow; Br = brown; P = pink; LGr = Lime green; l = light; d = dark; — = no color.

Phenothiazines	*Chromogenic reagents*								
	TCNE[a]	*TNF*	*CNTNF*[a]	*TetNF*	*Chloranil*[b]	*TCNQ*[a]	*TNB*	*HNS*	*TACOT*
I	dGr→Gy	Gr	Br	Gr	lGr→Gr	Br	Br→Gy	Gy	P
II	Gr→Gy	Gy	Gr	Gr	lGr	Br→Br edge	Br	Gy	P
III	dGr→Gy	Gr	Br	Gr	lBr	Gy→Gr	Gy	Gy	P
IV	Gr→Gy	Gy	Gy	Gy	lGy	lBr→lGy	Br	P	P
V	BrY→Y	lGy→Gy	lGy→Gy	lGy→Gy	—→lGy	—	Br	—	P
VI	BrY→Y	lGy→Gy	lGy	lGy→Gy	—→Gy	—	Br	P	P
VII	BrY→Y	Gy	Gy	Gy	—→lGy	—	Br	P	P
VIII	LGr→Br	Gy	Gy	Gy	Gy	Br	Br	P	P
IX	LGr→Br	Gy	Gy	Gy	—→Gy	Br	Br	P	P
X	LGr→Br	Gy	Gy	Gy	—→Gy	Br	Br	P	P
XI	LGr→Br	Gy	Gy	Gy	—→Gy	Br	Br	P	P
XII	LGr→Br	Gy	Gy	Gy	—→Gy	Br	—	P	P
XIII	LGr→Br	Gy	Br→Gy	Gy	—→Gy	Br	Br	P	P

[a] Yellow background.
[b] Pale yellow-green background.



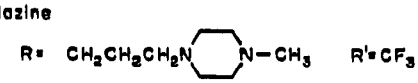
I Phenothiazine R = H R' = H

II 2—Chlorophenothiazine R = H R' = Cl

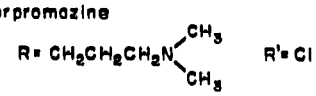
III 2—Methoxyphenothiazine R = H R' = CH_3O

IV 2—Trifluoromethylphenothiazine R = H R' = CF_3

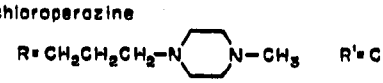
V Stelazine



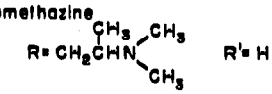
VI Chlorpromazine



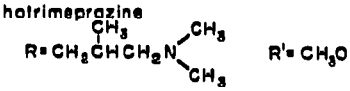
VII Prochloroperazine



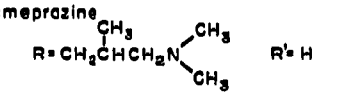
VIII Promethazine



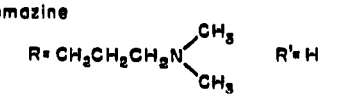
IX Methotrimeprazine



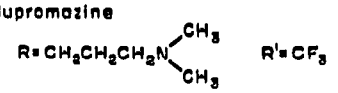
X Trimeprazine



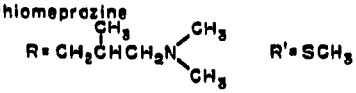
XI Promazine



XII Triflupromazine



XIII Methiomeprazine



studied and one reagent, chloranil, only gave weak colors which were slow to develop), the detection limits which were as low as 0.1 μg in some cases were very good and in the same order as some of the best of the more conventional reagents. The electron-acceptor reagents are useful for the detection of phenothiazines not only because of their sensitivity, but also because of their non-destructive action. The formation of electron-donor–acceptor complexes enables differential mass spectrometry to be carried out on the complex, the phenothiazines being more volatile than the complexing agents used. The mass spectra of the phenothiazine component can clearly be obtained (*cf.* the use of this type of reagent to detect certain aromatic ethers[33]). The crystalline electron-donor–acceptor complexes which form by the interaction of phenothiazine, its 2-methoxy derivative and CNTNF and those that form between TNF and 2-methoxyphenothiazine (or 2-chlorophenothiazine) were prepared and examined by mass spectrometry. In all cases differential sublimation of the complexes occurred and in each case the mass spectrum of the phenothiazine derivative was seen at lower temperatures (150–180°), whilst at higher temperatures (200–230°) the mass spectrum showed either the presence of ions due to both species of the complex, or those due to the electron-acceptor reagent alone.

In an attempt to find some even less volatile electron acceptors, two temperature-insensitive high explosives (*cf.* ref. 34), which are both polynitro compounds and which should be good electron acceptors (HNS and TACOT) were investigated. Both compounds in question were quite suitable as spray reagents; TACOT in particular was quite sensitive to the phenothiazines which appear as pink spots on a pale yellow highly fluorescent background. The relatively low solubilities of HNS and TACOT in non-polar solvents meant, however, that only relatively dilute solutions of these reagents could be used. This fact, together with evidence that some reaction occurred between the phenothiazines and the TACOT, indicated that further work will be needed to fully evaluate the utility of these two new potentially valuable electron-acceptor spray reagents.

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