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Note

The use of electron-acceptor reagents for the detection of some hallucinogens*,**

The widespread use (or misuse) of a number of chemical compounds known to modify mood, consciousness and perception broadly known as hallucinogens has, in recent years, created many medico-socio-legal problems. This has intensified the need for improved analytical procedures for the detection and identification of microquantities of these substances. Such procedures are important for the accurate and rapid identification of the chemical nature of illicit material seized by law enforcement agencies, or for clinical diagnosis in the case of psychiatric emergencies due to drug-induced toxic states.

Numerous methods have, in fact, been reported in the literature for the identification and analysis of the hallucinogens. Some of the methods widely used for the analysis of hallucinogenic substances have been reviewed recently by MORTON[1], BROWN *et al.*[2], KAISTHA[3], and SPERLING[4].

The majority of the methods that are widely used for the detection and identification of the hallucinogens generally involve an extraction step, followed by some form of chromatographic separation and identification. Many different types of chemical compounds have been reported to be hallucinogenic, and this has necessitated the use of numerous different chromatographic separation procedures and chromogenic reagents.

Electron-acceptor reagents have been extensively employed recently for the detection of "π-excessive" (or "electron-rich") compounds on thin-layer chromatograms. Such reagents have proved to be of considerable value for the detection of many types of organic molecules including indoles[5,6], phenols[7], aromatic amines[7], aromatic ethers[8], pesticides[9] and phenothiazines[10]. We now wish to report the use of a selection of electron-acceptor reagents as chromogenic reagents for the detection of some hallucinogenic drugs.

Experimental

Hallucinogens. Mescaline hydrochloride, 3,4-methylenedioxyamphetamine [β-(3,4-methylenedioxyphenyl)isopropylamine], desoxyephedrine (N,α-dimethylphenethylamine, *i.e.* "Speed"), α-methyltryptamine, and ibogaine hydrochloride were obtained from the Aldrich Chemical Co. Ltd. 5-Methoxy-N^{ω},N^{ω}-dimethyltryptamine, N^{ω},N^{ω}-dimethyltryptamine, 6-methoxyharmalan, N^{ω}-methyltryptamine, α-methyltryptamine and harmaline hydrochloride dihydrate were obtained from the Regis Chemical Co. N^{ω},N^{ω}-Diethyltryptamine was obtained from the California Corporation for Biochemical Research. D-Lysergic acid amide and D-isolysergic acid amide were gifts from Sandoz AG.

A sample of *trans*-2-(3,4,5-trimethoxyphenyl)cyclopropylamine hydrochloride (TMT) was a gift from Dr. P. D. COOPER, University of Montreal, Montreal, P.Q.

* Issued as NRCC No. 13114.

** The name hallucinogen used in this communication for this group of compounds is often used interchangeably with others such as: psychotomimetic, psychedelic, psychodysleptic and a variety of others.

D-Lysergic acid diethylamide bitartrate (LSD), 2,5-dimethoxy-4-methylamphetamine (STP), 5-methoxy-3,4-methylenedioxyamphetamine (MMDA), 2,5-dimethoxyamphetamine hydrochloride, and 2,3-dimethoxyamphetamine hydrochloride were gifts from the Department of National Health and Welfare, Ottawa. 3,4,5-Trimethoxyamphetamine was a gift from Dr. O. HUTZINGER.

Chromogenic reagents. The following reagents were 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); fluoranil — a solution of fluoranil (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 tetranitro-2,3:5,6-dibenzo-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.*[11].

Color development. The hallucinogens were applied in 0.1-, 0.5-, 1.0-, 3.0-, 5.0- and 10.0-μg quantities as the free bases* from solution in a suitable solvent (10 mg of free base in 10 ml of solvent) 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.

Preparation of N^{ω},N^{ω}-dimethyltryptamine–CNTNF complex for mass spectrometry. N^{ω},N^{ω}-Dimethyltryptamine (10 mg) was spotted on a Brinkmann cellulose (without fluorescent indicator) plate (thickness 0.10 mm) and chromatographed in the solvent system isopropanol–ammonia–water (8:15:5). The plate was sprayed with a solution of CNTNF and the area corresponding to the complex scraped off and introduced directly into the mass spectrometer.

Preparation of the N-tricyanovinyl derivative of mescaline (XX). Mescaline (500 mg) and tetracyanoethylene (303 mg) were dissolved in the minimum amount of ethyl acetate and heated under reflux for 2 h 30 min. On cooling the solution was applied to two Brinkmann PLC Silica Gel F_{254} (thickness 2 mm) plates and chromatographed in the solvent system chloroform–ethanol–ammonia (15:85:0.4). The main band (purple to ninhydrin) was removed and eluted with ethanol. On evaporation this solution produced a red-brown residue which on recrystallization from benzene gave brown-pink** crystals of N-tricyanovinylmescaline m.p. 147–148°. *Anal-*

* Where necessary the hallucinogen was obtained as the free base by decomposing the appropriate salt with 20% aqueous ammonia solution and extracting the liberated free base with ether. In the case of LSD bitartrate, the free base was liberated *in situ*, by carrying out a chromatographic run in a suitable basic solvent.

** The pink-brown color of this compound is of interest, since the compound would be expected to be colourless when pure. The colour is probably due to some form of intramolecular complex formation between the tricyanovinyl group and the π-electron system of the trimethoxyphenyl moiety of the molecule.

ysis. Calculated for $C_{16}H_{16}O_3N_4$: C, 61.53; H, 5.16; N, 17.94. Found: C, 61.40; H, 5.14; N, 17.96 %.

Results and discussion

The colors obtained from twenty hallucinogens on Silica Gel G by the action of nine different spray reagents are shown in Table I.

In the majority of cases studied the hallucinogens could be detected on silica gel layers by one or more of the reagents used and usually the detection limits were very good, in some instances being in the order of 0.1 μg. As would have been expected the polycyclic and consequently more "electron-rich" hallucinogens, such as the harmine derivatives VIII and IX gave stronger colors than the simple monocyclic β-phenylethylamine derivatives. Fluoranil was the only satisfactory reagent for the β-phenylethylamine derivatives, such as mescaline (I), 3,4,5-trimethoxyamphetamine (TMA, II), *trans*-2-(3,4,5-trimethoxyphenyl)cyclopropylamine (TMT, III), 3,4-methylenedioxyamphetamine (MDA, VII), N-methylamphetamine (*i.e.*, desoxyephedrine, "Speed", XI), 2,5-dimethoxy-4-methylamphetamine ("STP", "DOM", XII), 5-methoxy-3,4-methylenedioxyamphetamine (MMDA, XIII), 2,5-dimethoxyamphetamine (XIV), and 2,3-dimethoxyamphetamine (XV).

The tryptamine derivatives N^{ω},N^{ω}-dimethyltryptamine (DMT, V), N^{ω},N^{ω}-diethyltryptamine (DET, VI), 5-methoxy-N^{ω},N^{ω}-dimethyltryptamine (IV), N^{ω}-methyltryptamine (XIX) and α-methyltryptamine (XVII) reacted strongly with most of the reagents studied except HNS; the colors produced were relatively weak and slow to develop with chloranil. Fluoranil was the best reagent with detection limits in the 0.1-μg range.

The β-carboline derivatives 6-methoxyharmalan (VIII) and harmaline (IX) gave intense colors with all reagents studied and once again, with some reagents, the detection limits were $\sim$ 0.1 μg.

The alkaloids ibogaine (X) and the lysergic acid derivatives D-lysergic acid diethylamide (LSD, XVI), D-lysergic acid amide (LA, XVIIIa) and D-isolysergic acid amide (isoLA, XVIIIb) were easily detected with these reagents. LSD reacted immediately with all the spray reagents used except HNS, however, a weak pink color was observed with this reagent after 24 h.

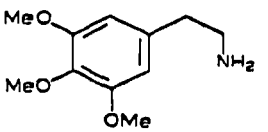
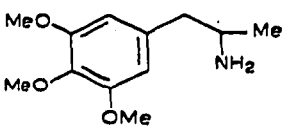
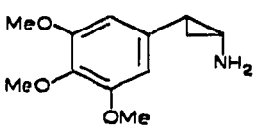
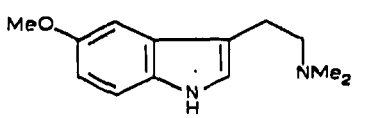
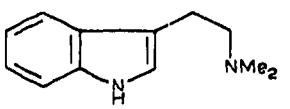
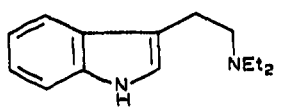
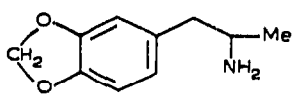
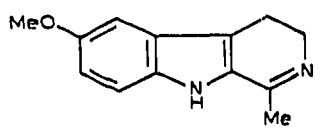
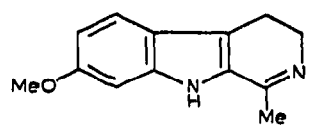
The electron-acceptor reagents are useful for the detection of the hallucinogens not only because of their sensitivity, but also because of their generally "non-destructive" action, *i.e.* a definite chemical reaction does not occur between the hallucinogen and the chromogenic reagent on the thin-layer plate. The formation of electron-donor–acceptor complexes enables differential mass spectrometry to be carried out on the complex, the hallucinogens being in general more volatile than the complexing agents used. The mass spectra of the hallucinogens (*i.e.*, the "donor") component can clearly be obtained (*cf.* the use of this type of reagent to detect several types of compound, *e.g.* certain aromatic ethers[8]). A good example is shown in Fig. 1. The colored product formed from N^{ω},N^{ω}-dimethyltryptamine (V) and CNTNF can be introduced directly into the mass spectrometer (if cellulose is used for the thin-layer chromatography (TLC) no prior elution of the colored spot is required as in the case of the use of silica layers). The mass spectrum at relatively low temperatures (*i.e.* 121°) is that of DMT (V) only, but at higher temperatures (198°) the mass spectrum is essentially only that of the complexing reagent CNTNF.

TABLE I

COLOR REACTIONS OF SOME HALLUCINOGENS WITH ELECTRON-ACCEPTOR SPRAY REAGENTS ON SILI GEL PLATES

The colors reported are those observed by viewing the developed chromatograms (after spraying) daylight. The initial color produced by the particular reagent is reported together with any subseque major color changes. Abbreviations: Bl = blue, Br = brown, Gr = green, Gy = grey, Li = lil O = orange, P = purple, Pk = pink, Y = yellow; (f) = fading, l = light.

Hallucinogens

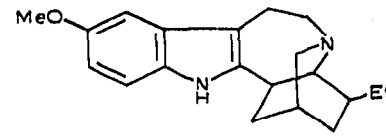
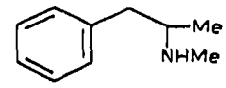
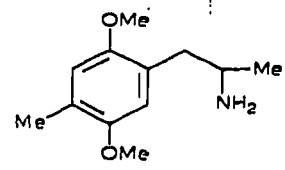
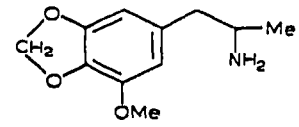
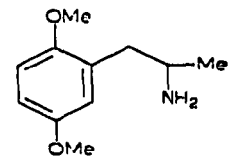
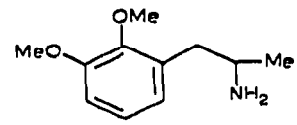
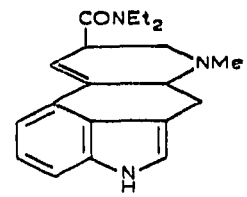
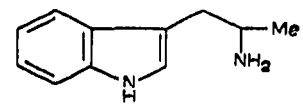
I	Mescaline	
II	3,4,5-Trimethoxyamphetamine (TMA)	
III	*trans*-2-(3,4,5-Trimethoxyphenyl)cyclopropylamine (TMT)	
IV	5-Methoxy-N^{ω},N^{ω}-dimethyltryptamine	
V	N^{ω},N^{ω}-Dimethyltryptamine (DMT)	
VI	N^{ω},N^{ω}-Diethyltryptamine (DET)	
VII	3,4-Methylenedioxyamphetamine (MDA)	
VIII	6-Methoxyharmalan	
IX	Harmaline	

Chromogenic reagents								
TCNE[a]	*TNF*	*CNTNF*[a]	*TetNF*	*Chloranil*[a]	*Fluoranil*[b]	*TNB*	*HNS*	*TACOT*[c]
—	—	—	—	—	P	—	—	—
—	—	—	—	—	P	—	—	—
—	lBr	Gy→Y	PkBr→Br	—	lBr→lP	lGy→Br	—	lPk
lGr (f)	lBr	Gy	lP	—	P	Y→Br	—	lPk
lBr (f)	lBr	lGy	lBr	—	P	Y→Br	—	lPk
lBr (f)	lBr	lGy	lBr	—	P	Y→Br	—	lPk
—	lBr	—	—	—	P	—	—	lPk
Gy	Br	Gy→Gr	Gy	Br	Y→GrBr	Br	Y	O→Br
Gy	Y→Gr	Gy	Gr	Br	YBr→Br	Br→YBr	Y	Y→Or→YBr

(*Continued on p. 246*)

TABLE I (*continued*)

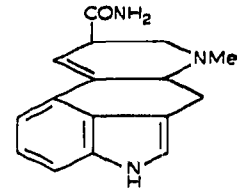
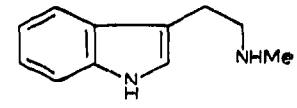
Hallucinogens

X	Ibogaine	
XI	N-Methylamphetamine (desoxyephedrine, "Speed")	
XII	2,5-Dimethoxy-4-methylamphetamine ("STP", "DOM")	
XIII	5-Methoxy-3,4-methylenedioxyamphetamine (MMDA)	
XIV	2,5-Dimethoxyamphetamine	
XV	2,3-Dimethoxyamphetamine	
XVI	Lysergic acid diethylamide (LSD)	
XVII	α-Methyltryptamine	

vomogenic reagents								
'NE[a]	*TNF*	*CNTNF*[a]	*TetNF*	*Chloranil*[a]	*Fluoranil*[b]	*TNB*	*HNS*	*TACOT*[c]
→Y	Br	Gy→P	GyBr→Br	—	—	Br→lBr	—	lP→--
	—	lGy→P	—	—	Bl→lGy	—	—	—
	—	lGy→P	—	—	P→—	—	—	—→Pk
	—	lGy→P	—	—	lP→—	—	—	—→Pk
	—	lGy→P	—	—	lP→—	Br→lBr	—→lBr	Pk→—
	—	lGy→P	—	—	lP→—	—	—	—
→—	Br	Gy→Br	lGy→Gy	lBr→Br	P→lBr	Br	—	Pk
	lBr	Gy	Br	—→Gy	P	lBr	—	OPk→lPk

(*Continued on p. 248*)

TABLE I (*continued*)

Hallucinogens		
XVIIIa,b [d]	Lysergic acid amide	
XIX	N^{ω}-Methyltryptamine	

[a] Yellow background.
[b] Light purple background.
[c] Background shows weak pink/yellow fluorescence in ultraviolet light.
[d] XVIIIa (D-lysergic acid amide) and XVIIIb (D-isolysergic acid amide) gave the same colors w the chromogenic reagents used. The formula in the table does not show the stereochemical differen at position 5 between these two amides.

It must be pointed out, however, that care must be taken in the choice of the electron-acceptor reagent used, especially when mass spectrometry of the complex is to be attempted. For example tetracyanoethylene (TCNE), which forms charge-transfer complexes with some compounds, has been found to react with others

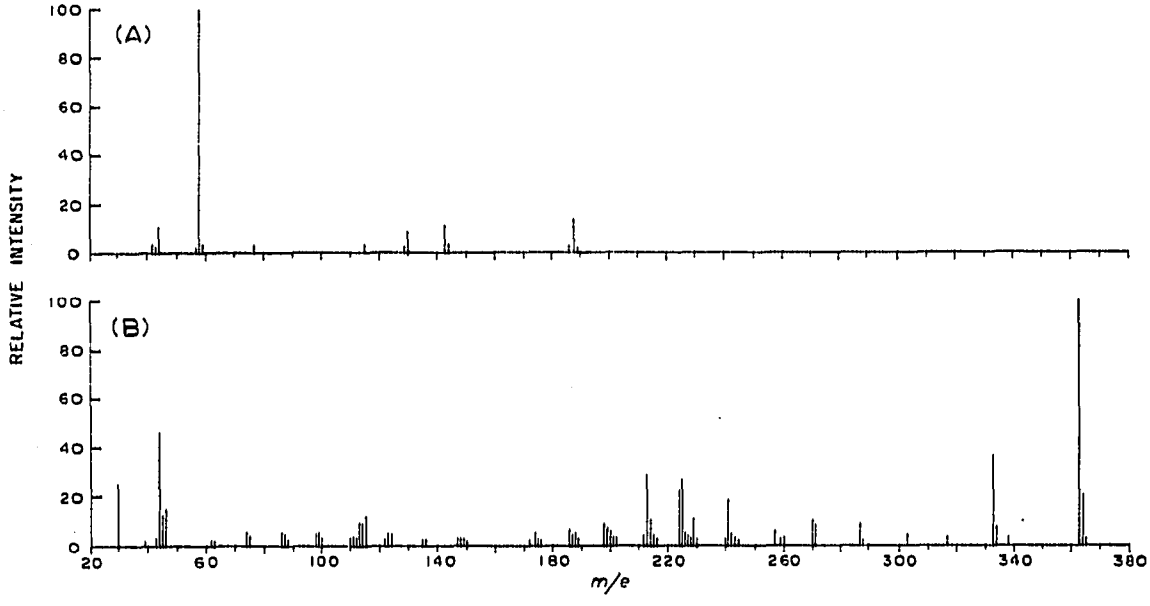


Fig. 1. The 70-V mass spectra of the N^{ω},N^{ω}-dimethyltryptamine-CNTNF complex at different sample temperatures: (A) spectrum at 121° (N^{ω},N^{ω}-dimethyltryptamine) and (B) spectrum at 198° (CNTNF).

vonogenic reagents								
'NE[a]	*TNF*	*CNTNF*[a]	*TetNF*	*Chloranil*[a]	*Fluoranil*[b]	*TNB*	*HNS*	*TACOT*[c]
	Gy	Gy→Br	Gy	—	Li	lBr	—	OPk→Pk
	Br	Gy	Br	lGy→Gy	Bl	Y→lY	—	OPk→Pk

particularly on the surface of TLC plates (*cf.* reactions with indoles, HEACOCK *et al.*[12]) to form tricyanovinyl derivatives. In the case of mescaline (I) it was found that a well defined product, N-tricyanovinylmescaline (XX), is formed, as shown by its mass spectrum in Fig. 2. This compound has also been prepared in solution and its structure determined unambiguously by chemical and physical means.

In an attempt to find some even less volatile electron acceptors, two temperature-insensitive high explosives (*cf.* ref. 13), which are both polynitro compounds and which should be good electron acceptors (HNS, *i.e.*, 2,2′,4,4′,6,6′-hexanitrostilbene, A, and TACOT, *i.e.*, tetranitro-2,3:5,6-dibenzo-1,3a,4,6a-tetraazapentalene, B) were investigated. Both compounds in question were quite suitable as spray reagents; TACOT in particular was quite sensitive to the hallucinogens, if somewhat nonspecific, which appear as pink spots on a pale yellow highly fluorescent background. The

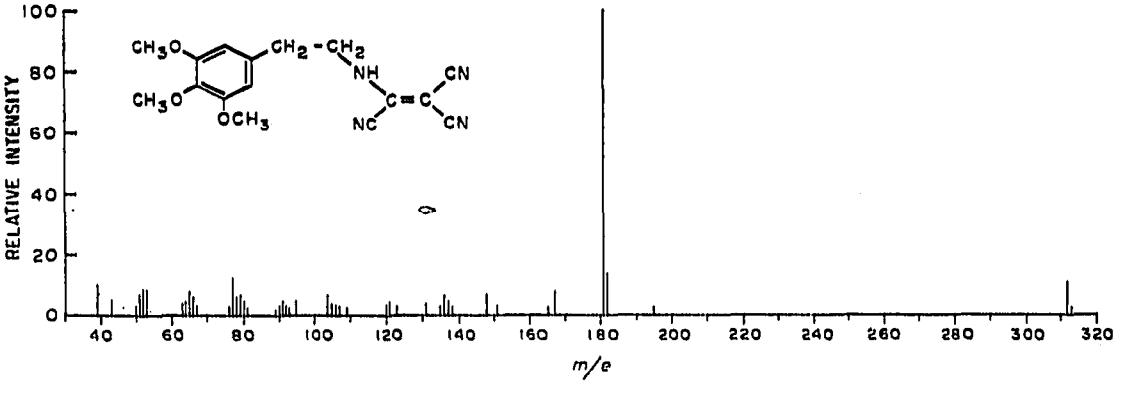


Fig. 2. The 70-V mass spectrum of N-tricyanovinylmescaline (XX) at 180°.

relatively low solubilities of A and B 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 some of the hallucinogens investigated and 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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