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Note

Selective detection of tertiary N-ethyl drugs on thin-layer chromatograms

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There are many reagents available for the detection of basic drugs in general on paper and thin-layer chromatograms[1–5]. However, reagents selective for specific groups of basic drugs are few and usually differentiate compounds with grossly different structural features. There are no reagents available for differentiating tertiary N-ethyl drugs from their N-methyl analogues. These compounds are common in pharmaceuticals and are frequently prescribed in the same dosage form (*e.g.*, cough mixture containing an N-ethyl antitussive with an N-methyl antihistamine or an ephedrine analogue). N-Methyl and N-ethyl compounds usually have close pK_a values and similar solubility characteristics[4,6], which often makes their clear separation in paper chromatography (PC) or thin-layer chromatography (TLC) impracticable. The identification of either type from their R_F values alone is not decisive.

The purpose of this investigation was to develop a selective method for the detection of N-ethyl drugs. This was achieved by utilizing chloranil as the detection reagent, which selectively oxidizes, then condenses with, the two-carbon chain of the tertiary N-ethyl moiety, yielding blue aminovinylquinone derivatives. N-Methyl and other N-alkyl analogues were found not to interfere.

EXPERIMENTAL

All drugs examined were of pharmaceutical grade (DAB 7), obtained as gifts from various manufacturers, and were utilized as working reference compounds without further treatment. Chloranil (Merck, Darmstadt, G.F.R.) "for synthesis" was crystallized twice from benzene (charcoal) and had a melting point of 289° (sublimation). All solvents used were of analytical-reagent grade.

For TLC, pre-coated (0.25 mm) silica gel G plates (Merck) without fluorescent indicator were used after heating for 10 min at 105°. Drugs were applied in chloroform solution (bases) or in 70% aqueous methanol (salts) at a concentration of 0.02 *M* (corresponding to 5 mg/ml for a drug with an average molecular weight of 250) (2–5 μl). The developing solvent was methanol–concentrated ammonia solution (100:1.5). The spray reagent was applied as a 0.2% solution of chloranil in acetonitrile followed by heating the plates at 105–110° for 2 min. The chromatograms were observed in daylight.

For PC, Whatman No. 1 paper (W.B. Whatman, London, Great Britain) was used. The solvent system was toluene–methanol–concentrated ammonia solution

(90:10:0.5) and the ascending technique was used with a distance of 10 cm (20–30 min). The chromatograms were sprayed with a 1% solution of chloranil in benzene followed by heating at 105–110° (5 min).

RESULTS AND DISCUSSION

The colours produced on spraying various drugs, chromatographed on paper or thin layers, with chloranil are given in Table I. With tertiary amine drugs with two flexible N-ethyl groups, blue colours against a pale yellow background were

TABLE I

COLOURS OF TLC AND PC SPOTS OF DRUGS WITH CHLORANIL SPRAY REAGENT

Me = Methyl group; Et = ethyl group; Bu = *n*-butyl group.

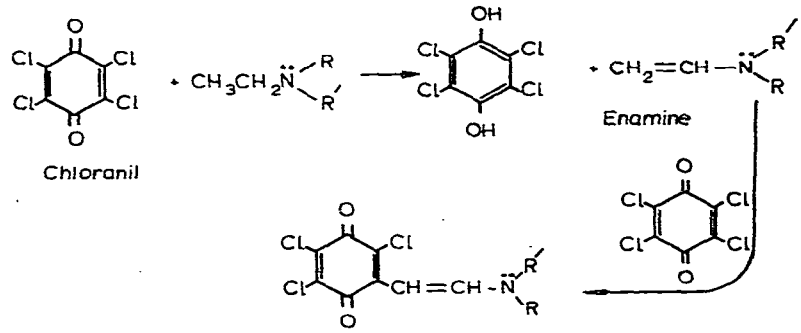
No.	*Drug*	$R_F \times 100$*	*Colour***	*Terminal N-alkyl moiety*
1	Ciclonium bromide	5	Brown-violet	$-NMeEt_2$
2	Etilefrine	28	Grey-brown	$-NHEt$
3	Chloroquine	31	Blue-green	$-NEt_2$
4	Camylofine	34	Brown	$-NH(CH_2)_2NEt_2$
5	Phenglutarimide	38	Blue	$-NEt_2$
6	Etafedrine	38	Grey	$-NMeEt$
7	Metoclopramide	40	Pale blue	$-NEt_2$
8	Ethylamphetamine	42	Grey-brown	$-NHEt$
9	Myrtecaine	43	Blue	$-NEt_2$
10	Carbochromen	44	Pale blue	$-NEt_2$
11	Oxeladine	45	Deep blue	$-NEt_2$
12	Carbetapentane	46	Blue	$-NEt_2$
13	Chloropyramine	48	Violet-brown	$-NMe_2$
14	Etamiphylline	49	Vivid blue	$-NEt_2$
15	Fenfluramine	50	Brown	$-NHEt$
16	Clofenciclan	51	Blue	$-NEt_2$
17	Hexahydroadiphenine	55	Blue	$-NEt_2$
18	Tetracaine	57	Grey-violet	$-NHBu$, $-NMe_2$
19	Fencamfamine	60	Grey-brown	$-NHEt$
20	Adiphenine	61	Blue	$-NEt_2$
21	Procaine	61	Blue-violet	$-NEt_2$
22	Fluorazepam	62	Faint blue	$-NEt_2$
23	Butethamate	63	Blue	$-NEt_2$
24	Levallorphan	63	Brown	$-NCH_2CH=CH_2$
25	Bietamiverine	65	Blue	$-NEt_2$
26	Dicycloverine	66	Blue	$-NEt_2$
27	Chlordiazepoxide	67	Brown	$-NHMe$
28	Tolycaine	70	Blue	$-NEt_2$
29	Lidocaine	71	Blue	$-NEt_2$
30	Amfepramone	71	Brown-violet	$-C(=O)CH(CH_3)NEt_2$
31	Propanidide	73	Brown	$-C(=O)NEt_2$
32	Diazepam	75	Brown	$-N(C=O)Me$
33	Prazepam	83	Brown	-N-cyclopropyl
34	Crotamitone	83	Grey	$-N(C=O)Et$

* R_F values in TLC (methanol–concentrated ammonia solution, 100:1.5).

** Limit of detection for N-ethyl drugs yielding a positive blue colour varied from 5 μg per 50 mm² for those drugs designated "deep blue" or "vivid blue" to about 50 μg per 50 mm² for drugs giving "pale" or "faint" colours.

obtained. Other drugs tested gave grey, brown or violet-brown colours distinct from the blue colour formed with the N-ethyl analogues.

Reactions between tertiary amines containing flexible N-ethyl groups and some halogenated quinones have been previously studies in the course of synthesis[7] and in the course of investigating molecular complexes[8]. The interaction can result in dehydrogenation to enamines, which condense with a second molecule of the haloquinone to yield blue dialkylaminovinylquinones:



It can be seen from the structures that a terminal two-carbon alkyl group is necessary for the formation of the blue quinone. This explains the selectivity of blue colour formation to N-ethyl drugs and the failure of N-methyl and other N-alkyl analogues to give this colour (Table I, compounds 13, 18, 24, 27, 32 and 33). Parallel to the finding in the course of synthesis[7], secondary N-ethyl compounds did not yield the blue quinone (compounds 2, 8, 15 and 19). However, these compounds possibly interact directly with chloranil by nucleophilic attack on one of the chlorine atoms, yielding aminoquinones, which are known[9,10] to have orange-red colours. This probably explains the brown colours shown by these compounds and their methyl secondary amine analogue (Table I, compound 27).

No blue colour was given by N-ethylamides or quaternized N-ethyl drugs (compounds 31, 34 and 1). Similarly, salts of tertiary diethylamino drugs, which gave blue colours after chromatography with alkaline developing solvents, afforded only a faint blue hue, or no colour at all, when sprayed on the baseline without chromatography. These findings suggest that the utilization of the lone pair of electrons on the nitrogen atom by amidation, quaternization or salt formation decreases the basicity required for the oxidation step with chloranil according to the suggested mechanism[7,8].

Some exceptions to the above findings were shown by the failure of compounds 6 and 30 to yield the blue colours although they have a tertiary N-ethyl moiety. The reason for the negative response of etafedrine may be related to the presence of only one ethyl (the other being methyl) group in this compound, and also to the possible steric hindrance imposed by the branched chain. The oxidation–condensation leading to the blue vinylquinone is reported to require a "flexible" N-ethyl grouping[7]. This steric factor is probably also the reason for the failure of formation a blue colour with amfepramone (compound 30), in addition to the presence of a carbonyl group in close proximity to the nitrogen atom in this compound, which would also decrease the basicity required for oxidation in a manner analogous to amidation.

With camylofine (compound 4) the brown colour formed suggests that condensation of the secondary amine function with chloranil took priority over the oxidation–condensation reaction of the NEt_2 group, as the compound possesses two functions.

In general, the blue colours were stable for at least 48 h. They may acquire a violet tinge on storage, particularly when the atmosphere of the laboratory contains ammonia vapour.

The colours were more vivid and more stable on paper chromatograms (distinct blue colours persisted for more than 3 months). However, as no single solvent system was convenient for separating all of the drugs tested, as is the case in TLC, paper chromatograms were developed with toluene–methanol–concentrated ammonia solution (90:10:0.5) for a short distance to allow the release of bases from salt combinations in order to obtain distinct colours. The separation of individual classes of the above large group of drugs is also feasible by PC and has been reported previously[4,5].

Silica gel G layers with a fluorescent indicator gave indistinct colours, especially when not activated before chromatography, and most basic drugs tended to give an additional violet tinge which could obscure the colour differentiation, particularly with heavy spraying.

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REFERENCES

1 G. E. Bainlescu and T. Constantinescu, *Anal. Chem.*, 47 (1975) 2156.
2 I. Sunshine, *Handbook of Analytical Toxicology*, Chemical Rubber Co., Cleveland, Ohio, 1969.
3 H. Kröger, *Beiträge zum toxikologischen Nachweis basicher Arzneistoffe, Ph.D. Dissertation*, Universität Münster, 1975.
4 E. G. C. Clarke (Editor), *Isolation and Identification of Drugs*, Pharmaceutical Press, London, 1969.
5 K. Macek (Editor), *Pharmaceutical Applications of Thin-Layer and Paper Chromatography*, Elsevier, Amsterdam, 1972.
6 M. Windholtz, *The Merck Index*, Merck, Sharp and Dohme, Rahway, N.J., U.S.A., 9th ed., 1976.
7 D. Buckley, S. Dunstan and H. Henbest, *J. Chem. Soc.*, (1957) 4880.
8 R. Foster, *Rec. Trav. Chim. Pays-Bas*, 83 (1964) 711.
9 K. Wallenfels and W. Draber, *Tetrahedron*, 20 (1964) 1889.
10 N. P. Buu-Hoi, R. Roger and B. Eckert, *Rec. Trav. Chim. Pays-Bas*, 71 (1952) 1059.