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SELECTIVE CHROMATOGRAPHIC DETECTION OF TERTIARY N-ETHYL DRUGS

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There are 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 to differentiate tertiary N-ethyl drugs from their N-methyl analogues. These compounds have common occurance among pharmaceuticals and are frequently prescribed in the same dosage form (e.g, cough mixture containing N-ethyl antitussive with 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]. This renders clear-cut separation in paper chromatography (PC) or thin layer chromatoraphy (TLC) not always feasible. Thus identification of either type through R_f-values alone is not decisive.

The purpose of this investigation was to develop a selective method of detection of N-ethyl drugs. This has been achieved by utilising chloranil as detecting reagent which selectively oxidises, then condenses with, the two-carbon chain of the tertiary N-ethyl moiety yielding blue amino vinylquinone 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. They were utilised as working reference compounds without further treatment . Chloranil (Merck, Darmstadt, west Germany)was crystallized twice from benzene (charcoal) and had a m.p. of 289° (subl.) All

solvents used were of the reagent analytical grade.

For TLC, precoated (0.25 mm) silica gel G plates (Merck) without fluorescent indicator were used with short prior activation (10 minutes at 105°). Drugs were applied in chloroform solution (bases) or in 70% aqueous methanol (salts) at a concentration of 0.02 M. The developing solvent was methanol -strong ammonia (100 : 1.5). The spray reagent was applied as 0.2% solution of chloranil in acetonitrile followed by heating the plates at 105-110° for two minutes. Observe in daylight.

For PC, Whatman No. 1 paper for chromatography (W.B. Whatman, London, England) was used. Solvent system was toluene-methanol-strong ammonia (90 : 10 : 0.5), ascending technique for 10 cm (20-30 minutes). Chloranil spray for PC was 1% solution in benzene followed by heating at 105-110° (5 minutes).

Results and Discussion

The colors produced on spraying various drugs, chromatographed on paper or thin layers, with chloranil are given in Table I With tertiary amine drugs having two flexible N-ethyl groups, blue colors were formed against a pale yellow background. Other drugs tested gave grey, brown or violet-brown colors.

Reactions between tertiary amines containing flexible N-ethyl grouping and some halogenated quinones have been previously studied in the course of synthesis[7] as well as 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 yielding blue dialkylaminovinylquinones:

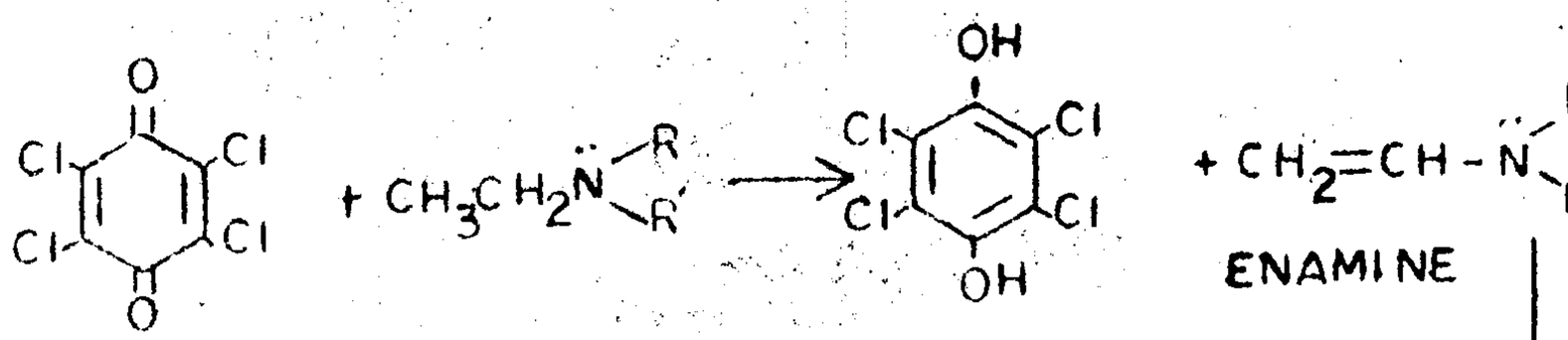
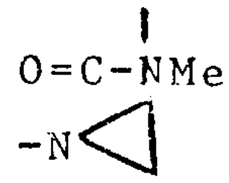
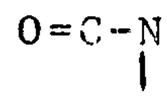
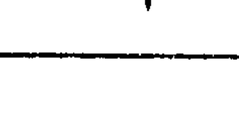


Table I : Colours of TLC and PC spots of drugs with chloranil

NO	*Drug*	R_f X 100 §	*Colour* §§	*Terminal N-alkyl moiety.*
1	Ciclonium bromide	05	brown-violet	$-\overset{+}{N}MeEt_2$
2	Etilefrine	28	grey brown	-NH-ET
3	Chloroquine	31	blue-green	$-NEt_2$
4	Camylofine	34	brown	$-NH(CH_2)-NEt_2$
5	Phenglutarimide	38	blue	$-NEt_2$
6	Etafedrine	38	grey	-NMeEt
7	Metoclopramide	40	pale blue	$-NEt_2$
8	Ethylamphetamine	42	grey-brown	-NH-Et
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	-NH Et
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	-NH-Et
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	Clordiazepoxide	67	brown	-NH-Me
28	Tolycaine	70	blue	$-NEt_2$
29	Lidocaine	71	blue	$-NEt_2$
30	Amfepramone	71	brown-violet	$O=C.CH(CH_3)NEt_2$
31	Propanidide	73	brown	$O=C-NEt_2$

Table I (Continued)

32	Diaxepam	75	brown	
33	Prazepam	83	brown	
34	Grotamitone	83	grey	

§ R_f-values on TLC (methanol-strong ammonia 100 : 1.5)

§§ Limit of detection for N-ethyl drugs yielding positive blue colour varied from 5 μg/50 mm^2 for those drugs designated "deep blue" or "vivid blue" to about 50 μg/50 mm^2 for drugs giving "pale" or "faint" colours .

Since a terminal two-carbon alkyl group is necessary for the formation of the blue quinone , the selectivity of this colour reaction to the N-ethyl drugs is evidently reflexed on the data given in Table I. Parallel to the finding in the course of synthesis[7], secondary N-ethyl compounds did not yield the blue quinone (Table I, compounds No. :2, 8, 15, 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 No. 27).

No blue colour was given by N-ethyl amides or quaternized N-ethyl drugs (Table I, compounds No.:31, 34 and 1). Similarly, salts of tertiary diethylamino drugs, which gave blue colours after chromatograchy with the alkaline developing solvents, afforded only faint blue hue, or no colours at all, when sprayed on the base line without chromatography. These findings suggest that the engagment of the nitrogen lone electron pair in amide bonding ,quaternization or salt formation decreases the basicity required for oxidation by chloranil according to the suggested mechanism[7,8].

Some exceptions to the above findings were shown by the failure of compounds No. 6 and 30 to yield the blue colours though having a tertiary N-ethyl mioety. The reason for the negative response of

etafedrine may be related to the presence of only one ethyl (the other being methyl) grouping in this compound, as well as 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 behind the failure of blue colour formation with amfepramone(compound No. 30, Table I), 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,.

With camylofine(compound No.4), the brown colour formed suggests, that condensation of the secondary amine function with chloranil took priority over the oxidation/condensation reaction of the $N\text{-}Et_2$, moiety.

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

Colours were also more vivid and more stable on paper chromatograms(distinct blue colours persisted for more than three months). However, no single solvent system was found convenient to separate all drugs tested . Hence, paper chromatograms were developed with toluene-methanol-strong ammonia (90:10:0.5) to a short distance to allow the release of bases from salt combinations.Separation of individual classes of the above large group of drugs is also feasible by PC[4,5] .

Silica gel G layers with fluorescent indicator afforded poor distinction of colours, specially when not acivated before chromatography. Most basic drugs tended to give an additional violet tinge which could obscure the colour differentiation, particularly with heavy spraying.

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طريقة كروماتوجرافية انتقائية لكشف على الادوية ثلاثية ايثيل النيتروجين

على محمود طه ـ محمود عاطف عبدالقادر
قسم الكيمياء الصيدلية ـ كلية الصيدلة ـ جامعة اسيوط

سجلت المراجع عدة كواشف للكشف على الادوية القاعدية بصفة عامة سواء على كروماتوجرافيا الورق او الطبقة الرقيقة.

وعلى أية حال فان كانت انتقائية هذه الكواشف لبعض المجموعات فى الادوية القاعدية تعتبر ضئيلة فانها من الممكن عادة ان تفرق بين المركبات ذات التراكيب المختلفة اختلافا جوهريا.

لم تشمل المراجع اى من الكواشف يمكنها التفرقة بين الادوية ثلاثية ايثيل النيتروجين وبين شبيهاتها من ميثيل النيتروجين حيث يوجد كلاهما فى المركبات الصيدلية ضمن الصياغة الصيدلية الواحدة ومثال ذلك فان المخاليط المضادة للسعال قد تحتوى على مركب ايثيل نيتروجينى مضاد للهستامين اومشابه للافدرين.

وحيث ان المركبات التى تحتوى على ايثيل اوميثيل النيتروجين تكون ذات قيم متقاربة من حيث ثابت التشتت كما ان خواصها الذوبانية تكون متشابهة فان امكانية فصل هذه المركبات عن بعضها سواء بواسطة كروماتوجرافيا الورق او الطبقة الرقيقة تكون دائما ضعيفة.