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ARTIFACTS PRODUCED BY USING DICHLOROMETHANE IN THE EXTRACTION AND STORAGE OF SOME ANTIHISTAMINIC DRUGS

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SUMMARY

The use of dichloromethane for the extraction of amines may lead to the formation of artifacts. Dichloromethane reacts rapidly (37.5°) with brompheniramine, diphenylpyraline, cyclizine, cyproheptadine but not with antazoline and lignocaine. This difference in reactivity as well as the thin-layer chromatographic, gas–liquid chromatographic, nuclear magnetic resonance, and mass spectrometric characteristics of cyclizine and diphenylpyraline chloromethochlorides are investigated.

INTRODUCTION

Halogenated hydrocarbon solvents are known to react with some alkaloids and amines during extraction procedures. Anhydrorosmar inecine, strychnine and brucine react with trace impurities of chlorobromomethane and dichloromethane in chloroform to form quaternary salts[1–4].

Phillipson and Bisset[5] demonstrated that with strychnine and brucine, not only can the quaternary chloromethochlorides be formed but also oxidation products, N-oxides and 16-hydroxy compounds. It has also been suggested that 7β-hydroxy-1-spiro (2′, 2′-dichlorocyclopropyl)-8β-pyrrolizidine was an artifact resulting from the storage of 7β-hydroxy-1-methylene-8β-pyrrolizidine[6].

Williams[7–10] reported the interaction of ephedrine with chloroform and its aldehydic impurities. Pethidine and dextromethorphan interaction products with dichloromethane have been identified by Vaughan[11] as the chloromethochloride salts.

We now report the interaction of dichloromethane with some antihistaminic drugs (lignocaine, a local anaesthetic, was also used) and the synthesis and characteristics of diphenylpyraline (II) and cyclizine (III) chloromethochlorides (VII, VIII) (see Fig. 1).

EXPERIMENTAL

Compounds and solvents

The compounds used in this study were kindly supplied by various firms:

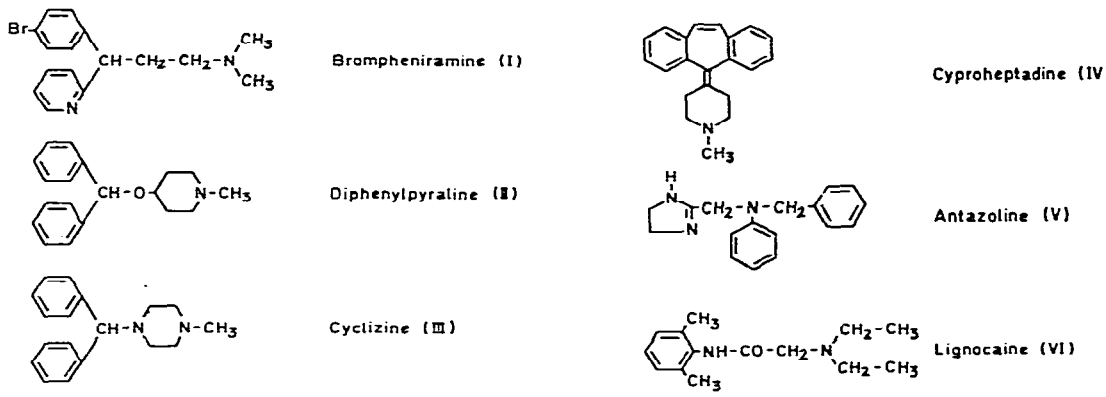


Fig. 1. Chemical structures of the compounds tested for interaction with dichloromethane.

brompheniramine maleate (A. H. Robins, Richmond, U.S.A.) diphenylpyraline hydrochloride (Smith Kline & French Labs., Philadelphia, Pa., U.S.A.), cyclizine hydrochloride (Burroughs Wellcome, N.C., U.S.A.), cyproheptadine hydrochloride (Laboratories Almirall-Barcelona, Spain) antazoline hydrochloride (Ciba, Basle, Switzerland), lignocaine base (Macfarlan Smith, Edinburgh, Great Britain). All the compounds were checked for purity by gasliquid chromatography (GLC). Dichloromethane was bought from May & Baker (Dagenham, Great Britain) and diethyl ether from Fisons (Cough borough, Great Britain). The solvents were distilled before use.

Physical measurements

Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ using a Perkin-Elmer R-10 NMR spectrometer and a Northern Scientific 544 CAT; TMS was the internal standard. Mass spectra were recorded using a Perkin-Elmer Model 270 gas chromatograph–mass spectrometer system at an ionization potential of 70 eV. Column B, a 1 m × 0.6 cm O.D. glass column [2% OV-17 on Chromosorb Q (100–120 mesh), oven temperature 200° and helium as the carrier gas 0.7 kg/cm²].
The direct-inlet technique was also used.

Gas–liquid chromatography

A Perkin-Elmer Model FII gas chromatograph equipped with a flame-ionization detector and a Hitachi/Perkin-Elmer Model 56 recorder. Column A and conditions: 1 m × 0.6 cm O.D. glass tubing, 1% OV-17 on Chromosorb G AW DMCS (80–100 mesh): oven temperature 165°; injection block temperature 250°; nitrogen, 120 ml/min; helium, 1.75 kg/cm²; air, 0.7 kg/cm².

Thin-layer chromatography (TLC)

Glass plates (20 × 20 cm) were spread to a thickness of 0.25 mm with a mixture of silica gel G (E. Merk, Darmstadt, G.F.R.) and water (1:2). The plates were first allowed to dry at room temperature and heated for 1 h at 110° before use. The solvent system used was: benzene–diethylamine–methanol (8:1:1). The various spots were visualized by Dragendorff's reagent.

Conductimetric titration was carried out using a CM25 conductivity meter. Diphenylpyraline chloromethochloride (VII, 51.9 mg) and cyclizine chloromethochloride (VIII, 62.1 mg) were separately dissolved in distilled water (50 ml) and titrated against silver nitrate (0.05 *M*) at 28.5°.

Cyclizine and diphenylpyraline chloromethochlorides (VIII and VII)

Cyclizine base (III, 2 g) was refluxed in dichloromethane (200 ml, dried over anhydrous sodium carbonate) for 24 h. The reaction mixture was evaporated to dryness under reduced pressure. The residue was washed with sodium-dry ether (5 × 50 ml) to remove the unreacted base and recrystallized from dichloromethane and ether to give a white hygroscopic powder. TLC of the methanolic solution gave only one spot. Found: C, 62.7; H, 7.0; N, 7.7; Cl, 19.1. Calculated for $C_{19}H_{24}N_2Cl_2$: C, 65.0; H, 6.8; N, 8.0; Cl, 20.2; mass spectrum (solid inlet) *m/e* 167 (100), 207 (70), 165 (56), 50 (33), 152 (28), 168 (28), 194 (28), 84 (27), 56 (25), 99 (25), 252 (24), 208 (23), 195 (20), 196 (17), 266 (17), 52 (14), 36 (13), 35 (4); NMR ($CDCl_3$) δppm 2.3 (S, 3, CH_3, base), 2.8 (S, 3, CH_3, hydrochloride salt), 3.5 (S, 3, CH_3, chloromethochloride), 6.1 (S, 2, N-CH_2-Cl only in the chloromethochloride); equivalent weight (conductimetric titration). Found: 354.8; calculated: 350.0.

Diphenylpyraline chloromethochloride (VII) was synthesized by the same procedure and gave only one spot on TLC. Found: C, 63.0; H, 6.7; N, 3.6; O, 9.0; Cl, 17.7. Calculated for $C_{20}H_{27}NO_2Cl_2$ (for one molecule of water): C, 62.7; H, 7.0; N, 3.6; O, 8.4; Cl, 18.3; mass spectrum (solid inlet) *m/e* 167 (100), 99 (62), 50 (54), 105 (37), 98 (33), 165 (31), 114 (27), 36 (27), 77 (25), 281 (25), 70 (21), 52 (19),168 (19), 152 (16), 184 (12), 35 (16); NMR ($CDCl_3$) δppm 2.2 (S, 3, CH_3, base), 2.7 (S, 3, CH_3, hydrochloride salt), 3.4–3.6 (S, 3, CH_3, chloromethochloride), 5.8–6.2 (S, 2, N-CH_2Cl) only in the chloromethochloride); equivalent weight (conductimetric titration). Found: 364.2; calculated: 365.4.

Interaction of amines with dichloromethane on storage

A 10-ml volume of an aqueous solution (1 mg/ml) of compounds (I, II, III, IV, VI, salts) was separately made alkaline with sodium hydroxide (20%, 0.5 ml). A 10-ml volume of an aqueous solution (1 mg/ml) of antazoline hydrochloride (V)

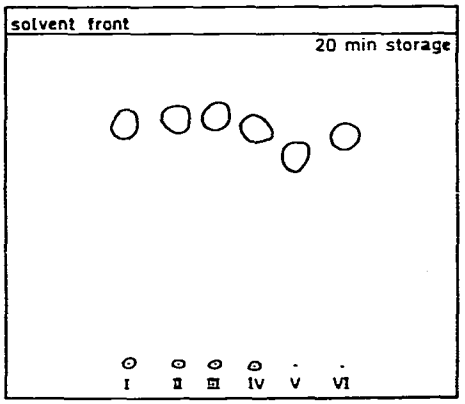


Fig. 2. Thin-layer chromatography of compounds I, II, III, IV, V and VI free bases in dichloromethane stored for 20 min. Temperature 37.5°.

was made alkaline (pH 9.0) with dilute ammonia solution to avoid its decomposition in strong alkaline solutions[12]. These alkaline aqueous solutions were extracted with freshly distilled diethyl ether (5 × 5 ml); the ether was removed by evaporation on a water bath (42°). The resultant free bases were then separately dissolved in dichloromethane (10 ml) in 25-ml conical flasks. The flasks were stoppered and placed in a shaking incubator (37.5°) for various time intervals (0, 5, 20, 35, 55, 120 min). The dichloromethane extracts were analysed by TLC (visualized by Dragendorff's reagent) (see Fig. 2).

RESULTS AND DISCUSSION

The products of the reaction of diphenylpyraline (II) and cyclizine (III) with dichloromethane were identified as their chloromethochlorides as follows.

(a) Elemental analysis of both (VII and VIII) confirmed the presence of the chlorine atoms. However, the difference between the found and the calculated values was most likely due to the fact that these quaternary salts were highly hygroscopic and thus liable to decomposition on handling.

(b) The equivalent weights of (VII and VIII) obtained were near to the theoretical values. Moreover, the fact that it was possible to titrate these chloromethochlorides against silver nitrate confirmed the presence of ionizable chlorine.

(c) TLC (Fig. 3) showed that (VII and VIII) were more polar than their respective bases since both chloromethochlorides stayed on the base line in contrast to the free bases in a comparatively non-polar solvent. The hydrochloride salts of diphenylpyraline (II) and cyclizine (III) ran similar to the free bases due to the alkalinity of the solvent system. However, the different behaviour of (VII and VIII) in the same solvent system indicated that these products are not simple salts.

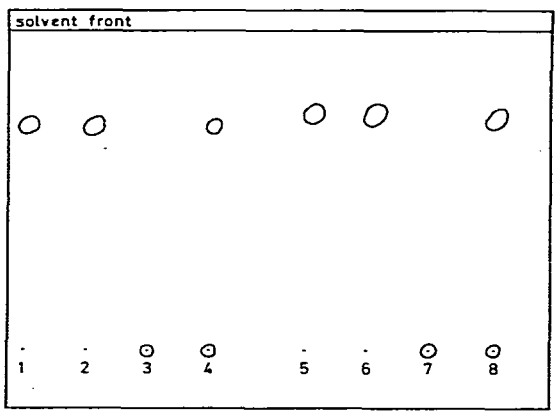


Fig. 3. Thin-layer chromatography of diphenylpyraline (II) and cyclizine (III) ran as: free bases, hydrochloride salts, chloromethochlorides and free bases concentrated in dichloromethane (visualized by Dragendorff's reagent). 1 = II free base in methanol; 2 = II hydrochloride in methanol; 3 = VII chloromethochloride in methanol; 4 = II free base concentrated in dichloromethane [5 ml (20 μg/ml in dichloromethane) were evaporated down (45°) to ca. 50 μl]; 5 = III free base in methanol; 6 = III hydrochloride in methanol; 7 = VIII chloromethochloride in methanol; 8 = III free base concentrated in dichloromethane [5 ml (20 μg/ml in dichloromethane) were evaporated down (45°) to ca. 50 μl].

(d) The mass spectrum of (VII and VIII) confirmed the presence of both organic and inorganic chlorine (*m/e* 50 for CH_3Cl^+ and *m/e* 36 for HCl^+. The presence of *m/e* 281 in VII, 252 and 266 in VIII corresponding to the dealkylated products was a result of a thermal degradation process. This pattern of fragmentation is known to occur in quaternary ammonium salts[13].

(e) The proton resonance of the N-methyl group has undergone a progressive paramagnetic downfield shift in going from the base, to the hydrochloride, to the chloromethochloride in both VII and VIII. This pattern is consistent with the expected inductive effect controlling the resonance of the N-methyl protons in the case of the hydrochloride and the chloromethochloride salts. Similar behaviour has been reported for pethidine chloromethochloride[11] and N-methyl cyproheptadine[14]. The signal at δ 6.1 in VIII and δ 5.8–6.2 in VII could well indicate the presence of two methylene protons adjacent to a quaternary nitrogen atom and a non-proton bearing electronegative atom.

TABLE I

GLC OF CYCLIZINE (III), NORCYCLIZINE (IX), CYCLIZINE CHLOROMETHOCHLORIDE (VIII), DIPHENYLPYRALINE (II) AND DIPHENYLPYRALINE CHLOROMETHOCHLORIDE (VII)

Compound	t_R *(min)*	
	Column A (165°)	*Column B (200°)*
Cyclizine (II)	4.0	5.6
Norcyclizine (IX)	5.2	10.4
Cyclizine chloromethochloride (VIII)	4.0(A), 5.2(B)	5.6(A), 10.4(B)
Diphenylpyraline (II)	6.0	9.2
Diphenylpyraline chloromethochloride (VII)	6.0(C), 8.0(D)	9.2(C), 22.4(D)

When either diphenylpyraline (VII) or cyclizine (VIII) chloromethochlorides were injected into the gas chromatograph two peaks were present (Table I). This was consistent with the expected thermal decomposition of quaternary ammonium compounds. Vaughan[11] has shown that methadone and dextromethorphan chloromethochlorides decompose on GLC columns to give methadone and dextromethorphan respectively. Strychnine and brucine chloromethochlorides were shown to follow a similar thermal decomposition pattern[5]. However, in the case of VII and VIII, not only the tertiary amines (diphenylpyraline and cyclizine) were produced upon thermal decomposition on the GLC column but also the secondary amines (column A: t_R 8.0 min, column B: t_R 22.4 min for nordiphenylpyraline; column A: t_R 5.2 min, column B: t_R 10.4 min for norcyclizine) (Fig. 4). In the case of cyclizine chloromethochloride (VIII) the GLC–(mass spectrometric (MS) characteristics (t_R and *m/e*) of its elimination products were identical to those of reference compounds (Fig. 4c and d; Fig. 5a and b). The elimination product (column A: t_R 6.0 min, column B: t_R 9.2 min) from diphenylpyraline chloromethochloride (VII) was identified as diphenylpyraline (II) by comparison to the reference compound (Fig. 4a and b; Fig. 6c). The second GLC peak (column A: t_R 8.0 min, column B: t_R 22.4 min) gave MS fragments characteristic of nordiphenylpyraline (Fig. 6d). The similarity of diphenylpyraline and cyclizine chloromethochlorides (VII and VIII) in their thermal decomposition pattern follows closely their related chemical structures.

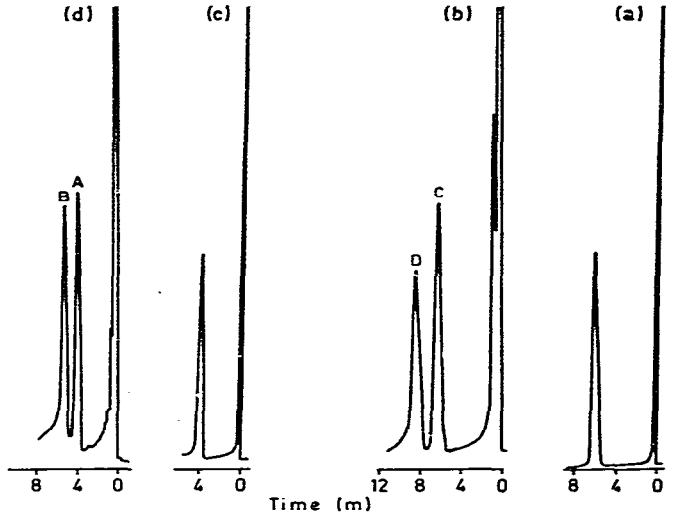


Fig. 4. Chromatograms of (a) authentic diphenylpyraline, (b) diphenylpyraline chloromethochloride, (c) authentic cyclizine, (d) cyclizine chloromethochloride. (GLC column A).

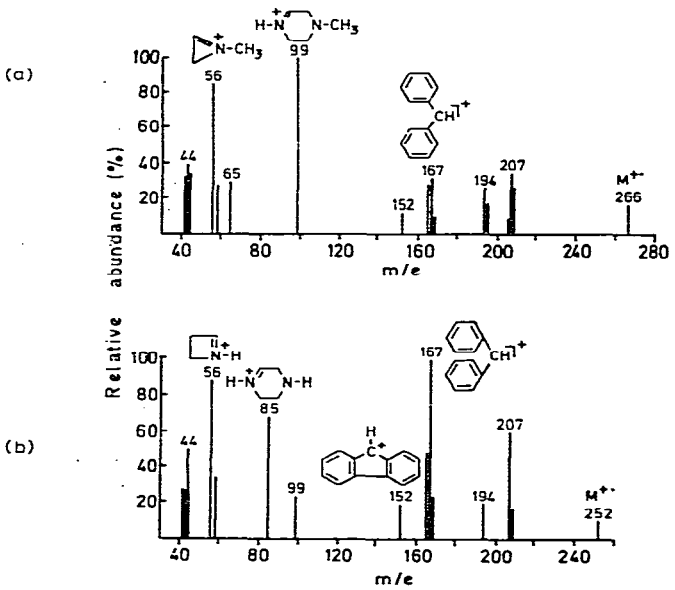


Fig. 5. GLC mass spectra of (a) the elimination product A(t_R, 4 and 5.6 min) and (b) the elimination product B(t_R, 5.2 and 10.4 min) obtained after injection of cyclizine chloromethochloride on GLC columns A and B, respectively.

The significance of studying such thermal decomposition products lies in the fact that if tertiary amines are allowed to react with dichloromethane and GLC is used for the analysis it may not be realized that quaternary artifacts are present.

Antazoline (V, pK_a 10.06) and lignocaine (VI, pK_a 7.85) did not react with dichloromethane (Table II) in contrast to brompheniramine (I, pK_a 9.16), diphenyl-

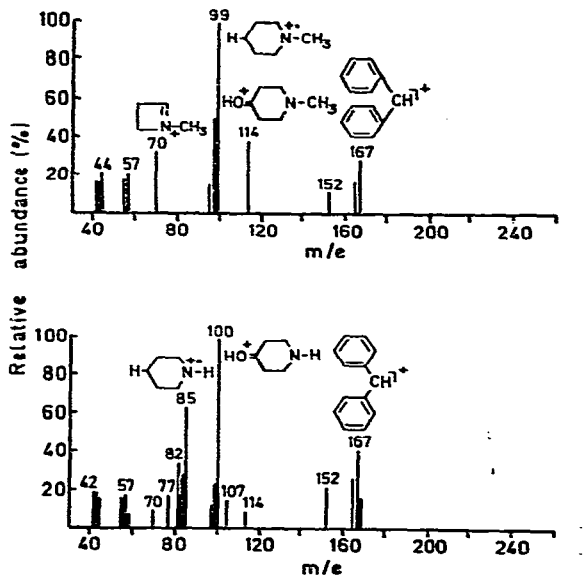


Fig. 6. GLC mass spectra of (a) the elimination product C(t_R, 6 and 9.2 min) and (b) the elimination product D(t_R, 8 and 22.4 min) obtained after the injection of diphenylpyraline chloromethochloride on GLC columns A and B, respectively.

TABLE II

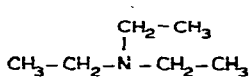
TLC OF COMPOUNDS I, II, III, IV, V and VI FREE BASES IN DICHLOROMETHANE STORED FOR VARIOUS TIME INTERVALS (37.5°)

+ = interaction; − = no interaction.

Compounds	*Storage time (min)*					
	0	*5*	*20*	*35*	*55*	*120*
Brompheniramine	−	+	+	+	+	+
Diphenylpyraline	−	+	+	+	+	+
Cyclizine	−	+	+	+	+	+
Cyproheptadine	−	+	+	+	+	+
Antazoline	−	−	−	−	−	−
Lignocaine	−	−	−	−	−	−

pyraline (II), cyclizine (III, pK_a 8.16) and cyproheptadine (IV). The non-reactivity of antazoline (V) and lignocaine (VI) is most likely due to steric factors as their tertiary nitrogen atoms are sterically hindered to a higher degree than in any of the other compounds studied. The effect of steric hindrance on the rate of quaternization of amines has been previously demonstrated for quinuclidine (X) and triethylamine (XI)[15]. Although both (X) and (XI) were comparatively similar in their nucleophilic character, quinuclidine (X) was found to be 57 times more reactive than triethylamine (XI) when both compounds (X and XI) were reacted with methyl iodide.

X



XI

The halogenated hydrocarbon solvents and in particular dichloromethane and chloroform are often used in the extraction of drugs from biological fluids. When dichloromethane was used to extract cyproheptadine and its metabolic products from rat and mice urine two very polar metabolites were reported but attempts to identify them were not successful[16]. In view of our results one of these metabolites might be an artifact resulting from the use of dichloromethane.

Such interactions will have an effect on the results of drug analysis and their interpretations specially when the compounds to be analysed are in small quantities as is often the case in metabolic studies. It is therefore important that thorough examination of organic solvents to be carried out before their use in such studies.

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