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INTERACTION OF CHLOROFORM WITH SOME AMINES DURING GAS–LIQUID CHROMATOGRAPHY ON AN ALKALINE CARBOWAX 20M SYSTEM

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SUMMARY

The use of chloroform as an extracting solvent complicates gas–liquid chromatographic analyses of mexiletine and N-benzylaniline when polyethylene glycol (Carbowax 20M) liquid phase coated with potassium hydroxide is used. Artifacts are produced in side reactions catalysed by polyethylene glycol. Two such artifacts have been identified using gas chromatography–mass spectrometry. The results are compared with those obtained when alternative stationary phases such as alkaline Apiezon L and neutral silicone OV-101 are used. Lignocaine does not undergo reaction with chloroform under the same conditions. Dichloromethane has been shown to be a good alternative solvent for gas–liquid chromatographic analyses of mexiletine and N-benzylaniline using an alkaline Carbowax 20M chromatographic system.

INTRODUCTION

Chlorinated hydrocarbons are commonly used in extraction procedures. Various artifacts however may occur with some amines and alkaloids when chloroform is used as an extracting agent[1–3].

The decomposition of chloroform under alkaline conditions leads to the formation of dichlorocarbene[4]:

$$CHCl_3 + B^- \leftrightarrows CCl_3^- + BH \quad (1)$$

$$CCl_3^- \rightarrow :CCl_2 + Cl^- \quad (2)$$

The process of decay of the trichloromethyl anion is the rate-limiting step but in the presence of an acceptor, the equilibrium may be shifted to the right. Without an acceptor, formation of dichlorocarbene is slow but can be accelerated by crown ethers[5], trialkylamines[6], quaternary ammonium salts[7] or tetraethyleneglycol dimethyl ether (Tetraglym)[8]. When primary amines are treated with chloroform under alkaline conditions, an isonitrile derivative is formed[9]:

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$$RNH_2 + :CCl_2 \rightarrow \left[\overset{+}{R}NH_2\overset{-}{C}Cl_2 \rightarrow RNHCHCl_2 \right] \rightarrow RNC \qquad (3)$$

Under similar conditions, secondary amines form di-substituted formamides[10,11] but tertiary amines yield less predictable products, frequently via ylides which may undergo further transformations[4,12]:

$$RRNH + :CCl_2 \rightarrow \left[\overset{+}{R}R\overset{-}{N}HCCl_2 \right] \rightarrow RRNCHCl_2 \overset{H_2O}{\rightarrow} RRNCHO \qquad (4)$$

$$RRRN + :CCl_2 \rightarrow \left[\overset{+}{R}R\overset{-}{R}NCCl_2 \right] \rightarrow \text{Products} \qquad (5)$$

where R = aryl or alkyl.

The present study was initiated by significant practical problems which arose in this laboratory during improvement of existing methods for gas-chromatographic analyses of the anti-arrhythmic drug, mexiletine, in human blood plasma[13] (Fig. 1).

Interactions of chloroform with mexiletine and N-benzylaniline during gas chromatographic analyses using a column packing coated with 5% Carbowax 20M–5% potassium hydroxide are described. The results are compared with those obtained after extraction with dichloromethane and the use of other column-packing materials.

EXPERIMENTAL

Gas–liquid chromatography

Analyses were performed using a Varian 3700 gas chromatograph fitted with a flame-ionization detector. Hydrogen and air flow-rates were 38 and 380 ml/min,

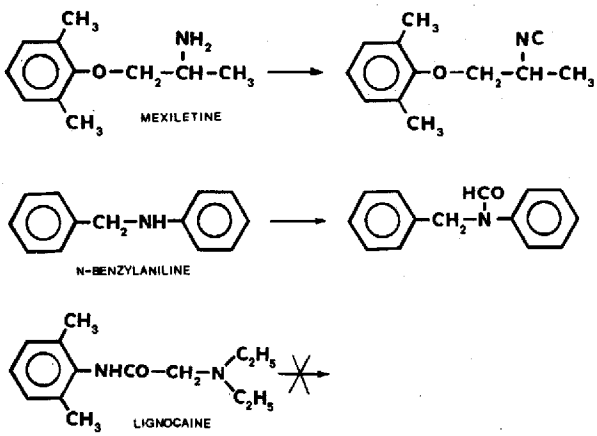

Fig. 1. Structural formulae of mexiletine, N-benzylaniline and lignocaine and artifacts observed during gas–liquid chromatographic analyses using alkaline Carbowax 20M packing and chloroform.

respectively, and the temperatures of the detector and injector port were 250 and 200°C, respectively.

The following column packing materials were used: 5% Carbowax 20M–5% potassium hydroxide on Chromosorb W HP, 100–200 mesh (Microchem, Australia) (CBW-20M); 10% Apiezon L–2% potassium hydroxide on Chromosorb W AW, 80–100 mesh (SGE Scientific, Australia) (APL); 5% OV-101 on Chromosorb W HP, 100–200 mesh (Microchem) (OV-101). All glass tubing (2 mm I.D.) was silanised before packing. Specifications of the column used, together with oven temperatures and nitrogen flow-rates are listed in Table I. To minimize any possible contamination of the column, the oven temperature was raised to 210°C at 10°C/min after each injection and was maintained at the final temperature for 10 min.

Gas chromatography–mass spectrometry (GC–MS)

The analyses were carried out on a Hewlett-Packard Model 5992 A gas chromatograph–mass spectrometer. Gas chromatographic separations were performed on a silanised glass column (2 m × 2 mm I.D.) packed with 5% Carbowax 20M–5% potassium hydroxide on Chromosorb W HP, 100–200 mesh (Microchem). Helium was used as carrier gas at a flow-rate of 20 ml/min. The injector port and jet separator were maintained at 260°C. The oven temperature was 180°C for studies with mexiletine and 200°C for N-benzylaniline analyses. Retention times (t_R) for mexiletine and an artifact were 2.1 and 4.2 min, respectively, and for N-benzylaniline and an artifact, t_R = 8.6 and 15.9 min, respectively. The mass spectrometer was set to scan from m/e 40 to 400. The ionization energy was 70 eV, and the electron-multiplier energy ranged from 2 to 3 kV.

Reagents

Mexiletine hydrochloride was obtained from Boehringer (Ingelheim, F.R.G.), N-benzylaniline from Light (U.K.) and lignocaine from Sigma (U.S.A.). Chloroform, dichloromethane and other chemicals were of analytical-reagent grade. Acridine (Hopkins & Williams, U.K.) and 2,4-dimethylquinoline (Aldrich, Gillingham, U.K.) were used as internal standards.

Extraction procedures

Stock solutions of all the amines investigated and the relevant internal standards were prepared in concentrated aqueous sodium chloride (5 *M*) containing 0.05

TABLE I

SPECIFICATIONS OF THE SIX CHROMATOGRAPHIC SYSTEMS

System	*Packing material*	*Column length (m)*	*Oven temp. (°C)*	*Nitrogen flow-rate (ml/min)*
A-1	CBW-20M	1	125	43
A-2	CBW-20M	1	185	36
B-1	OV-101	2	135	30
B-2	OV-101	2	180	22
C-1	APL	2	170	36
C-2	APL	2	210	30

M hydrochloric acid. The extractions were performed in duplicate using chloroform and dichloromethane, respectively.

Mexiletine. Mixtures of 0.1 ml of mexiletine stock solution (48 μg/ml), 0.1 ml of 2,4-dimethylquinoline internal standard stock solution (48 μg/ml) and 0.1 ml saturated phosphate buffer (pH 11.0) were extracted separately with 0.1 ml of chloroform and 0.1 ml of dichloromethane. The final aqueous pH was 9.8. The mixtures were vortex-mixed for 1 min and 1-μl aliquots of the organic layers were analysed on the A-1, B-1 and C-1 gas chromatographic systems (Table I). The retention times (t_R) for mexiletine were 3.7 min (A-1), 4.5 min (B-1) and 3.3 min (C-1), and for the internal standard were 7.2 min (A-1), 5.2 min (B-1) and 5.4 min (C-1).

N-Benzylaniline. Mixtures of 0.1 ml of N-benzylaniline stock solution (40 μg/ml), 0.1 ml of acridine internal standard solution (28 μg/ml) and 0.1 ml of saturated phosphate buffer were extracted separately with chloroform and dichloromethane as described above. The final aqueous pH was 10.1. Aliquots (1 μl) of the organic layers were analysed on the A-2, B-2 and C-2 chromatographic systems. The retention times for N-benzylaniline were 4.7 min (A-2), 3.7 min (B-2) and 5.2 min (C-2), and for acridine were 6.7 min (A-2), 5.2 min (B-2) and 9.2 min (C-2).

Lignocaine. Volumes (each 100 μl) of the lignocaine stock solution (54 μg/ml) were extracted with chloroform and dichloromethane using the same procedure as for N-benzylaniline. The final aqueous pH was 10.1. Samples (each 2 μl) of the organic layers were analysed on the A-2, B-2 and C-2 chromatographic systems. The retention times for lignocaine were 5.5 min (A-2), 7.3 min (B-2) and 7.5 min (C-2).

The efficiency of the extraction procedures was assessed by comparison of the extracts with aqueous standards. Recoveries for mexiletine, N-benzylaniline, lignocaine and the internal standards ranged from 94 to 98% in both chloroform and dichloromethane extracts.

Preparation of the isonitrile derivative of mexiletine

Mexiletine was converted into the corresponding isonitrile by the Hoffmann "Carbylamine Reaction"[9]. Chloroform (0.1 ml) containing 24 μg of mexiletine was mixed with 0.4 ml of heptane. The mixture was treated with 5 mg of powdered potassium hydroxide for 10 min at 50°C. A portion (2 μl) of the solution was analysed on the A-1 chromatographic system; the retention time for the isonitrile was 9.5 min.

Formylation of N-benzylaniline

N-Benzylaniline was formylated using a procedure similar to that described by Weygand and Hilgetag[10]. Chloroform (0.5 ml) containing 20 μg of the compound was warmed for 15 min at 50°C in the presence of powdered potassium hydroxide (5 mg). A 2-μl volume of the solution was chromatographed on the A-2 system; the retention time for the N-formyl derivative was 8.1 min.

RESULTS

Dichloromethane and chloroform extracts of the primary amine, mexiletine, the secondary amine, N-benzylaniline and the tertiary amine, lignocaine were chromatographed in six systems as described in Table I. Acridine and 2,4-dimethylquinoline were used as internal standards as heterocyclic amines have been reported to

TABLE II

CHROMATOGRAPHIC COMPARISON OF STOCK SOLUTIONS OF MEXILETINE (MEX), N-BENZYLANILINE (NBA) AND LIGNOCAINE (LIG) IN SIX CHROMATOGRAPHIC SYSTEMS

The columns represent peak height ratios of the amines to the appropriate internal standard (I.S.). The last column on the right of the table shows peak height ratios when pure chloroform (1 μl) was re-injected 30 sec after the original injection of chloroform solution of the relevant amine.

Amine	*Chromatographic system*	*Peak height ratio*		
		Amine/I.S. in dichloromethane	*Amine/I.S. in chloroform*	*Amine/I.S. in chloroform, one re-injection of chloroform*
MEX	A-1	1.14	0.60	0.40
MEX	B-1	1.02	1.01	
MEX	C-1	0.97	0.95	0.93
NBA	A-2	1.32	1.20	0.78
NBA	B-2	1.23	1.24	
NBA	C-2	1.51	1.53	1.51
LIG	A-2	0.98	1.00	0.97
LIG	B-2	0.65	0.66	
LIG	C-2	0.86	0.88	0.87

be stable in the presence of dichlorocarbene[4,12]. Solutions of these amines in chloroform were analysed on the A-1, A-2, C-1, C-2 and B-2 chromatographic systems. Chloroform solutions of acridine and 2,4-dimethylquinoline were also treated with powdered potassium hydroxide at 50°C for 1 h and then analysed on system B-2. No decomposition was evident.

Peak ratios of mexiletine, N-benzylaniline and lignocaine to internal standard in six chromatographic systems are summarised in Table II.

Mexiletine

Differences in mexiletine content were observed when dichloromethane and chloroform extracts of the drug were compared on the A-1 chromatographic system (Table II). When mexiletine dissolved in chloroform was analysed, a major new peak eluting after mexiletine and 2,4-dimethylquinoline (t_R = 9.5 min, Fig. 2b) appeared. The new peak was not present when dichloromethane extracts of the amine and the internal standard were chromatographed (Fig. 2a).

A more marked difference in the peak ratio of mexiletine and the internal standard occurred when an additional volume of chloroform (1 μl) was re-injected on the column 30 sec after the first injection (Fig. 2b).

To explain this phenomenon mexiletine was transformed into an isonitrile using the Hoffmann "Carbylamine Reaction"[9], and the reaction mixture was analysed without purification on the A-1 chromatographic system (Fig. 3b). The mexiletine peak, t_R = 3.7 min, disappeared almost completely and the new peak, t_R = 9.5 min, appeared. The new peak was identified as the relevant isonitrile using GC–MS (Fig. 4b).

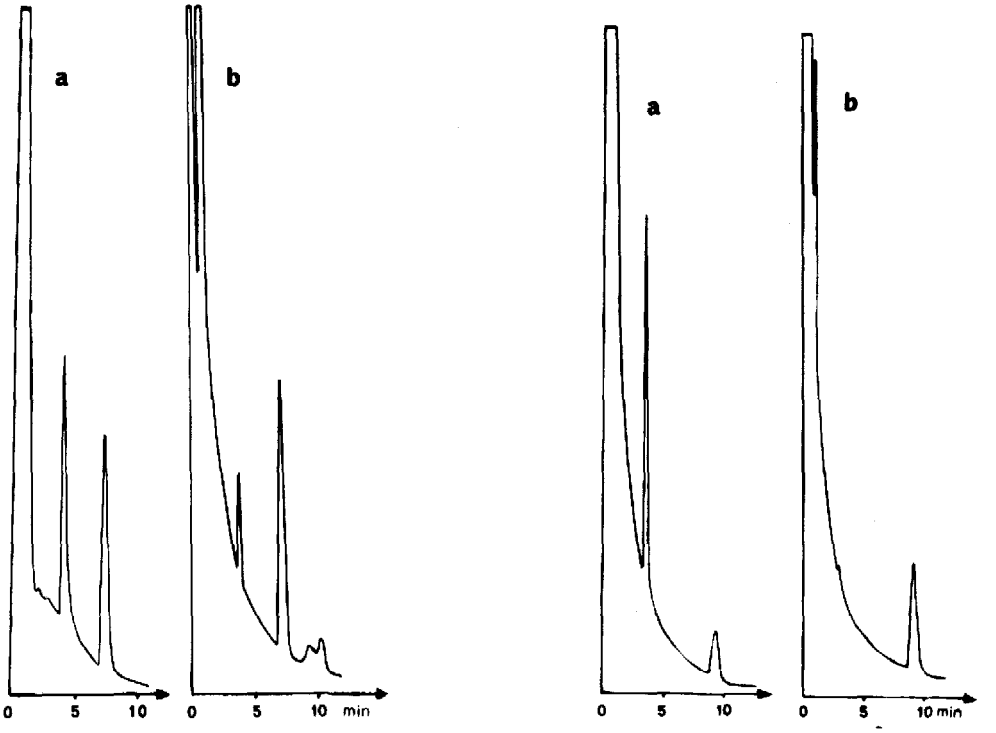

Fig. 2. Chromatograms of mexiletine and an artifact analysed on the A-1 chromatographic system. (a) Dichloromethane extract containing 48 μg/ml of mexiletine and 48 μg/ml of 2,4-dimethylquinoline as an internal standard. (b) Chloroform extract containing 48 μg/ml of mexiletine and 48 μg/ml of the internal standard. After 30 sec 1 μl of chloroform was re-injected. The double peak at t_R = 9.5 min represents the artifact. Retention times for mexiletine and the internal standards were 3.7 and 7.3 min, respectively.

Fig. 3. Chromatograms of mexiletine and its artifact analysed on the A-1 chromatographic system in chloroform solution. (a) Chloroform solution containing 90 μg/ml of mexiletine, t_R = 3.7 min. The peak at t_R = 9.5 min represents the artifact. (b) The reaction mixture of the independently synthesised isonitrile derivative. A 2-μl volume of the chloroform solution was injected onto the column. The retention time of the product was 9.5 min.

Mexiletine, t_R = 2.8 min (GC–MS system, Fig. 4a) showed the following mass spectrum: *m/e* 41 (13% abundance), 44 (100), 58 (55), 77 (4), 91 (3), 105 (3), 107 (2), 121 (4) and 179 (2, M^+). The artifact formed in chloroform solution of mexiletine, t_R = 4.1 min, (GC–MS system, Fig. 4b) showed *m/e* 41 (81% abundance), 42 (24), 51 (38), 57 (100), 65 (31), 69 (49), 77 (91), 91 (68), 92 (40), 105 (81), 107 (52), 120 (69), 121 (62), 122 (29), 145 (37), 157 (27) and 189 (28, M^+). The synthesised isonitrile, t_R = 4.2 min (GC–MS system, Fig. 4c) showed *m/e* 41 (86% abundance), 51 (36), 57 (93), 65 (30), 68 (49), 77 (100), 91 (73), 92 (36), 105 (89), 106 (58), 120 (70), 121 (33), 145 (30), 159 (32) and 189 (29, M^+).

The mass spectrum of the artifact formed from mexiletine during chromatography was essentially the same as that obtained using the independently synthesised isonitrile derivative (Fig. 4b and c). No changes were observed in mexiletine content when dichloromethane and chloroform solutions of the drug were analysed using the

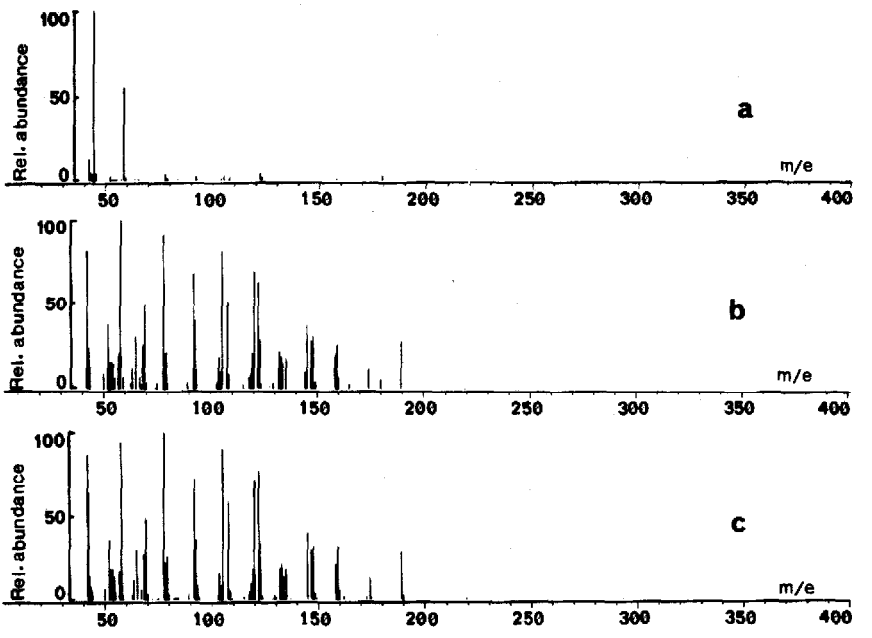

Fig. 4. Mass spectra obtained during analyses of mexiletine solutions using GC–MS (5% Carbowax 20M–5% potassium hydroxide): (a) mass spectrum of pure mexiletine dissolved in dichloromethane, t_R = 2.8 min (*cf.* Fig. 2a); (b) analysis of mexiletine dissolved in chloroform; the mass spectrum represents the artifact peak, t_R = 4.1 min (*cf.* Fig. 3a); (c) mass spectrum of the independently synthesised isonitrile, a derivative of mexiletine, t_R = 4.2 min (*cf.* Fig. 3b). Retention times relate to the GC–MS system.

B-1 neutral chromatographic system, and only a slight decrease of the drug content was observed when an alternative alkaline C-1 chromatographic system was used (Table II).

N-Benzylaniline

Interaction of N-benzylaniline with chloroform occurred when chromatographic system A-2 (Table II) was used but this was less marked than in the case of mexiletine. The amount of N-benzylaniline detected was further lowered when 1 μl of chloroform was re-injected 30 sec after the first injection of the amine in chloroform solution (Fig. 5a and b), and a new peak, t_R = 8.1 min, appeared. No interactions were observed using solutions of N-benzylaniline in dichloromethane.

In an independent experiment, N-benzylaniline was warmed in chloroform in the presence of powdered potassium hydroxide, and the reaction mixture was analysed using the A-2 chromatographic system (Fig. 5c). The mass spectra of the peak at t_R = 8.1 min on the A-2 system and the synthesised N-formyl derivative were identical.

N-Benzylaniline showed the following mass spectrum: *m/e* 44 (16% abundance), 51 (21), 65 (29), 77 (26), 91 (100), 106 (29), 182 (33) and 183 (64, M^+). The additional peak appearing in chloroform solution (Fig. 6b) showed *m/e* 44 (17% abundance), 51 (19), 65 (27), 77 (19), 91 (100), 182 (11) and 211 (49, M^+). The

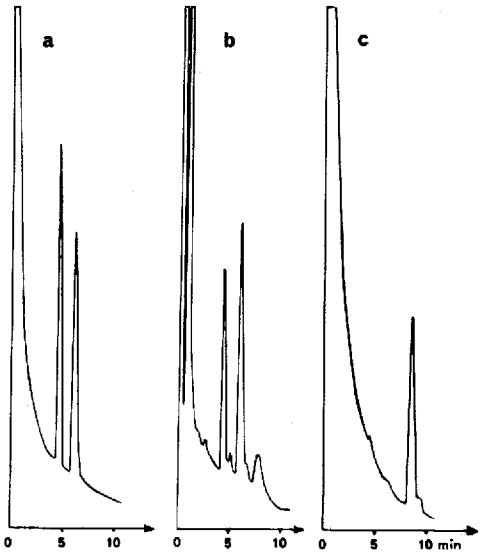

Fig. 5. Chromatograms of N-benzylaniline analysed on the A-2 system. (a) Dichloromethane extract containing 40 μg/ml of N-benzylaniline and 28 μg/ml of acridine as an internal standard. The retention times are 4.7 and 6.7 min respectively. (b) Chloroform extract containing 40 μg/ml of N-benzylaniline (t_R = 4.7 min), 28 μg/ml of acridine (t_R = 6.7 min). After 30 sec, 2 μl of chloroform was re-injected. An additional peak appears at t_R = 8.1 min. (c) Independently synthesised N,N-formylbenzylaniline, t_R = 8.1 min.

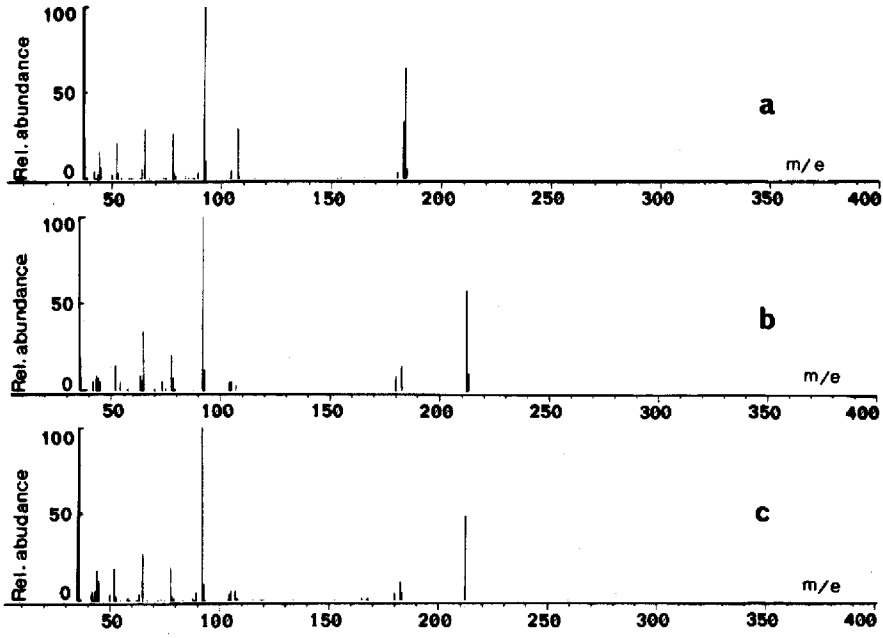

Fig. 6. Mass spectra obtained from analyses of N-benzylaniline solutions on 5% Carbowax 20M coated with 5% potassium hydroxide. (a) Pure N-benzylaniline analysed in dichloromethane solution. t_R = 8.6 min (*cf.* Fig. 5a). (b) The chloroform solution of N-benzylaniline. The spectrum results from an additional peak, t_R = 15.9 min (*cf.* Fig. 5b). (c) Mass spectrum of independently synthesised N,N-formylbenzylaniline, t_R = 15.9 min (*cf.* Fig. 5c). Retention times relate to the GC–MS system.

formylated N-benzylaniline (Fig. 6c) showed *m/e* 44 (26% abundance), 51 (17), 65 (28), 77 (19), 91 (100), 181 (11) and 211 (52, M^+).

Lignocaine

Analysis of dichloromethane and chloroform extracts of lignocaine using the A-2, B-2 and C-2 chromatographic systems (Table II) produced similar results. No interactions were observed.

DISCUSSION

The phenomena of the formation of artifacts from mexiletine and N-benzylaniline were observed only with chloroform extracts of the amines and using the Carbowax 20M coated with potassium hydroxide. It is surprising that both compounds apparently did not react with chloroform using the alkaline Apiezon L system. This could be compared to the situation where chloroform undergoes very slow degradation in aqueous alkaline conditions but, when a catalyst is added, the process is tremendously accelerated[12]. Although it is difficult to draw the parallels between the mechanisms occurring in liquid–liquid two-phase catalytic and gas–liquid chromatographic systems, the fact is that the products formed from mexiletine and N-benzylaniline during gas–liquid chromatographic analyses are identical with those obtained by an independent method. There are a number of reports concerning catalytic properties of polyethylene glycols in various reactions[14]. Their properties are comparable to those which quaternary ammonium salts and crown ethers exhibit. In a recent publication, Kimura and Regen[14] demonstrated a catalytic effect of polyethylene glycols on the dehydrohalogenation of (2-bromoethyl)benzene in a liquid–liquid two-phase alkaline system. These authors suggested a catalytic action "18-crown-like" for the polyethylene glycols used in the process. Robinson[8] used a solid–liquid two-phase system for the generation of dichlorocyclopropanes from chloroform and olefins in the presence of anhydrous sodium hydroxide and tetraethyleneglycol dimethyl ether.

In the light of these reports, the results presented in this paper strongly suggest that the liquid phase, polyethylene glycol, is responsible for the transformation of chloroform to dichlorocarbene and, consequently, for the formation of unexpected products during analyses of mexiletine and N-benzylaniline using the A-1 and A-2 gas chromatographic systems. There is a possibility that the catalytic effect of the liquid phase on the degradation of chloroform under the chromatographic conditions could be similar to that exhibited by crown ethers[7,14].

With the three amines investigated, mexiletine was the most vulnerable to the attack by dichlorocarbene. This is consistent with the results of previous studies concerning the reactivity of dichlorocarbene with ammonia derivatives and the stability of nitrogen ylides[4,6,11,12]. The process of formation of an isonitrile is believed to involve initial addition of electrophilic dichlorocarbene to the nitrogen atom forming an unstable ylide which undergoes proton transfer from nitrogen to carbon yielding an N-substituted aminodichloromethane. Further elimination of two molecules of hydrochloric acid in the presence of base yields the isonitrile (eqn. 3).

A similar mechanism is proposed for the transformation of secondary amines to N,N-disubstituted formamides (eqn. 4). The inertness of the tertiary amine, lig-

nocaine, under the chromatographic conditions described could be explained by more difficult migration of an alkyl group from the cationic nitrogen of the ylide to the anionic carbon, after initial addition of dichlorocarbene (eqn. 5). The facts that mexiletine is stable in dichloromethane on the A-1 chromatographic system and lignocaine is stable in chloroform solution on the A-2 system have been successfully applied in improving existing methods of measurement of these drugs in human plasma[13,15].

Understanding the nature of the interactions investigated in the presented work has valuable practical meaning for an analytical organic chemist. A number of factors, therefore, influence the process of formation of artifacts during gas–liquid chromatographic analyses of mexiletine and N-benzylaniline on the alkaline Carbowax 20M system. The most important are the presence of chloroform and the character of the amine. In view of these findings, chloroform should be excluded as an extracting solvent for analyses of primary and secondary amines in the above-mentioned gas–liquid chromatographic system.

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