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THE NATURE OF SOME SOLVENT-DERIVED ARTIFACT SPOTS OBSERVED DURING THE CHROMATOGRAPHY OF ADRENALINE*

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SUMMARY

Several spots are often detected on chromatograms obtained from solutions of a single pure β-(3,4-dihydroxyphenyl)ethanolamine derivative, such as adrenaline, particularly when the base is dissolved in strong acids prior to chromatography. The formation of some of these multiple spots can be explained by either the formation of complexes or salts, involving the catecholamine and the acids employed, or by the chemical modification of the catecholamine prior to chromatography. It has now been shown that substances responsible for certain artifact spots are formed by the interaction of one of the components of the chromatographic solvent system used with a reactive intermediate derived from the β-phenylethanolamine derivative being chromatographed. For example, adrenaline *n*-butyl ether is formed during the chromatography of adrenaline in *n*-butanol–hydrochloric acid solvent mixtures and β-(*p*-hydroxyphenyl)-β-(3′,4′-dihydroxyphenyl)ethylmethylamine is one of the artifact spots observed when adrenaline is chromatographed in phenol–hydrochloric acid systems.

INTRODUCTION

Several spots have often been detected on chromatograms of solutions of a single pure catecholamine, such as adrenaline (1). This phenomenon was first reported by CRAWFORD[1] in 1951 and a number of different explanations of this behaviour have subsequently been advanced. Some workers have suggested the formation of complexes or salts between the catecholamine and the acids employed, either in the extraction procedure or in the chromatographic solvent system used[2–7]. Other workers have proposed that distinct new chemical compounds are formed from the catecholamines by the action of acids prior to chromatography[1,8–12]. It has been suggested[11,12] that

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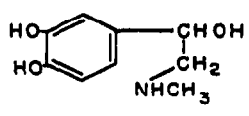
two of the new compounds detected when solutions of adrenaline in 10 *N* hydrochloric acid were chromatographed in a phenol–0.1 *N* hydrochloric acid system, were identical with the compounds described as "diadrenaline ether"[13,14] (2) and adnamine[15] (*i.e.*, 5-methylaminomethyl-2,3,7,8-tetrahydroxydibenzo[*a*,*e*]cycloheptatriene (3)). Both of these compounds can be obtained as crystalline solids by the action of hydrochloric acid on adrenaline[13–16]. However, it has recently been shown that the symmetrical ether structure proposed for the former is incorrect[17,18]. This compound is, in fact, 6-(3′,4′-dihydroxymethylaminomethylbenzyl)adrenaline[17,18] (4) and the trivial name adrepine was proposed for this product[17,18].

Other workers have investigated the action of strong mineral acids on β-phenylethanolamines in general and report the formation of different types of product including: β-phenylnaphthalene derivatives[19,20], phenylacetaldehyde and phenylacetone derivatives[21–23]. It is also known that in the presence of acids, aliphatic alcohols form ethers with the secondary alcohol function in the β-phenylethanolamines[13,14,24–27].

When solutions of adrenaline in 10 *N* hydrochloric acid, which have been stored for varying periods of time, are examined chromatographically, several spots are observed on the chromatograms obtained. The number (which varies up to a maximum of 7 or 8) and nature of the spots observed depend on several factors, including acid strength and time and temperature of storage of the solution. In cases where the same



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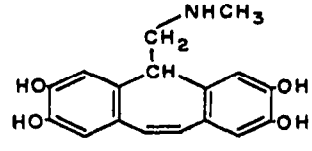

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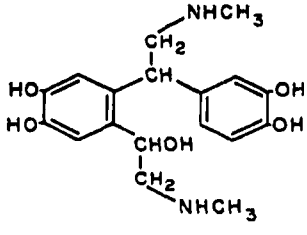

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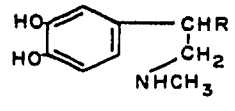
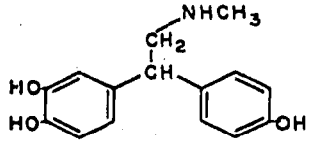


5 : R = H
8 : R = Cl



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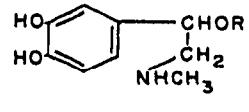

6 : R = CH_3
7 : R = $CH_2CH_2CH_2CH_3$
10 : R = C_6H_5

acid (*i.e.* hydrochloric acid) is used in the developing solvent it is unlikely that the new spots observed arise as a result of physical interactions of the type described previously by other workers[2–7].

In fact the new spots are due to chemical modification of the adrenaline molecule, and two of the substances which are eventually formed appear to be, as would be expected, adnamine and adrepine[11,28]. The nature of all the compounds formed when adrenaline is dissolved in 10 *N* hydrochloric acid and which may be at least partly responsible for the observed multispot phenomena is currently under investigation in these laboratories[28].

β-Phenylethylamine derivatives lacking a β-hydroxyl group have previously been shown not to produce spots analogous to adnamine and adrepine[9]. This has been confirmed by current investigations[28]. The most likely first step in the formation of adnamine and adrepine from adrenaline in the presence of strong mineral acids would be the elimination of the β-hydroxyl group with the formation of a highly reactive carbonium ion. This species would be expected to react readily with aliphatic hydroxyl groups to form the corresponding alkoxy derivatives and it is well known that reactions of this type do in fact occur[13,14,24–26]. In view of these facts it was decided to investigate the possibility that one or more of the extra spots observed when adrenaline was chromatographed using hydroxylic solvents, containing hydrochloric acid, were due to the formation of β-alkoxy or β-aryloxy derivatives. Two solvent systems that have been used extensively for the chromatography of adrenaline and related compounds are phenol containing 15 % v/v 0.1 *N* hydrochloric acid, and *n*-butanol saturated with 2 *N* hydrochloric acid. These systems have been used in this investigation.

EXPERIMENTAL

General

The melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The IR and UV spectra were recorded on Perkin-Elmer Model 237 and Beckman DK-2 recording spectrophotometers, respectively. The NMR spectra were obtained on a Varian A-60-A instrument using tetramethylsilane as an external reference. The mass spectral data were obtained on a Bell and Howell/C.E.C. Model 21-110 instrument.

Paper chromatography

Radial development on Whatman No. 1 paper discs (diameter 32.0 cm) was used throughout these investigations.

Adrenaline, epinine (5) and adrenaline methyl ether (6)[29] (100 μg) were chromatographed from aqueous solution (as their hydrochlorides) or from solution in 10 *N* hydrochloric acid (10 mg/ml). The developing solvents used were: S_1, phenol containing 15 % v/v 0.1 *N* hydrochloric acid, and S_2, *n*-butanol saturated with 2 *N* hydrochloric acid.

The spots were located by viewing the developed chromatograms in UV light, before and after exposure of the papers to ammonia fumes and by the use of the following chromogenic reagents: (a) 4-aminoantipyrine followed by potassium ferri-

cyanide[30], (b) potassium ferricyanide, followed by exposure to ammonia fumes, and (c) ninhydrin (the papers were heated after spraying).

In some experiments phenol (10 mg/ml) or *n*-butanol (0.4 ml/ml) was added to the aqueous and 10 *N* hydrochloric acid solutions of the amines prior to chromatography.

Adrepine hydrochloride[14,17,18], adnamine hydrochloride[16], adrenaline *n*-butyl ether (7) hydrochloride and β-(*p*-hydroxyphenyl)epinine hydrochloride (β-(4-hydroxyphenyl)-β-(3′,4′-dihydroxyphenyl)-N-methylethylamine (9)) hydrochloride (the last two compounds were prepared by the methods described below), were also chromatographed from solutions in distilled water and 10 *N* hydrochloric acid.

Solutions of methylaminomethyl-3,4-dihydroxyphenylchloromethane (8) hydrochloride[29] in 10 *N* hydrochloric acid and in the two chromatographic solvents mentioned above (*i.e.*, S_1 and S_2) were prepared and chromatographed in turn in these same two solvent systems.

Chemical

β-(p-Hydroxyphenyl)epinine hydrochloride (9)

A solution of L-adrenaline (10 g) and phenol (10 g) in 20% aqueous hydrochloric acid (200 ml) was maintained at 40° for 48 h. The reaction mixture was then cooled to 4° and allowed to stand overnight at this temperature. A crystalline precipitate (13.75 g) was obtained, which afforded β-(*p*-hydroxyphenyl)epinine hydrochloride monohydrate in colourless prisms (m.p. 131°) on recrystallisation from 70% aqueous alcohol.

Anal. Calcd. for $C_{15}H_{18}NO_3Cl \cdot H_2O$: C, 57.40; H, 6.42; N, 4.46; Cl, 11.29. Found: C, 57.85; H, 6.55; N, 4.61; Cl, 11.60%. Anhydrous β-(*p*-hydroxyphenyl)-epinine hydrochloride (m.p. 198°) was obtained on drying the monohydrate at 120° *in vacuo* for 1 h. $\lambda_{max}^{EtOH} = 278$ mμ; $\varepsilon_{max}^{EtOH} = 5200$. Anal. Calcd. for $C_{15}H_{18}NO_3Cl$: C, 60.90; H, 6.13. Found: C, 60.71; H, 6.16%. This compound has previously been obtained by KAPPE AND ARMSTRONG[31] from the interaction of β-(4-hydroxyphenyl)-N-methylethanolamine and catechol in 2 *N* hydrochloric acid at 100°. The m.p. reported[31] (126–128°) was presumably that of the monohydrate.

NMR. τ(D_2O): 2.62–3.24 (7H, m, aromatic, including 4H AA′BB′ centered at 3.20, $J_{AB} = 9.0$ Hz); 5.70 (1H, t, methine H, $J = 8.5$ Hz); 6.35 (2H, d, $-CH_2-$); 7.16 (3H, s, $>N-CH_3$).

Mass spectral data. M = 259; 100% peak 215; *m/e* (% relative height): 259 (34); 255 (10); 216 (31); 215 (100); 196 (10); 168 (11); 159 (19); 141 (12); 139 (18); 131 (11); 110(20); 102 (11); 92 (28); 91 (61); 89 (12); 77 (19); 76 (15); 64 (21); 63 (16); 58 (11); 55 (12); 52 (10); 51 (20); 45 (29); 44 (90).

Adrenaline n-butyl ether hydrochloride (7). A suspension of methylaminomethyl-3,4-dihydroxyphenylchloromethane hydrochloride (1.0 g, prepared by the action of pure thionyl chloride on adrenaline by the method of HUKKI AND SEPPÄLÄINEN[29]) in *n*-butanol (20 ml) was heated, under reflux, with stirring, at 85–95°. The clear solution which was obtained after 4–5 min heating was heated under reflux for a further 10 min. The reaction mixture was cooled and evaporated to dryness *in vacuo* (below 40°); a pale yellow oil was obtained which slowly formed a yellow solid (m.p. *ca.* 140°) on trituration with acetone. Adrenaline *n*-butyl ether hydrochloride was eventually obtained as a pale yellow crystalline solid (m.p. 140–142°) by repeated recrystal-

lisation from *n*-butanol–acetone mixtures. $\lambda_{max}^{EtOH} = 283$ mμ; $\varepsilon_{max}^{EtOH} = 4600$.

Anal. Calcd. for $C_{13}H_{22}NO_3Cl$: C, 56.60; H, 8.04; N, 5.09; Cl, 12.85. Found: C, 56.48; H, 7.96; N, 5.04; Cl, 12.89 %.

NMR. $\tau(D_2O)$: 2.79–2.90 (3H, m, aromatic); the $>CH-CH_2-N<$ protons occurred as an ABX system, with the signal due to the X proton centered at 5.17 (1H, dd, methine), the A proton of the AB at 6.30 (1H, d, $J = 6.0$ Hz) and the B proton at 6.5 (1H, d, $J = 7.5$ Hz); 6.45 (2H, dd, $-O-CH_2-$); 6.95 (3H, s, $>N-CH_3$); 8.00–9.00 (7H, m, $-CH_2CH_2CH_3$).

Mass spectral data. M = 239; 100 % peak 139; *m/e* (% relative height): 239 (12); 196 (26); 195 (62); 166 (11); 165 (10); 139 (100); 138 (11); 137 (18); 124 (12); 123 (10); 93 (11); 56 (14); 44 (92).

RESULTS AND DISCUSSION

The results of the chromatographic studies are summarised in Table I. The chromatography of adrenaline, epinine and adrenaline methyl ether hydrochlorides (100 μg) from solution in distilled water (10 mg/ml) resulted in the formation of dis-

TABLE I

R_F VALUES OF SOME AMINES AND THEIR SOLVENT-DERIVED ARTIFACTS

Amine[a]	*Added component (to amine solutions)*	*Developing solvent*[b]	R_F *values*[c] *from solutions*[a] *in distilled water*	R_F *values*[c,d] *of spots obtained from solutions in 10 N HCl*						
Adrenaline	—	S_1	0.49	0.00	0.56[e]	0.67[f]		0.80	0.91	
	phenol	S_1	0.49		0.59[e]	0.66[f]		0.80		
	n-butanol	S_1	0.50	0.00		0.66[f]		0.79		0.94
	—	S_2	0.31	0.00	0.19[e]	0.38[f]		0.83		
	phenol	S_2	0.32			0.38[f]	0.73			
	n-butanol	S_2	0.31	0.00		0.37[f]		0.85		
Epinine	—	S_1	0.65			0.81[f]				
	phenol	S_1	0.66			0.80[f]				
	n-butanol	S_1	0.65			0.80[f]				
	—	S_2	0.43			0.51[f]				
	phenol	S_2	0.44			0.52[f]				
	n-butanol	S_2	0.41			0.50[f]				
Adrenaline methyl ether	—	S_1	0.78	0.00	0.56[e]	0.68[f]		0.81	0.92	
	phenol	S_1	0.77		0.59[e]	0.68[f]		0.82		
	n-butanol	S_1	0.77	0.00		0.66[f]		0.79		0.94
	—	S_2	0.55	0.00	0.19[e]	0.38[f]		0.84		
	phenol	S_2	0.57			0.38[f]	0.73			
	n-butanol	S_2	0.55	0.00		0.36[f]		0.83		

[a] Solutions of the amine hydrochlorides in distilled water.
[b] S_1 = phenol containing 15% v/v 0.1 *N* HCl; S_2 = *n*-butanol saturated with 2 *N* HCl.
[c] Radial development.
[d] Single underlining = average intensity spot; no underlining = weak spot; broken underlining = intense spot.
[e] Adrepine.
[f] Parent amine.

crete spots in both solvent systems used in this investigation. The mean R_F values were 0.32, 0.43 and 0.55, respectively, in the *n*-butanol–hydrochloric acid solvent and 0.49, 0.65 and 0.78, respectively, in the phenol–hydrochloric acid solvent.

When chromatographed (100 μg) from solution in 10 *N* hydrochloric acid (10 mg/ml) epinine still only produced one spot, however, the mean observed R_F values were somewhat higher (0.51, *n*-butanol–hydrochloric acid; 0.80, phenol–hydrochloric acid) than those observed when the amine was chromatographed from aqueous solution. By contrast adrenaline and adrenaline methyl ether both produced several spots when chromatographed (100 μg) from solution in 10 *N* hydrochloric acid (10 mg/ml); it was impossible, however, to distinguish between the chromatographic behaviour of these two amines under such conditions and in each case the principal spot, due to adrenaline, had R_F values of 0.37 (*n*-butanol–hydrochloric acid) and 0.66 (phenol–hydrochloric acid) which were again higher than the R_F values for adrenaline obtained following chromatography of aqueous solutions. Three additional spots were obtained in the *n*-butanol–hydrochloric acid solvent system and four additional spots were observed with the phenol–hydrochloric acid solvent system. Retention of some products at the point of application (R_F 0.00) was common to both solvent systems. Colour reactions and comparisons with reference chromatograms allowed the spot having an R_F value of 0.19 in the *n*-butanol–hydrochloric acid system and 0.56 in the phenol–hydrochloric acid system to be identified as adrepine.

In the *n*-butanol–hydrochloric acid solvent system the unidentified spot (R_F 0.83) was probably a solvent-derived artifact. When *n*-butanol was added to the solution of adrenaline in hydrochloric acid prior to chromatography, the intensity of this spot was markedly increased. It therefore seemed most likely that the compound responsible for this spot was adrenaline *n*-butyl ether which has now been synthesised by an adaptation of the method used by HUKKI AND SEPPÄLÄINEN for the synthesis of other simple ethers of adrenaline[20]. The structure of this compound was confirmed by consideration of its mass and NMR spectra. The mass spectrum of adrenaline *n*-butyl ether showed the required molecular ion at *m/e* 239 as well as the expected fragment ions at *m/e* 195 [M — 44] and at *m/e* 139 [M — 44–56]. The NMR spectrum of adrenaline *n*-butyl ether showed the multiplet typical of an *n*-butyl ether grouping between τ 8.0 and τ 9.0 due to the $CH_3CH_2CH_2$– group and the triplet of the $-CH_2-O-$ group at τ 6.45 (J = 6.0 Hz). The other protons were observed at: (i) three aromatic protons as a multiplet between τ 2.79 and τ 2.90, (ii) the $>CH-CH_2-N<$ protons as an ABX system with the signal due to the X proton centred at τ 5.17 (J values: 6.0 and 7.5 Hz), the A proton at τ 6.30 (J = 6.0 Hz) and the B proton at τ 6.50 (J = 7.5 Hz), and (iii) the $>N-CH_3$ protons as a singlet at τ 6.95.

The chromatographic properties (*i.e.*, R_F values and colour reactions) of the synthetic adrenaline *n*-butyl ether were identical with those of the artifact spot with the same R_F value (0.83) observed when adrenaline solutions in 10 *N* hydrochloric acid are chromatographed in *n*-butanol–hydrochloric acid solvent systems. This assignment was verified by comparison of the IR spectra of a sample of the authentic material with a sample obtained by eluting the spot in question from a suitable chromatogram. In the phenol–hydrochloric acid solvent system the adrenaline *n*-butyl ether had an R_F value of 0.94, *i.e.* the same as that of the extra spot produced by addition of *n*-butanol to the adrenaline solution in hydrochloric acid prior to chromatography in the phenol–hydrochloric acid solvent system.

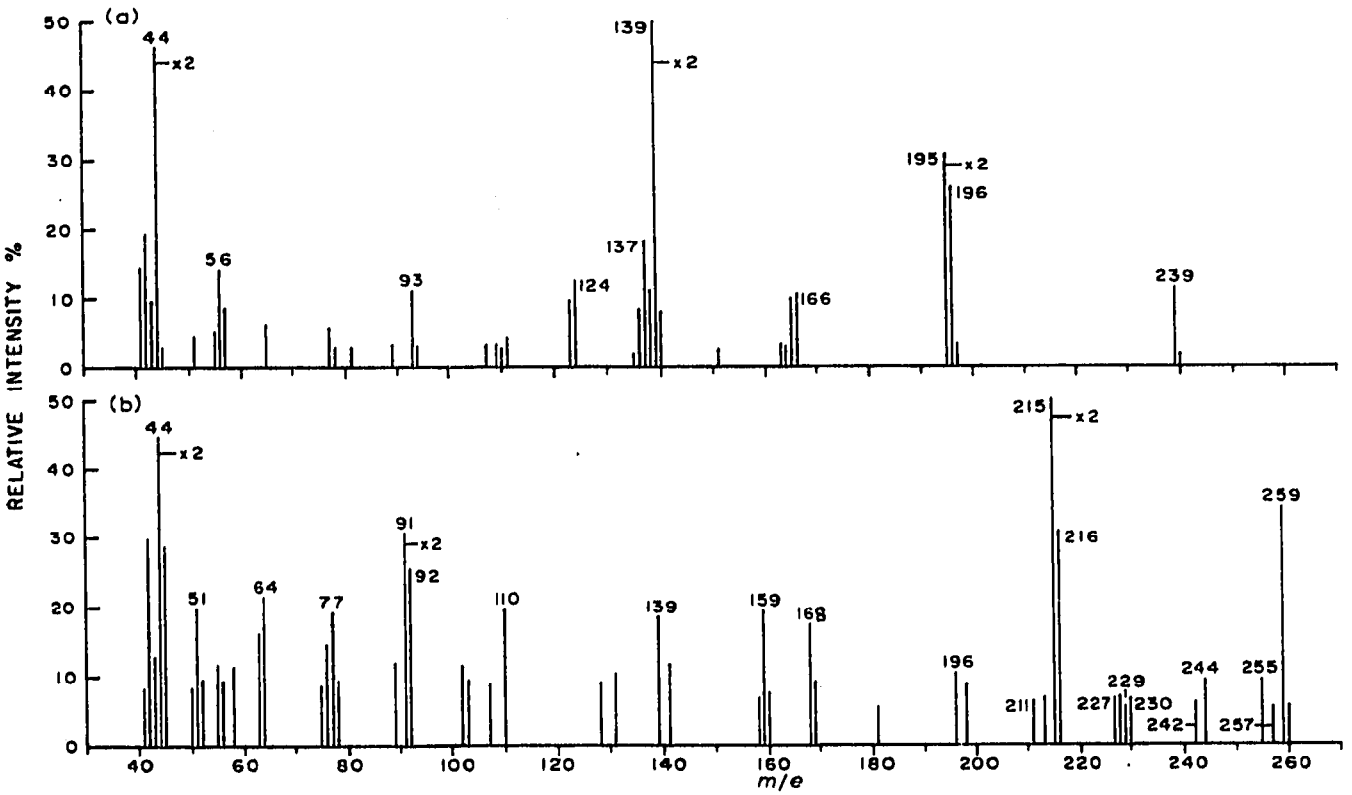

Fig. 1. Mass spectra of (a) adrenaline *n*-butyl ether (7) and (b) β-(*p*-hydroxyphenyl)epinine (9) hydrochlorides.

The two unidentified spots (R_F values *ca.* 0.80 and 0.90) observed on chromatograms of adrenaline or adrenaline methyl ether in the phenol–hydrochloric acid solvent system were possibly solvent-derived artifacts. Addition of phenol to the hydrochloric acid solution of adrenaline prior to chromatography resulted in the formation of an intense spot (R_F 0.80) as well as two weaker spots with R_F values of 0.59 (*i.e.* adrepine) and 0.66 (*i.e.* adrenaline). The spot at R_F 0.80 therefore appeared to be a compound derived from the reaction of adrenaline with phenol. In the *n*-butanol–hydrochloric acid solvent system an intense spot with R_F 0.73 and a weak spot with R_F 0.38 (adrenaline) were observed on chromatography of the adrenaline in hydrochloric acid solution which had been treated with phenol.

In some experiments involving the addition of phenol to the adrenaline–10 *N* hydrochloric acid systems a white crystalline product was eventually obtained after the reaction mixtures had stood at room temperature for several days. This product had the same R_F values in the two solvent systems as the artifact spot believed to be derived from the presence of phenol in the system. By employing higher concentrations of adrenaline and phenol and higher reaction temperatures the crystalline product could readily be obtained in good yield.

Microanalysis of the product obtained from phenol and adrenaline indicated that the compound had an empirical formula of $C_{15}H_{17}NO_3 \cdot HCl$, *i.e.* suggesting a 1:1 condensation had occurred between adrenaline and phenol with the loss of the elements of water. (The product was initially obtained as a monohydrate but the water of crystallisation could readily be removed *in vacuo*.) Whilst it was tempting

to assume that the product was a phenyl ether of adrenaline (10) the NMR spectrum of the compound (in D_2O) indicated that this could not be the case. The NMR spectrum showed the presence of: (i) seven aromatic protons in the region τ 2.5–3.0; the signals included those due to a definite AA'BB' system, centered at τ 2.75 (J = 9.0 Hz); (ii) a single >CH–CH_2– grouping occurring as an AB_2 system with the B_2 protons at τ 6.35 and the A proton at τ 5.72 with a coupling constant of about 8.5 Hz; and (iii) one N-methyl group as a singlet at τ 7.2.

The presence of signals due to seven aromatic protons, including an AA'BB' system in the NMR spectrum, indicates that the phenol nucleus had been substituted in the *para* position and suggests that this product is not the phenyl ether 10, but is, in fact, β-(4-hydroxyphenyl)-β-(3',4'-dihydroxyphenyl-*N*-methylethylamine (9) produced by the electrophilic attack of the carbonium ion, obtained from the adrenaline by the loss of the side-chain hydroxyl group in strongly acid media, at the *para* position of the phenol molecule.

The mass spectrum of compound 9 confirmed that it has a basic β,β-diphenylethylamine structure. The molecular ion is observed as a strong peak at *m/e* 259. The 100% peak is observed at *m/e* 215 (*i.e.* [M — 44]) and the corresponding peak at *m/e* 44 is also present in the spectrum indicating that, similarly to other compounds of this type (*cf.* refs. 18 and 32–34), the expected fragmentation β to the nitrogen atom had occurred.

Methylaminomethyl-3,4-dihydroxyphenylchloromethane hydrochloride was a possible intermediate in the formation of the solvent-derived artifacts from adrenaline, and can be readily obtained by the action of HCl on adrenaline. Solutions of this compound (prepared by the method of HUKKI AND SEPPÄLÄINEN[29]) in 10 *N* hydrochloric acid and in the *n*-butanol–hydrochloric acid and phenol–hydrochloric acid solvent systems, were chromatographed in these solvents. The results obtained from these experiments indicated that the solvent-derived artifact spot obtained in the phenol-containing solvent having an R_F value of *ca.* 0.75–0.80 and that obtained in the case of the *n*-butanol–hydrochloric acid solvent having an R_F value of *ca.* 0.85 were the same as those derived from adrenaline or adrenaline methyl ether.

In contrast to the behaviour of adrenaline, the methyl ether and the chloro compound which reacted with both phenol and *n*-butanol in hydrochloric acid, the chromatographic behaviour of acid solutions of epinine was unaffected by the addition of phenol or *n*-butanol. As well, the chromatographic behaviour of aqueous solutions of adrenaline, epinine and adrenaline methyl ether hydrochlorides was unaffected by the presence of phenol or *n*-butanol in the distilled water used to prepare the solutions. It would appear from the results presented above that the presence of *n*-butanol or phenol in acid solutions of the catecholamines which have a substituent, such as halogen or oxygen on the β-carbon, decidedly modifies their composition as observed when they are chromatographed.

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