RESEARCH PAPERS

CHARACTERISTICS OF CARBAMATE FORMATION BY ALKYLOXY-1-PHENYLETHYLAMINES IN RELATION TO DEVELOPMENT OF ANALGESIC ACTIVITY WITHIN THE SERIES

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STRONGLY basic primary amines absorb carbon dioxide to give bicarbonates and carbamates[1]. Addition of bicarbonate or carbon dioxide to solutions of primary arylalkylamines caused marked reduction in recoverable amine when the solution was basified and extracted with a solvent[2]. Apparently the retained base was held in solution as the carbamate ion. Previously it had been observed that the amount of carbon dioxide absorbed by 1-(*p-cyclo*hexyloxyphenyl)ethylamine base, unlike the corresponding *iso*propyl ether, was appreciably less than the theoretical requirement for formation of a bicarbonate or carbamate. This implied the formation of a distinct molecular species by the *cyclo*hexyl ether. These two ethers had distinct pharmacological properties[3] not accounted for by differences in their physical properties, e.g., they had almost identical pKa numbers, 8·67 and 8·75 at 37° C., and a similar gross distribution pattern in rat tissues[4]. They seemed to be metabolised to the same extent. Since carbon dioxide and bicarbonate are present in biological fluids, the properties of the carbamates formed by these two bases in solution have been investigated in detail while seeking differences between them sufficient to explain the pharmacological distinction.

EXPERIMENTAL

Carbamate formation by pure bases. Freshly distilled bases, from pure hydrochlorides, solidified and attained a constant weight increase after several days exposure to carbon dioxide in closed vessels. The amines chosen were non-volatile at room temperature and pressure. The products regenerated the bases when distilled under reduced pressure. Increases in weight were expressed as the number of moles of base absorbing one mole of carbon dioxide or its equivalent (bicarbonate formation requires absorption of water). Figure 1 shows that three types of amine were distinguishable by their weight increases within the series; those that combined in ratios greater than, equal to, or less than two moles of amine to one mole equivalent of carbon dioxide. This criterion also roughly classified them into those that had marked, doubtful and no analgesic activity respectively as assessed by the method of D'Amour and Smith[5] in rats. The ratio is less than two when bicarbonate is produced, and is equal to two for the substituted ammonium carbamate. A ratio greater

than two was explicable only by postulating partial enolisation of the carbamate to give a proportion of the species $RN = C(ORNH_3)_2$.

Attempts to analyse the products for total and non-carbamate amine were not wholly successful due to decomposition in aqueous-ethanolic solution. The product from 1-(*p-cyclo*hexyloxyphenyl)ethylamine gave

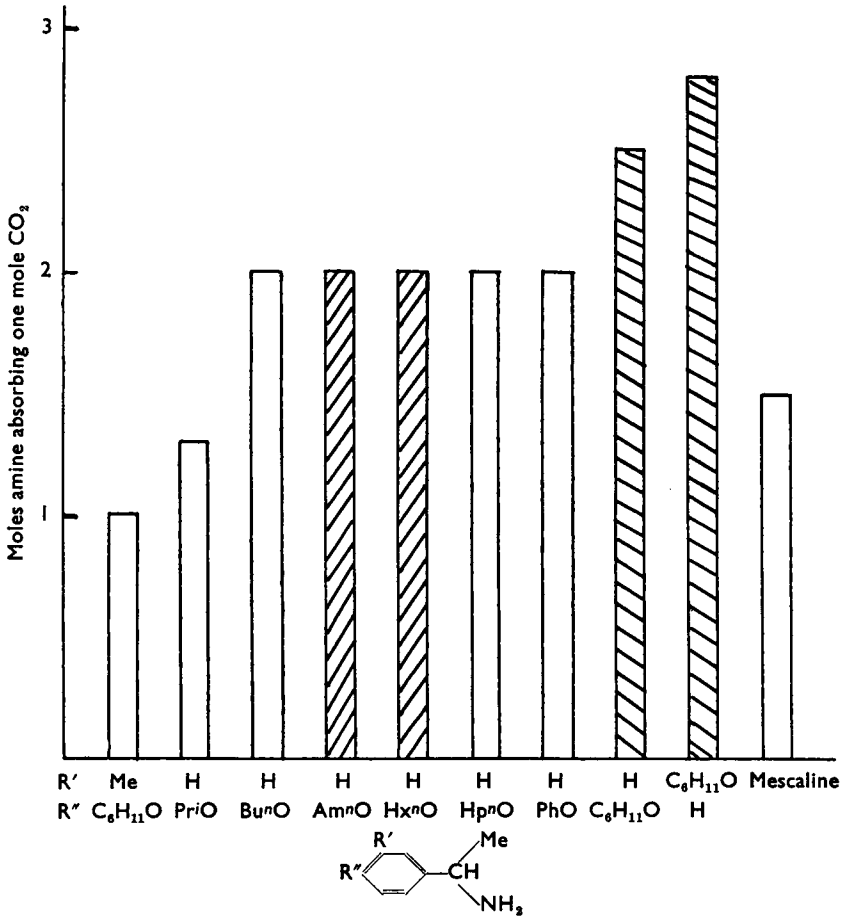

FIG. 1. Absorption of carbon dioxide by primary arylalkylamines. L–R upward hatching = slight analgesic activity; downward hatching = marked analgesic activity; □ = inactive.

92·5 per cent. total base after basifying a solution in acid ethanol, but only 64 per cent. after careful solution in dilute ethanolic sodium hydroxide.

Carbamate formation in solution. Little information exists on carbamate formation between amines and bicarbonate in solution. Reaction of free amino groups in hæmoglobin with carbon dioxide has been studied to explain certain features of carbon dioxide uptake and release by blood[6]. Formation of histamine carbamate has been investigated in relation to its action on isolated intestine[7].

Experiments were confined to the morphine-like 1-(*p-cyclo*hexyloxyphenyl)ethylamine and the corresponding but inactive *iso*propyl ether. Since the analgesic action followed tissue but not plasma concentration[4] there was little basis for using plasma as solvent and initial experiments have been made using simple aqueous solutions.

Solutions of the amine hydrochlorides (10^{-5} to 10^{-2}M) in sodium bicarbonate (0 to 0·6M) or phosphate buffer under different partial pressures of carbon dioxide were incubated at 37° C. to constant pH. Excess of 5N sodium hydroxide was added to retain carbamate and the liberated base was extracted with ether for estimation by the salicylaldehyde method[2]. In a few experiments the aqueous residues were acidified and carbon dioxide removed in a current of nitrogen. After basifying the liberated amine was extracted with ether. Total recovery was almost quantitative.

Results

In phosphate buffer under carbon dioxide without added sodium bicarbonate, the recovery of the *cyclo*hexyl ether decreased, that of the *iso*propyl ether increased, with falling pH. To explain this behaviour many hypothetical equilibria were analysed but only the following sequence could give a quantitative basis to this result and those with added bicarbonate.

$$RN^+H_3 + HCO_3^- \overset{k_1}{\rightleftharpoons} RNH_3HCO_3 \quad .. \quad .. \quad .. \quad .. \quad I$$

$$RN^+H_3 + HCO_3^- \overset{k_2}{\rightleftharpoons} RNHCOOH + H_2O \quad .. \quad .. \quad .. \quad II$$

$$\left.\begin{matrix} RN^+H_3 \\ RNHCOOH \end{matrix}\right\} \begin{matrix} + CO_2 \\ + H^+ \end{matrix} \overset{k_3}{\rightleftharpoons} RN^+H{=}C(OH)_2 \text{ or } RN^+H_2COOH \quad .. \quad III$$

$$RN^+H{=}C(OH)_2 + HCO_3^- \overset{k_4}{\rightleftharpoons} RNH{=}C(OH)_2{\cdot}HCO_3 \quad .. \quad .. \quad .. \quad IV$$

$$RNHCOOH \rightleftharpoons RNHCOO^- + H^+ \quad .. \quad .. \quad .. \quad V$$

Measurement of carbon dioxide evolution after tipping the solid hydrochlorides into equilibrated sodium bicarbonate solutions under carbon dioxide (100 per cent.) at 37° C. in closed constant pressure systems, gave the pKa of the carbamic acid; 8·38 and 8·75 respectively for the *cyclo*hexyl and *iso*propyl ethers. Within the range of pH used, the contribution of hydrogen ion from the substituted ammonium ion was negligible. At pH $< 7{\cdot}5$ it was therefore permissible to neglect reaction V and the sequence I to IV was summarised in the hyperbolic function VI

$$r = \frac{1}{A}\left\{\frac{[H^+] + B}{C[H^+] + D}\right\} \quad .. \quad .. \quad .. \quad VI$$

where r is the ratio of recovered to retained amine, $A = [CO_2]$; $B = k_1k_6[CO_2]$; $C = k_3(1 + k_4[HCO_3^-])$; $D = k_2k_6$; k_6 = the first dissociation constant of carbonic acid.

Calculation of the constants. The values of the equilibrium constants $k_1 - k_4$ given in Table I were calculated from data derived from the hyperbola relating r to $1/[H^+]$. Due to the enormous elongation of the abscissæ relative to r, this relation was approximately linear over the range of pH used and could be equated to the tangent at an intermediate point within the range (Fig. 2). There was good agreement between found and calculated values for recovered amines under a variety of conditions (Table II). The slope of the tangent to this hyperbola is negative or positive depending on whether B is less or greater than ADr when all constants are positive, as they must be in this example. This relation is solely dependent on the value of k_1 and k_2 and is independent of $[H^+]$. The apparent stabilising effect of hydrogen ion on the carbamate of the *cyclo*hexyl ether shown by the positive slope in Figure 2 is due solely to using $[H^+]$ as a convenient measure of the relative amounts of bicarbonate and carbon dioxide in solution. A less apparent difference in behaviour between the two carbamic acids was reflected in the nature of constant C. For the *iso*propyl ether this could be equated to k_3 under all conditions, i.e., $k_4 = 0$, whereas for the *cyclo*hexyl ether, C contained a $[HCO_3^-]$ dependent term, and a definite value could be ascribed to k_4. It was inferred that the carbamic acid derived from the *cyclo*hexyl ether formed a product capable of association with bicarbonate ion, as was indicated by the break in the curve between pH 6 and 7 (Fig. 2) for recovery of *cyclo*hexyl ether from phosphate buffer under carbon dioxide but without added bicarbonate. Presumably carbon dioxide formed little bicarbonate in phosphate buffer below pH 6. The evidence of Figure 1 where a combining ratio of about 2·5 to 1 implied partial formation of the species $RN = C(ORNH_3)_2$ suggested that the species $RN^+H = C(OH)_2$ formed part of the carbamic acid derived from the *cyclo*hexyl ether in solution (reaction III). The carbamic acid from the *iso*propyl ether, also associated with hydrogen ion, could

TABLE I

VALUES OF EQUILIBRIUM CONSTANTS FOR REACTIONS OF THE *cyclo*HEXYL AND *iso*PROPYL ETHERS OF 1-(*p*-HYDROXYPHENYL)-ETHYLAMINE WITH BICARBONATE AND CARBON DIOXIDE

	*cyclo*Hexyl ether	*iso*Propyl ether
k_1	$10^{2\cdot755}$	$10^{0\cdot592}$
k_2	$10^{2\cdot160}$	$10^{0\cdot839}$
k_3	$10^{2\cdot154}$	$10^{1\cdot479}$
k_4	$10^{2\cdot810}$	0

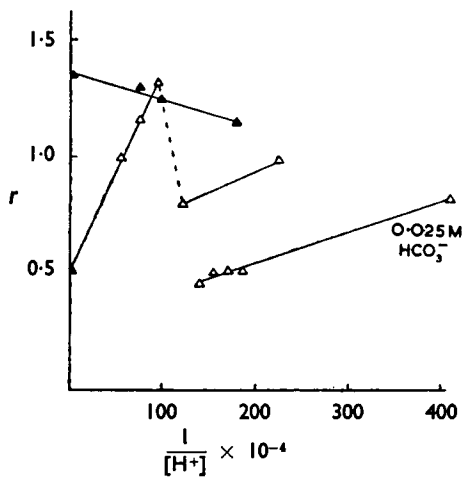

FIG. 2. Relation between ratio of recovered and retained amine, *r*, to $1/[H^+]$ in buffered solutions. △ = 1-(*p-cyclo*hexyloxyphenyl)ethylamine; ▲ = 1-(*p-iso*propyloxyphenyl)ethylamine. Phosphate buffer (except where bicarbonate is indicated) under 100 per cent. carbon dioxide.

TABLE II

RECOVERY OF ETHERS OF 1-(*p*-HYDROXYPHENYL)ETHYLAMINE FROM BICARBONATE—CARBON DIOXIDE SOLUTIONS IN EQUILIBRIUM AT 37° C.

Molar concentrations		Carbon dioxide per cent.	pH	Recovery per cent	
Amine	Bicarbonate			Found*	Calc.
		*cyclo*Hexyl ether			
10^{-4}	0	5	6·90	78	82
10^{-4}	0·013	5	7·30	81	77
10^{-4}	0·063	5	8·00	74	77
10^{-4}	0·125	5	8·31	73	77
8×10^{-3}	0·059	50	6·97	49	55
5×10^{-3}	0	100	5·98	60	58
2×10^{-4}	0·025	100	6·18	36	36
10^{-4}	0·025	0	8·66	89	81
		*iso*Propyl ether			
10^{-4}	0	50	4·41†	74	73
10^{-4}	0	50	5·98†	78	72
10^{-4}	0	50	6·19†	74	71
10^{-3}	0·100	5	7·82	81	79
10^{-3}	0·375	50	7·57	43	49
10^{-3}	0·147	50	7·40	57	53
10^{-3}	0·058	50	7·00	61	61

* Calculated to 5 per cent. (*cyclo*hexyl ether) or 10 per cent. (*iso*propyl ether) experimental loss.
† In phosphate buffer.

have the structure RN^+H_2COOH. A partial analogy may exist in the relation between urea and urethane, the former probably existing partly as $N^+H_2 = C\begin{matrix}O^-\\ \diagdown\\ \diagup\\ NH_2\end{matrix}$ and capable of salt formation with acids. Urethane behaves as an amide and does not form salts with acids.

DISCUSSION

There seems to be little doubt that the carbamic acids derived from the two amines have a distinct difference, best interpreted on the available evidence by the postulate that the *cyclo*hexyl ether gives a carbamic acid that can exist as its enolic form. This species seems to bear more resemblance to the "ortho" form of a ketone than to a carboxylic acid. Whether such a difference is related to the pharmacological distinction between the two can only be conjectural at this stage but the following observations support the conclusion. Among a long series of 1-(alkyloxyphenyl)ethylamines studied, only *m*- and *p-cyclo*hexyl ethers have so far shown marked analgesic activity. Others have often been strong depressants and slight activity could be due to this. The *p-cyclo*pentyl ether had no activity. Models showed that an axial hydrogen[8] of the *cyclo*hexyl ring in the "chair" form could form a hydrogen bond with carbonyl oxygen of the carbamic acid group. C——H——O bonds are poorly substantiated but the ease of elimination of axial hydrogen from a *cyclo*hexyl ring suggests that it may have sufficient degree of polar character to allow it to participate in hydrogen bond formation. This could be a driving force to induce enolisation. Such a bond is not possible for the *iso*propyl ether. Moreover, steric interference by alkyl substituents

ortho to the ether group prevent the bonding and 1-(3-methyl-4-*cyclo*-hexyloxyphenyl)ethylamine had no analgesic activity. Figure 1 indicates that this ether reacted with carbon dioxide in a similar manner to the *iso*propyl ether.

SUMMARY

1. Reaction of 1-(alkyloxyphenyl)ethylamines with carbon dioxide and bicarbonate or with bicarbonate alone, in solution can lead to formation of carbamic acids.

2. Some differences in the behaviour of two such acids in solution are discussed in relation to the analgesic activity of 1-(*p-cyclo*hexyloxyphenyl)-ethylamine.

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