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THE DIURETIC EFFECTS AND *IN VITRO* STABILITY OF ALKYL NN-ETHYLENE CARBAMATES

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Abstract—The methyl, ethyl, propyl, isopropyl and butyl esters of NN-ethylene carbamic acid have been prepared and assessed for diuretic activity in the rat. With the exception of the methyl ester all caused polyuria when doses of less than half the LD_{50} were used. All derivatives yielded ethyleneimine when incubated with rat plasma but it was not possible to correlate the rate of ethyleneimine production with diuretic activity.

A NUMBER of ethyleneimine derivatives have been described which bring about a water diuresis in non-hydrated rats.[1] One of the effective substances was ethyl NN-ethylene carbamate (NN-ethyleneurethane). This paper is concerned with an investigation of the methyl, ethyl, propyl, isopropyl and butyl esters with a view to comparing the relationship between the diuretic activity of these homologues and their *in vitro* rates of hydrolysis.

MATERIALS AND METHODS

The alkyl NN-ethylene carbamates (Table 1) were synthesised by the reaction of ethyleneimine (1·1 M) with the appropriate alkyl chloroformate (1 M) and triethylamine (1 M) at −20° using ether as solvent. The compounds were purified by distillation and characterised by elemental analysis.

TABLE 1. COMPARATIVE TOXICITY AND DIURETIC POTENCY OF THE METHYL, ETHYL, PROPYL, ISOPROPYL AND BUTYL ESTERS OF NN-ETHYLENE CARBAMIC ACID

Ester	H_2C / H_2C N.COOR, R	LD_{50} i.p. 28 day (mg/kg)	0–3 day cumulative diuresis at ½ LD_{50} (ml/kg)	Ethyleneimine equivalent to ½ LD_{50} (mg/kg)
Methyl	$-CH_3$	8·5	65	1·8
Ethyl	$-C_2H_5$	10·0	245	1·9
n-Propyl	$-C_3H_7$	17·5	270	2·9
iso-Propyl	$-CH(CH_3)_2$	17·5	280	2·9
n-Butyl	$-C_4H_9$	15·0	210	2·3
Ethyleneimine		3·8	220	1·9
Control		—	80	—

Animal techniques

LD_{50} levels were determined using groups of 5 male Wistar rats (260 ± 20 g) injected i.p. with the compound in arachis oil. Deaths were recorded up to 4 weeks. Diuretic effects were assessed as previously described,[1] the 0–3 day cumulative urine excretion at the half LD_{50} level being determined after administration of graded doses of each compound.

In vitro *studies*

The esters (2 mg/ml) were incubated in a range of phosphate buffers only (0·067 M; pH 5·3–8·0) and in the presence of 10% v/v of either rat plasma or plasma preheated for 5 min at 100°. Ethyleneimine formation was measured at half-hour intervals up to 2 hr using the technique previously described by Craig, Jackson and James.[2]

RESULTS

Toxicity and diuretic effects

Toxicity was found to be maximal with the methyl derivative (8·5 mg/kg) and minimal with the propyl and isopropyl derivatives (17·5 mg/kg; Table 1). The methyl ester failed to promote diuresis except at near-lethal dose levels. At the half LD_{50} level the propyl and isopropyl esters were rather more effective than the ethyl and butyl derivatives with a fourfold increase in urine output compared with the control (Table 1). In contrast to the propyl and isopropyl derivatives the toxicities of the methyl, ethyl and butyl esters seemed to bear a relation to their ethyleneimine 'content'.

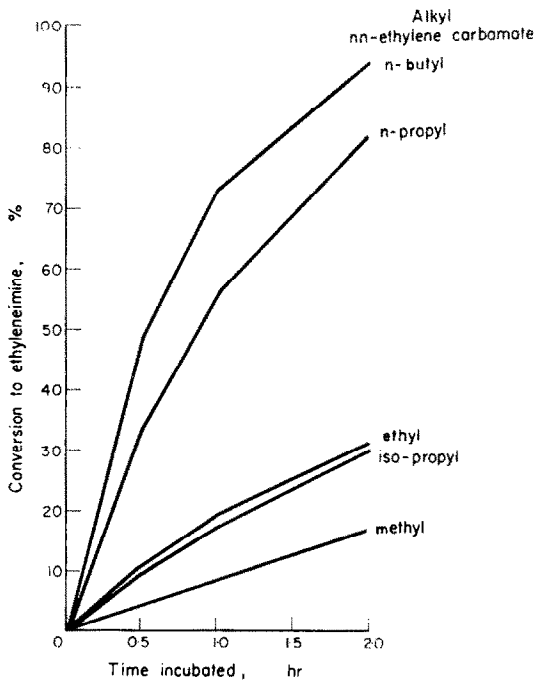


FIG. 1. Rate of conversion of various alkyl NN-ethylene carbamates (2 mg/ml) to ethyleneimine on incubating in pH 7 phosphate buffer containing 10% v/v of rat plasma. Values corrected for ethyleneimine hydrolysis.

In vitro *studies*

Since ethyleneimine (e.g. 0·5 mg/ml) slowly disappears from buffer (3 per cent/hr at pH 7) or buffer containing plasma (10 per cent/hr at pH 7), the data presented have been corrected to allow for this and permit clearer assessment of results.

Over the range of pH 5·3 to 8 the esters were stable in phosphate buffer alone. However, the presence of rat plasma promoted ethyleneimine production. The rate of hydrolysis was related to the nature of the alkyl substituent (Fig. 1), breakdown being most rapid with the butyl compound (94 per cent in 2 hr) and slowest with the methyl derivative (16 per cent in 2 hr); the isopropyl ester was comparable to the ethyl ester (30 per cent in 2 hr). No hydrolysis occurred on incubation with preheated plasma. The rate of liberation of ethyleneimine from ethyl NN-ethylene carbamate in the presence of normal plasma, increased with pH (Table 2).

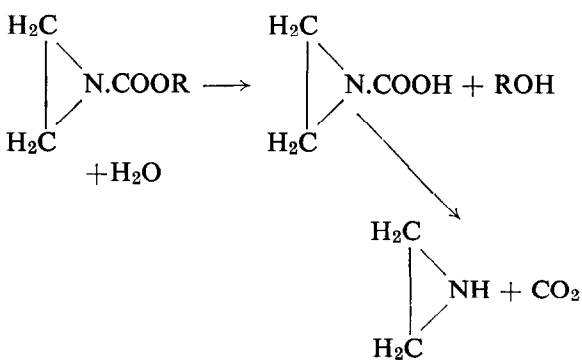
TABLE 2. THE EFFECT OF pH ON THE LIBERATION OF ETHYLENEIMINE FROM ETHYL NN-ETHYLENE CARBAMATE (2 mg/ml) INCUBATED WITH NORMAL RAT PLASMA (10% v/v). RESULTS CORRECTED FOR ETHYLENEIMINE HYDROLYSIS

Solution (in 0·067 M phosphate) buffer)	% converted to ethyleneimine		
	hr ½	1	2
Control pH 5·3 – 8·0	0	0	0
pH 5·3	1	3	6
pH 6·2	2	9	14
pH 7·0	11	19	31
pH 8·0	5	17	34

DISCUSSION

It has been suggested by Jackson and James,[1] that the diuresis brought about by a number of ethyleneimines might be due to the *in vivo* production of ethyleneimine. The series of NN-ethylene carbamic esters described in this paper promote diuresis and form ethyleneimine *in vitro* in the presence of plasma, although the more slowly hydrolysed methyl derivative only showed diuretic activity at near-lethal levels.

Hydrolysis of these esters would be expected to result in ethyleneimine formation via the unstable carbamic acid thus:



That enzymic hydrolysis of the alkyl NN-ethylene carbamates occurs is confirmed by the fact that it no longer proceeds when heat denatured plasma is employed.

The comparable rates of ethyleneimine formation by ethyl and propyl NN-ethylene carbamates and the almost complete hydrolysis of the butyl derivative in 2 hr (Fig. 1) indicate that the mechanism responsible functions more efficiently as the length of the carbon chain of the alkyl substituent is increased. The isopropyl ester is more stable than the propyl derivative yet as effective a diuretic agent, suggesting that simple hydrolysis to ethyleneimine may not be the entire reason for the diuretic action.

The failure of methyl NN-ethylene carbamate to induce polyuria at low doses is difficult to explain as its toxicity together with those of the ethyl and butyl derivatives seem to be correlated with ethyleneimine content (Table 1). As previous data have shown that only small amounts of ethyleneimine (1·2 mg/kg) are required to cause diuresis[1] it could be that this base is only a minor metabolite of the methyl ester and is not responsible for its toxicity.

Differences in biological activities of the methyl and ethyl NN-ethylene carbamates are not surprising since methyl carbamate is pharmacologically inert compared with ethyl carbamate (Urethane), the latter being hypnotic, antileukaemic, carcinogenic and mutagenic. Such differences may result from differences in metabolism. When injected into rats (0·5 g/kg) some 5–10 per cent of methyl carbamate is excreted unchanged in the urine and the rest is metabolised at about half the rate of the ethyl ester.[3] In the rat, Urethane is almost completely metabolised.[4, 5]

The information presented here supports the possibility that ethyleneimine or one of its metabolites is responsible for the diuresis initiated by certain ethyleneimine derivatives in non-hydrated rats. It is however, not always possible to correlate the rate of ethyleneimine production, under standard conditions *in vitro*, with the property of inducing diuresis. This is presumably due to an interdependence on such factors as solubility, absorption and the rate and mode of metabolism in the intact animal.

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