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STABILITY OF CERTAIN ETHYLENEIMINES IN RELATION TO DIURETIC ACTION

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Abstract—The breakdown of several ethyleneimine derivatives to ethyleneimine has been studied in relation to pH and the presence of tissues and plasma. In neutral solution NN-ethyleneurea hydrolysed at a rate unaffected by rat liver, kidney or plasma. Hydrolysis of NNN'N'-diethyleneurea was apparently enzyme dependent whilst that of di(aziridin-1-yl)sulphoxide was activated by the phosphate ion. The N'-isopropyl and N'N'-dimethyl homologues of NN-ethyleneurea were stable in buffered solution but there was evidence suggesting their breakdown in the presence of tissue. Triethylenephosphoramide yielded ethyleneimine at pH 5·3. The relationship between chemical stability of these compounds and their diuretic action is discussed.

INTRODUCTION

POLYURIA occurs in non-hydrated rats after administration of ethyleneimine and a number of its derivatives.[1,2] Partial metabolism of NNN'N'-diethyleneurea and di(aziridin-1-yl)sulphoxide to ethyleneimine in rats and mice,[3] suggests that the latter compound is responsible for the diuresis. The current work is concerned with the formation of ethyleneimine from several ethyleneureas, di(aziridin-1-yl)sulphoxide and triethylenephosphoramide (Fig. 1). Studies were undertaken to observe the relation between stability and diuretic activity since effective esters of NN-ethylene carbamic acid yield ethyleneimine on incubation with rat plasma.[2]

MATERIALS AND METHODS

Di(aziridin-1-yl)sulphoxide and NN-ethylene-N'-isopropyl urea (Fig. 1) were prepared as described previously[1] and the remaining compounds using the methods of Bestian.[4] Ethyleneimine formation *in vitro* was measured colorimetrically following reaction with 1,2 naphthaquinone-4-sodium sulphonate.[5]

N,2-hydroxyethylurea was prepared by adding an equimolar amount of ethanolamine to isocyanic acid in ether, the reaction mixture being cooled with powdered solid carbon dioxide. The urea derivative recrystallised from aqueous alcohol (m.p. 96°).

Each ethyleneimine derivative was incubated (2 mg/ml) in 0·067 M KH_2PO_4/Na_2HPO_4 buffer solution over the range of pH 5·3 to 8·0 and the amount of ethyleneimine produced measured at $\frac{1}{2}$ hr intervals up to 3 hr. Also, the rate of ethyleneimine production at pH 7 was studied on incubation (37°) with rat liver or kidney slices (10%w/v) or plasma 10% v/v). Since phosphate ion promotes the hydrolysis of di(aziridin-1-yl)sulphoxide[5] comparative studies were carried out with NN-ethyleneurea and NNN'N'-diethyleneurea at pH 7 using both phosphate and 0·1 M sodium diethyl barbiturate/HCl buffers.

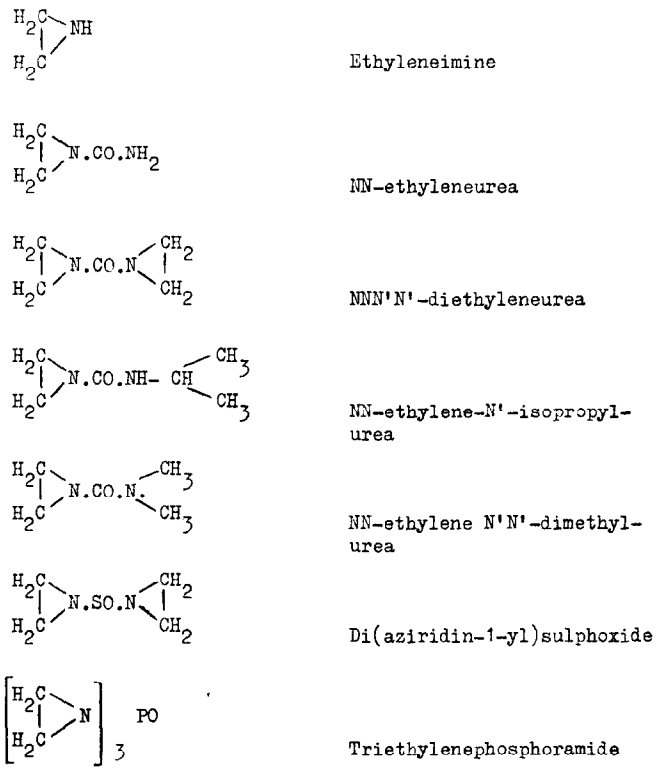


FIG. 1. Ethyleneimine Derivatives.

RESULTS

The effect of pH on hydrolysis rate. Ethyleneimine (0·5 mg/ml) was more stable at pH 5·3 than at pH 8·97 and 91% respectively being unchanged after 3 hr incubation. To correct for the breakdown of ethyleneimine produced in other experiments, all results have been adjusted using data obtained from control experiments with the base (0·5 mg/ml). Thus, at pH 5·3 the mean rate was 0·5%/½ hr and 1·5%/½ hr at pH 8. Ethyleneimine formation by NN-ethyleneurea (Table 1) was more rapid at pH 6·2–8·0 (22–25% in 3 hr) than at pH 5·3 (9% in 3 hr). In contrast the diethyleneurea yielded only 6% of ethyleneimine at pH 8 (3 hr) and was stable in this respect at acid pH. Breakdown of di(aziridin-1-yl)sulphoxide at pH 5·3 and 6·2 was maximal by 1 hr (about 72%) but only reached a peak figure of 51% after 2 hr at pH 7·4 and 41% in 3 hr at pH 8. Triethylenephosphoramide did not yield ethyleneimine at pH 8 but gave 21% of the available base in 3 hr at pH 5·3. Over the same period NN-ethylene-NN'-dimethylurea and NN-ethylene-N'-isopropylurea (2 mg/ml) failed to produce ethyleneimine.

Two-dimensional chromatography (water saturated butan-1-ol and 3 methyl butan-1-ol) demonstrated the partial conversion of NN-ethyleneurea to N,2-hydroxy-ethylurea on incubation at pH 7. The *Rf* values of these two substances in water-saturated butan-1-ol were 0·39 and 0·26 respectively and in water-saturated 3-methyl butan-1-ol, 0·27 and 0·15.

TABLE 1. ETHYLENEIMINE PRODUCTION BY A NUMBER OF ITS DERIVATIVES ON INCUBATION*

Compound	pH	Ethyleneimine production as percentage of theoretical yield			
		hr ½	1	2	3
NN-ethyleneurea	5·3	6	8	9	9
	6·2	8	13	17	22
	7·4	8	14	21	25
	8·0	8	16	23	25
NNN'N'-diethyleneurea	5·3	0	1	0	0
	6·2	0	1	1	1
	7·4	1	1	2	3
	8·0	1	3	4	6
di(aziridin-1-yl)sulphoxide	5·3	63	74	68	67
	6·2	56	71	69	67
	7·4	22	41	51	46
	8·0	10	17	30	41
Triethylenephosphoramide	5·3	7	11	16	21
	6·2	0	1	4	6
	7·4	1	1	1	1
	8·0	0	0	0	0

* (2 mg/ml) in 0·067 M KH_2PO_4/Na_2HPO_4 buffer (pH 5·3-8·0). Results corrected for hydrolysis of ethyleneimine (see text).

TABLE 2. COMPARISON OF THE RATES OF LIBERATION OF ETHYLENEIMINE*

Compound	Buffer pH7	Tissue	Ethyleneimine production as percentages of theoretical yield			
		hr	½	1	1½	2
NN-ethyleneurea	Barbitone	Control	7	13	18	19
		Liver	7	14	17	20
		Kidney	7	14	17	20
		Plasma	7	14	19	20
	Phosphate	Control	7	13	19	21
		Liver	7	13	15	19
		Kidney	7	13	18	21
		Plasma	5	9	15	20
NNN'N'-diethyleneurea	Barbitone	Control	0	0	0	1
		Liver	21	30	35	54
		Kidney	7	12	19	30
		Plasma	9	15	20	24
	Phosphate	Control	1	2	2	2
		Liver	22	28	34	51
		Kidney	5	15	19	29
		Plasma	6	12	16	20
Di(aziridin-1-yl)sulphoxide	Barbitone	Control	2	2	2	2
		Liver	4	10	17	18
		Kidney	2	5	8	10
		Plasma	0	0	0	0
	Phosphate	Control	37	55	64	67
		Liver	36	61	70	70
		Kidney	36	61	70	70
		Plasma	27	56	69	72

*From NN-ethyleneurea, NNN'N'-diethyleneurea and di(aziridin-1-yl)sulphoxide on incubation (2 mg/ml) with rat liver (1 g/10 ml) or kidney (1 g/10 ml) or plasma (10% v/v) in pH 7 phosphate or barbitone buffer. Values corrected for hydrolysis of ethyleneimine.

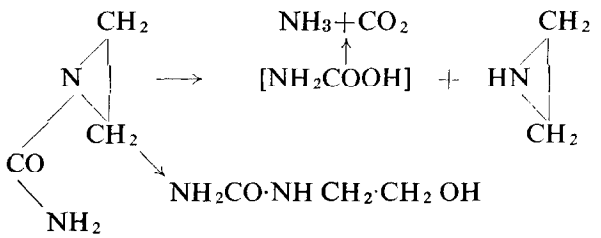
Incubation n the presence of rat liver, kidney or plasma. Ethyleneimine disappeared more rapidly in the presence of tissue than from buffer alone, the relative rates per ½ hr at pH 7 being 5% and 1·2% which were taken into account when calculating results. The production of ethyleneimine from NN-ethyleneurea in either phosphate or barbitone buffer was not accelerated by tissue or plasma (Table 2). Hydrolysis of NNN′N′-diethyleneurea in both buffers was particularly increased by rat liver, more than 50% of the available ethyleneimine being liberated in 2 hr. Kidney and plasma also promoted the rate of breakdown. In the presence of slices of rat liver pre-heated for 5 min at 100°, ethyleneimine formation was reduced to the control value (Table 2). With fresh tissue the hydrolysis rate decreased with pH. Thus, after 2 hr at pH 5·3, 13% of the available ethyleneimine was liberated compared with 51% at pH 7 and 65% at pH 8.

In phosphate buffer the rapid hydrolysis of di(aziridin-1-yl)sulphoxide was not significantly affected by liver, kidney or plasma. Incubation of the sulphoxide in barbitone buffer alone, resulted in very little ethyleneimine formation. However, in the presence of liver and kidney, 18 and 10% conversion respectively occurred within 2 hr. Ethyleneimine formation in the latter instances was most likely promoted by phosphate ion present in the tissue.

Triethylenephosphoramide was quite stable in this series of experiments. NN-ethylene-N′N′-dimethylurea and NN-ethylene-N′-isopropylurea yielded bases other than ethyleneimine, perhaps dimethylamine and isopropylamine respectively, the amounts of which could not be determined.

DISCUSSION

The present results demonstrate that ethyleneimine derivatives may hydrolyse with the liberation of ethyleneimine and that enzymic action is sometimes involved, as is the case with the esters of NN-ethylene carbamic acid.[2] An alternative route involves opening of the ethyleneimine ring with the production of 2-hydroxyethylamino-compounds. Thus with NN-ethyleneurea the possible reactions are:—



N,2-hydroxyethylurea

The more rapid disappearance of ethyleneimine in the presence of tissue is presumably due to metabolism including the possibility of alkylating reactions. Although technical difficulties prevented measurement of ethyleneimine formed from isopropyl and dimethyl ethyleneureas the information so far available is that all compounds promoting polyuria in rats yield ethyleneimine *in vitro*. Triethylene-phosphoramide (TEPA), which does not cause diuresis, only produced ethyleneimine at higher hydrogen ion concentration. The *in vitro* stability of TEPA (pH 4) is discussed by Beroza and Borkovec,[6] who found the compound to yield ethyleneimine and 2-hydroxyethylaminophosphoramide derivatives. Sinces ethyleneimine formation

occurs at pH 5·3 (Table 1), hydrolysis might take place in appropriate parts of the renal tubule. In the rat, Craig and Jackson,[7] found that 80–90% of triethylenephosphoramide (1 mg/kg) was excreted unchanged in 24 hr. Thus the inability of this phosphoramide to promote polyuria in rats implicates ethyleneimine in the diuretic process. The site of metabolism appears important since TEPA, which is partially converted to ethyleneimine in the mouse kidney, fails to cause diuresis in this species.[3]

The principal factor affecting breakdown of NN-ethyleneurea, NNN′N′-diethyleneurea and di(aziridin-1-yl)sulphoxide is different for each compound. Hydrolysis of NN-ethyleneurea is primarily pH dependent, that of di(aziridin-1-yl)sulphoxide is promoted by the phosphate ion whilst active metabolism of NNN′N′-diethyleneurea and the N′-alkyl NN-ethyleneureas probably occurs.

Increased resistance to hydrolysis could account for the reduced diuretic activity of NN-ethylene-N′N′-dimethyl urea and NN-ethylene-N′-isopropylurea.[1] Since these compounds respectively, have approximately 1/40 and 1/18 of the toxicity of diethyleneurea, it would appear that their principal metabolic pathway does not involve ethyleneimine production as only small amounts of this base (e.g. 1·5 mg/kg) are required to promote diuresis in the rat.[1] Available evidence suggests that the compounds bringing about diuresis are metabolised to yield ethyleneimine *in vitro*. In order to establish the mode of action of ethyleneimine, information is required concerning its metabolism and site of action. In particular, knowledge of any effect it has on the pituitary-renal relationship should be of considerable interest.

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