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Potent xanthine oxidase inhibitors—4(or 5)-diazoimidazole-5(or 4)-carboxamide and two related compounds

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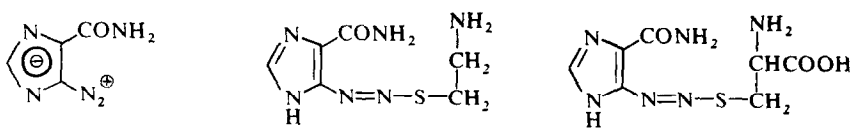
IN RECENT years, a series of triazenoimidazoles had been synthesized as possible antitumor agents and some of them have been shown to be effective.[1–6] In preliminary experiments, we examined the effects of these compounds and their synthetic intermediate, 4(or 5)-diazoimidazole-5(or 4)-carboxamide (Diazo-ICA) in suppression of xanthine oxidase (xanthine: O_2 oxidoreductase; EC 1. 2. 3. 2) of rat liver *in vitro*, and found that Diazo-ICA inactivated this enzyme. Accordingly we tested the inhibitory effect of analogues of Diazo-ICA on cream xanthine oxidase.

Some purine analogues have been demonstrated by many investigators to inhibit xanthine oxidase. Among these, allopurinol (4-hydroxypyrazolo(3,4-*d*)pyrimidine), a potent xanthine oxidase inhibitor,[7,8] has recently been used for the relief of hyperuricemia associated with gout,[9,10] and for retardation of metabolic breakdown of 6-mercaptopurine, used in treatment of neoplastic diseases.[11] Imidazole derivatives, however, have not been found to act in this way. For this reason also it was thought desirable, to evaluate the new type of inhibitor, Diazo-ICA and the two related compounds.

The present communication shows that Diazo-ICA and the two new, related compounds, are potent inhibitors, both *in vivo* and *in vitro*, of rat liver xanthine oxidase and of cream xanthine oxidase, which converts hypoxanthine to xanthine and the latter to uric acid. The new related compounds were 4(or 5)-(2-aminoethylthio-azo)imidazole-5(or 4)-carboxamide (cysteaminyl-Diazo-ICA) and S-(5(or 4)-carbamoyl-4(or 5)-imidazolyl azo) cysteine (cysteinyl-Diazo-ICA) which were prepared by coupling the diazonium salt with the corresponding SH-compounds, in our laboratory. The physico-chemical properties of these compounds will be described elsewhere. The chemical structures of these compounds are given in Fig. 1.



(I) Diazo-ICA (II) Cysteaminyl-Diazo-ICA (III) Cysteinyl-Diazo-ICA

FIG. 1. Chemical structures of 4(or 5)-diazoimidazole-5(or 4)-carboxamide and related compounds
(I) Diazo-ICA: 4(or 5)-diazoimidazole-5(or 4)-carboxamide
(II) Cysteaminyl-Diazo-ICA: 4(or 5)-(2-aminoethylthio azo) imidazole-5(or 4)-carboxamide
(III) Cysteinyl-Diazo- ICA: S-[5(or 4)-carbamoyl-4 (or 5) -imidazolyl azo] cysteine

In vitro study: Bovine milk xanthine oxidase which was prepared according to the method of Klenow, was used. The enzyme activity was measured by the increase in E_{293} due to formation of uric acid from hypoxanthine for 10 min at 23° in Shimazu QV-50 spectrophotometer.

Table 1 shows the relative potencies of several imidazole analogues as inhibitors of xanthine oxidase *in vitro*; for comparison the inhibitory effects of allopurinol, 8-azaguanine, 6-mercaptopurine, and 2-azahypoxanthine are also shown. Diazo-ICA and the two related compounds, cysteaminyl-Diazo-ICA and cysteinyl-Diazo-ICA were found to be even more effective inhibitors of cream xanthine oxidase than allopurinol.

The concentration of Diazo-ICA causing 50 per cent inhibition of xanthine oxidase activity after aerobic incubation for 10 min at 23° was 8×10^{-7} M, while that of allopurinol was 2×10^{-6} M. The inactivation was proportional to the concentration of these compounds present. Cysteaminyl-Diazo-ICA and cysteinyl-Diazo-ICA were both as effective inhibitors as Diazo-ICA.

The inhibitory effect of 4(or 5)-(dimethyltriazeno)midazole-5(or 4)-carboxamide (Dimethyl-TICA), which was also synthesized in our laboratory, was approximately the same as that of 2-azahypoxanthine.

The other diazonium compound tested, diazobenzene sulfonamide, which was synthesized in our laboratory, was much less inhibitory.

No or only slight inhibition was observed with 4(or 5)-aminoimidazole-5(or 4)-carboxamide (AICA), 1-β-D-ribofuranosyl-4(or 5)-aminoimidazole-5(or 4)-carboxamide (AICA riboside), 4(or 5)-aminotriazole-5(or 4)-carboxamide (Aza-AICA), 1-β-D-ribofuranosyl-5-aminoimidazole-4-thiocarboxamide (Thio-AICA riboside) or 4(or 5)-formylaminoimidazole-5(or 4)-thiocarboxamide (Formylthio-AICA).

Rat liver xanthine oxidase was also found to be markedly inhibited by Diazo-ICA. Allopurinol (100 mg/kg, i.p.) inhibited the enzyme activity about 66 per cent relative to that of control rats. Much higher inhibitions were obtained with cysteaminyl-Diazo-ICA and cysteinyl-Diazo-ICA at doses of 50 mg/kg (i.p.). About 50 per cent inhibition of enzyme activity was obtained by intraperitoneal administration of Diazo-ICA at a dose of 20 mg/kg. These inhibitors were injected i.p. 24 hr before testing enzyme activity in excised liver.

On intraperitoneal administration Diazo-ICA and the two related compounds caused stretching responses which lasted for about 2–3 hr after administration of the drugs at the doses used.[12] No other apparent behavioral changes were observed.

Diazo-ICA has been found to couple with sulfhydryl compounds or SH groups in biological preparations.[13] Moreover, P-chloromercurobenzoate and iodosobenzoate[14] have recently been shown to inactivate cream xanthine oxidase chiefly by interaction with the SH groups in the enzyme. Accordingly, studies were made to see whether the inhibition by Diazo-ICA was due to its effect on sulfhydryl groups in the enzyme preparation.

In the present experiments, these coupling compounds were shown to be as potent inhibitors of xanthine oxidase as Diazo-ICA. In addition, it was indicated that 50 per cent inactivation of the oxidizing enzyme by Diazo-ICA could not be reversed by simultaneous addition of excess cysteine. A well-known blocker of sulfhydryl group, *N*-ethylmaleimide did not inhibit cream xanthine oxidase at concentrations of 1×10^{-5} M to 1×10^{-7} M as shown in Table 1. These results suggest that the inactivation of xanthine oxidase preparations by Diazo-ICA does not seem to be due to modification of SH-groups, but to some other mechanisms.

The contradictory findings on the correlation between interference with SH groups and enzyme inhibition by SH-blockers will be discussed elsewhere.

Studies on the biochemical mechanisms of the effects of Diazo-ICA and related compounds on the enzyme are now in progress in our laboratory using cream xanthine oxidase.

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TABLE 1. *IN VITRO* INHIBITION OF CREAM XANTHINE OXIDASE BY 4 (OR 5)-DIAZOIMIDAZOLE-5 (OR 4)-CARBOXAMIDE AND RELATED COMPOUNDS

Compound	Per cent inhibition* (average of values of 5 experiments)	
	1×10^{-6} (M)	1×10^{-5} (M)
Diazo-ICA[a]	70	100
Cysteaminyl-Diazo-ICA[b]	51	100
Cysteinyl-Diazo-ICA[c]	48	100
AICA·HCl[d]	—	0
AICA riboside[e]	—	0
Aza-AICA[f]	—	5
Thio-AICA riboside[g]	—	1
Formylthio-AICA[h]	—	9
Allopurinol[i]	39	94
2-Azahypoxanthine	—	17
6-Mercaptopurine	—	3
8-Azaguanine	—	3
Dimethyl-TICA·HCl[j]	8	21
Diazo-BS[k]	—	16
N-Ethylmaleimide	—	0

a, 4(or 5)-diazoimidazole-5(or 4)-carboxamide; b, 4(or 5)-(2-aminoethylthio azo)-imidazole-5(or 4)-carboxamide; c, S-(5(or 4)-carbamoyl-4(or 5)-imidazolyl azo) cysteine; d, 4(or 5)-aminoimidazole-5(or 4)-carboxamide hydrochloride; e, 1-β-D-ribofuranosyl-5-aminoimidazole-4-carboxamide; f, 4(or 5)-aminotriazole-5(or 4)-carboxamide; g, 1-β-D-ribofuranosyl-5-aminoimidazole-4-thiocarboxamide; h, 4(or 5)-formylaminoimidazole-5(or 4)-thiocarboxamide; i, 4-hydroxypyrazolo(3,4-d)pyrimidine; j, 4(or 5)-(dimethyltriazeno)imidazole-5(or 4)-carboxamide hydrochloride; k, diazobenzene sulfonamide benzenesulfonate.

* The inhibition measurements were carried out by the addition of graded amounts of the various compounds 0·4 ml to incubation vessel containing glycylglycine (0·1 M, pH 7·5) 2 ml, EDTA (1 μmole/ml) 0·4 ml, hypoxanthine (1·25 μmole/ml) 0·2 ml, the enzyme (20 unit) 0·5 ml and water 0·4 ml.

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Anti-convulsant action of anabolic steroids

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MUTUAL antagonism in rats between leptazol (pentylenetetrazol)-induced convulsions and the steroids, progesterone and desoxycorticosterone, has been reported.[1] It has also been shown that several steroids effectively antagonize electroshock seizures in rats by elevating the electroshock