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Department of Pharmacology,
University of Göteborg,
S-400 11 *Göteborg* 33, *Sweden*
and
Department of Histology,
Karolinska Institutet,
S-104 01 *Stockholm* 60, *Sweden*

JAMES L. MEEK†
KJELL FUXE
ARVID CARLSSON

† Present address: National Institute of Mental Health, Division of Special Mental Health Research, Saint Elizabeth's Hospital, WAW Building, Washington D.C. 20032.

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The action of some ω-diazoacetophenones on purine biosynthesis, as compared with the action of L-azaserine

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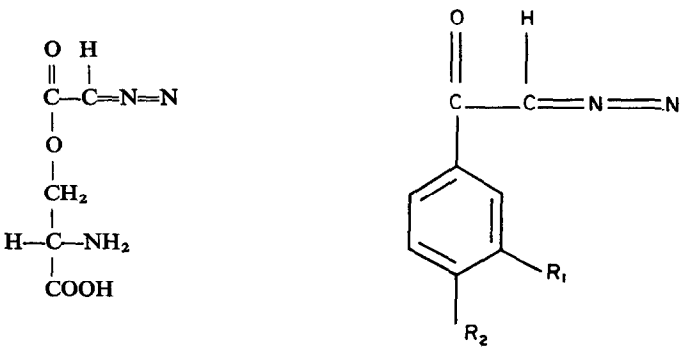
THE SUBSTITUTED L-serine, *O*-diazoacetyl-L-serine (L-azaserine),[1] has been shown to be similar in structure to L-glutamine,[2] and to interfere in the biosynthesis of the purines by competing with L-glutamine in the amination of formyl-glycinamide ribotide (FGAR) to formyl-glycinamidine ribotide (FGAM).[3,4] In spite of the high degree of specificity and potency shown by L-azaserine in *in vitro* tests, clinical studies of L-azaserine against neoplastic conditions showed it to have little beneficial effect.[5,6] It appeared that L-azaserine was extensively inactivated *in vivo*. We thought it possible that analogous ω-diazoacetophenones (Fig. 1) might retain the specific enzyme blocking action of L-azaserine, whilst being more stable to metabolic breakdown, and further if degraded, the breakdown products might have antineoplastic activity.



L-azaserine ω-diazoacetophenone

R_1 = F, *m*—fluoro diazoacetophenone
R_2 = F, *p*—fluoro diazoacetophenone
R_2 = MeO, *p*-methoxy diazoacetophenone
R_2 = $COCHN_2$, di-diazoketone of terephthalic acid

FIG. 1. A comparison of the structure of the antimetabolite L-azaserine, with analogous ω-diazoacetophenones, synthesised by the method of Bradley and Robinson.[20]

Accordingly, we have tested the action of ω-diazoacetophenone, and a short series of substituted ω-diazoacetophenones on the activity of the enzyme, 2-formamido-*N*-ribosylacetamide-5′-phosphate-amido-ligase (ADP) (EC 6.3.5.3) which causes amido transfer in the reaction

$$\text{FGAR} \xrightarrow{\text{Zn}\ \text{Mg}} \text{FGAM}$$
$$\text{L-glutamine} \xrightarrow{\text{NH}} \text{glutamic acid}$$

in the biosynthesis of the purines.

Materials and methods

The activity of the enzyme was measured indirectly, by converting the product of the reaction to the next product of the synthesis, aminoimidazole ribotide (AIR), with the enzyme, 2-formamido-*N*-ribosylacetamidine-5′-phosphate-cyclo-ligase (ADP) (EC 6.3.3.1). The AIR formed was estimated as diazotizable amine by modified Bratton and Marshall reagents.[7–9]

The first substrate, FGAR, was enzymatically synthesised from pigeon liver extract,[10,11] and collected as the barium salt. The enzyme 2-formamido-*N*-ribosylacetamide-5′-phosphate-amido-ligase (ADP) was prepared from chicken liver,[12] and the enzyme 2-formamido-*N*-ribosylacetamidine-5′-phosphate-cyclo-ligase (ADP) was prepared from pigeon liver.[13] Each active precipitate was dissolved in 0·01 M phosphate buffer, pH 7·4, containing 0·1 M potassium chloride, frozen at −25°, and used within 2 days.

Incubation procedure with L-*azaserine, and ω-diazoacetophenones*

Incubations were carried out at 38° for 30 min, in glass tubes that had been rinsed in 10^{-4} M EDTA, prior to use. The incubations contained the following reagents, in a total volume of 0·3 ml.: tris buffer 6·0 μM (0·02 ml, pH 8·0), ATP 3·0 μM (0·02 ml), L-glutamine 0·3 μM (0·02 ml), magnesium chloride 3·0 μM (0·02 ml), potassium chloride 12 μM (0·02 ml), 2-formamido-*N*-ribosylacetamide-5′-phosphate-amido-ligase (ADP) (20 mg protein/ml) (0·05 ml), 2-formamido-*N*-ribosylacetamidine-5′-phosphate-cyclo-ligase (ADP), 50 mg protein ml (0·03 ml), 0·05 ml FGAR equivalent to 10 μM of barium acetate (0·02 ml), and water 0·10 ml. The reaction was stopped by the addition of 20% trichloracetic acid, and the diazotizable amine formed was reacted with modified Bratton and Marshall reagents, read at 500 nM, and expressed as equivalent μM of 5-amino-4-imidazolecarboxamide (AICAR).

The 2-formamido-*N*-ribosylacetamide-5′-phosphate-amido-ligase fraction was preincubated with L-azaserine and the ω-diazoacetophenones for 3 min before the other reagents were added to the incubation mixture. ω-diazoacetophenone and *p*-methoxy-diazoacetophenone were added to the incubations in the same order of concentration as L-azaserine, the more soluble *p*-fluoro, and *m*-fluoro compounds were added at higher concentration. The ω-diazoacetophenones were estimated in phosphate buffer by polarography.[14]

Results and discussion

It can be seen from Table 1, that the ω-diazoacetophenones, unlike L-azaserine, produced no obvious inhibition of the enzyme 2-formamido-*N*-ribosylacetamide-5′-phosphate-amido-ligase. Although it has been shown[15] that L-azaserine, and ω-diazoacetophenone have the common action of causing an anti-diuresis in mice, these results suggest that ω-diazoacetophenones do not combine irreversibly with this enzyme in the same fashion as L-azaserine. Buchanan[16] showed that the length of the carbon chain in L-azaserine analogs was important if these compounds were to function as specific L-glutamine antagonists. It was assumed that both L-glutamine, and L-azaserine bind to the enzyme by the α carboxyl, and α amino groups, so that the carboxyl carbon is in the correct position to interact with a sulphydryl group of a cystein residue.[17] On this proposed theory, the ω-diazoacetophenones lack any obvious mechanism for primary binding to this enzyme.

However, Ancill,[18] using ω-diazoacetophenone has achieved delayed blast cell mitosis in guinea pigs after treatment with this compound. If this action is not at the FGAR ⟶ FGAM level,

TABLE 1

Inhibitor	Final conc. inhibitor in incubation (μM)	AIR formed expressed as 5-amino-4-imidazole-carboxamide 10^{-7}M*
Control		0·95 ± 0·04
L-azaserine	0·24	0·07 ± 0·03
ω-diazoacetophenone	0·24	0·85 ± 0·07
ω-diazoacetophenone	0·48	0·87 ± 0·10
di-diazoketone of terephthalic acid	0·03	0·92 ± 0·16
p-methoxy-diazoacetophenone	0·42	0·89 ± 0·08
p-fluoro-diazoacetophenone	18·00	0·86 ± 0·08
m-fluoro-diazoacetophenone	39·00	0·88 ± 0·12

* Mean of three incubations, with difference of highest and lowest readings.

In incubation mixtures containing the elements for the enzymatic conversion of FGAR ⟶ FGAM ⟶ AIR, the above table shows the AIR formed, measured as diazotizable amine, in incubations containing the antimetabolite L-azaserine, and some analogous ω-diazoacetophenones.

The AIR formed was measured by the modified method of Bratton and Marshall,[7–9] at 500 nM. and the concentration expressed in terms of μM of 5-amino-4-imidazolecarboxamide, read at 540 nM, then we suggest it may be through the reaction of the carbonium ion ($R\text{-}CO\text{-}CH_2$),[19] formed during the acid hydrolysis of these compounds, with nucleophilic groups in the cell, for example the N-7 in the guanine molecule of DNA. In the case of ω-diazoacetophenone, this would result in the introduction of a substituted aryle group in the DNA molecule, thus giving an antimitotic effect independent of purine synthesis.

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Pharmacology Group,
School of Pharmacy,
Bath University of Technology,
Bath, Somerset, U.K.

A. J. COLLINS
R. J. ANCILL

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Purification and substrate specificity of indoleamine-*N*-methyl transferase

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An enzyme, present in rabbit lung, which transfers the methyl group from *S*-adenosylmethionine to the amino group of a variety of indoleamines and phenylethylamines, has been described by Axelrod.[1] Recently, a similar indoleamine-*N*-methyl transferase was isolated from brain, although it appears to be more specific in that it does not *N*-methylate phenylethylamines.[2] Although serotonin was reported to be the most active substrate in both cases, the studies presented herein on partially purified indoleamine-*N*-methyl transferase from rabbit lung demonstrate that *N*-methyltryptamine is the substrate with the lowest K_m. These results raise the possibility that a physiological role of the enzyme may be the formation of dimethyltryptamine.

For the purification of indoleamine-*N*-methyl transferase, the 40–60% ammonium sulfate fraction was prepared from albino male rabbit lung according to the procedure of Axelrod.[1] A 12-ml aliquot of the dialyzed enzyme preparation containing about 150 mg protein was placed on a Sephadex G-150 column (3 × 45 cm) equilibrated with 1×10^{-3}M potassium phosphate buffer (pH 7·0) con-