Inhibition of poly(ADP-Rib) polymerase by different agents

Inhibitor	V_{max} (nmoles/mg protein)	K_m (μM)	K_i (μM)	K_i/K_m
Ethidium bromide	98.4	42.2	143.8	3.4
Formycin B	96.0	43.7	68.9	1.6
Showdomycin	96.3	43.0	107.8	2.5
1-Methyl adenine	95.9	41.9	226.6	5.4

The standard enzyme mixture (see in legend to the Figure) with different NAD concentrations (in the range between 9 and 83 μM) has been used; the mixture was incubated for 10 min. K_m and K_i were determined according to LINEWEAVER et al.[8].

The substrate analogues related to the adenosine moiety of NAD exert a different inhibitory activity in the poly (ADP-Rib) polymerase assay. The natural analogues Ade, Ado, dAdo, AMP, ADP, ATP, dAMP, dADP and dATP are without influence on the reaction. The effects of some unusual analogues have been tested; without influence were: 9-β-D-arabinofuranosyladenine (Mack), 9-β-D-arabinofuranosyladenine-5′-monophosphate, -diphosphate and -triphosphate (Terra Marine), α-β-ATP-methylene diphosphonate (Terra Marine), β-γ-ATP-methylene diphosphonate (Terra Marine), adenosine-5′-*O*-(3-thiotriphosphate) (Boehringer, Mannheim), cordycepin (Sigma), coformycin and tubercidin (Sigma). Three analogues have been found to affect the activity of poly(ADP-Rib) polymerase in different strengths (Table): Formycin B (Calbiochem), showdomycin (Calbiochem) and 1-methyl-adenine (Sigma). These 3 compounds inhibit the enzyme competitively to the NAD substrate. The inhibition kinetics obtained are perfectly linear, both in LINEWEAVER and BURK plot[8] and in DIXON plot[9]. One example is shown for formycin B in the Figure. The highest affinity among the three inhibitors to the enzyme is found in the case of formycin B; showdomycin and 1-methyl adenine are less effective.

The three different enzyme poisons tested are without influence on the activity of poly(ADP-Rib) polymerase: Rifamycin (Calbiochem; final concentration in the assay 0.2 mg/ml), α-amanitin (Boehringer Ingelheim; final concentration 0.1 mg/ml) and 2-phenylethanol (Mack; final concentration 1 mg/ml). To rule out the possibility that these compounds which are capable of inhibiting poly(ADP-Rib) polymerase are contaminated with NMN adenylyltransferase, the compound solutions were assayed for this enzyme; no enzyme activity could be detected[10]. NMN adenylyltransferase could convert NAD into NMN, a potent inhibitor of poly(ADP-Rib) polymerase[11].

Summary. The activity of poly(ADP-Rib) polymerase is enhanced in the presence of spermine and spermidine. Among the adenosine-like antibiotics tested, only formycin B and showdomycin cause an inhibition of the enzyme, which is competitive to NAD. The activity of poly(ADP-Rib) polymerase is not reduced by rifamycin, α-amanitin and 2-phenylethanol.

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14 March 1975.

[8] H. LINEWEAVER and D. BURK, J. Am. Chem. Soc. *56*, 408 (1934).
[9] M. DIXON and E. C. WEBB, *The Enzymes*, 2nd edn. (Longmans and Green, London 1966), p. 315.
[10] M. R. ATKINSON, J. F. JACKSON and R. K. MORTON, Biochem. J. *80*, 318 (1961).
[11] J. PREISS, R. SCHLAEGER and H. HILZ, FEBS Lett. *19*, 244 (1971).
[12] W. L. MCGUIRE and B. W. O'MALLEY, Biochim. biophys. Acta *157*, 187 (1968).
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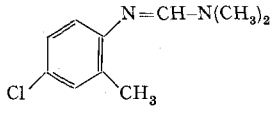
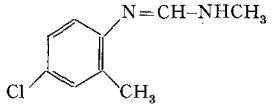
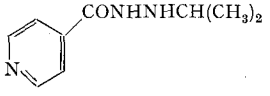
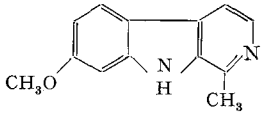
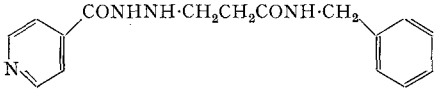
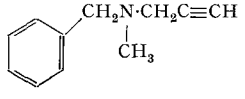
Chlorodimeform and its Effect on Monoamine Oxidase Activity in the Cattle Tick, *Boophilus microplus*

Galecron (active ingredient chlorodimeform) is a relatively new acaricide which is particularly effective against cattle ticks, including resistant strains[1]. Its mode of action is uncertain but it is known not to involve inhibition of acetylcholinesterase[2], the mode of action of organophosphorus and carbamate acaricides. Two different modes of action for chlorodimeform have been proposed. ABO-KHATWA and HOLLINGWORTH[3,4] using mitochondria from rat liver and from cockroach thoracic muscle found that chlorodimeform uncoupled respiratory-chain phosphorylation and stimulated ATP-ase. However, as pointed out by BEEMAN and MATSUMURA[5] this uncoupling action would not give rise to chlorodimeform's known action on the central nervous system. AZIZ and KNOWLES[6] and BEEMAN and MATSUMURA[5,7] have shown chlorodimeform to be a potent in vitro inhibitor of monoamine oxidase (MAO) from both rat liver and cockroach thoracic muscle and this led them to suggest that inhibition of MAO may be the mode of acaricidal action.

[1] C. O. KNOWLES and W. J. ROULSTON, J. econ. Entomol. *66*, 1245 (1973).
[2] V. DITTRICH, J. econ. Entomol *59*, 889 (1966).
[3] N. ABO-KHATWA and R. M. HOLLINGWORTH, Life Sci. *11*, 1181 (1972).
[4] N. ABO-KHATWA and R. M. HOLLINGWORTH, Pest. Biochem. Physiol. *3*, 358 (1973).
[5] R. W. BEEMAN and F. MATSUMURA, Pest. Biochem. Physiol. *4*, 325 (1974).
[6] S. A. AZIZ and C. O. KNOWLES, Nature, Lond. *242*, 417 (1973).
[7] R. W. BEEMAN and F. MATSUMURA, Nature, Lond. *242*, 273 (1973).

Table I. In vitro inhibition of cattle tick monoamine oxidase by chlorodimeform and other compounds

Compound	Structure	I_{50} (M) (Mean of 4 experiments)
Chlorodimeform (I)		3.75×10^{-6}
N-Desmethylchlorodimeform (II)		2.75×10^{-6}
Iproniazid		1×10^{-4}
Harmine		3×10^{-4}
Nialamide		6×10^{-4}
Pargyline		3×10^{-8}

Although chlorodimeform is used principally as an acaricide, the majority of studies on its biochemical mode of action have used rats[6] or insects such as the cockroach *Periplaneta americana*[5]. Our own studies have been with the cattle tick *Boophilus microplus*, and in particular we looked to see if MAO inhibition could be related to the toxicity of the compound to the tick.

Susceptible strains of the larvae of the cattle tick, *Boophilus microplus*, were kindly donated by Dr F. S. DOWNING (ICI Pharmaceuticals Division). After freezing for 30 min the larvae were weighed and a 10% homogenate prepared in 0.25 M sucrose/0.002 M calcium chloride, containing dithiothreitol (100 mg/l). The homogenate was filtered through cheese cloth and used immediately for assay of MAO activity using the radioactive method described by WURTMAN and AXELROD[8]. Tryptamine bisuccinate (side chain-2-^{14}C; specific activity 2 mCi/mmol and 10^{-6} M) was used as substrate. The standard incubation mixture consisted of 100 µl 10% enzyme homogenate 200 µl of 0.3 M potassium phosphate buffer, pH 7.4 and 100 µl substrate. Inhibitors were added to the reaction mixture in 10 µl of ethanol and pre-incubated with the enzyme for 30 min. The reaction was started by addition of substrate. Following incubation at 33 °C for 60 min, the reaction was stopped by addition of 400 µl 3 N hydrochloric acid. The non-basic products were extracted into 4 ml of toluene by shaking (blanks containing boiled enzyme were used to correct for the small amount of substrate extracted by the toluene). After centrifugation, the radioactivity in 1 ml of the organic layer was measured by liquid scintillation counting using a Packard Tri-Carb Scintillation counter fitted with an absolute activity analyzer. Results are expressed as the concentration of inhibitor in mol/l giving 50% inhibition of MAO activity (I_{50}) and are the mean of 4 experiments.

To study the effect of chlorodimeform and its metabolite, N-desmethylchlorodimeform[9] on the in vivo levels of MAO, cattle tick larvae were enclosed in vials containing filter paper impregnated with 500 µg of the chemicals. To study the effect of piperonyl butoxide, the filter paper was impregnated with a mixture of toxicant (500 µg) and synergist (1,000 µg). At various times after exposure larvae were removed and their MAO levels determined. The percentage mortality was also assessed by visual examination at times up to 48 h after the initial exposure.

Table I shows the in vitro inhibition of MAO from susceptible strains of cattle tick larvae by chlorodimeform (I), N-desmethylchlorodimeform (II), and some classical MAO inhibitors. Both amidines are potent inhibitors of cattle tick MAO. The effect of piperonyl butoxide on the toxicity of the compounds to the tick was examined. The results presented in Table II show that the toxicity of chlorodimeform was antagonized by the piperonyl butoxide, the ticks showing no symptoms of poisoning up to 72 h after treatment. In contrast chlorodimeform, in the absence of piperonyl butoxide caused 100% mortality 24 h after treatment. N-Desmethylchlorodimeform caused 100% mortality whether the chemical modifier was present or not and no synergism was observed. Control experiments were carried out using the standard MAO inhibitors iproniazid, nialamide and pargyline at a concentration of 1000 µg. However such compounds failed to produce any inhibition of MAO

[8] R. J. WURTMAN and J. AXELROD, Biochem. Pharmac. *12*, 1439 (1963).

[9] S. AHMAD and C. O. KNOWLES, Comp. gen. Pharmac. *2*, 189 (1971).

Table II. In vivo inhibition of MAO from cattle tick larvae after treatment with chlorodimeform and N-desmethylchlorodimeform

Time (h)	Treatment (% inhibition)			
	Chlorodimeform	Chlorodimeform and piperonyl butoxide	N-desmethylchlorodimeform	N-desmethylchlorodimeform and piperonyl butoxide
0	0	0	0	0
5	74	65	60	54
24	81	81	68	65
48	82	81	75	74
Mortality (%)				
5 h	0	0	0	0
24 h	100	0	100	100

Results are the mean of up to 5 separate experiments.

in vitro nor was there any detectable mortality using such compounds. This can probably be attributed to their lack of penetration of the cattle tick larvae.

These results suggest that inhibition of MAO cannot be the primary mode of action of chlorodimeform in cattle ticks. Chlorodimeform and its metabolite N-desmethylchlorodimeform are equally good inhibitors of MAO in vivo, and yet ticks survive with low MAO activities when metabolism of chlorodimeform is inhibited by the presence of piperonyl butoxide. Our results also support the view that in the tick N-desmethylchlorodimeform rather than chlorodimeform may be the actual toxicant[1].

The mode of action of chlorodimeform could still involve an interference with neuroactive amines, either by inhibition of other regulatory enzyme systems or by interference with the uptake processes for monoamines. We are continuing our investigations along these lines.

Summary. The action of the acaricide, chlorodimeform and its metabolite, N-desmethylchlorodimeform, on the activity monoamine oxidase (MAO) from the cattle tick, *Boophilus microplus*, were studied, Both compounds were found to be potent in vitro and in vivo inhibitors of the enzyme. However the inhibition of MAO does not seem to be related to the toxic action of the acaricide.

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α^5 Pyridoxalacetic Acid and α^5 Pyridoxyl-L-Phenylalanine Acetic Acid: Their Action on some B_6-Dependent Enzymes

The interaction between pyridoxal-P and apoenzymes pyridoxal-P dependent has been studied using a variety of analogues of pyridoxal-P in an attempt to investigate the role of functional groups of the coenzyme in binding and in catalysis[1].

The results of Groman et al.[2] on coenzymatic activity of 6 analogues with the position 5'-modified on 3 bacterial enzymes tryptofanase, D-serine dehydratase, arginine decarboxylase, support the view that 5'-phosphate group, in addition to contributing importantly to binding, is also important for precise positioning of the coenzyme on the enzyme surface and that the requirement for achieving this precise positioning also must vary from one enzyme to another.

In the present paper we describe the interaction between 4 apoenzymes pyridoxal-P dependent, tyrosine transaminase from rat liver (TAT), tyrosine decarboxylase from *Streptococcus faecalis* (TDC), aspartate transaminase from pig heart and aspartate transaminase from wheat germ (AAT) and an analogue of pyridoxal-P, the α^5-pyridoxalacetic acid, and a pyridoxalacetic acid derivative, the α^5-pyridoxyl-L-phenylalanine acetic acid, compound with structure similar to that generally postulated for the initial substrate-coenzyme complex formed during the enzymic reaction catalyzed by B_6-dependent enzymes[3].

Materials and methods. The purification of TAT[4,5], AAT from wheat germ[6,7] and from pig heart[8,9] and TDC[10], their conversion to their respective apoproteins, and assay of their enzymatic activities were performed as described in the cited references. The pyridoxal-P analogue which contains the substituent CH_2CH_2COOH in the 5'-position has been synthesized according to Iwata and Metzler[11]: further purification was achieved

[1] E. E. Snell, Vitams Horm. *28*, 265 (1970).
[2] E. Groman, Y. Z. Huang, T. Watanabe and E. E. Snell, Proc. natn. Acad. Sci., USA *69*, 3297 (1972).
[3] C. Turano, C. Borri, A. Orlacchio and F. Bossa, FEBS Symposium *18*, 123 (1970).
[4] S. C. Hayashi, D. K. Granner and G. M. Tomkins, J. biol. Chem. *242*, 3998 (1967).
[5] T. I. Diamondstone, Analyt. Biochem. *16*, 395 (1966).
[6] M. Martinez-Carrion, C. Turano, E. Chiancone, F. Bossa, A. Giartosio, F. Riva and P. Fasella, J. biol. Chem. *242*, 2397 (1967).
[7] V. Scardi, P. Scotto, M. Iaccarino and E. Scarano, Biochem. J. *88*, 172 (1963).
[8] A. Orlacchio and C. Turano, Congr. Soc. ital. Biochim., Pavia, Com. (1970), p. 206.
[9] A. Karmen, J. clin. Invest. *34*, 131 (1955).
[10] H. Muruyama and D. B. Coursin, Int. Symposium on Pyridoxal Enzymes, Japan (1967), p. 235.
[11] C. Iwata and D. E. Metzler, J. heter. Chem. *41*, 319 (1967).