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SOME PYRAZOLES AS INHIBITORS OF PURINE BIOSYNTHESIS *DE NOVO**

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Abstract—The inhibitory effect of some pyrazole derivatives on purine biosynthesis was studied in a pigeon liver cell-free system. It was demonstrated that 3-amino-4-carbethoxypyrazole, 3-amino-4-carboxypyrazole and 3-(3',3'-bis-β-chloroethyltriazenyl-1')-4-carbethoxypyrazole were inhibitors, while *N*-β-hydroxyethyl-3-amino-4-carbethoxypyrazole was almost inactive. A possible mechanism of action is discussed.

We found previously [1, 2, 3] that some pyrazole derivatives inhibit the growth of several strains of microorganisms. The compounds were structural analogues of 5-amino-4-imidazolecarboxamide the ribose-5-phosphate derivative of which is a precursor of purine biosynthesis *de novo*. Previously [1] it was shown that aminoimidazole carboxamide did not reverse the inhibitory effect of aminocarbethoxypyrazole on the growth of microorganisms. The compounds tested belong to four different series (Fig. 1 A, B, C, D). The first three of them differ in the *N*-2 substituent in the pyrazole ring, and the fourth in the alkyltriazeno group, replacing the 3-amino group. It was established that the triazeno-derivatives (series D) were the most active [3], followed by the compounds of series A with no substituent at *N*-2. The remaining compounds from series B and C with a β-hydroxyethyl or phenyl substituent at *N*-2 inhibited only slightly the growth of the microorganisms tested. The triazene derivatives are not very stable on prolonged storage and especially when exposed to light. However, the rate of decomposition is very low and under our experimental conditions no measurable degradation of 3-(3',3'-bis-β-chloroethyltriazenyl-1')-4-carbethoxypyrazole was detected.

We suggested that the compounds investigated interfered with purine biosynthesis. To check this assumption some of the analogues were tested in a pigeon liver cell-free system. The compounds were selected on the basis of the results from the microbiological screening. From the series A and B the most active compounds, i.e. the esters of the corresponding *N*-unsubstituted and *N*-β-hydroxyethyl substituted aminopyrazole carboxylic acids were tested. The less active 3-aminopyrazole carboxylic acid was also tested for comparison. The ester of *N*-phenyl substituted aminopyrazole carboxilic acid, as well as the other compounds of series C were water-insoluble and for this reason were not studied in the cell-free system. From series D, 3-(3',3'-bis-β-chloroethyltriazenyl-1')-4-carbethoxypyrazole was tested.

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† Abbreviation used: ATP, adenosine-5'-triphosphate; FGAR, 5'-phosphoribosyl-*N*-formylglycinamide; GAR, 5'-phosphoribosylglycinamide.

MATERIALS AND METHODS

The acetone powder was prepared from pigeon liver according to the procedure described by Goldthwait and Greenberg [4]. The enzyme system was isolated before use by extraction of the powder with 10 volumes of veronal buffer, pH 7.5.

D,L-Homocystein was obtained *ex tempore* by reduction of homocystine with tin shavings in HCl (0.1 g of homocystine, 1 g Sn, 10 ml HCl). The reduction was carried out at 70–80° for 30 min. After dilution of the initial volume of 10 ml to 60 ml with distilled water the solution was saturated with H_2S. The precipitate was filtered out and the filtrate was evaporated to dryness under reduced pressure. The homocystein obtained was measured spectrophotometrically [3].

The inhibitors 3-amino-4-carbethoxypyrazole (ACEP), 3-amino-4-carboxypyrazole (ACP) and *N*-β-hydroxyethyl-3-amino-4-carboxypyrazole (*N*-β-hydroxyethyl-ACEP) were obtained by the method of Schmidt and Druey [6] and Schmidt *et al.* [7]. 3-(3',3'-Bis-β-chloroethyl-triazenyl-1')-4-carbethoxy pyrazole (triazeno-CEP) was synthesized as described previously [3].

[^{14}C]Formate was purchased from Isocommerz (DDR), 3-phosphoglyceric acid from Koch-Light, ATP from Kyova, Hanko & Kogio Co Ltd, glycine and glutamine from Reanal and ribose-5-phosphate from Boehringer. All other substances used were analytical grade reagents.

The action of the compounds on purine biosynthesis was tested by measuring the *in vitro* incorporation of [^{14}C]formate into inosinic acid using a slight modification of the technique of Goldthwait and Greenberg [4] and Slavikova [8]. The incubation mixture contained in a final volume of 2.3 ml the following compounds in μmoles: glutamine, 10; ATP, 5; ribose-5-phosphate, 50; 3-phosphoglyceric acid, 7.3; $MgCl_2$, 5; D,L-homocystein, 5; glycine, 5; veronal buffer (pH 7.5), 50; boiled extract of pigeon liver, 0.20 ml; enzyme extract, 0.50 ml; [^{14}C]formate, 0.5 μCi; inhibitors, 20, 10, 5, 1, 0.5 or 0.1 μmoles. The samples were incubated at 37° for 40 min and then 1 ml of 10% CCl_3COOH was added to precipitate the proteins. The veronal buffer and CCl_3COOH were removed by successive ether extractions. Hypoxanthine, 0.5 mg, was added to each sample as a carrier

Table 1. The inhibitory effect of 5 μmoles of 3-amino-4-carbethoxypyrazole (ACEP), 3-(3′,3′-bis-β-chloroethyl-triazenyl-1′)-4-carbethoxypyrazole (triazeno-CEP), 3-amino-4-carboxypyrazole (ACP) and *N*-β-hydroxyethyl-3-amino-4-carbethoxy pyrazole (*N*-β-hydroxyethyl-ACEP) on the *in vitro* incorporation of [^{14}C]formate into inosinic acid (see text).

Pyrazole analogue	Inhibition (%)	Number of experiments
ACEP	91 ± 4	4
Triazeno-CEP	90 ± 2	5
ACP	77 ± 5	4
N-Hydroxyethyl-ACEP	5 ± 1	4

The results are expressed as Mean ± S.E.M. 4 or 5 experiments in which the cpm were in the range 700–1500 for the controls (taken as 100 per cent) and 50–100 for the blank samples.

and hydrolysis was carried out with 0.3 ml of 2% $FeCl_3$ in 2 N HCl in a boiling water bath for 40 min. The samples were evaporated to dryness, dissolved in 0.5 ml of water and chromatographed on Whatman No. 3 paper. The chromatograms were developed in water-saturated *n*-butanol and dried. The hypoxanthine spots were cut out, extracted with 2 ml of 0.1 N HCl, mixed with 10 vol of toluene–Triton X-100 scintillation fluid and counted with a Packard Tricarb 3200 liquid scintillation spectrometer [9].

RESULTS AND DISCUSSION

The pyrazole derivatives tested inhibited the purine biosynthesis to a varying extent. As shown in Table 1 the most active inhibitors were ACEP and triazeno-CEP. ACP had a lower activity, while at the same concentration, *N*-β-hydroxyethyl-ACEP was almost inactive.

The dose-dependence of the inhibitory action of the analogues is shown in Fig. 2. The inhibitory effect of ACEP, ACP and *N*-β-hydroxyethyl-ACEP correlates with their effect on the growth of microorganisms. Although triazeno-CEP and ACEP displayed almost equal activities in the cell-free system, all triazeno derivatives were considerably more active, when tested on bacteria. This discrepancy may be explained by the probable alkylating action of the alkyltriazeno-group in the cells of the microorganisms.

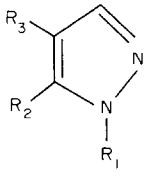

Fig. 1. Pyrazole derivatives: A—R_1—H; R_2—NH_2; R_3—COOH, —COOEt, —$CONH_2$, —$CONHNH_2$, —CN, —$C(:NOH)NH_2$; B—R_1—CH_2CH_2OH; R_2—NH_2; R_3—COOH, —COOEt, —$CONH_2$, —$CONHNH_2$, —CN, —$C(:NOH)NH_2$; C—R_1—C_6H_5; R_2—NH_2; R_3—COOH, —COOEt, —$CONH_2$ —$CONHNH_2$, —CN, —$C(:NOH)NH_2$; D—R_1—H, —CH_3, —CH_2CH_2OH; R_2—$N:NN(CH_2CH_2Cl)_2$, —$N:NN(CH_3)_2$, —$N:NN(C_2H_5)_2$; R_3—COOEt.

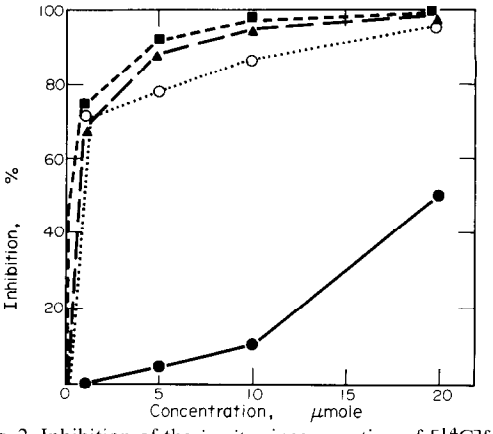

Fig. 2. Inhibition of the *in vitro* incorporation of [^{14}C]formate into inosinic acid by different pyrazole analogues (see text); --■--■--, 3-amino-4-carbethoxypyrazole (ACEP); --▲--▲--, 3-(3′,3′-bis-β-chloroethyl-triazenyl-1′)-4-carbethoxypyrazole (triazeno-CEP); ····○····○··, 3-amino-4-carboxypyrazole (ACP); ——●——●——, *N*-β-hydroxyethyl-3-amino-4-carbethoxypyrazole (*N*-β-hydroxyethyl-ACEP).

Analysis of the incorporation of labeled precursor into inosinic acid demonstrated that the pyrazole derivatives tested were *in vitro* inhibitors of purine biosynthesis *de novo*. It was found that the compounds unsubstituted at the N-2 position of the pyrazole ring were more active in the cell-free system as well as on microorganisms. Some purine nucleotides and their analogues are known to inhibit the first steps of purine biosynthesis [10, 11] up to the step of FGAR formation. It is likely, therefore, that the compounds tested act at some of these stages, and as corresponding ribose-5-phosphates. Two facts support this assumption: (a) the *N*-2-substituted compounds are almost inactive in a cell-free system and (b) those active in a cell-free system and on most microorganisms are not active on *E. coli* for which Le Gal *et al.* [12] reported GAR synthesis from ammonia and ribose-5-phosphate.

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