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Structural and Biological Links between Urea- and Purine-cytokinins

Structural and biological correlation between two classes of cytokinins, purine- and urea-cytokinins, were discussed. The biological response in the presence of competitive antagonists of cytokinins suggested that benzyladenine and N-(4-pyridyl)-N′-phenylurea have a common active site for the growth of tobacco callus.

Keywords——cytokinin; tobacco callus; tissue culture; N-(4-pyridyl)-N′-phenylurea; cytokinin antagonist; structure-activity relationship

In the accompanying paper we described the high cytokinin activity of N-(4-pyridyl)-N′-phenylureas (**1**: X=Y=H: 4PU) with an electronegative substituent(s) at positions 2 and/or 6 of the pyridine ring in the standard tobacco callus bioassay.[1] In particular, N-(2,6-dichloro-4-pyridyl)-N′-phenylurea (**1**: X=Y=Cl) has a surprising high activity which highly exceeds that of purine cytokinins such as benzyladenine (**2**: BA) and zeatine. The high activity of these urea-cytokinins prompted us to examine the relationship between urea- and purine-cytokinins. This structural relationship was discussed by Leonard and Skoog,[2] who studied ureidopurines. Our approach to clarifying the relationship was to examine the effect of deletion of parts of BA.[3] Deletion of the pyrimidine ring followed by side chain modification led to formation of imidazole carbanilide (**3**) and isonicotinic acid anilide (**4**) (Correlation A in Fig. 1). These two compounds were active (10^{-6}—10^{-7} M) in the tobacco callus bioassay. Combination of **4** with cytokinin active N,N′-diphenylurea (DP) gave 4PU.[3] On the other hand, deletion of the imidazole ring of the purine showed that 4-aminopyrimidine derivatives (**5**: R=benzyl, isopentenyl or benzoyl) have cytokinin activity (10^{-6} M) (Correlation B in Fig. 1).[4] Through 4-benzoylaminopyrimidine and N-(4-pyrimidyl)-N′-phenylurea (**5**: R=PhNHCO–), 4PU is correlated to BA. One or both the correlations may be important, or on the contrary both may be superficial. However, in any case, there is apparant correlation between BA and 4PU, and the latter compound is closely related to N,N′-diphenylurea.

The two classes of cytokinins appear to have very similar biological activities covering a broad range of tissues and species. All the biological activities so far tested, such as cell divi-

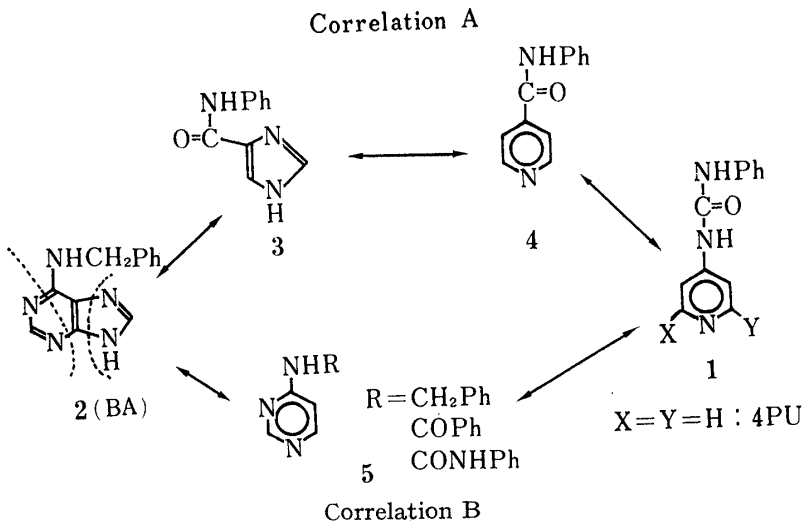

Fig. 1. Structural Correlation between Benzyladenine and N-(4-Pyridyl)-N′-phenylurea

sion, shoot formation, chlorophyll retention, inhibition of root growth, and inhibition of flower shedding, were similar, the principal differences between them being in the concentrations required for activity. These results support the idea that the two classes of compounds have a common active site. But one argument against urea- and purine-cytokinins acting at the same site is that at first glance the chemical structures of these compounds are so different, though this possibility seems to be ruled out by the structural correlation discussed above.

Another hypothesis is that urea-cytokinins do not exert biological activity directly, but stimulate the production of purine-cytokinins. The physiological level of cytokinin may be controlled by rates of synthesis or destruction, or of its transport. In these cases the active sites of urea- and purine-cytokinins must be different. It is another possibility that urea- and purine-cytokinins act separately, and accordingly that their active sites are different, although many of their biological effects are the same.

Cytokinin antagonists[5,6] are very useful for obtaining information about the active sites of urea- and purine-cytokinins. 4-(Cyclobutylamino)-2-methylpyrrolo[2,3-*d*]pyrimidine (CP) is a strong antagonist developed by Iwamura.[6] CP is a competitive antagonist of BA, reversibly inhibiting the effect of BA on cell division. N-Benzyl-N′-(4-bromophenyl)urea (BP) was found to inhibit DP and BA when examined by tests on chlorophyll retention.[7] If the active site of the two groups of cytokinins are same, CP should inhibit urea-cytokinins as well as BA, and BP should inhibit the purine-cytokinins as well as urea-cytokinins. In addition the mode of inhibition should be competitive: further addition of the cytokinins should restore the activity to that without the inhibitor. We confirmed this cross competitive inhibition by BP and CP in an experiment on callus growth (Fig. 2). Growth curves a in Fig. 2 show the activities of 4PU and BA, respectively, in the absence of inhibitors. Curves b and c were obtained in the presence of BP (3.3×10^{-5} M) and CP (5×10^{-7} M), respectively. Both BP and CP inhibited the growth induced by BA and 4PU, but higher concentrations of BA and 4PU restored their activities to the maximal levels without inhibitors. The shifts of the corresponding curves of the two compounds were similar. This inhibition experiment shows that the active site of BA is competitively occupied by a urea derivative and the active site of 4PU is competitively occupied by a purinoid compound. These results favor the idea that urea- and purine-cytokinins have a common active site.

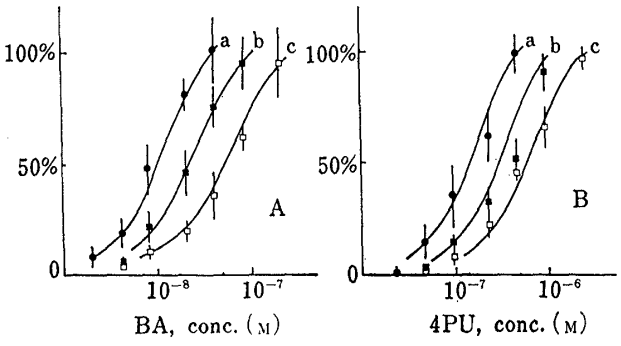

Fig. 2. Cross Competitive inhibitions of Cytokinin Activity of 4PU and BA by Antagonists BP and CP

A: 6N-benzyladenine. B:N-(4-pyridyl)-N′-phenylurea. Curves a (●), growth curves with BA and 4PU in the absence of antagonists. Curves b (■) and c (□), growth curves with BA and 4PU in the presence of BP (3.3×10^{-5} M) and CP (0.5×10^{-6} M), respectively. The maximum yield of callus in the presence of BA or 4PU is shown as 100%. The maximum yields in the presence of the antagonists were identical with the best yield without antagonists.

In this paper we discussed the structure-activity relationship between urea- (in particular 4-pyridylureas) and purine-cytokinins and report evidence for a common active site in the two classes of cytokinins obtained using cytokinin antagonists. Any mechanism for the action of purine-cytokinins must be the same time as that for the action of urea-cytokinins, because these two types of cytokinins bind to the same active site. This guide should be useful in studies on the true cytokinin receptor.

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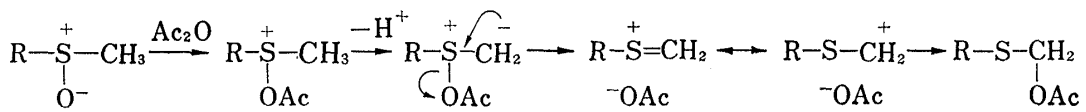
Reaction of Benzene with Diphenyl Sulfoxides

Diphenyl sulfoxide reacted with benzenes in the presence of trifluoroacetic anhydride and trifluoromethanesulfonic acid to yield triphenylsulfonium ions in good yields. A new electrophilic species was proposed.

Keywords——electrophilic aromatic substitution; diphenyl sulfoxide; triphenylsulfonium salt; trifluoromethanesulfonic acid; benzene

The most widely known reaction of benzene is the substitution by electrophilic species.[1] The quest for new electrophiles has been overcome by employing strong acids or suitable leaving groups.[1] The iminium-benzenium dication produced from N-phenylhydroxylamine in the reaction catalyzed by trifluoromethanesulfonic acid (TFSA) is an example of new electrophiles which react with benzene under mild conditions.[2] The phenoxenium ion prepared by elimination of sulfonamide as the nucleofungal leaving group, also reacts with benzene.[3] This paper describes a discovery of a new electrophilic species which can react with benzene easily.

Sulfoxide, on acylation, gives an acyloxysulfonium ion. In the Pummerer type reactions, a proton abstraction on α-carbon atom and the subsequent elimination of an acyloxy group gives ion pairs; carbonium ion and acetate anion. A nucleophilic attack of acetate anion to the carbonium ion gives the product (eq. 1).[4]



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