STRUCTURE-ACTIVITY RELATIONSHIP OF CYTOKININS: CRYSTAL STRUCTURE AND CONFORMATION OF 6-FURFURYLAMINOPURINE (KINETIN)

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Received February 24,1975

Summary. The crystal structure of 6-furfurylaminopurine(kinetin) was determined from three-dimensional x-ray diffraction data. The N^6-substituent is distal to the imidazole ring. The molecules are linked across centers of inversion by pairs of N(6)-H$\cdots$N(7) and N(9)-H$\cdots$N(3) hydrogen bonds, utilizing the Hoogsteen sites for base pairing. From an analysis of the conformational preferences of cytokinins, an "active conformation" favourable for eliciting maximal cytokinin activity is proposed. The loss of cytokinin activity due to various chemical modifications such as the substitution at N^1, alteration of the methylene bridge, is correlated to the inability of cytokinins to achieve the active conformation.

Introduction. Kinetin[a] is a highly potent growth factor(cytokinin) which, along with other plant growth substances, promotes cell division and ensures orderly growth and development of plants (for recent reviews, see (1-3)). The discovery of this growth factor by Miller, Skoog and co-workers (4) led to the investigation of the range of the biological activity of kinetin and several of its analogues, and the structure-activity relationship of these compounds (3,5). Most active compounds contain a mono-substituted adenine ring with a side chain on N^6, but there are some exceptions (3) (see also (6) and references therein). These studies indicate that the degree of cytokinin activity conferred by the N^6-side chain of adenine derivatives depends on the physicochemical properties of the side chain such as dimension, conformation, presence of polar groups, saturation of double bonds etc., rather than the presence of a specific "functional" group. Consequently, the three-dimensional structure and conformation of kinetin will be of interest

[a] Abbreviations used are: Kinetin, 6-furfurylaminopurine; $6Pe^iAde$, N^6-(Δ^2-isopentenyl)-adenine; $2MeS6Pe^iAde$, the 2-methylthio analog of $6Pe^iAde$.

especially since kinetin is used as a reference compound for comparing cytokinin activities of other cytokinins and for deducing structure-activity relationships. This paper forms a part of our investigation relating the three-dimensional structure of cytokinins with their biological activity, and describes the results of our studies on kinetin.

Methods. Single crystals of kinetin suitable for X-ray diffraction analysis were obtained from hot ethanol solutions by slow cooling. The crystals of kinetin ($C_{10}H_9N_5O_1$) are triclinic, space group $P\bar{1}$ with cell constants at (22± 3)°C: $\underline{a}$ = 7.874(3)Å, $\underline{b}$ = 12.526(3)Å, $\underline{c}$ = 4.947(1)Å, α = 91.15(4)°, β = 99.15(6)°, γ = 96.35(4)°, Z = 2, $\rho_{obsd.}$ = 1.49 g.cm^{-3}, ρ_{calc} = 1.50 g.cm^{-3}. Three-dimensional intensity data (2213 reflections to the limit 2θ = 165° for CuK_α radiation) were collected using a GE XRD-6 diffractometer and Ross filters by the stationary crystal-stationary counter method (7). The structural solution was obtained by the multisolution technique (8,9) followed by a translation search procedure (10,11) to place the molecule at appropriate distances from the crystallographic centers of inversion. The structural parameters were refined to an R of 0.06 using the least-squares method with block-diagonal approximation. Individual anisotropic thermal parameters were applied to the non-hydrogen atoms. The hydrogen atoms were located from electron density difference maps; their positional and individual isotropic thermal parameters were included in the refinement.

Results and Discussion: Figure 1 illustrates the conformation of the kinetin molecule. The molecular conformation can be best described in terms of two planes; one passing through the ten atoms of the adenine nucleus, and the other through the furfuryl group. The dihedral angle between the two planes is ±79°. The orientation of the substituent on N^6 is distal (12,13) to the imidazole ring leading to the Hoogsteen (14) sites for base-pairing. The bases are paired across crystallographic centers of inversion by N(6)-H ···N(7) and N(9)-H···N(3) hydrogen bonds resulting in continuous ribbons of purine bases with the furfuryl ring pointing up and down from these sheets. The distal orientation of the N^6-substituent with respect to the imidazole ring is found to occur in several 6-substituted adenines and the importance of this conformation has been discussed (12,13,15). A comparison of the conformation of the three cytokinins, kinetin, $6Pe^iAde$ (16) and its 2-methylthio

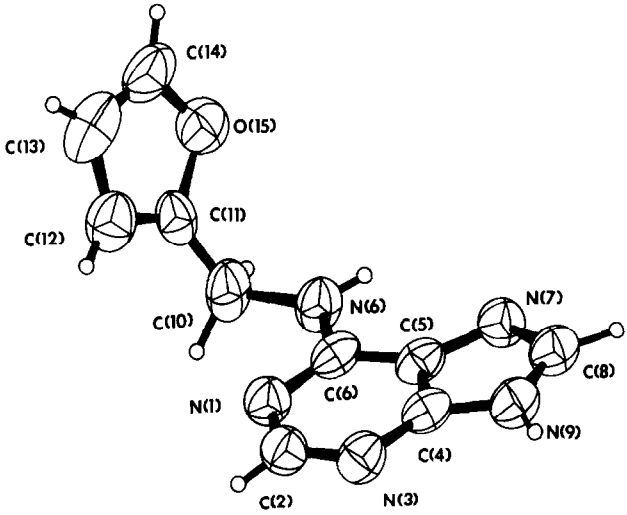

Figure 1
Conformation of kinetin molecule.

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analog (17) for which X-ray crystallographic studies have been carried out reveal some common and interesting structural and conformational features. In the crystal structure, these molecules are hydrogen bonded in a very similar manner, and even crystallize in the same space group ($P\bar{1}$). The plane through the adenine ring is inclined approximately perpendicular to the plane through those atoms of the side chain that take part in π-bonding, namely the isopentenyl group in 6Pe^{i}Ade and 2MeS6Pe^{i}Ade and the furfuryl group in kinetin. The dihedral angle between these two planes is ±79°for kinetin, ±72°for 6Pe^{i}Ade (16) and ±91°for 2MeS6Pe^{i}Ade(17). In order to discover whether any preferred conformations exists for the relative orientation of these two planes through the adenine and the side chain we studied the conformation of 126 compounds containing the group $\diagdown$X-CH_2-Y- (X is a C or N atom having sp^2 hybridization, Y is any atom) and for which the three-dimensional structures are known from X-ray crystallographic analysis. Our studies showed that in a majority of these molecules, the torsion angle ϕ_1 around the X-CH_2 bond with respect to the normal to the sp^2-plane of X is densely distributed ±20° around ϕ_1= 0 (and 180°)(Figure 2). This indicates a preference for a conformation in which the CH_2-Y bond nearly eclipses the $p_z$orbital of the atom X with sp^2-hybridization.

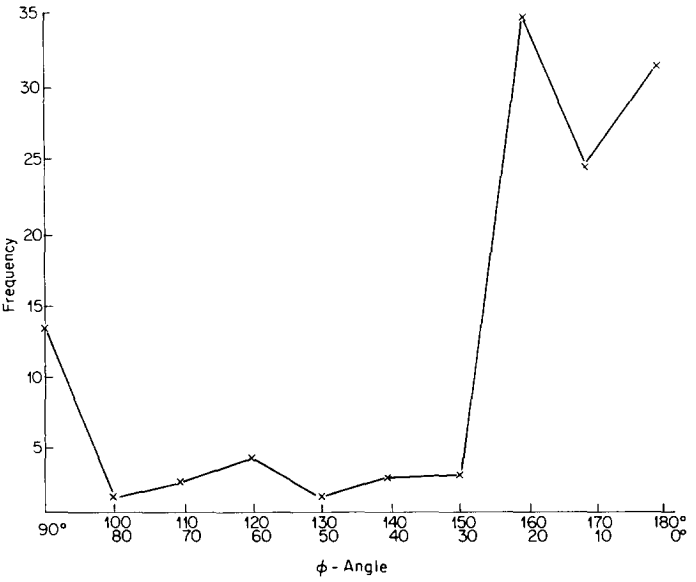

Figure 2
Distribution of the torsion angle ϕ about the $X-CH_2$ bond in 126 compounds containing the $>X-CH_2-Y-$ group.

This conformation will minimize any possible 1,4 or 1,5 type of non-bonded interactions between Y and any substituent on X. In 6Pe^{i}Ade, 2MeS6Pe^{i}Ade and kinetin, not only X but also Y is sp^2 hybridized: X is C(sp^2) and Y is N^6(sp^2). Consequently, the torsion angle ϕ_2 for X about the CH_2-Y bond with respect to the sp^2-plane of Y also needs to be considered. The preferred conformation of ϕ_2, analogous to ϕ_1 is distributed $\pm 20^\circ$ around $\phi_2 = 0$ (or 180°). The observed values for the pairs of torsion angles ϕ_1 and ϕ_2 for 6Pe^{i}Ade, 2MeS6Pe^{i}Ade and kinetin are respectively, $\pm 207.4^\circ, \pm 3.2^\circ$; $\pm 53.5^\circ, \pm 13.6^\circ$ and $\pm 197.9^\circ, \pm 10^\circ$. These values indicate that 6Pe^{i}Ade and kinetin will have their π-electrons of the N^6-substituent at approximately the same location relative to the adenine ring. 2MeS6Pe^{i}Ade does not exhibit such a conformation in the crystal (17); if the orientation of the isopentenyl group in 2MeS6Pe^{i}Ade were similar to that of 6Pe^{i}Ade, a somewhat short contact of 3.41 Å will result between a methyl group of the side chain and the S-methyl group. 2MeS6Pe^i-Ade could take up a conformation similar to 6Pe^{i}Ade if the methyl group on S is trans to N(1), and not cis as found in the crystal

structure. Since the 2-methylthio substituent has little influence on the cytokinin activity (18) and since the presence of this group will not necessarily prevent the 6Pe^{i}Ade-like conformation for 2Me-S6Pe^{i}Ade we shall propose (as a working hypothesis) that the conformation found for kinetin and 6Pe^{i}Ade may be regarded as favourable for eliciting cytokinin activity ("active conformation"). We shall investigate the consequence of this working hypothesis by studying the observed changes in biological activity due to the structural modifications and show that when the modified molecule is unable to assume this active conformation, it loses its cytokinin activity. By this procedure, it cannot be asserted that it is the conformation that determines the biological activity: extensive studies by several workers (see 5,3,19,20) have strongly indicated that it is the physical characteristics of the side chain on N^6 that determines the biological activity, and the molecular conformation is an important physical characteristic.

Since all (adenylate) cytokinins are N^6-substituted, the orientation of the side-chain has to be distal (12,13). In view of the conformational preferences of the substituents on the N(sp^2)-CH_2-C(sp^2) link about the N-CH_2 and CH_2-C bonds, 6-(trans-γ-hydroxy-methyl-γ-methylallylamino)-purine (zeatin) and N^6-benzylaminopurine may be expected to assume a conformation similar to 6Pe^{i}Ade and kinetin. But 6-(α,α-dimethylallylamino)purine cannot have its C_α-C_β bond nearly eclipsing the p_z orbital of N^6, since such an orientation will place one of the methyl group on C_α less than 2.5Å (from molecular model studies) from N(1) of adenine. Consequently 6-(α,α-dimethylallylamino)purine cannot assume the "active conformation": accordingly, its cytokinin activity is greatly reduced compared to 6-(γ,γ-dimethylallylamino)purine (5). Any substituent on N(1) will severely restrict the conformational freedom about the N^6-CH_2 bond because of the steric repulsion between the CH_2-group and the substituent on N^1: if the molecule were to assume the active conformation, one of the hydrogens on the -CH_2-group will make a very short contact with the substituent on N^1 (less than 2.0 Å from the methyl carbon, if a methyl group is on N^1). Consequently the active conformation is least likely, if not impossible, for the N^1-substituted analogs. This result is in complete accord with the structure-activity studies (5) that show that the 1-position of adenine must be free for cytokinin activity. The relative orientation of the side chain on N^6 with respect to adenine in the acti-

ve conformation depends on the $N(sp^2)-CH_2-C(sp^2)$ link and the conformational preferences of $N-CH_2$ and CH_2-C bonds. Any alteration in this link will impair this relative orientation and may be expected to affect adversely the cytokinin activity. This conclusion is in agreement with chemical studies (5) which show that bridges such as $-O-CH_2-$, $-NH-CH_2-CH_2-$, -NH-CO- or the removal of the methylene carbon, all lowered the biological activity considerably.

In addition to their role as plant hormones, certain cytokinins also occur as components of tRNA from several species (3,21), and possess antitumour activity in animal cells (22,23,24,25). Structure-activity relationships (25,26,27) show that N^6-substituted adenines which possess cytokinin activity, yield nucleosides which inhibit the growth of tumour cells, while the corresponding adenines are inactive. There is a remarkable similarity between the structural requirements of the N^6-side chain that confers cytokinin and antitumour properties, and hence our analysis of the conformation of cytokinins may be applied to these antitumour agents also.

Aeknowledgements. We are thankful to Drs D. Harker, G.B. Chheda, M.H. Fleysher and B.I.S. Srivastava for several discussions. This work in part was supported by a grant from the National Cancer Institute (CA-16844). M.S. thanks the Consejo Nal. de Ciencía y Tecnología de México (CONACYT) for a fellowship.

References.

1. Helgeson, J.P. (1968) Science 161, 974-981.
2. Skoog, F., and Armstrong, D.J. (1970) Ann. Rev. Plant Physiol. 21, 359-384.
3. Hall, R.H.(1973) Ann. Rev. Plant Physiol. 24, 415-444.
4. Miller, C.O., Skoog, F., von Saltza, M.H., and Strong, F.M. (1955) J. Amer. Chem. Soc. 77, 1392.
5. Skoog, F., Hamzi, H.Q., Szweykowska, A.M., Leonard, N.J., Carraway, K.L., Fuji, T., Helgeson, J.P., and Loeppky, R.N. (1967) Phytochem. 6, 1169-1192.
6. Tovigoe, Y., Akiyama, M., Hirobe,M., Okamoto, T., and Isogai, Y. (1971) Phytochem. 11, 1623-1630.
7. Furnas,Jr, T.C., and Harker, D. (1955) Rev. Sci. Instr. 26, 449-453.
8. Karle, J., and Karle, I. (1966) Acta Cryst. 21, 849-859.
9. Germain, G., Main, P., and Woolfson, M.M. (1971) Acta Cryst. A27, 368-376.
10. Karle, J. (1972) Acta Cryst. B28, 820-824.

11. Soriano-Garcia, M., and Parthasarathy, R., in preparation.
12. Parthasarathy, R., Ohrt, J.M., and Chheda, G.B. (1974) Biochem. Biophys. Res. Commun. 57, 649-653.
13. Parthasarathy, R., Ohrt, J.M., and Chheda, G.B. (1974) J. Amer. Chem. Soc. 96, 8087-8094.
14. Hoogsteen, K. (1959) Acta Cryst. 12, 822-823.
15. Parthasarathy, R., Ohrt, J.M., and Chheda, G.B. (1974) Biochem. Biophys. Res. Commun. 60, 211-218.
16. Bugg, C.E., and Thewalt, U. (1972) Biochem. Biophys. Res. Commun. 46, 779-784.
17. McMullan, R.K., and Sundaralingam, M. (1971) Biochem. Biophys. Res. Commun. 43, 1158-1163.
18. Hecht, S.M., Leonard, N.J., Schmitz, R.Y., and Skoog, F. (1970) Phytochem. 9, 1907-1913.
19. Schmitz, R.Y., Skoog, F., Hecht, S.M., Leonard, N.J. (1971) Phytochem. 10, 275-280.
20. Letham, D.S. (1967) Ann. Rev. Plant Physiol. 18, 349-364.
21. Stewart, P.R., and Letham, D.S. " The Ribonucleic Acids" , Springer- Verlag, N.Y. 1973, pp. 80-106.
22. Grace, Jr, J.T., Hakala, M.T., Hall, R.H., and Blakeslee, J. (1967) Proc. Am. Assoc. Cancer Res. 8, 23.
23. Suk, D., Simpson, C.L., and Mihich, E. (1968) Proc. Am. Cancer Res. 9, 69.
24. Jones, Jr, R., Grace, Jr, J.T., Mittleman, A., and Woodruff, W. (1968) Proc. Am. Cancer Res. 9, 35.
25. Fleysher, M.H., Hakala, M.T., Bloch, A., and Hall, R.H. (1968) J. Med. Chem. 11, 717-720.
26. Fleysher, M.H., Bloch, A., Hakala, M.T., and Nichol, C.A. (1969) J. Med. Chem. 12, 1056-1061.
27. Fleysher, M.H. (1972) J. Med. Chem. 15, 187-191.