Crystallographic Studies on Urea Cytokinins

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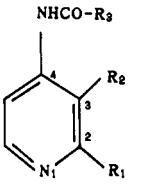
Some heteroaromatic ureas, such as *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (I) and *N*-(4-pyridyl-*N'*-phenylurea (II), exhibit strong cytokinin activity. However, the structure–activity relationship of urea cytokinins remains unclear. To elucidate this problem, we have performed X-ray crystal structure analyses of the above-mentioned compounds. It was found that all the free urea molecules take an extended trans conformation, whereas the *N*-methylurea molecule which is completely inactive takes a folded cis conformation in the crystalline state. We concluded that the trans extended conformation is required for cytokinin activity in 4-pyridylurea derivatives. However, the difference between the potent cytokinin activities of I and II as compared with the much weaker activities of IV and III, respectively, could not be explained in terms of the steric structures around the urea moiety.

INTRODUCTION

Cytokinins are plant growth hormones that control the proliferation (Skoog and Armstrong, 1970) and differentiation (Hall, 1973) of plants. In the course of our work on plant growth regulators, various aromatic urea derivatives (Bruce and Zwar, 1969) have been synthesized and assayed, and strong cytokinin activity was found in some heteroaromatic ureas. *N*-(4-Pyridyl)-*N'*-phenylurea (II) showed a strong cytokinin activity in the tobacco callus growth test, and *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (I, Forchlorofenuron) has even higher activity than zeatin, a potent cytokinin (Takahashi et al., 1978).

Our results can be summarized as follows: (1) the molecular requirements for high cytokinin activity include the presence of a 4-pyridyl, but not a 2- or 3-pyridyl moiety, and the presence of an *N'*-phenyl group (most substituents on the phenyl ring reduce the activity); (2) N-methylation abolishes the activity; (3) compounds with an electronegative nonpolar substituent (F, Cl, CN, CF_3, OCH_3) at position 2 of the pyridine ring have high activity, while polar substituents suppress the activity (Okamoto et al., 1981). Quantitative structure–activity analysis by the Hansch method was applied to clarify the structural factors contributing to the cytokinin activity of the aromatic ureas. The results showed that the major contributor is the electronic effect (σ_m) of the substituents and the contribution of their polarity is minor. No correlation between activity and steric size of the substituents was observed. Though this may have implications for the shape of the active site or the critical binding site of cytokinins, we do not have any definite stereochemical structural information on these urea compounds. Nevertheless, the active site of purine and urea cytokinins is common (Kurosaki et al., 1981), and a significant structural feature common to both groups of cytokinins seems to the flatness of a part of the molecules. We hypothesized therefore that the biologically active molecular shape or a part of the active site may be approximately planar or constructed from two planes, so that no special molecular shape other than flatness is required for activity, provided that the compound falls within a certain range of molecular size.

This paper describes the detailed structural studies of five representative aromatic urea cytokinins by X-ray crystallography to shed light on the above hypothesis as well as to provide a basis for consideration of the stereochemical structure–activity relationships. The generic chemical structure and cytokinin activity of this series of compounds are shown in Figure 1, together with the aromatic numbering.



	R1	R2	R3	optimum conc. M
(I)	Cl	H	NH-phenyl	4.0×10^{-9}
(II)	H	H	NH-phenyl	4.7×10^{-7}
(III)	3-pyridyl		NH-phenyl	4.7×10^{-6}
(IV)	H	Cl	NH-phenyl	4.0×10^{-6}
(V)	H	H	N(CH_3)-phenyl	inactive

Figure 1. Chemical structures of I–V in generic form.

EXPERIMENTAL PROCEDURES

The X-ray crystal structure analyses were performed on crystals of *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (I), *N*-(4-pyridyl)-*N'*-phenylurea (II), *N*-(3-pyridyl)-*N'*-phenylurea (III), *N*-(3-chloro-4-pyridyl)-*N'*-phenylurea (IV), and *N*-methyl-*N*-phenyl-*N'*-4-pyridylurea (V). A crystal of V contains two independent molecules in the asymmetric unit, and IV crystallizes as the monohydrate, while the crystals of all the other compounds simply contain one molecule in the asymmetric unit. Details of the crystal parameters, data collection, and refinement are listed in Table I. Intensity data were collected with a Rigaku AFC5 or Philips PW1100 diffractometer using graphite-monochromated Cu $K\alpha_1$ radiation (λ = 1.54050 Å) by the θ–2θ scan method. The typical ω scan width was $(1.3 + 0.41 \tan \theta)°$. Intensities were corrected for Lorentz and polarization factors, but no correction was made for absorption.

Structures were solved by using the program package SAPI85 (Yao et al., 1085) version of MULTAN (Main et al., 1980). The refinement was carried out by the full-matrix least-squares method with anisotropic thermal parameters for non-hydrogen atoms. The function minimized was $\sum w(|(|F_o|)^2 - (|F_c|)^2|)^2$ with $w = 1/[\sigma^2(F_o) + 0.02(F_o)^2]$, while $\sigma(F_o)$ was determined from counting statistics. All H atoms located from the difference map and from theoretical calculations were refined. All calculations were performed by using a Panafacom computer with the RCRYSTAN (Rigaku Corp., 1985) X-ray analysis program system. The

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0021-8561/91/1439-0793$02.50/0

Table I. Details of Cell Dimensions, Data Collection, and Structure Refinement

	I	II	III	IV	V
formula	$C_{12}H_{10}N_3OCl$	$C_{12}H_{11}N_3O$	$C_{12}H_{11}N_3O$	$C_{12}H_{10}N_3OCl{\cdot}H_2O$	$C_{13}H_{13}N_3O$
weight	247.7	213.2	213.2	265.5	227.3
space group	$P2_1/a$	$P2_1/a$	$P2_1/nb$	$Pna2_1$	$P\bar{1}$
cell dimensions					
lattice a, Å	12.619 (1)	12.553 (3)	10.631 (1)	11.617 (1)	10.766 (1)
b	11.572 (1)	11.866 (3)	19.821 (3)	15.990 (1)	14.445 (1)
c	7.794 (1)	7.297 (2)	5.074 (1)	6.997 (1)	8.724 (1)
α, deg	90.00	90.00	90.00	90.00	100.33 (1)
β	93.45 (1)	93.22 (1)	90.00	90.00	97.16 (1)
γ	90.00	90.00	90.00	90.00	115.92 (1)
V, Å^3	1142.5 (2)	1085.3 (5)	1069.4 (5)	1299.7 (2)	1168.4 (2)
Z	4	4	4	4	4
D_x, Mg m^{-3}	1.440	1.305	1.324	1.358	1.292
μ, mm^{-1}	2.853	0.714	0.725	2.6, 2	0.696
$F(000)$	512	448	448	552	480
data collection					
diffractometer	AFC5	PW1100	AFC5	PW1100	AFC5
crystal size, mm	0.10 × 0.15 × 0.05	0.40 × 0.30 × 0.50	0.45 × 0.60 × 0.05	0.40 × 0.20 × 0.35	0.50 × 0.15 × 0.50
max $(\sin\theta)/\lambda$, Å^{-1}	0.56	0.58	0.56	0.58	0.56
no. of reflections	1928	1176	1001	972	3240
independent	1650	1174	845	960	2965
$[F > 3\sigma(F)]$	1088	1134	799	949	2965
refinement					
R	0.057	0.045	0.050	0.038	0.059
wR	0.073	0.052	0.055	0.062	0.057
G^a	1.101	1.208	1.560	1.435	2.038
$(\Delta/\sigma)_{max}$	0.26	0.16	0.23	0.29	0.38

^{a} $G = \sum w[(|(|F_o|)^2 - (|F_c|)^2|)^2/(N_r - N_v)]^{1/2}$.

atomic scattering factors were taken from the *International Tables for X-ray Crystallography* (1974). The final atomic coordinates and thermal parameters for the crystals are deposited as supplementary material.

RESULTS AND DISCUSSION

The molecular structures of the five urea compounds are shown as stereoviews of the ellipsoid plots in Figure 2. A distinct difference of molecular shape is seen in the *N*-methylurea. All the free urea molecules (I–IV) adopt an extended conformation, whereas the *N*-methylurea molecule (V) adopts a folded conformation. The characteristic differences can be ascribed mainly to the differences in torsion angles at the *N*-methylurea site of the urea moiety.

Bond lengths and angles relevant to the urea bonds in the five molecules are listed in Table II. As can be seen from the bond lengths, C_1–N in V seems to be significantly longer than those in the other molecules. As for the bond angles, β_1 in V takes a significantly larger value than those in the other molecules, while β_3 shows the opposite tendency. These results can be ascribed to steric crowdedness due to the cis urea conformation. A comparison of the 3- and 2-chloro compounds (IV and I) reveals rather similar values. The urea C–N bonds show partial double-bond character in each compound, as evidenced by the rather short amide linkages [the N–C* and N′–C* distances ranged from 1.364 (6) to 1.389 (4) and from 1.361 (4) to 1.387 (6) Å, respectively. However, the degree of this contribution is smaller than in diphenylurea [1.340 (2) and 1.357 (2) Å] (Dannecker and Kopf, 1979). This indicates that the double-bond character of the C=O bond should be increased. In fact, compound I shows the shortest C*–O distance of 1.199 (6) Å within this series of compounds, indicating a higher double-bond contribution compared to the others. Planar hybridization at the nitrogen atoms can be observed in each compound, further supporting the double-bond character of the urea bonds. As for the hydridization character of the urea nitrogen of the *N*-methylurea, the sum of the three bond angles around nitrogen is close to 360°, suggesting sp^2 character for the nitrogen atom, although quantitative comparisons of the geometries with those of the free urea compounds could not be made due to the poor reliability of the atomic coordinates of hydrogens obtained from X-ray analyses. Deviations of the neighboring atoms from the least-squares planes, calculated from urea nitrogen, carbonyl carbon, and oxygen, are listed in Table III. The highest deviation is observed in I among the five ureas. These results are reflected in the torsion angles around the urea bonds. The values of the torsion angles are summarized in Table IV [ϕ_1, ω_1, ω_2, and ϕ_2 are defined as the torsion angles around C_1—N(H), N(H)—C*(=O), C*(=O)—N′(H) and N′(R)—C_1′, respectively]. All the free ureas take trans conformation with ω_2 of −5.5 (8)°, 9.1 (5)°, 1.0 (7)°, and −7.2 (9)° for I–IV, respectively, whereas the *N*-methylurea takes cis conformation with ω_2 of −164.8 (3)° [and −153.2 (3)°]. Thus, the deviations of the angle (ω_2) from 0° in the free ureas are within 10°, whereas in the *N*-methylurea the deviation from 180° is larger. A difference of interplanar angles between the ureas and the phenyl group between the free ureas and the *N*-methylurea is also found, reflecting the steric bulkiness of the methyl moiety. In the case of ω_1, deviations range from −12.5 (8)° to 11.3 (5)°, all being in the trans amide form. The small differences among these compounds may be due to crystal packing forces rather than intrinsic conformational stability. As can be seen from Table IV, the torsion angles ϕ_2 deviate significantly from 0°, indicating that resonance between the urea group and the neighboring benzene rings is rather small and does not contribute much to the overall stability of the compounds. On the other hand, no distinct characteristics were found in the distribution of ϕ_1 angle values except that the deviations from 0° are rather small, ranging from −0.2 (5)° to −13.9 (5)°. This can probably be ascribed to the intramolecular hydrogen bond between the hydrogen at C_3 and the carbonyl oxygen as a proton acceptor (Taylor and Kennard, 1982).

Because the pyridyl nitrogen acidifies the hydrogen, the interaction is stronger than the normal interaction in diphenylurea (C–H···O: 2.49 and 2.66 Å) and resembles

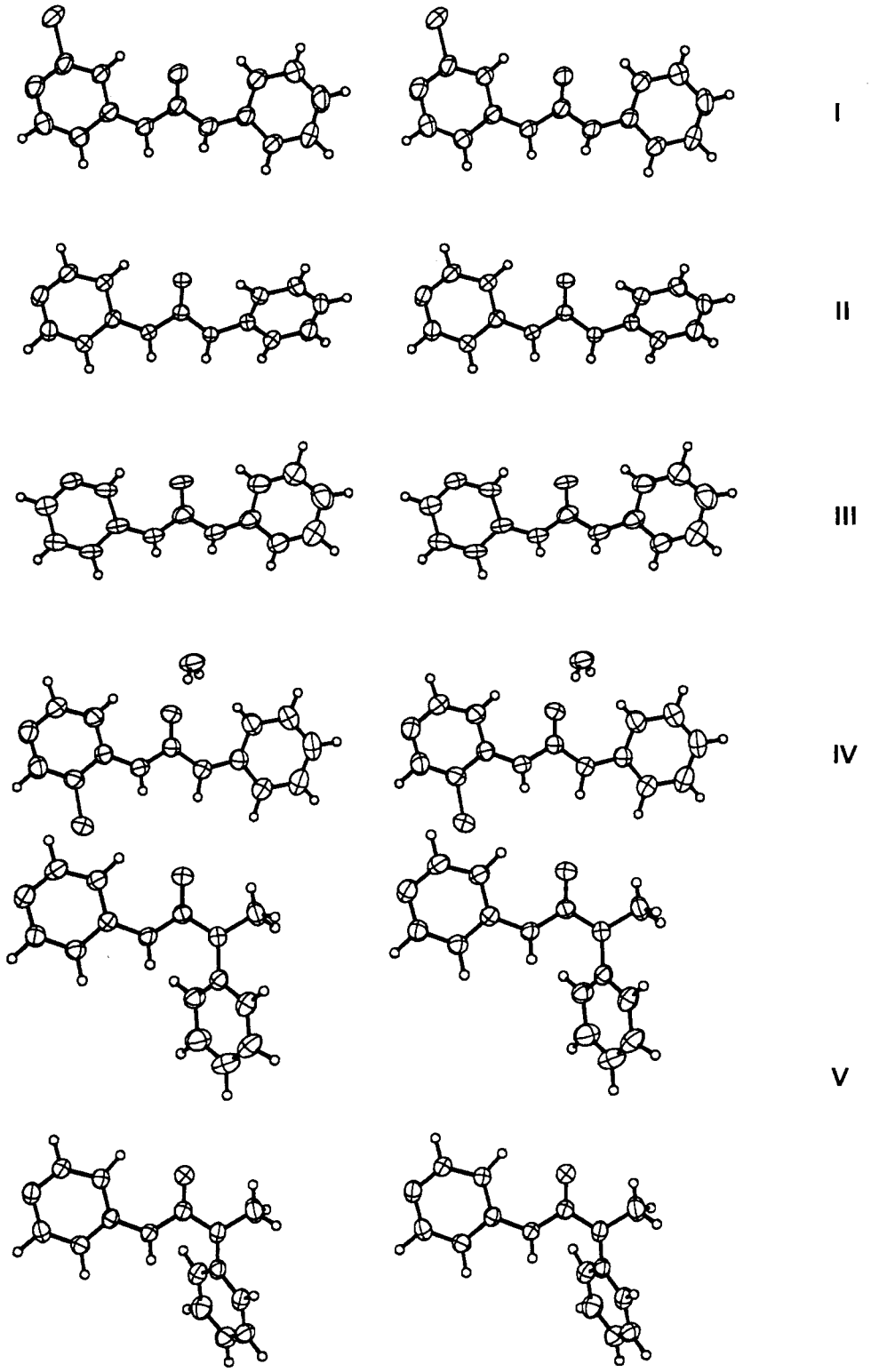
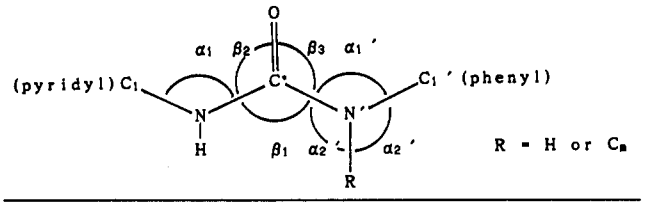


Figure 2. Molecular structures of I–V drawn as stereoviews of the ellipsoid plots.

that in the case of nitrodiphenylureas (Etter and Panunto, 1988). This is reflected by the short C–H···O distances in I–V: 2.45 (5), 2.19 (3), 2.34 (4), 2.26 (5), and 2.20 (3) Å [and 2.28 (3) Å], respectively. In the crystal structure, strong intermolecular hydrogen bonding between pyridyl nitrogen and carbonyl oxygen was observed in each compound except IV. Between the pyridyl nitrogen and the neighboring urea NH, intermolecular hydrogen bond distances of 2.24 (5) and 2.18 (3) Å [and 2.14 (3) Å] were observed for I and V, respectively, and distances of 2.04 (3) and 2.34 (4) Å were observed between pyridyl nitrogen and the urea N′H located on the phenyl side for II and III. In the case of crystalline IV, in which a water molecule is located in the asymmetric unit, intermolecular hydrogen bonds between the aqua hydrogen and the pyridyl nitrogen (N_1) and between the other aqua hydrogen and the carbonyl oxygen were observed.

The distances of C–H···N and O–H···O are 1.99 (5) and 1.96 (7) Å. Both I and IV in the crystalline states exist in an extended trans structure, though their biological activities are very different. This change could be explained by the stereochemistry of the urea moieties. However, we should note the difference of the relative orientations of the location of the chlorine atom and of the carbonyl group: In compound I the positions of the chlorine and the carbonyl are on the same side; this

Table II. Bond Lengths (Angstroms) and Angles (Degrees) Related to the Urea Bond for the Five Crystals[a]



	C_1–N_1	N–C*	C*–O	C*–N′	N′–C_1′
I	1.381 (6)	1.382 (7)	1.199 (6)	1.387 (6)	1.403 (7)
II	1.393 (4)	1.389 (4)	1.206 (4)	1.361 (4)	1.422 (4)
III	1.374 (6)	1.364 (6)	1.216 (4)	1.361 (7)	1.382 (6)
IV	1.389 (6)	1.377 (6)	1.220 (7)	1.365 (6)	1.415 (6)
V	1.399 (4)	1.378 (5)	1.221 (4)	1.368 (4)	1.324 (5)
	1.401 (4)	1.378 (4)	1.217 (4)	1.379 (3)	1.405 (5)
DPU[b]	1.417 (4)	1.357 (2)	1.234 (4)	[1.340 (2)]	[1.422 (4)]

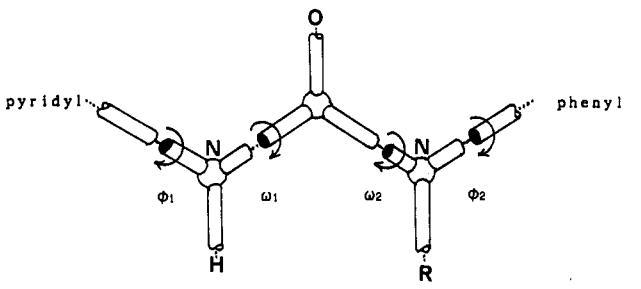
	α_1	β_1	β_2	β_3	α_1'	α_2'	α_3'
I	125.1 (4)	112.0 (4)	124.1 (4)	123.7 (4)	127.0 (4)		
II	127.1 (2)	11.4 (2)	123.0 (2)	125.4 (2)	123.8 (2)		
III	128.6 (3)	114.3 (3)	122.4 (4)	123.1 (4)	129.5 (3)		
IV	126.9 (4)	111.0 (4)	123.8 (4)	125.0 (4)	127.8 (4)		
V	125.6 (3)	115.0 (2)	123.1 (2)	121.8 (2)	125.4 (2)	116.5 (3)	117.2 (2)
	126.2 (2)	114.5 (3)	123.8 (2)	121.6 (3)	123.6 (3)	115.8 (3)	115.3 (2)

[a] Estimated standard deviations are shown in parentheses. [b] *N,N′*-Diphenylurea.

Table III. Deviations (Angstroms) of Neighboring Atoms to the Urea Bonds from the Corresponding Amide Plane

plane:	N, C*, O		N′, C, *O	
atoms:	C_1	N′	C_1′	C_m
I	−0.244	0.028	0.108	
II	0.144	0.000	−0.168	
III	−0.105	0.014	−0.020	
IV	−0.037	0.025	−0.143	
V	0.222	−0.034	−0.111	−0.537
	−0.221	0.050	0.116	0.478

Table IV. Torsion Angles (Degrees) Relating to the Urea Bond for the Five Crystals[a]



	ϕ_1	ω_1	ω_2	ϕ_2
I	−7.9 (7)	−12.5 (8)	−5.5 (8)	17.3 (7)
II	−1.1 (5)	6.5 (5)	9.1 (5)	35.3 (4)
III	−4.6 (7)	−5.6 (7)	1.0 (7)	−6.1 (7)
IV	−13.0 (8)	1.9 (8)	−7.2 (9)	12.9 (8)
V	−13.9 (5)	11.3 (5)	−164.8 (3)	−133.4 (3)
	−0.2 (5)	8.7 (5)	−153.2 (3)	−138.6 (3)

[a] Estimated standard deviations are shown in parentheses.

plausibly results from a certain interaction of the proton at the more acidic position 3 and carbonyl (Etter and Panunto, 1988). In compound IV the chlorine atom and the carbonyl are located in opposite directions. Since the importance of the position of the chlorine atom is the point, as in the case of phenoxyacetic acids (Wain and Wightman, 1953), this problem should be further studied.

CONCLUSIONS

The *N*-methylurea molecule (V) adopts the cis urea conformation in the crystalline state, whereas the free ureas (I–IV) take the trans urea conformation. We concluded that the trans extended conformation is required for cytokinin activity in 4-pyridylurea derivatives. However, the potent cytokinin activities of the 2-chloro compound (I) as compared with the 3-chloro compound (IV) and of the 4-pyridyl compound (II) as compared with the 3-pyridyl compound (III) could not be explained in terms of the steric structures around the urea moiety through these studies. Though a structure in crystals can be regarded as one of possible (local minimum) conformation, the crystalline structure does not always reflect the active structure on the receptor. The contribution of another possible conformation as well as the electronic factors to the activity is under investigation by computational methods using molecular mechanics, molecular dynamics, and molecular orbital calculations based on the structure analyses, presented here.

ACKNOWLEDGMENT

This work was supported by Toray Scientific Foundation for which we are greatly appreciative.

Supplementary Material Available: Listing of crystallographic data, atomic coordinates, and thermal parameters for the five compounds (10 pages). Ordering information is given on any current masthead page.

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Received for review July 16, 1990. Accepted October 29, 1990.

Registry No. I, 68157-60-8; II, 1932-35-0; III, 2000-55-7; IV, 76947-82-5; V, 80194-82-7.