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CYTOKININS: SYNTHESIS AND BIOLOGICAL ACTIVITY OF UREIDOPURINES*

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Abstract—Series of 6-aryl- and 6-alkylureidopurines have been prepared by the reaction of the appropriate isocyanate with adenine protected at the 9-position by the 1-ethoxyethyl group, followed by removal of this blocking group. The representative 6-ureidopurines were compared with 6-benzylamino-, 6-furfurylamino-, and 6-phenylaminopurine and with *N,N'*-diphenylurea for cytokinin (growth-promoting) activity in the tobacco bioassay. Seven of the compounds [6-phenylureidopurine, 6-*o*-tolylureidopurine, 6-*m*-chlorophenylureidopurine, 6-isopropylureidopurine, 6-allylureidopurine, and *N*-ethyl-*N'*-phenyl-*N*-purin-6-ylurea] ranged in activity in the order listed from *ca.* 10% to 0·5% that of 6-benzylaminopurine (activity starting in the range from 3×10^{-2} to 1 μM). All were equal to or more active than *N,N'*-diphenylurea and induced maximal yields of tissue. One, *p*-tolylureidopurine, was only weakly active, starting at 1 μM, and failed to give maximum yield at any concentration. 6-Phenylureido-9-β-D-ribofuranosylpurine was also synthesized. Its activity, starting at *ca.* 0·1 M was $<10\%$ that of 6-benzylamino-9-β-D-ribofuranosylpurine. The three most active 6-ureidopurines [6-phenylureidopurine, 6-*o*-tolylureidopurine, and 6-*m*-chlorophenylureidopurine] promoted bud formation in tobacco callus cultures, and their action may differ from that of the 6-alkylamino- or 6-arylaminopurine type.

INTRODUCTION

THE ISOLATION of diphenylurea from coconut milk and its cytokinin activity were reported first by Shantz and Steward,[1] and subsequently the relation between structure and cytokinin activity of a series of substituted phenylurea derivatives was investigated by Bruce, Zwar and Kefford.[2–5] These results suggested to us that phenylureidopurines would be of special interest as growth and cell-division factors. We have synthesized and tested the substituted ureidopurines XI–XVII because of their accessibility, the spread of activities in the correspondingly substituted phenylureas, and, in the case of XVII, the gross structural similarity to 6-(3-methyl-2-butenylamino)purine.[6] The natural occurrence of a ureidopurine,

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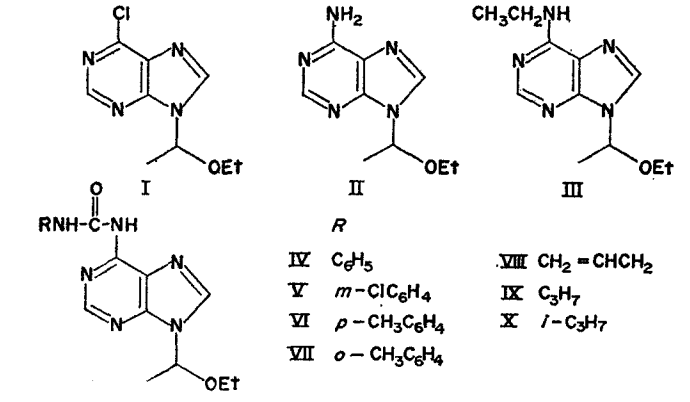
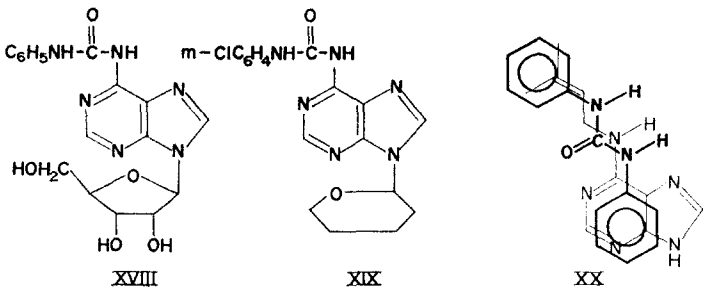
[1] E. M. SHANTZ and F. C. STEWARD, *J. Am. Chem. Soc.* **77**, 6351 (1955).
[2] M. I. BRUCE, J. A. ZWAR and N. P. KEFFORD, *Life Sci.* **4**, 461 (1965).
[3] N. P. KEFFORD, M. I. BRUCE and J. A. ZWAR, *Planta Berl.* **68**, 292 (1966).
[4] N. P. KEFFORD, J. A. ZWAR and M. I. BRUCE, *Planta Berl.* **67**, 103 (1965).
[5] M. I. BRUCE and J. A. ZWAR, *Proc. R. Soc.* (B) **65**, 245 (1966).
[6] F. SKOOG, H. Q. HAMZI, A. M. SZWEYKOWSKA, N. J. LEONARD, K. L. CARRAWAY, T. FUJII, J. P. HELGESON and R. N. LOEPPKY, *Phytochem.* **6**, 1169 (1967).

N-(purin-6-ylcarbamoyl)-threonine,[7–9] as the riboside in various tRNA's corresponding to codons starting with A, was reported while our tests were in progress, making the ureidopurines of greater immediate interest.





RESULTS AND DISCUSSION

The desired aryl- or alkylureidopurines were prepared by the reaction of the appropriate isocyanate with 9-protected adenine, followed by removal of the protecting group. We found the procedure employing a blocking group at 9 to have advantage over the direct treatment of adenine with, for example, phenylisocyanate.[10,11] The use of the 1-ethoxyethyl (EOE) blocking group on nitrogen can be specifically recommended for providing solubility, for the ease with which the EOE group can be attached and removed, and for its advantage

[7] G. B. CHHEDA, R. H. HALL, D. I. MAGRATH, J. MOZEJKO, M. P. SCHWEIZER, L. STASIUK and P. R. TAYLOR, *Biochem.* **8**, 3278 (1969).
[8] M. P. SCHWEIZER, G. B. CHHEDA, L. BACZYNSKYJ and R. H. HALL, *Biochem.* **8**, 3283 (1969).
[9] H. ISHIKURA, Y. YAMADA, K. MURAO, M. SANEYOSHI and S. NISHIMURA, *Biochem. Biophys. Res. Commun.* **37**, 990 (1969).
[10] A. S. JONES and J. H. WARREN, *Tetrahedron* **26**, 791 (1970).
[11] G. HUBER, *Angew. Chem.* **69**, 642 (1957).

over the tetrahydropyranyl protecting group in making a more distinctive contribution to the nuclear magnetic resonance spectrum. 6-Chloropurine reacts readily with acetal at reflux to give 6-chloro-9-(1-ethoxyethyl)purine (I). The reaction is more efficient with acetal than with ethyl vinyl ether and *p*-toluenesulfonic acid. Compound I can then be treated with amines to give the corresponding 6-substituted amino derivatives or with ethanolic ammonia to give 9-(1-ethoxyethyl)adenine [6-amino-9-(1-ethoxyethyl)purine] (II).[12] The latter can also be made by direct reaction of acetal with adenine,[12] and, for example, 9-(1-ethoxyethyl)-6-ethylaminopurine (III) results when 6-ethylaminopurine is used.

The substituted 6-ureido-9-EOE-purines (IV–X) were readily synthesized by reaction of the appropriate isocyanate with II. Complete reaction of II with aromatic isocyanates (in slight excess) was effected in refluxing tetrahydrofuran within 5–10 hr. Reaction with the non-aromatic isocyanates required larger excess of isocyanate, raised pressure, and longer time (see Table 3 in Experimental for yields and physical data). The u.v. spectra of the 6-ureido-9-EOE-purines are distinctive. For example, the spectrum of 9-(1-ethoxyethyl)-6-phenylureidopurine (IV) in 95% ethanol shows a maximum at 279 nm which, upon basification, shifts to 314 nm with a 56% increase in extinction coefficient (Table 4 in Experimental). 6-Allylureido-9-(1-ethoxyethyl)purine (VIII) undergoes a shift from 269 nm (sh 275 nm) in 95% ethanol to 310 nm on basification. The u.v. spectra of the aliphatic ureido examples all display an isosbestic point at 238–239 nm. Together with the NMR data (see Experimental) the u.v. data indicated that basification removes the N^6 proton. This was confirmed by the u.v. spectrum of *N*-ethyl-*N'*-phenyl-*N*-[9-(1-ethoxyethyl)]purin-6-ylurea (IVa = IV, Et on N^6), which was made by the action of phenylisocyanate on III and has no N^6-hydrogen. There was essentially no change in the u.v. maximum of this compound in going from 95% ethanol (289 nm) to basic solution (288 nm).

The 9-EOE derivatives IV–VIII were easily hydrolyzed to the corresponding 6-ureidopurines XI–XV by refluxing for a short period in aq. ethanol acidified with a drop of HCl. The intermediates IX and X were hydrolyzed preferably by stirring in 50% aq. acetic acid for a few hours. In many cases the unblocked purinylurea precipitated analytically pure (Table 5 of Experimental). The u.v. spectra of XI–XIV exhibited minor variations with the change in substituent on the phenyl ring. The striking feature in the spectra of the 6-alkylureidopurines XV–XVII was the double peak in the spectrum taken in neutral ethanol. For the 6-ureidopurines no bathochromic, hyperchromic shift in u.v. maximum was observed on basification. 6-Phenylureido-9-β-D-ribofuranosylpurine (XVIII) was synthesized for comparison with other ribonucleosides possessing cytokinin activity, and *m*-chlorophenylureido-9-(2-tetrahydropyranyl)purine (XIX) was made for comparison of synthetic methods.

Biological Activity

The relative cytokinin activities of eight 6-ureidopurines [compounds XI–XVII and XIa (XIa = XI, Et on N^6)] have been determined and compared with the activities of 6-benzylamino-, 6-furfurylamino-, and 6-phenylaminopurine and with *N,N'*-diphenylurea in the tobacco bioassay (Fig. 1). The activities of 6-benzylamino-, 6-furfurylamino-, and 6-phenylaminopurine declined in that order, as shown in Fig. 1, and are in agreement with values reported earlier.[6] All eight 6-ureidopurines had lower activities, but used in adequate concentrations all except 6-*p*-tolylureidopurine promoted vigorous growth and maximum yields of callus.

[12] N. J. LEONARD, J. J. MCDONALD and M. E. REICHMANN, *Proc. Natl. Acad. Sci. U.S.* **67**, 93 (1970).

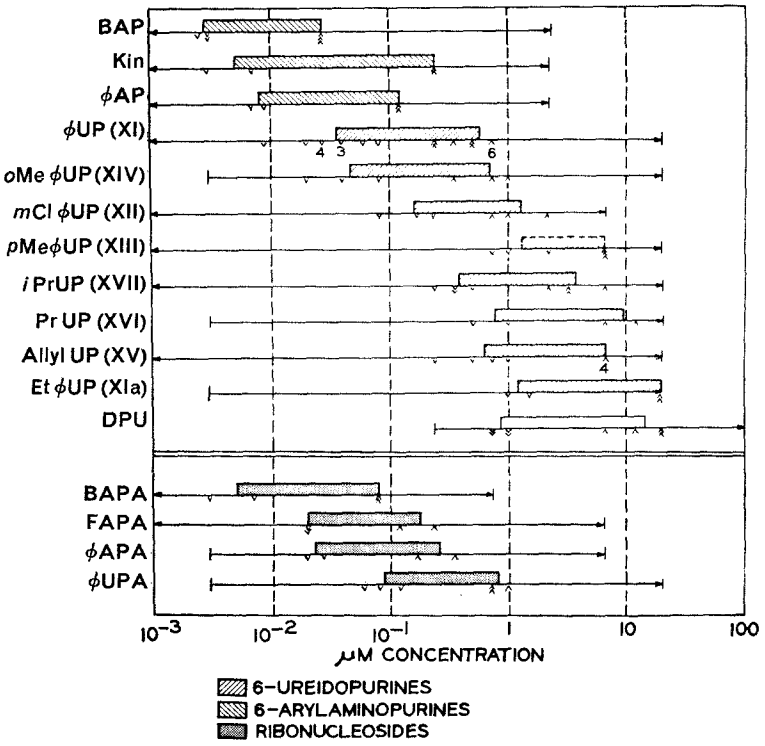


FIG. 1. CYTOKININ ACTIVITIES OF 6-ARYLAMINOPURINES, 6-UREIDOPURINES, DIPHENYLUREA, AND CERTAIN RIBONUCLEOSIDES.

The bars represent average values of the range in which growth increases as a linear function of $\log_{10}$ of the concentration. (A discontinuous outline indicates less than maximum response obtained with all concentrations.) The base lines represent tested concentration ranges, and the arrows under the base lines represent the start and end points of the linear growth response in individual experiments. Note that the starting points of the bars are somewhat higher than the limit of biologically detectable concentrations. The compounds tested are as follows: BAP, 6-benzylaminopurine; Kin, kinetin, 6-furfurylaminopurine; φAP, 6-phenylaminopurine; φUP (XI), 6-phenylureidopurine; *o*MeφUP (XIV), 6-*o*-tolylureidopurine; *m*ClφUP (XII), 6-*m*-chlorophenylureidopurine; *p*MeφUP (XIII), 6-*p*-tolylureidopurine; *i*PrUP (XVII), 6-isopropylureidopurine; PrUP (XVI), 6-*n*-propylureidopurine; AllylUP (XV), 6-allylureidopurine; EtφUP (XIa), 6-N-ethyl-*N'*-phenylureidopurine (*N*-ethyl-*N'*-phenyl-*N*-purin-6-ylurea); DPU, *N,N'*-diphenylurea; BAPA, 6-benzylamino-9-β-D-ribofuranosylpurine; FAPA, 6-furfurylamino-9-β-D-ribofuranosylpurine; φAPA, 6-phenylamino-9-β-D-ribofuranosylpurine, and φUPA, 6-phenylureido-9-β-D-ribofuranosylpurine.

It is known, in the case of the phenyl group, that a one-carbon-atom link joining the ring to N^6 of the adenine confers the highest activity and that bridges of two or more carbon atoms reduce the activity greatly.[6,13] Accordingly, on the basis of length alone, the ureido group, contributing an additional NH–CO moiety, would be expected to reduce markedly the activity of 6-phenylureidopurine as compared to 6-benzylaminopurine. In comparative tests 6-phenylureidopurine actually averaged 8% of the activity of 6-benzylaminopurine.

In the diphenylurea series, Bruce and Zwar[5] reported that substitution on one of the phenyl groups of *N,N'*-diphenylurea generally gave products with activities in the order: *meta* > *para* > *ortho*. In the present tests the substituted 6-phenylureidopurines showed the

[13] S. KURAISHI, *Sci. Papers Coll. Gen. Educ., Univ. Tokyo* **9**, 67 (1959).

following decreasing order of activity: *o*-tolyl > > *m*-chlorophenyl > > > *p*-tolyl (i.e. XIV > > XII > > > XIII in Fig. 1). This is in contrast to the order *m*-chlorophenyl > *p*-tolyl > *o*-tolyl reported by Bruce and Zwar for the corresponding *N,N'*-diphenylurea derivatives. Thus, despite the fact that the only difference between the two series is the replacement of a phenyl group by a purin-6-yl group, this replacement both enhances the degree of cytokinin activity and changes the effect of substitution in the (other) phenyl ring on the activity. In each series the most active derivative equalled or slightly exceeded its parent compound, while the other two had distinctly lower activities. Considering the 6-phenylureidopurines as disubstituted urea derivatives it should be noted that *N'*-phenyl-*N*-purin-6-ylurea (XI) is *ca.* 25 times more active than *N,N'*-diphenylurea (8% *vs.* 0·3% as active as 6-benzylaminopurine) in the present tobacco bioassay.

The addition of an ethyl group as a second substituent on N^6 of 6-phenylureidopurine (XI) to give *N*-ethyl-*N'*-phenyl-*N*-purin-6-ylurea (XIa) resulted in a drastic (97%) loss in activity. This decrease is in agreement with results of disubstitution on N^6 of other cytokinin-active adenine derivatives.[6]

The activities of the ribonucleosides are in the same decreasing order as listed for the corresponding bases (Fig. 1). The nucleoside of 6-benzylaminopurine shows activity starting around 5×10^{-3} μM; those of kinetin and 6-phenylaminopurine are both roughly 20%, and that of 6-phenylureidopurine (XVIII) only about 5% as active. It should be noted that possible degradation of the ribonucleosides to form the more active free bases during the test period makes it difficult to obtain meaningful cytokinin-activity values.

Effects of Ureides on Bud Formation

In early experiments on the effects of 6-phenylureidopurine on the growth of tobacco callus, a striking increase in bud formation was obtained with this compound as compared with 6-(3-methyl-2-butenylamino)purine or kinetin. Results obtained with the former two substances and with diphenylurea in one experiment are shown in Table 1. Further testing showed that this effect varied with conditions and appeared to be dependent on a relatively high 3-indoleacetic acid concentration. Effects on budding of various cytokinin-active ureidopurines and other cytokinins are summarized in Table 2. Other experiments also suggest that the budding response of the callus tissue to 6-phenylureidopurines is different

TABLE 1. EFFECTS OF 6-(3-METHYL-2-BUTENYLAMINO)PURINE (iP), 6-PHENYLUREIDOPURINE (φUP) AND *N,N'*-DIPHENYLUREA (DPU) ON BUD FORMATION IN TOBACCO CALLUS CULTURES*

Concentration (μM)	iP			φUP (XI)			DPU		
	A[a]	B[a]	C[a]	A	B	C	A	B	C
19·7	94	4	1·8	94	4	0·12	0	0	0
6·6	0	0	0	65	8	2·4	0	0	0
2·2	0	0	0	0	0	0	0	0	0
0·73	0	0	0	0	0	0	0	0	0

* Linsmaier and Skoog Revised Medium (1965) with 11 μM indoleacetic acid. Growth period 20 September 1968 to 19 November 1968.

Key: A[a] = Percentage of callus pieces with buds; B[a] = Average number of buds per callus; C[a] = Length in cm of longest shoot (24 callus pieces used for each treatment).

TABLE 2. EFFECT OF CYTOKININ-ACTIVE 6-UREIDOPURINES ON BUD FORMATION IN TOBACCO CALLUS CULTURED ON LINSMAIER AND SKOOG REVISED MEDIUM (1965). I, WITHOUT, II WITH 3 μM INDOLEACETIC ACID.*,†

	Concentration (μM)	Zeatin			iP			ϕUP (XI)			*o*MeϕUP (XIV)			*m*ClϕUP (XII)			*p*MeϕUP (XIII) AllylUP (XV) PrUP (XVI) *i*PrUP (XVII)		
		A[a]	B[a]	C[a]	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
I	19·7	100	10	0·5	17	10	2	42	>20	0·3	92	>20	4	0	0	0	0	0	0
	6·6	100	10	8	84	12	14	22	>20	0·2	25	>20	1·2	0	0	0	0	0	0
	2·2	100	10	10	100	12	12	17	2	0·15									
	0·73	25	3	12	67	7	18	8	2	0·2									
	0·24	0	0	0	0	0	0	0	0	0									
II	19·7	100	>20	0·8	100	>20	4	100	>20	2	88	>20	6	17	6	0·8	0	0	0
	6·6	100	>20	2	84	6	3	84	>20	1·2	0	0	0	0	0	0	0	0	0
	2·2	8	>20	0·2	17	1	1	17	1	0·8									
	0·73	0	0	0	33	1	0·5	0	0	0									
	0·24	0	0	0	0	0	0	0	0	0									

* Growth periods: I, 14 May 1969 to 2 July 1969; II, 29 May 1969 to 17 July 1969.
† Names corresponding to symbols and numbers of compounds are given in legend to Fig. 1.
Key: A[a], B[a] and C[a] as in legend to Table 1 (12 callus pieces per treatment).

from the response to cytokinins which are not ureides. However, the responses, especially to combined treatments with the two types of cytokinins, were too complex to reveal a clear distinction between the action of the ureides and the other cytokinins.

In experiments on the induction of bud formation in *Funaria protonemata*, which respond only weakly to the urea derivatives alone, a synergistic action of the two types of cytokinins is often observed.[14]

More experimental evidence is required to determine what the respective actions of the urea-containing and other cytokinins may be, but the available evidence on bud formation suggests that their actions are distinct, despite gross structural similarities which may exist (*cf.* XX).

EXPERIMENTAL

Synthesis of Test Substances

6-*Chloro*-9-(1-*ethoxyethyl*)*purine* (*I*). *Method A.* A suspension of 400 mg (2·6 mmoles) of 6-chloropurine in 50 ml of acetal (Aldrich) was refluxed for 5 hr until complete solution occurred and no starting material could be observed by TLC. The acetal was removed *in vacuo* and the remaining oil was recrystallized several times from hexane with charcoal treatment to yield colorless chunks of I: NMR ($CDCl_3$) τ 1·17 (s, 1, purine H), 1·50 (s, 1, purine H), 3·91 (q, 1, J = 6 Hz, N—CH—O), *ca.* 6·6 (m, 2, O—C$\underline{H}_2$CH$_3$), 8·16 (d, 3, J = 6 Hz, CH$_3$), 8·80 (t, 3, J = 7 Hz, O—CH$_2$C$\underline{H}_3$); other data in Table 3.

Method B. In an adaptation of a literature method for 6-chloro-9-(2-tetrahydropyranyl)purine,[15] 2·8 g (40 mmoles) of ethyl vinyl ether was added dropwise to a solution of 3·0 g (20 mmoles) of 6-chloropurine and 50 mg of *p*-toluenesulfonic acid in 50 ml of ethyl acetate at 55°. After the solid had dissolved completely, the solution was cooled to room temp. and extracted four times with a solution of 2 ml conc. NH_4OH in 25 ml of water and then once with 25 ml of water. The solution was dried over Na_2SO_4 and concentrated *in vacuo* to an oil which crystallized on standing. The solid was recrystallized from pentane-ether with charcoal decolorization to yield 2·96 g of I, mp 52–53°.

9-(1-*Ethoxyethyl*)*adenine* (*II*). This compound was made by reaction of 6-chloropurine with acetal (Aldrich) followed by treatment with ethanolic ammonia (Method A) in 74% yield or by direct reaction of adenine with acetal (Eastman) (Method B) in 22% yield.[12]

9-(1-*Ethoxyethyl*)-6-*ethylaminopurine* (*III*). A solution of 0·25 g (1·5 mmoles) of 6-ethylaminopurine[16] in 25 ml of acetal (Eastman) was heated at reflux for 3 days. The solution was concentrated to dryness *in vacuo* to a yellow oil which solidified upon refrigeration. The crude product was recrystallized from hexane with charcoal treatment to give III as colorless needles: NMR ($CDCl_3$) τ 1·61 (s, 1, purine H), 2·00 (s, 1, purine H), 3·65 (broad, 1, NH), 4·10 (q, 1, J = 6 Hz, N—CH—O), 6·18 and *ca.* 6·5 (q and m, 4, NC$\underline{H}_2$CH$_3$ and OC$\underline{H}_2$CH$_3$), 8·24 (d, 3, J = 6 Hz, CH$_3$), 8·68 and 8·83 (both t, 6, both J = 7 Hz, NCH$_2$C$\underline{H}_3$ and OCH$_2$C$\underline{H}_3$); other data in Table 3.

6-*Arylureido*-9-(1-*ethoxyethyl*)*purines* (*IV–VII*). A solution of 1·0 g (4·8 mmoles) of II and 5·0 to 6·0 mmoles of the isocyanate was refluxed under anhydrous conditions in 50 ml of dry tetrahydrofuran for 4–8 hr until complete conversion to product was indicated by TLC. The solvent was removed *in vacuo*, and the remaining solid was washed with dry hexane before recrystallization from 95% ethanol. Ureidopurine VII was filtered analytically pure from the concentrated THF solution and washed with hexane. The NMR spectra ($CDCl_3$) were fully consistent with the assigned structures. Analytical data are reported in Table 3.

6-*Alkylureido*-9-(1-*ethoxyethyl*)*purines* (*VIII–X*). A solution of 1·0 g (4·8 mmoles) of II and 20 mmoles of the isocyanate in 50 ml of dry tetrahydrofuran was heated in a pressure bottle at 90° for 24 hr. After evaporation of the THF, the solid residue was recrystallized with charcoal treatment from solvents noted in Table 3. The NMR spectra ($CDCl_3$) were fully consistent with assigned structures.

6-*Arylureidopurines* (*XI–XIV*). A solution of the appropriate 6-substituted 9-EOE purine in 95% ethanol containing a few drops of conc. aq. HCl was heated at reflux for 10 min. The precipitate which appeared on cooling was removed by filtration and recrystallized from aq. ethanol. Ureidopurine XII started to precipitate analytically pure from a refluxing solution of 15% aq. ethanol after treatment with HCl. The full yield of precipitate was realized after cooling to room temp. None of the compounds melted or colored upon heating to 330°. The NMR spectra (TFA) were consistent with the assigned structures. Analytical data are reported in Table 5.

[14] H. Simon, R. Y. Schmitz and F. Skoog (unpublished).
[15] R. K. Robins, E. F. Godefroi, E. C. Taylor, L. R. Lewis and A. Jackson, *J. Am. Chem. Soc.* **83**, 2574 (1961).
[16] G. B. Elion, E. Burgi and G. H. Hitchings, *J. Am. Chem. Soc.* **74**, 411 (1952).

TABLE 3. 6-SUBSTITUTED 9-(1-ETHOXYETHYL)PURINES

6-Substituent	Yield (%)	M.p.	Formula	Carbon, % Calc.	Carbon, % Found	Hydrogen, % Calc.	Hydrogen, % Found	Nitrogen, % Calc.	Nitrogen, % Found
Chloro (I)	62	53–54°	$C_9H_{11}ClN_4O$	47·69	47·54	4·98	4·95	24·71	24·42
Amino (II)	74	172–173°	$C_9H_{13}N_5O$						
Ethylamino (III)	34	71–71·5°	$C_{11}H_{17}N_5O$	56·15	56·24	7·28	7·12	29·76	29·52
Phenylureido (IV)	79	163–163·5°	$C_{16}H_{18}N_6O$	58·88	59·05	5·56	5·60	25·75	25·48
m-Chlorophenylureido (V)	84	175–176°	$C_{16}H_{17}ClN_6O$	53·25	51·59	4·75	4·88	23·29	23·28
p-Tolylureido (VI)	83	169–170°	$C_{17}H_{20}N_6O$	59·98	59·96	5·92	5·92	24·69	24·65
o-Tolylureido (VII)	96	185–186°	$C_{17}H_{20}N_6O$	59·98	60·05	5·92	6·23	24·69	24·54
Allylureido (VIII)	71	156–157°*	$C_{13}H_{18}N_6O$	53·78	53·69	6·25	6·16	28·95	28·69
n-Propylureido (IX)	39	134–135·5°†	$C_{13}H_{20}N_6O$	53·41	53·65	6·90	7·01	28·75	28·59
Isopropylureido (X)	67	131–132°‡	$C_{13}H_{20}N_6O$	53·41	53·36	6·90	6·86	28·75	28·86

* From ethyl acetate-hexane. Compound VIII has also been found to stimulate the growth of soya bean tissue (see W. H. DYSON, C. M. CHEN, S. N. ALAM and R. H. HALL, *Science*, **170,** 328 (1970)).

† From acetonitrile.

‡ From hexane.

TABLE 4. ULTRA-VIOLET ABSORPTION MAXIMA OF COMPOUNDS I–XIX*

Compound	0·1 *N* HCl nm ($\epsilon \times 10^{-3}$)	95% EtOH nm ($\epsilon \times 10^{-3}$)	0·1 *N* NaOH nm ($\epsilon \times 10^{-3}$)
I	264 (10·1)	264 (10·1)	264 (10·1)
II	257 (13·3)	254 (13·2)	257 (13·2)
III	263 (18·1)	266 (17·4)	266 (17·4)
IV	287 (23·9)	279 (28·2)	314 (43·7)
V	286 (26·7)	278 (31·2)	317 (46·1)
VI	288 (22·7)	280 (27·1)	314 (42·3)
VII	282 (22·5)	278 (25·2)	312 (40·0)
VIII	277 (20·6)	269 (22·6)†	300 (32·7)
IX	277 (22·2)	269 (24·3)†	300 (31·9)
X	277 (20·7)	269 (22·3)†	301 (31·3)
XI	288 (23·9)	282 (27·1)	285 (26·6)
XII	287 (26·2)	283·5 (28·4)	287 (26·4)
XIII	288 (22·5)	281 (26·8)	286 (28·4)
XIV	284 (20·2)	279 (22·5)	286 (24·0)
XV	277·5 (20·4)	269 (18·9) 276 (17·9)	278·5 (17·3)
XVI	277·5 (20·4)	269 (18·9) 276 (17·9)	278·5 (17·3)
XVII	278 (20·4)	269 (18·9) 277 (17·8)	279 (17·3)
XVIII	282·5 (25·3)	278 (28·1)	313 (45·0)
XIX	284 (27·6)	277 (31·4)	315·5 (50·1)

* For analytical u.v. data, a solution of a known amount of compound in absolute ethanol was diluted with an appropriate amount of 2 N HCl, H_2O, or 2 N NaOH to a final strength of 95% ethanol; According to N. J. LEONARD, K. L. CARRAWAY and J. P. HELGESON, *J. Heterocyl. Chem.* **2**, 291 (1965).

† Pronounced shoulder at 276 nm.

6-Alkylureidopurines (XV–XVII). Urea XV was obtained from acidified 95% ethanol as above. Ureas XVI and XVII were obtained after stirring IX and X in 50% aq. acetic acid for 12 and 5 hr, respectively. The solutions were concentrated to dryness *in vacuo* and the solids remaining were recrystallized. The NMR spectra taken in TFA were consistent with the assigned structures. Analytical and mp data are reported in Table 5.

6-Phenylureido-9-β-D-ribofuranosylpurine (XVIII). A solution of 1·0 g (2·5 mmoles) of 2′,3′,5′-tri-*O*-acetyladenosine[17] and 0·36 g (3 mmoles) of phenylisocyanate in 50 ml of dry 1,2-dimethoxyethane was heated at reflux for 4 hr. The solvent was removed *in vacuo* leaving a colorless oil with a u.v. spectrum characteristic of a 9-substituted-6-ureidopurine. Deblocking was achieved by dissolving the oil in 60 ml of methanol previously saturated with anhydrous ammonia at 0° and allowing the solution to stand overnight at room temp. Evaporation of the solvent *in vacuo* and recrystallization of the solid residue from 95% ethanol yielded 0·79 g (80%) of XVIII in two crops: m.p. 200·5–201·5°; NMR (DMSO-d_6) τ −1·68 (s, 1, NH), 0·03 (s, 1, NH), 1·30 and 1·33 (both s, 2, purine H), 2·3–3·0 (m, 5, C_6H_5), 3·97 (d, $J \sim 5{\cdot}5$ Hz, $C_{1'}$—H), 4·49 (d, 1, $J = 6$ Hz, OH), 4·84 (m, 2, OH), 5·38 (m, 1, $C_{2'}$—H), 5·86 (m, 2, $C_{3'}$ and $C_{4'}$—H) 6·33 (unresolved m, 2, $C_{5'}$—H). All absorption assigned to NH and OH is wiped out upon equilibration with D_2O.

Anal. Calc. for $C_{17}H_{18}N_6O_5$: C, 52·84; H, 4·70; N, 21·75. Found: C, 52·98; H, 4·78; N, 22·02.

6-m-Chlorphenylureido-9-(2-tetrahydropyranyl)purine (XIX). A solution of 0·55 g (2·5 mmoles) of 6-amino-9-(2-tetrahydropyranyl)purine[15] and 0·42 g (2·7 mmoles) of *m*-chlorophenylisocyanate in 20 ml of dry THF was refluxed overnight. The solution was concentrated to dryness *in vacuo*, and the solid residue was washed with hot hexane. Recrystallization of the crude product from 75 ml of 95% ethanol gave 0·51 g (55%) of XIX as colorless needles: m.p. 185–187°; NMR ($CDCl_3$) τ 0·40 (broad, 1, NH), 1·18 (s, 1, purine H), 1·30 (s, 1, purine H), 2·0–2·9 (m, 4, aromatic H), 4·13 (m, 1, N—CH—O), 5·5–6·5 (m, 2, OCH_2), 7·6–8·5 (broad, 6, CH_2).

[17] M. IKEHARA, *Bull. Chem. Soc. Japan* **8**, 367 (1960).

TABLE 5. 6-UREIDOPURINES

Ureidopurine	Yield (%)	Formula	Carbon, % Calc.	Carbon, % Found	Hydrogen, % Calc.	Hydrogen, % Found	Nitrogen, % Calc.	Nitrogen, % Found
6-Phenyl (XI)	86	$C_{12}H_{10}N_6O$	56·68	56·96	3·96	4·19	33·05	32·86
6-*m*-Chlorophenyl (XII)	92	$C_{12}H_9ClN_6O$	49·92	49·64	3·14	3·28	29·12	29·03
6-*p*-Tolyl (XIII)	99	$C_{13}H_{12}N_6O$	58·20	58·27	4·51	4·79	31·33	31·46
6-*o*-Tolyl (XIV)	65	$C_{13}H_{12}N_6O$	58·20	58·05	4·51	4·77	31·33	31·41
6-Allyl (XV)	75*	$C_9H_{10}N_6O$	49·53	49·81	4·62	4·77	38·52	38·32
6-*n*-Propyl (XVI)	77†, ‡	$C_9H_{12}N_6O$	49·08	49·28	5·49	5·66	38·16	37·93
6-Isopropyl (XVII)	64†, §	$C_9H_{12}N_6O$	49·08	49·36	5·49	5·64	38·16	38·04

* From 80% ethanol; did not melt or color up to 330°.
† From ethanol–ethyl acetate (1:1).
‡ Changes from microneedles to prisms at 235°; does not melt again or color under 330°.
§ Changes from microneedles to prisms at 220°; unchanged to 330°.

Anal. Calc. for $C_{17}H_{17}ClN_6O_2$: C, 54·76; H, 4·60; N, 22·54. Found: C, 54·72; H, 4·70; N, 22·73.

N-*Ethyl*-N'-*phenyl*-N-[9-(1-*ethoxyethyl*)]*purin*-6-*ylurea* (*IV, Et in place of* N^6—H). A solution of 0·77 g (3·3 mmoles) of III and 0·47 g (4·0 mmoles) of phenylisocyanate was refluxed in hexane for 6 hr. After cooling to room temp. the solution deposited, upon refrigeration, 0·95 g (81%) of analytically pure colorless needles: m.p. 92–93°; NMR ($CDCl_3$) consistent with the assigned structure. $\lambda_{max}^{95\%}$ EtOH 289 nm (ϵ 26,600); (H^+) 289 (24,900); (OH^-) 288 (26,700).

Anal. Calc. for $C_{18}H_{22}N_6O_2$: C, 61·00; H, 6·26; N, 23·72. Found: C, 61·19; H, 6·25; N, 23·69.

N-*Ethyl*-N'-*phenyl*-N-*purin*-6-*ylurea* (*XI, Et in place of* N^6—H). Deblocking was accomplished by heating in 50% acetic acid to effect solution and then stirring at room temp. for 1 hr. Analytically pure, colorless needles precipitated in 90% yield: m.p. 168–168·5°; NMR (TFA) τ 0·77 (s, 1, purine H), 0·97 (s, 1, purine H), 1·35 (s, 1, NH), 2·50 (s, 5, C_6H_5), 5·27 (q, 2, $J = 7$ Hz, CH_2), 8·31 (t, 3, $J = 7$ Hz, CH_3); $\lambda_{max}^{95\%}$ EtOH 288 nm (ϵ 25,100); (H^+) 290 (22,400); (OH^-) 294 (23,900).

Anal. Calc. for $C_{14}H_{14}N_6O$: C, 59·56; H, 5·00; N, 29·77. Found: C, 59·31; H, 4·91; N, 29·86.

NMR Spectra. The NMR spectrum of each of the following compounds is typical of its class of ureidopurine.

9-(1-*Ethoxyethyl*)-6-*phenylureidopurine* (*IV*): τ ($CDCl_3$) −1·48 (s, 1, NH), 0·62 (s, 1, NH, exchangeable with D_2O), 1·25 (s, 1, purine H), 1·39 (s, 1, purine H), *ca.* 2·7 (m, 5, C_6H_5), EOE resonances as for I–III.

6-*Allylureido*-9-(1-*ethoxyethyl*)*purine* (*VIII*): τ ($CDCl_3$) 0·28 (t, 1, allyl-NH), 0·60 (s, 1, NH, exchangeable with D_2O), 1·49 (s, 2, purine H's) *ca.* 4·0 (m, CH_2=C<u>H</u>), *ca.* 4·7 (m, 2, C<u>H</u>$_2$=CH), 5·91 (t, 2, allylic CH_2), EOE resonances as for I–III. In the NMR spectra of IX and X, the alkyl-NH protons, which are not exchanged in D_2O, are present as a triplet and a doublet, respectively.

6-o-*Tolylureidopurine* (*XIV*): τ (TFA) 0·91 (s, 2, purine H's), 1·59 (s, 1, NH), 2·57 (s, 4, C_6H_4), 7·57 (s, 3, CH_3).

6-*Isopropylureidopurine* (*XVII*): τ (TFA) 0·91 (s, 2, purine H's), 3·4 (s, 1, NH), 5·8 (s, 1, CH), 8·57 (d, $J = 6$ Hz, CH_3).

Bioassay Procedures

The cytokinin activity of the compounds was measured by the ability to promote growth (increase in fresh weight) of tobacco callus tissue in the tobacco bioassay.[6] In order to avoid degradation by heat the test substances were dissolved in dimethyl sulfoxide and added to the cooling autoclaved media.[18] This procedure permitted the testing of compounds, such as diphenylurea, which are difficult to dissolve in water in the required concentrations. A series of three-fold dilutions was made and the activity observed over the full range of concentrations which promoted callus growth. The range of concentrations over which there was a nearly linear relationship between the increase in fresh weight and the logarithmic increase in cytokinin concentration was then determined and compared for the various compounds (see legend to Fig. 1).

In budding experiments observations of growth and bud counts were made at weekly intervals over a 7 to 9 weeks growth period.

[18] R. Y. Schmitz and F. Skoog, *Plant Physiol.* **45**, 537 (1970).