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RETARDATION OF LEAF SENESCENCE BY UREA CYTOKININS IN *RAPHANUS SATIVUS**

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Key Word Index—*Raphanus sativus*; Cruciferae; cytokinin; urea derivatives; phenylureas; radish; leaf senescence; cell division; chemical structure and activity.

Abstract—Approximately 500 urea derivatives and related compounds were tested for ability to retard leaf senescence as measured by chlorophyll retention in radish (*Raphanus sativus*) leaf discs. Of the 90 compounds found to be active, some had activity at 10^{-6} M of the same order as kinetin. There was a high correlation between ability to promote chlorophyll retention and initiation of cell division. Highly active compounds had a planar ring and a HNCONH bridge; substitution with a HNCSNH bridge reduced activity and all other tested arrangements of the bridge gave inactive compounds. Substitution of both amino hydrogen atoms on one or both sides of the bridge reduced or removed activity. Some *N*-substituted phenyl ureas were highly active. Introduction of a *N'*-phenyl ring to a *N*-phenyl urea increased activity except where one ring was substituted in the *para* position with chloro, bromo or iodo. The activities of symmetrical disubstituted ureas were generally less than the corresponding *N*-monosubstituted derivative. The results suggest that the receptor site for cytokinin activity is the same for senescence retadation and cell division initiation.

INTRODUCTION

ABOUT 300 derivatives of urea, of 500 tested, have been found to be active in the induction of cell division in isolated tobacco stem pith.[1,2] In addition to inducing cell division, urea derivatives are active in promoting pea bud development,[3] the retardation of senescence in leaf discs of radish[1] and in whole leaves of carrot and beet,[4] the promotion of cucumber cotyledon expansion,[4] the inhibition of root development on excised cucumber cotyledons and mung bean shoot cuttings[4] the alteration of sex expression in *Luffa*,[5] and bud formation in *Funaria* protonemata.[6] This spectrum of biological activities qualifies urea derivatives for classification as growth regulators of the type named cytokinin.[7]

The relationship between chemical structure and biological activity of urea cytokinins has been explored only in connection with the induction of cell division.[2] A survey using a second test system for cytokinin activity is of interest for the comparison of the primary sites of cytokinin activity and of secondary factors in activity that may be operating differently in the two systems. Further data on the relationships between cytokinin structure

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and activity are also desirable in view of the cytokinin activity of two classes of compounds, namely urea derivatives and purine derivatives, which have little apparent chemical relationship.[8]

The present paper is based upon activities of about 500 urea derivatives in the retardation of leaf senescence using discs of radish leaf as a test tissue. The same collection of compounds was tested for activity in cell division induction by Bruce and Zwar.[2] As Bruce and Zwar provided a complete list of the compounds, those tested but found inactive in chlorophyll retention are not all mentioned in the present paper.

RESULTS

Chlorophyll retention is used as a measure of senescence retardation in radish leaf discs, and results are expressed as an index of chlorophyll retention. To obtain this index the amount by which the chlorophyll in urea derivative treated discs exceeded that of water treated discs was expressed as a percentage of the amount by which chlorophyll in 10^{-5} M kinetin treated discs exceeded that of water treated discs. Kinetin at 10^{-5} M achieved its maximal effect upon senescence retardation and at 10^{-7} M had perceptible activity.[9] Each of the 500 urea compounds was tested at least at concentrations of 10^{-6} and 10^{-5} M plus 5×10^{-5} or 10^{-4} M depending upon the water solubility of the compound. A compound

TABLE 1. UREA DERIVATIVES FOUND MOST ACTIVE IN SENESCENCE RETARDATION

Compound No.	Compound RNHCONHR′ R	R′	Chlorophyll index* at concentrations (M) 10^{-6}	5×10^{-6}	10^{-5}	$2{\cdot}5 \times 10^{-5}$	5×10^{-5}	10^{-4}	Cell division index†
I	4-$(CO_2H)C_6H_4$	C_6H_5	(66)‡	62	124	128	149	116	1
II	3-Cl-4MeC_6H_3	C_6H_5	(52)	122	142	93	82		1
III	3-FC_6H_4	C_6H_5	(58)	89	88	91	81		1
IV	C_6H_5	5-quinolyl	(53)	87	104	153	143	137	1
V	4-NO_2-3-$CF_3C_6H_3$	C_6H_5	37		105		89		<1
VI	4-Me-3-$NO_2C_6H_3$	C_6H_5	35		85		76		<1
VII	3-FC_6H_4	4-MeC_6H_4	(33)	70	68	59	66		33
VIII	4-BrC_6H_4	H		60	89	118	115		1
IX	3-FC_6H_4	3-MeC_6H_4		70	68	59	66		3
X	3,4-$Cl_2C_6H_3$	H		50	89	108	103		1
XI	C_6H_5	C_6H_5		52	69	77	101		0·5
XII	C_6H_5	4-C_5H_4N		69	91	109	90		0·3
XIII	4-IC_6H_4	H		36	70	70	107		6
XIV	3-FC_6H_4	4-FC_6H_4	37		67		73		<3

* A chlorophyll index $\geqslant 30$ is considered to indicate an active compound.

† The cell division index is the lowest concentration (in ppm) of the compound which would produce new cells on explants of tobacco stem pith (see Ref. 2). This index is quoted as less than a certain concentration when the amount of cell division produced by that concentration, the lowest tested, was more than minimal. The lower is the cell division index, the greater is the activity of the compound.

‡ Index values in parentheses were obtained on a different occasion from the other index values shown or the compound.

was considered active only if it produced a chlorophyll index of 30 or more at any one of the concentrations tested. Activities of ureas in cell division, as determined by Bruce and Zwar[2] using tobacco pith tissue, are included for comparison.

The compounds with the highest activities in chlorophyll retention were I–XIV in Table 1. Amongst these, some (I–IV) were clearly active at concentrations of 10^{-6} M, and at

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TABLE 2. ACTIVITIES OF SOME UREA DERIVATIVES IN SENESCENCE RETARDATION

Compound No.	Compound RNHCONHR′ R	R′	Chlorophyll index at concentrations (M) 10^{-6}	10^{-5}	5×10^{-5}	Cell division index†
XV	C_6H_5	H	9	9	16§	75
XVI	C_6H_5	Me	13	10	78§	1
XVII	C_6H_5, Me	H	5	21	21§	n.a.
XVIII	C_6H_5	Et	−7	51	129§	10
XIX	C_6H_5	*n*-C_3H_7	8	56	71§	33
XX	4-MeC_6H_4	*n*-C_3H_7	40	45	68	33
XXI	C_6H_5	*trans*-2-$C_6H_5 \cdot C_3H_5$	−29	51	66	2
XXII	H	3-$(C_6H_5)C_6H_5$	13	12	66	3
XXIII	C_6H_5	3-C_5H_4N	−2	38	67§	1
XXIV	C_6H_5	5-indolyl	5	46	82§	3
XXV	4-ClC_6H_4	(Me_2=HR′)	28	20	25§	33
XXVI	3,4-$Cl_2C_6H_3$	Me, nC_4H_9	−2	4	18§	20
XXVII	3,4-$Cl_2C_6H_3$	(Me_2=HR′)	1	11	95§	100
XXVIII	Me_2	$(C_6H_5)_2$	1	10	17	n.a.
XXIX	Me	$(C_6H_5)_2$	14	8	24	n.a.
XXX	Me_2	C_6H_5	−1	−3	3§	n.a.
XXXI	$(C_6H_5)_2$	H_2	−5	10	25	n.a.
XXXII	C_6H_5	Me,OH	23	17	4§	n.a.
XXXIII	2-C_5H_4N	H	38	42	72§	13
XXXIV	3-C_5H_4N	H	15	10	32§	n.a.
XXXV	4-C_5H_4N	H	5	17	85§	n.a.
XXXVI	3-FC_6H_4	C_6H_5 (S)	15	29	61	<20
XXXVII	C_6H_5	4C_5H_4N (S)	9	45	68	67
XXXVIII	4-$(CO_2H)C_6H_4$	H	−3	12	32§	n.a.
XXXIX	4-$(CO_2Et)C_6H_4$	H	−2	4	19	n.a.
XL	4-$(CO_2Et)C_6H_4$	C_6H_5	14	94	133	1
XLI	4-$(CO_2EtMe)C_6H_4$	C_6H_5	20	9	46	<1
XLII	3-$(CO_2Et)C_6H_4$	C_6H_5	25	41	84	33
XLIII	3-(CO_2H)-4-$(C_2H_3)C_6H_3$)	C_6H_5	16	24	54	20
XLIV	4-$(CNMe)C_6H_4$	H	0	2	−5	n.a.
XLV	4-$(CNMe)C_6H_4$	C_6H_5	−1	3	59	2
XLVI	3-$OEtC_6H_4$	H	26	4	10§	33
XLVII	3-$OEtC_6H_4$	C_6H_5	0	20	41	<1
XLVIII	4-$OEtC_6H_4$	H	27	18	−3§	n.a.
XLIX	4-$OEtC_6H_4$	C_6H_5	10	28	50	60
L	3-$OMeC_6H_4$	H	13	−2	20§	67
LI	3-$OMeC_6H_4$	C_6H_5	35	45	72§	<1
LII	4-$OMeC_6H_4$	H	6	−4	13§	n.a.
LIII	4-$OMeC_6H_4$	C_6H_5	−4	12	38§	3
LIV	2-OMe-4$NO_2C_6H_3$	H	32	15	25	33
LV	2-OMe-4$NO_2C_6H_3$	C_6H_5	40	41	79	1
LVI	2-OMe-5$NO_2C_6H_3$	H	8	−5	6	50
LVII	2-OMe-5$NO_2C_6H_3$	C_6H_5	11	29	44	1
LVIII	4-OMe-2$NO_2C_6H_3$	H	18	1	0	33
LIX	4-OMe-2$NO_2C_6H_3$	C_6H_5	5	34	54	10
LX	4-NO_2-3-$CF_3C_6H_3$	H	11	20	32	20
LXI	2-$CF_3C_6H_4$	H	2	0	1§	67
LXII	3-$CF_3C_6H_4$	H	13	30	60§	10

Also active at 10^{-5} M were: ethyl-4-(phenylureido)benzene sulphonate, 2-(phenylureido)benzophenone and 4-(4′-phenylureidophenylazo)benzoic acid; and at 10^{-4}M: 4-(phenylureido)salicyclic acid.

*,† As in Table 1.

§ Value obtained at 10^{-4} M.

n.a. = not active.

5×10^{-6} M approached the level of activity shown by 10^{-5} M kinetin. Compounds V–XIV were less active in that they were not as active at 10^{-6} M and concentrations of $2{\cdot}5 \times 10^{-5}$ M were required to achieve the level of chlorophyll retention of 10^{-5} M kinetin. Urea derivatives therefore have the same potential as kinetin in the retardation of senescence. With the exception of *N*-3-fluorophenyl-*N'*-4-tolyl urea (VII), there is a strong correspondence between the level of activity in chlorophyll retention and the level of activity in cell division induction.

The sulphur analogues of two of the highly active ureas, III and XII, namely *N*-3-fluorophenyl-*N'*-phenyl thiourea (XXXVI) and *N*-phenyl-*N'*-4-pyridyl thiourea (XXXVII), were found active in senescence retardation (Table 2), thus replacement of the carbonyl oxygen in urea with a sulphur atom did not remove activity, rather activity was reduced. A series of compounds, such as anilides, without an intact –N–C–N-bridge were inactive in chlorophyll retention as were a series of variants of phenylurea, *N,N'*-diphenyl urea, and urea; complete lists of the compounds tested are in Tables 2 and 3 of Bruce and Zwar,[2] but examples of activities are in Table 4. Hence as with cell division activity, the –N–C–N– bridge could not be altered significantly without destroying chlorophyll retaining activity.

TABLE 3. ACTIVITIES IN SENESCENCE RETARDATION AND CELL DIVISION OF SOME PHENYLUREA DERIVATIVES CONTAINING HALOGEN OR METHYL SUBSTITUENTS

Compound No.	Compound RNHCONHR′ R	R′	Chlorophyll index at concentrations (M)* 10^{-6}	10^{-5}	5×10^{-5}	Cell division index‡
LXIII	2-FC_6H_4	H	−8	18	−3§	n.a.
LXIV	2-FC_6H_4	C_6H_5	31	22	33	33
LXV	3-FC_6H_4	H	13	18	76§	1
LXVI	3-FC_6H_4	3-ClC_6H_4	0	15	66	<20
LXVII	3-FC_6H_4	4-ClC_6H_4	−5	1	−5	1
LXVIII	3-FC_6H_4	4-$EtOC_6H_4$	6	47	37	10
LXIX	3-FC_6H_4	3-$NO_2C_6H_4$	6	36	43	<1
LXX	4-FC_6H_4	H	22	3	13	n.a.
LXXI	4-FC_6H_4	C_6H_5	15	55	107	<1
LXXII	4-FC_6H_4	4-FC_6H_4	17	25	44	20
LXIII	4-FC_6H_4	4-ClC_6H_4	0	0	1	20
LXXIV	4-FC_6H_4	3-C_5H_4N	11	23	61	20
LXXV	2-ClC_6H_4	H	4	4	−6	20
LXXVI	2-ClC_6H_4	C_6H_5	7	4	12	33
LXXVII	2-ClC_6H_4	2-ClC_6H_4	26	17	70	33
LXXVIII	3-ClC_6H_4	H	3	19	47§	3
LXXIX	3-ClC_6H_4	C_6H_5	30	65	44	0·1
LXXX	3-ClC_6H_4	4-C_5H_4N	−9	16	40	67
LXXXI	3-ClC_6H_4	3-ClC_6H_4	32	34	106	20
LXXXII	3-ClC_6H_4	4-ClC_6H_4	15	−4	2	50
LXXXIII	3-ClC_6H_4	3,5-$Cl_2C_6H_3$	27	30	62	<20
LXXXIV	3-ClC_6H_4	2-$NO_2C_6H_4$	3	53	57	20
LXXXV	4-ClC_6H_4	H	1	30	110	1
LXXXVI	4-ClC_6H_4	C_6H_5	17	26	17	0·1
LXXXVII	4-ClC_6H_4	4-ClC_6H_4	14	14	3	50
LXXXVIII	4-ClC_6H_4	3,4-$Cl_2C_6H_3$	−4	5	5	0·6
LXXXIX	4-ClC_6H_4	3-$NO_2C_6H_4$	−2	15	84	33
XC	2,5-$Cl_2C_6H_3$	3-$NO_2C_6H_4$	25	9	52	n.a.
XCI	3,4-$Cl_2C_6H_3$	C_6H_5	−7	32	61	3
XCII	3,4-$Cl_2C_6H_3$	3,4-$Cl_2C_6H_3$	18	3	38	2
XCIII	3,5-$Cl_2C_6H_3$	H	11	7	3	n.a.

TABLE 3—*continued*

Compound No.	Compound RNHCONHR′ R	R′	Chlorophyll index at concentration (M)* 10^{-6}	10^{-5}	5×10^{-5}	Cell division index‡
XCIV	$3,5\text{-Cl}_2C_6H_3$	C_6H_5	3	41	71	<1
XCV	$3,5\text{-Cl}_2C_6H_3$	$3\text{-(CF}_3)C_6H_4$	35	60	48	<20
XCVI	2-BrC_6H_4	H	6	12	12	3
XCVII	2-BrC_6H_4	C_6H_5	16	4	45	33
XCVIII	3-BrC_6H_4	H	9	9	86§	0·1
XCIX	3-BrC_6H_4	C_6H_5	18	40	52	1
C	4-BrC_6H_4	C_6H_5	−8	−5	10	33
CI	4-BrC_6H_4	4-BrC_6H_4	7	17	11	n.a.
CII	2-IC_6H_4	H	3	−3	9	n.a.
CIII	2-IC_6H_4	C_6H_5	−13	−2	5	67
CIV	4-IC_6H_4	C_6H_5	28	27	159	6
CV	3-Cl-4-MeC_6H_3	H	15	15	106§	33
CVI	2-MeC_6H_4	H	−5	−18	9§	n.a.
CVII	2-MeC_6H_4	$n\text{-C}_3H_7$	7	13	−10	n.a.
CVIII	2-MeC_6H_4	C_6H_5	37	61	35	33
CIX	2-MeC_6H_4	3-C_5H_4N	24	21	88	50
CX	2-MeC_6H_4	2-MeC_6H_4	8	15	−1	n.a.
CXI	2-MeC_6H_4	$3\text{-NO}_2C_6H_4$	35	41	44	10
CXII	2-MeC_6H_4	$4\text{-NO}_2C_6H_4$	22	9	78	20
CXIII	3-MeC_6H_4	H	37	45	59	20
CXIV	3-MeC_6H_4	$n\text{-C}_3H_7$	4	25	31	<20
CXV	3-MeC_6H_4	C_6H_5	55	77	41	0·6
CXVI	3-MeC_6H_4	2-C_5H_4N	28	88	59	56
CXVII	3-MeC_6H_4	3-MeC_6H_4	17	62	64	33
CXVIII	3-MeC_6H_4	$4\text{-NO}_2C_6H_4$	19	5	39	50
CXIX	4-MeC_6H_4	H	49	45	80	100
CXX	4-MeC_6H_4	$n\text{-C}_3H_7$	40	45	68	33
CXXI	4-MeC_6H_4	C_6H_5	37	45	79	20
CXXII	4-MeC_6H_4	4-C_5H_4N	3	9	61	25
CXXIII	4-MeC_6H_4	4-MeC_6H_4	5	7	8	n.a.
CXXIV	4-MeC_6H_4	$4\text{-NO}_2C_6H_4$	21	12	106	n.a.
CXXV	$3,4\text{-Me}_2C_6H_3$	H	−4	7	82§	n.a.
CXXVI	$3,4\text{-Me}_2C_6H_3$	C_6H_5	26	46	62	3
CXXVII	$3,5\text{-Me}_2C_6H_3$	H	14	32	56§	6
CXXVIII	$4\text{-Me-2-NO}_2C_6H_3$	H	−9	24	13	n.a.
CXXIX	$4\text{-Me-2-NO}_2C_6H_3$	C_6H_5	10	55	57	<1
CXXX	$4\text{-Me-3-NO}_2C_6H_3$	H	14	87	96	

*,†,§ As in Tables 1 and 2.

As variants in the phenyl urea structure, a number of alkyl, saturated cyclic (Table 4), and heterocyclic aromatic groups were substituted for the phenyl group. Out of the 19 compounds of this type listed in Table 4 of Bruce and Zwar,[2] *N*-2-pyridyl and *N*-4-pyridyl ureas (XXXIII and XXXV) were active in chlorophyll retention (Table 2). *N*-4-pyridyl urea, which is inactive in cell division initiation, is an exception to the general rule that compounds inactive in cell division are inactive in chlorophyll retention.

All of the highly active compounds (Table 1) are derivatives of *N*-phenyl urea, but *N*-phenyl urea (XV) itself did not produce detectable chlorophyll retention activity. Monosubstitution of the phenyl ring with halogen (LXXV, LXXVIII, LXXXV, XCVI, XCVIII, C), methyl (CVI, CXIII, CXIX) and trimethylfluoromethyl (LXI, LXII) groups in the

meta and *para* positions produced active compounds, but substitution in the *ortho* positions was ineffective in inducing activity (Tables 2 and 3). Dichloro- and dimethyl-phenyl urea isomers were also tested (Table 3) and compounds X, XXVII, XC, XCV, CXXV, CXXVII were active; the 3,4-isomers in particular had significant activity.

Three of the highly active compounds (VIII, X, XIII) listed in Table 1 are *N*-phenyl ureas with substituents on the ring, hence such compounds have all of the structural requirements for activity in chlorophyll retention as is the case for cell division initiation.

TABLE 4. ACTIVITIES IN SENESCENCE RETARDATION OF UREAS CONTAINING SATURATED RINGS OR OF COMPOUNDS WITHOUT AN INTACT N–C–N– BRIDGE

Compound No.	Compound	Chlorophyll index concentrations (M)* 10^{-6}	10^{-5}	5×10^{-5}	Cell division index†
CXXXI	*N*-Cyclohexyl urea	−5	−4	0§	n.a.
CXXXII	*N*-Cyclohexyl-*N'*-phenyl urea	2	0	−13	n.a.
CXXXIII	*N*-Cyclopentyl urea	5	−3	8§	n.a.
CXXXIV	*N*-Cyclopentyl-*N'*-phenyl urea	−4	8	10	n.a.
CXXXV	*N,N'*-Diphenylguanidine	19	5	6	n.a.
CXXXVI	*N,N'*-Methylenedianiline	−5	2	9§	n.a.
CXXXVII	Phenylacetanilide	−3	0	9	n.a.
CXXXVIII	Phenyl-3-bromanilide	0	4	12	n.a.

*,†,§ As in Tables 1 and 2.

Phenylurea may be postulated to be capable of effectively occupying a cytokinin receptor site in the cell and, if its ring is appropriately substituted, to produce a complex with the receptor that is highly active in initiating cytokinin activities.

To further explore the relations of chemical structure to chlorophyll retention activity, substitutions were made in the free amino group of compounds of the type RC_6H_4NH-$CONH_2$. In compounds of the type $RC_6H_4NHCONHR'$ it was found that when R = H, chlorophyll retention activity was detected when R' was methyl (XVI), ethyl (XVIII), propyl (XIX), phenyl (XI), *trans*-2-phenylcyclopropyl (XXI), 3-pyridyl (XXIII), and 4-pyridyl (XII) (Table 2). Compounds containing a number of other groups (see Table 4 in Ref. 2) were inactive and some of these were active in cell division induction.[2] An increase in chlorophyll retention activity resulting from substitution of the second nitrogen of phenyl urea, on the opposite side of the bridge, conformed with the general relation of structure to cell division activity although a broader range of groups produced the effect on cell division than on chlorophyll retention. Further substitution of either nitrogen atom of the formula RNH–CO–NHR reduced activity in both chlorophyll retention and cell division induction (XXV–XXXII, Table 2).

The substitution of both nitrogens of urea on opposite sides of the bridge with appropriate ring systems produced highly active compounds, however the presence of one ring, if appropriately substituted, is sufficient for high activity. It is unlikely that two molecules of a phenyl urea are metabolically converted in the cell to an active diphenylurea because in some cases (LXXXVII, CI, CXXIII) the activities of symmetrical *N,N'*-disubstituted ureas are less than those of the *N*-monosubstituted (Table 3). Further, some highly active *N*-monosubstituted ureas, for example *N*-3,4-dichlorophenyl urea (X), show reduced activity when any ring system is added to the second nitrogen (LXXXVIII, XCII, XCI).

For cell division activity, Bruce and Zwar[2] reported that usually there was an increase in activity with the introduction of a second *N*-phenyl group into the free amino group of compounds of the type $RC_6H_4NHCONH_2$. The rule was found to hold for chlorophyll activity when R = H (XV, XI), 3,4-dimethyl (CXXV, CXXVI), 3,5-dichloro (XCIII, XCIV), 3-chloro-4-methyl (CV, II), 4-methyl-2-nitro (CXXVIII, CXXIX), 4-nitro-3-trifluoromethyl (LX, V), 3-methoxy (L, LI), 4-methoxy (LII, LIII), 3-ethoxy (XLVI, XLVII), 4-ethoxy (XLVIII, XLIX, 2-methoxy-4-nitro (LIV, LV), 2-methoxy-5-nitro (LVI, LVII), 4-methoxy-2-nitro (LVIII, LIX), 4-carboxy (XXXVIII, I), 4-carbethoxy (XXXIX, XL), and 4-cyanomethylphenyl (XLIV, XLV). In all of these cases, except 3,4-dimethyl and 3-chloro-4-methyl, the substituted urea with only one phenyl group was inactive. Bruce and Zwar[2] also noted exceptions to the general rule when one of the phenyl rings had bromo- or iodo- groups as substituents in the *para* position. Such exceptions were more evident with chlorophyll activity (Table 3). In some cases, the introduction of a second phenyl group into a highly active compound, such as *N*-4-bromopnenyl urea (VIII, C) and *N*-4-iodophenyl urea (XII, CIV), drastically reduced activity. The introduction of 4-chlorophenyl as the second substituted phenyl group was even more effective in removing activity (Table 3).

DISCUSSION

This survey of the activities of urea derivatives in senescence retardation and the comparison with cell division activities shows basic similarities in the requirements of chemical structure for activity in both processes of plant development. A –NH–CO–NH– bridge, and a planar aromatic ring (Table 4) are requirements of both, and changes in activity arising from variations upon this basic structure are similar. The range of compounds for which chlorophyll retention activity could be detected was less than for cell division activity and therefore restricted the scope for detailed quantitative comparison of activities in the two test systems. In addition, comparisons of the responses of different tissues to exogenous chemicals are always prone to confusion by the effects of secondary factors such as uptake, movement and metabolism. Nevertheless the evidence favors the conclusion that urea derivatives have similar receptor sites for affecting senescence retardation in radish leaf as for initiating cell division in tobacco stem pith.

The relationships of the chemical structure of phenylurea derivatives to their biological activity are complicated, not only by the secondary biological factors listed above, but also because many of the active compounds are likely to be inherently bilateral with respect to receptor sites in the cells of any tissue. In the simplest model system, a urea derivative and a receptor in the cell interact, and the complex so formed (if active) initiates the developmental response. Ureas with one ring are postulated to have the elements of structure necessary for successful attachment and the formation of an active complex. In those active compounds with two rings, it may be presumed that only one ring will be attached to the receptor at any time and the other ring will act to modify the attachment, or the activity of the complex. In such circumstances, the biological response induced by a urea with two rings could be the outcome of attachment by one ring, the other ring or both. Further, the attachment function of each ring may be expected to be different from its function in the modification of the activity of the complex. Thus the overall biological response of a urea with two rings would at best reflect the sum of these functions. The problems of the interpretation of effects of structural modifications in even this simple model are apparent.

Two modes of function for the two ring elements of *N,N'* disubstituted ureas were indicated by the activities of symmetrical *N,N'* disubstituted ureas. Compounds of this type were tested (see Table 9 in Bruce and Zwar[2]) and only ureas symmetrically substituted with phenyl (XI), 2-chlorophenyl (LXXVII), 3-chlorphenyl (LXXXI), and 3-tolyl (CXVII) groups had chlorophyll activity, despite the inclusion of potentially highly active groups such as 3,4-dichlorophenyl (XCII).

A minor sorting of the attachment and complex-activity functions of ring structures appears possible through the antagonism of cytokinin-induced senescence retardation by derivatives of *N*-benzylurea. Such studies by Kefford *et al.*[9] showed *N*-benzyl-*N'*-phenyl urea to be a strong antagonist of an active phenylurea, such as *N*-3,4-dichlorophenyl urea. This antagonism may be presumed to be chiefly concerned with competition between the phenyl ring of *N*-benzyl-*N'*-phenylurea and the 3,4-dichlorophenyl urea ring in the attachment function, because *N*-benzyl urea itself is not an antagonist. The antagonistic effects of the *ortho*, *meta* and *para* analogues of chloro- and *N*-benzyl-*N'*-nitrophenyl ureas showed that the order of increasing antagonism was also the order of increasing cytokinin activity of the chloro- and nitro-phenyl ureas. Thus for the attainment of activity through the substitution of a phenyl ring with chloro or nitro groups, *ortho* substitution appears least favorable to attachment. This is supported by the activities of derivatives of *N*-1-propyl-*N'*-tolyl urea (CVII, CXIV, CXX) for which the propyl group cannot be directly involved in attachment.

Complex formation between a phenyl urea and a receptor in a cell could occur through at least two types of interaction: (a) ring attachment, presumably involving the aromatic π-electrons, and (b) hydrogen-bond formation by the peptide link –NH–CO–. Both types of interaction would be affected by substitution, in particular *meta* and *para* substitution of the ring would be expected to influence the potential for hydrogen bonding. The observed inhibition of activity by *ortho* substituents,, appears most likely to be steric, by preventing the phenylurea molecule from assuming the necessary conformational relationship with the receptor.

The common inhibitory effects of *ortho* substitution of phenylurea derivatives has relevance to some ureidopurines[6] recently found to have some cytokinin activity. This finding might suggest that phenyl urea derivatives achieve activity through providing an *N*-6-substituent for a 6-aminopurine. However, the structure-activity relationships found for the ureidopurines do not parallel those found for substituted ureas. For example, McDonald *et al.*[6] found the substituted 6-phenylureidopurines showed a decreasing order of activity: *o*-tolyl $\gg$ *m*-chlorophenyl $\ggg$ *p*-tolyl, which contrasts with the order: *m*-chlorophenyl > *p*-tolyl > *o*-tolyl reported by Bruce and Zwar for the analogous *N,N'*-diphenyl urea derivatives.[2]

Urea cytokinins and purine cytokinins have the same regulating effects upon a variety of developmental processes suggesting that they act at the same site in the cell. This possibility is supported by the effects of benzyl urea antagonists of cytokinin activity[9] and by the fact that interaction of urea and purine cytokinins with the primary site for cell division induction may require metabolic predisposition of both types of molecules.[10] Investigations of the relations of the chemical structure of purine cytokinins to activity[6,8,11,12] have not produced a definition of the requirements for activity that would be of aid to speculations on structure-activity relations for urea cytokinins or the nature of the receptor site in the cell.

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[11] LETHAM, D. S. and YOUNG, H. (1971) *Phytochemistry* **10**, 23.

EXPERIMENTAL

The methods of preparation, purification and identification of the urea derivatives and related compounds used in this investigation have been described by Bruce and Zwar.[2] The biological assay using chlorophyll retention in discs from leaves of radish (*Raphanus sativus*) as an index of senescence retardation and the derivation of the chlorophyll index through reference to the relative effects of water and 10^{-5} M kinetin, were described by Kefford *et al.*[9]

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[12] SCHMITZ, R. Y., SKOOG, F., HECHT, S. M. and LEONARD, N. J. (1971) *Phytochemistry* **10,** 275.