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CYTOKININS: SYNTHESIS OF 2-, 8-, AND 2,8-SUBSTITUTED 6-(3-METHYL-2-BUTENYLAMINO)PURINES AND THEIR RELATIVE ACTIVITIES IN PROMOTING CELL GROWTH

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Key Word Index—Cytokins; 2-, 8- and 2,8-substituted 6-(3-methyl-2-butenylamine) purines; relative activity; growth activities.

Abstract—We have synthesized 2- and 8-monosubstituted and 2,8-disubstituted derivatives of the cytokinin 6-(3-methyl-2-butenylamino)purine (N^6-isopentenyladenine) and have shown the dependence of growth-promoting activity in the tobacco bioassay upon the position, number, and type of substituent. The representative substituent groups were MeS, Me, $MeSO_2$, $C_6H_5CH_2S$, HS and Cl. The 8-methyl derivative was exceptional in being more active than the unsubstituted parent compound. In general, substitution in the 8-position decreases activity less than substitution in the 2-position, with the exception of the electron-attracting methylsulfonyl. Substitution in both the 2- and 8-positions lowers the activity more than substitution at either single position on the adenine nucleus, with the exception of the 2,8-dimethyl derivative. The chloro and methylthio derivatives show activity in the same range as the methyl derivatives, and the mercapto compounds, which exist mainly as C=S tautomers, show somewhat less activity than the corresponding methylthio compounds. Bulky ($C_6H_5CH_2S$ and $MeSO_2$) and strongly electron-attracting ($MeSO_2$) substituents cause relatively great reduction in cytokinin activity.

INTRODUCTION

STRUCTURE-ACTIVITY relationships for cytokinins have received renewed attention in anticipation of the discovery of new cytokinins, in attempting to relate cytokinin activity to other kinds of biological activity, and in providing a large number of compounds from which to choose in probing the nature of cytokinin activity.[1–5] Modification of the N^6-sidechain on adenine has received major attention[3,6–15] because of the natural occurrence of the cytokinins: 6-(3-methyl-2-butenylamino)purine [N^6-(Δ^2-isopentenyl)adenine,

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i^{6}Ade or 2iP],[3,16] 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosyl purine [N^6-(Δ^2-isopentenyl)adenosine, i^{6}Ado or 2iPA],[3,17 21] 6-(4-hydroxy-3-methyl-*trans*-2-butenylamino) purine [zeatin],[3] 6-(4-hydroxy-3-methylbutylamino)purine [dihydrozeatin],[3] and 6-(4-hydroxy-3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine [ribosylzeatin] as the *cis*.[3,16,22] and *trans*[3,22] isomers. However, with the identification of 6-(3-methyl-2-butenylamino)-2-methyl thioadenosine [ms2iPA][3,19-21,23,24] and 6-(4-hydroxy-3-methyl-2-butenyl)-2-methylthioadenosine [ms-ribosyl-*cis*- and *trans*-zeatins][3,19-21,25] as components of several *t*RNAs, the possibility of finding other cytokinins with ring modification, including precursor groups to MeS, was recognized. In addition, it seemed desirable to remedy the lack of information concerning the comparative effects on biological activity of 2-, 8-, and 2,8-substitution on the 6-(3-methyl-2-butenylamino)purine structure.

The finding that zeatin had the highest activity of any known cytokinin had offered the challenge of synthesizing more active compounds. The challenge was only partially met when it was found that of the 2-substituted zeatins, 2-chlorozeatin was at least as active and possibly more active than zeatin itself,[26] and that the other 2-substituted derivatives were less active than their unsubstituted counterparts.[15,26,27] For both the zeatin and the 2iP derivatives, the activities were in decreasing order for 2-substituents: Cl > NH_2 $\geqslant$ MeS $\gg$ OH. By contrast, in senescence (chlorophyll retention) tests the 8-methyl derivatives of 6-benzylaminopurine and kinetin (6-furfurylaminopurine) showed enhanced cytokinin activity over the unsubstituted compounds.[28] The 2- and 8-monosubstituted and 2,8-disubstituted isopentenyladenines (Ia–r) synthesized for comparison of activities in this study were planned in groups of three with the same substituent. By selection of a variety of substituents, the information to be derived included the effects of different substituents at a single position and of changes in the position and number of the same substituent.

RESULTS AND DISCUSSION

Syntheses

Most of the substituted N^6-(Δ^2-isopentenyl)adenines (**1**) were prepared in a straightforward manner by heating at reflux the suitable substituted purine precursor with 3-methyl-2-butenylamine,[29] 3-methyl-2-butenylamine in ethanol, or 3-methyl-2-butenylamine hydrochloride in *n*-butanol with triethylamine.[30] The difference in reactivity of the 2-, 6-, and 8-positions toward nucleophilic displacement was advantageous, in that a good

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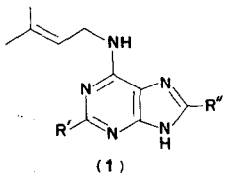
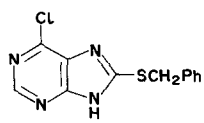
leaving group such as chloro or methylsulfonyl at the 6-position is more reactive than the same or a poorer leaving group at either the 2- or the 8-position.[31] Conditions could thus be selected which led to selective displacement by the amine at the 6-position. In most cases the substituted purine reactant was synthesized by published procedures (Table 1). The 6-chloro compounds **2** and **3**, which had not been described previously, were synthesized from 8-benzylthio-6-hydroxypurine[32] and 2,8-bis-(benzylthio)-6-hydroxypurine, by treatment with phosphorus oxychloride. The latter was prepared by reaction of 2,8-dimercapto-6-hydroxypurine[33] with benzyl chloride in aqueous base. The mercapto-substituted compounds (**1**m–o) were synthesized via the benzylthio-substituted compounds (**1**j–l) by removal of the benzyl group with sodium and liquid ammonia, which did not effect cleavage of the isopentenyl–N bond.

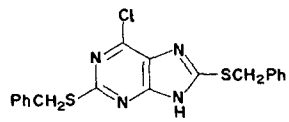


(1)

(1a) R′ = SMe; R″ = H
(1b) R′ = H; R″ = SMe
(1c) R′ = SMe; R″ = SMe
(1d) R′ = Me; R″ = H
(1e) R′ = H; R″ = Me
(1f) R′ = Me; R″ = Me
(1g) R′ = SO_2Me; R″ = H
(1h) R′ = H; R″ = SO_2Me
(1i) R′ = SO_2Me; R″ = SO_2Me
(1j) R′ = SCH_2Ph; R″ = H
(1k) R′ = H; R″ = SCH_2Ph
(1l) R′ = SCH_2Ph; R″ = SCH_2Ph
(1m) R′ = SH; R″ = H
(1n) R′ = H; R″ = SH
(1o) R′ = SH; R″ = SH
(1p) R′ = Cl; R″ = H
(1q) R′ = H; R″ = Cl
(1r) R′ = Cl; R″ = Cl



(2)



(3)

Cytokinin activity

Cytokinin activities were determined by the tobacco bioassay,[34] on the basis of fresh weight yields of callus cultured on serial concentrations of the test substances. From results presented in Fig. 1, it is apparent that the 2-, 8-, and 2,8-substituted derivatives of 2iP generally have lower cytokinin activities than 2iP itself. However, as shown clearly in Fig. 2, the 8-methyl derivative is more active than unsubstituted 2iP. This accords with the finding of Kulaeva *et al.*[28] cited above. Generally, substitution in the 8-position decreases activity less than substitution in the 2-position. The only exception found thus far is in the case of the electron-withdrawing substituent, methylsulfonyl, for which all three of the substituted derivatives are much less active than 2iP, and the 8-substituted derivative is at least ten times less active than the 2-substituted isomer.

In general, the 2,8-disubstituted derivatives are definitely less active than the 2- or 8-substituted derivatives. The one exception is the 2,8-dimethyl derivative, which reflects in part the activity-enhancing effect of the methyl group in the 8-position alone.

Certain generalizations can be made on the basis of the relative effectiveness of various substituent groups. The chloro and methylthio derivatives show activity in the same range as the methyl derivatives. The unique effect of 2-chloro substitution in equalizing (2iP series) or enhancing (zeatin series) activity has been reported previously.[26]

The mercapto compounds, which exist mainly as ketonic tautomers, show somewhat less activity than the corresponding methylthio compounds. Although the mercapto derivatives are susceptible to oxidation, we assumed them to be in the reduced form for the sake

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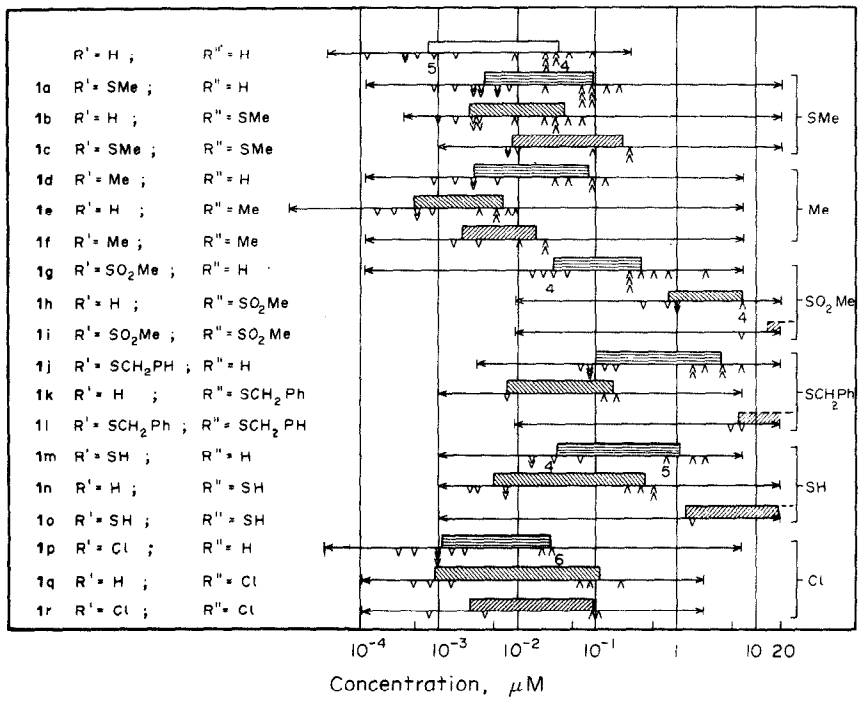

FIG. 1. SUMMARY OF CYTOKININ ACTIVITIES OF 2-, 8, AND 2,8-SUBSTITUTED DERIVATIES OF 2iP.
The symbols refer to the derivatives as described in the text and Scheme 1. For convenience the nature and positions of the substituents are also indicated in the margins, and the positions are further specified by the shading of the bars. The base line represents the tested concentration range for each compound, while the bar represents the mean range over which growth increases as a nearly linear function of the log of concentration of the tested compound. Arrows indicate start and end points of the linear ranges for individual experiments. Open-ended bars indicate that maximum growth was not obtained at the highest tested concentration.

of the comparison of bioassay results. It should be noted that substitution of the mercapto group on the 2-position has about the same influence on activity as the hydroxyl group.[26]

The relatively great reduction in cytokinin activity caused by the benzylthio and methylsulfonyl groups is ascribed to the bulkiness of these substituents as well as to the electronegativity of the latter group. It was further observed that differences in activity between the 2-, 8- and 2,8-derivatives are much greater for benzylthio and methylsulfonyl substituents than for the other substituents. This effect, illustrated for the benzylthio derivatives in a typical experiment in Fig. 3, is striking, particularly when compared with the methyl derivatives illustrated in Fig. 2.

While the data for the entire series accurately reflect the growth-promoting activities under the standard test conditions, and these show dependence upon the position, number, and type of substituent, it is recognized that at least in part the various substituents may be exerting their effects by alteration in the rates of absorption and metabolism of the exogenous cytokinins. Nevertheless, the present results are in general agreement and extend earlier results on the structure-activity relationships of N^6-(Δ^2-isopentenyl) adenine derivatives and further indicate the importance of individual loci as well as the overall structure for the attainment of the highest order of biological activity. A total understanding of the mode of action of this class of compounds must take into account the fact that among the many that exhibit activity there are both specific and non-specific ring substituent effects.

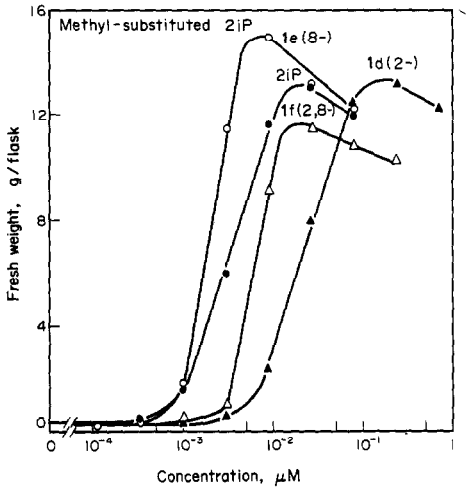

FIG. 2. EFFECT OF THE POSITION OF THE METHYL SUBSTITUENT ON CYTOKININ ACTIVITY IN THE TOBACCO BIOASSAY.
Symbols as in Scheme 1. Experiment C 115, 5 October to 11 November 1970.

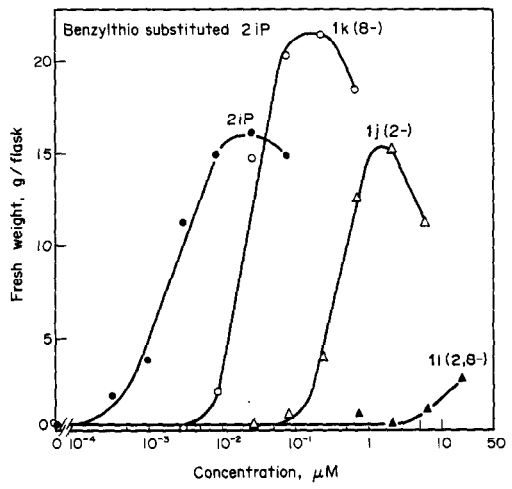

FIG. 3. EFFECT OF THE BENZYLTHIO SUBSTITUENT ON CYTOKININ ACTIVITY IN THE TOBACCO BIOASSAY.
Symbols as in Scheme 1. Experiment C 111, 11 September to 15 October 1970.

EXPERIMENTAL

Synthesis of test substances. All m.ps are uncorrected. Synthesis of 6-(3-methyl-2-butenylamino)-2-methylthiopurine (**1a**)[21] and 2-chloro-6-(3-methyl-2-butenylamino)purine[26] has been described elsewhere.

General procedure for the preparation of the substituted 6-(3-*methyl*-2-*butenylamino*)*purines in Table* 1. A soln of the suitably substituted 6-chloro(6-methylsulfonyl for **1g**)-purine with 3-methyl-2-butenylamine hydrochloride[30] (~ 1·2 mmol/mmol purine) and Et_3N (~ 1 ml/mmol purine) in *n*-butanol (10 ml/mmol purine)[35] was heated at reflux for the time period specified in Table 1. Evaporation to dryness, followed by shaking the residue with H_2O[36] and filtering, gave the crude product. Purification was effected by recrystallization from the solvents listed in Table 1, with decolorization.[37] Quantitative UV absorption spectra for the new substituted 6-(3-methyl-2-butenylamino)purines are listed in Table 2.

6-(3-*Methyl*-2-*butenylamino*)-8-*methylthiopurine* (**1b**). NMR δ [$(CD_3)_2SO$]: 1·7 (6H, *s*, $(Me)_2C$), 2·69 (3H, *s*, MeS), 4·14 (2H, *t*, C–CH_2–N), 5·35 (1H, *m*, C=CH–C), 7·23 (1H, *t*, N^6H, exchanged by D_2O), 8·12 (1H, *s*, PuC_2–H) (Found: C, 52·72; H, 5·95; N, 28·17. Calc. for $C_{11}H_{15}N_5S$: C, 52·98; H, 6·06; N, 28·09%).

6-(3-*Methyl*-2-*butenylamino*)-2,8-*bis*(*methylthio*)*purine* (**1c**). NMR δ [$(CD_3)_2SO$]: 1·7 (6H, *s*, $(Me)_2C$), 2·48, 2·65 (6H, 2-*s*, SCH_3s), 4·1 (2H, *t*, C–CH_2–N), 5·33 (1H, *m*, C=CH–C), 7·34 (1H, *t*, N^6H, exchanged by D_2O) (Found: C, 48·56; H, 5·71; N, 23·93. Calc. for $C_{12}N_{17}N_5S_2$: C, 48·79; H, 5·80; N, 23·70%).

2-*Methyl*-6-(3-*methyl*-2-*butenylamino*)*purine* (**1d**). NMR δ [$(CD_3)_2SO$–D_2O]: 1·71 (6H, *s*, $(Me)_2C$), 2·42 (3H, *s*, PuC_2–Me), 4·15 (2H, *d*, C–CH_2–N), 5·33 (1H, *m*, C=CH–C), 7·98 (1H, *s*, PuC_8–H) (Found: C, 60·87; H, 7·12; N, 32·25. Calc. for $C_{11}H_{15}N_5$: C, 60·81; H, 6·96; N, 32·23%).

8-*Methyl*-6-(3-*methyl*-2-*butenylamino*)*purine* (**1e**). NMR δ [$(CD_3)_2SO$]: 1·7 (6H, *s*, $(Me)_2C$), 2·46 (3H, *s*, PuC_8–Me), 4·1 (2H, *t*, C–CH_2–N), 5·33 (1H, *m*, C=CH–C), 7·23 (1H, *m*, N^6H, exchanged by D_2O), 8·12 (1H, *s*, PuC_2–H) (Found: C, 60·85; H, 6·87; N, 32·12. Calc. for $C_{11}H_{15}N_5$: C, 60·81; H, 6·96; N, 32·23%).

2,8-*Dimethyl*-6-(3-*methyl*-2-*butenylamino*)*purine* (**1f**). NMR δ [$(CD_3)_2SO$–D_2O]: 1·7 (6H, *s*, $(Me)_2C$), 2·39, 2·41 (6H, 2-*s*, PuC_2– and C_8–CH_3's), 4·07 (2H, *d*, C–CH_2–N), 5·29 (1H, *m*, C=CH–C) (Found: C, 62·54; H, 7·53; N, 30·19. Calc. for $C_{12}H_{17}N_5$: C, 62·31; H, 7·41; N, 30·28%).

6-(3-*Methyl*-2-*butenylamino*)-2-*methylsulfonylpurine* (**1g**). NMR δ [$(CD_3)_2SO$–D_2O]: 1·73 (6H, *s*, $(Me)_2C$), 3·31 (3H, *s*, SO_2Me), 4·17 (2H, *d*, C–CH_2–N), 5·35 (1H, *m*, C = CH–C), 8·31 (1H, *s*, PuC_8–H) (Found: C, 47·17;

[35] Compound **1g** separates analytically pure from the reaction mixture on cooling if twice this amount of *n*-BuOH is used.

[36] For compound **1h**, the mixture was acidified to pH 1 with dil. HCl and then refrigerated for several hours. For **1i**, the residue was taken up in a small amount of EtOH before adding water, then treated as for **1h**.

[37] Compound **1b** was obtained pure by washing the crude solid with chilled ether.

TABLE 1. SYNTHESIS OF SUBSTITUTED 6-(3-METHYL-2-BUTENYLAMINO)PURINES (1)

Compound	Starting purine	Reflux time (hr)	Recrystallization solvent	Yield (%)	M.P. (°C)
1b	6-Cl[38]	3	—	92	196–197
1c	6-Cl[33]	4·5	EtOH	77	183–184
1d	6-Cl[39]	2·5	EtOH	86	252·5–253
1e	6-Cl[40]	2·5	EtOH	53	219–220
1f	6-Cl[41]	3	EtOH–H_2O	35	203–204
1g	6-SO_2Me[33]	20	—	74	261–262
1h	6-Cl[33]	4	EtOH	58	243–244
1i	6-Cl[33]	3	EtOAc	39	165–167
1k	6-Cl[42]	3	EtOH–H_2O	76	201–203 dec.
1l	6-Cl[42]	4	EtOH	68	122·5–124 dec.

TABLE 2. UV MAXIMA AND MINIMA, nm($\epsilon \times 10^{-3}$), FOR COMPOUNDS 1

	95% EtOH 0·1 N HCl		95% EtOH		95% EtOH 0·1 N NaOH	
	λ_{max}	λ_{min}	λ_{max}	λ_{min}	λ_{max}	λ_{min}
1b	235 (12·7) 302 (25·1)	257 (5·1)	225 (27·1) 286 (23·1)	250 (5·1)	291 (23·8)	254 (3·8)
1c	255 (21·9) 313 (29·2)	230 (14·8) 278 (6·3)	241 (27·1) 297 (24·6)	260 (7·8)	233 (30·7) 302 (23·4)	261·5 (4·0)
1d	281 (20·0)	238 (3·8)	270 (18·7)	229 (2·2)	277 (17·2)	242·5 (3·9)
1e	280 (19·4)	239 (3·7)	268·5 (20·6)	230 (2·5)	276·5 (19·6)	242·5 (3·4)
1f	282·5 (21·3)	240 (4·1)	270 (20·0)	231 (3·0)	278 (18·2)	244 (3·7)
1g	270·5 (13·4)	239 (3·2)	268 (15·0)	239 (3·8)	274 (12·1) 292 st sh*	252 (5·2)
1h	281·5 (17·8)	241 (3·6)	294 (19·4)	245 (3·0)	292 (20·0)	244 (3·2)
1i	222 (26·5) 278 sh* 300 (14·2)	246 (3·8)	222 (24·1) 299 (15·8)	251 (4·5)	301·5 (15·6)	253 (2·6)
1j	253 (23·3) 292 (16·1)	232 (12·2) 278 (13·5)	242 (27·0) 282 (17·1)	223 (12·7) 260 (11·5)	288 (16·5)	257 (7·4)
1k	303 (26·9)	258 (6·2)	288 (22·4)	250 (6·9)	293 (24·4)	255 (5·3)
1l	257 (20·4) 316 (30·5)	237 (17·8) 282 (7·6)	242·5 (27·9) 302 (24·6)	230·5 (23·1) 265 (9·8)	305·5 (24·7)	266 (7·1)
1m	251 290		265 317·5		260 308	
1n	246 (14·0) 315 (23·3)	267·5 (4·2)	241 (19·3) 305 (28·9) 312 (31·7)	260 (4·2)	309 (24·7)	262 (4·1)†
1o	244 (26·6) 313 (29·9) 322 (32·5)	267 (7·8)	244 (26·7) 313 (30·2) 322 (33·0)	266 (7·6)	255 (19·7) 318 (22·6)	279 (12·0)†
1q	278 (17·6)	237·5 (3·7)	275·5 (18·3) 307 sh*	240 (3·6)	277 (20·7) 284 (16·2) sh*	241 (3·3)
1r	275 (20·4)	236 (3·4)	277 (19·9)	240·5 (4·0)	280·5 (20·5) 287 sh*	244 (3·8)

* sh = Shoulder. † 2-Mercaptoethanol added to UV solutions and blanks (10–25 μl/100 ml).

H, 5·38; N, 24·79. Calc. for $C_{11}H_{15}N_5O_2S$: C, 46·96; H, 5·37; N, 24·89%).

6-(3-*Methyl*-2-*butenylamino*)-8-*methylsulfonylpurine* (**1h**). NMR δ [$(CD_3)_2SO$–D_2O]: 1·76 (6H, *s*, $(Me)_2C$), 3·35 (3H, *s*, SO_2Me), 4·29 (2H, *d*, C–CH_2–N), 5·38 (1H, *m*, C=CH–C), 8·24 (1H, *s*, PuC_2–H) (Found: C, 46·74 H, 5·48; N, 24·89. Calc. for $C_{11}H_{15}N_5O_2S$: C, 46·96; H, 5·37; N, 24·89%).

6-(3-*Methyl*-2-*butenylamino*)-2,8-*bis*-(*methylsulfonyl*)*purine* (**1i**). NMR δ [$(CD_3)_2SO$]: 1·73 (6H, *s*, $(Me)_2C$), 3·30, 3·47 (3H each, 2-*s*, SO_2CH_3's), 4·15 (2H, *m*, C–CH_2–N), 5·33 (1H, *m*, C=CH–C) (Found: C, 40·12; H, 4·87; N, 19·44. Calc. for $C_{12}H_{17}N_5O_4S_2$: C, 40·10; H, 4·77; N, 19·48%).

8-*Benzylthio*-6-(3-*methyl*-2-*butenylamino*)*purine* (**1k**). NMR δ [$(CD_3)_2SO$–D_2O]: 1·72 (6H, *s*, $(Me)_2C$), 4·15 (2H, *d*, C–CH_2–N), 4·53 (2H, *s*, $PhCH_2S$), 5·38 (1H, *m*, C=CH–C), 7·36 (5H, *m*, PhH's), 8·17 (1H, *s*, PuC_2–H) (Found: C, 62·50; H, 5·78; N, 21·40. Calc. for $C_{17}H_{19}N_5S$: C, 62·74; H, 5·88; N, 21·52%).

2,8-*Bis*-(*benzylthio*)-6-(3-*methyl*-2-*butenylamino*)*purine* (**1l**). NMR δ [$(CD_3)_2SO$–D_2O]: 1·68 (6H, *s*, $(Me)_2C$), 4·1 (2H, *d*, C–CH_2–N), 4·37, 4·48 (4H, 2-*s*, $PhCH_2S$'s), 5·3 (1H, *m*, C = CH–C), 7·3 (10H, *m*, PhH's) (Found: C, 64·36; H, 5·79; N, 15·86. Calc. for $C_{24}H_{25}N_5S_2$: C, 64·40; H, 5·63; N, 15·64%).

2-*Benzylthio*-6-(3-*methyl*-2-*butenylamino*)*purine* (**1j**). To 1·0 g (2·75 mmol) 2,6-bis-(benzylthio)purine[43] was added 5·0 g 3-methyl-2-butenyl amine.[29] The resulting solution was heated at reflux for 20 hr and cooled. Water was then added to precipitate the crude product which was filtered and washed with H_2O. Trituration with hot EtOH left 580 mg (65%) of a white solid, m.p. 259–260·5°. NMR δ [$(CD_3)_2SO$]: 1·7 (6H, *s*, $(Me)_2C$), 4·16 (2H, *t*, C–CH_2–N), 4·43 (2H, *s*, $PhCH_2S$), 5·34 (1H, *m*, C = CH–C), 7·39 (5H, *m*, PhH's), 7·73 (1H, *m*, N^6H, exchanged by D_2O), 8·03 (1H, *s*, PuC_8–H) (Found: C, 62·52; H, 5·77; N, 21·73. Calc. for $C_{17}H_{19}N_5S$: C, 62·74; H, 5·88; N, 21·52%).

2-*Mercapto*-6-(3-*methyl*-2-*butenylamino*)*purine* (**1m**). To a soln of 325 mg (1·0 mmol) of **1j** in 50 ml liq. NH_3 was added sodium metal in small increments until a blue color persisted for more than 15 min (*ca.* 100 mg). The NH_3 was allowed to evaporate and 35 ml Et_2O was added to the residue. After being warmed slightly until evolution of gas subsided, the Et_2O layer was extracted with two 25-ml portions H_2O. The aqueous extracts were then acidified with AcOH and refrigerated for several hr. The ppt was filtered, washed with H_2O, and dried *in vacuo* at 65° giving 201 mg (86%) of a while solid, m.p. 262–263°. NMR δ [$(CD_3)_2SO$]: 1·71 (6H, *s*, $(Me)_2C$), 4·23 (2H, *m*, C–CH_2–N), 5·34 (1H, *m*, C=CH–C), 8·02 (1H, *s*, PuC_8–H) (Found: C, 50·75; H, 5·54; N, 29·56. Calc. for $C_{10}H_{13}N_5S$: C, 51·04; H, 5·57; N, 29·76%).

8-*Mercapto*-6-(3-*methyl*-2-*butenylamino*)*purine* (**1n**). To 650 mg (2·0 mmol) of **1k** dissolved in *ca.* 120 ml liq. NH_3 was added Na metal in small increments until a blue color persisted for more than 20 min (about 400 mg). The reaction was cleared by the addition of a small amount of NH_4Cl, then allowed to evaporate. To the residue was added 70 ml Et_2O and the whole was extracted with three 40-ml portions H_2O. The aqueous extracts were treated with decolorizing carbon, filtered, and then carefully acidified with AcOH. After refrigeration overnight the product was filtered, washed with H_2O, and dried *in vacuo* giving 280 mg (60%) of a white solid, m.p. 254–256° dec. NMR δ [$(CD_3)_2SO$]: 1·73 (6H, *s*, $(Me)_2C$), 4·06 (2H, *t*, C–CH_2–N), 5·33 (1H, *m*, C=CH–C), 6·68 (1H, *t*, N^6H, exchanged by D_2O), 8·17 (1H, *s*, PuC_2–H) (Found: C, 50·78; H, 5·75; N, 29·57. Calc. for $C_{10}H_{13}N_5S$: C, 51·04; H, 5·57; N, 29·76%).

2,8-*Dimercapto*-6-(3-*methyl*-2-*butenylamino*)*purine* (**1o**). To 600 mg of sodium dissolved in *ca.* 100 ml liq. NH_3 was added dropwise 1·2 g (2·68 mmol) of **1l** dissolved in 7 ml of 1,2-dimethoxyethane, with stirring. After stirring 10 min the reaction mixture became colorless. The NH_3 was allowed to evaporate and to the residue was added 50 ml H_2O and 50 ml of Et_2O. The aqueous layer was separated and washed with a further 50-ml portion of Et_2O. Acidification with AcOH precipitated a tan solid which was filtered after several hours of refrigeration and dried. The crude product was dissolved in 1 N NaOH and decolorized. The filtrate was acidified with AcOH and refrigerated for several hours. The product was filtered, washed sequentially with H_2O, 1,2-dimethoxyethane, and Et_2O, giving 480 mg (67%) of a white solid, m.p. 283–284° dec. NMR δ [$(CD_3)_2SO$–D_2O]: 1·58 (6H, *s*, $(Me)_2C$), 3·87 (*d*, C–CH_2–N), 5·03 (1H, *m*, C=CH–C) (Found: C, 45·16; H, 5·07; N, 26·29. Calc. for $C_{10}H_{13}N_5S_2$: C, 44·92; H, 4·90; N, 26·19%). Disulfide formation can be detected by λ_{max}^{EtOH} at 280 and 352 nm.

8-*Chloro*-6-(3-*methyl*-2-*butenylamino*)*purine* (**1q**). To 945 mg (5·0 mmol) 6,8-dichloropurine[38] in 4 ml EtOH was added 4·0 g 3-methyl-2-butenylamine. The solution was heated at reflux for 12 hr and cooled. The solution was poured into 40 ml 10% AcOH and refrigerated overnight. The solid was collected, washed with H_2O, then cold EtOH, and recrystallized from EtOH–DMF with decolorization, yield 350 mg (30%), m.p. 228–229° (dec). NMR δ [$(CD_3)_2SO$–D_2O–NaOD]: 1·69 (6H, *s*, $(Me)_2C$), 4·01 (2H, *d*, C–CH_2–N), 5·25 (1H, *m*, C=CH–C), 7·86 (1H, *s*, PuC_2–H) (Found: C, 50·76; H, 5·18; N, 29·68. Calc. for $C_{10}H_{12}ClN_5$: C, 50·53; H, 5·09; N, 29·46%).

2,8-*Dichloro*-6-(3-*methyl*-2-*butenylamino*)*purine* (**1r**). Prepared from 2,6,8-trichloropurine,[44] 506 mg (2·5 mmol), in a parallel procedure to **1q** using a 2-hr reflux period. The crude solid after washing with H_2O was dissolved

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[42] See Experimental.

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in EtOAc and dried. Addition of light petrol. (30–60°) to the EtOAc soln gave 560 mg (84%) of a white solid, m.p. 199–200 (dec). NMR δ [$(CD_3)_2SO$–D_2O]: 1·7 (6H, *s*, $(Me)_2C$), 4·0 (2H, *d*, C–CH_2–N), 5·27 (1H, *m*, C=CH–C) (Found: C, 44·43; H, 4·28; N, 25·34. Calc. for $C_{10}H_{11}Cl_2N_5$: C, 44·14; H, 4·07; N, 25·73%).

8-*Benzylthio*-6-*chloropurine* (**11**). 10 g (38·7 mmol) 8-benzylthio-6-hydroxypurine,[32] 250 ml $POCl_3$ and 20 ml of *N*,*N*-diethylaniline were heated at reflux with stirring for 3 hr and cooled. The excess $POCl_3$ was removed under reduced pressure and the residue was poured into 400 ml of ice water with rapid manual stirring. The solution was made strongly basic with 10 N NaOH, then allowed to stand 15 min in the cold before acidifying to pH 1 with conc. HCl. After refrigeration overnight the solid was filtered, washed with H_2O, and recrystallized from EtOH with decolorization, yield 5·65 g (53%). A further recrystallization from EtOH, with decolorization, gave an analytical sample, m.p. 171–172° dec. UV: λ_{max}^{EtOH} 297; λ_{max}^{EtOH} (pH < 3) 255, 296; λ_{max}^{EtOH} (pH > 10) 302·5. NMR δ from TMS [CF_3CO_2H]: 4·84 (2H, *s*, $ArCH_2S$), 7·45 (5H, *s*, ArH's), 9·11 (1H, *s*, PuC_2–H) (Found: C, 52·20; H, 3·40; N, 20·40. Calc. for $C_{12}H_9ClN_4S$: C, 52·08; H, 3·28; N, 20·24%).

2,8-*Bis*-(*benzylthio*)-6-*hydroxypurine*. To a stirred solution of 6-hydroxy-2,8-purinedithiol,[33] 20 g (0·1 mol), in 300 ml H_2O containing 8 g (0·2 mol) NaOH was added dropwise 28 g benzyl chloride over a 0·5 hr period. Stirring was continued for 24 hr, when the solid was filtered, washed with H_2O, and dried *in vacuo*, yield 36 g (95%), m.p. over a wide range (*ca.* 110°) with dec. UV: λ_{max}^{EtOH} 282·5; λ_{max}^{EtOH} (pH < 3) 285; λ_{max}^{EtOH} (pH > 10) 295. NMR δ from TMS [$(CD_3)_2SO$–D_2O]: 4·43, 4·49 (4H, 2-*s*, $ArCH_2S$'s), 7·32 (10H, *m*, ArH's). The compound was sufficiently pure for the following step.

2,8-*Bis*-(*benzylthio*)-6-*chloropurine* (**3**). 20 g (52·5 mmol) 2,8-bis-(benzylthio)-6-hydroxypurine, and 350 ml $POCl_3$ were heated at reflux with stirring for 3 hr and cooled. The excess $POCl_3$ was removed under reduced pressure and the residue was poured onto ice with manual stirring. The soln was made strongly basic with 10 N NaOH, then the pH was adjusted to 5 with AcOH. After refrigeration for 1 hr the ppt was filtered, washed with H_2O, and dried *in vacuo*. The crude reddish solid was dissolved in hot C_6H_6, decolorized, then precipitated by the addition of light petrol. (b.p. 30–60°). Dissolving the evaporated mother liquors in smaller amounts of C_6H_6 followed by precipitation as above gave additional crops, total yield 11·8 g (56%). Reprecipitation from C_6H_6 solution with light petrol. gave an analytical sample, m.p. 159–161°. UV: λ_{max}^{EtOH} 232, 318; λ_{max}^{EtOH} (pH < 3) 230, 262, 318; λ_{max}^{EtOH} (pH > 10) 250, 320. NMR δ from TMS [CF_3CO_2H]: 4·56, 4·77 (4H, 2-*s*, $ArCH_2S$'s), 7·42 (10H, *m*, ArH's) (Found: C, 57·21; H, 3·86; S, 16·19; Cl, 8·68. Calc. for $C_{19}H_{15}ClN_4S_2$: C, 57·20; H, 3·79; S, 16·07; Cl, 8·88%).

Bioassay procedures. The determination of cytokinin activities was based on the tobacco bioassay as described by Linsmaier and Skoog.[34] The medium contained the mineral salts specified in Table 6, part A, of this reference, and the following organic constituents: 30 g/l. sucrose, 10 g/l. Difco agar, 560 μM *myo*-inositol, 11·4 μM indole-3-acetic acid, and 1·2 μM thiamine hydrochloride. In order to avoid possible degradation of the compounds by heat and to increase solubility, each compound to be tested was dissolved in $(Me)_2SO$. A series of 3-fold dilutions was made, and small aliquots were then added to the cooling agar media. The final concentration of $(Me)_2SO$ did not exceed 0·05%, which does not affect the biological activity in this assay system.[45]

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