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CYTOKININS: INFLUENCE OF SIDE-CHAIN PLANARITY OF N^6-SUBSTITUTED ADENINES AND ADENOSINES ON THEIR ACTIVITY IN PROMOTING CELL GROWTH*

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Abstract—Eight compounds have been synthesized to define the relationship between side-chain planarity and cytokinin activity within a series of N^6-substituted adenines and adenosines. These compounds and closely related ones have been tested for relative promotion of growth in the tobacco bioassay. It has been concluded from the examples available that side-chain planarity is important in imparting the highest order of cytokinin activity.

INTRODUCTION

FOUR COMPOUNDS with cytokinin[1-5] (cell division, growth) activity, namely 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine (I),[6-20] 6-(3-methyl-2-butenylamino)-2-methyl-

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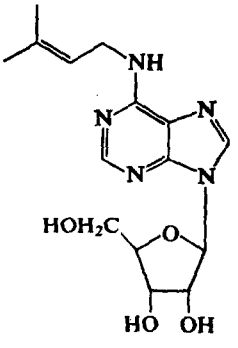

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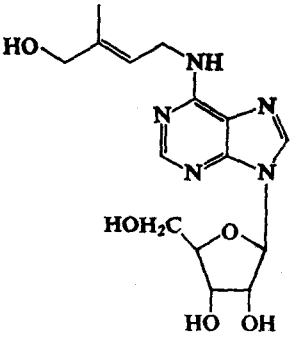

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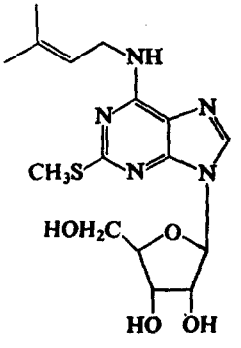

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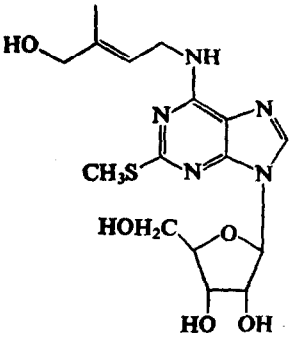

(IV)

thio-9-β-D-ribofuranosylpurine (III),[6,8,21-24] 6-(4-hydroxy-3-methyl-*cis*-2-butenylamino)-9-β-D-ribofuranosylpurine (*cis* isomer of II),[17] and 6-(4-hydroxy-3-methyl-2-butenylamino)-2-methylthio-9-β-D-ribofuranosylpurine (IV),[25] have been isolated as components of tRNA. These four cytokinins have in common their planar or periplanar isopentenyl side-chains. Therefore, the recent activity study by Hall and Srivastava,[26] which indicated that the naturally occurring compound of presumed structure 6-(4-hydroxy-3-methyl-*cis*-2-butenylamino)-9-β-D-ribofuranosylpurine was less active than its corresponding synthetic *trans* isomer (II), was of interest since the most obvious consequence of a change in geometrical configuration is a possible distortion of side-chain planarity. Our interest in the structure-activity relationship for cytokinins prompted us to consider the effect of side-chain planarity, or steric bulk, on cytokinin activity.

RESULTS AND DISCUSSION

Chemistry of Test Substances

The synthesis of 6-(3-chloro-*trans*-and *cis*-2-butenylamino)purines (XI, XII; *trans* is pictured) and their 9-β-D-ribosyl derivatives (XIII, XIV; *trans* is pictured) was effected start-

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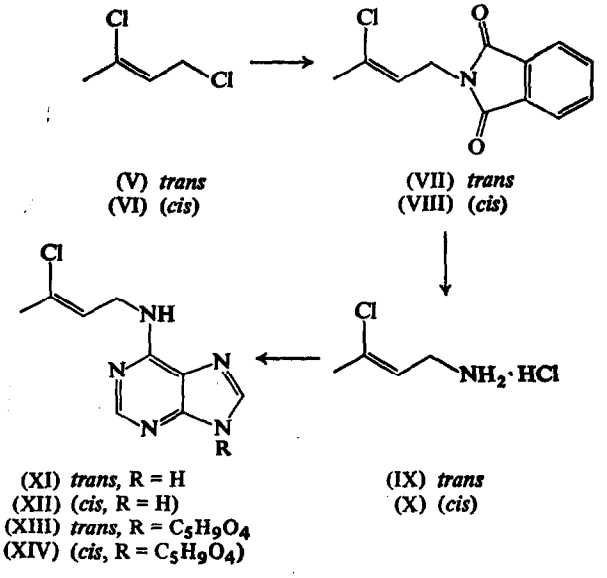
ing from the 1,3-dichloro-*trans*-and *cis*-2-butenes.[27] The latter were converted to the corresponding 3-chloro-*trans*-and *cis*-2-butenylamines via the Gabriel amine synthesis. Condensation of the amines with 6-chloropurine and 6-chloro-9-β-D-ribofuranosylpurine converted the amines to the corresponding *trans* and *cis* purines and their 9-β-D-ribosyl derivatives,



(V) *trans*
(VI) (*cis*)

(VII) *trans*
(VIII) (*cis*)

(XI) *trans*, R = H
(XII) (*cis*, R = H)
(XIII) *trans*, R = $C_5H_9O_4$
(XIV) (*cis*, R = $C_5H_9O_4$)

(IX) *trans*
(X) (*cis*)

respectively. 6-(3,3-Dimethylbutylamino)purine (XVIII) and its corresponding ribonucleoside (XIX) were prepared in the same general manner, starting from 1-chloro-3,3-dimethylbutane.

The synthesis of 6-(2-bromo-3-methyl-2-butenylamino)purine (XXIII) and its riboside, 6-(2-bromo-3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine (XXIV), was accomplished starting from *N*-(3-methyl-2-butenyl)phthalimide (XX).[28] Treatment of XX with purified N-bromosuccinimide in carbon tetrachloride gave *N*-(2-bromo-3-methyl-2-butenyl)-phthalimide (XXI) in good yield. The reaction gave a number of minor products as well, the relative proportions being highly susceptible to reaction scale and conditions. Hydrazinolysis of this phthalimide gave the corresponding free amine which was converted to XXIII and XXIV by condensation with the appropriate chlorinated purine precursors.

Cytokinin Activity

The average relative cytokinin activities of the eight new compounds, and of six related compounds, in the tobacco bioassy are summarized in Fig. 1. Of particular interest is the three-fold greater activity of the *trans* versus the *cis* forms of 6-(3-chloro-2-butenylamino)-purine and of their 9-β-D-ribosyl derivatives. This finding is consistent with the probable greater activity of *trans*- versus *cis*-zeatin riboside, which can be inferred from a comparison of our data with those of Hall and Srivastava,[26] who determined the activity of *cis*-zeatin riboside (*cis* isomer of II) and of I in the tobacco pith bioassay. The chloro group, which

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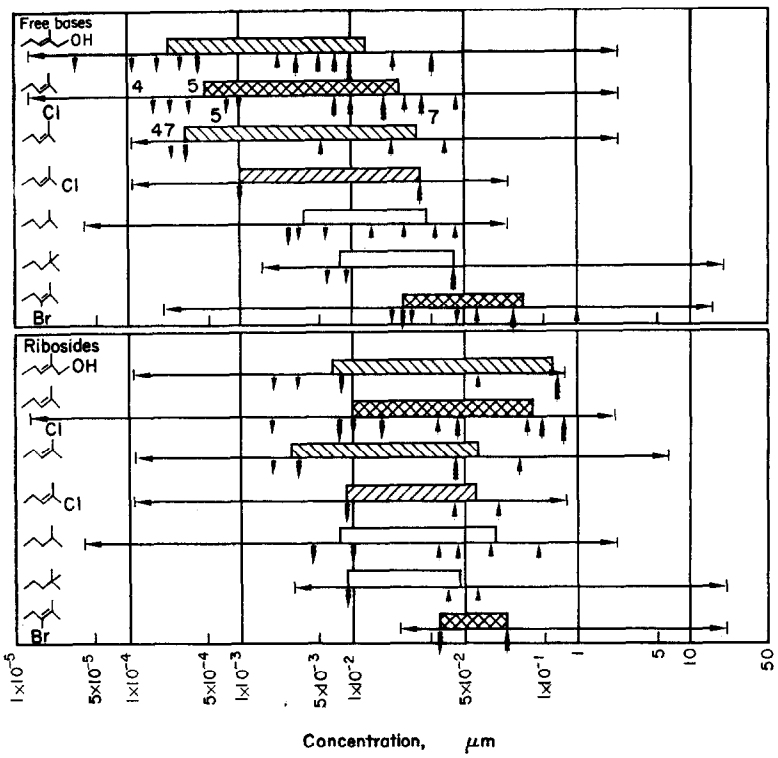
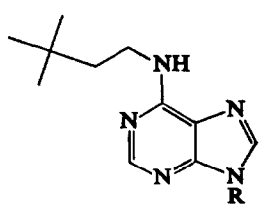
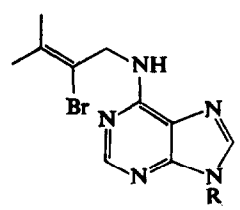


FIG. 1. RELATIVE CYTOKININ ACTIVITIES OF A SERIES OF N^6-SUBSTITUTED ADENINES AND ADENOSINES.
The compounds are designated by the structures of the N^6-substituents on adenine or adenosine. The *bars* represent average values of the ranges in which growth increases as a linear function of the log of concentration.[5] 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. Numbers have been substituted when more than three arrows occur at one point.

has been shown to provide less steric interference than a methyl group (e.g. in hindered biphenyls),[29] should disturb side-chain planarity less in compounds XI and XIII, with the *trans*-2-butene configuration, than in compounds XII and XIV. Similarly, the methyl group in II offers less steric interference with the α_N-methylene than does the CH_2OH-group in the *cis*-2-butenoid isomer of II.



(XVIII) R = H
(XIX) R = $C_5H_9O_4$



(XXIII) R = H
(XXIV) R = $C_5H_9O_4$

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Even more striking is the result that 6-(2-bromo-3-methyl-2-butenylamino)purine (XXIII) is only 1% as active as 6-(3-methyl-2-butenylamino)purine. The substitution of the bulky bromine group for hydrogen as the fourth substituent on the double bond disturbs side-chain planarity appreciably. The resulting markedly lower cytokinin activity can be explained at least partially by this steric factor.

If side-chain planarity is disturbed by adding substituents to the double bond, activity is also lowered. Thus, 6-(3-methyl-2-butenylamino)purine is much more active than 6-isopentylaminopurine, which is more active than 6-(3,3-dimethylbutylamino)purine (Fig. 1). In addition, we have previously shown that 6-(4-hydroxy-3-methyl-*trans*-2-butenylamino)-purine is more active than its saturated analog, 6-(4-hydroxy-3-methylbutylamino)purine.[5]

The chief consistency of these reproducible testing results lies in the fact that, among the compounds tested, side-chain planarity imparts high cytokinin activity.

EXPERIMENTAL

Synthesis of Test Substances

N-(3-*Chloro*-trans-2-*butenyl*)*phthalimide* (VII). To a stirred suspension of 33·0 g (0·18 mole) of potassium phthalimide in 500 ml of $HCONMe_2$ was added dropwise 18·2 g (0·15 mole) of 1,3-dichloro-*trans*-2-butene.[27] The mixture was heated at 75° for 2 hr, and the cooled product was poured into 1·5 l. of ice water and maintained at 0° for several hours. The suspension was filtered and the white solid was air-dried and recrystallized from ethanol, yield 26·8 g (78%), m.p. 80–81·5°; NMR δ from TMS (CCl_4): 2·12 (3H, d, CH_3C), 4·34 (2H, m, $C—CH_2—N$), 5·55 (1H, m, C=CH), 7·69 (4H, m, C_6H_4). (Found: C, 60·98; H, 4·19; N, 5·76. Calc. for $C_{12}H_{10}ClNO_2$: C, 61·16; H, 4·28; N, 5·94%.)

N-(3-*Chloro*-cis-2-*butenyl*)*phthalimide* (VIII). The *cis* isomer was synthesized from 1,3-dichloro-*cis*-2-butene by analogy with the synthesis of the *trans* isomer. The product was isolated as colorless crystals, yield 81%, m.p. 77–79°, wide depression on admixture with the *trans* isomer; NMR δ (CCl_4): 2·27 (3H, s, CH_3C), 4·21 (2H, d, $C—CH_2—N$), 5·70 (1H, m, C=CH), 7·72 (4H, m, C_6H_4). (Found: C, 60·91; H, 4·29; N, 5·99. Calc. for $C_{12}H_{10}ClNO_2$: C, 61·16; H, 4·28; N, 5·94%.)

3-*Chloro*-trans-2-*butenylamine hydrochloride* (IX). To 15·0 g (64 mmoles) of *N*-(3-chloro-*trans*-2-butenyl)-phthalimide (VII) suspended in 50 ml of absolute methanol was added 3·7 ml of 85% aq. hydrazine hydrate. The solution was stirred and heated at reflux for 2 hr, after which 25 ml of water was added to the cooled product. The methanol was removed under diminishing pressure and 125 ml of water was added to the reaction mixture which was adjusted to pH 1 with HCl. The precipitate that formed was filtered, washed well with water, and discarded. The filtrate was evaporated, leaving a white solid which was recrystallized from ethanol as colorless plates, yield 5·8 g (64%), m.p. 215–217°; NMR δ (DMSO-d_6-D_2O): 2·13 (3H, s, CH_3C), 3·50 (2H, d, $C—CH_2—N$), 5·72 (1H, t, C=CH). (Found: C, 33·76; H, 6·35; N, 9·88. Calc. for C_4H_9ClN: C, 33·82; H, 6·39; N, 9·86%.)

3-*Chloro*-cis-2-*butenylamine hydrochloride* (X). The *cis* isomer was synthesized by the same procedure as the corresponding *trans* isomer. The product consisted of colorless crystals, yield 47%, m.p. 242·5–243·5°, wide depression on admixture with the *trans* isomer; NMR δ (D_2O): 2·17 (3H, s, CH_3C), 3·69(2H, d, $C—CH_2—N$), 5·77 (1H, m, C=CH). (Found: C, 34·04; H, 6·40; N, 9·80. Calc. for C_4H_9ClN: C, 33·82; H, 6·39; N, 9·86%.)

6-(3-*Chloro*-trans-2-*butenylamino*)*purine* (XI). To 40 ml of *n*-butanol was added 710 mg (5·0 mmoles) of 3-chloro-*trans*-2-butenylamine hydrochloride (X), 580 mg (3·75 mmoles) of 6-chloropurine and 2·5 ml of NEt_3. The mixture was heated at reflux for 1 hr. The cooled product was concentrated under diminished pressure and 25 ml of water was added to the residue, which was adjusted to pH 8 and refrigerated. The suspension was filtered and the solid product was recrystallized from ethanol several times (decolorization) to afford white crystals of XI, yield 318 mg (38%), m.p. 221·5–222·5°; λ_{max}^{EtOH} (pH 7) 268 nm (ε 18,900), λ_{min} 228 (1900); λ_{max}^{EtOH} (pH 1) 278 (18,300), λ_{min} 237 (3000); λ_{max}^{EtOH} (pH 10) 284 (sh), 276 (17,900), λ_{min} 242 (3300); NMR δ (DMSO-d_6): 2·12 (3H, s, CH_3C), 4·31 (2H, m, $C—CH_2—N$), 5·83 (1H, t, C=CH), 8·12, 8·25 (2H, s, Ad-$C_{2,8}$-H's). (Found: C, 48·20; H, 4·46; N, 31·10. Calc. for $C_9H_{10}ClN_5$: C, 48·33; H, 4·51; N, 31·31%.)

6-(3-*Chloro*-cis-2-*butenylamino*)*purine* (XII). This compound was synthesized in the same manner as the *trans* isomer. The product was obtained as white crystals in 66% yield, m.p. 254·5–255·5°, wide depression on admixture with the *trans* isomer; λ_{max}^{EtOH} (pH 7) 268 nm (ε 19,100), λ_{min} 228 (2300); λ_{max}^{EtOH} (pH 1) 278 (18,600), λ_{min} 236 (3400); λ_{max}^{EtOH} (pH 10) 284 (sh), 276 (18,200), λ_{min} 241 (3800); NMR δ (DMSO-d_6): 2·21 (3H, s, CH_3C), 4·22 (2H, m, $C—CH_2—N$), 5·84 (1H, t, C=CH), 8·15, 8·28 (2H, s, Ad-$C_{2,8}$-H's). (Found: C, 48·61; H, 4·54; N, 30·96. Calc. for $C_9H_{10}ClN_5$: C, 48·33; H, 4·51; N, 31·31%.)

6-(3-*Chloro*-trans-2-*butenylamino*)-9-β-D-*ribofuranosylpurine* (XIII). To 20 ml of absolute ethanol was added 483 mg (3·4 mmoles) of 3-chloro-*trans*-2-butenylamine hydrochloride, 744 mg (2·6 mmoles) of 6-chloro-9-β-D-ribofuranosylpurine and 2·0 ml NEt_3. The mixture was heated at reflux for 2 hr. The cooled product was concentrated under diminished pressure and the residue was treated with 20 ml of water, adjusted to pH 8 and refrigerated. The suspension was filtered and the solid product was recrystallized from ethanol to give colorless crystals of XIII, yield 348 mg (38%), m.p. 160·5–162·5°; λ_{max}^{EtOH} (pH 7) 267 nm (ε 19,600), λ_{min} 230 (1300); λ_{max}^{EtOH} (pH 1) 266 (18,300), λ_{min} 237 (3500); λ_{max}^{EtOH} (pH 10) 268 (19,900), λ_{min} 234 (2900); $[\alpha]_D^{25}$ −41° (*c*. 0·71, abs. EtOH); NMR δ (DMSO-d_6-D_2O): 2·12 (3H, s, CH_3C), 3·72 (2H, m, C-5′ protons), 4·21 (4H, m $C—CH_2—N$, C-3′ and C-4′ protons), 4·65 (1H, m, C-2′ proton), 5·88 (2H, m, C=CH and C-1′ protons), 8·26, 8·36 (2H, s, Ad-$C_{2,8}$-H's). (Found: C, 47·36; H, 5·36; N, 19·49. Calc. for $C_{14}H_{18}ClN_5O_4$: C, 47·27; H, 5·10; N, 19·68%.)

6-(3-*Chloro*-cis-2-*butenylamino*)-9-β-D-*ribofuranosylpurine* (XIV). This compound was synthesized by analogy with the *trans* isomer. The product was isolated as colorless crystals, yield 40%, m.p. 156·5–158°, wide depression in admixture with the *trans* isomer; λ_{max}^{EtOH} (pH 7) 266 nm (ε 20,000), λ_{min} 229 (2500); λ_{max}^{EtOH} (pH 1) 266 (18,700), λ_{min} 235 (4300); λ_{max}^{EtOH} (pH 10) 267 (20,500), λ_{min} 233 (3700); $[\alpha]_D^{25}$ −61° (*c*. 1·07, abs. EtOH); NMR δ (DMSO-d_6-D_2O): 2·22 (3H, s, CH_3C), 3·78 (2H, m, C-5′ protons), 4·25 (4H, m, $C—CH_2—N$, C-3′ and C-4′ protons), 4·71 (1H, m, C-2′ proton), 5·92 (2H, m, C=CH and C-1′ proton), 8·32, 8·40 (2H, s, Ad-$C_{2,8}$-H's). (Found: C, 47·38; H, 5·04; N, 19·72. Calc. for $C_{14}H_{18}ClN_5O_4$: C, 47·27; H, 5·10; N, 19·68%.)

N-(3,3-*Dimethylbutyl*)*phthalimide* (XVI). To a stirred suspension of 65 g (0·35 mole) of potassium phthalimide in 1 l. of dimethylformamide was added, dropwise, 35 g (0·29 mole) of 1-chloro-3,3-dimethylbutane (XV). The mixture was heated at reflux for 30 min with continuous stirring. The cooled mixture was poured into 3 l. of ice water. The suspension was maintained at 0° for a few hours and then filtered. The solid was air-dried and recrystallized from ethanol–water as white crystals of XVI, yield 60·0 g (90%), m.p. 84–85°; NMR δ (CCl_4): 1·07 (9H, s, $(CH_3)_3C$), 1·62 (2H, m, $C—CH_2—C$), 3·82 (2H, m, $C—CH_2—N$), 8·11 (4H, m, C_6H_4). (Found: C, 72·54; H, 7·60; N, 6·01. Calc. for $C_{14}H_{17}NO_2$: C, 72·70; H, 7·31; N, 6·06%.)

3,3-*Dimethylbutylamine hydrochloride* (XVII). To 26·6 g (115 mmoles) of *N*-(3,3-dimethylbutyl)phthalimide (XVI) suspended in 75 ml of absolute methanol was added 6·5 ml of 85% aq. hydrazine hydrate. The solution was heated at reflux for 2 hr. The cooled solution was treated with 50 ml of water and concentrated under diminished pressure. An additional 250 ml of water was added and the pH was adjusted to 1. The solid that formed was filtered, washed well with water and discarded. The filtrate was evaporated to afford a white solid which was recrystallized from ethanol–ether to give white plates of XVII, yield 7·6 g (48%), m.p. > 320° (sublimed above 310°); NMR δ (D_2O): 0·99 (9H, s, $(CH_3)_3C$), 1·68 (2H, m, $C—CH_2—C$), 3·19 (2H, m, $C—CH_2—N$). (Found: C, 52·37; H, 11·69; N, 10·32. Calc. for $C_6H_{16}ClN$: C, 52·35; H, 11·72; N, 10·18%.)

6-(3,3-*Dimethylbutylamino*)*purine* (XVIII). To 40 ml of *n*-butanol was added 690 mg (50mmoles) of 3,3-dimethylbutylamine hydrochloride (XVII), 580 mg (3·8 mmoles) of 6-chloropurine and 2·5 ml NEt_3. The mixture was heated at reflux for 1 hr, and the cooled solution was concentrated under diminished pressure. Water (25 ml) was added to the residue and the resulting suspension was adjusted to pH 8 and refrigerated. The solid product was filtered and recrystallized from ethanol to give white crystals of XVIII, yield 425 mg (52%), m.p. 282·5–284°; λ_{max}^{EtOH} (pH 7) 267 nm (ε 17,400), λ_{min} 228 (2200); λ_{max}^{EtOH} (pH 1) 275 (16,100), λ_{min} 235 (3200), λ_{max}^{EtOH} (pH 10) 284 (sh), 275 (17,300), λ_{min} 240 (3600); NMR (DMSO-d_6): 0·96 (9H, s, $(CH_3)_3C$), 1·53 (2H, m, $C—CH_2—C$), 3·58 (2H, m, $C—CH_2—N$), 8·05, 8·18 (2H, s, Ad-$C_{2,8}$-H's). (Found: C, 60·36; H, 7·65; N, 32·10. Calc. for $C_{11}H_{17}N_5$: C, 60·25; H, 7·81; N, 31·94%.)

6-(3,3-*Dimethylbutylamino*)-9-β-D-*ribofuranosylpurine* (XIX). To 20 ml of absolute ethanol was added 468 mg (3·4 mmoles) of 3,3-dimethylbutylamine hydrochloride (XVII), 744 mg (2·6 mmoles) of 6-chloro-9-β-D-ribofuranosylpurine and 2·0 ml NEt_3. The mixture was heated at reflux for 2 hr. The cooled solution was concentrated and the residue was treated with 20 ml of water, adjusted to pH 8 and refrigerated. The oil that separated was isolated and crystallized from ethanol in a dry-ice bath. Recrystallization from ethanol afforded colorless crystals of XIX, yield 166 mg (18%), m.p. 72–74°; λ_{max}^{EtOH} (pH 7) 267 nm (ε 16,400), λ_{min} 230 (2300); λ_{max}^{EtOH} (pH 1) 264 (16,200), λ_{min} 235 (4000); λ_{max}^{EtOH} (pH 10) 267 (17,600), λ_{min} 235 (4600); NMR δ (DMSO-d_6-D_2O): 0·95 (9H, s, $(CH_3)_3C$), 1·59 (2H, m, $C—CH_2—C$), 3·72 (4H, m, $C—CH_2—N$ and C-5′ protons), 4·19 (2H, m, C-3′ and C-4′ protons), 4·68 (1H, m, C-2′ proton), 5·97 (1H, d, C-1′ proton), 8·27, 8·36 (2H, s, Ad-$C_{2,8}$-H's). (Found: C, 53·17; H, 7·02; N, 18·90. Calc. for $C_{16}H_{25}N_5O_4{\cdot}\frac{1}{2}H_2O$: C, 53·32; H, 7·27; N, 19·43%.)

N-(2-*Bromo*-3-*methyl*-2-*butenyl*)*phthalimide* (XXI). To 2·0 g (9·4 mmoles) of N-(3-methyl-2-butenyl) phthalimide (XX) was added 10 ml of carbon tetrachloride and 1·8 g of *N*-bromosuccinimide, which had been recrystallized from water. The mixture was subjected to strong incandescent illumination for 1·5 hr and then cooled to −18° for 30 min and filtered. The filtrate was concentrated under diminished pressure to leave a crystalline residue which was recrystallized from ethanol–water as colorless needles, yield 1·0 g (37%), m.p. 122–124°; NMR δ ($CDCl_3$): 1·92 (3H, s, CH_3C), 2·08 (3H, s, CH_3C), 4·67 (2H, s, $CH_2—N$), 7·78 (4H, m, C_6H_4). (Found: C, 52·77; H, 4·17; N, 4·69. Calc. for $C_{13}H_{12}BrNO_2$: C, 53·08; H, 4·11; N, 4·76%.)

2-*Bromo*-3-*methyl*-2-*butenylamine hydrochloride* (XXII). To 1·7 g (5·8 mmoles) of N-(2-bromo-3-methyl-2-butenyl)phthalimide (XXI) was added 25 ml of absolute methanol and 0·34 ml of 85% aq. hydrazine hydrate. The reaction mixture was heated at reflux for 2·5 hr and the cooled product was treated with 15 ml of water.

The methanol was removed under diminished pressure and an additional 75 ml of water was added. The solid that formed upon acidification of the solution to pH 1 was filtered, washed well with water and discarded. The solution was concentrated to afford a yellow solid which was recrystallized from ethanol–ether (decolorization) to give white plates of XXII, yield 655 mg (57%) m.p. 185–186·5°; NMR δ (D_2O): 1·88 (3H, s, CH_3C), 1·91 (3H, s, CH_3C), 4·05 (2H, s, C—CH_2—N). (Found: C, 30·01; H, 5·66; N, 7·03. Calc. for $C_5H_{11}BrClN$: C, 29·95; H, 5·53; N, 6·99%.).

6-(*2-Bromo-3-methyl-2-butenylamino)purine* (XXIII). To 8 ml of *n*-butanol was added 200 mg (1·0 mmole) of 2-bromo-3-methyl-2-butenylamine hydrochloride, 116 mg (0·75 mmole) of 6-chloropurine and 0·50 ml NEt_3. The mixture was heated at reflux for 1 hr. The cooled product was concentrated under diminished pressure and 5 ml of water was added to the product which was adjusted to pH 8 and refrigerated. The solid that formed was filtered, air-dried and recrystallized from ethanol to give white crystals of XXIII, yield 132 mg (62%), m.p. 239–241·5°; λ_{max}^{EtOH} (pH 7) 268 nm (ϵ 18,200), λ_{min} 228 (1500); λ_{max}^{EtOH} (pH 1) 279 (19,000), λ_{min} 236 (2800); λ_{max}^{EtOH} (pH 10) 284 (sh), 275 (17,700), λ_{min} 245 (4100); NMR δ (DMSO-d_6-D_2O): 1·89 (3H, s, CH_3C), 2·03 (3H, s, CH_3C), 4·64 (2H, s, C—CH_2—N), 8·21, 8·31 (2H, s, Ad-$C_{2,8}$-H's). (Found: C, 42·63; H, 4·41; N, 24·65. Calc. for $C_{10}H_{12}BrN_5$: C, 42·57; H, 4·29; N, 24·82%.)

6-(*2-Bromo-3-methyl-2-butenylamino*)-9-β-D-*ribofuranosylpurine* (XXIV). To 10 ml of absolute ethanol was added 340 mg (1·7 mmoles) of 2-bromo-3-methyl-2-butenylamine hydrochloride, 372 mg (1·3 mmoles) of 6-chloro-9-β-D-ribofuranosylpurine and 1·0 ml NEt_3. The solution was heated at reflux for 2 hr, cooled and concentrated under diminished pressure. The residue was treated with 10 ml of water and the suspension was adjusted to pH 8 and refrigerated. The solid product was filtered and recrystallized from ethanol to afford white crystals of XXIV, yield 304 mg (57%), m.p. 168·5–169·5° λ_{max}^{EtOH} (pH 7) 267 nm (ϵ 12,600), λ_{min} 232 (1600); λ_{max}^{EtOH} (pH 1) 268 (12,000), λ_{min} 237 (3000); λ_{max}^{EtOH} (pH 10) 267 (12,800), λ_{min} 234 (2500); $[\alpha]_D^{25}$ −56° (*ca.* 1·33, abs. EtOH); NMR δ (DMSO-d_6-D_2O): 1·86 (3H, s, CH_3C), 1·99 (3H, s, CH_3C), 3·68 (2H, m, C-5′ protons), 4·15 (2H, m, C-3′ and C-4′ protons), 4·62 (3H, m, C—CH_2—N and C-2′ protons, 5·95 (1H, d, C-1′ proton), 8·26, 8·36 (2H, s, Ad-$C_{2,8}$-H's). (Found: C, 43·44; H, 4·89; N, 16·87. Calc. for $C_{15}H_{20}BrN_5O_4$: C, 43·49; H, 4·87; N, 16·90%.)

Bioassay Procedures

The cytokinin activities were determined in the tobacco bioassay.[1] These compounds were treated with a small amount of dimethylsulfoxide (DMSO) to facilitate solution, diluted in filter-sterilized water solutions and added to the cooling agar media. The final concentration of DMSO in the media did not exceed 0·02% (v/v), well below the concentrations that affect tissue growth.[30]

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[30] R. Y. Schmitz and F. Skoog, *Plant Physiol.* in press.