*CYTOKININS: SYNTHESIS OF 6-(3-METHYL-3-BUTENYLAMINO)-9-β-D-RIBOFURANOSYLPURINE (3iPA), AND THE EFFECT OF SIDE-CHAIN UNSATURATION ON THE BIOLOGICAL ACTIVITY OF ISOPENTYLAMINOPURINES AND THEIR RIBOSIDES**

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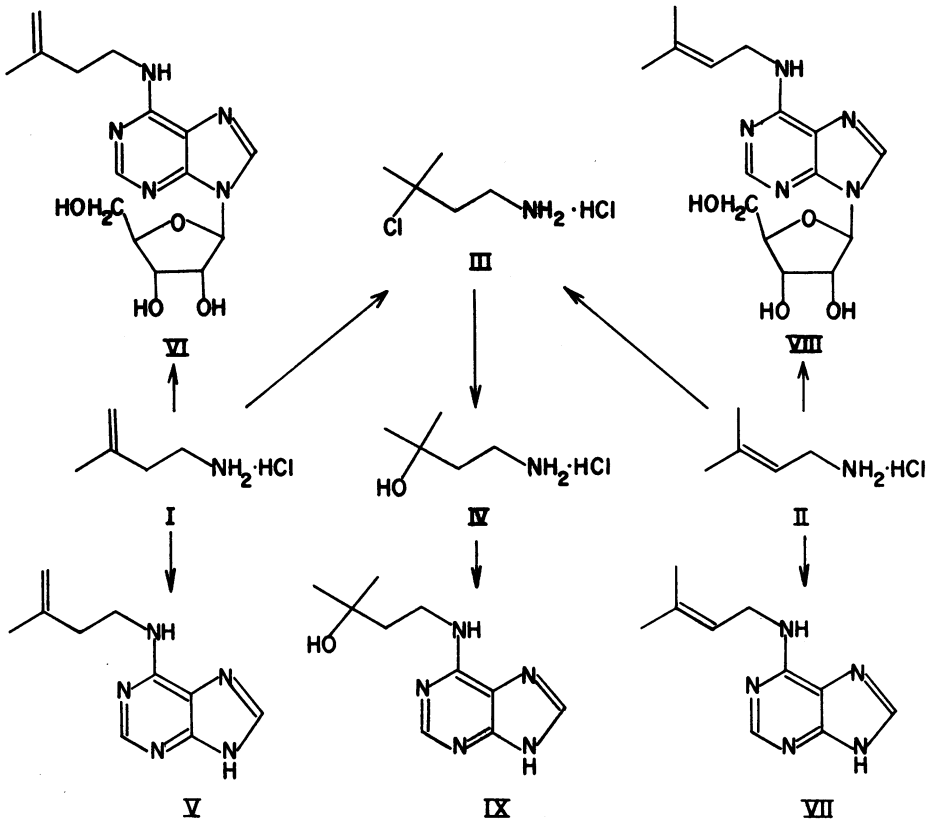
6-(γ,γ-Dimethylallylamino)purine (VII), 2iP, which was initially obtained synthetically,[1, 2] has been shown to be a highly active,[3–7] naturally occurring cytokinin.[8, 9] The corresponding ribosyl derivative, 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosylpurine (VIII), 2iPA, is also biologically active[10–12] and has been shown to occur naturally in serine and tyrosine tRNA's.[11–20] These findings suggested that 6-(3-methyl-3-butenylamino)purine (V),[21] 3iP, and 6-(3-methyl-3-butenylamino)-9-β-D-ribofuranosylpurine (VI), 3iPA, might also possess cytokinin activity because of the biological equivalence of the Δ^2- and Δ^3-isopentenyl groups, at least at the pyrophosphate stage in biosynthesis.[22–25]

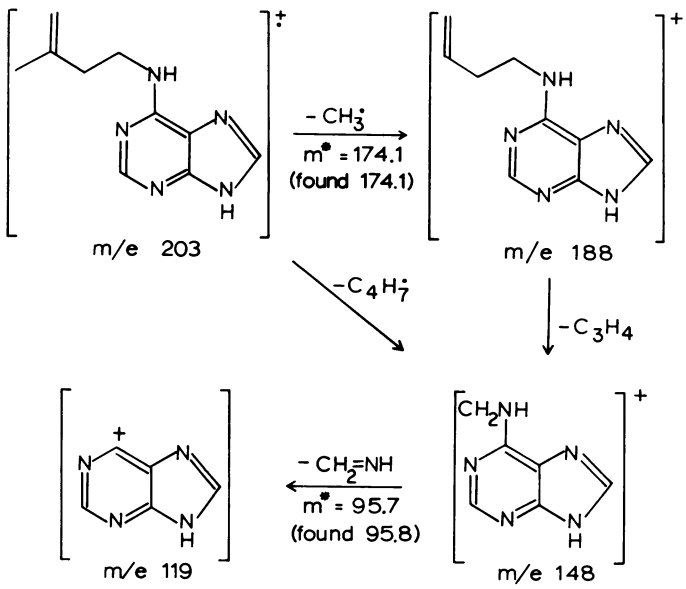
In amplification of the outline provided for the synthesis of 3iP (V),[21] 3-methyl-3-buten-1-yl *p*-toluenesulfonate was prepared by treating the corresponding alcohol with *p*-toluenesulfonyl chloride in pyridine at 0° for 24 hours, followed by destruction of the excess acid chloride with water, extraction of the products from the aqueous phase with ether, and treatment of the ether extracts sequentially with acid, base, water, and drying agent. Concentration of the ether solution afforded a liquid (92% yield), characterized by microanalysis (calculated for $C_{12}H_{16}SO_3$: C, 59.97; H, 6.71; found: C, 60.03; H, 7.09) and by the NMR spectrum (CCl_4), δ from TMS: 1.64 (3H, s, CH_3), 2.32 (2H, t, C—CH_2—C), 2.43 (3H, s, CH_3Ar), 4.08 (2H, t, C—CH_2—O), 4.73 (2H, d, =CH_2), 7.35 and 7.73 (2H each, d, C_6H_4). The tosylate was treated with potassium phthalimide in DMF at 80° for two hours. The cooled mixture was poured into ice water to give a precipitate of N-(3-methyl-3-butenyl)phthalimide, yield 92 per cent, colorless needles from petroleum ether, mp 51–53°; NMR δ ($CDCl_3$): 1.82 (3H, s, CH_3), 2.43 (2H, t, C—CH_2—C), 3.86 (2H, t, C—CH_2—N), 4.75 (2H, s, =CH_2), 7.78 (4H, m, C_6H_4). (*Analysis:* Calculated for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.34; H, 6.36; N, 6.29.)

The phthalimide was heated at reflux for 1.5 hours in absolute methanol containing an equimolar amount of 85 per cent hydrazine hydrate (aq.). The cooled solution was concentrated under diminished pressure and water was added. While the temperature was maintained at 0°, hydrochloric acid was added cautiously to pH 1.0 (pH meter). The mixture was allowed to stand at 0° for a few minutes, and the solid was removed by filtration. The aqueous phase was lyophilized, and the residue was recrystallized from ethanol-ether to give 3-methyl-

3-butenylamine hydrochloride (I), mp 187–189°, yield 48%; NMR δ (DMSO-d_6-D_2O): 1.76 (3H, s, CH_3), 2.43 (2H, t, C—CH_2—C), 3.01 (2H, m, C—CH_2—N), 4.88 (2H, s, =CH_2). (*Analysis:* Calculated for $C_5H_{12}ClN$: C, 49.30; H, 9.95; N, 11.52. Found: C, 49.14; H, 9.79; N, 11.25.)



Condensation with 6-chloropurine was effected in *n*-butanol and triethylamine at reflux for one hour. After the cooled reaction mixture was concentrated under diminished pressure, water was added and the pH was adjusted to 8. The solid that formed upon refrigeration was filtered, dried, and recrystallized from 1:1 ethanol–acetonitrile to give 6-(3-methyl-3-butenylamino)purine (V), mp 180.5–182°, yield 62%, λ_{max}^{EtOH} (pH 1) 276 mμ (ϵ 15,400), λ_{min} 237 (3,300); λ_{max}^{EtOH} (pH 7) 268 (16,500), λ_{min} 228 (2,100); λ_{max}^{EtOH} (pH 10) 283 (sh), 275 (16,300), λ_{min} 241 (3,400); NMR δ (DMSO-d_6-D_2O): 1.80 (3H, s, CH_3), 2.42 (2H, t, C—CH_2—C), 3.76 (2H, t, C—CH_2—N), 4.82 (2H, s, =CH_2), 8.18, 8.28 (2H, s, Ad-$C_{2,8}$-H's). (*Analysis:* Calculated for $C_{10}H_{13}N_5$: C, 59.09; H, 6.45; N, 34.46. Found: C, 59.28; H, 6.64; N, 34.36.) The mass spectra of 2iP (VII)[9] and 3iP (V) are compared in Figure 1. The substantial differences in the spectra of these double-bond isomers are noteworthy, as is the relatively simple fragmentation pattern of the latter in the high m/e range, which may be represented as in Scheme I.



SCHEME I

The riboside 3iPA (VI) was prepared by condensing compound I with 6-chloro-9-β-D-ribofuranosylpurine in ethanol and triethylamine at reflux for 2.5 hours and was purified by recrystallization from ethanol-ether, mp 148–150°, yield 67%; $C_{15}H_{21}N_5O_4$ (M^+ calculated: 335.1576; found: 335.159); λ_{max}^{EtOH} (pH 1) 265 mμ (ε 17,200), λ_{min} 235 (4,700); λ_{max}^{EtOH} (pH 7) 267 (17,900), λ_{min} 229 (2,900); λ_{max}^{EtOH} (pH 10) 267 (17,800), λ_{min} 232 (3,600); $[\alpha]_D^{25}$ − 43° (c 1.00, abs. EtOH); mass spectrum: m/e 335.159 (M^+), 280.105, 203.118, 148.062, and 119.036 (cf. Scheme I) NMR δ (DMSO-d_6-D_2O): 1.78 (3H, s, CH_3), 2.38 (2H, t, C—CH_2—C), 3.77 (4H, m, C—CH_2—N and C-5′ protons), 4.1–4.2 (2H, m, C-3′ and C-4′ protons), 4.80 (3H, m, =CH_2 and C-2′ protons), 6.00 (1H, d, C-1′ proton), 8.28, 8.38 (2H, s, Ad-$C_{2,8}$-H's).

If, in the work-up leading to compound I, too much hydrochloric acid was used or the temperature of the acidified solution was allowed to rise, a mixture resulted. The hydrochloride mixture contained compound I, 3-chloro-3-methylbutylamine hydrochloride (III), and 3-hydroxy-3-methylbutylamine hydrochloride (IV). Although the mixture was not easily separated into its components, each of the three could be synthesized independent of the other two, as we have seen with I. The preparation of III was effected by heating I or II[26] in aqueous solution containing excess hydrochloric acid. Evaporation of the cooled solution under reduced pressure yielded 3-chloro-3-methyl-butylamine hydrochloride, colorless plates from ethanol-ether, mp 196–199°; NMR δ (DMSO-d_6-D_2O): 1.62 (6H, s, $(CH_3)_2C$), 2.16 (2H,t,C—CH_2—C), 3.00 (2H, t, C—CH_2—N). (*Analysis:* Calculated for $C_5H_{13}Cl_2N$: C, 37.99; H, 8.29. Found: C, 37.67; H, 8.23.)

Compound IV was obtained by heating III in water and subsequently evaporating the solution under reduced pressure to yield (~100%) 3-hydroxy-3-

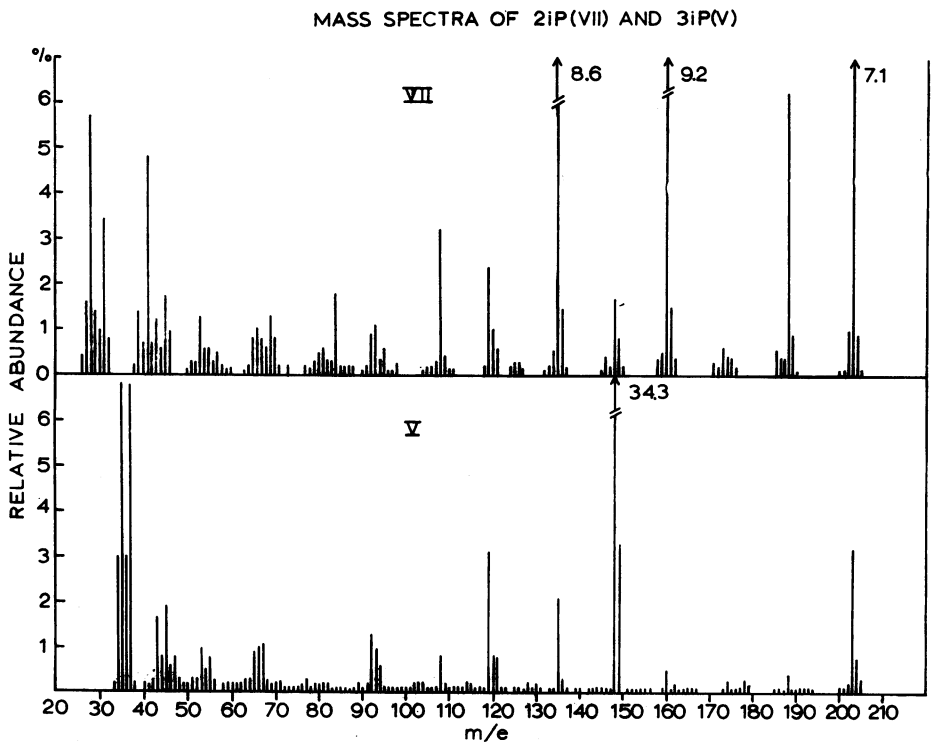


FIG. 1.—Mass spectra of 2iP and 3iP at 70 ev.

methylbutylamine hydrochloride (IV) as the dihydrate, a liquid; NMR δ (DMSO-d_6-D_2O): 1.16 (6H, s, $(CH_3)_2C$), 1.65 (2H, t, C—CH_2—C), 2.87 (2H, t, C—CH_2—N).

The availability of authentic IV permitted the direct synthesis of pure 6-(3-hydroxy-3-methylbutylamino)purine (IX) via the normal route of condensation of IV with 6-chloropurine in *n*-butanol and triethylamine at reflux for one hour. Purification by chromatography on silica gel, elution with 3:1 ethyl acetate–propanol, and recrystallization from 1:1 ethanol–acetonitrile yielded (43%) IX, mp 180.5–181.5° (lit 173–174°,[11] 166–171°[12]);[27] λ_{max}^{EtOH} (pH 1) 274 mμ (ϵ 13,500), λ_{min} 234 (2,000); λ_{max}^{EtOH} (pH 7) 267 (14,700), λ_{min} 227 (300); λ_{max}^{EtOH} (pH 10) 283 (sh), 275 (14,500), λ_{min} 241 (2,700); NMR δ (DMSO-d_6-D_2O): 1.19 (6H, s, $(CH_3)_2C$), 1.76 (2H, t, C—CH_2—C), 3.74 (2H, t, C—CH_2—N), 8.21, 8.32 (2H, s, Ad-$C_{2,8}$-H's). (*Analysis:* Calculated for $C_{10}H_{15}N_5O$: C, 54.28; H, 6.83; N, 31.66. Found: C, 54.12; H, 6.88; N, 31.29.) This compound, which is isomeric with dihydrozeatin,[28] may be regarded as the hydration product of both 3iP (V) and 2iP (VII), so that comparison of the cytokinin activities of these three compounds was of interest along with the activities of the pair 3iPA (VI) and 2iPA (VIII). Also tested was the hydrogenation (Pd/C) product of both 3iPA and 2iPA, 6-isopentylamino-9-β-D-ribofuranosylpurine, mp 154.5–156°; λ_{max}^{EtOH} (pH 1) 264 mμ (ϵ 16,800), λ_{min} 235 (3,600); λ_{max}^{EtOH} (pH 7) 268 (16,700), λ_{min} 228 (1,100); λ_{max}^{EtOH} (pH 10) 267 (17,200), λ_{min} 229 (1,900); $[\alpha]_D^{25}$ −42° (c 1.03, abs.

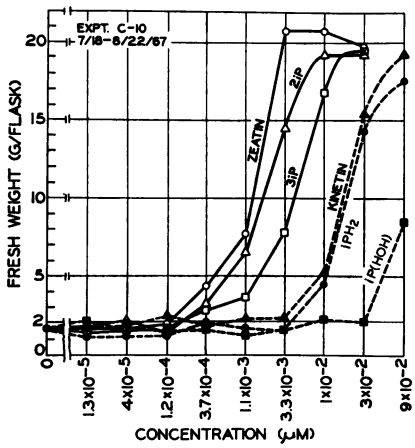


FIG. 2.—Effects of zeatin, 2iP, 3iP, and related bases on yields of tobacco callus.

EtOH); NMR δ (DMSO-d_6-D_2O): 0.94 (6H, d, $(CH_3)_2C$), 1.58 (3H, m, C—CH_2—CH), 3.75 (4H, m, C—CH_2—N and C-5′ protons), 4.20 (2H, m, C-3′ and C-4′ protons), 4.71 (1H, m, C-2′ proton), 5.98 (1H, d, C-1′ proton), 8.20, 8.32 (2H, s, Ad-$C_{2,8}$-H's). (*Analysis:* Calculated for $C_{15}H_{23}N_5O_4$: C, 53.40; H, 6.87; N, 20.76. Found: C, 53.32; H, 7.16; N, 21.03.)

Cytokinin Activity.—Biological activities were determined in terms of promotion of growth (fresh weight yields) in the tobacco bioassay.[10] To avoid breakdown from heating, the test samples were filter-sterilized and added to the medium when it was close to the gelation point. In earlier tests of synthetic samples of 2iP and zeatin, both were about ten times more active than kinetin,[10] and, in the most sensitive tests for each, both were detected in concentrations down to 5×10^{-5} μM. Often, however, somewhat higher concentrations were required, and on the average zeatin was slightly more active than 2iP. Present tests (Fig. 2 and Table 1) confirm this and show that 3ip is nearly but not quite as active as 2iP, whereas the saturated derivatives 6-(isopentylamino)purine,

TABLE 1. *Summary of cytokinin activities of 6-isopentenylaminopurines, their ribosides, and other derivatives.*

Substance	Concentration (μM) Required for	
Free bases	Detection	Maximum yield
Zeatin	1.7×10^{-4} (5)*	1.8×10^{-2} (5)
2iP	3.6×10^{-4} (8)	2.6×10^{-2} (8)
3iP	1.1×10^{-3} (5)	3.9×10^{-2} (5)
iPH_2	4×10^{-3} (2)	$>9 \times 10^{-2}$ (2)
iP(HOH)	3.6×10^{-2} (3)	2.3 (1)
Kinetin	4×10^{-3} (3)	$>9 \times 10^{-2}$ (3)
9-Ribofuranosides		
Zeatin	—	—
2iP	1.4×10^{-2} (6)	6×10^{-1} (5)
3iP	6×10^{-3} (3)	$>3 \times 10^{-1}$ (3)
iPH_2	1×10^{-2} (2)	5×10^{-1} (2)
ip(HOH)	—	—
Kinetin	1×10^{-2} (2)	5×10^{-1} (2)

* Number of determinations given in parentheses.

iPH_2, and 6-(3-hydroxy-3-methylbutylamino)purine, iP(HOH), are much less active. The curves in Figure 2 show the order of decreasing activity and the concentration ranges required for detectable and maximum increases in fresh weight yield consistently obtained in three experiments in which the above four bases were compared with zeatin and kinetin. The data in Table 1 show the average values from all tests of each substance. It should be noted that the introduction of a hydroxyl group in the 3-position of 2iP or 3iP, giving iP(HOH), lowers the activity to such an extent that the highest tested concentration (0.9 μM, in Fig. 2) was less than would have been required for maximum yield with this substance. In a repeat experiment with higher concentrations, a normal curve was obtained with a maximum at 2.3 μM. It is of interest that the introduction of a second hydroxyl in the 2-position, to give 6-(2,3-dihydroxy-3-methylbutylamino)purine,[10] lowers the activity to the point of no detectability below 0.1 μM.

The ribosides, 2iPA and 3iPA, are at best only a few per cent as active as the free bases in the tobacco bioassay (Fig. 3 and Table 1). Furthermore, no clear

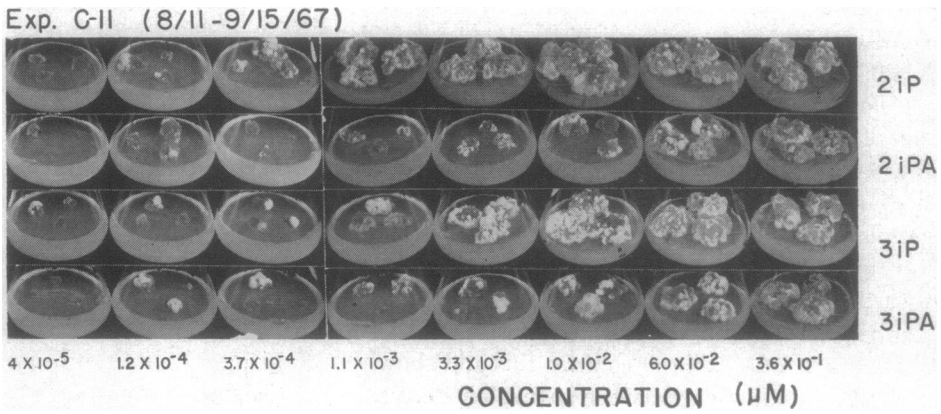


FIG. 3.—Comparison of effects of serial concentrations of 2iP, 2iPA, 3iP, and 3iPA on growth of tobacco cultures.

distinction could be made in these tests between the activities of the two ribosides, nor between these and that of the 6-(isopentylamino)purine riboside (iPH_2-A). All three seem to be active in about the same range as established earlier for kinetin riboside (0.01–0.5 μM). Possibly more closely spaced tests over the low concentration range would reveal differences, but on the basis of the present assays it is impossible to conclude whether the ribosides are active as such or the measured activity is due to the free bases which may have been liberated in the course of the assay. The closeness and shape of the yield/concentration curves for the ribosides rather suggest that the purine bases of 2iPA and 3iPA may be released and/or utilized in reduced or otherwise altered form, even in the absence of heat or extreme pH conditions. In general, then, the tests show that 2iPA and 3iPA and/or the saturated bases to which they may give rise are much less

active than the 6-isopentenylaminopurines themselves. Nevertheless, all these substances still must be considered as potent cytokinins.

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The abbreviations 3iPA for 6-(3-methyl-3-butenylamino)-9-β-D-ribofuranosylpurine and 2iPA for 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine are used following the suggestions of Dr. Waldo E. Cohn. The bases related to these nucleosides are abbreviated 3iP for 6-(3-methyl-3-butenylamino)purine and 2iP for 6-(3-methyl-2-butenylamino)purine or 6-(γ,γ-dimethylallylamino)purine. Other abbreviations used are: iPH_2 for 6-(isopentylamino)-purine or 6-(3-methylbutylamino)purine; iP(HOH) for 6-(3-hydroxy-3-methylbutylamino)-purine.

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