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Relative Cytokinin Activity of 3-Methyl Substituted Adenylate Cytokinins†

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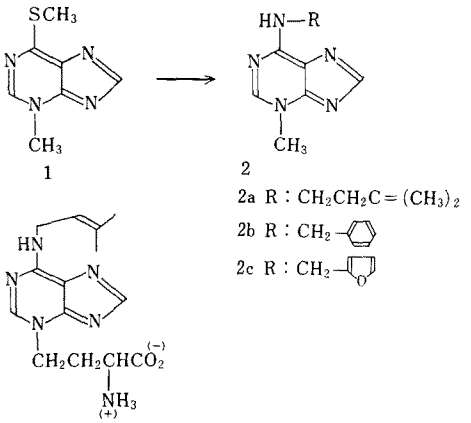
A large number of N^6-monosubstituted adenines and their derivatives have been tested for cytokinin activity to determine relationship between the chemical structure and their biological activity in tobacco callus bioassay.

Structural requirements for a high order of cytokinin activity of N^6-monosubstituted adenines (adenylate cytokinins) generally include an intact adenine nucleus with N^6-side chain of moderate size. Additional substitution on the purine ring, especially, on the 1-position or the 3-position lead to drastic decrease of the activity.[1,2] Skoog *et al.* observed that substitution of a methyl of benzyl group on the 3-position of 6-benzylaminopurine does practically eliminate cytokinin activity.[1] Fox *et al.*[3] synthesized glucopyranosides and xylofuranosides of 6-benzylaminopurine and found that only the 3-glucopyranoside isomer surprisingly exhibits good activity in both tobacco and soybean tests. However, the high activity of the 3-isomer was conceivably ascribed to the susceptibility to glucosidase which produce rapidly the free base *in vivo*.

Recently, we reported that the new natural purine derivative named discadenine, 3-(3-methyl-3-carboxypropyl)-6-(3-methyl-2-butenylamino)purine (3), has potent cytokinin activity in the tobacco callus bioassay.[4] This finding apparently suggest differential influences of substitution on the 3-position of adenylate cytokinins.

In the present paper we describe the syntheses of the 3-methyl substituted derivatives of the potent adenylate cytokinins, 6-(3-methyl-2-butenylamino)purine (2iP), 6-benzylaminopurine and 6-furfurylaminopurine (kinetin) and tests for cytokinin activity compared with the corresponding parent compounds.

Syntheses. The 3-methyl substituted 2iP, 6-benzylaminopurine and kinetin were prepared in a straightforward manner by heating at reflux 3-methyl-6-(methylthio)-3H-purine (**1**)[5] with 3-methyl-2-butenylamine, benzylamine and furfurylamine, respectively, in dry methanol. The ultraviolet spectra and mass spectra confirmed the identity of the products. The UV spectral evidences, the hyperchromic shift in acidic solution on long wave side of the maxima and the negative values for λ_{min}^{pH2}-λ_{min}^{pH7}, clearly indicated that the products are the 3-substituted N^6-monosubstituted adenine derivatives.[6] Three compounds showed a intense molecular ion peak. The major fragmentation pathways of the molecular ion were i) loss of the N^6-side chain involving transfer of side chain hydrogen to the base to giving 3-methyladenine ion *m/e* 149 and ii) elimination of $RCH_2N\cdot$ from the N^6-substituted amino group to affording 3-methylpurine ion *m/e* 134. Other peaks take place along the side chain were accounted for.



Cytokinin activity. Cytokinin activity was determined in the tobacco callus bioassay on the basis of fresh weight yields of callus cultured on serial concentration of the test substances. The results summarized in Fig. 1 (A and B) indicate that the replacement of a methyl group on the 3-position of 6-benzylaminopurine caused about 100-fold loss in activity in a concentration range between 0.01 μM and 1.0 μM. Our result was consistent with the finding of Skoog *et al.* cited above. On the other hand, 3-methyl-2iP was about equally active as 2iP itself in a concentration range between 0.1 μM and 1.0 μM. However, the 3-methyl derivative required more high concentration for detectable response. In the case of kinetin, substitution of a methyl group on the 3-position lowered the activity and the yield obtained at 1.0 μM was about one-fourth that produced by kinetin at the same concentration.

The present results indicate that the introduction of substituents on the 3-position of N^6-substituted adenylate cytokinins tend to decrease the activity, but the

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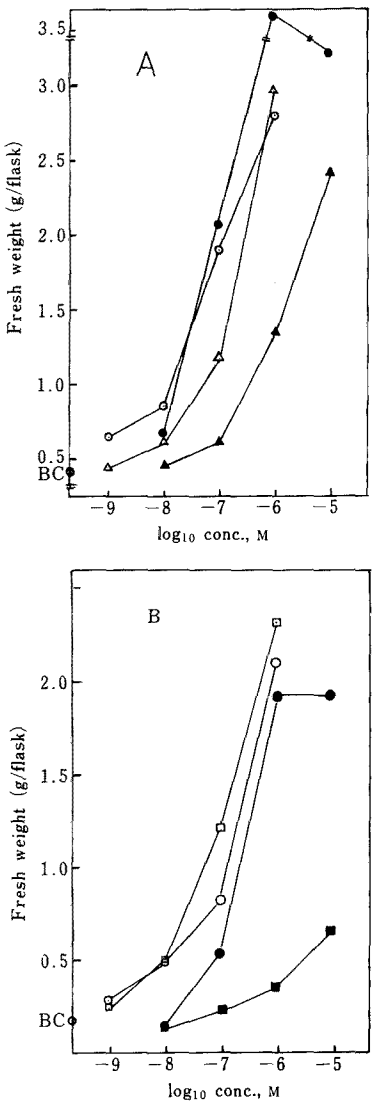

FIG. 1 (A and B). Effect of 2iP, 6-Benzylaminopurine (BA), Kinetin and Their 3-Methyl Derivatives (Compound 2a, 2b and 2c) on Fresh Weight Yield of Tobacco Callus.

○, 2iP; ●, 3-Me-2iP (2a); □, BA; ■, 3-Me-BA (2b); △, kinetin; ▲, 3-Me-kinetin (2c).

magnitude of the influence seems to be widely different among each cytokinin as illustrated between the 3-methyl derivative of 2iP and 6-benzylaminopurine. Although insufficient information is available at present, we presume that the potent cytokinin activity of 3-methyl-2iP may be due to its own activity because it has high activity in the same range as 2iP. In connection with this, it is of interest to recall our finding concerning cytokinin activity of discadenine, 2iP derivative with a large substituent on the 3-position.

EXPERIMENTAL

Bioassay. Callus derived from the pith of tobacco plant (*Nicotiana tobacum* var. Wisconsin No. 38) was cultured aseptically on the revised agar medium of Murashige and Skoog (RM–1965)[7] with 2 mg/liter of IAA. The test solution was added through a sterilised membrane filter syringe to avoid breakdown by heating. Two or three pieces of callus were planted per flask with 20 ml of agar medium and 10 flasks were used per treatment. These flasks were incubated at 30°C in dark for 3 weeks and fresh weight yields of callus were determined.

Test substances. All mps are uncorrected. Mass spectra were acquired with an LKB 9000 mass spectrometer under the following condition: Sample introduced by direct probe, ion source temperature 270°C, ionizing energy 70 eV. A solution of 3-methyl-6-(methylthio)-3H-purine (0.5 g) and 0.5 g of 3-methyl-2-butenylamine, benzylamine and furfurylamine, respectively, in dry methanol (10 ml) was heated under reflux for 8～10 hr. The reaction solution was evaporated to dryness under diminished pressure. The resulting residue was extracted with *n*-hexane and the extract was discarded. The crystalline solid was recrystallised from suitable solvents.

3-Methyl-6-(3-methyl-2-butenylamino)purine (**2a**). Colorless prism from ethyl acetate, mp 175～6°C. UV $\lambda_{max}^{H_2O}$ nm (ε): (pH 2) 285 (22400), (pH 7) 287 (18000), (pH 12) 288 (17600); $\lambda_{min}^{H_2O}$ nm (ε): (pH 2) 240 (2800), (pH 7) 247 (2500), (pH 12) 248 (2800). IR ν_{max}^{Nujol}: 3200, 1635, 1540, 1170, 1155, 775 and 650 cm^{-1}. Mass *m/e*: 217 (M^+), 202, 174, 149 (base peak), 134, 121 and 94.

3-Methyl-6-benzylaminopurine (**2a**). Colorless needles from ethyl acetate, mp 210～3°C. UV $\lambda_{max}^{H_2O}$ nm (ε): (pH 2) 284 (23700), (pH 7) 287 (18900), (pH 12) 288 (18100); $\lambda_{min}^{H_2O}$ nm (ε): (pH 2) 241 (3150), (pH 7) 247 (3440), (pH 12) 247 (3220). IR ν_{max}^{Nujol}: 3400, 1630, 1540, 1220, 1180, 1165, 775, 690 and 650 cm^{-1}. Mass *m/e*: 239 (M^+ base peak), 224, 162, 134 and 91.

3-Methyl-6-furfurylaminopurine (**2c**). Colorless crystals from ethyl acetate-chloroform, mp 234～6°C. UV $\lambda_{max}^{H_2O}$ nm (ε): (pH 2) 284 (22000), (pH 7) 286 (17700), (pH 12) 287 (17200); $\lambda_{min}^{H_2O}$ nm (ε): (pH 2) 240 (4140), (pH 7) 247 (3570), (pH 12) 247 (1720). IR ν_{max}^{Nujol}: 3400, 1630, 1540, 1330, 1220, 1180, 1010, 740 and 650 cm^{-1}. Mass *m/e*: 229 (M^+ base peak), 200, 149, 134, 119, 107 and 81.

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