*N*⁶-Alkylthiomethyl and *N*⁶-Arylthiomethyl Derivatives of Adenosine and Their Cytokinin Activity

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Five new derivatives of adenosine, *N*⁶-[(1-methylethyl)thiomethyl]-(**1**), *N*⁶-methylthiomethyl-(**2**), *N*⁶-phenylthiomethyl-(**3**), *N*⁶-[(3-amino-3-carboxypropyl)thiomethyl]-(**4**), and *N*⁶-[(2-amino-2-carboxyethyl)thiomethyl]adenosine (**5**), were synthesized and their cytokinin activity was tested in the *Amaranthus* betacyanin assay and the soybean callus growth.

1, **2**, and **3** were active in the former assay and all five compounds were active in the latter assay. The activities of the compounds were, however, weaker than those of the reference derivatives, in which sulfides were replaced by methylenes, *N*⁶-isopentyl-, *N*⁶-*n*-propyl-, *N*⁶-benzyl-, and *N*⁶-(5-amino-5-carboxypentyl)adenosine. This fact indicates that the sulfide structure introduced into the *N*⁶-side chains had the effect of reducing cytokinin activity.

Recently we found the reaction of adenine nucleotides with formaldehyde and thiols to give *N*⁶-alkylthiomethyl derivatives of the nucleotides and applied it to prepare polymer-bound ADP and ATP useful as immobilized cofactors for enzyme reactors or as affinity adsorbents.¹⁾ Cytokinin activity of many species of *N*⁶-substituted adenine derivatives have been reported.²⁾ The unique structure of the products synthesized by the reaction prompted us to test their cytokinin activity to know the effects of the sulfide structure introduced into *N*⁶-side chains on the cytokinin activity.

*N*⁶-[(1-Methylethyl)thiomethyl]-(**1**), *N*⁶-methylthiomethyl-(**2**), *N*⁶-phenylthiomethyl-(**3**), *N*⁶-[(3-amino-3-carboxypropyl)thiomethyl]-(**4**), and *N*⁶-[(2-amino-2-carboxyethyl)thiomethyl]adenosine (**5**) were synthesized by treating adenosine* with formaldehyde and the following thiols: 2-propanethiol for **1**, methanethiol for **2**, thiophenol for **3**, *N*-acetyl-DL-homocysteine for **4**, and *N*-acetyl-L-cysteine for **5**.

In the case of **4** and **5**, the acetyl group in their intermediates was removed with an aminoacylase.

The presence of *N*⁶-alkylthiomethyl substituent in compounds **1**, **2**, **4**, and **5** was confirmed by their UV spectra having the same features ($\lambda_{\text{max}} = 272$ nm in neutral solution and a hypochromic shift at the maximum in acidic solution) as those of *N*⁶-(2-carboxyethyl)-thiomethyl 9-substituted adenines reported previously.¹⁾ The data on elemental analyses and PMR spectroscopy for the compounds were all consistent with the structures expected from their syntheses. The compounds **4** and **5** proved to be positive with the ninhydrin reagent in TLC, showing that the protective group in their intermediates was deblocked by the aminoacylase.

The compound **3** is the first example of *N*⁶-

* Adenosine but not adenine was used as the starting material in spite of the fact that *N*⁶-substituted adenines are usually more active cytokinins than their 9-ribosides,³⁾ since an additional modification with formaldehyde is expected to occur at the *N*⁹ site of adenine.

arylthiomethyl adenine derivatives to be reported. Structural evidence of the N^6 -side chain was obtained from the PMR spectrum of **3** which showed a multiplet (5H) at δ 7.23, a triplet (1H, $J=7$ Hz) at δ 8.54, and a broad singlet (2H) at δ 5.16 assignable to phenyl, NH, and the methylene (NCH_2S), respectively. The presence of phenyl group is clearly reflected in the low-field shift of the singlet due to the methylene (NCH_2S) compared with that (at δ 4.7) in the spectra of **1** or **2**.

In the previous work¹⁾ the N^6 -alkylthio-methyl linkage was shown to be gradually hydrolyzed even in physiological conditions to yield the parent adenine nucleotides. Therefore, stability of the synthesized compounds was examined by measuring UV spectral changes or by high-performance liquid chromatography (HPLC). Compounds **1**, **2**, and **4** were fairly stable and remained undecomposed in over 90% (for **1** and **2**) or over 75% (for **4**) after one month at 25°C in 0.1 M phosphate buffer (pH 7.0), but **3** and **5** were very labile, the half-life period of their decomposition being about 1 and 11 days, respectively, in the above conditions (Fig. 1). The decomposition of **3** was accompanied by regeneration of adenosine and thiophenol, which were identified by HPLC and GC, respectively. The lability of **3** and **5** may be interpreted in terms of the electron-withdraw-

FIG. 1. Stability of the Synthesized Compounds in 0.1 M Phosphate Buffer (pH 7.0) at 25°C.

Log (residual %) is plotted against incubation time. Symbols: ●, **1**; ○, **2**; ▽, **3** (50 μM); ▾, **3** (100 μM); ■, **4**; □, **5**. Initial concentration of **1**, **2**, **4**, and **5** was 40 μM.

ing effect of phenyl (for **3**) or $-\text{NH}_3^+$ group (for **5**) which makes each compound to easily dissociate into two ions ($\text{R}-\text{NH}=\text{CH}_2^+$ and $^-\text{S}-\text{R}'$).

All the compounds including the very unstable compound **3** were tested on cytokinin

FIG. 2. Cytokinin Activities of the Synthesized Compounds in the *Amaranthus* Betacyanin Test (A) and in the Soybean Callus Growth (B).

The vertical axis in A indicates amounts of betacyanin produced. The control values were obtained using only the solvents of samples (MeOH or water for A and dimethylsulfoxide for B). Adenosine and thiophenol showed no effect on both the two assays in the above concentration ranges.

Symbols: ●, **1**; ▲, **2**; ■, **3**; ▽, **4**; ○, **5**; □, **6**; △, **7**; ▾, **8**; □, N^6 -benzyladenosine; ⊙, N^6 -benzyladenine.

activity in the *Amaranthus* betacyanin test and the soybean callus growth. The following compounds were used as references: *N*⁶-benzyladenine, *N*⁶-isopentyl- (6), *N*⁶-*n*-propyl- (7), *N*⁶-(5-amino-5-carboxypentyl)- (8), and *N*⁶-benzyladenosine.* Results are shown in Fig. 2.

In the *Amaranthus* assay, compounds 1, 2, and 3 were active whereas 4 and 5 having amino acid residues were inactive. In the callus growth, all five compounds were active and the order of activities was 1, 3 > 2 >> 4, 5.

The compounds 1, 2, 4, and 5 were less active on both bioassay systems than the reference derivatives in which sulfides were replaced by methylenes (6, 7, and 8), except for the case of 2 and 7 in the callus growth. By comparing the activity of 1 with that of 6 in the *Amaranthus* assay, it was shown that the substitution of a sulfur for a methylene group in the *N*⁶-side chains required an over 100 times higher concentration for a detectable response. In the callus growth also 1 was at least 10 times less active than 6. The effect of the sulfide structure in lowering cytokinin activity may be attributed to the properties (atomic size, bond angle, hydrophobicity, and so on) of the bivalent sulfur which are different from those of a methylene group.

Besides, the activity of 3 is worthy to note. The fact that the compound showed the clear cytokinin activity in the callus growth (Fig. 2B) in spite of the rapid decomposition in aqueous media (Fig. 1) can be interpreted in two ways: (1) the activity might be caused by the remaining compound (at least 0.01% on the 13th day, if half-life period of decomposition is 1 day), suggesting the compound to be a very potent cytokinin; (2) the callus might be stimulated by the compound before most of it was decomposed within a few days from start of the assay and its growth might continue, if initiated, without the compound. In other words, the contact of callus with cytokinins for such a short period might be enough to

induce cell division.* Further work is in progress on the activity of compound 3.

EXPERIMENTAL

All melting and decomposing points were determined on a hot stage and are uncorrected. UV spectra were obtained in 0.1 M phosphate buffer (pH 7.0) and in 0.1M KCl-HCl buffer (pH 1.1), or in MeOH. PMR spectra were taken with a Hitachi R-24B high resolution NMR spectrometer (60 MHz). *N*-Acetyl-DL-homocysteine thiolactone and *N*-acetyl-L-methionine were purchased from Fluka AG Chemische Fabrik (Swiss) and *N*-acetyl-L-cysteine, from Wako Pure Chemical Industries Ltd (Japan). *N*^α-Acetyl-L-lysine, 6-chloropurine 9β-D-ribose, *N*⁶-benzyladenine, and *N*⁶-benzyladenosine were obtained from Sigma Chemical Co, U.S.A.

*N*⁶-Isopentyladenosine and *N*⁶-*n*-propyladenosine were synthesized by condensing 6-chloropurine 9β-D-ribose with isoamylamine or *n*-propylamine according to the method of Fleischer *et al.*⁴⁾

Syntheses of 1, 2, and 3. The compound 1 was synthesized as follows and 2 and 3 were synthesized in analogous way.

A solution containing 2 g of adenosine and 9.6 ml of 35% formalin (128 mmol of formaldehyde; determined by the method cited previously¹⁾) in about 50 ml of water was prepared, and adjusted to pH 12 with 2 M NaOH. To the solution was added 5.6 g of 2-propanethiol with vigorous stirring. The pH was readjusted to 12 with 2 M NaOH and the total volume was brought to 75 ml by adding water. The thiol was completely dissolved by adding 10 ml of EtOH. The solution was kept at 50°C overnight and then extracted five times with EtOAc (150 ml each). The EtOAc and the remaining thiol were removed by vacuum distillation and the residue was crystallized from a mixture of EtOH (20 ml), ether (100 ml), and hexane (600 ml). The crude product was twice recrystallized from EtOH to give 2.39 g (90% yield) of 1 as needles, dec 123~133°C; odor of the thiol was generated at the melting. *Anal.* Found: C, 46.88; H, 5.90; N, 19.39; S, 8.99. Calcd. for C₁₄H₂₁N₅O₄S: C, 47.30; H, 5.97; N, 19.71; S, 9.02%. UV $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ nm(ε): 272 (22.0 × 10³) at pH 7.0 and 272.5 (19.0 × 10³) at pH 1.1. PMR (DMSO-*d*₆) δ: 1.23 (6H, d, *J*=6.6 Hz, -CH(CH₃)₂), 3.12 (1H, quintet, *J*=6.6 Hz, -CH(CH₃)₂), 3.60 (2H, m, H-5'), 3.98 (1H, m, H-4'), 4.15 (1H, m, H-3'), 4.59 (1H, q, *J*=6 Hz, H-2'), 4.70 (2H, broad s, NCH₂S), 5.0~5.4 (3H, undissolved m, OH × 3), 5.87 (1H, d, *J*=6 Hz, H-1'), 8.19 (1H, s, H-2), 8.32 (1H, s, H-8), and 8.35

* Among the reference compounds, 8 is a new compound.

* Both adenosine and thiophenol (the decomposition products of 3) were found to be inactive in the assay.

(1H, broad s, NH). 2. Needles from EtOAc-EtOH in 68% yield, dec 139~142°C. *Anal.* Found: C, 43.65; H, 5.18; N, 21.15; S, 9.69. *Calcd.* for $C_{12}H_{17}N_6O_4S$: C, 44.02; H, 5.24; N, 21.40; S, 9.79%. UV $\lambda_{\max}^{H_2O}$ nm(ϵ): 272 (21.7×10^3) at pH 7.0 and 272 (19.0×10^3) at pH 1.1. PMR (DMSO- d_6) δ : 2.10 (3H, s, SCH₃), 3.57 (2H, m, H-5'), 3.94 (1H, m, H-4'), 4.15 (1H, m, H-3'), 4.55 (1H, q, $J=6$ Hz, H-2'), 4.69 (2H, broad s, NCH₂S), 5.0~5.4 (3H, undissolved m, OH $\times 3$), 5.84 (1H, d, $J=6$ Hz, H-1'), 8.14 (1H, s, H-2), 8.29 (1H, s, H-8), and 8.37 (1H, t, $J=7.2$ Hz, NH). 3. Plates from EtOH in 27% yield, dec 164~166°C. *Anal.* Found: C, 51.75; H, 4.90; N, 17.66; S, 8.00. *Calcd.* for $C_{17}H_{10}N_5O_4S$: C, 52.42; H, 4.93; N, 17.99; S, 8.23%. UV $\lambda_{\max}^{MeOH}$ nm(ϵ): 274 (22.2×10^3). PMR (DMSO- d_6) δ : 3.61 (2H, m, H-5'), 3.98 (1H, m, H-4'), 4.19 (1H, m, H-3'), 4.58 (1H, q, $J=5.4$ Hz, H-2'), 4.9~5.4 (5H, undissolved m, NCH₂S and OH $\times 3$), 5.88 (1H, d, $J=5.4$ Hz, H-1'), 7.23 (5H, m, phenyl), 8.21 (1H, s, H-2), 8.32 (1H, s, H-8), and 8.54 (1H, t, $J=6$ Hz, NH); addition of two drops of D₂O to the sample caused the signals due to OH and NH to disappear, leaving a singlet (2H, NCH₂S) at δ 5.16.

Aminoacylase. A strain of *Streptovorticillium parvisporogenes* (IFO 13394) was cultured in a medium (5 $\times$ 100 ml) of 1.5% glucose, 0.5% polypeptone, 0.5% meat extract, 0.5% yeast extract, and 0.5% NaCl (pH 7) with shaking at 30°C for 3 days. After removing the cells by centrifugation, ammonium sulfate was added to the supernatant to 70% of saturation. The precipitate was collected by centrifugation, dissolved in about 20 ml of 0.02 M phosphate buffer, pH 7.0, and dialyzed against the buffer at 4°C for 2 days. The dialyzed solution (30 ml) contained 28 U of aminoacylase and was used in the subsequent preparations of 4 and 5. The enzyme activity was assayed at 30°C for 30 min with a mixture containing 20 μ mol of *N*-acetyl-L-methionine, 0.1 mmol of barbital/HCl buffer, and enzyme in a total volume of 1.0 ml, pH 8.3. The reaction was stopped by heating at 100°C for 2 min, and then the liberated L-methionine was determined by the ninhydrin method.⁵⁾

Syntheses of 4 and 5. To a solution containing 2 g of adenosine and 9.6 ml of 35% formalin in about 40 ml of water was dissolved 11.9 g of *N*-acetyl-DL-homocysteine thiolactone. To hydrolyze the thiolactone, NaOH (granules) was added in limited amounts to the mixture under stirring until the pH reached 12. Water was added to bring the total volume to 75 ml and the pH was readjusted to 12 with 2 M NaOH. The mixture was kept at 50°C overnight. After neutralization with 8 M HCl, the mixture was diluted with water to about 150 ml. Then the solution was applied to a Dowex 1 $\times$ 2 column (Cl⁻, 3-cm diameter $\times$ 34 cm). Elution

was performed with a linear LiCl gradient obtained by placing 1 liter of 0.2 M LiCl, pH 3.5, in the mixing chamber and 1 liter of 1 M LiCl, pH 1.5, in the reservoir. Fractions (No. 77~115, each volume=14 ml) were pooled, adjusted to pH 6.5, and concentrated on a rotary evaporator at 35°C to about 30 ml. To the concentrate was added 400 ml of EtOH-acetone (1:1) and then 1 liter of ether. The solution was allowed to stand at -20°C overnight to give a viscous precipitate. The supernatant was removed by decantation and the residue was washed with a small amount of acetone. After drying in vacuum, the residue was dissolved in 65 ml of 0.02 M phosphate buffer, pH 7.2, containing 14 U of the aminoacylase. The mixture was incubated at 30°C for 2 days to give a white precipitate, which was collected by filtration and again dissolved in 50 ml of dilute HCl (pH 1). After removing insoluble materials by filtration, the solution was adjusted to pH 5.5 with 2 M NaOH to afford a white precipitate. The crude product was crystallized from water (1.5 liter, 80°C) to give 0.62 g (20% yield) of 4 as needles, dec 174~179°C. *Anal.* Found: C, 42.94; H, 5.37; N, 20.04; S, 7.58. *Calcd.* for $C_{15}H_{22}N_6O_6S$: C, 43.46; H, 5.36; N, 20.28; S, 7.73%. UV $\lambda_{\max}^{H_2O}$ nm(ϵ): 272 (22.1×10^3) at pH 7.0 and 272.5 (18.9×10^3) at pH 1.1. PMR (DMSO- d_6) δ : 2.05 (2H, m, SCH₃), 2.74 (2H, m, CH₂CH(CO)N), 3.34 (1H, m, CH(CO)N), 3.60 (2H, m, H-5'), 3.95 (1H, m, H-4'), 4.14 (1H, m, H-3'), 4.7 (3H, undissolved m, H-2' and NCH₂S), 5.5 (6H, broad s, OH $\times 3$ and NH₃⁺), 5.85 (1H, d, $J=6$ Hz, H-1'), 8.15 (1H, s, H-2), 8.29 (1H, s, H-8), and 8.45 (1H, broad s, NH).

Compound 5 was synthesized analogously to 4, using *N*-acetyl-L-cysteine. The *N*-acetylated intermediate was treated with the aminoacylase, and the product was purified on a Dowex 1 (Cl⁻) column by eluting with water followed by a linear LiCl-gradient elution (from water to 0.25 M LiCl, pH 2). Fractions containing the product were combined, neutralized, and concentrated on a rotary evaporator at 40°C. Addition of an excess amount of EtOH gave a white precipitate, which was collected by centrifugation and triturated with boiling MeOH. The residual solid was dissolved in dilute HCl (pH 1) and precipitated by neutralizing with 2 M LiOH followed by the addition of MeOH. The procedure was repeated to give 5 as white powderes (29% yield), dec 150~151°C. The product showed a single spot in TLC [*R*_f: 0.64 on a cellulose F-coated aluminum sheet (Merck) developed with water and *R*_f: 0.74 on a PEI-cellulose F-coated glass plate (Merck) developed with 0.1 M LiCl; spots were detected under UV light (254 nm) and/or by the ninhydrin-spray method]. *Anal.* Found: C, 41.61; H, 5.07; N, 20.60; S, 8.05. *Calcd.* for $C_{14}H_{20}N_6O_6S$: C, 41.99; H, 5.04; N, 20.99; S, 8.01%. UV $\lambda_{\max}^{H_2O}$ nm(ϵ): 272 (21.7×10^3) at pH 7.0 and 272 (18.5×10^3) at pH 1.1. PMR (DMSO- d_6) δ : 3.04 (2H, m, SCH₃), 3.60 (3H, undissolved m, H-5' and

$\text{CH}(\text{CO})\text{N}$), 3.95 (1H, m, H-4'), 4.17 (1H, m, H-3'), 4.7 (3H, undissolved m, H-2' and NCH_2S), 5.5 (6H, broad s, $\text{OH} \times 3$ and NH_3^+), 5.87 (1H, d, $J=6$ Hz, H-1'), 8.19 (1H, s, H-2), 8.3 (1H, broad s, NH), and 8.32 (1H, s, H-8).

Synthesis of 8. A mixture of 150 mg (0.52 mmol) of 6-chloropurine 9 β -D-ribose, 100 mg (0.53 mmol) of *N* α -acetyl-L-lysine, 50 mg of Na_2CO_3 , and 1 ml of water was heated in a sealed tube at 100°C for 3 hr. The solution was mixed with 4 ml of 0.02 M phosphate buffer (pH 7.2) containing 3.8 U of the aminoacylase and then incubated at 25°C for 2 days. A white precipitate appeared, but it was redissolved by acidifying the whole solution with 1 M HCl. The solution was diluted to about 10 ml with water, adjusted to pH 5 with 2 M NaOH, and immediately applied to a Dowex 1 $\times$ 2 column (Cl^- , 9-mm diameter $\times$ 20 cm). Elution was performed with water (40 ml) followed by a dilute HCl solution (pH 3, 100 ml) and the eluate was collected as 10-ml fractions. Fractions No. 4~9 were combined and concentrated on a rotary evaporator at 40°C to afford a white precipitate. The crude product was recrystallized from water to give 97 mg (46% yield) of **8** as needles, dec 197~203°C. *Anal.* Found: C, 47.70; H, 6.16; N, 20.90. *Calcd.* for $\text{C}_{16}\text{H}_{24}\text{N}_8\text{O}_8 \cdot \frac{1}{2}\text{H}_2\text{O}$: C, 47.39; H, 6.23; N, 20.73%. UV $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ nm (ϵ): 267 (18.5×10^3) at pH 7.0 and 263 (19.0×10^3) at pH 1.1. PMR ($\text{DMSO}-d_6$) δ : 1.50 (6H, undissolved m, CH_2CH_2), 3.19 (1H, m, $\text{CH}(\text{CO})\text{N}$), 3.59 (4H, undissolved m, H-5' and NCH_2), 3.93 (1H, m, H-4'), 4.12 (1H, m, H-3'), 4.50 (1H, m, H-2'), 4.7~6.0 (broad s, OH and NH_3^+), 5.82 (1H, d, $J=6$ Hz, H-1'), 7.69 (1H, t, $J=6$ Hz, NH), 8.07 (1H, s, H-2), and 8.21 (1H, s, H-8).

Stability experiments. For compounds **1**, **2**, **4**, and **5**—Solutions of each derivative (40 μM) in 0.1 M phosphate buffer (pH 7.0) containing chloroform (3%, as a bactericide) were prepared and allowed to stand at 25°C. Aliquots were withdrawn at time intervals and their UV absorptions were measured at 259 and 272 nm. Residual percentages of each derivative were calculated in the same way as described previously⁶⁾ using the following molar absorption coefficients: $\epsilon_{259}=15.4$ and $14.4 \text{ mm}^{-1}\text{cm}^{-1}$ and $\epsilon_{272}=8.0$ and $22.0 \text{ mm}^{-1}\text{cm}^{-1}$ for adenosine and the compounds, respectively.

For compound **3**—The method of measuring UV absorption changes is not adequate for this compound, since a decomposition product, thiophenol, which was identified by GC as described later on, has an UV absorption maximum near 260 nm and moreover it is inclined to be lost from aqueous solutions for its volatility and insolubility in water. Thus, stability of **3** was examined by HPLC. Solutions of **3** (50 and 100 μM) in 0.1 M phosphate buffer (pH 7.0) were prepared

by adding the compound dissolved in dimethylsulfoxide (10 mg/ml) to the buffer containing chloroform (3%). The solutions were kept at 25°C and aliquots (20 μl each) were subjected at time intervals to HPLC: Toyo Soda HLC-802UR chromatograph equipped with a column (4-mm i. d. $\times$ 30 cm) packed with Nucleosil 7 C₁₈ (7.5 μm , Macherey-Nagel, Germany); solvent, MeOH/H₂O (50%, v/v); pressure, 250 kg/cm²; flow rate, 1 ml/min; detection, UV at 254 nm. Retention times were 13.2 min for compound **3** and 3.0 min for adenosine. Residual percentages were calculated from peak areas of **3**.

After incubation for one week, the solutions were extracted with benzene. The organic layer was concentrated on a rotary evaporator at 40°C and subjected to GC: Hitachi 063 gas chromatograph with a hydrogen flame-ionization detector; columns, stainless steel columns (3-mm i. d. $\times$ 1 m) packed with 3% SE-30 on Chromosorb W or with 2% OV-1 on Chromosorb W and that (3-mm i. d. $\times$ 2 m) packed with 2.5% Ucon oil 50HB 5100 on Chromosorb W; carrier gas, helium (flow rate of 30 ml/min). Retention times of thiophenol (in the sample and authentic) were as follows: 3.1 min on SE-30 at 80°C, 2.1 min on OV-1 at 80°C, and 2.9 min on Ucon oil at 115°C.

Bioassays. Cytokinin activity was assayed by the *Amaranthus* (*Amaranthus caudatus* L.) betacyanin test⁷⁾ and the soybean (*Glycine Max* Acme) pith callus growth using the modified Miller's soybean callus medium.⁸⁾ In the latter case, each of the compounds was dissolved in dimethylsulfoxide in a series of ten-fold dilutions covering the range of cytokinin activity, and the solutions were then added by 20 μl to the autoclaved agar media (40 ml each) using a sterilized microsyringe when the media were close to the gelation point. Five pieces of the callus were planted per flask and cultured at 25°C in the dark for one month.

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REFERENCES

- 1) Y. Yamazaki and H. Suzuki, *Eur. J. Biochem.*, **92**, 197 (1978); *idem*, *Nucl. Acids Res.* (Special Publication No. 5), s 291 (1978).
- 2) F. Skoog, H.Q. Hamzi, A.M. Szwedkowska, N.J. Leonard, K. L. Carraway, T. Fujii, J. P. Helgeson and R. N. Loeppky, *Phytochem.*, **6**, 1169 (1967).
- 3) R. Y. Schmitz, F. Skoog, S. M. Hecht, R. M. Bock and N. J. Leonard, *Phytochem.*, **11**, 1603 (1972).
- 4) M. H. Fleysheer, M. T. Hakala, A. Bloch and R.H.

- Hall, *J. Med. Chem.*, **11**, 717 (1968).
- 5) H. Rosen, *Arch. Biochem. Biophys.*, **67**, 10 (1957).
- 6) Y. Yamazaki, H. Maeda and H. Suzuki, *Biotechnol. Bioeng.*, **18**, 1761 (1976).
- 7) N. L. Biddington and T. H. Thomas, *Planta*, **111**, 183 (1973).
- 8) D. E. Fosket and J. G. Torrey, *Plant Physiol.*, **44**, 871 (1969).
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