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Cytokinin-Like Activities of Nucleocyclitols

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Abstract. Although the 9-substituted adenines are commonly inactive as cytokinins, the nucleocyclitol 3-(-adenin-9-yl)-3-deoxy-1,5,6-tri-0-(methylsulfonyl)-*muco*-inositol (NI) proved to be active in the following bioassays: cell proliferation in soybean cotyledon callus tissue, cell expansion in excised radish cotyledons, and delay of senescence in detached leaves. In these assays, the effect of the compound, applied at the same molar concentration as benzyl adenine, was lower or less uniform than BA. NI completely failed to promote germination of lettuce seeds in conditions of secondary dormancy or thermodormancy, whre BA is effective. NI can substitute for BA in some though not all of the numerous responses evoked by cytokinins.

The synthesis of carbohydrates condensed with purine and pyrimidine bases has received attention because they exhibit physiological activities as antitumor, antibiotic, and antivirial agents. Isopentenyl adenosine, a compound known to act as a cytokinin in plant cells (Letham and Palni 1983) also has antileukemic properties (Rathbone and Hall 1972) and influences mitosis (Gallo et al. 1969) and RNA synthesis (Haeker and Feldbush 1971) in lymphocytes. Nitrogen bases condensed with inositols have been investigated less; these derivatives, not rigorously analogous in structure to nucleosides, were designated by the general term nucleoinositols or nucleocyclitols (Cadenas et al. 1984). One of them, 3-(adenin-9-yl)-3-deoxy-1,5,6 tri-0-(methylsulfonyl)-*muco*-inositol (NI, Fig. 1a), exhibits antitumoral and immunosuppressor effects on animal tissues (Canabal et al. 1985). On this basis, we investigated the possible cytokinin-like activities of this compound on plant cells.

The relationships between structure and function of cytokinins were studied by Skoog et al. (1967), who defined function as "promotion of cytokinesis in plant cells of diverse origin" and established that activity is limited to 6-substituted purines. Naturally occurring cytokinins are structurally related to isopentenyl adenine.

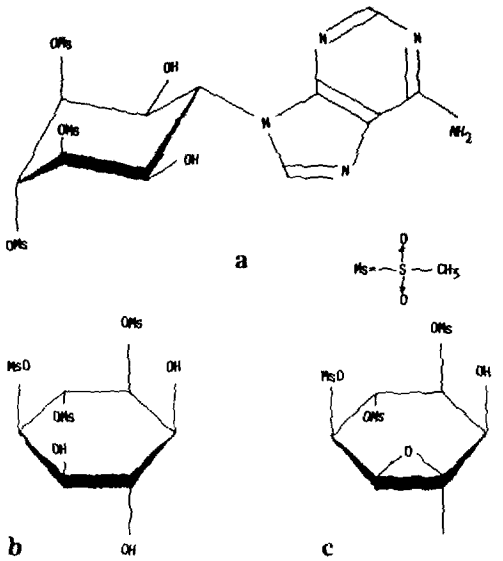

Fig. 1. Chemical structure of the tested compounds. (a) 3-(Adenin-9-yl)-3-deoxy-1,5,6-tri-0- (methylsulfonyl)-*muco*-inositol. (b) Trimesil inositol. (c) Epoxy inositol. Ms, mesil-methyl sulfonyl.

Materials and Methods

Growth of Soybean Callus Tissue

Pieces of callus tissue originated from cotyledons of *Glycine max* var. Acme, weighing 15–20 mg fresh weight, were subcultured in 50 ml of basic medium (Miller 1963) containing 5.0 mg/L IAA and 0.5 mg/L BA or 1.0 mg/L NI. Fresh weight of callus pieces was determined after 3 weeks of growth in the dark at 25°C.

Expansion of Excised Radish Cotyledons

This bioassay was performed according to Letham (1971). Radish seeds were imbibed in distilled water and left for 36 h at 25°C in darkness. The smaller cotyledon was then excised from each seedling. Three samples of 10 cotyledons were incubated on filter paper in small Petri dishes containing 5 ml of buffer at pH 6.0 plus 0.04 mM BA or NI. After 3 days at 25°C under continuous weak fluorescent light, the cotyledons were weighed individually.

Chlorophyll Retention in Senescing Barley Leaves

The procedure was based on that of Schistad and Nissen (1984). Four-centimeter segments of expanded leaves from 8- to 10-day-old barley plants were excised, placed in test tubes with 5 ml distilled water or 0.01 mM solutions of BA or NI, and kept in the dark at 25°C. Chlorophyll was extracted in 80% ethanol in an 80°C water bath from samples of five leaf segments, and its absorbance at 650 nm was determined.

Table 1. Growth of soybean callus tissue. Fresh weight after 3 weeks in the dark at 25°C. Means of 10 callus pieces ± SE.

Medium	Weight (mg)
Basic medium	75.3 ± 5.3
BA 0.5 mg/L	492.6 ± 8.7
NI 1.0 mg/L	360.8 ± 16.5

Germination Tests

Duplicated samples of 50 seeds of lettuce cv. Grand Rapids were sown in the dark in small plastic trays with 2 ml of solutions of the growth regulators (ABA, GA, BA, NI) according to Khan (1980/81). The samples were incubated in the dark at 25°C for 3 days before germination counts were made. In the presowing infusion method (Khan 1977), samples of 50 seeds were submerged for 1 h in 3 ml of solutions of GA (1 mM), BA (0.5 mM), or NI (0.5 mM) in acetone, air-dried, and sown in the dark in 2 ml of distilled water. After 3 days at 30°C the percentage of germination was recorded.

NI Solutions

NI was synthesized as described in Cadenas et al. (1984) and was pure by chromatographic and spectroscopic standards. On preparation of culture medium, NI, being very stable on heating, was added before autoclaving.

RESULTS

NI, in the same molar concentration as BA, promoted growth in the soybean callus tissue (Table 1). The mean fresh weight was lower than in the BA medium, and the increase in growth of individual pieces was not uniform, as in the case of BA.

The bioassays based on chlorophyll retention in senescing leaves of grasses (maize, barley, and wheat) consistently showed activity of NI. The results were usually more variable than those obtained with BA. Figure 2 shows the typical results obtained on one assay with barley leaves. Inositol was included in this case, because it, like other organic compounds, may possess some senescence-retarding action. When applied in the same molar concentration as NI or BA, inositol had no effect, and only when its concentration was raised to 0.3 M was the effect comparable to that of BA. The mean absorbance in the case of NI was usually lower than in the case of BA, in the concentrations used in this test, but NI considerably delayed the degradation of chlorophyll in all cases. Complete yellowing of the leaf sections was only visible 3–5 days later than in controls. BA-treated leaf sections remained green 5–7 days longer than controls. The results with the excised cotyledons bioassay (Fig. 3) indicate a significant promotion of expansion by NI.

All the germination tests performed gave negative results with respect to NI

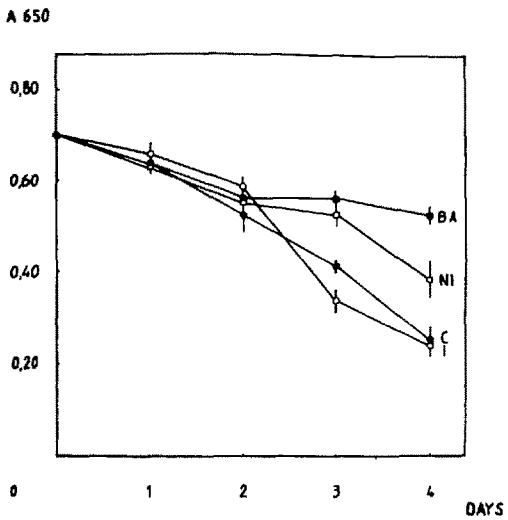

Fig. 2. Retention of chlorophyll in senescing barley leaves. Absorbance at 650 nm of the extracted chlorophyll. Samples of five detached leaves were incubated in distilled water, BA, inositol, or NI 0.01 mM. The SE of triplicate samples is indicated.

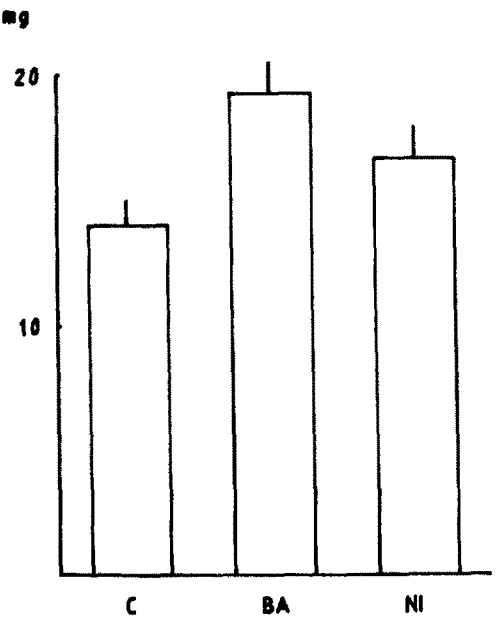

Fig. 3. Expansion of excised radish cotyledons. Fresh weight in milligrams per cotyledon after 3 days in continuous light at 25°C in 2 ml of buffer solution, BA, or NI 0.04 mM. Vertical bars represent SE.

activity. NI replaced BA neither in the reversion of ABA suppression of GA promotion (Table 2a) nor in the alleviation of high-temperature inhibition when added together with GA (Table 2b).

In an attempt to define the influence of chemical structure on the above-mentioned effects, compounds related to NI, namely epoxyinositol (Fig. 1c) and trimesil inositol (Fig. 1b), structurally representing part of the NI mole-

Table 2. Effect of growth regulators on the germination of lettuce seeds, cv. Grand Rapids.

(a) Growth regulators were provided in the incubation solution to seeds previously imbibed 4 days in distilled water. Percent germination was recorded after 3 days at 25°C.

Incubation solution	Percent germination
Control	0
GA 1 mM	70
GA 1 mM + ABA 0.04 mM	0
GA 1 mM + ABA 0.04 mM + BA 0.1 mM	60
GA 1 mM + ABA 0.04 mM + NI 0.1 mM	0

(b) Growth regulators were provided by the presowing infusion technique. Germination was recorded after 4 days at 30°C in distilled water.

Presowing infusion treatment	Percent germination
Control	0
GA 1 mM	0
GA 1 mM + BA 0.5 mM	64
GA 1 mM + NI 0.5 mM	0

cule, were also tested. They proved to be inactive in the promotion of growth of callus tissue and only weakly active in the delay of senescence or expansion of cotyledons (data not shown).

Discussion

The bioassays most commonly used for cytokinins evaluate different responses to this growth regulator. The callus tissue assay is advantageous because it is performed under sterile conditions, and it is very sensitive and specific (Miller 1963). Delay of senescence in detached leaves is a sensitive bioassay although not quite specific. The expansion of cotyledons is a rapid assay, and only GA shows similar activity to cytokinins (Letham 1971).

There are no references to 9-substituted adenine compounds behaving similarly to cytokinins (Skoog et al. 1967). Rogozinska et al. (1964) found that activity of triacanthine (3-dimethyl-allyl adenine) was probably due to a heat-induced change from the 3 to the 6 position of the allyl group. Skoog et al. (1967) found that 3-substituted purines were active in the tobacco callus test after they were converted to the corresponding N^6 isomers, whereas 9-derivatives undergo conversion much less readily and are thus inactive.

In our work a significant action of NI could be demonstrated in three different bioassays, in conditions where adenine is known to be ineffective, and inositol or trimesil inositol proved to be inactive at least in the low concentration provided. The somehow irregular activity of NI as compared to BA at the same molar concentrations could be attributed to impediments in absorption or mobilization due to the size or the structure of the molecules. However, as the bioassays employed excised tissue during extended periods, it does not seem

probable that activity would have been restricted by uptake. Furthermore, higher concentrations of NI did not increase its effect.

Germination is a complex process highly dependent on other growth regulators as well as light or temperature. Conditions as prescribed by Khan (1980/81) provide a reliable test for cytokinins, and the presowing infusion technique would ensure the uptake and the transport to the site of action of the growth regulators. NI was completely inactive in the germination tests. Kuraishi and Yamaki (1967) found that 4-benzylamino-benzimidazol protected chlorophyll from degradation and promoted expansion of leaf disks but failed to induce germination in tobacco seeds. The authors suggsted that this compound was destroyed during the bioassay period. In our case, as the germination of lettuce seeds was detectable in less than 48 h, destruction of NI can hardly explain the lack of response.

It is known that the range of activity of zeatin, BA, and kinetin differs according to the test used to evaluate it, and zeatin proved to be inactive in germination tests (Biddington and Thomas 1976). Hall (1973) emphasized the difficulties in relating a "fact" as structure and a "diffuse phenomenon" as function. Since the mechanisms involved in the regulation of development processes or the site of action of cytokinins are yet to be elucidated, it is conceivable that some compounds play no action in a particular process, whereas they evoke biological responses in other processes.

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