Photoaffinity-labeled Cytokinins

SYNTHESIS AND BIOLOGICAL ACTIVITY

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ABSTRACT

Two new azidopurine derivatives, 2-azido-N*-{(Δ^5-isopentenylnyl)adenine and 2-azido-N*-benzyladenine, have been synthesized as potential photoaffinity labels for probing cytokinin-binding sites. The preparation and the biological activity of these compounds are described.

The diverse effects of cytokinins on plant physiological processes, including cell growth and differentiation and the flow of assimilates and nutrients through the plant, presumably stem from a primary "mode of action" which has not yet been determined (6, 10). Recent approaches to definition of cytokinin-binding sites have included isolation and partial purification of a cytokinin-binding protein by affinity chromatography (13), utilization of radioactive substrates of very high specific activity for probing plant cell substructures (12), and synthesis of "stretched-out" analogs of cytokinins as potential fluorescent probes (11).

Photoaffinity-labeling reagents designed for probing cytokinin-binding sites would not only be nondestructive but would also be capable of reacting with any nearby components of the binding site. In addition, the reactivity of the photoaffordable functional group can be controlled to maximize labeling selectivity (4).

In examining complex systems by photoaffinity-labeling techniques, a high relative binding affinity is desirable so that the distribution of bound substrate favors specific sites over nonspecific sites. Whereas 2- or 8-substitution on the purine ring of zeatin or N-e{(Δ^5-isopentenylnyl)adenine generally lowers the cytokinin activity, substitution at a position by chlorine does not reduce the activity appreciably (2, 3). The 2-chloro compounds are particularly favorable in this regard. The similarity between the chloro group and the azido group in terms of inductive and hydrophobic characteristics, together with previous applications of photoaffordable azide compounds (1), suggested the 2-azido derivatives as the initial candidates for investigation. We have, therefore, prepared 2-azido-N*-{(Δ^5-isopentenylnyl)adenine (Ie) and 2-azido-N*-benzyladenine (IIe) as photoaffinity-labeled cytokinins and have tested their activity in the tobacco bioassay.

\[
\text{Ia} \quad \text{RNH} \quad \text{Ib} \quad \text{IIa} \quad \text{IIb} \quad \text{Ie} \quad \text{IIe} \quad \text{N}_3
\]

MATERIALS AND METHODS

General. All melting points are uncorrected. PMR\(^3\) spectra were recorded on Varian Associates A-60A and HA-100 spectrometers using tetramethylsilane (TMS) as an internal standard. Ultraviolet absorption spectra were measured on a Beckman Acta model MVI spectrophotometer. Mass spectra were obtained on a Varian-MAT CH-5 mass spectrometer coupled with a 620i computer and STATOS recorder. Infrared spectra were determined on a Perkin-Elmer model 337 spectrophotometer. Microanalyses were performed by Mr. Josef Nemeth and his associates.

6-Benzylamino-2-chloropurine (IIa). To 1 g (5.3 mmol) of 2,6-dichloropurine (Aldrich) in 35 ml of 1-butanol were added 2 g (18.7 mmol) of benzylamine. The solution was heated at reflux for 3 hr and cooled. Solvent was removed in vacuo, 45 ml water were added to the residue, and the sample was refrigerated for several hours. The product was collected by filtration, washed well with water, and dried under reduced pressure. An analytical sample was obtained by trituration with two 30-ml portions of hot ethanol, 1.07 g (78%) yield, melting point 245 to 246 C.

\[
\text{C}_{13}\text{H}_{12}\text{ClIN}_3
\]

Calculated: C 55.49, H 3.89, N 26.97

Found: C 55.56, H 3.83, N 27.26

2-Hydrazino-6-(3-methyl-2-butenylamino)purine (Ib). A solution of 1 g (4.2 mmol) of 6-chloro-6-(3-methyl-2-butenylamino)purine (Ia) (3) in 15 ml of an 85% solution of hydrazine hydrate in water was heated at reflux for 1 hr and then allowed to cool to room temperature. A white crystalline precipitate was collected by filtration, washed well with water, and recrystallized from absolute ethanol to give 750 mg (76%) of colorless crystals, melting point 213 to 215 C, PMR [(CD\text{3})\text{SO}] δ 7.68 (s, 1, 8-H), 7.06-7.26 (m, 2, N-H's), 5.30 (t, 1, J = 6 Hz, =C-H),

1 Abbreviation: PMR, proton magnetic resonance.

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4.05 (t, 2, J = 6 Hz, CH₃), 1.67 (s, 6, CH₃₂'s). UV \( \lambda_{\text{max}} \) (E1%1cm) 283 nm; \( \lambda_{\text{max}} \) (E1%1cm) (pH 1) 283, 252, 226 nm; \( \lambda_{\text{max}} \) (E1%1cm) (pH 11) 284, 245 (sh) nm.

C₁₀H₁₈N₄
Calculated: C 51.47, H 4.69, N 42.03
Found: C 51.77, H 6.62, N 42.11

6-Benzylamino-2-hydrazinopurine (IIb). This compound was prepared in the same manner as Ib from 6-benzylamino-2-chloropurine (Ia); 87% yield, melting point 252 C, PMR ([CD₃]₂SO) δ 7.76 (t, 1, J = 6 Hz, pur-NH), 7.70 (s, 1, 8-H), 7.08-7.50 (m, 5, C₆H₅), 4.69 (d, 2, J = 6 Hz, CH₃), 3.5-2.9 (b, 3, N-H's). UV \( \lambda_{\text{max}} \) (E1%1cm) 283 nm; \( \lambda_{\text{max}} \) (E1%1cm) (pH 1) 283, 252, 225 nm; \( \lambda_{\text{max}} \) (E1%1cm) (pH 11) 286 nm.

C₁₀H₁₈N₄
Calculated: C 56.45, H 5.14, N 38.41
Found: C 56.53, H 4.96, N 38.43

2-Azido-6-(3-methyl-2-butenylamino)purine or 2-Azido-N°-(\( \Delta^2 \)-isopentenyl) adenine (Ic). To 750 mg (3.3 mmol) of 2-hydrazino-6-(3-methyl-2-butenylamino)purine (IIb) suspended in 50% ethanol 630 mg (81%) of a solid, UV \( \lambda_{\text{max}} \) (pH 11) 286, 241 nm; MS (70 eV) m/z 244 (M⁺).

C₁₂H₁₇N₄O
Calculated: C 56.53, H 4.84, N 38.42
Found: C 54.19, H 4.96, N 38.42

Bioassay Procedure. Cytokinin activities of the test compounds were determined by the tobacco bioassay as described by Linmaier and Skoog (8). The medium contained the following salts specified in Table 6, part A, of reference (8) and the following organic constituents: sucrose, 30 g/l; Difco agar, 10 g/l; myo-inositol, 560 μM; IAA, 11.4 μM; and thiamine hydrochloride, 1.2 μM. For reasons of solubility and to avoid possible degradation by heat, the test compounds were dissolved in dimethylsulfoxide, a series of 3-fold dilutions made, and aliquots added to the cooling, autoclaved agar media. The final concentration of dimethylsulfoxide did not exceed 0.05% by vol, a concentration which does not affect biological activity in this assay (9).

In order to protect the photolabile azido group of the test compounds, all manipulations were done in a light-tight room under a photographic safe red lamp. The tissues were cultured in this room in the dark except for brief exposures to red light for observation during the 5-week growth period. A General Electric "germicidal lamp" (2537 A) held 20 cm above the exposed material was used for UV radiation.

RESULTS AND DISCUSSION

The azido-substituted cytokinin candidates Ic and IIc were synthesized by a conventional route involving hydrazines and azides.


