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Chemistry

The structural characterization of endogenous factors from *Vinca rosea* crown gall tumors that promote cell division of tobacco cells

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ABSTRACT **The ability of two compounds, a cytokinin and an auxin, to stimulate tobacco cell growth and differentiation has been known for >30 years, but the molecular mechanism of this activation is still unknown. Previous reports of factors endogenous in crown gall tumors of *Vinca rosea* that could replace the cytokinin requirement in tobacco cell culture has motivated an investigation of these tissues. The optimization of a reverse-phase isolation scheme has led to the purification of sufficient material to allow for the identification of six different related compounds. The structures of two of these compounds have been assigned as a set of epimeric dehydrodiconiferyl alcohol β-D-glycosides. The structure of these compounds suggests that they would most likely be derived from the plant cell wall.**

The induction of crown gall tumors in dicotyledonous plants by *Agrobacterium tumefaciens* involves the transfer and incorporation of oncogenic DNA into the host cell (1, 2). The resulting biochemical changes that allow these cells to grow continuously on defined medium without exogenous phytohormones have been studied extensively (3, 4), and recently genes transferred to the host have been found that code for enzymes involved in the biosynthetic production of cytokinins (5) and auxins (6). The transformed cell continuously produces these hormones and grows autonomously at a state of differentiation specified by the bacterial strain (7). While it is well established that the endogenous hormones can account for the tissue growth (8, 9), the molecular basis of this process is not yet known. Previous reports (10–12) of other growth factors present in *Vinca rosea* crown gall tumors have stimulated this investigation, which has resulted in the isolation and characterization of compounds capable of replacing the cytokinin requirement for tobacco cell growth in culture.

MATERIALS AND METHODS

Tissue Cultures. Axenic cultures of *V. rosea* crown gall tumors were grown on White's basic medium (13) containing 1% Bacto-Agar (Difco). Six pieces of tumor tissue (≈0.5 g each) were each placed in a culture dish (25 × 100 mm). Tumors were grown under constant illumination. After 4 weeks, at which time the tissue was at logarithmic phase growth, a small portion was used for subculture and the remainder was harvested for isolation.

Bioassay. Stems of *Nicotiana tabacum* cv. Havana 425 were surface-sterilized by washing with Ivory soap, washed three times each with 7% bleach and 70% EtOH/H_2O (1 min each), and three times with sterile distilled H_2O. Pith was isolated from mature internode sections, cut into ≈10-mg explants, and placed in shell vials (90 × 25 mm) containing 5 ml of agar-solidified LS medium (14) with 1.0 μM naphthalene acetic acid and filter-sterilized zeatin riboside or the dehydrodiconiferyl glucoside (DCG) isomer as indicated. The tissues were grown at 25°C under cool white fluorescent lights with a 16-hr light cycle and were harvested and weighed after 21 days.

Isolation and Analysis. All solvents were HPLC grade (J.T. Baker Chemical, Phillipsburg, NJ). The water was double-distilled from an all glass system. Octadecyl silanized silica (ODS) (particle size, 40 μm) was purchased from J.T. Baker. Analytical size HPLC columns, Zorbax C8 and Zorbax ODS (particle size, 5 μm; 25 × 0.45 cm), were purchased from DuPont. The HPLC system consisted of a DuPont 8800 Pump Module and a Beckman Model 160 Absorbance Detector with 254-nm detection.

A 1-kg batch of harvested tumor cells was extracted twice with equivalent amounts (wt/wt) of 50% MeOH/H_2O. The filtrate (2–3 liters) was concentrated *in vacuo* at 35°C to a thick syrup. The crude extract was partitioned in CH_2Cl_2/MeOH/H_2O (7:13:8, vol/vol), the organic layer was discarded, and the aqueous layer was filtered through a bed of celite. The solvent was removed *in vacuo*, and the sample was resuspended in a minimal volume of H_2O and applied to an ODS column (1 × 4 cm). This column was eluted with a step gradient of 0%, 25%, 50%, 75%, and 100% MeOH/H_2O (40 ml each) and collected separately. The 50% MeOH/H_2O fraction was concentrated to dryness and resuspended in a minimal amount (<2 ml) of 40% MeOH/H_2O. A flash column (1.5 × 15 cm) of ODS gel (particle size, 40 μm) was equilibrated in 40% MeOH/H_2O. The sample was applied and eluted isocratically under low N_2 pressure. Fractions (5 ml) were collected and two bands of activity (typically fractions 10–15 and 16–30) were found. The separately pooled fractions were each further purified on HPLC over a C8 column in 35% MeOH/H_2O, and both were resolved by HPLC on an ODS column. The early-eluting fraction gave 95% resolution of two components in 25% MeOH/H_2O, labeled A and B. The later-eluting fraction, which showed less biological activity, resolved into four components, D–G, in 15% tetrahydrofuran/H_2O with >98% resolution. Clean samples of each were collected by peak shaving. One kilogram of tumor tissue yielded ≤1 mg of each component.

Spectral Characterization. ^1H NMR were recorded at 500 MHz on an instrument built at The University of Chicago using an Oxford magnet and a Nicolet 1280/293C data system. All NMR solvents were "100 atom % ^2H" in 0.5-ml ampules (Aldrich). Correlation spectroscopy (15) and two-dimensional exchange spectroscopy (16) utilized the $(\pi/2\text{-}\Delta\text{-}t_1\text{-}\pi/2\text{-}\Delta\text{-}t_2)_n$ and $(\pi/2\text{-}t_1\text{-}\pi/2\text{-}t_1/4\text{-}\tau_m/2\text{-}\pi\text{-comp-}\tau_m/2\text{-}\pi/2\text{-}t_2)_n$ pulse sequences, respectively, and in both experiments the data sets were symmetrized (17). Proton-decoupled ^{13}C NMR spectra were recorded on a Varian XL400 operating at a carbon frequency of 100 MHz. Low- and high-resolution

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Abbreviations: DCG, dehydrodiconiferyl glucoside; CAD, collision-activated decomposition.

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mass spectra were taken using a VG model 70-250. Tandem mass spectrometry (18) was performed on a VG 7070 EBQQ; selected ions were allowed to undergo collision-activated decomposition (CAD) with N_2 in the first quadrapole cell and the daughter ions were analyzed in the second. Ultraviolet spectra were taken on a Perkin–Elmer Lambda 5 UV-Visible spectrometer. Circular dichroism spectra were recorded on a Cary-60 Spectro-Polarimeter.

Acetylation reactions used pyridine/acetic anhydride (2:1; vol/vol) at 23°C overnight. Solvent and reagent were removed *in vacuo* and the samples were repurified on HPLC using an analytical ODS column in 30% H_2O/MeOH. Silylation utilized pyridine/*N*,*O*-bis(trimethylsilyl)trifluoroacetamide (1:1; vol/vol) (Pierce). Solvent and reagent were removed *in vacuo*.

DCG-A. UV (H_2O), λ_{max} (ε), 274 (18,300), 310 sh (6000). ^1H NMR (^2H_2O) δ 7.18 (1H, d, J = 9.1 Hz, H-5′), 7.11 (1H, s, H-5 or -7), 7.10 (1H, d, J = 2.0 Hz, H-2′), 7.06 (1H, s, H-5 or -7), 7.01 (1H, dd, J = 1.8/8.9 Hz, H-6′), 6.57 (1H, d, J = 15.5 Hz, H-10), 6.28 (1H, dt, J = 15.5/5.1 Hz, H-11), 5.68 (1H, d, J = 5.6 Hz, H-2), 5.12 (1H, m, H-1″), 4.28 (2H, d, J = 5.1 Hz, H-12), 3.94 (3H, s, H-14), 3.87 (3H, s, H-7′), 3.5–3.9 (9H, H-3, H-13a, H-13b, H-2″, H-3″, H-4″, H-5″, H-6a″, H-6b″); (peracetylated, $C_6{}^2H_6$) δ 7.15 (1H, d, J = 8.9 Hz, H-5′), 6.94 (1H, d, J = 1.8 Hz, H-2′), 6.90 (1H, dd, J = 1.8/8.3 Hz, H-6′), 6.77 (1H, s, H-7), 6.76 (1H, s, H-5), 6.48 (1H, d, J = 15.9 Hz, H-10), 6.09 (1H, dt, J = 15.9/6.5 Hz, H-11), 5.57 (1H, dd, J = 7.8/7.8 Hz, H-2″), 5.45 (1H, dd, J = 7.8/7.8 Hz, H-3″), 5.43 (1H, d, J = 6.9 Hz, H-2), 5.29 (1H, dd, J = 7.8/7.9 Hz, H-4″), 4.90 (1H, d, J = 7.7 Hz, H-1″), 4.66 (2H, d, J = 6.5 Hz, H-12), 4.32 (1H, dd, J = 5.2/11.2 Hz, H-13a), 4.20 (1H, dd, J = 4.8/12.3 Hz, H-6a″), 4.12 (1H, dd, J = 7.5/11.2 Hz, H-13b), 3.99 (1H, dd, J = 2.3/12.3 Hz, H-6b″), 3.59 (1H, m, H-3), 3.47 (3H, s, H-14), 3.31 (3H, s, H-7′), 3.25 (1H, m, H-5″), and 1.9–1.6 (six acetate singlets). ^{13}C NMR (^2H_2O) δ 148.9 (C-4′), 147.1 (C-9), 145.5 (C-3′), 143.7 (C-8), 136.3 (C-1′), 131.5 (C-4), 131.1 (C-10), 128.5 (C-6), 126.3 (C-11), 118.7 (C-6′), 115.9 (C-5′), 115.5 (C-5), 110.7 (C-7), 110.2 (C-2′), 100.5 (C-1″), 87.8 (C-2), 76.2 (C-3″), 75.6 (C-5″), 72.9 (C-2″), 69.3 (C-4″), 62.9 (C-13), 62.5 (C-12), 60.5 (C-6″), 56.0 (C-14 or 7′), 55.9 (C-14 or 7′), 52.9 (C-3); mass spectrum (trimethylsilylated), m/z (%), M^+ 952.412 (2) (calculated for $C_{44}H_{80}O_{11}Si_6$, 952.432), 574 (39), 502 (25), 484 (20), 450 (15), 361 (83), 217 (39), 73 (BP). CD (H_2O) $\Delta\varepsilon_{270}$, +4.3; $\Delta\varepsilon_{285}$, +5.7.

DCG-B. UV (H_2O), λ_{max} (ε), 274 (18,300), 310 sh (6000). ^1H NMR (^2H_2O) δ 7.06 (1H, d, J = 9.1 Hz, H-5′), 7.01 (1H, s, H-2′), 7.00 (2H, s, H-5 and -7), 6.91 (1H, br d, J = 9.1 Hz, H-6′), 6.52 (1H, d, J = 15.5 Hz, H-10), 6.22 (1H, dt, J = 15.5/5.1 Hz, H-11), 5.58 (1H, d, J = 5.6 Hz, H-2), 5.01 (1H, m, H-1″), 4.13 (2H, d, J = 5.1 Hz, H-12), 3.80 (3H, s, H-14), 3.74 (3H, s, H-7′), 3.4–3.8 (9H, H-3, H-13a, H-13b, H-2″, H-3″, H-4″, H-5″, H-6a″, H-6b″); (peracetylated, $C_6{}^2H_6$) δ 7.15 (1H, d, J = 8.9 Hz, H-5′), 6.94 (1H, d, J = 1.8 Hz, H-2′), 6.90 (1H, dd, J = 1.8/8.3 Hz, H-6′), 6.76 (2H, br s, H-5 and -7), 6.48 (1H, d, J = 15.9 Hz, H-10), 6.09 (1H, dt, J = 15.9/6.5 Hz, H-11), 5.57 (1H, dd, J = 7.8/7.8 Hz, H-2″), 5.45 (1H, dd, J = 7.8/7.8 Hz, H-3″), 5.43 (1H, d, J = 6.9 Hz, H-2), 5.29 (1H, dd, J = 7.8/7.8 Hz, H-4″), 4.90 (1H, d, J = 7.7 Hz, H-1″), 4.66 (2H, d, J = 6.5 Hz, H-12), 4.32 (1H, dd, J = 5.9/11.0 Hz, H-13a), 4.21 (1H, dd, J = 5.0/12.4 Hz, H-6a″), 4.11 (1H, dd, J = 7.5/11.1 Hz, H-13b), 4.00 (1H, dd, J = 3.5/12.4 Hz, H-6b″), 3.59 (1H, m, H-3), 3.47 (3H, s, H-14), 3.31 (3H, s, H-7′), 3.25 (1H, m, H-5″), and 1.9–1.6 (six acetate singlets). ^{13}C NMR (^2H_2O) δ 148.9 (C-4′), 147.1 (C-9), 145.5 (C-3′), 143.7 (C-8), 136.3 (C-1′), 131.5 (C-4), 131.1 (C-10), 128.5 (C-6), 126.3 (C-11), 118.7 (C-6′), 115.9 (C-5′), 115.5 (C-5), 110.7 (C-7), 110.2 (C-2′), 100.5 (C-1″), 87.8 (C-2), 76.2 (C-3″), 75.6 (C-5″), 72.9 (C-2″), 69.3 (C-4″), 62.9 (C-13), 62.5 (C-12), 60.5 (C-6″), 56.0 (C-14 or -7′), 55.9 (C-14 or -7′), 52.9 (C-3); mass spectrum (trimethylsilylated), m/z (%), M^+ 952 (.6), 574 (19), 502 (12), 484 (9), 450 (8), 361 (53), 217 (28), 73 (BP). CD (H_2O) $\Delta\varepsilon_{270}$, −3.3; $\Delta\varepsilon_{285}$, −4.5.

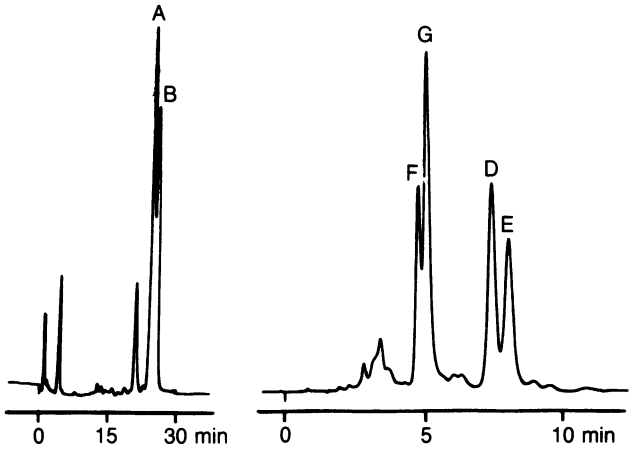

FIG. 1. ODS chromatographic elution of the individual isomers A, B, D, and E after initial C-8 chromatography of the ODS flash column resolved materials. Each chromatogram was run with the solvent at 2 ml/min and was detected at 254 nm. Solvents: *Left*, 25% MeOH/H_2O; *Right*, 15% tetrahydrofuran/H_2O.

RESULTS

Isolation. The levels of the biologically active compounds were low within the tumor tissue, and efforts were made to streamline the purification as much as possible. After 3–4 weeks of growth under continuous illumination, 1 kg of tumor tissue was extracted, and solvent was partitioned and purified using the selectivity of different reverse-phase chromatographic supports. This approach[§] gave a rapid and efficient purification of these factors (Fig. 1) that could be completed in <3 days. No evidence for any contamination by known cytokinins has ever been detected, nor would they be expected to chromatograph favorably (19) in the unbuffered and non-ion pairing reverse-phase conditions of this scheme.

Establishment of Structure. The six factors appeared to be structurally related and because of the limited amount of material, the approach to the assignment relied on this similarity to provide information about the basic structural framework. The common and distinctive UV chromophore (λ_{max}, 274 nm; 310-nm shoulder) suggested that the assignment of the substituents around a similar base would be the main structural challenge. Mass spectrometric analysis showed these compounds to be nonvolatile, but both acetylated and silylated derivatives gave apparent molecular ions under electron impact conditions. An m/z 772 ion was seen as both a CI positive (CH_4) and negative (N_2O) ion for the acetylated factors, suggesting a stable fragment or a molecular ion capable of facile electron transfer. The observation that four different samples (A, B, D, and E) gave the same molecular species and two samples (F and G) produced ions at 30 mass units higher was taken as evidence that these were molecular ions. This suggestion was further confirmed with the ^{13}C NMR spectra of A and B, indicating 26 carbon

[§]Small quantities of material isolated by the original isolation procedure contained UV transitions and, on acetylation, mass spectral molecular ions that correspond to the present isolated samples. These fractions provided enough information on the spectral and physical properties of the compounds to allow for the optimization of the purification scheme that we now report. We cannot attribute the previously reported biological activity exclusively to these compounds; however, these materials were certainly present in the original preparation.

resonances, and by exact mass measurements of hexatrimethylsilylated A, giving a composition of $C_{26}H_{32}O_{11}$ for the underivatized material.

Tandem mass spectrometry (Fig. 2) resolved individual fragmentation pathways. The positive ion (CI, CH_4) spectra of the peracetylated derivatives gave base peaks at m/z 331 and CAD of this ion (data not shown) gave a spectrum identical to the CAD of the m/z 331 ion obtained from the acetylated methylglucopyranoside. The aglycone fragment, m/z 441, was very weak under both positive and negative ion conditions and CAD analysis of the m/z 772 molecular ion gave no m/z 441, but rather two ions at m/z 381 and 322. Support for the assignment of these ions as (M-glucose-acetic acid)$^-$ and (M-glucose-acetic acid-acetate)$^-$ came from the silylated derivatives. CAD of the molecular ion, m/z 952, gave major ions at m/z 574, which arose from the loss of the sugar with a silyl group transfer, and m/z 502 as the aglycone. The m/z 574 ion readily lost silyloxy to m/z 484, whereas the aglycone fragment gave ions at m/z 412 (M-glucose-silyloxy)$^+$ and m/z 322 (M-glucose-2-silyloxy)$^+$. The m/z 322 ion, which was more intense in the main beam of the acetylated derivative, on CAD showed two losses 15 atomic mass units to m/z 307 and 292, indicative of methyl substituents and also an ion at exactly one-half the mass of the parent at m/z 161. The resistance of the aglycone fragments to further fragmentation or low-energy rearrangements was indicative of a stable highly unsaturated base. This assignment was further supported by the UV spectrum and the composition data.

The ^{1}H NMR spectra of fractions A and B at 500 MHz were well resolved and essentially identical under all conditions. Correlation spectroscopy (20) of the peracetylated derivative of A allowed for the complete ^{1}H-NMR assignment and identified five isolated spin systems in addition to two *O*-methyls and six *O*-acetates. The regional assignment of the protons on the two aromatic rings, the configuration of the hexose, and the olefin geometry were based on the magnitude of the spin–spin coupling constants. The final spin system consisted of four protons assigned to a three-carbon fragment. The downfield shift of the α proton in the unacetylated spectra, its small change on acetylation (−0.25 ppm) relative to the H-13 protons (+0.5 ppm), and the single degree of unsaturation remaining after the other structural assignments, relegated it to a fragment joining the two aromatic rings.

The molecular relationship between these isolated spin systems was most efficiently determined with two-dimensional exchange spectroscopy (Fig. 3). The placement of the glucose residue was established by the dipolar coupling seen between the anomeric proton (1″) of the glucose and one of the *ortho*-coupled protons of the trisubstituted aromatic ring. The *ortho*-coupled partner is dipolar coupled to a methine proton of the three-carbon fragment. This methine, in turn, is also dipolar coupled to the *meta* spin–spin coupled proton (H-2′) of the same aromatic spin system. The additional dipolar coupling observed between H-2′ and the downfield methoxy substituent completes the assignment of each substituent on the aromatic ring. The same aromatic proton, H-2′, is also dipolar coupled to the H-3 proton of the three-carbon fragment, which is likewise coupled to the H-5/H-7 protons of the other aromatic system. These two protons are not resolved enough at 500 MHz to be distinguished in the two-dimensional map, but the observation that they are *meta*-coupled (seen in C^2HCl_3 spectra), dipolar coupled to H-3, H-10, H-11, and the high-field methoxy leaves the only assignment as that seen in Fig. 3. The orientation of the substituents on the dihydrobenzofuran was assigned based on the observations of dipolar coupling between H-3 and H-2′, H-2 and H-13, and the absence of such coupling between H-2 and H-3. This same assignment is supported by nuclear Overhauser effect difference experiments and allows for compound A to be assigned as the 4′-β-D-glucoside of dehydrodiconiferyl alcohol—DCG.

The mass spectral fragmentation further supports this structure assignment. The mass spectra of both the silylated and acetylated derivatives show a facile loss of the hexose and one equivalent of the derivatizing agent to a common ion at m/z 322. This ion would be expected to have a structure that on CAD would fragment either the methoxy groups or give an ion at m/z 161. Both the mass spectrometry and the

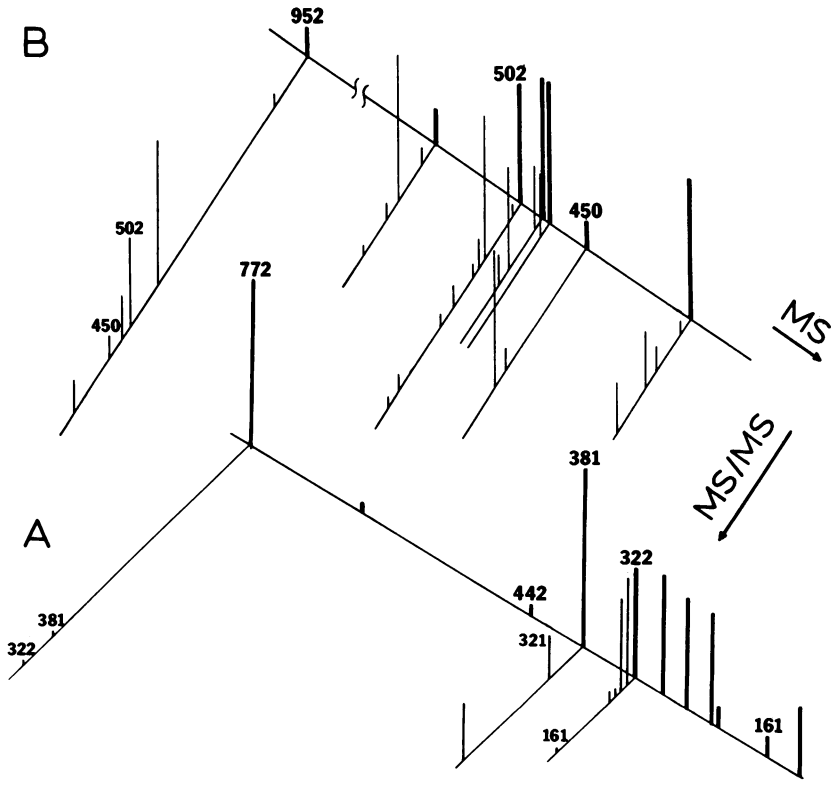

FIG. 2. Tandem mass spectrometry (MS/MS) of fraction A. The main beam spectra are indicated in boldface; the daughter ion spectra are shown as branches from their respective parent ions. (*A*) The negative ion spectra of the peracetylated derivative, CI(N_2O). (*B*) The positive ion spectra of the pertrimethylsilated derivative, EI (70 eV).

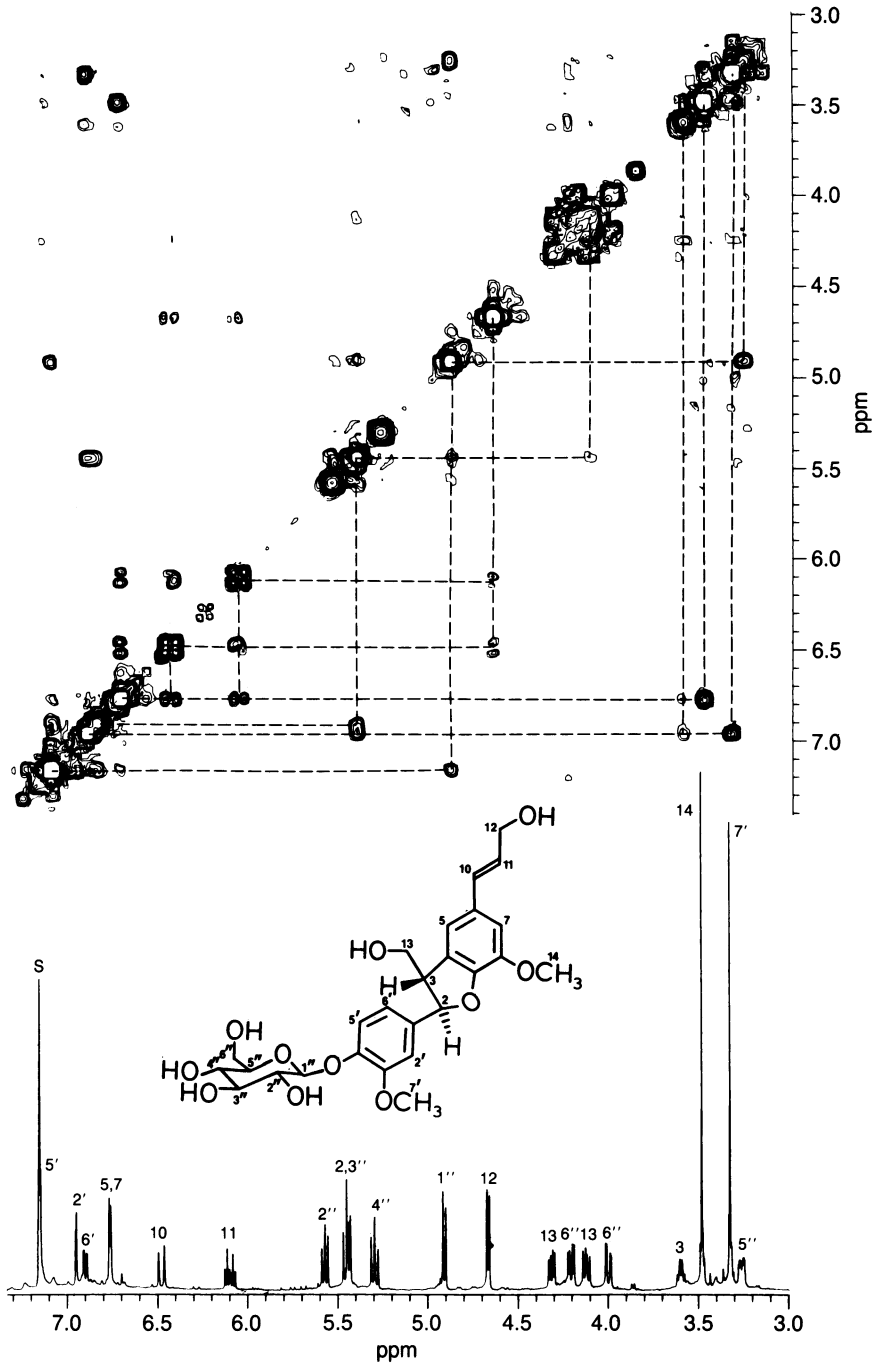

FIG. 3. The two-dimensional exchange spectrum of peracetylated fraction A using a mixing time of 0.8 sec. The map consists of 512 × 512 data points and the corresponding one-dimensional spectrum (16,000 data points) is shown below. The structure of DCG-A is shown.

^{1}H NMR data are then consistent with the assignment of the phenyl propanoid dimers shown in Fig. 3.

The individual spin systems and their spatial orientation as determined by ^{1}H NMR and the mass spectral fragmentation of A and B are identical. The only difference was apparent in the CD spectra, where the intense transition at 285 and 270 nm were of equal intensity but of opposite sign. DCG-B can then be assigned as containing the enantiomeric base of A.

Biological Activity. The activity of a 1:1 mixture of the DCG isomers A and B as they exist in the tumor was compared to zeatin riboside over a wide range of concentrations in the tobacco pith assay (Fig. 4). The results demonstrate some stimulation of growth at 1 μM DCG-A/-B and stimulation with 10 μM equal to that seen with 0.1 μM zeatin riboside. Even though higher concentrations were required, these endogenous tumor factors could completely replace the cytokinin requirement for tobacco pith cell growth. The further characterization of the individual isomers and the relationship of their biological activity to that of the cytokinins will be dealt with in a subsequent paper (21).

DISCUSSION

Since the discovery of the cytokinins, their mechanism of action and the control of their biosynthesis have been extensively studied, but as yet, no clear description of the process has emerged (21–23). Like many of the mammalian hormones, their activity is pleiotropic in that they act very differently on a variety of different tissues (24). Crown gall tissue contains specifically incorporated genes for perpetual hormone expression and provides an opportunity to screen for other factors that may be controlled by these hormones. The previous reports (10–12) of factors that could replace the cytokinins in the tobacco pith assay suggest that these compounds might provide some insight into the pleiotropic effects of the cytokinins.

Our purification methods provided sufficient amounts (1–3 mg) of purified compounds for spectroscopic identification

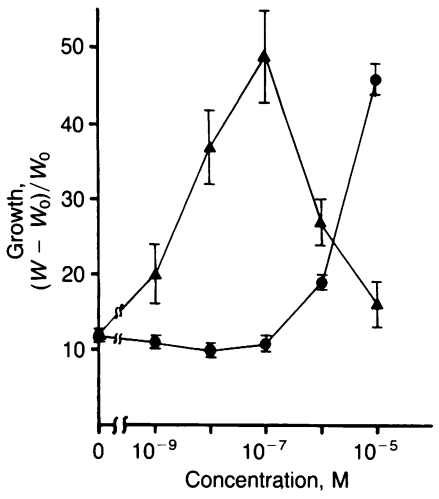

FIG. 4. Response of *N. tabacum* cv. Havana 425 pith to zeatin riboside (▲) or DCG-A-B mixture (●) in the presence of 1 μM naphthalene acetic acid. Fresh weight (W) measured after 21 days. Growth is expressed as ($W - W_0/W_0$), where W_0 = initial fresh weight of the explant (11 mg); mean ± SEM (n = 5).

and biological testing. The combination of mass spectrometry and ^{1}H NMR allowed for the assignment of different domains of the molecule as well as the interconnections between these domains. These data, together with the CD spectra, established the assignment of the structures on a small enough scale so that sufficient material was available for further biological testing. Two of these factors, A and B, together in a 1:1 mixture as they exist in the tumor can completely replace the cytokinin requirement for growth in fresh tobacco explants. The further documentation of the activity of these factors in the stimulation of cell division and differentiation will be dealt with in a subsequent paper (21).

The role of phenylpropanoids in plants has been found to be quite diverse. They serve as an integral part of the structural matrix of lignin (25, 26), in plant protection as phytoalexins (22) and allelopathic agents (27, 28), and have been shown to dramatically inhibit plant cell growth (29). Both dehydrodiconiferyl alcohol (30) and the monomeric dihydroconiferyl alcohol have been isolated from plant tissue. The monomer has been isolated from lettuce seedlings (31) as a gibberellin synergist in induction of lettuce hypocotyl elongation (32) and has been shown to stimulate auxin-induced elongation of cucumber hypocotyl sections (33). The same compound has been found in sycamore sap (34) and demonstrated to stimulate growth in the soybean callus and tobacco callus assays and the radish leaf senescence assay (35). It is striking that this phenylpropanoid, which appears to have a specific role in hypocotyl elongation and stimulates cell growth in general, would constitute a portion of the structure of the DCGs.

The establishment of the structure of these factors as diastereomeric phenylpropanoids associates them biosynthetically with the nonstereospecific polymerization of phenylpropanoid monomers found in lignins and cell wall structural components. Host cell wall components are involved in the regulation of organogenesis in parasitic plants (36, 37) and small phenolic compounds released from wounded or metabolically active plant cells induce *vir* gene activation in *A. tumefaciens* (38, 39). Such cell wall phenolic compounds may be generally associated with the stimulation of growth and development in plant cells. Wall carbohydrates have also been suggested to play a role in plant cell differentiation (40) and it may be that these phenolic compounds play a complementary role with the carbohydrates in directing growth and differentiation.

This paper is dedicated to the memory of Armin C. Braun (deceased Sept. 2, 1986). We thank Mayland Chang for growing the cell cultures, Aaron Panner for his assistance in the isolation, and Andrew Binns and Manfred Ruddat for advice and assistance with the assays. We gratefully acknowledge the support of the National Institutes of Health (GM 33585) and The University of Chicago Cancer Center. D.G.L. is a Sloan Foundation Fellow and a Camille and Henry Dreyfus Teacher–Scholar.

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