Convenient Synthesis of 2,4-Diacetylphloroglucinol, a Natural Antibiotic Involved in the Control of Take-All Disease of Wheat

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2,4-Diacetylphloroglucinol (DAPG) is an antibiotic with broad-spectrum antibacterial and antifungal activities. It is a major determinant in the biological control of several plant diseases. DAPG is produced by *Pseudomonas fluorescens* both in vitro and in the rhizosphere of wheat. It is involved in the natural suppression of take-all disease known as take-all decline, which develops in soils following extended monoculture of wheat or barley. A one-step synthesis of DAPG from the commercially available 2-acetylphloroglucinol is described. This reaction involves the direct alkylation of 2-acetylphloroglucinol using acetic anhydride as the acetylation reagent, with boron trifluoride−etherate as the catalyst. This synthesis is simple and produces higher yields of DAPG (90%) as compared with previously described procedures. As ecological concerns are gaining equal status with agricultural concerns, the demand for natural biocontrol measures is increasing. There is tremendous pressure from society on agriculture to reduce the use of pesticides. A discussion is given on the agricultural and ecological importance of this natural antibiotic and its application as an alternative to reduce the use of synthetic pesticides.

Keywords: *O-Acetylated; total intensity chromatogram (TIC); photodiodide array UV spectroscopy; high-pressure liquid chromotograpy (HPLC); mass spectrum (MS); retention time (RT); melting point (mp); phloroglucinol (Phl); 2-acetylphloroglucinol (MAPG); 2,4-diacetylphloroglucinol (DAPG); boron trifluoride−etherate; Pseudomonas fluorescens*

INTRODUCTION

American agriculture is under increasing pressure to reduce the use of synthetic chemical pesticides for the control of plant diseases, insect pests, and weeds because of concerns about the adverse impact of pesticides on public health and the environment. Thus, there is considerable interest in finding and developing natural products with pesticidal activity. Phloroglucinol (Phl) antibiotics are phenolic metabolites produced by bacteria and plants with broad-spectrum antibacterial, antifungal, antiviral, anthelmintic, and phytotoxic properties (Thomashow and Weller, 1996). Incontrovertible evidence has accumulated that the polyketide antibiotic 2,4-diacetylphloroglucinol (DAPG) is a major determinant in the biological control of several plant diseases by certain strains of *Pseudomonas fluorescens*. A genetic approach was used to demonstrate the role of DAPG in the suppression of black root rot of tobacco (*Thielaviopsis basicola*) and take-all of wheat (*Gaeumannomyces graminis* var. *tritici*) by *P. fluorescens* CHA0 (Keel et al., 1990, 1992), take-all of wheat by *P. fluorescens* Q2-87 (Harrison et al., 1993; Vincent et al., 1991), and damping-off of sugar beet (*Pythium ultimum*) by *P. fluorescens* F113 (Fenton et al., 1992; Shanahan et al., 1992). Mazzola et al. (1995) reported that DAPG-producing strain Q2-87 suppressed take-all caused by three DAPG-sensitive isolates of *G. graminis* var. *tritici* but failed to suppress take-all caused by two isolates of the pathogen that were insensitive to DAPG at 3.0 μg/mL. Furthermore, DAPG-producing fluorescent *Pseudomonas* spp. have been shown to be responsible for take-all decline, a natural biological control of take-all which develops in soils following extended monoculture of wheat or barley (Raaijmakers et al., 1997; Raaijmakers and Weller, 1998). The positive role for DAPG in the biological control of plant diseases demonstrated by the use of genetic approaches was confirmed by direct isolation of the antibiotic from the rhizosphere of wheat. When seeds of wheat were coated with *P. fluorescens* Q2-87 and then sown in raw Shano silt loam and Ritzville silt loam, 0.16 and 0.33 μg of DAPG/10^7 colony-forming units (CFU) of Q2-87/g of root plus rhizosphere soil, respectively, were isolated (Bonsall et al., 1997). The amount of DAPG produced in the rhizosphere recently was shown to be directly related to the population size of DAPG producers present (Raaijmakers et al., 1999).

DAPG can be readily isolated in milligram quantities from cultures of fluorescent *Pseudomonas* spp. containing the DAPG biosynthetic locus strains (Keel et al., 1996), and production of DAPG can be enhanced when plant roots are added to the culture (Aino et al., 1995). The DAPG biosynthetic locus has been cloned (Bangera and Thomashow, 1996, 1999) and sequenced (Genbank accession no. U41818). However, no efficient chemical synthesis for DAPG has been described. The purpose

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of this study was to develop a simple, convenient, and affordable synthesis of DAPG to facilitate studies of this natural product for plant disease control. 2-Acetylphloroglucinol (MAPG) was used as the starting material because it is commercially available and inexpensive.

MATERIALS AND METHODS

General Experimental Procedures. Solvents were of either HPLC grade (CH_3CN, MeOH) or ACS grade (EtOAc, hexanes) from J. T. Baker. Column and analytical thin layer chromatographic separations (Al Si G/UV 254) were performed using silica gel 60 (230−400 mesh, Whatman). IR spectra were recorded on a Perkin-Elmer (Spectrum 2000) FTIR spectrometer, and NMR spectra were obtained using a Bruker ARX500 (500 MHz) spectrometer. All NMR spectra were recorded in acetone-d_6 using TMS as internal reference, with chemical shifts (δ) expressed in parts per million.

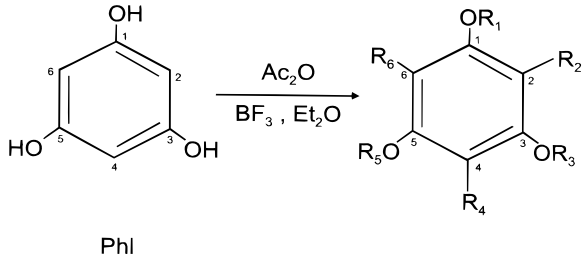
Electron ionization mass spectrometry (EI/MS) analyses were carried out with an HPLC/MS (Waters, Integrity). The Waters Integrity system consisted of an Alliance 2690 separation module with a 996 photodiode array detector and an electron ionization Thermabeam mass detector (TMD). The separation was achieved using reversed phase column chromatography (Waters, Symmetry C-18; 150 × 3.0 mm). Solvent conditions included a flow rate of 0.35 mL/min with a 2 min initialization at 10% CH_3CN in water with 2% v/v acetic acid, followed by a 20 min linear gradient to 100% CH_3CN (2% v/v acetic acid). MS analyses of total intensity chromatograms (TIC) were performed from *m/z* 70 to 355 at a rate of 1 scan s^{-1}. The nebulizer, ion source, and expansion region temperatures were 84 , 220, and 80 °C, respectively. These temperatures were optimized using commercial MAPG (Aldrich) and natural DAPG as references. High-resolution mass spectrometry (HRMS) analyses employed a VG 7070 EHF mass spectrometer at 70 eV. Melting points are uncorrected and were recorded on a Büchi apparatus B-540. Elemental analysis was conducted by Desert Analytics (Tucson, AZ).

Procedure for Synthesis of DAPG. Acetic anhydride (10 mL, 106.5 mmol, 1.8 equiv) was added dropwise at room temperature to MAPG (Aldrich; 10 g, 59.5 mmol), dried at 80 °C under vacuum for 12 h in a 250-mL round-bottom flask. Boron trifluoride−etherate ($Et_2O{\cdot}BF_3$) as a catalyst (50 mL, 39.7 mmol) was slowly added with a syringe to the stirring mixture, followed by continuous stirring for 24 h at room temperature. Water (30 mL) was then slowly added, maintaining vigorous stirring, followed by 60 mL of water/methanol v/v (1:1). Potassium carbonate (K_2CO_3; 40 g, 290 mmol) was then carefully added followed by the addition of water/methanol 1:1 (~20 mL) to maintain stirring of the solution. The basic solution was stirred overnight, followed by neutralization/acidification (pH 5−6) with 10% HCl. Methanol was evaporated from the mixture by rotor evaporation under vacuum (40 °C). Distilled water (500 mL) was added to the moist residue followed by stirring for 5 min and then filtration through a Büchner funnel apparatus. This operation was repeated twice to remove salts from the crude extract. The residue was dried overnight in a desiccator under vacuum at room temperature. The residue was dissolved in a minimum amount of acetone, subjected to silica column chromatography (400 × 40 mm), and eluted with hexanes/EtOAc v/v (4:1); DAPG was eluted first (monitored by TLC; UV = 254 nm). The elutant was rotor-evaporated under vacuum (50 °C maximum) to dryness, followed by recrystallization from benzene/petroleum ether v/v 2:1 (15 mL), suction filtered through a Büchner funnel, and dried overnight in a desiccator under vacuum. To recover the remainder of DAPG, the recrystallization filtrate was rotor-evaporated to dryness under vacuum (50 °C maximum) and purified by silica gel column chromatography (300 × 20 mm) as described above. The white solid DAPG fractions (obtained from recrystallization and the second silica gel purification) were combined to give a total mass of 11.2 g (53.3 mmol) at a 90% yield. TLC: $R_f = 0.55$ (hexanes/EtOAc v/v [1:1]).

Table 1. Phloroglucinol and C- or O-Acetylated Derivatives

compound	R_1[a]	R_2	R_3	R_4	R_5	R_6	MW (*m/z*)[b]	RT[c]
Phl	H	H	H	H	H	H	126	3.59
MAPG	H	Ac	H	H	H	H	168	12.35
DAPG	H	Ac	H	Ac	H	H	210	18.19
TAPG	H	Ac	H	Ac	H	Ac	252	25.02
1-*O*-acetyl-Phl	Ac	H	H	H	H	H	168	9.87
1,3-di-*O*-acetyl-Phl	Ac	H	Ac	H	H	H	210	13.79
1,3,5-tri-*O*-acetyl-Phl	Ac	H	Ac	H	Ac	H	252	16.56
1-*O*-2-diacetyl-Phl	Ac	Ac	H	H	H	H	210	14.43
5-*O*-2-diacetyl-Phl	H	Ac	H	H	Ac	H	210	15.66
3-*O*-2,4-triacetyl-Phl	H	Ac	Ac	Ac	H	H	252	

[a] Substituent groups on the phloroglucinol ring as shown in Figure 1. [b] Molecular weight based on the parent molecular weight ion as determined by mass spectrometry. [c] RT, retention time.



Phl

Figure 1. Direct acetylation of Phl using acetic anhydride (Ac_2O) and $Et_2O{\cdot}BF_3$ as catalyst.

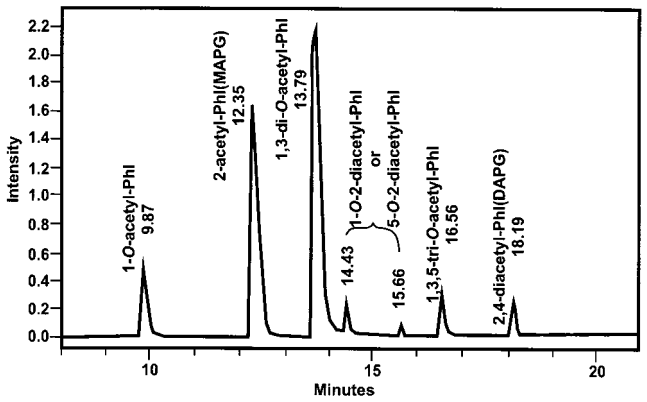


Figure 2. TIC of the synthesis of DAPG from Phl. TIC was performed from *m/z* 70 to 355 at a rate of 1 scan/s. Numbers shown are retention times. Peaks at retention times of 14.43 and 15.66 are either 1-*O*-2-diacetyl-Phl or 5-*O*-2-diacetyl-Phl because both peaks exhibit similar mass spectra.

RESULTS

Synthesis of DAPG from Phl. Dean and Robertson (1953) reported a synthesis of DAPG involving the acetylation of Phl (Table 1; Figure 1). As a first step in our study, we repeated their procedures. LC/MS analysis revealed the formation of many different side products due to random *O*-acetylation of Phl and MAPG together with the formation of MAPG and DAPG (Table 1; Figure 1) production. However, from the total intensity chromatogram (TIC), the peak that corresponded to DAPG (M^+ at $m/z = 210$) was a minor peak when compared to that of the *O*-acetylated compounds and MAPG produced (Figure 2).

O-Acetylation of Phl. 1-*O*-acetyl-Phl (Table 1; Figure 1; RT = 9.87, Figure 2) has a molecular ion M^+ at *m/z* 168 (which is the expected M^+ mass for MAPG) but exhibits a TIC RT (Figure 2) and a mass spectrum (Figure 3D) different from those of MAPG (Figure 3B).

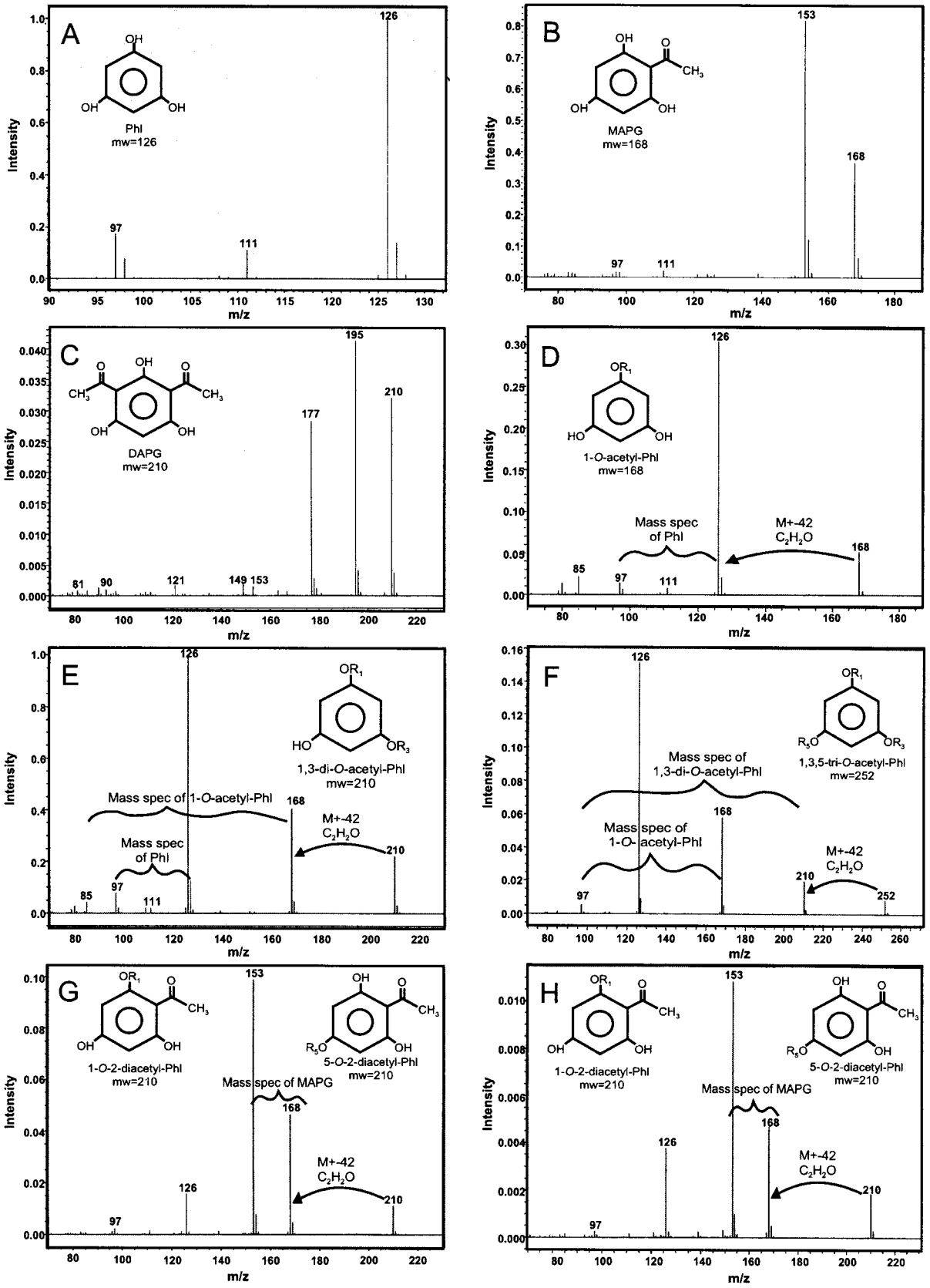


Figure 3. Mass spectra of Phl, MAPG, DAPG, and O-acetylated forms of Phl and MAPG: (A) Phl [*m/z* (relative intensity) 126 (100, $[M]^+$), 111 (8, $[M - CH_3]^+$)]; (B) MAPG [*m/z* (relative intensity) 168 (45, $[M]^+$), 153 (100, $[M - CH_3]^+$)]; (C) DAPG [*m/z* (relative intensity) 210 (90, $[M]^+$), 195 (100, $[M - CH_3]^+$), 177 (75, $[M - H_2O - CH_3]^+$); 149 (10, $[M - H_2O - COCH_3]^+$)]; (D) 1-*O*-acetyl-Phl [*m/z* (relative intensity) 168 (16, $[M]^+$), 126 (100, $[M - CH_3]^+$), 111 (4, $[M - CH_3CO]^+$), 97 (6)]; (E) 1,3-di-*O*-acetyl-Phl [*m/z* (relative intensity) 210 (25, $[M]^+$), 168 (40, $[M - CH_3CO]^+$), 126 (100, $[M - 2(CH_3CO)]^+$), 111 (5), 97 (10)]; (F) 1,3,5-tri-*O*-acetyl-Phl [*m/z* (relative intensity) 252 (6, $[M]^+$), 210 (12, $[M - CH_3CO]^+$), 168 (37, $[M - 2(CH_3CO)]^+$), 126 (100, $[M - 3(CH_3CO)]^+$), 97 (5)]; (G, H) either 1-*O*-2-diacetyl-Phl or 5-*O*-2-diacetyl-Phl [*m/z* (relative intensity) 210 (10, $[M]^+$), 168 (55, $[M - CH_3CO]^+$), 153 (100, $[M - CH_3CO - CH_3]^+$), 126 (20, $[M - 2(CH_3CO)]^+$), 97 (3) or 210 (22, $[M]^+$), 168 (48, $[M - CH_3CO]^+$), 153 (100, $[M - CH_3CO - CH_3]^+$), 126 (36, $[M - 2(CH_3CO)]^+$), 97 (3)].

This peak was identified as 1-*O*-acetyl-Phl because the molecular ion M^+ of *m*/*z* 168 (Figure 3D) was followed by a mass spectrum pattern identical to that of Phl (Figure 3A), with a neutral fragment loss of C_2H_2O ($M^+ - 42$ at *m*/*z* 126).

1,3-Di-*O*-acetyl-Phl (Table 1; Figure 1; RT = 13.79, Figure 2) exhibited a mass spectrum with a molecular ion M^+ at *m*/*z* 210, which is the expected mass for DAPG. Mass spectra analysis (Figure 3E) revealed that the molecular ion M^+ at *m*/*z* 210 was followed by a mass spectrum pattern identical to that obtained for 1-*O*-acetyl-Phl (Figure 3D) and Phl (Figure 3A), with a neutral fragment loss of C_2H_2O ($M^+ - 42$ at *m*/*z* 168).

1,3,5-Tri-*O*-acetyl-Phl (Table 1; Figure 1; RT = 16.56, Figure 2) exhibited a mass spectrum with a molecular ion M^+ at *m*/*z* 252, which is the expected mass for 2,4,6-triacetylphloroglucinol (TAPG; Table 1; Figure 1). The mass spectrum (Figure 3F) of this peak showed a neutral fragment loss of C_2H_2O ($M^+ - 42$ at *m*/*z* 210) similar to those of 1,3-di-*O*-acetyl-Phl (Figure 3E) and 1-*O*-acetyl-Phl (Figure 3D).

O-Acetylation of MAPG. 1-*O*-2-Diacetyl-Phl (Table 1; Figures 1 and 2) and 5-*O*-2-diacetyl-Phl (Table 1; Figures 1 and 2) also exhibit a mass spectrum with a molecular ion M^+ at *m*/*z* 210, which is the expected mass for DAPG. Because the MS spectra (Figure 3G,H) of both peaks at RT = 14.43 and 15.66 (Figure 2) are similar, it was impossible to assign which peak belonged to what product. However, these two peaks are related to the *O*-acetylation of MAPG, due to the fact that the molecular ion at *m*/*z* 210 (Figure 3G,H) is followed by a mass spectrum pattern identical to that of MAPG (Figure 3B) with a neutral fragment loss of C_2H_2O ($M^+ - 42$ at *m*/*z* 168). Therefore, the only two possibilities that can exist are 1-*O*-2-diacetyl-Phl (Table 1; Figure 1) and 5-*O*-2-diacetyl-Phl (Table 1; Figure 1) for the two peaks found at RT = 14.43 and 15.66 (Figure 2).

Production of MAPG and DAPG from Acetylation of Phl. The peaks at RT = 12.35 (MAPG) and 18.19 (DAPG) on the LC/MS profile (Figure 2) gave UV and mass spectra that matched our referenced MAPG (Figures 4A and 3B) and DAPG (Figures 4B and 3C). Again, the amount of DAPG formed was a very minor fraction (3%) when compared to the total amount of *O*-acetylated products and MAPG formed (Figure 2).

Synthesis of DAPG from MAPG. Due to the problems with the *O*-acetylated compounds that were encountered by the synthesis of DAPG from Phl, a small-scale conversion was set up using MAPG as the starting material that could be later scaled up (Figure 5). It should be mentioned that extending the reaction time (48 h) of the conversion gives *O*-acetylation of DAPG yielding 3-*O*-2,4-triacetyl-Phl (Table 1; Figure 1). This conclusion was made on the basis of MS analysis that exhibited a molecular ion M^+ of *m*/*z* 252 followed by an MS spectrum pattern similar to that of DAPG, with a neutral fragment loss of C_2H_2O ($M^+ - 42$ at *m*/*z* 210) (data not shown). In addition, this was confirmed by ^{13}C NMR, which exhibited a symmetrical spectrum pattern: only one ^{13}C peak for both C-2 and C-4, as expected for a substitution position C-3 (data not shown). Later, it was observed that DAPG could be recovered from the *O*-acetylated product by simple basic treatment with K_2CO_3 in aqueous methanol. In conclusion, the conversion of MAPG to DAPG with basic treatment will yield only nonconverted MAPG with DAPG as products, which can be separated by column

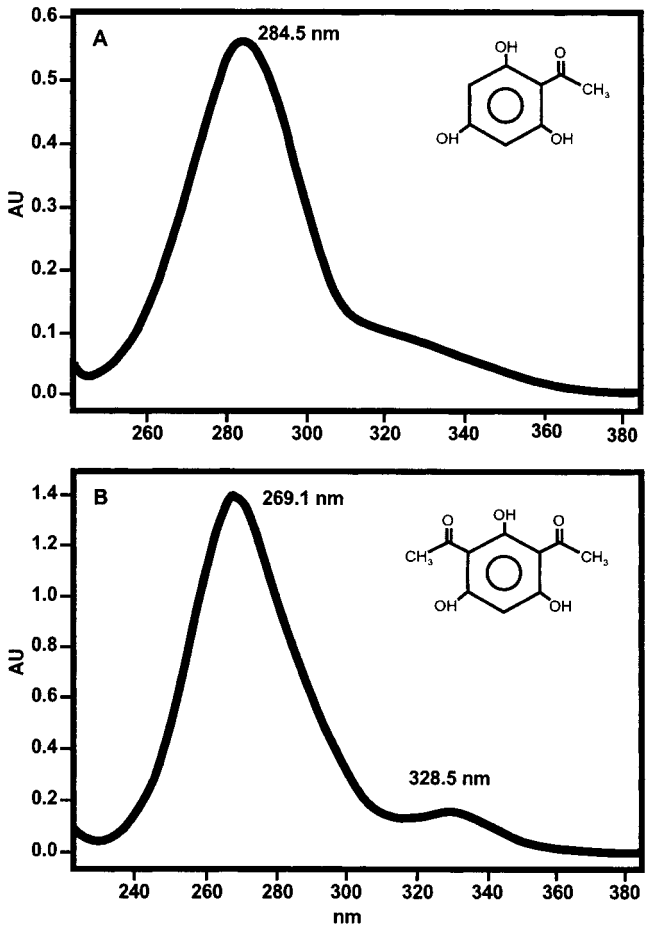


Figure 4. Photodiode array UV spectra of (A) MAPG and (B) DAPG.

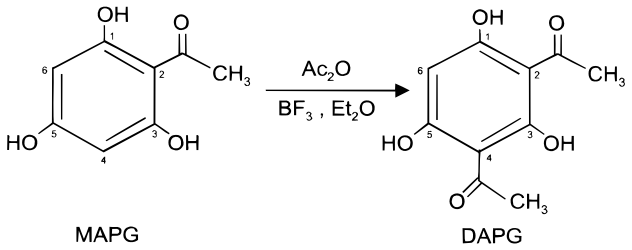


Figure 5. Conversion of MAPG to DAPG using acetic anhydride (Ac_2O) and $Et_2O{\cdot}BF_3$ as catalyst.

chromatography (silica gel). The purity of DAPG after silica gel column chromatography was demonstrated by HPLC/MS using TIC (Figure 6).

The proton NMR spectra, ^{13}C NMR spectra, EI mass spectra, high-resolution mass spectra (HRMS), Fourier transform infrared spectra (FTIR), photodiode array UV spectra, elemental analysis, and melting point (mp) data for the properties of DAPG are as follows: ^1H NMR (CD_3COCD_3) δ 5.87 (m, 1H CHAr), 3.90−3.30 (br s, 3H, OH), 2.61 (s, 3H, CH_3); ^{13}C NMR (CD_3COCD_3) δ 205.1 (C=O), 173.4 (s, Cq-OH: C-1 and C-5), 170.8 (s, Cq-OH: C-3), 105.3 (s, Cq-C=O), 96.3 (d, CH) 33.5 (q, CH_3); EI/MS, *m*/*z* (relative intensity) 210 (90, $[M]^+$), 195 (100, $[M - CH_3]^+$), 177 (75, $[M - H_2O - CH_3]^+$), 149 (10, $[M - H_2O - COCH_3]^+$); HRMS calcd for $C_{10}H_{10}O_5$ 210.0528, found 210.0509; FTIR ν (cm^{-1}) 3400−3000 (OH br) 1610, 1562, 1468 (aromatic conjugated C=O with intramolecular hydrogen bonds and aromatic C=C), 1364 (C−OH), 1283 (C−O); photodiode array analysis (UV spectra) λ_{max} = 270, 330 nm. Anal. calcd for $C_{10}H_{10}O_5$ C,

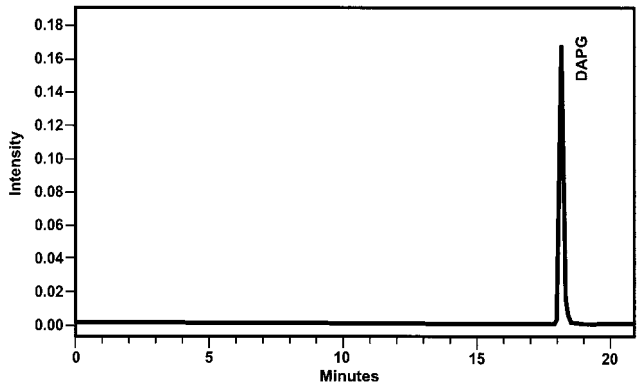


Figure 6. TIC of the synthesis of DAPG from MAPG. TIC was performed from *m/z* 70 to 355 at a rate of 1 scan s^{-1}.

57.14; H, 4.80. Found: C, 56.30; H, 4.99 (taking into account 0.18 molecule of water associated with one molecule of DAPG C, 57.18; H, 4.81). mp = 173 °C [lit. 173−174 °C (Nowak-Thompson et al., 1994)].

Because Phl and MAPG are commercially available as the dihydrate and monohydrate (Phl, $2H_2O$; MAPG, H_2O), respectively, the water content of DAPG was investigated by elemental analysis and by thermal gravimetric analysis. This was done to obtain information concerning the real molecular weight and water content of DAPG for an accurate determination of a percent yield of synthesis from MAPG. Elemental analysis of synthetic DAPG at room temperature revealed C, 56.30, and H, 4.99. This value was compared with theoretical values for the molecular formula of $C_{10}H_{10}O_5$, $X = 0$ (C, 57.14; H, 4.80) and $C_{10}H_{12}O_6$, $X = 1$ (C, 52.63; H, 5.30). It was concluded that synthesized DAPG does not contain any molecules of water associated with it (maximum hydration + 0.18 molecule of water). Reconfirmation by thermal gravimetric analysis on recrystallized DAPG also revealed no degrees of hydration (<0.5% H_2O). Thus, the molecular $C_{10}H_{10}O_5$ formula for DAPG was confirmed with a molecular mass of 210 g mol^{-1}.

DISCUSSION

There have been a limited number of attempts to chemically synthesize DAPG in which Phl has been the preferred starting material (Dean and Robertson, 1953; Mani et al., 1991; Ranjaneyulu et al., 1987). We began our study by duplicating the synthesis of DAPG from Phl as described by Dean and Robertson (1953). Their study involved the *C*-acetylation of Phl and *C*-methyl Phl together with their mono- and dimethyl ethers, using boron trifluoride−etherate as catalyst, to produce DAPG, 2,4-diacetyl-6-methylphloroglucinol, and their corresponding methyl ethers. We demonstrated that the direct alkylation of Phl resulted in many *O*-acetylated molecules, with the same molecular weight (M^+ at *m/z* 210) and with the same theoretical elemental analysis ($C_{10}H_{10}O_5$) as those of DAPG. As a result, the credibility of the synthesis described by Dean and Robertson (1953) was further investigated. In their paper, assessing the purity of the crude extract involved only elemental analysis and no mass spectral or ^1H and ^{13}C NMR data. These *O*-acetylated compounds have the same molecular weights that would be expected for MAPG, DAPG, and TAPG. Therefore, straight elemental analysis that is not reconfirmed by mass spectrometry and ^1H and ^{13}C NMR would mislead the investigator to believe that they had pure quantities of MAPG and DAPG (as the major products) with TAPG (as the minor product) from the synthetic reaction.

In our study, using MAPG as the starting material, purity was assessed after recrystallization (on the whole mass), using both spectroscopic (UV, IR, NMR, MS) and elemental analysis. In addition, direct alkylation of MAPG by our method yields pure DAPG with no *O*-acetylated products, as determined by mass spectral analysis on the HPLC/MS profile, using TIC (Figure 6). Our synthesis of DAPG gave a melting point of 173 °C, which was identical to that reported by Nowak-Thompson et al. (1994; 173−174 °C), whose sample was analyzed by ^1H and ^{13}C NMR. In contrast, Dean and Robertson (1953) and Mani et al. (1991) reported a lower melting point of 168 °C indicating impure material. Also questionable is the purity of synthetic DAPG claimed by Ranjaneyulu et al. (1987) because they obtained purple crystals in the reaction with $Ac_2O/ZnCl_2$ instead of the white crystals that we obtained. In addition, the previously reported yields for MAPG and DAPG appear to be in error. Ranjaneyulu et al. (1987) reported a yield of MAPG of 50%; however, the actual yield should be 36% because they did not consider that MAPG will crystallize with one molecule of water (MAPG, H_2O; $C_8H_{10}O_5$; commercially available form of MAPG). In addition, their report of a 25% yield of DAPG should actually be 20.5% (Ranjaneyulu et al., 1987). Mani et al. (1991) made the same type of error, such that the reported yield of 85% should be 51%.

Our chemical synthesis using MAPG as the starting material with basic (K_2CO_3) treatment and silica column chromatography separation produces only DAPG as the final product (Figure 6). However, the crystals of this extract are slightly yellow in color and, after recrystallization (benzene/petroleum ether), gave pure white DAPG crystals. This reaction is a simple one-step reaction at low cost yielding high quantities of DAPG with no *O*-acetylated side products as contaminants. Purity was demonstrated by a melting point of 173 °C, elemental analysis, photodiode array, mass spectrometry, FTIR, and both ^1H and ^{13}C NMR. Furthermore, by elemental and thermal gravimetric analyses it was determined that there were no degrees of hydration associated with synthetic DAPG.

Biological production of DAPG (Aino et al., 1995; Nowak-Thompson et al., 1994) is slow and results in very low yields (120 mg/L in 2 days with a maximum of 1.1 g/L in 15 days). Moreover, the need of a multistep purification process involving extractions and column chromatography with further repetitive separations by HPLC makes obtaining even small quantities of DAPG tedious. Compared to the biological production of DAPG, the chemical synthesis described in this paper is more efficient, quicker, and less costly with a 90% yield.

DAPG is an antibiotic known to be active against a broad spectrum of microorganisms and is involved in the suppression of many plant diseases. It is particularly important in the suppression of take-all disease of wheat (*G. graminis* var. *tritici*) by both naturally occurring and introduced strains of *P. fluorescens*. Take-all is considered by agriculturists to be among wheat's most damaging root diseases, causing millions of dollars in losses annually in U.S. wheat production alone. In infested fields, crop yields drop 10−50%, but the diseases can "take-all" of the crop.

Traditionally, take-all has been controlled by a combination of crop rotation and tillage. However, modern wheat farming practices, including direct seeding and growing several wheat crops before a break, exacerbate take-all. There are no varieties of wheat with resistance to take-all, and methods of chemical control are ineffective. A natural suppression of take-all, known as take-all decline, will develop in the soil following extended wheat or barley monoculture; however, few growers have been able to make use of this biocontrol measure because they cannot sustain the severe disease losses that occur until decline develops. In nature, wheat roots in take-all suppressive soils become colonized by DAPG-producing bacteria, which produce small amounts of the antibiotic in the rhizosphere and inhibit the pathogen.

This paper provides a means of synthesizing large quantities of DAPG quickly with scale-up possibilities. The chemical synthesis avoids the long and tedious extractions and purifications that are associated with biologically produced samples. The availability of pure, synthetic DAPG provides a standard reference for positive identification of DAPG from microorganisms and for calculating the amounts of the antibiotic naturally present in the rhizosphere of plants. It also facilitates studies concerning the stability of the antibiotic in soils from soil-borne pathogens and in research on DAPG analogues concerning biosynthetic pathway elucidation. Perhaps most important, the availability of synthetic DAPG makes it possible to assess the activity of this natural product as seed and foliar treatments against plant diseases in the field.

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