A Cyanoallyl Glucoside from *Alliaria petiolata*, as a Feeding Deterrent for Larvae of *Pieris napi oleracea*

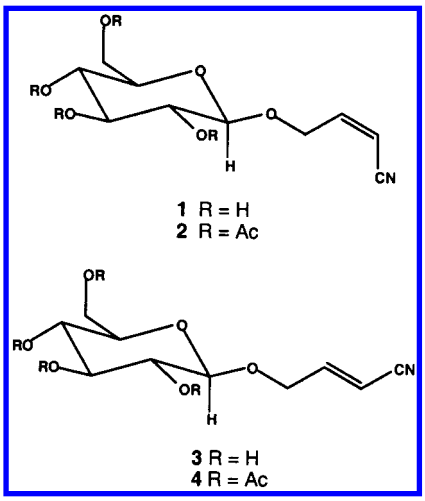
Meena Haribal,*,† Zhicai Yang,‡ Athula B. Attygalle,‡ J. Alan A. Renwick,† and Jerrold Meinwald‡

Boyce Thompson Institute, Tower Road, Ithaca, New York 14853, and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853

Received November 17, 2000

Alliarinoside, a feeding inhibitor against early instar larvae of *Pieris napi oleracea*, was isolated from the foliage of *Alliaria petiolata* and characterized as (2*Z*)-4-(*β*-D-glucopyranosyloxy)-2-butenenitrile (**1**) by spectroscopic methods. The structural assignment was confirmed by synthesis of peracetylated alliarinoside (**2**) and its 2*E* isomer (**3**). A sample of synthetic **1** was isolated by preparative HPLC from the hydrolysis of the 2*Z* acetate. Feeding inhibition assays showed comparable activity for the synthetic and natural glycosides.

Alliaria petiolata Cavara & Grande (Cruciferae) (garlic mustard), an introduced weed from Europe, is rapidly replacing many native species in the flora of the northeastern United States.[1] From extracts of the leaves of this plant, we reported previously an apigenin derivative, isovitexin-6″-*O*-*β*-D-pyranoglucoside, as a feeding deterrent to fourth instar larvae of *Pieris napi oleracea*.[2] In addition to this activity, water and *n*-butanol extracts of foliage were found to inhibit feeding of early instars of *P. napi oleracea* (Renwick et al. unpublished). An HPLC separation of these extracts, in conjunction with bioassays of the fractions, revealed the presence of an additional feeding inhibitor, which we designate as alliarinoside. We now describe the identification and synthesis of this compound, (2*Z*)-4-(*β*-D-glucopyranosyloxy)-2-butenenitrile (**1**).



Results and Discussion

Alliarinoside (**1**) was isolated by repeated semipreparative HPLC of an aqueous extract of young rosette leaves of *A. petiolata*. Choice bioassays of this compound with neonates of *P. napi oleracea* confirmed the activity of this compound as a feeding inhibitor (Table 1, Supporting Information). Compound **1** was highly water-soluble and formed a hygroscopic, gummy, and transparent material upon drying under vacuum. Its IR spectrum (neat) showed absorptions at 3300 (OHs), 2260 and 2130 (CN), 1680 (unsaturation) 1630, 855, 790, 750, and 720 cm^{-1}. On the basis of positive ion accurate-mass spectroscopy, the molecular formula of alliarinoside was determined to be $C_{10}H_{15}O_6N$.

^{13}C NMR and DEPT spectra of **1a** showed the presence of two methylene, six methine, and two quaternary carbons. The signals at δ 5.73 (1H, d, J = 11.2 Hz) and 6.76 (1H, ddd, J = 11.2, 6.0 and 6.4 Hz) in the ^1H NMR spectrum suggested the presence of an olefinic moiety. The multiplet signal at δ 6.76 correlated to the proton signals at δ 4.35 (1H, dd, J = 6.4 and 14 Hz) and 4.49 (1H, dd, J = 6 and 14 Hz) of methylene protons. The signals at δ 4.20, 2.98, 3.16, 3.13, 3.05, 3.40, and 3.60 together with signals at δ 4.92, 4.97, and 5.07, which collapsed to produce broad singlets at δ 4.75, 5.10, and 5.23 on addition of D_2O, were assigned to a hexose moiety. The coupling constant of 8 Hz for the anomeric proton at δ 4.20 suggested that the hexose is an α-substituted sugar. The signal at δ 116.6 in the ^{13}C NMR spectrum due to a quaternary carbon further complemented the IR spectral evidence for the presence of a nitrile function. The HMBC spectrum showed the presence of two isolated spin systems, one of which could be assigned to a hexose moiety and the other to an allylic group with a terminal nitrile functional group. Thus, on the basis of the NMR spectra, we concluded that **1** was a cyanoallyl glycoside.

The positive-ion ESIMS showed a quasi-molecular ion $(M + H)^+$ at *m*/*z* 246. Other significant fragment ions were observed at *m*/*z* 214 $(M + H - 32)^+$ [a loss of CH_3OH from the primary alcohol moiety], 163 $((M + H - 83)^+$ [loss of aglycone], and 84 $(M + H - 162)^+$ [loss of dehydrohexose].

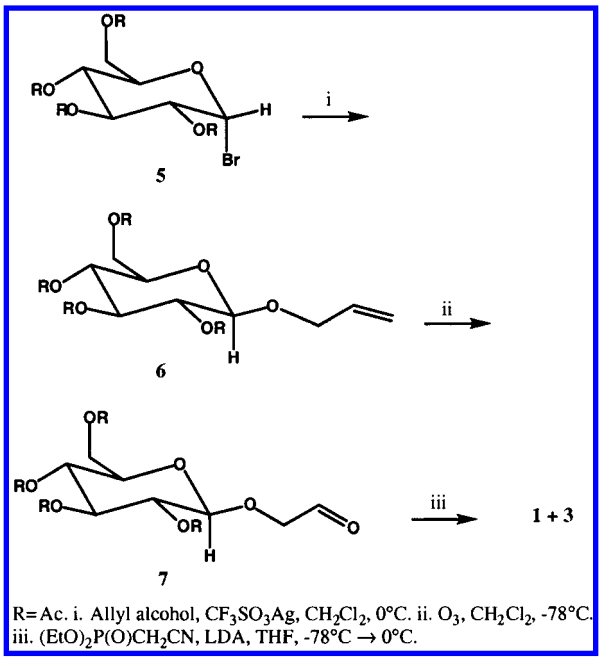
Alliarinoside (**1**) was acetylated with $(Ac)_2O$-pyridine to give a peracetylated derivative (**2**). The positive-ion ESIMS of the derivative showed a quasi-molecular ion $(M + H)^+$ at *m*/*z* 414, indicating the presence of four free OH groups in **1**. This was further confirmed by the presence of four singlets at δ 2.12, 2.06, 2.04, and 2.01 for the $COCH_3$ groups in the ^1H NMR spectrum of **2**.

Compound **1** was hydrolyzed with 1 M HCl, and the products were partitioned between aqueous and organic solvents. The aqueous layer gave a hexose sugar along with other compounds. The hexose was identified as glucose by comparing the GC retention time of its TMS derivative with that of the derivative of authentic glucose. Subsequent

* To whom correspondence should be addressed. Tel: 607-254-5414. Fax: 607-254-2598. E-mail: mmh3@cornell.edu.
† Boyce Thompson Institute.
‡ Cornell University.

10.1021/np000534d CCC: $20.00
Published on Web 03/21/2001

Scheme 1



R=Ac. i. Allyl alcohol, CF_3SO_3Ag, CH_2Cl_2, 0°C. ii. O_3, CH_2Cl_2, -78°C. iii. $(EtO)_2P(O)CH_2CN$, LDA, THF, -78°C → 0°C.

NMR spectrometry confirmed this conclusion. As expected, the organic layer did not yield any appreciable amount of cyanoallyl alcohol as the aglycone.

Typical coupling constant values for *cis* protons in 1,2-disubstituted double bonds are around 10 Hz (the range is from 6 to 12), whereas those for *trans* are around 17 Hz (but range from 12 to 18). The observed value of 11.2 Hz for olefinic protons did not allow for unambiguous assignment of the configuration. Therefore, we undertook chemical synthesis of both *cis* and *trans* isomers.

Both *cis*-allyl nitrile **2** and *trans*-allyl nitrile **4** tetraacetates were synthesized as described in Scheme 1. Condensation of tetra-*O*-acetyl glucosyl bromide **5** with allyl alcohol in the presence of silver triflate provided the β-glucoside tetracetate **6** in good yield.[3,4] Ozonolysis of the allyl alcohol **6** afforded the aldehyde **7** in excellent yield. A Horner−Emmons reaction of the aldehyde **7** with diethyl cyanomethylenephosphonate yielded both *cis*- and *trans*-allyl nitriles **2** and **4** in the ratio of 3:2.[5] The ^{1}H NMR spectrum revealed coupling constants $J_{2,3} = 11.2$ Hz for **2** and $J_{2,3} = 16$ Hz for **4**, and both MS and NMR data of **2** were congruent with those of the 2,3,4,6-tetra-*O*-acetyl-alliarinoside. Deacetylation with K_2CO_3 in dry MeOH at room temperature produced at least two major compounds, and one of these products showed an HPLC retention time identical to that of **1**. The ESIMS of this product was indistinguishable from that of the natural **1**. This compound (**1**) was isolated by repeated HPLC and tested for its activity by a choice bioassay. The activity of the synthetic **1** was comparable to that of natural **1** (Table 1, Supporting Information). The ^{1}H NMR spectrum of the *trans* peracetylated synthetic compound (**4**) was very similar to that of the *cis* compound, except for the peaks of *trans* olefinic protons, which were shifted downfield by about 0.15 ppm. In fact, ^{1}H NMR analysis of **1** also showed the presence of 2−3% of the *trans* compound. However, we were not able to determine whether the *E* isomer is natural or an artifact formed during the isolation process.

The presence of cyanogenic glycosides (cyanohydrins) in many plant families is well established.[6] In addition, a few noncyanogenic glycosides formed from aglycones with nongeminal hydroxy and nitrile groups are also known from plants and animals. For example, such glycosides have been reported from a variety of plant families including Celastraceae,[7] Crassulaceae,[8] Sapindaceae,[9] Euphorbiaceae,[10] Gramineae,[11] etc. Recently a related aglycone has been reported from Cruciferae.[12] Noncyanogenic glycosides have also been reported from many unpalatable insects, some of which feed on the above plant families. These compounds are known to have a range of biological activities to herbivores such as deterrency,[7−9] and phytotoxicity;[12] thus they may play a role in plant defense. Specialist herbivores[7−9] and organisms such as powdery mildews[12] have utilized these chemicals for their own defense by sequestering them.

Experimental Section

General Experimental Procedures. GC−MS analysis was conducted using a capillary column (DB-5, 30 m × 0.23 mm) installed in a HP5890 GC linked to a Hewlett-Packard 5970 mass selective detector. UV spectra were recorded on a Perkin-Elmer Lambda 5 UV/vis spectrophotometer. IR spectra were recorded on a Perkin-Elmer 297 infrared spectrophotometer. ^{1}H NMR spectra were recorded at 399.99 MHz, and ^{13}C NMR at 25.59 MHz in DMSO-d_6 or in acetone-d_6 unless otherwise stated, on a INOVA 400 Varian instrument. 2D NMR spectra were recorded on a 499.99 MHz INOVA 500 Varian instrument using standard software. ESI mass spectra were recorded on a Micromass (Manchester, UK) Quattro 1 instrument, and the samples were introduced by a direct infusion method. HPLC was performed on a Varian Vista 5500 (Limmerick, Ireland) instrument, attached to a HP 1040A diode array detector (Hewlett-Packard, Palo Alto, CA) monitored at 218 and 254 nm. Melting points were uncorrected. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter with a 1 dm cell. High-resolution mass spectra were recorded on a Micromass Autospec instrument operated in the positive-ion ESI mode. The positive-ion ESI MS−MS were recorded on a VG Quattro instrument. Tetra-*O*-acetyl-α-glucosyl bromide was purchased from Sigma. TRI-SIL 'Z' was purchased from Pierce Chemical Company (Rockford, IL).

Plant Material. Seedlings of *A. petiolata* were collected from Eastern Heights area in Ithaca (Tompkins County, NY) in November 1996 and were grown in a greenhouse under controlled conditions (16:8:L:D photoperiod and RH of 65%).

Extraction and Isolation. Young rosette leaves (50 g) of *A. petiolata* were extracted with 200 mL of 95% boiling EtOH. The resulting mixture was homogenized, and the insoluble plant material was removed by filtration. The extract was concentrated to 50 mL, diluted with an equal amount of H_2O, and vortexed. The insoluble material was removed by centrifugation, and the supernatant was evaporated to dryness, redissolved in water, and diluted to 50 mL. Compound **1** was collected from the fraction that eluted as a single peak at 11.2 min by HPLC on a reversed-phase column (C_{18} Bondex 10, 5 μm; 300 × 7.0 mm, Phenomenex, Torrance, CA), using a water−acetonitrile gradient at a flow rate of 2 mL/min (0−5 min 0% acetonitrile; 25% acetonitrile at 40 min; 100% acetonitrile at 50 min) monitored by a diode-array detector at 218 and 254 nm.

Alliarinoside [(2*Z*)-4-(*β*-D-glucopyranosyloxy)-2-butenenitrile] (1): colorless gummy material; UV (MeOH) λ_{max} 218 nm; IR (as neat and KBR pellet) ν_{max} at 3300, 2260, 2130, 1680, 1630, 855, 790, 750, and 720 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.76 (1H, ddd, $J = 11.2$, 6.0, 6.4 Hz, H-3), 5.73 (1H, d, $J = 11.2$ Hz, H-2), 4.49 (1H, dd, $J = 6.0$, 14.0 Hz, H-4a), 4.35 (1H, dd, $J = 6.4$, 14.0 Hz, H-4b), 4.20 (1H, d, $J = 8.0$ Hz, H-1′), 3.60 (1H, d, $J = 11.8$ Hz, H-6b′), 3.40 (1H, dd, $J = 11.8$, 6.0 Hz, H-6a′), 3.16 (1H, dd, $J = 6.8$, 16.4 Hz, H-3′), 3.13 (1H, m, H-4′), 3.05 (1H, m, H-5′) 2.98 (1H, dd, $J = 8.0$, 16.4 Hz, H-2′); ^{13}C NMR (DMSO-d_6) δ 151.8 (C-3), 116.6 (C-1), 103.0 (C-1′), 100.9 (C-2), 77.4 (C-4′), 76.8 (C-3′), 73.6 (C-2′), 70.0 (C-5′), 67.3 (C-4a), 67.3 (C-4b), 61.3 (C-6a), 61.3 (C-6b); positive-ion ESIMS *m/z*

246 $(M + H)^+$, 214, 168, 145, 84 $(M + H - 162)^+$; HRESIMS $(M + Na)^+$, *m/z* 268.0807; calcd for $C_{10}H_{15}O_6NNa$ 268.0797.

Acid Hydrolysis of Alliarinoside (1). Alliarinoside (5 mg) was heated with 1 M HCl (2 mL) at 80 °C for 3 h. The reaction mixture was extracted with 6 mL (3 × 2 times) of $CHCl_3$ and EtOAc each. The aqueous solution was evaporated to dryness under reduced pressure and used for preparation of a TMS derivative. The TMS derivative was prepared by adding 1 mL of TRI-SIL 'Z' to the residue and heating the mixture at 60 °C for 20 min. The reaction mixture was directly injected into GC. TMS glucose was confirmed by comparison with an authentic material prepared in similar a manner. Another batch of compound (1 mg) was hydrolyzed in the same way, and the aqueous fraction was directly injected into the mass spectrometer for analysis of hydrolysis products.

Peracetylated Alliarinoside (2). Alliarinoside (5 mg) was heated in Ac_2O−pyridine (1:1, 3 mL) at 60 °C for 4 h. The reaction mixture was worked up in the usual way to obtain **2** as a colorless crystalline compound: UV (MeOH) λ_{max} 218 nm; ^{1}H NMR ($CDCl_3$) δ 6.58 (1H, ddd, J = 11.2, 6.8, 5.6 Hz, H-3), 5.48 (1H, dd, J = 11.2, 1.6 Hz, H-2), 5.22 (1H, dd, J = 9.6, 9.6 Hz, H-3′), 5.10 (1H, dd, J = 9.6, 9.6 Hz, H-4′), 5.02 (1H, dd, J = 9.6, 8.0 Hz, H-2′), 4.59 (1H, ddd, J = 14.4, 5.6, 1.6 Hz, H-4a), 4.57 (1H, d, J = 8.0 Hz, H-1′), 4.53 (1H, dd, J = 14.4, 6.8 Hz, H-4b), 4.26 (1H, dd, J = 12.4, 4.8 Hz, H-6a′), 4.17 (1H, dd, J = 12.4, 2.4 Hz, H-6b′), 3.73 (1H, ddd, J = 9.6, 4.8, 2.4 Hz, H-5′), 2.12, 2.06, 2.04, 2.01 (3H, s each, CO*CH*$_3$); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.4, 170.1, 169.7 (*C*O), 150.5 (C-3), 116 (C-1), 102.1 (C-1′), 100.9 (C-2), 73.5 (C-2′), 72.6 (C-3′), 72.0 (C-4′), 69.4 (C-4a), 69.4 (C-4b), 67.9 (C-5′), 62.7 (C-6a′), 62.7 (C-6b′), 20.7, 20.7, 20.6, 20.6 (CO*CH*$_3$); positive-ion ESIMS *m/z* 414 $(M + H)^+$, 383 $(M + H - 31)^+$, 372 $(M + H - 42)^+$, 84 $(M + H - 330)^+$.

1-*O*-Allyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (6). To a mixture of tetra-*O*-acetyl-α-glucosyl bromide (**5**) (206 mg, 0.5 mmol), allyl alcohol (44 mg, 0.75 mmol), and 4 Å molecular sieves (150 mg) in CH_2Cl_2 (5 mL) was added silver triflate (193 mg, 0.75 mmol) at 0 °C. The reaction mixture was stirred vigorously at 0 °C for 40 min, and the mixture was filtered through a pad of silica gel. The filtrate was washed with saturated $NaHCO_3$ and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc−hexanes, 1:2) to afford the glucoside **6** (106 mg, 55%) as colorless prisms, mp 86−88 °C, $[\alpha]_D$ −22.6° (*c*, 1.8, $CHCl_3$): ^{1}H NMR ($CDCl_3$) δ 5.81 (1H, dddd, J = 17.2, 10.4, 6.4, 4.8 Hz), 5.24 (1H, ddd, J = 17.2, 3.2, 1.6 Hz), 5.17 (1H, ddd, J = 10.4, 3.2, 1.6 Hz), 5.16 (1H, d, J = 9.6 Hz), 5.06 (1H, t, J = 9.6 Hz), 4.99 (1H, dd, J = 9.6, 8.0 Hz), 4.53 (1H, d, J = 8.0 Hz), 4.30 (1H, ddt, J = 13.2, 4.8, 1.6 Hz), 4.23 (1H, dd, J = 12.4, 4.8 Hz), 4.11 (1H, dd, J = 12.4, 2.4 Hz), 4.06 (1H, tt, 6.4, 1.6 Hz), 3.66 (1H, ddd, J = 9.6, 4.8, 2.4 Hz), 2.05 (3H, s), 2.01 (3H, s), 1.99 (3H, s), 1.97 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.3, 169.4, 169.3, 133.3, 117.6, 99.6, 72.9, 71.8, 71.3, 70.0, 68.4, 61.9, 20.7, 20.7, 20.6, 20.6.

2-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)ethanal (7). The olefin **6** (24 mg, 0.062 mmol) in CH_2Cl_2 (2 mL) was cooled to −78 °C, and ozone was bubbled into the solution at −78 °C until the solution appeared blue. The reaction mixture was purged with nitrogen as it warmed to room temperature. Dimethyl sulfide (20 μL) was added, and the reaction mixture was stirred at room temperature overnight. After washing with water (2 mL) and brine (2 mL), the solution was dried ($MgSO_4$), and the solvent was removed in vacuo. The residue was purified by chromatography (EtOAc−hexanes, 1:1) to afford aldehyde **7** (20 mg, 83%) as colorless oil, $[\alpha]_D$ −23.5° (*c*, 0.7, $CHCl_3$): ^{1}H NMR ($CDCl_3$) δ 9.68 (1H, dd, J = 1.6, 0.8 Hz), 5.24 (1H, t, J = 9.6 Hz), 5.10 (1H, dd, J = 9.6, 8.0 Hz), 5.09 (1H, t, J = 9.6 Hz), 4.60 (1H, d, J = 8.0 Hz), 4.28 (1H, dd, J = 17.2, 0.8 Hz), 4.25 (1H, dd, J = 12.4, 5.2 Hz), 4.19 (1H, dd, J = 17.2, 1.6 Hz), 4.12 (1H, dd, J = 12.4, 2.4 Hz), 3.72 (1H, ddd, J = 9.6, 5.2, 2.4 Hz), 2.09 (3H, s), 2.09 (3H, s), 2.04 (3H, s), 2.02 (3H, s); ^{13}C NMR (in $CDCl_3$) δ 199.8, 170.6, 170.2, 160.4, 160.3, 100.9, 74.1, 72.5, 72.1, 70.9, 68.3, 61.3, 20.7, 20.7, 20.6, 20.6.

2,3,4,6-Tetra-*O*-acetylalliarinoside and Its *trans*-Isomer (2 and 4). To a solution of diethyl cynomethylphosphonate (100 mg, 0.564 mmol) in THF (15 mL) was added LDA (1.5 M, 0.38 mL) at −78 °C. The reaction mixture was stirred at −78 °C for 1 h, and then the aldehyde **7** (220 mg, 0.564 mmol) in THF (5 mL) was added at −78 °C. The reaction mixture was stirred, slowly warmed to 0 °C, and quenched with saturated aqueous NH_4Cl. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (5 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc−hexanes, 2:1) to yield the *cis*-alkenyl nitrile **2** (118 mg, 51%) as colorless prisms, mp 84−86 °C, $[\alpha]_D$ −4° (*c*, 0.4, $CHCl_3$): ESIMS 414 $(M + H^+)$, 331, 271, 169, 113, 98, 87, 79, 73, 60; HRCIMS *m/z* 414.1390 (calcd for $C_{18}H_{24}N_1O_{10}$ 414.1400); ^{1}H NMR and ^{13}C NMR identical to that of **2** obtained from natural **1**. Compound **2** was formed together with the *trans*-alkenyl nitrile **4** (80 mg, 34%) as colorless needles, mp 86−88 °C, $[\alpha]_D$ −23.1° (*c*, 0.3, $CHCl_3$): ^{1}H NMR ($CDCl_3$) δ 6.71 (1H, dt, J = 16.0, 4.0 Hz, H-3), 5.63 (1H, dt, J = 16.0, 2.4 Hz, H-2), 5.23 (1H, dd, J = 9.6, 9.6 Hz, H-3′), 5.10 (1H, dd, J = 9.6, 9.6 Hz, H-4′), 5.06 (1H, dd, J = 9.6, 8.0 Hz, H-2′), 4.56 (1H, d, J = 8.0 Hz, H-1′), 4.52 (1H, ddd, J = 16.8, 4.0, 2.4 Hz, H-4a), 4.26 (1H, dd, J = 12.4, 4.8 Hz, H-6a′), 4.23 (1H, ddd, J = 16.8, 4.0, 2.4 Hz, H-4b), 4.16 (1H, dd, J = 12.4, 2.4 Hz, H-6b′), 3.72 (1H, ddd, 9.6, 4.8, 2.4 Hz, H-5′), 2.10, 2.07, 2.04, 2.03 (s 3H each CO*CH*$_3$); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.4, 169.5, 169.4 (*C*O), 149.0 (C-3), 117.1 (C-1), 100.5 (C-1′), 100.4 (C-2), 72.7 (C-3′), 72.3 (C-2′), 71.2 (C-4′), 68.4 (C-4a), 68.4 (C-4b), 67.5 (C-5′), 61.9 (C-6a′), 61.9 (C-6b′), 20.9, 20.9, 20.8, 20.8 (CO*CH*$_3$); ESIMS 414 $(M + H^+)$, 331, 271, 169, 113, 98, 87, 79, 73, 60; HRCIMS *m/z* 414.1405 (calcd for $C_{18}H_{24}N_1O_{10}$ 414.1400).

Hydrolysis of 2 and 4. The acetylated compound (**2**, 5−6 mg) was dissolved in 0.5 mL of dry MeOH, and anhydrous K_2CO_3 was added. The mixture was stirred at room temperature for 4 h. The reaction was monitored by ESIMS to detect the formation of **1**. The resultant mixture was filtered through glass wool and separated by repeated HPLC to give **1**.

Bioassays. Choice bioassays for feeding deterrent/inhibitor activity were conducted with neonate larvae of *P. napi oleracea*. Two 1.5 cm disks of cabbage leaves were used as test substrate. One disk was treated with the sample to be tested, and the other was treated with solvent alone. Five to eight replicates were used for each test. In the case of the natural compound 0.1 g leaf equivalent of sample dissolved in 20 μL of MeOH was applied on both sides of each test disk. For the synthetic compound, although the amount was not verified, a sample of UV absorbance similar to that for the natural compound was used (to ensure comparable amount of natural and synthetic alliarinoside). Two disks were mounted on insect pins that were affixed to the bottom of waxed paper cups, 10 cm wide and 5 cm deep, lined with moistened filter paper. The pins were placed in a way that there was a slight overlap of two disks to enable neonate larvae to move from one disk to the other. Eggs from *P. napi oleracea* were collected on a Parafilm layer so that the hatching larvae had no contact with the host plants. Either four or five freshly emerged neonates were transferred to the disks. The cups were covered with an airtight lid and placed in a larger box lined with moistened paper napkins to keep the humidity high to prevent the disks from desiccating. This box was kept in an incubator for 18 h at 28 °C. Activity was expressed as numerical values based on the area that was consumed. This was done visually as follows: 0 = no feeding; 1 = <5% of the disk; 2 = >5% and <10%; and 3 = >10%. Ranks were assigned to both control and treated disks.

Statistical Analyses. Ranks assigned to disks were compared by Wilcoxon's nonparametric sign rank test for paired samples (Siegel and Castallen).[13]

Acknowledgment. This work was supported by NSF grant no. DEB-9419797 to Dr. Alan Renwick and NIH grant no. GM53830 to Dr. Jerrold Meinwald. We thank Dr. Bruce Ganem for the use of the Perkin-Elmer IR spectrophotometer.

Supporting Information Available: Table of feeding deterrent/inhibition activity of natural and synthetic **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Nuzzo, V. In *Biological Pollution: Control and Impact of Invasive Exotic Species:* Proceedings of a symposium held at the University Place Conference Center; Indiana University-Purdue University at Indianapolis on October 25 & 26, 1991; McKnight B. N., Ed.; Indiana Academy of Sciences, 1993; pp 137−141.
(2) Haribal, M.; Renwick, J. A. A. *Phytochemistry* **1998**, *48*, 1237−1240.
(3) Ueda, M.; Sawai, Y.; Yamamura, S. *Tetrahedron Lett*. **1999**, *40*, 3757−3760.
(4) Shigemori, H.; Miyoshi, E.; Shizuri, Y.; Yamamura, S. *Tetrahedron Lett*. **1989**, *30*, 6389−6392.
(5) Zhang, T. Y.; O'Toole, J. C.; Dunigan, J. M. *Tetrahedron Lett*. **1998**, *39*, 1461−1464.
(6) Nahrstedt, A. In *Biologically Active Natural Products*; Annual Proceedings of the Phytochemical Society of Europe; Hostettmann, K., Lea, P. J., Eds.; Clarendon Press: Oxford, New York, 1987; Vol. 27, pp 213−234.
(7) Nishida, R.; Rothschild, M.; Mummery, R. *Phytochemistry* **1994**, *36*, 37−38.
(8) Nishida, R.; Rothschild, M. *Experientia* **1995**, *51*, 267−269.
(9) Braekman, J. C.; Daloze, D.; Pasteels, J. M. *Biochem. Syst. Ecol*. **1982**, *10*, 355−364.
(10) Van DenBerg, A. J. J.; Horsten, S. F. A. J.; Van-Den, K.; Jantina, B. J.; Kroes, B. H.; Labadie, R. P. *Phytochemistry* **1995**, *40*, 597−598
(11) Pourmohseni H.; Ibenthal, W. D.; Machinek, R.; Remberg, G.; Wray, V. *Phytochemistry* **1993**, *33*, 295−297.
(12) Vaughn, S. F.; Berhow, M. A. *J. Chem. Ecol.* **1998**, *24*, 1117−1126.
(13) Siegel, S.; Castellan, N. J., Jr. *Nonparamatric Statistics for the Behavioral Sciences*, 2nd ed.; McGraw-Hill: New York, 1988; pp 87−89.

NP000534D