Carbohydrate Research 394 (2014) 13–16

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

Synthesis of the allelochemical alliarinoside present in garlic mustard (*Alliaria petiolata*), an invasive plant species in North America

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ARTICLE INFO

Article history:
Received 14 January 2014
Received in revised form 2 May 2014
Accepted 10 May 2014
Available online 17 May 2014

Keywords:
Allyl β-D-glucopyranoside tetra-acetate
Epoxidation
Trimethylsilyl cyanide
α,β-Unsaturated nitriles
Deprotection

ABSTRACT

The allelochemical alliarinoside present in garlic mustard (*Alliaria petiolata*), an invasive plant species in North America, was chemically synthesized using an efficient and practical synthetic strategy based on a simple reaction sequence. Commercially available 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose was converted into prop-2-enyl 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranoside and subjected to epoxidation. In a one-pot reaction, ring-opening of the epoxide using TMSCN under solvent free conditions followed by treatment of the formed trimethylsilyloxy nitrile with pyridine and phosphoryl chloride, afforded the acetylated β-unsaturated nitriles (*Z*)-4-(2′,3′,4′,6′-tetra-*O*-β-D-glucopyranosyloxy)but-2-enenitrile and its isomer (*E*)-4-(2′,3′,4′,6′-tetra-*O*-β-D-glucopyranosyloxy)but-2-enenitrile. Deacetylation of *Z*- and/or *E*-isomers afforded the target molecules alliarinoside and its isomer.

Plants produce a large number of allellochemicals that when liberated in the environment affect the growth, survival, and reproduction of other organisms to the plants own benefit.[1,2] They may exert their effects, for example, by preventing seeds from other plant species to germinate or by inhibiting the growth of other plants.[3] Allellochemical production may thus offer a plant species unique opportunities to obtain higher abundance and to spread to different types of plant communities. Many of these allellochemicals are produced in low amounts or as exudates from roots and thus difficult to isolate in amounts required for studies of the effect of the specific allellochemical in different biological systems. In many of such cases chemical synthesis of the allellochemical constitutes the obvious alternative.

In this study we report an easy and cheap route to the synthesis of alliarinoside (**5**) and its l stereoisomer **6**. Alliarinoside is an allellochemical produced by garlic mustard (*Alliaria petiolata*).[4] It was initially isolated from the foliage of garlic mustard[5] and has not been described to be present in any other plant species. Garlic mustard is a common native plant at most continents and one of the oldest known herbs in Europe selected based on its mild garlic and mustard flavor.[6] For the very same reason garlic mustard was introduced into North America in the 1860s. Unfortunately it has now spread as an invasive species on most of the North American continent where it is established as a predominant plant in numerous different environments.[7] Mustard garlic produces allellochemicals that prevent the growth of the mycorrhizal fungi species that facilitate the ability of other plant species to take-up phosphorous ions from the soil.[8,9] This enables garlic mustard to slow down the growth of native plants. Likewise, the insect herbivores feeding on garlic mustard in its original native environments are not present in North America which further adds to its property as an invasive species.

Garlic mustard belongs to the mustard family, Brassicaceae.[4] The main class of secondary metabolites produced by the mustard family is the glucosinolates and garlic mustard produces glucosinolates derived from homomethionine, dihomomethionine, phenylalanine, homophenylalanine, and tryptophan.[10,11] 2-Propenyl glucosinolate (sinigrin) derived from homomethionine is the most dominant glucosinolate in aerial tissues of garlic mustard and is the glucosinolate showing the closest structural similarity to alliarinoside. However, alliarinoside is not a glucosinolate and belongs to the related class of compounds denoted as hydroxynitrile glycosides.[12] Except for *Carica papaya*,[13] other glucosinolate producing plants are not known to produce hydroxynitrile glucosides. Hydroxynitrile glucosides are widespread in the plant kingdom

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http://dx.doi.org/10.1016/j.carres.2014.05.006
0008-6215/

with cyanogenic glucosides (α-hydroxynitrile glucosides) being present in pteridophytes, gymnosperms and angiosperms as well as in a few arthropods.[12,14,15] Alliarinoside belongs to the γ-hydroxynitriles which together with β-hydroxynitriles typically co-occur with cyanogenic glucosides but in a restricted number of plant species.[12,16]

The only previously reported chemical synthesis of **5** and **6** is the small scale synthesis by Haribal and co-worker,[5] based on ozonolysis of the allyl glucoside **2** to produce the corresponding aldehyde which in a Horner–Emmons reaction with diethylcyanomethylenephosphonate afforded the protected glucoside **4a** and **4b** which provided **5** and **6** after deprotection. However, our need for alliarinoside (**5**) and its stereoisomer **6** for use in large scale biological experiments prompted us to develop an easier synthetic approach. The synthetic strategy is outlined in Scheme 1.

Commercially available 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**1**) was converted into prop-2-enyl 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranoside (**2**) using $ZnCl_2$ as described by Yuasa and Yuasa[17] as the most convenient procedure among several alternative options.[17–20] Epoxidation of **2** was accomplished using *m*-chloroperbenzoic acid (*m*-CPBA) according to reported procedures[21–23] except for use of 2 equiv of *m*-CPBA instead of 3 equiv[22] and reflux in $C_2H_2Cl_2$ instead of $CHCl_3$ or CH_2Cl_2 for 30 min to obtain (2*R*)- and (2*S*)-2,3-epoxypropyl 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranoside (**3**) in near 100% yield and an epimeric ratio of 3:2. In a one-pot reaction, ring-opening of epoxide **3** using TMSCN under solvent free conditions[24] followed by treatment of the formed trimethylsilyloxy nitrile with pyridine and phosphoryl chloride[25] afforded the acetylated α,β-unsaturated nitriles (*Z*)-4-(2′,3′,4′,6′-tetra-*O*-β-D-glucopyranosyloxy)but-2-enenitrile (**4a**), (33% yield) and, (*E*)-4-(2′,3′,4′,6′-tetra-*O*-β-D-glucopyranosyloxy)but-2-enenitrile (**4b**), (43% yield) which were separated using chromatography on silica in a total yield of 77% (*Z*/*E* = 3:4). Deprotection of **4a** and/or **4b** with triflic acid in MeOH afforded the target molecules **5** and **6** after chromatographic purification on silica gel. The overall yield of **5** and **6** was 14% and 19%, respectively, calculated from **1**.

All the synthesized compounds were characterized and their identity verified using electrospray ionization mass spectrometry and ^1H and ^{13}C NMR. Typical coupling constant values for *cis* protons in 1,2-disubstituted double bonds are known to be around 10 Hz (the range is from 6 to 12), whereas those for *trans* protons in 1,2-disubstituted double bonds are around 17 Hz (the range from 12 to 18). The observed value of 11.1 and 11.4 Hz for the olefinic protons of **4a** and **5**, respectively did not allow for unambiguous assignment of their *cis*-configuration. However, the observed value of 16.4 Hz for the olefinic protons of **4b** and **6** confirmed the assignment of their *trans*-configuration and thus the *cis*-configuration of the olefinic protons in **4a** and **5** as previously shown.[5]

In conclusion, an efficient, practical, and stereospecific synthesis of alliarinoside **5** and its isomer **6** was developed starting from β-D-glucopyranose pentaacetate via a simple reaction sequence (allyl glucosidation, epoxidation, one-pot α,β-unsaturated nitrile formation and deacetylation). The method offers a straightforward route to the synthesis and isolation of alliarinoside (**5**) and its stereoisomer **6**.

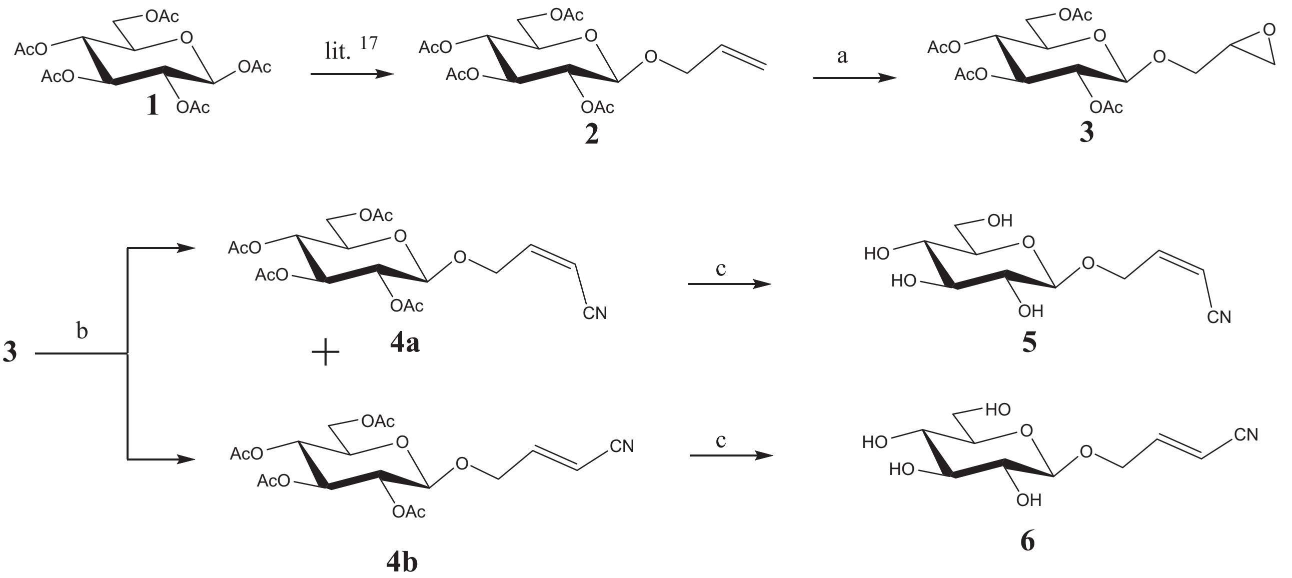
1. Experimental

1.1. General methods

Melting points were determined using a Mettler FP81 MBC Cell connected to a Mettler FP80 Central processor unit. Optical rotations were measured at 21 ± 2 °C with an Optical Activity Ltd AA-1000 Polarimeter. All reactions were monitored by TLC on aluminum sheets coated with silica gel 60F254 (0.2 mm thickness, E. Merck, Darmstadt, Germany) and the components present were detected by charring with 10% H_2SO_4 in MeOH. Column chromatographies were carried out using silica gel 60 (particle size 0.040–0.063 mm, 230–400 mesh ASTM, E. Merck). Solvent extracts were dried with anhyd $MgSO_4$ unless otherwise specified. The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 and 101 MHz, respectively. δ-Values are relative to internal TMS; coupling constants (*J*) are given in Hz. HR-ESI-MS spectra were recorded on a Bruker micrOTOF-Q mass spectrometer.

1.2. Prop-2-enyl 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranoside (2)[17]

β-D-Glucose pentaacetate **1** (15.0 g, 38.4 mmol), allyl alcohol (10.0 mL, 117.1 mmol), and $ZnCl_2$ (5.3 g, 38.9 mmol) in toluene (120.0 mL) were reacted as previously described.[17] The product



Scheme 1. Reagents and conditions: (a) *m*-CPBA, DCE, 85 °C, 1 h, quant.; (b) TMS-CN, $LiClO_4$, $3H_2O$, 85 °C 1 h, Py/$POCl_3$, 85 °C, 2 h, 77% yield; (c) TfOH, MeOH, rt, 3 h, Amberlyst-A26 (OH^- form), 85% yield.