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MEGOURAPHIN GLUCOSIDES: TWO YELLOWISH PIGMENTS FROM THE APHID *MEGOURA CRASSICAUDA*

Mitsuyo Horikawa,[a,*] Daisuke Kikuchi,[a] Toshihito Imai,[a] Masami Tanaka,[a] Hiroto Kaku,[a] Takeshi Nishii,[a] Makoto Inai,[a] Shigeru Takahashi,[b] and Tetsuto Tsunoda [a,*]

[a]Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan. [b]Faculty of Agriculture, Utsunomiya University, Utsunomiya 321-8505, Japan. E-mail: horikawa@ph.bunri-u.ac.jp or tsunoda@ph.bunri-u.ac.jp

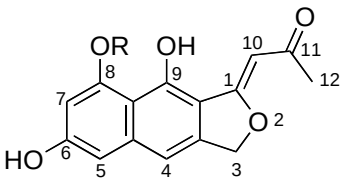
Abstract – Two new yellow pigments, megouraphin glucosides A (**1**) and B (**2**), were isolated from the aphid *Megoura crassicauda*. Their structures were established by detailed analysis of their 1D and 2D NMR spectra and via chemical conversion.

INTRODUCTION

Aphids produce novel pigments, such as the protoaphins,[1-7] furanaphin,[8] and the uroleuconaphines,[9,10] viridaphin A_1 glucoside,[11] which may possess interesting biological activities such as cytotoxicity.[8,9,11,12] The presence of pigments is also important for expressing aphid body color, and it is presumed that subtle differences body coloration (color polymorphism) affect predator-prey interactions.[13] Therefore, the unique structures and potentially important biological activities of aphid pigments are of interest.

As the first step, we have been studying the chemical structures of pigments in aphids.[8-11] In the present manuscript, we describe our studies of the aphid *Megoura crassicauda* (Figure 1)[14] and the isolation of two fluorescent yellow pigments named megouraphin glucosides A (**1**) and B (**2**) (Figure 2). Their chemical structures are described in detail.

Figure 1. *Megoura crassicauda*



1 : R = β-D-glucopyranosyl
2 : R = 6'-*O*-acetyl β-D-glucopyranosyl

Figure 2. Structures of megouraphin glucosides (**1** and **2**)

RESULTS AND DISCUSSION

The green aphid *Megoura crassicauda* was observed feeding on *Vicia sativa*, in Tokushima Prefecture, Japan. The aphids were removed from the plant with a soft paintbrush and collected in a plastic Erlenmeyer flask equipped with a plastic funnel. In the laboratory the aphids were crushed and washed repeatedly with a mixture of hexane and MeOH. The MeOH-soluble portion was separated and evaporated under reduced pressure. Two fluorescent yellowish pigments, compounds **1** and **2**, were isolated by repeated chromatographic purification using Sephadex LH-20, silica gel, and preparative TLC.

The more polar pigment, **1**, was obtained as yellow powder, mp 171 °C (decomp). Its molecular formula was established as $C_{21}H_{22}O_{10}$ by FAB-HRMS (*m*/*z* 435.1275, $[M+H]^+$; Δ -1.6 mmu). The IR spectrum of **1** indicated the presence of a hydroxy group (3357 cm^{-1}, br) and a conjugated ketone (1650 cm^{-1}). The ^1H NMR spectrum of **1** in DMSO-d_6 (Table 1) showed characteristic signals for an acetyl methyl group at δ_H 2.31 (3H, s), methylene protons at δ_H 5.57 (2H, s), an olefinic proton at δ_H 6.17 (1H, s) and three aromatic protons at δ_H 6.85 (1H, d, J = 2.1), 7.00 (1H, d, J = 2.1), and 7.19 (1H, s). Furthermore, the presence of a sugar moiety was suggested by the observation of seven protons at δ_H 3.26, 3.37, 3.42, 3.45, 3.57, 3.79, and 5.10. The ^{13}C NMR spectrum of **1** displayed 13 sp^2-carbon signals and 8 sp^3-carbon signals. The heteronuclear multiple quantum coherence (HMQC) spectrum (Table 1) revealed the presence of an oxygen-bearing methylene carbon (δ_H/δ_C 5.57/75.0) and four aromatic and/or olefinic methine carbons [(δ_H/δ_C 6.17/100.6), (δ_H/δ_C 7.00/104.1), (δ_H/δ_C 6.85/104.7), and (δ_H/δ_C 7.19/108.9)]. The combination of a carbon signal at δ_C 31.0 with the proton signal at δ_H 2.31 (3H, s) was assigned to an acetyl methyl group. Furthermore, the presence of the sugar moiety was confirmed by the observation of 6 carbon signals (δ_C 60.6, 69.6, 73.4, 76.2, 77.8, and 103.0). Analysis of the heteronuclear multiple bond coherence (HMBC) and NOESY spectra led to the proposed structure **1** and enabled the complete assignment of the ^1H and ^{13}C NMR spectra (Table 1). Figure 3 shows the structure of pigment **1** along with the ^{13}C-^1H long-range correlations identified through analysis of the HMBC and NOESY spectra.

At this stage, the geometry of the olefinic bond could not be determined and the structure of the sugar moiety could not be identified. Therefore, **1** was methylated using diazomethane to afford dimethyl ether,

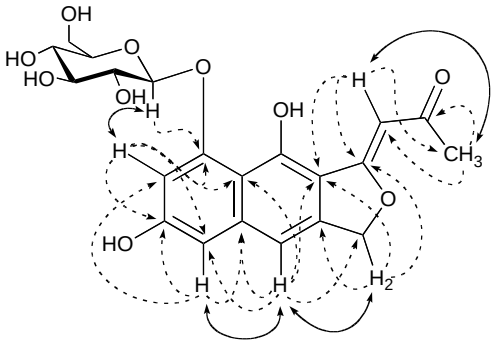
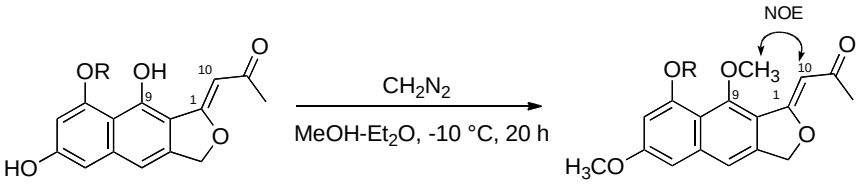


Figure 3. Selected HMBC (dotted line) and key NOESY (solid line) correlations for compound **1**

Table 1. NMR spectroscopic data (600 MHz) for compounds **1** and **2**

	1 in DMSO-d_6		**2** in methanol-d_4	
position	δ_C	δ_H	δ_C	δ_H
1	166.0		170.4	
3	75.0	5.57 (2H, s)	76.6	5.53 (2H, s)
3a	140.3		141.8	
4	108.9	7.19 (1H, s)	110.0	7.09 (1H, s)
4a	139.6		141.8	
5	104.7	6.85 (1H, d, 2.1)	106.2	6.80 (1H, d, 2.1)
6	157.8		159.5	
7	104.1	7.00 (1H, d, 2.1)	105.5	6.98 (1H, d, 2.1)
8	156.6		158.2	
8a	108.9		110.8	
9	152.5		155.0	
9a	111.3		112.9	
10	100.6	6.17 (1H, s)	101.4	6.38 (1H, s)
11	195.3		200.9	
12	31.0	2.31 (3H, s)	30.9	2.40 (3H, s)
1'	103.0	5.10 (1H, d, 8.0)	104.2	5.11 (1H, d, 8.0)
2'	73.4	3.42 (1H, dd, 8.8, 8.0)	74.8	3.61 (1H, dd, 9.1, 8.0)
3'	76.2	3.37 (1H, ddd, 9.6, 8.8, 5.2)	78.0	3.53 (1H, dd, 9.1, 8.8)
4'	69.6	3.26 (1H, ddd, 9.8, 9.6, 5.5)	71.6	3.44 (1H, dd, 9.7, 8.8)
5'	77.8	3.45 (1H, ddd, 9.8, 6.0, 1.9)	76.1	3.77 (1H, ddd, 9.7, 6.9, 2.2)
6'	60.6	3.57 (1H, ddd, 11.8, 6.0, 5.8)	64.6	4.31 (1H, dd, 11.8, 6.9)
		3.79 (1H, ddd, 11.8, 5.8, 1.9)		4.50 (1H, dd, 11.8, 2.2)
6'-OAc (C=O)	——		172.8	
6'-OAc (CH_3)	——		20.7	2.13 (3H, s)
2'-OH		5.96 (1H, br s)		
3'-OH		5.26 (1H, d, 5.2)		
4'-OH		5.17 (1H, d, 5.5)		
6'-OH		4.70 (1H, t, 5.8)		
Other OH		10.34 (1H, br s)		
		10.28 (1H, br s)		

3, for which an NOE correlation between the hydrogen at the C-10 position and the methoxy protons at the C-9 position revealed a double bond with *Z* geometry (Scheme 1). Next, **1** was hydrolyzed under acidic conditions to afford a sugar that was identified as glucose by TLC using (3-aminopropyl)triethoxysilane-treated silica-gel 60 with a developing solution comprising $CH_3CN/MeOH/H_2O$ (7:2:1, three developments). The resulting sugar was acetylated with acetic anhydride in pyridine and then purified. The isolated pentaacetate was identified as D-glucose by comparison with the optical rotation ($[\alpha]_D^{20}$) of the pentaacetate of standard L-glucose.



1: R = β-D-glucopyranosyl **3** : R = β-D-glucopyranosyl

Scheme 1. Methylation of compound **1**

The less polar pigment **2** was also isolated as yellow crystals, mp 147 °C (decomp). Its molecular formula was established as $C_{23}H_{24}O_{11}$ by FAB-HRMS (*m/z* 477.1420 $[M+H]^+$; Δ +2.3 mmu). The data of ^1H and ^{13}C NMR spectra of **2** were listed in Table 1 comparing with those of **1**. HMBC correlations of methylene protons (δ_H 4.31 and 4.50) at C-6′ to acetyl carbonyl carbon (δ_C 172.8) suggested the presence of an acetyl group at C-6′ position. Furthermore, since the methylation of **2** using diazomethane gave compound **3**,[15] the structure of sugar moiety of **2** was determined to be β-D-glucopyranose as compound **1**.

Thus, the structures of megouraphin glucosides A (**1**) and B (**2**) were determined. Further work on the biological activities of compounds **1** and **2**, and structural determination of other interesting aphid pigments are in progress.

EXPERIMENTAL

General. Melting points were determined on a Yanaco MP-3 apparatus and are uncorrected. Optical rotations were obtained on JASCO DIP-1000 and P-1030 polarimeters. UV-visible spectra were measured on a Shimadzu UV-1650pc spectrophotometer. IR spectra were measured on a JASCO FT/IR-410 spectrophotometer. ^1H NMR spectra were recorded on a Varian Unity-600 (600 MHz) NMR spectrometer with TMS as an internal standard in solvent. ^{13}C NMR spectra were recorded on a Varian Unity-600 (150 MHz) NMR spectrometer; chemical shifts were referenced to the residual solvent signal (DMSO-d_6: δ_C 39.5, methanol-d_4: δ_C 49.0). Signal multiplicities were established with DEPT experiments.

Mass spectra including HRMS were recorded on a JEOL JMS-700 spectrophotometer. For column chromatography, silica gel (Kanto Chemical Co., Inc., 60N 63-210 μm) and Sephadex^{TM} LH-20 (Amersham Biosciences) were used. For TLC analysis, Merck precoated silica gel plates (60F and RP-18 WF_{254S}) was used. Acetic anhydride and pyridine were purchased from Nacalai Tesque Inc. Pyridine was used after distillation from CaH_2. Diazomethane was prepared from *N*-nitrosomethylurea.

Material. The aphid *Megoura crassicauda* was collected as they fed on *Vicia sativa* in Tokushima Prefecture, Japan, in June 2011.

Extraction and Isolation. The aphids (21 g) were crushed in hexane and MeOH several times. The combined MeOH solutions were evaporated under reduced to give crude extracts (554 mg). The extracts were subjected to repeated chromatographic purification over Sephadex LH-20 (MeOH), silica gel ($CHCl_3$/MeOH = 5:1-2:1), and preparative TLC to afford the fluorescent yellow pigment **1** (7.3 mg) and pigment **2** (1.4 mg). Same experiments were repeated to obtain more pigments **1** and **2**.

Megouraphin Glucoside A (1): a yellow solid, mp 171 °C (decomp); $[\alpha]_D^{20}$ -136.3 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 237 (4.27), 291 (4.38), 304 (4.42), 373 (4.24) nm; IR (ATR) ν_{max} 3357 (-OH), 1650 (C=O), 1604, 1367, 1263, 1018 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) data provided in Table 1; FAB-MS *m/z* 435 ([M+H]$^+$); FAB-HRMS *m/z* 435.1275 (calcd for $C_{21}H_{23}O_{10}$, 435.1291).

Megouraphin Glucoside B (2): a yellow solid, mp 147 °C (decomp); $[\alpha]_D^{20}$ -65.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 238 (4.19), 278 (4.10), 292 (4.26), 305 (4.29), 350 (4.06), 374 (4.11) nm; IR (ATR) ν_{max} 3358 (-OH), 1734 (C=O), 1650 (C=O), 1602, 1457, 1373 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data provided in Table 1; FAB-MS *m/z* 477 ([M+H]$^+$); FAB-HRMS *m/z* 477.1420 (calcd for $C_{23}H_{25}O_{11}$ 477.1397).

Methylation of Compound 1. A suspension of **1** (4.8 mg) in MeOH (1.5 mL) was treated with a diazomethane-diethyl ether solution. The resulting mixture was stirred at -10 °C for 20 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (3.0 g, $CHCl_3$/MeOH = 10:1) to give 3.7 mg of the ether **3** as a yellow amorphous solid; $[\alpha]_D^{21}$ -65.7 (*c* 0.12, MeOH); IR (ATR) ν_{max} 3373, 1581 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) δ 2.46 (3H, s, H-12), 3.46 (1H, dd, *J* = 9.6, 9.1 Hz, H-4′), 3.52-3.55 (2H, m, H-3′ and 5′), 3.70 (1H, dd, *J* = 9.3, 7.7 Hz, H-2′), 3.73 (1H, dd, *J* = 12.2, 5.6 Hz, H-6′), 3.91 (3H, s, 6-OC*H*$_3$), 3.93 (1H, dd, *J* = 12.2, 2.2 Hz, H-6′), 3.96 (3H, s, 9-OC*H*$_3$), 5.14 (1H, d, *J* = 7.7 Hz, H-1′), 5.60 (2H, s, H-3), 6.36 (1H, s, H-10), 6.97 (1H, d, *J* = 2.2 Hz, H-7), 6.98 (1H, d, *J* = 2.2 Hz, H-5), 7.57 (1H, s, H-4); ^{13}C NMR (CD_3OD, 150 MHz) δ 31.2 (C-12), 56.1 (6-O*C*H$_3$), 62.5 (C-6′), 63.3 (9-O*C*H$_3$), 71.3 (C-4′), 75.1 (C-2′), 76.5 (C-3), 78.1(C-3′ or 5′), 78.5 (C-3' or 5′), 101.8 (C-10), 102.4 (C-5), 102.7 (C-1′), 105.1 (C-7), 116.5 (C-4), 117.1 (C-8a), 121.2 (C-9a), 141.3 (C-3a), 142.5 (C-4a), 156.6 (C-9), 156.9 (C-8), 161.5 (C-6), 168.9 (C-1), 201.1 (C-11); FAB-MS *m/z* 463

([M+H]$^+$); FAB-HRMS *m/z* 463.1578 (calcd for $C_{23}H_{27}O_{10}$ 463.1604).

Methylation of Compound 2. A suspension of **2** (4.8 mg) in MeOH (1.0 mL) was treated with a diazomethane-diethyl ether solution. The resulting mixture was stirred at -10 °C for 16 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (4.5 g, $CHCl_3$/MeOH = 12:1) to give 2.4 mg of the ether **3** as a yellow amorphous solid; $[\alpha]_D^{21}$ -58.1 (*c* 0.20, MeOH). The data of IR, ^{1}H NMR, ^{13}C NMR, FAB-HRMS were the same with those of the compound derived from **1**.

Hydrolysis of Megouraphin Glucoside A (1) and Determination of the Structure of the Resulting Sugar. Compound **1** (4.4 mg) was heated in a mixture of 0.5 M H_2SO_4 (2 mL) and dioxane (2 mL) at 100 °C for 1.5 h. After cooling, the reaction mixture was neutralized with $Ba(OH)_2$ and a white precipitate was filtered off. The filtrate was evaporated in vacuo and analyzed by TLC using (3-aminopropyl)triethoxysilane-treated silica gel 60 (MeCN/MeOH/H_2O = 7:2:1, three developments). The R_f value (0.18) of the sample was identical to that of standard glucose. Next, a pyridine (1 mL) solution of the resulting sugar was treated with 500 μL of acetic anhydride at ambient temperature for 24 h. After addition of 2 M HCl (4 mL), the mixture was extracted with CH_2Cl_2 (3 mL × 2) and the organic layers were dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by silica gel column chromatography (2 g, hexane/EtOAc = 5:1–3:1–1:1) to give 2.9 mg of the pentaacetate of the sugar as a white powder with an $[\alpha]_D^{20}$ +42.2 (*c* 0.20, $CHCl_3$) {pentaacetate of standard L-glucose, $[\alpha]_D^{22}$ -43.8 (*c* 2.1, $CHCl_3$)}. This finding suggested that compound **1** contained D-glucose.

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