Tetrahedron 64 (2008) 5515–5518

Contents lists available at ScienceDirect

Tetrahedron

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

Uroleuconaphins A_{2a}, A_{2b}, B_{2a}, and B_{2b}: four yellowish pigments from the aphid *Uroleucon nigrotuberculatum* (Olive)

Mitsuyo Horikawa *, Masami Tanaka, Hiroto Kaku, Takeshi Nishii, Tetsuto Tsunoda *

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan

ARTICLE INFO

Article history:
Received 3 March 2008
Received in revised form 24 March 2008
Accepted 27 March 2008
Available online 3 April 2008

Keywords:
Structure determination
Pigment
Uroleuconaphins
Aphid
Uroleucon nigrotuberculatum (Olive)

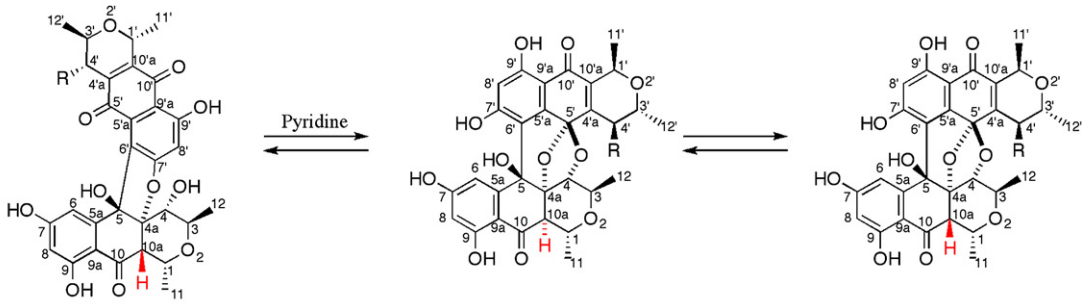
ABSTRACT

Four novel yellowish pigments, uroleuconaphins A_{2a}, A_{2b} (**3a**, **3b**), and B_{2a}, B_{2b} (**4a**, **4b**), were isolated from the aphid *Uroleucon nigrotuberculatum* (Olive). Their structures were established by detailed analyses of 1D and 2D NMR spectra and mechanistic consideration of the interconversion between **3** (or **4**) and uroleuconaphin A_1 (**1**) (or B_1 (**2**)).

1. Introduction

In our previous paper,[1] we reported that the red aphid *Uroleucon nigrotuberculatum* (O.), feeding on *Solidago altissima* L., produced mainly two red pigments, uroleuconaphins A_1 (**1**) and B_1 (**2**), whose structures were determined by a single crystal X-ray analysis. Furthermore, it was revealed that the pigments possessed interesting biological activity, such as cytotoxicity.[1,2] We also isolated a biologically active yellow pigment, furanaphin, from the yellowish aphid *Aphis spiraecola* P.[3] The investigation of aphid pigments is worthwhile due to their unique structures and interesting biological activities.

As part of our continuing efforts toward determination of chemical structures of pigments in aphids, we reinvestigated the aphid *U. nigrotuberculatum* (O.), and isolated four yellow pigments, uroleuconaphins A_{2a}, A_{2b} (**3a**, **3b**), and B_{2a}, B_{2b} (**4a**, **4b**), which are minor components of the aphid (Fig. 1). In this paper, we describe their structural elucidation in detail.



1 (uroleuconaphin A_1): R = H
2 (uroleuconaphin B_1): R = OH

3a (uroleuconaphin A_{2a}): R = H
4a (uroleuconaphin B_{2a}): R = OH

3b (uroleuconaphin A_{2b}): R = H
4b (uroleuconaphin B_{2b}): R = OH

Figure 1. Structures of uroleuconaphins.

* Corresponding authors. Tel.: +81 88 622 9611; fax: +81 88 655 3051.
E-mail addresses: horikawa@ph.bunri-u.ac.jp (M. Horikawa), tsunoda@ph.bunri-u.ac.jp (T. Tsunoda).

0040-4020/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2008.03.100

Table 1
^{13}C NMR (150 MHz) and ^{1}H NMR (600 MHZ) data of **4a** and **3a** in acetone-d_6

Position	**4a**		**3a**	
	δ_C	δ_H	δ_C	δ_H
1	71.1	4.34 (1H, dq, 10.6, 6.2)	71.1	4.36 (1H, dq, 10.6, 5.9)
3	66.8	3.90 (1H, dq, 9.9, 6.2)	66.8	3.88 (1H, dq, 9.9, 5.9)
4	77.8	4.19 (1H, d, 9.9)	77.6	4.22 (1H, d, 9.9)
4a	85.9		85.7	
5	75.0		75.3	
5a	Not assigned		139.2 or 148.1	
6	109.4	6.34 (1H, d, 2.2)	109.5	6.35 (1H, d, 2.2)
7	166.2		166.2	
8	103.4	6.32 (1H, d, 2.2)	103.3	6.31 (1H, d, 2.2)
9	167.6		167.6	
9a	108.8		108.7	
10	199.3		199.0	
10a	53.8	3.33 (1H, d, 10.6)	54.2	3.10 (1H, d, 10.6)
11	23.0	1.55 (3H, d, 6.2)	23.0	1.55 (3H, d, 5.9)
12	18.9	1.19 (3H, d, 6.2)	18.9	1.20 (3H, d, 5.9)
9-OH		13.15 (1H, s)		13.15 (1H, s)
1′	67.7	4.58 (1H, qd, 6.8, 1.1)	68.0	4.66 (1H, qd, 6.6, 1.8)
3′	68.7	3.81 (1H, dq, 8.1, 6.2)	62.6	3.95 (1H, m)
4′	68.0	4.12 (1H, ddd, 8.1, 7.3, 1.1)	30.4	2.08 (1H, ddd, 19.4, 10.3, 1.8) 2.48 (1H, dd, 19.4, 3.3)
4′-OH		4.05 (1H, br d, 7.3)		—
4′a	Not assigned		146.2	
5′	99.2		98.3	
5′a	Not assigned		139.2 or 148.1	
6′	113.7		114.2	
7′	164.6		165.0	
8′	104.5	6.55 (1H, s)	104.6	6.52 (1H, s)
9′	164.9		165.0	
9′a	106.6		106.4	
10′	187.8		186.8	
10′a	142.0		139.7	
11′	19.4	1.55 (3H, d, 6.8)	19.9	1.49 (3H, d, 6.6)
12′	18.8	1.21 (3H, d, 6.2)	21.8	1.20 (3H, d, 6.2)
9′-OH		12.08 (1H, s)		12.22 (1H, s)
Other OH		5.84 (1H, s)[a]; 9.85 (1H, s)[a]; 10.71 (1H, s)[a]		Not found
	Not assigned carbon: 140.0, 145.4, 147.8			

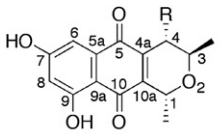
[a] These signals were observed by 300 MHz-NMR.

2. Results and discussion

The pigments, in the form of yellow-hued powders, were obtained as described in the previous paper.[1] Two yellowish spots (R_f=0.23 and 0.16, hexane/AcOEt=1:1) were detected by TLC in addition to the red pigments **1** (R_f=0.39) and **2** (R_f=0.20). Each yellowish substance **3** and **4** was separated by silica gel column chromatography. However, **3** and **4** showed unusual and complex chemical behaviors. For example, yellow substance **4** consisted of a 5:1 mixture of two yellowish compounds **4a** and **4b**, which were equilibrated on silica gel or in a polar solvent, such as CH_3CN/H_2O (approximately 1 day). Furthermore, a small amount of a red compound was also detected under the same conditions. A mixture of **4a** and **4b** was also obtained by thermal treatment of the red pigment **2** at 50 °C in pyridine for 2 h. After considerable effort, pure **4a** was obtained by C18 reversed phase column chromatography using acetonitrile and water as an eluent with a gradient of 1:2–1:1. Unfortunately, however, **4b** could not be purified by any method. The yellowish substance **3**, which consisted of a 3.5:1 mixture of two yellowish compounds **3a** and **3b**, exhibited chemical behavior similar to that of **4**. Thus, treatment of red pigment of **1** in pyridine at 55 °C afforded a mixture of **3a** and **3b**.

The infrared spectrum of **4a**, yellow-hued crystals, mp 156 °C (dec), indicated the presence of hydroxyl (3235 cm^{-1}, br) and carbonyl (1607 and 1623 cm^{-1}) groups. The molecular formula of **4a** was established as $C_{30}H_{28}O_{12}$ by the HR-FABMS (m/z 579.1501, $[M-H]^-$).

In the ^{1}H NMR spectra of the major component uroleuconaphin B_{2a} (**4a**), two hydrogen-bonded O–H signals were observed at 13.15 and 12.08 ppm. The heteronuclear multiple quantum coherence (HMQC) spectra (Table 1) revealed the presence of four methyl groups on carbons bearing oxygen [C 18.8/H 1.21 (3H, d), C 18.9/H 1.19 (3H, d), C 19.4/H 1.55 (3H, d), and C 23.0/H 1.55 (3H, d) ppm], six oxygen-bearing methine carbons [C 66.8/H 3.90 (1H, dq), C 67.7/H 4.58 (1H, qd), C 68.0/H 4.12 (1H, ddd), C 68.7/H 3.81 (1H, dq), C 71.1/H 4.34 (1H, dq), and C 77.8/H 4.19 (1H, d) ppm], three aromatic protons [C 103.4/H 6.32 (1H, d), C 104.5/H 6.55 (1H, s), C 109.4/H 6.34 (1H, d) ppm], and one methine [C 53.8/H 3.33 (1H, d) ppm]. Moreover, the ^{13}C NMR spectra (Table 1) indicated the presence of 3 oxygen-bearing quaternary carbons (C 75.0, 85.9, and 99.2 ppm), 11 quaternary carbons (C 106.6, 108.8, 113.7, 140.0, 142.0, 145.4, 147.8, 164.6, 164.9, 166.2, and 167.6 ppm), and 2 carbonyl carbons (C 187.8 and 199.3 ppm). A signal at 99.2 ppm was assigned to an acetal carbon. These data and the HMBC spectra suggested the presence of two units of quinone A (**5a**) (Fig. 2) moiety in **4a**.



5a (quinone A): R = OH
5b (dehydroxy quinone A): R = H

Figure 2. Structures of quinone A (**5a**) and dehydroxy quinone A (**5b**).

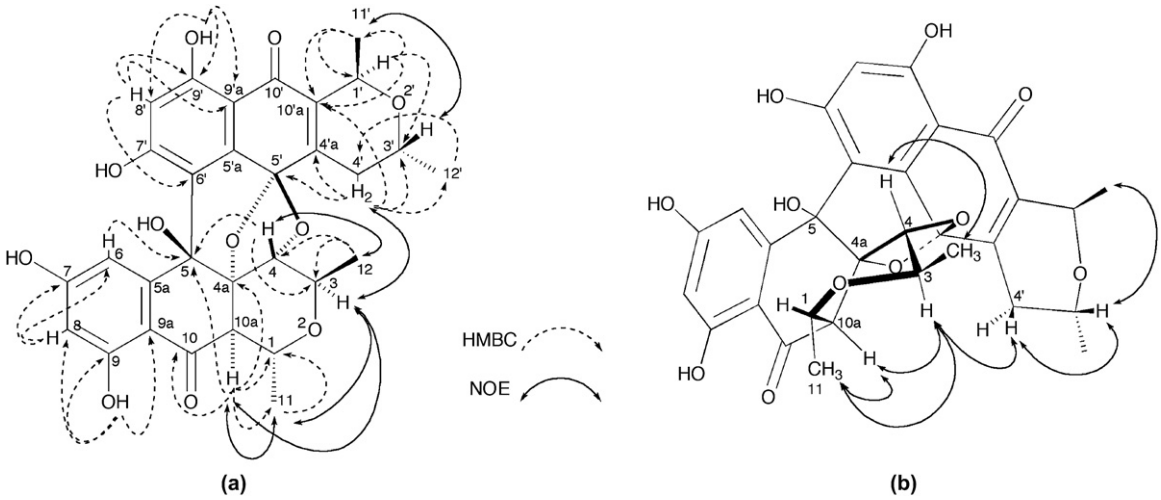


Figure 3. HMBC and NOESY correlations of **3a** in acetone-d_6.

In contrast, the ^{1}H and ^{13}C NMR spectra, HMQC, HMBC, and NOESY spectra of uroleuconaphin A_{2a} (**3a**) were obtained by careful measurement of the 3.5:1 mixture of **3a** and **3b**. The NMR spectra of **3a** strongly resembled those of **4a**, except that the hydroxyl group at the C-4′ position was absent (Table 1). The molecular formula of **3a** was established as $C_{30}H_{28}O_{11}$ by HR-FABMS (*m*/*z* 565.1681, $[M+H]^+$). Thus, yellowish compound **3a** may consist of **5a** and its dehydroxy derivative **5b**, which have the same carbon framework with **4a**. HMBC correlation between the ^{13}C signal at 98.3 ppm (C-5′ position) and the ^{1}H signal at 2.48 ppm (C-4′ position) in **3a** revealed that the C-5 carbonyl carbon of the **5b** unit was converted to the acetal carbon.

In conclusion, two C15 units (**5a** or **5b**) were linked by a 1,3-dioxolane ring system and a C–C bond, which was also present in the carbon framework of uroleuconaphins A_1 (**1**) and B_1 (**2**). The structures of **3a** and **4a** are shown in Figure 1. ^{13}C–^{1}H long-range correlations in HMBC and NOESY spectrum of **3a** are illustrated in Figure 3. Unfortunately, we could not observe evidence of the binding position between the two monomers, such as HMBC correlation between the ^{1}H signal at C-4 position and the ^{13}C signal at C-5′. However, NOESY correlation between the ^{1}H signals at C-3 and C-4′ (see Fig. 3b) supported the structure of **3a**. The stereochemistry at C-10a position of the compounds **3a** was also confirmed by the NOESY spectra of the ^{1}H signals at C-10a, C-11, and C-3 (see Fig. 3b).

All spectral data of minor components **3b** and **4b** suggested that their structures are the epimers of **3a** and **4a**, respectively, at the α-position (C-10a) of the carbonyl group. The correlations between structure and chemical behavior of **1**, **3a**, and **3b** (also **2**, **4a**, and **4b**), are illustrated in Figure 1.

The mechanism of the isomerization of **1** to **3a**, **b** (**2** to **4a**, **b**) is proposed as follows (Scheme 1). Pyridine accelerates the retro-Michael reaction of phenolic ether to afford **6**. The hydroxyl group at the C-4 position attacks the C-5′ carbonyl carbon intramolecularly, then carbonyl oxygen connects to the C-4a carbon via Michael addition to give **3a** and **3b**.

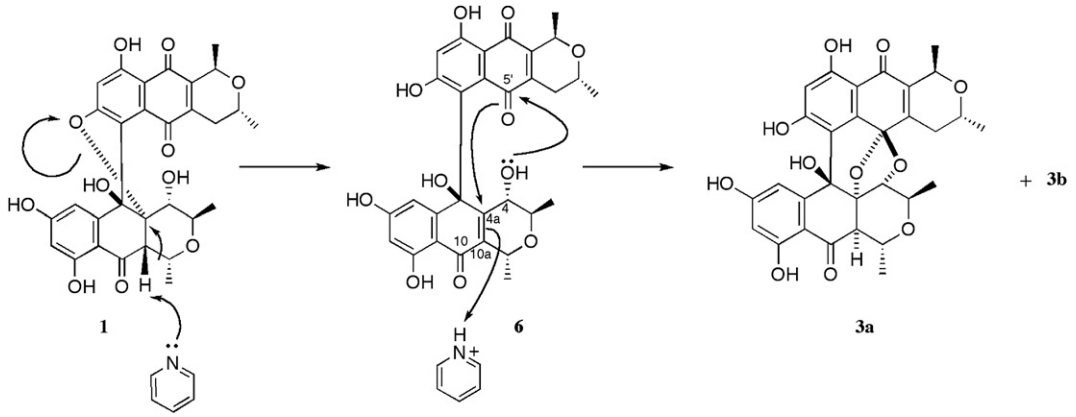
3. Conclusion

In conclusion, the chemical structures and reaction of aphid pigments are of much interest, although we do not know yet the reason for the production of such novel compounds. Further studies of the biological activities of **3** and **4** and structural determination of other interesting aphid pigments are currently in progress.

4. Experimental

4.1. General

Melting points were determined on a Yanaco MP-3 apparatus and were uncorrected. IR spectra were measured on a JASCO FT/IR-410 spectrophotometer. UV–visible spectra were measured on a Shimadzu UV-1650pc spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Unity-600 (600 MHz) and a Varian Mercury-300 (300 MHz) with tetramethylsilane as an internal standard



Scheme 1. Mechanism of isomerization from **1** to **3a**.

in acetone-d_6. ^{13}C NMR spectra were taken on a Varian Unity-600 (150 MHz), chemical shifts were referenced to the residual solvent signal (acetone-d_6: δ_C 29.8 ppm). Signal multiplicities were established by DEPT experiments. Mass spectra including high-resolution mass spectra were recorded on a JOEL JMS-700 spectrophotometer. For column chromatography, silica gel (Kanto Chemical Co. Inc, 60N 63–210 μm), C18 reversed phase silica gel (Nacalai Tesque Inc., Cosmosil 75C_{18} OPN), and Sephadex™ LH-20 (Amersham Biosciences) were used. Merck precoated silica gel 60F and RP-18 WF_{254S} were used for TLC. Pyridine was purchased from Nacalai Tesque Inc. and used without any purification.

4.2. Material

The aphid *U. nigrotuberculatum* (Olive) feeding on *S. altissima* L. was collected in Tokushima Prefecture, Japan in June 1999.

4.3. Extraction and isolation

The aphid (200 g) was squashed in diethyl ether, and then the ethereal supernatant solution was separated by decantation. The residue was washed with several portions of fresh ether. The combined ethereal solutions were dried over Na_2SO_4 and were evaporated to give a crude extract (21.9 g). The reddish residue was subjected to silica gel column chromatography (600 g) using hexane/AcOEt (3:1–1:3) as an eluent to give four fractions. The fraction 2 (3.2 g) was subjected to silica gel column chromatography (150 g) using hexane/AcOEt (4:1–0:1) as an eluent to afford **1** (1.2 g) and a mixture of yellow pigments (780 mg). These yellow pigments were subjected to repeated chromatographic purification over Sephadex LH-20 (MeOH/AcOEt (1:1)) and silica gel (hexane/AcOEt (4:1–0:1)) to afford a mixture (3.5:1) of **3a** and **3b** (210 mg). The fraction 3 (1.3 g) was subjected to silica gel column chromatography (60 g) using hexane/AcOEt (4:1–1:1) as an eluent to afford **2** (755 mg) and a mixture of other yellow pigments (76 mg). These yellow pigments were purified by C18 reversed phase column chromatography eluting with CH_3CN/H_2O (1:2–1:1) to afford **4a** (18 mg) and a mixture of **4a** and **4b**.

4.3.1. A mixture of uroleuconaphins A_{2a} (**3a**) and A_{2b} (**3b**)

Yellow solid. Mp 188 °C (dec). $[\alpha]_D^{21}$ +329.9 (*c* 0.50, CH_3CN). UV (CH_3CN): λ_{max} 233 (log ε 4.32), 289 (log ε 4.16), 327 (log ε 4.03) nm. IR (ATR): ν_{max} 3234 (–OH), 1605, 1622 (C=O) cm^{-1}. MS (FAB) *m*/*z* 565 ($[M+H]^+$). HRMS (FAB) calcd for $C_{30}H_{29}O_{11}$ 565.1710 ($[M+H]^+$), found 565.1681.

4.3.2. Uroleuconaphin B_{2a} (**4a**)

Yellow solid. Mp 156 °C (dec). $[\alpha]_D^{21}$ +311.6 (*c* 0.72, CH_3CN). UV (CH_3CN): λ_{max} 289 (log ε 4.12), 322 (log ε 3.98) nm. IR (ATR): ν_{max} 3480 (–OH), 1607, 1623 (C=O) cm^{-1}. MS (FAB) *m*/*z* 579 ($[M-H]^-$). HRMS (FAB) calcd for $C_{30}H_{27}O_{12}$ 579.1502 ($[M-H]^-$), found 579.1501.

4.3.3. A mixture of uroleuconaphins A_{2a} (**3a**) and A_{2b} (**3b**) from **1**

A solution of **1** (47 mg) in pyridine (3 mL) was stirred at 55 °C for 24 h. The resulting mixture was poured into CH_2Cl_2 and washed with 1 N HCl (15 mL) and water (10 mL). The organic layer was dried over Na_2SO_4. After evaporation of the solvent, the crude residue was purified by silica gel column chromatography (3.5 g, hexane/AcOEt=5:1–1:1) to give 8.4 mg of a mixture of **3a** and **3b** as a yellow solid. ^1H NMR (300 MHz, acetone-d_6) of **3b**: δ 1.23 (3H, d, *J*=6.0 Hz), 1.29 (3H, d, *J*=6.3 Hz), 1.49 (3H, d, *J*=6.9 Hz), 1.58 (3H, d, *J*=6.6 Hz), 2.16 (1H, ddd, *J*=18.9, 10.2, 2.1 Hz), 2.56 (1H, dd, *J*=18.9, 3.6 Hz), 3.86–4.02 (2H, m), 4.24 (1H, d, *J*=5.7 Hz), 4.28 (1H, d, *J*=6.3 Hz), 4.60–4.70 (2H, m), 6.28 (1H, d, *J*=2.0 Hz), 6.48 (1H, d, *J*=2.0 Hz), 6.49 (1H, s), 12.18 (1H, s), 12.80 (1H, s).

4.3.4. A mixture of uroleuconaphins B_{2a} (**4a**) and B_{2b} (**4b**) from **2**

A solution of **2** (39 mg) in pyridine (3 mL) was stirred at 50 °C for 2 h. The resulting mixture was poured into $CHCl_3$ and washed with NH_4Cl aq (30 mL) and water (9 mL). The organic layer was dried over Na_2SO_4. After evaporation of the solvent, the crude residue was purified by silica gel column chromatography (16 g, hexane/acetone=3:1–1:2) and reversed phase silica gel column chromatography (15 g, H_2O/acetonitrile=2:1–1:1) to give 16.8 mg of a mixture of **4a** and **4b** as a yellow solid. ^1H NMR (300 MHz, acetone-d_6) of **4b**: δ 1.24 (3H, d, *J*=6.0 Hz), 1.46 (3H, d, *J*=7.2 Hz), 1.55 (3H, d, *J*=6.6 Hz), 1.55 (3H, d, *J*=6.9 Hz), 3.83 (1H, dq, *J*=7.5, 6.3 Hz), 4.12–4.18 (2H, m), 4.19 (1H, d, *J*=8.1 Hz), 4.43 (1H, d, *J*=3.3 Hz), 4.47 (1H, d, *J*=1.5 Hz), 4.59 (1H, qd, *J*=6.3, 0.6 Hz), 4.71 (1H, dq, *J*=8.1, 6.6 Hz), 6.20 (1H, s), 6.31 (1H, d, *J*=2.3 Hz), 6.36 (1H, d, *J*=2.3 Hz), 6.52 (1H, s), 9.79 (1H, s), 10.57 (1H, s), 12.07 (1H, s), 12.88 (1H, s).

Acknowledgements

We thank Professor Shigeru Takahashi (Utsunomiya University, Japan) for the identification of the aphid. This work was supported partially by a Grant-in-Aid for Scientific Research (C, 19590028) from MEXT (the Ministry of Education, Culture, Sports, Science and Technology of Japan). We are also thankful to MEXT.HAITEKU, 2003–2007.

References and notes

1. Horikawa, M.; Hashimoto, T.; Asakawa, Y.; Takaoka, S.; Tanaka, M.; Kaku, H.; Nishii, T.; Yamaguchi, K.; Masu, H.; Kawase, M.; Suzuki, S.; Sato, M.; Tsunoda, T. *Tetrahedron* **2006**, *62*, 9072.
2. Suzuki, S.; Tomita, M.; Hyodo, M.; Horikawa, M.; Tsunoda, T.; Sato, M. *Biol. Pharm. Bull.* **2006**, *29*, 2383.
3. Horikawa, M.; Noguchi, T.; Takaoka, S.; Kawase, M.; Sato, M.; Tsunoda, T. *Tetrahedron* **2004**, *60*, 1229.