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Isofuranonaphthoquinone derivatives from cultures of the lichen *Arthonia cinnabarina* (DC.) Wallr.

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Abstract

Two isofuranonaphthoquinone derivatives, named arthoniafurones A (1-acetyl-8-hydroxynaphtho[2,3-c]furan-4,9-dione) and B [1-acetyl-4,8-dihydroxynaphtho[2,3-c]furan-9(4*H*)-one], were isolated from a spore-derived culture of the mycobiont of the lichen *Arthonia cinnabarina*, that is new to Japan. Bostrycoidin and 8-*O*-methylbostrycoidin were also identified in the *A. cinnabarina* culture.

Keywords: Lichen; *Arthonia cinnabarina*; Arthoniales; Isofuranonaphthoquinone; Arthoniafurone A; B; Bostrycoidin; 8-*O*-Methylbostrycoidin

1. Introduction

Lichens are symbiotic associations of algal and fungal partners. They contain many characteristic phenols such as depsides, depsidones, dibenzofurans and pulvinates produced by their fungal components (Culberson, 1969). Lichen mycobiont cultures without the algal partner can also synthesize novel and extraordinary constituents under stress conditions such as high osmotic pressure. Examples are cristazarins (*Cladonia cristatella*) (Yamamoto et al., 1996), graphislactones (*Graphis scripta* var. *pulverulenta*) (Tanahashi et al., 1997), graphenone (*Graphis scripta*) (Miyagawa et al., 1994), and graphisquinone (*Graphis desquamescens*) (Miyagawa et al., 1994), and dibenzofurans [(*Evernia esorediosa* (Miyagawa et al., 1993), *Stereocaulon japonicum* (Miyagawa et al., 1997) and *Usnea orientalis* (Kon et al., 1997)]. In the course of our search for new bioactive compounds from cultures and natural thalli of lichens, we successfully cultured the mycobiont of *Arthonia cinnabarina* (DC.) Wallr. derived from its spores. We report herein the isolation and identification of four compounds produced by this mycobiont, two of which were previously unknown.

2. Results and discussion

A. cinnabarina is a lichen belonging to the Arthoniales. It has a whitish crustose thallus with dark brown apothecia. The apothecium has a cinnabar-coloured pruina. The species is widely distributed in Europe and North America, and is new to Japan. Several more or less brightly red pigments are found in certain species in the Arthoniales. The structures of most of these compounds are still unknown, but chiodectonic acid, parietin, rhodocladonic acid and skyrin have been isolated (Grube, 1998; Thor, 1990) The genus *Arthonia* Ach. is currently one of the largest genera of crustose lichens, comprising more than 500 species (Grube et al., 1995), and reddish K^+ reacting ascomatal pigments are currently known from several *Arthonia* species (Grube and Matzer, 1997). The pigments are chemically different, as noted by differences in TLC run lengths and K^+

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reactions, and they vary both in color (yellow, orange, or red) and consistency (Grube and Matzer, 1997). Until now, however, none of the pigments have been identified.

We isolated the mycobiont culture from spores discharged from apothecia of *A. cinnabarina* collected in Japan in 1995. The mycobiont was cultured on malt-yeast extract agar-medium for 10 months, then extracted with $CHCl_3$ and subjected to silica gel chromatography to yield two yellow pigments **1** and **2**, and two red pigments **3** and **4** (Fig. 1).

The yellow pigment (**1**) had a molecular formula $C_{14}H_8O_5$ as determined by HR-EIMS and confirmed by ^{13}C NMR and DEPT analyses. The IR spectrum of **1** indicated the presence of hydroxyl (3450 cm^{-1}) and carbonyl (1690, 1640 cm^{-1}) groups. The ^1H NMR spectrum showed a methyl resonance at δ 2.75 (*s*), four aromatic protons at δ 7.38 (*dd*, $J=8.0$ and 1.0 Hz), 7.69 (*dd*, $J=8.0$ and 1.0 Hz) , 7.81 (*dd*, $J=8.0$, 8.0 Hz) and 8.96 (*s*), and a hydrogen-bonded hydroxy proton at δ 12.45 (*s*). The ^{13}C NMR spectra revealed 14 carbon signals, which were assigned by DEPT as one methyl, four methine and nine quaternary carbons including two carbonyl carbons. In HMQC and ^2J and ^3J HMBC experiments, correlations between all protons and carbons were elucidated (Fig. 2). The hydroxy proton signal at δ 12.45 (*s*) suggested the presence of a hydrogen bond with a carbonyl group at either C-4 or C-9, but the position of the hydroxyl group could not be determined from these data. The yellow pigment (**2**), however, was slowly converted into **1** over a few days in DMSO-d_6. Since the structure of **2** was determined as described below, the hydroxy group of **1** was assigned to C-8. Substance **1** was thus determined to be a new isofuranonaphthoquinone, 1-acetyl-8-hydroxynaphtho[2,3-c]furan-4,9-dione, and named arthoniafurone A.

The yellow pigment **2** had a molecular formula $C_{14}H_{10}O_5$ as determined by HR-EIMS and confirmed by ^{13}C NMR and DEPT analyses. The IR spectrum of 2 indicated the presence of hydroxyl (3410 cm^{-1}) and carbonyl (1650 and 1630 cm^{-1}) groups. The ^1H NMR spectrum showed a methyl at δ 2.71 (*s*), four aromatic protons at δ 6.97 (*dd*, $J=7.9$ and 0.9 Hz), 7.27 (*dt*, $J=7.9$ and 0.9 Hz) , 7.66 (*dd*, $J=7.9$, 7.9 Hz) and 8.26 (*d*, $J=1.2$ Hz), one methine proton at δ 5.72 (*brs*) and a hydrogen-bonded hydroxy proton at δ 12.47 (*s*). The ^{13}C NMR spectra gave 14 carbon signals, which were assigned by DEPT to one methyl, four methine and nine quaternary carbons including one carbonyl carbon. The methine proton at δ 5.72 (*brs*) was correlated with the hydroxyl-bearing carbon at δ 60.2 in the HMQC spectrum. From these data, **2** has one secondary hydroxyl group in place of one carbonyl group on comparison with **1**. In HMQC and ^2J and ^3J HMBC experiments, the correlation between all protons and carbons were elucidated as shown in Figs. 2 and 3. Moreover in the NOE difference spectra, enhancement of the methine protons at δ 7.27 (*dt*, $J=7.9$ and 0.9 Hz) and 8.26 (*d*, $J=1.2$ Hz) were observed by irradiation of the methine proton at δ 5.72 (*brs*) (Fig. 3). The combination of these data assigned the hydroxyl group to position at C-4. Accordingly, the structure of **2** was established as 1-acetyl-4,8-dihydroxynaphtho[2,3-c]furan-9(4*H*)-one and named arthoniafurone B. Although **2** was optically active with $[\alpha]_D$ -14.6°, the stereochemistry at C-4 was not determined.

Naturally occurring isofuranonaphthoquinones previously reported are nectriafurone from cultures of the non-lichenized pyrenomycetes *Nectria haematocca* (Parisot et al., 1983) and *Fusarium oxysporum* (Tatum et al., 1987) and ventilones from the root bark of plants *Ventilago goughii* (Jammula et al., 1991), *V. maderaspatana* (Hanumaiah et al., 1985) and *V. vitiensis* (Ali et al., 1994). Since both nectriafurone and bostrycoidin display antimicrobial activity (Baker et al., 1990), it should be investigated whether arthoniafurones also have such properties. Arthoniafurones are the first isofuranonaphthoquinone derivatives from lichens. Furanonaphthoquinone derivatives such as chiodectonic and rhodocladonic acids were previously isolated as red pigments (Huneck and Yohsimura, 1996), but on the whole few naphthoquinones have been isolated from lichens (Culberson, 1969).

EIMS analysis proved that the red pigment (**3**) had a nitrogen atom in the molecule because its molecular ion was at *m/z* 285. Pigment **3** had a molecular formula $C_{15}H_{11}NO_5$ as determined by HR-EIMS analysis and confirmed by ^{13}C NMR and DEPT analyses. The IR

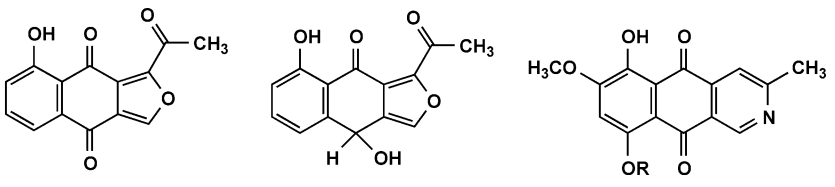

Fig. 1. Structures of isolated compounds **1**–**4**.

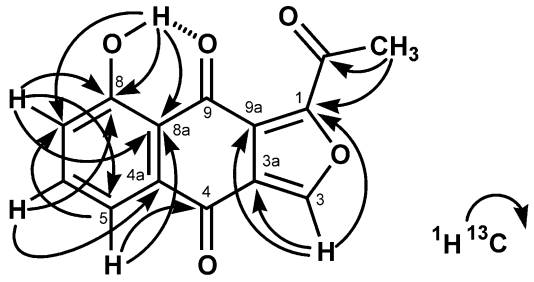

Fig. 2. HMBC of arthoniafurone A (**1**).

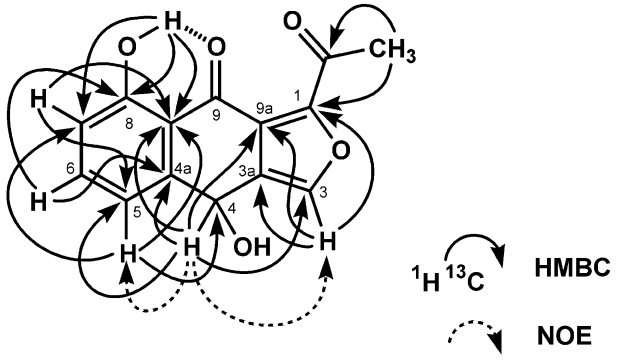

Fig. 3. HMBC and NOE of arthoniafurone B (**2**).

spectrum of **3** exhibited a hydroxyl (3400 cm^{-1}) and a carbonyl (1620 cm^{-1}) group. The ^{1}H NMR spectrum showed a methyl at δ 2.80 (*s*), an *O*-methyl at δ 4.02 (*s*) and two hydrogen-bonded hydroxy protons at δ 13.20 (*s*) and 13.49 (*s*), respectively. The ^{13}C NMR spectra revealed 15 carbon signals, which were identified by DEPT as two methyls (including an *O*-methyl), three methine and 10 quaternary carbons. In ^{2}J and ^{3}J HMBC experiments, the correlations between all protons and carbons were assigned. The hydroxyl protons at δ 13.20 (*s*) and 13.49 (*s*) suggested the presence of hydrogen bonds with the carbonyl groups at C-9 and C-10. Moreover, in the NOE difference spectra, enhancement of the methine protons at δ 7.96 was observed by irradiation of methyl protons at δ 2.80 (*s*). These data suggested that the red pigment was bostrycoidin; this had been previously isolated from cultures of the non-lichenized pyrenomycet *Fusarium bostrycoide* (Cajori et al., 1954), *F. solani* (Arsenault, 1965) and *F. moniliforme* (Steyn et al., 1979) as an antibiotic (Hamilton et al., 1953). Substance **3** was thus identified as bostrycoidin [5,8-dihydroxy-6-methoxy-3-methyl-2-aza-9,10-anthraquinone]. Since the ^{1}H and ^{13}C NMR spectra data of bostrycoidin (**3**) have not been reported, assignments of the ^{1}H and ^{13}C NMR spectra for **3** are described herein.

By the same method, spectral data was compared with the reported data and the red pigment (**4**) was identified as 8-*O*-methylbostrycoidin. Although these pigments have previously been identified from cultures of the non-lichenized pyrenomycet *F. moniliforme* (Steyn et al., 1979), this is their first isolation from cultures of lichen.

Arthoniafurones, bostrycoidin and 8-*O*-methylbostrycoidin, are extraordinary metabolites of *A. cinnabarina* mycobiont, which are likely to be biosynthesized from the same heptaketide precursors. After the establishment of the naphthoquinone moiety, their side chains may be modified to form the furan ring substituted with an acetyl group, as in the case of arthoniafurones, and with a pyridine ring by introduction of a nitrogen atom from an ammonia molecule in the case of bostrycoidin derivatives.

3. Experimental

3.1. General experimental procedure

Melting points were determined on a Yanagimoto MP micromelting point apparatus. The IR spectra were measured with a Jasco A-102 IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded using a Jeol GSX 400 (^{1}H 400 and ^{13}C 100 MHz) and a Jeol JNM LA500 (^{1}H 500 and ^{13}C 125 MHz) spectrometer in DMSO-d_6 or $CDCl_3$ with TMS as the int. standard. Column chromatography was carried out on 70–230 mesh silica gel (Merck).

3.2. Material and culture

A. cinnabarina thallus (collection no. YY95051611) was collected in May, 1995, in Wakayama Pref., Japan by Yamamoto, and air-dried at room temperature overnight. The collected sample is now in the herbarium of the Natural History Museum and Institute, Chiba. One week after collection, a section with an apothecium was excised from the thallus and submerged for 1 h in sterile water in a Petri dish at room temperature. The section was aseptically fixed with silicone grease (Toray Silicone Inc., Tokyo) inside the center of the lid of a 60 mm diam. petri dish containing 5 ml water–agar (WA) medium composed of distilled water and 2% (w/v) agar. The apothecium discharged spores to the agar-plate, with these being incubated at 15 °C in the dark, and after 1 day they germinated. One month after incubation, the mycelia derived from the spores had grown to form a small colony. A small agar block with a mycobiont colony was removed and transferred to fresh MY medium (5 ml) in a 60 mm diam. petri dish. The mycobiont (strain no. 0025M) was subcultured in the dark at 15 °C every 10 months in the culture collection of Akita Prefectural University.

3.3. Extraction and isolation

The colonies of mycobiont were collected and extracted with $CHCl_3$. The $CHCl_3$ extract (243.4 mg) was subjected to Si gel column chromatography using a stepwise gradient [$CHCl_3$–MeOH (100:1–1:1)] and then by HPLC [silica-4251-N 1 × 25 cm, Si gel treated with 3% oxalic acid, UV detection at 254 nm, flow rate 3 ml/min, $CHCl_3$–acetone (50:1)], to yield two yellow powders **1** (18.6 mg) and **2** (23.7 mg) and two red powders **3** (3.0 mg) and **4** (12.9 mg), respectively, which were then individually crystallised from either $CHCl_3$ or EtOH.

3.3.1. Arthoniafurone A (*1*)

Orange prisms ($CHCl_3$), mp 191–192 °C; UV (EtOH) nm (log ε) 223 (4.81), 250 (sh, 4.67), 310 (4.28), 395 (4.23); IR υ_{max} (KBr): 3450, 1740, 1690, 1640, 1530, 1450, 1310, 1260, 1220, 880, 860 cm^{-1}; HREIMS *m/z* 256.0375 (calcd for $C_{14}H_8O_5$, 256.0372); EIMS *m/z* (rel. int.%) 258 (M + 2, 3), 257 (M + 1, 16), 256 (M^+, 100), 241 (36), 228 (61); ^1H NMR ($CDCl_3$): δ 2.75 (3H, *s*, H-11), 7.38 (1H, *dd*, *J* = 8.0, 1.0 Hz, H-7), 7.69 (1H, *dd*, *J* = 8.0, 1.0 Hz, H-5), 7.81 (1H, *dd*, *J* = 8.0, 8.0 Hz, H-6), 8.96 (1H, *s*, H-3), 12.45 (1H, *s*, OH-8), ^{13}C NMR ($CDCl_3$): δ 150.9 (1-C), 148.4 (3-C), 124.0 (3a-C or 9a-C), 178.1 (4-C), 134.2 (4a-C), 118.8 (5-C), 137.1 (6-C), 124.6 (7-C), 162.3 (8-C), 117.8 (8a-C), 184.2 (9-C), 121.8 (9a-C or 3a-C), 186.6 (10-C), 29.0 (11-C).

3.3.2. Arthoniafurone B (*2*)

Orange prisms ($CHCl_3$), mp 141–144 °C; $[\alpha]_D^{20}$: −14.6° (DMSO, *c* = 0.12); UV (EtOH) nm (log ε): 205 (4.10), 220 (sh, 4.03), 250 (sh, 3.88), 322 (sh, 3.77), 370 (3.53); IR υ_{max} (KBr): 3410, 2930, 1650, 1630, 1610, 1590, 1510, 1450, 1390, 1325, 1300, 1290, 880, 770 cm^{-1}; HREIMS *m/z* 258.0526 (calcd for $C_{14}H_{10}O_5$, 258.0528); EIMS *m/z* (rel. int.%) 258 (M^+, 100), 230 (20), 215 (47); ^1H NMR (DMSO-d_6): δ 2.71 (3H, *s*, H-11), 5.72 (*brs*), 6.97 (1H, *dd*, *J* = 7.9, 0.9 Hz, H-7), 7.27 (1H, *dt*, *J* = 7.9, 0.9 Hz, H-5), 7.66 (1H, *dd*, *J* = 7.9, 7.9 Hz, H-6), 8.26 (1H, *d*, *J* = 1.2 Hz, H-3), 12.47 (1H, *s*, OH-8); ^{13}C NMR (DMSO-d_6): δ 150.0 (1-C), 143.3 (3-C), 121.0 (3a-C or 9a-C), 60.1 (4-C), 145.9 (4a-C), 119.0 (5-C), 136.7 (6-C), 116.5 (7-C), 162.1 (8-C), 116.0 (8a-C), 185.1 (9-C), 129.6 (9a-C or 3a-C), 186.6 (10-C), 28.9 (11-C).

3.3.3. Bostrycoidin (*3*)

Red powder, mp 242–244 °C; UV (EtOH) nm (log ε): 250 (4.64), 320 (3.99), 497 (4.07), 525 (4.06); IR υ_{max} (KBr): 3450, 1620, 1590, 1260, 1090, 1020, 800 cm^{-1}; EIMS *m/z* (rel. int.%): 285 (M^+, 100), 267 (12), 242 (10), 211 (7); ^1H NMR ($CDCl_3$): δ 9.50 (1H, *s*, H-1), 2.80 (3H, *s*, H-3), 7.96 (1H, *s*, H-4), 13.49 (1H, *s*, OH-5), 4.02 (3H, *s*, H-6), 6.76 (1H, *s*, H-7), 13.20 (1H, *s*, OH-8); ^{13}C NMR ($CDCl_3$): δ 143.3 (1-C), 165.4 (3-C), 25.3 (3-CH_3), 118.0 (4-C), 138.7 (4a-C), 186.4 (5-C), 151.2 (6-C), 157.9 (7-C), 56.8 (7-CH_3), 107.9 (8-C), 161.3 (9-C), 183.9 (9-C), 124.6 (10a-C).

3.3.4. 8-O-Methylbostrycoidin (*4*)

Red powder, mp 214–216 °C; UV (EtOH) nm (log ε): 205 (4.42), 245 (4.54), 315 (3.96), 480 (3.87); IR υ_{max} (KBr): 3500, 1655, 1595, 1470, 1430, 1375, 1310, 1265, 1210, 1130, 1025, 920, 800 cm^{-1}; EIMS *m/z* (rel. int.%): 299 (M^+, 100), 270 (52).

References

Ali, S., Read, R.W., Sotheeswaran, S., 1994. Benzisochromanquinones and an isofuranonaphtho-quinone from *Ventilago vitiensis* (Rhamnaceae). Phytochemistry 35, 1029–1034.

Arsenault, G.P., 1965. The structure of bostrycoidin, a β-aza-anthraquinone from *Fusarium solani* D_2 purple. Tetrahedron Letters 45, 4033–4037.

Baker, R.A., Tatum, J.H., Nemec Jr., S., 1990. Antimicrobial activity of naphthoquinones from Fusaria. Mycopathologia 111, 9–15.

Cajori, F.A., Otani, T.T., Hamilton, M.A., 1954. The isolation and some properties of an antibiotic from *Fusarium bostrycoides*. Journal of Biological Chemistry 208, 107–114.

Culberson, C.F., 1969. Chemical and Botanical Guide to Lichen Products. The University of North Carolina Press, Chapel-Hill.

Grube, M., 1998. Classification and phylogeny in the Arthoniales (lichenized ascomycetes). Bryologist 101, 377–391.

Grube, M., Matzer, M., Hafellner, J., 1995. A preliminary account of the lichenicolous *Arthonia* species with reddish K^+ active pigments. Lichenologist 27, 25–42.

Grube, M., Matzer, M., 1997. Taxonomic concepts of lichenicolous *Arthonia* species. Bibliotheca Lichenologica 68, 1–17.

Hamilton, M.A., Knorr, M.S., Cajori, F.A., 1954. Experimental studies of an antibiotic derived from *Fusarium bostrycoides*. Antibiotics and Chemotherapy 3, 853–860.

Hanumaiah, T., Rao, G.S.R., Rao, C.P., Rao, K.V.J., Cowe, H.J., Cox, P.J., Howie, R.A., Marshall, D.S., Thomson, R.H., 1985. Isofuranonaphthoquinones from *Ventilago maderaspatana*. Tetrahedron 41, 635–642.

Huneck, S., Yoshimura, I., 1996. Identification of Lichen Substances. Springer-Verlag, Berlin.

Jammula, S.R., Pepalla, S.B., Telikepalli, H., Rao, K.V.J., Thomson, R.H., 1991. An acetonaphthone and an isofuranonaphthoquinone from *Ventilago goughii*. Phytochemistry 30, 2427–2429.

Kon, Y., Kashiwadani, H., Wardlaw, J.H., Elix, J.A., 1997. Effects of culture conditions on dibenzofuran production by cultured mycobionts of lichens. Symbiosis 23, 97–106.

Miyagawa, H., Hamada, N., Sato, M., Ueno, T., 1994. Pigments from the cultured lichen mycobionts of *Graphis scripta* and *G. desquamescens*. Phytochemistry 36, 1319–1322.

Miyagawa, H., Hamada, N., Ueno, T., 1993. Hypostrepsilic acid, a new dibenzofuran from the cultured lichen mycobiont of *Evernia esorediosa*. Phytochemistry 34, 589–591.

Miyagawa, H., Yamashita, M., Hamada, N., 1997. Hypostrepsilalic acid from a cultured lichen mycobiont of *Stereocaulon Japonicum*. Phytochemistry 46, 1289–1291.

Parisot, D., Devis, M., Frzou, J-P., Barbier, M., 1983. Pigments from *Nectria haematococca:* anhydrofusarubin lactone and nectriafurone. Phytochemistry 22, 1301–1303.

Steyn, P.S., Wessels, P.L., Marasas, W.F.O., 1979. Pigments from *Fusarium moniliforme* sheldon. Tetrahedron 35, 1551–1555.

Tanahashi, T., Kuroishi, M., Kuwahara, A., Nagakura, N., Hamada, N., 1997. Four phenolics from the cultured lichen mycobiont of *Graphis scripta* var. *pulverulenta*. Chemical and Pharmaceutical Bulletin 45, 1183–1185.

Tatum, J.H., Baker, R.A., Berry, R., 1987. Naphthofurans produced by *Fusarium oxysporum* isolated from citrus. Phytochemistry 26, 2499–2500.

Thor, G., 1990. The lichen genus chiodection and five allied genera. Opera Botanica 103, 1–92.

Yamamoto, Y., Matsubara, H., Kinoshita, Y., Kinoshita, K., Koyama, Takahashi, K., Ahmadjian, V., Kurokawa, T., Yoshimura, I., 1996. Naphthazarin derivatives from cultures of the lichen *Cladonia cristatella*. Phytochemistry 43, 1239–1242.