Brominated Depsidones from *Acarospora gobiensis*, a Lichen of Central Asia

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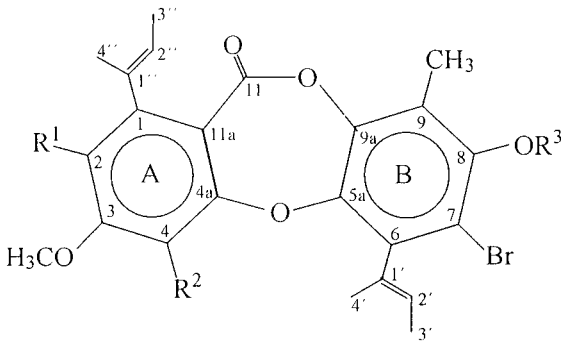
Two new antimicrobial polysubstituted brominated depsidones, acarogobien A (**1**) and acarogobien B (**2**), were isolated from the lichen *Acarospora gobiensis*. Their structures were established by ^{1}H and ^{13}C NMR, MS, IR, and UV spectroscopy.

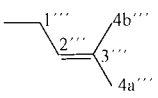
Lichens occur worldwide and in most habitats. They can protect themselves from extreme environmental conditions by synthesizing a number of secondary products.[1] The role of some lichen metabolites as mineral-releasing agents and lichen surface sealants, their antibiotic, allelopathic, and antiherbivore potentials, and the role of some lichen substances in gathering and excluding radiation energy has been established.[2]

At the present time more than 600 lichen secondary products are known, including aliphatic acids, chromones, xanthones, naphthoquinones, anthraquinones, depsides, depsidones, and dibenzofurans.[2] Most of the known depsidones are lichen metabolites, and a few are chloro substituted.[3−6]

The lichen *Acarospora gobiensis* H. Mogn. (Acarosporaceae) is a typical mountain species, colonizing rock substrates. Although some lichen acids, for example, rhyzocarpic, psoromic, norstictic, and gyrophoric acids, have been found in species of the *Acarospora* genus,[7,8] no phytochemical information about *A. gobiensis* is available. In this paper, characterization of two new brominated polysubstituted depsidones [acarogobiens A (**1**) and B (**2**)] isolated from *A. gobiensis* is presented, and their antimicrobial activity is discussed.



1 $R^1 = R^3 = H$, $R^2 = CH_3$

2 $R^1 = Br$, $R^2 = CHO$, $R^3 =$ 

HPLC of an extract from the dried thallus of *A. gobiensis* yielded two compounds that gave a green positive color reaction with Beilstein's test. The molecular formula of **1** was $C_{24}H_{25}O_5Br$ by HREIMS. The MS of **1** showed the molecular ions at *m/z* 472 and 474, respectively, in the ratio of about 1:1, indicating the presence of one bromine atom in **1**. The presence of an ester carbonyl was revealed by an IR absorption at 1715 cm^{-1}.

In the ^{1}H NMR spectrum of **1**, the aryl-coupled methyl olefinic proton pairs at δ 1.89−5.22 and δ 1.94−5.49 coupled with methyl protons at δ 1.72 and 1.83, respectively. These substituents formed two sets of 1-methyl-1-propenyl groups. In addition to these, one singlet aromatic proton (δ 6.49), one phenolic proton (δ 5.31), and two aromatic methyls (δ 2.21 and 2.30) were also revealed. Among 24 carbon signals identified in the ^{13}C NMR spectrum, those with directly bonded protons were assigned by analysis of the ^{1}H−^{13}C COSY spectrum.

The location of substituents on each aromatic ring was determined by analysis of ^{1}H−^{13}C long-range couplings in the HMBC spectrum. The observable ^{1}H−^{13}C long-range correlations are restricted to the proton−carbon pairs that are separated by two or three bonds. The presence of long-range coupling between C-1 (δ 136.6) and H-2 (δ 6.49) indicated that one 1-methyl-1-propenyl group was ortho to H-2. Carbon signals at δ 160.9 and 116.0, which were long-range coupled with H-2, were assigned to C-3 and C-4, respectively, to account for the upfield shifts of C-2 (δ 108.1) and C-4 (δ 116.0) caused by the ether substituent on C-3 (δ 160.9).

The remaining carbon signal (δ 122.8), which was also long-range coupled with H-2, was assigned to C-11a. One of the methyl groups resonating at δ 2.30 was placed at C-4 due to the ^{1}H−^{13}C long-range correlation with C-3 and C-4. Assignment of C-4a, based on the long-range correlation with methyl protons at δ 2.30 alone, was ambiguous due to the coexistence of the 9-Me signal at the same position. However, it seemed appropriate to assign its signal at δ 161.4, considering the meta-disubstitution of ether groups on ring A. Placement of the ether substituent on C-4a would explain the upfield shifts of C-4 and C-11a. Thus, ring A was established as shown. Ring B was assigned from analysis of the ^{1}H−^{13}C long-range correlation essentially in the same fashion as described above. The substituents of ring B were methyl, hydroxy, 1-methyl-1-propenyl, and a bromine atom. Assignments of the carbon chemical shifts and the determination of the position of the functional groups on B ring were carried out mainly on the basis of the signal multiplicity in the proton-coupled ^{13}C NMR spectrum and the additivity rule of substituent effects on the benzene ring.[9] To determine the position of functional groups in **1**, we first considered the shift effects of ortho-disubstituted phenols.[10] Characteristic upfield shifts of carbon and those of C-1 *ortho*, *meta*, and *para* to the

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Table 1. Inhibitory Effects of Acarogobiens Against *B. subtilis* and *S. aureus*

compounds	*B. subtilis* IC_{50} (μM)	*S. aureus* IC_{50} (μM)
acarogobien A (**1**)	17	22
acarogobien B (**2**)	1.5	2.8
penicillin G	0.7	0.03
streptomycin	3.1	0.5

hydroxy group were comparable to those of the ortho-disubstituted phenols. It was thus determined that the OH group of **1** was at C-8.

The ortho-substitution of −OR and −O(C═O)R groups at C-5a and C-9a was suggested by their chemical shifts. The A and B rings of **1** were connected using the NOE cross-peaks between 4-Me and 4′, 3′-Me, and between 9-Me and 4″-Me observed in the NOESY spectrum. The trans configuration of both 1-methyl-1-propenyl groups was also derived from the NOEs observed between 3′-Me and 4′-Me and between 3″-Me and 4′-Me.

Acarogobien B (**2**) showed molecular ions at *m/z* 632, 634, and 636 in the proportions of about 1:2:1, suggesting the presence of two bromine atoms in this compound. HRMS of **2** confirmed the molecular formula as $C_{29}H_{30}O_6Br_2$. Compound **2** showed signals of two 1-methyl-1-propenyl groups, one aromatic methyl, one methoxy group, one aldehydic hydrogen, and carbon. NMR analysis indicated that **2** also had an *O*-prenyl group [δ_H 1.77 and 1.79 s, each 3H, 4.63 (2H) and 5.53 m (1H), δ_C 18.3 q, 26.0 q, 66.2 t, 121.4 d, and 137.5 s, respectively]. The absorption maximum at 1720 cm^{-1} in the IR spectrum and the ^{13}C NMR signal at C-8 (δ 163.9) confirmed the presence of an ester function in structure B. The above data indicated that **2** was structurally related to **1**, with an additional bromine atom replacing the aromatic hydrogen at the 2 position. Long-range $^1H-^{13}C$ correlations observed in the HMBC spectrum of **2** allowed assignment of the structure shown.

The crude extract of *A. gobiensis* exhibited antibiotic activity against *Bacillus subtilis* and *Staphylococcus aureus*, and the pure acarogobiens (**1** and 2) inhibited trypsin. Acarogobien B (2) was more effective than acarogobien A (1) and accounted for most of the toxicity to *B. subtilis* and *S. aureus* (1 and 10 μg/disk, respectively) (Table 1). Both **1** and **2** were tested with respect to their inhibition of proteolytic enzymes, and the highest anti-trypsin effect was exhibited by 2 (IC_{50} value 1×10^{-4} mol/L).

Both **1** and **2** are new lichen depsidones. They are probably related to emuguisines,[3] nidulin, or unguinol,[5] previously isolated from fungi. Acarogobiens A and B are the first examples of brominated depsidones isolated from natural sources. To our knowledge, no natural brominated depsidones have been described previously.[6,11]

Experimental Section

General Experimental Procedures. UV spectra were measured by a Cary 118 (Varian) apparatus in heptane within the range of 200−350 nm. A Perkin−Elmer model 1310 (Perkin−Elmer, Norwalk, CT) IR spectrophotometer was used for scanning IR spectroscopy of depsidones as neat films. NMR spectra were recorded on a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^1H), 125.7 MHz (^{13}C). HRMS and LRMS were recorded using a VG 7070E−HF spectrometer (70 eV).

Plant Material. The specimens of *Acarospora gobiensis* H. Mogn. were collected by hand in July 1996, along the shore of lake Issyk Kul, Tian-Shan mountain, Uzbekistan. The voucher specimens are deposited in the lichen collection under curatorship of the second author (I.A.G.).

Extraction and Isolation. The 100-g of air-dried lichen were extracted by the method of Blight and Dyer.[12] The total lipid extract, a viscous dark oil, was subjected to a Sephadex LH-20 column with Me_2CO−EtOAc (1:5) and was further separated by semipreparative reversed-phase HPLC on a C_{18} reversed-phase column (5 μm, 7.8 × 250 mm, Supelco, USA). A linear gradient of A [80% H_2O (pH ∼ 3, adjusted with H_3PO_4) and 20% acetonitrile] and B [20% water (pH ∼ 3, adjusted with H_3PO_4) and 80% acetonitrile] with 50% B at the start, rising to 100% B over 25 min, flow rate 2 mL/min, was used to separate all of the compounds in the crude extract. Depsidones **1** and **2** were detected by UV absorption at 280 nm.

Biological Tests. The test organisms were *Bacillus subtilis* (CCM 2216) and *Staphyloccocus aureus* (CCM 2551) (CCM = Czechoslovak Collection of Microorganisms, Brno). Antibacterial assays were carried out according to the literature method.[13] Applied amounts were 50 μg of compound per test disk, see Table 1.

Trypsin activity was determined according to Bergmeyer.[14] The percentage of inhibition of trypsin activity by isolated compounds and reference substances was as follows: acarogobien A (**1**), 64% (5×10^{-4} mol/L), 27% (1×10^{-4}), and 5% (5×10^{-5} mol/L); acarogobien B (**2**), 81% (5×10^{-4} mol/L), 39% (1×10^{-4} mol/L), and 12% (5×10^{-5} mol/L); benzamidine·HCl (standard), 33% (5×10^{-4} mol/L) and 8% (1×10^{-4} mol/L).

Acarogobien A (1): 7.6 mg, colorless crystals (toluene), mp 172 °C; UV (EtOH) (log ϵ) 210 (4.57), 278 (4.06), and 310 (3.45) nm; IR (KBr) ν_{max} cm^{-1} 3400 (OH), 1715 (COO), 1670, 1428, 1279, 1260, 1175; ^1H NMR ($CDCl_3$, 500.1 MHz) δ 1.72 (3H, br d J = 6.4 Hz, H-3′), 1.83 (3H, dd J = 6.9, 1.0 Hz, H-3″), 1.89 (3H, br s, H-4′), 1.94 (3H, s, H-4″), 2.21 (3H, s, 9-Me), 2.30 (3H, s, 4-Me), 3.85 (3H, s, 3-OMe), 5.22 (1H, br q J = 6.9 Hz, H-2′), 5.31 (1H, s, *H*O-8), 5.49 (1H, qq J = 6.7, 1.0 Hz, H-2″), 6.49 (1H, s, H-2); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 8.4 (q, 4-Me), 9.0 (q, 9-Me), 14.0 (q, C-3′), 14.7 (q, C-3″), 17.1 (q, C-4′), 17.5 q, C-4″), 57.1 (q, C-3-OMe), 107.1 (s, C-7), 108.1 (d, C-2), 116.0 (s, C-4), 117.1 (s, C-9), 122.8 (s, C-11a), 123.8 (d, C-2″), 127.8 (d, C-2′), 133.7 (s, C-6), 135.0 (q, C-1′), 136.6 (s, C-1), 136.9 (s, 1″), 142.9 (s, C-9a), 144.1 (s, C-5a), 152.3 (s, C-8), 160.9 (s, C-3), 161.4 (s, C-4a), 164.1 (s, C-11), 136.9 (s, C-1″), 142.9 (s, C-9a); EIMS *m/z* 472 and 474 [M^+]; HREIMS *m/z* 472,4621 [M^+], (calcd $C_{24}H_{25}O_5{}^{79}Br$ 472.4639).

Acarogobien B (2): 11.8 mg; colorless needles (toluene), mp 197 °C; UV (EtOH) (log ϵ) 210 (4.34), 280 (4.18), and 310 (3.27) nm; IR (KBr) ν_{max} cm^{-1} 3400 (OH), 1720 (COO), 1440; ^1H NMR ($CDCl_3$, 500.1 MHz) δ 1.74 (3H; dd J = 6.6, 1.1 Hz. H-3″), 1.77 (3H; s, H-4b‴), 1.79 (3H; s, H-4a‴), 1.84 (3H; br d J = 6.8 Hz, H-3′), 1.96 (3H; br s, H-4″), 2.03 (3H; s, H-4′), 2.18 (3H, s, 9-Me), 3.79 (3H; s, 3-OMe), 4.63 (2H; d J = 6.7 Hz, H-1‴), 5.37 (1H; br q J = 6.4 Hz, H-2′), 5.49 (1H; dd J = 6.5, 0.9 Hz, H-2″), 5.53 (1H; dd J = 6.7 1.8 Hz, H-2‴), 9.91 (1H; s, 4-CHO); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 9.0 (q, 9-Me), 14.2 (q, C-3′), 14.9 (q, C-3″), 16.9 (q, C-4′), 17.3 (q, C-4″), 18.3 (q, C-4b‴), 25.8 (q. C-4a‴), 58.3 (q, 3-OMe), 66.2 (t, C-1‴), 102.7 (s, C-2), 111.4 (s, C-4), 115.4 (s, C-7), 121.4 (d, C-2‴), 124.0 (d, C-2″), 124.9 (s, C-11a), 125.3 (s, C-9), 128.1 (d, C-2′), 133.8 (s, C-6), 134.8 (s, C-1′), 135.7 (s, C-1″), 137.5 (s, C-3‴), 138.2 (s, C-1), 142.9 (s, C-9a), 146.4 (s, C-5a), 155.6 (s, C-8), 160.4 (s, C-4a), 163.3 (s, C-3), 163.9 (s, C-11), 191.0 (d, 4-CHO); EIMS *m/z* 632, 634 and 636 [M^+]; HREIMS *m/z* 632.5587 [M^+], (calcd $C_{29}H_{30}O_6{}^{79}Br_2$ 632.5539.

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