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Gergory K. Poch, and James B. Gloer

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AURANTICINS A AND B: TWO NEW DEPSIDONES FROM A MANGROVE ISOLATE OF THE FUNGUS *PREUSSIA AURANTIACA*

GREGORY K. POCH and JAMES B. GLOER*

Department of Chemistry, University of Iowa, Iowa City, Iowa 52242

ABSTRACT.—Auranticins A [**1**] and B [**2**], two new antimicrobial depsidones, have been obtained from a mangrove isolate of the fungus *Preussia aurantiaca*. The structures were determined through analysis of selective INEPT, decoupling, COSY, and NOESY experiments.

Our interest in fungi collected from marine environments as sources of new biologically active secondary metabolites (1,2) led us to examine a mangrove isolate of *Preussia aurantiaca* (ATCC 14745). *Preussia* spp. are commonly found in terrestrial environments, so the occurrence of *P. aurantiaca* in mangroves is likely to be fortuitous. However, recent discoveries of interesting chemistry associated with three isolates from this rarely studied genus [3–5] also stimulated the investigation of *P. aurantiaca*. Extracts of culture filtrates produced by this organism were found to exhibit antibiotic activity against *Bacillus subtilis* and *Staphylococcus aureus*. Chemical studies of these extracts have led to the isolation and structure determination of two new antimicrobial depsidones that we have called auranticins A and B.

Si gel chromatography of the EtOAc-Me_2CO (9:1) extract from liquid cultures of *P. aurantiaca* afforded auranticins A [**1**] and B [**2**] as major constituents that accounted for the antibiotic activity of the extract. Analysis of the more active component (auranticin A) by hrfabms suggested that it has the molecular formula $C_{24}H_{24}O_8$. The ir and ^{13}C-nmr data revealed the presence of two carboxyl groups, at least one of which must be a carboxylic acid. The ^{1}H-nmr spectrum (DMSO-d_6, Table 1) contains three aromatic or vinylic singlets, one vinylic quartet, one vinylic methyl doublet, and singlets for a -CH_2O- unit, a methoxy group, and three vinylic or aromatic methyl groups. A series of homonuclear proton decoupling experiments established the presence of a 1-methylpropenyl group and a 3-substituted 3-methylpropenoic acid unit. The ^{13}C-nmr data (Table 1) confirmed the presence of 24 carbons, including sixteen aromatic or olefinic carbons, and was consistent with the conclusions drawn from the ^{1}H-nmr data. The multiplicities of all ^{13}C-nmr signals were assigned through a DEPT experiment, which also revealed the presence of three exchangeable protons. Treatment of auranticin A with CH_2N_2 afforded a dimethylated product **3** (nmr data, Table 1), indicating that only two of these exchangeable protons are associated with acid or phenol groups. The -CH_2O- unit must therefore be present as an hydroxymethyl group, and this was confirmed by the appearance of a previously unobserved OH proton as an exchangeable triplet coupled to the -CH_2O- group in the ^{1}H-nmr spectrum of **3**. The ^{13}C-nmr spectrum of this compound also showed a small confirmatory change in the chemical shift for the carboxyl carbon of the esterified methylpropenoic acid side-chain.

The presence of two pentasubstituted aromatic rings (partial structures **a** and **b**) was established primarily by analysis of a series of selective INEPT long-range CH correlation experiments (Table 2). Irradiation of the upfield Me-group protons located the position of attachment of this methyl (C-9), and identified the oxygenated carbons C-8 and C-9a as being attached to C-9. Irradiation of the MeO protons revealed this group's connection to C-8, while NOESY and COSY experiments showed H-7 to be ortho to this substituent. Irradiation of H-7 afforded polarization transfer to H-1′, even when optimized for large J values, indicating that the 1-methylpropenyl substituent is ortho

TABLE 1. ^{1}H- and ^{13}C-nmr Data for Auranticin A [1], Auranticin B [2], and Dimethylauranticin A [3].[a]

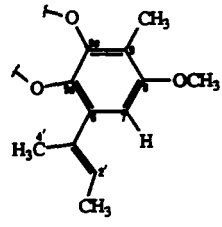
Position	Compound					
	1		2		3	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1		148.24		153.20		148.37
2	6.65 (s)	112.77	6.83 (s)	114.88	6.87 (s)	108.80
3		161.01[b]		163.68[b]		161.40[b]
4		118.65		111.89		120.52
4a		161.78[b]		165.04[b]		160.95[b]
5a		142.29		141.31		142.14
6		135.83		135.85		135.91
7	6.55 (s)	107.67	6.60 (s)	107.88	6.56 (s)	107.73
8		154.19		154.38		154.22
9		116.30		116.82		116.34
9a		142.75		142.27		142.56
11		162.87		161.53		162.74
11a		110.13		110.83		112.07
4-CH_2-O	4.58 (s)	52.27	—	—	4.55 (s)	51.41
4-CHO	—	—	9.85 (s)	191.62	—	—
3-OMe					3.88 (s)	56.41[c]
3″-OMe					3.66 (s)	51.05[c]
8-OMe	3.77 (s)	55.95	3.78 (s)	56.03	3.77 (s)	55.93
9-Me	2.10 (s)	8.18	2.11 (s)	8.82	2.10 (s)	8.18
1′		133.15		132.18		133.12
2′	5.55 (qq; 6.8, 1.4)	125.74	5.47 (qq; 6.8, 0.8)	126.19	5.55 (qq; 6.8, 1.5)	125.84
3′	1.78 (d; 6.8)	17.60	1.78 (d; 6.8)	17.90	1.78 (d; 6.8)	17.61
4′	2.05 (br s)	13.72	2.05 (br s)	13.52	2.03 (br s)	13.79
1″		154.66		154.58		155.94
2″	5.60 (d; 1.3)	119.48	5.65 (d; 1.3)	120.38	5.79 (d; 1.3)	118.52
3″		166.77		166.41		165.70
4″	2.25 (br s)	19.94	2.23 (br s)	19.39	2.34 (br s)	20.34

[a]Data were recorded in DMSO-d_6 at 360 and 90.7 MHz, respectively. Carbon multiplicities were determined through DEPT experiments, and are consistent with the assignments.

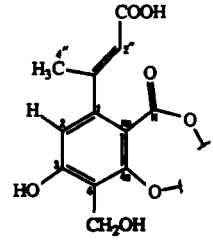
[b,c]Assignments in the same column with identical superscripts may be interchanged.

to H-7. These experiments also resulted in appearance of a signal for C-5a, which must be the remaining carbon of the aromatic ring. Both C-5a and C-9a must be oxygenated in order to account for their chemical shifts. These results indicated the presence of partial structure **a**.

Selective INEPT irradiation of the hydroxymethyl CH_2 proton signal indicated the attachment of this substituent to a second aromatic ring (at C-4) and identified carbons C-3 and C-4a as being attached to C-4. The position of attachment of the CH_2OH



a



b

TABLE 2. Selective INEPT Long-Range CH Correlations for Auranticin A [1].[a]

Proton signal irradiated	Carbon signals observed	Proton signal irradiated	Carbon signals observed
H-2	1[c], 1″, 4, 11[b,c], 11a	H-2′	3′[d], 4′[d], 6
H-7	1′[d], 5a, 6[c], 8, 9[d]	H-3′	1′, 2′
4-CH_2O-	3, 4, 4a	H-4′	1′[d], 2′[d], 6
8-OMe	8	H-2″	1[d], 3″[c], 4″[d]
9-Me	8, 9, 9a	H-4″	1, 1″, 2″[d]

[a]Each proton signal analyzed with the selective INEPT technique was subjected to three or four separate experiments, optimizing for 4, 7, 10, or 14 Hz. Signals separated by less than 25 Hz were irradiated off-center in the appropriate direction to avoid ambiguous results.

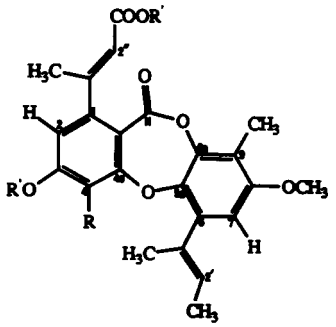
[b]Denotes a four-bond coupling.

[c]Signal observed only at 4 Hz.

[d]Signal absent at 4 Hz, but observed at 10 or 14 Hz.

group must be C-4, because the chemical shifts of C-3 and C-4a require their attachment to oxygen atoms. Signals for the remaining three ring carbons were observed upon irradiation of H-2. The methylpropenoic acid side-chain was shown to be connected to one of these carbons (C-1) on the basis of a selective INEPT correlation between the C-4″ proton signal and the signal for C-1. This side-chain must be ortho to H-2 to account for observation of a correlation between H-2 and C-1″ in a selective INEPT experiment optimized for 7 Hz. The only remaining carbon of this ring must be attached to the carbonyl carbon (the only other substituent not yet accounted for) rather than an oxygen in order to rationalize its chemical shift. The position of the phenolic OH and the relative disposition of the substituents connected to C-1, C-2, and C-11a were ascertained by analysis of nmr data for the methylation product **3**. The phenolic OH was determined to be ortho to H-2 due to weak coupling between H-2 and one of the new methoxy groups, as detected by signal sharpening in decoupling experiments. Because the methylpropenoic acid side chain was already determined to be ortho to H-2, this ring must be substituted as shown in partial structure **b**.

Assignments of *E* geometries for the C-1 and C-6 side chains were based on a combination of NOESY data and chemical shift comparisons. Evidence supporting the *E* geometry for the C-1 side chain is provided by the sterically influenced ^{13}C-nmr shift of the C-4″ signal (19.9 ppm) as compared to the literature values for the corresponding carbon signals of the two isomers of α-methyl cinnamic acid (*E*-isomer 18.3 ppm, *Z*-



1 R=CH_2OH, R′=H
2 R=CHO, R′=H
3 R=CH_2OH, R′=Me

isomer 27.5 ppm) (6). The lack of a strong NOESY correlation between these proton signals is consistent with the assignment. The protons on C-3′ and C-4′ exhibit upfield shifts expected for cis methyl groups and exhibit a strong NOESY correlation.

Subunits **a** and **b** could be linked in only two possible ways, either of which would form a depsidone ring system. The possibilities were distinguished on the basis of nOe effects. Although difference nOe experiments involving relevant signals did not show conclusive enhancements, a NOESY correlation was observed between the C-3′ and the 4-CH_2O proton signals, resulting in assignment of the structure of auranticin A as **1**, a member of the depsidone class with a new substitution pattern. All of the proton and carbon chemical shifts could be assigned on the basis of these data except for those of C-2, C-3, C-4a, and C-7. Assignments for C-2 and C-7 were made through single frequency heteronuclear decoupling experiments. Only the unprotonated carbons C-3 and C-4a could not be differentiated. Carbon shift calculations and ^{13}C-nmr assignments for known depsidones (7) are consistent with the assignments in Table 1.

The data for compound **2** differ from those for auranticin A [**1**] only in the absence of a ^{1}H-nmr singlet for a CH_2O- unit, the presence of a ^{1}H singlet corresponding to an aldehyde moiety, and a difference of two mass units in mol wt. Analysis of ^{1}H- and ^{13}C-nmr data (Table 1) and the results of decoupling experiments for auranticin B indicated that this component has structure **2** and is simply an oxidized analogue of **1**.

This study serves to further illustrate the utility of selective INEPT experiments in making structural assignments, even for highly substituted aromatics, without extensive reliance on comparison to model compounds. Although most known depsidones have been reported as lichen metabolites, auranticins A and B are members of an increasing number of depsidones isolated from non-lichen sources (7–10). Auranticin A is much more potent than auranticin B in antimicrobial assays and accounts for most of the *B. subtilis* and *S. aureus* activity of *P. aurantiaca* (5 and 50 μg/disc, respectively).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—General procedures and instrumentation employed in this work have been described previously (11,12).

CULTIVATION.—A culture of *P. aurantiaca* (ATCC 14745) was obtained from the American Type Culture Collection, Rockville, Maryland. Nine 2-liter Erlenmeyer flasks, each containing 350 ml of potato dextrose broth (Difco), were inoculated with several 1-cm^2 plugs taken from 1-week-old Petri dish cultures of *P. aurantiaca*. Flask cultures were incubated at 25–28° and aerated by agitation on an orbital shaker at 200 rpm. Concentrations of the active metabolites were monitored by bioassay of the culture filtrate, and antibiotic activity of the culture filtrate reached a maximum after 12 days. The use of an artificial seawater medium resulted in production of the same major metabolites.

ISOLATION AND CHARACTERIZATION OF AURANTICINS A AND B.—The filtered broth (3150 ml) was extracted with EtOAc-Me_2CO (9:1) (6 × 200 ml), and the crude extract was dried ($MgSO_4$) and evaporated to afford 400 mg of a yellow oil. The crude oil was triturated with Me_2CO, and the residual insoluble white solid (94.0 mg) was found to account for most of the biological activity of the crude extract. The solid was chromatographed on a column of Si gel (4 × 25 cm) using a gradient of 0–50% MeOH in $CHCl_3$. The major component (a white crystalline solid, 65.3 mg) eluted at 30% MeOH and showed a single peak by hplc [Rt 13.4 min; 5μ C_{18} column, 250 × 10 mm, MeOH-H_2O (70:30), 2.0 ml/min, 215 nm]. This compound, auranticin A [**1**], gave the following data: mp 264° (dec); ir (neat) 3387, 3335 (br, COOH), 2962, 1721, 1708 (br), 1640, 1616, 1565 cm^{-1}; uv max (MeOH) 284 nm (ε 23823); fabms (glycerol) *m/z* $[M+H]^+$ 441 (36% rel. int.), 423 (49), 405 (21), 377 (19), 361 (14), 349 (9.5), 240 (25), 217 (20), 203 (23), 189 (48), 150 (100), 131 (29); ^{1}H and ^{13}C nmr see Table 1; hrfabms observed *m/z* $[M+H]^+$ 441.1527, calcd for $C_{24}H_{24}O_8$ + H, *m/z* 441.1529.

The Me_2CO-soluble portion of the crude extract described above (306 mg) showed some residual antibiotic activity and was chromatographed on a column of Si gel (4 × 25 cm) using a gradient of 0–25% MeOH in $CHCl_3$. Late fractions were combined to afford 44.5 mg of a yellow oil that was then chromatographed on a column of Sephadex LH-20 (2 × 16 cm) eluting with CH_2Cl_2-hexane (4:1). The major component **2** (auranticin B, 24.8 mg) was isolated as an orange oil which showed a single, broad peak by hplc

(retention time 8.4 min; same conditions as above). Compound **2** gave the following data: ir (neat) 3264 (br, COOH) 2915, 2851, 1734, 1717, 1653, 1616, 1416, 1265, 1130 cm^{-1}; uv max (MeOH) 350 nm (ε 5957), 266 (ε 18578); eims (70 eV) *m/z* $[M]^+$ 438 (3.7% rel. int.), 410 (0.9), 393 (31), 377 (3.2), 365 (7.2), 323 (2.5), 307 (7.2), 241 (1.0), 217 (24), 203 (6.2), 191 (53), 129 (39), 115 (18); ^1H and ^{13}C nmr see Table 1.

METHYLATION OF AURANTICIN A.—A sample of compound **1** (16.3 mg) was treated with an ethereal solution of CH_2N_2. The solution was allowed to stand for 30 min, and the solvent was then evaporated to afford compound **3** (16.7 mg) as a yellow oil: eims (70 eV) *m/z* $[M]^+$ 468 (4.4% rel. int.), 450 (20), 405 (8.1), 391 (100), 303 (24), 261 (6.9), 203 (7.3), 189 (21), 173 (11), 129 (22), 115 (31); ^1H and ^{13}C nmr see Table 1.

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