Full Papers

Isolation and Structural Studies on the Chemical Constituents of *Skimmia laureola*

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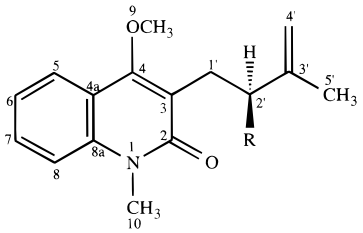
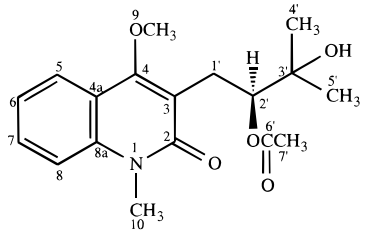
Studies on the chemical constituents of the aerial parts of *Skimmia laureola* have led to the isolation of four new alkaloids, ptelefoliarine (**1**), acetoxyptelefoliarine (**2**), acetoxyedulinine (**3**), and orixiarine (**4**). Their structures were established by spectroscopic studies.

Skimmia laureola Hook. (Rutaceae) is an aromatic gregarious evergreen shurb that is widely found in the Western Himalayas and Kashmir. The leaves of this plant are used for the treatment of smallpox.[1] *Skimmia* species contain triterpenoids that are generally of the lupane type. The leaves of *S. laureola* are known to contain lupenone, lupeol, β-sitosterol, scopoletin, umbelliferene, bergapten, and isopimpinellin. The bark of the plant is reported to contain bergapten, linalool, and sucrose. A number of bioactive constituents have been reported from *Skimmia laureola*. Four alkaloids isolated from this plant, named chimanines A−D, were found to be active in vitro against *Leishmania braziliensis* and *Trypanosoma cruzi*. A poisonous crystalline alkaloid skimmianine has been isolated from this plant.[1] Our studies on the leafy shoots have led to the isolation of four new alkaloids, **1**−**4**, from the aerial parts of *S. laureola*.



1) R = OH
2) R = $OCOCH_3$



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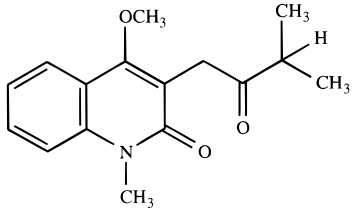

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Results and Discussion

Compound **1** was obtained as a yellow gummy substance. Its empirical formula was derived as $C_{16}H_{19}NO_3$ from the HREI MS (M^+ *m/z* 273.1365, calcd 273.1364) indicating eight double-bond equivalents in the molecule. The UV spectrum of **1** showed absorptions at 230, 275, 285, and 330 nm characteristic of 4-alkoxy-2-quinolone alkaloids.[2,3] The IR spectrum featured strong absorptions at 1625 (lactam carbonyl) and 1580 (conjugated C=C) cm^{-1}.

The EI MS of **1** furnished a characteristic strong peak at *m/z* 256 due to the loss of a hydroxyl group from the M^+, confirming the presence of hydroxyl group. The presence of hydroxyl group on the side chain was further supported from the ion at *m/z* 202 corresponding to $C_{12}H_{12}NO_2$, which was generated by the loss of the side chain (71 amu C_4H_7O) from the M^+. Another strong peak at *m/z* 258 was due to the loss of a methyl group from the M^+.

The ^1H NMR spectrum of **1** showed signals for 19 protons. Three proton singlets resonating at δ 3.93, 3.74, and 1.85 were ascribed to OCH_3, NCH_3, and vinylic methyl protons, respectively.[4] The downfield one-proton double doublet at δ 7.83 ($J_{5,6}$ = 8.0 Hz, $J_{5,7}$ = 1.5 Hz) was assigned to H-5. The doublet of double doublet resonating at δ 7.58 ($J_{7,8}$ = 8.5 Hz, $J_{7,6}$ = 7.0 Hz, $J_{7,5}$ = 1.5 Hz) was assigned to H-7. Another doublet of double doublet resonating at δ 7.29 ($J_{6,5}$ = 8.0 Hz, $J_{6,7}$ = 7.0 Hz, $J_{6,8}$ = 1.0 Hz) was assigned to H-6. The downfield 1H doublet at δ 7.40 was assigned to H-8, which showed coupling with H-7 ($J_{8,7}$ = 8.5 Hz). The assignment of aromatic protons was also confirmed by the COSY-45° spectrum. The C-5 proton (δ 7.83) showed a cross-peak only with the C-6 proton (δ 7.29). The C-6 proton showed COSY interactions with C-5 and C-7 protons. Similarly, the C-7 proton (δ 7.58) showed cross-peaks only with C-6 (δ 7.29) and C-8 (δ 7.40), while the C-8

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **2**

	1			**2**		
C	δ^{13}C	^{13}C-multiplicity	attached proton (HMQC) δH (J = Hz)	δ^{13}C	^{13}C-multiplicity	attached proton (HMQC) δH (J = Hz)
2	165.9	−C−		163.6	−C−	
3	117.7	−C−		117.4	−C−	
4	161.8	−C−		161.7	−C−	
4a	120.1	−C−		118.8	−C−	
5	123.7	CH	7.83 (dd, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.5)	123.5	CH	7.81 (dd, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.5)
6	122.5	CH	7.29 (ddd, $J_{6,5}$ = 8.0, $J_{6,7}$ = 7.0, $J_{6,8}$ = 1.0)	121.8	CH	7.28(ddd, $J_{6,5}$ = 7.6, $J_{6,7}$ = 5.6)
7	130.8	CH	7.58 (ddd, $J_{7,8}$ = 8.5, $J_{7,6}$ = 7.0, $J_{7,5}$ = 1.5)	130.4	CH	7.56 (ddd, $J_{7,8}$ = 8.6, $J_{7,6}$ = 7.1, $J_{7,5}$ = 1.5)
8	114.5	CH	7.40 (d, $J_{8,7}$ = 8.5)	114.2	CH	7.37 (d, $J_{8,7}$ = 8.4)
8a	139.1	−C−		139.4	−C−	
9	62.4	OCH_3	3.93 (s)	61.9	OCH_3	3.95 (s)
10	38.4	NCH_3	3.74 (s)	29.7	NH_3	3.70 (s)
1′	32.1	CH_2	3.06 (dd, $J_{1'\alpha,1'\beta}$ = 14.0, $J_{1'\alpha,2'\alpha}$ = 9.0), 2.94 (dd, $J_{1'\beta1'\alpha}$ = 14.0, $J_{1'\beta,2'\alpha}$ = 3.0)	29.5	CH_2	3.08 (dd, $J_{1'\alpha,1'\beta}$ = 11.4, $J_{1'\beta,2'\alpha}$ = 5.1)
2′	76.2	CH	4.31 (dd, $J_{2'\alpha,1'\beta}$ = 2.5, $J_{2'\alpha,1'\alpha}$ = 9.0)	75.5	CH	5.60 (dd, $J_{2'\alpha,1'\alpha}$ = 8.5, $J_{2'\alpha,1'\beta}$ = 5.1)
3′	147.9	−C−		143.7	−C−	
4′	110.2	CH_2	5.05 (dd,$J_{4'a,4'b}$ = 2.50, $J_{4'a,2'}$ = 1.5), 4.83 (dd, $J_{4'b,4'a}$ = 3.0, $J_{4'b,2'}$ = 1.5)	112.2	CH_2	4.97 (s) 4.85 (dd, $J_{4'b,4'a}$ = 3.0, $J_{4'b,2'}$ = 1.5)
5′	18.0	CH_3	1.85 (s)	18.4	CH_3	1.86 (s)
6′				169.9	C=O	
7′				21.1	CH_3	1.91 (s)

proton (δ 7.40) showed interactions with the C-7 proton (δ 7.58), thereby confirming the chemical shifts of aromatic protons on ring A. The C-5 methine proton showed cross-peaks with H-8 and H-7 in the HOHAHA spectrum. Two AB double doublets at δ 2.94 and 3.06 were assigned to the C-1′ methylene protons, which showed vicinal couplings with H-2′ ($J_{1'\alpha,2'\alpha}$ = 9.0 Hz). The C-2′ proton at δ 4.31 geminal to the OH group resonated as a double doublet ($J_{2'\alpha,1'\alpha}$ = 9.0 Hz, $J_{2'\alpha,1'\beta}$ = 2.5 Hz) due to vicinal couplings with the C-1′ protons. Vinylidine $H_{4'a}$ and $H_{4'b}$ protons appeared as two double doublets at δ 5.05 ($J_{4'a,4'b}$ = 2.50 Hz, $J_{4'a,2'}$ = 1.5 Hz, H-4′a) and 4.83 ($J_{4'b,4'a}$ = 3.0 Hz, $J_{4'b,2'}$ = 1.5 Hz, H-4′b), respectively. The mass spectral fragments and ^{1}H NMR spectrum of **1** were almost superimpossible with those of the known quinoline alkaloids.[5]

The nature of the C-3 side chain was also investigated by using 2D-NMR spectroscopic techniques. The COSY spectrum showed that the downfield C-2′ proton (δ 4.31) was vicinally coupled to the C-1′ methylene protons (δ 3.06). In the HOHAHA spectra, the vinylic methyl resonating at (δ 1.85) showed long-range couplings with the C-4′ vinylidene protons (δ 4.83, 5.05). This suggested the presence of an isopropenyl group. The C-1′ methylene protons resonating at δ 3.06 and 2.94 showed cross-peaks with the C-4′ methylene protons resonating at δ 5.05 and 4.83 and with the methine proton resonating at δ 4.31 in the HOHAHA spectrum. The absolute configuration at C-2′ was established as *S* by Horeau's procedure (see the Experimental Section).

The broad-band-decoupled ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of **1** showed signals for all 16 carbons. The DEPT spectra[6,7] showed the presence of two methylenes, five methines, and three methyls and, hence, by difference from the broad-band spectrum, six quaternary carbons (Table 1). The downfield signals at δ 110.2 and 147.9 were assigned to the olefinic C-4′ and C-3′, respectively.[5] Other downfield signals at δ 161.8 and 117.7 were due to the C-4 and C-3 olefinic carbons. The three methyl carbons at δ 62.4, 38.1, and 18.0 were assigned to the OMe, NMe, and vinylic CH_3 carbons, respectively. The methylenic signal resonating at δ 32.1 was assigned to C-1′. The downfield chemical shift of C-2′ (δ 76.2) is consistent with the presence of an attached hydroxyl substituent. Compound **1** seems to be closely identical to didemethoxyptelefoline and (demethylenedioxy)ptelefolidine. The chemical shift assignments to the various carbons are presented in Table 1.

The heteronuclear multiple quantum coherence (HMQC) spectrum[8] of **1** displayed cross-peaks between the directly coupled carbon−proton pairs. The C-1′ protons at δ 3.06 showed a cross-peak with C-1′ (δ 32.1), while the C-2′ proton resonating at δ 4.31 was coupled with C-2′ (δ 76.2). Other HMQC interactions are presented in Table 1.

The heteronuclear multiple bond connectivity (HMBC) spectrum[9] of **1** helped to determine the position of the double bond between C-3 and C-4. Moreover, C-3 (δ 117.7) and C-4 (δ 161.8) showed long-range interactions with H-1′ (δ 3.06) and H-2′ (δ 4.31). The C-3′ also showed coupling with H-4′ (δ 5.05). The vinylic C-5′ methyl protons showed interactions with C-3′, C-2′, and C-4′ protons, respectively, confirming the position of the side chain. From an analysis of all of the above data, the structure of compound **1** was established as ptelefoliarine.

Compound **2** was isolated as a light yellow gummy substance. Its IR spectrum displayed strong absorptions at 1738 (ester C=O) and 1640 (lactam carbonyl) cm^{-1}. The HREI MS spectrum of **2** showed the M^+ at *m*/*z* 315.146 (calcd 315.167). Its molecular formula was therefore derived as $C_{18}H_{21}NO_4$, indicating the presence of nine double bond equivalents in the molecule. The M^+ of **2** was 42 amu higher than that of **1**. The mass fragmentation pattern of **2** was similar to that of **1** except that the ion at *m*/*z* 274 appeared at 42 amu higher than the corresponding ion in compound **1** (*m*/*z* 232). An important peak at *m*/*z* 256.1310 corresponding to the formula $C_{16}H_{18}O_2N$ appeared due to the loss of a $C_2H_3O_2$ unit from the M^+, thus indicating the presence of an acetate unit at C-2′ of the side chain.

The ^{1}H NMR spectrum of **2** was very similar to that of **1** except that it showed the downfield 1H double doublet for the C-2′ methine proton at δ 4.31 in **1** now appeared at δ 5.60 ($J_{2'\alpha,1'\alpha}$ = 8.5 Hz, $J_{2'\alpha,1'\beta}$ = 5.1 Hz). This was indicative of the presence of an acetate group

Table 2. ^{1}H and ^{13}C NMR Data of Compounds 3 and 4

	3			**4**		
C	δ^{13}C	^{13}C-multiplicity	attached proton (HMQC) δH (J = Hz)	δ^{13}C	^{13}C-multiplicity	attached proton (HMQC) δH (J = Hz)
2	164.2	−C−		165.1	−C−	
3	117.4	−C−		118.6	−C−	
4	161.8	−C−		163.9	−C−	
4a	119.4	−C−		118.2	−C−	
5	123.5	CH	7.78 (dd, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.5)	124.5	CH	7.87 (dd, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.5)
6	122.1	CH	7.24 (ddd, $J_{6,5}$ = 8.0, $J_{6,7}$ = 7.0, $J_{6,8}$ = 1.0)	123.6	CH	7.33 (ddd, $J_{6,5}$ = 8.00, $J_{6,7}$ = 7.0, $J_{6,8}$ = 1.0)
7	130.5	CH	7.54 (ddd, $J_{7,8}$ = 8.5, $J_{7,6}$ = 7.0, $J_{7,5}$ = 1.5)	132.1	CH	7.64 (ddd, $J_{7,8}$ = 8.5, $J_{7,6}$ = 7.0, $J_{7,5}$ = 1.5)
8	114.4	CH	7.37 (d, $J_{8,7}$ = 8.5)	115.9	CH	7.59 (d, $J_{8,7}$ = 8.0)
8a	139.3	−C−		140.5	−C−	
9	61.9	OCH_3	3.91 (s)	62.7	OCH_3	3.88 (s)
10	29.8	CH_3	3.70 (s)	30.7	CH_3	3.70 (s)
1′	25.9	CH_2	3.04 (d, $J_{1'\alpha,2'\alpha}$ = 6.5)	37.8	CH_2	3.86 (s)
2′	78.4	CH	5.18 (dd, $J_{2'\alpha,1'\alpha}$ = 7.0, $J_{2'\alpha,1'\beta}$ = 5.5)	214.1	−C−	
3′	72.2	−C−		42.0	CH	2.89 (m)
4'	26.6	CH_3	1.26 (s)	30.3	CH_3	1.18 (d,$J_{4',3'}$ = 2.5)
5′	25.5	CH_3	1.31 (s)	18.7	CH_3	1.19 (d, $J_{5',3'}$ = 2.5)
6′	176.0	C═O				
7'	21.0	CH_3	1.91 (s)			

instead of a hydroxyl group. To confirm the stereochemistry at C-2′, compound **2** was subjected to hydrolysis followed by Horeau's esterification (see the Experimental Section) which established the *S* configuration at C-2′. To establish that compound **2** was a genuine natural product and not an artifact, a comparative TLC of compound **2** against the crude ethanolic extract of the fresh plant was studied, thus establishing that compound **2** was a naturally occurring compound as it was present in the crude extract with the same TLC mobilities (R_f = 0.23 in $CHCl_3$/MeOH 95:5). On the basis of this spectroscopic evidence, compound **2** was identified as the naturally occurring *O*-acetyl derivative of **1**.

Compound **3** was isolated as a light brown substance. Its empirical formula was derived as $C_{18}H_{23}NO_5$ from the HREI mass spectrum (*m/z* 333.1553, calcd 333.1576) indicating the presence of eight double-bond equivalents in the molecule. The IR spectrum (KBr) showed absorption bands at 1590 (conjugated olefin), 1625 (six-membered lactam C═O), and 1722 ester (carbonyl group) cm^{-1}. The UV spectrum of **3** showed absorptions at 325, 292, 278, 231, and 229. The M$^+$ for **3** was 18 amu higher than that of **2**. An important peak at *m/z* 274.1135 corresponding to the formula $C_{16}H_{20}NO_3$ was due to the loss of a $-C_3H_7O$ unit from the M$^+$, thus indicating the loss of a hydroxyl-bearing isopropyl group. The mass spectrum of **3** contained peaks at *m/z* 318 and 289 that were higher by 18 amu in comparison to **2**. The ions at *m/z* 214, 259, and 274 containing the side chain indicated the presence of a hydroxyl-bearing isopropyl group.

The ^{1}H NMR spectrum of **3** was similar to that of **2** except that it showed the upfield shift of the C-4′ protons to δ 1.26 (s) and the C-5′ protons to δ 1.31 (s) due to the absence of olefinic carbons as was in **2**. The stereochemistry of the acetate group at C-2′ was confirmed by acylation of the hydrolyzed compound using Horeau's procedure (see the Experimental Section).

The ^{13}C NMR spectrum of **3** exhibited the upfield shift of C-4′ to δ 26.6 and C-5′ to δ 25.5 due to the presence of a hydroxyl group at C-3′ and the absence of olefinic carbons as found in **2**. Compound **3** also shared the same R_f with the crude extract before further processing exhibiting the same TLC mobilities (R_f = 0.66 in $CHCl_3$/MeOH 94:6). On the basis of this evidence, compound **3** (acetoxyedulinine) was identified as the naturally occurring hydrated analogue of **2**.

Compound **4** (orixiarine)was obtained as a yellow substance. Its empirical formula was derived as $C_{16}H_{19}NO_3$ from the HREI mass spectrum (M$^+$ observed *m/z* 273.1353, calcd 273.1364, indicating the presence of eight double-bond equivalents in the molecule. The mass fragmentation pattern of **4** was similar to that of **1** except for the ion that appeared at *m/z* 230 (*m/z* 232 in **1**), which arose by the loss of 43 amu (C_3H_7), thus indicating the presence of an isopropyl unit instead of a isopropenyl unit.

The ^{1}H NMR spectrum (500 MHz) of **4** was similar to that of **1** except that it showed the downfield shift of the C-1′ methylene protons to δ 3.86 (s) and the C-3′ proton to δ 2.89 (m) due to the presence of the C-2′ oxo group (Table 2).

The ^{13}C NMR spectrum (125 MHz) afforded downfield shifts of C-1′ to δ 37.8, C-3 to δ 42.0, and C-2′ to δ 214.1, in comparison to those of the corresponding carbon atoms in compound **1**, indicating the presence of an oxo function at C-2′. The ^{1}H- and ^{13}C NMR spectra of **4** were found to be consistent with the proposed structure (Table 2).

Experimental Section

General Experimental Procedures. The mass spectra were recorded on a JEOL HX-110 instrument. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 500 and 125 MHz, respectively, on a Bruker AM-500 NMR spectrometer. The UV and IR spectra were recorded on Shimadzu UV-240 and JASCO A-302 spectrophotometers, respectively. Optical rotations were measured on a Polatronic D polarimeter. The purity of the compounds was checked on TLC (Si gel, Merck PF 254, 0.25 mm thickness).

Plant Material. The aerial parts of *S. laureola* was collected from Azad Kashmir in 1992. A voucher specimen is deposited in the Herbarium of Department of Botany, University of Karachi.

Extraction and Isolation. Air-dried aerial part (20 kg) of *S. laureola* was extracted with EtOH (100 L). The

EtOH extract of the whole plant was concentrated to a gum (822 g), dissolved in distilled water, and extracted thoroughly with petroleum ether (40−60 °C) (45 L). The petroleum ether-soluble portion was evaporated under reduced pressure to a gum (67 g). The remaining aqueous layer was extracted with $CHCl_3$ at neutral pH. The $CHCl_3$-soluble portion was evaporated under reduced pressure to a gum (20.0 g) and chromatographed on a Si gel column. Elution of this column with pure chloroform yielded a mixture (50 mg) containing mainly **1** and **2**. This mixture was chromatographed on a Si gel column (Merck, 70−230 mesh), elution being carried out with 50% petroleum ether and 50% chloroform (2.5 L). The first eight fractions were found to contain compound **1** (20 mg), while fractions 11−19 contained compound **2** (25 mg).

Compound **1** was purified by preparative TLC (Si gel) using $CHCl_3$−MeOH (97:3) to afford the pure compound (15 mg) 7.5×10^{-5}% yield with $R_f = 0.53$. Compound **2** was purified by preparative TLC on Si gel using $CHCl_3$−MeOH (95:5) to afford pure **2** (18 mg) in 9.0×10^{-5}% yield.

The remaining aqueous layer was acidified with acetic acid to pH 3, and the aqueous acidic layer was then extracted with $CHCl_3$. The aqueous acidic layer was made alkaline with NH_4OH to pH 12 and extracted with $CHCl_3$ (40 L). The $CHCl_3$-soluble portion was dried over Na_2SO_4, filtered, and evaporated to dryness in a vacuum to afford a crude alkaloidal mixture (224 g). This mixture was chromatographed on a Si gel column (Merck, 70−230 mesh), elution being carried out with petroleum ether containing increasing ratios of $CHCl_3$. Subsequent elution of this column with 96% $CHCl_3$−MeOH yielded an impure mixture containing compounds **4** and **3**. This mixture was chromatographed on a SiO_2 gel column (Merck, 70−230 mesh), which was first eluted with (hexane−$CHCl_3$ 20:80) (2.5 L). Fractions 9−20 were found to contain compound **4** (20 mg). Fractions 9−20 were combined and again chromatographed on a Si gel column using $CHCl_3$−MeOH (98:2). Compound **4** was purified by preparative TLC (Si gel) using $CHCl_3$−MeOH (97:3) to afford pure **4** (15 mg, 7.5×10^{-5}% yield with $R_f = 0.66$). Fractions 30−42 obtained by elution with $CHCl_3$/MeOH (94:6) were combined and again chromatographed on a silica gel column to afford pure compound **3** (23 mg), 1.1×10^{-4}% yield.

Ptelefoliarine (1): light brown gum; $[\alpha]^{29}_D$ −8° (c = 0.12, $CHCl_3$); UV λ_{max} (MeOH) 232 (log ϵ 4.39) nm; IR ($CHCl_3$) ν_{max} 1625 (lactam ring), 1580 (C═C, conju) cm^{-1}; EI MS m/z (rel int) 273 $[M]^+$, 131 (3) 159 (3), 188 (67), 203 (100), 232 (3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.83 (1H, dd, $J_{5,6}$ = 8.0 Hz, $J_{6,7}$ = 7.0 Hz, $J_{5,7}$ = 1.5 Hz, H-5), 7.58 (1H, ddd, $J_{7,8}$ = 8.5 Hz, $J_{7,6}$ = 7.0 Hz, $J_{7,5}$ = 1.5 Hz, H-7), 7.29 (1H, ddd, $J_{6,5}$ = 8.0 Hz, $J_{6,8}$ = 1.0 Hz, H-6), 7.40 (1H, d, $J_{8,7}$ = 8.5 Hz, H-8), 3.74 (3H, s, NCH_3), 3.94 (3H, s, OCH_3), 3.06 (1H, dd, $J_{1'\alpha,1'\beta}$ = 14.0 Hz, $J_{1'\alpha,2'\alpha}$ = 9.0 Hz, H-1′a), 2.94 (1H, dd, $J_{1'\beta,1'\alpha}$ = 14.0 Hz, $J_{1'\beta,2'\alpha}$ = 3.0 Hz, H-1′b), 4.31 (1H, dd, $J_{2'\alpha,1'\alpha}$ = 9.0 Hz, $J_{2'\alpha,1'\beta}$ = 2.5 Hz, H-2′), 1.85 (3H, s, CH_3), 5.05 (1H, dd, $J_{4'a,4'b}$ = 2.0 Hz, $J_{4'a,2'}$ = 1.0 Hz, H-4′a), 4.83 (1H, dd, $J_{4'b,4'a}$ = 3.0 Hz, $J_{4'b,2'}$ = 1.5 Hz, H-4′b); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.9 (C-2), 117.7 (C-3), 161.8 (C-4), 120.1 (C-4a), 123.7 (C-5), 130.8 (C-7), 122.5 (C-6), 114.5 (C-8), 139.1 (C-8a), 32.1 (C-1′), 76.2 (C-2′), 147.9 (C-3′), 110.2 (C-4′), 62.4 (OCH_3), 38.1 (NCH_3), 18.0 (CH_3).

Acetoxyptelefoliarine (2): Light brown amorphous substance; $[\alpha]^{29}_D$ −8° (c = 0.13, $CHCl_3$); UV λ_{max} (MeOH) 230 (log ϵ 4.16) nm; IR ($CHCl_3$) ν_{max} 3000 (OH), 1738 (C═O), 1640 (six-membered lactam ring); EI MS m/z (rel int) 315 (3), 274 (2), 256 (4), 202 (47), 172 (8), 188 (31), 158 (10);^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81 (1H, dd, $J_{5,6}$ = 8.0 Hz, $J_{5,7}$ = 1.5 Hz, H-5), 7.56 (1H, ddd, $J_{7,6}$ = 7.1 Hz, $J_{7,8}$ = 8.6 Hz, $J_{7,5}$ = 1.5 Hz, H-7), 7.28 (1H, ddd, $J_{6,5}$ = 7.6 Hz, $J_{6,7}$ = 5.6 Hz, H-6), 7.37 (1H, d, $J_{8,7}$ = 8.4 Hz, H-8), 3.70 (3H, s, NCH_3), 3.95 (3H, s, OCH_3), 3.08 (1H, dd, $J_{1'\alpha,1'\beta}$ = 11.4, $J_{1'\beta,2'\alpha}$ = 5.1 Hz, H-1′α,β), 5.60 (1H, dd, $J_{2',1'a}$ = 8.5 Hz, $J_{2',1'b}$ = 5.1 Hz, H-2′), 1.86 (3H, s, CH_3), 4.97 (1H, s, H-4′a), 4.85 (1H, dd, $J_{4'b,4'a}$ = 3.0 Hz, $J_{4'b,2'}$ = 1.5 Hz, H-4′b), 1.96 (3H, s, $COCH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.6 (C-2), 117.4 (C-3), 161.7 (C-4), 118.8 (C-4a) δ 123.5 (C-5), 130.4 (C-7), 121.8 (C-6), 114.2 (C-8), 139.4 (C-8a), 29.7 (NCH_3), 61.9 (OCH_3), 29.5 (C-1′), 75.5 (C-2′), 143.7 (C-3′), 112.2 (C-4′), 18.4 (C-5′), 169.9 (C═O), 21.1 ($CO\mathit{C}H_3$).

Acetoxyedulinine (3): light brown amorphous substance; $[\alpha]^{29}_D$ −40° (c = 0.025, $CHCl_3$); UV λ_{max} (MeOH) 229 (log ϵ 4.55), 231 (log ϵ 4.57) nm; IR ($CHCl_3$) ν_{max} 1625, 1722 cm^{-1}; EIMS m/z (rel int) 274 (3.4), 232 (56), 202 (22), 214 (100), 188 (20), 59 (36); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.78 (1H, dd, $J_{5,6}$ = 5.5 Hz, $J_{5,7}$ = 1.5 Hz, H-5), 7.24 (1H, ddd, $J_{6,5}$ = 8.0 Hz, $J_{6,8}$ = 1.0 Hz, H-6), 7.54 (1H, ddd, $J_{7,8}$ = 8.5 Hz, $J_{7,6}$ = 7.00 Hz, $J_{7,5}$ = 1.5 Hz, H-7), 7.37 (1H, d, $J_{8,7}$ = 8.5 Hz, H-8), 3.70 (3H, s, CH_3), 3.91 (3H, s, OCH_3), 3.04 (2H, d, $J_{1'\alpha,2'\alpha}$ = 6.5 Hz, H-1′), 1.26 (3H, s, CH_3, H-4′), 1.31 (3H, s, CH_3, H-5′), 5.18 (1H, dd, $J_{2'\alpha,1'\alpha}$ = 7.0 Hz, $J_{2'\alpha,1'\beta}$ = 5.5, H-2′), 1.91 (3H, s, C−CH_3); ^{13}C NMR (CD_3OD, 125 MHz) δ; 164.2 (C-2), 117.4 (C-3), 161.8 (C-4), 119.4 (C-4a), 123.5 (C-5), 122.1 (C-6), 130.5 (C-7), 114.4 (C-8), 139.3 (C-8a), 25.9 (C-1′), 78.4 (C-2′), 72.2 (C-3′), 26.6 (C-4′), 25.5 (C-5′), 61.9 (OCH_3), 21.0 (CO−C-7′), 29.8 (CH_3).

Orixiarine (4): light brown amorphous substance; UV λ_{max} (MeOH) 236 (log ϵ 4.25), 232 (log ϵ 4.24) nm; IR ($CHCl_3$) ν_{max} 1722 (C═O) and 1628 (six-membered lactam ring) cm^{-1}; EIMS m/z (rel int) 273 $[M^+]$, 104 (15), 131 (9), 143.9 (27), 171.9 (36), 188 (33), 202 (100), 230 (56); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.87 (1H, dd, $J_{5,6}$ = 8.0 Hz, $J_{5,7}$ = 1.5, Hz, H-5), 7.64 (1H, ddd, $J_{7,8}$ = 8.5 Hz, $J_{7,6}$ = 7.0 Hz, $J_{7,5}$ = 1.5 Hz, H-7), 7.33 (1H, ddd, $J_{6,5}$ = 8.0 Hz, $J_{6,7}$ = 7.0 Hz, $J_{6,8}$ = 1.5 Hz, H-6), 7.59 (1H, d, $J_{8,7}$ = 8.0 Hz, H-8), 3.70 (3H, s, CH_3), 3.88 (3H, s, OCH_3), 3.86 (2H, s, H-1′), 1.18 (3H, d, $J_{1,2}$ = 2.5 Hz, H-4′), 1.19 (3H, d, $J_{1,2}$ = 2.5 Hz, H-5′), 2.89 (1H, m, H-3′); ^{13}C NMR (CD_3OD, 125 MHz) δ 165.1 (C-2), 118.6 (C-3), 163.9 (C-4), 118.2 (C-4a), 124.5 (C-5), 132.1 (C-7), 123.6 (C-6), 115.9 (C-8), 140.5 (C-8a), 37.8 (C-1′) 214.1 (C-2′), 42.0 (C-3′), 30.3 (C-4′), 18.7 (C-5′), 62.7 (C-9).

Horeau's Procedure. The sample compound (5 mg, ca. 001 mmol) was added to a solution of racemic 2-phenylbutanoic anhydride (0.1 mL) in 0.5 mL of pyridine. The resulting mixture was stirred overnight at room temperature. Distilled water (0.3 mL) was added and the reaction mixture allowed to stand for 30 min. NaOH (0.1 M) was then added dropwise until the pH became 9, and the solution was then extracted with $CHCl_3$. The aqueous layer was acidified to pH = 3 using 1 M HCl and the acidic layer extracted with C_6H_6 (10

mL). The benzene extract was evaporated to adjust the volume to 1 mL. The optical rotation of the resulting 2-phenylbutanoic acid in aqueous solution was found to be positive (*R*), thereby establishing the *S*-configuration of the hydroxyl group at C-2′ in compound **1**.

References and Notes

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