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PANICEINS AND RELATED SESQUITERPENOIDS FROM THE MEDITERRANEAN SPONGE *RENIERA FULVA*

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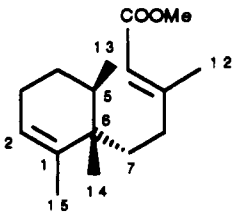
ABSTRACT.—Two novel sesquiterpenes, fulvanin 1 [**1**] and fulvanin 2 [**2**], have been isolated from the Mediterranean sponge *Reniera fulva*. Compound **2** is a sesquiterpenoid linked to a hydroquinone. Also found in *Reniera fulva* are four known paniceins, a group of aromatic sesquiterpenoids linked to a quinol, isolated previously from *Halichondria panicea*. The total assignments of ^{13}C-nmr spectra of paniceins have been made with the aid of 2D ^{1}H-^{13}C correlation techniques, confirming their structures. The paniceins were tested in the in vitro disease-oriented primary antitumor screen at NCI, Bethesda. Paniceins C and B3 showed cytotoxicity against NCI-H522 non-small lung cancer cells and CCRF-CEM leukemia cells, while panicein A hydroquinone revealed more selective cytotoxicity against the latter ones.

A diverse array of secondary metabolites have been isolated from *Reniera* sp. The group of three arylcarotenoids, renieratin, isorenieratin, and renierapurpurin, were isolated from *Reniera japonica* (1–4). Another *Reniera* sp. collected in the vicinity of Isla Grande, Mexico contained an antimicrobial isoquinoline quinone, renierone (5), along with the dimeric renieramycins (6,7), which supposedly have an actinomycete origin associated with the *Reniera* sp. The Mediterranean *Reniera sarai* was the source of a unique group of pentacyclic alkaloids, the sarains (8–11). *Reniera fulva* (Topsent) (family Renieridae), collected in the bay of Naples, contains five diacetylene metabolites (12). In June 1989 during an underwater expedition near Favignana, Egadi Islands, we collected samples of *R. fulva* from which we isolated two new sequiterpenes, designated fulvanin 1 [**1**] and fulvanin 2 [**2**], along with four known compounds: panicein B3 [**3**], panicein B2 [**4**], panicein A hydroquinone [**5**], and panicein C [**6**] previously isolated from *Halichondria panicea* (13).

The Me_2CO extract of the sponge was partitioned between H_2O and Et_2O. The Et_2O fraction was chromatographed on Si gel using hexane and increasing amounts of EtOAc as eluents. The first eluted fractions contained the sesquiterpene **1** and were further purified by hplc on a Whatman Partisil 10 column. The more polar fractions contained the prenylated hydroquinones **2–6**, which were separated by reversed-phase hplc.

Fulvanin 1 [**1**], viscous oil, $[\alpha]_D$ +7.9°, has the molecular formula $C_{16}H_{26}O_2$, as deduced from eims and ^{13}C nmr (Table 1). The ir bands at 1720 and 1645 cm^{-1} and uv absorption at 233 nm (ϵ=14000) were in agreement with an α,β-unsaturated ester. The ^{1}H-nmr spectrum contained signals at δ 3.68 (3H, s), 5.67 (1H, s), and 2.17 (3H, brs)



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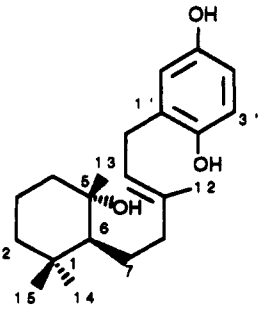

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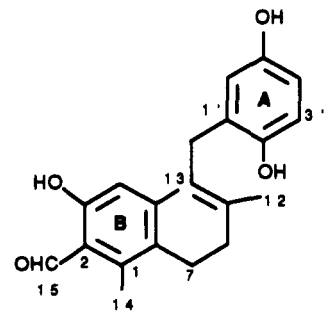

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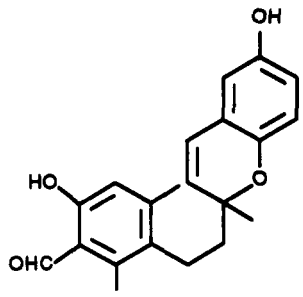

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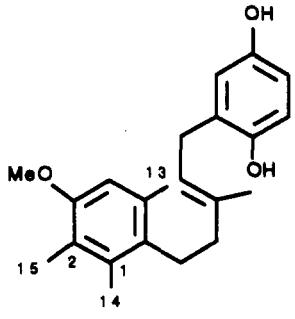

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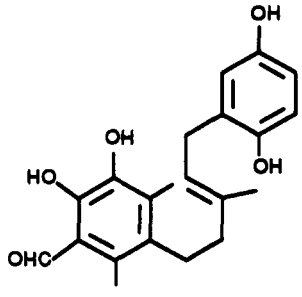

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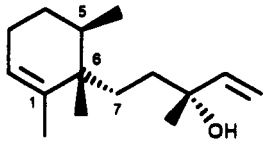
assigned to Me-C=CH-CO_2Me part structure (Me\$CO_2$Me cis) (14). In the ^{1}H-^{1}H 2D COSY spectrum, the olefinic proton signal at δ 5.44 (brs) gave a cross peak with the vinyl methyl resonance at δ 1.60 (3H, brs), and two other methyl signals at δ 0.86 (3H, d, J=7 Hz) and 0.87 (3H, s). These structural parts can be readily accommodated in a rearranged mono-cyclofarnesane skeleton as in **1**. The intense ion at *m/z* 123 (base peak) in the mass spectrum of **1**, also seen in striatol [**7**] (15) and microcionin 2 [**8**] (16), gave support to this rearranged structure. The chemical shifts of the methyl signals at C-5 and C-6 (δ 0.86 and 0.87), however, clearly favored the relative stereochemistry of striatol [**7**] (δ 0.87 and 0.88) over that of microcionin 2 [**8**] (δ 0.99 and 1.08). A comparison of the ^{13}C-nmr spectra of **1** (Table 1) with that of striatol [**7**] revealed an excellent correlation for the relevant signals. HETCOR experiments helped assign the carbon resonances for **1** (Table 1). With the assignment of **1** established, the assignments for the striatol [**7**] and the related diterpene ageline A [**9**] (17), which also possess the same 6-alkyl-1,5,6-trimethyl-1-cyclohexene ring, were subsequently revised as in Table 1. The absolute configuration of **1** is probably 5*R*,6*S* since the sign of the molecular rotation, [M]D +19.8°, is of the same sign of striatol [**7**], [M]D +100°, and opposite that of ageline A [**9**], [M]D −35°.

Fulvanin 2 [**2**] in its fabms in the negative ion mode exhibited a molecular ion at *m/z* 331 $[M-H]^-$. The uv band at 295 nm and ^{1}H-nmr signals at δ 6.57 (dd, J=8.3, 2.8 Hz), 6.67 (d, J=2.8 Hz), and 6.68 (d, J=8.3 Hz) are consistent with a monosubstituted 1,4-quinol structure. The ^{1}H nmr also contained four methyl signals at δ 1.76 (s), 1.15 (s), 0.92 (s), and 0.82 (s), and a vinyl proton signal at δ 5.30 (brt, J=7.2 Hz), which was



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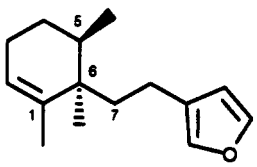

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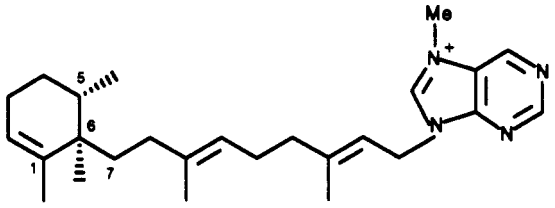

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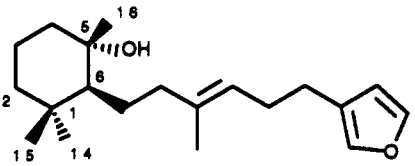
coupled to the signals at δ 3.24 (dd, J=15.2, 7.2 Hz) and 3.33 (dd, J=15.2, 7.2 Hz), assigned to the benzylic methylene. The ^{13}C-nmr spectrum contained signals at δ 139.1 (quaternary) and 121.0 (CH), assigned to a trisubstituted olefin, and at 74.3 (quaternary) due to a tertiary alcohol carbon. On the assumption that we are dealing with a regular sesquiterpene skeleton, we assigned the structure **2** to fulvanin 2. The signals in the ^{13}C-nmr spectrum of fulvanin 2 (Experimental) were assigned by comparison with data for model compounds (18). The chemical shift of the olefinic methyl signal at 16.3 ppm indicated that the olefinic bond has the *E* geometry. A comparison of the ^{1}H and ^{13}C

TABLE 1. ^{13}C and ^{1}H nmr Spectral Data of Fulvanin 1 [**1**] ($CDCl_3$, 125 and 500 MHz)[a] Compared with the Relevant ^{13}C Spectral Data for Striatol [**7**] and Ageline A [**9**].

Position	Compound					
	1				**7**	**9**
	^{13}C	^{1}H			^{13}C	^{13}C
	δ	δ	mult.	J (Hz)	δ	δ
1	139.0				139.3	139.5
2	124.7	5.44	bs		124.0	122.4
3	25.5	1.95	m		25.4[b]	25.5[b]
4	27.0	1.45	m		27.0[b]	27.0[b]
5	33.3	1.68	m		33.2	33.1
6	40.5				40.0	40.3
7	34.4	1.53	m			
8	35.8	1.79	dt	14, 5.7		
		2.08	dt	14, 5.7		
9	161.6					
10	114.7	5.67	s			
11	167.3					
12	19.1	2.17	s			
13	15.8	0.86	s		15.7[b]	15.8[b]
14	21.0	0.87	s		21.0[b]	21.0[b]
15	19.1	1.60	bs		19.0[b]	19.2[b]
OMe	50.8	3.68	s			

[a]Assignments made by H-C correlation experiments (HETCOR).
[b]Revised assignments on the basis of spectral data of **1**.

spectra of **2** with that of ambliol A [**10**], a major diterpene isolated from *Dysidea amblia* (19), revealed an excellent correlation for the relevant signals (δ_H 0.82 s, 0.92 s, and δ_C 33.0, 21.4 assigned to the gem-methyl groups, and δ_H 1.15 and δ_C 23.3 to the methyl on the oxygen-bearing carbon in **2**; in ambliol A the same signals were observed at δ_H 0.80 s, 0.93 s, and 1.10 and δ_C 33.0, 21.6, and 23.5 ppm, respectively). On this basis we propose the relative stereochemistry as shown in **2**, with the hydroxyl group and the side chain both equatorial with respect to the cyclohexane ring.



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The structures of paniceins were elucidated from chemical and ir, uv, and ^{1}H-nmr spectral data (13). In particular, the relative positions of the substituents in the aromatic sesquiterpenoid ring B of panicein B3 [**3**] were assigned by spin decoupling and nOe experiments. Two decades have passed since this report, and with the advent of 2D nmr techniques in recent years, it is now possible to re-investigate the structures of these compounds. In the present work we report the total assignments of the ^{13}C-nmr spectrum of **3** (Table 2) on the basis of 2D one bond and long range proton-carbon shift correlation spectroscopy using ^{13}C detection (HETCOR and COLOC). We have confirmed the substituent assignment on the sesquiterpenoid ring B. The ^{13}C-nmr spectrum of **3** exhibited the expected 21 carbon resonances (Table 2). While the side chain and hydroquinol carbon resonances assignments could be made unequivocally on the basis of ^{13}C chemical shift arguments and HETCOR spectra, the ring B carbon resonance assignments required COLOC (20). Long-range correlations observed in the COLOC experiments are summarized in Table 3.

The ^{13}C chemical shifts of the chromenol panicein B2 [**4**] have been now made by comparison with **3**.

Compound **5** is the the quinol corresponding to panicein A and the assignments of the relative positions of the substituents of ring B were supported by chemical correlation with **3** (13). Assignment of the ^{13}C spectrum of panicein A hydroquinone [**5**] (Table 2), present in the mixture in small amounts, has now been made with the help of heteronuclear chemical shifts correlation experiments: HMQC (21) and HMBC (22,23).

Finally, the ^{13}C nmr chemical shifts assignments of the minor constituent panicein C [**6**] have been made by comparison with **3**.

Paniceins have been submitted to the in vitro disease-oriented primary antitumor screen at NCI. The screen consists of 60 tumor cell lines against which compounds are tested at a five concentrations (from 10^{-4} to 10^{-8} M) (24). Paniceins C [**6**] and B3 [**3**] showed some cytotoxicity, most active against the CCRF-CEM leukemia cells (panicein C $\log_{10}$ GI50=−5.48, $\log_{10}$ TGI=−5.02; panicein B3 $\log_{10}$ GI50=−5.35, $\log_{10}$ TGI=−4.52), and the NCI-H522 non-small lung cancer cells (panicein C $\log_{10}$ GI50=−5.28, $\log_{10}$ TGI=−4.67, $\log_{10}$ LC50=−4.20; panicein B3 $\log_{10}$ GI50=−5.00, $\log_{10}$ TGI=−4.63, $\log_{10}$ LC50=−4.26). Panicein A hydroquinone revealed an interesting selective cytotoxicity against the CCRF-CEM leukemia cells ($\log_{10}$ GI50=−5.11, $\log_{10}$ TGI>−4.60, $\log_{10}$ LC50>−4.60).

TABLE 2. ^{13}C- and ^{1}H-nmr Spectral Data of Paniceins **3–6** (CD_3OD, 125 and 500 MHz).[a]

Position	Compound															
	3				**4**				**5**				**6**			
	^{13}C	^{1}H			^{13}C	^{1}H			^{13}C	^{1}H			^{13}C	^{1}H		
	δ	δ	Mult.	*J* (Hz)	δ	δ	Mult.	*J* (Hz)	δ	δ	Mult.	*J* (Hz)	δ	δ	Mult.	*J* (Hz)
1	140.8				140.8				136.3				133.6			
2	118.5				118.6				123.2				117.8			
3	162.2				162.2				156.4				149.8			
4	117.5	6.63	s		117.5	6.62	s		111.2	6.60	s		142.4			
5	148.4				148.9				134.4				130.2			
6	132.1				131.9				132.0				132.1			
7	28.7	2.78	m		26.0	2.80	m		29.8	2.76	m		29.0	2.78	m	
8	40.4	2.16	m		41.0	1.77	m		40.8	2.14	m		40.6	2.15	m	
9	136.3				78.0				137.1				136.5			
10	124.5	5.42	t	7.4	131.1	6.42	d	10.1	123.8	5.44	t	7.4	124.3	5.43	t	7.4
11	29.2	3.30	d	7.4	124.3	5.77	d	10.1	29.0	3.31	d	7.4	29.1	3.30	d	7.4
12	16.2	1.85	s		23.9	1.46	s		16.2	1.85	s		16.2	1.85	s	
13	21.3	2.40	s		21.2	2.32	s		20.4	2.33	s		13.1	2.30	s	
14	13.8	2.56	s		13.7	2.55	s		15.7	2.25	s		13.0	2.50	s	
15	197.3	10.36	s		197.2	10.38	s		11.9	2.13	s		197.7	10.34	s	
1′	129.8				123.0				130.0				129.8			
2′	151.0				142.2				151.0				150.9			
3′	116.4	6.62	d	9.2	117.3	6.51	d	8.5	116.4	6.63	d	8.5	116.4	6.63	d	9.2
4′	113.8	6.49	dd	9.2; 3.3	113.7	6.60	dd	8.5; 3.0	113.7	6.49	dd	8.5; 2.8	113.8	6.49	dd	9.2; 3.3
5′	148.9				152.1				148.8				148.8			
6′	117.0	6.58	d	3.3	116.3	6.66	d	3.0	117.0	6.59	d	2.8	117.0	6.59	d	3.3
OMe									55.8	3.79	s					

[a]In compounds **3** and **5** assignments made by H-C correlation experiments (HETCOR and COLOC in **3** and HMQC and HMBC in **5**); in compounds **4** and **6** assignments made by comparison with **3**.

TABLE 3. Selected Data from COLOC Experiment of Panicein B3 [**3**][a] and from HMBC Experiment of Panicein A Hydroquinone [**5**][b] in CD_3OD.

Compound			
3		**5**	
Position	COLOC	Position	HMBC
H-4 (δ 6.63)	*117.5* (C-4), 21.3 (C-13), 118.5 (C-2), 132.1 (C-6), 162.2 (C-3).	H-4 (δ 6.60)	20.4 (C-13), 123.2 (C-2), 132.0 (C-6), 156.4 (C-3).
H-15 (δ 10.36)	*197.3* (C-15), 118.5 (C-2), 162.2 (C-3).	H-15 (δ 2.13)	123.2 (C-2), 136.3 (C-1), 156.4 (C-3).
H-14 (δ 2.56)	*13.8* (C-14), 118.5 (C-2), 132.1 (C-6), 140.8 (C-1).	H-14 (δ 2.25)	123.2 (C-2), 132.0 (C-6), 136.3 (C-1).
H-13 (δ 2.40)	*21.3* (C-13), 117.5 (C-4), 132.1 (C-6), 148.4 (C-5).	H-13 (δ 2.33)	111.2 (C-4), 132.0 (C-6), 134.4 (C-5).

[a]The shifts in italics correspond to the carbons one-bond correlated; the experiment was optimized for long range couplings with a fixed delay $\Delta=83$ msec.

[b]The experiment was optimized for long range couplings with a fixed delay $\Delta=60$ msec. The low pass *J*-filter in the experiment to eliminate responses from direct ($^1J_{CH}$) pairs was optimized for 150 Hz.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were obtained on a Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer, using the UXNMR software package. 2D homonuclear proton chemical shift correlation (COSY) and heteronuclear one bond correlation experiments (HETCOR) experiments were performed by employing the conventional sequence. 2D heteronuclear multiple bond correlation experiment (COLOC) was performed according to H. Kessler (20). The ^{1}H-detected (^{1}H-^{13}C) shift correlation experiments (at 305 K) utilized a 5-mm probe with reverse geometry, and the sample was not spun. ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) experiment was performed according to Bax and Subramanian (21), using an initial BIRD pulse to suppress ^{1}H resonances not coupled to ^{13}C and a GARP sequence for ^{13}C decoupling during data acquisition. ^{1}H-detected heteronuclear multiple bond correlation (HMBC) spectroscopy was performed according to Bax and co-workers (22,23). Fabms were obtained on a VG ZAB mass spectrometer equipped with fab source (in glycerol matrix; Xe atoms of energy of 2–6 keV). Eims were obtained on a Kratos MS 50 instrument. Optical rotations were done on a Perkin-Elmer 141 polarimeter, using a sodium lamp operating at 589 nm. Ir spectra were from a Bruker IFS-48 spectrometer. Uv spectra were from a Beckman DU70 spectrometer. Hplc operations were performed with a Waters Model 6000 A pump with reflective index detection.

EXTRACTION AND ISOLATION.—The sponge *R. fulva* was collected near Favignana (Egadi Islands) in June 1989 and identified by Professor M. Sarà (Zoological Institute, University of Genova). A zoological sample is kept at Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli, under the reference number FA8985. The animals (0.85 kg) were extracted (3×) with Me_2CO at room temperature for 2 days. The combined extracts (3 liters) were concentrated under reduced pressure, and the remaining aqueous solution was extracted with Et_2O (1.5 liters). The Et_2O solution was evaporated to dryness to give an oily residue (2.3 g), which was chromatographed on a Si gel column (Merck Kieselgel 60, 230–400 mesh, 200 g). Elution with hexane allowed the separation of fulvanin 1 [**1**] from the remaining sesquiterpenoid hydroquinones **2–6**, which were eluted with hexane and increasing amounts of EtOAc up to 4:6. Fulvanine 1 [**1**] was further purified by hplc on a Whatman Partisil 10 column (50 cm×10 mm i.d.) using *n*-hexane–EtOAc (98:2) as the eluent. The hydroquinones **2–6** were fractioned by reversed-phase hplc on a Waters μ Bondapak C18 column (30 cm×7.8 mm i.d.) using MeOH-H_2O (65:35) as the eluent.

Fulvanin 1 [**1**].—$[\alpha]_D$ +9° ($CHCl_3$, c=0.4); $[M]_D$ +22.5°, eims *m/z* (rel. int.) $[M]^+$ 250 (4), $[M-Me]^+$ 235 (7), $[M-C\text{-}H_{11}O_2]^+$ 123 (100); uv λ max (MeOH) 233 nm (ε=14000); ir ν max (KBr) 3400–3200 (OH), 1720 (C=O), 1460 and 1380 (Me) cm^{-1}.

Fulvanin 2 [**2**].—Negative ion fabms $[M-H]^-$ 331; uv λ max (MeOH) 295 nm (ε=3200); ir ν max (KBr) 3400–3200 (OH), 1570 (skeletal C=C ring) cm^{-1}; ^{1}H nmr δ ($CDCl_3$, 500 MHz) 6.68 (H-3′, d, J=8.3 Hz), 6.67 (H-6′, d, J=2.8 Hz), 6.57 (H-4′, dd, J=8.3, 2.8 Hz), 5.67 (H-10, t, J=7.2 Hz), 3.33 (H-11, dd, J=15.2, 7.2, Hz), 3.29 (H-11, dd, J=15.2, 7.2 Hz), 1.76 (H-12, s), 1.15 (H-13, s), 0.92 (H-14, s), 0.82

(H-15, s); ^{13}C nmr δ ($CDCl_3$, 125 MHz) 149.5 (C-5′), 147.9 (C-2′), 139.1 (C-9), 128.4 (C-1′), 121.9 (C-10), 116.7 (C-4′), 116.7 (C-6′), 113.6 (C-3′) 74.3 (C-5), 55.9 (C-6), 43.4 (C-2 or C-4 or C-8), 42.4 (C-2 or C-4 or C-8), 41.5 (C-2 or C-4 or C-8), 35.7 (C-1), 33.0 (C-14), 29.6 (C-11), 24.1 (C-7), 23.3 (C-13), 21.4 (C-15), 20.4 (C-3), 16.3 (C-12).

Panicein B3 [**3**].—Eims *m/z*, (rel. int.) $[M]^+$ 340 (10), $[M-177]^+$ 163 (100); positive ion fabms $[M+H]^+$ 341; uv λ max (MeOH) 276 nm (ε=12600), 294 (5100), 346 (3400) ir ν max ($CHCl_3$) 3600 (OH unbonded), 3400–3200 (OH bonded), 2900–2600 (OH chelated), 2825 and 2720 (CHO), 1640 (C=O), 1570 (skeletal C=C rings) cm^{-1}.

Panicein B2 [**4**].—$[\alpha]_D$ +0°; eims *m/z* (rel. int.) $[M]^+$ 338 (8), $[M-175]^+$ 163 (20), $[M-177]^+$ 161 (100); uv λ max (MeOH) 273 nm (ε= 8100), 340 (2800); ir ν max (liquid film) 3600–3200 (OH), 2825 and 2720 (CHO), 1635 (C=O), 1570 (skeletal C=C rings), 1460 and 1380 (Me) cm^{-1}.

Panicein A hydroquinone [**5**].—Eims *m/z* (rel. int.) $[M]^+$ 340 (6), $[M-177]^+$ 163 (100), $[M-179]^+$ 161 (12); uv λ max (MeOH) 275 nm (ε=9200); ir ν max (liquid film) 3600–3100 (OH), 1580 (skeletal C=C rings), 1460 and 1380 (Me), 1290 and 1115 (OMe) cm^{-1}.

Panicein C [**6**].—Eims *m/z* (rel. int.) $[M]^+$ 356.1 (13), $[M-177]^+$ 179.1 (100); positive ion fabms $[M+H]^+$ 357; uv λ max (MeOH) 291 nm (ε=15300), 380 (2100); ir ν max (liquid film) 3600–3100 (OH), 2900–2600 (OH chelated), 2825 and 2710 (CH aldehyde), 1630 (C=O), 1580 (skeletal C=C rings), 1540 and 1380 (Me) cm^{-1}.

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