

## Secondary Mould Metabolites: Part 45.<sup>1</sup> Isolation and Structure Elucidation of Sulcatine G, a Novel Sesquiterpene from *Laurilia Sulcata*

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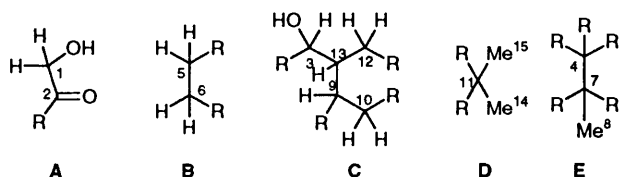
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The structure and the relative configuration of sulcatine G **1a**, a new sesquiterpene isolated from still cultures of *Laurilia sulcata*, has been assigned on the basis of <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy studies; sulcatine G possesses a new ring skeleton which may be derived by contraction of the six-membered ring of a protoilludene sesquiterpenoid.

During our continuing search for biologically active sesquiterpene metabolites produced by *Basidiomycetes* fungi, we reported the isolation of sulcatines A–F, armillol and 5-*epi*-armillol.<sup>2–5</sup> From this fungus we have now isolated a new sesquiterpene, sulcatine G, to which structure **1a** is assigned on the basis of NMR evidence. The compound **1a** was isolated as an oil, [ $\alpha$ ]<sub>D</sub> +44.5 10<sup>-1</sup> deg cm<sup>2</sup> g<sup>-1</sup> (*c* 0.15, CHCl<sub>3</sub>). The IR spectrum (liquid film) exhibited absorption bands at 3410 and 1690 cm<sup>-1</sup> due to hydroxy and carbonyl groups while the molecular formula was established to be C<sub>15</sub>H<sub>24</sub>O<sub>3</sub> by electron impact mass spectrometry (M<sup>+</sup>, 252) and elemental analysis. The two strong peaks observed in the mass spectrum at *m/z* 221 and 193, could be rationalized as being due to sequential loss of CH<sub>2</sub>OH and CO groups from the C-4 side-chain.

The broad-band <sup>1</sup>H-decoupled <sup>13</sup>C NMR spectrum of sulcatine G **1a** (Table 1) showed the presence of 15 signals which, on the basis of their chemical shift and multiplicity determined using DEPT experiments, were assigned to three methyl, five methylene, three methine and three quaternary sp<sup>3</sup>-hybridized carbon atoms and to one carbonyl carbon atom (C-2;  $\delta$  212.96).

The corresponding <sup>1</sup>H NMR spectrum (Table 2) revealed that the three methyl groups are bonded to quaternary carbons as they appeared as singlets, albeit slightly broadened, while the presence of one primary and one secondary hydroxy proton, vicinally coupled to the methylene protons at  $\delta$  4.47 and 4.21 (1-H<sub>2</sub>) and to the methine proton at  $\delta$  4.25 (3-H), was inferred from the disappearance of the resonances at  $\delta$  3.61 and 4.11 upon addition of D<sub>2</sub>O to the sample. The fact that the methylene protons presented vicinal couplings only with the hydroxy proton and exhibited a geminal coupling of 19.0 Hz strongly indicates that the CH<sub>2</sub>OH group is bonded to the sp<sup>2</sup> C-2 quaternary carbonyl carbon<sup>6</sup> as depicted in fragment A.

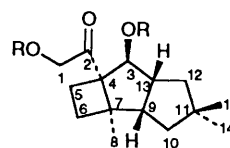


Moreover, the <sup>1</sup>H NMR spectrum showed an AA'MM'-like spin system which was assigned to the protons of an isolated CH<sub>2</sub>-CH<sub>2</sub> moiety (fragment B) while the first order analysis of the remaining multiplets established the presence of the fragment C. These findings, together with the two- and three-bond (<sup>13</sup>C, <sup>1</sup>H) interactions observed in the partial COLOC spectrum, permitted us to construct fragments D and E and to connect the different structural units.

Thus, the cross-peaks observed for both the 14- and 15-

methyl protons with the quaternary C-11 carbon and the C-10 and C-12 methylene carbons require the presence in sulcatine G of a *gem*-dimethyl group linked at C-11 and establish the link between fragments C and D to form a cyclopentane moiety. Similarly, the cross-peaks observed for the 8-methyl protons and C-4, C-6, C-7 and C-9 imply the presence of the fragment E and define the mode of linking of the fragments B, C and E via the formation of the C-6–C-7 and C-7–C-9 bonds.

To obtain the gross structure of sulcatine G **1a** we must then connect the quaternary C-4 carbon to C-2, C-3 and C-5.



**1a** R = H  
**1b** R = Ac

Additional evidence in favour of structure **1a** followed from the value of 135 Hz, typical of a cyclobutane ring, observed in the fully <sup>1</sup>H-coupled <sup>13</sup>C NMR spectrum for the direct (<sup>13</sup>C, <sup>1</sup>H) couplings of both the C-5 and C-6 carbons, and from the long-range (<sup>13</sup>C, <sup>1</sup>H) coupling of 5 Hz observed between C-5 and 3-H upon selective irradiation of 3-H, which is consistent with a three-bond interaction.

The relative configuration of the five chiral centres in sulcatine G was determined by the NOE difference experiments carried

**Table 1** <sup>13</sup>C NMR data for compounds **1a** and **1b** in [<sup>2</sup>H<sub>6</sub>]acetone

Carbon atom	<b>1a</b>		<b>1b</b> <sup>a</sup>
	$\delta_c$	<sup>1</sup> J(C,H)/Hz	
1	68.43 t	142	68.33
2	212.96 s		203.79
3	84.49 d	142	88.34
4	64.27 s		62.90
5	14.40 t	135	14.44
6	32.36 t	135	31.27
7	52.17 s		53.98
8	21.22 q	125	21.03
9	55.64 d	130	55.36
10	44.78 t	128	44.01
11	40.86 s		41.00
12	45.08 t	128	45.46
13	51.50 d	131	50.24
14	32.15 q	124	31.80
15	31.20 q	124	30.88

<sup>a</sup> The carbonyl and methyl carbon atoms of the acetate groups resonate at  $\delta_c$  171.60, 170.30 and 20.66, 20.49.

**Table 2**  $^1\text{H}$  NMR data for compounds **1a** and **1b** in  $[\text{C}_2\text{H}_6]$ acetone

Proton	$\delta_{\text{H}}$		$J(\text{H,H})$	$J/\text{Hz}$	
	<b>1a</b>	<b>1b</b>		<b>1a<sup>a</sup></b>	<b>1b</b>
1a	4.47	5.25	1a, 1b	19.0	16.7
1b	4.21	4.66	1a, 1-OH	4.8	
3	4.25	4.96	1b, 1-OH	5.1	
5 $\alpha$	2.24	2.43	3, 13	9.2	8.8
5 $\beta$	2.24	1.95	3, 3-OH	5.6	
6 $\alpha$	1.83	1.82	9, 10 $\alpha$	11.5	12.7
6 $\beta$	1.83	1.95	9, 10 $\beta$	8.4	7.0
8	0.95	1.03	9, 13	7.3	7.6
9	2.21	2.27	10 $\alpha$ , 10 $\beta$	12.4	12.5
10 $\alpha$	1.38	1.51	10 $\alpha$ , 15	0.7	0.7
10 $\beta$	1.32	1.33	10 $\beta$ , 12 $\beta$	0.8	1.1
12 $\alpha$	1.67	1.64	10 $\beta$ , 13	0.8	1.1
12 $\beta$	1.76	1.80	12 $\alpha$ , 12 $\beta$	13.6	13.8
13	2.68	2.97	12 $\alpha$ , 13	2.3	2.3
14	1.15	1.15	12 $\alpha$ , 15	0.6	0.6
15	0.98	0.99	12 $\beta$ , 13	7.6	8.1
1-OR	3.61	2.09 <sup>b</sup>			
3-OR	4.11	2.02 <sup>b</sup>			

<sup>a</sup>  $J_{5\alpha,6\alpha} + J_{5\alpha,6\beta} \sim 17.2$  Hz. <sup>b</sup> Assignments may be interchanged.

**Table 3** Selected connectivities for compound **1b** established by NOE difference experiments in  $[\text{C}_2\text{H}_6]$ acetone

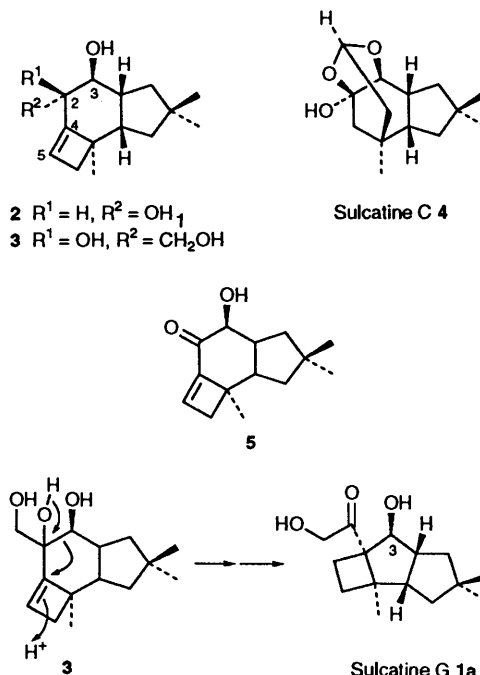
Proton irradiated	Protons affected (%)
1a	1b (17), 3 (1), 5 $\alpha$ (3.5)
1b	1a (18), 5 $\alpha$ (2), 10 $\alpha$ (1)
3	1a (2), 1b (0.5), 10 $\alpha$ (3.5), 12 $\alpha$ (4.5)
8	1a (2), 1b (1.5), 3 (0.5), 6 $\alpha$ (3), 9 (5), 10 $\alpha$ (3.5), 10 $\beta$ (3.5)
9	6 $\beta$ (2.5), 8 (0.5), 10 $\beta$ (3.5), 13 (5), 15 (1.5)
10 $\alpha$	1b (1.5), 3 (6), 8 (0.5), 10 $\beta$ (9.5), 14 (0.5)
10 $\beta$	9 (4), 10 $\alpha$ (8.5), 15 (0.5)
12 $\alpha$	3 (8.5), 12 $\beta$ (9), 13 (1), 14 (1)
13	5 $\beta$ and/or 6 $\beta$ (2), 9 (3.5), 12 $\beta$ (2)
14	3 (2.5), 10 $\alpha$ (4.5), 10 $\beta$ (0.5), 12 $\alpha$ (3.5), 12 $\beta$ (0.5)
15	9 (7), 10 $\alpha$ (0.5), 10 $\beta$ (4.5), 12 $\alpha$ (0.5), 12 $\beta$ (4)

out on the diacetate **1b** (Table 3). In particular, the mutual NOEs observed between 9-H, assumed as  $\beta$ , and 15-H<sub>3</sub> (1.5 and 7%) and 9-H and 13-H (5 and 3.5%) require that these protons are on the same  $\beta$ -side of the molecule whereas the NOEs observed between 14-H<sub>3</sub> and 3-H (2.5%), 8-H<sub>3</sub> and 3-H (0.5%) and 8-H<sub>3</sub> and 1-H<sub>2</sub> (1.5 and 2%) indicate that these protons are on the  $\alpha$ -face.

Having assumed that C-9 has the  $S^*$  configuration, it follows that sulcatine G must have the  $3S^*$ ,  $4S^*$ ,  $7R^*$ ,  $9S^*$ ,  $13R^*$  relative configuration.

As previously reported,<sup>2,3</sup> sulcatines A **2** and B **3** are the first example of protoilludene sesquiterpenoids having a double bond at C-4/C-5 position. These compounds may be possible precursors of sulcatine C **4** which belongs to a new class of sesquiterpenes for which we have proposed the name norisooludalanes,<sup>4</sup> and of sulcatine G **1a**, which contains a new *cis, anti, cis* tricyclic 4.5.5 skeleton. In fact, the formation of sulcatine C may proceed *via* a retro-aldol cleavage of the C-4=C-5 double bond of the keto derivative **5** while sulcatine G may arise by rearrangement of the protoilludene skeleton promoted by acidic bio-catalysis (see Scheme 1). In this context, the conservation of configuration at C-3 is significant.

Like other sulcatines, compound **1a** exhibits antifungal activity on *Cladosporium cladosporioides* and *C. cucumerinum*.<sup>4</sup>

**Scheme 1**

## Experimental

M.p.s were determined on a Kofler apparatus and are uncorrected. IR and UV spectra were recorded with a Perkin-Elmer 177 Instrument and a Jasco Uvidec-510 spectrophotometer, respectively; optical rotations on a Jasco DIP-181 polarimeter; mass spectra on a Finnigan-MATT TSQ70 spectrometer. NMR Spectra were acquired on a Bruker AC 250L spectrometer operating at 250.1 MHz for  $^1\text{H}$  and 62.9 MHz for  $^{13}\text{C}$ . Chemical shifts are in ppm ( $\delta$ ) from  $\text{SiMe}_4$  as internal standard, and  $J$ -values in Hz. DEPT and COLOC spectra were performed using the DEPT and COLOC pulse sequences of the AC 250L software. TLC and PLC were performed with Merck  $\text{HF}_{254}$  silica gel. Owing to the complexity of the purification procedure, we report the  $R_f$ -values in hexane-EtOAc (1:1) and  $\text{CH}_2\text{Cl}_2$ -MeOH (15:1), respectively.

**Isolation and Purification of Metabolite 1a.**—A strain of *Laurilia sulcata* (Burt) Pouzar (CBS 365.49) was maintained on MPGA (malt, peptone, glucose, agar; 20:4:20:15 g dm<sup>-3</sup>) slants and grown as previously described;<sup>3</sup> the extracts were chromatographed on a column of flash silica gel with hexane-EtOAc (2:1) as eluent to yield, after the separation from other sulcatines, sulcatine G **1a** (25 mg);  $R_f$  0.3 and 0.2 (Found: C, 71.1; H, 9.4.  $\text{C}_{15}\text{H}_{24}\text{O}_3$  requires C, 71.39; H, 9.59%);  $\lambda/\text{nm}$  200 ( $\epsilon$  3300);  $m/z$  (EI) 252 ( $\text{M}^+$ )(2%), 234(5), 221(100), 203(20), 193(37) and 175(39);  $m/z$  (CI, isobutane) 235( $\text{MH}^+ - 18$ ), 217, 199, 189 and 173;  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectroscopic data are reported in Tables 1 and 2.

**Acetylation of Sulcatine G 1a.**—Sulcatine G (15 mg) was dissolved in dry pyridine (0.3 cm<sup>3</sup>) containing  $\text{Ac}_2\text{O}$  (0.6 cm<sup>3</sup>) and the solution was kept at 0 °C for 6 h. The mixture was poured into ice-water and extracted with  $\text{CH}_2\text{Cl}_2$ . Evaporation of the solvent followed by PLC in hexane-EtOAc (4:1) gave the diacetate **1b** as a solid (diethyl ether-hexane), m.p. 62 °C;  $m/z$  (FAB, tioglycerine) 337 ( $\text{MH}^+$ )(12%), 336(17), 276(95), 235(67) and 217(100);  $^{13}\text{C}$  and  $^1\text{H}$  NMR data are in Tables 1 and 2.

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**References**

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