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SULCATINE, A NORSESQUITERPENE FROM THE FUNGUS *LAURILIA SULCATA**

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Key Words Index—*Laurilia sulcata*; *Echinodontium*; Basidiomycetes; fungus; secondary metabolites; protoilludane derivatives; structural determination; absolute configuration.

Abstract—The structure of sulcatine, a novel protoilludene norsesquiterpene isolated from a culture of *Laurilia sulcata*, has been assigned on the basis of a detailed study of its ^{1}H NMR and ^{13}C NMR spectra and chemical evidence. The relative configuration was deduced from the observed ^{1}H–{^{1}H} nuclear Overhauser effects (NOEs) and from the values of ^{1}H–^{1}H coupling constants. The application of the exciton chirality method on the dibenzoate permitted establishment of the absolute configuration of sulcatine as 2*S*,3*R*,7*S*,8*S*,9*R*.

INTRODUCTION

As a part of our studies on secondary fungal metabolites, we have screened during the last few years a number of strains belonging to the Basidiomycetes [1–4]. Species of this class has proved a rich source of new sesquiterpenoids possessing antibacterial activity. In particular a number of new biogenetically related compounds with the protoilludane skeleton have been isolated from *Armillaria mellea* [1, 5] and *Clitocybe* spp. [2].

The investigation of the crystalline material produced in pure culture by *Laurilia sulcata* (Burt) Pouzar, a rare basidiomycetous fungus occurring in Norwegian woods, permitted the isolation of a new norsesquiterpene for which the name sulcatine was proposed. This compound, which possesses a weak antibacterial activity, was isolated from dried mycelium obtained by cultivation in stationary liquid cultures with malt-extract solution as medium. Its structure, Δ^5-norprotoilluden-7,8-diol, was deduced from a detailed study of its ^{1}H NMR and ^{13}C NMR spectra.

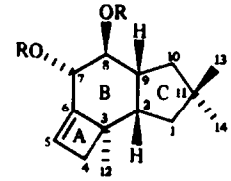
RESULTS AND DISCUSSION

The molecular formula, $C_{14}H_{22}O_2$, of sulcatine (**1**) mp 155–160°, $[\alpha]_D^{20}$ −61.3° (*c* 2.4; $CHCl_3$) was established by elemental analysis and by means of high-resonance mass spectrometry. Additionally the mass spectrum of the acetylated derivative (**2**) indicated the presence of two hydroxyl groups.

The ^{13}C NMR spectrum of sulcatine (**1**) showed 14 carbon atom resonances. Two signals were indicative of the presence of a trisubstituted double bond (C-5 and C-6)* while 12 resonances were attributed to three methyl (C-12, C-13 and C-14), three methylene (C-1, C-4 and C-10), four methine (C-2, C-7, C-8 and C-9), two of them oxygen-bearing and two quaternary (C-3 and C-11) sp^3-hybridized carbon atoms. Chemical shift criteria and the analysis of ^{1}H-^{13}C coupling constants in the fully ^{1}H-coupled ^{13}C NMR spectrum, in conjunction with low-power specific ^{1}H decouplings which enabled us to correlate ^{1}H and ^{13}C resonances, permitted their assignment (Table 1).

The ^{1}H NMR spectrum contained three broadened singlets at δ1.18, 1.10, and 0.94 which were assigned to tertiary methyl groups. The remainder of the spectrum exhibited extensive fine structure. First order analysis of these multiples yielded the chemical shifts and the coupling constants (Table 2) which permitted, in conjunction with ^{13}C NMR spectral data, the fragments (**5**) and (**6**) to be constituted.

The chemical shift values and the magnitude of the geminal and vicinal coupling constants possessed by H_2-1, H-2, H-7, H-8, H-9 and H_2-10, as corroborated by extensive homonuclear decoupling experiments, led to the constitution of fragment **5**. In fact the characteristic downfield shift experienced by H-7 and H-8 of the



1 R = H
2 R = COMe
3 R = COPh
4 R = H; 5,6-dihydro

*Part XVIII in the series "Secondary Mould Metabolites". For part XVII see Assante, G. and Nasini, G. (1987) *Phytochemistry* **26**, 703.

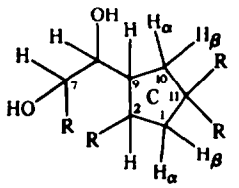
*The numbering system is that used for the protoilludane skeleton [6].

Table 1. ^{13}C NMR spectral data of sulcatine 1 (75.47 MHz, $CDCl_3$)

C			C		
1	42.69	*t* (128)*	8	79.12	*d* (143)
2	46.81	*d* (129)	9	44.14	*d* (128)
3	44.43†	*s*	10	46.95	*t* (128)
4	45.46	*t* (139)	11	39.62†	*s*
5	129.81	*d* (172)	12	23.01	*q* (126)
6	154.38	*s*	13	26.78	*q* (125)
7	71.13	*d* (145)	14	29.26	*q* (125)

*Values in parentheses are directly bonded ($^1J_{C,H}$) coupling constants in Hz.

†Assignments may be interchanged.



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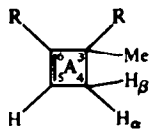

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*Due to the complexity of this spectrum a detailed analysis was not performed.

diacetate 2 relative to the corresponding proton in sulcatine (1) ($\Delta\delta$ 1.17 and 1.61 ppm) placed the hydroxy groups at C-7 and C-8. Moreover the additional small coupling of 2.1 Hz between H-1β and H-10β suggested that the corresponding carbon atoms are linked to the quaternary C-11 to form the cyclopentane ring C, as appreciable four-bond coupling is observed along a W-type path in related saturated systems [1, 7].

The presence of a cyclobutene moiety (6) was inferred from the ^{13}C NMR data. In fact the magnitude of the directly bonded ^{13}C–^{1}H coupling constants ($^1J_{C,H}$) of 172 and 139 Hz observed for the resonances at δ129.81 and 45.46, which have been correlated with the vinylic proton at δ6.04 (H-5) and the methylene protons at δ2.21 and 2.12 (H_2-4), is diagnostic for proton-bearing cyclobutene carbon atoms [8]. In addition, the value of the couplings between H-5 and H_2-4 is in agreement with a vicinal position of these protons [8]. The location of the H_3-12 at C-3 followed from the W-type long-range coupling between H_3-12 and H-4β.

Evidence for the linkage of C-6 with C-7 was provided by the allylic coupling between H-5 and H-7 (^4J = 0.9 Hz) and the homoallylic couplings between H_2-4 and H-7 (^5J = 1.1 and 2.1 Hz). Further support of this structural assignment came from the ^{1}H NMR spectrum of the compound 4, obtained by reduction of sulcatine (1) with Pd 10% on $BaSO_4$, in which H-7 presented a vicinal coupling (J = *ca* 8 Hz) with the newly formed H-6*. The mode of linkage of the two remaining methyl groups at C-11 followed from the long-range couplings observed between H_3-13 and H-1α and H-10α, and between H_3-14 and H-1β and H-10β. Finally the linkage between C-2 and

Table 2. ^{1}H NMR spectral data of compounds 1–3 (300.13 MHz, $CDCl_3$, TMS as internal standard)

H	1	2	3	*J*	1 (Hz)	2 (Hz)*
1α	1.32	1.36 (1.41)†	1.45	1α,1β	12.4	12.4
1β	1.44	1.45 (1.49)	1.51	1α,2	11.5	11.2
2	2.37	2.40 (2.48)	2.54	1α,13	0.7	0.7
4α	2.21	2.23 (2.20)	2.28	1β,2	7.8	7.8
4β	2.12	2.14 (2.18)	2.23	1β,10β	2.1	2.1
5	6.04	6.07 (6.03)	6.22	1β,14	*ca* 0.2	*ca* 0.2
7	3.98	5.15 (5.11)	5.50	2,9	11.5	11.8
8	3.69	5.30 (5.31)	5.81	4α,4β	13.4	13.5
9	2.10	2.28 (2.35)	2.54	4α,5	0.9	1.0
10α	1.16	1.17 (1.17)	1.33	4α,7	1.1	1.3
10β	1.78	1.55 (1.57)	1.64	4β,5	1.1	1.2
12	1.18	1.21 (1.21)	1.31	4β,7	2.1	2.0
13	0.94	0.92 (0.95)	0.96	4β,12	0.5	n.a.
14	1.10	1.07 (1.07)	1.09	5,7	0.9	ca 1
7-OR	2.35	2.03 (1.99)‡	7.3–8.1	7,8	8.4	9.0
8-OR	2.35	2.03 (1.96)‡	7.3–81.	8,9	11.3	11.4
				8,10α	*ca* 0.2	n.a.
				9,10α	10.3	10.3
				9,10β	7.5	7.4
				10α,10β	12.3	12.6
				10α,13	0.8	0.8
				10β,14	*ca* 0.2	*ca* 0.2

*Coupling constants obtained in acetone-d_6.

†Values in parentheses are chemical shifts in acetone-d_6.

‡Assignments may be interchanged.

n.a., Not assigned.

C-3 gave the overall gross structure of sulcatine (1).

NOE experiments and the anaiysis of the ^{1}H–^{1}H coupling constants performed on the diacetate 2 established as *cis* the junction between rings B and C and indicated that these two rings adopt a boat-like and *exo*-envelope geometry respectively. Sulcatine (1) and its diacetate 2 presented similar ^{1}H–^{1}H coupling constants (Table 2). Therefore the stereochemical and conformational results obtained for 2 also hold for compound 1. Moreover if it is assumed that C-2 has the *S*-configuration, i.e. H-2 is β-orientated, the relative conformation of sulcatine (1) is 2*S*,3*R*,7*S*,8*S*,9*R*. The NOE connectivity pattern and the preferred conformation of the diacetate 2 are depicted in Fig. 1.

Irradiation of H_3-13 resulted in enhancement of H-2β and H-9β and this NOE experiment proved the *cis*-junction between rings B and C. As a consequence the relative configuration of C-9 is *R*. The same irradiation of the β-disposed H_3-13 also enhanced H-1β and H-10β which must be equatorially orientated because they are W-type long-range coupled, whilst irradiation of the α-disposed H_3-14 enhanced H-1α and H-10α in addition to H-1β and H-10β. Thus H-1α and H-10α must be axially positionated since the vicinal couplings of 11.5 and 10.3 Hz with H-2β and H-9β, respectively, account for dihedral angles of *ca* 150°. On the other hand the vicinal couplings of H-1β with H-2β, and H-9β with H-10β (^{3}J = 7.8 and 7.5 Hz, respectively) are in agreement with dihedral angles of *ca* 30°. These results indicate that the cyclopentane ring C preferentially adopts the *exo*-envelope conformation shown in Fig. 1. The 1,3-*cis*-diaxial orientation of H-7β and H-9β followed from the values of the vicinal coupling constants between these two protons and H-8 (^{3}J = 8.4 and 11.3 Hz, respectively) which must be then α-axially disposed. These findings, in conjunction with the NOEs between H-8α and H_3-12, and H-7β and H-9β, require that the six-membered ring B adopts a boat-like conformation and that C-3, C-7 and C-8 possess a (*R*,*S*,*S*)-relative configuration, respectively.

The absolute configuration of C-7 and C-8 was deduced as (*S*,*S*) from a CD spectrum carried out on the dibenzoate 3. According to the dibenzoate rule [9], the CD curve exhibited a positive Cotton effect around 238 nm, this fact indicating the right-handedness of the orientation of the two benzoate groups. Sulcatine must, therefore, have the absolute configuration as shown in 1, *viz.* 2*S*,3*R*,7*S*,8*S*,9*R*.

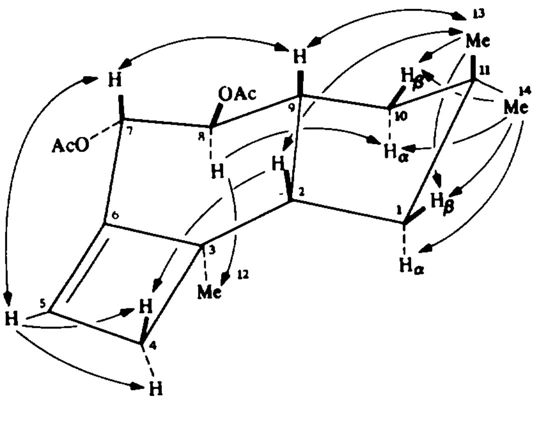

Fig. 1. NOE connectivity pattern and the preferred conformation of diacetate 2.

The isolation of sulcatine further confirms the presence of sesquiterpenoids possessing a protoilludane skeleton in many Basidiomycetes, i.e. *Armillaria* spp. [1,2,5], *Clitocybe* spp. [2, 10], *Omphalotus olearius* [6], *Fomitopsis* spp [6], and now *Laurilia*. From a taxonomic point of view the genus *Laurilia* Poùzar is incorporated in the genus *Echinodontium* [11].

EXPERIMENTAL

Mps are uncorr. Flash chromatography was performed with Merck silica gel (0.040–0.063 nm) and TLC with Merck HF_{254} silica gel. UV spectra were measured in 95% EtOH. CD spectra were recorded on a Jasco 500A spectropolarimeter. Mass spectra were taken on a VG-ZAB2 instrument at 70 eV. ^{1}H(300.13 MHz) and ^{13}C(75.47 MHz) NMR spectra were recorded on a Bruker CXP-300 spectrometer. Chemical shifts are in ppm (δ) from TMS as internal standard. NOE difference spectra were obtained by subtracting alternatively right-off resonance-free induction decays (FIDS) from right-on resonance-induced FIDS.

Isolation and purification of sulcatine (1). The strain of *Laurilia sulcata* (Burt) Pouzar [*Echinodontium sulcatum* (Burt) Gross = *Stereum sulcatum* Burt] received from Laboratorio Central de Fitopatologia, Buenos Aires (LCF 618) was originally a strain (71688-R) of the Division of Forest Phathology, U.S. Department of Agriculture. It was maintained on cherry-agar slants and subcultured in liquid stationary malt extract broth 4% in 50 Erlenmeyer flasks (100 ml) for 6 weeks at 24°; the mycelium separated from culture filtrates was dried and extracted twice with EtOAc containing 1% of MeOH. The extracts were dried and evaporated under red. pres. to give a mixture (0.8 g) of crude metabolites that was chromatographed on a column of silica gel using hexane–EtOAc (3:1) as eluant; the main fraction (80 mg) corresponding to sulcatine 1 was crystallized from Et_2O–hexane to give the pure compound.

Sulcatine (1), as white crystalline needles, had mp 155–160°; MS *m/z* 222 $[M]^+$, 204 $[M-18]^+$, and 189; (found: *m/z* 222.1622; $C_{14}H_{22}O_2$; calc. 222.1620) (found: C, 75.4; H, 9.8%; $C_{14}H_{22}O_2$ requires C, 75.63; H, 9.97%); IR ν_{max}^{Nujol} cm^{-1}: 3350 (OH), 1470, 1460 and 1380.

^{13}C NMR and ^{1}H NMR data are reported in Tables 1 and 2 respectively.

Sulcatine diacetate (2). Compound 1 (20 mg) was dissolved in 0.5 ml of dry pyridine and treated with Ac_2O (0.5 ml). The soln was left to stand at 0° for 24 hr. Standard work-up yielded compound 2 (18 mg) as white powder, Mp 61–63°; MS *m/z* 306 $[M]^+$, 264 $[M-42]^+$, 246 $[M-60]^+$, 204. ^{1}H NMR data are reported in Table 2.

Benzoylation of sulcatine. Compound 1 (50 mg) was treated with pyridine (1 ml) and benzoyl chloride (0.25 ml). After 30 min H_2O (5 ml) was added and the ppt was filtered, washed with H_2O, dried and crystallized from hexane–Et_2O. Compound 3 was obtained as white crystals, mp 125–130°; MS *m/z* 430 $[M]^+$, 325 $[M-105]^+$, 308, 293, 280, and 203 (found, C, 77.7; H, 7.0%; $C_{28}H_{30}O_4$ requires C, 78.11; H, 7.02%): UV $\lambda_{max}^{95\%\ EtOH}$ nm: 197, 225, 278 and 287sh (ε 22000, 24800, 1600 and 1400); IR ν_{max}^{KBr} cm^{-1}: 1725 (aryl ester); CD (*c* 4 × 10^{-4} gl, EtOH) 223 and 238 nm ($\Delta\varepsilon$ −8.14 and +8.14). ^{1}H NMR data are reported in Table 2.

Hydrogenation of sulcatine. Compound 1 (20 mg) was dissolved in EtOAc (5 ml) and hydrogenated in the presence of Pd 10% on $BaSO_4$ (10 mg). Filtration, evapn of the solvent and prep. TLC in hexane–EtOAc (2:1) gave 4, Mp 110–112° (hexane); MS *m/z* 224 $[M]^+$, 196 and 178; ^{1}H NMR (300 MHz, $CDCl_3$): δ3.56 and 3.22 (2H, *m*, H-7 and H-8), 2.3 (2H, OH), 2.4–1.2 (11H, *m*, alicyclic protons), 1.14, 1.10 and 0.98 (9H, *s*, Me).

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REFERENCES

1. Arnone, A., Cardillo, R. and Nasini, G. (1986) *Phytochemistry* **25**, 471.
2. Arnone, A., Cardillo, R. and Nasini, G. (1985) Int. Symposium on Organic Chemistry of Medicinal Nat. Prod., Shanghai, B-033.
3. Donnelly, D. M. X., O'Reilly, J., Polonsky, J. and Van Eijk, G. W. (1982) *Tetrahedron Letters* **23**, 5451.
4. Van Eijk, G. W., Roeijmans, H. J. and Verwiel, P. E. J. (1984) *Exp. Mycol.* **8**, 273.
5. Donnelly, D. M. X., Coveney, D. J. and Polonsky, J. (1985) *Tetrahedron Letters* **26**, 5343.
6. Ayer, W. A. and Browne, L. M. (1981) *Tetrahedron* **37**, 2199.
7. Sternhell, S. (1969) *Quart. Rev.* **23**, 236.
8. Hill, E. A. and Roberts, J. D. (1967) *J. Am. Chem. Soc.* **89**, 2047.
9. Harada, N. and Nakanishi, K. (1983) in *Circular Dichroic Spectroscopy*. Oxford University Press, Oxford.
10. Roberts, J. S. and Bryson, I. (1984) *Nat. Prod. Rep.* 105.
11. Stalpers, J. A. (1980) *Taxon* **29**, 289.