Tsugicoline E, a New Polyoxygenated Protoilludane Sesquiterpene from the Fungus *Laurilia tsugicola*[1]

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Tsugicoline E (**3**) has been isolated from cultures of the Basidiomycetous fungus *Laurilia tsugicola* and its structure deduced from ^{1}H and ^{13}C NMR and single-crystal X-ray diffraction studies. The suggested absolute configuration is consistent with biogenetic considerations.

In the course of a screening program for biologically active novel metabolites from Basidiomycetous fungi, we found that still liquid cultures of the mushroom *Laurilia tsugicola* (Echinodontiaceae) are a rich source of new sesquiterpenes having the protoilludane skeleton, such as tsugicolines A (**1**) −D.[2] Compound **1** was successively converted via chemical reactions into the corresponding sterpurane derivative **2**.[3] In this paper we describe the isolation of a related compound, tsugicoline E (**3**), and present its structure elucidation using mass spectrometry, NMR spectroscopy, and single-crystal X-ray diffraction.

Results and Discussion

Tsugicoline E (**3**) was isolated as white crystals, mp 219−221 °C, $[\alpha]_D$ −38° (*c* 0.1, MeOH). Elemental analysis and FABMS (MH^+, 285) established $C_{15}H_{24}O_5$ as the molecular formula, indicating the presence in **3** of a molecule of water more than compound **1**. Strong peaks observed in the mass spectrum at *m*/*z* 267, 249, and 231 were assumed due to sequential loss of three molecules of water.

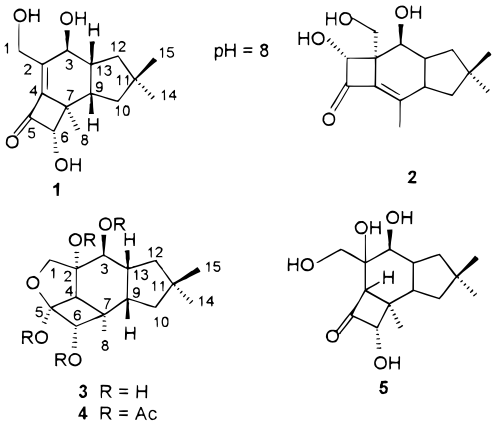
A comparison of the ^{13}C and ^{1}H NMR data of compounds **1** and **3** (Table 1 and Experimental Section)[2] suggested that the two metabolites share the same basic protoilludane skeleton, the only relevant differences being the carbons of the α,*β*-unsaturated ketone moiety. In fact, the sp^2 carbons resonating at *δ* 153.0, 144.0, and 200.1 (C-2, C-4, and C-5) in **1** were replaced by sp^3 carbons resonating at *δ* 80.3, 55.6, and 107.8. The lack of vicinal coupling between the new CH proton and H_2-1 indicates that it must be placed at C-4, while the chemical shift values exhibited by C-2 and C-5 are in agreement with alcoholic and hemiketal carbons. Formation of the tetraacetyl derivative **4** confirmed the presence of four hydroxy groups. NOE enhancement observed for H-4 (12%), by irradiation of H_3-8, and those observed for H-6 (3%) and H-9 and -13 (2%), by irradiation of H*β*-1 at *δ* 3.78, permitted us to assign the stereochemistry as 2*R*,4*R*,5*S*.

A possible scheme of formation of **3** *in vivo* could involve a Michael addition of water onto the unsaturated ketone system of **1**, followed by intramolecular acetalization. Although the polycyclic structure of **3** may appear rather cumbered, the preference for this structure over the corresponding open form **5** is apparent from molecular modeling. In fact, structure **3** is the more stable according to calculations performed with the molecular mechanics program DISCOVER (about 29 kcal/ mole) or with MOPAC (about 24 kcal/ mole). A signal at *δ* 107.3 in the ^{13}C NMR spectrum of tetraacetate **4**, attributable to the C-5 ketal carbon, indicates that **3** retains the closed structure even after acetylation.

Table 1. ^{1}H NMR Data for Compound **3** in $(CD_3)_2SO$

proton[a]	*δ*H	$J_{(H,H)}$/H$_z$
1α[b]	3.66 d	11.0
1*β*	3.78 d	11.0
3	3.38 d	11.2
4	1.73 s	
6	3.92 s	
8	0.87 s	
9	1.99 m	13.0, 6.6, 6.5
10α	1.24 dd	13.0, 12.5
10*β*	1.33 dd	12.5, 6.6
12α	1.79 dd	13.8, < 0.5
12*β*	1.46 dd	13.8, 7.2
13	1.96 m	11.2, 7.2, 6.5, < 0.5
14	1.08 s	
15	0.97 s	

[a] The hydroxy protons resonated at *δ* 6.49, 4.80, 4.75, and 4.68. [b] Numbering system is that used for the protoilludane skeleton.



A view of **3** showing its relative configuration (2*R*,3*S*,4*R*,5*S*,6*S*,7*R*,9*S*,13*R*) and numbering scheme is shown in Figure 1. The structure of **3** is, to our knowledge, the first presenting a cyclobutane ring fused to both a cyclohexane and a furanoid ring. Each of these three rings is fused cis to both the other two, and, in addition, there is a cyclopentane ring also cis-fused only to the cyclohexane ring. Bond lengths and angles in **3** are normal; the O(5)−C(5) bond distance [1.373(5) Å] is somewhat short com-

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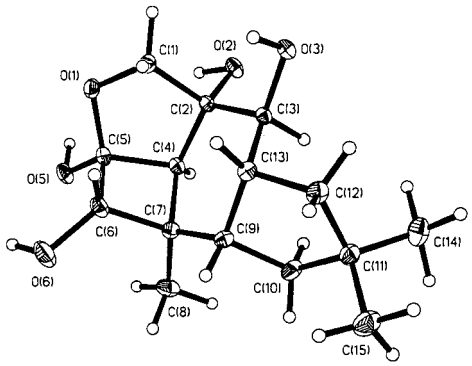

Figure 1. Crystalline conformation of **3** showing its probable absolute configuration.

pared to standard hemiacetal bond distances (1.401 Å)[4] because of the increased *s*-character in the exocyclic bonding orbitals. The cyclobutane ring shows an intermediate puckering ($q = -0.202$ Å); the average values of ring bond lengths (1.553 Å), bond angles (89.1°), and ring torsion angles (14.8°) are in agreement with literature values[5] for cyclobutane rings with similar puckering. Rather than a twisted conformation, the furanoid ring adopts an envelope conformation, with the oxygen in the apical position, consistent with a φ_2 value[6] of 348°. The cyclohexane ring is found in a distorted conformation, intermediate between a chair and a half-chair, while the cyclopentane, fused only to the six-membered ring, displays a φ_2 value (168°) quite typical of an envelope conformation with C(9) in the apical position.[6] The crystal structure shows a weak intramolecular hydrogen bond that results in a five-atom ring formed by the hydrogen on O(6) and O(5) as acceptor [O(5)···H(O6) 2.217 Å; O(5)···H(O6) 2.703 Å; O(5)···H(O6)—O(6) 129°] (Figure 1). A 2D network orthogonal to *c* of strong intermolecular hydrogen bonds involves the hydrogens on the O(5) and O(3) hydroxyls with O(2) and the hemiacetal oxygen O(1), respectively.

Work is in progress to study the compounds obtained from the reaction of compound **1** in the presence of nucleophiles.[7] Tsugicoline E (**3**) is inactive against *Bacillus cereus*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* at a concentration of 100 μg on disks.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler apparatus and are uncorrected; optical rotations were obtained on a JASCO DIP-181 polarimeter; MS were obtained with a Finnigan-MAT TSQ70 spectrometer. NMR spectra were acquired on a Bruker AC 250L spectrometer operating at 250.1 MHz for ^{1}H and 62.9 MHz for ^{13}C. TLC and PLC were performed with Merck HF_{254} Si gel. Owing to the complexity of the purification procedure, we report the R_f value on RP-18 plates in Me_2CO-H_2O (2:1).

Molecular modeling calculations were performed using the program INSIGHT II, 2.3.5 (Biosym Technologies, San Diego, CA) and with MOPAC 6.00 included in the same program.

Organism. A strain of *Laurilia tsugicola* (CBS 248.51) received from Centraal Bureau voor Schimmel Cultures, Baarn, was cultured as described previously[2] in (40) Erlenmeyer flasks (250 mL).

Isolation and Purification of 3. EtOAc extracts of the cultures of *L. tsugicola* were chromatographed on a column of flash Si gel with a gradient of CH_2Cl_2—MeOH as eluent; after the elution of tsugicolines A—D[2] compound **3** (15 mg) was eluted with a ratio (7:1), and the fractions collected and evaporated were crystallized from Me_2CO.

Tsugicoline (3): R_f 0.7; IR (KBr) ν_{max} 3300 (OH), 2954, 2929, 1452, 1286, and 1182 cm^{-1}; *anal.* C 63.3%, H 8.4%, calcd for $C_{15}H_{24}O_5$, C 63.36%, H 8.51%; CIMS *m/z* 267 ($MH^+ - 18$); FABMS *m/z* 285 (MH^+, 7%), 267 (100), 249 (20), 231 (12), 191 (14), 173 (12); ^{1}H NMR spectroscopic data reported in Table 1; selected NOE experiments [$(CD_3)_2SO$] {Hα-1} enhanced Hβ-1 (13%), H-4 (1%); {Hβ-1} enhanced Hα-1 (13%), H-6 (3%), H-9 and -13 (2%); {H-6} enhanced Hβ-1 (4%), H-9 and -13 (4%); {H_3-8} enhanced H-4 (12%), Hα-10 (2.%), Hβ-10 (3%); {H-9 and -13} enhanced Hβ-1 (3%), H-6 (6%), H_3-8 (0.5%), Hβ-10 (3%) Hβ-12 (3.5%), H_3-15 (1%); {H_3-14} enhanced H-3 (3.5%), Hα-10 (2.5%), Hα-12 (4.5%); {H_3-15} enhanced H-9 and -13 (2.5%), Hβ-10 (3.5%), Hβ-12 (4.5%); ^{13}C NMR [$(CD_3)_2SO$] δ 107.5 (s, C-5), 80.3 (s, C-2), 73.7 and 73.4 (d, $^1J = 140$ and 142 Hz, C-3 and -6), 72.9 (t, $^1J = 146$ Hz, C-1), 55.6 (d, $^1J = 146$ Hz, C-4), 46.0 and 42.8 (d, $^1J = 128$ Hz, C-9 and -13), 43.1 and 43.0 (t, $^1J = 128$ Hz, C-10 and -12), 35.5 and 35.4 (s, C-7 and -11), 32.5 and 32.4 (q, $^1J = 125$ Hz, C-14 and -15), and 19.2 (q, $^1J = 127$ Hz, C-8).

Acetylation of 3. Compound **3** (25 mg) was dissolved in dry pyridine (0.4 mL), Ac_2O was added (0.8 mL), and the solution was kept at 0 °C for 2 days. The mixture was then poured into ice-water and extracted with CH_2Cl_2. Evaporation of the extract followed by PLC in hexane—EtOAc (1:1) of the residue gave tetraacetate **4** as a solid (20 mg): mp 123—125 °C; $[\alpha]_D$ −17° (*c* 0.07, MeOH); FABMS *m/z* 453 [MH^+] (10%), 393 [$MH^+ - 60$] (24), 351 (18), 307 (20), 291 (22), 273 (43), 231 (63), 215 (100); CIMS *m/z*, 393; ^{1}H NMR ($CDCl_3$) δ 5.72 (1H, br d, $J = 11.3$ Hz, H-3), 5.09 (1H, br s, H-6), 4.55 and 4.16 (2H, d, $J = 10.8$ Hz, H_2-1), 3.09 (1H, br s, H-4), 2.38 and 2.36 (2H, m, H-9 and -13), 2.12, 2.09, 2.08, and 2.00 (12H, s, 4 × OAc), 1.74, 1.55, 1.51, and 1.38 (4H, m, H_2-10 and -12), 1.11, 1.09, and 1.01 (9H, s, H_3-8, -14, and -15); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.7, 169.7, and 168.6 (s, Me*C*O_2), 107.3 (s, C-5), 87.1 (s, C-2), 77.4 and 70.7 (d, C-3 and -6), 75.8 (t, C-1) 54.6 (d, C-4), 46.9 and 40.7 (d, C-9 and -13), 42.9 and 42.5 (t, C-10 and -13), 37.5 and 35.3 (s, C-7 and -11), 32.6, 32.2, 21.8, 21.3, 21.1, 20.8, and 19.8 (q, Me).

Crystal Data and X-ray Crystal Structure Determination of Tsugicoline E (3). Crystals of **3**, suitable for X-ray analysis, were obtained by crystallization from Me_2CO.

Crystal data: $C_{15}H_{24}O_5$; $M = 284.34$: Orthorombic, $a = 6.014(1)$, $b = 9.810(1)$, $c = 24.330(2)$ Å, $V = 1435.4(3)$ Å^3, space group $P2_12_12_1$, $Z = 4$, $D_x = 1.316$ Mg m^{-3}, $\mu = 0.804$ mm^{-1}, $F(000) = 616$; colorless prismatic crystals, dimension 0.1 × 0.2 × 0.5 mm^3.

Data Collection. Siemens P4 diffractometer, $\theta-2\theta$ scan technique, graphite-monochromated Cu Kα radiation; 2338 reflections measured ($3.63 < \theta < 56.91°$, $+h$, $+k$, $+l$ and $-h$, $-k$, $-l$), 1946 unique. Three standard reflections measured every 100 reflections showed no significant decay. Data were corrected for Lorentz and polarization effects. An empirical absorption correction was also applied.

Structure Analysis and Refinement. The crystal structure was solved by direct methods (SIR 92)[8] and refined by full-matrix least squares on F^2 values (SHELXL-93).[9] Nonhydrogen atoms were refined with anisotropic temperature factors. Hydrogen atoms were included at calculated positions and refined in the riding mode. Final values of the residuals *R* and *wR*2 [for 1701 reflections with $I > 4\sigma(I)$] were, respectively, 0.0596 and 0.1649. The highest and the lowest peaks in final difference Fourier map were 0.186 and −0.272e Å^{-3}. The refined value of Flack's *x* parameter[10] 0.0(5), and its standard deviation, suggested the absolute configuration as (2*R*,3*S*,4*R*,5*S*,6*S*,7*R*,9*S*,13*R*) consistent by biogenetic reason.[11]

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References and Notes

(1) Secondary Mould Metabolites Part 55. For Part 54, see Arnone, A.; Nasini, G.; Vajna de Pava, O. *Phytochemistry* **1997**, *46*, 1099—1101.
(2) Arnone, A.; Brambilla, U.; Nasini, G.; Vajna de Pava, O. *Tetrahedron* **1995**, *51*, 13357—13364.

(3) Arnone, A.; De Gregorio, C.; Nasini, G.; Vajna de Pava, O. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1523−1525.
(4) *International Tables for Crystallography*, Vol. C, Wilson A. J., Ed., Kluwer Academic: Dordrecht, 1992.
(5) Allen, F. H. *Acta Crystallogr.* **1984**, *B40*, 64−72.
(6) Cremer, D.; Pople, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 1354−1358.
(7) Arnone, A.; De Gregorio, C.; Nasini, G.; Vajna de Pava, O. Manuscript in preparation.
(8) Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A. *J. Appl. Crystallogr.* **1993**, *26*, 343−350.
(9) Sheldrick, G. M SHELXL93 *Program for Crystal Structure Refinement*, University of Göttingen, Germany, 1993.
(10) Flack, H. D. *Acta Cryst.* **1983**, *A39*, 876−881.
(11) Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.

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