Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 5033–5038

TETRAHEDRON

Isolation and structure elucidation of new sesquiterpenes of protoilludane origin from the fungus *Clavicorona divaricata*[☆]

Alberto Arnone, Gabriele Candiani, Gianluca Nasini* and Roberta Sinisi

Dipartimento di Chimica, Materiali ed Ingegneria Chimica 'Giulio Natta' del Politecnico, CNR-Istituto di Chimica del Riconoscimento Molecolare, Sezione 'Adolfo Quilico'; via Mancinelli 7, I 20131 Milano, Italy

Received 13 November 2002; revised 14 April 2003; accepted 8 May 2003

Abstract—Four novel sesquiterpenes of protoilludane origin, divaricatines A **3a** and B **3b**, 7-*epi*tsugicoline H **4a** and tsugicoline M **5a** have been isolated from agar cultures of the fungus *Clavicorona divaricata* (Basidiomycetes). Their structures were elucidated by means of NMR studies and chemical correlations. All the metabolites are weakly active on bacteria but inhibited the germination of the water cress *Lepidium sativum*. A possible mechanism of their formation from the protoilludane tsugicoline A **1** is suggested. A fifth metabolite, the norsesquiterpene tsugicoline L **2b**, was also isolated from the same fungus together with the known tsugicoline I **2a**. © 2003 Elsevier Science Ltd. All rights reserved.

1. Introduction

In the course of a program aimed at identifying new bioactive metabolites produced in culture by Basidiomycetes, we have studied *Laurilia tsugicola* (*Echinodontium tsugicola*) a decay agent on *Tsugae* and *Abies*; when the fungus was grown in still liquid cultures (malt–peptone–glucose) (MPG), the protoilludane sesquiterpenes tsugicolines A(**1**)–E were isolated[2] whereas in agar cultures (MPGA) the more oxygenated furosesquiterpenes tsugicolines F–H and the norsesquiterpene tsugicoline I **2a** were formed.[3]

The easy transformation of tsugicoline A **1** into the sterpurane derivative **6**[4] and the fact that the formation of clavicoronic acid **7** (isolated from the Basidiomycetae *Clavicorona pyxidata*) was ascribed to a fragmentative opening of a hypothetical sterpurane intermediate,[5] prompted us to look for protoilludane sesquiterpenes among the metabolites of the fungus *Clavicorona divaricata*, so far not investigated. In this paper we describe the isolation and structure elucidation of new sesquiterpenes named tsugicolines L **2b** and M **5a**, 7-*epi*tsugicoline H **4a**, divaricatines A **3a** and B **3b**, together with the known tsugicolines I **2a** and H **4c**.

2. Results and discussion

The strain of *C. divaricata* was grown on MPGA medium for three weeks and the metabolites were extracted with EtOAc; four main metabolites, tsugicolines I **2a** and L **2b** and divaricatines A **3a** and B **3b**, were isolated by silica gel chromatography from the neutral fraction (see Section 3) and three, 7-*epi*tsugicoline H **4a**, tsugicolines H **4c** and M **5a**, from the acidic fraction.

Tsugicoline L **2b** was obtained as a cream powder, mp 168–170°C; $[\alpha]_D=-1.6$ (*c* 0.1; MeOH) and analysed for $C_{14}H_{16}O_3$ (M^+, 232), indicating a norsesquiterpene derivative; the IR spectrum (KBr) exhibited a strong absorption band at 1742 cm^{-1}, suggesting the presence of a lactone moiety. The ^1H NMR spectrum in $[^2H_6]$acetone (Tables 1 and 2) showed the presence of four broadened singlets in a 1:2:3:3 ratio which were assigned to one methine (H-11), one methylene (H_2-1) and two methyl groups (H_3-13 and -14), two AB and one AA′X spin systems attributable to two methylene (H_2-7, and -9) and one C(15)H_2OH ($^3J_{AX}={}^3J_{A'X}$=5.0 Hz) groups.

Comparison of ^{13}C NMR data in $CDCl_3$ of **2a** and **2b** (Table 3) indicated a close similarity between the two compounds, the only difference being the presence in **2b** of a C(15)H_2OH moiety in place of a Me group. The downfield shifts experienced by C-8 ($\Delta\delta$ 4.84 ppm, β effect) and C-15 ($\Delta\delta$ 40.36 ppm; α effect) and the upfield shifts experienced by C-7, -9 and -14 ($\Delta\delta$ 4.47–5.32 ppm, γ effect) together with the COLOC and NOE results reported in the Section 3 are in agreement with a C-15 hydroxy substitution. The formation of the monoacetate **2c** with the

---

[☆] See Ref. 1.

Keywords: sesquiterpenes; *Clavicorona divaricata*; protoilludane.

* Corresponding author; e-mail: nasini@dept.chem.polimi.it

0040–4020/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0040-4020(03)00738-5

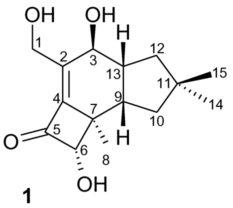
Table 1. ^{1}H NMR chemical shifts for compounds **2–5** and **8b** in $CDCl_3$

Proton	δ_H									
	2a	**2b**	**2c**	**3a**	**3b**	**4b**	**4d**	**5a**[a]	**5b**	**8b**
1a	5.17	5.17 (5.20)[a]	5.18 (5.21)	5.07 (5.23)[a]	5.08 (5.23)[a]	7.54 (7.67)[a]	7.54 (7.62)[a]	7.41	7.37	7.32
1b	5.17	5.17 (5.20)	5.18 (5.21)	4.98 (4.73)	4.94 (4.73)					
3				6.85 (6.95)	6.85 (6.95)	4.61 (4.58)	4.43 (4.48)	4.42	4.48	2.83
4				5.21 (5.16)	5.20 (5.16)					
7a	2.71	2.95 (2.97)	2.91 (2.94)							
7b	2.71	2.54 (2.59)	2.69 (2.72)							
8				2.55 (2.52)	2.54 (2.51)	1.69 (1.64)	1.59 (1.54)	2.10	2.10	2.10
9a	2.80	3.06 (3.08)	3.02 (3.06)			2.60 (2.53)	2.72 (2.71)			
9b	2.80	2.69 (2.68)	2.77 (2.81)							
10a				2.73 (2.73)	2.92 (2.96)	1.18 (1.19)	1.05 (1.07)	2.28	2.29	2.27
10b				2.73 (2.73)	2.64 (2.59)	1.77 (1.67)	1.60 (1.55)	2.28	2.29	2.27
11	7.05	7.09 (7.20)	7.07 (7.20)							
12a				2.77 (2.79)	3.00 (3.02)	1.51 (1.48)	1.04 (1.08)	1.43	1.41	1.31
12b				2.77 (2.79)	2.69 (2.64)	1.77 (1.73)	1.74 (1.74)	1.96	1.96	1.82
13	2.57	2.54 (2.52)	2.57 (2.52)			2.39 (2.44)	2.57 (2.59)	2.76	2.78	2.69
14	1.17	1.17 (1.16)	1.21 (1.26)	1.16 (1.16)	1.17 (1.14)	1.07 (1.04)	1.00 (1.00)	1.16	1.18	1.15
15a	1.17	3.46 (3.45)	4.04 (4.60)	1.16 (1.16)	3.52 (3.43)	1.05 (1.03)	0.98 (0.99)	0.99	0.99	0.99
15b		3.46 (3.45)	4.01 (4.60)		3.52 (3.43)					
3-R						1.80 (2.90)	3.40 (4.92)	5.00	3.80	1.97
4-OH				4.45 (6.16)	3.00 (6.23)					
6-OR						3.96 (3.92)	3.93 (3.88)	5.00	3.89	9.60
7-OH						5.60 (5.40)	4.92 (5.55)			
15-OR		4.24 (4.00)	2.08 (2.02)		3.00 (4.01)					

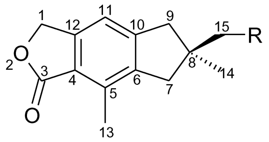
[a] In [$^2H_6$]acetone.

observed downfield shift of the 15-methylene protons gave further support to the proposed structure of tsugicoline L (**2b**).

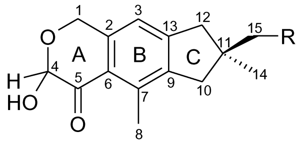
Tsugicoline L **2b** was optically active; previously, we have determined the absolute configuration of tsugicolines A **1** and G **8a**[3] and since there are biogenetic and chemical correlations between the large number of tsugicolines,[2–4] we would tentatively assign to C-8 the stereochemistry *R*, the same observed in tsugicoline G.



1



2 a R = H
2 b R = OH
2 c R = OAc



3 a R = H
3 b R = OH

Table 2. ^{1}H NMR coupling constants for compounds **2–5** and **8b**

	2a[a]	2b[b,c]	2c[b]	3a[d]	3b[b,e]	4b[b]	4d[b,f]	5a[b]	5b[a]	8b[b,g]
1, 3				0.9	0.9	1.0	0.9	1.7	1.7	1.9
1, 11	1.0	1.0	1.0							
1a, 1b				15.5	15.5					
3, 13						6.0	5.2	11.6	11.7	13.7
7a, 7b	n.d.	16.5	16.5							
9, 10a						12.8	11.2			
9, 10b						6.5	7.4			
9, 13						7.3	10.2			
9a, 9b	n.d.	16.7	16.7							
10a, 10b				n.d.	16.0	12.8	12.4	n.d.	n.d.	n.d.
12a, 12b				n.d.	16.0	13.0	12.4	12.2	12.2	11.8
12a, 13						5.0	9.9	11.0	11.0	10.8
12b, 13						7.2	7.4	7.4	7.4	7.0

[a] In $CDCl_3$.
[b] In [$^2H_6$]acetone.
[c] $J_{15,15\text{-OH}}$=5.0 Hz.
[d] $J_{4,4\text{-OH}}$=5.8 Hz.
[e] $J_{4,4\text{-OH}}$=5.8 Hz, $J_{15,15\text{-OH}}$=5.3 Hz.
[f] $J_{3,3\text{-OH}}$=5.4 Hz.
[g] $J_{3b,13}$=6.2 Hz. n.d.=not determined.

Table 3. ^{13}C NMR data for compounds **2**

Carbon atom	**2a**[a]				**2b**[b]				**2c**		
	δ_C[d]		^1J(C,H)	$>^1J$(C,H)	δ_C[d]		^1J(C,H)	$^{>1}J$(C,H)	δ_C[a]	δ_C[c]	
1	68.40	Td	151.5	3	68.98	Td	152	3	(68.37)[a]	68.40	T
2											
3	171.62	Sdt		1, 2.5	171.62	Sdt		1, 2.5	(171.53)	171.42	S
4	121.30	Sdtq		6.5, 2.5, 5.5	121.95	Sdtq		6.5, 2.5, 5.5	(120.96)	121.66	S
5	135.27	Sbrtq		2, 6.5	135.27	Sbrtq		2, 6.5	(134.95)	135.56	S
6	144.24	Sm			144.47	Sm			(143.71)	142.92	S
7	45.26	Tm	129		40.79	Tm	129.5		(39.94)	40.76	T
8	40.20	Sm			45.87	Sm			(45.04)	43.29	S
9	48.14	Tm	129		43.61	Tm	129.5		(43.02)	43.25	T
10	150.83	Stt		5, 5	151.18	Stt		5, 5	(150.44)	149.33	S
11	115.27	Dbrs	161.5		116.66	Dbrs	162.5		(115.45)	115.42	D
12	146.35	Sbrt		4.5	147.89	Sbrt		4.5	(146.33)	146.62	S
13	13.91	Qs	128		13.68	Qs	128		(13.79)	13.94	Q
14	28.80	Qttq	125	4.5, 4.5, 4.5	24.56	Qttt	125.5	5.5, 4, 4	(24.33)	24.41	Q
15	28.80	Qttq	125	4.5, 4.5, 4.5	69.63	Tm	139.5		(69.16)	70.87	T

[a] In $CDCl_3$.

[b] In [$^2H_6$]acetone.

[c] The OAc carbons resonate at 171.23 and 20.91 ppm.

[d] Capital letters refer to the pattern resulting from directly bonded (C,H) couplings [^1J(CH)] and small letters to that from (C,H) couplings over more than one bond [$^{>1}J$(CH)]. S=Singlet, D or d=doublet, T or t=triplet, Q or q=quartet, m=multiplet, and br=broad.

Divaricatine B **3b** was isolated as an oil, [α]=−94 (*c* 0.4, $CHCl_3$) from the more polar chromatographic fractions and the molecular formula was determined by HR-EIMS as $C_{15}H_{18}O_4$; the IR spectrum ($CHCl_3$) exhibited absorption bands at 3440 and 1687 cm^{-1} indicative of the presence of an hydroxy and of a conjugated ketonic carbonyl groups; the mass spectrum presented a molecular peak, EIMS *m*/*z* 262 (M^+), 30 mass units more than **2b** corresponding to a CH_2O fragment. The ^{1}H NMR spectrum of **3b** in [$^2H_6$]acetone showed two additional vicinally coupled protons (^3J=5.8 Hz), one of which exchanged upon addition of D_2O, attributable to a C(4)HOH group; similarity, the ^{13}C NMR spectrum of **3b** (Table 4) contained one additional resonance at 94.28 ppm characteristic of an oxygen-bearing carbon atom. The ^{1}H and ^{13}C NMR resonances of rings B and C were very similar to those exhibited by the corresponding rings of **2b**, this fact suggesting that the additional C(4)HOH group is part of ring A. The NOEs observed between H_2-1 and H-3 permitted us to link C-1 at C-2 while the chemical shift value of 192.50 ppm exhibited by C-5, typical of an α,β-unsaturated carbonyl carbon, allowed us to join C-5 at C-6. As a consequence, C-4, which presented three-bond ^{1}H, ^{13}C coupling constants of 6.5 and 3.5 Hz with H_2-1, must be connected to the oxygen of the C(1)H_2O moiety and to C-5 to form the six-membered ring A.

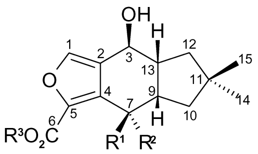
Divaricatine A **3a** is an oil; [α]$_D$=+38 (*c* 0.2, MeOH) and had an analysis consistent with its formulation as $C_{15}H_{18}O_3$; EI mass spectroscopy gave a molecular peak at *m*/*z* 246 (M^+), 16 mass units less than **3b** and shows a strong peak at *m*/*z* 200 consistent with the loss of water and CO (18+28); the ^{1}H and ^{13}C NMR data of **3a** and **3b** revealed that the two compounds show the same basic structure, the only relevant difference being the presence in **3a** of a methyl group assigned to H_3-15 in place of the C(15)H_2OH moiety. The upfield shifts experienced by C-11 ($\Delta\delta$ 4.75 ppm) and C-15 ($\Delta\delta$ 40.29 ppm) and the downfield shifts experienced by

Table 4. ^{13}C NMR data for compounds **3** in [$^2H_6$]acetone

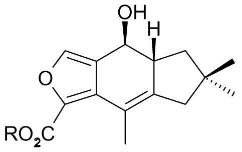
Carbon atom	**3a**				**3b**				
	δ_C[a]		^1J(C, H)	$^{>1}J$(C, H)	δ_C	δ_C[a]		^1J(C, H)	$^{>1}J$(C, H)
1	62.58	Tdd	145.5	5.5, 5.5	(65.25)[b]	62.17	Tdd	145.5	5.5, 5.5
2	142.84	St		4	(141.97)	142.36	St		4
3	118.89	Dbrs	158.5		(117.98)	118.51	Dbrs	158.5	
4	94.59	Ddd	168	6.5, 3.5	(93.87)	94.28	Ddd	167	6.5, 3.5
5	192.89	Sbrs			(193.41)	192.50	Sbrs		
6	125.49	Sm			(124.59)	125.01	Sm		
7	137.77	Sbrtq		2, 6.5	(137.64)	137.45	Sbrtq		2, 6.5
8	18.08	Qs	128		(17.77)	17.80	Qs	128	
9	143.81	Sm			(143.42)	143.09	Sm		
10	46.76	Tm	128		(46.18)	41.49	Tm	128.5	
11	39.99	Sm			(39.50)	44.74	Sm		
12	48.83	Tm	129		(48.38)	43.50	Tm	129	
13	150.35	Stt		5, 5	(150.62)	149.71	Stt		5, 5
14	29.02	Qttq	126	4.5, 4.5, 4.5	(28.84)	24.27	Qttt	126	5.5, 4, 4
15	29.02	Qttq	126	4.5, 4.5, 4.5	(28.84)	69.31	Tm	139.5	

[a] See note of the Table 3.

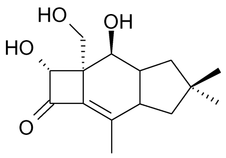
[b] In $CDCl_3$.



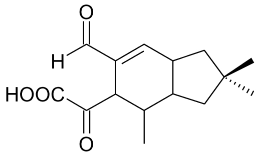
4 a $R^1 = \overset{8}{\text{Me}}$; R^2 = OH ; R^3 = H
4 b R^1 = Me ; R^2 = OH ; R^3 = Me
4 c R^1 = OH ; $R^2 = \overset{8}{\text{Me}}$; R^3 = H
4 d R^1 = OH ; R^2 = Me ; R^3 = Me



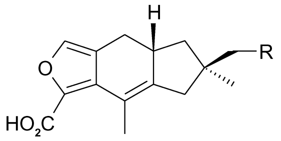
5 a R = H
5 b R = Me



6



7



8 a R = OH
8 b R = H

C-10, -12 and -14 ($\Delta\delta$ 4.75–5.33 ppm) in conjunction with the NOE experiments reported in the Section 3 are entirely in accord with the proposed structure of divaricatine A **3a**.

7-*Epi*tsugicoline H **4a** was obtained as methyl ester **4b** upon reaction with CH_2N_2 (Section 3); compound **4b** exhibited the same molecular weight of **4d** and similar ^1H and ^{13}C NMR spectra (Tables 1, 2 and 5), the only significant differences being the chemical shift and the magnitude of the ^1H, ^1H coupling constants of some cyclopentane protons. NOE experiments carried out on **4b** (Section 3) indicated that H-9, H-13, H_3-8 and H_3-15 are on the same β-side of the molecule and H-3 and H_3-14 are on the α-side. On the hypothesis that the absolute configuration at C-3, C-9 and C-13 is the same as that established for **4d**, it follows that compounds **4b** and **4d** are epimer at C-7.

It must be noted that in **4d** the value of 10.2 Hz observed between H-9 and H-13 requires a nearly eclipsed relationship of these protons; as a consequence the cyclohexane ring adopts a boat-like disposition in which the two hydroxy groups OH-3 and OH-7 are *cis*-axially disposed forming an intramolecular hydrogen bonding. In **4b** the value of 7.3 Hz observed between H-9 and H-13 is in agreement with a dihedral angle of ca. 30°; thus the cyclohexane ring assumes a chair-like conformation in order to relieve steric interactions between H_3-8 and OH-3.

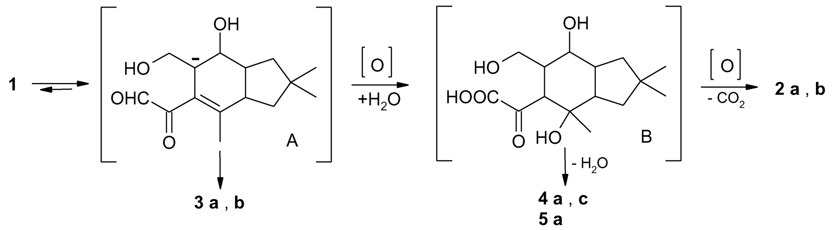
Tsugicoline M **5a** analysed for $C_{15}H_{18}O_4$ and gave the corresponding methyl ester **5b** upon treatment with CH_2N_2. Comparison of the ^1H and ^{13}C NMR spectra of **5b** with those of the methyl ester of the 7-*epi*tsugicoline H **4b** and of the tsugicoline F[3] **8b** indicated that the main structure of **5b** differed from those of **4b** and **8b** in that the $-C(7)MeOH-C(9)H<$ moiety in **4b** and one proton at C-3 have been replaced by a vinylic Me group and one hydroxy group, respectively. Although 7-*epi*tsugicoline H **4a** slowly affords tsugicoline M **5a** through loss of H_2O, we deem that this latter compound is not an artifact being present in high yield in the crude extract.

The anomalous presence of a carbonyl function in the four

Table 5. ^{13}C NMR data for compounds **4**, **5** and **8b**

Carbon atom	$4b^{a,b}$			$4d^{a,c}$				$5b^{d,e}$			8b		
	$\delta_C{}^f$		^1J(C, H)	δ_C	$\delta_C{}^f$		^1J(C, H)	$\delta_C{}^f$		^1J(C, H)	δ_C		^1J(C, H)
1	143.96	D	205	(142.80)^{d,g}	141.96	D	205	138.51	D	204	139.15	D	201.5
2	128.69	S		(126.51)	129.75	S		129.51	S		125.28	S	
3	65.31	D	146	(65.66)	66.32	D	145	71.40	D	144	25.02	T	130
4	141.42	S		(140.21)	139.17	S		132.74	S		134.83	S	
5	139.42	S		(138.71)	139.22	S		136.86	S		135.63	S	
6	162.12	S		(161.21)	161.02	S		159.71	S		163.73	S	
7	69.62	S		(69.20)	70.65	S		147.68	S		151.27	S	
8	32.80	Q	127.5	(32.63)	27.38	Q	127.5	16.94	Q	128	17.09	Q	127.5
9	52.12	D	131	(51.32)	51.27	D	132	119.86	S		119.21	S	
10	43.82	T	130.5	(42.72)	44.26	T	131	45.14	T	130	45.13	T	130
11	37.13	S		(36.68)	38.46	S		38.48	S		38.26	S	
12	46.72	T	130	(45.84)	46.49	T	132.5	45.23	T	130	47.43	T	130
13	47.52	D	133.5	(47.43)	45.87	D	132.5	49.79	D	127	40.47	D	128
14	31.32	Q	124	(31.27)	29.70	Q	124	29.38	Q	125	29.48	Q	125
15	29.56	Q	124	(30.40)	27.48	Q	124	28.07	Q	125	28.14	Q	125

[a] In $[^2H_6]$acetone.
[b] The OMe carbon resonates at 52.64 ppm.
[c] The OMe carbon resonates at 52.31 ppm.
[d] In $CDCl_3$.
[e] The OMe carbon resonates at 51.79 ppm.
[f] See note d of Table 3.
[g] The OMe carbon resonates at 52.65 ppm.



Scheme 1. The scheme shows a possible mechanism of formation of the metabolites of *Clavicorona divaricata* from the protoilludane **1** via the intermediates A and B.

membered ring of the protoilludane tsugicoline A **1**, is the key to the reactivity of this interesting metabolite and led to an easy opening of the 6–7 bond with the formation of a large number of new nor-and sesquiterpenes;[2–4] the isolation of **1** from a still culture of *C. divaricata* on MPG medium supported this hypothesis. Furthermore, the rearrangement of **1** under the basic conditions[4] led to a compound with a furan ring, very similar to metabolites **4a**,**c** and **5a**. The skeleton of divaricatines A and B was never found among the sesquiterpenes of protoilludane origin; in particular it may be formed by acetalization of the intermediate A (Scheme 1).

Compounds **2a**,**b**, **4a**,**c** and **5a** showed weak antibacterial activity against *Bacillus cereus, B. subtilis* and *Sarcinea lutea* (50 μg/disc). All the new metabolites inhibited the growth of *Lepidium sativum*;[6] after 48 h the inhibition of the root elongation for compounds **4a**,**c** and **5a** was ca. 90%.

3. Experimental

3.1. General

Mps were determined on a Kofler apparatus and are uncorrected; the IR spectra on a Perkin–Elmer 177 spectrophotometer; mass spectra on a Finnigan-MAT-TSQ70 spectrometer; optical rotations on a JASCO-500 DIP-18 polarimeter. NMR spectra were recorded on a Bruker ARX 400 spectrometer operating at 400.1 MHz for ^1H and 100.6 MHz for ^{13}C (δ) from $SiMe_4$ as internal standard, and J-values are given in Hz. Flash column chromatography was performed with Merck silica gel (0.04–0.063 mm), and TLC and PLC with Merck HF_{254} silica gel. HPLC analysis were performed with a LiChrocart (250×4 cm) column RP-18 and a Perkin–Elmer 1100 chromatograph.

3.2. Isolation and purification of metabolites 2a, 2b, 3a, 3b, 4a, 4c and 5a

A strain of *C. divaricata* (ATCC 22500) received from American Type Culture Collection-Rockville, was maintained on MPGA (malt, peptone, glucose, agar, 20:4:20:15 g dm^{-3}) slants and a mycelium suspension was inoculated into a 40 Roux flasks containing MPGA (100 cm^3). After four weeks the cultures were extracted twice with EtOAc containing 1% of MeOH and the extracts were dried (Na_2SO_4) and concentrated to 500 cm^3; the EtOAc was treated with a solution of 5% NaOH (250 cm^3) and the organic layer was washed with water, dried and evaporated to yield 0.6 g of the neutral fraction containing a mixture of **2a**, **2b**, **3a** and **3b** metabolites. The basic solution was acidified with diluted HCl and extracted with EtOAc; the solvent was dried and evaporated to obtain 0.5 g of a mixture of the sesquiterpenes **4a**, **4c** and **5a**. The neutral extract was chromatographed on a silica gel column using hexane–EtOAc (gradient) as eluant to give in order of elution tsugicoline I **2a** (80 mg)[3], divaricatine A **3a** (10 mg), divaricatine B **3b** (4 mg) and tsugicoline L **2b** (30 mg). The acidic fraction was separated on silica gel column with CH_2Cl_2–MeOH as eluent (ratio 9:1) to obtain tsugicoline M **5a** (30 mg) and a mixture of compounds **4a** and **4c** (95 mg); HPLC: gradient solvent system, MeCN–H_2O–TFA (20:60:0.1/40:60:0.1), flow rate 0.6 cm^3/min, T=30°C-**4c**, rt 16.8 min, 26.6%; **4a**, rt 17.3 min, 73.34%. Due to the difficulty of separating the acids **4a**,**c** even by preparative HPLC, the mixture was successively treated with CH_2N_2 to yield after PLC in CH_2Cl_2–MeOH (20:1) the methylesters **4b** (30 mg) and **4d** (10 mg), respectively.[3]

3.2.1. Tsugicoline L 2b. UV: λ_{max} 230, 278 and 288sh (ε 133.500, 31.650 and 28.300). (Found: C, 72.1; H, 7.0. $C_{14}H_{16}O_3$ requires C, 72.40; H, 6.94%); HR EIMS, *m/z* 232.1087 (calcd for $C_{14}H_{16}O_3$, 232.1099); EIMS, *m/z* 232 (58%), 214 (18), 185 (35), 155 (45), 43 (62) and 31 (100). Selected COLOC correlations: H_2-1 showed cross peaks with C-3 and -12, H_2-7 with C-5, -6, -8, -9, -10, -14 and -15, H_2-9 with C-6, -7, -8, -10, -14 and -15, H-11 with C-1, -4 and -6, H_3-13 with C-4, -5 and -6, H_3-14 with C-7, -8, -9 and -15, H_2-15 with C-7, -8, -9 and -14. Selected NOE experiment (^{2}[H_6]acetone): {H-11} enhanced H_2-1 (1%) and H_2-9 (1%).

3.2.2. Acetylation of tsugicoline L 2b. Compound **2b** (30 mg) was dissolved in dry pyridine (0.2 cm^3) and treated with Ac_2O (0.5 cm^3) overnight at 0°C. Standard work-up followed by PLC on silica gel in hexane–EtOAc (2:1) gave the acetate derivative **2c** as an oil, EIMS, *m/z* 274, 214 (M^+-60), 185, 155 and 128.

3.2.3. Divaricatine A 3a. ν_{max} ($CHCl_3$)/cm^{-1}, 3400 (OH), 1688 (conj. CO), 1605 and 1455. (Found: C, 72.9; H, 7.4. $C_{15}H_{18}O_3$ requires C, 73.14; H, 7.36%); HR EIMS, *m/z* 246.1264 (calcd for $C_{15}H_{18}O_3$, 246.1255); CIMS, *m/z* 247 (MH^+) (93%), 229 (100), 217 (38) and 200 (17).

3.2.4. Divaricatine B 3b. (Found: C, 68.5; H, 6.9. $C_{15}H_{18}O_4$ requires C, 68, 68; H, 6.92%); HR EIMS, *m/z* 262.1212 (calcd for $C_{15}H_{18}O_4$, 262.1205). Selected COLOC correlations: H_2-1 showed cross peaks with C-2 and -4, H-3 with C-1, -6, -9 and -12, H-4 with C-1, H_3-8 with C-6, -7, and -9, H_2-10 with C-7, -9, -11, -12, -13 and -14, H_2-12 with C-9, -11, -13 and -14, H_3-14 with C-10, -11, -12 and -15, H_2-15 with C-10, -11, -12 and -14.

3.2.5. Methyl ester of 7-*epi*tsugicoline H. Compound **4b**, as an oil, had $[\alpha]_D=+54.2°$ (*c*, 0.5, $CHCl_3$). (Found: C, 65.3; H, 7.5. $C_{16}H_{22}O_5$ requires C, 65.28; H, 7.53%); HR EIMS, *m*/*z* 294.1465 (calcd for $C_{16}H_{22}O_5$, 294.1467); CIMS, *m*/*z* 277 (MH^+-18). Selected NOE experiments ($CDCl_3$): {H-3} enhanced H-1 (2.5%), H-10α (2.5%), H-12α (2.5%), H-13 (2%), H_3-14 (1%); {H_3-8} enhanced H-9 (9.5%) and H-13 (6%); {H-9} enhanced H_3-8 (1%), H-10β (3%), H-13 (2.5%), H_3-15 (0.5%); {H-13} enhanced H-3 (1.5%), H_3-8 (1%), H-9 (3%), H-12β (2%).

3.2.6. Tsugicoline M 5a. Mp 105–110°C, $[\alpha]_D=+34°$ (*c* 0.2, MeOH). (Found: C, 68.8; H, 7.0. $C_{15}H_{18}O_4$, requires C, 68.68; H, 6.92%); HR EIMS, *m*/*z* 262.1215 (calcd for $C_{15}H_{18}O_4$ 262.1205); EIMS, *m*/*z* 262 (M^+), 244, 200 and 185.

3.2.7. Methyl ester of tsugicoline M 5a. Compound **5a** (15 mg) in 8 cm^3 of CH_2Cl_2 was methylated with CH_2N_2. Evapn of the solvent and PLC using hexane–EtOAc (2:1) as eluent gave ester **5b** as an oil; CIMS, *m*/*z* 277 (MH^+).

References

1. Part 62 in the series 'Secondary Mould Metabolites' for part 61 see: Nasini, G.; Arnone, A.; Assante, G.; Candiani, G.; Vajna de Pava, O. *Tetrahedron Lett.* **2002**, *43*, 1665–1668.
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. *Tetrahedron* **1998**, *54*, 10199–10204.
4. Arnone, A.; De Gregorio, C.; Nasini, G.; Vajna de Pava, O. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1523–1525.
5. Erkel, G.; Anke, T.; Gimenez, A.; Steglich, W. *J. Antibiot.* **1992**, *45*, 29–37.
6. Arnone, A.; Assante, G.; Nasini, G.; Vajna de Pava, O. *Phytochemistry* **1990**, *29*, 613–616.