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Isolation and Structure Elucidation of Tsugicolines A-D, Novel Protoilludane Sesquiterpenes from *Laurilia tsugicola*[1]

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Abstract: Four novel sesquiterpenes, tsugicolines A-D (**1a, 2, 3a,** 4), have been isolated from still cultures of the fungus *Laurilia tsugicola* (Basidiomycetae). Their structures were elucidated by means of chemical correlations and NMR studies and the relative configurations were established through a series of NOE difference spectra. The absolute configuration of tsugicoline A **1a** (3-*epi*-illudol-5-one) was determined as 3S,6S,7R,9R,13S by the 'partial resolution' method of Horeau.Treatment of tsugicoline A **1a** with triethylamine in MeOH gave the metabolite 4; a possible mechanism is reported. Tsugicoline A is inactive on bacteria and fungi but inhibits the germination of the water cress *Lepidium sativum*.

Introduction

The fungal genus Basidiomycetes is a rich source of new, interesting secondary metabolites of sesquiterpenic type. In particular, we have recently isolated a number of sesquiterpenes with a protoilludane skeleton from *Armillaria*[2], *Clitocybe*[3] and *Laurilia spp.*[4] and a new class of compounds, the nor-*iso*-illudalanes from *Laurilia sulcata.*[5]

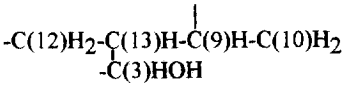
In this paper we describe the isolation and structure elucidation of four protoilludane sesquiterpenes, named tsugicolines A-D, **1a-4**, produced by *L. tsugicola (Echinodontium tsugicola)* a decay agent on *Tsugae* and *Abies.*The fungus was grown for one month in still liquid cultures (malt-peptone-glucose) and the metabolites were extracted with EtOAc.

Results and Discussion

Tsugicoline A **1a** was isolated by filtration of the insoluble part of an Et_2O washing of the extracts as white crystals (CH_2Cl_2-hexane), m.p. 168-170°C; $[\alpha]_D$ -156° (*c* 0.15, MeOH) and analysed for $C_{15}H_{22}O_4$ (M^+, 266); chemical ionization mass spectroscopy (isobutane) gave a distinct peak at *m/z* 267 (MH^+) and a fragment was found at *m/z* 249 [(MH^+)-H_2O; base peak] due to the ready loss of water; in addition, strong peaks were observed at *m/z* 231 [(MH^+)-$2H_2O$] and 203 (231-28). The IR spectrum (KBr) exhibited a large absorption at 3350 and a band at 1730 cm^{-1}, due to hydroxy and carbonyl groups respectively, and the UV spectrum [λ_{max} 260 nm (ε 8500)] agreed with the presence of a conjugated system.

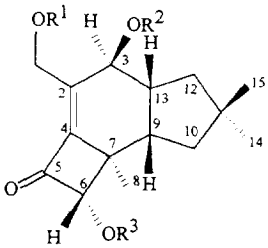
The broad-band ^1H-decoupled ^{13}C NMR spectrum of tsugicoline A **1a** (Table 1) revealed the presence of 15 signals attributable to 3 sp^2- and 12 sp^3-hybridized carbon atoms on the basis of chemical shift criteria and one-bond $^1H,^{13}C$ HETCOR correlations. The sp^2 signals were assigned to the carbons of a fully-substituted α,β -unsaturated ketone moiety (C-2,C-4 and C-5) while the sp^3 signals were assigned to three methyl (C-8,C-14 and C-15), three methylene (C-1,C-10 and C-12, one of them oxygen-bearing), four methine (C-3,C-6,C-9 and C-13, two of them oxygen-bearing) and two quaternary (C-7 and C-11) carbon atoms. The analysis of the corresponding ^1H NMR spectrum (Tables 2 and 3), as corroborated by selective decoupling experiments,

extended the above evidence through the appearance of three tertiary methyl groups, a -C(1)H_2OH and a -C(6)HOH moieties and a sequence like that shown below:



The presence of three hydroxyl groups was confirmed by the formation of the triacetate **1b**. In a COLOC experiment optimized for the observation of two-and three-bond couplings of *ca.* 6 Hz, 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 tsugicoline A of a *gem*-dimethyl group linked at C-11, it follows that the sequence C-12, C-13, C-9, C-10 reported above is closed through C-11 to form a cyclopentane ring. The long range couplings of 0.8 Hz observed between the 15-methyl protons and both Hα-10 and Hα-12 and that of 1.8 Hz observed between Hβ-10 and Hβ-12 gave further support. Moreover, additional correlations observed between the 8-methyl protons and C-4, C-6, C-7 and C-9 defined the formation of the four bonds between the quaternary C-7 carbon and C-4, C-6, C-8 and C-9 while the coupling of 5 Hz observed between C-5 and H-6 suggested that C-5 and C-6 are joined together.

Evidence for the linkage between C-4 and C-5, and not between C-2 and C-5, to form the α,β-unsaturated carbonyl moiety followed from the chemical shift values of 153.02 and 143.98 ppm exhibited by C-2 and C-4 which imply that these carbons are in β- and in α-position with respect to the carbonyl group.[6]



1a $R^1 = R^2 = R^3 = H$

1b $R^1 = R^2 = R^3 = Ac$

1c R^1–$R^2 = CMe_2$; $R^3 = H$

1d $R^1 = R^3 = (Me)_3C\text{-}CO$; $R^2 = H$

The relative configuration of the five chiral centres in tsugicoline A **1a** was determined by the use of NOE experiments (Experimental). In particular, the mutual NOEs observed between H-9, assumed as β, and H-6, H-13 and H_3-15 require that these protons are on the same β-side of the molecule whereas the NOEs observed between H-3 and H_3 -8 and H_3α-14 indicate that these protons are on the α-face. The absolute configuration of C-3 and C-6 was deduced as S for both carbons by application of the Horeau chirality rule to the acetonide derivative **1c** and to the 1,6-dipivalate **1d**, respectively.[7] In fact, it can be reasonably assumed that in **1d** C-13 with the neighbouring groups is smaller than C- 2, and that in **1c** the carbonyl C-5 is larger than C-7. Tsugicoline A must therefore have the absolute configuration as shown in structure **1a**, *i.e.* 3**S**,6**S**,7**R**,9**S**,13**R**, and represents a new member of the protoilludane sesquiterpenes being identified as 3-*epi*illudol-5-one.[8]

The above findings together with the magnitude of the coupling constants of the protons of the cyclopentane ring indicate that the tsugicoline A **1a** assumes in solution the preferred conformation in which the cyclopentane and the cyclohexene rings adopts an *exo*-envelope and a boat-like geometry, respectively ; the same result was obtained by a molecular model depicted in the Figure built by means of molecular mechanisms calculations. The second metabolite, tsugicoline B **2**, was obtained as an oil, $[\alpha]_D$ -71.5 (*c* 4.5, $CHCl_3$). It analysed for $C_{15}H_{22}O_3$ and presented UV and IR spectra very similar to those of compound **1a**. Comparison of the ^{13}C and ^1H NMR spectra of compound **2** with those of **1a** (Tables 1,2 and 3) indicated a close similarity between the two metabolites, the only significant difference being the presence in **2** of a C(6)H_2 group (δ_C 60.31; δ_H 2.82 and 2.75) instead of the C(6)HOH fragment (δ_C 90.33; δ_H 4.39 and 2.95).

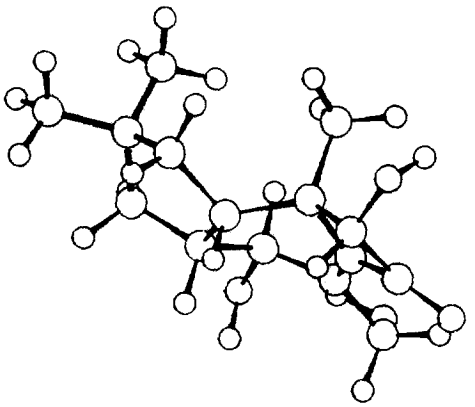
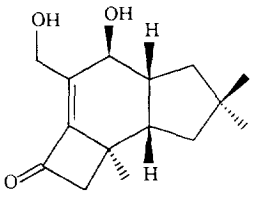
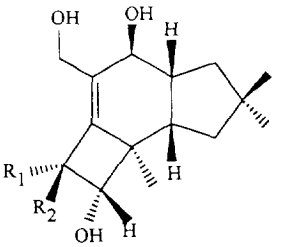


Fig. - Model of tsugicoline A **1a**, as derived by molecular mechanics

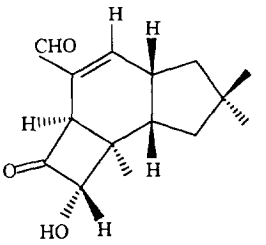


2



3a R^1= OH; R^2 = H

3b R^1 = H, R^2 =OH



4

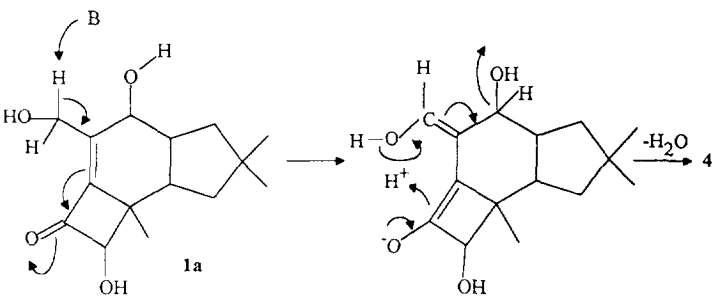
Tsugicoline C **3a** was obtained as white crystals, m.p. 74-76°C; [α] -39.6° (*c* 3, $CHCl_3$), and had an analysis consistent with its formulation as $C_{15}H_{24}O_4$; chemical ionisation mass spectroscopy gave a molecular peak at *m/z* 269 (MH^+) and a base peak at 233 due to the loss of two molecules of water. The ^{13}C and ^1H NMR data of compound **3a** were very similar to those exhibited by **1a**, the only relevant difference being the presence in **3a** of a C(5)HOH moiety (δ_C 70.90; δ_H 4.84 and 4.82) in place of a >C(5)=O group (δ_C 200.15).

Definitive confirmation of the structure came from the reduction of **1a** with $NaBH_4$ in methanol to give a mixture of the two C-5 epimers **3a** and **3b** in a 5:95 ratio. NOE difference experiments allowed us to assign the chirality of the C-5 carbons (Experimental). Specifically, irradiation of the 8-methyl protons led to enhancement of 5-H (10.5%) in compound **3b**, thus indicating as S its absolute configuration. Therefore the natural compound **3a**, where this effect is not observed, must have 5R configuration.

Tsugicoline D **4** was isolated as a white solid, m.p. 107-110°C; $[\alpha]_D$ +161.8° (*c* 0.1, $CHCl_3$); elemental analysis and CI mass spectroscopy indicated the formula $C_{15}H_{20}O_3$; the IR spectrum ($CHCl_3$) exhibited absorption bands at 3400 (OH) and contained carbonyl bands at 1780 and 1680 cm^{-1} indicative of the presence of a non conjugated and of a conjugated ketonic carbonyl groups; and the UV spectrum was consistent with presence of a conjugated carbonyl system since it exhibited absorptions at 215 and 240 nm (ε 6050 and 4800).

Table 1 ^{13}C NMR data for compounds **1-4**

	1a		**2**		**3a**		**3b**		**4**	
Carbon atom	δ_C	^1J(C,H)/Hz	δ_C	^1J(C,H)/Hz	δ_C	^1J(C,H)/Hz	δ_C	^1J(C,H)/Hz	δ_C	^1J(C,H)/Hz
1	61.45 t	144.5	61.17 t		59.43 t	140.5	60.50 t	144	193.97 t	176.5
2	153.02 s		149.99 s		139.59 s		136.55 s		134.54 s	
3	74.93 d	143.0	74.51 d		74.16 d	142.5	74.44 d	143	154.21 d	157.5
4	143.98 s		148.67 s		141.03 s		138.76 s		54.55 d	145
5	200.15 s		197.77 s		70.90 d	155	77.95 d	148	203.02 s	
6	90.33 d	146.5	60.31 t		76.92 d	151.5	85.54 d	150	85.83 d	144
7	42.70 s		36.35 s		51.03 s		42.49 s		38.38 s	
8	14.66 q	127.5	20.41 q		15.48 q	127	15.88 q	127	18.82 q	127
9	47.02 d	131.0	46.40 d		46.59 d	130	47.28 d	131	43.02 d	129
10	42.50 t	127.0	41.27 t		41.82 t	128	42.14 t	127.5	44.40 t	129
11	40.89 s		40.81 s		40.50 s		40.14 s		37.61 s	
12	47.51 t	128.5	46.56 t		47.70 t	128	47.20 t	127	47.26 t	130
13	50.90 d	134.0	52.45 d		51.58 d	129	50.16 d	129	40.72 d	130
14	29.79 q	124.5	29.46 q		29.87 q	124	30.04 q	124	32.00 q	125
15	27.23 q	124.5	26.82 q		27.35 q	124	27.60 q	124	31.61 q	125



Scheme -A possible mechanism of formation of metabolite **4** from **1a** in presence of Et_3N.

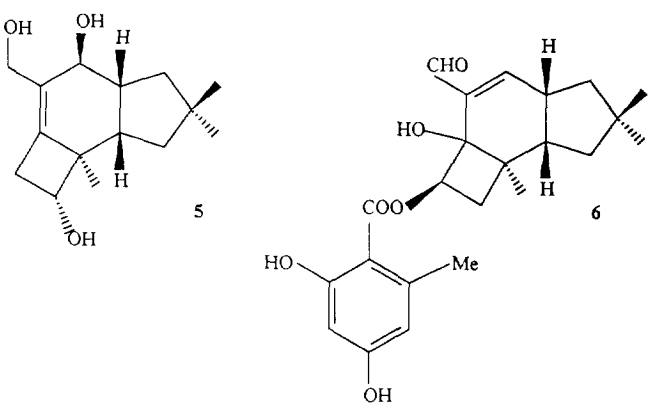


Table 2 ^{1}H NMR chemical shifts for compounds **1–4**

	δ_H							
Proton[a]	**1a**[b]		**1b**[c]	**1c**[b]	**2**[c]	**3a**[b]	**3b**[b]	**4**[b]
1a	4.46	(4.57)[d]	4.86	4.51	4.54	4.28	4.40	9.47
1b	4.42	(4.57)	4.72	4.44	4.50	4.26	4.40	
3	4.23	(4.24)	5.43	4.26	4.24	4.15	4.06	6.77
6	4.36	(4.39)	5.12	4.36	2.82	3.70	3.68	4.71
8	1.00	(1.02)	0.99	1.00	1.17	1.04	0.92	1.13
9	2.54	(2.51)	2.65	2.54	2.48[e]	2.30	2.30	2.64
10α	1.51	(1.48)	1.42	1.53	1.46	1.35	1.36	1.07
10β	1.55	(1.62)	1.67	1.57	1.56	1.39	1.42	1.59
12α	1.30	(1.23)	1.21	1.30	1.22	1.17	1.20	1.72
12β	1.86	(1.88)	1.65	1.80	1.85	1.78	1.77	2.10
13	2.32	(2.33)	2.48	2.33	2.44[e]	2.25	2.19	3.16
14	1.13	(1.15)	1.10	1.12	1.14	1.08	1.08	1.08
15	1.01	(1.00)	0.99	1.01	0.99	0.97	0.97	1.01
1-OR	3.82	(1.55[e])	2.12[e]		3.00[f]	3.97[e]	4.13[e]	
3-OR	4.57	(2.15[e])	2.14[e]		3.80[f]	4.50[e]	4.60[e]	
6-OR	5.16	(2.95[e])	2.15[e]	5.10		3.95[e]	5.15[e]	5.14

[a]Compound **1c** exhibited two methyl protons at 1.45 and 1.33; compound **2** exhibited the remaining 6-H at 2.75; compound **3a** exhibited 5-H and 5-OH at 4.84 and 4.82; compound **3b** exhibited 5-H and 5-OH at 4.65 and 4.60; and compound **4** exhibited 4-H at 3.28 ppm.[b] In [$^2H_6$] acetone. [c]In $CDCl_3$. [d]Values in parentheses are chemical shifts in $CDCl_3$.[e,f] Assignments within each column may be interchanged.

Table 3 ^{1}NMR coupling constants for compounds **1–4.**

	J/Hz						
J(H,H)[a]	**1a**[b]	**1b**[c]	**1c**[b]	**2**[c]	**3a**[b]	**3b**[b]	**4**[b]
1a,1b	16.0	16.0	16.5	17.6	13.4	e	
1a,3	1.4	1.6	1.4	1.6	1.0	1.1	
1b,3	1.5	1.6	2.6	1.9	1.2	1.1	
3,13	8.7	9.2	7.8	9.1[d]	8.5	8.9	2.0
9,10α	10.4	10.6	10.3	10.2[d]	10.2	10.6	12.7
9,10β	7.9	7.6	8.1	8.3[d]	8.2	8.0	6.9
9,13	11.5	12.0	11.8	11.9[d]	12.0	11.8	7.4
10α,10β	12.4	13.0	12.7	12.6	12.6	12.6	12.6
10α,15	0.8	0.8	0.8	0.8	0.8	0.8	~0
10β,12β	1.8	2.0	1.7	1.8	1.6	1.7	~0
12α,12β	12.2	12.5	12.5	12.3	12.6	12.6	13.5
12α,13	10.4	10.5	10.8	11.3[d]	10.2	9.9	2.3
12α,15	0.8	0.8	0.8	0.8	0.8	0.8	~0
12β,13	7.3	7.0	7.5	7.2	7.2	7.5	9.2

In compound **1a** 9-H exhibited J = 0.6 with both 3- and 6-H and the OH protons presented J = 5.8, 5.8, 5.5 and 7.4 with 1a-, 1b-, 3- and 6-H; in compound **3a** 5-H exhibited J = 0.7, 0.7, 1.3 and 5.8 with 1a-, 1b-, 3- and 6-H; in compound **3b**, 5-H exhibited J = 2.1, 2.1, 3.2 and 5.7 with 1a-, 1b-, 3- and 6-H; and in compound **4** 4-H exhibited J = 1.4, 2.9 and 2.3 Hz with 3-, 6- and 13-H, and 6-H a J = 7.8 Hz with 6-OH. [b]In [$^2H_6$] acetone. [c]In $CDCl_3$.[d]In [$^2H_6$] benzene. [e]Not assigned.

Comparison of the ^{13}C and ^{1}H NMR spectra of compound **4** with **1a** revealed the presence in **4** of the α,β-unsaturated aldehydic moiety OHC(1)-C(2)=C(3)H in place of the HOC(1)H_2-C(2)-C(3)HOH grouping. The ^{1}H NMR spectrum of **4** showed signals at δ 9.47 and 6.77 attributable to H-1 and -3, which were correlated with the aid of an HETCOR spectrum to the corresponding carbons resonating at δ 193.97 and 154.21, and only one hydroxy proton due to OH-6. Furthermore, the $^{13}C,^{1}H$ couplings of 27 and 9.5 Hz observed in the fully ^{1}H-coupled ^{13}C NMR spectrum between C-2 and H-1 and C-1 and H-3 were indicative of a two-bond interaction between the aldehydic proton and the vinylic carbon and of a *cis* geometry between the aldehydic carbon and the vinylic proton, respectively.[9] Finally, the ^{1}H NMR spectrum of **4** contained a signal at δ 3.28 which was assigned to H-4 since it presented coupling of 1.4 and 2.3 Hz with H-3 and -13 in accord with allylic and homoallylic relationships. Irradiation of H-4 in a NOE experiments (Experimental) led to enhancement of the 8-methyl protons (1%) thus permitting to indicate as **R** the stereochemistry of C-4.

Tsugicoline D was easily obtained by reaction of **1a** with MeOH/Et_3N ; a possible mechanism which may mimic the conversion *in vivo* between the two products **1a** and **4** is shown in the Scheme. Work is in progress to examine reactions of **1a** in the presence of different nucleophiles since the α,β-unsaturated carbonyl moiety is expected to behave as Michael acceptor.[10]

Tsugicoline A **1a** is the first reported sesquiterpene of protoilludane origin with carrying two oxidated functions in the four-membered ring.[8] It is therefore possible that it is a common intermediate in the biosynthesis of sesquiterpenes such as 3-*epi*illudol **5**[11] (isolated from *Clitocybe candicans*) and melleolide **6**[12] (from *Armillaria mellea) via* reduction processes.

Tsugicoline A **1a** is inactive against *Bacillus subtilis, B. cereus, Sarcinea lutea, Cladosporium cladosporioides* and *Saccharomyces cerevisiae* at a concentration of 100 μg $disc^{-1}$ but inhibited the growth of *Lepidium sativum*;[13] the same activity (allelopathic) was showed by 3,4-dihydroxybenzaldehyde isolated in poor yield during the purification of the tsugicolines.

Experimental

General.-M.p.s were determined on a Kofler apparatus and are uncorrected; IR spectra on a Perkin-Elmer 177 spectrophotometer; mass spectra on a Finnigan-MAT-TSQ70 spectrometer; optical rotations on a JASCO-500 DIP-181 polarimeter . NMR spectra were recorded on a Bruker AC 250L spectrometer operating at 250.1 MHz for ^{1}H and 62.9 MHz for ^{13}C. Molecular modelling was performed on a Silicon Graphics 4D-35GT, equipped with 16 Mb memory and 1 Gb hard disk, running Insight II & Discover package, version 2.10 (Biosym Technologies, San Diego, USA). A model was built by using standard bond lenghts and angles and by taking atomic potentials and charges as defined in the CVFF fragment library; the molecular model was then energy optimized. Chemical shifts are in ppm (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 preparative TLC (PLC) with Merck HF_{254} silica gel. Owing to the complexity of the purification procedure, we report the R_f values in hexane-EtOAc (1:1) and CH_2Cl_2-MeOH (15:1), respectively.

Isolation and Purification of Metabolites **1a, 2, 3a** and **4**.- A strain of *Laurilia tsugicola* (Henn. and Shirai)[*Echinodontium tsugicola*](CBS 248.51) received from Centraal Bureau voor Schimmel Cultures, Baarn, was maintained on MPGA (malt, peptone, glucose, agar, 20:4:20:15 g dm^{-3}) slants and sub-cultured in 40 stationary Erlenmeyer flasks (250 cm^3) containing a liquid medium MPG (50 cm^3) for 4 weeks at 24°C; the culture filtrates which were separated from the mycelium were extracted twice with EtOAc and the extracts were dried (Na_2SO_4) and evaporated to yield a mixture (2.5 g) of sesquiterpenes. The mixture was treated with Et_2O (50 cm^3); the residue (0.8 g) is formed of pure tsugicoline A **1a** and the mother liquid was chromatographed on a column of flash silica gel with hexane-EtOAc (2:1) as eluent to give a mixture of tsugicoline B **2** and D **4** that was further purified by PLC in CH_2Cl_2 (15:1) yielded the pure metabolites **2** (15 mg) and **4** (20 mg); with hexane-EtOAc (1:2) we obtained again compound **1a** (300 mg) and finally with EtOAc we have tsugicoline D **3a** (5 mg).

Tsugicoline A **1a**. R_f 0.1, 0.3 (Found: C, 67.5; H,8.3. $C_{15}H_{22}O_4$ requires C, 67.64; H, 8.33%); ^{13}C and ^1H NMR data are reported in Tables 1,2 and 3. Selected NOE experiments ([$^2H_6$] acetone + D_2O) : {H-3} enhanced H_3 -8 (1%), Hα-12 (4%) and H-13 (0.5%); {H-6} enhanced H_3-8 (0.5%) and H-9 (11.5%); {H-9} enhanced H-6 (10.5%), Hα-10 (1%), Hβ-10 (4%), H-13 (6%) and H_3-15 (1%); {H_3-8 and -15 } enhanced H-3 (9%), H-6 (0.5%), H-9 (6.5%), Hα-10 (6.5%), Hβ-10 (4%), Hβ-12 (5%) and H-13 (7%); {H-13} enhanced H-3 (1%), H-9 (5%), Hβ-12 (4%) and H_3-15 (1%); {H_3-14} enhanced H-3 (0.5%), Hα-10 (4.5%), Hβ-10 (3%), Hα-12 (3.5%) and Hβ-12 (3%).

Acetylation of Tsugicoline A **1a**.- Tsugicoline A **1a** (50 mg) was dissolved in dry pyridine (0.5 cm^3) and treated with Ac_2O (1 cm^3) overnight at 0°C. Standard work-up followed by PLC on silica gel in hexane-EtOAc (2:1) gave the triacetate derivative **1b** (40 mg) as an oil ; $[\alpha]_D$ -43.2 (*c* 0.1, $CHCl_3$); ν_{max} (film)/cm^{-1} 1750 (acetate) and 1670 (conj.CO) ; *m/z* (CI, isobutane) 393 (MH^+)(100%), 333 [(MH^+)-60](35), 273(50) and 231(30); ^1H NMR data are reported in Tables 2 and 3.

Acetonide Derivative of Tsugicoline A **1a**.- A solution of **1a** (50 mg) in dry acetone (5 cm^3) was treated with a trace of sulphuric acid during 2h at -20°C. The acetone was evaporated and ice was added. The mixture was then extracted with $CHCl_3$ and the extracts were washed with satd aqueous $NaHCO_3$ and dried (Na_2SO_4). Evaporation of the solvent gave the acetonide **1c** (40 mg) as white crystals, m.p. 146-148°C; *m/z* (CI, isobutane), 307 (MH^+)(14%) and 231(100); ^1H NMR data are reported in Tables 2 and 3.

Reaction of Tsugicoline A **1a** *with Pivaloyl Chloride*.- Tsugicoline A **1a** (100 mg), pivaloyl chloride (0.2 cm^3) and dry pyridine (1 cm^3) were left at -30°C for 0.5 h. Standard work-up followed by PLC on silica gel in hexane-EtOAc (4:1) afforded the dipivalate **1d** (20 mg) as the main product; m.p. 110-112°C; *m/z* (CI, isobutane), 435 (MH^+); δ_H ($CDCl_3$) 5.06 (1H, br s, H-6), 4.94 and 4.83 (2H, br d, J = 13.0 Hz, H_2-1), 4.10 (1H, br d, J = 8.7 Hz, H-3), 2.63 (1H, m, H-9), 2.30 (1H, m, H-13), 1.91, 1.69, 1.36 and 1.21 (4H, m, H_2-10 and -12), 1.6 (1H, br signal, OH-3), 1.22 (H-18, s, 2 x ^{t}bu), and 1.12, 1.00 and 0.90 (9H, s, H_3-8, -14 and -15).

Reaction of the Acetonide **1c** with (±)-*2-Phenylbutyric Anhydride*.- The acetonide **1c** (40 mg) and (±)-2-phenylbutyric anhydride (50 mg) were dissolved in dry pyridine (0.5 cm^{-3}) and the solution was kept for 20 h at room temperature. (+)-2-phenylbutyric acid $[\alpha]_D$ +3.5 (*c* 0.5, pyridine) was obtained upon work-up of the reaction mixture according to the literature method.[7]

Reaction of the dipivalate **1d** with (±)-2-Phenylbutyric Anhydride.- Compound **1d** (90 mg) in dry pyridine (0.5 cm^3) was treated with the anhydride; after normal work-up as above (-)-2-phenylbutyric acid $[\alpha]_D$ -31 (*c* 0.5, pyridine) was obtained.

Tsugicoline B **2**.- R_f 0.5; 0.3 (Found: C, 71.6; H,8.7. $C_{15}H_{22}O_3$ requires C, 71.97; H, 8.86%); UV: λ_{max} 254 nm (ε 5100); ν_{max} ($CHCl_3$)/cm^{-1}, 1730 (conj.CO); *m/z* (CI, isobutane) 351 (MH^+)(100%), 233 (MH^+-18)(95), 215(38), 205(30), 203(22), 187(28) and 173(25); ^1H and ^{13}C NMR data are reported in Tables 1, 2 and 3.

Tsugicoline C **3a**.- R_f 0.2; 0.05 (Found: C, 66.9; H, 8.9. $C_{15}H_{24}O_4$ requires C, 67.13; H, 9.02%); UV: λ_{max} 208sh (ε 5600); ν_{max} ($CHCl_3$)/cm^{-1}, 3400 (OH); ^{13}C and ^1H NMR data are reported in the Tables 1,2 and 3. Selected NOE experiments ([$^2H_6$] acetone + D_2O) : {H_3-8} enhanced H-3 (8.5%), H-6 (1.5%), H-9 (1%) and H_2-10 (5%); {H_3-14} enhanced H_2-10 (5%) and Hβ-12 (2.5%); {H_3-15} enhanced H-9 (5.5%), Hβ-10 (2%), Hβ-12 (2.%) and H-13 (5%).

Reduction of Tsugicoline A **1a**.- Tsugicoline A **1a** (100 mg) was treated with $NaBH_4$ (20 mg) in MeOH (5 cm^3); usual work-up gave a 95:5 mixture of two compounds, which were purified by PLC in CH_2Cl_2-MeOH (15:1) (3 runs) and identified as tsugicoline C **3a** (4 mg) and its C-5 epimer **3b** (91 mg).

5-epi-Tsugicoline C **3b**.-This compound was isolated as a solid, m.p. 85-87°C; $[\alpha]_D$ -59 10^{-1}deg cm^2g^{-1} (*c* 0.05, $CHCl_3$); *m/z* (CI, isobutane) 269 (MH^+); ^{13}C and ^1H NMR data are reported in Tables 1, 2 and 3. Selected NOE experiments ([$^2H_6$] acetone + D_2O): {H_3-8} enhanced H-3 (6%), H-5 (10.5%), H-6 (1%), H-9 (1%) and H_2-10 (3.5%); {H_3-14} enhanced H_2-10 (3%) and Hβ-12 (2%); {H_3-15} enhanced H-9 (5%), Hβ-10 (2%), Hβ-12 (3.5%) and H-13 (5%).

Tsugicoline D **4**.- R_f 0.7; 0.3; (Found: C, 72.2; H, 8.0. $C_{15}H_{20}O_3$ requires C, 72.55; H, 8.12%); *m/z* (CI, isobutane) 249 (MH^+)(80%), 231 (MH^+-18)(100), 220 (20) and 203 (25); ^{13}C and ^1H NMR data are reported in Tables 1, 2 and 3. Selected NOE experiments ([$^2H_6$] acetone) : {H-1} enhanced H-3 (11.5%) and H-4 (1.5%); {H-3} enhanced H-1 (18%), Hα-12 (4.5%) and H-13 (4.5%); {H-4} enhanced H-1 (1%) and H_3-8 (1%); {H-6} enhanced H-9 (3%) and H-13 (10.5%); {H-13} enhanced H-3 (3%), H-6 (7%), H-9 (4%) and Hβ-12 (3.5%).

Reaction of Tsugicoline A **1a** *with Triethylamine*.- Tsugicoline A **1a** (50 mg) dissolved in MeOH (3 cm^3) was treated with triethylamine (0.1 cm^3) at room temperature for 2 h; evaporation of the solvent and PLC with CH_2Cl_2-MeOH (15:1) gave a compound (20 mg) identical with the natural one **4** (^1H NMR, MS and TLC).

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References and Notes

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