Secondary mould metabolites. Part 58.[1] Modifications in basic conditions and Michael additions of the protoilludane sesquiterpene tsugicoline A; some implications for the biogenesis of other sesquiterpenoids produced by Basidiomycetes

Alberto Arnone, Cristiana De Gregorio, Andrea Mele, Gianluca Nasini * and Orso Vajna de Pava

Dipartimento di Chimica del Politecnico, Centro del CNR per le Sostanze Organiche Naturali, Politecnico di Milano, via Mancinelli 7, I-20131, Milano, Italy

Received (in Cambridge, UK) 9th November 1999, Accepted 17th January 2000

The protoilludane sesquiterpene tsugicoline A **1** rearranges at pH 13 into compound **8a** with a structure very similar to isolactarane sesquiterpenes and into compound **9a** similar to the natural furosesquiterpene tsugicoline H **9c**; under different basic conditions compounds **4a**, **5**, **6a** and **7a**,**b**, are formed. In particular, **4a** shows the same skeleton of the natural tsugicoline E **4c**. Compound **1** reacts with some thiols to give a Michael addition to α,β-conjugated carbonyl system, giving the adducts **10**–**13**. In the case of compounds **12a**,**b** a new tetrahydrothiophene ring is formed. The structures and stereochemistry of the products are discussed with the aid of NMR data; compounds **4a**,**c**, **10** and **12a**,**b** are correlated also with tandem MS studies. The implications of these results on current opinions on the biogenetic pathways of sesquiterpenes of Basidiomycetes are discussed.

A number of Basidiomycetes are known to produce sesquiterpenoids with a protoilludane skeleton.[2] Among these, in the course of continuing studies in search of biologically active constituents, we have studied extensively the secondary metabolic pathway of a strain of *Laurilia tsugicola*, a decay agent of *Tsuga* and *Abies*.

When the fungus was grown in liquid cultures, tsugicoline A **1** was produced in high yield;[3] it was transformed into the sterpurane derivative **2** at pH 8[4] while reaction in Et_3N–MeOH gave tsugicoline D (Scheme 1).[3] The polyoxygenated tsugicoline E **4c** was isolated from the more polar chromatographic fractions and the absolute configuration was deduced from X-ray analysis.[5] A possible scheme of formation *in vivo* of compound **4c** involves a Michael addition of water onto the unsaturated ketone system of **1**, followed by intramolecular acetalization. The growth of *L. tsugicola* in agar medium gave the furosesquiterpenes tsugicolines F–H together with the norsesquiterpene tsugicoline I **6b**.[6]

The isolation of compound **2** was the first example of the chemical conversion of a protoilludane into a sterpurane sesquiterpene (the formation of the latter skeleton normally is restricted to the fungi of the genera *Stereum* and *Merulius*) and prompted us to study the reactivity of **1** under a range of basic conditions.

In the present paper we describe the structure elucidation of compounds **4a**–**9a** formed in different basic conditions and of compounds **10**–**13** obtained by reaction of **1** with some thiols.

Results and discussion

Treatment of tsugicoline A **1** with MeOH saturated with ammonia at room temp. gave two products in a 5:1 ratio; the structure of the minor compound was readily assigned as it was identical on the basis of NMR evidence with that of the sterpurane derivative **2** formerly obtained by biotransformation from **1**.[4] The major compound **4a**, analysed for $C_{15}H_{25}NO_4$; FABMS (thioglycerol) gave a distinct peak at *m*/*z* 284 (MH^+) thus confirming the presence of a nitrogen atom in the molecule. The ^{13}C and ^1H NMR data of **4a**, reported in the Experimental section and in Tables 1 and 2, were similar to those exhibited by the known tsugicoline E **4c**,[5] the only relevant differences being the upfield shift of C-2 (δ_C 61.90 *vs.* 80.27) and the number and the chemical-shift values of the exchangeable protons. All these findings pointed to the presence, in **4a**, of an NH_2 group at C-2 in place of the OH function. This conclusion was supported by the ^1H NMR spectrum of the tetraacetyl compound **4b** since it contained a slow-exchanging signal at δ_H 5.79 attributable to the proton of the amidic moiety formed by acetylation of the 2-NH_2 group.

Subsequently, tsugicoline A **1** was treated with solid $NaHCO_3$ in MeOH to reflux; the first step of the reaction was the formation of compound **2** which, after five hours, gave in low yield, compound **5**; CIMS showed a peak at *m*/*z* 315 (MH^+) corresponding to a formula $C_{16}H_{26}O_6$ with 48 mass units more than in sterpurane **2** while the large absorption at 1727 cm^{-1} observed in the IR spectrum (liquid film) suggested the presence of a lactone group. The ^{13}C NMR spectrum of **5** lacked the vinylic carbons present in the spectrum of **2** and the carbonyl carbon underwent an upfield effect (δ_C 179.80 *vs.* 200.21), confirming its lactonic nature. These results, together with the presence in the ^1H NMR spectrum of a methoxy signal at δ_H 3.22 and of a methine proton at 2.90, assigned to 4-H, indicated the addition of one mole of MeOH to the C-4–C-7 double bond of **2**. The NOEs observed between 8-H_3 and 3-H (7.5%), 4-H and 1-H_2 (1.5%), 5-H and 9-H (6.5%) permitted the assignment of the chirality of C-2, -4, -5 and -7.

The formation of the new five-membered ring can be explained with a keto–enolic tautomerism in the cyclobutanone ring of compound **2** (see Scheme 2) followed by a Baeyer–Villiger oxidation.

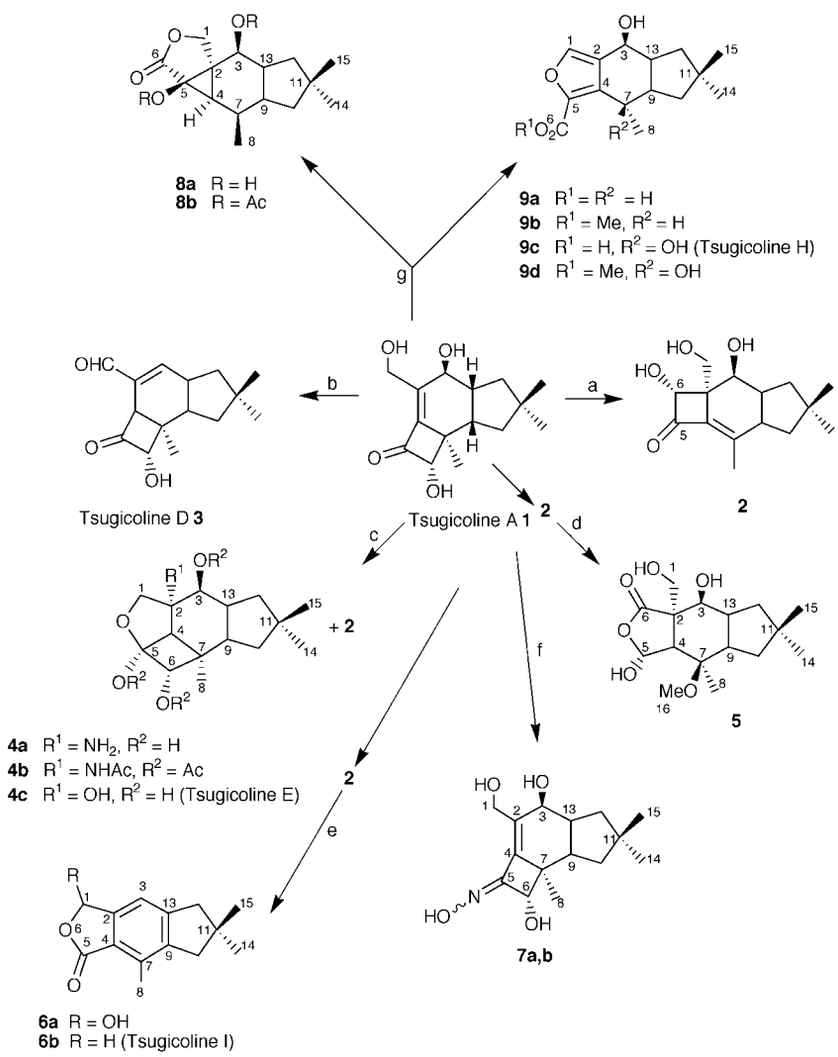
Treatment of compound **1** with a solution of 10% NaOH in MeOH at room temp. provided the norsesquiterpene **6a** *via* intermediate **2**. It was isolated as a white solid that was identified by analysis to be $C_{14}H_{16}O_3$. A comparison of the ^1H and ^{13}C NMR spectra of **6a** with those of tsugicoline I **6b**[6] revealed the presence in **6a** of a $\succ$C(1)HOH fragment (δ_H 6.50 and 6.79,

DOI: 10.1039/a908912h

Table 1 ^{1}H NMR data for compounds **4a**, **5**, **6a**, **8a**, **9b**, **10**, **11**, **12a**,**b**, **13**

	δ_H									
Proton[a]	**4a**[b]	**5**[c]	**6a**[c]	**8a**[c]	**9b**[c]	**10**[c]	**11**[b]	**12a**[b]	**12b**[b]	**13**[c]
1a	3.82	3.97 (4.20)[d]	6.50	4.50	7.64 (7.51)[d]	4.05	3.97 (4.12)[e]	3.92 (3.98)[e]	3.73	3.57
1b	3.50	3.82 (3.71)		3.91		3.91	3.77 (3.81)	3.64 (3.98)	3.25	3.06
3	3.38	3.75 (3.72)	7.25	4.15	4.56 (4.58)	3.66	3.97 (3.68)	3.69 (3.89)	3.54	3.59
4	1.66	2.90 (2.65)		1.15		1.74	1.84 (2.07)	2.30 (2.31)	2.12	3.03
6	3.94					4.15	3.97 (4.18)	4.14 (4.35)	4.53	5.26
8	0.87	1.31 (1.30)	2.51	1.11	1.41 (1.42)	1.00[e]	0.98 (1.03)	0.98 (1.11)	0.98	1.00
9	2.02	2.50 (2.53)		2.39	2.45 (2.43)	2.16[f]	2.03 (2.21)	2.02 (2.18)	2.07	2.74
10α	1.26	1.45 (1.32)	2.75	1.02	0.95 (0.97)	1.28	1.19 (1.32)	1.16 (1.30)	1.16	1.52
10β	1.32	1.52 (1.54)	2.75	1.78	1.59 (1.62)	1.42	1.34 (1.45)	1.27 (1.38)	1.28	1.59
12α	1.78	1.29 (1.20)	2.87	1.23	1.07 (1.04)	1.88	1.76 (1.90)	1.75 (1.90)	1.76	1.83
12β	1.46	1.76 (1.82)	2.87	1.89	1.62 (1.64)	1.56	1.46 (1.58)	1.42 (1.54)	1.43	1.68
13	1.99	2.12 (2.33)		2.44	2.58 (2.56)	2.20[f]	2.07 (2.25)	2.29 (2.49)	2.37	2.62
14	1.08	1.08 (1.08)	1.17	1.05	1.00 (0.99)	1.10	1.07 (1.12)	1.06 (1.11)	1.05	1.11
15	0.96	0.95 (0.94)	1.17	0.91	0.94 (0.96)	0.99[e]	0.97 (1.02)	0.98 (1.02)	0.97	1.04
1-OH		3.59 (4.20)	6.79					4.94 (4.35)	5.05	3.99
3-OH	3.50	4.81 (4.20)		4.05	4.16 (1.54)	3.80	4.86 (3.94)	4.80 (4.07)	4.79	3.39
5-OH	4.80	6.38 (4.20)		5.56		3.80	6.61 (5.80)	4.92 (4.40)	5.15	
6-OR	3.50				3.84 (3.90)	3.80	4.92 (4.05)	5.19 (4.35)	4.66	5.08

[a] In **4a** the NH_2 protons resonated at 3.50; in **5** 5-H and the OMe protons resonated at 5.83 (5.88) and 3.22 (3.26); in **8a** and **9b** 7-H resonated at 1.98 and at 3.20 (3.21); in **10** 16-H_2 resonated at 3.26 and 3.09; 17-H at 4.37; the NH_2 and OMe protons at 3.80 and 3.68; in **11** 16-H_2 and the OMe protons resonated at 3.55, 3.52 (3.53, 3.45) and at 3.62 (3.70) ppm. [b] In [$^2H_6$]DMSO. [c] In [$^2H_6$]acetone. [d] In $CDCl_3$. [e,f] Assignments may be interchanged.



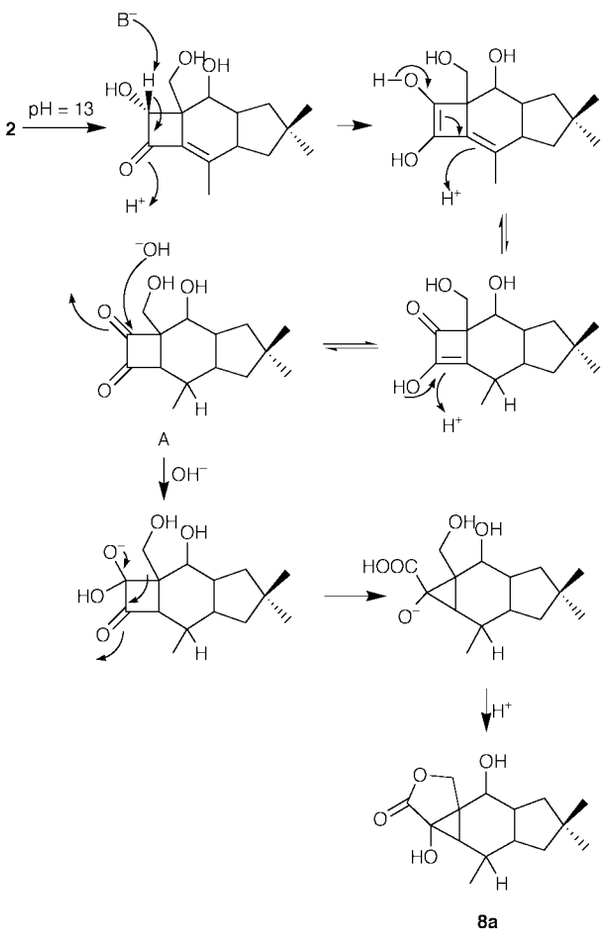
Scheme 1 Chemical transformations of tsugicoline A **1** under basic conditions. A non-systematic, biosynthetic numbering scheme is shown, and is used in the assignment of NMR spectral data. *Reagents and conditions*: (a) bioagents or buffer at pH 8; (b) MeOH–Et_3N; (c) MeOH–NH_3; (d) MeOH–$NaHCO_3$, reflux; (e) MeOH–NaOH (10%); (f) MeOH–NH_2OH; (g) DMSO–buffer at pH 13.

$J_{1\text{-H,1-OH}}$ 8.3 Hz; δ_C 97.04) instead of a CH_2 group (δ_H 5.17, δ_C 68.40), the remaining signals being very similar. The NOE observed between 1-H and 3-H (2%) confirmed that these protons are *peri* related. The formation of the γ-lactone ring of **6a** is evidently analogous to that postulated for the previous compound **5** while the aromatization of the cyclohexene ring pro-

Table 2 Coupling constants for compounds **4a**, **5**, **8a**, **9b**, **10**, **11**, **12a**,**b**, **13**

	J/Hz								
J[a]	**4a**	**5**	**8a**	**9b**	**10**	**11**	**12a**	**12b**	**13**
1a, 1b	10.5	10.1	8.8		11.2	11.2	10.5	10.8	10.6
3, 13	10.5	10.0	8.6	3.6	11.0	10.0	11.0	10.9	9.8
9, 10α	13.2	10.2	10.5	11.5	13.2	13.2	13.7	13.4	11.8
9, 10β	6.8	8.0	7.3	7.3	6.4	6.4	6.6	6.5	7.4
9, 13	[b]	11.8	8.0	8.6	[b]	6.5	5.7	6.3	[b]
10α, 10β	12.3	12.5	12.0	12.4	12.1	12.4	12.1	12.4	12.7
10β, 12β	<0.5	1.8	2.0	1.8	<0.5	<0.5	<0.5	<0.5	1.0
12α, 12β	13.5	12.6	12.2	12.4	13.6	13.8	13.7	13.6	13.5
12α, 13	1.0	10.3	10.4	8.5	1.2	1.2	1.2	1.5	4.2
12β, 13	6.5	7.6	7.6	7.3	6.9	6.8	7.1	7.4	7.6
1a, 1-OH		[b]					5.7	5.0	5.0
1b, 1-OH		[b]					5.7	5.0	6.0
3, 3-OH	[b]	[b]	[b]	3.6	[b]	7.5	5.4	5.3	8.2
6, 6-OH	[b]				[b]	6.0	6.3	6.0	7.6

[a] $J_{4,5}$ = 3.1, $J_{7,8}$ = 6.7 and $J_{7,9}$ = 10.4 Hz were observed in **8a**; $J_{7,8}$ = 7.2 and $J_{7,9}$ = 1.7 Hz were observed in **9b**; $J_{4,6}$ = 1.2, $J_{16a,16b}$ = 14.2, $J_{16a,17}$ = 9.1 and $J_{16b,17}$ = 3.6 were observed in **10**; $J_{16a,16b}$ = 14.8 Hz was observed in **11**; $J_{4,6}$ = 2.2 Hz was observed in **13**. [b] Not assigned.



Scheme 2 A possible mechanism of formation of compound **8a** from the sterpurane **2**.

ceeded presumably *via* the oxidation and decarboxylation of the CH_2OH group of compound **2**.

The reaction of **1** with NH_2OH gave the expected *syn* and *anti* oxime derivatives **7a**,**b**, the structure of which was confirmed by NMR data and MS results. The ^{13}C NMR data of the oximes **7a**,**b** when compared with those of tsugicoline A **1** presented signals at δ_C 155.68 and 151.30 in place of the C-5 carbonyl carbon at δ_C 200.15 and significant differences only on the chemical-shift values of the C-2, -4, -6 and -7 carbons.

Finally, we have studied the modification of compound **1** in a buffer solution (pH 13) at room temp. for four hours; the reaction mixture gave, after acidification, EtOAc extraction and chromatographic separation, two products **8a** and **9a** in a 2:1 ratio. The major compound **8a** was isolated as a white solid, mp 85–90 °C, and analysed for $C_{15}H_{22}O_4$, and therefore an isomer of sterpurane **2**; CIMS showed a peak at *m*/*z* 249 ($MH^+ - 18$) and the IR spectrum gave a signal at 1747 cm^{-1} attributable to a lactone function. The presence of two hydroxy groups in the molecule was then evidenced by the formation of the diacetate **8b**.

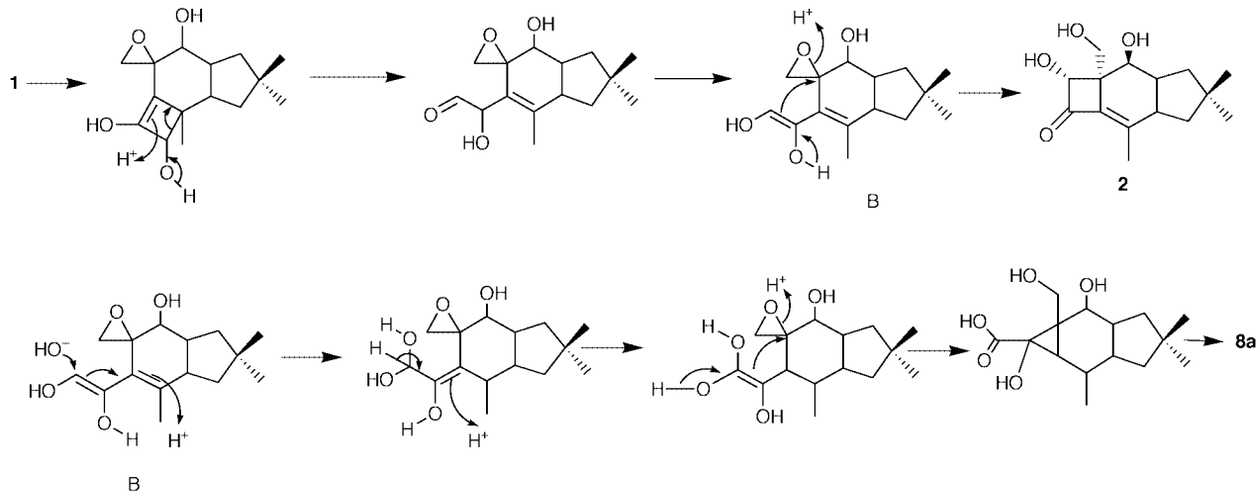
Analysis of the ^1H NMR spectrum of **8a** revealed the presence of >C(3)HOH and >C(4)H–C(7)HMe–C(9)H< moieties linked to the C-13 and -9 atoms of the unchanged cyclopentane ring. Moreover, the characteristic one-bond $^1H,^{13}C$ coupling constant of 161.5 Hz exhibited by C-4 implies that this carbon is part of a cyclopropane system, the other two carbons of the ring resonating as quaternary signals at δ_C 36.88 and 68.41. Finally, the NOEs observed between 1b-H and 4-H (2%), 3α-H and 1a-H (1%) and 5-OH and 9β- and 13β-H (2%) suggested that the remaining OH and CO_2CH_2 units are allocated as depicted in formula **8a** while the mutual NOEs observed between 3α-H and 7-H (2.5 and 2%) established that these protons are on the same (α) side of the cyclohexane ring.

Scheme 2 shows the formation of **8a** from the intermediate **2** through a tautomeric process to the diketone **A** followed by a classical benzylic rearrangement. A possible mechanism for the formation of compounds **2** and **8a** might involve the intervention of a 1,2- (or 2,3-) epoxide through the common intermediate **B**.†

From a biogenetic point of view, this interesting compound, presenting a cyclopropane ring fused with a cyclohexane, is assimilable to the isolactarane sesquiterpenes like, *e.g.*, isolactararufin isolated from *Lactarius rufus*.[2]

The minor compound **9a** was isolated as an acidic derivative from fractions eluted with CH_2Cl_2–MeOH 4:1 with added formic acid; subsequent treatment of **9a** with CH_2N_2 permitted the isolation of the methyl ester **9b**. A comparison of the NMR data of compound **9b**, which analysed for $C_{16}H_{22}O_4$, with those of the methyl ester **9d** of the natural tsugicoline H **9c**[6] indicated that the two compounds share the same basic structure, the only significant difference being the presence in the ^1H NMR spectrum of **9b** of a methine proton at C-7 vicinally coupled to 8-H_3 and 9-H in place of an hydroxy group. Accordingly, in the ^{13}C NMR spectrum of **9b**, the quaternary carbon at δ_C 70.65 has been replaced by a methine carbon at δ_C 30.27.[6]

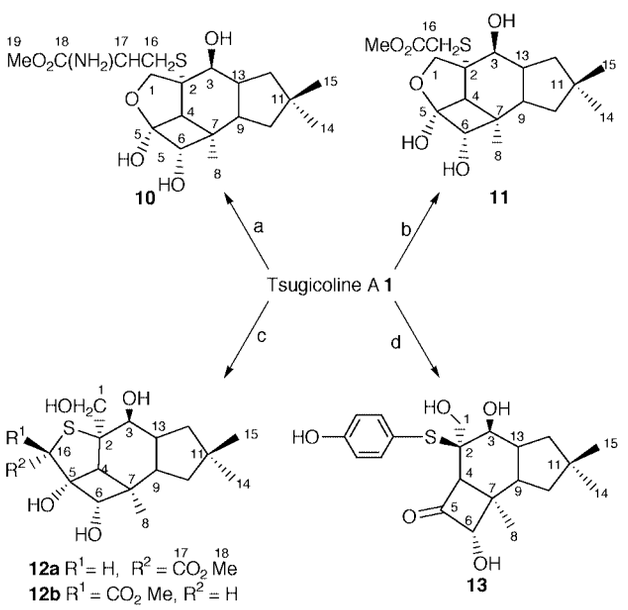
† We are indebted to the Referees for suggesting such a sequence: see Scheme 3.



Scheme 3 An alternative mechanism of formation of compounds **2** and **8a** from tsugicoline A **1**.

The α,β-unsaturated carbonyl moiety in tsugicoline A **1** would be expected to behave as a Michael acceptor toward thiols and therefore we examined the reaction of **1** with three mercapto group-containing compounds such as L-cysteine methyl ester, methyl thioglycolate and 4-mercaptophenol.

L-Cysteine methyl ester was found to react readily with **1** in the presence of sodium hydrogen carbonate giving only compound **10** (see Scheme 4) as a yellow solid, mp 150 °C; elemental



Scheme 4 Michael addition of some thiols to tsugicoline A **1**. *Reagents and conditions*: (a) L-cysteine methyl ester–$NaHCO_3$–MeOH, reflux; (b) methyl thioglycolate–MeOH, reflux; (c) methyl thioglycolate–MeOH–$NaHCO_3$; (d) 4-mercaptophenol–acetone–H_2O.

analysis and CIMS (MH^+, *m*/*z* 402) established the molecular formula as $C_{19}H_{31}NO_6S$. The ^1H NMR spectrum was similar to that exhibited by tsugicoline E **4c**[5] except for the presence in **10** of signals attributable to an $SCH_2CH(NH_2)CO_2Me$ moiety in place of a hydroxy group. In addition, the C-2 carbon resonated in **10** at higher field than in **4c** (δ_C 58.98 *vs.* 80.27).

More interesting was the behaviour of compound **1** in the presence of methyl thioglycolate. Compound **11** was obtained in a neutral medium, without added bases; a comparison of the ^1H and ^{13}C NMR spectra of **11** with those of **10** indicated that the two compounds possess the same basic skeleton and differ only in the nature of the C-2 side chain. The NOEs observed between the 2-SCH_2 protons and 4-H (5%) and between 4-H and 8α-H_3 (1%) confirmed that all these protons are on the same (α) side of the molecule.

If the reaction was performed in the presence of a catalytic amount of $NaHCO_3$ we obtained compounds **12a**,**b** in a 6:1 ratio. They revealed the same relative molecular mass as **11** and showed ^1H NMR spectra similar to that exhibited by **11**, the only relevant differences being the presence in **12a**,**b** of one additional hydroxy proton vicinally coupled to the 1-methylene protons and of only one proton linked to the sulfur-bearing C-16 carbon. The NOEs observed in both compounds **12a**,**b** between 8α-H_3 and 4-H and between 4-H and 1-H_2 indicated that the CH_2OH group is α oriented and, as a consequence, that the $SCHRCO_2Me$ grouping is on the β side of the molecule.

All these findings, together with the upper-field values exhibited by C-5 in the ^{13}C NMR spectra of **12a**,**b** with respect to **11** (δ_C 88.84, 88.30 *vs.* 107.81 ppm), require that in each of the two compounds **12a**,**b** C-16, and not O-1, be linked to C-5 to form two tetrahydrothiophene rings, epimeric at C-16, whose chirality followed from the NOE observed in **12b**, but not in **12a**, between 1b-H and 16-H (3.5%). In this case, the acidity of the CH_2 group α to the sulfur was enough to trigger an aldolic condensation on the C-5 carbonyl.

Finally, tsugicoline A **1** was treated with 4-mercaptophenol to give the easy formation of the sesquiterpene derivative **13** which was isolated as a white solid. The ^{13}C NMR spectrum showed, in addition to the signals due to the thiophenol moiety, a methine and a quaternary sp^3 carbon in place of the vinylic carbons contained in tsugicoline A **1**. The methine resonance was assigned to C-4 since in the ^1H NMR spectrum 4-H presented no vicinal coupling constants with 1-H_2 and the thiophenol ring was then allocated at C-2 in a β position since the 2′- and 6′-H protons gave mutual NOEs with 6β-H (1 and 0.5%).

The attempted reactions of compound **1** with other nucleophiles such as phenylalanine, glycinemethyl ester, 4-hydroxy-6-methyl-2-pyrone and ethyl cyanoacetate were unsuccessful.

Some clarification is necessary on the Michael addition: if the nucleophilic reagent forms the adduct from the α side of the unsaturated carbonyl plane, followed by intramolecular acetalization of the CH_2OH group, we have the formation of compounds **4a**, **10** and **11**. The formation of triacetate **4b** indicates that **4a** retains the closed structure even after acetylation. Weakly basic conditions (compounds **12a**,**b**) or steric hindrance (**13**) promote the β attack of the reagent.

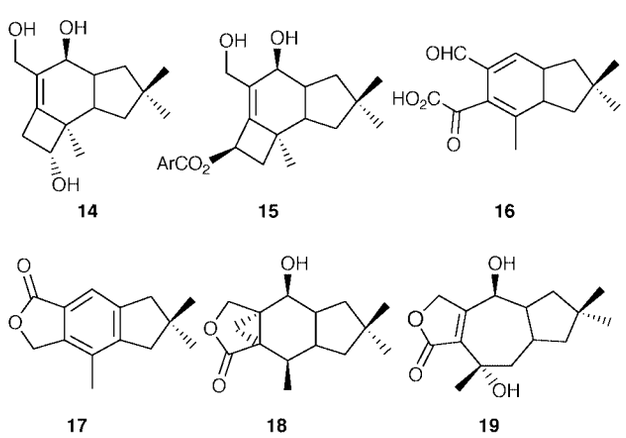
Mass spectrometric characterization of compounds 4a,c, 10 and 12a,b

Compounds **4a**,**c**, **10** and **12a**,**b** were characterized by using FABMS and tandem MS. All the examined compounds

provided appreciably intense signals due to protonated analyte $[M + H]^+$ at *m/z* 284 (relative intensity 100%), 285 (10), 402 (40) and 373 (10), respectively. Compound **4c** underwent loss of neutral H_2O affording $[M - H_2O + H]^+$ at *m/z* 267 as the base peak. The existence of a mutual polycyclic frame shared by compounds **4a**,**c** and **10** was confirmed by tandem MS experiments. Indeed, protonated **4a**,**c** and **10** undergo efficient loss of neutral molecules under FABMS conditions, all affording the fragment at *m/z* 249. The latter can be assigned to $[M - H_2O - NH_3 + H]^+$ for **4a**, $[M - 2H_2O + H]^+$ for **4c** and [M − (L-cysteine methyl ester) $- H_2O + H]^+$ for **10**. Three different experiments of collision-activated decomposition were carried out on the ion at *m/z* 249 generated by **4a**,**c** and **10**, respectively. In all the cases the same fragmentation pattern was observed, irrespective of the origin of the precursor ion, confirming that compounds **4a**,**c** and **10** differ only in functionalization and side chains, but share the same polycyclic molecular building.

The mass spectrometric response of compounds **12a**,**b** pointed to significant differences with respect to the above described trend. Indeed, collision-activated decomposition of the ion at *m/z* 373 (corresponding to protonated **12a**,**b**) provided some series of fragment ions: i) *m/z* 355, 337, 319, 301, assigned to $[M - (H_2O)_n + H]^+$, with $n = 1$, 2, 3 and 4; ii) *m/z* 305, 287, assigned to $[M - (H_2O)_n - S + H]^+$, with $n = 1$, 2; iii) *m/z* 277, 259 assigned to $[M - (H_2O)_n - S - CO + H]^+$, with $n = 2$, 3. The formal loss of elemental sulfur, as reported in ii) and iii), was not observed at all in the fragmentation pattern of sulfur-containing compound **10**, suggesting that the S atom is in two completely different environments in compounds **12** and **10**, consistent with intra-ring S in **12** and extra-ring S in **10**. The information from tandem MS and NMR spectroscopy (*vide supra*) allowed us to confirm the tetrahydrothiophene frame of compounds **12a**,**b**.

The reactivity of tsugicoline A **1** confirms the important role played by the protoilludanes in the biosynthetic pathways of the sesquiterpenoids from Basidiomycetes.[2] For example, the easy formation of compound **2** suggests that the sterpuranes may arise directly *via* protoilludanes rather then by the proposed pathways through bulleranes or illudanes. It is therefore possible that **1** may be a common intermediate in the biosynthesis of 3-epiilludol **14**,[7] armillylorsellinate **15**, melleolides



and melledonals isolated by several groups from *Armillaria mellea*.[8,9] **1** can be transformed into a derivative of clavacoronic acid **16** isolated from the Basidiomycete *Clavicorona pyxidata*,[4,10] and the isolation of a new class of compounds, the furosesquiterpenes tsugicolines F–H, from solid cultures of the fungus *L. tsugicola*, suggested their formation from **1**. Compound **6a** is quite similar to the norsesquiterpene isolactarufin **17**, isolated from *Lactarius strobilatus*.[11] Further, the analogy of compound **8a** obtained from **1** with the isolactarane isolactarorufin **18**, and the co-occurrence of **18** with the lactarane lactarorufin **19** in *Lactarius rufus*, pose an interesting problem from a biogenetic point of view.[2]

To conclude, in our opinion the presence of the two oxygen functions in the four-membered ring in **1** is the key to the reactivity of this interesting possible intermediate and may offer new biogenetic implications in the pathways so far accepted for the formation of sesquiterpenes of protoilludane origin.

Experimental

Mps 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 were obtained at 20 °C on a JASCO Dip.-181 polarimeter and $[a]_D$-values are given in 10^{-1} deg cm^2 g^{-1}. FABMS and tandem MS spectra were obtained on a Finnigan-MAT TSQ70 triple-stage quadrupole machine equipped with an Ion-Tech (Teddington, UK) atom gun with Xe as bombarding gas. The emission current was typically set at 2 mA with an accelerating voltage of 8 keV. For all experiments the source was kept at room temperature. CsI was used for mass calibration. Tandem MS experiments were performed using Ar as collision gas ($p = 8 \times 10^{-4}$ Torr, 1 Torr = 133.3 Pa, nominal collision energy $E_{lab} = 10$ eV). Product ion scan experiments[12] were performed by using the first quadrupole as parent mass selector, the second as collision cell and the third as mass analyser (typically, *m/z* scan range 100 to 2000, scan rate 500 amu s^{-1}). All the spectra were acquired in centroid mode and calculated over an average of 20 to 60 scans using standard Finnigan software.

Samples were prepared by dissolving a small amount of analyte in a suitable matrix. For all the examined compounds the best results were obtained by using 3-mercaptopropane-1,2-diol (thioglycerol) previously acidified with HCl vapour as matrix.

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. Chemical shifts are in ppm (δ) from $SiMe_4$ as internal standard, and *J*-values are given in Hz. TLC and preparative TLC (PLC) were performed with Merck HF_{254} silica gel.

For biogenetic reasons, the formulae numbering system is that used for the protoilludane skeleton;[2] the names of compounds were deduced by Chemical Abstracts index name.

Reaction of (1*S*,4*S*,4a*R*,7a*S*,7b*R*)-1,4,4a,5,6,7,7a,7b-octahydro-1,4-dihydroxy-3-hydroxymethyl-6,6,7b-trimethyl-2*H*-cyclobut-[*e*]inden-2-one 1 with ammonia

40 mg of tsugicoline A[3] **1** were treated with 15 cm^3 of MeOH saturated with NH_3; the solution was kept for 1 h at room temp., the solvent evaporated off and the mixture purified with PLC using CH_2Cl_2–MeOH (7:1)–triethylamine 1% v/v as eluent. Elution of the upper band gave 2,2a,3,3a,4,5,6,6a-octahydro-2-hydroxy-2a-hydroxymethyl-5,5,7-trimethyl-1*H*-cyclobuta[*f*]inden-1-one **2** (4 mg; R_f 0.3), identical (TLC and ^{1}H NMR) with a sample previously isolated.[4]

3a-Aminododecahydro-6,6,7b-trimethylcyclobuta[cd]cyclopent[f]isobenzofuran-1,1a,4-triol **4a** was also obtained (27 mg), mp 160–165 °C (from acetone–hexane); R_f 0.1; ν_{max}(KBr)/cm^{-1} 3430, 1635 and 1385; $[a]_D$ 8 (*c* 0.5, MeOH) (Found: C, 63.5; N, 4.8; H, 8.8. $C_{15}H_{25}NO_4$ requires C, 63.58; N, 4.94; H, 8.89%); the ^{1}H NMR data are reported in Tables 1 and 2; δ_C ([$^2H_6$]-DMSO) 107.54 (s, C-5), 73.62 (d, C-6), 73.37 (d, C-3), 72.49 (t, C-1), 61.90 (s, C-2), 54.70 (d, C-4), 46.14 and 42.02 (d, C-9 and -13), 42.93 and 42.88 (t, C-10 and -12), 35.38 and 34.99 (s, C-7 and -11), 32.41 and 32.35 (q, C-14 and -15), 19.32 (q, C-8).

Acetylation of 3a-aminododecahydro-6,6,7b-trimethylcyclobuta-[*cd*]cyclopent[*f*]isobenzofuran-1,1a,4-triol 4a

Compound **4a** (15 mg) was dissolved in dry pyridine (0.3 cm^3) containing Ac_2O (0.6 cm^3) and the solution was kept at 0 °C

for two days. The mixture was then poured into ice–water and extracted with CH_2Cl_2. Evaporation of the extract followed by PLC with CH_2Cl_2–MeOH (15:1) of the residue gave the tetraacetyl derivative **4b** (12 mg) as a solid, mp 95–97 °C (from CH_2Cl_2–hexane); FABMS *m/z* 392 ($MH^+ - 60$); δ_H ($CDCl_3$) 5.79 (1H, s, 2-NH), 5.70 (1H, d, *J* 11.2, 3-H), 5.08 (1H, d, *J* 1.3, 6-H), 4.50 and 4.00 (2H, d, *J* 10.5, 1-H_2), 3.08 (1H, d, *J* 1.3, 4-H), 2.35 (2H, m, 9- and 13-H), 2.12, 2.10, 2.09 and 1.93 (12H, s, 4 × Ac), 1.76, 1.55, 1.49 and 1.35 (4H, m, 10- and 12-H_2), 1.12 (3H, s, 8-H_3), 1.10 (3H, s, 14-H_3), 1.00 (3H, s, 15-H_3); selected NOE experiments ($CDCl_3$) {1-H^a} enhanced 1-H^b (6%), 6-H (2%), 9-H (2.5%), 13-H (2.5%), {3-H} enhanced 4-H (2%), 8-H_3 (1.5%), 12-H^α (2%), {4-H} enhanced 3-H (2.5%), 8-H_3 (0.5%), {6-H} enhanced 1-H^a (2%), 9-H (5%), {8-H_3} enhanced 3-H (5%), 4-H (8%), {9- and 13-H} enhanced 1-H^a (5%), 6-H (13%), 10-H^β (6%), 12-H^β (6%), {15-H_3} enhanced 9- and 13-H (3%), 10-H^β (4%) and 12-H^β (4%); δ_C ([$^2H_6$]DMSO) 171.07, 170.60, 169.95, 168.87 (s, 4 × Me*C*O), 107.63 (s, C-5), 77.34 and 71.93 (d, C-3 and -6), 75.83 (t, C-1), 64.25 (s, C-2), 55.61 (d, C-4), 46.75 and 42.95 (d, C-9 and -13), 42.55 and 40.21 (t, C-10 and -12), 36.36 and 35.36 (s, C-7 and -11), 32.63, 32.04, 23.82, 21.43, 21.06, 20.50 and 19.88 (q, 7 × Me).

3,3a,4,4a,5,6,7,7a,8,8a-Decahydro-3,8-dihydroxy-4-methoxy-4,6,6-trimethyl-1*H*-indeno[5,6-*c*]furan-1-one 5

Compound **1** (100 mg) was dissolved in MeOH (8 cm^3), $NaHCO_3$ (100 mg) was added, and the mixture stirred for 1 h at room temp. until the formation of product **2**; 50 mg of $NaHCO_3$ were added and the reaction was kept at reflux for 5 h; after filtration, and evaporation of the mixture, the residue was diluted with water, acidified with 5% HCL and extracted with EtOAc. PLC with CH_2Cl_2–MeOH (9:1) gave 16 mg of compound **5** as a white solid, mp 53–55 °C (from CH_2Cl_2–hexane); IR ν_{max}(liquid film)/cm^{-1} 1727 (lactone); HRMS [Found: 314.377 (M^+). $C_{16}H_{26}O_6$ requires *M*, 314.382]. The ^1H NMR data are reported in Tables 1 and 2; selected NOE experiments ([$^2H_6$]acetone + D_2O) {3-H} enhanced 8-H_3 (1.5%), 12-H^α (2.5%), {4-H} enhanced 1-H_2 (1.5%), 5-H (1%), 8-H_3 (1.5%), 7-OMe (1%), {5-H} enhanced 4-H (2%), 8-H_3 (<0.5%), 9-H (6.5%), 7-OMe (0.5%), {8-H_3} enhanced 3-H (7.5%), 4-H (6.5%), 7-OMe (2%), {7-OMe} enhanced 4-H (3.5%), 5-H (0.5%), 8-H_3 (1.5%), 9-H (2%); δ_C ($CDCl_3$) 179.80 (s, C-6), 98.72 (d, C-5), 76.05 (s, C-7), 70.82 (d, C-3), 66.04 (t, C-1), 54.96 (s, C-2), 54.90, 43.02 and 41.99 (d, C-4, -9 and -13), 49.31 (q, OMe), 46.11 and 43.18 (t, C-10 and -12), 38.35 (s, C-11), 29.11 and 27.14 (q, C-14 and -15), 22.18 (q, C-8).

3,5,6,7-Tetrahydro-3-hydroxy-6,6,8-trimethyl-1*H*-indeno[5,6-*c*]-furan-1-one 6a

Tsugicoline A **1** (50 mg) was dissolved in MeOH (5 cm^3) and treated with 10% aq. NaOH (5 cm^3); the mixture was kept for 6 h at room temp., and dilution with water, acidification with 5% HCl and extraction with EtOAc gave, after PLC with CH_2Cl_2–MeOH (15:1), compound **6a**, mp 195–200 °C (from acetone–hexane); IR ν_{max}(KBr)/cm^{-1} 3243, 1728 (lactone), 1334, 1159 and 938; CIMS (methane) *m/z* 233 (MH^+, 53%), 215 ($MH^+ - 18$, 100) and 186 (68) (Found: C, 72.5; H, 6.8. $C_{14}H_{16}O_3$ requires C, 72.39; H, 6.94%); the ^1H NMR data are reported in Table 1; selected NOE experiments ([$^2H_6$]acetone) {3-H} enhanced 1-H (2%), 12-H_2 (1.5%), {8-H_3} enhanced 10-H_2 (1.5%); δ_C ([$^2H_6$]acetone) 169.73 (s, C-5), 151.59, 148.24, 146.41, 134.95 and 120.20 (s, C-2, -4, -7, -9 and -13), 117.83 (d, C-3), 97.04 (d, C-1), 48.55 and 45.79 (t, C-10 and -12), 40.76 (s, C-11), 28.93 (q, C-14 and -15), 13.88 (q, C-8).

Oximes of tsugicoline A 1 (7a,b)

A solution of **1** (20 mg) in MeOH (5 cm^3), $NH_2OH{\cdot}HCl$ (10 mg) and NaH_2PO_4 (10 mg) was kept at room temp. for 20 h and the product was subjected to PLC with CH_2Cl_2–MeOH (9:1) to give a mixture of oximes **7a**,**b** (15 mg) as a solid, mp >300 °C (decomp.) (from acetone–hexane) EIMS *m/z* 263 ($M^+ - 18$) (Found: C, 63.9; H, 8.0; N, 4.8. $C_{15}H_{23}NO_4$ requires C, 64.03; H, 8.24; N, 4.98%); δ_H ([$^2H_6$]acetone + D_2O) 4.67 (4.42)‡ (1H, dd, *J* 13.5 and 1.0, 1-H^a), 4.56 (4.33) (1H, dd, *J* 13.5 and 1.5, 1-H^b), 4.36 (4.52) (1H, s, 6-H), 4.28 (4.26) (1H, ddd, *J* 8.5, 1.5 and 1.0, 3-H), 2.38 (2.38) (1H, ddd, *J* 12.0, 10.0 and 8.0, 9-H), 2.31 (2.31) (1H, dddd, *J* 12.0, 10.2, 8.5 and 7.2, 13-H), 1.84 (1.83) (1H, ddd, *J* 12.5, 7.2 and 2.2, 12-H^β), 1.49 (1.48) (1H, ddd, *J* 13.0, 8.0 and 2.2, 10-H^β), 1.44 (1.43) (1H, dd, *J* 13.0 and 10.0, 10-H^α), 1.25 (1.24) (1H, dd, *J* 12.5 and 10.2, 12-H^α), 1.10 (1.10) (3H, s, 14-H_3), 0.99 (1.01 and 0.99) (6H, s, 8- and 15-H_3); δ_C ([$^2H_6$]acetone) 155.68 (151.30),‡ 143.04 (141.65) and 134.65 (136.18) (s, C-2, -4 and -5), 81.39 (80.90) (d, C-6), 75.61 (75.08) (d, C-3), 60.70 (60.50) (t, C-1), 50.72 (51.20) (d, C-13), 47.72 (47.64) (t, C-12), 47.64 (46.55) (s, C-7), 46.26 (45.90) (d, C-9), 42.29 (42.04) (t, C-10), 40.87 (40.73) (s, C-11), 29.77 (29.77) (q, C-14), 27.20 (27.20) (q, C-15), 14.30 (14.39) (q, C-8).

3b,4,4a,5,6,7,7a,8-Octahydro-3a,8-dihydroxy-4,6,6-trimethyl-3*H*-indeno[5′,6′:1,3]cyclopropa[1,2-*c*]furan-3(3a*H*)-one 8a and 4a,5,6,7,7a,8-hexahydro-4-hydroxy-6,6,8-trimethyl-4*H*-indeno-[5,6-*c*]furan-1-carboxylic acid 9a

The metabolite **1** (100 mg) as a solution in DMSO (1 cm^3) was added to 20 cm^3 of a buffer solution [25 cm^3 KCl (0.2 M), 66 cm^3 NaOH (0.2 M), and water to 100 cm^3] at pH 13 and at room temp.; after 4 h the mixture was acidified with 5% HCl to pH 1 and extracted twice with EtOAc. The combined organic phases were dried (Na_2SO_4) and evaporated; the residue was chromatographed on a flash silica gel column using CH_2Cl_2–MeOH (9:1) as solvent to give compound **8a** (20 mg), $[a]_D$ −6 (*c* 0.6, MeOH), ν_{max}(KBr)/cm^{-1} 3388 (OH), 1747 (lactone), 1149 and 1077 (Found: C, 67.6; H, 8.4. $C_{15}H_{22}O_4$ requires C, 67.64; H, 8.33%); the ^1H NMR data are reported in Tables 1 and 2; selected NOE experiments ([$^2H_6$]acetone) {1-H^a} enhanced 1-H^b (13.5%), {1-H^b} enhanced 1-H^a (11%) and 4-H (2%), {3-H} enhanced 1-H^a (1%), 7-H (2.5%), 12-H^α (4.5%), {7-H} enhanced 3-H (2%), 4-H (6%), 8-H_3 (1.5%), 10-H^α (5%), {15-H_3} enhanced 9- and 13-H (3.5%), 10-H^β (2%), 12-H^β (2%), {5-OH} enhanced 9- and 13-H (2%); δ_C ([$^2H_6$]acetone) 177.50 (s, C-6), 72.46 (d, $^1J_{C,H}$ 142, C-3), 70.46 (t, $^1J_{C,H}$ 152, C-1), 68.41 (s, C-5), 51.35 and 50.11 (t, $^1J_{C,H}$ 127, C-10 and -12), 44.30 and 43.70 (d, $^1J_{C,H}$ 131.5, C-9 and -13), 39.27 and 36.88 (s, C-2 and -11), 36.23 (d, $^1J_{C,H}$ 161.5, C-4), 34.58 (d, $^1J_{C,H}$ 126, C-7), 30.23 and 28.15 (q, $^1J_{C,H}$ 124, C-14 and -15), 21.82 (q, $^1J_{C,H}$ 125.5, C-8).

The more polar chromatographic fractions (eluent CH_2Cl_2–MeOH 4:1; formic acid 0.5% v/v) were treated with CH_2N_2 to give a mixture that was successively purified by PLC (CH_2Cl_2–MeOH 20:1) to yield the methyl ester **9b** (8 mg) of the acid **9a**.

Compound 9b

Oil (Found: C, 68.9; H, 7.9. $C_{16}H_{22}O_4$ requires C, 69.04; H, 7.97%); CIMS *m/z* 279 (MH^+, 100%), 261 ($MH^+ - 18$, 87), 231 (15), 205 (18) and 173 (44); the ^1H NMR data are reported in Tables 1 and 2; selected NOE experiments ([$^2H_6$]acetone) {1-H} enhanced 3-H (1.5%), {3-H} enhanced 1-H (1.5%), 12-H^α (1.5%), 13-H (2%), 3-OH (2%); δ_C ($CDCl_3$) 159.58 (s, C-6), 142.49 (d, C-1), 139.68 and 136.81 (s, C-4 and -5), 125.90 (s, C-2), 64.91 (d, C-3), 51.65 (q, 6-OMe), 48.36 and 45.80 (t, C-10 and -12), 43.50 and 43.15 (d, C-9 and -13), 36.92 (s, C-11), 30.27 (d, C-7), 29.68 and 28.04 (q, C-14 and -15), 23.33 (q, C-8).

Acetylation of compound 8a

Compound **8a** (10 mg) was acetylated as above with pyridine and Ac_2O for one day and the solution was then poured into ice–water and extracted with CH_2Cl_2. Evaporation of the

‡ Values in parentheses refer to the minor isomer.

extract followed by PLC with hexane–EtOAc (1:1) gave the diacetate **8b** (8 mg) as an oil; δ_H ([$^2H_6$]acetone) 5.47 (1H, d, *J* 8.6, 3-H), 4.24 and 4.07 (2H, d, *J* 8.8, 1-H_2), 2.21 and 2.06 (6H, s, 2 × OAc), 2.2–2.0 (3H, m, 7- , 9- and 13-H), 1.81 and 1.76 (2H, ddd, *J* 12.2, 6.8, 1.9 and 12.2, 7.2, 1.9, 10- and 12-H^{β}), 1.59 (1H, d, *J* 3.0, 4-H), 1.3–1.0 (2H, m, 10- and 12-H^{α}), 1.18 (3H, d, *J* 6.5, 8-H_3), 1.06 (3H, s, 14-H_3), 0.94 (3H, s, 15-H_3).

Reaction of compound 1 with L-cysteine methyl ester

To a stirred solution of compound **1** (80 mg) in MeOH (5 cm^3) were added L-cysteine methyl ester (52 mg) and 10% aq. $NaHCO_3$ (0.5 cm^3). The mixture was refluxed for 3 h, then diluted with water and extracted with EtOAc. After concentration the crude product was chromatographed on a flash silica gel column using, as eluent, a mixture of CH_2Cl_2–MeOH (9:1) to give 60 mg of *S-(dodecahydro-1,1a,4-trihydroxy-6,6,7b-trimethylcyclobuta[cd]cyclopent[f]isobenzofuran-3a-yl)cysteine methyl ester* **10**, mp 150 °C; $[a]_D$ +17 (*c* 0.5, MeOH); CIMS (methane) *m*/*z* 402 (MH^+, 40%), 267 (MH^+ − 135, 18), 249 (100), 231 (62), 203 (44), 176 (55), 136 (100) (Found: C, 56.8; H, 7.6; N, 3.4; S, 8.0. $C_{19}H_{31}NO_6S$ requires C, 56.83; H, 7.78; N, 3.49; S, 7.99%); the ^1H NMR data are in Tables 1 and 2; δ_C ([$^2H_6$]acetone) 171.23 (s, C-18), 107.51 (s, C-5), 74.92 and 74.82 (d, C-3 and -6), 71.03 (t, C-1), 64.17 (d, C-17), 58.98 (s, C-2), 55.90 (d, C-4), 52.48 (q, C-19), 47.39 and 42.66 (d, C-9 and -13), 44.49 and 44.18 (t, C-10 and -12), 37.53 and 36.42 (s, C-7 and -11), 34.31 (t, C-16), 32.80 and 32.71 (q, C-14 and -15), 19.63 (q, C-8).

Methyl (dodecahydro-1,1a,4-trihydroxy-6,6,7b-trimethylcyclobuta[*cd*]cyclopent[*f*]isobenzofuran-3a-ylthio)acetate 11

To a solution of **1** (10 mg) in MeOH (5 cm^3) was added methyl thioglycolate (120 mg) and the solution was kept at room temp. for 24 h; the mixture was then refluxed for 3 h. PLC of the residue with CH_2Cl_2–MeOH (9:1) gave compound **11** as an oil; ν_{max}(Nujol)/cm^{-1} 3435 (OH), 1725 (ester) and 1634; CIMS (isobutane) *m*/*z* 373 (MH^+, 100%), 355 (26), 337 (4); the ^1H NMR data are reported in Tables 1 and 2; selected NOE experiments ([$^2H_6$]acetone + D_2O) {4-H} enhanced 8-H_3 (1%) and 16-H_2 (3%), {9- and 13-H} enhanced 1-H^a (6%), 6-H (7.5%), 8-H_3 (1%), 10-H^{β} (3%), 12-H^{β} (3%) and 15-H_3 (1%), {16-H_2} enhanced 1-H^b (2%) and 4-H (5%); δ_C ([$^2H_6$]acetone) 172.41 (s, C-17), 107.81 (s, C-5), 75.07 and 72.46 (d, C-3 and -6), 71.93 (t, C-1), 59.39 (s, C-2), 55.46 (d, C-4), 52.72 (q, C-18), 47.46 and 42.73 (d, C-9 and -13), 44.37 and 43.99 (t, C-10 and -12), 37.41 and 36.40 (s, C-7 and -11), 32.94 and 32.90 (q, C-14 and -15), 19.41 (q, C-8).

Methyl dodecahydro-1,1a,4-trihydroxy-3a-hydroxymethyl-6,6,7b-trimethylcyclobuta[*cd*]cyclopenta[*f*]benzothiophene-2-carboxylate 12a,b

150 mg of compound **1**, 8 cm^3 of MeOH, 70 mg of methyl thioglycolate and 1 cm^3 of saturated aq. sodium hydrogen carbonate, were stirred for 6 h at room temp. until the starting material disappeared. The residue was chromatographed on a flash silica gel column using a mixture of hexane–EtOAc (1:2) as solvent to give compounds **12a**,**b** (100 mg) in the ratio 6:1, as white crystals from MeOH, mp 220 °C; $[a]_D$ −69.6 (*c* 0.5, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3276 (OH), 1730 (ester), 1458 and 1120; CIMS (methane) *m*/*z* 373 (MH^+, 10%), 355 (25), 337 (90), 319 (62), 209 (100), 173 (95) (Found: C, 63.9; H, 7.5; S, 8.4. $C_{18}H_{28}O_6S$ requires C, 64.10; H, 7.58; S, 8.59%); the ^1H NMR data are reported in Tables 1 and 2. For **12a**: selected NOE experiments ([$^2H_6$]acetone + D_2O) {1-H_2} enhanced 4-H (9.5%), {4-H} enhanced 1-H_2 (2.5%), 3-H (1.5%), 8-H_3 (1%) and 10-H^{α} (1%); {6-H} enhanced 9-H (4.5%), 13-H (10%) and 16-H (10%); {13-H} enhanced 6-H (7.5%), 9-H (4.5%), 12-H^{α} (1%) and 12-H^{β} (3.5%); {16-H} enhanced 6-H (7.5%); δ_C ([$^2H_6$]acetone) 173.94 (172.44)§ (s, C-17), 89.07 (88.84) (s, C-5), 72.74 and 69.54 (71.31 and 66.13) (d, C-3 and -6), 67.89 (64.60) (t, C-1), 65.99 (65.89) (s, C-2), 53.85, 52.32 and 48.38 (53.22, 50.73 and 47.05) (d, C-4, -13 and -16), 52.11 (51.37) (q, C-18), 43.55 and 43.41 (42.50 and 42.37) (t, C-10 and -12), 41.35 and 36.43 (39.85 and 35.33) (s, C-7 and -11), 41.23 (39.80) (d, C-9), 32.99 and 32.94 (32.39 and 32.25) (q, C-14 and -15), 19.85 (19.56) (q, C-8).*

For **12b**: selected NOEs ([$^2H_6$]DMSO + D_2O) {1-H^b} enhanced 4-H (2.5%) and 16-H (3.5%), {6-H} enhanced 9-H (2.5%), 13-H (4.5%) and 16-H (1%), {13-H} enhanced 6-H (6%), 9-H (3.5%), 12-H^{α} (1%) and 12-H^{β} (2%), {14-H_3} enhanced 3-H (3.5%), 4-H and 12-H^{α} (3%), {8- and 15-H_3} enhanced 4-H (10%), 6-H (1%), 9-H (5%), 10-H^{β} (3%) and 12-H^{β} (2.5%); δ_C ([$^2H_6$]DMSO) 169.53 (s, C-17), 88.30 (s, C-5), 68.05 and 66.09 (d, C-3 and -6), 65.84 (t, C-1), 63.51 (s, C-2), 53.48, 50.53 and 46.90 (d, C-4, -13 and -16), 51.83 (q, C-18), 42.73 and 42.55 (t, C-10 and -12), 40.27 (d, C-9), 39.92 and 35.20 (s, C-7 and -11), 32.39 and 32.25 (q, C -14 and -15), 19.73 (q, C-8).

1,2a,3,4,4a,5,6,7,7a,7b-Decahydro-1,4-dihydroxy-3-hydroxymethyl-3-(4-hydroxyphenylthio)-6,6,7b-trimethyl-2*H*-cyclobut[*e*]inden-2-one 13

25 mg of compound **1** were treated with 20 mg of 4-mercaptophenol in acetone–H_2O (3:1) and the mixture was stirred for 5 h at room temp.; evaporation of the mixture and purification of the residue by PLC in hexane–EtOAc (1:1) gave compound **13** (18 mg), mp 150–155 °C (from acetone–hexane); ν_{max}(KBr)/cm^{-1} 3430 (OH), 1780 (CO band); CIMS (isobutane) *m*/*z* 393 (MH^+, 20%), 375 (MH^+ − 18, 100), 351 (25), 249 (55); the ^1H NMR data are reported in Tables 1 and 2; selected NOE experiments ([$^2H_6$]acetone) {6-H} enhanced 9-H (4%), 13-H (3%), 2′- and 6′-H (0.5%), {2′- and 6′-H} enhanced 1-H_2 (1%), 6-H (1%), 9-H (0.5%), 3′- and 5′-H (10%); δ_C ([$^2H_6$]acetone) 206.76 (s, C-5), 159.44 (s, C-4′), 140.24 (d, C-2′ and -6′), 118.86 (s, C-1′), 116.27 (d, C-3′ and -5′), 90.51 (d, C-6), 70.28 (d, C-3), 65.13 (t, C-1), 64.52 (d, C-4), 57.46 (s, C-2), 45.73 and 42.06 (d, C-9 and -13), 45.23 and 44.46 (t, C-10 and -12), 37.88 and 37.41 (s, C-7 and -11), 31.64 and 30.70 (q, C-14 and −15), 20.24 (q, C-8).

§ Values in parentheses are chemical shifts in [$^2H_6$]DMSO.

References

1 Part 57, L. Merlini, G. Nasini, L. Scaglioni, G. Cassinelli and C. Lanzi, *Phytochemistry*, in the press.
2 W. A. Ayer and L. M. Browne, *Tetrahedron*, 1981, **37**, 2199.
3 A. Arnone, U. Brambilla, G. Nasini and O. Vajna de Pava, *Tetrahedron*, 1995, **51**, 13357.
4 A. Arnone, C. De Gregorio, G. Nasini and O. Vajna de Pava, *J. Chem. Soc.*, *Perkin Trans. 1*, 1997, 1523.
5 A. Arnone, C. De Gregorio, S. V. Meille, G. Nasini and G. Sidoti, *J. Nat. Prod.*, 1999, **62**, 51.
6 A. Arnone, C. De Gregorio, G. Nasini and O. Vajna de Pava, *Tetrahedron*, 1998, **54**, 10199.
7 A. Arnone, R. Cardillo, V. Di Modugno and G. Nasini, *J. Chem. Soc.*, *Perkin Trans. 1*, 1995, 1989.
8 D. Donnelly, P. F. Quigley, D. J. Coveney and J. Polonsky, *Tetrahedron Lett.*, 1987, **26**, 3075, and references therein.
9 A. Arnone, R. Cardillo, G. Nasini and S. V. Meille, *J. Chem. Soc.*, *Perkin Trans. 1*, 1988, 503 and references therein.
10 G. Erkel, T. Anke, A. Gimenez and W. Steglich, *J. Antibiot.*, 1992, **45**, 29.
11 A. Bosetti, G. Fronza, G. Vidari and P. Vita-Finzi, *Phytochemistry*, 1989, **28**, 1427.
12 E. de Hoffmann, *J. Mass Spectrom.*, 1996, **31**, 129.

Paper a908912h