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ICHTHYOTOXIC SESTERTERPENOIDS FROM THE NEO GUINEAN SPONGE *CARTERIOSPONGIA FOLIASCENS*[*]

J.C. BRAEKMAN[***], D. DALOZE, M. KAISIN and B. MOUSSIAUX

Unité de Chimie Bio-organique, Faculté des Sciences

Université Libre de Bruxelles, 1050 - Brussels, Belgium

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ABSTRACT : Six 20,24-dimethylscalarane derivatives (5, 12, 15, 17, 19, and 21) have been isolated from the Neo Guinean sponge *Carteriospongia foliascens*. Compound 12 is identical with a C_{27} tetracyclic terpene previously isolated from an Australian specimen of the same sponge. The five other derivatives are new and their structures have been established on the basis of their spectral data. The structure of 5 was confirmed by single-crystal X-ray diffraction and that of 15 by chemical correlation with 12. The configuration at C-4 for all these compounds has been determined through ^{13}C NMR data. Evidence leading to reverse the configuration at this centre in previously reported C_{27} tetracyclic terpenes is discussed. An ecological function is suggested for these molecules.

In recent years, many new tetracyclic sesterterpenes containing the scalarane skeleton (1), together with derivatives bearing one or two extra carbon atoms, have been reported from marine sponges [1-23]. Metabolites of this class are unique to these animals and are confined to a small number of closely related genera of the order Dictyoceratida (see Table 1). The fact that 24-methylscalaranes have also been found in two dorid nudibranchs of the genus *Chromodoris*[24] is not contradictory to this assertion since it is logical to assume that these terpenes are of dietary origin for these animals.

Some of these tetracyclic terpenes exhibit significant antiinflammatory[2,4,6,21] or cytotoxic[13] activities. Moreover, Walker et al.[11] reported that heteronemin and 19-deacetyl-12,18-diepiscalaradial are toxic to several marine invertebrates indicating that they may be important in preventing predation and overgrowth of *Spongia idia*.

In the course of our systematic search for substances protecting the sponges against their predators and competitors[25], we noted that the dichloromethane extract of sun-dried specimens of *Carteriospongia foliascens*, collected around Laing Island (Papua-New Guinea), is toxic to *Lebistes reticulatus* (LD : 20 mg/l). The toxicity was found to be associated with a complex neutral fraction from which the tetracyclic terpenoids 5, 12, 15, 17, 19 and 21 could be separated. In this paper we want to report their structure determination.

[*]King Leopold III Biological Station, Laing Island, Papua-New Guinea. Contribution n°82.
[***]Maître de Recherches du F.N.R.S.

TABLE 1 : Distribution of the scalarane type tetracyclic sesterterpenoids in the Dictyoceratida.

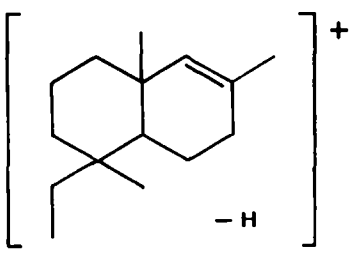
Species	Origin	S[1]	NS	MS1	MS2	MS3	Ref
Spongiidae							
Carteriospongia[2] *foliascens*	Great Barrier Reef (Australia)				X	X	(1)
C. foliascens	Okinawa (Japan)				X		(3) (4)
C. foliascens	Laing Island (Papua-New G.)				X	X	This work
C. radiata	Great Barrier Reef (Australia)			X	X		(1)
C. dendyi	Great Barrier Reef (Australia)			X			(2)
C. sp.	Great Barrier Reef (Australia)			X			(2)
C. sp.	Fiji Islands (Pacific)				X		(5)
Lendenfeldia sp	Great Barrier Reef (Australia)			X			(6)
Spongia officinalis	Bay of Naples (Italy)	X					(7)
S. nitens	Bay of Naples (Italy)	X					(8-10)
S. virgultosa	Bay of Naples (Italy)	X					(8)
S. idia	San Diego (California)	X					(11)
Thorectidae							
Cacospongia scalaris	Bay of Naples (Italy)	X					(12)
C. scalaris	Wakayama (Japan)	X					(13)
C. scalaris	Kagoshima (Japan)	X					(13)
C. mollior	Bay of Naples (Italy)	X					(14)
C. mollior	Bay of Taranto (Italy)	X					(15-18)
Heteronema[3] *erecta*	Great Barrier Reef (Australia)	X					(19)
H. erecta	Eilat (Red Sea)	X					(20)
H. erecta	Tonga (Pacific)	X	X				(21)
Dysideidae							
Dysidea herbacea	Eilat (Red Sea)			X	X		(22)
D. pallescens	Bay of Naples (Italy)	X					(23)

(1) S = Scalarane
NS = 25-norscalarane
MS1 = 24-methylscalarane
MS2 = 19(or 20),24-dimethylscalarane
MS3 = 19(or 20),24-dimethyl-25-norscalarane

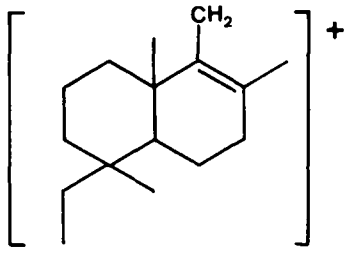
(2) *Carteriospongia* syn *Phyllospongia*

(3) *Heteronema* syn *Hyrtios*

The spectroscopic properties of these compounds and/or of their derivatives clearly suggested that they belong to the 20,24-dimethylscalarane (2) series. Of particular diagnostic value was the presence in the mass spectra of characteristic fragment ions at m/z 205 and 219 which may be attributed to ions a and b respectively (corresponding ions down-shifted by 14 mass units are usually found in the scalarane derivatives[7,12]) and in the ^{1}H NMR spectra of signals attributable to at least four tertiary and one primary methyl groups (see Table 2).



a



b

Comparison of the ^{13}C NMR spectra with those reported for the scalaranes[4,8-11,20,21] fully confirms this hypothesis. In particular, a very good correlation exists between the ^{13}C NMR chemical shifts of the carbon atoms C-1 to C-12, C-19 to C-22 and C-27 of our derivatives, and those of diacetylfoliaspongin (3)[4] (see Table 3). From evidence discussed later in this paper, it emerges that the 4α-ethyl configuration proposed by Kikuchi et al[4] for foliaspongin and its diacetyl derivative must be revised to that depicted by 4 and 3 where the ethyl group at C-4 is axial (β).

Assuming the 20,24-dimethylscalarane skeleton (2) which seemed most likely from the above evidence we could place an axial acetoxy group (α) at C-12 in all our derivatives. Indeed, the ^{1}H NMR spectra contained an ill resolved signal around δ 5 (1H,dd,J=1,1Hz) while the ^{13}C NMR spectra showed a signal at about δ 76 (d). An equatorial acetoxy group on that carbon atom would have given a well resolved double doublet (J=10,4Hz) and a slightly deshielded signal (∿82 ppm) respectively[4,8]. It follows that 5, 12, 15, 17, 19 and 21 are 12α-acetoxy-20,24-dimethyl-scalarane derivatives differing in the substitution pattern of ring D.

Initial separation on silica gel of the crude CH_2Cl_2 extracts led to a fraction of low polarity from which compound 5 could be isolated, by successive chromatographies, as its methyl ester 6 prepared by treatment of the crude fraction with an ethereal solution of CH_2N_2.

Exact mass measurement on the molecular ion of 6 (M=498) indicated a molecular formula of $C_{30}H_{42}O_6$. Next to the signals expected for the A, B and C ring substituents, the ^{1}H NMR spectrum of 6 contained 3 singlets at δ 2.40 (3H), 3.69 (3H) and 6.29 (1H) attributable to a methylketone, a methyl ester and a deshielded vinylic proton respectively. The ^{13}C NMR spectrum showed signals due to 4 carbonyl groups and 2 double bonds. The UV spectrum (λ_{max} : 244 nm) is compatible with an α,α'-dienone chromophore[26] and the IR spectrum showed carbonyl absorptions at 1745, 1715 and 1660 cm^{-1}. These evidences led us to propose structure 5, without configuration at C-4, for the natural derivative. This proposal has been confirmed by X-ray diffraction analysis[27]. Interestingly, and in contrast to the structure previously assigned to foliaspongin[4], the C-4 ethyl group was found to be axial (β). This discrepancy led us to look closer to the problem of the determination of the configuration at C-4 in the C_{27} tetracyclic terpenes.

With the exception of the X-ray diffraction analysis, no obvious method is known to solve this problem. For two C_{27} derivatives other than 6, namely 7[5] and 8[1], the relative configuration at C-4 has been established, without ambiguity, by X-ray diffraction analysis. In all other described C_{27} derivatives, the position of the ethyl group is either undetermined[22] or claimed to be equatorial only by analogy with the structure of 8[1,4].

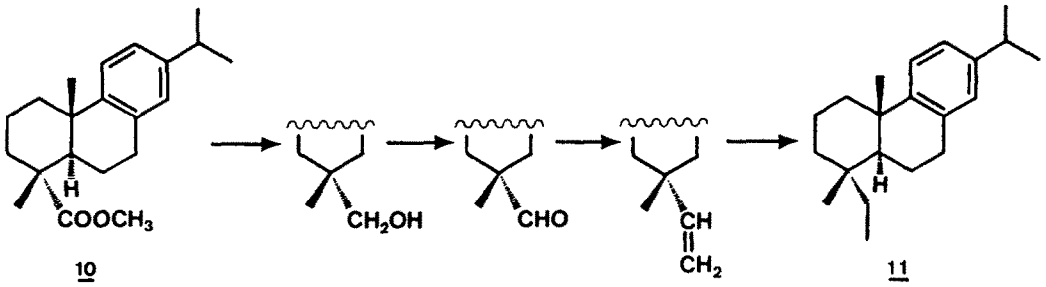
If we compare the ^{13}C NMR spectrum of 6 with that of the scalarane derivatives[4,8-11,20,21], it appears that the introduction of a methyl group at C-20 induces in the spectrum of 6 an impor-

tant upfield shift of the signal assigned to C-3 (24.7 ppm versus 42 ppm*). This can be attributed to a γ-gauche effect between C-27 and C-3.

Such a shift is observed in all our derivatives as well as in foliaspongin (4) and its diacetyl derivative (3). If this effect is not operating in the derivatives having an equatorial ethyl group, the C-3 chemical shift would be of diagnostic value for the determination of the configuration at C-4 in the C_{27} tetracyclic terpenes. Unfortunately, suitable derivatives having the adequate configuration at C-4 (α) are not available for comparison purposes. The only structurally related compound we found in the literature was the diterpene 9, isolated from the liverwort *Trichocoleopsis sacculata*, in which the C-3 absorbs at δ 39.4[29]. This value prompted us to synthesize the model compound 11, which could be prepared from dehydroabietic acid methyl ester (10) using the sequence of reactions described in figure 1.

The value of the C-3 chemical shift (36.3 ppm) measured for compound 11, clearly indicates that the strong shielding effect, observed for the C-4β ethyl derivatives, is not found when the ethyl groups is equatorial (α). Hence, the configuration at C-4 in derivatives having partial structure A (with R = alkyl) may be attributed on the basis of the C-3 chemical shift.

Application of this empirical rule led us to assign the 4-axial position (β) to the ethyl group of all our derivatives as well as to foliaspongin and diacetylfoliaspongin which thus should be represented by 4 and 3 respectively.

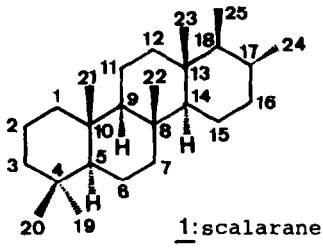


10 11

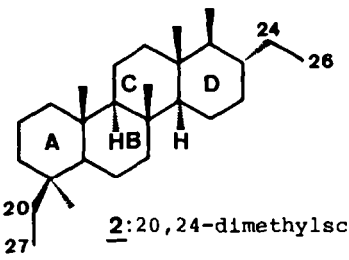
Figure 1 : Hemisynthesis of the model compound 11 from dehydroabietic acid methyl ester (10).

Compound 12 presents a molecular ion at m/z 474 compatible with the formula $C_{29}H_{46}O_5$. The IR spectrum indicated the presence of an acetate (1740, 1240 cm^{-1}) and hydroxyl groups (3450 cm^{-1}). The latter could be acetylated by treatment with the mixture pyridine/acetic anhydride 1:1, leading to the diacetyl derivative 13. Next to the signals expected for the structural moiety common to all our derivatives, the ^1H and ^{13}C NMR spectra of 13 contained signals due to a secondary acetoxy (1H, δ 4.67, dd (11,11,5) ; 3H, δ 2.21,s ; δ 74.9,d), a methylketone (3H, δ 2.34,s ; δ 211.1,s ; δ 32.9,q) and an aldehyde (1H, δ 9.48,s ; δ 202.4,d). The α-acetoxy proton signal is coupled (J=11Hz) to a signal at δ 3.08 (1H, dd (11,11)) coupled itself to a doublet at δ 3.19 (11Hz). These data are compatible with structure 12 for the natural derivative. This structure is identical, except for the configuration at C-4, to that of one of the C_{27} scalarane derivatives isolated by Hofheinz and Daly from an Australian specimen of *C. foliascens* [1]. Comparison of their ^1H NMR spectrum showed nevertheless that they were identical. In our mind, this infers that the configuration at C-4 for Hofheinz and Daly's compound should be reversed. Moreover, it is more than likely that this will also be the case for the other C_{27} derivatives isolated by these authors from the same sponge and for which detailed spectroscopic data are not available.

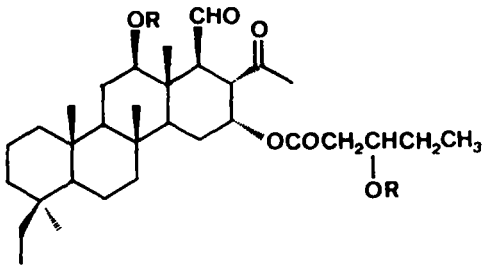
* Mean chemical shift measured for the C-3 of the scalarane derivatives [4,8-11,20,21] and for the model compound 14[28].



1:scalarane

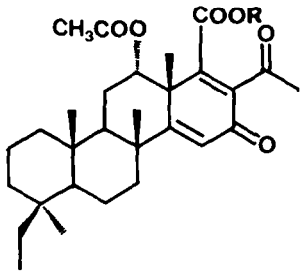


2:20,24-dimethylscalarane



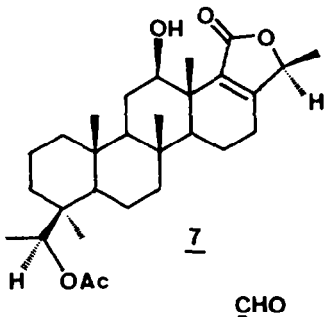
3:diacetylfoliaspongin R=$COCH_3$

4:foliaspongin R=H



5: R=H

6: R=CH_3



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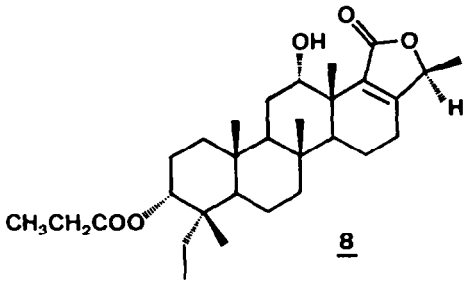

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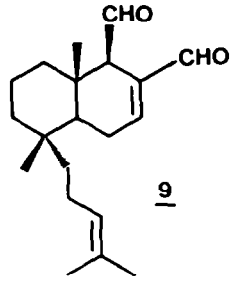

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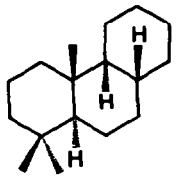
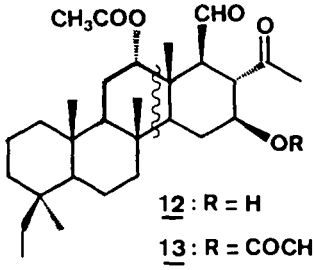
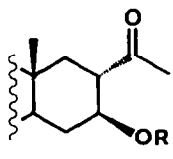


14



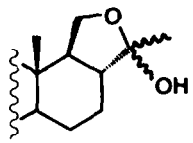
12: R=H

13: R=$COCH_3$



15: R=H

16: R=$COCH_3$



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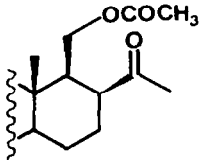

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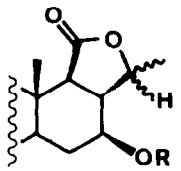
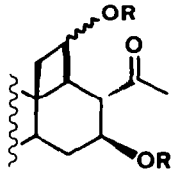


19: R=H

20: R=$COCH_3$



21: R=H

22: R=$COCH_3$

TABLE 2 : 250 MHz ^{1}H NMR data $CDCl_3$ (TMS, δ(Hz)).

		6	12	13	15	16	17	18	20	21	22
H_3C-19	s	0.83	0.82	0.81	0.81	0.80	0.81	0.81	0.79	0.71	0.70
H_3C-21	s	0.92	0.82	0.81	0.81	0.80	0.82	0.81	0.81	0.79	0.79
H_3C-22	s	1.29	0.85	0.83	0.82	0.80	0.85	0.85	0.83	0.80	0.79
H_3C-23	s	1.76	0.95	0.97	1.09	1.08	0.91	0.94	1.07	-	**
H_3C-26		2.40 s	2.40 s	2.34 s	2.14 s	2.12 s	1.42 s	2.15 s	1.36 d(6)	2.25 s	2.15 s
H_3C-27	tr(7)	0.76	0.75	0.74	0.74	0.73	0.74	0.74	0.73	0.73	0.73
HC-12	dd	5.31	5.10	5.11	4.76	4.74	4.61	4.88	5.37	5.05	5.09
		(1,1)	(2,2)	(1,1)	(2,2)	(1,1)	(1,1)	(1,1)	(1,1)	(2,2)	(1,1)
HC-15		6.29 s	-	-	-	-	-	-	-	-	-
HC-16		-	3.59 ddd	4.67 ddd	3.79 ddd	4.95 ddd	-	-	4.81 ddd	3.73 ddd	4.78 ddd
			(11,11,5)	(11,11,5)	(11,11,5)	(11,11,5)			(10,10,5)	(10,10,4)	(11,11,4)
HC-17		-	2.90 dd	3.08 dd	2.60 m	2.84 ddd	-	2.62 m	2.12 ddd	3.04 dd	3.16
			(11,11)	(11,11)		(11,10,3)			(10,10,14)	(10,10)	(10,11)
HC-18		-	3.10 d	3.19 d	-	-	-	2.24 ddd	2.50 d	-	2.54 ddd
			(11)	(11)				(4,7,11)	(14)		(10,7,5)
HC-24		-	-	-	-	-	-	-	4.23 dq	-	-
									(6,10)		
HC-25		-	9.48 s	9.48 s	-	-	ν_A 3.66(8,8)*	ν_A 4.13(4,11)*	-	4.43 ddd	5.05 ddd
							ν_B 3.48(8,10)	ν_B 3.67(7,11)		(7,7,7)	(8,7,7)
$COOCH_3$	s	1.97	2.19	2.03	2.07	1.98	2.11	2.14	2.05	2.10	2.01
	s	-	-	2.21	-	2.08	-	1.93	2.07	-	2.05
	s	-	-	-	-	-	-	-	-	-	2.13
$COOCH_3$	s	3.69	-	-	-	-	-	-	-	-	-

* AB part of an ABX system

** H_{23a} 2.39 dd (8,12)

H_{23b} 2.10 ddd (12,7,5)

TABLE 3 : 62.8 MHz ^{13}C NMR (BBD) $CDCl_3$ (TMS,δ)

*	**	3	6	11	13	15	16	18	20	21
1	t	40.1	40.3	38.7	40.3	40.2	40.2	40.2	40.2	40.3
2	t	18.2	18.2	19.1	18.1	18.1	18.0	18.1	18.2	18.1
3	t	24.5	24.7	36.3	24.6	24.5	24.6	24.6	24.5	24.6
4	s	36.1	36.1	35.5	36.2	36.1	36.1	36.1	36.1	36.2
5	d	50.7	49.6	47.0	50.9	50.0	50.8	50.7	50.1	52.8
6	t	18.2	18.5	18.7	18.6	18.2	18.2	18.2	18.1	18.3
7	t	41.7	40.7	30.4	41.8	41.6	41.5	42.0	42.0	40.7
8	s	37.0	41.7	-	37.0	37.5	37.5	37.9	37.0	37.0
9	d	57.0	58.2	-	52.9	53.1	53.1	52.6	53.3	53.5
10	s	37.6	37.8	37.5	38.2	37.0	37.0	36.9	37.8	38.1
11	t	26.9	21.9	-	21.8	22.4	22.3	21.9	21.1	22.3
12	d	82.1	75.2	-	76.0	76.8	76.5	75.0	78.8	75.6
13	s	43.8	46.8	-	40.6	37.7	37.3	40.1	38.0	38.2
14	d	52.5	141.9 (s)	-	49.3	53.9	49.6	53.9	48.4	47.2
15	t	23.3	123.8 (d)	-	26.0	28.2	25.6	30.2	26.8	29.2
16	d	69.0	184.6 (s)	-	74.9	71.2	73.8	19.7 (t)	73.8	71.3
17	d	58.3	166.1 (s)	-	58.6	58.6	58.6	58.6	52.4	58.7
18	d	58.6	141.9 (s)	-	58.7	38.9 (t)	39.2 (t)	43.5	58.6	45.0
19	q	25.1	28.5	36.4 (t)	28.5	28.5	28.4	28.5	28.4	28.5
20	t	36.6	36.5	20.7 (q)	36.7	36.7	36.7	36.7	36.6	36.7
21	q	17.1	17.0	25.3	16.8	16.6	16.6	16.9	16.9	16.8
22	q	17.1	23.7	-	16.9	16.8	16.8	17.3	16.8	15.1
23	q	8.6	27.5	-	17.2	20.7	21.0	15.4	19.6	37.3 (t)
24	s	206.1	201.3	-	211.1	212.6	209.0	211.7	73.6 (d)	214.2
25	d	202.1	175.1 (s)	-	202.4	-	-	63.1 (t)	172.8 (s)	63.7
26	q	28.5	30.5	-	32.9	29.2	29.1	29.5	16.5	30.3
27	q	9.4	8.6	7.7	8.6	8.6	8.6	8.6	8.6	8.6
$OCO\underline{C}H_3$	q	-	20.8	-	21.0,21.5	21.4	20.6,21.3	20.7,21.5	21.2,21.2	21.4
$COO\underline{C}H_3$	q	-	52.7	-	-	-	-	-	-	-
$O\underline{C}OCH_3$	s	-	169.6	-	169.7,170.4	170.4	169.8,170.3	170.4,170.2	169.4,170.3	171.0

* Carbon atom. Some of the attributions may be reversed.

**Type of carbon atoms unless otherwise noted : q = primary, t = secondary, d = tertiary, s = quaternary. The multiplicity of the different resonance lines was assigned by using the DEPT pulse sequence[33]. The signals were attributed to the various carbon atoms by comparison with the chemical shifts reported for the scalaranes[4,8-11,20,21] and the model compound 14[28], taking into consideration substituent effects.

The ^{1}H and ^{13}C NMR spectra of 15 ($C_{28}H_{46}O_4$; IR : 3420, 1745, 1720 and 1245 cm^{-1}) and its acetylated derivative 16 are very similar to those of 12 and 13 respectively, except that the signals due to the aldehyde group at C-18 in 12 and 13 are missing. Moreover the C-17 proton appears in the ^{1}H NMR spectrum of 16 as a ddd (J=11,10,3Hz) suggesting that in the latter, the aldehyde has been replaced by an hydrogen atom. To prove this relationship, compound 13 was transformed into 16 as follows. Oxidation of the aldehyde function of 13 by $KMnO_4$ in acetone yielded the acid 23 (yield : 60%) characterized as its methyl ester 24, obtained by treatment with an ethereal solution of CH_2N_2. The acid 23 was then treated with oxalyl chloride in pyridine to give the corresponding acyl chloride 25. The latter was easily decarboxylated using the radical decarboxylation method described by Barton et al.[30] (yield : 40%). The C_{26} derivative isolated is identical in all respects with compound 16. It follows that the natural compound 15 is 12α-acetoxy-16β-hydroxy-20,24-dimethyl-24-oxo-25-norscalarane.

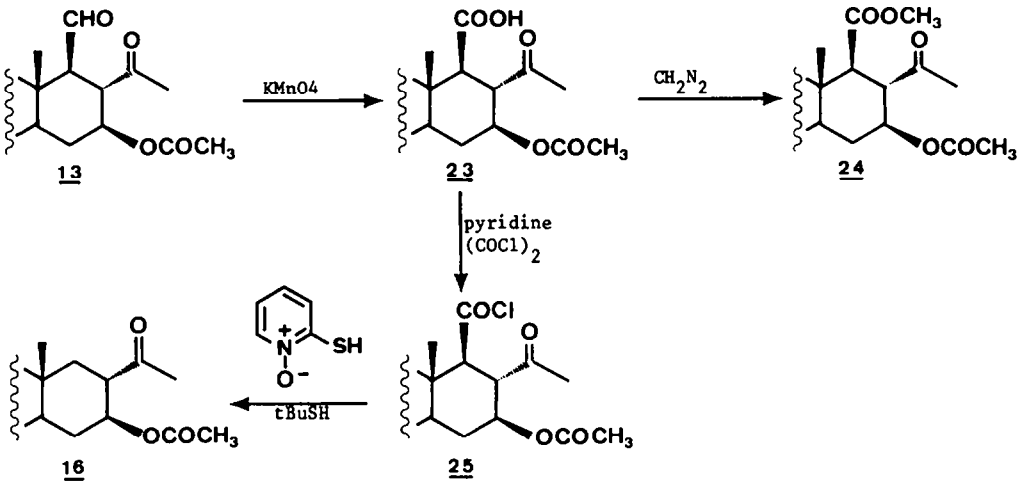


Figure 2 : Chemical correlation between 13 and 16.

The most characteristic feature in the ^{1}H NMR spectrum of compound 17 ($C_{29}H_{48}O_4$; IR : 3425, 1740 and 1240 cm^{-1}) is the presence of a 3H singlet at δ 1.42, and of signals pertaining to the AB part of an ABX system at δ 3.66 (J=8,8Hz) and 3.48 (J=8,10Hz). These data are best explained by assuming the formation of an hemiketal function between a primary alcohol at C-25 and a ketone at C-24. Treatment of 17 with acetic anhydride in pyridine at room temperature gave the keto ester 18 (IR : 1745, 1715 and 1240 cm^{-1}) whose ^{1}H NMR spectrum contained signals attributable to a methyl ketone and two acetoxy groups (3H singlets at δ 2.15, 2.14 and 1.93). Two double doublets (δ 4.13 (J=11,3Hz) and 3.67 (J=7,11Hz)) could be assigned to the AB part of an ABX system attributed to the C-25 methylene bearing an acetoxy group. These two protons are coupled to a ddd (J=4,7, 11Hz) at δ 2.24, attributable to H-18, coupled itself to H-17 (m at δ 2.62). The ^{13}C NMR spectrum of 18 strongly supports the proposed structure. The J_{17-18} (11Hz) determined by double irradiation experiments fixed the equatorial position of the substituents at C-17 and C-18. Compound 17 is thus 12α-acetoxy-24,25-epoxy-24-hydroxy-20,24-dimethylscalarane.

Compound 19 could be obtained pure only after transformation into its acetylated derivative 20. The IR spectrum of 20 ($C_{31}H_{48}O_6$) contained bands at 1780, 1745 and 1245 cm^{-1} assigned to a γ-lactone and acetoxy groups respectively. The ^{1}H NMR spectrum indicated, besides the structural elements common to all previously described derivatives, the presence of a 16β-acetoxy (1H ddd at δ 4.81 (J=10,10,5)) and a secondary methyl group (3H d at δ 1.36 (J=6Hz)) coupled to a deshielded proton (dq at δ 4.23 (J=6,10)), itself coupled to H-17 (δ 2.12, ddd, J=10,10,4). Irradiation of the latter collapsed H-16 into a double doublet (J=10,5Hz) and H-18 into a singlet. The measured coupling constants fixed the relative configuration at C-16, C-17 and C-18 as depicted in 20.

The proposed structure for compound 19 was compatible with all these spectral data as well as with the ^{13}C NMR spectrum. 19 is thus best represented by 12α-acetoxy-16β-hydroxy-20,24-dimethyl-25-norscalarane-18,24-carbolactone.

The IR spectrum of the last compound of the series (21 ; $C_{29}H_{46}O_5$) indicated the presence of hydroxyl (3380 cm^{-1}), acetate (1745 cm^{-1}) and keto (1720 cm^{-1}) groups. Two 1H ddd at δ 3.73 (J=10, 10,4Hz) and 4.43 (J=7,7,7Hz) shifted to 4.78 and 5.05 ppm respectively in the ^1H NMR spectrum of the triacetate 22, indicate that the two hydroxyl groups are secondary. Furthermore, 3H singlets at δ 2.10 and 2.25 as well as a characteristic 1H dd at 5.05 (J=2,2) in the ^1H NMR spectrum of 21 could be assigned to a 12α-acetoxy and to a methylketone at C-17. Interestingly, only three singlets attributable to angular tertiary methyl groups could be seen in the ^1H NMR spectra of 21 and 22. This, coupled to the fact that in the ^{13}C NMR spectrum of 21 the methyl signal attributable to the H_3C-23 is replaced by a methylene signal (δ 37.3) implies that C-23 is not only bonded to C-13 but also to another carbon atom of the skeleton. This became evident after extensive double irradiation experiments performed on 21 and 22, which clearly showed the sequence depicted in figure 3.

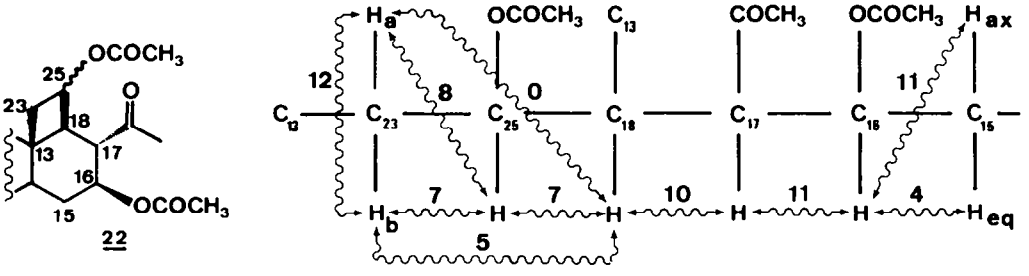


Figure 3 : Coupling constant values (Hz) measured for 22 by double irradiation experiments.

The long-range coupling constant values (J=5 and 0 Hz) between HC-18 and the C-23 methylene protons agree with a cyclobutane ring[31]. These experiments led also to the positioning of the hydroxyl groups at C-16 and C-25 and allowed to fix an axial position to the protons at C-16, C-17 and C-18. Compound 19 is thus best represented by 12α-acetoxy-23,25-cyclo-16β,25ξ-dihydroxy-20,24-dimethyl-24-oxoscalarane.

The isolated derivatives have been tested against the fresh water fish *Lebistes reticulatus* for their ichthyotoxicity. Compound 12 and 21 are the most toxic (LD 5 mg/l). Compound 15 and 17 showed moderate activities (LD 20 and 40 mg/l respectively) while 5 and 19 are both still inactive at 40 mg/l. These preliminary laboratory assays revealed that some of these tetracyclic terpenes may indeed be important in preventing predation and overgrowth of the sponge *C. foliascens* as in the case of *Spongia idia*[11].

Moreover, on a chemotaxonomical point of view, our results are in agreement with the finding that the 20,24-dimethylscalarane derivatives are unique to the closely related genera *Carteriospongia* and *Lendenfeldia*[32], although sesterterpenes deriving from the scalarane skeleton occur in several other Dendroceratida sponges (see table 1).

EXPERIMENTAL

The following instruments were used to measure the physical data. IR : Pye Unicam SP 1000 ; UV : Cary 14 ; ^1H and ^{13}C NMR : Bruker WM250 ; Rotation power : Perkin Elmer 141 ; MS : Finnigan 3000D and Micro-mass 7070F. The NMR spectra were recorded in $CDCl_3$ solution. Chemical shifts are quoted in δ values (ppm) downfield from TMS as internal standard. The tlc were performed on Polygram Sil G precoated plates (Macherey-Nagel). All described compounds were homogeneous in tlc. Column chromatographies were performed on Macherey-Nagel Kieselgel-60 (0.04-0.063 mm) using the flash chromatography technique.

Isolation of the compounds.

Sun-dried specimens (541 g) of the sponge *Carteriospongia foliascens* collected around Laing Island (Papua-New Guinea) were extracted with CH_2Cl_2. The CH_2Cl_2 extract was evaporated under reduced pressure to give a viscous oil (12.2 g - 2.2% dry weight - LD = 20 mg/l). Typically, 5.5 g of the crude oil was applied to a column of SiO_2 that was eluted with hexane, hexane/acetone 7:3 and acetone to obtain 3 major fractions. The intermediate ichthyotoxic fraction (2.29 g) was further chromatographed on SiO_2 column (eluent : hexane/acetone 8:2→0:10). Tlc monitoring afforded 5 fractions (F 2.1 to F 2.5). Rechromatography on SiO_2 column of the fraction F 2.1 (1.1 g) (eluent : benzene/ethylacetate 9:1→0:10) led to compounds 15 (90 mg) and 17 (32 mg) homogeneous in tlc as well as to a fraction (217 mg) containing 5 and 12. This mixture was treated with an ethereal solution of CH_2N_2 prepared from Diazald (Janssen Chimica). SiO_2 chromatography (eluent : benzene/ethylacetate 9:1) of the resulting mixture afforded 6 (11 mg) and 12 (70 mg).

Rechromatography on SiO_2 column of the fraction F 2.2 (450 mg) afforded crude 19 which was further purified as its acetate 20 (32 mg) after treatment with the mixture pyridine/acetic anhydride 1:1 at room temperature for 24 h and usual work up.

The fraction F 2.4 (320 mg) contained compound 21 (18 mg) that could be separated by several successive SiO_2 chromatographies.

Lethal dose (LD) determination.

The adequate amounts of the extracts, fractions or compounds to be tested, are each dissolved in 1 ml of EtOH. The resulting solutions are poured into beakers (250 ml) containing 100 ml of tap water. The lethal dose is determined by immersion of samples of 2 fishes (*Lebistes reticulatus*) in the different concentrations (generally 40 to 5 mg/l). For each concentration the number of dead fishes after 24 h is noted.

The LD of the CH_2Cl_2 extract of *C. foliascens* was found to be 20 mg/l while that of the isolated derivatives was > 40 mg/l for 5 and 19, 40 mg/l for 17, 20 mg/l for 15 and 5 mg/l for 21 and 12.

12α-acetoxy-20,24-dimethyl-16,24-dioxoscalar-14,17-dien-25-oic acid.

This derivative was isolated as its methyl ester (6).

6 : m.p. 173-4° ; $|\alpha|_{579}$ = +95° ($CHCl_3$, c = 1.5) ; HRMS : $M^{+\cdot}$ calculated for $C_{30}H_{42}O_6$: 498.2981, found 498.3026 ; MS : characteristic peaks at m/z 498 ($M^{+\cdot}$), 466, 456, 438, 424, 409, 395, 259, 217, 205, 191 ; IR (film) : $\nu_{C=O}$ at 1745, 1715 and 1660 cm^{-1} ; UV (CH_3OH) : λ max 203 nm (7415), 244 nm (4430) ; ^1H NMR : see table 2 ; ^{13}C NMR : see table 3 ; X-ray diffraction analysis (27).

12α-acetoxy-16β-hydroxy-20,24-dimethyl-24-oxoscalar-25-al.

12 : oil ; $C_{29}H_{46}O_5$; MS : characteristic peaks at m/z 474 ($M^{+\cdot}$), 456, 414, 368, 325, 219, 205 ; IR (film) : ν_{OH} at 3450 cm^{-1}, $\nu_{C=O}$ at 1740 and 1720 cm^{-1}, ν_{C-O} at 1240 cm^{-1} ; ^1H NMR : see table 2.

12α,16β-diacetoxy-20,24-dimethyl-24-oxoscalar-25-al.

13 : m.p. 179-182° ; $|\alpha|_{579}$ = +117° ($CHCl_3$, c = 0.77) ; $C_{31}H_{48}O_6$; HRMS : $M^{+\cdot}$-60 calculated for $C_{29}H_{44}O_4$: 456.3240, found 456.3267 ; MS : characteristic peaks at m/z 516 ($M^{+\cdot}$), 456, 428, 396, 378, 368, 325, 219, 205 ; IR (film) : $\nu_{C=O}$ at 1750 and 1725 cm^{-1}, ν_{C-O} at 1245 cm^{-1} ; ^1H NMR : see table 2 ; ^{13}C NMR : see table 3.

12α-acetoxy-16β-hydroxy-20,24-dimethyl-24-oxo-25-norscalarane.

15 : oil ; $|\alpha|_{579}$ = +36° (CH_2Cl_2, c = 0.78) ; $C_{28}H_{46}O_4$; MS : characteristic peaks at m/z 446 ($M^{+\cdot}$), 386, 368, 357, 353, 339, 325, 284, 219, 205 ; IR (film) : ν_{OH} at 3420 cm^{-1}, $\nu_{C=O}$ at 1745 and 1720 cm^{-1}, ν_{C-O} at 1245 cm^{-1} ; ^1H NMR : see table 2 ; ^{13}C NMR : see table 3.

12α,16β-diacetoxy-20,24-dimethyl-24-oxo-25-norscalarane.

16 : oil ; $C_{30}H_{48}O_5$; $|\alpha|_{579}$ = +38° (CH_2Cl_2, c = 0.80) ; MS : characteristic peaks at m/z 488 ($M^{+\cdot}$), 428, 399, 368, 353, 339, 325, 284, 219, 205 ; IR (film) : $\nu_{C=O}$ at 1740 and 1720 cm^{-1}, ν_{C-O} at 1240 cm^{-1} ; ^1H NMR : see table 2 ; ^{13}C NMR : see table 3.

12α-acetoxy-24,25-epoxy-24-hydroxy-20,24-dimethylscalarane.

17 : oil ; $|\alpha|_{579}$ = +65° (CH_2Cl_2, c = 0.61) ; $C_{29}H_{48}O_4$; MS : characteristic peaks at m/z 460 ($M^{+\cdot}$), 442, 400, 382, 367, 340, 286, 219, 205 : IR (film) : $\nu_{C=O}$ at 1740 cm^{-1}, ν_{C-O} at 1240 cm^{-1}, ν_{OH} at 3425 cm^{-1} ; ^1H NMR : see table 2.

12α,25-diacetoxy-20,24-dimethyl-24-oxoscalarane.

18 : m.p. 154-7° ; $|\alpha|_{579}$ = +67° (CH_2Cl_2, c = 0.52) ; $C_{31}H_{50}O_5$; MS : characteristic peaks at m/z 442 ($M^{+\cdot}$-60), 427, 413, 399, 382, 377, 353, 339, 219, 205 ; IR (film) : $\nu_{C=O}$ at 1745 and 1715 cm^{-1}, ν_{C-O} at 1240 cm^{-1} ; ^1H NMR : see table 2 ; ^{13}C NMR : see table 3.

12α,16β-diacetoxy-20,24-dimethyl-25-norscalarane-18,24-carbolactone.

20 : oil ; $|\alpha|_{579}$ = +44° ($CHCl_3$, c = 1.46) ; $C_{31}H_{48}O_6$; MS : characteristic peaks at m/z 473 ($M^{+\cdot}$-43), 456, 441, 427, 219, 205 ; IR (film) : $\nu_{C=O}$ at 1780 and 1745 cm^{-1}, ν_{C-O} at 1245 cm^{-1} ; ^1H NMR : see table 2 ; ^{13}C NMR : see table 3.

12α-acetoxy-23,25-cyclo-16β,25ξ-dihydroxy-20,24-dimethyl-24-oxoscalarane.

21 : m.p. 190-3° ; $|\alpha|_{579}$ = +46° ($CHCl_3$, c = 0.41) ; $C_{29}H_{46}O_5$; MS : characteristic peaks at m/z 474 ($M^{+\cdot}$), 456, 396, 381, 370, 367, 353, 327, 315, 309, 229, 219, 205 ; IR (KBr) : 3380, 1745, 1720 and 1250 cm^{-1} ; ^1H NMR : see table 2 ; ^{13}C NMR : see table 3.

12α,16β,25ξ-triacetoxy-23,25-cyclo-20,24-dimethyl-24-oxoscalarane.

22 : amorphous solid ; $C_{33}H_{50}O_7$; MS : characteristic peaks at m/z 558 ($M^{+\cdot}$), 498, 438, 396, 378, 335, 219, 205 ; IR (film) ; $\nu_{C=O}$ at 1745 and 1720 (sh) cm^{-1}, ν_{C-O} at 1245 cm^{-1} ; ^1H NMR : see table 2.

Chemical correlation between 13 and 16.

A solution of the aldehyde 13 (30 mg) and 1.4 equivalent of $KMnO_4$ in acetone (3 ml) was stirred at room temperature for 17 h. Addition of water (10 ml), extraction with CH_2Cl_2 and evaporation of the solvent, gave the acid 23 (20 mg) ($M^{+\cdot}$ at m/z 532 ; IR : $\nu_{C=O}$ at 1745, 1720 cm^{-1}, ν_{OH} at 3550 - 3400 cm^{-1}), that was purified by SiO_2 chromatography (eluent : hexane/acetone 8:2 containing 6% of MeOH).

Treatment of 23 (10 mg) with an ethereal solution of CH_2N_2 prepared from Diazald, during 24 h at room temperature, yielded the ester 24 (IR : $\nu_{C=O}$ at 1745, 1730 and 1720 (sh) cm^{-1} ; ^1H NMR : 3H singlets at δ 0.82, 0.82, 0.85, 1.03, 2.03, 2.14, 2.24 and 3.55, 3H triplet at δ 0.75, 1H doublet (11Hz) at δ 3.08, 1H double doublet at δ 3.30 (11,11Hz) and 4.63 (1,1Hz), 1H ddd at δ 4.69 (11,11,4Hz).

A solution of the acid 23 (14 mg) and 0.5 ml of freshly distilled oxalyl chloride in pyridine (2 ml) was stirred at room temperature for 3 h. Addition of water (10 ml), extraction with CH_2Cl_2 and evaporation of the solvent gave the acyl chloride 25 (10 mg ; $M^{+\cdot}$ at m/z 551 and 553 ; IR : $\nu_{C=O}$ at 1805, 1745 and 1720 (sh) cm^{-1} ; ^1H NMR : 3H singlets at δ 0.80, 0.82, 0.84, 1.07, 1.99, 2.07 and 2.08, 3H triplet at δ 0.74, 1H dd at δ 2.48 (13,10Hz) and 5.30 (1,1Hz), 1H doublet at δ 2.89 (13Hz), 1H ddd (10,10,4Hz).

A solution of 2-mercaptopyridine-N-oxide sodium salt (11 mg), t-butylmercaptan (3 ml) and N,N-dimethyl-4-aminopyridine (catalytic amount) in benzene (5 ml) was heated at 80° C . The acyl chloride 25 (8 mg) dissolved in benzene (1 ml) was added dropwise to this solution and the resulting mixture was refluxed for 20 h. Addition of water (20 ml), extraction with CH_2Cl_2, evaporation of the solvent and SiO_2 chromatography of the solid residue gave a compound (3 mg) identical in all respects with 14 (MS, ^1H NMR, IR, $[\alpha]$, tlc).

Hemisynthesis of 11 from dehydroabietic acid methyl ester (10).

Compound 10 (100 mg) was treated with LAH (5 eq.) in anhydrous diethylether (2 ml) for 1 1/2 h at room temperature. After destroying the excess of LAH with a saturated $MgSO_4$ solution, usual work-up followed by SiO_2 column chromatography (eluent : hexane/acetone 9:1) afforded the expected alcohol (78 mg ; M^+ 286 ; IR ; ν_{OH} at 3400 cm^{-1}). This alcohol (40 mg) was treated with pyridinium chlorochromate (1.5 eq.) in CH_2Cl_2 (3 ml) for 1 1/2 h at room temperature. The resulting mixture was filtered on a small SiO_2 column (eluent : CH_2Cl_2) and the solid residue obtained after evaporation of the solvent was submitted, without further purification, to a Wittig reaction.

Triphenylmethylphosphonium bromide (20 eq.) dissolved in anhydrous THF (10 ml) was added under N_2 to a solution of butyl lithium (18 eq.) in hexane. The resulting mixture was stirred at room temperature for 2 h. The crude aldehyde (40 mg), dissolved in anhydrous THF (5 ml), was added dropwise to this solution and the mixture was left at room temperature under stirring for 16 h. Extraction with CH_2Cl_2 and chromatography on SiO_2 (eluent : hexane) afforded an olefin (24 mg ; M^+ 282 ; IR : $\delta_{=CH_2}$ at 915 cm^{-1} ; ^1H NMR : olefinic protons at 5.7 (1H) and 4.9 ppm (2H)) which was catalytically hydrogenated (ethyl acetate, Pd on carbon, 2 h, room temperature) affording 19 mg of compound 11.

11 : oil ; $C_{21}H_{32}$; MS : 284 (M^+), 269, 173, 159 ; ^1H NMR : 6.9-7.2 (3H Arom), 3H singlets at 1.21 and 0.89, 6H doublet at 1.22 (J=7Hz), 3H triplet at 0.77 ppm (J=7.5Hz) ; ^{13}C NMR : see table 3.

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