FULL PAPER

Two New Retigerane-Type Sesterterpenoids from the Lichen *Leprocaulon microscopicum*

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Two new sesterterpenes, **1** and **2**, have been isolated from the lichen *Leprocaulon microscopicum.* In addition to classic chromatographic methods, a liquid-liquid chromatography technique, namely centrifugal partition chromatography (CPC) was applied for the purification of compound **2**. The structures were determined by analyses of mass spectrometry and 1D- and 2D-NMR data. The relative configuration of the isolated compounds was assigned on the basis of 2D-NOESY experiments. The two compounds possess a rare pentacyclic carbon skeleton typical for lichen metabolism, and quite unusual in the vegetal kingdom.

Introduction. – Naturally occurring sesterterpenes were first encountered less than sixty years ago and new sesterterpenes are constantly being discovered from natural sources. With 21 major carbon frameworks, the chemical diversity of sesterterpenes is surprising [1–4]. Considering their large range of polarity features, isolation and purification require various adsorbents and eluents through column chromatography and thin layer chromatography [5]. Mainly marine organisms, but also fungi and lichens are able to biosynthesize such metabolites. Only one sesterterpene, retigeranic acid, has been described in two lichen species belonging to the genus *Lobaria* [6]. *Leprocaulon microscopicum* (Vill.) Gams (syn. *L. quisquiliare*) is a common lichen, formerly included in the *Stereocaulon* genus, despite its primary thallus which possesses a great similarity to that of the *Lepraria* genus. Then, because of different chemistry and lacking of reproductive sexual structures, the *Leprocaulon* genus has been related to the imperfect lichens. Recently, thanks to molecular phylogenetic analyses, the *Leprocaulon* genus has been recognized as a new family, Leprocaulaceae, and order, Leprocaulales [7]. The highly complex chemistry shows variable combination of usnic acid, atranorin, rangiformic acid, zeorin, and unidentified substances [8]. Our previous study on the chemical composition of *L. microscopicum* from the French region Limousin displayed an abundance of dibenzofuran derivatives, including (−)-usnic acid, (−)-isousnic acid, (−)-placodiolic acid and (−)-9-*O*-methylplacodiolic acid [9]. In our continuing study on minor non-aromatic secondary metabolites from *L. microscopicum*, we describe for the first time, the isolation and structure elucidation of compounds **1** and **2**, two new sesterterpenoid derivatives in this genus. In addition to the common purification methods that are often time-consuming and also accompanied with sample loss on solid supports, we applied a new liquid-liquid type chromatography, namely Centrifugal Partition Chromatography (CPC), for the purification of compound **2**.

Results and Discussion. – The dried lichen *L. microscopicum* was extracted successively with acetone and an aqueous MeOH mixture yielding two extracts. The acetone extract was subjected to centrifugation affording a yellow precipitate, as well as a residual acetone fraction. The yellow precipitate afforded (−)-usnic acid [9] and a triterpene which was identified as zeorin on the basis of mass spectral data and by comparison of their ^{1}H- and ^{13}C-NMR spectral data with literature values [10].

The residual acetone fraction was then submitted to successive MPLC and preparative TLC on silica gel of the apolar fraction yielding compound **1**. To optimize the purification of the complex fractions of the extract, *Fr. 4* was submitted to centrifugal partition chromatography. Indeed, CPC allows the use of a wide range of biphasic systems and fractionation to be carried out, in the same experiment, in normal-phase mode followed by a reversed-phase mode (or *vice versa*) called dual-mode [11]. Hence, after selection of the appropriate biphasic systems (Arizona system *Y* heptane/AcOEt/MeOH/H_2O 19:1:19:1) and operating conditions (rotor speed 1500 rpm; flow rate 6 ml/min, dual mode descending then ascending modes), the dibenzofuran placodiolic acid, as well as compound **2**, was isolated.

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The MeOH/H_2O extract was subjected to solvent partition, column chromatography on *Sephadex LH-20*, reversed-phase MPLC and preparative TLC to afford compound **2**. Their structures were elucidated by 1D- and 2D-NMR spectroscopy (COSY, NOESY, HSQC, HMBC).

Compound **1** was isolated as colorless crystalline needles, and its molecular formula was established as $C_{25}H_{42}O$ from the HR-TOF-ESI-MS spectrum which displayed a *pseudo*-molecular ion at *m*/*z* 357.3163 ($[M-H]^-$). This molecular formula implies five degrees of unsaturation. The ^{1}H-NMR spectrum of **1** (*Table*) showed several *multiplet*s between 1.05 and 1.91 ppm standing for 24 aliphatic H-atoms, as well as three secondary Me signals at δ(H) 0.82 (*d*, *J* = 6.2), 0.92 (*d*, *J* = 6.2), and 0.96 (*d*, *J* = 6.3), and three tertiary Me *singlet*s at δ(H) 0.85 (*s*), 0.96 (*s*), and 1.16 (*s*). The ^{13}C-NMR and DEPT spectra exhibited 25 signals (*Table*), including those of six Me, eight CH_2, seven CH, and four quaternary C-atoms. Among the quaternary C-atoms, two of them were strongly shifted downfield to δ(C) 71.3 and 82.2 suggesting the proximity of O-atoms. The hypothesis of the presence of an epoxy pattern in the structure was formulated but rapidly refuted due to the incompatibility between the DEPT signals and the corresponding molecular formula. Thus, the quaternary C-atom at δ(C) 82.2 was supposed to be substituted by a OH group, whereas the quaternary C-atom at δ(C) 71.3 was assigned to be the junction between a tricyclic skeleton endowed with a ring strain explaining the downfield chemical shift. This second hypothesis was strengthened by the observation that this tricyclic ring pattern belongs to retigerane, the only pentacyclic triquinane sesterterpene carbon skeleton described for lichens (*Fig. 1*). The combination of HSQC, COSY, and extensive HMBC analysis of **1** served to delineate its structure as shown in *Fig. 2*. The ^{1}H,^{1}H-COSY between two Me *doublet* signals at δ(H) 0.82 and 0.92 with a CH H-atom at δ(H) 1.60 – 1.66 (H–C(19)) is in favor of the presence of an i-Pr unit in the proposed structure. Correlations between H-

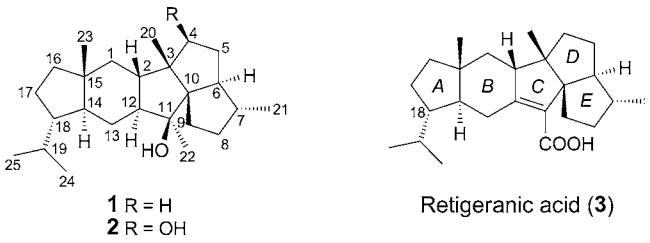


Fig. 1. *Structures of compounds* **1** – **3**

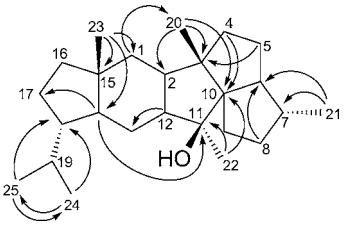


Fig. 2. *Key HMBCs of* **1**

Table. *^{1}H- and ^{13}C-NMR Data* (400 MHz, $CDCl_3$) *of* **1** *and* **2**. δ in ppm, *J* in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.44 – 1.51, 1.14 – 1.16 (2*m*)	40.8 (*t*)	1.49 – 1.51, 1.11 – 1.14 (2*m*)	40.8 (*t*)
2	1.76 (*d*, *J* = 3.0)	46.5 (*d*)	1.83 – 1.97 (*m*)	46.4 (*d*)
3	–	50.7 (*s*)	–	53.9 (*s*)
4	1.20 – 1.33, 1.67 – 1.70 (2*m*)	36.1 (*t*)	3.83 (*t*, *J* = 5.6)	78.1 (*d*)
5	1.52 – 1.59, 1.86 – 1.91 (2*m*)	27.7 (*t*)	1.42 (*t*, *J* = 5.9), 1.83 – 1.97 (*m*)	40.3 (*t*)
6	1.52 – 1.59 (*m*)	58.3 (*t*)	1.55 – 1.69 (*m*)	54.3 (*t*)
7	1.44 – 1.51 (*m*)	42.3 (*d*)	1.83 – 1.97 (*m*)	41.7 (*d*)
8	1.44 – 1.51, 1.44 – 1.51 (2*m*)	30.9 (*t*)	1.55 – 1.69, 1.83 – 1.97 (2*m*)	28.3 (*t*)
9	1.14 – 1.16 (*m*)[a], 1.52 – 1.59 (*m*)	36.1 (*t*)	1.20 – 1.38, 1.83 – 1.97 (2*m*)	35.3 (*t*)
10	–	71.3 (*s*)	–	71.4 (*s*)
11	–	82.2 (*s*)	–	81.4 (*s*)
12	1.08 (*dd*, *J* = 3.2, 1.9)	54.5 (*d*)	1.07 (*dd*, *J* = 3.5, 1.8)	52.8 (*d*)
13	1.71 (*t*, *J* = 3.2), 1.20 – 1.33 (*m*)	23.9 (*t*)	1.69 – 1.76, 1.20 – 1.38 (2*m*)	23.4 (*t*)
14	1.44 – 1.51 (*m*)	52.8 (*d*)	1.44 – 1.48 (*m*)	52.4 (*d*)
15	–	42.9 (*s*)	–	42.7 (*s*)
16	1.52 – 1.59 (*m*), 0.91 – 0.94 (*m*)[a]	40.9 (*t*)	1.55 – 1.69 (*m*), 0.88 (*d*, *J* = 2.6)	40.6 (*t*)
17	1.60 – 1.66, 1.80 – 1.85 (2*m*)	28.3 (*t*)	1.55 – 1.69, 1.80 – 1.83 (2*m*)	28.3 (*t*)
18	1.20 – 1.33 (*m*)	31.5 (*d*)	1.20 – 1.38 (*m*)	31.4 (*d*)
19	1.60 – 1.66 (*m*)	46.6 (*d*)	1.55 – 1.69 (*m*)	46.5 (*d*)
20	0.96 (*s*)	26.7 (*q*)	0.99 (*s*)	18.7 (*q*)
21	0.96 (*d*, *J* = 6.3)	19.7 (*q*)	0.97 (*d*, *J* = 6.9)	19.8 (*q*)
22	1.16 (*s*)	22.3 (*q*)	1.16 (*s*)	22.7 (*q*)
23	0.85 (*s*)	20.0 (*q*)	0.84 (*s*)	19.8 (*q*)
24	0.92 (*d*, *J* = 6.2)	24.1 (*q*)	0.93 (*d*, *J* = 6.4)	24.1 (*q*)
25	0.82 (*d*, *J* = 6.2)	22.4 (*q*)	0.82 (*d*, *J* = 6.4)	22.4 (*q*)

[a]) Signal partially obscured.

atoms at δ(H) 0.92 (*d*, *J* = 6.0, Me(24)) and 0.82 (*d*, *J* = 6.2, Me(25)) with the C-atom at δ(C) 31.5 indicated the attachment of the i-Pr chain to the five-membered ring *A*. Indeed, COSY and HMBC couplings established the linkages of C(18)–C(17), C(17)–C(16), and C(1)–C(2).

The positions of the angular Me groups, Me(23)–C(15) and Me(20)–C(3), were established regarding the HMBCs between H-atoms at δ(H) 0.85 (Me(23)) and C-atoms at δ(C) 52.8 (C(14)), 42.9 (C(15)), 40.9 (C(16)), and 40.8 (C(1)), and between H-atoms at δ(H) 0.96 (Me(20)) and C-atoms at δ(C) 46.5 (C(2)), 50.7 (C(3)), 36.1 (C(4)), and 71.3 (C(10)). Finally, the CH signal of ring junction at δ(H) 1.44 – 1.51 (H–C(14)) displayed COSY couplings with H–C(18) and H–C(13) and HMBCs with C(18) (δ(C) 31.5) and C(23) (δ(C) 20.0) and led to the confident assignments of rings *A* and *B*. The HMBCs between the Me group at δ(H) 1.16 (Me(22)) and δ(C) 82.2 (C(11)) clearly indicated that the Me and OH substituents were attached at the quaternary C(11) (δ(C) 82.2) on ring *C*. This was confirmed by HMBCs between δ(H) 1.16 (*s*, Me(22)) and δ(C) 71.3 (C(10)). HMBCs between δ(H) 0.96 (*s*, Me(21)) and δ(C) 58.3 (C(6)) and 42.3 (C(7)) confirm the substitution of the pentacyclic ring *E* by a Me group at C(7).

The structure of retigeranic acid (**3**) was previously established by *Kaneda et al.* by X-Ray analysis and confirmed by total synthesis of the racemate [6][12]. On the basis of the above results, compound **1** was characterized as retigeran-11-ol reported for the first time. It differs from retigeranic acid (**3**) by the lack of the COOH function and the unsaturation at the ring *C*.

The relative configurations of the stereogenic centers of **1** were assigned on the basis of 2D-NOESY experiments (*Fig. 3*). The lack of a NOE correlation between Me(23) and H–C(14) and between H–C(12) and H–C(2) suggested a *trans*-ring junction between rings *A* and *B*, as well as rings *B* and *C*. Furthermore, it has been assumed that this configuration is thermodynamically the more stable [13]. The NOE effect observed between δ(H) 0.85 (Me(23)), 1.76 (H–C(2)), and 0.96 (Me(20)) suggested the β-orientation of both Me(23) and Me(20). The NOE effects observed between the bridgehead H-atom at δ(H) 1.52 – 1.59 (H–C(6)) and the Me H-atoms at δ(H) 0.96 (Me(21)) and 1.16 (Me(22)) established that the two Me groups were located in the opposite face. This observation was corroborated by NOE interactions between H-atoms at δ(H) 1.44 – 1.51 (H–C(14)), 1.08 (H–C(12)), and 1.16 (Me(22)). Consequently, the β-orientation of the OH group at the same C-atom at δ(C) 82.2 (C(11)), other than of C(22), connected to a *singlet* at δ(H) 1.16 (Me(22)), is established. This observation is strengthened by the fact that a OH-bearing C-atom is less deshielded by an adjacent axial OH group, than by an equatorial one [14]. The configuration of the i-Pr chain was determined following the observation of a strong NOE effect between δ(H) 0.92 (Me(24)) and δ(H) 1.71 (H_α–C(13)). This observation suggested an α-orientation of the i-Pr chain. *Fig. 3* shows the main NOE correlations after 3D optimization of the structure by a theoretical quantum mechanic calculation [15].

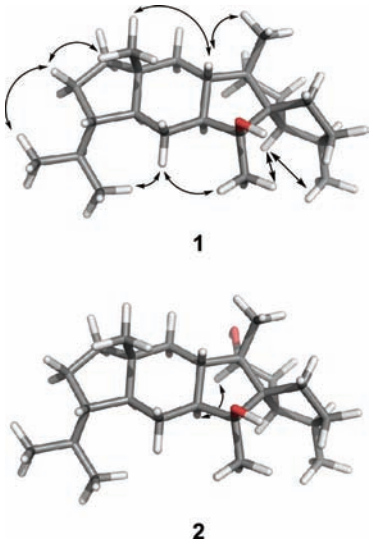


Fig. 3. *Optimized conformations of* **1** *and* **2** *as calculated with DFT/B3LYP/6-31 + G(d,p), and key NOESY correlations*

The molecular formula of compound **2** was established as $C_{25}H_{42}O_2$ by HR-ESI-MS (*m*/*z* 397.3081 ($[M + Na]^+$)). This difference of 16 amu clearly indicated the presence of one more O-atom compared to compound **1**. By comparison of both ^{1}H-NMR spectra, the disappearance of the CH_2 group at C(4) and the presence of one signal at δ(H) 3.83, which correlated in the HSQC spectrum with δ(C) 78.1, clearly indicated the presence of a secondary OH function. HMBCs between δ(H) 3.83 (H–C(4)) and δ(C) 46.4 (C(2)) and 54.3 (C(6)) indicated that this additional OH function is located at C(4) (*Table*). The presence of the OH function at C(4) is confirmed by its influence on the chemical shift of the neighborhood C-atoms, C(3) ($\Delta\delta$ + 3.2), C(4) ($\Delta\delta$ + 42.0), C(5) ($\Delta\delta$ + 12.6), C(6) ($\Delta\delta$ − 4.0), and C(20) ($\Delta\delta$ − 8.0). Considering the biosynthetic fact that compound **2** was isolated together with **1** from *L. microscopicum* and regarding the ^{13}C and NOE correlations, the relative configuration of **2** was deduced to be the same as that of **1**. Like compound **1**, the NOE effects observed between δ(H) 0.84 (Me(23)), 1.83 – 1.97 (H–C(2)), and 0.99 (Me(20)) suggested that Me(23) and Me(20) were located on the β-face of the molecule. The β-orientation of the additional OH–C(4) was deduced through the observation of a strong NOE effect between H–C(4) (δ(H) 3.83) and the bridgehead H-atom H–C(12) (δ(H) 1.07).

Vibrational circular dichroism (VCD) experimental spectrum was performed on compound **2** to determine its absolute configuration. Due to the chiral complexity of the molecules, theoretical spectra have been established by calculation for only two diasteroisomers. Unfortunately, no significant differences have been observed between the two spectra and VCD seems to be inappropriate in this case. Thus, X-ray remains the only possibility to clearly establish

the absolute configuration as it has been carried out for retigeranic acid. Due to the small quantity obtained, it was not possible to provide suitable crystals of compounds **1** and **2**.

Conclusions. – The above mentioned results complete the chemical composition of *L. microscopicum* and the chemotaxonomic data available presently on the lichen. Zeorin was isolated previously from *Lecanora muralis* and were already identified in *Leprocaulon microscopicum* only by TLC [16]. This work described for the first time the presence of sesterterpenoids in this genus and gave the complete NMR assignments of these retigerane derivatives. Sesterterpenes (C_{25}) are the rarest of the terpenoid classes of secondary metabolites isolated from terrestrial fungi, marine organisms, and more occasionally from insects, higher plants or lichen [2]. The carbon framework of known sesterterpenes shows that retigeranic acid (**3**) was the only sesterterpene reported in lichens and was isolated from *Lobaria retigera* [6][17][18]. A possible pathway for the biosynthesis of **1** is suggested in the *Scheme*. As classically observed in the terpenoid family, these derivatives resulted from a series of cyclization steps from the precursor geranylfarnesyl pyrophosphate (GFPP) led to transient intermediates **A**, **B**, and **C** [19]. Fusaproliferin, isolated from *Fusarium proliferatum*, was identified as an intermediate in the biosynthetic pathway of lichen sesterterpenes, and results from the oxidation, followed by esterification of the intermediate **A** [20]. Oxidation of intermediate **C** may lead either to the previously known retigeranic acid (**3**) or to compound **1** *via* a different oxidation mode of the ethylenic C=C bond.

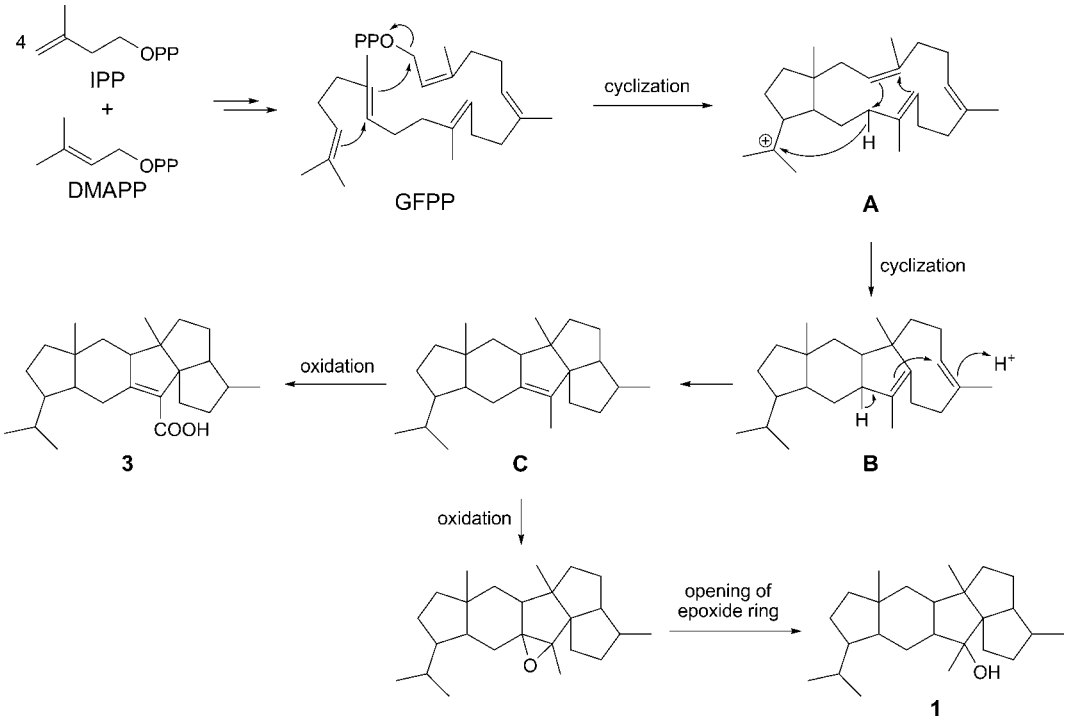
To conclude, two new sesterterpenes, retigeran-11-ol (**1**) and 4-hydroxyretigeran-11-ol (**2**), together with the known triterpene zeorin, were isolated from the lichen *L. microscopicum* from Limousin. In addition to classic chromatography methods, centrifugal partition chromatography was successfully applied to the purification of 4-hydroxyretigeran-11-ol (**2**), and compound **2** was thus obtained with a good repeatability and a better purification rate. The new structures were determined by extensive 1D- and 2D-NMR spectroscopic analysis and HR-ESI-MS. The NMR assignments of this quite unusual pentacyclic skeleton have been provided here for the first time.

Experimental Part

General. Thin layer chromatography (TLC): precoated silica-gel aluminium sheets (SiO_2; *Kieselgel 60 F_{254}*, 0.20 mm, *Merck*). Column chromatography (CC): SiO_2 *60 H* (35 – 70 μm, *Merck*), SiO_2 *RP-18* (15 – 25 μm, *Merck*), and *Sephadex LH-20®* gel (*Sigma–Aldrich*). MPLC: *Büchi* pump model *C-605*, *C-615*. Centrifugal partition chromatography (CPC): *CPC® C 50 Kromaton Technologies* apparatus using a rotor made of 800 cells for a 57-ml total volume at r.t.; solvents were pumped by a HPLC pump *422* (*Kontron Instruments*). The sample was introduced into the CPC column *via* 6-port medium pressure injection valve *Upchurch Scientific*. Fractions of 2 ml were collected by a mini-collector *MC30* (*Köhler Technische Produkte*). ^{1}H- and ^{13}C-NMR: *Bruker* NMR spectrometer at 400 and 100 MHz, resp., in $CDCl_3$; δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-MS: *MICROMASS ZabspecTOF* spectrometer for chemical ionization at Centre Régional de Mesures Physiques de l'Ouest; in m/z.

Lichen Material. Leprocaulon microscopicum (Vill.) Gams ex D. Hawksw (syn. of *L. nanum* (Ach.) Nyl. ex Lamy) was collected on rocks along the Vienne River in Limoges center (N 45°48′4408″, E 1°30′4121″), in December 2009 and was identified by Prof. *Botineau*

Scheme. *Plausible Biogenetic Pathway for Compound* **1**



(Lab. Botanic, University of Limoges). A voucher sample (HL-L14) was deposited with the Laboratory of Pharmacognosy, Faculté de Pharmacie, Université de Limoges, France.

Extraction and Isolation. The dried thallus of lichen (110 g) was extracted with acetone (1000 ml, 3 ×) at r.t. The acetone extract was concentrated under reduced pressure to give 6.5 g of a brown gum. The remaining material was extracted with a mixture of MeOH/H_2O 20 : 80 (400 ml, 2 ×) at r.t. The acetone extract was dissolved in acetone (5 ml) and submitted to centrifugation (3000 tr/min) to give a yellow precipitate (3.6 g) and a brown residual extract (2.9 g). The brown residual extract was separated on MPLC (SiO_2; 90 g, column: 50 × 3 cm), hexane/$CHCl_3$ 10 : 0, 8 : 2, 0 : 10 (400, 2600, and 1400 ml, resp.), 3 ml/min; followed by $CHCl_3$/AcOEt 3 : 3, 3 : 7, 0 : 10 (450 ml each) to yield ten fractions, *Frs. 1 – 10*. Terpenoid compounds were identified by TLC (SiO_2; toluene/AcOEt/HCOOH 70 : 20 : 5; anisaldehyde reagent) due to their pink color after anisaldehyde/sulfuric acid spray and without any UV absorption, only in *Frs. 1* and *2* and in the yellow precipitate. *Fr. 2* (12.3 mg) was purified by prep. TLC (hexane/AcOEt 9 : 1) to yield compound **1** (1.2 mg). The yellow precipitate was a combination of the well-known usnic acid and the triterpene zeorin. A small part of the precipitate (300 mg) was purified on prep. TLC (toluene/AcOEt/HCOOH 70 : 20 : 5) to obtain zeorin (150 mg) and (−)-usnic acid (132 mg). CPC has been conducted on 50 mg of the fraction *Fr. 4*. The separation was performed with a system heptane/AcOEt/MeOH/H_2O 19 : 1 : 19 : 1, in the isocratic mode. The rotor was first filled with the upper phase of the solvent system, as the stationary phase. The apparatus was rotated at 1500 rpm and the upper mobile phase of the solvent mixture was then pumped into the inlet of the column at a flow rate of 6 ml/min in the descending mode. *Fr. 4* (50 mg) was diluted in a mixture of 1.5 ml of the upper phase and 1.5 ml of the lower phase. It was loaded in the 5 ml injection-loop, and injected in the column in a 'sandwich' mode, *i.e.*, at the same time than the mobile phase. The stationary phase retention at the end of the separation represented 48% of the column volume (57 ml). Elution first occurred in the descending mode (reversed-phase mode): the rotor was filled with the upper apolar phase of the solvent mixture, and the pumped mobile phase is the polar lower phase. After collecting 40 ml into a flask, the switching valve is turned to the ascending mode, and the pumped mobile phase is the upper one this time. Hence, after collecting firstly 40 ml into a flask, we collected in thirty tubes each containing 2 ml (*Frs. 4.1 – 4.30*). Extrusion was performed by pumping the upper phase in descending mode to eject the totality of the lower phase out of the rotor. The content of each fraction was then offline monitored by TLC analysis. *Frs. 4.2 – 4.9* and *4.17 – 4.24* were combined to give placodiolic acid (26 mg) and compound **2** (2.7 mg), resp. The MeOH extract was concentrated under reduced pressure to give 3.7 g of a brown gum which was partitioned between BuOH/H_2O 6 : 4 to afford 700 mg of a BuOH-soluble extract. The extract was subjected to *Sephadex LH-20* column (55 × 2 cm) using CH_2Cl_2/MeOH 2 : 1 as eluent to afford twelve fractions. *Fr. 4* (320 mg) was subjected to MPLC on C_{18} SiO_2 (50 × 1.5 cm), eluted by MeOH/H_2O 3 : 7, 4 : 6, 5 : 5, 6 : 4, 7 : 3, 8 : 2, 9 : 1, and 10 : 0 (100 ml each) to give ten fractions, *Frs. 4.1′ – 4.10′*. *Fr. 4.5′* (48 mg) was purified by prep. TLC (hexane/$CHCl_3$/HCOOH 50 : 50 : 0.3) to give compound **2** (2.8 mg).

Retigeran-11-ol (=*(3*R*,3a*S*,5a*S*,5b*S*,6a*R*,9*S*,9a*S*,10a*S*,11*S*,11a*S*)-Hexadecahydro-3,5a,6a,11-tetramethyl-9-(1-methylethyl)-1*H*-pentaleno[1,6a-*a*]-s-indacen-11-ol*; **1**). White translucent needles ($CHCl_3$). ^{1}H- and ^{13}C-NMR ($CDCl_3$): see the *Table*. HR-ESI-MS: 357.3163 ([M − H]$^-$, $C_{25}H_{41}O^-$; calc. 357.3163).

4-Hydroxyretigeran-11-ol (=*(3*R*,3a*S*,5*S*,5a*S*,5b*S*,6a*R*,9*S*,9a-S*,10a*S*,11*S*,11a*S*)-Hexadecahydro-3,5a,6a,11-tetramethyl-9-(1-methylethyl)-1*H*-pentaleno[1,6a-*a*]-s-indacene-5,11-diol*; **2**). White translucent needles ($CHCl_3$). ^{1}H- and ^{13}C-NMR ($CDCl_3$): see the *Table*. HR-ESI-MS: 397.3081 ([M + Na]$^+$, $C_{25}H_{42}NaO_2^+$; calc. 397.3077).

Supplementary Data. Experimental procedures and scans of 1D- and 2D-NMR spectra of compounds **1** and **2** are available as Supporting Information.

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REFERENCES

[1] J. R. Hanson, *Nat. Prod. Rep.* **1996**, *13*, 529.
[2] L. Wang, B. Yang, X.-P. Lin, X.-F. Zhou, Y. Liu, *Nat. Prod. Rep.* **2013**, *30*, 455.
[3] P. Crews, S. Naylor, *Prog. Chem. Org. Nat. Prod.* **1985**, *48*, 203.
[4] J. D. Connolly, R. A. Hill, 'Dictionary of Terpenoids', Chapman & Hall, London, 1991, p. 1107 – 1117.
[5] T. S. Agostini-Costa, R. F. Vieira, H. R. Bizzo, D. Silveira, M. A. Gimenes, 'Secondary Metabolites, Chromatography and Its Applications', Ed. S. Dhanarasu, 2012.
[6] M. Kaneda, R. Takahashi, Y. Iitaka, S. Shibata, *Tetrahedron Lett.* **1972**, *45*, 4609.
[7] J. C. Lendemer, B. Hodkinson, *Mycologia* **2013**, *105*, 994.
[8] M. Lamb, A. Ward, *J. Hattori Bot. Lab.* **1974**, *38*, 499.
[9] M. Millot, M. Kaouadji, Y. Champavier, A. Gamond, A. Simon, A. J. Chulia, *Phytochem. Lett.* **2013**, *6*, 31.
[10] G. M. König, A. D. Wright, *Phytochem. Anal.* **1999**, *10*, 279.
[11] C. Roullier, M. Chollet-Krugler, A. Bernard, J. Boustie, *J. Chromatogr.* **2009**, *877*, 2067.
[12] E. J. Corey, M. C. Desai, T. A. Engler, *J. Am. Chem. Soc.* **1985**, *107*, 4339.
[13] L. A. Paquette, J. Wright, G. J. Drtina, R. A. Roberts, *J. Org. Chem.* **1987**, *52*, 2960.
[14] S. B. Mahato, A. P. Kundu, *Phytochemistry* **1994**, *37*, 1517.
[15] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. N. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, M. J. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian 09, Revision A.02, Wallingford CT, 2009.
[16] V. Marcano, L. Galiz, S. Mohali, A. M. Méndez, E. Palacios-Prü, *Tropical Bryology* **1997**, *13*, 47.
[17] H. Sugawara, A. Kasuya, Y. Iitaka, S. Shibata, *Chem. Pharm. Bull.* **1991**, *39*, 3051.
[18] S. Huneck, I. Yoshimura, 'Identification of Lichen Substances', Springer, Berlin-Heidelberg, 1996, p. 1.
[19] Y. Wang, M. Dreyfuss, M. Ponelle, L. Oberer, H. Riezman, *Tetrahedron* **1998**, *54*, 6415.
[20] A. Santini, A. Ritieni, V. Fogliano, G. Randazzo, L. Mannina, A. Logrieco, E. Benedetti, *J. Nat. Prod.* **1996**, *59*, 109.

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