Two New Retigerane-Type Sesterterpenoids from the Lichen Leprocaulon microscopicum

Marion Millot, Marie Martin-De-Lassalle, Marylene Chollet-Krugler, Yves Champavier, Lengo Mambu, José-Albert Chulia, Marie-Aleth Lacaille-Dubois

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

By **Marion Millot[*a)], Marie Martin-de-Lassalle[a)], Marylène Chollet-Krugler[b)], Yves Champavier[c)], Lengo Mambu[a)], José-Albert Chulia[a)], Marie-Aleth Lacaille-Dubois[d)]**

[a)] LCSN – EA 1069, Laboratoire de Pharmacognosie, Faculté de Pharmacie, 2 rue du Dr Marcland, 87025 Limoges, France
[b)] UMR CNRS ISCR 6226 "Institut des Sciences chimiques de Rennes" Equipe PNSCM "Produits Naturels-Synthèse-Chimie Médicinale", UFR des Sciences Pharmaceutiques et Biologiques, Université Européenne de Bretagne, Université de Rennes 1, 2 Avenue du Pr Léon Bernard, 35043 Rennes Cedex, France.
[c)] Service Commun de Recherche et d'Analyse des Biomolécules de Limoges, 2 rue du Dr Marcland, 87025 Limoges, France
[d)] EA 4267, FDE/UFC, Laboratoire de Pharmacognosie, UFR des Sciences de Santé, Université de Bourgogne-Franche-comté, BP 87900, 7 Bd Jeanne d'Arc, 21079 Dijon Cedex, France.

[*] Dr. Marion Millot, Laboratoire de Chimie des Substances Naturelles (EA 1069), Faculté de Pharmacie, Université de Limoges, 2 rue du Dr. Marcland, 87025 Limoges cedex, France. E-mail: marion.millot@unilim.fr Phone: + 33 (0) 5 55 43 58 35 Fax: + 33 (0) 5 55 43 59 10

Two new sesterterpenes **1**-**2**, have been isolated from the lichen *Leprocaulon microscopicum.* In addition to classic chromatography 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. Relative configuration of the isolated compounds was assigned on the basis of 2D NOESY experiments. The two compounds possess a rare pentacyclic carbon skeleton specific to lichen metabolism, quite unusual in the vegetal kingdom.

Keywords: Lichens, Sesterterpenes, NMR, Retigerane, Centrifugal partition chromatography

Introduction. - Naturally occurring sesterterpenes were first encountered less than sixty years ago and new sesterterpenes are constently discovered from natural sources. With 21 major carbon frameworks, the chemical diversity of sesterterpenes is surprising [1-4]. Considering their large range of polarity feature, isolation and purification require various adsorbents and eluents through column chromatography and thin layer chromatography [5]. Mainly found in marine organisms and fungi, lichens are also able to biosynthetize 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 *Stereocaulon* genus, despite its primary thallus which possesses a great similarity to that of *Lepraria* genus. Then, because of different chemistry and lacking of reproductive sexual structures, *Leprocaulon* genus has been related to imperfect lichens. Recently, thanks to molecular phylogenetic analyses, *Leprocaulon* genus have been recognized in 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 French Limousin, shows the abundance of dibenzofuran derivatives including (–)-usnic acid, (–)-isousnic acid, (–)-placodiolic acid and (–)-9-*O*-methylplacodiolic acid [9]. In our continuing study of minor non aromatic secondary metabolites from *L. microscopicum*, we describe for the first time in this Genus, the isolation and structure elucidation of compounds **1**-**2**, two new sesterterpenoid derivatives. In addition to their common purification methods often time-consuming with also sample loss on solid support, we applied a liquid-liquid 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 methanol mixture yielding two extracts. The acetone extract was subjected to centrifugation affording a yellow precipitate as well as a residual acetonic 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 acetonic fraction was then submitted to successive MPLC and preparative TLC on silica gel of the apolar fraction yielding compound **1**. In order to optimize the purification of the complex fractions of the extract, Fr. 4 has been 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 Hept/EtOAc/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** were isolated.

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

Compound **1** was isolated as crystalline needles and its molecular formula was established as $C_{25}H_{42}O$ from the HR-ESI-TOF-MS spectrum showing 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 1*) showed several multiplets between 1.05 and 1.91 ppm standing for 24 aliphatic protons as well as three secondary methyl 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 methyl singlets at δ(H) 0.85 s, 0.96 s and 1.16 s. The ^{13}C-NMR and DEPT spectra exhibited 25 signals (*Table 1*), including those of six methyls, eight methylenes, seven methines and four quaternary carbons. Among the quaternary carbons, two of them were strongly downfield shifted at δ(C) 71.3 and δ(H) 82.2 suggesting the proximity of an oxygen. 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 molecular formulae. Thus, the quaternary carbon at δ(C) 82.2 was ascribe to be substituted by an hydroxyl group whereas the quaternary carbon at δ(C) 71.3 was asssign to be a junction between a tricyclic skeleton 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 in lichen (*Fig. 1*). Combination of HSQC, COSY and extensive HMBC correlation analysis of **1** served to delineate its structure (*Fig. 2*). The ^{1}H-^{1}H correlation (COSY) between two methyl doublet signals at δ_H 0.82 and 0.92 with a methine proton at δ(H) 1.60 – 1.66 (H(19)) are in favor of an isopropyle chain in the structure. Correlations between protons at δ(H) 0.92 (d, *J*=6.0, Me(24)) and 0.82 (d, *J*=6.2, Me(25)) with carbon at δ(C) 31.5 indicated the attachment of the isopropyl chain to the five membered ring A. Indeed, COSY and HMBC couplings established the linkages between C(18) and C(17); C(17) and C(16); C(1) and C(2). The positions of the angular methyl at C(15), Me(23) and C-3, Me(20) were established regarding the HMBC correlations between proton at δ(H) 0.85 (Me(23)) and carbons at δ(C) 52.8 (C(14)), 42.9 (C(15)), 40.9 (C(16)) and 40.1 (C(1)), and between proton at δ(H) 0.96 (Me-20) and carbons at δ(C) 46.5 (C(2)), 50.7 (C(3)), 36.1 (C(4)) and 71.3 (C(10)). Finally, the ring junction methine signal at δ(H) 1.44 – 1.51 (H(14)), displayed COSY couplings with H(18) and H(13) and HMBC correlations with C(18) (δ(C) 46.6) and C(23) (δ(C) 20.0) and led to the confident assignments of rings A and B. The

HMBC correlations between the methyl group at δ(H) 1.16 (Me(22)) and δ(C) 82.2 (C(11)) clearly indicated that the methyl and hydroxyl substituents were attached at the quaternary C(11) (δ 82.2) on ring C. This was confirmed by HMBC correlations between δ(H) 1.16 (s, Me(22)) and δ(C) 71.3 (C(10)). HMBC correlations between δ_H 0.96 (s, Me(21)) and δ(C) 58.6 (C(6)) and 42.3 (C(7)) confirm the substitution of the pentacyclic ring E by a methyl group at C(7).

The structure of retigeranic acid was previously established by Kaneda et al. (1972) only by X-Ray analysis and confirm 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 by the lack of the carboxylic function and the insaturation at the C-ring.

The relative configurations of the stereocenters of **1** were assigned on the basis of 2D NOESY experiments (*Fig. 3*). The lack of NOE correlation between the Me(23) and H(14) and between H(12) and H(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 stereochemistry is thermodynamically the more stable. [13] The NOE effect observed between δ(H) 0.85 (Me(23)), δ(H) 1.76 (H(2)) and δ(H) 0.96 (Me(20)) suggesting that the *β*-orientation methyl groups Me(23) and Me(20). The NOE effects observed between the bridgehead proton δ(H) 1.52 – 1.59 (H(6)) and the methyl protons δ(H) 0.96 (Me(21)) and δ_H 1.16 (Me(22)) established that the two methyl groups were located in the opposite face. This observation was corroborated by NOE interactions between protons at δ(H) 1.44 – 1.51 (H(14)), δ(H) 1.08 (H(12)) and δ(H) 1.16 (Me(22)). Consequently, the *β*-orientation of the hydroxyl group bearing by the same carbon at δ 82.2 (C(11)) than δ(H) 1.16 (Me(22)) is established. This observation is strengthened with the fact that a hydroxyl bearing carbon is less deshielded by the adjacent axial hydroxyl than the equatorial one [14]. The configuration of the isopropyl

chain is determined following the observation of a strong NOE effect between δ(H) 0.92 (Me(24)) and δ(H) 1.71 (H(13α)). This observation suggested the *α*-orientation of the isopropyl chain. *Fig. 2* shows the main NOE correlations after 3D optimization of the structure by a theoretically quantum mechanic calculation [15].

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

Vibrational circular dichroism (VCD) experimental spectrum has been realized on compound **2** in order to determine the 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 made for retigeranic acid. Due to the small quantity obtained it was not possible to access to the mono-cristal of compound **1** and **2**.

Conclusion. - These results complete the chemical composition of *L. microscopicum* and the chemotaxonomic data available on the lichen. Zeorin has previously been isolated 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 (C25) are the rarest of the terpenoid classes of secondary metabolites isolated from terrestrial fungi, marine organisms and more occasionnaly from insects, higher plants or lichen [2]. The carbon framework of known sesterterpenes shows Retigeranic acid was the only sesterterpenes reported in lichens and was isolated from *Lobaria retigera* [6, 17-18]. A possible pathway for the biosynthesis of **1** is suggested in *Fig. 4*. As classically observed in terpenoid family, these derivatives resulted from a series of cyclisation steps from 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 led either to the previously known retigeranic acid or to compound **1** *via* another oxidation of the ethylenic double bond.

To conclude, two new sesterterpenes (C25), retigeran-11-ol (**1**) and 4-hydroxy-retigeran-11-ol (**2**), together with a 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** has been obtained with a good repeatability and a better

purification rate. The new structures have been determined by extensive 1D and 2D NMR spectroscopic analysis and HRESIMS. The NMR assignments of this quite unusual pentacyclic skeleton have been provided here for the first time.

Experimental Part

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR were recorded at 400 and 100 MHz respectively, on a Bruker NMR spectrometer, using $CDCl_3$ (TMS as internal standard). High resolution mass spectrometric measurements for exact mass determination (HRMS) were performed on a MICROMASS ZabspecTOF spectrometer for chemical ionization at Centre Régional de Mesures Physiques de l'Ouest. TLC was performed on precoated silica gel aluminium sheets (Kieselgel 60 F_{254}, 0.20 mm, Merck). Chromatographic separation was performed using column chromatography on silica gel 60H (35-70 µm, Merck) silica gel RP-18 (15-25 µm, Merck) and Sephadex LH-20® gel (Sigma-Aldrich). MPLC was carried out using the Buchi pump model C-605, C-615. CPC were performed at room temperature on a CPC® C 50 Kromaton Technologies apparatus using a rotor made of 800 cells for a 57 mL total volume, solvents were pumped by a HPLC pump 422 from Kontron Instruments. 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).

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 Pr. Botineau (Lab. Botanic, University of Limoges). A voucher sample (HL-L14) was deposited in 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 times) at room temperature. The acetone extract was concentrated under reduced pressure to give 6.5 g of a brown gum. The resulted marc was extracted with a hydro-methanolic mixture (20/80) (400 mL, 2 times) at room temperature. The acetonic 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 residual extract was separated on a silica gel MPLC (90 g, column: 50 x 3 cm), *n*-hexane-$CHCl_3$ (10:0, 8:2, 0:10, 400 mL, 2600 mL and 1400 mL respectively), 3 mL/min; followed by $CHCl_3$-EtOAc (3:3, 3:7, 0:10, 450 mL each) to yield ten fractions ($F_A - F_J$). Terpenoids compound were identified by TLC (SiO_2, Toluene/EtOAc, HCOOH 70:20:5 ; Anisaldehyde reagent) thanks to their pink color after anisaldehyde sulfuric spray and without any UV absorption, only in F_A and F_B and in the yellow precipitate. Fr. 2 (12.3 mg) was purified by preparative TLC (*n*-hexane/EtOAc 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. Small part of the precipitate (300 mg) was purified on preparative TLC (Toluene/EtOAc/Formic acid 70:20:5) to obtain zeorin (150 mg) and (-)-usnic acid (132 mg). CPC has been conducted on 50 mg of the fraction F_D. The separation was performed with a system Hept/EtOAc/MeOH/H_2O 19:1:19:1 v/v/v/v, 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. F_D (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 (reverse-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 (F_{D1} - F_{D30}). Extrusion was performed by pumping the upper phase in descending mode to eject the totality of the lower phase out of the rotor. Content of each fraction was then offline monitored by TLC analysis. F_{D2} - F_{D9} and F_{D17} - F_{D24} were combined to give respectively placodiolic acid (26 mg) and compound **2** (2.7 mg). The methanolic extract was concentrated under reduced pressure to give 3.7 g of a brown gum which was partitioned between butanol-H_2O (6:4) to afford 700 mg of a butanol-soluble extract. The extract was subjected to Sephadex LH-20 column (55 x 2 cm) using CH_2Cl_2/MeOH (2:1) as eluent to afford twelve fractions. F_D (320 mg) was subjected to MPLC on C18 silica gel (50 x 1.5 cm), eluted by MeOH-H_2O (3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0, 100 mL each) to give ten fractions (F_{D1} – F_{D5}). The fraction F_{D5} (48 mg) was purified by preparative TLC (Hexane/$CHCl_3$/Formic acid 50:50:0.3) to give compound **2** (2.8 mg).

Retigeran-11-ol (**1**): white translucent needle ($CHCl_3$); ^{1}H and ^{13}C-NMR ($CDCl_3$): see *Table 1*; HRESIMS: *m*/*z* 357.3163 ($C_{25}H_{41}O$, calcd 357.3157).

4-Hydroxyretigeran-11-ol (**2**): white translucent needle ($CHCl_3$); ^{1}H and ^{13}C-NMR ($CDCl_3$): see *Table 1*; HRESIMS: m/z 397.3081 ($C_{25}H_{41}O_2$, calcd 397.3082).

This work was financially supported by Région of Limousin. We are grateful to Pr. M. Botineau for lichen identification. The authors are grateful to J-V. Naubron (Spectropole, Université Aix-Marseille) for VCD measurement and theoretical calculation. We express our

gratitude to G. Fabre for structure optimization by quantum mechanic calculations and to J. Cook-Moreau for linguistic corrections.

Supplementary data

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

References

[1] J. R. Hanson, *Nat. Prod. Rep.* **1996**, *13*, 529.
[2] L. Wang, B. Yang, X.-P. Lin, X.-F. Zhou and Y. Liu, *Nat. Prod. Rep.* **2013**, *30*, 455.
[3] P. Crews and S. Naylor, *Progress in the Chemistry of Organic Natural Products* **1985**, *48*, 203.
[4] J. D. Connolly and R. A. Hill, *Dictionary of Terpenoids*, Chapman & Hall, London, **1991**, p. 1107-1117.
[5] T. Agostini-Costa, R. F. Vieira, H. R. Bizzo, D. Silveira and M. A. Gimenes in *Vol.* (Ed. D. S.), **2012**, Secondary Metabolites, Chromatography and Its Applications, Dr. Sasikumar Dhanarasu (Ed.) pp. 131.
[6] M. Kaneda, R. Takahashi, Y. Iitaka and S. Shibata, *Tetrahedron Let.* **1972**, *45*, 4609.
[7] J. C. Lendemer and B. Hodkinson, *Mycologia* **2013**, *105*, 994.
[8] M. Lamb and A. Ward, *J. Hattori Bot. Lab* **1974**, *38*, 499.
[9] M. Millot, M. Kaouadji, Y. Champavier, A. Gamond, A. Simon and A. J. Chulia, *Phytochem. Let.* **2013**, *6*, 31.
[10] G. M. König and A. D. Wright, *Phytochem. Anal.* **1999**, *10*, 279.
[11] C. Roullier, M. Chollet-Krugler, A. Bernard and J. Boustie, *J. Chromatogr.* **2009**, *877*, 2067.
[12] E. J. Corey, M. C. Desai and T. A. Engler, *Journal of the Amercian Chemical Society* **1985**, *107*, 4339.
[13] L. A. Paquette, J. Wright, G. J. Drtina and R. A. Roberts, *J. Org. Chem.* **1987**, *52*, 2960.
[14] S. B. Mahato and A. P. Kundu, *Phytochemistry* **1994**, *37*, 1517.
[15] M. Frisch, G. Trucks, H. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. L. Caricato, X., H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa and M. N. Ishida, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, M. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. , *Gaussian 09, Revision A.02* Wallingford CT, **2009**.
[16] V. Marcano, L. Galiz, S. Mohali, A. M. Méndez and E. Palacios-Prü, *Tropical Bryology* **1997**, *13*, 47.

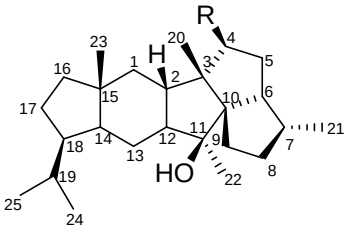
[17] H. Sugawara, A. Kasuya, Y. Iitaka and S. Shibata, *Chem. Pharm. Bull.* **1991**, *39*, 3051.
[18] S. Huneck and I. Yoshimura, *Identification of lichen substances*, Springer, Berlin-Heidelberg, **1996**, p. 1.
[19] Y. Wang, M. Dreyfuss, M. Ponelle, L. Oberer and H. Riezman, *Tetrahedron* **1998**, *54*, 6415.
[20] A. Santini, A. Ritieni, V. Fogliano, G. Randazzo, L. Mannina, A. Logrieco and E. Benedetti, *Journal of Natural Products* **1996**, *59*, 109.

Table 1. NMR Spectroscopic Data (400 MHz, $CDCl_3$) for retigeran-11-ol (**1)** and 4-hydroxyretigeran-11-ol (**2**)

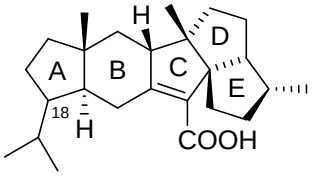
	Retigeran-11-o1 (**1**)		4-hydroxyretigeran-11-ol (**2**)	
Position	δ(C)	δ(H)	δ(C)	δ(H)
1a	40.8 (CH_2)	1.44 – 1.51 (*m*)	40.8 (CH_2)	1.49 – 1.51 (*m*)
1b		1.14 – 1.16 (*m*)		1.11 – 1.14 (*m*)
2	46.5 (CH)	1.76 (*d*, *J* = 3.0)	46.4 (CH)	1.83 – 1.97 (*m*)
3	50.7 (C)	-	53.9 (C)	-
4a	36.1 (CH_2)	1.20 – 1.33 (*m*)	78.1 (CH)–	3.83 (*t*, *J* = 5.6)
4b		1.67 – 1.70 (*m*)		
5a	27.7 (CH_2)	1.52 – 1.59 (*m*)	40.3 (CH_2)	1.42 (*t*, *J* = 5.9)
5b		1.86 – 1.91 (*m*)		1.83 – 1.97 (*m*)
6	58.3 (CH_2)	1.52 – 1.59 (*m*)	54.3 (CH_2)	1.55 – 1.69 (*m*)
7	42.3 (CH)	1.44 – 1.51 (*m*)	41.7 (CH)	1.83 – 1.97 (*m*)
8a	30.9 (CH_2)	1.44 – 1.51 (*m*)	28.3 (CH_2)	1.55 – 1.69 (*m*)
8b		1.44 – 1.51 (m)		1.83 – 1.97 (*m*)
9a	36.1 (CH_2	1.14 – 1.16 (m^b)	35.3 (CH_2)	1.20 – 1.38 (*m*)
9b		1.52 – 1.59 (*m*)		1.83 – 1.97 (*m*)
10	71.3 (C)	-	71.4 (C)	-
11	82.2 (C)	-	81.4 (C)	-
12	54.5 (CH)	1.08 (*dd*, *J* = 3.2, 1.9)	52.8 (CH)	1.07 (*dd*, *J* = 3.5, 1.8)

13a	23.9 (CH_2)	1.71 (*t*, *J* = 3.2)	23.4 (CH_2)	1.69 – 1.76 (*m*)
13b		1.20 – 1.33 (*m*)		1.20 – 1.38 (*m*)
14	52.8 (CH)	1.44 – 1.51 (*m*)	52.4 (CH)	1.44 – 1.48 (*m*)
15	42.9 (C)	-	42.7 (C)	-
16a	40.9 (CH_2)	1.52 – 1.59 (*m*)	40.6 (CH_2)	1.55 – 1.69 (*m*)
16b		0.91 – 0.94 (*m*[b])		0.88 (*d*, *J* = 2.6)
17	28.3 (CH_2)	1.60 – 1.66 (*m*)	28.3 (CH_2)	1.55 – 1.69 (*m*)
		1.80 – 1.85 (*m*)		1.80 – 1.83 (*m*)
18	31.5 (CH)	1.20 – 1.33 (*m*)	31.4 (CH)	1.20 – 1.38 (*m*)
19	46.6 (CH)	1.60 – 1.66 (*m*)	46.5 (CH)	1.55 – 1.69 (*m*)
20	26.7 (CH_3)	0.96 (*s*)	18.7 (CH_3)	0.99 (*s*)
21	19.7 (CH_3)	0.96 (*d*, *J* = 6.3)	19.8 (CH_3)	0.97 (*d*, *J* = 6.9)
22	22.3 (CH_3)	1.16 (*s*)	22.7 (CH_3)	1.16 (*s*)
23	20.0 (CH_3)	0.85 (*s*)	19.8 (CH_3)	0.84 (*s*)
24	24.1 (CH_3)	0.92 (*d*, *J* = 6.2)	24.1 (CH_3)	0.93 (*d*, *J* = 6.4)
25	22.4 (CH_3)	0.82 (*d*, *J* = 6.2)	22.4 (CH_3)	0.82 (*d*, *J* = 6.4)

[b]Signal partially obscured.



(**1**) R = H
(**2**) R = OH



(3) Retigeranic acid

Fig. 1. Structures of compound **1** and **2** and retigeranic acid.

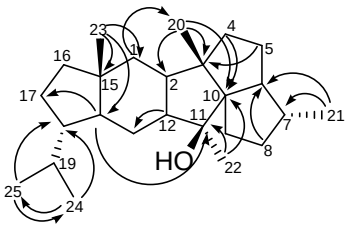


Fig. 2. Key HMBC correlations of **1**.

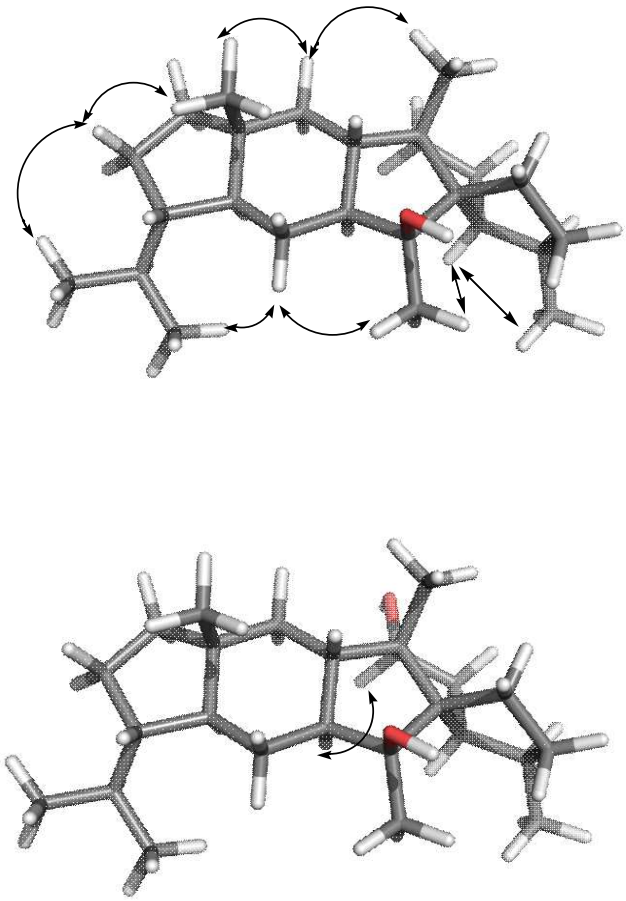
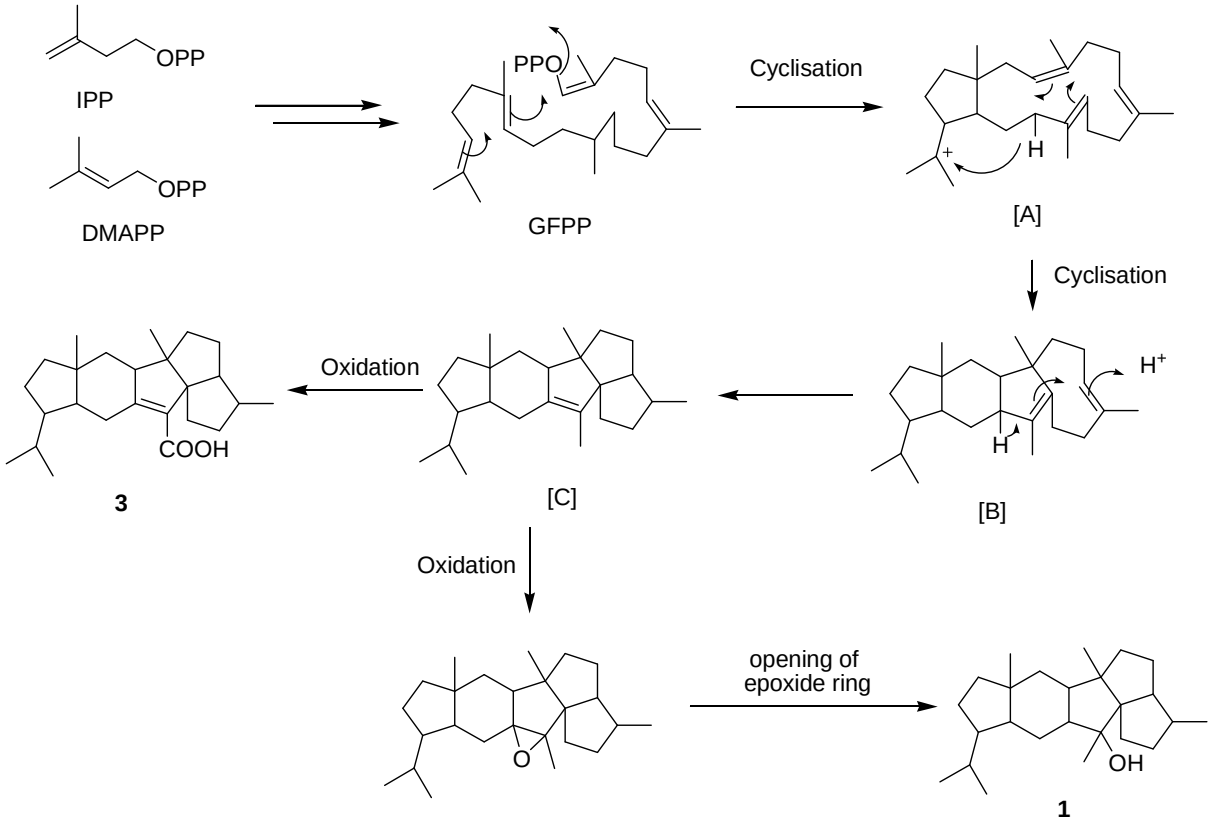


Fig. 3. The optimized conformation of **1** and **2** calculated with DFT/B3LYP/6-31+G(d,p) and NOESY key correlations.



b

Fig. 4. Plausible biogenetic pathway for compound (**1**).