Potamopholide, a New Sesquiterpene Lactone From *Vernonia potamophila*

Babady-Bila,[a] Amuri Kilonda,[a] Shetonde Mihigo,[a] Osomba Lohohola,[a] Tshibangu Sha Tshibey,[a] Frans Compernolle,*[b] Suzanne Toppet,[b] and Georges J. Hoornaert[b]

Keywords: Natural products / Terpenoids / Lactones / Structure elucidation

A new sesquiterpene lactone, named potamopholide, was isolated along with the known triterpene lupeol from the leaves of *Vernonia potamophila*. The stereochemical structure of the new compound, (3*R**,4*S**,5*E*,8*S**,10*S**)-3,8-epoxy-3,4,13-trihydroxy-1-oxogermacra-5,7(11)-dien-6,12-olide, was elucidated by mass spectrometric (CIMS, EIMS, HREIMS) and NMR spectroscopic (HMQC, HMBC, NOESY) analyses and by comparison with closely related compounds. The analysis was confirmed by MM+ geometry optimisation of the conformational structure. This 3,8-O-bridged 10-membered ring hemiacetal seems to be related structurally to known 1,4-O-bridged compounds.

Introduction

In the course of a search for bioactive principles from plants used in folk medicine in the Democratic Republic of Congo,[1] we have examined *Vernonia potamophila* Klatt. This small tree grows in Central and Southern regions of Africa,[2,3] but has not been studied previously for its bioactive components. The leaves of *Vernonia potamophila* are used in African folk medicine for the treatment of wounds and of cancer.[4] A considerable number of chemical species have been investigated from the large genus *Vernonia*, which has more than 1000 taxa. The most widespread components identified from this previous work are highly oxygenated sesquiterpene lactones containing a 7(11)-double bond and an acyl group on C-12.[5] The present work deals with the isolation and structure elucidation of a new sesquiterpene lactone from the leaves of *Vernonia potamophila.* The known triterpene lupeol also was identified in the extract.

Results and Discussion

A triterpene and a new sesquiterpene lactone **1** (Figure 1) were isolated from the leaves of *Vernonia potamophila* by dissolution of a crude chloroform extract in methanol followed by careful chromatographic separation of the methanol-soluble fraction on silica gel. Mass spectral analysis and ^{1}H and ^{13}C NMR spectroscopic data of the triterpene revealed that it was lupeol. Compound **1** was obtained as white crystals. Its IR spectrum showed absorptions at 3453 (OH), 1750 (γ-lactone), 1709 (>C=O), 1658, and 1631 (>C=C<) cm^{-1}. The molecular formula of compound **1** was established as $C_{15}H_{18}O_7$ on the basis of DEPT ^{13}C

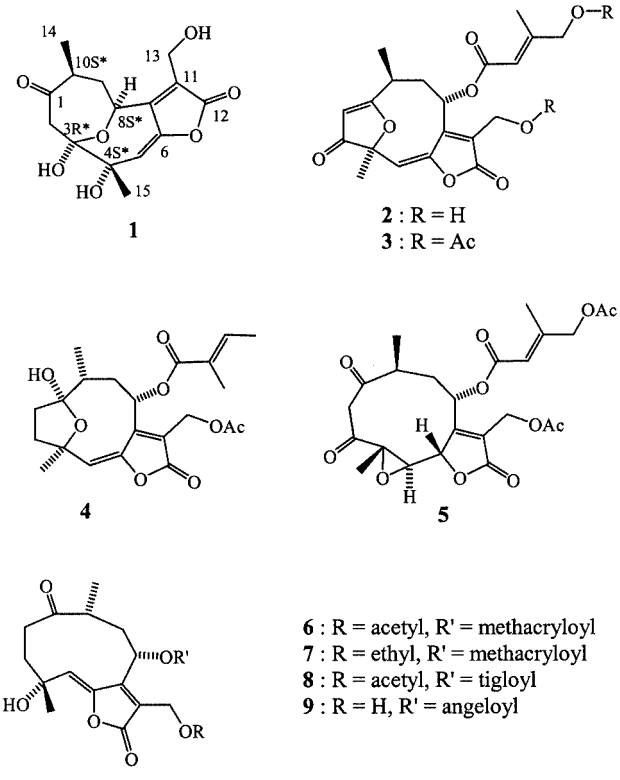


Figure 1. Structures of **1** and related sesquiterpene lactones

[a] Laboratoire de Chimie des Substances Naturelles, Faculté des Sciences, Université de Kinshasa
B. P. 190 Kinshasa XI, République Démocratique du Congo
[b] Laboratorium voor Organische Synthese, Departement Chemie, K. U. Leuven,
Celestijnenlaan 200F, 3001 Leuven, België
Fax: (internat.) + 32-16/327-990
E-mail: Frans.Compernolle@chem.kuleuven.ac.be

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of compound **1** in [D_6]DMSO (* intensity: 2 protons; ** intensity: 3 protons)

	^{1}H NMR		^{13}C NMR		
	δ [ppm]	Multiplicity, *J* [Hz]		δ [ppm]	DEPT
2α-H	2.35	d, ^2J = 14.5 Hz	C-1	209.7	C
2β-H	3.23	dd, ^2J = 14.5 Hz, ^4J = 2.3 Hz	C-2	46.1	CH_2
3-OH	5.85	d, ^4J = 2.3 Hz	C-3	100.9	C
4-OH	5.29	s	C-4	74.3	C
5-H	5.84	s	C-5	117.7	CH
8-H	5.65	dd, ^3J = 10.2, 8.5 Hz	C-6	155.1	C
9α-H	2.52	ddd, ^2J = 14 Hz, ^3J = 10.2, 2.3 Hz	C-7	146.6	C
9β-H	2.01	ddd, ^2J = 14 Hz, ^3J = 13, 8.5 Hz	C-8	68.5	CH
10-H	2.71	dqd, ^3J = 13, 7.1, 2.3 Hz	C-9	37.7	CH_2
13-H*	4.24	d, ^3J = 5 Hz	C-10	40.3	CH
13-OH	5.24	t, ^3J = 5 Hz	C-11	126.2	C
14-H**	1.02	d, ^3J = 7.1 Hz	C-12	167.9	C
15-H**	1.23	s	C-13	53.4	CH_2
			C-14	15.1	CH_3
			C-15	25.4	CH_3

NMR spectroscopic data and the chemical ionisation mass spectrum, which displays a pseudo-molecular ion at *m*/*z* = 311 $[M + H]^+$ and a base peak at *m*/*z* = 293 resulting from the elimination of water. In the high-resolution EI mass spectrum a similar fragment ion $[M - H_2O]^{+\cdot}$ is observed at *m*/*z* (%) = 292.0939 (2) indicating the elemental composition $C_{15}H_{16}O_6$. The molecular formula $C_{15}H_{18}O_7$ combined with the ^{13}C NMR spectrum displaying signals for two carbonyl units and two ethylenic double bonds, infers a tricyclic structure.

According to the DEPT data, only 15 hydrogen atoms are bound to carbon atoms. In agreement with the strong absorption observed at 3453 cm^{-1} in the IR spectrum, the remaining three hydrogen atoms were attributed to the presence of three hydroxy groups. In the ^{1}H NMR spectrum of compound **1** (Table 1), this assignment was confirmed by three signals centered at δ = 5.85 (d), 5.29 (s), and 5.24 (t, CH_2OH) ppm that disappear on presaturation of the DOH signal. Six of the seven oxygen atoms can be attributed to a ketone and a lactone function and three hydroxy groups. The seventh oxygen atom is likely to be part of either an ether or a (hemi)acetal group; such a hemiacetal group indeed was suggested by the appearance of a peak at δ = 100.9 ppm in the ^{13}C NMR spectrum (Table 1).

^{1}H NMR decoupling experiments allowed the determination of the skeleton fragment C-8−C-9−C-10−C-14, which is similar to that of related compounds isolated from other species of the *Vernonia* genus, namely **2**,[6] **3**−**6**,[7] **7**,[5] **8**,[8] and **9**.[9] The main spectral difference observed between the fragment C-8−C-14 of compound **1** and those of compounds **2**−**9** was the magnitude of the coupling constants measured for protons 8-H. Whereas the spectra of **2**−**9** each displayed both a large and a small coupling, two large couplings (10.2, 8.5 Hz) were observed for proton 8-H of compound **1**. This finding implies a small dihedral angle between 8-H and the *cis*-disposed 9-H methylene proton, suggesting that 8-H (δ = 5.65 ppm) is located at the bridgehead position of an oxygen bridge. (From a conformationally optimised model of **1** a dihedral angle of ca. 16° was estimated for H−C-8−C-9−H, which corresponds to the larger value ^3J = 10.2 Hz: see Figure 2, top). Further support for the location of the oxygen bridge at C-8 was provided by comparing the patterns observed for the signals of the hydroxymethylene protons 13-H of compound **1** with those reported for the related compounds **2**−**9**. For compound **1** a doublet was observed at δ = 4.24 ppm corresponding to a single coupling of the equivalent protons 13a-H and 13b-H with 13-OH. This finding implies that the oxygen bridge at C-8 allows free rotation about the C-

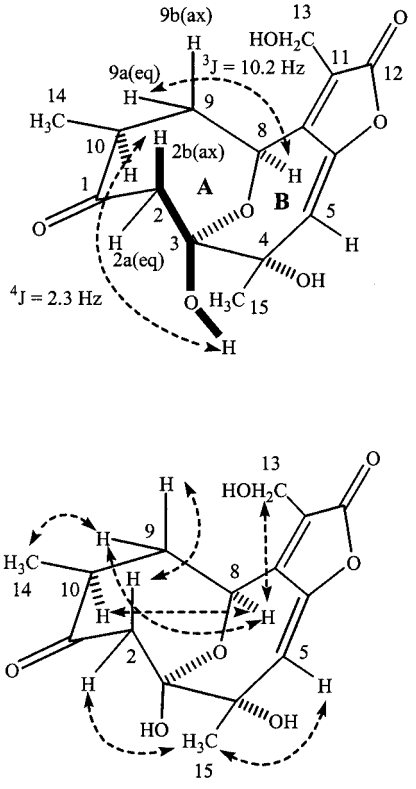


Figure 2. Conformational structure of **1** with pertinent coupling constants and NOESY correlations

13−C-11 linkage. By contrast, hindered rotation about this linkage was induced by the bulky 8-O substituents of compounds **2**−**9**, resulting in the observation of AB coupling patterns for 13a-H and 13b-H. The doublets of the AB system are less well separated in the absence of such a bulky group.[10]

Fragment C-1−C-2 of compound **1** is similar to that observed in compound **5**. Instead of the simple AB coupling pattern observed for the C-2 methylene group of **5**, however, the spectrum of **1** displays a further long-range coupling of the axial proton 2β-H with 3-OH. The signal attributed to 2β-H (δ = 3.23 ppm, ^2J = 14.5 Hz, ^4J = 2.3 Hz) was transformed into a doublet (^2J = 14.5 Hz) on presaturation of the DOH signal. The long-range coupling (^4J = 2.3 Hz) and its disappearance reveal clearly the location of the hydroxy group on the tetrasubstituted carbon atom C-3 and its *anti* (*trans*-diaxial) orientation relative to 2β-H in ring A. The four σ-bonds between protons 3-OH and 2β-H are connected in a W conformation (Figure 2, top), which is observed often in bridged rings or rigid systems.[11,12] We concluded that 3-OH was part of a hemiacetal group, and consequently C-3 was presumed to be the second bridgehead position of a C-3−O−C-8 oxygen bridge. This presumption was verified by the HMBC data, which showed a correlation between the hemiketal carbon atom C-3 at δ = 100.9 ppm with the *anti*-oriented proton 8-H at δ = 5.65 ppm.

The relative stereochemistry of compound **1** was determined from the coupling values in the ^{1}H NMR spectrum (Table 1) and from the correlations found by NOESY analysis (Table 2). From this analysis, we derived the conformationally optimised model depicted in Figure 2, top. The seven-membered ring A containing the oxo function adopts a boat conformation having all three carbon substituents C-4, C-7, and C-14 in equatorial positions. Protons 10-H and 9β-H have a *trans*-1,2-diaxial relationship (^3J = 13 Hz) while the quasi-axial proton 8-H displays dihedral angles of ca. 16 and 131° with the equatorial proton 9α-H^{cis} and the axial 9β-H^{trans} (see before). The cross peaks observed between the protons 8-H, 9α-H^{eq}, and 10-H confirm their common α-orientation and the nearly 1,3-diaxial disposition of 8-H and 10-H. Likewise, a cross peak was detected between the axial protons 9β-H and 2β-H and between the *trans*-disposed equatorial proton 9α-H^{eq} and the equatorial 10-Me group. The other seven-membered ring B assumes a half-chair conformation with a *trans*-diaxial disposition of C-2 and 4-OH and a *trans*-diequatorial orientation for 3-OH and 4-Me with respect to ring B. The close spatial vicinity of the equatorial 4-Me group with 5-H and 2α-H^{eq} was revealed by relevant NOE cross peaks observed in the NOESY spectrum (Figure 2, bottom). As can be deduced from the above data, the structure and relative configuration of compound **1** is (3*R**,4*S**,5*E*,8*S**,10*S**)-3,8-epoxy-3,4,13-trihydroxy-1-oxogermacra-5,7(11)-dien-6,12-olide. To the best of our knowledge, **1** is a new compound, which we have named potamopholide.

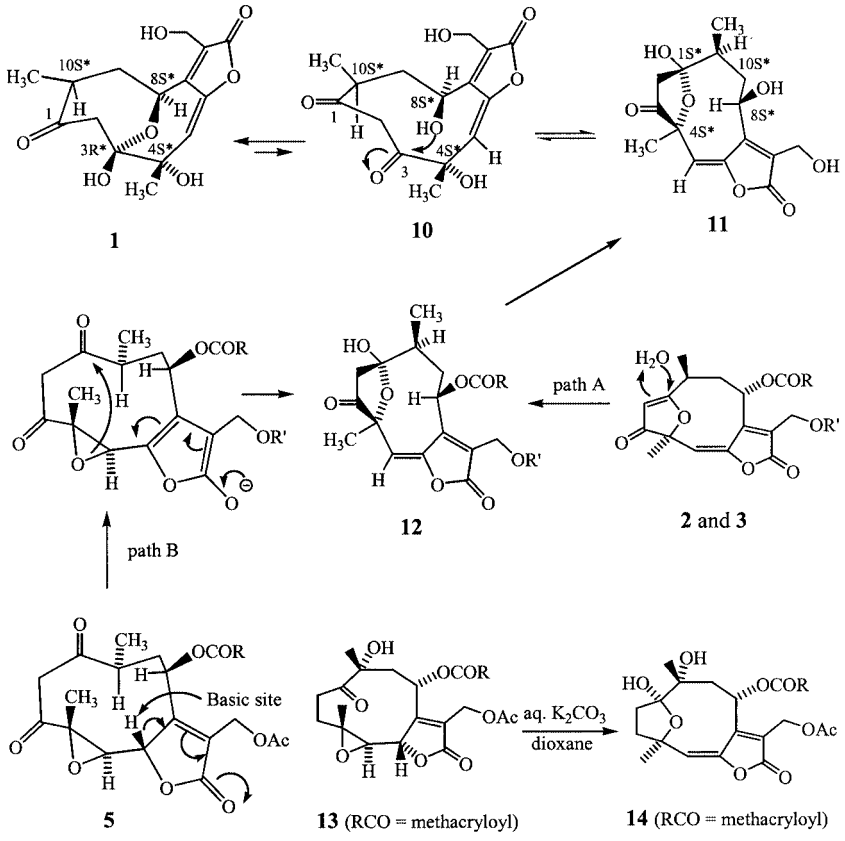
Compound **1** can be related to its ring-chain tautomer, β-diketone **10**, and to the isomeric 1,4-O-bridged hemiacetal form **11** and similar ring systems (e.g., **12**) where equilibration with the 3,8-O-bridged structure of **1** is blocked by an 8-*O*-acyl substituent (Scheme 1). From MM+ molecular mechanics calculations, **11** was determined to be less stable than **1** by ca. 4 kcal/mol. Since the structure of **12** is similar to that of several 8-*O*-acylated sesquiterpene lactones isolated from other species of *Vernonia*, it is suggested that compound **1** may originate from the exergonic biotransformation of such an 8-*O*-acyl precursor, such as the 1,4-O-bridged compounds **2**, **3** (path A), or the *trans*-epoxide **5** (path B). As depicted in path A, hydration of the dihydrofuran ring of **2** and **3**, followed or preceded by *O*-deacylation at the O-8 and O-13 positions, could provide hemiacetal **11**, which then may equilibrate to form isomer **1** via the common β-diketone intermediate **10**. In path B, abstraction of proton 6-H from *trans*-epoxide **5** may result in formation of a stable aromatic furan enolate anion and opening of the epoxide ring by a nonconcerted *syn*-elimination (a torsion angle of 43° was calculated for H−C-6−C-5−O from a conformationally optimised model of **5**, which agrees with the ^{1}H NMR and NOE data reported by Jakupovic et al.[7]). This process may lead to formation of hemiacetal **12**, which could then be transformed into compound **11** by *O*-deacylation at 8-O and 13-O. A similar conversion of epoxide **13** into hemiacetal **14** was effected chemically by heating **13** with potassium carbonate in dioxane.[8]

Table 2. NOE and HMBC correlations of compound **1** in [D$_6$]DMSO

	NOESY		HMBC
Proton	Correlated proton(s)	δC	Correlated proton
5-H	15-H	209.7	2α-H, 14-H
15-H	5-H, 2α-H	167.9	13-H
2α-H	15-H, 2β-H	155.1	5-H, 8-H, 13-H, 9β-H
2β-H	2α-H, 9β-H	146.6	5-H, 8-H, 15-H
9β-H	2β-H, 9α-H	126.2	13-H
9α-H	9β-H, 8-H, 14-H	117.7	15-H
8-H	9α-H, 10-H, 13-H	100.9	5-H, 8-H, 2α-H, 15-H
10-H	8-H, 14-H	74.3	15-H
		68.5	−
		53.4	−
		46.1	5-H
		40.3	2α-H, 9α-H, 14-H
		37.7	8-H, 14-H
		25.4	5-H, 4-OH
		15.1	−

Conclusion

In this work, a new sesquiterpene lactone, named potamopholide, was isolated from the leaves of *Vernonia potamophila* along with the known triterpene lupeol. The stereochemical structure of the new compound (3*R**,4*S**,5*E*,8*S**,10*S**)-3,8-epoxy-3,4,13-trihydroxy-1-oxogermacra-5,7(11)-dien-6,12-olide, was determined by a detailed NMR analysis and confirmed by MM+ geometry optimisation of the conformational structure. This 3,8-O-bridged 10-membered



Scheme 1

ring hemiacetal can be related to known 1,4-O-bridged compounds.

Experimental Section

General Remarks: Melting points are uncorrected. The optical rotations were measured with a Propol polarimeter fitted with a 7-cm cell. IR spectra were recorded as KBr pellets with a Perkin−Elmer 297 grating IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in [D$_6$]DMSO with a Bruker AMX 400 instrument operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The ^{1}H and ^{13}C chemical shifts are reported in ppm relative to tetramethylsilane as an internal reference. The *J* values are reported in Hz. Mass spectra were run with Kratos MS50 and Hewlett−Packard instruments; the ion source temperature was varied between 150 and 250 °C as required. Exact mass measurements were performed at a resolution of 10000. Analytical thin layer chromatography was performed using Merck silica gel 60 PF-224. Column chromatography was carried out using 70−230 mesh Merck silica gel 60.

Computational Details: Conformational calculations were carried out using Hyperchem™ (version 4.5; MM+ force-field).

Plant Material: Leaves of *Vernonia potamophila* Klatt were collected at Menkao situated in the region of Kinshasa. They were authenticated from a voucher specimen (A. Carlier 150 of August 31, 1955) kept at the herbarium of the INERA, Faculty of Sciences, University of Kinshasa.

Extraction: Powdered leaves of *Vernonia potamophila* (2160 g) were soaked in $CHCl_3$ (2 × 4 L) for 8 d. After filtration and evaporation of the $CHCl_3$ solvent, a black residue (188.6 g) was obtained. This residue was dissolved in EtOH at 60 °C and extracted with petroleum ether. After evaporation of the solvent, the residue was redissolved in $CHCl_3$ and the solution filtered. Evaporation of the $CHCl_3$ left a residue that was treated with MeOH. The insoluble material was discarded and the MeOH solution was concentrated to dryness to give a black residue (62.4 g) of terpene products. A part of this residue (9 g) was subjected to silica gel column chromatography. The column was eluted with a mixture of petroleum ether and ethyl acetate (9:1, 7:1, 4:1, then 1:1), followed by pure ethyl acetate and pure MeOH. Fractions eluted with the 9:1 mixture of petroleum ether and ethyl acetate were rechromatographed using the same eluent to afford a white solid (0.870 g) that was identified as lupeol by means of spectroscopic data.[13] Fractions eluted with EtOAc were further purified by silica gel column chromatography with the same eluent to furnish compound **1** as a white solid (0.300 g); m.p. 214−215 °C, $[\alpha]_D^{25}$ = +168.0 (*c* = 0.017, MeOH). For ^{1}H and ^{13}C NMR spectroscopic data, see Table 1. CI MS: *m/z* (%) = 311 (9) $[M + H]^+$, 293 (100) $[M + H - H_2O]^+$. HR MS: calcd. for $C_{15}H_{16}O_6$ $[M - H_2O]^{+\cdot}$ *m/z* = 292.0947, found *m/z* (%) = 292.0939 (2), calcd. for $C_{15}H_{14}O_5$ $[M - 2\,H_2O]^{+\cdot}$ *m/z* = 274.0841, found *m/z* (%) = 274.0837 (12).

[1] Babady-Bila, T. E. Gedris, W. Herz, *Phytochemistry* **1996**, *41*, 1441−1443.
[2] K. Kalala, S. Lisowsky, *Le genre Vernonia (Asteraceae) dans la flore d'Afrique Centrale* (Zaïre, Rwanda, Burundi), Krakow, Poland, **1995**, pp. 696−697.
[3] Y. Tailfer, *La flore dense d'Afrique Centrale, identification prat-*

ique des principaux arbres, CTA, The Netherlands, **1989**, vol. 2, p. 492.
[4] K. Kambu, *Eléments de phytothérapie comparée*, CRP, Kinshasa, **1990**, p. 86.
[5] C. A. N. Catalán, D. I. A. De Iglesias, J. Kavka, V. E. Sosa, W. Herz, *Phytochemistry* **1988**, *27*, 197−202.
[6] F. Bohlmann, N. G. Ates, J. Jakupovic, *Phytochemistry* **1983**, *22*, 1159−1162.
[7] J. Jakupovic, S. Banerjee, V. Castro, F. Bohlmann, A. Schuster, J. D. Mssonthi, S. Keeley, *Phytochemistry* **1986**, *25*, 1359−1364.
[8] A. Bardón, N. I. Kamiya, C. A. De Ponce De Léon, C. A. N. Catalán, J. G. Diaz, W. Herz, *Phytochemistry* **1992**, *31*, 609−613.
[9] J. Jakupovic, G. Schmeda-Hirschmann, A. Schuster, C. Zdero, F. Bohlmann, R. M. King, H. Robinson, J. Pickardt, *Phytochemistry* **1986**, *25*, 145−158.
[10] P. L. Cowall, J. M. Cassady, C.-J. Chang, J. F. Kozlowski, *J. Org. Chem.* **1981**, *46*, 1108−1114.
[11] R. M. Silverstein, C. G. Bassler, T. C. Morrill, *Spectroscopic identification of organic compounds*, 3rd ed., John Wiley & Sons, Inc., London, **1974**, pp. 191−192.
[12] D. H. Williams, I. Fleming, *Spectroscopic methods in organic chemistry*, 4th ed., McGraw-Hill, London, **1987**, p. 99.
[13] W. F. Reynolds, S. McLean, J. Poplawski, R. G. Enriquez, I. Laura, I. Leon, *Tetrahedron* **1986**, *42*, 3419−3428.

Received July 11, 2002
[O02384]