A New Labdane Diterpenoid from *Renealmia alpinia* Collected in the Suriname Rainforest[1]

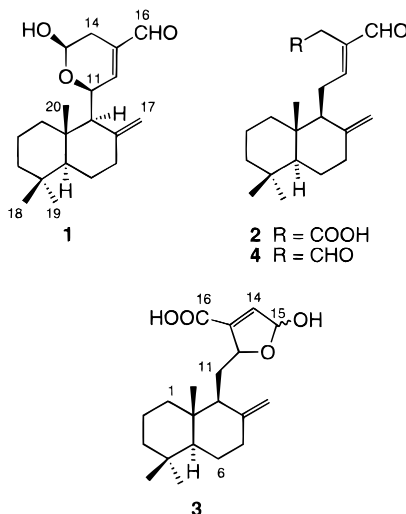
Shu-Wei Yang,[†,Δ] Bing-Nan Zhou,[†] Stan Malone,[‡] Marga C. M. Werkhoven,[§] Frits van Troon,[⊥] Jan H. Wisse,[‖] and David G. I. Kingston*[,†]

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212, Conservation International Suriname, Burenstrasse 7, Paramaribo, Suriname, The National Herbarium of Suriname, P. O. Box 9212, Paramaribo, Suriname, Asindopo Village, Suriname, and Bedrijf Geneesmiddelen Voorziening Suriname, Commissaris Roblesweg, Geyersvlijt, Suriname

Received February 15, 1999

Continuation of a previous study on *Renealmia alpinia* resulted in the isolation of the new labdane diterpenoid **3**, together with two known diterpenoids. The structure of the new diterpenoid was determined by a combination of NMR techniques and HRFABMS.

In continuation of our search for anticancer and other bioactive agents from the Suriname rainforest,[2] an extract of an unknown plant was identified as possessing activity in our Sc-7 yeast assay for cytotoxic agents. Fractionation was thus initiated on this extract using liquid−liquid partition followed by column chromatography on Sephadex LH-20, on Si gel, and by preparative TLC on Si gel. As fractionation proceeded it became clear that the active constituent of this extract was identical to the labdane diterpenoid **1** obtained previously from *Renealmia alpinia*,[2a] and further investigation led to the identification of known diterpenoid **2** as well as a new diterpenoid **3**. Compounds **1** and **2** were identified by comparison of their spectroscopic data with those previously reported for these metabolites[2a] and by direct comparison of samples by TLC. When the identification of the plant was made, it unsurprisingly turned out to be *Renealmia alpinia* (Rottb.) Maas (Zingiberaceae).



The molecular formula of compound **3** was determined as $C_{20}H_{30}O_4$ by HRFABMS and ^{13}C NMR data. In the ^1H NMR spectrum of **3**, three methyl singlets (δ 0.68, 0.80, and 0.88) and two olefinic signals (δ 4.89, 4.71) had chemical shifts very similar to the corresponding signals of compounds **1** and **2**,[2a] suggesting that the skeleton of **3** was also of the labdane type. The ^{13}C NMR spectrum of compound **3** confirmed this assignment, as the signals for C-1 to C-10 and for C-17 to C-20 matched the corresponding signals of the previously isolated compounds **2** and **4**[3] very well. An HMBC experiment (Table 1) provided further confirmation of the similarity between these systems. However, a downfield doublet (δ 4.56, $J = 11.2$) and two singlets (δ 6.12 and 7.04) were observed in the ^1H NMR spectrum of **3,** which did not correspond with signals in any of the compounds previously isolated from *R. alpinia*.[2a]

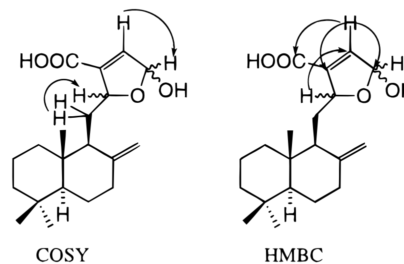


Figure 1.

The ^{13}C NMR data of **3** suggested the presence of an anomeric carbon, a carboxylic acid, a double bond (>C= CH−), an oxygenated methine carbon, and a methylene carbon. As noted previously, the ^{13}C NMR shifts for C-8, C-9, and C-10 were similar to those of the corresponding carbons in compounds **2** and **4**, but not **1**, indicating that C-11 must be a methylene carbon. The presence of an anomeric carbon (δ 97.0), together with the presence of four oxygen atoms, indicated that the side chain is cyclized through a hemiacetal linkage, as in diterpenoid **1**. $^1H-^1H$ COSY correlations between H-14 (δ 7.04) and H-15 (δ 6.12) and between H-11 (δ 1.89 and 1.68) and H-12 (δ 4.56) (Figure 1) indicate that the oxygenated methine carbon is adjacent to a methylene group and that the double bond is next to the anomeric carbon. These findings suggest that the cyclization on the side chain of **3** is through a five-membered hemiacetal ring. The H−C long-range correlations of H-14 to C-13, C-15, and C-16 (Figure 1) confirmed the relative location of the double bond to the anomeric carbon and showed that the carboxylic acid is located on C-13. The NOE observed between protons H-14/H-15 and

* To whom enquiries should be addressed: Tel: (540) 231-6570. Fax: (540) 231-7702. E-mail: dkingston@vt.edu.
† Virginia Polytechnic Institute and State University.
‡ Conservation International Suriname.
§ The National Herbarium of Suriname.
⊥ Asindopo Village.
‖ Bedrijf Geneesmiddelen Voorziening Suriname.
Δ Present address: Phytera Inc., 377 Plantation Street, Worcester, MA 01605.

10.1021/np990055i CCC: $18.00 © 1999 American Chemical Society and American Society of Pharmacognosy
Published on Web 06/23/1999

Table 1. ^{13}C NMR, ^{1}H NMR, HMBC, and NOESY Data for Compound **3** (in $CDCl_3$)

position	δ_H	δ_H	HMBC (H to C)	ROESY
1eq	1.68, m	39.3 (t)		
1ax	1.04, m			
2	1.53, m	19.3 (t)		
3eq	1.19, m	42.0 (t)		
3ax	1.41, m			
4		33.6 (s)		
5	1.15, m	55.5 (d)		
6eq	1.75, m	24.3 (t)		
6ax	1.35, m			
7β, eq	2.42, br d, $J = 13.0$	39.0 (t)		H-17a
7α, ax	2.02, m			
8		148.1 (s)		
9	2.05, m	51.8 (d)		
10		38.2 (s)		
11	1.89, m	30.1 (t)		H-17b
	1.68, m			
12	4.56, d, $J = 11.2$	65.5 (d)	C-13, C-14	
13		143.0 (s)		
14	7.04, s	142.8 (d)	C-13, C-15, C-16	H-15
15	6.12, s	97.0, 96.8 (d)	C-13, C-14	H-14
16		170.1 (s)		
17a	4.89, s	107.2 (t)	C-7, C-8, C-9	H-7β
17b	4.71, br s		C-7, C-8, C-9	H-11
18-Me	0.88, s	33.6 (q)	C-3, C-4, C-5, C-19	
19-Me	0.80, s	21.7 (q)	C-3, C-4, C-5, C-18	
20-Me	0.68, s	14.6 (q)	C-1, C-5, C-9, C-10	

H-11/H-17b also supported the proposed structure. Based on these spectroscopic data, the structure of the new diterpenoid was thus assigned as **3**.

The ^{1}H and ^{13}C NMR resonances of **3** were assigned unambiguously by spectral data including $^{1}H-^{1}H$ COSY, HMBC, HMQC, and NOESY, and are listed in Table 1. The observation of a pair of ^{13}C resonances for C-15 (δ 97.0, 96.8) indicated that **3** exists in solution as a pair of epimers in a ratio of 1:1, presumably at the anomeric carbon. Due to the limited sample available we were not able to assign the stereochemistry of **3** at C-12.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in $CDCl_3$ on a Varian Unity 400 NMR instrument at 399.951 MHz for ^{1}H and 100.578 MHz for ^{13}C, using standard Varian pulse sequence programs. LRMS were obtained on a VG 7070E-HF at VPI&SU. Exact mass measurements were obtained at the Nebraska Center for Mass Spectrometry. Other conditions were as previously described.[2]

Plant Material. The plant, *Renealmia alpinia* (Rottb.) Maas (Zingiberaceae), was collected near Asindopo village, Suriname. A herbarium specimen has been deposited in the National Herbarium of Suriname. The plant was ground to a powder and extracted at Bedrijf Geneesmiddelen Voorziening Suriname (BGVS) with EtOAc followed by MeOH to yield EtOAc and MeOH extracts as BGVS E950118 and M950118, respectively.

Isolation and Purification. Dried extract M 950118 (2.3 g) was distributed between hexane and 60% aqueous MeOH to give 1.1 g of hexane-soluble material. The aqueous MeOH was then adjusted to 50% aqueous MeOH and partitioned with CH_2Cl_2 to give 210 mg of aqueous MeOH-soluble material and 440 mg of the CH_2Cl_2 fraction. This CH_2Cl_2 fraction was loaded onto a column of Sephadex LH-20 and eluted with EtOH. Compound **1** (1 mg) was obtained from LH-20 fraction 6 (41 mg) by preparative TLC on Si gel developed by $CHCl_3$−MeOH (10:1). Fraction 5 (138 mg), containing most of the diterpenoids from the LH-20 column, was applied to a Si gel column and eluted with hexane−Me_2CO (6:1); compound **2** was obtained by preparative TLC of fraction 6 from this column. Fractions 15−17 (12 mg) from the LH-20 column were combined and applied to a preparative TLC plate, which was developed with *n*-hexane−Me_2CO (4:1). The first UV-absorbing band was collected, washed, and dried to yield compound **3** as a colorless gum (2 mg). Compounds **1** and **2** were identified by comparison of their NMR data and their TLC behavior with those of authentic samples.

Compound 3: colorless gum; ^{1}H NMR ($CDCl_3$), ^{13}C NMR ($CDCl_3$), HMBC, and NOESY data, see Table 1; unambiguous spectral assignments achieved based on $^{1}H-^{1}H$ COSY, HMQC, HMBC, and NOESY; FABMS (pos.) 357 $[M + Na]^+$; HRFABMS *m/z* 357.20421 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_4Na$, 357.20418).

Yeast Bioassay. The manipulated Sc-7 yeast assay was performed on a nine-well agar plate as previously described.[2a] Compound **3** gave IC_{12} values of 150 and 200 μg/mL in duplicate determinations.

Acknowledgment. This work was supported by an International Collaborative Biodiversity Grant, No. U01 TW/CA-00313 from the Fogarty Center, NIH, and this support is gratefully acknowledged. MS were provided by Mr. Kim Harich of Virginia Polytechnic Institute and State University and the Nebraska Center for Mass Spectroscopy in the Department of Chemistry, University of Nebraska.

References and Notes

(1) Biodiversity Conservation and Drug Discovery in Suriname. Part 5. For Part 4, see Yang et al.[2d]

(2) (a) Zhou, B.-N.; Baj, N. J.; Glass, T. E.; Malone, S.; Werkhoven, M. C. M.; van Troon, F.; David, M.; Wisse, J.; Bursuker, I.; Neddermann, K.; Kingston, D. G. I. *J. Nat. Prod.* **1997**, *60*, 1287−1293. (b) Abdel-Kader, M. S.; Wisse, J. H.; Evans, R.; van der Werff, H.; Kingston, D. G. I. *J. Nat. Prod.* **1997**, *60*, 1294−1297. (c) Abdel-Kader, M. S.; Bahler, B. D.; Malone, S.; Werkhoven, M. C. M.; van Troon, F.; David, M.; Wisse, J. H.; Burkuser, I.; Neddermann, K. M.; Mamber, S. W.; Kingston, D. G. I. *J. Nat. Prod.* **1998**, *61*, 1202−1208. (d) Yang, S.-W.; Zhou, B.-N.; Wisse, J.; Evans, R.; van der Werff, H.; Miller, J. S.; Kingston, D. G. I. *J. Nat. Prod.* **1998**, *61*, 901−906.

(3) Morita, H.; Itokawa, H. *Planta Med.* **1988**, *54*, 117−120.

NP990055I