Bioactive Labdane Diterpenoids from *Renealmia alpinia* Collected in the Suriname Rainforest[1]

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The preservation of tropical rainforests is an important goal both for the intrinsic value of their cultural and biological diversity as well as for the well-being of the peoples who make these forests their home. In addition, tropical forests are potential sources of new pharmaceutical products that can only be found by chemical prospecting in Nature's genetically encoded combinatorial library. As part of an effort to integrate biodiversity conservation and drug discovery with economic development, we have initiated a collaborative program to discover potential pharmaceuticals in the rainforest of Suriname. The plant *Renealmia alpinia* (Zingiberaceae) was selected for investigation based on its ethnomedical use as a febrifuge, but testing in the yeast Sc-7 assay gave a positive response, indicative of cytotoxic activity. Using this bioassay, the two new labdane diterpenes, 11-hydroxy-8(17),12(*E*)-labdadien-15,-16-dial 11,15-hemiacetal (**1**) and 16-oxo-8(17),12(*E*)-labdadien-15-oic acid (**2**), and the known diterpene, 8(17),12(*E*)-labdadien-15,16-dial (**3**), have been isolated. Their structures were elucidated by 1D and 2D NMR techniques (DEPT, COSY, HETCOR, HMBC, and NOESY) and IR, UV, and MS spectra, and the absolute stereochemistry of **1** was established by CD spectroscopy and by the formation and NMR analysis of α-methoxyphenylacetyl esters. The hemiacetal **1** was cytotoxic to M109 cells, with an IC_{50} value of 2.6 μg/mL.

The search for biologically active natural products from plants has been remarkably successful, to the extent that 57% of the top-selling prescription drugs in the United States contained natural products, derivatives, or analogues of natural products.[2] Because sales of prescription drugs in 1990 were approximately $147 billion, natural products can be seen to contribute to sales some $80 billion.[3] In support of these figures, a review of new drugs has shown that 39% of all drugs approved between 1983 and 1994 in 33 different disease areas were natural products or derived from natural products, while in the anticancer area the amount reached 59%.[4]

Interest in natural product research in the pharmaceutical industry has varied from company to company, but the publicity surrounding blockbuster drugs such as lovastatin and related compounds and paclitaxel rekindled interest in natural product research.[5] Although competing drug discovery methods such as combinatorial chemistry will clearly make a significant contribution to drug discovery, Nature's genetically encoded combinatorial library remains an unparalleled assembly of complex compounds that could not readily be obtained in any other way. A further advantage of the natural products approach is that the compounds obtained by it are all inherently active in some way, and it has been cogently argued that "all natural products have evolved under the pressure of natural selection to bind to specific receptors."[6]

Despite the benefits and potential of drug discovery through the natural product approach, serious problems remain to be solved if new drugs are to continue to be developed by this route. Chief among these is that species are disappearing at an alarming rate.[7,8] In terms of plant biodiversity, tropical flora account for more than 60% of the estimated 250 000 currently known higher plant species,[9] and yet the tropical forests that originally covered 16% of the earth's land surface had been reduced to 7% of its surface by 1990, and in some regions such as Madagascar, coastal Ecuador, and coastal Brazil, tropical forests have been reduced to less than 10% of their former extent.[10] It is clear that if present patterns of use continue, vast tracts of tropical rainforest and their unique biodiversity will be lost in the foreseeable future.[8,10]

In recent years it has become apparent that ethical use of the genetic resources of the rainforest must include appropriate payments to the owners of these resources, however they are identified in any particular case, and it has been suggested that such payments for "chemical prospecting" could be linked to biodiversity conservation in a mutually reinforcing way.[11] With this in mind, a consortium of U.S. Government agencies (the National Science Foundation, the National Institutes of Health, and the U. S. Agency for International Development) established the International Cooperative Biodiversity Group (ICBG) Program in 1992.[12] This program included funding not only for chemical prospecting and drug discovery, but also for work related to training, economic development, and biodiversity conservation, and it thus provides a model for future

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cooperative efforts at drug discovery in the tropical rainforests of the world. A review of the overall ICBG Program has recently appeared.[13]

As one contribution to the ICBG Program, a cooperative group was assembled to promote the aims of the program in Suriname.[12] The nation of Suriname (formerly Dutch Guiana) was selected for this effort because it has a large area of undisturbed neotropical Amazonian forest, with a diverse flora in a wide variety of ecological life zones. In addition, Suriname has a unique culture, including intact communities of Bushnegroes (Maroons) descended from runaway African slaves who have preserved an African culture in South America. The collection methods used included ethnobotanical strategies in addition to botanical strategies, so as to derive the maximum benefit from this unique culture.

As a part of the ethnobotanical strategy to discover bioactive extracts, a team from Conservation International−Suriname interviewed native healers in the Saramakan village of Asindopo to learn those plants that are used for healing purposes; the Saramakan tribe is the major tribal group in the interior of Suriname. In the course of this study it was learned that leaves of the plant *Renealmia alpinia* (Rottb.) Maas (Zingiberaceae) are used as a febrifuge.[14] The fresh leaves of *R. alpinia* ("gran masusa") are boiled together with leaves of the lime tree (*Citrus* sp.) and soursop (*Annona* sp., "alukutu" in the Saramakan tribal language), and the decoction is used for hot baths against fever and fatigue. A decoction of the freshly boiled leaves is also used for hot baths for women for three months after childbirth. A sample of *R. alpinia* was thus collected for further study.

Renealmia alpinia has not been extensively investigated in the past, and studies of lipids[15] and terpenes[16] from its seed oil are the only publications in recent years; the major constituent of the seed oil was identified as 8(17),12(*E*)-labadadiene-15,16-dial. The same labdadienal was also isolated from the related plant *R. guianensis*.[17]

Results and Discussion

A sample of the leaves of *R. alpinia*, together with samples of many other plants, was extracted by workers at Bedrijf Geneesmiddelen Voorziening Suriname (BGVS) and sent to Virginia Polytechnic Institute and State University (VPI&SU) Bioassay at VPI&SU was carried out in the Sc-7 yeast strain, which has been shown to respond to cytotoxic agents.[18] Activities in this assay are recorded as IC_{12} values, which are the concentrations (in μg/mL) required to give an inhibition zone 12 mm in diameter around a 100-μL well in a 4-mm agar layer plated with Sc-7 yeast. The EtOAc extract of *R. alpinia* leaves gave an IC_{12} value of 590 μg/mL in this assay, and this extract was thus selected for fractionation studies.

The EtOAc extract (4.67 g) was partitioned between *n*-hexane and 60% MeOH, and the bioactive *n*-hexane part was subjected to column chromatography on SiO_2 eluted with *n*-hexane−Me_2CO (6:1) to give eight fractions. Compound **1** (3.9 mg) was isolated from the bioactive fraction 2 by repeated chromatography on SiO_2 (*n*-hexane−Me_2CO, 6:1) and reversed-phase columns (RP-18, MeOH−H_2O, 9:1). Compound **2** (4.8 mg) and additional quantities of **1** (5.0 mg) were isolated from the active fraction 4 by repeated column chromatography on SiO_2 (*n*-hexane−Me_2CO, 5:1) and preparative TLC (SiO_2, *n*-hexane−Me_2CO 4:1, developed twice). Compound **3** (23.3 mg) was obtained from the active fraction 1 by column chromatography on SiO_2 (*n*-hexane−Me_2CO, 10:1) and on RP-18 (MeOH−H_2O, 9:1).

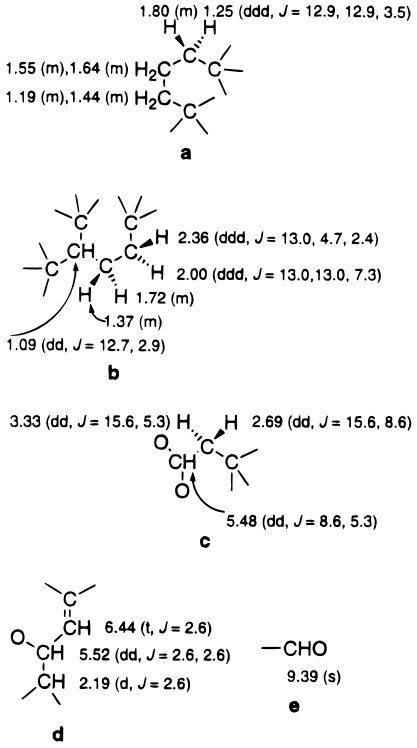
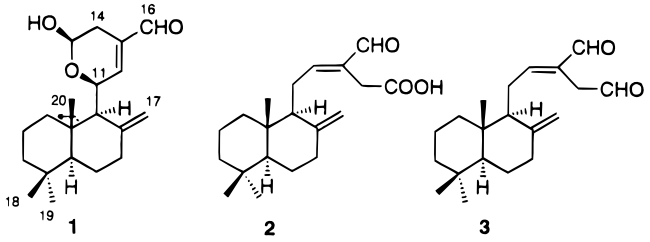


Figure 1. Partial structures **a**−**e** of compound **1**.



Compound **1**, obtained as a gum, did not give a molecular ion on mass spectrometry. Its composition was deduced to be $C_{20}H_{30}O_3$ on the basis of its ^{1}H- and ^{13}C-NMR data and on the MS data of its reduction product **5** (see below). Its UV spectrum showed λ_{max} at 233 nm, close to the calculated 237 nm for an α,β-disubstituted enone.[19] Its DEPT and HETCOR NMR spectra indicated the presence of three tertiary methyls, six methylenes, two methines, two quaternary carbons, an aldehyde group, one trisubstituted double bond, one $-C{=}CH_2$ group, and two oxygenated methines. Because these groups account for three of the six unsaturations, **1** is tricyclic.

The ^{1}H-NMR spectrum of **1** was analyzed with the aid of a TOCSY spectrum. The highfield portion of this spectrum could be analyzed in terms of two major spin systems, assignable to the groups **a** and **b** shown in Figure 1, while the lowfield portion could also be assigned to the two spin systems **c** and **d** and the isolated aldehyde to group **e** (Figure 1). The chemical shift of the methine proton in spin system **c** (δ_H 5.48

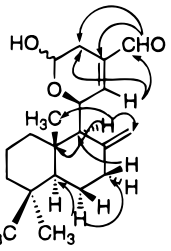


Figure 2. Selected HMBC data for **1**.

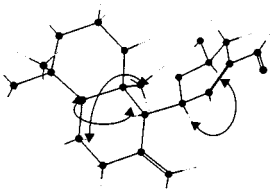


Figure 3. Selected NOE enhancements observed for **1**.

ppm) and of its corresponding carbon (δ_C 101.9) suggested it must be on a carbon bearing two oxygen substituents, while that of the allylic methine proton in system **d** (δ_H 5.52 ppm) and its corresponding carbon (δ_C 85.8) place it on a singly oxygenated carbon β to a carbonyl group. These assignments are consistent with the presence of an unsaturated cyclic hemiacetal bearing an aldehyde group in **1**.

The connections between the partial structures **a**−**e** were established by an HMBC spectrum; the key correlations established by this spectrum are shown in Figure 2. Structure **1** thus represents the basic structure of the new bioactive substance, which belongs to the class of labdane diterpenoids.

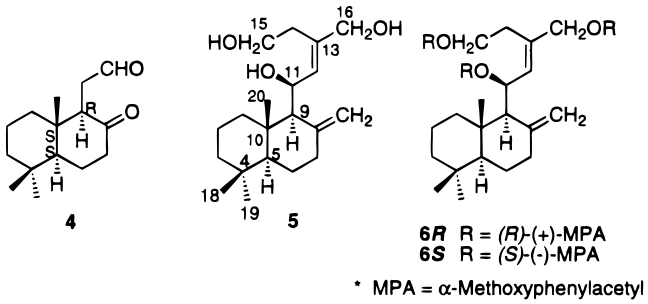
The relative stereochemistry of ring C could not be determined by NOE experiments in $CDCl_3$ because of the very similar chemical shifts of H-11 and H-15 (5.52 and 5.48 ppm, respectively). In C_6D_6, however, the signals for H-11 and H-15 appeared at 5.30 and 5.01 ppm, respectively, presumably due to the anisotropic effect of the π-electrons of the benzene ring, and an NOE was observed between these two protons, indicating that both of them were in the pseudoaxial position (Figure 3). This conclusion was supported by the coupling constants observed for H-15 ($J_{14ax-15ax}$ = 8.6 Hz, $J_{14eq-15ax}$ = 5.3 Hz). Interestingly, the equatorial orientation of the hemiacetal hydroxyl group in **1** required by this assignment is in contrast to that predicted by the anomeric effect;[20] presumably the α,β-unsaturated aldehyde function adds constraints to the system that reverse the normal preference of the hydroxyl group for an axial orientation.

These data establish the structure and relative stereochemistry of **1** as indicated as 11-hydroxy-8(17),12-(*E*)-labdadien-15,16-dial 11,15-hemiacetal. The absolute stereochemistry and the stereochemistry at C-11 were established as described below on the basis of CD and data from Mosher esters.

Compound **2**, a gum-like substance, was assigned the molecular formula $C_{20}H_{30}O_3$ by HREIMS. Its UV spectrum, with λ_{max} 236 nm, indicated the presence of an α,β-disubstituted enone moiety in the molecule.[19] Its ^{1}H- and ^{13}C-NMR spectra, and its DEPT spectrum indicated the presence of three singlet methyl signals, seven methylenes, two methines, two quaternary carbons, one −C═CH_2 group [δ_H 4.39 (1H, d, J = 1.0 Hz), 4.86 (1H, d, J = 1.2 Hz); δ_C 148.0 (s), 107.9 (t)], one −C═CH− group [δ_H 6.70 (t, J = 6.6 Hz); δ_C 135.6 (s), 159.5 (d)], one carboxylic acid group [δ_C 174.4], and one aldehyde group [δ_H 9.37 (s), δ_C 193.7]. Comparison of the above data with that published for the known 8(17),-12(*E*)-labdadien-15,16-dial (**3**),[17,21−23] indicated that compound **2** differed from **3** only in having a carboxyl group in place of one of the aldehyde groups of **3**. The aldehyde group was assigned to C-16 because the aldehyde proton gave a sharp singlet signal at 9.37 ppm in the ^{1}H-NMR spectrum; this conclusion was supported by the long-range couplings observed in the HMBC spectrum. The stereochemistry of the $\Delta^{12,13}$ double bond was assigned as *E* based on cross peaks in the NOESY spectrum between the aldehyde proton and H-12. Cross peaks between H-6_{ax} and both CH_3-18 and CH_3-20 indicated both methyl groups to be axial. Correlations between H-5 and H-9 as well as between H-5 and CH_3-19 indicated that H-5 and H-9 are axial and CH_3-19 is equatorial. An NOE enhancement was also observed between H-11_b and CH_3-20, indicating that the side chain is equatorial and cis to CH_3-20. Compound **2** was thus assigned as 16-oxo-8(17),12(*E*)-labdadien-15-oic acid.

Compound **3** was a gum-like substance. Its DEPT NMR spectrum and HREIMS supported the molecular formula $C_{20}H_{30}O_2$, and its ^{1}H- and ^{13}C-NMR spectra as well as its physical constants were identical with those of the known 8(17),12(*E*)-labdadiene-15,16-dial.[19,23−25] The structural assignment of **3** was supported by its 2D NMR spectra (TOCSY, HETCOR, HMBC, and NOESY), but these data also indicated that some minor reassignments of NMR signals were necessary. Thus, the ^{1}H-NMR signals at 0.89 ppm and at 0.73 ppm were assigned to CH_3-18_{ax} and CH_3-20_{ax}, respectively, and the ^{13}C-NMR signals for C-7 and C-14 were assigned as 37.8 and 39.3 ppm, respectively.

The availability of the known compound **3** provided an opportunity to determine the absolute stereochemistry of compounds **1** to **3**. The dialdehyde **3** underwent clean ozonolysis to yield the keto aldehyde **4** ($C_{15}H_{24}O_2$ by HRFABMS) in acceptable yield, and a CD spectrum obtained on **4** showed a negative Cotton effect at 289 nm, consistent with the assignment of **3** as a labdane diterpenoid rather than as an enantiomeric *ent*-labdane diterpenoid.[22] It is thus reasonable to assume that the diterpenoids **1** and **2** also have the labdane stereochemistry.



The assignment of stereochemistry of **1** at the C-11 position was carried out by the Mosher ester method. The original Mosher paper[24] describes the use of mandelate, *O*-methylmandelate or α-methoxyphenyl acetate (MPA), and α-methoxy-α-trifluoromethylphenyl acetate (MTPA) esters, but most subsequent users have selected

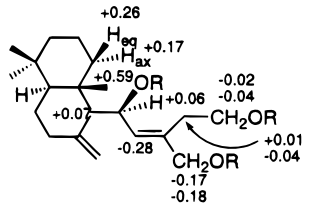
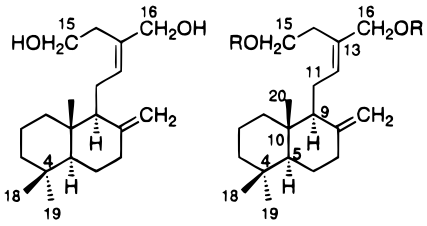


Figure 4. $\Delta\delta^{RS}$ ($\delta^R - \delta^S$) values for the (*R*)- and (*S*)-MPA esters **6*R*** and **6*S*** in $CDCl_3$.

MTPA esters because the chiral acid lacks a proton α to the carbonyl group and is thus resistant to racemization. The application of MTPA has been made more secure by the use of highfield NMR and the comparison of the NMR signals of as many protons as can be assigned rather than just two, as in the original paper.[25] Despite this bias in favor of MTPA esters, a recent paper demonstrates that α-methoxyphenylacetic acid has significant advantages over MTPA because its esters exist in one main conformer, in contrast to MTPA, which has a more complex conformational analysis in which shielding and deshielding effects tend to cancel each other out. The authors conclude "in the case of secondary alcohols MTPA is clearly less reliable than MPA".[26,27] Because of these considerations, we selected the MPA esters **6*R*** and **6*S*** of the triol **5** for our study.

Because the proposed MPA ester would be that of a triol, it became important to determine the effect, if any, of acylation of the two primary hydroxyl groups of **5** with MPA. The diol **7** was thus prepared as a model compound by reduction of the dialdehyde **3**, and converted to its (*R*)- and (*S*)-MPA esters **8*R*** and **8*S***.



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8*R* R = *(R)*-(+)-MPA
8*S* R = *(S)*-(-)-MPA

* MPA = α-Methoxyphenylacetyl

The ^{1}H-NMR spectra of **8*R*** and **8*S*** showed essentially zero chemical shift differences for all the assignable protons, with $\Delta\delta$ values ranging from −0.03 to +0.03 ppm. This experiment thus demonstrated that acylation at the C-15 and C-16 primary hydroxyl groups has little effect on $\Delta\delta$ values and, thus, that the triesters **6*R*** and **6*S*** can safely be used to make stereochemical assignments.

Reduction of hemiacetal **1** with $NaBH_4$ afforded the triol **5** ($C_{20}H_{34}O_3$ by HRFABMS) in a clean reaction with no detectable reduction of the conjugated double bond. Acylation with both (*R*)- and (*S*)-α-MPA yielded the (*R*)-ester (**6*R***: $C_{47}H_{58}O_9$ by HRFABMS) and the (*S*)-ester (**6*S***: $C_{47}H_{58}O_9$ by HRFABMS). The chemical shift difference values ($\Delta\delta^{RS} = \delta^R - \delta^S$)[26] of the individual protons of **6*R*** and **6*S*** are shown in Figure 4. The systematic arrangement of positive and negative $\Delta\delta^{RS}$ values indicated that the absolute configuration of C-11 is *R*, as indicated by the structure **1**. Due to the NOE relationship between H-11 and H-15, C-15 could also be assigned as *R*. Because the labdane stereochemistry for **1**, **2**, and **3** was demonstrated by the similarities of their NOE spectra and by the CD spectrum of **4** as noted above, the complete stereochemistry of **1** is established by this work as (11*R*, 15*R*)-11-hydroxy-8(17),12(*E*)-labdadien-15,16-dial 11,15-hemiacetal.

Compound **1** showed moderately strong activity against the Sc-7 yeast strain with an IC_{12} of 3 μg/mL, and it was also cytotoxic to M109 cells with an IC_{50} of 2.6 μg/mL. Compound **2** was weakly active in the Sc-7 assay, with an IC_{12} of 200 μg/mL, and compound **3** was slightly more active, with an IC_{12} value of 46 μg/mL in the Sc-7. Neither compound **2** nor **3** was active in the M109 cytotoxicity assay. Compound **3** has been reported as showing antifungal activity (MIC 6.25 μg/mL against *Candida tropicalis* and *Candida guilliermondii*) and cytotoxicity (ED_{50} 40 μg/mL against KB cells).[22]

Experimental Section

General Experimental Procedures. The CD spectrum was recorded on a JASCO J720 spectropolarimeter. 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 taken on a VG 7070 E-HF at VPI&SU, and exact mass measurements were obtained at the Nebraska Center for Mass Spectrometry. Other conditions were as previously described.[28]

Yeast Bioassay. The bioassay was carried out by determining growth inhibition against the Sc-7 yeast strain. The Sc-7 strain of *Saccharomyces cerevisiae* (BMRIT #109) was obtained from Bristol-Myers Research Institute, Tokyo, Japan, via Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, and was maintained on yeast extract peptone dextrose (YEPD) broth (Difco and BBL) at 4 °C. The broth culture was maintained by weekly aseptic transfer to fresh media, which was incubated at 30 °C for 2 days on a shaker prior to refrigeration. Inoculum was prepared by resuspending the yeast in the liquid culture and aseptically adding a portion to sterile distilled H_2O to an OD of 0.12 (25% transmittance) at 600 nm. Yeast Morphology Agar (Difco) plates were prepared and flooded with 2.5 mL of inoculum. After a brief interval the excess inoculum was pipeted off the plates (approximately 2 mL is removed) and the plates allowed to dry under a biological hood leaving a uniform lawn of yeast cells. After drying, wells of 6−7 mm in diameter were cut in the agar layer, and samples to be tested were dissolved in DMSO−MeOH (1:1) and added to the wells in 100-mL aliquots. Nystatin (Sigma) was used as a positive control at 20 mg/mL. The plates were incubated at 30 °C for 36−48 h, and the resulting zones of inhibitions were measured in millimeters. Activity was determined from a dose−response curve and reported as an IC_{12} value, which is the dosage (in μg/mL) required to produce a zone of inhibition 12 mm in diameter.

Cytotoxicity Bioassay. The in vitro antitumor cytotoxicity assays were performed at Bristol-Myers Squibb Pharmaceutical Research Institute using the Madison Lung Carcinoma (M109)[29] murine cell line as previously described.[30]

Plant Materials. The leaves of *Renealmia alpinia* (Rottb.) Maas (Zingiberaceae) were collected from Asindopo, Suriname, in June 1994. A herbarium speci-

Table 1. ^{1}H- and ^{13}C-NMR Data (δ in ppm, J in Hz) for Compounds **1**−**3** in $CDCl_3$

	compound **1**				compound **2**		compound **3**	
position	δ_H	δ_C[a]	HMBC (H to C)	NOESY[b]	δ_H	δ_C[a]	δ_H	δ_C[a]
1_{eq}	1.80 (m)	39.0 (2)	C-3, C-5		1.72 (m)	39.2 (2)	1.69 (m)	39.2 (2)
1_{ax}	1.25 (ddd, 12.9, 12.9, 3.5)			$H\text{-}5_{ax}$				
					1.08 (ddd, 12.7, 12.7, 4.0)		1.06 (ddd, 12.5, 12.5, 4.2)	
2	1.55 (m)	19.2 (2)			1.59 (m)	19.3 (2)	1.56 (m)	19.3 (2)
	1.64 (m)				1.53 (m)		1.52 (m)	
3_{eq}	1.44 (m)	42.0 (2)	C-1, C-5		1.21 (m)	42.0 (2)	1.20 (m)	42.0 (2)
3_{ax}	1.19 (m)				1.42 (m)		1.42 (m)	
4		33.6 (0)				33.6 (0)		33.6 (0)
5	1.09 (dd, 12.7, 2.9)	55.9 (1)		$H\text{-}1_{ax}$, $H\text{-}7_{ax}$, $H\text{-}9_{ax}$, H-19	1.14 (dd, 12.6, 2.6)	55.4 (1)	1.13 (dd, 12.6, 2.7)	55.4 (1)
6_{eq}	1.72 (m)	24.0 (1)	C-5, C-7		1.74 (m)	24.1 (2)	1.75 (m)	24.1 (2)
6_{ax}	1.37 (m)			H-18, H-20	1.35 (m)		1.35 (m)	
7_{eq}	2.36 (ddd, 13.0, 2.4, 4.7)	38.0 (2)	C-5, C-9	H-17b	2.42 (m)	37.8 (2)	2.42 (ddd, 13.0, 2.4, 4.6)	37.8 (2)
7_{ax}	2.00 (ddd, 13.0, 13.0, 7.3)			$H\text{-}5_{ax}$	2.03 (ddd, 13.0, 12.8, 5.0)		2.03 (ddd, 13.0, 12.8, 4.6)	
8		144.9 (0)				148.0 (0)		148.0 (0)
9	2.19 (br d, 2.6)	62.6 (1)	C-8, C-10, C-17, C-20	$H\text{-}5_{ax}$, H-12	1.92 (dd, 11.0, 3.1)	56.4 (1)	1.90 (dd, 11.0, 3.1)	56.4 (1)
10		39.1 (0)				39.6 (0)		39.6 (0)
11	5.52 (dd, 2.6, 2.6)	85.8 (1)		H-15, H-17a, H-20	2.56 (ddd, 16.9, 6.6, 3.1)	24.6 (2)	2.58 (ddd, 16.9, 6.5, 3.1)	25.7 (2)
					2.38 (ddd, 16.9, 11.0, 6.6)		2.38 (ddd, 16.9, 11.0, 6.5)	
12	6.44 (t, 2.6)	155.9(1)	C-11, C-14, C-16	H-9, H-16, H-17a, H-20	6.70 (t, 6.6)	159.5 (1)	6.76 (t, 6.5)	160.0 (1)
13		136.4 (0)				135.6 (0)		134.8 (0)
14_{eq}	3.33 (dd, 15.6, 5.3)	28.0 (2)	C-12, C-13		3.40 (d, 16.5)	29.5 (2)	3.46 (d, 17.1)	39.3 (2)
14_{ax}	2.69 (dd, 15.6, 8.6)				3.34 (d, 16.5)		3.39 (d, 17.1)	
15	5.48 (dd, 8.6, 5.3)	101.9 (1)		H-11		174.4 (0)	9.40 (s)	197.3 (1)
16	9.39 (s)	192.4 (1)	C-13, C-14	H-12	9.37 (s)	193.7 (1)	9.63 (s)	193.4 (1)
17a	4.83 (br.s)	109.2 (2)	C-7, C-9	H-11, H-12	4.39 (d, 1.0)	107.9 (2)	4.36 (d, <1.0)	107.8 (2)
17b	4.79 (d, 1.4)			$H\text{-}7_{eq}$	4.86 (d, 1.2)		4.86 (d, <1.0)	
18	0.87 (s)	33.7 (3)	C-3, C-4, C-5, C-19	$H\text{-}6_{ax}$, H-20	0.89 (s)	33.6 (3)	0.89 (s)	33.6 (3)
19	0.84 (s)	21.6 (3)	C-3, C-4, C-5, C-18	H-5	0.83 (s)	21.7 (3)	0.82 (s)	21.7 (3)
20	0.98 (s)	16.7 (3)	C-1, C-5, C-9, C-10	$H\text{-}6_{ax}$, H-11, H-12, H-18	0.75 (s)	14.4 (3)	0.73 (s)	14.4 (3)

[a] Carbon type as determined by DEPT spectra: 0 = quaternary, 1 = methine, 2 = methylene, 3 = methyl. [b] The NOESY spectrum was determined in C_6D_6.

men, number CI 0034, was deposited in the National Herbarium of Suriname.

Plant Extraction. *Renealmia alpinia* leaves were extracted with EtOAc and MeOH at BGVS; extraction of 300 g of dried plant material with EtOAc yielded 4.67 g of extract as BGVS E-940072.

Isolation of Labdanes 1−3. The EtOAc extract E-940072 (4.67 g, IC_{12} 590 μg/mL in the Sc-7 assay) was partitioned between *n*-hexane and 60% aqueous MeOH to give a bioactive *n*-hexane fraction (0.7 g, IC_{12} 120 μg/mL). This fraction was then subjected to repeated chromatography on Si gel with elution by *n*-hexane−Me_2CO, 6:1, to give eight fractions after combination of similar fractions as judged by TLC. Bioactivity was detected in fractions 2 (15.7 mg, IC_{12} 9 μg/mL) and 4 (34 mg, IC_{12} 16 μg/mL); the very small fraction 3 was not investigated. Repeated chromatography of fraction 2 on Si gel (*n*-hexane−Me_2CO, 6:1) and RP-18 Si gel (MeOH−H_2O, 9:1) yielded compound **1** (3.9 mg, IC_{12} 3 μg/mL). Chromatography of fraction 4 on Si gel (*n*-hexane−Me_2CO, 5:1) followed by preparative TLC on Si gel (*n*-hexane−Me_2CO, 4:1, developed twice) yielded compound **2** (4.8 mg, IC_{12} 200 μg/mL) and additional compound **1** (5.0 mg). Compound **3** (23.3 mg, IC_{12} 46 μg/mL) was isolated from fraction 1 (194 mg) by chromatography on Si gel (*n*-hexane−Me_2CO, 10:1) and on RP-18 Si gel (MeOH−H_2O, 9:1).

Compound 1: colorless gum-like substance; $[\alpha]^{25}_D$ −40.0° (*c* 0.43, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 233.5 nm (4.13); IR (KBr) ν_{max} 3396 (−OH), 2933, 2873, 2842, 1682 (conjugated −CHO), 1645, 1643, 1462, 1442 1214, 1167, 1083, 1049, 898, 667 cm^{-1}; ^{1}H-NMR, ^{13}C-NMR, HMBC, and NOESY data, see Table 1.

Compound 2: colorless gum-like substance, $[\alpha]^{25}_D$ +10.8° (*c* 0.24, CH_3OH), UV (MeOH) λ_{max} (log ϵ) 236.5 nm (4.13); ^{1}H-NMR and ^{13}C-NMR data, see Table 1; EIMS *m/z* 318 $[M]^+$, 300 $[M - 18]^+$, 285, 137 (100%), 123, 109 95, 81, 69; HREIMS *m/z* 318.2184 (calcd for $C_{20}H_{30}O_3$, 318.2194).

Compound 3: colorless gum-like substance, $[\alpha]^{25}_D$ +16.5° (*c* 0.43, $CHCl_3$), UV (MeOH) λ_{max} (log ϵ) 234 nm (3.91); ^{1}H-NMR and ^{13}C-NMR data, see Table 1; EIMS *m/z* 302 $[M]^+$; CIMS *m/z* 303 $[M + 1]^+$; HRFABMS *m/z* 309.2406 (calcd for $C_{20}H_{30}O_2$ + Li, 309.2406).

Ozonolysis of Compound 3. Compound **3** (8.15 mg) in 2 mL of MeOH was connected to a microscale ozone generator[31] and ozonized for 3 min at −70 °C, after which the reaction mixture was treated with Zn (14 mg) and HOAc (0.3 mL) for 1 h at room temperature. The crude product was purified by preparative TLC (*n*-hexane−Me_2CO, 6:1) to give keto aldehyde **4** (6.0 mg, 94%); spectroscopic data (^{13}C NMR and ^{1}H NMR) identical with literature;[22] CD, negative Cotton effect at λ 289 nm, ($\Delta\epsilon$ −3.40, *c* 0.01M, MeOH); HRFABMS *m/z* 237.1857 (calcd for $C_{15}H_{24}O_2$ + H, 237.1855).

Reduction of Compound 3 with $NaBH_4$. Compound **3** (32 mg, 0.106 mmol) was treated with $NaBH_4$ (40 mg, 10.5 mmol) in MeOH (2 mL) at room temperature for 30 min. The reaction mixture was worked up by the usual procedure and the crude product purified

by preparative TLC (*n*-hexane−Me_2CO, 10:1) to yield diol **7** (27 mg, 84%): ^{1}H NMR ($CDCl_3$) δ 0.70 (3H, s, CH_3-20), 0.81 (3H, s, CH_3-19), 0.87 (3H, s, CH_3-18), 3.74 (2H, t, CH_2-15), 4.01 (2H, s, CH_2-16), 4.44 (1H, s, H-17$_a$), 4.82 (1H, s, H-17$_b$), 5.50 (1H, t, J = 6.3 Hz, H-12); ^{13}C NMR (CDCl3) δ 14.41 (q, C-20), 19.35 (t, C-2), 21.74 (q, C-19), 22.29 (d, C-11), 24.10 (t, C-6), 32.79 (t, C-14), 33.60 (s, C-4), 33.60 (q, C-18), 38.07 (t, C-7), 39.15 (t, C-1), 39.55 (s, C-10), 42.08 (t, C-3), 55.40 (d, C-5), 57.10 (d, C-9), 61.50 (t, C-15), 68.60 (t, C-16), 107.38 (t, C-17), 132.39 (d, C-12), 135.43 (s, C-13), 148.49 (s, C-8).

Preparation of (*R*)-(−)-α-Methoxyphenyl Acetate (8*R*) of Diol 7. Diol **7** (13.4 mg, 0.044 mmol) was treated with (*R*)-(−)-α-methoxyphenylacetic acid (73.0 mg, 0.44 mmol), and DCC (70 mg, 0.34 mmol) in the presence of 4-pyrrolidinopyridine (catalytic amount) in CH_2Cl_2 (1 mL), and the mixture was stirred at room temperature for 8 h. The di-[(*R*)-(−)-α-methoxyphenyl acetate] **8*R*** (15.0 mg, 57%) was obtained by preparative TLC on SiO_2 (*n*-hexane−$(CH_3)_2CO$, 20:1): ^{1}H NMR ($CDCl_3$) δ 0.68 (3H, s, CH_3-20), 0.81 (3H, s, CH_3-19), 0.87 (3H, s, CH_3-18), 0.99 (1H, ddd, J = 12.8, 12.8, 4.1 Hz, H-1$_{ax}$), 1.49 (1H, m, H-1$_{eq}$), 1.17 (1H, ddd, J = 13.3, 13.3, 4.2 Hz, H-3$_{ax}$), 1.40 (1H, m, H-3$_{eq}$), 1.07 (1H, dd, J = 12.5, 2.6 Hz, H-5), 1.58 (1H, m, H-7$_{ax}$), 1.95 (1H, m, H-7$_{eq}$), 2.36 (1H, m, H-9), 2.19 (1H, br s, H-11$_a$), 1.95 (1H, m, H-11$_b$), 5.43 (2H, t, J = 7.2 Hz, H-12), 2.24 (2H, m, CH_2-14), 4.10 (1H, m, H-15$_a$), 3.92 (1H, m, H-15$_b$), 4.42 (2H, s, CH_2-16), 4.77 (1H, br s, H-17$_a$), 4.32 (1H, br s, H-17$_b$). The α-methoxyphenylacetic acid part had δ 7.20−7.40 (10H, m, aromatic protons), 4.72 (2H, s), 3.38 (6H, s, 2 × CH_3O-).

Preparation of (*S*)-(+)-α-Methoxyphenyl Acetate (8*S*) of Diol 7. Diol **7** (13.54 mg, 0.044 mmol) was treated with (*S*)-(+)-α-methoxyphenylacetic acid (78 mg, 0.046 mmol), and DCC (75 mg, 0.36 mmol) in the presence of 4-pyrrolidinopyridine (catalytic amount) in CH_2Cl_2 (1 mL), and the mixture was stirred at room temperature for 8 h. The di-[(*S*)-(+)-α-methoxyphenyl acetate] **8*S*** (17.7 mg, 66.5%) was obtained by preparative TLC on SiO_2 (*n*-hexane−Me_2CO, 20:1): ^{1}H NMR ($CDCl_3$) δ 0.68 (3H, s, CH_3-20), 0.81 (3H, s, CH3−19), 0.87 (3H, s, CH3−18), 0.97 (1H, ddd, J = 12.8, 12.8, 3.8 Hz, H-1$_{ax}$), 1.48 (1H, m, H-1$_{eq}$), 1.16 (1H, ddd, J = 13.3, 13.2, 3.8 Hz, H-3$_{ax}$), 1.40 (1H, m, H-3$_{eq.}$), 1.07 (1H, dd, J = 2.6, 12.5 Hz, H-5), 1.59 (1H, m, H-7$_{ax}$), 1.96 (1H, m, H-7$_{eq.}$), 2.36 (1H, m, H-9), 2.19 (1H, m, H-11$_a$), 1.96 (1H, m, H-11$_b$), 5.45 (1H, t, J = 6.4 Hz, H-12), 2.23 (2H, m, CH_2-14), 4.07 (1H, m, H-15$_a$), 3.90 (1H, m, H-15$_b$), 4.43 (2H, s, CH_2-16), 4.78 (1H, br s, H-17$_a$), 4.35 (1H, br s, H-17$_b$). The α-MPA part had δ 7.20−7.40 (10H, m, aromatic protons), 4.72 (2H, s), 3.38 (6H, s, 2 × CH_3O).

Reduction of 1 with $NaBH_4$. Compound **1** (8.56 mg, 0.026 mmol) was treated with $NaBH_4$ (17.9 mg) in MeOH (2 mL) at room temperature for 30 min. The reaction mixture was worked up by the usual procedure and the crude product purified by preparative TLC (hexane−Me_2CO, 6:1) to yield triol **5** (7.0 mg, 86.6%): ^{1}H NMR ($CDCl_3$) δ 0.83 (3H, s, CH_3-19), 0.86 (3H, s, CH_3-18), 1.01 (3H, s, CH_3-20), 1.05 (1H, dd, J = 12.7, 3.1 Hz, H-5$_{ax}$), 1.41 (1H, m, H-6$_{ax}$), 1.81 (1H, d, J = 4.7 Hz, H-9), 1.89 (1H, m, H-1$_{eq}$), 2.02 (1H, ddd, J = 13.1, 13.1, 5.8 Hz, H-7$_{ax}$), 2.32 (1H, m, H-14$_b$), 2.37 (1H, m, H-7$_{eq}$), 2.65 (1H, ddd, J = 14.2, 9.8, 4.8 Hz, H-14$_a$), 3.71 (1H, ddd, J = 10.3, 9.8, 4.8 Hz, H-15$_b$), 3.81 (1H, ddd, J = 10.3, 5.0, 4.8 Hz, H-15$_a$), 4.09 (2H, s, CH_2-16), 4.82 (1H, dd, J = 8.5, 4.7 Hz, H-11), 5.01 (1H, br s, H-17$_b$), 5.06 (1H, br s, H17$_a$), 6.05 (1H, d, J = 8.5 Hz, H-12); ^{13}C NMR ($CDCl_3$) δ 16.58 (q, C-20), 19.26 (t, C-2), 21.81 (q, C-19), 24.49 (t, C-6), 31.48 (t, C-14), 33.58 (s, C-4), 33.80 (q, C-18), 38.78 (t, C-7), 40.02 (t, C-1), 40.35(s, C-10), 42.03 (t, C-3), 55.53 (d, C-5), 60.48 (t, C-15), 61.30 (d, C-9), 65.13 (d, C-11), 67.23 (t, C-16), 109.82 (t, C-17), 132.67 (d, C-12), 137.66 (s, C-13), 146.65 (s, C-8); HRFABMS *m/z*: 345.2406 (calcd for $C_{20}H_{34}O_3$ + Na, 345.2406).

Preparation of the (*R*)-(−)-α-Methoxyphenyl Acetate of Triol 5. Triol **5** (3.49 mg, 0.0109 mmol) was treated with (*R*)-(−)-α-MPA (9.88 mg, 0.0595 mmol), and DCC (12.24 mg, 0.0594 mmol) in the presence of 4-pyrrolidinopyridine (catalytic amount) in CH_2Cl_2 (1 mL) and the mixture stirred at room temperature for 8 h. The tri-[(R)-(−)-α-methoxyphenyl acetate] **6*R*** was obtained by column chromatography on SiO_2 (CH_2Cl_2−EtOAc, 2:1) and preparative TLC on RP-18 (MeOH−H_2O, 9:1): $[\alpha]_D$ −63.7° (*c* 0.09, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.06 (1H, ddd, J = 12.6, 11.0, 4.0 Hz, H-1ax), 1.75 (1H, ddd, J = 12.6, <2, <2 Hz, H-1eq), 1.15 (1H, ddd, J = 12.9, 12.9, 4.7 Hz, H-3ax), 0.99 (1H, dd, J = 13.0, 3.0 Hz, H-5ax), 1.90 (1H, ddd, J = 13.1, 13.1, 5.1, H-7$_{ax}$), 2.26 (1H, m, H-7$_{eq}$), 1.67 (1H, br s, H-9), 5.93 (1H, dd, J = 9.3, 2.4 Hz, H-11), 5.53 (1H, d, J = 9.3 Hz, H-12), 2.51 (1H, ddd, J = 14.5, 7.1, 7.1 Hz, H-14a), 2.26 (1H, m, H-14b), 4.12 (1H, ddd, J = 10.9, 7.1, 7.1 Hz, H-15a), 3.94 (1H, ddd, J = 10.9, 7.1, 7.1 Hz, H-15b), 4.28 (1H, d, J = 13.1 Hz, H-16a), 4.17 (1H, d, J = 13.1 Hz, H-16b), 4.83 (1H, br s, H-17a), 4.70 (1H, br s, H-17b), 0.83 (3H, s, CH_3-18), 0.80 (3H, s, CH_3-19), 0.79 (3H, s, CH_3-20). The (α)-MPA part had δ 7.20−7.50 (15-H, m, aromatic protons), 3.35 (3H, s, −OCH_3), 3.37 (3H, s, −OCH_3), 3.38 (3H, s, −OCH_3), 4.66 (2H, s), 4.74 (1H, s); ^{13}C NMR ($CDCl_3$) δ 16.19 (q, C-20), 19.11 (t, C-2), 21.72 (q, C-19), 24.27 (t, C-6), 29.70 (t, C-14), 33.56 (s, C-4), 33.69 (q, C-18), 39.00 (t, C-7), 39.19 (t, C-1), 40.20 (s, C-10), 41.83 (t, C-3), 55.90 (d, C-5), 63.20 (t, C-15), 67.92 (d, C-11), 68.80 (t, C-18), 110.29 (t, C-17), 129.13 (d, C-12), 132.60 (s, C-13), 135.84, 136.03, 144.38 (s, C-8). The (α)-MPA part had δ 57.29 (q, CH_3−O−), 57.35 (q, CH_3O−), 57.35 (q, CH_3O−), 82.37 (d, −CH−O−), 82.42 (d, −CH−O−), 82.70 (d, −CH−O−), 127.12, 127.20, 127.22, 128.53, 128.64, 128.74, 135.84, 136.03, 136.03 (aromatic carbons) 169.66 (s, −COOR), 169.95 (s, −COOR), 170.35 (s, −COOR); HRFABMS *m/z* 789.3969 (calcd for $C_{47}H_{58}O_9$ + Na, 789.3978).

Preparation of the (*S*)-(+)-α-Methoxyphenyl Acetate of Triol 5. Triol **5** (2.45 mg, 0.0077 mmol) was treated with (*S*)-(+)-α-MPA acid (11.5 mg, 0.0692 mmol) and DCC (8.59 mg, 0.0417 mmol) in the presence of 4-pyrrolidinopyridine (catalytic amount) in CH_2Cl_2 (1 mL) at room temperature with stirring for 8 h. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2−EtOAc, 2:1) and by RP-18 preparative TLC (MeOH−H_2O, 9:1) to give the tri-[(*S*)-(+)-α-methoxyphenyl acetate] **6*S***: ^{1}H NMR ($CDCl_3$) δ 0.89 (1H, m, H-1ax), 1.49 (1H, m, H-1eq), 1.05 (1H, ddd, J = 13.1, 13.1, 5.5 Hz, H-3ax), 0.86 (1H, dd, H-5ax), 1.86 (1H, ddd, J = 13.1, 12.7, 5.0 Hz, H-7ax), 2.25 (1H, m, H-7eq), 1.60 (1H, d, J = 2.0 Hz, H-9), 5.87 (1H, dd, J = 9.6, 2.0 Hz, H-11), 5.81 (1H, d, J = 9.6 Hz, H-12), 2.55 (H-14a), 2.25 (H-14 b), 4.16 (1H, ddd, J = 10.9, 7.0, 7.0 Hz, H-15a),

3.96 (1H, ddd, J = 10.9, 7.0, 7.5 Hz, H-15b), 4.45 (1H, d, J = 13.3 Hz, H-16a), 4.35 (1H, d, J = 13.3 Hz, H-16b), 4.91 (1H, br s, H-17a), 4.86 (1H, br s, H-17b), 0.69 (3H, s, CH_3-18), 0.79 (3H, s, CH_3-19), 0.20 (3H, s, CH_3-20). The (α)-MPA part had δ 7.20−7.50 (15H, m, aromatic protons), 4.61 (1H, s), 4.73 (1H, s), 4.75 (1H, s), 3.38 (6H, s, 2 × $-OCH_3$), 3.33 (3H, s, $-OCH_3$); ^{13}C NMR ($CDCl_3$) δ 15.19 (q, C-20), 18.95 (t, C-2), 21.62 (q, C-19), 24.01 (t, C-6), 29.70 (t, C-14), 33.45 (s, C-4), 33.60 (q, C-18), 38.79 (t, C-7), 39.00 (t, C-1), 39.81 (s, C-10), 41.83 (t, C-3), 55.65 (d, C-5), 60.71 (d, C-9), 63.38 (t, C-15), 67.85 (d, C-11), 68.95 (t, C-16), 110.13 (t, C-17), 129.49 (d, C-12), 132.60 (s, C-13), 144.53 (s, C-8). The (α)-MPA part had δ 57.14 (q, CH3O−), 57.30 (q, CH3O−), 57.38 (q, CH_3O−), 82.36 (d, −CH−O−), 82.42 (d, −CH−O−), and 82.79 (d, −CH−O−), 127.20, 127.22, 127.64, 128.52, 128.62, 128.66, 128.72, 128.75, 128.79, 135.45, 135.45, and 136.11 (aromatic carbons), 169.51 (s, −COOR), 170.00 (s, −COOR), and 170.38 (s, −COOR); HRFABMS *m*/*z* 789.3984 (calcd for $C_{47}H_{58}O_9$ + Na, 789.3978).

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