New Trichothecenes Isolated from *Holarrhena floribunda*

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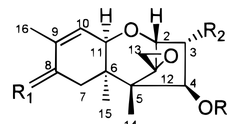
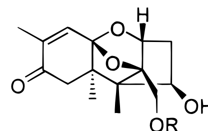
Bioassay-guided fractionation of an extract of *Holarrhena floribunda* stem, has led to the isolation of the new trichothecenes, 8-dihydrotrichothecinol A (**1**), loukacinol A (**2**), and loukacinol B (**3**), and the known compounds, trichothecolone (**4**), trichothecin (**5**), trichothecinol A (**6**), rosenonolactone (**7**), 6β-hydroxyrosenonolactone (**8**), and rosololactone (**9**). The structures were determined by spectral and chemical methods, and absolute configurations were established by a modified Horeau's method using HPLC. Compounds **1** and **6** exhibited significant cytotoxicity against several human tumor cell lines, whereas compound **8** showed moderate and weak antileishmanial activity toward extracellular and intracellular *Leishmania donovani*, respectively.

During the course of screening medicinal plant extracts for antileishmanial, antimicrobial, and antitumor activity, an ethanolic extract of *Holarrhena floribunda* (G. Don) T. Durand & Schinz (Apocynaceae) stem exhibited significant cytotoxicity against KB (squamous carcinoma), SK-Mel 28 (melanoma), A-549 (lung carcinoma), and MDA-MB 231 (breast carcinoma) cell lines. Despite extensive phytochemical and pharmacological investigation of *H. floribunda*,[2,3] there are no ethnomedical records on the use of this species in "cancer" medicine, and so far, no cytotoxicity has been reported for its known constituents (alkaloids, triterpenes, phenolic acids, flavonoids). The exception is progesterone, previously isolated from the leaves,[4] which is reported to possess mammary-carcinoma inhibiting potential.[5] In a recent publication, the isolation of several cytotoxic and leishmanicidal aminoglycosteroids and aminosteroids from the species *H. curtisii* was reported.[6]

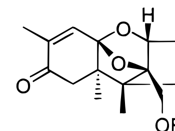
HPLC fractionation of the extract resulted in enrichment of activity in several chromatographic fractions,[1] which also showed a lethal effect against *Artemia salina*.[7] Further investigation of the extract led to the isolation of three new trichothecenes, 8-dihydrotrichothecinol A (**1**), loukacinol A (**2**), and loukacinol B (**3**), along with the known trichothecenes **4**−**6** and compounds **7**−**9**. In this paper we describe the isolation and structure elucidation of these metabolites, as well as the antitumor evaluation of **1**−**6** and the synthetic derivative **2a**. Compound **8** was assayed for its leishmanicidal activity against *Leishmania* parasites.



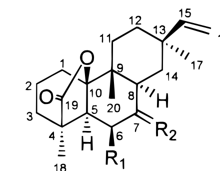
1 $R_1 = H_2$ $R_2 = OH$ $R_3 = C(O)CH{=}CHCH_3$ (1′, 2′, *Z*, 3′, 4′)
4 $R_1 = O$ $R_2 = H$ $R_3 = H$
5 $R_1 = O$ $R_2 = H$ $R_3 = C(O)CH{=}CHCH_3$
6 $R_1 = O$ $R_2 = OH$ $R_3 = C(O)CH{=}CHCH_3$



2 R = H
2a R = Ac



3 R = H
3a R = Ac



7 $R_1 = H$ $R_2 = O$
8 $R_1 = OH$ $R_2 = O$
9 $R_1 = OH$ $R_2 = H_2$

Results and Discussion

Following a partition process, the alkaloid constituents of *H. floribunda* stem were removed from the extract and concentrated in a fraction that did not display activity in the antitumor assays. The nonalkaloid fraction exhibited significant cytotoxic activity.

The cytotoxic fraction was chromatographed on Sephadex LH-20, affording two subfractions (I and II). A combination of flash Si gel and RP_{18} column chromatography (CC) of fraction I, followed by preparative TLC, yielded compounds **2**, **3**, and **4**. Fraction II afforded compounds **1**, **5**, **6**, and **7**−**9**. Compounds **4** ($C_{15}H_{20}O_4$, M^+ 264) and **5** ($C_{19}H_{24}O_5$, M^+ 332) displayed ^1H and ^{13}C NMR spectra exhibiting features characteristic of 12,13-epoxytrichothec-9-en-8-ones,[8] and TLC analysis showed color characteristics of trichothecenes.[9,10] These two metabolites were identified as trichothecolone and trichothecin by comparison with authentic samples whose complete spectral data have been previously reported.[11−14]

Compound **6** ($C_{19}H_{24}O_6$, M^+ 348), whose ^1H and ^{13}C NMR spectra closely resemble those of **5**, showed an IR hydroxyl absorption, suggesting that **6** is a hydroxylated derivative of **5**. The observed vicinal coupling of H-4 with a low-field proton at δ 4.28 linked to a carbon at δ 78.9 proved that the hydroxyl group was attached at C-3. NMR resonances (Tables1 and 2) were in good agreement with those reported for trichothecinol A, previously isolated from the fungus *Trichothecium roseum*.[15] The stereochemistry of **6** was assigned by the NOESY spectrum in conjunction with

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Table 1. ^{1}H NMR Spectral Data for Compounds **1**, **2**, **2a**, **3**, **3a**, and **6**[a]

	compound					
proton	**1**	**2**	**2a**	**3**	**3a**	**6**
2β	3.71 (d, 4.5)	4.10 (d, 5.1)	4.27 (d, 5.0)	4.31 (d, 5.1)	4.16 (br s)	3.80 (d, 7.8)
3α		2.57 (dd, 15.7; 7.0)	2.58 (dd, 15.7; 7.0)	1.95 (m)	1.89 (m)	
3β	4.21 (s)	1.90 (ddd, 15.7; 5.1; 2.7)	1.88 (ddd, 15.7; 5.1; 2.7)	1.95 (m)	1.89 (m)	4.28 (dd, 4.6; 2.1)
4α	5.00 (d, 1.6)	4.30 (dd, 7.0; 2.7)	4.18 (dd, 7.9; 2.7)	1.50 (m)	1.56 (m)	4.95 (d, 2.1)
4β				1.98 (m)	2.01 (m)	
7α	1.42 (m)	2.28 (d, 14.8)	2.28 (d, 15.0)	2.20 (d, 14.8)	2.24 (d 15.2)	2.31 (d, 14.8)
7β	2.00 (m)	2.76 (d, 14.8)	2.80 (d, 2.8)	2.73 (d, 14.8)	2.73 (d, 15.2)	2.95 (d, 14.8)
8	2.00 (s)					
10	5.52 (d, 3.1)	6.38 (s)	6.40 (s)	6.45 (s)	6.45 (s)	6.60 (d, 5.2)
11α	4.00 (d, 4.0)					4.40 (d, 5.2)
13α	3.04 (d, 3.5)	4.06 (m)	4.54 (m)	4.12 (d, 12.4)	4.46 (d, 12.8)	3.08 (d, 3.7)
13β	2.78 (d, 3.5)	4.06 (m)	4.54 (m)	3.74 (d, 12.4)	4.37 (d, 12.8)	2.80 (d, 3.7)
14	0.77 (s)	1.06 (s)	0.97 (s)	0.97 (s)	0.98 (s)	0.77 (s)
15	0.92 (s)	0.99 (s)	1.04 (s)	0.98 (s)	1.00 (s)	1.04 (s)
16	1.72 (s)	1.84 (s)	1.84 (s)	1.88 (s)	1.84 (s)	1.84 (s)
2′	5.87 (d, 11.4)					5.88 (d, 11.2)
3′	6.41 (m)					6.45 (m)
4′	2.17 (d, 6.7)					2.18 (d, 7.1)
OH	3.3 (s)					3.42 (br s)
OAc			2.12 (s)		2.12 (s)	

[a] Values were recorded at 400 MHz (in $CDCl_3$ in the δ scale, with *J* values in Hz in parentheses).

Table 2. ^{13}C NMR Data of Compounds **1**, **2**, **2a**, **3**, and **6**[a]

C	**1**	**2**	**2a**	**3**	**6**
2	79.0	75.0	80.7	82.6	79.3
3	78.7	41.7	40.8	27.3	78.9
4	84.1	82.9	74.9	31.0	83.3
5	48.9	54.7	55.2	50.3	48.9
6	41.3	54.3	54.1	54.6	44.4
7	24.5	48.0	48.0	47.7	42.0
8	28.0	198.9	198.5	198.0	198.8
9	139.8	141.3	141.3	140.9	137.8
10	118.9	133.8	133.6	134.1	137.2
11	71.5	106.0	106.0	105.2	71.0
12	64.6	95.7	94.7	96.3	64.5
13	47.1	59.3	60.3	58.6	46.7
14	6.1	11.3	11.1	17.1	5.9
15	16.0	17.4	17.4	17.6	18.4
16	23.2	15.4	15.5	15.5	15.4
1′	167.9				168.0
2′	120.2				119.9
3′	146.5				147.1
4′	15.5				15.6
*C*H$_3$CO			20.9		
CH$_3$*C*O			170.5		

[a] Values in parts per million (δ) were recorded at 100.61 MHz (in $CDCl_3$).

Table 3. Absolute Configuration of Secondary Alcohols **1**, **2a**, **4**, **6**, **8**, and **9**

compound	(1*R*,2′*S*)/(1*R*,2′*R*)	absolute configuration
1	59/41	*R*
2a	52/48	*R*
4	70/30	*R*
6	69/31	*R*
8	40/60	*S*
9	88/12	*R*

the determination of absolute configuration at C-3 by modified Horeau's method using HPLC.[16] Reaction of **6** with racemic 2-phenylbutanoic anhydride, followed by addition of (*R*)-(+)-1-(1-naphthyl)ethylamine afforded a mixture of (1*R*,2′*S*)- and (1*R*,2′*R*)-*N*-[1-(1-naphthyl)ethyl]-2-phenylbutanamides, in the proportion of 69:31 as calculated by HPLC (Table 3), which is indicative of an *R* configuration of the secondary hydroxy group of **6**.

HREIMS of **1** was consistent with a molecular formula of $C_{19}H_{26}O_5$, while initial inspection of its ^{13}C NMR, DEPT, and HMQC spectra revealed the absence of a ketone functionality at C-8 that appears at approximately δ 198 in **4**−**6**. There were several carbon resonances identical to those observed for trichothecinol A: four methine at δ 79.0 (C-2), 78.7 (C-3), 84.1 (C-4), 71.5 (C-11); one methylene at δ 47.1 (C-13); one quaternary at δ 64.6 (C-12) linked to oxygen; and the characteristic signals of the −C(O)CH═CHCH$_3$ moiety at C-4. Comparison of C-7, C-10, and C-16 chemical shifts with those of **6** indicated significant upfield shifts of C-7 (−17.5 ppm) and C-10 (−18.8 ppm) and a downfield shift of C-16 (+7.8 ppm), due to absence of the 8-oxo-group. The HMBC spectrum showed $^{2}J_{CH}$ and $^{3}J_{CH}$ correlations of C-9 and C-10 with two methylenic protons at δ 2.0, which are attributed to H-8 (Table 4). The H-7 methylene protons, which appear in the ^{1}H NMR spectrum of trichothecinol A as two well-resolved doublets at δ 2.31 and 2.95 ($J = 14.8$ Hz), are shifted to δ 1.42 (H-7α) and 2.0 (H-7β) in compound **1**. The NOESY spectrum suggested the β-orientation of H-3. This proton shows a unique cross-peak with H-2, which in turns interacts with one epoxy proton at δ 3.04 (H-13$_{\alpha}$) (Figure 1). The other methylene proton of the epoxide ring, at δ 2.78 (H-13$_{\beta}$), displays a NOE with CH$_3$-14. The absolute configuration at C-3 was confirmed as *R* on the basis of modified Horeau's method (Table 3). Therefore, this new compound was determined to be 8-dihydrotrichothecinol A.

Compound **2** was analyzed for $C_{15}H_{20}O_5$ by HREIMS. The IR spectrum showed hydroxyl and ketone absorptions, whereas DEPT and HMQC spectra indicated the presence of three methyl groups, two sp^2 olefinic carbons, two primary −CH$_2$− carbons, two quaternary carbons, two oxygen-bearing quaternary carbons, two methines, one methylene, and a carbonyl. The HMBC spectrum showed cross-peaks of the carbonyl carbon with two methylenic protons, one methyl, and a vinyl proton, thus establishing the substitution pattern of ring A. The ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2), when compared to those of compounds **4**−**6**, revealed some significant differences: the vinyl proton H-10 appears as a singlet without any observable cross-peak in the $^{1}H-^{1}H$ COSY spectrum, proving that C-11 is tetrasubstituted; C-11 has an unusual downfield chemical shift (δ 106.0) that suggests an acetal function; the characteristic AB signal arising from the two protons of the 12,13-epoxide is replaced by a two-proton multiplet centered at δ 4.06, attached to a carbon at δ 41.7; the resonance of C-14 that ranges from δ 5.7 to 6.1 in compounds **1**−**3** is shifted to δ 11.3; and finally, the tetrasubstituted C-12 has a downfield chemical shift (δ 95.7) from the corresponding carbon of the 12,13-epoxytrichothecenes. The above data, supported by HMBC long-range correlations, suggested the presence of a primary alcohol bonded to C-12, and a hydroxyl group at C-4, as

Table 4. ^{1}H NMR Data for Compounds **7**−**9** and ^{13}C NMR Data for Compound **8**, in $CDCl_3$

	compound			
	7	**8**		**9**
position	^{1}H	^{1}H	^{13}C	^{1}H
1	1.20−1.70 (m)	1.20−1.70 (m)	31.2	2.16 (m)
2	1.70 (m); 1.92 (m)	1.80 (m); 1.95 (m)	19.8	1.70 (m); 1.85 (m)
3	1.61 (m): 1.70 (m)	1.60 (m); 1.80 (m)	36.4	1.60 (m); 1.75 (m)
4			45.6	
5	2.25 (m)	2.30 (d, 4.8)	54.9	1.8 (m)
6	1.90 (m); 2.20 (m)	3.95 (d, 4.8)	68.6	4.20 (m)
7			212.4	1.90 (m); 2.0 (m)
8	2.38 (m)	2.60 (dd, 3.9;11.8)	47.1	1.30−1.80 (m)
9			40.6	
10	2.10 (m); 2.35 (m)		86.4	
11	2.10 (m); 2.35 (m)	2.10 (m); 2.35 (m)	31.6	1.3−1.8 (m)
12	1.20−1.70 (m)	1.20−1.70 (m)	31.4	1.3−1.8 (m)
13			35.0	
14	1.20−1.70 (m)	1.20−1.70 (m)	31.3	1.20 (m); 1.40 (m)
15	5.80 (dd, 17.6; 10.8)	5.80 (dd, 17.6; 10.8)	149.5	5.80 (dd, 17.6; 10.8)
16	4.90 (d, 10.8); 4.95 (d, 17.6)	4.90 (d, 10.8); 4.99 (d, 17.6)	110.0	4.80 (d, 10.8); 4.9 (d, 17.6)
17	0.90 (s)	0.97 (s)	21.6	0.99 (s)
18	1.08 (s)	1.4 (s)	17.0	1.30 (s)
19			179.6	
20	0.93 (s)	1.1 (s)	16.4	1.23 (s)
OH		2.70 (s)		

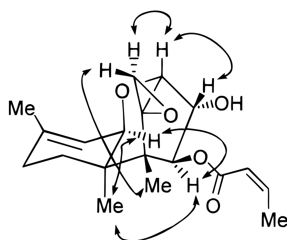

Figure 1. Important NOE interactions of **1**.

indicated by the $^3J_{CH}$ coupling and NOE effect of the methine proton H-4 (δ 4.3) with C-6 (δ 54.3) and CH_3-15, respectively. These assignments were confirmed by selective acetylation of the primary alcohol with acyl cyanide to form compound **2a**, whose ^{1}H NMR spectrum exhibited an expected downfield shift (+ 0.48 ppm) of the $-CH_2OAc$ protons. Application of modified Horeau's method using HPLC to the secondary alcohol of **2a**, allowed us to establish the absolute configuration (*R*) at C-4. Stereochemical requirements of the 1,3-dioxolane ring system imply the exo (β) orientation of the 11,12-ether bridge. The new metabolite **2** can thus be regarded as an 11-*epi*-12-*epi*-trichothecane derivative, and it appears to be related to sambucinol.[17,18]

Loukacinol B (**3**) was determined to have a molecular formula $C_{15}H_{20}O_4$ by HREIMS. The IR spectrum showed a ketone and a band attributed to a hydroxyl group. ^{1}H and ^{13}C NMR data (Tables 1 and 2) indicated the characteristic resonances for ring A of trichothec-9-en-8-one group, two tetrasubstituted and one secondary oxygen-bearing carbons at δ 105.2, 96.3, and 82.6. The last is attached to a methine proton (δ 4.31), which is part of a five-spin system $-OCH(R)-CH_2-CH_2-C(R)_2-$ as proved by the ^{1}H−^{1}H COSY and HMQC spectra. Comparison of the above data, which were supported by HMBC correlations, with those of compounds **1**, **2**, and **4**−**6**, revealed that C-3 and C-4 lack the hydroxyl substituent. The presence of two methylene protons (δ 3.74 and 4.12) placed on a carbon (δ 58.6) adjacent to C-12 (δ 96.3), suggested a similar dioxolane ring system for **2** and **3**. In compounds **2** and **2a** these protons overlap in a multiplet, whereas in **3** they are well resolved in an AB signal with a geminal coupling $J = 12.4$ Hz. These nuances in the form of H-13 methylene resonances, which have been previously reported for sambucinol,[17] its acetyl derivative,[17] and sporol,[19] are a result of a conformational change. The conclusive structure characterization followed from the mild acetylation of **3** to form **3a**, which caused a marked downfield shift of 0.34 and 0.63 ppm of the H-13 protons (Table 1). This deshielding effect, when compared to that observed in the acetylation of **5** (+ 0.48 ppm), indicates that the shifted protons are α to the hydroxyl group.

Three diterpenes of molecular formulas $C_{20}H_{28}O_3$ (**7**), $C_{20}H_{28}O_4$ (**8**), and $C_{20}H_{30}O_3$ (**9**) were also isolated from the active fraction. The IR and ^{13}C NMR spectra of **7** indicated the presence of γ-lactone and ketone groups. An additional hydroxyl band appeared in the IR spectra of **8** and **9** (3.418 and 3.522 cm^{-1}, respectively), whereas the latter lacked the ketone absorption. The ^{13}C and mass spectra of **7** were identical to those reported for the rosane diterpenoid rosenonolactone.[13,20−22] ^{1}H and ^{13}C NMR spectra of **8** (Table 4) resembled those of rosenonolactone in many respects, but showed an additional secondary hydroxy group attached to a carbon at δ 68.6. The methine proton (δ 3.95) correlates with H-5 (δ 2.3) and C-10 (δ 86.4) in the COSY and HMBC spectra, respectively, thus confirming the position of the hydroxyl group at C-6. Its axial orientation was deduced from the coupling constant ($J_{5-6} = 4.8$ Hz) and from the observed cross-peak between H-6 and the equatorial C-18 in the NOESY spectrum. The structure of **8**, whose carbon and complete proton NMR data are here reported for the first time, corresponds to the known 6β-hydroxyrosenonolactone.[23,24] Compound **9** appeared to be the 7-deoxy derivative of **8**, and its carbon resonances were in good agreement with the previously reported data for rosololactone.[20]

The C-6 absolute configuration of compounds **8** and **9** was established as for the preceding cases (Table 3). These rosane lactones have previously been isolated only from several species of *Trichothecium* fungi.[25]

The cytotoxicity of compounds **1**−**6** and **2a** against KB, SK-MEL 30, A-459, and MCF-7 cancer cell lines was evaluated, and the results are shown in Table 5. The high activity exhibited by compound **6** is in accordance with results previously reported by Iida et al., which indicated an antitumor-promoting effect of trichothecinol A.[15] Compound **1** was also markedly cytotoxic, whereas the remaining compounds were moderately active. These results agree with generalized conclusions about the structure−cytotoxicity relationship of trichothecenes[26,27] and indicate that

Table 5. Cytotoxicity Data for Compounds **1**, **2**, **2a**, and **3**−**6**

compound	cell lines EC_{50} (μg/mL)			
	KB	SK-MEL 30	A-549	MCF-7
1	0.10	0.11	0.11	0.11
2	13.9	16.4	21.4	15.8
2a	13.6	18.0	26.6	15.9
3	11.8	13.6	15.2	13.3
4	10.6	8.6	7.1	10.6
5	35.0	14.0	16.0	19.0
6	0.014	0.013	0.021	0.014

the C-3 α-hydroxy substituent in **1** and **6** is responsible for the increase of activity, compared to that of trichothecolone and trichothecin. A similar effect was observed in the brine shrimp assay, with LC_{50} values of 2.2 and 0.52 μg/mL for **5** and **6**, respectively.

6β-Hydroxyrosenonolactone (**8**) was evaluated for leishmanicidal activity against promastigotes of *L. donovani*, *L. major*, *L. infantum*, and *L. enrietii*, and amastigote forms of *L. donovani* residing within murine macrophages. The results showed only moderate and weak activity against extracellular and intracellular *L. donovani*, respectively, and low toxicity against macrophage host cells.

The source of these trichothecenes and diterpenoids in *H. floribunda* remains to be explained. It is well-known that nonmacrocyclic trichothecenes are secondary metabolites produced by molds, especially various species of *Fungi imperfecti* (*Fusarium*, *Stachybotrys*, *Trichothecium*, *Myrothecium*, *Cephalosporium*, etc).[28,29] We cannot exclude that the compounds isolated from the stem of *H. floribunda* result from fungal contamination, although inspection of the plant material did not reveal any obvious contamination. Furthermore, the compounds comprise ca. 0.03% of the dry plant material, a rather high percentage for such putative contamination. To test the presence of *Fusarium* in the plant, small pieces of stem were placed on modified Nash−Snyder medium, which is selective for this fungus.[30] There was little fungal growth from any of the pieces cultured, and no *Fusarium* was recovered. Another hypothesis is that these metabolites were produced by fungi in soil and then absorbed and translocated in the plant.

Experimental Section

General Experimental Procedures. Melting points were determined on a Reichert microscope. Optical rotations were recorded on a Perkin-Elmer 241-MC polarimeter. UV spectra were recorded in EtOH on a Milton Roy Spectronic 1201 spectrophotometer, and FTIR spectra were measured on a Perkin−Elmer 157G infrared spectrophotometer. EIMS spectra (70 eV) were obtained on a Shimadzu QP-1000EX and HREIMS/HRFABMS were obtained on a Finnigan MAT 711. ^1H (400 MHz) and ^{13}C (100.61 MHz) 1D and 2D NMR spectra were recorded on a Bruker ARX-400 spectrometer. Determination of absolute configuration by modified Horeau's method using HPLC[16] was carried out on a Spectra Physics 100 chromatograph, equipped with a UV detector and a Nucleosil-100 column (5 μm, 25 cm × 4.6 mm i.d.). The following Merck chromatographic supports were used: Si gel, 230−400 mesh, and LiChroprep RP_{18}, 40−63 μm, for flash and reversed-phase column chromatography, respectively; Si gel plates, 0.25 and 2 mm thick, for analytical and preparative HPTLC, respectively; HPTLC DIOL $F_{254}S$ plates, 0.2 mm thick; and RP_{18} $F_{254}S$ plates, 0.25 mm thick.

Plant Material. *Holarrhena floribunda* was collected in January 1994, at Contuboel, Guinea-Bissau, and identified at the Herbarium of Botany Centre (LISC), voucher specimen no. 866.

Extraction and Isolation. The air-dried stem of *H. floribunda* was ground and extracted with 95% EtOH using a Soxhlet apparatus. The resulting extract (33.3 g) was partitioned between hexane and EtOH−H_2O (9:1). The aqueous EtOH fraction residue (19.2 g) was treated with 2 N HCl and then extracted with $CHCl_3$. The acid aqueous layer was adjusted to pH 8 with 25% NH_4OH and extracted with $CHCl_3$, to yield an alkaloid fraction A (239 mg), as indicated by TLC analysis (EtOAc−hexane−Et_2NH, 75:24:6, Dragendorff spray). The organic layer was further washed with H_2O to yield a nonalkaloid fraction B (6.1 g) that showed strong toxicity in the brine shrimp assay and significant cytotoxicity against a panel of human tumor cell lines. The active fraction B was chromatographed on Sephadex LH-20, with MeOH−CH_2Cl_2 (60:40), and the eluted fractions were combined in two groups (I and II) according to their composition.

Fraction I was subjected to Si gel column chromatography using a $CHCl_3$−MeOH gradient system. The eluate with $CHCl_3$−MeOH (95:5 to 90:10) was further purified by repeated Si gel and RP_{18} CC using gradient systems of hexane−EtOAc and CH_2Cl_2−MeOH, to afford compounds **4** (41 mg), **2** (37 mg), and **3** (5 mg), whose purification was achieved by preparative TLC using CH_2Cl_2−EtOAc (80:20) as the mobile phase.

Fraction II was fractionated by RP_{18} cm^3 using a step gradient of CH_3CN (0−100%) in H_2O. The fraction eluted with H_2O−CH_3CN (4:6) was chromatographed on a Si gel column (CH_2Cl_2−EtOAc gradient), to afford compound **3** (32 mg), which was purified by RP_{18} preparative TLC (MeOH−H_2O, 7:3), and a mixture of **6** (4 mg) and **8** (7 mg), further separated by HPTLC DIOL (CH_2Cl_2−hexane, 7:3). The fraction eluted with H_2O−CH_3CN (3:7) was further submitted to Si gel column chromatography (hexane−EtOAc, 7:3), to afford 81 mg of **5** after preparative TLC purification (hexane−EtOAc, 7:3). The fraction eluted with H_2O−CH_3CN (2:8) was applied to a Si gel column, using a CH_2Cl_2−EtOAc gradient system, yielding compound **7** (176 mg). The fraction eluted with H_2O−CH_3CN (1:9 to 0:10) was submitted to successive Si gel and RP_{18} CC using gradient systems of hexane−EtOAc and CH_2Cl_2−MeOH, to afford 66 mg of **9**.

8-Dihydrotrichothecinol A (1): amorphous white solid (4 mg, 0.011% of extract); mp 119−120 °C, $[\alpha]_D$ −25° (*c* 0.35, CH_2Cl_2); UV (MeOH) λ_{max} (ε) 223 (2989) nm; IR ν_{max} (NaCl) 3429, 2920, 2850, 1716, 1645, 1442, 1374, 1227, 1170, 1076, 954 and 815 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive FABMS (*m*/*z*, %) 335 $[M + H]^+$ (22), 69 $[C_4H_5O]^+$ (100); negative FABMS (*m*/*z*, %) 333 $[M - H]^-$ (22), 265 $[C_{15}H_{21}O_4]^-$ (7), 85 $[C_{15}H_{21}O_4]^-$ (100); HREIMS *m*/*z* 334.17814 (calcd for $C_{19}H_{26}O_5$, 334.17803).

Loukacinol A (2): amorphous white solid (37 mg, 0.11% of extract); mp 118−120 °C, $[\alpha]_D$ +16.5° (*c* 1.1, CH_2Cl_2); UV (MeOH) λ_{max} (ε) 224 (3543) nm; IR ν_{max} (NaCl) 3270, 2927, 1682, 1448, 1379, 1282, 1103, and 1048 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 280.13105 (calcd for $C_{15}H_{20}O_5$, 280.13108); positive FABMS (*m*/*z*, %) 281 $[M + H]^+$ (54), 267 (15), 219 (25), 175 (21), 137 (46), 109 (83), 43 (100); negative FABMS (*m*/*z*, %) 279 $[M - H]^-$ (90), 231 (8), 153 (27), 136 (100).

13-Acetoxyloukacinol A (2a). Compound **2** (5 mg) was dissolved in a mixture of acetonitrile (2 mL) and triethylamine (1 mL), and acyl cyanide (0.17 mmol) was added. After standing at room temperature for 2.5 h, the reaction was worked up by addition of H_2O followed by extraction with CH_2Cl_2. After evaporation of the solvent, the residue was filtered through a small column of Si gel. Elution with CH_2Cl_2−EtOAc (9:1) gave the corresponding monoacetate **2a** (6 mg, 94%), $[\alpha]_D$ +6.5° (*c* 0.18, CH_2Cl_2); UV (MeOH) λ_{max} (ε) 223 (5390) nm; IR ν_{max} (NaCl) 3435, 2924, 2853, 1740, 1682, 1454, 1369, 1240, and 1057 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS(*m*/*z*, %) 322 $[M]^+$ (12), 249 (1), 221 (2), 207 (2), 201 (7), 191 (5), 175 (7), 165 (15), 149 (14), 137 (27), 121 (27), 43 (100).

Loukacinol B (3): amorphous white solid (5 mg, 0.014% of extract); mp 91−92 °C, $[\alpha]_D$ +19.9° (*c* 0.32, CH_2Cl_2); UV (MeOH) λ_{max} (ε) 229 (2696) nm; IR ν_{max} (NaCl) 3435, 2924, 1682, 1461, 1378, 1281, 1068, 1027, and 994 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 264.13613 (calcd for $C_{15}H_{20}O_4$, 264.13616).

13-Acetoxyloukacinol B (3a). Compound **3** (1.3 mg) was dissolved in a mixture of Ac_2O (0.4 mL), pyridine (0.4 mL),

and DMAP, and left overnight at room temperature. Solvents were removed under reduced pressure and the residue purified by column chromatography (CH_2Cl_2) to give **3a** (90%). ^{1}H NMR data, see Table 1; EIMS (*m/z*, %) 306 $[M]^+$(5), 291(2), 263 (1), 203 (8), 149 (9), 108 (33), 43 (100).

Trichothecolone (4): amorphous white solid (4 mg, 0.012% of extract); mp 145−150 °C, $[\alpha]_D$ +14.8° (*c* 1.0, CH_2Cl_2) {lit.[31] mp 183 °C, $[\alpha]_D$ +22.5° (*c* 1, $CHCl_3$)}; spectral data identical to those of an authentic sample of trichothecolone (Sigma).

Trichothecin (5): amorphous white solid (81 mg, 0.24% of extract); mp 114 °C, $[\alpha]_D$ +29.3° (*c*, 1.59, CH_2Cl_2) {lit.[32] mp 118 °C, $[\alpha]_D$ +44°}; spectral data identical to those of an authentic sample of trichothecin (Sigma).

Trichothecinol A (6): amorphous white solid (32 mg, 0.094% of extract); mp 84 °C, $[\alpha]_D$ +13.6° (*c* 0.25, CH_2Cl_2) {lit.[15] $[\alpha]_D$ +81.5° (*c* 0.7, MeOH)}; HREIMS *m/z* 348.15722 (calcd for $C_{19}H_{24}O_6$, 348.15729); positive FABMS (*m/z*, %) 371 $[M + Na]^+$ (4), 349 $[M + H]^+$ (8), 242 (3), 227 (3), 176 (12), 120 (19), 69 (100); IR, UV, ^{1}H and ^{13}C NMR spectra are in good agreement with reported data.[16]

Rosenonolactone (7): amorphous white solid (176 mg, 0.53% of extract); mp 198 °C, $[\alpha]_D$ −1.6° (*c* 1.9, CH_2Cl_2) {lit.[33] mp 214 °C, $[\alpha]_D$ −107.5° (*c* 1.2 $CHCl_3$)};^{1}H NMR data, see Table 4; UV, IR, and EIMS data identical to those reported in the literature.[13,24,33]

6*β*-Hydroxyrosenonolactone (8): amorphous white solid (7 mg, 0.02% of extract); mp 174 °C, $[\alpha]_D$ −3.5° (*c* 0.34, CH_2Cl_2) {lit.[24] mp 180 °C, $[\alpha]_D$ −162°)}; ^{1}H and ^{13}C NMR data, see Table 4; UV, IR, and EIMS data identical to those reported in the literature.[23]

Rosololactone (9): amorphous white solid (66 mg, 0.19% of extract); mp 185 °C, $[\alpha]_D$ −3° (*c* 1.4, CH_2Cl_2) {lit.[34] mp 186 °C $[\alpha]_D$ +6.3°)}; UV and IR data identical to those reported in the literature;[34] ^{1}H NMR data, see Table 4; HREIMS *m/z* 318.21963 (calcd for $C_{20}H_{30}O_3$, 318.21950).

Determination of Absolute Configuration of 1, 2, 4, 6, 8, and 9. To a solution of **1** (2.56 μmol) in pyridine (25.8 μL) was added (±)-2-phenylbutanoic anhydride (1.6 mg) in toluene (25.9 μL), followed by addition of (dimethylamino)pyridine (78.9 μg) in toluene (6.5 μL). The mixture was kept at room temperature for 1.5 h, and then the solution was treated with (*R*)-(+)-1-(1-naphthyl)ethylamine (2.6 μg) in toluene (77.6 μL), kept at room temperature for 30 min and poured into a 4.7 M solution of perchloric acid in acetonitrile (50 μL). After workup, the crude product was diluted in hexane−EtOAc 9:1 (30 μL) and analyzed by HPLC, using hexane−EtOAc 9:1 as eluent. Ratios of (1*R*,2′*S*)- and (1*R*,2′*R*)-*N*-[1-(1-naphthyl)ethyl]-2-phenylbutanamides are indicated in Table 3. Identical experimental procedures were followed for compounds **2**, **4**, **6**, **8**, and **9**.

Parasites. *L. donovani* LV9, *L. infantum* (strain D.SCH. isolated 1995 at the Bernhard Nocht-Institut, Hamburg, Germany, from a case of infant VL), *L. enrietii*, and *L. major* LV39 organisms were maintained by animal passage (except *L. infantum*) and cryopreserved in liquid nitrogen. Promastigotes were cultured in growth medium at 25 °C, 5% CO_2 in a humidified incubator. The cultures were passaged every 3−4 days. Culture media were prepared according described procedures.[35]

Bioassays. The brine shrimp (*Artemia salina*) lethality assay was used at all steps of the fractionation process as described in the literature.[7] Cytotoxicities to human solid-tumor cell lines were evaluated for squamous carcinoma (KB), melanoma (SK-Mel 28), lung carcinoma (A-549), and breast carcinoma (MCF-7), according to reported experimental procedures.[1] Assays for extracellular and intracellular leishmanicidal activities are described in detail elsewhere.[36] In parallel to the assay for intracellular leishmanicidal activity, the cytotoxicity toward host cells was carried out with noninfected C57BL/10ScSn mice bone marrow-derived macrophages (BMMΦ). The ED_{50} of compound **8** against intracellular and extracellular *L. donovani* and BMMΦ was 37.2, 10.3, and 19.5 μg/mL, respectively. No activity was found against *L. major*, *L. infantum*, or *L. enrietti* (ED_{50} > 50 μg/mL). Pentamidine−isethionate and amphothericin B were used as reference.

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Supporting Information Available: HMBC data of compounds **1**, **2**, **2a**, and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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