New Cytotoxic Bis 5-Alkylresorcinol Derivatives from the Leaves of *Oncostemon bojerianum* from the Madagascar Rainforest[1]

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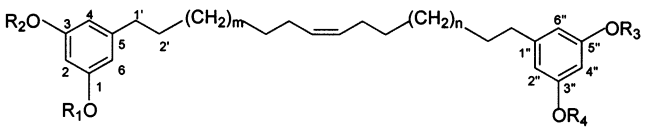
Bioassay-directed fractionation of a CH_2Cl_2−MeOH extract of the leaves of *Oncostemon bojerianum* resulted in the isolation of eight new 5-alkylresorcinols, named oncostemonols A−H (**1**−**8**), and two known derivatives, (8′*Z*)-1,3-dihydroxy-5-[16′-(3″,5″-dihydroxyphenyl)-8′-hexadecen-1′-yl]benzene (**9**) and (8′*Z*)-1,3-dihydroxy-5-[14′-(3″,5″-dihydroxyphenyl)-8′-tetradecen-1′-yl]benzene (**10**). The structures of the new compounds **1**−**8** were elucidated on the basis of extensive 1D and 2D NMR spectroscopic interpretation and chemical derivatization. All the compounds exhibited cytotoxic activity against the A2780 ovarian cancer cell line.

As a part of our continuing research on the isolation of bioactive compounds from the Suriname and Madagascar rainforests[2] as a part of the mission of the Suriname-Madagascar International Cooperative Biodiversity Group (ICBG), we obtained a sample of the CH_2Cl_2−MeOH extract of the leaves of *Oncostemon bojerianum* A. DC (Myrsinaceae) from a collection made in Madagascar. *Oncostemon* A. Juss. is a member of the Myrsinaceae, a family of about 33 genera and 850 mostly tropical species. One of the largest genera in the family, *Oncostemon*, is endemic to Madagascar, the Comores, and the Mascarenes. Although about 100 species have been described, the genus is tremendously complex and in serious need of taxonomic revision, as it has not been treated since the Flore de Madagascar,[3] and undoubtedly many species remain undescribed. The present species was identified as *O. bojerianum* A. DC by the collectors, but the name must be accepted with some reservation pending further taxonomic study.

The extract of *O. bojerianum* was selected for bioassay-guided fractionation on the basis of both its cytotoxicity, with an IC_{50} 15.7 μg/mL against the A2780 ovarian cancer cell line, and the absence of any reported phytochemistry of this genus. Many 5-alkylresorcinols have been reported from the Myrsinaceae and from other plant sources,[4−7] but none from *Oncostemon*. The crude extract after extensive chromatography followed by reversed-phase HPLC furnished the eight new bioactive 5-alkylresorcinols, oncostemonols A−H (**1**−**8**), in addition to the two known 5-alkylresorcinols **9** and **10**.



1 $R_1 = R_4 = Ac$; $R_2 = R_3 = H$; $m = n = 3$

2 $R_1 = Ac$; $R_2 = R_3 = R_4 = H$; $m = n = 3$

3 $R_1 = R_4 = H$; $R_2 = R_3 = Me$; $m = n = 3$

4 $R_2 = Me$; $R_1 = R_3 = R_4 = H$; $m = n = 3$

5 $R_2 = R_3 = R_4 = Me$; $R_1 = H$; $m = n = 3$

7 $R_1 = R_2 = R_3 = R_4 = H$; $m = n = 5$

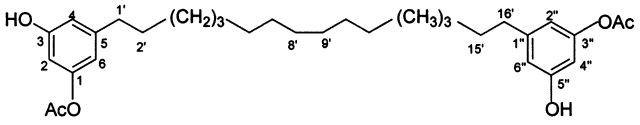
8 $R_1 = R_2 = R_3 = R_4 = H$; $m = 5$; $n = 3$

9 $R_1 = R_2 = R_3 = R_4 = H$; $m = n = 3$

10 $R_1 = R_2 = R_3 = R_4 = H$; $m = 3$; $n = 1$

11 $R_1 = R_2 = R_3 = R_4 = Ac$; $m = n = 3$

12 $R_1 = R_2 = R_3 = R_4 = Me$; $m = n = 3$



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Results and Discussion

Initial liquid−liquid partition of the crude extract indicated that the bioactivity was concentrated in the $CHCl_3$-soluble portion of a $CHCl_3$−aqueous MeOH partition. Chromatography of this fraction on a Sephadex LH-20 column followed by column chromatography over reversed-phase (LRP-2) silica gel and then finally by reversed-phase HPLC furnished the eight new bioactive 5-alkylresorcinols, oncostemonols A−H (**1**−**8**), in addition to the two known 5-alkylresorcinols **9** and **10**. The structures of the two known compounds were identified as (8′*Z*)-1,3-dihydroxy-5-[16′-(3″,5″-dihydroxyphenyl)-8′-hexadecen-1′-yl]benzene (**9**) and (8′*Z*)-1,3-dihydroxy-5-[14′-(3″,5″-dihydroxyphenyl)-8′-tetradecen-1′-yl]benzene (**10**), by comparison of their spectral data with values reported in the literature.[8]

Oncostemonol A (**1**) was isolated as a colorless viscous liquid whose molecular formula was established as $C_{32}H_{44}O_6$ from HRFABMS, ^{13}C NMR, and DEPT spectral data. Compound **1** gave a green coloration with methanolic $FeCl_3$, indicating the presence of a phenolic hydroxyl group, and this was supported by the observation of an absorption at 3450 cm^{-1} in its IR spectrum. The IR spectrum also showed the presence of a carbonyl group at 1735 cm^{-1}, and

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Table 1. ^{1}H NMR Data for Compounds **1**−**8** and **11**[a] (acetone-d_6, 500 MHz)

position	1	2	3	4	5	6	7	8	11
2	6.36 t 2.1	6.36 t 2.1	6.35 t 1.9	6.24 t 2.1	6.27 t 2.0	6.40 t 2.0	6.19 t 2.1	6.18 t 2.0	6.76 t 2.1
4	6.37 d 2.1	6.39 d 2.0	6.28 d 2.1	6.28 d 2.1	6.29 d 2.0	6.41 d 2.1	6.18 d 2.2	6.17 d 2.1	6.84 d 2.1
6	6.55 d 2.1	6.56 d 2.1	6.17 d 2.1	6.21 d 2.0	6.19 d 2.0	6.56 d 2.0	6.18 d 2.2	6.17 d 2.1	6.84 d 2.1
1′	2.49 t 7.5	2.48 t 7.6	2.52 t 7.6	2.49 t 7.5	2.51 t 7.6	2.54 t 7.5	2.42 t 7.5	2.42 t 7.5	2.60 t 7.5
2′	1.53 m	1.54 m	1.58 m	1.57 m	1.56 m	1.59 m	1.54 m	1.54 m	1.59 m
3′−5′	1.30 m	1.30 m	1.31 m	1.29 m	1.29 m	1.30 m	1.29 m	1.30 m	1.32 m
6′	1.53 m	1.54 m	1.58 m	1.57 m	1.56 m	1.30 m	1.29 m	1.30 m	1.59 m
7′	2.04 m	2.04 m	2.03 m	2.04 m	2.03 m	1.30 m	1.29 m	1.30 m	2.04 m
8′	5.34 t 4.6	5.33 t 4.5	5.33 t 4.5	5.34 t 4.5	5.33 t 4.6	1.30 m	1.54 m	1.54 m	5.34 t 4.6
9′	5.34 t 4.6	5.33 t 4.5	5.33 t 4.5	5.34 t 4.5	5.33 t 4.6	1.30 m	2.03 m	2.03 m	5.34 t 4.6
10′	2.04 m	2.04 m	2.03 m	2.04 m	2.03 m	1.30 m	5.34 t 4.6	5.33 t 4.5	2.04 m
11′	1.53 m	1.54 m	1.58 m	1.57 m	1.56 m	1.30 m	5.34 t 4.6	5.33 t 4.5	1.59 m
12′	1.30 m	1.30 m	1.31 m	1.29 m	1.29 m	1.30 m	2.03 m	2.03 m	1.32 m
13′	1.30 m	1.30 m	1.31 m	1.29 m	1.29 m	1.30 m	1.54 m	1.54 m	1.32 m
14′	1.30 m	1.30 m	1.31 m	1.29 m	1.29 m	1.30 m	1.29 m	1.30 m	1.32 m
15′	1.53 m	1.54 m	1.58 m	1.57 m	1.56 m	1.59 m	1.29 m	1.30 m	1.59 m
16′	2.49 t 7.5	2.48 t 7.6	2.52 t 7.6	2.49 t 7.5	2.51 t 7.5	2.54 t 7.5	1.29 m	1.30 m	2.60 t 7.5
17′							1.29 m	1.54 m	
18′							1.29 m	2.42 t 7.5	
19′							1.54 m		
20′							2.42 t 7.5		
2″	6.55 d 2.1	6.17 d 2.1	6.17 d 2.1	6.17 d 2.1	6.32 d 2.1	6.56 d 2.0	6.18 d 2.2	6.17 d 2.1	6.84 d 2.1
4″	6.36 t 2.1	6.18 t 2.2	6.35 t 1.9	6.15 t 2.2	6.26 t 2.2	6.40 t 2.0	6.19 t 2.1	6.18 t 2.0	6.76 t 2.1
6″	6.37 d 2.1	6.17 d 2.1	6.28 d 2.1	6.17 d 2.1	6.32 d 2.1	6.41 d 2.1	6.18 d 2.2	6.17 d 2.1	6.84 d 2.1
1,3″,3,5″-OH							8.01	8.02	
−OCOCH$_3$	2.19 s	2.18 s				2.19 s			2.18 s
−OCH$_3$			3.77 s	3.75 s	3.75 s				

[a] Assignments made on the basis of COSY and HMQC spectral data and comparison with the literature values.[8]

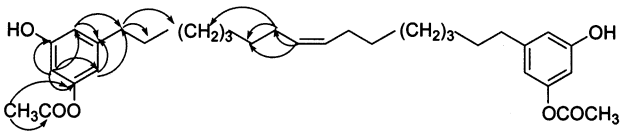

Figure 1. HMBC correlations for oncostemonol A (**1**).

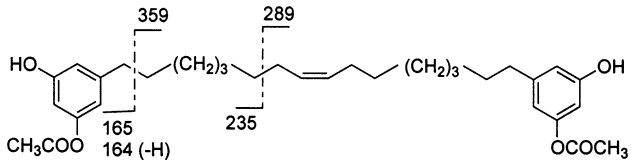

Figure 2. Key mass spectroscopic fragmentations for oncostemonol A (**1**).

this was confirmed as an acetoxyl group from its signals in the ^{1}H (δ 2.19, s) and ^{13}C (δ 168.7 and 20.2) NMR spectra, respectively. The fragment ions observed at *m*/*z* 464 and 404 in the EIMS indicated the successive loss of two acetic acid molecules from the molecular ion. The ^{1}H NMR spectrum contained two doublets at δ 6.37 (2H, J = 2.1 Hz) and 6.55 (2H, J = 2.1 Hz), three triplets at δ 6.36 (2H, J = 2.1 Hz), 2.49 (4H, J = 7.5 Hz), and 5.34 (2H, J = 4.6 Hz), two multiplets centered at δ 2.04 (4H) and 1.53 (8H), and a broad singlet at δ 1.30 (12H). This spectrum was almost identical to that of (8′*Z*)-1,3-dihydroxy-5-[16′-(3″,5″-dihydroxyphenyl)-8′-hexadecen-1′-yl]benzene (**9**),[8] except for differences in the chemical shift values of the protons in the aromatic region.

The absence of any terminal methyl group or carboxylic acid, coupled with the observation that some of the signals in the ^{13}C NMR spectrum of **1** integrated for more than one carbon, indicated that it was a symmetrical 5-alkylresorcinol. Since the mass spectrum of **1** had its molecular ion at *m*/*z* 524, which is 84 mass units higher than that of **9**, and since the singlet at δ 2.19 in its ^{1}H NMR spectrum integrated for six protons, the structure **1** was assigned as the diacetate derivative of **9**. The ^{1}H NMR signals of **1** at δ 6.36, 6.37, and 6.55, each corresponding to two protons, confirmed that the two aromatic rings in **1** are identical and, hence, that the two acetate groups are located at C-1 and C-3′. This conclusion is supported by the HMBC correlations shown in Figure 1.

The ^{13}C NMR values for all the carbons of **1** were assigned on the basis of HMQC and HMBC spectral data and were in good agreement with the assigned structure (Table 2). The mass fragments observed at *m*/*z* 359, 289, 235, and 165 in its EIMS (Figure 2) supported the structure further. The stereochemistry at the double bond between C-8′/C-9′ of **1** was assigned as *Z*, as in the case of **9**, on the basis of the close similarity of coupling constants and ^{13}C NMR values. Acetylation of **1** furnished the new tetraacetate (**11**), whose molecular formula was confirmed as $C_{36}H_{48}O_8$ by HRFABMS; the identical compound was also prepared by acetylation of **9**. Thus, the structure of oncostemonol A (**1**) was assigned as (8′*Z*)-1-acetoxy-3-hydroxy-5-[16′-(3″-acetoxy-5″-hydroxyphenyl)-8′-hexadecen-1′-yl]-benzene.

Oncostemonol B (**2**) was also obtained as a colorless liquid, and its molecular formula was assigned as $C_{30}H_{42}O_5$ by HRFABMS. From the molecular formula it was observed that compound **2** contained one acetate unit less than **1**. Its IR spectrum also showed the presence of hydroxyl (3425 cm^{-1}) and ester carbonyl (1745 cm^{-1}) groups, as for **1**. The ^{1}H NMR spectrum of **2** was almost identical to that of oncostemonol A (**1**), except for the presence of three new aromatic proton signals at δ 6.17 (2H, J = 2.1 Hz) and 6.18 (1H, J = 2.2 Hz) and a reduction of the integration of the three signals at δ 6.56, 6.36, and 6.39 from two protons to a single proton each. This indicated that the two aromatic rings in **2** were not identical. Similarly the singlet at δ 2.18 corresponded to three protons as compared with six in **1**, indicating the presence of only one acetate group in **2**. These data confirm the assignment of **2** as the monoacetate derivative of **9**; this was supported by an ion at *m*/*z* 422 formed by the loss of acetic acid from the molecular ion. A close comparison of the ^{1}H NMR values of **2** with those of **1** and **9** confirmed the structure completely. As final confirmation, acetylation of **2** with Ac_2O−pyridine furnished the tetraacetate **11**. As in the case of **1** and **9**, the

Table 2. ^{13}C NMR Data for Compounds **1**−**8** and **11**[a] (acetone-d_6, 125 MHz)

carbon	1	2	3	4	5	6	7	8	11
1	152.0	152.1	158.5	158.5	158.5	158.4	158.4	158.4	151.4
2	112.4[c]	112.5[c]	98.7	98.5	98.6	112.7	100.1	100.2	113.2
3	158.4	158.5	161.0	161.0	161.0	152.1	158.4	158.4	151.4
4	106.7	106.5	105.3	105.4	105.4	106.6	106.9	106.9	118.9
5	145.1	145.1	145.1	145.1	145.1	145.0	145.1	145.1	145.2
6	112.5[c]	112.7[c]	107.9	107.7	107.8	112.4	106.9	106.9	118.9
1′	35.6	35.6	35.9	36.0	36.1	35.7	35.8	35.8	35.3
2′	31.7	31.8	31.3	31.3	31.3	31.8	31.3	31.7	31.0
3′	29.0[d]	29.0[d]	28.5[c]	28.7[c]	28.c[b]	29.1[c]	28.7[c]	28.7[c]	28.9[c]
4′	29.2[d]	29.2[d]	28.7[c]	29.0[c]	29.0[c]	29.2[c]	28.9[c]	28.9[c]	29.0[c]
5′	29.4[d]	29.3[d]	29.1[c]	29.1[c]	29.2[c]	29.3[c]	29.1[c]	29.0[c]	29.1[c]
6′	29.5[d]	29.4[d]	29.4[c]	29.4[c]	29.3[c]	29.4[c]	29.2[c]	29.2[c]	29.2[c]
7′	26.9	27.0	26.9	26.9	27.0	29.6[c]	29.3[c]	29.4[c]	27.0
8′	129.9	129.8	129.8	129.8	129.8	29.6[c]	29.5[c]	29.5[c]	129.9[d]
9′	129.9	129.8	129.8	129.8	129.8	29.6[c]	27.0[d]	27.1[d]	129.7[d]
10′	26.9	27.0	26.9	26.9	27.0	29.6[c]	129.8[e]	129.8[e]	27.0
11′	29.0[d]	29.0[d]	28.5[c]	28.7[c]	28.c[b]	29.1[c]	129.7[e]	129.9[e]	28.9[c]
12′	29.2[d]	29.2[d]	28.7[c]	29.0[c]	29.0[c]	29.2[c]	26.9[d]	27.0[d]	29.0[c]
13′	29.4[d]	29.3[d]	29.1[c]	29.1[c]	29.2[c]	29.3[c]	29.5[c]	28.9[c]	29.1[c]
14′	29.5[d]	29.4[d]	29.4[c]	29.4[c]	29.3[c]	29.4[c]	29.2[c]	29.0[c]	29.2[c]
15′	31.6	31.8	31.2	31.3	31.3	31.8	29.1[c]	29.2[c]	27.0
16′	35.6	35.8	35.8	36.0	36.0	35.7	28.9[c]	29.4[c]	35.3
17′							28.7[c]	31.6	
18′							28.9[c]	35.8	
19′							31.2		
20′							35.8		
1″	145.1	145.1	145.1	145.1	145.1	145.0	145.1	145.1	145.2
2″	112.5	106.8	107.9	106.9	98.7	112.4	106.9	106.9	118.9
3″	152.0	158.5	158.5	158.5	161.0	158.4	158.4	158.4	151.4
4″	112.4	100.0	98.7	100.2	97.0	112.7	100.1	100.2	113.2
5″	158.4	158.5	161.0	158.5	161.0	152.1	158.4	158.4	151.4
6″	106.7	106.8	105.3	106.9	105.4	106.6	106.9	106.9	118.9
$OCOCH_3$	168.7	168.8				168.8			168.6
$OCOCH_3$	20.2	20.3				20.2			20.2
OCH_3			54.5	54.6	54.6				

[a] Assignments made on the basis of HMQC and HMBC and comparison with the literature data.[8] [b] Values having the same superscripts in the respective columns are interchangeable.

stereochemistry between the double bond at C-8′/C-9′ was assigned as *Z* on the basis of the almost identical coupling constants and ^{13}C NMR values of the signals for the C-8′/C-9′ protons and carbons in the three compounds. On the basis of the above spectral data, the structure of oncostemonol B (**2**) was established as 8(′*Z*)-1-acetoxy-3-hydroxy-5-[16′-(3″,5″-dihydroxyphenyl)-8′-hexadecen-1′-yl]benzene.

Oncostemonol C (**3**) was obtained as a colorless liquid and was determined to have the molecular formula $C_{30}H_{44}O_4$ by HRFABMS. Its IR spectrum showed the presence of a hydroxyl group (3430 cm^{-1}) and the absence of an ester carbonyl group. Its ^1H NMR spectrum was very similar to that of **1**, except for the absence of the acetate singlet at δ 2.19. Instead, the ^1H NMR spectrum showed the presence of a six-proton singlet at δ 3.77, indicating that the two acetate groups in **1** had been replaced by two methoxyl groups in **3**. This was supported by the presence of a carbon signal at δ 54.5 and by the observation of fragment ions at *m*/*z* 436 and 404 in its EIMS, formed by the successive loss of two molecules of methanol from the molecular ion. The ^{13}C NMR values for all the carbons were assigned on the basis of HMQC and HMBC spectra and were in good agreement with the structure (Table 2). Methylation of **3** with diazomethane furnished a product whose spectral data (^1H, ^{13}C NMR and MS) matched those of (8′*Z*)-1,3-dimethoxy-5-[16′-(3″,5″-methoxyphenyl)-8′-hexadecen-1′-yl]benzene (**12**),[8] confirming the structure. Oncostemonol C (**3**) was thus assigned as (8′*Z*)-1-hydroxy-3-methoxy-5-[16′-(3″-hydroxy-5″-methoxyphenyl)-8′-hexadecen-1′-yl]benzene.

The molecular formula of oncostemonol D (**4**) was determined to be $C_{29}H_{42}O_4$ by HRFABMS. The ^1H NMR spectrum of **4** was similar to that of oncostemonol C (**3**), except for minor differences in the chemical shifts of the protons in the aromatic region and the lack of a signal for one of the methoxy groups of **3**. Thus instead of the three proton signals at δ 6.17 (2H, J = 2.1 Hz), 6.28 (2H, J = 2.1 Hz), and 6.35 (2H, J = 1.9 Hz) of oncostemonol C (**3**), compound **4** showed three doublets at δ 6.17 (2H, J = 2.1 Hz), 6.21 (1H, J = 2.0 Hz), and 6.28 (1H, J = 2.1 Hz) and two triplets at δ 6.15 (1H, J = 2.2 Hz) and 6.24 (1H, J = 2.1 Hz), indicating that compound **4** is not a symmetrical structure like **3**. The molecular formula of **4** differed from that of **3** by one CH_2 group, confirming that it is a monomethyl ether derivative of **9**. This conclusion was supported by the observation of a fragment ion at *m*/*z* 422 in the EIMS, corresponding to the loss of only one molecule of methanol from the molecular ion. Methylation of **4** with CH_2N_2 yielded compound **12**, identical in all respects with that prepared from **3**. On the basis of the above spectral data, the structure of oncostemonol D (**4**) was established as (8′*Z*)-1-hydroxy-3-methoxy-5-[16′-(3″,5″-dihydroxyphenyl)-8′-hexadecen-1′-yl]benzene.

Oncostemonol E (**5**) was isolated as a colorless oil, with a molecular formula of $C_{31}H_{46}O_4$ (HRFABMS). A comparison of the ^1H NMR data of **5** with that of **3** and **4** indicated the similar nature of the three compounds, with the major difference being that the singlet at δ 3.75 corresponded to nine protons compared with three and six protons in oncostemonols D (**4**) and C (**3**), respectively. This indicated that compound **5** is the trimethyl ether of **9**, and this was supported by fragments at *m*/*z* 450, 418, and 386 observed in the EIMS, corresponding to the successive loss of three molecules of methanol from the molecular ion. Methylation

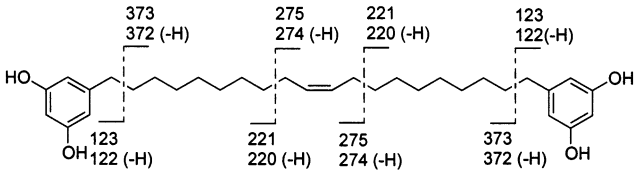

Figure 3. Key mass spectroscopic fragmentations for oncostemonol F (**7**).

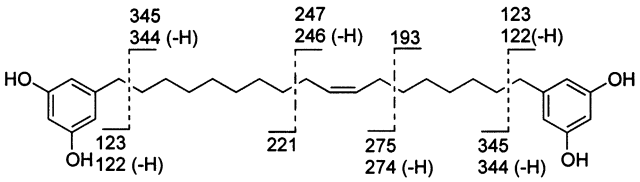

Figure 4. Key mass spectroscopic fragmentations for oncostemonol G (**8**).

Table 3. Cytotoxicities of Compounds **1**−**10** to A2780 Cells[a]

compound	IC_{50}, μg/mL
1	9.4, 10.2, 11.0
2	10.2, 11.2
3	9.8, 10.6, 11.4
4	10.0, 11.2
5	9.8, 10.6
6	9.5, 10.3
7	10.2, 11.4
8	9.4, 11.2
9	10.2, 11.0
10	9.8, 10.6, 11.2

[a] The concentration of compound that inhibited 50% (IC_{50}) of the growth of the A2780 mammalian cell line according to the procedure described.[1,10]

of **5** with diazomethane furnished a product identical to **12** in all spectral characteristics. The structure of oncostemonol E (**5**) was thus assigned as (8′*Z*)-1-hydroxy-3-methoxy-5-[16′-(3″,5″-dimethoxyphenyl)-8′-hexadecen-1′-yl]benzene.

The molecular formula of oncostemonol F (**6**) was deduced as $C_{32}H_{46}O_6$ by HRFABMS. The ^{1}H NMR data of **6** were almost identical to those of **1** (Table 1), except for the absence of signals for the double bond between C-8′ and C-9′. Thus the absence of a two-proton triplet at about δ 5.35, together with the observation of a broad singlet at δ 1.30 for 16 protons, indicated the saturated nature of the alkyl chain. This information, together with the mass spectrum which indicated a molecular weight two mass units greater than that of **1**, confirmed the structure of oncostemonol F as dihydro-oncostemonol A, or **6**. The ^{13}C NMR values of **6** were assigned on the basis of HMQC and HMBC correlations and were in good agreement with the structure. Thus, oncostemonol F (**6**) was assigned as 1-acetoxy-3-hydroxy-5-[16′-(3″-acetoxy-5″-hydroxyphenyl)-hexadecan-1′-yl]benzene.

Oncostemonol G (**7**) was isolated as a viscous liquid, and its molecular formula was established as $C_{32}H_{48}O_4$ by HRFABMS. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) were very similar to those of **9**, except for the integration of the broad singlet at δ 1.29, which corresponded to 20 protons compared with 12 protons in **9**. This suggested the presence of four additional methylene groups between the two aromatic rings of **9**. Previous studies indicated that the location of the double bond in similar compounds could be established on the basis of mass spectroscopic fragmentation patterns[6] as an alternative to ozonolysis[4] or oxidative degradation studies.[5] Since compound **7** was isolated in small quantity, ozonolysis or oxidative degradation experiments could not carried out, and hence the location of the double bond was determined on the basis of its mass spectroscopic fragmentation as shown in Figure 3. The fragments at *m*/*z* 373, 372, 275, 274, 221, 220, 123, and 122 observed in the EIMS (Figure 3) indicated the symmetrical nature of the molecule, confirming the presence of the double bond at C-10′/C-11′. The stereochemistry at the double bond between C-10′/C-11′ of **1** was assigned as *Z* on the basis of its almost identical coupling constants and ^{13}C NMR values with those of **9**. On the basis of these spectral data, the structure of oncostemonol G (**7**) was established as (10′*Z*)-1,3-dihydroxy-5-[20′-(3″,5″-dihydroxyphenyl)-10′-dodecen-1′-yl]benzene.

The molecular formula of oncostemonol H (**8**) was deduced as $C_{30}H_{44}O_4$ by HRFABMS. The ^{1}H NMR spectrum of **8** was almost identical to that of **7**, except that the signal at δ 1.30 corresponded to 16 protons, indicating that compound **8** had two fewer methylene units than **7**. The location of the double bond in **8** was also established as being between C-10′/C-11′ on the basis of its EI mass spectroscopic fragmentation pattern (Figure 4), with key fragment ions observed at *m*/*z* 345, 344, 275, 274, 247, 246, 221, 193, 123, and 122. Comparison with the spectra of **7** and **9** indicated a *Z* stereochemistry at C-10′/C-11′, and the structure of oncostemonol H (**8**) was thus assigned as (10′*Z*)-1,3-dihydroxy-5-[18′-(3″,5″-dihydroxyphenyl)-10′-octadecen-1′-yl]benzene.

The isolated compounds were tested for cytotoxicity against A2780 ovarian cancer cells. As shown in Table 3, all the isolated compounds (**1**−**10**) were found to be weakly cytotoxic, with IC_{50} values ranging between 9.4 and 11.4 μg/mL (Table 3). The fact that all the compounds isolated had similar activities suggests that the activity is mainly dependent on the basic skeleton of the 5-alkylresorcinols, rather than on any specific substituents thereon. It should also be noted that the compounds isolated represent only a fraction of the bioactivity of the starting extract. The bioactivity in this extract did however decline on storage, suggesting that the bioactive constituents may be unstable. This is consistent with the phenolic nature of the bioactive constituents, and it is likely that the compounds oxidized during the isolation process. It is also noteworthy that Hecht and his collaborators have found that 5-alkylresorcinols function as DNA-cleaving agents,[9] and it seems possible that these dialkylresorcinols act in the same way.

Experimental Section

General Experimental Procedures. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on a JEOL Eclipse 500 spectrometer. Mass spectra were obtained on a JEOL HX-110 instrument. The chemical shifts are given in δ (ppm) with TMS as internal reference and coupling constants in Hz. Sephadex LH-20 and reversed-phase Si gel (LRP-2, 200 μm) were used for column chromatography. Reversed-phase HPLC was performed on a Shimadzu LC-10AT instrument with an ODS C_{18} column.

Cytotoxicity Bioassays. The A2780 ovarian cancer cell line assay was carried out by the reported methods.[10]

Plant Material. The leaves of *Oncostemon bojerianum* A. DC. (Myrsinaceae) were collected by Stéphan Rakotonandrasana, S. Randrianasolo, C. Birkinshaw, and P. Antilahimena. The plants were collected in Madagascar in the province of Toamasina, near Antanandava, 2 km north of the village of Ankosy, just outside the Parc National de Zahamena in dense humid forest at 1000 m in elevation. Voucher specimens have been deposited at the herbaria in Antananarivo (TEF and CNRP), Paris (P), and the Missouri Botanical Garden, St. Louis (MO).

Extract Preparation. The plant samples were dried, ground, and extracted with CH_2Cl_2−MeOH (1:1) to give the dried extract MG 003.

Extraction and Isolation. The crude extract (0.75 g) was suspended in aqueous MeOH (MeOH−H_2O, 9:1, 200 mL) and extracted with *n*-hexane (3 × 200 mL). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with $CHCl_3$ (3 × 200 mL). The aqueous layer was concentrated, and the residue obtained was suspended in H_2O (25 mL) and extracted with *n*-BuOH (3 × 25 mL). The $CHCl_3$ extract was found to be the most cytotoxic and was fractionated over Sephadex LH-20 using *n*-hexane−EtOAc (100:0 to 0:100) and then MeOH−H_2O (100:0 to 40:60) to furnish eight fractions (A−H), of which fractions A−D were found to be the most active. Fraction A on column chromatography over LRP-2 Si gel using MeOH−H_2O (70:30) followed by reversed-phase HPLC with the mobile phase CH_3CN−H_2O (80:20) yielded the new 5-alkylresorcinol **6** (1.4 mg). Fraction B on reversed-phase preparative TLC (MeOH−H_2O, 70:30) followed by reversed-phase HPLC with the mobile phase CH_3CN−H_2O (70:30) yielded the new 5-alkylresorcinol **1** (1.8 mg). Fraction C on column chromatography over LRP-2 using MeOH−H_2O (80:20) followed by reversed-phase HPLC with mobile phase CH_3CN−H_2O (75:25) furnished the four new 5-alkylresorcinols **2** (1.7 mg), **3** (1.5 mg), **4** (1.2 mg), and **5** (1.1 mg). Fraction D on reversed-phase preparative TLC (MeOH−H_2O, 85:15) followed by reversed-phase HPLC with the mobile phase CH_3CN−H_2O (90:10) yielded the two new 5-alkylresorcinols **7** (0.9 mg) and **8** (0.7 mg) as well as the two known compounds **9** (4.2 mg) and **10** (2.3 mg). The two known compounds **9** and **10** were identified by comparison of their spectral data with literature values.[8]

Oncostemonol A [(8′*Z*)-1-acetoxy-3-hydroxy-5-[16′-(3″-acetoxy-5″-hydroxyphenyl)-8′-hexadecen-1′-yl]benzene] (1): colorless liquid; UV (MeOH) λ_{max} 276 nm (ϵ 14 600); IR ν_{max} 3450, 2960, 1735, 1645, 1615, 1453, 1128, 1065, 753 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* (rel int) 524 [M$^+$] (6), 507 (4), 464 (12), 404 (21), 359 (18), 289 (23), 235 (10), 205 (4), 165 (43), 164 (28), 123 (12), 61 (100); HRFABMS *m*/*z* 525.3213 [M + H]$^+$ (calcd for $C_{32}H_{45}O_6$, 525.3216).

Acetylation of Oncostemonol A (1). Oncostemonol A (**1**, 0.8 mg) was treated with Ac_2O−Py (1:1, 0.4 mL) at room temperature for 4 h. Usual workup gave product **11** (0.7 mg): ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* (rel int) 608 [M$^+$] (12), 548 (16), 488 (15), 291 (13), 277 (25), 207 (17), 205 (28), 165 (23), 123 (22), 61 (100); HRFABMS *m*/*z* 609.3439 [M + H]$^+$ (calcd for $C_{36}H_{49}O_8$, 609.3427).

Oncostemonol B [8(′*Z*)-1-acetoxy-3-hydroxy-5-[16′-(3″,5″-dihydroxyphenyl)-8′-hexadecen-1′-yl]benzene] (2): colorless liquid; UV (MeOH) λ_{max} 272 nm (ϵ 13 980); IR ν_{max} 3425, 2960, 1745, 1655, 1625, 1450, 1135, 1060, 750 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* (rel int) 482 [M$^+$] (8), 465 (12), 422 (15), 359 (24), 289 (26), 235 (21), 205 (6), 165 (48), 123 (17), 61 (100); HRFABMS *m*/*z* 483.3097 [M + H]$^+$ (calcd for $C_{30}H_{43}O_5$, 483.3110).

Acetylation of Oncostemonol B (2). Acetylation of oncostemonol B (**2**, 0.6 mg) as previously described and usual workup gave a product (0.5 mg) that was identical (TLC and ^{1}H NMR) with **11**.

Oncostemonol C [(8′*Z*)-1-hydroxy-3-methoxy-5-[16′-(3″-hydroxy-5″-methoxyphenyl)-8′-hexadecen-1′-yl]benzene] (3): colorless liquid; UV (MeOH) λ_{max} 268 nm (ϵ 14 100); IR ν_{max} 3430, 2965, 1642, 1450, 1120, 1065, 751 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* (rel int) 468 [M$^+$] (12), 450 (4), 437 (3), 436(21), 404 (17), 331 (46), 221 (21), 207 (16), 193 (16), 149 (36), 137 (100); HRFABMS *m*/*z* 469.3315 [M + H]$^+$ (calcd for $C_{30}H_{45}O_4$, 469.3318).

Methylation of Oncostemonol C (3). Oncostemonol C (**3**, 0.8 mg) was treated with excess ethereal CH_2N_2. Evaporation and purification by preparative TLC gave a product (0.6 mg) that was identical (^{1}H, ^{13}C NMR and MS) with compound **12**.

Oncostemonol D [(8′*Z*)-1-hydroxy-3-methoxy-5-[16′-(3″,5″-dihydroxyphenyl)-8′-hexadecen-1′-yl]benzene] (4): colorless liquid; UV (MeOH) λ_{max} 281 nm (ϵ 11 650); IR ν_{max} 3440, 2950, 1645, 1615, 1445, 1121, 1054, 745 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* (rel int) 454 [M$^+$] (9), 437 (4), 422 (11), 405 (13), 331 (38), 221 (18), 207 (14), 193 (11), 149 (23), 137 (100), 123 (72); HRFABMS *m*/*z* 455.3156 [M + H]$^+$ (calcd for $C_{29}H_{43}O_4$, 455.3161).

Methylation of Oncostemonol D (4). Methylation of oncostemonol D (**4**, 0.8 mg) as previously described gave a product (0.6 mg) that was identical (^{1}H NMR and MS) with **12**.

Oncostemonol E (5): colorless liquid; UV (MeOH) λ_{max} 279 nm (ϵ 13 600); IR ν_{max} 3450, 2953, 1635, 1451, 1116, 1050, 742 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* (rel int) 482 [M$^+$] (16), 465 (7), 450 (15), 418 (24), 386 (17), 331 (27), 221 (14), 151 (72), 136 (41), 123 (100); HRFABMS *m*/*z* 483.3462 [M + H]$^+$ (calcd for $C_{31}H_{47}O_4$, 483.3475).

Methylation of Oncostemonol E (5). Methylation of oncostemonol C (**5**, 0.8 mg) as described previously gave a product (0.6 mg) that was identical (^{1}H NMR and MS) with **12**.

Oncostemonol F (6): colorless liquid; UV (MeOH) λ_{max} 275 nm (ϵ 13 250); IR ν_{max} 3450, 2960, 1738, 1640, 1615, 1445, 1125, 1050, 1015, 750 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* (rel int) 526 [M$^+$] (5), 508 (6), 466 (17), 403 (48), 402 (28), 297 (6), 250 (21), 235 (13), 221 (17), 193 (14), 135 (32), 123 (35), 59 (100); HRFABMS *m*/*z* 526.3301 M$^+$ (calcd for $C_{32}H_{46}O_6$, 526.3293).

Oncostemonol G (7): colorless liquid; UV (MeOH) λ_{max} 291 nm (ϵ 13 900); IR ν_{max} 3390, 2945, 1640, 1450, 1125, 1055, 742 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* (rel int) 496 [M$^+$] (15), 479 (4), 461 (15), 373 (18), 372 (16), 289 (26), 288 (42), 275 (28), 274 (18), 235 (21), 221 (15), 220 (21), 207 (31), 206 (24), 137 (14), 123 (46), 122 (100); HRFABMS *m*/*z* 497.3618 [M + H]$^+$ (calcd for $C_{32}H_{49}O_4$, 497.3631).

Oncostemonol H (8): colorless liquid; UV (MeOH) λ_{max} 286 nm (ϵ 14 300); IR ν_{max} 3410, 2950, 1635, 1435, 1105, 1055, 750 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* (rel int) 468 [M$^+$] (11), 450 (6), 435 (10), 420 (8), 359 (15), 358 (23), 345 (34), 344 (17), 275 (19), 274 (21), 247 (18), 246 (15), 235 (17), 221 (28), 207 (19), 193 (14), 137 (23), 123 (72), 122 (100); HRFABMS *m*/*z* 469.3321 [M + H]$^+$ (calcd for $C_{30}H_{45}O_4$, 469.3318).

Acetylation of 9. Acetylation of **9** (1.5 mg) as described above gave a product (1.2 mg) that was identical (TLC and ^{1}H NMR) with **11**.

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