Three New Ellagic Acid Derivatives from the Bark of *Eschweilera coriacea* from the Suriname Rainforest[1]

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Received February 9, 1998

Bioassay-guided fractionation of *Eschweilera coriacea* collected in the lowland wet forest of Suriname yielded the new but only weakly active ellagic acid derivative eschweilenol A (**1**) and the two new but inactive ellagic acid derivatives eschweilenol B (**2**) and eschweilenol C (**3**). The four known compounds, sucrose, ellagic acid, 3-*O*-galloylepigallocatechin, and epigallocatechin, were also isolated. The structures of the three new compounds were determined by spectrometric methods, primarily from the HMQC, HMBC, NOESY, and ROESY NMR techniques, and chemical methods, including methylation and triethylsilylation. The location of a hydroxyl group in one ellagic acid derivative was determined by a new technique involving an NOE correlation of the protons of a triethylsilyl derivative with a proton on a neighboring aromatic ring.

In continuation of our search for anticancer and other bioactive agents from the Suriname forests,[2,3] an extract of *Eschweilera coriacea* (DC.) S. A. Mori (Lecythidaceae) collected near Asindopo village in the rainforest of Suriname was found to show weak but reproducible activity in a mechanism-based bioassay utilizing genetically engineered mutants of the yeast *Saccharomyces cerevisiae*.[4] The genus *Eschweilera* consists of 83 species distributed from Mexico through South America,[5] but no previous reports on the chemical constituents of any member of this genus have appeared in the literature. *E. coriacea* was thus selected for investigation, and a large extract was obtained for detailed study.

Bioassay-guided fractionation led to the discovery of the new weakly bioactive compound eschweilenol A (**1**), (IC_{12} 1000 μg/mL in the Sc-7 yeast strain[2,6]), along with two new inactive ellagic acid derivatives, eschweilenol B (**2**) and eschweilenol C (**3**), and four known compounds, sucrose, ellagic acid, 3-*O*-galloylepigallocatechin, and epigallocatechin.

Ellagic acid is a well-known compound with a rigid structure and poor solubility.[7] It is widely distributed in higher plants and has been found in 75 genera of dicotyledons but in only one monocotyledonous species, *Hypoxis filiformis*.[8] Several ellagic acid glycosides have been isolated,[7a] but ellagic acid conjugated with polyphenols has rarely been observed.[9,10]

To assist the structure elucidation of these highly oxygenated compounds, their methyl ethers **4**, **5**, and **6**, were prepared. Among those new compounds, the structure of eschweilenol B (**2**) was difficult to determine due to the existence of two possible regioisomers for the pentaoxygenated phenyl group conjugated to ellagic acid through a 1,4-dioxin linkage. A new method, using triethylsilyl (TES) derivatization combined with a ROESY experiment and molecular modeling, was developed to identify the isomeric structure of this type of compound.

Results and Discussion

The plant extract was distributed between *n*-hexane and aqueous MeOH (60% MeOH). The active aqueous MeOH fraction was further partitioned between 50% aqueous MeOH and CH_2Cl_2. The 50% aqueous MeOH fraction was partitioned with EtOAc. Among these fractions, only the aqueous MeOH fraction showed activity (IC_{12} 4000 μg/mL). After removing the aqueous MeOH solvent, the residue was passed through LH-20 using EtOH as eluent, and final purification was achieved by preparative TLC.

Compound **1** was found to have the molecular formula $C_{20}H_{10}O_{11}$ from HRFABMS. Only two singlets (δ 7.61 and 7.45) and two doublets (δ 6.31 and 6.61) were observed in its ^{1}H NMR spectrum. In its ^{13}C NMR spectrum 20 carbon signals were observed, including those for the two 7-carbon units of an ellagic acid skeleton and those for another highly oxygenated phenyl group. The existence of the ellagic acid moiety was confirmed by its HMBC correlations, in which the patterns of H−C correlations were similar to those of compound **3** (i.e., H-5 (δ 7.61) to C-3, C-4, C-6, and C-7; H-5′ (δ 7.45) to C-1′, C-3′, C-4′, C-6′, and C-7′, see Table 3). The proton and carbon signals of the ellagic acid skeleton were readily assigned from HMBC data (see Tables 1 and 2). The remaining signals indicated the existence of an extra phenyl ring containing four oxygen-bearing carbons, and this must be conjugated through one or two ether linkages to the ellagic acid skeleton. The number of free hydroxy groups was established as six by exact mass measurement and methylation of **1**, which suggested that only one ether linkage existed.

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S0163-3864(98)00046-9 CCC: $15.00
Published on Web 06/13/1998

Table 1. ^{1}H-NMR Data for Compounds **1**−**6**

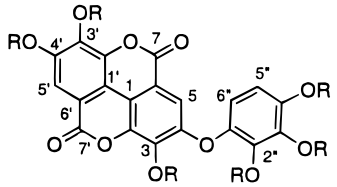
	compound					
^{1}H	**1**[a]	**2**[b]	**3**[b]	4[c]	**5**[a]	**6**[b]
5	7.61, s	7.48, s	7.72, s	7.38, s	7.60, s	7.77, s
5′	7.45, s	7.45, s	7.46, s	7.64, s	7.60, s	7.60, s
1″			5.44, br s			5.56, br s
2″			3.98, br s			3.94, m
3″			3.82, br d, J = 9.4			3.69, m
4″			3.27, dd, J = 9.4, 9.4			3.34, m
5″	6.31, d, J = 8.8		3.50, m	6.62, d, J = 8.8		3.48, m
6″	6.61, d, J = 8.8	6.14, s	1.12, d, J = 5.8	6.77, d, J = 8.8	6.39, s	1.12, d, J = 6.1
2″-OH						5.21, d, J = 4.3
3″-OH						4.86, d, J = 5.4
4″-OH						4.97, d, J = 6.3
3-OMe				4.23, s		4.06, s
3′-OMe				4.11, s	4.11, s	4.03, s
4′-OMe				3.96, s	3.93, s	3.99, s
2″-OMe				3.74, s		
3″-OMe				3.83, s	3.89, s	
4″-OMe				3.82, s	3.74, s	
5″-OMe					3.72, s	

[a] In MeOD. [b] DMSO-d_6. [c] In $CDCl_3$−MeOD (10:1).

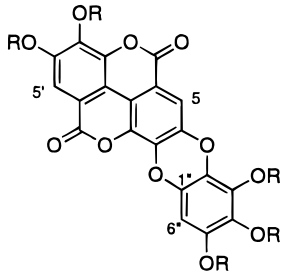
Table 2. ^{13}C-NMR Data for Compounds **1**−**4** and **6**

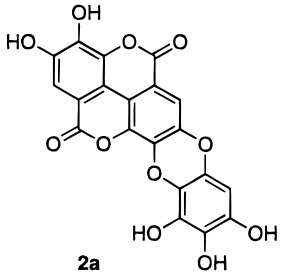
	compound				
^{13}C	**1**[a]	**2**[b]	**3**[b]	**4**[c]	**6**[b]
1	101.9, s	107.9, s	108.4, s	112.3, s	112.7, s
2	139.6, s	135.8, s	136.8, s	140.1, s	141.3, s
3	153.1, s	139.8, s	141.0, s	142.2, s	141.8, s
4	154.6, s	149.0, s	146.4, s	153.4, s	150.6, s
5	115.4, d	110.6, d	111.7, d	112.2, d	111.7, d
6	117.5, s	111.9, s	114.6, s	114.1, s	113.6, s
7	163.0, s	158.2, s	159.3, s	158.5, s	158.2, s
1′	107.6, s	111.3, s	107.8, s	111.2, s	112.4, s
2′	138.3, s	134.3, s	136.5, s	140.1, s	141.1, s
3′	146.7, s	135.2, s	139.5, s	141.7, s	140.9, s
4′	150.6, s	142.8, s	148.6, s	154.5, s	154.3, s
5′	110.3, d	110.9, d	110.5, d	107.8, d	107.6, d
6′	114.9, s	115.4, s	112.0, s	113.3, s	112.5, s
7′	163.0, s	158.3, s	159.1, s	159.0, s	158.2, s
1″	144.1, s	141.8, s	100.3, d	141.0, s	99.8, d
2″	140.3, s	132.3, s	69.9, d	145.6, s	70.0, d
3″	135.9, s	131.3, s	70.1, d	143.4, s	70.3, d
4″	140.1, s	123.0, s	71.8, d	151.4, s	71.5, d
5″	106.7, d	136.6, s	70.1, d	106.9, d	70.4, d
6″	112.9, d	94.1, d	18.0, q	116.1, d	17.9, q
3-OMe				61.9, q	61.6, q
3′-OMe				61.8, q	61.3, q
4′-OMe				56.6, q	56.8, q
2″-OMe				61.0, q	
3″-OMe				61.1, q	
4″-OMe				56.0, q	

[a] In MeOD. [b] DMSO-d_6. [c] In $CDCl_3$−MeOD (10:1).



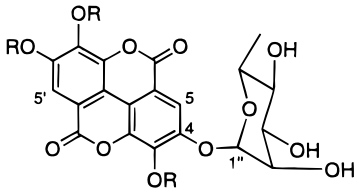
1 R = H
4 R = CH_3





2 R = H
2b R = C_6F_5CO
5 R = CH_3
7 R = TES

2a



3 R = H
6 R = CH_3

Coupling (J = 8.8) between H-5″ (δ 6.31) and H-6″ (δ 6.61) indicated that these two methine protons were adjacent. In the HMBC spectrum, the following two-bond and three-bond H−C correlations supported the tetraoxygenated phenyl ring moiety: H-5″ (δ 6.31) had correlations to C-1″ (δ 144.1) and C-3″ (δ 135.9), and H-6″ (δ 6.61) had correlations to C-2″ (δ 140.3) and C-4″ (δ 140.1). The position of the ether linkage could not be determined through HMBC, but it was established by the observation of an important positive NOE effect between H-5 (δ 7.61) and H-6″ (δ 6.61). This signal not only identified the location of the three contiguous hydroxy groups but also established the location of the ether linkage at the C-4 position.

Eschweilenol A thus has the structure **1**. This assignment was supported by the preparation of the methylation product **4**. In compound **4** six *O*-methyl groups (δ 4.23, 4.11, 3.96, 3.74, 3.83, and 3.82) were introduced into **1**, and these were observed in its ^{1}H and ^{13}C NMR spectra (see Tables 1 and 2). The observation of an NOE effect between H-5′ and 4′-OMe and between H-5″ and 4″-OMe supported the corresponding OH and ether linkage positions. No NOE effect was observed for H-6″ to any methyl group, providing negative evidence that H-6″ is next to the ether linkage. All of the ^{1}H and most of the ^{13}C chemical shifts were assigned unambiguously from the correlations obtained from NOESY, HMBC, and HMQC, except that a few quater-

Table 3. HMBC Data for Compounds **1**−**6**

	compound					
^{1}H	**1**	**2**	**3**	**4**[a]	**5**[a]	**6**[a]
5	C-1, C-3, C-4, C-6, C-7	C-1, C-3, C-4, C-6, C-7	C-1, C-3, C-4, C-6, C-7	C-3, C-4, C-6 (or C-1), C-7	C-3, C-4, C-6 (or C-1), C-7	C-3, C-4, C-6 (or C-1), C-7
5′	C-1′, C-3′, C-4′, C-6′, C-7′	C-1′, C-3′, C-4′, C-6′, C-7′	C-1′, C-3′, C-4′, C-6′, C-7′	C-3′, C-4′, C-6′ (or C-1′), C-7′	C-3′, C-4′, C-6 (or C-1′), C-7′	C-3′, C-4′, C-6′ (or C-1′), C-7′
1″			C-4, C-3″			C-4, C-3″
5″	C-1″, C-3 ″			C-1″, C-3″, C-4″		
6″	C-2″, C-4″	C-1″, C-2″, C-4″, C-5″	C-4″, C-5″	C-1″, C-2″, C-4″	C-1″, C-2″, C-4″, C-5″	C-4″, C-5″
3-OMe				C-3		C-3
3′-OMe				C-3′	C-3′	C-3′
4′-OMe				C-4′	C-4′	C-4′
2″-OMe				C-2″		
3″-OMe				C-3″	C-3″	
4″-OMe				C-4″	C-4″	
5″-OMe					C-5″	

[a] The chemical shifts of C-1 and C-6 and of C-1′ and C-6′ could not be distinguished by HMBC.

nary carbons (C-1, C-6, C-1′, C-6′) could not be assigned unambiguously.

Compound **2**, named eschweilenol B, was found to have the molecular composition $C_{20}H_8O_{11}$ from HR-FABMS. In the ^{1}H NMR spectrum, only three singlets (δ 7.48, 7.45, and 6.14) were observed. The ^{13}C NMR spectrum showed a total of 20 carbon signals, including signals for two 7-carbon units (ellagic acid skeleton) and a pentaoxygenated phenyl group, indicating that the skeleton of **2** is similar to that of **1**. The existence of the ellagic acid moiety was confirmed by HMBC correlations, in which the correlation patterns were similar to those of compounds **1** and **3** (see Table 3). The proton and carbon signals of the ellagic acid skeleton were assigned readily from HMBC data (see Tables 1 and 2). The remaining signals were for a pentaoxygenated phenyl ring, with ^{13}C-resonances observed at δ 141.8, 132.3, 131.3, 123.0, 136.6, and 94.1. The number of free hydroxy groups was confirmed by exact mass measurement and by methylation to the pentamethyl ether **5**. This confirmed the presence of five free hydroxy groups, which in turn indicated the existence of two ether linkages. The spectral evidence from ^{1}H−^{13}C correlations supporting this partial structure is described below.

In the HMBC spectrum of **2**, H-6″ (δ 6.14) had correlations to δ 141.8, 132.3, 123.0, and 136.6 (C-1″, C-2″, C-4″, and C-5″). The spectral data of the methylation product **5** revealed that it had three methoxy groups (3″−OMe at δ 3.89, 4″−OMe at δ 3.74, and 5″−OMe at δ 3.72) on the additional phenyl ring. This indicated that the other two oxygen atoms (1″−O and 2″−O) must be connected to C-3 and C-4 of the ellagic acid skeleton through ether linkages. The ^{1}H and ^{13}C chemical shifts of the five methyl groups of **5** were assigned by HMBC and NOESY (Tables 3 and 4, respectively). An NOE correlation of H-6″ (δ 6.39) to 5″−OMe (δ 3.72) of compound **5** indicated that H-6″ is between one ether linkage and one methoxy group, but NOESY and ROESY experiments on compounds **2** and **5** failed to show any correlations between H-5 and H-6″ or 3″−OMe. This evidence thus does not distinguish between the two possible structures **2** and **2a** for this novel ellagic acid derivative.

Structure **2** is the more likely structure based on the presumed biotransformation of compound **1** through oxidative cyclization between its 3-OH and H-6″ of **1**, but this cannot be assumed to be the correct structure. Two strategies were considered to solve the problem of the structural assignment of **2**. The first strategy was to link a bulky ester group containing a phenyl function to 3″−OH, in the expectation that it would cause a shielding effect on H-5 by a through-space interaction. This strategy had the disadvantage that the electronic effect of the ester substituent would also cause a downfield shift on the aromatic protons, and thus the two effects would need to be distinguished in some way. No suitable model was found in the literature to enable us to eliminate the electronic effect on this particular molecule. From molecular modeling distance calculations of our ester-substituted candidate using the MacSpartan program on the penta-*O*-(pentafluorobenzoate) **2b**, the pentafluorobenzoyl group (selected because it does not have aromatic protons that might interfere with the observation of the desired effect) did not have acceptable conformations to bring the aromatic ring close enough to H-5 to cause the desired shielding effect. After these considerations, this strategy was dropped.

The second strategy was to link a bulky alkyl group to the hydroxyl group at C-3″ through an ether linkage. The advantages of this approach are that the proton signals of the substituted alkyl group will not overlap with the signal for H-5, and that a suitable alkyl group would be expected to have several accessible conformations capable of NOE interactions with H-5. This is so because the bulky groups on C-3″, C-4″, and C-5″ would repel each other and force the alkyl group on C-3″ to

Table 4. NOESY (or ROESY) Data for Compounds **1** and **3**−**6**[a]

	compound				
^{1}H	**1**	**3**	**4**	**5**	**6**
5	H-6″	H-1″			H-1″
5′			4′-OMe	4′-OMe	4′-OMe
1″		H-5, H-2″			H-5, H-2″
2″		H-1″			H-1″, 2″-OH
4″		CH_3-6″			CH_3-6″
5″	H-6″	CH_3-6″	H-6″, 4″-OMe		CH_3-6″
6″	H-5, H-5″	H-5″, H-4″	H-5″	5″-OMe	H-5″, H-4″
4′-OMe			H-5′	H-5′	H-5′
4″-OMe			H-5″		
5″-OMe				H-6″	

[a] No NOE was observed in NOESY and ROESY spectra of compound **2**.

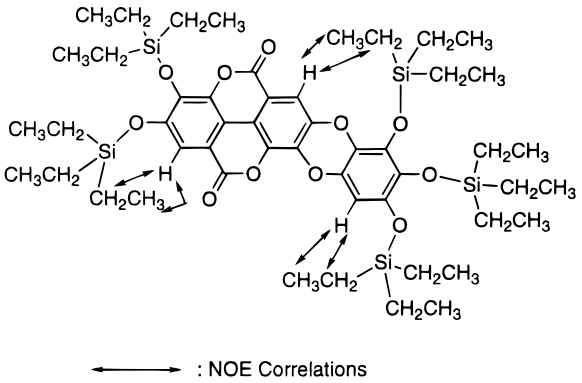


Figure 1. NOE correlations of **1** from a ROESY experiment.

approach H-5 (assuming that structure **2** is the correct structure). The TES ether was the best choice due to its steric bulk and its ease of introduction into the molecule. From an energy minimization (MacSpartan) of the proposed penta-*O*-(TES)ether derivative **7** of compound **2**, the distance between H-5 and the ethyl protons fell in the range of 2.3 to 2.8 Å in several accessible conformations, well within the range of the NOE effect.

The penta-*O*-(TES)ether derivative **7** was synthesized from compound **2** with excess TES chloride in the usual way. The ROESY spectrum of compound **7** showed NOE correlations of both the H-5 and H-5′ signals (δ 7.60 and 7.56) to signals of two ethyl groups (δ 0.99 and 0.84; δ 0.98 and 0.79, respectively) (Figure 1) on the TES group. This finding demonstrated that the ether linkage was as in structure **2** instead of structure **2a**. Molecular modeling (MacSpartan) of **2a** showed that the distance of H-5 to the ethyl group of the C-4″ TES group is always more than 5 Å, which is too great to cause an NOE effect. Thus, the structure of eschweilenol B was determined unambiguously as **2**.

Eschweilenol C (**3**), a poorly soluble compound in most common solvents except DMSO, was determined to have the composition $C_{20}H_{16}O_{12}$ from HRFABMS. In its ^{1}H NMR spectrum, only two singlets (δ 7.72 and 7.46) in the aromatic region and five oxygen-bearing methine protons were observed, along with a doublet methyl signal (δ 1.12) indicating the existence of one 6-deoxysugar. The glycoside was easily determined as rhamnose through analysis of the chemical shifts and coupling patterns of its proton signals (Table 1), which were connected by a ^{1}H−^{1}H COSY spectrum. In addition, the ^{13}C chemical shifts of the glycoside, δ 100.3, 69.9, 70.1, 71.8, 70.1, 18.0 (C-1″ to C-6″) (Table 2), resembled the corresponding signals of 1-substituted rhamnose glycosides.[11] Although the aglycon bears only two singlets (δ 7.72 and 7.46) in the aromatic region, there are 14 carbon signals in the lowfield region of its ^{13}C NMR spectrum, including two isolated olefin carbons (=CH−), two ester carbons (−COOR), six oxygen-bearing aromatic carbons, and four aromatic quaternary carbons (=C<). This information indicated that the main skeleton consisted of two symmetric, highly oxygenated, 7-carbon units that were conjugated to each other, again indicating an ellagic acid skeleton. After comparison of compound **3** to known ellagic acid glycosides,[12] the skeleton was determined as a monorhamnose-substituted ellagic acid glycoside. This proposed assignment was supported by the following HMBC and HMQC evidence. From the HMQC spectrum, aromatic carbons (δ 111.7 and 110.5) bearing singlet methine protons (δ 7.72 and 7.46, respectively) were correlated (C-5 and C-5′, respectively). In the HMBC spectrum, both vinyl proton signals (H-5 and H-5′) had similar patterns for their long-range correlations, which were to carbons with signals at δ 108.4 (C-1), 141.0 (C-3), 146.4 (C-4), 114.6 (C-6), 159.3 (C-7) and 107.8 (C-1′), 139.5 (C-3′), 148.6 (C-4′), 112.0 (C-6′), and 159.1 (C-7′), respectively. The location of the rhamnose substituent was confirmed by the observation of an NOE effect between H-5 (δ 7.72) and H-1″ (δ 5.44) in the NOESY spectrum, and the anomeric configuration was confirmed as α by the small coupling constant of the anomeric proton.

The methylation product of **3** was prepared by treatment with CH_2N_2. The resulting product had the structure **6**. All of the proton and most of the carbon chemical shifts were assigned from ^{1}H−^{1}H COSY, HMBC, HMQC, and NOESY spectra, except for a few quaternary carbons. The observation of an NOE between H-5 (δ 7.77) and H-1″ (δ 5.56) again proved the location of the rhamnose substituent.

In addition to the new compounds **1**−**3**, four known compounds (sucrose, ellagic acid, 3-*O*-galloylepigallocatechin, and epigallocatechin) were also isolated. Their stuctures were determined by comparison of their spectroscopic data with literature data.

In conclusion, the previously unstudied plant *E. coriacea* has yielded several flavonol and ellagic acid types of compounds, including the three new ellagic acid derivatives **1**−**3**. Two of them, **1** and **2**, have the unusual structure of a highly oxygenated phenyl ring conjugated to an ellagic acid skeleton. We developed a new method, a combination of triethylsilylation with a ROESY experiment and molecular modeling, to determine the regioisomer of a compound bearing a 1,4-dioxin linkage. Regrettably, none of the constituents showed good activity in our engineered yeast assay, but compounds **1** and **3** were weakly active against the Sc-7 yeast strain, with IC_{12} values of 1000 and 2000 μg/mL.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in various deuterated solvents ($CDCl_3$, DMSO-d_6, and CD_3OD) on a Varian Unity 400 NMR instrument at 399.951 MHz for ^{1}H and 100.578 MHz for ^{13}C, using standard Varian pulse sequence programs. LRMS were obtained on a VG 7070E-HF at Virginia Ppolytechnic Institute and State University or at the Nebraska Center for Mass Spectrometry. Exact mass measurements were obtained at the Nebraska Center for Mass Spectrometry. Other conditions were as previously described.[2]

Plant Material. The bark of *E. coriacea* was collected near Asindopo village, Suriname. Voucher specimens have been deposited in the National Herbarium of Suriname and at the Missouri Botanical Garden. The bark was ground to a powder and extracted with MeOH for 24 h at Bedrijf Geneesmiddelen Voorziening Suriname (BGVS), and then solvent was removed in vacuo. Extraction of 5 kg of dried plant material with MeOH yielded 400 g of extract as BGVS M940017 and 940021.

Extraction and Partition. Dried extract (12.5 g) was partitioned between *n*-hexane (200 mL) and 60%

aqueous MeOH (200 mL). The aqueous MeOH fraction was further diluted with H_2O to reach 50% aqueous MeOH, and then extracted with CH_2Cl_2 (200 mL). The aqueous MeOH layer was further extracted with EtOAc (300 mL). The solvents of all four fractions were removed in vacuo to yield 137 mg of the *n*-hexane fraction, 105 mg of the CH_2Cl_2 fraction, 4.0 g of the aqueous MeOH fraction, and 8.0 g of the EtOAc fraction.

Isolation and Purification. The active aqueous MeOH fraction (3 g, IC_{12} = 4000 μg/mL) was purified by chromatography on Sephadex LH-20 with elution by EtOH; a total of 20 fractions was collected. Fraction 2 (27 mg), containing sucrose, was further purified by column chromatography on LH-20 using the same conditions. The structure of sucrose was determined by comparing its ^{1}H and ^{13}C NMR to standard spectra.[13] A white precipitate was found in fractions 7 (69 mg) and 8 (112 mg) when the EtOH was removed and MeOH was added. The white powder was collected and washed with MeOH and then twice with Me_2CO-H_2O (1:1) to yield 15 mg of compound **3**. The active fractions 11−14 gave a pale yellow-green precipitate on treatment with MeOH. The precipitate from fraction 11 was collected and washed twice with MeOH to yield 10 mg of compound **2**. A 60-mg portion of the MeOH-soluble residue of fraction 11 was further purified by Si gel preparative TLC, developed twice with $CHCl_3$−MeOH (10:3). Three bands were recovered and were identified as epigallocatechin (12 mg),[14] 3-*O*-galloylepigallocatechin (6 mg),[15] and compound **1** (12 mg). Compound **1** was found to be unstable on standing for one week in MeOH or pyridine at room temperature. Ellagic acid was found as part of a mixture in fraction 7 and was purified as a tetra-*O*-methyl ellagic acid after methylation. Its structure was identified by comparing its NMR data to those of the known compound.[7c,11]

4-*O*-(2″,3″,4″-Trihydroxyphenyl)-ellagic acid (eschweilenol A) (1): yellow powder; UV λ_{max} (MeOH) (log ϵ) 250 (sh, 4.66), 258 (4.68), 352 (4.13) nm; IR (KBr) ν_{max} 3340, 1702, 1684, 1613, 1578, 1493, 1364, 1190, 1108, 1056, 1024 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; HMBC, see Table 3; NOESY, see Table 4; negative ion FABMS *m/z* 425 (M − H)$^-$; HREIMS *m/z* 425.0147 [M − H]$^-$ (calcd for $C_{20}H_{10}O_{11}$, 425.0145).

4-*O*-(2″,3″,4″-Trimethoxyphenyl)-3-*O*, 3′-*O*, 4′-*O*-trimethylellagic Acid (4). An excess of freshly prepared CH_2N_2 in ether−EtOH solution was added to an ether solution containing compound **1** (2 mg) at room temperature, and the mixture allowed to stand for 12 h. The CH_2N_2 residue was removed in hood, and the precipitated solid was collected and identified by ^{1}H and ^{13}C NMR and MS as the hexamethoxy product **4** (2 mg): white powder; UV λ_{max} (MeOH) (log ϵ) 251 (4.79), 357 (sh, 4.13), 370 (4.18) nm; IR (KBr) ν_{max} 1736, 1720, 1703, 1599, 1459, 1347, 1243, 1086 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; HMBC, see Table 3; NOESY, see Table 4; EIMS *m/z* 510 [M]$^+$ (100%), 358 (8); HREIMS *m/z* 510.1167 [M]$^+$ (calcd for $C_{26}H_{22}O_{11}$, 510.1162).

Eschweilenol B (2): pale yellow-green powder; UV λ_{max} (MeOH) (log ϵ) 251 (4.70), 270 (4.67), 276 (sh, 4.67), 334 (4.13), 353 (4.01), 371 (3.90) nm; IR (KBr) ν_{max} 3300, 1737, 1718, 1703, 1685, 1654, 1560, 1543, 1524, 1508, 1475, 1460, 1364, 1262, 1101 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; HMBC, see Table 3; NOESY: no correlations were observed; negative ion FABMS *m/z* 423 (M − H)$^-$; negative ion HRFABMS *m/z* 422.9973 [M − H]$^-$ (calcd for $C_{20}H_8O_{11}$, 422.9988).

Penta-*O*-methyleschweilenol B (5). Compound **2** (2 mg) was treated with CH_2N_2 as described above, except that the crude product was washed with MeOH. The dried powder (1 mg) was identified as the pentamethoxy product **5** by ^{1}H NMR and FABMS: yellow powder; UV λ_{max} (MeOH) (log ϵ) 243 (4.64), 276 (4.62), 335 (3.96), 350 (3.95), 371 (3.89) nm; IR (KBr) ν_{max} 1737, 1719, 1702, 1654, 1605, 1458, 1355, 1249, 1096 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR: δ 112.5 (d, C-5), 107.9 (d, C-5′), 96 (d, C-6″), 61.6 (q, 3′-OMe), 56.5 (q, 4′-OMe), 61.6 (q, 3″-OMe), 61.1 (q, 4″-OMe), 56.0 (q, 5″-OMe); quaternary carbons were not detected in the ^{13}C NMR spectrum due to poor solubility, but most of them were observed in an HMBC spectrum and were assigned as below: δ 111.8 (2 ×), 114.7 (2 ×) (C-1, C-1′ or C-6, C-6′), 134.6, 141.5 (C-3 and C-4), 141.3 (C-3′), 154.1 (C-4′), 158.0, 158.5 (C-7 and C-7′), 127.6, 135.7 (C-1″ and C-2″), 141.7 (C-3″), 139.5 (C-4″), 148.7 (C-5″; this assignment was not unambiguous due to the overlapping of H-5 and H-5′ signals); C-2 and C-2′ could not be observed and correlated; HMBC, see Table 3; ROESY, see Table 4; FABMS *m/z* 494 [M]$^+$; HRFABMS *m/z* 494.0845 [M]$^+$ (calcd for $C_{25}H_{18}O_{11}$, 494.0849).

Penta-*O*-(TES)eschweilenol B (7). Compound **2** (1 mg) was treated with TES chloride (0.5 mL) in anhydrous pyridine (0.5 mL) for 24 h at room temperature. The reaction mixture was diluted with *n*-hexane (8 mL) and filtered. The *n*-hexane and pyridine was removed in vacuo, and residual silyl reagents were removed under high vacuum over 3 days. The dried solid residue was identified by ^{1}H NMR as the penta-TES compound **7**: pale powder; ^{1}H NMR δ 7.60, 7.56 (two singlets, H-5 and H-5′), 6.27 (s, H-6″), 0.4−1.1 ppm (five ethyl groups on TES); ROESY δ 7.60 to 0.99 (CH_2) and 0.84 (CH_3); δ 7.56 to 0.98 (CH_2) and 0.79 (CH_3); 6.27 (H-6″) to δ 0.96 (CH_2) and 0.74 (CH_3) ppm.

4-(α-Rhamnopyranosyl)ellagic acid (eschweilenol C) (3): white powder; UV λ_{max} (MeOH) (log ϵ) 255 (4.72), 360 (4.01) nm; IR (KBr) ν_{max} 3270, 1737, 1720, 1704, 1619, 1496, 1439, 1345, 1187, 1101, 1051 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; HMBC, see Table 3; NOESY, see Table 4; FABMS *m/z* 448 [M]$^+$; negative ion HRFABMS *m/z* 447.0559 [M − H]$^-$ (calcd for $C_{20}H_{16}O_{12}$, 447.0642).

4-(α-Rhamnopyranosyl)-3,3′,4′-*O*,*O*,*O*-trimethylellagic Acid (6). The methylation product **6** (3.1 mg) was obtained by treating compound **3** (3 mg) with excess CH_2N_2 at room temperature for 12 h: white powder; UV λ_{max} (MeOH) (log ϵ) 251 (4.77), 355 (4.10), 368 (4.15) nm; IR (KBr) ν_{max} 3320, 1735, 1720, 1609, 1488, 1460, 1402, 1351, 1250, 1104, 986 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; HMBC, see Table 3; NOESY, see Table 4; FABMS *m/z* 491 [M + H]$^+$; HRFABMS *m/z* 491.1180 [M + H]$^+$ (calcd for $C_{23}H_{22}O_{12}$, 491.1189).

Yeast Bioassay. The manipulated yeast assay was performed on a 9-well agar plate as previously described.[2] The yeast was cultured with tested compounds in gradient concentrations for 48 h. The inhibition zones were measured, recorded, and the IC_{12} values were obtained from the average of three separate

individual experiments. Compounds **1** and **3** gave IC_{12} values of 1000 and 2000 μg/mL, respectively; all other compounds were inactive.

Acknowledgment. This work was supported by an International Cooperative Biodiversity Grant, number U01 TW/CA-00313 from the Fogarty Center, NIH, and this support is gratefully acknowledged, as is the advice and encouragement of Dr. Joshua Rosenthal of the Fogarty Center and Dr. James Rodman of the National Science Foundation. Mass spectra were obtained by Mr. Kim Harich of Virginia Polytechnic Institute and State University and the Midwest Center for Mass Spectroscopy in the Department of Chemistry, University of Nebraska. Thanks are due to Tom Glass, Virginia Polytechnic Institute and State University, for helpful discussions of some NMR programs. Ms. Yvette Merton, Mr. Reggy Nelson, and Mr. Richard Autar of Conservation International Suriname, and Ms. Lisa Famolare and Ms. Regina de Souza of Conservation International USA are thanked for their administrative assistance and support.

References and Notes

(1) Biodiversity Conservation and Drug Discovery in Suriname. Part 4. For Part 3, see Abdel-Kader et al.[6]
(2) Zhou, B.-N.; Baj, N. J.; Glass, T. E.; Malone, S.; Werkhoven, M. C. M.; van Troon, F.; David, M.; Wisse, J.; Bursuker, I.; Neddermann, K.; Kingston, D. G. I. *J. Nat. Prod.* **1997**, *60*, 1287−1293.
(3) Abdel-Kader, M. S.; Wisse, J. H.; Evans, R.; van der Werff, H.; Kingston, D. G. I. *J. Nat. Prod.* **1997**, *60*, 1294−1297.
(4) Nitiss, J.; Wang, J. C. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 7501−7505.
(5) Mori, S. A.; Prance, G. T. *Flora Neotropica, Monograph 21 (II); Lecythidaceae-Part II*. New York Botanical Garden: New York, 1990; pp 1−376.
(6) Abdel-Kader, M. S.; Bahler, B. D.; Malone, S.; Werkhoven, M. C. M.; van Troon, F.; David, M.; Wisse, J. H.; Burkuser, I.; Neddermann, K. M.; Mamber, S. W.; Kingston, D. G. I. *J. Nat. Prod.* in press.
(7) (a) *Dictionary of Natural Products*; Buckingham, J., Ed.; Chapman and Hall: London, 1992; Vol. 2, pp 2008−2009. (b)Vieitez, J.; Kingston, D. G. I.; Ballester, A.; Vieitez, E. *Tree Physiol.* **1987**, *3*, 247−255. (c) Nawwar, M. A. M.; Hussein, S. A. M.; Merfort, I. *Phytochemistry* **36**, 793−798, 1994.
(8) Bate-Smith, E. C. *Chem Ind. (London)* **1956**, *R*32−33.
(9) Nonaka, G.; Tanaka, T.; Nishioka, I. *J. Chem. Soc., Perkin Trans. 1* **1982**, *4*, 1067−1073.
(10) Schmidt, O. T.; Komarek, E. *Justus Liebigs Ann. Chem.* **1955**, *591*, 156−176.
(11) Bindra, R. S.; Satti, N. K.; Suri, O. P. *Phytochemistry* **1988**, *27*, 2313−2315.
(12) Nawwar, M. A. M.; Buddrus, J.; Bauer, H. *Phytochemistry* **1982**, *21*, 1755−1758.
(13) Pouchert, C. J.; Behnke, J. *The Aldrich Library of ^{13}C and ^{1}H FTNMR Spectra*; 1st ed., Aldrich Chemical Co.: Milwaukee, 1993; p 305C.
(14) Czochanska, Z.; Foo, L. Y.; Newman, R. H.; Porter, L. J. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2278−2286.
(15) Lee, M.-W.; Morimoto, S.; Nonaka, G.-I.; Nishioka, I. *Phytochemistry* **1992**, *31*, 2117−2120.

NP980046U