Alkaloids from *Alangium javanicum* and *Alangium grisolleoides* that Mediate Cu^{2+}-Dependent DNA Strand Scission

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Crude CH_2Cl_2−MeOH extracts prepared from *Alangium javanicum* and *A. grisolleoides* were found to induce DNA strand breakage in the presence of Cu^{2+} and were subjected to bioassay-guided fractionation to permit identification of the active principle(s). Javaniside (**1**), a novel alkaloid possessing an unusual monoterpenoid oxindole skeleton, was identified as an active principle contributing to the DNA cleavage activity observed for the crude extract of *A. javanicum*. Alangiside (**2**), a tetrahydroisoquinoline monoterpene glucoside widely distributed in the genus *Alangium*, was also isolated from *A. grisolleoides* as a new type of Cu^{2+}-dependent DNA cleavage agent. The relative configuration of the asymmetric centers in javaniside was established by analysis of $^1H-^1H$ coupling constants and NOESY correlations. Semisynthesis of javaniside from secologanin (**3**) established the absolute stereochemistry of javaniside.

The recognition that DNA serves as a target for small molecules both in the initiation of cellular disorders and also in the therapy of certain diseases has resulted in increased interest in the interactions of such molecules with DNA.[1−10] One such type of interaction involves DNA strand scission, which may occur through any of several mechanisms, including (i) oxidation of the deoxyribose sugar ring, (ii) alkylation or oxidation of the aromatic nucleobase, or (iii) hydrolysis of the phosphodiester backbone.[11]

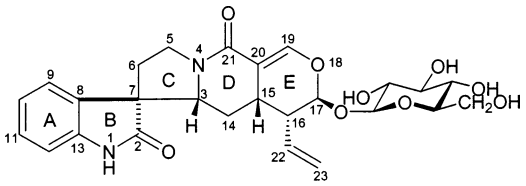
Since the discovery of the bleomycins as potent DNA strand scission agents,[12,13] considerable effort has been made to identify other naturally occurring molecules that are capable of mediating DNA strand scission, on the assumption that these may facilitate the identification of species that can also serve as useful clinical antitumor drugs. Types of natural products identified as a consequence of these efforts have included members of the flavanoid,[14] aurone,[14f] 5-alkylresorcinol,[14b,15] pterocarpan,[16] biphenyl,[17] stilbene,[18] anthrapyrone,[19] enediyne,[20] macroring lactam,[21] lignan,[14g] and phenolic amide[22] classes of compounds.

In a preliminary report, we previously described javaniside (**1**) from *A. javanicum* as an agent capable of Cu^{2+}-dependent DNA cleavage.[23] Described herein is the detailed bioassay-guided isolation and structural elucidation of **1** from *A. javanicum*, as well as the identification of structurally related alangiside (**2**) as an alkaloid from *A. grisolleoides* capable of mediating DNA cleavage. Also described is the semisynthesis of javaniside from secologanin, thereby establishing the absolute stereochemistry of javaniside.

Results and Discussion

During a survey of crude plant extracts for their ability to mediate DNA cleavage in an in vitro DNA strand scission assay, crude extracts of *A. javanicum* and *A. grisolleoides* exhibited considerable Cu^{2+}-dependent DNA strand scission activity. They were thus selected for bioassay-guided fractionation.

A crude 1:1 CH_2Cl_2−MeOH extract of *A. javanicum* was first applied to a polyamide column; elution was effected successively with H_2O, 1:1 MeOH−H_2O, 4:1 MeOH−CH_2Cl_2, 1:1 MeOH−CH_2Cl_2, and 9:1 MeOH−NH_4OH. The first two fractions, from the H_2O and 1:1 MeOH−H_2O washes, exhibited significant Cu^{2+}-dependent DNA cleavage activity. These two active fractions were combined and fractionated further on a C_{18} column using a MeOH−H_2O solvent system for elution. The 7:3 MeOH−H_2O fraction possessed the most potent activity in the DNA strand scission assay and was subjected to further HPLC fractionation. Compound **1** was finally isolated as the active principle responsible for the DNA cleavage activity of the crude extract.



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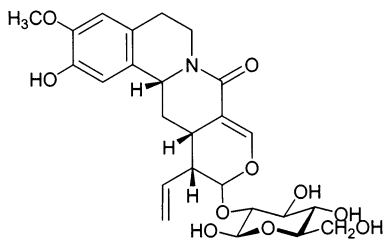

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Compound **1** was obtained as colorless amorphous powder; the positive ion CIMS displayed a quasi molecular ion at *m/z* 515 $[M + H]^+$. The HR−FAB mass spectrum of **1** exhibited a base peak at *m/z* 515.2015 $[M + H]^+$, indicating a molecular formula of $C_{26}H_{30}N_2O_9$. In the ^1H NMR spectrum, compound **1** displayed signals corresponding to a 1,2-disubstituted phenyl ring at δ 7.30 (br d, $J = 7.5$ Hz), 7.25 (td, $J = 7.5$ and 1.2 Hz), 7.07 (td, $J = 7.5$ and 1.2 Hz), and 6.90 (br d, $J = 7.5$ Hz). Also present were signals assigned to a terminal vinyl group at δ 5.49 (dt, $J = 16.8$ and 10.0 Hz), 5.19 (dd, $J = 16.8$ and 1.8 Hz), and 5.16 (dd, $J = 10.0$ and 1.8 Hz), an olefinic proton at δ 7.38 (d, $J = 2.4$ Hz), and a hemi-acetal proton at δ 5.42 (d, $J = 1.8$ Hz). The ^{13}C and DEPT spectra revealed the presence of two

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Table 1. ^{1}H and ^{13}C NMR Spectral Data for Javaniside (**1**) in CD_3OD (500 and 125 MHz)

C	δ_H [a] (*J*, Hz)	δ_C [b]
2		180.9
3	4.09 (dd, 11.6, 3.0)	65.5
5_α	4.04 (ddd, 11.4, 10.5, 7.4)	45.6
5_β	3.76 (br t, 11.4, 11.2)	
6_α	2.23 (dd, 13.2, 7.4)	33.4
6_β	2.40 (ddd, 13.2, 11.2, 10.5)	
7		58.0
8		129.5
9	7.30 (br d, 7.5)	123.9
10	7.07 (td, 7.5, 1.2)	123.8
11	7.25 (td, 7.5, 1.2)	130.0
12	6.90 (br d, 7.5)	110.9
13		143.6
14_α	1.27 (td, 12.2, 12.4)	27.0
14_β	1.37 (dt, 12.2, 3.8, 3.8)	
15	2.94 (dddd, 12.4, 5.5, 3.8, 2.4)	28.7
16	2.56 (ddd, 9.8, 5.5, 1.8)	44.6
17	5.42 (d, 1.8)	97.3
19	7.38 (d, 2.4)	148.2
20		108.9
21		165.8
22	5.49 (dt, 16.8, 10.0)	133.8
23	5.16 (dd, 10.0, 1.8)	120.4
23	5.19 (dd, 16.8, 1.8)	
1′	4.64 (d, 8.0)	99.5
2′	3.14 (dd, 9.0, 8.0)	74.8
3′	3.36 (m)	77.9
4′	3.26 (m)	71.5
5′	3.28 (m)	78.3
6′	3.63 (dd, 12.0, 5.6)	62.6
6′	3.83 (dd, 12.0, 2.0)	

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz.

carbonyl groups (δ 180.9 and 165.8), six aromatic carbons (δ 143.6, 130.0, 129.5, 123.9, 123.8, and 110.9), two aliphatic terminal vinyl carbons (δ 133.8 and 120.4), and one trisubstituted double bond (δ 148.2 and 108.9). Additionally, signals corresponding to a glucopyranose moiety were also identified from ^{13}C NMR experiments (Table 1).[24]

Using ^{1}H−^{1}H DQ-COSY and TOCSY experiments, the assignment of four separated spin−spin coupling systems was achieved as described previously,[23] including (A) H-9 (δ 7.30)−H-10 (δ 7.07)−H-11 (δ 7.25)−H-12 (δ 6.90); (B) H-3 (δ 4.09)−H-14 (δ 1.27, 1.37)−H-15 (δ 2.94)−H-16 (δ 2.56)−H-17 (δ 5.42)−H-22 (δ 5.49)−H-23 (δ 5.16, 5.19); (C) H-5 (δ 4.04, 4.09)−H-6 (δ 2.23, 2.40); and (D) H-1′ (δ 4.64)−H-2′ (δ 3.14)−H-3′ (δ 3.36)−H-4′ (δ 3.26)−H-5′ (δ 3.28)−H-6′ (δ 3.63, 3.83). The assignment of carbon signals corresponding to each proton signal was achieved by a HMQC experiment (Table 1). The spectral features of coupling systems B, C and the α,β-unsaturated carboxyl group deduced from HMBC signals indicated structural similarity of **1** to strictosamide,[25] a known monoterpenoid indole alkaloid. However, significant differences were noted for the indole ring carbon signals. The typical chemical shifts of C-2 and C-7 in monoterpenoid indole alkaloids are 135 and 108 ppm, respectively, whereas the C-2 and C-7 signals of **1** were at 180.9 and 58.0 ppm, respectively, which strongly suggested the existence of an oxoindole ring in **1** rather than the indole ring in monoterpenoid indole alkaloids, such as strictosamide.[25,26] Long range ^{1}H−^{13}C correlations between C-2−H-3, C-2−H-5, C-2−H-6, C-7−H-5, C-7−H-14, C-8−H-3, and C-8−H-6 suggested C-7 as a spiro-carbon to link the oxoindole ring and the pyrrole ring, and this linkage was further confirmed by the comparison of the ^{13}C NMR data with that of similar oxoindole alkaloids.[27]

The stereochemistry of **1** was determined through a careful analysis of all proton coupling constants and NOE effects. The coupling constants between H-16−H-17 (J = 1.8 Hz) and H-16−H-15 (J = 5.5 Hz) strongly suggested the $\beta/\beta/\alpha$ orientation of H-15, H-16, and H-17. The β configuration of H-3 was supported by the coupling constants between H-3 and H-14 protons ($J_{3,14\alpha}$ = 11.6 Hz, $J_{3,14\beta}$ = 3.0 Hz). The coupling constants indicated that **1** has the relative configuration illustrated in the structural formula above.[28] This configuration was further supported by a number of NOE signals deduced from NOESY experiments.[23] Further evidence for the stereochemistry of **1** came from a comparison of its ^{13}C NMR data with that in the literature.[25b,28a,29] On the basis of these data, the structure of **1** was identified as that of a novel monoterpenoid oxoindole alkaloid and was named javaniside.

Several monoterpenoid indole alkaloids have been isolated from terrestrial plants.[25,26,30] Javaniside (**1**) represents a new type of nitrogenous natural product from the genus *Alangium*. Further, the occurrence of this unusual oxindole glucoside provides some insight into the likely biosynthesis of the monoterpenoid indole alkaloid.

The bioassay-guided fractionation of the crude extract of *A. grisolleoides* was performed in a fashion similar to that of the crude extract of *A. javanicum*. Following the removal of most polyphenols on a polyamide column, successive fractionations of the extract on C_{18} and C_8 open columns, and then on reversed-phase HPLC columns, resulted in the isolation of active principle **2**. Compound **2** was also obtained as a colorless, amorphous powder; its positive ion CIMS displayed a quasi molecular ion at *m/z* 506 $[M + H]^+$. ^{1}H and ^{13}C NMR signals revealed patterns typical of a tetrahydroisoquinoline monoterpene glycoside. These NMR data, compared with data from the literature, established the structure of **2** as that of alangiside, a known alkaloid glucoside.[29,31]

To confirm the structure of javaniside and to determine its absolute configuration, a semisynthesis of javaniside and its isomer, 7-*epi*-javaniside, was performed (Scheme 1). The natural compound secologanin (**3**), whose absolute stereochemistry is known,[32] was first acetylated in 51% yield by treatment with Ac_2O in pyridine at room temperature, following the reported procedure.[33] Peracylated compound **4** was then treated at reflux in methanol with 2-oxotryptamine (**5**),[34] the latter of which had been prepared from tryptamine by treatment with dimethyl sulfoxide and hydrochloric acid, to provide isomeric **6** (51%) and **7** (17%) in ~3:1 ratio by a previously reported method.[35]

The stereochemistry of isomers **6** and **7** was determined by vicinal ^{1}H−^{1}H coupling constants and confirmed by analysis of NOESY experiments.[35] This analysis established the absolute configuration as 3*R*,7*S* for **6** and 3*R*,7*R* for **7**. Compounds **6** and **7** were then treated with sodium methoxide in methanol to provide compounds **1** (68%) and **8** (66%), respectively.[36] The stereochemistry of **1** and **8** was confirmed by analysis of vicinal ^{1}H−^{1}H coupling constants. The main differences between **1** and **8** in the NOESY experiments were the correlations of H-9 to H-3 in **1** and of H-9 to H-5b and H-14a in **8**. By comparison of NMR data and of optical rotations, synthetic compound **1** was shown to be identical to naturally derived javaniside. The absolute configuration of javaniside was thus determined as 3*R*,7*S*, 15*S*,16*R*,17*S*.

Plants in the genus *Alangium* have been recognized to be rich in different kinds of nitrogenous secondary metabolites, including ipecac alkaloids represented by emetine and cephaeline,[37] tetrahydroisoquinoline monoterpene glycosides, such as alangiside,[29,31] and several other types of

Scheme 1. Synthesis of Javaniside from Secologanin

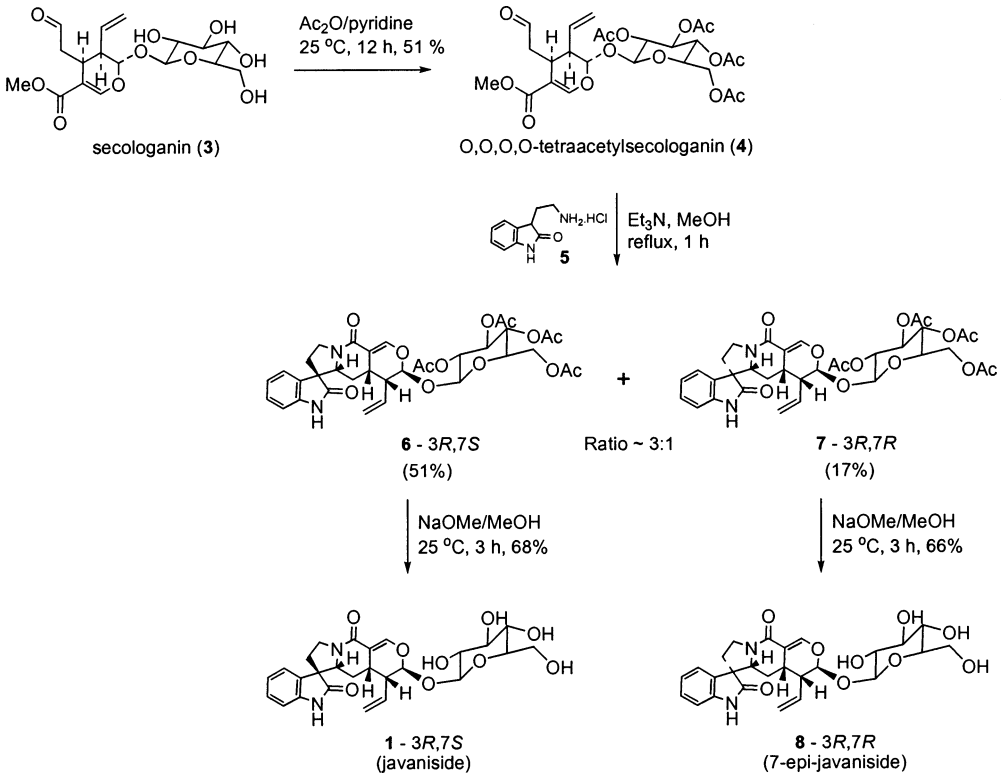

Table 2. Cu^{2+}-Dependent DNA Strand Scission by Compounds **1** and **2**

compound	Cu^{2+} (20 μM)	Form II DNA (%)
none	−	2
none	+	3
javaniside (**1**, 200 μM)	−	3
javaniside (**1**, 200 μM)	+	58
javaniside (**1**, 100 μM)	+	29
javaniside (**1**, 50 μM)	+	20
javaniside (**1**, 25 μM)	+	16
javaniside (**1**, 10 μM)	+	10
alangiside (**2**, 500 μM)	−	3
alangiside (**2**, 500 μM)	+	42
alangiside (**2**, 200 μM)	+	30
alangiside (**2**, 100 μM)	+	25
alangiside (**2**, 50 μM)	+	18
alangiside (**2**, 10 μM)	+	14

Table 3. Cu^{2+}-Dependent DNA Strand Scission by Synthetic Javaniside (**1**) and 7-*epi*-Javaniside (**8**)[a]

compound	Form II DNA (%)
none	6
javaniside (natural)	
200 μM	43
100 μM	28
10 μM	0
javaniside (synthetic)	
200 μM	35
100 μM	16
10 μM	0
7-*epi*-javaniside (synthetic)	
200 μM	7
100 μM	5
10 μM	0

[a] Carried out in the presence of equimolar (*epi*)javaniside and Cu^{2+}. The data were corrected for DNA cleavage observed in the presence of Cu^{2+} alone.

alkaloids.[38] However, the isolation of alangiside from *A. grisolleoides* has not been reported previously.

The abilities of **1** and **2** to effect DNA strand scission were evaluated using a supercoiled, covalently closed, circular DNA as a substrate in the absence and presence of Cu^{2+}. In the presence of Cu^{2+}, dose-dependent DNA relaxation of the supercoiled pBR322 plasmid DNA was observed for both **1** and **2** (Table 2). Consistent with the behavior of the crude extracts from which they were isolated, neither **1** nor **2** exhibited DNA strand scission activity in the presence of Fe^{2+}, nor in the absence of added metal ion.

In the presence of 20 μM Cu^{2+}, compounds **1** and **2** clearly mediated DNA strand scission in a concentration-dependent fashion. About 58% conversion of Form I to Form II DNA was apparent when **1** was employed at 200 μM concentration, whereas 42% Form II DNA was produced when 500 μM **2** was used. However, even when the concentrations utilized were as low as 10 μM, both **1** and **2** still possessed detectable DNA cleavage activity (Table 2). This is the first time that oxindole and tetrahydroisoquinoline alkaloids have been identified as agents capable of DNA strand scission. Also compared was the extent of DNA cleavage mediated by synthetic versus naturally derived javaniside. As shown in Table 3, when tested in the presence of equimolar Cu^{2+}, the cleavage by the synthetic and naturally derived samples was similar, although the natural sample gave slightly more Form II DNA. In contrast, 7-*epi*-javaniside effected the relaxation of the supercoiled DNA weakly, if at all. Thus, the cleavage of DNA by javaniside seems likely to have resulted from a specific interaction of javaniside with the DNA substrate.

Experimental Section

General Experimental Procedures. Polyamide 6S was purchased from Serva Electrophoresis GmbH. Silica C_{18} (40 μm) was obtained from J. T. Baker Chemicals. A Higgins Kromasil 100 C_{18} column (250 × 10 mm, 5 μm) was used for reversed-phase HPLC. ^{1}H and ^{13}C NMR spectroscopic experi-

ments were performed on Varian Unity Inova 300 and 500 spectrometers. Low-resolution chemical ionization mass spectra were recorded on a Finnigan MAT 4600 mass spectrometer. Optical rotations were determined on a JASCO P-1020 polarimeter. Ethidium bromide, bromophenol blue, and Trizma base were purchased from Sigma Chemicals. Boric acid was obtained from EM Sciences. (Ethylenedinitrilo)tetraacetic acid (EDTA) disodium salt was obtained from J. T. Baker. Cupric chloride and glycerol were from Mallinckrodt, Inc.; ultrapure agarose was from Bethesda Research Laboratories. The pBR322 plasmid DNA was purchased from New England Biolabs. Pierce microdialysis cassettes were used to remove EDTA from the pBR322 plasmid DNA.

Plant Material. Leaves of *A. javanicum* (Wang) were collected in Sabah, Malaysia on October 17, 1987. Branches of *A. grisolleoides* (Capuron) were collected in Madagascar on June 19, 1996. Voucher specimens of both plants (Q66O5281 and Q66V4326, respectively) are preserved in the Botany Department of the U.S. National Arboretum Herbarium, Washington, D.C.

Extraction and Isolation. Bioassay-Guided Fractionation of a Crude Extract of *A. javanicum*. Dried leaves of *A. javanicum* were steeped in 1:1 methylene chloride−methanol overnight at room temperature, then drained, washed with methanol, and freed of solvent to obtain dry crude extract. The crude extract of *A. javanicum* displayed significant Cu^{2+}-dependent DNA cleavage activity in a plasmid relaxation assay. In a typical experiment, the crude extract (1.5 g) was first applied to a polyamide 6S column, which was washed successively with H_2O, 1:1 MeOH−H_2O, 4:1 MeOH−CH_2Cl_2, 1:1 MeOH−CH_2Cl_2, and 9:1 MeOH−NH_4OH. With the removal of most polyphenols, the H_2O and MeOH−H_2O fractions (233 mg and 105 mg, respectively) induced potent DNA strand scission in an agarose gel assay at 100 and 50 μg/mL. These two fractions were combined and fractionated further on a C_{18} column using MeOH−H_2O for elution. The 7:3 MeOH−H_2O fraction (30 mg) showed the greatest potency in DNA strand scission and was applied to a C_{18} reversed-phase HPLC column (250 × 10 mm, 5 μm); elution was effected with a linear gradient of CH_3CN−H_2O (1:19 → 2:3) over a period of 35 min at a flow rate of 4.0 mL/min (UV monitoring at 254 nm). One particularly active fraction (11.3 mg) was obtained from the reversed-phase HPLC fractionation. Purification of this active fraction, employing the same HPLC conditions, afforded active compound **1** (8.0 mg).

Bioassay-Guided Fractionation of a Crude Extract of *A. grisolleoides*. The crude extract of *A. grisolleoides* was prepared from dried branches using the same extraction protocol for the crude extract of *A. javanicum*. The crude extract of *A. grisolleoides* also displayed fairly strong Cu^{2+}-dependent DNA cleavage activity. Typically, the crude extract (1.25 g) was first applied to a polyamide 6S column, which was washed successively with H_2O, 1:1 MeOH−H_2O, 4:1 MeOH−CH_2Cl_2, 1:1 MeOH−CH_2Cl_2, and 9:1 MeOH−NH_4OH. After the removal of most polyphenols, the H_2O fraction (906 mg) still induced significant in vitro DNA strand scission in the plasmid DNA relaxation assay at 100 and 50 μg/mL. The active fraction was fractionated further on a C_{18} column using a MeOH−H_2O solvent system. The 2:3 and 3:2 MeOH−H_2O fractions (19 and 10 mg, respectively) showed the most promising result in both the fractionation experiment and the DNA strand scission assay. Further fractionation of the combined mixture of the two fractions on a C_8 column provided one active fraction (12 mg) that eluted from the column in the water wash. Subsequently, the H_2O fraction was applied to a reversed-phase C_{18} HPLC column (250 × 10 mm, 5 μm); elution with a linear gradient of 1:99 → 2:3 CH_3CN−H_2O over a period of 35 min at 3.0 mL/min (UV monitoring at 215 nm) gave good separation of the active principle. Purification of the active fraction (2.5 mg) from this step, employing the same HPLC conditions, afforded active compound **2** (2.0 mg).

Javaniside (1): colorless amorphous powder; $[\alpha]^{21}_D$ −50.2° (*c* 0.2, MeOH); ^{1}H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 1; positive ion CIMS *m/z* (rel int.) 516 $[M + 2H]^+$ (23), 515 $[M + H]^+$ (100), 353 $[M + H + H_2O - \text{glucose}]^+$ (21); HRFABMS *m/z* 515.2015 $[M + H]^+$ (calcd for $C_{26}H_{31}N_2O_9$, 515.2030).

Alangiside (2): colorless amorphous powder; $[\alpha]^{21}_D$ −104.6° (*c* 1.00, MeOH), lit[31] $[\alpha]^{26}_D$ −105° (*c* 1.0, MeOH); ^{1}H and ^{13}C NMR data were identical with the literature data;[29,31] positive CIMS *m/z* (rel int.) 506 $[M + H]^+$ (100), 344 $[M + H + H_2O - \text{glucose}]^+$ (21).

Synthesis of Javaniside and 7-*epi*-Javaniside from Secologanin. *O,O,O,O*-Tetraacetylsecologanin (4).[33] A solution containing 215 mg (0.55 mmol) of secologanin (**3**) in 2 mL of dry pyridine was treated with 2 mL of Ac_2O. After stirring at 25 °C for 24 h, ice was added to the reaction mixture. The solution was extracted with five 10-mL portions of CH_2Cl_2. The combined organic extract was dried over $MgSO_4$ and concentrated under diminished pressure. The crude product was purified by SiO_2 chromatography (7:3 hexanes−Et_2O) to provide **4** as a colorless solid: yield 157 mg (51%); $[\alpha]^{20}_D$ −79.9° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.90 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.09 (s, 3H), 2.38 (ddd, 1H, *J* = 17.9, 7.8, 1.4 Hz), 2.80 (ddd, 1H, *J* = 9.6, 5.8, 3.0 Hz), 2.91 (ddd, 1H, *J* = 17.9, 5.8, 1.4 Hz), 3.29 (m, 1H), 3.67 (s, 3H), 3.73 (ddd, 1H, *J* = 10.6, 4.4, 2.1 Hz), 4.14 (dd, 1H, *J* = 12.3, 2.1 Hz), 4.28 (dd, 1H, *J* = 12.3, 4.4 Hz), 4.88 (d, 1H, *J* = 8.1 Hz), 5.01−5.25 (m, 5H), 5.27 (d, 1H, *J* = 3.0 Hz), 5.49 (m, 1H), 7.41 (d, 1H, *J* = 2.1 Hz), 9.70 (dd, 1H, *J* = 1.4, 1.4 Hz); mass spectrum (EIMS), *m/z* 557 $(M + H)^+$.

2-Oxo-2,3-dihydrotryptamine Hydrochloride (5).[34] Tryptamine (0.5 g, 3.13 mmol) was dissolved in 0.29 g (3.72 mmol) of DMSO, and 0.36 mL (3.72 mmol) of concentrated hydrochloric acid was added slowly. After stirring at 25 °C for 3 h, the precipitate was collected by filtration. The solid product was precipitated from EtOH to give **5** as a colorless solid: yield 0.43 g (65%); mp 234−235 °C; (lit[34] mp 235−239 °C); ^{1}H NMR (DMSO-d_6) δ 2.08 (dt, 2H, *J* = 15.2, 6.8 Hz), 2.92 (dt, 2H, *J* = 12.5, 6.8 Hz), 3.60 (dd, 1H, *J* = 6.8, 6.8 Hz), 6.85 (d, 1H, *J* = 7.7 Hz), 6.96 (t, 1H, *J* = 7.7 Hz), 7.18 (t, 1H, *J* = 7.7 Hz), 7.23 (d, 1H, *J* = 7.7 Hz), 8.22 (br s, 3H), 10.56 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 28.7, 36.9, 43.4, 110.2, 122.2, 124.7, 128.7, 129.5, 143.3, 179.1.

***O,O,O,O*-Tetraacetyljavaniside (6) and *O,O,O,O*-Tetraacetyl-7-*epi*-javaniside (7).**[35] To a solution of 100 mg (0.18 mmol) of **4** in 2 mL of dry methanol were added 76 mg (0.36 mmol) of 2-oxotryptamine hydrochloride and 49 μL (0.36 mmol) of Et_3N. The mixture reaction was heated at reflux for 1 h. The solution then was concentrated under diminished pressure, and the crude product was purified by SiO_2 chromatography (7:3 $CHCl_3$−acetone) to give compounds **6** and **7** as colorless solids: yield 62.5 mg (51%) for **6** and 20.8 mg (17%) for **7**.

Compound 6 (3*R*,7*S*): $[\alpha]^{20}_D$ −18.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.34 (m, 2H), 2.01 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 2.31 (m, 2H), 2.52 (ddd, 1H, *J* = 10.0, 5.5, 1.8 Hz), 2.68 (m, 1H), 3.74 (ddd, 1H, *J* = 9.7, 4.6, 2.2 Hz), 3.82 (br dd, 1H, *J* = 11.6, 5.8 Hz), 3.97 (dd, 1H, *J* = 10.8, 3.4 Hz), 4.11 (dd, 1H, *J* = 12.4, 2.2 Hz), 4.12 (m, 1H), 4.28 (dd, 1H, *J* = 12.4, 4.6 Hz), 4.90 (d, 1H, *J* = 8.1 Hz), 4.97 (dd, 1H, *J* = 9.7, 8.1 Hz), 5.05 (dd, 1H, *J* = 10.0, 1.4 Hz), 5.07 (dd, 1H, *J* = 9.7, 9.7 Hz), 5.13 (dd, 1H, *J* = 17.3, 1.4 Hz), 5.20 (d, 1H, *J* = 1.8 Hz), 5.24 (dd, 1H, *J* = 9.7, 9.7 Hz), 5.44 (m, 1H), 6.90 (d, 1H, *J* = 7.3 Hz), 7.10 (t, 1H, *J* = 7.3 Hz), 7.23 (d, 1H, *J* = 7.3 Hz), 7.28 (t, 1H, *J* = 7.3 Hz), 7.42 (d, 1H, *J* = 2.4 Hz), 7.86 (s, 1H); mass spectrum (EIMS), *m/z* 683 $[M + H]^+$.

Compound 7 (3*R*,7*R*): $[\alpha]^{20}_D$ −51.6° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85 (ddd, 1H, *J* = 12.7, 12.7, 11.4 Hz), 1.38 (ddd, 1H, *J* = 12.7, 3.7, 3.7 Hz), 1.99 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 2.49−2.60 (m, 2H), 2.77 (m, 1H), 3.73 (ddd, 1H, *J* = 10.0, 4.5, 2.2 Hz), 3.89 (m, 1H), 4.00 (ddd, 1H, *J* = 12.7, 12.1, 7.8 Hz), 4.10 (dd, 1H, *J* = 12.1, 3.5 Hz), 4.11 (dd, 1H, *J* = 12.4, 3.7 Hz), 4.12 (dd, 1H, *J* = 12.5, 2.2 Hz), 4.28 (dd, 1H, *J* = 12.5, 4.5 Hz), 4.89 (d, 1H, *J* = 8.1 Hz), 4.99 (dd, 1H, *J* = 9.5, 8.0 Hz), 5.00 (dd, 1H, *J* = 9.5, 2.0 Hz), 5.06 (dd, 1H, *J* = 17.1, 2.0 Hz), 5.08 (dd, 1H, *J* = 9.6, 9.6 Hz), 5.21 (d, 1H, *J* = 1.5 Hz), 5.26 (ddd, 1H, *J* = 17.1, 9.5, 9.5 Hz), 5.29 (dd, 1H, *J* = 9.6, 9.5 Hz), 6.91 (d, 1H, *J* = 7.5 Hz), 6.92 (d, 1H,

J = 7.5 Hz), 7.02 (dt, 1H, J = 7.5, 1.1 Hz), 7.25 (dt, 1H, J = 7.5, 1.1 Hz), 7.43 (d, 1H, J = 2.4 Hz), 7.83 (s, 1H); mass spectrum (EIMS), *m/z* 683 $[M + H]^+$.

(3*R*,7*S*) − Javaniside (1). To a solution of 50 mg (0.07 mmol) of tetra-*O*-acetyl javaniside (**6**) in 1.0 mL of absolute MeOH was added 1.6 mg (0.03 mmol) of NaOMe. After stirring at 25 °C for 1 h, the reaction mixture was neutralized with Amberlite IRC 50 resin (H^+ form). The reaction mixture was filtered, and the filtrate was concentrated under diminished pressure. The crude product was purified by C_{18} reversed-phase HPLC, eluting with a linear gradient from 15 to 35% (v/v) of CH_3CN in H_2O over a period of 30 min at a flow rate of 4 mL/min (UV monitoring at 255 nm) to afford **1** as a colorless solid: yield 25.6 mg (68%); $[\alpha]^{20}_D$ −55.9 ° (*c* 0.2, MeOH); mass spectrum (EIMS), *m/z* 515 $[M + H]^+$.

(3*R*,7*R*) − 7-*epi*-Javaniside (8). The same procedure employed for the conversion of **6** → **1** was applied for tetra-*O*-acetylated glycoside **7**. Compound **8** was obtained as a colorless solid in 66% yield; $[\alpha]^{20}_D$ −76.2° (*c* 0.2, MeOH); ^1H NMR (CD_3OD-d_4) δ 0.89 (ddd, 1H, J = 12.5, 12.5, 11.4 Hz), 1.40 (ddd, 1H, J = 12.5, 3.4, 3.4 Hz), 2.03 (ddd, 1H, J = 12.6, 7.6, 1.0 Hz), 2.48 (ddd, 1H, J = 12.6, 10.9, 9.9 Hz), 2.54 (ddd, 1H, J = 10.3, 5.5, 1.8 Hz), 3.07 (m, 1H), 3.16 (dd, 1H, J = 9.2, 7.9 Hz), 3.26 (dd, 1H, J = 9.6, 8.7 Hz), 3.28 (m, 1H), 3.36 (dd, 1H, J = 9.2, 8.7 Hz), 3.64 (dd, 1H, J = 12.0, 5.5 Hz), 3.82 (br d, 1H, J = 12.0 Hz), 3.87 (br dd, 1H, J = 12.0, 9.9 Hz), 3.96 (ddd, 1H, J = 12.0, 10.9, 7.6 Hz), 4.08 (dd, 1H, J = 11.4, 3.4 Hz), 4.64 (d, 1H, J = 7.9 Hz), 4.98 (dd, 1H, J = 10.3, 1.8 Hz), 5.07 (dd, 1H, J = 17.2, 1.8 Hz), 5.30 (ddd, 1H, J = 17.2, 10.3, 10.3 Hz), 5.42 (d, 1H, J = 1.8 Hz), 6.90 (d, 1H, J = 7.5 Hz), 6.97 (d, 1H, J = 7.5 Hz), 7.02 (dt, 1H, J = 7.5, 1.0 Hz), 7.26 (dt, 1H, J = 7.5, 1.0 Hz), 7.41 (d, 1H, J = 2.4 Hz); ^{13}C NMR (CD_3OD-d_4) δ 27.2, 28.2, 34.3, 44.5, 45.5, 58.8, 62.6, 64.8, 71.5, 74.7, 77.8, 78.3, 97.3, 99.5, 108.7, 111.5, 120.4, 123.7, 124.8, 129.8, 131.2, 133.4, 142.5, 148.5, 166.1, 170.3; mass spectrum (EIMS), *m/z* 515 $[M + H]^+$; mass spectrum (FAB), *m/z* 515.2028 $[M + H]^+$ ($C_{26}H_{31}N_2O_9$ requires 515.2030).

DNA Strand Scission Assay. For the DNA strand scission assay in Table 2, supercoiled pBR322 plasmid DNA (500 ng) in the absence or presence of 20 μM Cu^{2+} in 25 μL of 10 mM Tris-HCl buffer, pH 8.0, was treated with crude extracts or fractions (dissolved in DMSO, with a final DMSO concentration no greater than 5%). Each set of experiments included one blank control (DNA alone) and one metal ion control (DNA + Cu^{2+}). After incubation at 37 °C for 60 min, the reaction solution was mixed with 5 μL of 30% glycerol−0.01% bromophenol blue and then analyzed by electrophoresis in a 1.0% agarose gel containing 0.7 μg/mL ethidium bromide. Electrophoresis was carried out in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3) at 110−120 V for 2−3 h. Following electrophoresis, the gel was photographed under ultraviolet light. The extent of conversion of Form I to Form II DNA was determined by densitometry of the gel. The experiment shown in Table 3 was run in the same fashion, except that equimolar Cu^{2+} and **1** (**8**) were used in 10 mM Na cacodylate buffer, pH 7.0, containing supercoiled pSP64 plasmid DNA. The reaction was carried out at 25 °C for 2 h.

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