A Dihydroflavonol Glucoside from *Commiphora africana* that Mediates DNA Strand Scission

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A crude CH_2Cl_2−MeOH extract prepared from *Commiphora africana* was found to mediate Cu^{2+}-dependent relaxation of supercoiled plasmid DNA. Bioassay-guided fractionation of this extract was carried out and was monitored by the use of an in vitro DNA strand scission assay. The dihydroflavonol glucoside phellamurin (**1**) was identified as the active principle responsible for the DNA cleavage activity of the crude extract.

With the discovery that DNA can act as a cellular target for small molecules in the initiation of cellular disorders and the therapy of certain diseases such as cancer, there has been substantial effort directed toward characterizing the interaction of such molecules with DNA. Well-characterized noncovalent binding interactions include intercalation, partial intercalation, and binding to the minor and major grooves of DNA.[1−10] In addition, some of the molecules that bind to DNA also induce DNA strand scission. DNA strand breakage by such agents has been proposed to occur through one of several mechanisms including oxidation of the deoxyribose sugar ring, alkylation or oxidation of the aromatic nucleobase, or hydrolysis of the phosphodiester backbone of DNA.[11,12]

Bleomycin may be considered to be the prototype sequence-selective DNA strand scission agent.[13] Bleomycin-mediated DNA cleavage requires a metal ion such as Fe^{2+} or Cu^{2+} as well as dioxygen to effect strand scission.[14] The mechanism of bleomycin-induced DNA strand scission is known to involve the oxidative destruction of sugar moieties in DNA, initiated by abstraction of the C′-4 H atom from the minor groove of DNA.[14] Bleomycin has realized substantial utility in the clinic as an antitumor agent;[13,15] not surprisingly, there has been a substantial effort to identify other small molecules that can mediate similar effects. Efforts in this area have included the identification of other natural products capable of DNA degradation, in the hope that they may lead to the development of antitumor agents with improved properties. As a consequence of these efforts, members of numerous classes of agents have been found to induce DNA strand scission in the absence or presence of metal ions.[16−25] While the mechanism of DNA strand scission has not been studied in detail for all of these agents, at least some have been reported to involve free radical mechanisms.[18]

As part of an ongoing effort to identify compounds from natural sources that mediate DNA strand scission, a crude CH_2Cl_2−MeOH extract from *Commiphora africana* was found to mediate DNA strand scission in the presence of Cu^{2+}. Bioassay-guided fractionation of this crude extract led to the isolation of the dihydroflavonol glucoside phellamurin (**1**). Described herein is the isolation and structure identification of phellamurin **1** and its characterization as an agent capable of mediating Cu^{2+}-dependent DNA strand scission.

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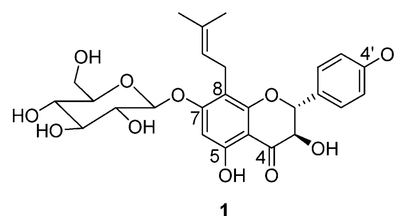

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The crude 1:1 CH_2Cl_2−MeOH extract of *C. africana* was applied to a polyamide 6S column, which was washed successively with H_2O, 1:1 MeOH−H_2O, 4:1 MeOH−CH_2Cl_2, 1:1 MeOH−CH_2Cl_2, and then 9:1 MeOH−NH_4OH. The 1:1 MeOH−H_2O and 4:1 MeOH−CH_2Cl_2 fractions exhibited significant Cu^{2+}-dependent DNA cleavage activity. These two active fractions were combined and fractionated further on a C_{18} reversed-phase column that was eluted with MeOH−H_2O mixtures. The 1:4, 2:3, and 3:2 MeOH−H_2O eluates possessed the strongest activity in the in vitro DNA strand scission assay. These fractions were combined and applied to a C_8 reversed-phase column, which was also eluted with MeOH−H_2O mixtures. The 4:1 MeOH−H_2O fraction exhibited the greatest DNA strand scission in the presence of Cu^{2+}. Fractionation of this material by C_{18} reversed-phase HPLC resulted in the isolation of compound **1** as the active principle responsible for the DNA cleavage activity of the crude extract.

Compound **1** was obtained as a pale yellow powder; positive ion chemical ionization mass spectrometry indicated the presence of the molecular ion at m/z 518 $[M]^+$. The ^1H and ^{13}C NMR features of **1** strongly suggested that **1** was a dihydroflavonol glycoside.[26] In the ^1H NMR spectrum, signals typical of a 5,7,4′-trihydroxydihydroflavonol were observed at δ 7.35 (2H, d, J = 8.5 Hz), 6.82 (2H, d, J = 8.5 Hz), 6.28 (1H, s), 4.99 (1H, d, J = 11.5 Hz), and 4.58 (1H, d, J = 11.5 Hz). Signals characteristic of a prenyl moiety were also observed at δ 5.22 (1H, br t, J = 7.0 Hz), 3.38 (2H, br d, J = 7.0 Hz), 1.78 (3H, s), and 1.64 (3H, s). In addition, the ^{13}C NMR spectrum not only revealed corresponding signals of the dihydroflavonol skeleton and prenyl group but also contained signals characteristic of a glucosyl residue at δ 101.3, 78.3, 78.0, 74.8, 71.2, and 62.4.[27] From these data, **1** was concluded to be a C-prenyl derivative of 5,7,4′-trihydroxydihydroflavonol.[26,28] Another ^1H NMR spectrum was recorded in acetone-d_6 in an effort to determine the position of attachment of the prenyl group by observing the proton signal of the hydrogen-bonded hydroxyl group at the C-5 position. The resonance at 11.98 ppm, assigned to the hydroxyl

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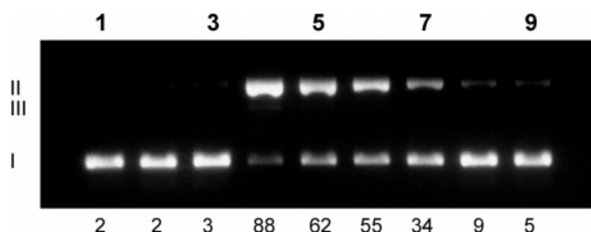

Figure 1. DNA strand scission by compound **1**, quantified after agarose gel electrophoresis. Lane 1, pBR322 plasmid DNA alone; lane 2, DNA + 20 μM Cu^{2+}; lane 3, 100 μM **1**; lanes 4–9, 100, 50, 20, 10, 5, and 1 μM **1** + 20 μM Cu^{2+}, respectively. The percent Form II DNA present is shown below each lane.

proton at C-5, strongly suggested that the prenyl moiety was attached to C-8.[29] This assignment was further supported by the observation of a long-range $^{1}H-^{13}C$ correlation between a carbon bearing hydrogen (δ 95.3) and the hydroxyl proton at δ 11.98 in the HMBC spectrum, whereas no signal was observed between a prenylated carbon (δ 112.1) and the corresponding hydroxyl proton. Consequently, compound **1** was identified as 3,5,7,4′-tetrahydroxy-8-(3-methylbut-2-enyl)flavanone-7-*O*-β-glucoside, which is identical to phellamurin.[26] The absolute configuration was assumed to be the same as that reported for phellamurin.

The activity of **1** in the induction of DNA strand breakage in the absence and presence of Cu^{2+} ion was evaluated using pBR322 plasmid DNA, a supercoiled, covalently closed, circular DNA. In the presence of Cu^{2+}, single-strand cleavage of the supercoiled pBR322 plasmid DNA was observed (Figure 1).[17b] In common with the crude extract from which **1** was isolated, compound **1** displayed no DNA strand scission activity in the presence of Fe^{2+} or in the absence of added metal ion.

In the presence of 20 μM Cu^{2+}, compound **1** induced DNA strand scission in a concentration-dependent fashion. About 62% conversion of Form I (supercoiled) to Form II (nicked circular) DNA was observed when **1** was employed at 50 μM concentration. Further, even when the concentration of **1** was as low as 1 μM, some DNA cleavage activity was still detectable (Figure 1). Moreover, when compound **1** was employed at 100 μM concentration, a small amount of Form III (linear duplex) DNA was produced in addition to nicked circular (Form II) DNA.

No phytochemical study has been carried out on this species; this is the first report of the isolation of a dihydroflavonol-type secondary metabolite from *C. africana* and also the first time that **1** has been reported to mediate Cu^{2+}-dependent DNA strand scission. Phellamurin (**1**) has been reported previously to regulate differential oviposition by swallowtail butterflies on a rutaceous plant, *Phellodendron amurense*.[26b]

Experimental Section

General Experimental Procedures. Polyamide 6S was purchased from Serva Electrophoresis GmbH, and silica C_{18} (40 μm) was from J. T. Baker Chemicals. A Higgins Kromasil 100 C_{18} reversed-phased column (250 × 10 mm, 5 μm) was used for HPLC. ^{1}H and ^{13}C NMR spectroscopy was performed on Varian Unity Inova 300 and 500 spectrometers. Low-resolution chemical ionization mass spectra were recorded on a Finnigan MAT 4600 mass spectrometer. Ethidium bromide, bromophenol blue, and Trizma were purchased from Sigma Chemicals. Boric acid was obtained from EM Sciences. (Ethylenediamino)tetraacetic acid (EDTA), disodium salt was purchased from J. T. Baker; cupric chloride and glycerol were from Mallinckrodt, Inc. Ultrapure agarose was purchased from Bethesda Research Laboratories. Supercoiled pBR322 plasmid DNA was purchased from New England Biolabs. A Pierce microdialysis cassette was used to remove EDTA from the pBR322 plasmid DNA.

Plant Material. Wood stem of *C. africana* was collected in December 1988 in Tanzania. A voucher specimen of *C. africana* (Q66T0524) is at the U.S. National Arboretum Herbarium, Washington, DC.

Extraction and Isolation. Dried wood stems of *C. africana* were steeped in 1:1 MeOH–CH_2Cl_2 overnight at room temperature, then drained and washed with MeOH. The combined organic solution was concentrated under diminished pressure to afford the crude extract, which displayed significant Cu^{2+}-dependent DNA cleavage activity. In a typical experiment, the crude extract (2.89 g) was 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 then 9:1 MeOH–NH_4OH. The 1:1 MeOH–H_2O and 4:1 MeOH–CH_2Cl_2 fractions (268 and 430 mg, respectively) induced DNA strand scission strongly when employed at 100 and 50 μg/mL concentrations. These two fractions were combined and fractionated further on a C_{18} reversed-phase column using MeOH–H_2O mixtures for elution. The 1:4, 2:3, and 3:2 MeOH–H_2O fractions (6.7, 55, and 176 mg, respectively) exhibited potent DNA cleavage. These fractions were combined and then applied to a C_8 reversed-phase column, which was eluted with MeOH–H_2O mixtures. The 4:1 MeOH–H_2O fraction from this step displayed the most potent DNA strand scission activity and was fractionated further on a C_{18} reversed-phase HPLC column (250 × 10 mm, 5 mm); elution was with a linear gradient of 1:3 → 11:9 CH_3CN–H_2O over a period of 100 min at 3.0 mL/min (UV monitoring at 275 nm). One particularly active fraction (5.8 mg) was obtained from the reversed-phase HPLC fractionation. Purification of the active fraction, employing the same HPLC conditions, afforded active compound **1** (3.0 mg).

Phellamurin (1): pale yellow amorphous powder; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.35 (2H, d, J = 8.5 Hz, H-2′ and H-6′), 6.82 (2H, d, J = 8.5 Hz, H-3′ and H-5′), 6.28 (1H, s, H-6), 5.22 (1H, br t, J = 7.0 Hz, H-2″), 5.01 (1H, d, J = 8.0 Hz, H-1‴), 4.99 (1H, d, J = 11.5 Hz, H-2), 4.58 (1H, d, J = 11.5 Hz, H-3), 3.88 (1H, dd, J = 11.5, 1.9 Hz, H-6‴), 3.79 (1H, dd, J = 11.5, 6.0 Hz, H-6‴), 3.64 (1H, m, H-4‴), 3.48 (1H, m, H-5‴), 3.46 (1H, m, H-3‴), 3.38 (2H, br d, J = 7.0 Hz, H-1″), 3.26 (1H, m, H-2‴), 1.78 (3H, s, H-5″), and 1.64 (3H, s, H-4″); ^{13}C NMR (CD_3OD, 125 MHz) δ 199.6 (C-4), 164.9 (C-7), 162.4 (C-8a), 161.4 (C-4′), 159.3 (C-5), 131.9 (C-1′), 130.4 (C-2′ and C-6′), 129.2 (C-3″), 123.7 (C-2″), 116.1 (C-3′ and C-5′), 112.1 (C-8), 103.2 (C-4a), 101.4 (C-1‴), 95.3 (C-6), 85.2 (C-2), 78.3 (C-5‴), 78.0 (C-3‴), 74.8 (C-2‴), 73.9 (C-3), 71.2 (C-4‴), 62.4 (C-6‴), 25.9 (C-5″), 22.1 (C-1″), 18.0 (C-4″); positive ion CI-MS (methane) m/z (rel int) 519 $[M + H]^+$ (26), 518 $[M]^+$ (100), 356 (15), 355 (40), 337 (31).

DNA Strand Scission Assay. A DNA strand scission assay was performed as follows: 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 the final DMSO concentration at no more than 5% in the 25 μL reaction solution). Each batch of experiments included one blank control (DNA alone) and one metal control (DNA + Cu^{2+}). After being incubated at 37 °C for 60 min, the reaction mixture was treated with 5 μL of 30% glycerol–0.01% bromophenol blue and was analyzed by electrophoresis in a 1.0% agarose gel containing 0.7 μg/mL ethidium bromide. The electrophoresis was carried out in TBE buffer (89 mM Tris, 89 mM boric acid, containing 2 mM EDTA, pH 8.3) at 110–120 V for 2–3 h. Following electrophoresis, the gel was photographed under ultraviolet light.

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