

A New Antimicrobial Prenylated Benzo-lactone from the Rhizome of *Cissus cornifolia*

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ABSTRACT

Background: Medicinal plants remain one of the largest reservoirs of new bioactive compounds. In this study, a new prenylated benzo-lactone (4, 6-dihydroxy-5-methoxy-3-(1, 2, 3, 4, 5-pentahydroxypentyl)-2-benzofuran-1(3H)-one) was isolated from the acetone extracts of the rhizome of *Cissus cornifolia*. The antimicrobial activity of the compound was evaluated against some microorganisms including *Staphylococcus aureus*, *Salmonella typhi*, and *Candida albicans*. **Materials and Methods:** The acetone extracts of the rhizome of *C. cornifolia* was separated and purified by various chromatographic techniques. The structure of the isolated compound was characterized by analysis of spectral data including one and two-dimensional nuclear magnetic resonance. **Results:** The isolated compound was characterized as (4, 6-dihydroxy-5-methoxy-3-(1, 2, 3, 4, 5-pentahydroxypentyl)-2-benzofuran-1(3H)-one), it showed activity against 6 out of 10 tested clinical isolates of some microorganisms including *S. aureus*, *S. typhi*, and *C. albicans*. The inhibition zones ranged between 17 mm and 25 mm. The inhibition zones observed compare favorably with the positive control used. **Conclusion:** The compound could serve as a lead for the development of more potent antimicrobial agent. To the best of our knowledge, this is the first report of the isolation and characterization as well as antimicrobial screening of the compound.

Key words: Antimicrobial, Benzo-Lactone, *Cissus cornifolia*, Prenylated

INTRODUCTION

Cissus cornifolia (Bak.) Planch (*Vitaceae*) commonly called “ivy grape” is found in the rocky environment of bushy Savanna regions of Ghana, Nigeria, and other parts of Africa. Locally, it is called “*Riigarbirii* (robe of the monkey)” or “*Tsìwààwùùnn birii*” among the Hausa speaking people of northern Nigeria.^[1] In folk medicine, it has been used as a remedy for gonorrhoea, malaria, septic tonsil, and pharyngitis.^[1] It is also used as sedative in patients with mental derangement.

Preliminary phytochemical screening of the methanol leaf extract revealed the presence of alkaloids, saponins, flavonoids, steroids/triterpenoids, and tannins.^[2] The same authors reported the neuropharmacological activity

of the leaf extract.^[2] Furthermore, the analgesic and anti-inflammatory activities of the methanol leaf extracts of the plant have also been reported.^[3,4] Following the ethnomedicinal report on the use of the plant in the treatment of some infectious disease; we have now carried out chemical investigation into the rhizome of the plant, which led to the isolation and characterization of a new prenylated benzo-lactone with antimicrobial activity.

To the best of our search, this is the first report on the isolation of any compound from the rhizome of this plant.

MATERIALS AND METHODS

General procedures

Nuclear magnetic resonance (NMR)-spectra were recorded on a Bruker Avance spectrometer (400 MHz) for ¹H-and (100 MHz) for ¹³C-NMR, internal standard was residual solvent signal with methanol as a solvent. The IR spectrum was measured on a Shimadzu FT-IR8 400S

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fourier transform infrared spectrophotometer. For thin layer chromatography (TLC) analysis, silica gel 60 F₂₅₄ (Merck) was used, column chromatography was performed using Merck silica gel (60–120) mesh while gel filtration chromatography was performed using Sephadex LH-20 (Sigma, Spruce street, St. Louis, USA). Spots on TLC plates were visualized by spraying with 10% H₂SO₄ followed by heating at 100°C for 5 min.

Plant material

The plant material, (Rhizome and leaves of *C. cornifolia* (Bak.) Planch) was collected from Kufena Village, Zaria Kaduna State Nigeria; in the month of July 2007. The herbarium sample was identified by Mallam Musa, M. of the herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria – Nigeria; where a voucher specimen (No. 024) was deposited. The Rhizome was cut into small pieces, air dried for 14 days, and crushed into coarse powder.

Extraction and isolation

The powdered dried rhizome of *C. cornifolia* (2 kg) was extracted by maceration with acetone (3 L) at room temperature to afford the acetone extract upon evaporation of the solvent at reduced pressure using Rotary evaporator. The extract (7 g) was chromatographed on a silica gel column and eluted with gradient solvent systems of chloroform/methanol mixtures to yield 8 fractions (Fr-1–Fr-8). Compound 1 (11 mg) was obtained from fraction Fr-2 (eluate of chloroform/methanol = 9:1) upon repeated gel filtration chromatography using Sephadex LH-20 eluting with methanol.

Antimicrobial assay

The isolated compound was tested for antimicrobial activity against clinical isolates of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium ulcerans*, *Bacillus subtilis*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Candida albicans* as described previously.^[5-7] Sparfloxacin and fluconazole were used as positive standards. The agar well diffusion was used. The standardized inocula of the isolates were uniformly streaked onto freshly prepared Mueller-Hinton agar plates. Using a sterile cork borer (6 mm in diameter), appropriately labeled wells were punched into each agar plate. 0.2 mL of the appropriate compound concentration was added in each well and then allowed to diffuse into the agar. The plates were incubated at 37°C for 24 h for bacteria, while the incubation period was 48 h at 25°C for fungi. The antimicrobial activities were expressed as diameter of inhibition zones produced by the compounds. The experiment was done in duplicates.

RESULTS AND DISCUSSION

Compound 1 was isolated as a white crystalline solid; its melting point was 225–229°C. The IR spectrum indicated the presence of carbonyl (1750 cm⁻¹), hydroxyl (3350 cm⁻¹), and aromatic (1550–1480 cm⁻¹) groups. The aromatic region of the proton NMR spectrum of compound 1 revealed the presence of only a single proton signal at δ_{H} 6.99 (1H, s, H-3), suggesting the presence of a pentasubstituted benzene ring. In addition, the proton NMR [Table 1] showed a methoxy proton signal at δ_{H} 3.78 (3H, s, -OCH₃), five oxidized methine protons at δ_{H} 5.04 (1H, d, H-7), δ_{H} 3.96 (1H, t, H-1'), δ_{H} 3.59 (1H, m, H-2'), δ_{H} 3.22 (1H, m, H-3'), δ_{H} 3.68 (1H, m, H-4'), and an oxymethylene proton at δ_{H} 3.41 (2H, s, H-5'). The ¹³C spectrum of 1 revealed the presence of 14 carbon atoms, which were assigned to an ester carbonyl carbon at δ_{C} 163.30; six unsaturated carbons at δ_{C} 151.07, 149.02, 141.56, 118.01, 115.85, 109.41 three of which are obviously oxygenated (151.07, 149.02, and 141.56); five oxymethine carbons δ_{C} 81.72, 79.74, 73.04, 72.05, 70.65; an oxymethylene carbon at δ_{C} 61.06 and a methoxy carbon at 59.77 ppm. Considering that the proton NMR spectrum revealed only a single aromatic proton, the six unsaturated (olefinic) carbons could be assigned to a penta-substituted benzene ring joined to a five-membered lactone (furanone) ring, with the three significantly deshielded carbons being oxygenated while two of the remaining up-field aromatic carbon atoms form part of the furanone ring, with the remaining carbon bearing the single aromatic proton. The oxymethine carbon atom at δ_{C} 72.05 ppm together with the ester carbonyl carbon and the oxygen atom of the ester functionality complete the furanone ring structure. The four remaining oxymethine carbon atoms were assigned to the polyhydroxy carbon chain which terminated with the oxymethylene carbon at δ_{C} 61.06 ppm.

Table 1: NMR spectral data for compound 1 (CD₃OD, 400 MHz)

No.	δ_{H} [mult., J (Hz)]	δ_{C} (mult.)	HMBC
2	-	163.30 (C)	-
3	6.99 s	109.41 (CH)	3, 9, 5, 4, 2
4	-	149.02 (C)	-
5	-	141.56 (C)	-
6	-	151.07 (C)	-
7	5.04 d	72.05 (CH)	7, 2', 9, 6, 2
8	-	118.01 (C)	-
9	-	115.85 (C)	-
1'	3.96 t	79.74 (CH)	3, 1', 9
2'	3.59 m	81.72 (CH)	3', 3
3'	3.22 m	70.65 (CH)	5', 3', 4', 2'
4'	3.68 m	73.04 (CH)	3'
5'	3.41 s	61.06 (CH ₂)	3'
5-OCH ₃	3.78 s	59.77 (CH ₃)	5

HMBC=Heteronuclear multiple bond correlation; NMR=Nuclear magnetic resonance

The ^1H and ^{13}C assignments were further supported by the two-dimensional (2D) NMR data including ^1H - ^1H COSY, HSQC and heteronuclear multiple-bond correlation (HMBC) experiments. In the five carbon polyhydroxy carbon chain, ^1H - ^1H COSY experiment indicated the following connectivity; H-1'/H-2', H-2'/H-3', H-3'/H-4' suggesting that the oxymethine carbons were adjacent to one another. Furthermore, H-7 of the benzo-lactone ring showed ^1H - ^1H COSY with H-1' of the polyhydroxy carbon chain, thus establishing the connection of the chain to the ring. This connectivity was further supported by the HMBC correlations of H-1'/C-7, H-7'/C-2' and H-1'/C-9. The structure of the benzo-lactone ring was established by the HMBC correlations of H-3/C-2, C-4, C-5, C-7, C-9, and H-7/C-2, C-3, C-6, C-9. The HMBC correlation of H-6'/C-5 established the attachment of the methoxy group to C-5 carbon of the aromatic ring. These and other HMBC correlations are shown in Figure 1.

Based on these spectroscopic evidences, the structure of 1 [Figure 2] was elucidated as 4, 6-dihydroxy-5-methoxy-3-(1, 2, 3, 4, 5-pentahydroxypentyl)-2-benzofuran-1(3H)-one.

The antimicrobial activity of the isolated compound was determined against ten clinical isolates of microorganisms including *S. aureus*, *S. pyogenes*, *C. ulcerans*, *B. subtilis*, *E. coli*, *Shigella dysenteriae*, *S. typhi*, *P. mirabilis*, *P. aeruginosa*, and *C. albicans*. The result of the antimicrobial activity as expressed by the zone of inhibition of growth of the microorganism is as presented in Table 2. The compound was active against six out of the ten tested microorganisms, *B. subtilis*, *S. pyogenes*, and *S. aureus* showed the highest sensitivity to the compound with zones of inhibition values of 25 mm, 22 mm, and 20 mm, respectively. Other microorganisms that showed sensitivity to the compound are *S. typhi* (19 mm), *S. dysenteriae* (17 mm), and *C. albicans* (17 mm). However, *C. ulcerans*, *E. coli*, *P. mirabilis*, and *P. aeruginosa* were all resistant to the isolated compound. When compared to the standard antibiotic and antifungal drugs used, the standard antibiotic was active against five microorganism as was the isolated compound. However, the inhibition zones exhibited by the standard was slightly higher. The standard antifungi used were active against all three fungi tested whereas the isolated compound was

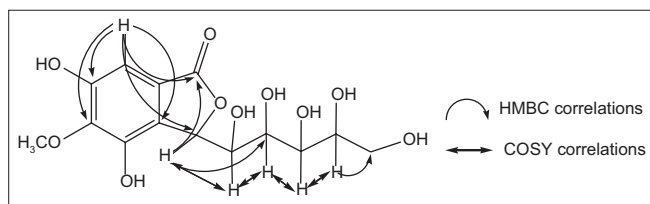


Figure 1: Some major heteronuclear multiple-bond correlation correlations of compound 1

active against only one of the tested fungi. The inhibition zone was also smaller for the isolated compound.

Prenylation is an interesting and important structural feature that has been observed to increase the pharmacological and biological activity of various bioactive compounds.^[8] It is also a well-established fact that the biological activities of various phenolic compounds have been greatly enhanced by the presence of hydroxyl groups on the ring.^[9] These two structural features play differing roles in enhancing the biological activity of molecules bearing them, the former increases the lipophilicity of a molecule while the latter increases the affinity of the molecule for proteins and may inhibit microbial enzymes.^[9] The prenyl chain in the isolated benzo-lactone has poly-hydroxy functionality which could affect the biological as well as pharmacological activities of the compound. Therefore, the observed antimicrobial activity of the isolated benzo-lactone which incorporated two interesting structural features viz; C-prenylation and poly-hydroxylation might be attributed to these structural features.

Although the antimicrobial activity of *C. cornifolia* rhizome has not been studied, the isolation of this antimicrobial lactone from the rhizome of the plant is consistent with the reported antimicrobial activity observed in other species of *Cissus* such as *C. quadrangularis*,^[10] *C. sicyoides*,^[11] *C. multistriata*,^[12] *C. vitiginea*,^[13] and *C. polyantha*.^[14]

Table 2: Antimicrobial activity of benzo-lactone

Test organism	Antimicrobial activity of benzo-lactone (50 µg/mL)		
	D5	Fluconazole (5 µg/mL)	Sparfloxacin (5 µg/mL)
<i>Staphylococcus aureus</i>	20	NT	35
<i>Streptococcus pyogenes</i>	22	NT	-
<i>Corynebacterium ulcerans</i>	0	NT	37
<i>Bacillus subtilis</i>	25	NT	40
<i>Escherichia coli</i>	0	NT	35
<i>Shigella dysenteriae</i>	17	NT	34
<i>Salmonella Typhi</i>	19	NT	-
<i>Proteus mirabilis</i>	0	35	NT
<i>Pseudomonas aeruginosa</i>	0	38	NT
<i>Candida albicans</i>	17	37	NT

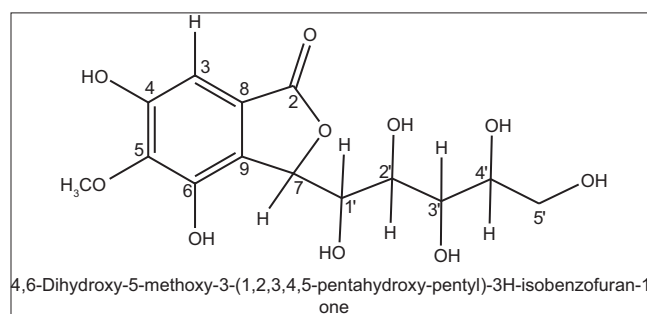


Figure 2: 4, 6-dihydroxy-5-methoxy-3-(1, 2, 3, 4, 5-pentahydroxy-pentyl)-2-benzofuran-1-(3H)-one

CONCLUSION

The present study is the first reported investigation into the chemistry of *C. cornifolia* rhizome from Nigeria. Interestingly and to the best of our knowledge, it led to the isolation of a new benzo-lactone with some fascinating structural features and antimicrobial activity.

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