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

Aliyu M. Musa, Nasir Tajuddeen[1], Abdullahi Y. Idris, Abdurahman Y. Rafindadi, Musa I. Abdullahi[2], Abubakar B. Aliyu[1], Mikhail S. Abdullahi[3], Mohammed A. Ibrahim[4]

Departments of Pharmaceutical and Medicinal Chemistry, [1]Chemistry and [4]Biochemistry Ahmadu Bello University, [3]Directorate of research, Nigeria Institute of Leather and Science Technology, Zaria, [2]Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodio University, Sokoto, Nigeria

Submitted: 12-01-2015 Revised: 18-02-2015 Published: 21-10-2015

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.

Access this article online

Website: www.phcogres.com

DOI: 10.4103/0974-8490.159572

Quick Response Code:

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 "*Tsùwààwùùn birii"* among the Hausa speaking people of northern Nigeria.[1] In folk medicine, it has been used as a remedy for gonorrhea, 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.

Address for correspondence:
Mr. Nasir Tajuddeen, Department of Chemistry Ahmadu Bello University, Zaria, Nigeria.
E-mail: ntajuddeen@yahoo.com

MATERIALS AND METHODS

General procedures

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

fourier transform infrared spectrophotometer. For thin layer chromatography (TLC) analysis, silica gel 60 F_{254} (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% H_2SO_4 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 dysentriae, 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,-OCH_3), 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 ^{13}C 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 (CD_3OD, 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 ^{1}H and ^{13}C assignments were further supported by the two-dimensional (2D) NMR data including ^{1}H-^{1}H COSY, HSQC and heteronuclear multiple-bond correlation (HMBC) experiments. In the five carbon polyhydroxy carbon chain, ^{1}H-^{1}H 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 ^{1}H-^{1}H 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. subtitlis, 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. dysentriae* (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 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)
Staphylococcus aureus	20	NT	35
Streptococcus pyogenes	22	NT	-
Corynebacterium ulcerans	0	NT	37
Bacillus subtilis	25	NT	40
Escherichia coli	0	NT	35
Shigella dysenteriae	17	NT	34
Salmonella Typhi	19	NT	-
Proteus mirabilis	0	35	NT
Pseudomonas aeruginosa	0	38	NT
Candida albicans	17	37	NT

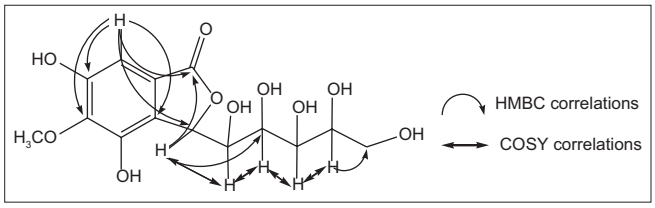

Figure 1: Some major heteronuclear multiple-bond correlation correlations of compound 1

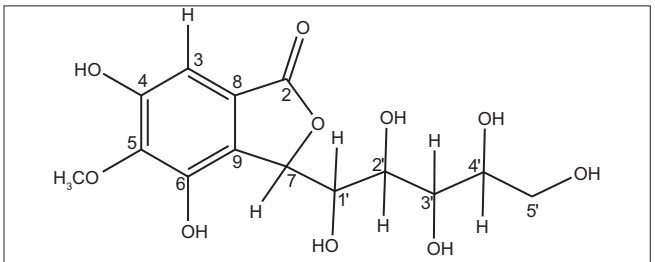
4,6-Dihydroxy-5-methoxy-3-(1,2,3,4,5-pentahydroxy-pentyl)-3H-isobenzofuran-1-one

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.

ACKNOWLEDGEMENT

We would like to acknowledge Professor Simon Gibbons of the Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, University of London, for helping to run the 1 and 2D NMR.

REFERENCES

1. Burkill HM. The Useful Plants of West Tropical Africa. 2nd ed. Kew: Royal Botanical Garden; 2000. p. 293-4.
2. Musa AM, Yaro AH, Usman H, Magaji MG, Habu, M. Phytochemical and some neuropharmacological studies on the methanol leaf extract of *Cissus cornifolia* (*Vitaceae*) in mice. Int J Pharmacol 2008;4:145-8.
3. Anuka JA, Yaro AH, Malami S, Mohammed M. Antinociceptive and antiinflammatory effects of *Cissus cornifolia* planch (*Vitaceae*). Biol Environ Sci J Trop 2009;6:56-9.
4. Malami S, Yaro AH, Aliyu M, Adamu M. Anti-nociceptive and anti-inflammatory effects of methanol root bark extract of *Cissus cornifolia* planch in laboratory animals. Niger J Pharm Sci 2009;8:66-1.
5. Volleková A, Kostálová D, Sochorová R. Isoquinoline alkaloids from *Mahonia aquifolium* stem bark are active against *Malassezia spp*. Folia Microbiol (Praha) 2001;46:107-11.
6. Ramesh LL, Umesh MK, Kirankumar S, Jayashree VH. Phytochemical screening and *in vitro* antimicrobial activity of *Typha angustifolia* linn leaves extract against pathogenic gram-negative microorganisms. J Pharm Res 2013;6:280-3.
7. Bonev B, Hooper J, Parisot J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. J Antimicrob Chemother 2008;61:1295-301.
8. Epifano F, Genovese S, Menghini L, Curini M. Chemistry and pharmacology of oxyprenylated secondary plant metabolites. Phytochemistry 2007;68:939-53.
9. Prusky D, Keen NT. Involvement of preformed antifungal compounds in the resistance of subtropical fruits to fungal decay. Plant Dis 1993;77:114.
10. Chidambara Murthy KN, Vanitha A, Mahadeva Swamy M, Ravishankar GA. Antioxidant and antimicrobial activity of *Cissus quadrangularis* L. J Med Food 2003;6:99-105.
11. Flavio LB, Griesiele LP, Dani LD, Prado OF, Roberto BB, Aparicio GC. Evaluation of the antidiabetic and antimicrobial activity of *Cissus Sicyoides*. Braz Arch Biol Technol 2002;45:21-5.
12. Adegoke SA, Opata OM, Olajide JE. Antimicrobial activity of the aqueous, methanol and chloroform leaf extracts of *Cissus multistriata*. Afr J Biotechnol 2002;9:1168-72.
13. Subramani V, Kamaraji M, Ramachandran B, Jerome JJ. Screening of phytochemical constituents, trace metals and antimicrobial efficiency of *Cissus vitiginea*. Int J Phytopharmacy 2014;4:96-8.
14. Sani YM, Musa AM, Abdullahi SM, Atiku I, Abdullahi MS, Hanwa UA, *et al*. Phytochemical and antimicrobial studies of methanol crude extract of the leaf of *Cissus polyantha*. Niger J Pharm Sci 2013;12:35-40.

Cite this article as: Musa AM, Tajuddeen N, Idris AY, Rafindadi AY, Abdullahi MI, Aliyu AB, *et al*. A New Antimicrobial Prenylated Benzo-lactone from the Rhizome of *Cissus cornifolia*. Phcog Res 2015;7:363-6.

Source of Support: Nil, **Conflict of Interest:** None declared.