

NOTE

In vitro DPPH Radical Scavenging Activity of α -Pyrones from *Odontonema strictum* (Acanthaceae)

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Abstract: An ethyl acetate leaf extract from *Odontonema strictum* has been reported to have potent antihypertensive activity by inhibiting coronary artery contractions in porcine heart. However, the phytochemistry of the active fraction was unknown. Here we report, for the first time, the isolation and characterization of four known α -pyrones from the active fraction. The antioxidant activity of umuravumbolide ($IC_{50} = 55.7 \pm 0.027 \mu\text{g/mL}$), deacetylumuravumbolide ($IC_{50} = 0.24 \pm 0.0002 \mu\text{g/mL}$), dideacetylboronolide ($IC_{50} = 149 \pm 0 \mu\text{g/mL}$) and deacetylboronolide ($IC_{50} = 24 \pm 0 \mu\text{g/mL}$) was evaluated *in vitro* against 2,2-diphenyl-1-picrylhydrazyl radicals. Ascorbic acid was used as a positive control ($IC_{50} = 1.73 \times 10^{-3} \pm 0.3 \mu\text{g/mL}$). The presence of 6-substituted 5,6-dihydro- α -pyrones and phenylpropanoid glucosides in the active fraction was suggested to be responsible for the antihypertensive activity. This is the first time that the antioxidant potential of these phytochemicals has been evaluated, and the results indicate that *O. strictum* has potential as an herbal medicine. Thus, further chemotaxonomic studies among the genera *Odontonema* and *Tetradenia*, a known source of α -pyrones, are recommended.

Key words: α -pyrone, antioxidant, *Odontonema strictum*, anti-hypertensive

1 Introduction

Phytochemicals containing 6-substituted 5,6-dihydro- α -pyrones have been identified in many families in the plant kingdom, including Lamiaceae, Piperaceae, Lauraceae and Annonaceae^{1,2)}. This class of secondary metabolites exhibits a large spectrum of biological activities, including antimicrobial, antifungal and cytotoxicity against human cancer cells^{3,4)}. In addition, these compounds have been reported to inhibit HIV protease⁵⁾. The genus *Tetradenia* (Lamiaceae) is a known source of α -pyrones. Boronolide was isolated from *T. barbarae* and *T. fruticosa*⁶⁾. *T. riparia*, known as Mutozo in the Democratic Republic of the Congo (DRC) and Umuravumba in Rwanda, is used as a folk medicine to treat malaria, diarrhoea, fever, tuberculosis, and pain⁷⁻¹⁰⁾. Umuravumbolide (1), deacetylumuravumbolide (2), boronolide, dideacetylboronolide (3) and deacetylboronolide (4) were isolated from *T. riparia* and characterised by Van Puyvelde and co-workers in 1979 (Fig. 1)¹¹⁾. Since then, several researchers have synthesized and evaluated the pharmaceutical potential of these active terpenes¹²⁻¹⁵⁾.

Odontonema strictum (Fig. 2) of the family Acanthaceae is a medicinal plant from tropical regions. Traditional medicine practitioners in Burkina Faso use the aqueous extract of the leaves to treat high blood pressure. The bio-guided fractionation of the leaf extract indicated that the active molecules against hypertension are abundant in the ethyl acetate (EtOAc) extract; however, the phytochemistry of the active fraction was unknown¹⁶⁻¹⁸⁾.

The aim of this study was to isolate and characterize secondary metabolites from the EtOAc leaf extract of *O. strictum* and to evaluate, for the first time, the DPPH radical scavenging activity. Compared to other models for scavenging free radicals, the method used here is simple and rapid¹⁹⁾.

The identification of 6-substituted 5,6-dihydro- α -pyrones and phenylpropanoid glucosides (verbascoside 5 and isoverbascoside 6, Fig. 1) in the EtOAc fraction may support the previously observed anti-hypertensive proper-

Abbreviations: DPPH; 2,2-diphenyl-1-picrylhydrazyl, DCM; dichloromethane, HRMS; high resolution mass spectrometry, IC_{50} ; 50% inhibition concentration

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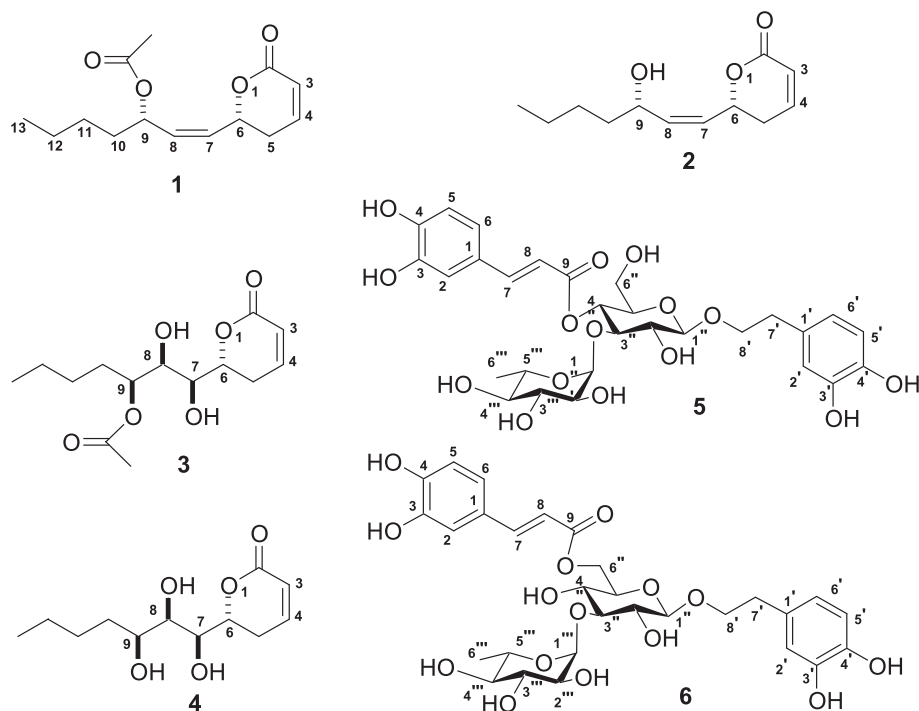


Fig. 1 Chemical structures of antioxidant molecules from the EtOAc leaf extract of *O. strictum*.



Fig. 2 *O. strictum*.

ties of the leaf extract of *O. strictum*. This is the first time that these compounds have been isolated from genus *Odontonema*. Thus, there is a need to investigate the chemotaxonomy between *Odontonema* and *Tetradenia*, a known source of α -pyrones.

2 Experimental Procedures

2.1 Solvents and reagents

MeOH and H₂O in high performance liquid chromatography (HPLC) grade were purchased from Kishida Chemical

(Osaka, Japan). DPPH was purchased from TCI (Tokyo, Japan). Methanol-d₄ for nuclear magnetic resonance (NMR) analysis was purchased from Eurisotop (Saint-Aubin, France).

2.2 Instruments

Reverse phase-HPLC: RP-HPLC system (Shimadzu, Kyoto, Japan) equipped with a quaternary pump (LC-20A $\times$ 2 units), a degasser, a thermostatic auto-sampler (SIL-20AC), a photodiode array detector (DAD SPD-20AV) and Column oven (CTO-20AC) was used.

UV analysis: JASCO (Tokyo, Japan) V-730BIO Spectrophotometer was used. FTIR analysis: JASCO FT/IR-4100 (Fourier Transform Infrared Spectrometer) was used. Optical rotation analysis: JASCO P-2200 Polarimeter was used. NMR: NMR spectra were recorded on a JEOL (Tokyo, Japan) JNM-ECA 500 spectrometer (500 MHz). ESI-MS: ESI-MS spectra were recorded on a JEOL JMST100LC instrument.

2.3 Plant materials

Plant specimens (leaves, stems and roots) were collected in Kinshasa (DRC) in June-July 2021 and dried in the shade at room temperature for two weeks. The collected plant materials were compared to a previous voucher specimen PLL3 identified by Dr. Chuba and deposited in the *Herbarium* of the Department of Biological Sciences at the University of Zambia and later sent to the Laboratory at Sophia University.

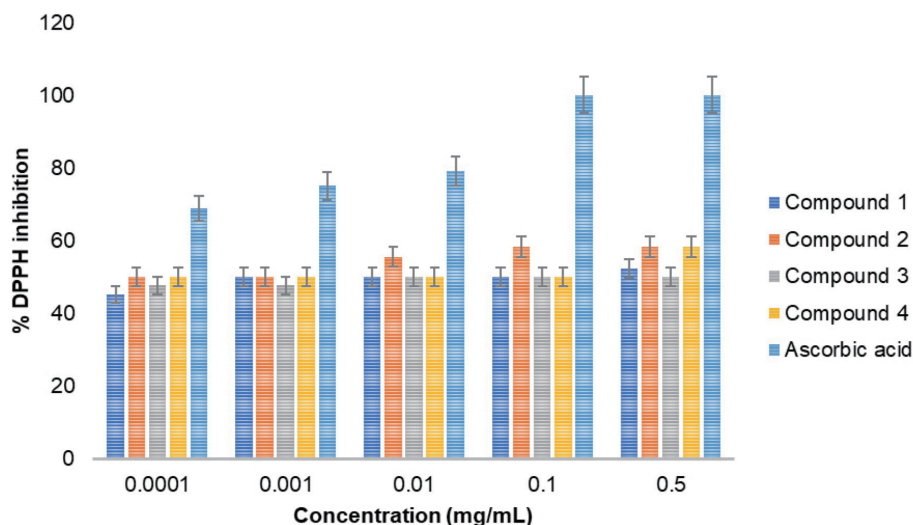


Fig. 3 Inhibitory activity of α -pyrones from the EtOAc leaf extract of *O. strictum*.

2.4 Preparation, extraction, and isolation procedures

Dried crushed samples, 365 g of leaves and 89 g of roots were extracted separately three times with a mixture of 1 L of methanol (MeOH) and 1 L of DCM at room temperature for 72 hours ($3 \times$, 2L). The MeOH-DCM solution was filtered through a cotton hood and evaporated under a vacuum at $<40^\circ\text{C}$ to afford 47.45 g of leaf crude extract (13% of yield) and 5 g of root crude extract (6% of yield). Further, crude extracts (leaf and root, separately) were dissolved in H_2O (20% MeOH) and later fractionated by *n*-hexane ($3 \times$), DCM ($3 \times$), EtOAc and *n*-butanol (*n*-BuOH, $3 \times$).

The EtOAc fraction (10.97 g) was purified by normal phase silica gel column chromatography and the gradient system for the elution was *n*-hexane-DCM-EtOAc-MeOH to afford 5 subfractions (E-1 to E-5). The first three subfractions were mixed and renamed E1-3 (3210 mg) and purified by RP-HPLC using a gradient system of MeOH (0.1% of formic acid) (A) and H_2O (0.1% of formic acid) (B) in 30 min. Sample injection was 100 μL , the flow rate 1.0 mL/mL and the peaks of interest were monitored at λ_1 230 nm and λ_2 320 nm. Four subfractions E1-3A, E1-3B, E1-3C and E1-3D were collected, and solvent evacuated under vacuum. Compound 1 (25 mg) was identified and characterized from E1-3A, compound 2 (20 mg) from E1-3B, compound 3 (8 mg) from E1-3C and finally E1-3D afforded compound 4 (4 mg).

2.5 Isolation RP-HPLC conditions

The isolation of compounds from E1-3 was performed using a RP-HPLC system equipped with a quaternary pump (LC-20A $\times 2$ units), a degasser, a thermostatic auto-sampler (SIL-20AC), a photodiode array detector (DAD SPD-20AV) and Column oven (CTO-20AC). The analysis was carried out with C_{18} column (5C₁₈-AR-II, 250 mm) and a C_{18}

guard column (5C₁₈-MS-II, 20 mm). The column was set at 40°C and the injection volume, 250 μL . The binary phase consisted of 0.1% of formic acid in water (B) and 0.1% of formic acid in MeOH (A). The flowrate was kept constant at 1.0 mL/min for a total run time of 38 min. Starting with 95% of B, in 5 min 50% of B and kept in 50% of B for 5 min, 50% to 0% of B in 20 min, and 0% to 95% of B in 38 min. Peaks of interest were monitored at λ 320 nm.

3 Results

The EtOAc fraction (E1-3, 3210 mg) was subjected to RP-HPLC, and 6 fractions were collected (A-F). Verbascoside and isoversbacoside were previously isolated and identified from fractions A and B²⁰. Fractions C to F, named E1-3A, E1-3B, E1-3C and E1-3D, were submitted for spectroscopic analysis (UV, IR, NMR, MS).

Compound 1 (25 mg) was isolated from the least polar fraction F as a colourless oil with $[\alpha]_D^{25} = +37$ (c 0.025 CHCl_3). HRMS calcd for $\text{C}_{14}\text{H}_{20}\text{NaO}_4$, 275.1259, found 275.1257 $[\text{M} + \text{Na}]^+$, suggesting 5 degrees of unsaturation. UV λ_{max} nm: 241 nm. IR (ATR): 3428, 1707, 1251, and 1020 cm^{-1} (21). COSY, HMQC, HMBC and NOESY correlations were established and compound 1 was found to be in good agreement with umuravumbolide (5,6-dihydro-6-(3-acetoxy-1-heptenyl)-2-pyrone) (Fig. 3), a compound previously isolated in 1979 from *T. riparia*^{11, 13}.

Compound 2 (20 mg) was obtained from fraction E as a colourless oil with $[\alpha]_D^{25} = -5.7$ (c 0.025, CHCl_3). HRMS calcd for $\text{C}_{12}\text{H}_{18}\text{NaO}_3$, 233.1154, found 233.1163 $[\text{M} + \text{Na}]^+$, suggesting 4 degrees of unsaturation. UV λ_{max} nm: 242 nm. IR (ATR): 3428, 1707, 1251, and 1020 cm^{-1} . In a search of the literature, compound 2 was identified as deacetylumuravumbolide, isolated for the first time in 1979 from *T.*

Table 1 Free DPPH radical scavenging activities of α -pyrones.

Compounds	IC ₅₀ (μ g/mL)
Umuravumbolide (1)	55.7 $\pm$ 0.027
Deacetylumuravumbolide (2)	0.24 $\pm$ 0.021
Dideacetylboronolide (3)	149 $\pm$ 0
Deacetylboronolide (4)	24 $\pm$ 0
Ascorbic acid	1.73 $\times 10^{-3}$ $\pm$ 0.0003

*riparia*¹¹⁾.

Compound **3** was obtained from fraction D as a colourless oil with $[\alpha]_D^{25} = +43$ (*c* 0.025 CHCl₃). HRMS calcd for C₁₄H₂₂NaO₆, 309.1314, found 309.1345 [M + Na]⁺, suggesting 4 degrees of unsaturation. UV $\lambda_{\max}$ nm: 243 nm. IR (ATR): 1707, 1251, and 1020 cm⁻¹. The NMR data of compound **3** was in good agreement with dideacetylboronolide, isolated from *T. riparia* by Van Puyvelde and co-workers in 1979¹¹⁾.

Compound **4** was obtained from fraction C as a colourless oil with $[\alpha]_D^{25} = +28$ (*c* 0.025, CHCl₃). HRMS calcd for C₁₂H₁₉O₅, 243.1233, found 243.1245 [M - H]⁻, suggesting 3 degrees of unsaturation. UV $\lambda_{\max}$ nm: 248 nm. IR (ATR): 3428, 1707, 1251, and 1020 cm⁻¹. The NMR data of compound **4** was in full agreement with the structure of deacetylboronolide, first isolated by Van Puyvelde and co-workers in 1979¹¹⁾.

The antioxidant activity of compounds **1-4** was then evaluated *in vitro* against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and the results are shown in Table 1. The IC₅₀ values of the α -pyrones from the leaf extract of *O. strictum* ranged from 0.24 to 149 μ g/mL.

4 Discussion

Deacetylumuravumbolide **2** was found to be the most potent DPPH free radical scavenger (IC₅₀ = 0.24 $\pm$ 0.21 μ g/mL) of the compounds examined. All 4 compounds exhibited notable hydrogen-donating ability, evidenced by the inhibition of 50% of DPPH radicals at a very low concentration (0.0001 mg/mL) (Fig. 3). These results clearly confirm that α -pyrones are potent antioxidant inhibitors, and may play a key role in preventing oxidative stress in various pathological conditions, such as hypertension, inflammation, cancer, aging, Parkinson's disease, Alzheimer's disease, and rheumatoid arthritis²²⁻²⁵⁾.

In 2005, a group of researchers from Burkina Faso evaluated the effect of an EtOAc leaf extract from *O. strictum* using a rat model. The extract inhibited coronary artery contractions in porcine heart (IC₅₀ = 1854 mg/kg), and it was suggested that the active molecules were flavones, a type of flavonoid. The extract showed low toxicity accord-

ing to the Hodge and Sterner scale and acted as an antihypertensive agent through its vasodilator effect. However, no pure compound was isolated and characterized^{26, 27)}. We report here, for the first time, the presence of umuravumbolide **1**, deacetylumuravumbolide **2**, dideacetylboronolide **3** and deacetylboronolide **4** in the EtOAc leaf extract of *O. strictum*. Two phenylpropanoid glucosides, verbascoside **5** (IC₅₀ = 0.09 $\pm$ 0.03 μ g/mL) and isoverbascoside **6** (IC₅₀ = 0.16 $\pm$ 0.07 μ g/mL) were previously isolated from the same fraction¹⁹⁾. It is possible that these six potent antioxidant molecules may act individually or in a synergetic manner to produce the observed antihypertensive activity^{28, 29)}. Further *in vitro* and *in vivo* investigations are needed to confirm this hypothesis.

Compounds containing α,β -unsaturated δ -lactones have been demonstrated to exhibit anti-cancer and anti-microbial activities. Interestingly, α -pyrones and fostriecin, a phosphate monoester originally isolated from *Streptomyces pulveraceus*, have shown similarities in chemical structure. According to Buck and co-workers, the lactone moiety is suggested to be a critical electrophile part of the pharmacophore. The high ability to inhibit protein phosphatase 2A was attributed to the unsaturated lactone^{30, 31)}. Sabitha and co-workers evaluated the anticancer activity of umuravumbolide **1** and deacetylumuravumbolide **2** on HeLa, MDA-MB-231, MCF7 and A549 cancer cell lines. Umuravumbolide **1** showed the most potent inhibitory effect on the tested human tumour cell lines, with IC₅₀ values of 0.24-1.92 μ M, while deacetylumuravumbolide **2** showed higher inhibitory activity (IC₅₀ values of 1.54-8.19 μ M) towards A549, HeLa and MCF7 cancer cell lines at 24 h incubation¹²⁾.

The antimicrobial activity of umuravumbolide **1** and deacetylumuravumbolide **2** was tested against eight food-borne bacterial pathogens (*Shigella sonnei*, *S. flexneri*, *Salmonella enterica* subsp. *enterica*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Listeria innocua*), and moderate antimicrobial activity was observed (IC₅₀ of 176.1-521.4 μ g/mL and 212.9-637.7 μ g/mL, respectively)³²⁾.

The above results reveal that *O. strictum*, as a novel source of 6-substitued 5,6-dihydro- α -pyrones, has potential as a nutraceutical medicine. In fact, this plant is already used in folk medicine without negative side effects.

5 Conclusion

This study identified, for the first time, four 6-substitued 5,6-dihydro- α -pyrones and two phenylpropanoid glycosides in the EtOAc extract of *O. strictum*. The potent antioxidant molecules umuravumbolide **1**, deacetylumuravumbolide **2**, dideacetylboronolide **3** and deacetylboronolide **4**, as well as verbascoside **5** and isoverbascoside **6** may play a key

role in the anti-hypertensive activity observed previously by researchers in Burkina Faso²⁶). The isolated compounds **1** to **4** were evaluated in the laboratory conditions for their ability to scavenge DPPH free radicals. IC₅₀ values ranged between 0.24 to 149 μ g/mL. Deacetylmuravumbolide **2** was found to be the most potent antioxidant α -pyrones. This is the first report on the antioxidant property α -pyrones in the genus *Odontonema*.

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Supporting Information

This material is available free of charge via the Internet at doi: 10.5650/jos.ess22319

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