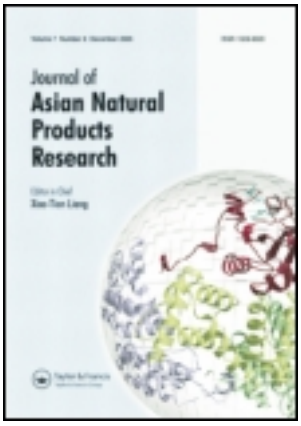

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A new depsidone and antibacterial activities of compounds from *Usnea undulata* Stirton

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NOTE

A new depsidone and antibacterial activities of compounds from *Usnea undulata* Stirton

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Usnea undulata Stirton (Usneaaceae) is a fruticose lichen used locally in ethnoveterinary medicine to treat mammary infections in cattle while human beings use it for the treatment of wounds in Eastern Cape, South Africa. Bioactivity-guided fractionation of its extracts led to the isolation and characterization of a new depsidone, 2′-*O*-methylhypostictic acid (**8**), together with seven known compounds, i.e. methyl β-orsellinate (**1**), norstictic acid (**2**), menegazziaic acid (**3**), (+) usnic acid (**4**), hypoconstictic acid (**5**), salazinic acid (**6**), and galbinic acid (**7**). The structures of the compounds were elucidated on the basis of their spectral analysis including homo- and hetero-nuclear correlation NMR experiments (COSY, NOESY, HMQC, and HMBC) and mass spectra as well as by comparison with available data in the literature. The minimum inhibitory concentrations (MICs) values of the compounds against six bacteria were determined. Compound **8** showed inhibitory activity against *Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcus epidermidis* with MICs of 31, 62.5, 62.5 μg/ml, respectively. (+) Usnic acid was most active against *B. subtilis*, *B. cereus*, *Staphylococcus aureus*, and *Escherichia coli* with MICs of 8, 8, 31, and 31 μg/ml, respectively, while other compounds exhibited moderate activity.

Keywords: *Usnea undulata*; antibacterial activity; depsidones; (+) usnic acid; β-orcinol

1. Introduction

Lichens are formed through symbiosis between fungi (mycobionts) and algae and/or cyanobacteria (photobionts). The morphology of these organisms can differ perceptibly, from crustose lichens growing on rocks to foliose or fruticose lichens growing on tree trunks, soil, or other substrates. *Usnea undulata* Stirton (Usneaaceae) is a fruticose lichen that grows epiphytically on tree trunks and branches in the moist Hogsback forest of the Eastern Cape Province, South Africa.

Lichens have been used for medicinal purpose since ancient times and are known to produce unique secondary metabolites. Many species of *Usnea* are used medicinally [1–4]. The *Formulary of Al-kindi*, *ca.* AD 850, documents the use of *Usnea*. Because of their long tradition of use as antimicrobial agents by indigenous peoples, lichens attracted attention early in the search for antibiotics. By 1944, as many as half the lichens studied contained lichen acids that exhibited variable antibiotic activity [5]. Among the lichen substances, the most widely distributed

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and the most extensively investigated one, without doubt, is usnic acid.

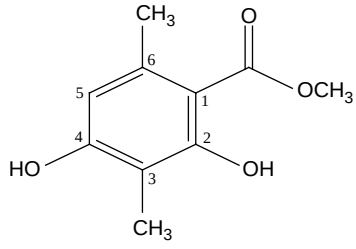
U. undulata is used locally in ethnoveterinary medicine to treat mammary infections in cattle while human beings use it for the treatment of wounds. While a few phytochemical works have been carried out on the Australian and Indian species [6,7], to our knowledge there was no report on the constituents of South African species before the commencement of this work. The purpose of this study was to isolate and identify bioactive compounds from *U. undulata*.

Bioactivity-guided fractionation of its extracts led to the isolation and characterization of a new depsidone 2′-*O*-methylhypostitic acid and seven known compounds, i.e. methyl β-orsellinate, norstictic acid, menegazziaic acid, (+) usnic acid, hypoconstictic acid, salazinic acid, and galbinic acid (Figure 1). The minimum inhibitory concentration (MIC) of each compound was determined by the microplate serial dilution method using 96-well microtiter plates [8] against four Gram-positive (*Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) and two Gram-negative (*Escherichia coli* and *Shigella sonnei*) bacteria.

2. Results and discussion

The known compounds (**1**–**7**) were identified by comparison of their physical and spectral data with literature values as methyl β-orsellinate [9], norstictic acid [6,10], menegazziaic acid [11], (+) usnic acid [12], hypoconstictic acid [11], salazinic acid [6,12], and galbinic acid [6].

Compound **8** was obtained as a white amorphous solid with optical rotation $[\alpha]_D$



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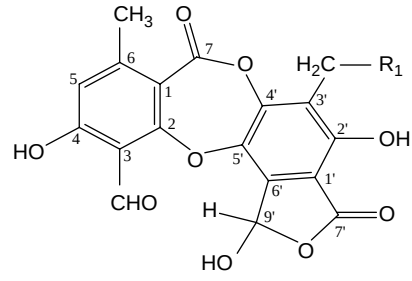
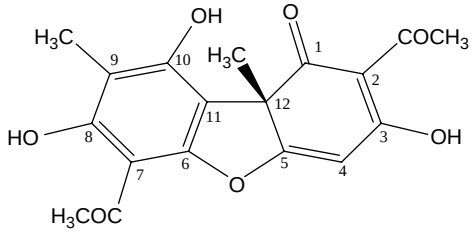


2. R_1 = H, **6**. R_1 = OH
7. R_1 = OAc



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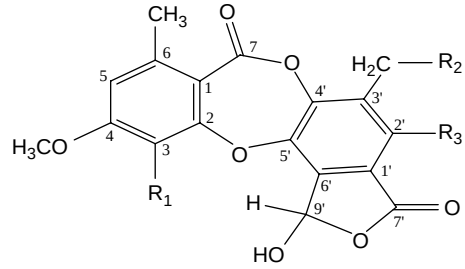

3. R_1 = OH, R_2 = H, R_3 = OH
5. R_1 = CH_3, R_2 = OH, R_3 = OH
8. R_1 = CH_3, R_2 = H, R_3 = OCH_3

Figure 1. Structures of compounds **1**–**8** isolated from *U. undulata*.

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data and key HMBC correlations for compound **8** (δ values in $CDCl_3$).

Position	^{1}H	^{13}C	HMBC
1		115.3	
2		161.5	
3		117.1	
4		151.1	
5	6.45 (s)	114.6	C-1, C-3, C-4, 6-CH_3
6		140.0	
7		159.7	
1′		107.9	
2′		156.7	
3′		119.5	
4′		153.4	
5′		140.0	
6′		135.0	
7′		166.8	
8′	2.42 (s)	8.3	C-2′, C-3′, C-4′
9′	6.52 (s)	107.9	C-1′, C-5′, C-7′
3-CH_3	2.11 (s)	8.6	C-3
4-OCH_3	3.80 (s)	55.3	C-4
6-CH_3	2.58 (s)	19.6	C-1, C-5, C-6
2′-OCH_3	3.82 (s)	61.6	C-2′

-4.8 ($c = 0.21$ in $CHCl_3$ at 27°C). The UV spectrum showed the absorption maxima at 314 and 239 nm. The IR spectrum (liquid film) showed absorption bands at 3540 cm^{-1} for hydroxyl and 1745 cm^{-1} for carbonyl group corresponding to the lactone five-membered ring [12], 1695 cm^{-1} for seven-membered ester carbonyl group. The HR-ESI(+) TOF-MS showed $[M + H]^+$ peak at *m/z* 387.2891 which revealed the molecular formula $C_{20}H_{18}O_8$.

The ^{1}H and ^{13}C NMR spectral data (Table 1) were identical to hypostatic acid [11] except for the presence of one more methoxyl group. The ^{1}H NMR spectrum exhibited signals corresponding to three aromatic methyl groups at δ 2.11 (3-CH_3), 2.58 (6-CH_3), and 2.42 (3′-CH_3), two methoxyl protons at δ 3.82 (4-OCH_3), and 3.80 (2′-OCH_3), methine proton at δ 6.52 (9′-H). The presence of only one aromatic proton at δ 6.45 (H-5) was supportive of the ether bridge between C-2 and C-5′ leading to a depsidone skeleton [13].

The ^{13}C NMR spectrum (Table 1) of compound **8** showed 20 carbons including two carbonyl group signals at δ 166.8 and 159.7, two methoxyl carbons resonated at δ 55.3 that meant that neither or only one of the *ortho* position was substituted and at δ 61.6 indicating that both positions ortho to the methoxyl were substituted [14]. It showed three methyl signals at δ 8.6 (3-CH_3), 19.6 (6-CH_3), and 8.3 (C-8′). The degree of protonation of each atom was determined by DEPT experiment, while the HMQC spectrum allowed the complete correlation of the protonated carbon resonances with those of the ^{1}H NMR spectrum. The determined molecular formula and the above NMR data analysis and comparison with those in literature [11] suggested that compound **8** was hypostictic acid with a methoxyl group. The positions of groups were confirmed by HMBC correlations.

In the HMBC experiment (Table 1), the aromatic proton at δ 6.45 (H-5) showed HMBC correlations with C-1 (115.3), C-3 (117.1), C-4 (δ 151.1), and 6-CH_3 (19.6).

The methine proton (δ 6.52, 9′-H) showed HMBC correlations with the carbonyl carbon C-7′ (δ 166.8) and quaternary carbons C-1′ (δ 107.9) and C-5′ (δ 140.0). The methoxyl proton (δ 3.80) showed the HMBC correlation with oxygenated carbon C-4 (δ 151.1). This was also confirmed by NOE observed between the methoxyl and H-5. The second methoxyl also showed the HMBC correlation with oxygenated carbon at C-2′ (δ 156.7). On this basis, compound **8** was determined as 2′-*O*-methylhypostictic acid. This compound appears to be novel.

The inhibitory activity of *n*-hexane, ethyl acetate (EtOAc), and isolated pure compounds was demonstrated using bioautographic assay on thin layer chromatography (TLC) [15,16]. All compounds showed significant antibacterial activity against *B. subtilis*. Compound **8** showed inhibitory activity (Table 2) against *B. cereus*, *B. subtilis*, and *S. epidermidis* with the MICs of 31, 62.5, and 62.5 μg/ml, respectively. (+) Usnic acid was most active against *B. subtilis*, *B. cereus*, *S. aureus*, and *E. coli* with MICs of 8, 8, 31, and 31 μg/ml respectively, while others exhibited moderate activity.

In addition to antimicrobial activity usnic acid has been shown to exhibit antiviral, antiprotozoal, antiproliferative, anti-inflammatory, and analgesic activities [17]. The antibacterial activity of the isolated new and known compounds in this study coupled with the well-reported medicinal importance of usnic acid, which is abundant in this species, may justify the traditional uses of *U. undulata* for the treatment of various infectious diseases.

The most unique lichen and usnea metabolites belong to the chemical classes of depsides, depsidones, and benzofurans [17], and therefore chemically *U. undulata* may be viewed as typical of the genus.

Table 2. Antibacterial activities of compounds **1**–**8** isolated from *U. undulata*.

	MIC of compounds (μg/ml)									
Bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	*	DMSO
Gram (+)										
B. cereus	125	62.5	250	8	250	125	62.5	31	4	>250
B. subtilis	125	62.5	250	8	250	125	62.5	62.5	4	>250
S. aureus	62.5	250	>250	31	250	>250	250	250	2	>250
S. epidermidis	125	125	>250	>250	31	>250	>250	62.5	2	>250
Gram (−)										
E. coli	62.5	125	31	31	62.5	125	125	125	4	>250
S. sonnei	125	250	>250	>250	>250	>250	>250	125	4	>250

Note: *Streptomycin.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 polarimeter in different solvents. General laboratory solvents were distilled from glass before use. The UV spectra were run in methanol using a Beckman DU-7400 spectrophotometer. The IR data for all compounds were obtained from thin films on NaCl disks using a Perkin-Elmer FT-IR spectrometer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AMX400 instrument, with chemical shift data reported in ppm relative to the solvent used. Two-dimensional NMR spectra were recorded on the same instruments using field gradient broad band inverse (inverse) probe. Mass spectra were determined by TOF-MS at Micromass LCT premier K146 and EI-MS at Micromass 70-70E. Vacuum liquid chromatography (VLC) and column chromatography (CC) were performed using Merck silica gel 60H (15 μm) and silica gel (0.063–0.2 mm), respectively. Silica gel 60 F_{254} coated on aluminum plates for TLC and silica gel 60 F_{254} coated on glass plates (20 cm × 20 cm) for preparative TLC, all were supplied by Merck (Darmstadt, Germany). Sephadex LH-20 (25–100 μm) for gel filtration chromatography (GFC) was obtained from Fluka/Sigma (Steinheim, Germany; Product of Sweden).

3.2 Plant materials

U. undulata was collected from trunks and branches of the trees in the moist Hogsback forest of the Eastern Cape Province, South Africa and air-dried. The lichen was confirmed by Dr Simone Houwhoff, Kew Botanical Garden, London. A voucher specimen was prepared (Afol/03) and deposited at the Giffen Herbarium, UFH.

3.3 Extraction and isolation

The dried lichen (750 g) was extracted by shaking in methanol at room temperature for 3 days. The extract was filtered and evaporated to a gummy mass in a rotary evaporator under vacuum at 40°C. The gummy mass (57.9 g) was partitioned between water and *n*-hexane and the aqueous part was further portioned between EtOAc and water.

The *n*-hexane extract (6.01 g) was fractionated by VLC over silica gel 60 H (15 μm), eluting with *n*-hexane–EtOAc (0–100%) and then with EtOAc–MeOH (0–15%). A total of 26 fractions (200 ml each) were collected, of which 24 showed antimicrobial activity. The fractions eluted with 25% and 30% EtOAc in *n*-hexane were combined (300 mg) and chromatographed over a Sephadex LH-20 column using $CHCl_3$ (400 ml) followed by $CHCl_3$:MeOH (95:5, 500 ml, 25 fractions, 25 ml each) to give methyl β-orsellinate (**1**, 10 mg). The fractions (600 mg) obtained with 35–40% EtOAc in *n*-hexane were combined (200 mg) and subsequently subjected to GFC (Sephadex LH-20), eluted with $CHCl_3$ and then $CHCl_3$–MeOH mixtures. It was finally purified by CC over silica gel (0.063–0.2 mm) using $CHCl_3$ and $CHCl_3$–MeOH (95:5, v/v) gradient to yield norstictic acid (**2**, 100.0 mg). The fraction (200 mg) obtained with 45% EtOAc in *n*-hexane and similarly fractionated by GFC (Sephadex LH-20) was eluted with $CHCl_3$ and then $CHCl_3$–MeOH mixture to give six fractions. Subfraction 02 was purified over a silica gel (0.063–0.2 mm) CC using $CHCl_3$–MeOH (90:5, v/v) for elution and afforded menegazziaic acid (**3**, 15.2 mg). The fractions (1.2 g) eluted with 50–55% EtOAc in *n*-hexane was similarly subjected to GFC (Sephadex LH-20) to yield usnic acid (**4**, 270.9 mg) and hypoconstictic acid (**5**, 7 mg). Compounds were finally purified by recrystallization. The fractions obtained with 65–75% EtOAc in *n*-hexane

were combined (700 mg) and subjected to GFC (Sephadex LH-20) and CC over silica gel (0.063–0.2 mm) using $CHCl_3$:MeOH (90:10, v/v) gradient to yield salazinic acid (**6**, 48.5 mg). Finally, the fractions obtained with 7–15% MeOH in EtOAc were combined (900 mg) and further treated by GFC and CC over silica gel (0.063–0.2 mm) using $CHCl_3$–MeOH (90:10, v/v) gradient to yield galbinic acid (**7**, 28.5 mg) and 2′-*O*-methylhypostictic acid (**8**, 70 mg).

The EtOAc extract (15.58 g) was subjected to the same fractionation procedure as above. A total of 30 fractions (250 ml each) were collected, of which 29 fractions showed activity. The fraction (1.6 g) eluted with 40–45% EtOAc in *n*-hexane gave needle-like crystals. It was finally purified by recrystallization which was usnic acid (**4**, 1.3 g). The fractions obtained with 55–65% EtOAc in *n*-hexane were combined (700 mg) and subjected to GFC using $CHCl_3$:MeOH (90:10, v/v) gradient to give three fractions and then purified by elution through a silica column using $CHCl_3$:MeOH (90:10, v/v) to give menegazziaic acid (**3**, 20.2 mg) and hypoconstictic acid (**5**, 10 mg).

*3.3.1 2′-O-methylhypostictic acid (**8**)*

A colorless amorphous solid, $[\alpha]_D$ −4.8 ($c = 0.21$ in $CHCl_3$). UV λ_{max} (MeOH) nm: 314, 239 nm. IR ν_{max} (liquid film): 790, 896, 934, 988, 1018, 1450, 1695, 1745, 2970, 3540 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR spectral data are shown in Table 1; HR-ESI (+) TOF-MS: *m/z* 387.2891 $[M + H]^+$.

3.4 Antibacterial assay

Laboratory strains of *B. subtilis*, *B. cereus*, *S. aureus*, *S. epidermidis*, *E. coli*, and *S. sonnei* were obtained from the Microbiology Department, Rhodes University, South Africa. During the extraction and purification procedure, bioautographic assay as described earlier [15,16] was performed on TLC plates using *B. subtilis*. The MIC values of the pure compounds were determined with a microplate dilution method as described earlier [8,16] against four *Gram*-positive (*B. subtilis*, *B. cereus*, *S. aureus*, and *S. epidermidis*) and two *Gram*-negative (*E. coli* and *S. sonnei*) bacteria using 96-well microtiter plates. MIC values were recorded as the lowest concentration resulting in complete inhibition of bacterial growth. Each treatment was replicated thrice. Streptomycin, solvents, and sample-free solutions were used as standard and blank controls.

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References

[1] K.O. Vartia, in *The Lichens*, edited by V. Ahmadjian and M.E. Hale (Academic Press, New York, 1973), pp. 547–561.
[2] D.H.S. Richardson, in *CRC Handbook of Lichenology*, Vol. 3, edited by M. Galun (CRC Press, Boca Raton, FL, 1988), pp. 93–108.
[3] J.D. Lawrey, *Bryologist* **92**, 326 (1989).
[4] J.A. Elix, in *Lichen Biology*, edited by T.H. Nash III (Cambridge University Press, Cambridge, 1996), pp. 154–181.
[5] C. Hobbs, *Usnea: The Herbal Antibiotic* (Botanica Press, Capitola, CA, 1986), p. 913.
[6] J.A. Elix and U. Engkaninan, *Aust. J. Chem.* **28**, 1793 (1975).
[7] P. Ramesh and E.S.A. Baig, *Ind. J. Chem., Sect. B: Org. Chem. Including Med. Chem.* **33B**, 899 (1994).
[8] J.N. Eloff, *Planta Med.* **64**, 711 (1998).
[9] P.J. Hylands and K. Ingolfsdottir, *Phytochemistry* **24**, 127 (1985).
[10] J.A. Elix, M.T. Adler, and J.H. Wardlaw, *Aust. J. Chem.* **49**, 1175 (1996).
[11] S. Huncek and I. Yoshimura, *Identification of Lichen Substances* (Springer,

Berlin, Heidelberg, New York, 1996), pp. 313–353.
[12] V.L. Eifler-Lima, A. Sperry, S. Sinbandhit, J. Boustie, S. Tomasi, and E. Schenkel, *Magn. Reson. Chem.* **38**, 472 (2000).
[13] P. Papadopoulou, O. Tzakou, C. Vagias, P. Kefalas, and V. Roussis, *Molecules* **12**, 997 (2007).
[14] N. Sultana, T.G. Hartley, and P.G. Waterman, *Phytochemistry* **50**, 1249 (1999).
[15] A.J. Slusarenko, A.C. Longland, and I.M. Whitehead, *Bot. Helv.* **99**, 203 (1989).
[16] N. Sultana and A.J. Afolayan, *Nat. Prod. Res.* **21**, 889 (2007).
[17] K. Ingolfsdottir, *Phytochemistry* **61**, 729 (2002).