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# β-Orcinol depsidones from the lichen *Usnea* sp. from Sri Lanka

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Two β-orcinol depsidone lactones, the methyl ethers of menegazziaic acid and stictic acid were isolated along with glyceryl trilinolate and usnic acid from an *Usnea* sp. new to Sri Lanka growing on rotting trees of *Acacia decurrans*. Usnic acid exhibited potent antitermite activity against a common pest of tea, *Glyptotermes dilatatus*, at low elevations.

*Keywords:* Lichen; *Usnea* sp.; Methyl ether of menegazziaic acid; Methyl ether of stictic acid; Glyceryl trilinolate; Usnic acid; Antitermite activity

## 1. Introduction

Lichens are the symbiotic organisms of fungi (mycobionts) and algae (photobionts) distributed worldwide [1]. Lichens accumulate large concentrations of products, particularly aromatic phenolic compounds, sometimes exceeding 20% of dry weight. The majority of these compounds originate from the mycobiont. The general resistance of lichens to insects and microbial attack is attributed to the presence of lichen compounds [2]. The cortical presence of yellow-coloured compounds, such as pulvinic acid derivatives in lichens play a defensive role against the non-visually oriented small invertebrate herbivores [3]. We report herein the isolation of two depsidones, one of which is unknown, along with glyceryl trilinolate and the antitermite activity of usnic acid, isolated from the lichen, *Usnea* sp.

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## 2. Results and discussion

*Usnea* sp. described in this study was collected from the surface of a rotting tree, identified as *Acacia decurrans*, in the Ambewela area (elevation 6000 ft above sea-level) in the Central Province of Sri Lanka, in November 1996. Its vegetative body (thallus) is shrubby and has a hanging, radial structure (fruticose); the colour ranges from greenish-gray to yellow. The lichen is attached to the substrate by a basal holdfast. Earlier we had reported the isolation of Ambewelamides A and B, two antineoplastic epidithiapiperazinediones [4] from the same lichen.

The MeOH extract of the lichen yielded two colourless depsidone lactones (**1**) and (**2**). Compound (**1**) had a molecular formula $C_{19}H_{16}O_9$, determined by HR-EIMS and confirmed by the $^{13}C$ NMR and DEPT analysis. The IR spectrum of (**1**) indicated two ester carbonyl groups at 1740, 1760 $cm^{-1}$, characteristic of a depsidone and a $\gamma$-lactone, respectively. The $^{13}C$ NMR spectrum of (**1**) contained 19 clearly resolved peaks. The $^1H$ NMR of compound (**1**) showed four singlets in the downfield region at $\delta$ 6.40, 6.51, 6.61 and 7.67, of which two disappeared upon addition of $D_2O$ ($\delta$ 6.40 and 7.67) suggesting the presence of two phenolic OH groups. The HMBC data (figure 2) readily identified the upfield proton at $\delta$ 6.61 ($^{13}C$ $\delta$ 111.4 (C-5)) as an uncoupled aromatic hydrogen being flanked by a methoxy group ($^1H$ $\delta$ 3.91, s, $OCH_3$-C-4; $^{13}C$ $\delta$151.7 (C-4), 56.4 ($OCH_3$)), and a C-methyl, which was adjacent to an ester carbonyl ($^1H$ $\delta$ 2.44, s, H-8; $^{13}C$ $\delta$ 20.7 (C-8), 134.5 (C-6), 114.2 (C-1), 160.8 (C-7)). Furthermore, the phenolic OH at C-3 correlated with the methoxy bearing carbon (C-4) and with C-2 bearing an oxygen ($^{13}C$ $\delta$ 147.2 (C-2), 113.5 (C-3). Thus, it was possible to identify a $\beta$-orcinol moiety for the ring A of the molecule.

The HMBC data further indicated another aromatic C-methyl, adjacent to the second phenolic OH group described above ($\delta$ 7.76) and an oxygen ($^1H$ $\delta$ 2.26, s, H-9′; $^{13}C$ $\delta$ 9.1 (C-9′), 121.2 (C-3′), 152.1 (C-2′), 149.6 (C-4′); significantly, the OH proton coupled with C-1′ ($^{13}C$ $\delta$107.2 (C-1′)), thus establishing the contiguous nature of C-1′ to C-4′. In addition, a second methoxy group was attached to a methine carbon ($^1H$ $\delta$ 3.76, s, $OCH_3$-C-8′; $^{13}C$ $\delta$ 57.4 ($OCH_3$) 102.0 (C-8′)) bearing the fourth upfield hydrogen described above at $\delta$ 6.64 (s). Significantly, this methine hydrogen showed further correlation to the $\gamma$-lactone carbonyl ($^{13}C$ $\delta$ 168.7 (C-7′)). The EIMS further corroborated the presence of the methoxy group by exhibiting an intense ion at $m/z$ 356 for the loss of MeOH (figure 1) further proving that the $\gamma$-lactone carbonyl was at C-1′ adjacent to the phenolic OH at C-2′. The foregoing data established a second $\beta$-orcinol aromatic ring where a $\gamma$-lactone moiety (bearing a $OCH_3$ at C-8′) originated from C-1′ and C-6′ ($^{13}C$ $\delta$ 138.7). Furthermore, it was reasonable to construct the seven-membered ring of a depsidone, between the two $\beta$-orcinol moieties described above, where C-4′ oxygen links up with the C-7 carbonyl forming a lactone functionality, and C-5′ ($^{13}C$ $\delta$ 163.7) and the oxygen at C-2 are connected by an ether linkage. Compound (**1**) was thus determined to be a new $\beta$-orcinol depsidone containing a methoxy lactone moiety. The parent 8′-hydroxyl compound, menegazziaic acid has been previously reported from the lichens *Menegazzia asahinae* and *M. terebrata* [5].

The more polar compound (**2**) exhibited a peak at 400.0793 in HR-EIMS corresponding to an elemental composition of $C_{20}H_{16}O_9$, which was corroborated by $^{13}C$ (20 peaks) and DEPT evidence. The IR spectrum of compound (**2**) had the characteristic ester carbonyl absorptions of a depsidone and a $\gamma$-lactone at 1740 and 1770 $cm^{-1}$, respectively, along with an aldehyde group (1690 $cm^{-1}$). Of the three $^1H$

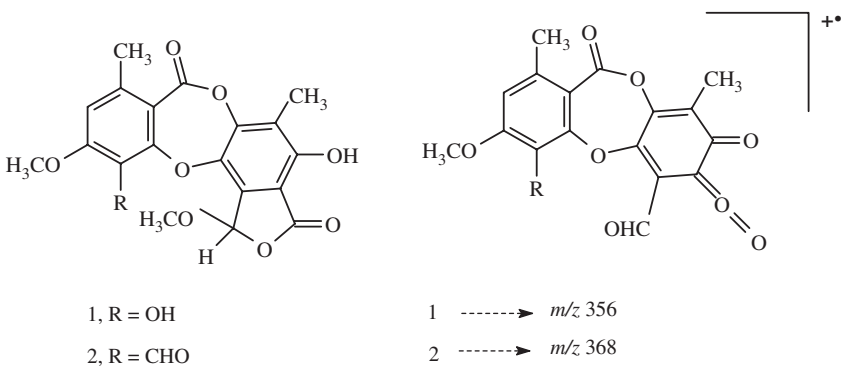


Figure 1. Structures of isolated depsidones (**1**) and (**2**); EIMS ($M^+$- MeOH) peaks from **1** and **2**.

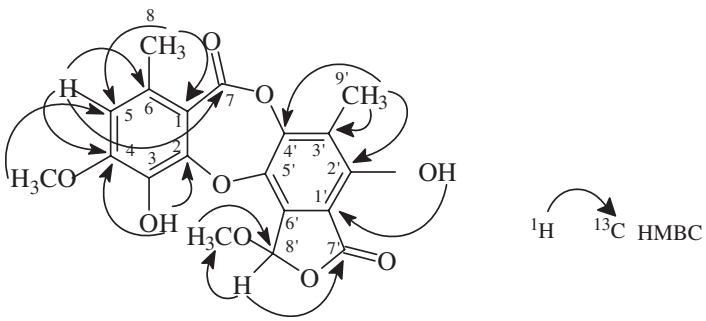


Figure 2. HMBC of depsidone (**1**).

resonances in the aromatic region at $\delta$ 6.39, 6.71 and 7.87, the latter disappeared upon the addition of $D_2O$, suggesting that compound (**2**) contained only one phenolic OH. The HMBC data showed that the ring A of compound (**2**) had, similar to compound (**1**), an uncoupled hydrogen (H-5) flanked by a methoxy group ($^1$H $\delta$ 6.71, s, H-5, 3.95 s, $OCH_3$-C-4; $^{13}$C $\delta$ 111.9 (C-5), 163.5 (C-4), 57.0 ($OCH_3$)), and a C-methyl which was adjacent to an ester carbonyl ($^1$H $\delta$ 2.53, s, H-9; $^{13}$C $\delta$ 22.4 (C-9), 134.4 (C-6), $\delta$ 114.2 C-1), 160.7 (C-7)). Furthermore, the COSY spectrum of compound (**2**) showed that H-5 correlated with the allylic methyl group (C-9) and with the homoallylic aldehydic hydrogen ($^1$H $\delta$ 10.46, s, H-8; $^{13}$C $\delta$ 186.8 (C-8)). Moreover, the NOE difference spectra showed that H-5 correlated with both the OMe group at C-4 and the methyl group at C-6. The foregoing data established the modified $\beta$-orcinol A-ring of the molecule with an aldehyde group at C-3.

The HMBC data (figure 3) for the second aromatic ring of compound (**2**), coupled with the EIMS peak at *m/z* 368 for the loss of MeOH (figure 1) showed that C-1′ through C-6′ had a substituent pattern identical to the depsidone (**1**) (figure 2). Compound (**2**) was thus determined to be the rare depsidone 8′-*O*-methylstictic acid previously reported only from the lichen *Lobaria oregana* [6].

Depsidones with a hydroxylactone ring undergo reaction with alcohols at elevated temperatures. Thus, stictic acid, when refluxed in MeOH gives 8′-*O*-methylstictic acid in low yield [7]. In order to rule out that both the compounds (**1**) and (**2**), are

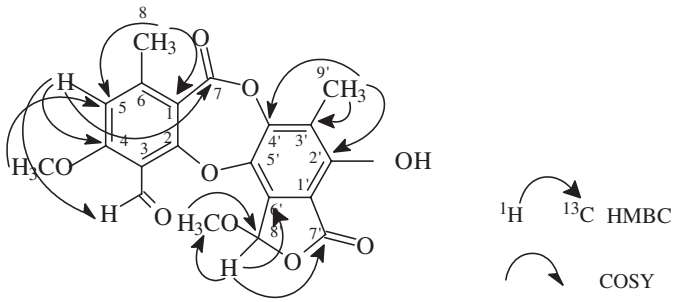


Figure 3. HMBC and COSY of depsidone (**2**).

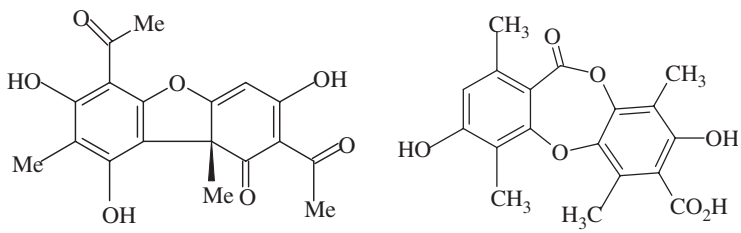

Figure 4. Usnic acid and hypoprotocetraric acid.

not 8′-*O*-methyl ethers produced during extraction with MeOH, the lichen was extracted into acetone at room temperature. The acetone extract also yielded 8′-*O*-methylstictic acid (**1**) and the methyl ether of menegazziaic acid (**2**), thus confirming that both the compounds are natural metabolites of the lichen. However, the measured optical rotation of compounds (**1**) and (**2**) were 0°, indicating that C-8′ in both are racemic.

The *β*-orcinol depsidones are formed biosynthetically from the common precursor hypoprotocetraric acid (figure 4) by stepwise oxidation reactions [8,9]. Minor biosynthetic variations within a lichen genus or a group of related species give rise to a variety of structurally similar substances referred to as a chemosyndrome [10]. In the case of *β*-orcinol depsidones, such structural variations include various oxidation states of the alkyl groups at C-3, C-6, C-3′ and C-6′ and various degrees of methylation of phenolic and other OH groups. Biosynthetic variations of the aromatic moieties within the stictic acid chemosyndrome produce a vast array of naturally occurring derivatives, such as norstictic acid, stictic acid, methylstictic acid, cryptostictic acid, constictic acid, *O*-acetylconstictic acid and menegazziaic acid [11]. Compounds (**1**) and (**2**) represent two rare examples of methylation of the hydroxylactone moiety; this is the first report of such compounds within the stictic acid chemosyndrome from the genus *Usnea*. This fact and the first time co-occurrence of two methylated depsidone lactones from a single lichen species are chemotaxonomically significant.

The hexane extract of the lichen yielded glyceryl trilinolate (**3**), which showed spectral data identical with those reported for the same compound [12]; it has been previously isolated from the lichen *Umbilicaria antarctica* [12]. However, this is the first report of this compound from an *Usnea* species. The $CH_2Cl_2$ extract furnished yellow needles

of the dibenzofuran, (+)-usnic acid (**4**) (2.59 g). The compound exhibited physical properties (TLC, co-TLC, mp and mmp) identical with an authentic sample and its spectral data compared well with those reported for the same compound [11].

Importantly, (+)-usnic acid (**4**) showed significant antitermite activity (80% mortality at 10 mg) against *Glyptotermes dilatatus*, which is a major live-wood attacking tea-termite at low elevations. Although usnic acid is a well-known antimicrobial compound [13], there is only one report of the insecticidal activity of usnic acid against the polyphagous herbivorous insect *Spodoptera littoralis* [14]. Currently, the control methods of *G. dilatatus* are focused on the development of resistant clones and there are no chemical methods for controlling the pest [15].

## 3. Experimental section

### 3.1. *General experimental procedures*

The melting points (uncorrected) were determined on an Electrothermal 9200 instrument. The UV absorptions were measured with a Shimadzu 160 UV spectrophotometer. The IR spectra were recorded on a Shimadzu 160 spectrophotometer on KBr pellets. The $^{1}H$ and $^{13}C$ NMR spectra were recorded using a Varian 300 ($^{1}H$ 300 and $^{13}C$ 75 MHz) and a Jeol 500 ($^{1}H$ 500 and $^{13}C$ 125 MHz) in $CDCl_3$ with TMS as the internal standard. Low-resolution electron impact mass spectra were recorded on a Kratos/AE1 MS-902 spectrometer.

## 4. Collection, extraction and isolation

Cleaned and dried *Usnea* sp. (940 g) was sequentially extracted with hexane followed by $CH_2Cl_2$ and MeOH. The crude MeOH extract (50 g) was fractionated, via MPLC (step gradient: from $CH_2Cl_2$ to 75% MeOH/$CH_2Cl_2$) and then flash chromatography (eluent: $CH_2Cl_2$) followed by preparative TLC (eluent $CH_2Cl_2$) to give the depsidone lactone (**1**) (33 mg) as a colourless powder; a more polar fraction on MPLC (eluent: step gradient from $CH_2Cl_2$ to 10% MeOH/$CH_2Cl_2$) and preparative TLC yielded the depsidone lactone (**2**) (20 mg) as a colourless powder. The hexane extract (1.2 g) was fractionated via MPLC (eluent: hexane to 100% $CH_2Cl_2$). The third fraction upon gravity column chromatography (eluent: 30% $CH_2Cl_2$/hexane to 40% $CH_2Cl_2$/hexane) yielded the yellow oil, glyceryl trilinolate (**3**) (17 mg). The $CH_2Cl_2$ extract upon MPLC (eluent: step gradient $CH_2Cl_2$ to 5% MeOH/$CH_2Cl_2$) yielded a yellow pigment, which was recrystallized with $CH_2Cl_2$ to give the pure needles of the dibenzofuran (+)-usnic acid (**4**) (2.59 g).

*Depsidone (1)* Colourless needles ($CH_2Cl_2$), mp 230–231°C; $[\alpha]_D^{25}$ 0° (MeOH, $c = 0.4$); IR $\nu_{max}$ (KBr): 1060, 1135, 1190, 1220, 1250, 1300, 1380, 1445, 1490, 1510, 1600, 1740, 1760, 2850, 2925 and 3450 $cm^{-1}$; UV ($CHCl_3$) nm (log $\varepsilon$): 306.0 (0.952), 275.2 (1.028); $^{1}H$ NMR (500 MHz, $CDCl_3$): $\delta$ 2.26 (3H, s, Me-8′), 2.44 (3H, s, Me-8), 3.76 (3H, s, OMe-C-8′), 3.91 (3H, s, OMe-C-4), 6.40 (1H, s, OH-C-3), 6.51(1H, s, H-8′), 6.61 (1H, s, H-5), and 7.67 (1H, s, OH-C-2′); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 114.2 (C-1), 147.2 (C-2), 113.5 (C-3), 151.7 (C-4), 111.4 (C-5), 134.5 (C-6), 160.8 (C-7), 20.7 (C-8), 56.4 (OMe-C-4), 107.2 (C-1′), 152.1 (C-2′), 121.2 (C-3′), 149.6 (C-4′), 163.7

(C-5′), 138.7 (C-6′), 168.7 (C-7′), 9.1 (C-9′), 102.0 (C-8′) and 57.4 (OMe-C-8′); EIMS $m/z$ (rel. int. %): 388 (28), 356 (100), 328 (16), 300 (42), 285 (10), 272 (20), 244 (10), 151 (28), 83 (20): EI-HRMS $m/z$ 388.0797 (calcd for $C_{19}H_{16}O_9$, 388.0794).

*Methyl ether of stictic acid (2)* Colourless needles ($CH_2Cl_2$), mp 228–231°C; $[\alpha]_D^{25}0°$ (MeOH, $c = 0.4$); IR $\nu_{max}$ (KBr) $cm^{-1}$: 1079, 1235, 1290, 1380, 1435, 1485, 1550, 1600, 1690, 1740, 1770, 2950 and 3450; UV ($CHCl_3$) nm (log $\varepsilon$): 294.0 (0.674), 268.0 (0.723); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 2.27 (3H, s, Me-9′), 2.53 (3H, s, Me-9), 3.76 (3H, s, OMe-C-8′), 3.95 (3H, s, OMe-C-4), 6.39 (1H, s, H-8′), 6.71 (1H, s, H-5), 7.87 (1H, broad s, OH-C-2′) and 10.46 (1H, s, CHO); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 114.2 (C-1), 151.3 (C-2), 114.8 (C-3), 163.5 (C-4), 111.9 (C-5), 134.4 (C-6), 160.7 (C-7), 186.8 (C-8), 22.4 (C-9), 56.0 (OMe-C-4), 107.7 (C-1'), 152.4 (C-2'), 121.1 (C-3′), 149.4 (C-4′), 162.7 (C-5′), 138.8 (C-6′), 169.5 (C-7′), 9.1 (C-9′), 102.8 (C-8′) and 56.0 (OMe-C-8′); EIMS $m/z$ (rel. int. %): 400 (100), 368 (60), 355 (36), 340 (36), 312 (22), 300 (14), 285 (16), 191 (30), 83 (40); EI-HRMS 400.0793 (calcd. for $C_{20}H_{16}O_9$, 400.0794).

### 4.1. *Antitermite assay [16]*

Test compound (10 mg) was dissolved in a minimum amount of $CH_2Cl_2$ and mixed with cellulose (1.25 g). The mixture was air-dried under a current of warm air to evaporate the organic solvents. To the resultant mixture, distilled water (10 ml) was added and heated (40–45°C) for 30 min to produce a slurry. An agar solution (1.0 g in 15 ml of distilled water) was heated (50–55°C) and mixed well with the cellulose/compound slurry. The resultant mixture was compressed using a pellet block to prepare approximately five pellets (thickness: 1 cm; diameter: 2.5 cm). Pellets for the control experiment were prepared in the same manner without the test compound. Each pellet (five with the test compound and two without) was transferred to a petri dish and *Glyptotermes dilatatus* (10 each), which had been starved for 24 h, were introduced. Mortality counts were recorded after five days from the start of the experiment and were continued for 30 days. Kampferol was used as the positive control, which had a 100% mortality in 23 days under the above conditions.

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