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Ambewelamides A and B, Antineoplastic Epidithiapiperazinediones Isolated from the Lichen *Usnea* sp.[1]

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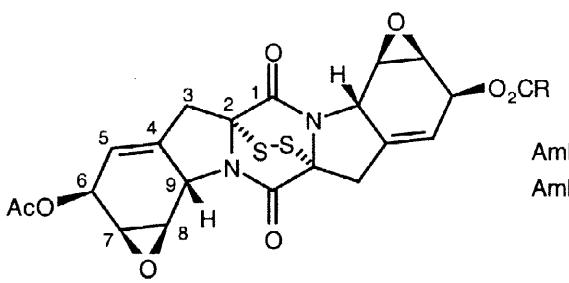
Abstract: Two potently cytotoxic epidithiapiperazinediones, ambewelamides A (**1**) and B (**2**), have been isolated from the lichen *Usnea* sp. collected in Sri Lanka. The structures were determined by a combination of X-ray crystallographic and spectroscopic analyses.

The recent introduction of paclitaxel into clinical use and the current excitement surrounding the discovery of the antimitotic compounds epothilone,[2] eleutherobin,[3] and discodermolide[4] illustrate that natural products continue to be an important source of novel bioactive chemotypes for anticancer drug development.[5] As part of a collaborative program designed to explore unique marine[6] and terrestrial natural product sources for new anticancer drug leads, it was found that extracts of the lichen *Usnea* sp., collected in Sri Lanka, exhibited potent in vitro cytotoxicity. Bioassay guided fractionation of the *Usnea* sp. extracts led to the isolation of ambewelamides A (**1**) and B (**2**), whose structures have been elucidated via a combination of single crystal X-ray diffraction and spectroscopic analyses.



Ambewelamide A **1** R = -$(CH_2)_2CH_3$
Ambewelamide B **2** R = -$(CH_2)_4CH_3$

Specimens of the *Usnea* sp. were collected from the surface of a rotting tree, identified as *Acacia decurrens*, in Ambewela, Sri Lanka. The dried lichen (950 g) was exhaustively extracted with CH_2Cl_2 to give a crude gum (5.1 g) that was fractionated via silica gel MPLC (eluent: step gradient from hexane to MeOH/CH_2Cl_2 (4:96)), silica gel preparative TLC (eluent: CH_2Cl_2), and Sephadex LH-20 chromatography (eluent: CH_2Cl_2/MeOH (2:8)) to give a crystalline fraction that contained a mixture of ambewelamides A (**1**) and B (**2**). Final purification was accomplished using normal phase HPLC (Waters Radial PAK cartridge; eluent: CH_2Cl_2/EtOAc (20:1)) to give 13.4 mg of ambewelamide A (**1**)[7] and 1.4 mg of ambewelamide B (**2**),[8] both as optically active clear crystalline plates.

The FABMS data obtained for ambewelamide A (**1**) gave ambiguous information that failed to clearly identify the molecular formula. In positive ion mode, the HRFABMS of **1** gave a relatively intense ion at m/z 501.1336 corresponding to an elemental composition of $C_{24}H_{25}N_2O_8S_1$, while in negative ion mode the HRFABMS gave an intense ion at m/z 533.1065 corresponding to an elemental composition of

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$C_{24}H_{25}N_2O_8S_2$. The ^{13}C NMR spectrum of ambewelamide A (**1**) contained only 17 clearly resolved resonances suggesting that the molecule had some element of symmetry. COSY and HMBC data readily identified acetyl (^1H δ 2.12, s, 3H: ^{13}C δ 170.6 (C-10), 20.7 (C-11)) and butanoyl (^1H δ 2.35, m , H-13; 1.67, m, H-14; 0.95, t, J = 7.4 Hz, H-15: ^{13}C δ 173.3 (C-12), 35.8 (C-13), 18.4 (C-14), 13.6 (C-15)) fragments in ambewelamide A (**1**). Analysis of the HMQC, COSY, and ^1H NMR integration data showed that all of the ^1H resonances, except those belonging to the acetyl and butanoyl groups, could be assigned to two virtually identical fragments in ambewelamide A (**1**). Therefore, the molecule appeared to contain a symmetrical central core that was acylated at two equivalent sites with acetyl and butanoyl fragments, respectively, to give a pseudosymmetric compound.

HMBC correlations observed between a ^1H resonance at δ 5.66 (H-6/ H-6') and ^{13}C resonances assigned to the acetyl (δ170.6) and butanoyl (δ173.3) carbonyls indicated that both acyl groups were present as esters of secondary alcohols. The δ 5.66 proton resonance showed HMQC correlations to a pair of ^{13}C resonances (δ65.0/64.7 (C-6/C-6'), that had nearly identical chemical shifts, consistent with the presumption of pseudosymmetry. COSY and HMBC data showed that the methine carbons (C-6/C-6') bearing the acetate and butanoate substituents were flanked by trisubstituted olefin (^1H δ 5.88, bs (H-5/H-5'): ^{13}C δ 120.3/120.1 (C-5/C-5'); 137.4/137.3 (C-4/C-4')) and disubstituted epoxide groups (^1H δ 3.61, m, (H-7/H-7'), 4.32, dd, J = 6.5, 3.5 Hz (H-8/H-8'): ^{13}C δ 51.0/51.0 (C-7/C-7'), 54.8 (C-8/C-8')), and that this array of functionality was incorporated into a cyclohexene ring (^1H δ 4.42, bs (H-9/H-9'): ^{13}C δ 56.2/56.2 (C-9/C-9')) having an exocyclic methylene group (^1H δ 3.69, d, J = 16.4 Hz (H-3a/H-3a'); 2.79, d, J = 16.4 Hz (H-3b/H-3b'): 13C δ 36.9 (C-3/C-3')) as the third olefinic substituent. The NMR data failed to completely define the remaining features of the molecule. Therefore, the overall structure of ambewelamide A (**1**) was determined using single crystal X-ray diffraction techniques,[9] and the final result is shown in Figure 1. While the molecular connectivity and relative stereochemistry was clear, the quality of the structure is limited by the low resolution and noisy data. Comparison of the CD spectrum of ambewelamide A with literature values for related epidithiapiperazinediones[10c] showed that the absolute stereochemistry is as shown in **1**. All of the remaining spectroscopic data were in complete accord with structure **1**.

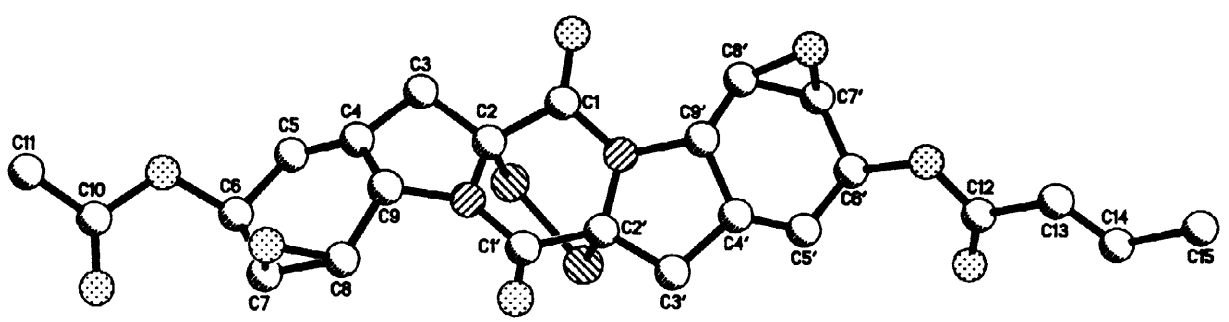
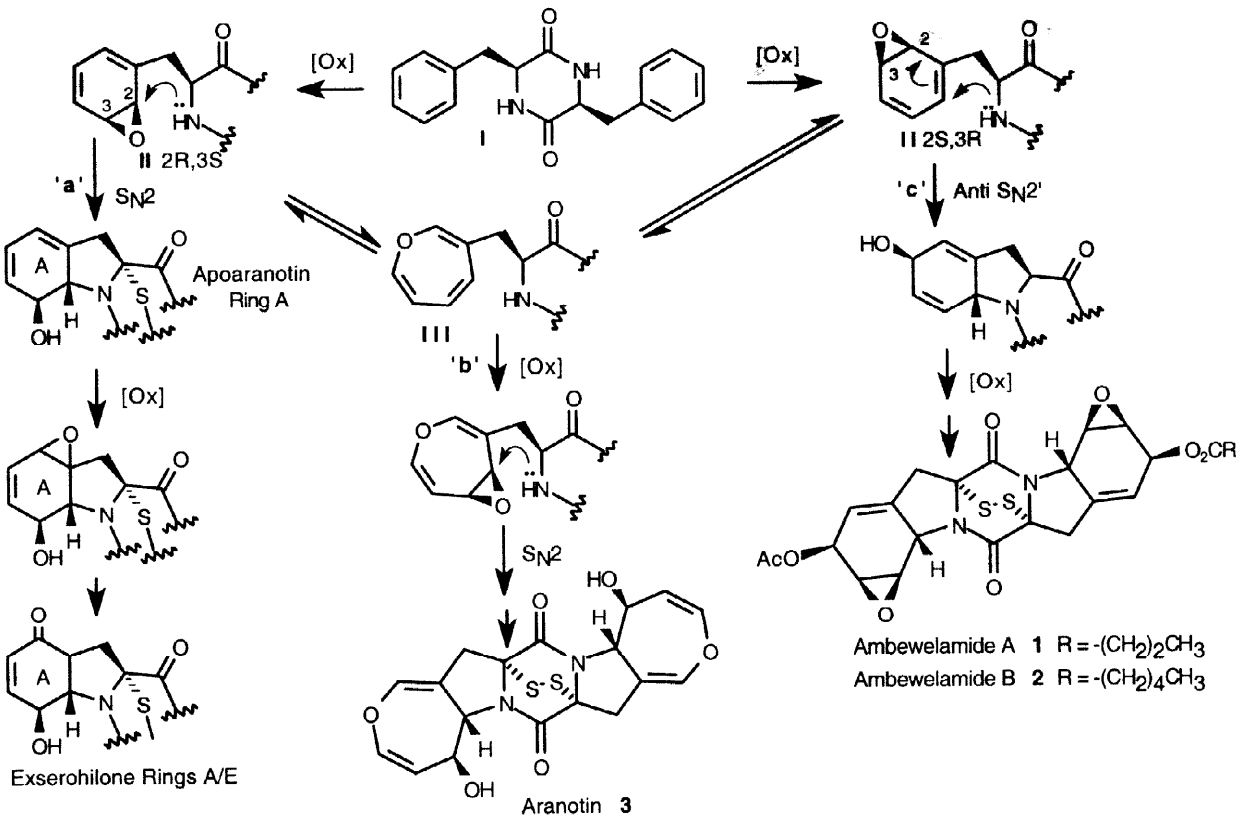


Figure 1. A perspective drawing of the final X-ray model of ambewelamide A (**1**). The absolute configuration shown is arbitrary and hydrogens have been omitted for clarity.



Scheme 1

Ambewelamide B (**2**) gave an intense ion at m/z 561.1369 in the negative ion HRFABMS corresponding to an elemental composition of $C_{26}H_{29}N_2O_8S_2$. The NMR data obtained for **2**[8] showed that it differed from ambewelamide A (**1**) simply by replacement of the butanoyl residue in **1** with a hexanoyl residue in **2**.

Ambewelamides A (**1**) and B (**2**) are new members of a family of highly modified phenylalanine diketopiperazines that includes the known fungal metabolites aranotin (**3**),[10] apoaranotin,[10] emethallicins,[11] exserohilone,[12] epoxyexserohilone,[13] and SCH64847.[14] The ambewelamides are the first examples of this family isolated from a lichen. Scheme 1 outlines a proposed biosynthesis for the four basic types of ring A and ring E functionality found to date in these modified diketopiperazines. The key step in this biosynthetic manifold is formation of the arene oxide **II**. The 2R,3S stereoisomer of **II** leads to the apoaranotin and exserohilone ring A functionality via pathway **'a'** and to the aranotin ring A/E functionality via the oxepin tautomer **III** and pathway **'b'**. Alternatively, the 2S,3R arene oxide **II**, which can be formed directly from the diketopiperazine **I** or by tautomeric equilibrium from the oxepin **III**, leads to the ambewelamide ring A/E functionality via an anti-S_N2' reaction as shown in pathway **'c'**.

Ambewelamide A (**1**) exhibited potent in vitro cytotoxicity (murine leukemia P388: IC_{50} = 8.6 ng/mL) and it also showed significant in vivo antineoplastic activity (P388: %T/C 140 @ 160 μg/Kg). There are reports of antiallergy,[11] antiviral, [10] EGF receptor binding,[14] and phytotoxic[12] activities for members of this family of diketopiperazines, but the ambewelamides are the first analogs to show potent cytotoxicity. Ambewelamides A (**1**) and B (**2**) are the only members of this family that have ring A/E epoxides and a diketopiperazine epidisulfide bridge, suggesting that this combination of functionality might be required for cytotoxicity.

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References and Footnotes

1) This paper is dedicated to Professor Edward Piers on the occasion of his 60th birthday
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7) Ambewelamide A (**1**): $[\alpha]^{25}_D$ +424° (c 0.5, CH_2Cl_2); mp 216-218°C; UV (4:1 MeCN/H_2O) λ max 218 (ε 33,000) nm; negative ion HRFABMS (thioglycerol matrix) $[M+2H-1H]^-$ *m/z* 533.1065 ($C_{24}H_{25}N_2O_8S_2$, calcd 533.1054); positive ion HRFABMS (thioglycerol matrix) $[M+H-S]^+$ *m/z* 501.1336 ($C_{24}H_{25}N_2O_8S_1$, calcd 501.1333), [M+H-2S]; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.69 (d, J = 16.4 Hz, H-3a/H-3a'), 2.79 (d, J = 16.4 Hz, H-3b/H-3b'), 5.88 (m, H-5/H-5'), 5.66 (m, H-6/H-6'), 3.61 (m, H-7/II-7'), 4.32 (dd, J = 6.5, 3.5 Hz, H-8/H-8'), 4.42 (bs, H-9/H-9'), 2.12 (s, H-11), 2.35 (m, H-13), 1.67 (m, H-14), 0.95 (t, J = 7.4 Hz, H-15) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 162.8 (C-1/C-1'), 75.9 (C-2/C-2'), 36.9 (C-3/C-3'), 137.4/137.3 (C-4/C-4'), 120.3/120.1 (C-5/C-5'), 65.0/64.7 (C-6/C-6'), 51.0/51.0 (C-7/C-7'), 54.8 (C-8/C-8'), 56.2/56.2 (C-9/C-9'), 170.6 (C-10), 20.7 (C-11), 173.3 (C-12), 35.8 (C-13), 18.4 (C-14), 13.6 (C-15) ppm.
8) Ambewelamide B (**2**): $[\alpha]^{25}_D$ +620° (c 0.07, CH_2Cl_2); mp 212-214°C; UV (4:1 MeCN/H_2O) λ max 216 (ε 44,000) nm; negative ion HRFABMS (thioglycerol matrix) $[M+2H-1H]^-$ *m/z* 561.1369 ($C_{26}H_{29}N_2O_8S_2$, calcd 561.1367); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.70 (d, J = 16.4 Hz, H-3a/H-3a'), 2.80 (d, J = 16.4 Hz, C-3b/C-3b'), 5.88 (m, H-5/H-5'), 5.67 (m, H-6/H-6'), 3.61 (m, H-7/H-7'), 4.33 (dd, J = 6.5, 3.4 Hz, H-8/H-8'), 4.42 (bs, H-9/H-9'), 2.13 (s, H-11), 2.37 (m, H-13), 1.65 (m, H-14), 1.31 (m, H-15), 1.31 (m, H-16), 0.88 (bt, J = 6.8 Hz, H-17) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.9 (C-1/C-1'), 75.9 (C-2/C-2'), 36.9 (C-3/C-3'), 137.4/137.3 (C-4/C-4'), 120.3/120.1 (C-5/C-5'), 65.0/64.7 (C-6/C-6'), 51.0/51.0 (C-7/C-7'), 54.8 (C-8/C-8'), 56.2/56.2 (C-9/C-9'), 170.6 (C-10), 20.7 (C-11), 173.5 (C-12), 34.0 (C-13), 24.6 (C-14), 31.2 (C-15), 22.3 (C-16), 13.9 (C-17) ppm.
9) Only a single thin plate (0.4 x 0.4 x 0.003 mm^3) of ambewelamide A (**1**) was available for single crystal X-ray analysis. Crystal: monoclinic space group $P2_1$ with *a* = 9.569(2), *b* = 8.423(2), *c* = 17.770(4) Å and β = 93.14(3)°, Z = 2. Data collection: Bruker diffractometer with a 1k ccd detector, λ = 0.71073 Å, -100 °C, 1.1 Å resolution limit, broad lumpy peak profiles, Rsym = 11.4% (3878 independent reflections, 4991 measurements). The structure was solved and refined using Bruker SHELXTL (v 5.07) software package, and in addition to the structure shown, a disordered methylene chloride was also observed. Isotropic refinement using full-matrix least-squares techniques and hydrogens included at all calculated positions have converged to a conventional R = 11.4% (gof = 0.958) Crystallographic data have been deposited with the Cambridge Crystallographic Data File.
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