# Insulicolide A: A New Nitrobenzoyloxy-Substituted Sesquiterpene from the Marine Fungus *Aspergillus insulicola*

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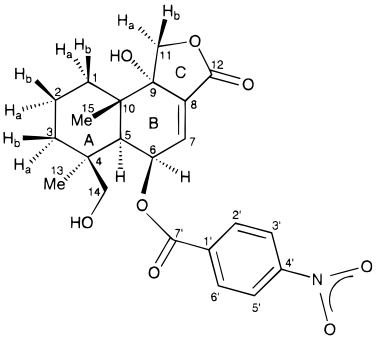
Insulicolide A (6$\beta$-[(4-nitrobenzoyl)oxy]-9$\alpha$,14-dihydroxycinnamolide) has been isolated from the chemically unexplored fungus *Aspergillus insulicola* and the structure determined by X-ray crystallography and NMR. The metabolite production of marine and terrestrial specimens of the fungus has been compared by HPLC diode-array analysis.

Nitro compounds are relatively rare in nature; the most well known is probably the antibiotic chloramphenicol, which has been isolated from bacteria. We have isolated a new nitrobenzoyloxy-substituted sesquiterpene, insulicolide A (**1**), from the chemically unexplored fungus *Aspergillus insulicola*. Low temperature X-ray crystallography established that **1** is the 6-*p*-nitrobenzoate of 6$\beta$,9$\alpha$,14-trihydroxycinnamolide (Figure 1). *A. insulicola* is one of the few known marine aspergilli[2] and has been accepted as a distinct species by several authors.[3−5] It is related to the ascomycete genus *Petromyces* in the Eurotiales.




**1**

Five strains of *A. insulicola* were isolated from marine sources, collected in the Bahamas in 1993. The strains were fermented on two or three different media: CYA (yeast extract 5 g/L, sucrose 30 g/L), YES (yeast extract 20 g/L, sucrose 150 g/L), and SB (potato starch 75 g/L, soybean meal 40 g/L, sea salts 40 g/L), and the mycelia and media were extracted with EtOAc. The neurotoxin asteltoxin[6] and **1** were isolated by liquid chromatography of extracts from two strains grown on CYA. The metabolite production of the five strains of *A. insulicola* was compared by HPLC diode-array analysis. Production of **1** and asteltoxin was shown to be dependent on medium but not on the origin of the fungi. Formation of **1** was established from the cultures on CYA and SB, but only in very small amounts or not at all on YES medium. Asteltoxin was detected only on CYA and SB media.

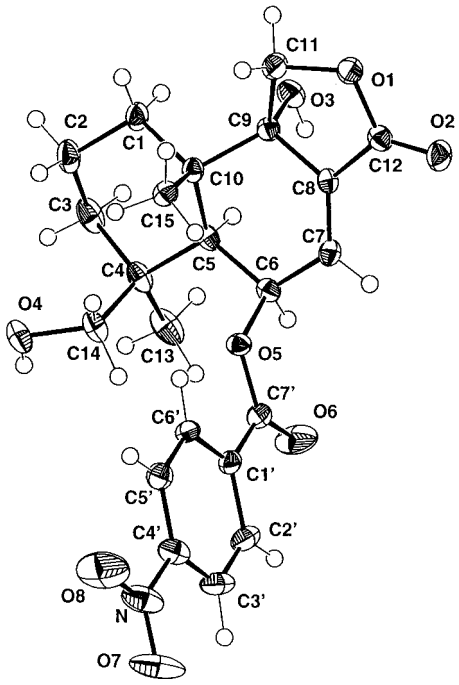



**Figure 1.** ORTEP[1] drawing of **1**.

HPLC diode-array analyses of extracts of several terrestrial isolates of the same species of fungus[7] were compared with those of marine isolates. According to these analyses, penicillic acid,[8] 4-hydroxymellein,[9] xanthomegnin,[10] viomellein,[11] vioxanthin,[12] asteltoxin,[6] and some unknown compounds are consistently expressed by *A. insulicola*. One of the major compounds has now been isolated and named insulicolide A (**1**). According to HPLC analysis, **1** was also produced by terrestrial isolates of *A. bridgeri* and some isolates of *A. sclerotiorum*, but not by any other species related to the genus *Petromyces* (*Aspergillus* subgenus *Circumdati* section *Circumdati*, formerly the *Aspergillus ochraceus* group). Insulicolide A is thus a good chemotaxonomic marker for the three species (details to be reported elsewhere).

The tricyclic sesquiterpene skeleton in **1** has not previously been thoroughly characterized by X-ray.[13]

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**Table 1.** NMR Data for Insulicolide A (**1**)

| no. | $^{13}C^a$ | $^1H^b$ | HMBC[c] |
|---|---|---|---|
| 1a | 31.8 | 1.21 (m) | |
| 1b | | 2.10 (td, 13.0, 4.8) | 1.17 |
| 2a | 17.2 | 1.53 (m) | |
| 2b | | 1.47 (m) | |
| 3a | 35.4 | 0.92 (td, 13.1, 3.4) | |
| 3b | | 2.01 (br d, 13.9) | 0.99, 3.41, 3.96, 2.36 |
| 4 | 38.9[d] | | 0.99, 2.36, 3.41, 5.97, 3.96 |
| 5 | 46.2 | 2.36 (d, 4.4) | 0.99, 1.17, 6.69, 5.96 |
| 6 | 67.6 | 5.97 (t, 4.2) | |
| 7 | 132.3 | 6.69 (d, 4.0) | 5.96 |
| 8 | 133.0 | | 4.18 |
| 9 | 76.0 | | 1.17, 2.36, 4.18, 6.69, 4.36 |
| 10 | 39.0[d] | | 1.17, 2.36, 5.97 |
| 11a | 75.0 | 4.18 (d, 9.9) | |
| 11b | | 4.36 (d, 9.9) | |
| 12 | 169.3 | | 4.18, 6.69 |
| 13 | 64.4 | 3.96 (d, 11.0) | 0.99, 2.36 |
| | | 3.41 (d, 11.0) | |
| 14 | 26.4 | 0.99 (s) | 2.36, 3.41, 3.96 |
| 15 | 21.1 | 1.17 (s) | 2.36, 2.10 |
| 1′ | 134.7 | | 8.20, 5.97 |
| 2′, 6′ | 130.6 | 8.04 (dt, 9.0, 2.0) | 8.04 |
| 3′, 5′ | 123.5 | 8.20 (dt, 9.0, 2.0) | 8.20 |
| 4′ | 150.4 | | 8.04, 8.20 |
| 7′ | 163.4 | | 5.97, 8.04 |

[a] $CDCl_3$ $\delta$ 77.0; $CD_3OD$ $\delta$ 48.7. [b] $CDCl_3$ $\delta$ 7.25; $CD_3OD$ $\delta$ 3.23; exchangeable protons are not discernable from the signal from traces of water in the solvent (br s, $\delta$ 3.71). The spatial position of H15 and H5 was decided by X-ray and protons at position 1, 2, 3, and 11 were assigned a and b by NOE-difference experiments according to these spatial placements: NOE effect from H15 to H11b, from H15 to H1b, from H1b to H2b, from H5 to H3a, and from H3a to H2a. [c] The HMBC spectrum was optimized on a long-range coupling constant of 7 Hz. [d] Values are interchangeable.

Structurally, it resembles the B, C, and D rings of the steroid skeleton. Ring A adopts a chair conformation, whereas the double bond in the B ring confers a half-chair conformation. Although the five-membered ring shows significant puckering (torsion angle C11−C9−C8−C12 = −20°), it is not as pronounced as in the D ring of steroids. Conjugation within the O1−C12−O2−C8−C7 fragment was anticipated, but all bond lengths correspond closely to localized single or double bonds. The absolute configurations determined from the X-ray study are 4S, 5S, 6S, 9S, and 10S. NMR data are given in Table 1.

Compound **1** is structurally related to astellolides A and B isolated from *Aspergillus variecolor* and/or *A. parasiticus*[14] and drimane sesquiterpene esters from *Aspergillus ustus* var. *pseudodeflectus*.[15]

## Experimental Section

**General Experimental Procedures.** NMR spectra were recorded in $CDCl_3/CD_3OD$ solution on a Varian 400 FT-NMR spectrometer at 400.0 and 100.6 MHz for $^1H$- and $^{13}C$-NMR spectra, respectively. The electrospray mass spectrum (ESMS) was obtained on a SCIEX API III Perkin-Elmer spectrometer. The circular dichroism spectrum was measured on a JASCO J-710 spectropolarimeter and the UV spectrum on a Hewlett-Packard 8452A diode-array spectrophotometer. The HPLC data were obtained on an HPLC system combined with a Millenium 996 photodiode-array detector from Waters.

**X-ray Data Collection and Refinement.** A single crystal (size 0.21 × 0.11 × 0.07 mm) was selected for the diffraction experiment. The data collection was performed at 122(2) K using a CAD-4 diffractometer with graphite-monochromatized Cu Kα radiation, $\lambda$ = 1.5418 Å. The unit cell dimensions, $a$ = 7.157(2), $b$ = 14.432(3), and $c$ = 19.684(3) Å, were determined from the centering of 20 reflections with 37.33 < $\theta$ < 38.14°. Using the $\omega-2\theta$ scan mode, intensities were measured for 12 938 reflections with 1 < $\theta$ < 75° ($hk \pm 1$, $-h - k \pm 1$, and $h - k \pm 1$(partial).

Analysis of the diffraction pattern showed that the crystal belonged to the space group $P2_12_12_1$. Data reduction was performed with the DREADD[16] program package. Reflections were averaged according to the symmetry of the crystal class 222 to give 4186 reflections. The structure was solved with SHELXS-86,[17] and the refinement using anisotropic thermal displacement parameters for all non-hydrogen atoms was performed with SHELXL-93,[18] w = $[\sigma^2(F_o2) + 0.03P^2]^{-1}$ where $P$ = [max $(F_o2,0) + 2F_c2]/3$. Hydrogen atoms were included in the refinement with isotropic thermal parameters $U_i = 1.2 \times U_{eq}$ of the adjacent atom, except for methyl groups where $U_i = 1.5 \times U_{eq}$ of the adjacent atom was used. The number of parameters was 355. Final $R_1$ and w$R_2$ were 0.032 and 0.074 for 3840 data with $F_o > 4\sigma(F_o)$ and 0.037 and 0.076 for all 4186 reflections. The final Fourier map had $(\Delta\rho)_{max} = 0.209$ and $(\Delta\rho)_{min} = -0.172$ e Å$^{-3}$. The absolute configuration was confirmed by the refined Flack parameter −0.08-(15).[19]

**Collection, Isolation, and Fermentation.** The five strains of *A. insulicola* were isolated from various samples of plant material collected in the Bahamas in 1993. The isolations were done on site within 24 h of the sampling time. Prior to isolation, the material was surface sterilized (sterile seawater, 70% EtOH, air drying). High- and low-nutrient agars, with 4% added sea salt, were used as isolation media. Incubation temperature was approximately 21 °C. Subcultures of the organisms are deposited at Novo Nordisk A/S, Microbiology, Novo Alle, DK-2880 Bagsværd, Denmark.

Isolate 1 (strain 121-5) originated from an unidentified green alga (Acklins Island, 2 ft), isolates 2 and 3 (strains 197-1 and 197-2, respectively) from a decaying leaf of *Rhizophora mangle* (collected in red mangrove, Little San Salvador, 2−3 ft), isolate 4 (strain 211-3) from a green alga, *Penicillus* sp. (collected in black mangrove, Sweetings Cay, Grand Bahama, 2−6 ft), and isolate 5 (strain 5-4) from a green alga, *Batophora* sp. (Eleuthera Pt., 5 ft).

The fungi were grown on solid medium, CYA for approximately 12 days, at 26 °C. In addition, they were fermented in one of two liquid media: YES broth and/or SB. The fermentations were carried out in 500-mL Erlenmeyer flasks containing 100 mL of broth for 7 days at 26 °C at 150 rpm.

**Extraction and Separation.** At the end of the incubation period, the mycelia and media were extracted with EtOAc. Extracts of isolates 1 and 2 fermented on CYA were subjected to purification by liquid chromatography, and fractions were combined when it was obvious that the same compounds were involved. Only the procedure for isolate 2 will be discussed. The extract (100 mg) was separated by vacuum liquid chromatography with EtOAc followed by EtOH. The EtOAc fraction (50 mg) was further purified on a Lobar LiChroprep Si 60 (40−63 μm) column from Merck (EtOAc−EtOH−heptane 30:5:65, 254 nm) giving nine fractions. One of these fractions was pure asteltoxin

(6 mg). Another fraction gave 3 mg of **1** when subjected to HPLC (LiChroCART LiChrospher Si 60 (10 $\mu$m), EtOAc−$CHCl_3$−heptane 60:5:35, 260 nm).

**Insulicolide A (1):** white crystals; mp 184−185 °C; $[\alpha]^{22}_D$ −360° (c 0.0025, EtOH); UV (EtOH) $\lambda$ max (log $\epsilon$) 254 (4.17); CD $\lambda$ ext (*c* 0.0025, EtOH) ($\Delta\epsilon$) 218 (−18.60), 256 (−3.53); ESMS *m*/*z* 431.0 ± 0.5 amu; NMR, Table 1.

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