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Isolation and Structure of Nodulisporic Acid A_1 and A_2, Novel Insecticides from a *Nodulisporium Sp.*

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Abstract: The isolation and structure elucidation of two novel indole terpene insecticides nodulisporic acid A_1(**2**) and A_2 (**3**) from a Nodulisporium sp. are reported. © 1999 Elsevier Science Ltd. All rights reserved.

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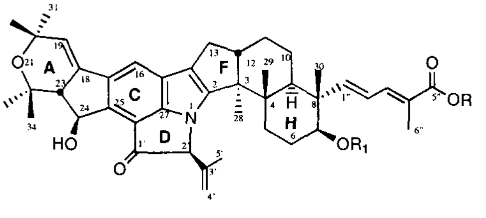
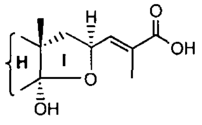
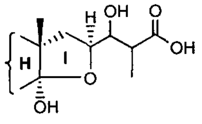
In our ongoing screening program for biologically active natural products we previously isolated the novel insecticide, nodulisporic acid A (**1a**) from a *Nodulisporium sp.* [1]. Its structure and relative stereochemistry were firmly established on the basis of spectroscopic evidence including the computerized INADEQUATE analysis of Dunkel [2-4] and X-ray diffraction analysis of the *p*-bromobenzoate methyl ester derivative **1b**. The absolute stereochemistry was determined by application of the advanced Mosher method [5]. We now wish to report the isolation and structure determination of two fermentation congeners, nodulisporic acids A_1 (**2**) and A_2 (**3**), which exhibit similar biological profiles to nodulisporic acid A.



1a: R = R_1 = H; nodulisporic acid A
1b: R = CH_3, R_1 = p-BrC_6H_4CO



2: nodulisporic acid A_1



3: nodulisporic acid A_2

Nodulisporic acid A_1 (**2**) has an LD_{50} of 0.3 - 1 μg/mL against Lucilia, similar to that of **1a** while **3** was slightly less active with an LD_{50} of 0.6 - 1.5 μg/mL. Previously, some

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structural similarities were noted among several indole diterpenes, such as shearinines and janthitrems, to nodulisporic acid A [1]. Several other indole diterpenes, including paspalines [6], lolitrems [7] and terpendoles [8], have a certain resemblance to A$_1$ and A.

The methyl ethyl ketone extract of whole broth (pH 5.0) of a 24-day fermentation was evaporated to dryness under vacuum. Nodulisporic acids A$_1$ and A$_2$ were isolated from the extract using a silica gel column with 1:1 MeOH:CH_2Cl_2. The fractions were dried, chromatographed on a Sephadex LH20 size exclusion column followed by a final purification on a preparative Zorbax C-18 HPLC column (22.5 mm x 250 mm) in 80% CH_3CN with 0.1% aqueous TFA which gave 1.8 mg of **2,** $[\alpha]^{22}$D -10.5 (c 0.6, MeOH) and 1.0 mg of **3,** $[\alpha]^{22}$D -21.05 (c 0.38, MeOH).

Nodulisporic acid A$_1$: High resolution FAB-MS of nodulisporic acid A$_1$ indicated the molecular formula $C_{43}H_{53}NO_7$ (found m/z 695.3806; calcd m/z 695.3822) which was supported by the carbon and carbon-bound proton counts from ^{13}C NMR and DEPT spectra (Table 1), respectively, assuming three exchangeable protons. The UV sprectum of **2** in MeOH gave absorption bands at λ_{max} 237 (ε 3883), 269 (ε 2767) and 384 (ε 721) nm. The infrared spectrum (ZnSe) showed major absorption bands at ν_{max} 3000, 2975, 1710, 1600, 1550, 1470, 1395, 1240, 1090, 1020 and 980 cm^{-1}. The ^{1}H NMR comparison with that of the major component **1a** showed the H7 carbinol resonance missing and modification of the dienoic acid moiety which was confirmed by an extensive number of HMBC correlations indicating the same "western hemisphere" comprising rings A-G in both components (Figure 1). COSY data suggested an ABMX spin system -CH$_A$H$_B$-CH$_M$(O)-CH$_X$= [J_{AB} = 12.6, J_{AM} = 9.3, J_{BM} = 7.3, J_{MX} = 8.8 Hz] which could be expanded by several HMBC correlations to the proposed structure **2**. For example, long-range correlations *via* the two methyl protons (Figure 1) unequivocally positioned the four-spin system with respect to the terminal isobutenoic acid and ring H in **2** and in particular places the hemiketal carbon (106.9 ppm) at C7. This is further corroborated by the three-bond correlations from H1" and H2" to C7 consistent with the presence of the five-membered tetrahydrofuran ring. Substitution of the carbinol group in **1a** by the hemiketal moiety in **2** satisfies the extra oxygen in the molecule and the same number of exchangeable OH protons in both components.

Nodulisporic acid A$_2$: Nodulisporic acid A$_2$ was shown to have the molecular weight 713 by ESI-MS (observed at m/z 736 as the $[M+Na]^+$ ion). High resolution EI-MS indicated the molecular formula $C_{43}H_{55}NO_8$ (found m/z 695.3768; calcd m/z 695.3822, $[M^+ - H_2O]$) up by a molecule of water from that of nodulisporic acid A$_1$ (**2**). This was supportedby the carbon and carbon-bound proton counts from ^{13}C NMR and DEPT spectra (Table. 1), respectively, assuming four exchangeable protons. The UV spectrum of **3** in MeOH gave absorption bands at λ_{max} 238 (ε 2740), 267 (ε 2420) and 384 (ε 500) nm. The infrared spectrum (ZnSe) showed major absorption bands at ν_{max}3000, 2975, 1710, 1590, 1470, 1380, 1250, 1090, 1070, 1035 and 1000 cm^{-1}. By a similar approach to **2**, structure **3** was proposed for nodulisporic acid A$_2$

Table 1. ^{1}H and ^{13}C NMR Assignments of Nodulisporic Acid A$_1$ (**2**) and A$_2$ (**3**) (CD$_2$Cl$_2$, 500 MHz)^a.

	2		**3**	
Carbon	δC	δH	δC	δH
2	154.8		154.7	
3	55.5		55.4	
4	39.5		39.6	
5	30.0	~1.90 m (2H)	30.7	~1.83 m ~2.00 m
6	30.4	~1.70 m ~1.91 m	30.1	
7	106.9		105.5	
8	49.6		49.2	
9	41.3	1.68 m	47.4	~1.70 m
10	24.8	~1.56 m (2H)	23.2	~1.48 m ~1.92 m
11	26.0	~1.9 (2H)	25.8	~1.78 m ~1.83 m
12	48.1	2.93 m	48.2	2.87 m
13	27.8	2.355 dd (10.8, 14.0) 2.755 dd (6.5, 14.0)	27.8	2.33 dd (10.8, 13.9) 2.77 dd (6.6, 13.9)
14	122.6		122.5	
15	121.8		121.8	
16	116.7	7.72 s	116.7	7.70 s
17	135.9		135.9	
18	134.0		134.0	
19	122.0	6.06 d (2.9)	122.0	6.06 d (2.9)
20	72.6		72.6	
22	73.9		73.9	
23	58.2	2.81 dd (2.9, 6.3)	58.2	2.81 dd (2.9, 6.3)
24	75.3	5.21 d (6.3)	75.3	5.21 d (6.3)
25	138.3		138.4	
26	113.2		113.2	
27	162.0		162.0	
28	29.9	1.34 s	29.9	1.33 s
29	32.0	1.31 s	32.0	1.31 s
30	23.4	1.122 s	23.4	1.118 s
31	30.1	1.42 s	30.0	1.42 s
32	15.3	0.95 s	15.1	0.92 s
33	16.7	1.06 s	16.7	1.05 s
34	17.7	1.126 s	17.6	1.112 s
1'	198.1		198.1	
2'	76.6 br	5.10 s	76.6	5.10 s
3'	140.1 br		140.1	
4'	117.8 br	5.0 br s 5.20 dq (~1.2)	117.9	5.00 br s 5.20 br s
5'	18.0 br	1.42 s	17.8	1.42 s
1"	44.5	1.73 dd (9.3, 12.6) 2.34 dd (7.3, 12.6)	40.3	1.48 dd (~9, ~13) 1.72 dd (7.5, 12.7)
2"	73.3	4.88 br dt (~8)	78.9	4.3 m
3"	147.1	6.93 dq (8.0, 1.3)	81.1	3.96 m^b
4"	126.8		40.4	2.70 m
5"	172.0		176.3 br	
6"	12.5	1.86 d (1.3)	13.2	1.09 d (7.0)

^{a} coupling constants are given in Hz in parentheses.^{b} δ3.80dd (3.1,8.3) spiked with DMSO-d$_6$

which makes sense biogenetically as it corresponds to hydration of the side-chain double bond in compound A$_1$ (**2**). In particular, the COSY evidence readily showed the contiguous array of protons $-CH_2-CH(O)-CH(OH)-CH(CH_3)-$ characterizing the side-chain at C8. Again, numerous HMBC correlations corroborated the proposed structure (cf. Figure. 1) especially the three (C3", C4", C5") and four (C7, C8, C9, C1") long-range correlations from the methyl groups at C4" and C8, respectively, H4" to C2", C3" and C5" and H3" to C1", C2", C4" and C4"-Me.

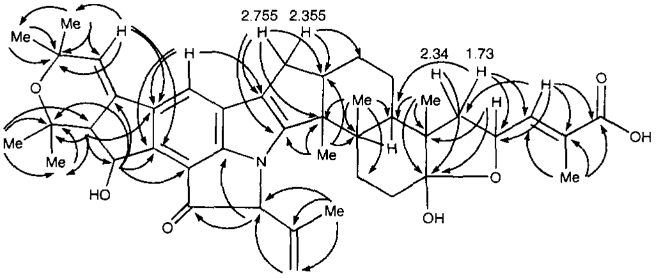

Figure 1: Some HMBC correlations of nodulisporic acid A_1 (**2**)

Stereochemistry: Besides many similar NOESY correlations (mixing time 0.5 sec, delay 2.5 sec) indicating the same relative stereochemistry of A (**1a**) and A_1 (**2**), the configuration at C2" as indicated followed readily from the strong cross peaks between the protons H1"α and H2"α on the α-face of the molecule and between the β oriented methyl group at C8 and the H1"β proton on the β-face. By analogy with **1a**, the complete absolute stereochemistry of **2** is therefore assumed to be the same as in **1a** with the *S* configuration at C2". Similarly, the vicinal couplings (Table.1) in the tetrahydrofuran ring and NOESY correlations in A_2 by comparison with A_1 suggest the same *S* configuration at C2".

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