Tetrahedron Letters, Vol. 34, No. 16, pp. 2569-2572, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

NEW PASPALININE DERIVATIVES WITH ANTIINSECTAN ACTIVITY FROM THE SCLEROTIA OF *ASPERGILLUS NOMIUS*

Gail M. Staub, Katherine B. Gloer, and James B. Gloer*,[1]

Department of Chemistry, University of Iowa, Iowa City, Iowa, 52242

Donald T. Wicklow and Patrick F. Dowd

Agricultural Research Service, National Center for Agricultural Utilization Research, USDA

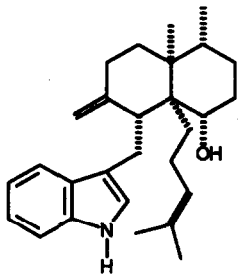
Peoria, IL, 61604

Abstract: 14-Hydroxypaspalinine (**3**) and 14-(N,N-Dimethyl-L-valyloxy)paspalinine (**4**) have been isolated from the sclerotia of *Aspergillus nomius*, and identified by analysis of 2D NMR data. Both compounds cause *ca.* 90% reduction in weight gain in assays against the corn earworm *Helicoverpa zea* at the 100 ppm (dry weight) dietary level. Paspalinine causes no effect at this concentration.

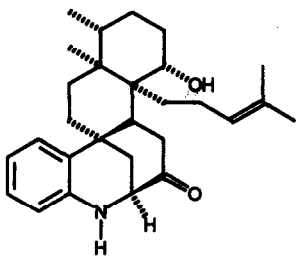
Studies of *Aspergillus* sclerotia as potential sources of new natural products with antiinsectan effects and other bioactivities continue to afford a variety of novel metabolites with activity against insects.[2a-e] Most of these compounds are indole-derived, and several of them contain previously undescribed or rare ring systems. For example, our earlier studies[2c,d] of sclerotial metabolites from *Aspergillus nomius* Kurtzman, Horn, and Hesseltine (NRRL 13137) led to the isolation of nominine (**1**) and aspernomine (**2**), both of which exhibit activity against the corn earworm *Helicoverpa zea*. Further studies of *A. nomius* metabolites are ongoing, and two new compounds (**3** and **4**) have now been isolated. These metabolites are closely related to the tremorgen paspalinine (**5**)[3] originally isolated from *Claviceps paspali*, but **3** and **4** are much more effective than paspalinine in assays against *H. zea*. Compound **4** contains an unusual N,N-dimethylvalyl unit, an amino acyl residue reported from only one other natural source. Details of the isolation, structure elucidation, and biological activity of compounds **3** and **4** are presented here.

Pentane- and chloroform-soluble extracts from sclerotia produced by *A. nomius* (NRRL 13137)[4] exhibited antiinsectan activity against *H. zea*. Chromatographic fractionation of these extracts by reversed-phase HPLC afforded compounds **3** and **4**,[5] as well as paspaline[6] and compounds **1**[2c], **2**[2d], and **5**. The numbering system shown for **3** and **4** was chosen to match that employed in the original literature report of the structure of paspalinine (**5**).[3]

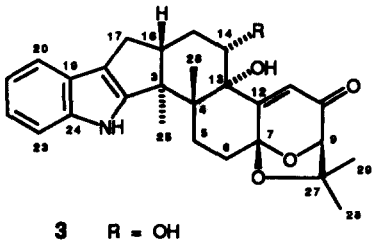
On the basis of HREIMS and ^{13}C NMR data, compound **3** was assigned the molecular formula $C_{27}H_{31}NO_5$. This formula differs from that of paspalinine by the addition of one oxygen atom. As expected, the NMR data revealed close similarities between the two compounds, specifically indicating that one of the five methylene carbons of **5** is replaced by an hydroxylated methine carbon (71.2 ppm) in **3**. The proton spin systems in **3** were identified by analysis of a ^1H-^1H COSY spectrum recorded at 600 MHz. However, these data did not permit unambiguous assignment of the position of hydroxylation, so further information was required. Shift assignments for carbons bound to hydrogen atoms were established on the basis of HMQC data. The remaining carbon NMR assignments and the compositions of the spin systems were determined



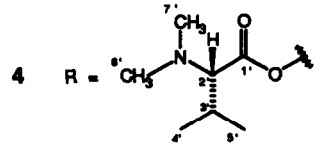
1



2



3 R = OH



4 R =

5 R = H

with the aid of an HMBC experiment. HMBC correlations of the methine proton at 4.25 ppm with signals for carbons 12, 13, 15, and 16 indicated that the secondary alcohol group must be located at position 14. All other HMBC correlations[6] are consistent with the proposed structure, and the remainder of the NMR and mass spectral data for this compound support the assignment of the structure as shown in 3.

The relative stereochemistry at the new chiral center was established through analysis of ^{1}H NMR *J*-values (Table I) and NOESY data. In the NOESY experiment, correlations of H-14 with H-16 and H_3-26 were observed. In addition, the signals for H-16 and H_3-26 showed correlations to H-14 and to each other. These data provided evidence for a 1,3,5-triaxial arrangement of H-14, H-16, and the methyl group H_3-26, indicating the relative stereochemistry at position 14 as shown. This stereochemistry is consistent with the *J*-values observed for H-14, which include an axial-axial coupling with H-15$_{ax}$ (10.7 Hz). The relative configurations at the other chiral centers are proposed to be the same as those of paspalinine, and the remainder of the NOESY data support this assignment. The absolute configuration (as shown in 3) is presumed to be analogous to that of other members of this class of compounds.[3,5]

Proton and carbon NMR spectra for the second new metabolite ($C_{34}H_{44}N_2O_6$ based on HRFABMS) contained signals that matched closely with those for compound 3. The only significant differences were a downfield shift of H-14 (from 4.25 to 5.36 ppm), an upfield shift of H-11 (from 6.24 to 5.60 ppm), and the presence of resonances accounting for the additional carbon atoms and protons indicated by the molecular formula. These observations suggested that the larger compound differed from 3 by acylation at the 14-OH with a $C_7H_{14}NO$ unit. The structure of this acyl subunit was identified by analysis of COSY, HMQC, and HMBC data for 4. The ^{13}C NMR signals associated with the acyl subunit consisted of a carboxyl carbon, four methyl carbons (two bound to nitrogen), and two methine carbons. ^{1}H NMR and COSY data demonstrated that the two methine protons are coupled to each other, and that the upfield methine (2.06 ppm) is part of an isopropyl group. HMBC correlations of the downfield methine proton (2.73 ppm) with the carboxyl, N-methyl, and isopropyl group signals, and correlation of the isopropyl methine with the carboxyl carbon signal, indicated that the acyl group is an N,N-dimethylvalyl unit. This assignment was confirmed by hydrolysis of 4 and isolation of N,N-dimethyl-L-valine from the hydrolyzate.[7] Connection of the acyl group to the oxygen at C-14 of the hydroxypaspalinine core structure was confirmed by analysis of a selective INEPT experiment, which afforded a 3-bond correlation of the H-14 resonance to the carboxyl carbon signal of the N,N-dimethylvalyl group (169.6 ppm). The only prior reports of the natural occurrence of the N,N-dimethylvalyl unit describe its presence as an amino acyl unit in the dolastatins, a family of peptide antineoplastic agents isolated from sea hares.[8]

Table I. ^{1}H (600 MHz) and ^{13}C (75 MHz) NMR Data for **3** and **4** in $CDCl_3$[a]

	3		4	
position	^{1}H	^{13}C	^{1}H	^{13}C
1	7.69 (s)	--	7.70 (s)	--
2	--	151.0	--	150.7
3	--	50.5	--	51.2
4	--	40.1	--	41.0
5 ax	2.68 (dd, 12.5, 10.4)	27.6	2.69 (m)	27.7
eq	1.79 (ddd, 12.7, 10.0, 8.9)		1.81 (m)	
6 ax	2.84 (m)	28.5	2.86 (m)	28.6
eq	2.00 (m)		1.99 (m)	
7	--	104.7	--	104.6
9	4.30 (br s)	88.1	4.29 (s)	88.4
10	--	197.8	--	196.1
11	6.24 (s)	120.3	5.60 (s)	119.5
12	--	167.0	--	166.0
13	--	79.3	--	79.1
14	4.25 (dd, 10.4, 5.7)	71.2	5.36 (dd, 10.5, 5.5)	74.6
15 ax	2.04 (ddd, 14.2, 12.4, 10.7)	31.9	2.28 (m)	28.9
eq	2.00 (m)		2.03 (m)	
16	2.84 (m)	45.2	2.86 (m)	45.1
17 eq	2.71 (dd, 13.2, 6.4)	27.2	2.74 (m)	27.2
ax	2.45 (dd, 13.2, 10.6)		2.45 (dd, 13.0, 10.7)	
18	--	117.3	--	117.5
19	--	125.0	--	125.0
20	7.41 (br d, 6.9)	118.5	7.42 (br d, 6.8)	118.6
21	7.06 (ddd, 7.0, 7.0, 1.6)	119.7	7.07 (m)	119.9
22	7.08 (ddd, 7.0, 7.0, 1.6)	120.7	7.09 (m)	120.9
23	7.27 (br dd, 7.0, 1.6)	111.5	7.28 (br d, 7.0)	111.6
24	--	139.8	--	140.0
25	1.36 (s)	16.2	1.40 (s)	16.4
26	1.21 (s)	23.1	1.26 (s)	23.2
27	--	78.4	--	78.5
28	1.42 (s)	28.8	1.41 (s)	28.6
29	1.17	23.2	1.15 (s)	23.1
1'	-	-	--	169.6
2'	-	-	2.73 (br d, 10.0)	74.4
3'	-	-	2.06 (m)	27.3
4'	-	-	0.96 (d, 6.6)	19.1
5'	-	-	0.94 (d, 6.6)	20.0
6'\7'	-	-	2.33 (s)	41.6

[a]Assignments are based on DEPT, COSY, HMBC, and HMQC data.

Compound **3** exhibits potent activity against the first instar larvae of *H. zea*. Incorporation of **3** into a standard pinto bean test diet at 100 ppm (dry weight)[12] caused a 91% reduction in weight gain of the test insects relative to controls. Compound **4** gives virtually the same result (88%). Interestingly, paspalinine itself (**5**) is inactive in this assay at the same concentration. To our knowledge, compound **4** is the first microbial metabolite reported to contain an N,N-dimethylvaline subunit, and **3** and **4** are the first representatives of the paspalinine/penitrem class to possess functionality at C-14.

Acknowledgment. This work was conducted under Cooperative Research Agreement No. 58-5114-O-1006 between the USDA ARS and the University of Iowa. Support provided by the National Science Foundation (CHE-8905894), and Biotechnology Research and Development Corporation is gratefully acknowledged.

References and Notes

1. Alfred P. Sloan Fellow (1990-94) and NIH Research Career Development Awardee (1990-95; K04 CA 01571).
2. (a) Wicklow, D. T.; Dowd, P. F.; TePaske, M. R.; Gloer, J. B. *Trans. Br. Mycol. Soc.* **1988**, *91*, 433. (b) Gloer, J. B.; TePaske, M. R.; Sima, J.; Wicklow, D. T.; Dowd, P. F. *J. Org. Chem.* **1988**, *53*, 5457. (c) Gloer, J. B.; Rinderknecht, B. L.; Wicklow, D. T.; Dowd, P. F. *J. Org. Chem.* **1989**, *54*, 2530. (d) Staub, G. M.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *J. Am. Chem. Soc.* **1992**, *114*, 1015. (e) Laakso, J. A.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *J. Org. Chem.* **1992**, *57*, 138.
3. Gallagher, R. T.; Finer, J.; Clardy, J.; Leutwiler, A.; Weibel, F.; Acklin, W.; Arigoni, D. *Tetrahedron Lett.* **1980**, *21*, 235.
4. *A. nomius* (NRRL 13137) was obtained from the USDA ARS Culture Collection, Peoria, IL. Sclerotia were prepared by solid substrate fermentation using procedures that have been previously described.[2a]
5. Sclerotia of *A. nomius* (59 g) were ground with a mortar and pestle and Soxhlet-extracted with pentane to afford 378 mg of a light yellow oil. Further extraction of the sclerotial solids with $CHCl_3$ (5 x 50 mL), and concentration of the combined $CHCl_3$ extracts afforded 214 mg of a yellow oil. A portion of the $CHCl_3$ extract (179 mg) was subjected to reversed-phase semipreparative HPLC (Beckman Ultrasphere 5μ C_{18} column; 10 x 250 mm; 8:2 MeOH:H_2O at 2.0 mL/min) to afford 5.8 mg of compound **3**, which gave: $[\alpha]_D^{29}$ = +127° (MeOH; c = 0.11 g/dL); ^1H and ^{13}C NMR data, Table I; HMBC correlations (^1H signal---^{13}C correlations): H-1---C-2, 18, 19, 24; H-5_{ax}---C-3, 4, 6, 7; H-5_{eq}---C-4, 6, 13; H-6_{ax}---C-4, 5, 7; H-6_{eq}---C-4, 7, 12; H-9---C-7, 10, 11, 28; H-11---C-7, 9, 12, 13; H-14---C-12, 13, 15, 16; H-15_{ax}---C-3, 13, 14, 16; H-15_{eq}---C-3, 13, 14, 16, 17; H-16---C-3, 4, 14, 15, 17, 25; H-17_{eq}---C-3, 16, 18; H-17_{ax}---C-15, 16, 18; H-20---C-18, 19, 22, 24; H-21---C-19, 23; H-22---C-20, 24; H-23---C-19, 21, 24; H-25---C-2, 3, 4, 16; H-26---C-3, 4, 5, 13; H-28---C-9, 27, 29; H-29---C-9, 27, 28. EIMS (70 eV) *m/z* 449 (M^+; rel. int. 3%), 434 (4), 358 (1), 285 (0.5), 265 (0.6), 212 (2), 182 (10), 168 (8), 130 (9), 100 (14), 44 (65), 43 (26). HREIMS, obsd. 449.2185, calcd. for $C_{27}H_{31}NO_5$, 449.2202.
Reversed-phase preparative HPLC separation of the pentane extract (Rainin Dynamax-60A 8 μ C_{18} column; 21.4 mm x 25 cm; 85:15 MeOH:H_2O at 11.2 mL/min) afforded 39.6 mg of compound **4**, which gave: $[\alpha]_D^{26}$ = +102° (MeOH; c = 0.44 g/dL); UV (MeOH) λ_{max} 228 (ε 15620), 279 (3110); IR (neat) 3399, 2936, 1734, 1690 cm^{-1}; ^1H and ^{13}C NMR data, Table I; FABMS (3-NBA matrix) *m/z* 577 [$(M + H)^+$, rel. int. 100%], 533 (3.9), 519 (5.2), 449 (2.1), 448 (4.2), 431 (3.8), 390 (4.4), 374 (6.7), 358 (5.2). HRFABMS obsd. 577.3278, calcd. for $C_{34}H_{45}N_2O_6$ $(M + H)^+$, 577.3276. Compound **4** showed most of the same HMBC correlations as **3**. Additional key correlations associated with the acyl unit of **4** include: H-14---C-1'; H-2'---C-1', 3', 4', 5', 6', 7'.
6. Springer, J. P.; Clardy, J. *Tetrahedron Lett.* **1980**, *21*, 231.
7. A 10-mg sample of **4** was placed in a screw-cap vial with 1.5 mL of 6N HCl. The vial was sealed and heated at 110 °C for 24 hr. The solution was filtered and extracted with $CHCl_3$ (3 X 1.5 mL) and EtOAc (3 X 1.5 mL). Evaporation of the aqueous solution afforded an off-white solid which was identified as N,N-dimethyl-L-valine on the basis of ^1H NMR, TLC, and $[\alpha]_D$ comparison with literature values,[9-11] and with values for an authentic standard prepared using a published method.[9]
8. Petit, G. R.; Herald, D. L.; Singh, S. B.; Thornton, T. J.; Mullaney, J. T. *J. Am. Chem. Soc.* **1991**, *113*, 6692.
9. Casella, L.; Gullotti, M. *Inorganic Chemistry*, **1983**, *22*, 242.
10. Hawkins, C. J.; Lawrance, G. A. *Aust. J. Chem.* **1973**, *26*, 1801.
11. Bowman, R. E.; Stroud, H. H. *J. Chem. Soc.* **1950**, 1342.
12. Dowd, P. F. *Entomol. Exp. Appl.* **1988**, *47*, 69.

(Received in USA 12 January 1993; accepted 10 February 1993)