Pergamon

Tetrahedron Letters, Vol. 38, No. 32, pp. 5655-5658, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01231-8

Asperparaline A, a New Paralytic Alkaloid from *Aspergillus japonicus* JV-23

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Abstract: A new paralytic alkaloid has been isolated from *Aspergillus japonicus* JV-23 and its structure was elucidated from NMR and X-ray crystallography data. The metabolite was designated asperparaline A. © 1997 Elsevier Science Ltd.

In our continuing studies on bioactive fungal metabolites using a bioassay with silkworm (*Bombyx mori*), we got from soil samples collected in Sakai an isolate *Aspergillus japonicus* JV-23[1)] which exhibited paralysis in silkworm. As a result of purification, we isolated a paralytic compound, asperparaline A (**1**), from *A. japonicus* JV-23 cultured with okara (the water insoluble residue of whole soybean). Herein we report the structure determination and paralytic activity of **1**.

Asperparaline A (**1**) was shown to have the molecular formula of $C_{20}H_{29}N_3O_3$ by the HR-EIMS together with NMR data (Table), indicative of eight degrees of unsaturation. Resonances at 171.9, 175.3, and 181.6 ppm in the ^{13}C-NMR spectrum of **1** indicated the presence of three carbonyl carbons, one of which must be an amido carbonyl and two other carbons were imido carbonyls in a five-membered ring on the basis of the IR data (1773 and 1698 cm^{-1}), showing **1** to be pentacyclic. In the ^{13}C-NMR spectrum, four quarternary carbons were observed besides three carbonyl carbons. In turn, the ^{1}H-NMR spectrum confirmed the presence of five methyls (two *N*-methyls, two tertially methyls and a secondary methyl), three isolated methylenes, a -CH_2-CH< linkage, and a -CH_2-CH_2-$CHCH_3$- linkage. For the connectivity of partial structures, HMBC experiments were carried out. The signal of H_3-23 (δ_H 2.98) was correlated with C-19 (δ_C 175.3) and C-21 (δ_C 181.6), indicating that the H_3-23 was a *N*-methyl in a succinimide moiety. The correlations between H_2-18 (δ_H 2.53 and 2.96) and C-10 (δ_C 44.5), C-11 (δ_C 57.8) and C-19, and

between H_2-12 (δ_H 1.64 and 2.77) and C-10, C-11, C-18 (δ_C 38.1) and C-21 supported the presence of the *N*-methylsuccimide moiety. These correlations also indicated that C-10 and C-12 were linked to C-11, which was linked to C-18 and C-21. The signals of H_3-16 (δ_H 1.09) and H_3-17 (δ_H 0.87) were correlated with each other, and both of them were correlated with C-6 (δ_C 53.2), C-10 and C-11, indicating that H_3-16 and H_3-17 were *geminal* methyls at C-10. In the ^1H-^1H COSY spectrum, the signal of H-6 (δ_H 2.83) was coupled with the signals of H_2-5 (δ_H 1.37 and 2.03), suggesting that C-5 and C-6 composed a -CH_2-CH< linkage. The signals of H_2-8 (δ_H 2.44 and 3.34) were correlated with C-7 (δ_C 64.4), the signal of H-6 (δ_H 2.83) with C-7 and C-8 (δ_C 59.1), the signals of H_2-12 with C-7 and C-8. These data suggested the presence of a cyclopentane ring made up with C-6, C-7, C-12, C-11 and C-10. The signal of H_3-22 (δ_H 2.98) was correlated with C-14 (δ_C 171.9), indicating that C-14 and N-15 composed a *N*-methyl amide group. The signal of H_3-22 was also correlated with C-7, indicative of the linkage between C-7 and N-15. Furthermore, the signal of H-1 (δ_H 3.16) was correlated with C-4 (δ_C 67.1), the signals of H_2-5 (δ_H 2.03 and 1.37) with C-4 and C-14, the signal of H-8 with C-1 and C-4, and the signal ofH_2-2 (δ_H 2.01) with C-4, strongly suggesting the presence of an indolizine moiety. Consequently, the plain structure of asperparaline A was determined. Furthermore, the confirmation of the structure of **1** was obtained by the application of X-ray crystallograpic analysis.[2] An ORTEP drawing of **1** is shown in Fig. 1.

The present study clarified the structure of asperparaline A (Fig. 2) as a sipro compound made up with a *N*-methylsuccimide and cyclopent[f]indolizine with a *N*-methylamide bridge. Paraherquamides[4] and sclerotiamide[5] are reported to have a cyclopent[f]indolizine ring system, and marcfortines[6] are known to contain a piperidine ring instead of a pyrrolidine ring in paraherquamides and sclerotiamide. All these compounds have a indole moiety in their structures, so asperparaline A has a quite unique structure.

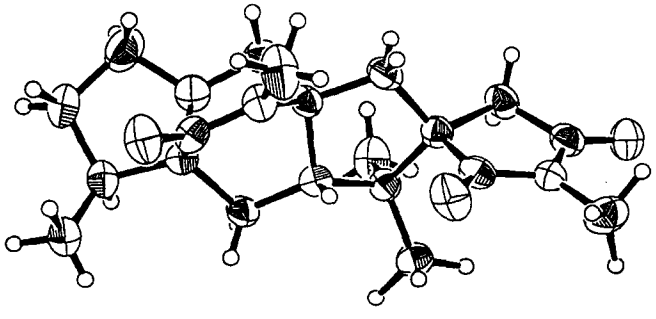

Fig. 1. ORTEP View of Asperparaline A (**1**)

Table NMR Data for Asperparaline A (1)[a]

position	^{13}C NMR δ_C (mult.)	^{1}H NMR δ_H (integral, mult., J Hz)	HMBC correlation (H to C)
1	53.1 (t.)	2.21 (1H, m.)	C-2, C-3,
		3.16 (1H, m.)	C-3, C-4
2	30.0 (t.)	1.80 (1H, m.)	C-3, C-13
		2.01 (1H, m.)	C-4
3	40.1 (d.)	1.90 (1H, m.)	C-5
4	67.1 (s.)		
5	27.7 (t.)	1.37 (1H, dd., 12.5, 9.8)	C-4, C-6, C-10, C-14
		2.03 (1H, dd., 12.5, 9.8)	C-4, C-6, C-7
6	53.2 (d.)	2.83 (1H, t., 9.8)	C-5, C-7, C-8, C-10, C-16, C-17
7	64.4 (s.)		
8	59.1 (t.)	2.44 (1H, d., 11.0)	C-1, C-7
		3.34 (1H, d., 11.0)	C-4
10	44.5 (s.)		
11	57.8 (s.)		
12	38.9 (t.)	1.64 (1H, d., 15.0)	C-6, C-7, C-8, C-11, C-18, C-21
		2.77 (1H, d., 15.0)	C-7, C-8, C-10, C-11, C-21
13	12.9 (q.)	1.38 (3H, d., 7.0)	C-2, C-3
14	171.9 (s.)		
16	19.9 (q.)	1.09 (3H, s.)	C-6, C-10, C-11, C-17
17	23.7 (q.)	0.87 (3H, s.)	C-6, C-10, C-11, C-16
18	38.1 (t.)	2.53 (1H, d., 18.6)	C-11, C-19
		2.96 (1H, d., 18.6)	C-10, C-11, C-19
19	175.3 (s.)		
21	181.6 (s.)		
22	24.7 (q.)*	2.98 (3H, s.)	C-7, C-14
23	25.3 (q.)*	2.98 (3H, s.)	C-19, C-21

[a] Taken in $CDCl_3$ at 500 MHz for ^{1}H and 125 MHz for ^{13}C;
* Assingments may be interchangeable.

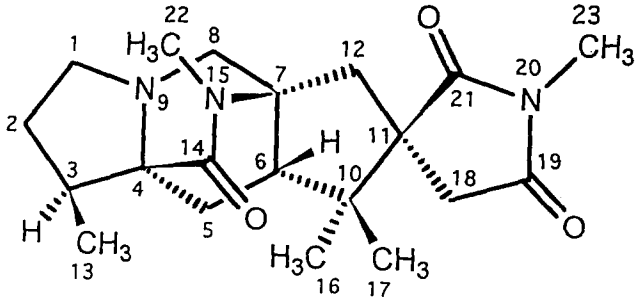

Fig. 2. Structure of Asperparaline A (1)

Biological activity of asparparaline A (**1**) was examined against fourth instar larvae of silkworm. Upon oral administration, **1** exhibited paralysis with a dose of 10 μg/g of diet within one hour and lasted for 7 to 10 hours. When injected with a microsyringe, **1** exhibited the paralysis at a dose of 3 μg/g of body within 20 minutes and lasted for 4 to 5 hours.

Acknowledgements. The authors express their thanks to Dr. K. Irie of the Department of Food Science and Technology at Kyoto University for the MS measurements. They are also grateful to Dr. T. Fujita of the Department of Applied Biological Chemistry at Osaka Prefecture University for the 500-MHz NMR measurements.

References and Notes

1) Identification of a strain JV-23 was carried out at Centraalbureau voor Schimmelcultures, the Netherland.

2) Data for **1**: colorless prisms; mp. 203-205 °C; $[\alpha]_D^{20}$ -8.5° (*c* 0.21, MeOH); IR (KBr) 1773, 1698, 1650, 1429 cm^{-1}; HR-EIMS $C_{20}H_{29}N_3O_3$ *m/z* 359.2195 (Δ −1.4 mmu); ^1H- and ^{13}C-NMR see Table.

3) Asperparaline A (**1**) was crystallized from toluene to afford colorless prisms. The crystals of 0.20 x 0.20 x 0.20 mm in size were orthorhombic ($P2_12_12_1$) with latice parameters a=16.153(4), b=17.728(5), c=6.744(3) Å, V=1931.2(10) $Å^3$, Z=4, and D_C=1.236 g/cm^3. All reflections with $2\theta_{max}$<120.1 ° were collected at 23 °C in the ω-2θ scan mode with a Rigaku AFC7R diffractometer (λ(CuKα)-1.54178 Å). Of the 1699 reflections collected, 1543 were judged to be observed after correction for Lorentz and polarization effect. The structure was determined by direct methods (SAPI91). Full-matrix least-squares refinement, with anisotropic temperature factors for the non-hydrogen atoms and isotropic factors for the hydrogen atoms, converged to a final R factor of 0.045 for the 1543 reflections.

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(*Received in Japan* 30 *April* 1997; *revised* 2 *June* 1997; *accepted* 18 *June* 1997)