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STRUCTURE OF ASZONALENIN, A NEW METABOLITE OF ASPERGILLUS ZONATUS

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Summary : Aszonalenin (1) has been isolated from Aspergillus zonatus together with LL-S490β (2) and the structure of 1 has been established by spectroscopic evidences and chemical transformation.

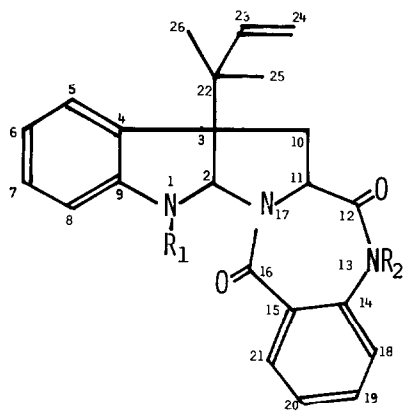
In the course of our screening for biologically active substances among fungal metabolites, we isolated a new compound from Aspergillus zonatus IFO 8817. We wish to report the isolation and structure of this compound (1) designated aszonalenin. In addition to 1, we isolated LL-S490β (2) which had been obtained from an unidentified Aspergillus species by Ellestad et al.[1)]

The fungus was stationarily cultured at 24°C for 21 days in the malt extract medium. The acetone extract from the dry mycelial mats was chromatographed on a column of silica gel and eluted with benzene-acetone (19:1, v/v). After elution of LL-S490β (2), aszonalenin (1) was obtained as a crude substance, which was rechromatographed over silica gel and eluted with benzene-ethyl acetate (19:1, v/v) to give colorless crystals in a yield of 51.2 mg/10 g dry mycelia.

Aszonalenin (1), $C_{22}H_{23}N_3O_2$ (EI-MS, m/z 373 M^+ and elementary analysis[2)]) mp. 244-247°C, $[\alpha]_D^{20}$ +53° (c=1.31, $CHCl_3$), forms colorless needles (from $CHCl_3$-MeOH). The physicochemical data of 1 are as follows. UV λ_{max}^{EtOH} nm(ε); 210 (44,700), 233 (sh, 25,100), 290 (5050). IR ν_{max}^{KBr} cm^{-1}; 3400 (NH), 1700 (C=O, amide), 1640 (C=O, amide), 1620 (C=C), 1578 (Ar). MS m/z; 373 (M^+), 304 (M^+-69, base peak), 130. ^{1}H-NMR spectrum among other spectra was very similar to that of 2. Though a signal at δ 2.59 due to methyl protons of acetyl group in 2 was not observed on the ^{1}H-NMR spectrum of 1, an additional signal at δ ca. 7.0 assignable to -NH- appeared in the ^{1}H-NMR spectrum of 1. In the ^{13}C-NMR spectrum of 1, signals at δ 24.2 (q) and 171.7 (s) due to acetyl group of 2 were not observed as mentioned below.

The presence of a 1,1-dimethyl-2-propenyl group in 1 was proved by the ^{1}H-NMR spectrum (δ 1.08, 1.16, 5.04, 5.07, 6.10)[3)] and the ^{13}C-NMR spectrum (δ 22.6, 22.9, 41.7, 114.3, 144.1)[4)] together with IR absorption band (1620

cm^{-1}). A peak at m/z 304 (M^+-69) in the mass spectrum supported the presence of a 1,1-dimethyl-2-propenyl ion.[5] 1 was positive to Ehrlich test. The prominent peak at m/z 130 was assigned to the indoline-3-methylene ion.[6] The UV spectrum (λ_{max}^{EtOH} :210, 233,290 nm) suggested the presence of indoline chromophore[7] together with indicating the presence of another chromophoric unit. In the ^{1}H-NMR spectrum, a signal at δ 8.77 was assigned to an amide proton by comparison with the spectrum of 2. From these results and the presence of eight aromatic protons between δ 6.55-7.92, another aromatic ring except for a benzene ring of indoline moiety must be present in 1. The IR spectrum of 1 showed absorption bands at 3240, 1700 and 1640 cm^{-1}, the latter two absorptions of which indicated the dipeptide system.[7b] The molecular formula and the above evidences led to the presence of 3,4-dihydro-4-methyl-1H-1,4-benzodiazepine-2,5-dione moiety [UV λ_{max}^{EtOH} nm (ε);215 (32,140), 291 (2180). IR ν_{max}^{Nujol} cm^{-1} ;1698, 1639. ^{1}H-NMR (in $CDCl_3$) δ ;7.0-7.9 (4 x Ar-H)].[8] From these results obtained above, the structure of 1 was determined as shown in Fig.



1 $R_1=R_2=H$

2 $R_1=COCH_3$, $R_2=H$

3 $R_1=R_2=COCH_3$

In the ^{13}C-NMR spectrum of 1, signals at δ 33.6 (t) and 57.5 (d) were assigned to C-10 and C-11 from the multiplicities and by comparison with the chemical shifts of echinulin (lit.,[4] 29.0 and 51.6). Also, two characteristic signals at δ 170.2 and 168.0 could be easily assigned to amide carbons of C-12 and C-16, respectively.[4] As mentioned above, signals due to the 1,1-dimethyl-2-propenyl carbons were observed. Twelve signals between δ 119.3-141.8 were assigned by comparison with the chemical shifts calculated for o-toluidine and o-tolyl-isocyanate from the equation of ^{13}C substituent effects of substituted benzene.[9] The remaining two signals at δ 82.0 and 61.1 were assigned to C-2 and C-3 from the chemical shifts and multiplicities. These signals were well coincident with those of 2 except for signals at δ 24.2 and 171.7 due to an acetyl group.

Table. ^{1}H-NMR and ^{13}C-NMR Spectral Data of Aszonalenin (1) and LL-S490β (2)[a]

		1			2			*Calcd.*
Position	Group	δ_H, ppm	J, Hz	δ_C, ppm	δ_H, ppm	J, Hz	δ_C, ppm	δ_C, ppm
1	NH	*ca.* 7.0	-	-	-	-	-	-
2	CH	5.57 s	-	82.0 d	6.10 s	-	82.4 d	-
3	-C-	-		61.1 s	-	-	60.7 s	-
4	=C-	-	-	131.5 s	-	-	134.4 s	133.8 s[b]
5	=CH	6.55		130.9 d	6.83		130.7 d	130.4 d[b]
6	=CH	| m	-	125.2 d	| m	-	124.8 d	125.6 d[b]
7	=CH			125.3 d			125.3 d	126.8 d[b]
8	=CH	7.92		121.3 d	8.17		124.8 d	124.8 d[b]
9	=C-	-	-	135.4 s	-	-	134.8 s	134.9 s[b]
10	CH_2	2.45 dd	14.0	33.6 t	2.42 dd	14.0	30.6 t	29.0 t[e]
			8.0			8.0		
		3.46 dd	14.0		3.39 dd	14.0		
			8.5			8.5		
11	CH	4.02 dd	8.0	57.5 d	3.87 dd	8.0	56.9 d	55.5 d[e]
			8.5			8.5		
12	C=O	-	-	170.2 s	-	-	169.5 s	168.0 s[e]
13	NH	8.77 s	-	-	8.62 s	-	-	-
14	=C-	-	-	149.3 s	-	-	141.8 s	146.6 s[c]
15	=C-	-	-	126.8 s	-	-	127.4 s	124.3 s[c]
16	C=O	-	-	168.0 s	-	-	167.6 s	165.8 s[e]
17	N	-	-	-	-	-	-	-
18	=CH	6.55		109.5 d	6.83		119.3 d	115.2 d[c]
19	=CH	|		133.0 d	|		132.8 d	133.6 d[c]
20	=CH	| m	-	118.9 d	| m	-	121.1 d	118.7 d[c]
21	=CH	7.92		128.5 d	8.17		129.1 d	129.5 d[c]
22	-C-	-	-	41.7 s	-	-	41.0 s	39.3 s[e]
23	=CH	6.10 dd	16.0	144.2 d	6.00 dd	16.0	143.6 d	144.1 d[e]
			10.0			10.0		
24	$=CH_2$	5.04 dd	16.0	114.3 t	5.20 dd	16.0	114.3 t	113.1 t[e]
			2.0			2.0		
		5.07 dd	10.0		5.25 dd	10.0		
			2.0			2.0		
25	CH_3	1.08 s	-	22.6 q	1.02 s	-	22.6 q	27.4 q[e]
26	CH_3	1.16 s	-	22.9 q	1.16 s	-	23.0 q	27.4 q[e]
27	C=O	-	-	-	-	-	171.7 s	-
28	CH_3	-	-	-	2.59 s	-	24.2 q	-

[a]Measured in $CDCl_3$ solution. [b]Calculated for o-toluidine. See reference 9). [c]Calculated for o-tolylisocyanate. See reference 9). [e]Obtained from reference 4).

Furthermore, the validity of this structure was proved by acetylation of 1. Treatment of 1 with acetic anhydride in pyridine at reflux for 3 hours gave mono- and di-acetates (2 and 3). Monoacetate of 1, mp. 242-244°C, was identified as LL-S490β in comparison with IR, UV, ^{1}H-NMR and MS spectra. The structure of diacetate [δ 1.38 (3H, s, CH_3CO-), 2.68 (3H, s, CH_3CO-)] was determined as 3[10] in which -NH-s of indoline and benzodiazepine moiety were acetylated. On acetylation with the same condition, LL-S490β also gave 3.

Application of 1 at a concentration of 50 μg/ml apparently induced the abnormal second cleavage of the sea urchin embryos.

References and Footnotes

1) G. A. Ellestad, P. Mirando and M. P. Kunstmann, J. Org. Chem., 24, 4204 (1973).

2) 1: *Anal.* Found :C, 73.68;H, 6.33;N, 11.28, Calcd. for $C_{23}H_{23}N_3O_2$:C, 73.97; H, 6.21;N, 11.25;O, 8.57 %.

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6) J. H. Beynon, R. A. Sanders and A. E. Williams, "The Mass Spectra of Organic Molecules", Elsevier, New York, N. Y., 1968, p.303.

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8) P. K. Martin, H. Rapoport, H. W. Smith and J. L. Wong, J. Org. Chem., 34, 1359(1969).

9) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemist", Wiley-Interscience, a Division of John Wiley & Sons, Inc., New York, N. Y., 1972, p.81.

10) 3: White amorphous mp. 218-221°C; UV λ_{max}^{EtOH} nm(ε): 206 (72,200), 237 (25,400), 277 (5000); IR ν_{max}^{KBr} cm^{-1}: 1735, 1720, 1688, 1650,1600; ^{1}H-NMR (in $CDCl_3$): 0.90 (3H, s), 1.12 (3H, s), 1.38 (3H, s), 2.68 (3H, s), 2.54 (1H, dd, J=14.0, 8.5 Hz), 3.12 (1H, d, J=14.0 Hz), 4.22 (1H, d, J=8.5 Hz), 5.08 (1H, dd, J=16.0 , 2.0 Hz), 5.13 (1H, dd, J=10.0, 2.0 Hz0, 5.86 (1H, dd, J=16.0, 10.0 Hz), 6.11 (1H, s), 7.02-8.02 (m, 8 x Ar-H); MS m/z: 457 (M^+), 415 (M^+-42), 346 (M^+-42-69), 304 (M^+-42-69-42), 130.

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