Short Communication

Structure of Aszonapyrone A,
a New Metabolite Produced by
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In the course of our screening for biologically active substances among the fungal metabolites, we isolated a new compound showing antibacterial activity from *Aspergillus zonatus* IFO 8817. We wish to report the isolation and structure of this compound **1** designated as aszonapyrone A.

The fungus was stationarily cultured at 24°C for 21 days in a malt extract medium. The acetone extract from the dry mycelial mats was chromatographed over silica gel and eluted with benzene-acetone (19:1, v/v). After evaporation of the solvent, the residual solid containing **1** was rechromatographed on a column of silica gel and eluted with a benzene-ethyl acetate mixture. After elution of aszonalenin,¹⁾ crude **1** was obtained from the eluate of benzene-ethyl acetate (9:1, v/v). Recrystallization from CHCl₃-MeOH afforded pure aszonapyrone A as colorless needles.

Aszonapyrone A (**1**), C₂₈H₄₀O₅ (EI-MS, *m/z* 456 M⁺ and elementary analysis), mp 242~244°C, [α]_D²⁰ +33° (*c* = 1.06, CHCl₃) was detected under a UV lamp. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹; 3400, 3090, 2940, 2850, 1728, 1665, 1640, 1580, 1445, 1410, 1380, 1255, 893. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ); 210 (16,900), 290 (7900). MS *m/z*; 456 (M⁺), 396, 317, 257, 192, 139, 135, 121, 107. In the ¹H-NMR spectrum (Pyridine-*d*₅, 400 MHz), the signals at δ 1.93 (3H, s) and 6.09 (1H, s) together with IR absorption bands at 3400,

1665 and 1580 cm⁻¹²⁾ and a UV maximum at 290 nm³⁾ indicated the presence of a 3'-substituted-4'-hydroxy-6'-methyl α -pyrone moiety **4** in **1**. In the ¹³C-NMR spectrum of **1**, the signals due to 28 carbons were observed. A doublet at δ 100.8 due to C-5' appeared and four singlet signals at δ 165.9, 103.2, 165.8, 159.4 due to C-2', -3', -4', -6' of an α -pyrone moiety in this order were assigned by comparison with those of known compounds.⁴⁾ The presence of an acetoxyl group was proved by the ¹H-NMR spectrum at δ 2.08 (3H, s) and the ¹³C-NMR spectrum at δ 21.0 (q) and 170.4 (s) together with IR absorption bands at 1728 and 1255 cm⁻¹. The IR absorption bands at 1640, 1445 and 890 cm⁻¹ indicated the presence of a terminal methylene group, appearing at δ 5.02 (1H, s) and 5.57 (1H, s), due to olefinic protons and at δ 107.0 (t) and 149.7 (s) due to carbons of this function.

From these results and the molecular formula, a tricyclic diterpene moiety was suggested to exist in the molecule of **1**. **1** was treated with OsO₄⁵⁾ followed by NaIO₄ to give a keto

compound **2**: mp 247~248°C. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ); 286 (9800). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} ; 3350, 2900, 2850, 1730, 1710, 1675, 1642, 1580, 1255, MS m/z ; 458 (M^+). In the ^1H -NMR spectrum, the signals due to terminal methylene protons were not observed. In the IR spectrum of **2**, a strong absorption band at 1710 cm^{-1} newly appeared, accompanied by the disappearance of the IR absorption bands due to a terminal methylene in **1**. These results proved the presence of an exomethylene of a six membered ring in **1**.

In the ^1H -NMR spectrum of **1**, the three protons indicating an ABX system were observed at δ 2.88 (1H, d, $J=13\text{ Hz}$), 3.12 (1H, d, $J=12\text{ Hz}$) and 3.18 (1H, dd, $J=12$ and 13 Hz), the former signal of which was assigned to an allylic proton and the latter two to methylene protons connected with an α -pyrone moiety from the chemical shifts. Further the signals in **1** at δ 2.05 (1H, m) and 2.39 (1H, m) assignable to allylic protons exhibited coupling with the methylene protons at δ 1.51 (2H, m), which also coupled with a double doublet proton at δ 1.10 (1H, dd, $J=2$ and 12 Hz). From these results, the partial structure **5** was elucidated as shown in the figure. The presence of **5** was further supported with the signals at δ 40.6, 55.4, 24.2, 38.4, 149.7, 60.0, 20.0 and 107.0 due to C-8, -9, -11, -12, -13, -14, -15 and -16 in this order which are well coincident with those of manool⁶⁾ and which contains the same partial structure.

Oxidation of **1** with alkaline hydrogen peroxide⁷⁾ was followed by chromium trioxide-pyridine oxidation to give a crude material, which was then methylated with diazomethane to afford a keto ester **3**; mp 169~171°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} ; 1740, 1710, 1640, 1440, 1270, 895. MS m/z ; 418 (M^+). UV absorption maximum due to the α -pyrone moiety in **1** was not observed. The presence of a $-\text{CH}(\text{COOCH}_3)_2$ group in **3** was deduced by carbon signals at δ 50.0 (d), 52.4 (q), 52.6 (q), 170.2 (s), 170.4 (s) and methoxycarbonyl protons at δ 3.80 (6H, s), together with IR absorption bands at 1740 and 1270 cm^{-1} . Also, the signals due to an acetyl group in **1** were not observed in the ^1H -

and ^{13}C -NMR spectra of **3**. However, a strong IR absorption band at 1710 cm^{-1} together with a newly appearing carbon signal at δ 217.8 (s), accompanied by the disappearance of a carbon signal at δ 80.8 (d) in **1**, indicated the occurrence of a six membered ring ketone in **3**. From these results, in the ^1H -NMR spectrum of **1**, the signal at δ 4.64 (1H, dd, $J=4$ and 12 Hz) was assigned to a methine proton bearing an acetoxyl group, which exhibited coupling with the signals at δ 1.69 (1H, m) and 2.09 (1H, m) due to methylene protons, both of which also coupled with the signal at δ 1.33 (2H, m). These results suggested the presence of the partial structure **6** which is often contained in the diterpens. Furthermore, the partial structure **6** was determined by the carbon signals of **1** at δ 38.6 (t), 23.9 (t), 80.8 (d), 37.8 (s), 55.3 (d), 38.1 (s), 19.3 (q) and 28.0 (q) assignable to C-1, -2, -3, -4, -5, -10, -17 and -18 in this order by comparison with those of leukamenin E.⁸⁾ The signals in **3** at δ 39.3 (t), 34.0 (t), 217.8 (s), 47.3 (s), 54.2 (d), 39.7 (s), 20.0 (q) and 26.7 (t) due to C-1, -2, -3, -4, -5, -10, -17 and -18 were also well coincident with those of the equivalent structure of leukamenin F.⁸⁾

From these results, 26 carbons among 28 carbons containing 5 methyl carbons at δ 15.5, 16.5, 16.7, 19.3 and 28.0 of **1** have been assigned so far and the remaining two carbons at δ 19.1 (t) and 40.6 (t) have not been assigned yet. However, one of these methylene carbons must be present as an adjacent one to C-5, a proton of which appeared at δ 1.62 (1H, dd, $J=4$ and 12 Hz).

On the basis of these results, the plain structure of aszonapyrone A was established as **1**.

Aszonapyrone A seemed to be biosynthesized in a combination of the mevalonate-geranyl-geranyl pyrophosphate route with the acetate-polyketide route as well as the biosynthesis of colletotrichins.⁹⁾

In the antimicrobial assay, **1** showed an M.I.C. of $6.3\text{ }\mu\text{g/ml}$ against *Staphylococcus aureus* 209 P and *S. aureus* res., by the method

of Lechevalier *et al.*¹⁰⁾

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