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ARTHRINONE, A NOVEL FUNGAL METABOLITE FROM *ARTHRINIUM* SP. FA 1744

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ABSTRACT.—A novel fungal metabolite, arthrinone [**1**], was isolated from the culture broth of *Arthrinium* sp. FA 1744 by chromatographic methods. The structure and relative stereochemistry of arthrinone were determined by spectral and single-crystal X-ray analyses.

Our research laboratories in Japan have recently reported the isolation and structural elucidation of a novel syncytium formation inhibitor, terpestacin, from *Arthrinium* sp. FA 1744 (ATCC 74132) (1,2). In a continuing chemical investigation of metabolites produced by the same fungal strain, we have isolated a novel aromatic metabolite, designated as arthrinone [**1**], and a known compound, norlichexanthone, along with terpestacin. This paper deals with the isolation and structural determination of arthrinone [**1**].

RESULTS AND DISCUSSION

From the EtOAc extract of the fermentation broth of *Arthrinium* sp., three metabolites were isolated by cc on Si gel and Sephadex LH-20 with various solvent systems, and finally by prep. hplc on a reversed-phase C_{18} column. Two compounds were identified as terpestacin and norlichexanthone by comparison with authentic samples and published spectral data (1,3,4). The third compound appeared to be a novel natural product and was designated as arthrinone [**1**].

The molecular formula of **1**, established as $C_{13}H_{12}O_7$ by high-resolution fabms, indicated eight degrees of unsaturation. The ir spectrum showed an absorption band at 1622–1635 cm^{-1}, implying that **1** contained a conjugated keto carbonyl, which was supported by the ^{13}C-nmr signal at δ 192.84. The uv absorption maxima at 290 and 330 nm were further suggestive of the presence of an aromatic carbonyl chromophore with possible ortho/para substitution by a hydroxyl group on the aromatic ring (5). The six ^{13}C-nmr signals at δ 167.07, 165.05, 143.62, 110.72, 107.05, and 101.48, as well as two meta-coupled ^1H-nmr signals at δ 6.51 and 6.33 (J=2.3 Hz each) demonstrated the presence of a 1,2,3,5-tetrasubstituted benzene ring. Because there were no other double-bond signals in the nmr spectra, the conjugated keto carbonyl should be attached to the benzene ring. The signals at δ 66.55 and 63.05 arising from two quaternary carbons suggested the presence of a fully substituted epoxy group. The ^1H-, ^{13}C-nmr, and HETCOR spectra (Table 1) revealed further the presence of a hydrogen-bonded hydroxyl group (δ 11.88), as well as a methoxyl (δ_C 55.87, δ_H 3.82), an oxymethylene (δ_C 64.71,

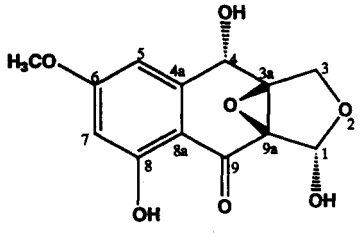

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TABLE 1. ^{1}H- and ^{13}C-Nmr Data and Key C-H Long-Range Correlations of Arthrinone [**1**] ($CDCl_3$, δ in ppm, *J* in Hz).

Position	^{13}C	^{1}H	Long-range C→H	Long-range H→C
1	93.81	5.75 (s)		C-3a
3	64.71	4.44 (d, J=10.4, H_A)	H-1	
		4.08 (d, J=10.4, H_B)		C-3a, C-9a
3a	66.55		H-3_B, H-4	
4	65.45	5.12 (s)	H-5	C-3a, C-4a, C-5, C-9a
4a	143.62		H-4	
5	110.72	6.51 (d, J=2.3)	H-4, H-7	C-4, C-6, C-7, C-8a
6	167.07		H-5, H-7, H-10	
7	101.48	6.33 (d, J=2.3)	H-5, 8-OH	C-5, C-6, C-8, C-8a
8	165.05		H-7, 8-OH	
8a	107.05		H-5, H-7, 8-OH	
9	192.84			
9a	63.05		H-4, H-3_B	
10	55.87	3.82 (3H, s)		C-6

δ_H 4.08 and 4.44), an oxymethine (δ_C 65.45, δ_H 5.12), and a hemiacetal (δ_C 93.81, δ_H 5.75) group. The above-mentioned structural fragments and functional groups represented six degrees of unsaturation, so the remaining two degrees of unsaturation were attributed to two additional rings in the molecule.

Detailed 2D nmr study (Table 1, Figure 1) of **1** led to the establishment of the key connectivities of the molecule, in spite of the presence of a number of quarternary carbons with few correlations. The connection of the hydrogen-bonded hydroxyl group to the aromatic carbon C-8 was established through the long-range couplings observed between the hydroxyl proton (δ 11.88) and C-8 (δ 165.05), C-8a (δ 107.05) and C-7 (δ 101.48) in the HMBC experiment. The attachment of the methoxy group to C-6 was clear from the observation of C-H long-range coupling between C-6 (δ 167.07) and the methoxyl protons (δ 3.82). One oxymethine group (C-4, δ 65.45, H-4, δ 5.12) was placed between the benzene ring and the epoxy group based on the long-range couplings between H-4 and the two epoxy carbons C-3a and C-9a (δ 66.55 and δ 63.05) as well as between H-5 (δ 6.51) and C-4. The long-range correlations observed for the hemiacetal proton (H-1, δ 5.75) vs. the oxymethylene carbon (C-3, δ 64.7) as well as one epoxyl carbon (C-3a), and the oxymethylene proton (H-3_B, δ 4.08) vs. both epoxyl carbons (C-3a, C-9a), indicated that the hemiacetal (C-1), oxymethylene (C-3), and

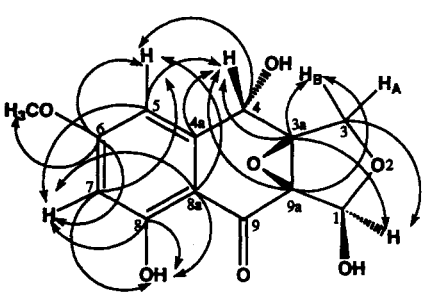

FIGURE 1. Key ^{13}C-^{1}H long-range correlations of **1**.

epoxyl groups share the same hydrofuran moiety, in which C-1 and C-3 are linked through the furan ring oxygen on one side and the epoxyl group on the other side.

The complete structure and relative stereochemistry of arthrinone [**1**] were established by X-ray crystallographic analysis. An ORTEP representation of the molecular structure is illustrated in Figure 2. The final atomic fractional coordinates and equivalent isotropic thermal parameters for non-hydrogen atoms are listed in Table 2.

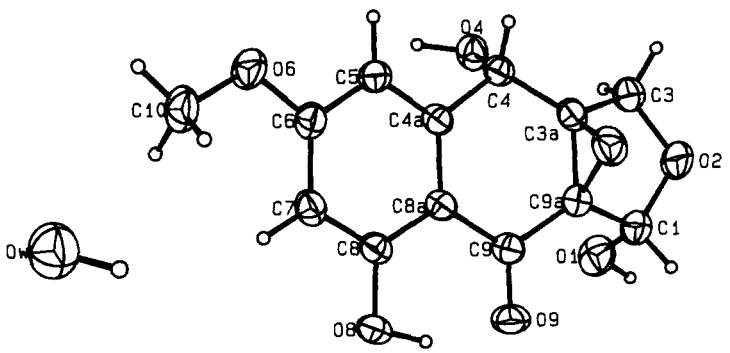

FIGURE 2. ORTEP drawing of **1**.

The X-ray crystallographic analysis showed that both hydroxyl groups at C-4 and C-1 were on the same side and anti to the oxygen of the epoxy ring. The hydroxyl group at C-8 formed an intramolecular hydrogen bond to the C-9 carbonyl oxygen (O-H···O=154.8°, O···O=2.583 Å and H···O=1.674 Å). Among the four fused rings, the cyclohexanone ring was slightly puckered and could be best described as a boat with flattened ends inasmuch as C-9 −0.116(3) Å and C-4 −0.225(3) Å deviated from the best least-squares plane formed by the other atoms in the ring. The flattened end at the

TABLE 2. Positional Parameters of Arthrinone [**1**] and Their Estimated Standard Deviations.

Atom	*x*	*y*	*z*	Beq
C-1	1.1114 (4)	0.8084 (4)	−0.12858 (7)	3.16 (6)
C-3	0.8853 (4)	0.5093 (4)	−0.15655 (6)	3.19 (7)
C-3a	0.8115 (3)	0.5141 (3)	−0.11567 (6)	2.38 (5)
C-4	0.6899 (3)	0.3383 (3)	−0.09066 (6)	2.19 (5)
C-4a	0.6896 (3)	0.3892 (3)	−0.04715 (6)	2.05 (5)
C-5	0.5407 (3)	0.2554 (3)	−0.02394 (6)	2.32 (5)
C-6	0.5356 (3)	0.2933 (3)	0.01671 (6)	2.36 (5)
C-7	0.6838 (3)	0.4585 (3)	0.03396 (6)	2.44 (5)
C-8	0.8342 (3)	0.5929 (3)	0.01072 (6)	2.22 (5)
C-8a	0.8384 (3)	0.5658 (3)	−0.03070 (6)	2.04 (5)
C-9	0.9834 (3)	0.7234 (3)	−0.05462 (6)	2.29 (5)
C-9a	0.9535 (3)	0.7007 (3)	−0.09838 (6)	2.46 (5)
C-10	0.3436 (4)	0.1977 (4)	0.07564 (7)	3.18 (6)
O-1	1.2697 (3)	0.7862 (3)	−0.11696 (5)	3.85 (5)
O-2	1.0276 (3)	0.7106 (3)	−0.16486 (4)	3.82 (5)
O-3a	0.7647 (2)	0.6663 (2)	−0.11166 (5)	3.08 (4)
O-4	0.7639 (2)	0.2063 (2)	−0.09609 (4)	2.78 (4)
O-6	0.3743 (3)	0.1561 (2)	0.03595 (4)	3.25 (4)
O-8	0.9758 (2)	0.7561 (2)	0.02875 (4)	2.78 (4)
O-9	1.1218 (3)	0.8727 (3)	−0.04103 (5)	3.36 (5)
O-w	0.5876 (4)	0.000	−0.167	3.39 (7)

C-9 carbonyl was required to provide proper geometry for the intramolecular hydrogen bond with the C-8 hydroxyl group. The five-membered tetrahydrofuran ring is also puckered and an envelope conformation was observed with the oxygen 0.405(2) Å above the best plane defined by the four carbon atoms. In crystals, the compound existed as a hydration complex of the formula $C_{13}H_{12}O_7 \cdot 0.5H_2O$ (mol wt 289.24) with the H_2O oxygen lying on a special position in this space group, a twofold axis. This water molecule played an important role in stabilizing the crystal structure through forming an extensive three-dimensional hydrogen bond network with arthrinone [**1**] molecules.

Arthrinone [**1**], to the best of our knowledge, is a novel natural product containing the epoxynaphtho[2,3-c]furan ring system. The only reported compound having the same ring system is cervic acid, which was a degradation product of the antitumor antibiotic cervicarcin produced by *Streptomyces ogaensis* (6). The epoxynaphthonone moiety of arthrinone appears to be rare among natural products; only a few antibiotics such as cervicarcin (6), nanaomycin E (7,8), and lactoquinomycin B (9) have this moiety. However, it should be noted that most of the related antibiotics were further oxidized to naphthoquinone, whereas arthrinone [**1**] and cervicarcin existed in a semiquinone form. Biosynthetic studies of this class of compounds have indicated that they are derived via the polyketide pathway by head-to-tail condensation of acetate, and the epoxy group is formed from a vinyl group at the corresponding position (8). It seems likely that arthrinone [**1**] is similarly biosynthesized.

EXPERIMENTAL

GENERAL EXPERMENTAL PROCEDURES.—Solvents used for extraction, solvent partition, and column chromatography were ACS grade. MeCN for hplc was Fisher hplc grade, and H_2O for hplc was in-house deionized using a Barnstead Nanopure II system. Tlc was performed on Kieselgel 60 F_{254} plates 0.2 mm thick (EM Science). For cc Si gel 60 (EM Science, particle size 40–63 μm) and Sephadex LH-20 (Pharmacia) were used. Hplc purification was performed on a C_{18} semi-prep. column, S-7 μm, 250×20 mm i.d. (YMC Co.). The uv spectrum was taken on a Shimadzu UV2100 spectrophotometer; the ir spectrum was recorded on a Perkin-Elmer Ft-ir 1800 spectrometer; the circular dichroism (cd) spectrum was measured on a Jasco J-500A spectropolarimeter. Mass spectra (ms) were taken on a Finnigan 4500 quadrupole spectrometer, the high-resolution ms analysis was performed with a Kratos MS50 mass spectrometer, and all ^{1}H- and ^{13}C-nmr spectra including COSY, HETCOR, COLOC, and HMBC were taken on a Bruker AM-500 spectrometer (^{1}H, 500 MHz; ^{13}C, 125 MHz).

EXTRACTION AND ISOLATION.—The fermentation broth of *Arthrinium* sp. FA 1744 was prepared as described by Oka *et al.* (1). Culture broth (20 liters) from shake flasks was extracted twice with EtOAc. The organic layers were combined and concentrated *in vacuo* to yield 9.1 g of a brown oil. The oil was first fractionated by cc on Si gel by eluting with CH_2Cl_2, then CH_2Cl_2-MeOH (95:5) followed by CH_2Cl_2-MeOH (80:20). Each of the fractions was monitored by tlc and hplc (YMC C_{18} reversed-phase column, MeCN-H_2O (1:1); uv detection at 290 nm). The fractions eluted with CH_2Cl_2-MeOH (80:20) were concentrated and further chromatographed on a Sephadex LH-20 column (MeCN-H_2O, 4:1) to give a residue (0.9 g), which was a mixture of three compounds according to tlc and hplc analyses. Separation of this mixture by a YMC semi-prep. hplc column, eluting with 50% MeCN, afforded 115 mg of arthrinone [**1**] (R_f 5.5 min), 410 mg of terpestacin (R_f 12.5 min) and 23 mg of norlichexanthone (R_f 19.5 min).

Arthrinone [**1**].—Yellowish crystals; mp 156–158° (dec); ir (KBr) ν max 3400, 1622–1635, 1390, 1300, 1200, 1100, 1020, 940, 790 cm^{-1}; uv λ max (log ε) (EtOH) 218 (4.06), 238 (3.77) 290 (3.96), 330 (3.78) nm; cd λ max (MeOH) 348 (Δε 1.47), 315 (Δε −6.43), 283 (Δε 2.74), 247 (Δε −5.42), 220.50 (Δε 5.83) nm; hrfabms *m/z* found 280.0651 [M]$^+$, calcd for $C_{13}H_{12}O_7$, 280.0660; ^{1}H- and ^{13}C-nmr data, see Table 1.

X-RAY CRYSTAL STRUCTURE ANALYSIS.—Light-brown crystals suitable for X-ray diffraction experiments were grown from a mixture of EtOH and EtOAc at room temperature. A fragment of approximately 0.25×0.33×0.33 mm size cut from a large crystal was used for diffraction intensity data collection. Experiments were carried out at room temperature on an Enraf-Nonius CAD4 diffractometer using graphite monochromated Cu Kα radiation (λ=1.5418 Å). The crystals are trigonal, space group $P3_121$, with unit cell constants $a=b=7.912(1)$, $c=33.763(2)$ Å, $\alpha=\beta=90$, $\gamma=120°$ and $V=1830.5(2)$ Å^3 obtained from a least-squares fit to data for 25 well-centered reflections in the range $18.50 \leq \theta \leq 29.74°$. There are six

molecules in the unit cell and the calculated crystal density Dx=1.574 g/cm^3. Intensity data were collected with $0 \leq h \leq 9$, $-9 \leq k \leq 9$, $0 \leq l \leq 41$ to $2\theta = 140°$. A total of 3826 reflections was measured using an $\omega/2\theta$ scan mode. The Lorentz and polarization effects were corrected and an empirical absorption correction was applied. After data reduction, the unique data set contained 1421 independent reflections (R_{int}=0.018); 1344 of them with $I \geq 3\sigma(I)$ were considered observed and were used for structure determination and refinements.

The structure was solved by direct methods using the program SHELXS-86 (10) and was refined by full-matrix least-squares techniques using the computer software MOLEN (11). The final refinements included 187 variables, a scale factor, an extinction coefficient, atomic coordinates, and anisotropic temperature factors for non-hydrogen atoms.[1] Hydroxyl hydrogens were located in difference Fourier maps and positions of the other hydrogens were calculated for an idealized geometry with standard bond lengths and angles. All hydrogen atoms were given isotropic temperature factors and were included in structure factor calculations with fixed parameters. The final R(F)=0.029, R_w(F)=0.029 and S=0.668 with unit weights for 1344 reflections. The final difference electron density map showed no recognizable residual features ($-0.019 \leq \Delta\rho \leq 0.019$ eÅ^{-3}).

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LITERATURE CITED

1. M. Oka, S. Iimura, O. Tenmyo, Y. Sawada, M. Sugawara, N. Ohkusa, H. Yamamoto, K. Kawano, S. Hu, Y. Fukagawa, and T. Oki, *J. Antibiot.*, **46**, 367 (1993).
2. M. Oka, S. Iimura, Y. Narita, T. Furumai, M. Konishi, T. Oki, Q. Gao, and H. Kakisawa, *J. Org. Chem.*, **58**, 1875 (1993).
3. D. Broadbent, R. Mabelis, and H. Spencer, *Phytochemistry*, **14**, 2082 (1975).
4. J. Santesson, *Acta Chem. Scand.*, **22**, 1698 (1968).
5. A.I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Pergamon Press, Oxford, UK, 1964, pp. 100–115.
6. S. Marumo, K. Sasaki, K. Ohkuma, K. Anzai, and S. Suzuki, *Agric. Biol. Chem.*, **32**, 209 (1968).
7. M. Kasai, K. Shirahata, S. Ishii, K. Mineura, H. Marumo, H. Tanaka, and S. Omura, *J. Antibiot.*, **32**, 442 (1979).
8. C. Kitao, H. Tanaka, S. Minami, and S. Omura, *J. Antibiot.*, **33**, 711 (1980).
9. T. Okabe, K. Nomoto, and N. Tanaka, *J. Antibiot.*, **39**, 1 (1986).
10. G.M. Sheldrick, C. Kruger, and R. Goddard, "Crystallographic Computing," Oxford University Press, London, 1986, Vol. 3, pp. 175–189.
11. MOLEN, An Interactive Structure Solution Procedure, Enraf-Nonius, Delft, The Netherlands, 1990.

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[1]Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1 EZ, UK.