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Structures of A88696 C, D and F: Gastric ATP-ase Inhibitors

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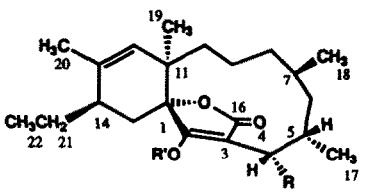
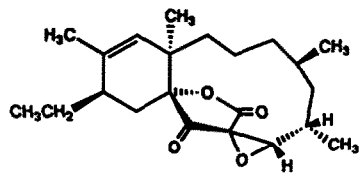
Abstract: The structures of A88696C (**1**), D (**2**) and F (**3**), isolated from *Streptomyces sclerotialus*, are described on the basis of spectroscopic analysis. **1** and **3** are spirotetronic acid-containing macrolides, and **2** is the β-dicarbonyl tautomer with a spiroepoxide at the α-site of the lactone ring.

Omeprazole® is the first gastric H^+/K^+ ATP-ase inhibitor to be approved for human use, and has been shown to treat gastric as well as duodenal ulcers, unlike H_2 antagonists such as Tagamet® and older therapies.[1] The desire to discover new gastric ATP-ase inhibitors with improved properties led us to screen culture broths from microorganisms, since microbes continue to be a rich source for the discovery of chemically novel compounds as inhibitors of enzymes. This effort resulted in the identification of the gastric ATP-ase inhibitory activity in the broth of culture A88696, *Streptomyces sclerotialus*.[2] In this paper we present the structures of the tetronic acids A88696 C, D, and F responsible for the observed inhibition of hog mucosal-derived gastric ATP-ase. [3]



1, R, R' = H, **A88696C**
3, R = OH, R' = H, **A88696F**
4, R = OH, R' = O-4(S)-phenyloxazolidylacetate ester



2, **A88696D**

The major metabolite, A88696C (**1**), has the empirical formula $C_{22}H_{34}O_3$ and an MH^+ of 347.2580 (calc. 347.2582). The UV spectrum in EtOH exhibited two maxima at 241 and 267 nm; upon addition of acid or base, only a single maximum was present at 241 nm or 267 nm, respectively. The 500 MHz ^1H NMR spectrum showed a lone olefinic singlet at 4.98 ppm and all other signals were in the 0.5-2.5 ppm region as given in Table 1; the ^{13}C NMR spectrum displayed two carbonyl signals at 178 and 181 ppm and three other sp^2 carbons at 101, 132 and 135 ppm. Suitable crystals of **1** were obtained from acetonitrile for determination of its crystal structure. **1** has a bicyclic system consisting of a spirotetronic acid as part of an eleven-

membered ring which is fused to a substituted cyclohexene. Other recently discovered spirotetronic acids such as tetronolide, the aglycone of the tetrocarcins produced by *Micromonospora chalcea* and kijanolide, the aglycone of kijanimycin produced by *Actinomadura kijaniata,* possess similar structural characteristics. [4] It is well documented that tetronic acids can tautomerize and this phenomenon is responsible for the observed UV shifts.[5] In fact, isolation of the base forms of **1** and **3** occurred when close attention was not paid to the pH of the aqueous portion of the mobile phase during HPLC purification.

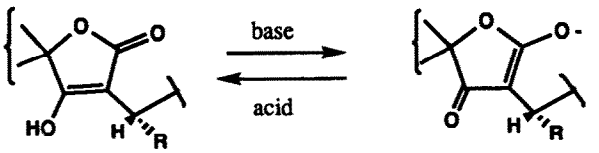


Figure 1. Tetronic acid tautomers

Derivatization of **1** by acylation of the tetronic acid with 4-S-phenyloxazolidyl acetyl chloride [6] afforded crystalline **4** from EtOH/H_2O. X-ray analysis of the ester **4**, as shown in Figure 2, allowed the determination of the absolute configuration at the chiral centers as follows: 1R, 5S, 7S, 11R, and 14R.[7]

A88696D, **2**, a minor factor, has a formula of $C_{22}H_{32}O_4$ and a molecular ion at m/z 360 (FD-MS). The UV spectrum indicated a λ_{max} at 260 nm in neutral EtOH but the compound decomposed under acidic and basic treatment. The ^1H and ^{13}C NMR data are shown in Table 1; the proton spectrum shows a doublet at 3.8 ppm and the carbon spectrum clearly shows a keto-carbonyl at 206 ppm in addition to an ester-type carbonyl at 169 ppm. The peaks at 57 and 77 ppm indicate attachment of the fourth oxygen atom. Again, a crystal structure was obtained which is shown in Figure 2.[8] Oxidation at C3 and C4 provides the a spiro-epoxide and the consequent bis-carbonyl in the neighboring spiro-γ-lactone. The absolute configuration at C-4 is (S).

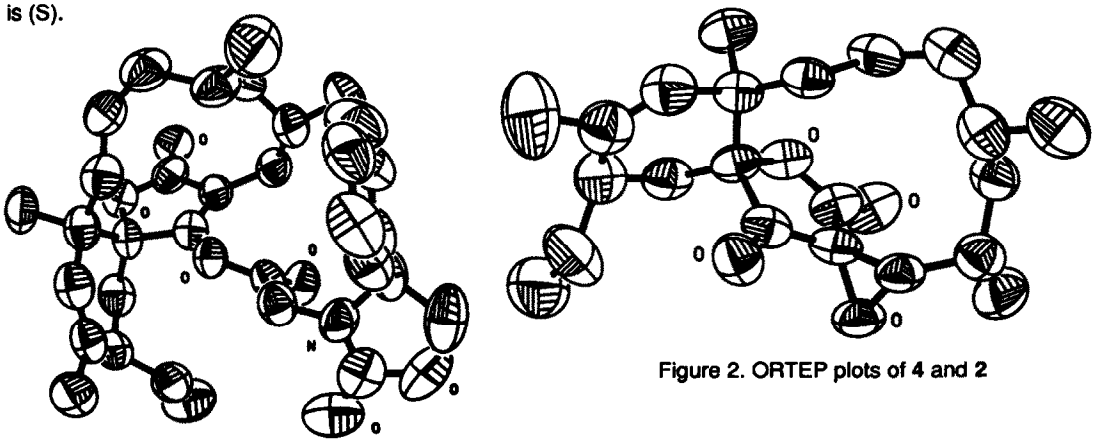


Figure 2. ORTEP plots of **4** and **2**

The final factor, A88696F, **3**, possessed a formula of $C_{22}H_{34}O_4$, two hydrogen atoms more than in **2**. A molecular ion could not be seen by either FD or FAB MS modes; however, an $(M+Li)^+$ = 369 and $(M-OH)^+$ =345 could be obtained using a Li^+/sulfolane matrix in the FAB-MS mode. The UV spectrum was similar to that of **1** and displayed two absorptions at 239 and 257 nm which collapsed to 234 nm in acid and 260 nm in base, thus establishing the presence of the tetronic acid. The ^1H NMR now displayed the C4 methine as a doublet at 4.4 ppm, which suggested attachment of an oxygen atom, and the ^{13}C NMR confirmed this with a

Table 1. 500 MHz NMR assignments for A88696C (**1**), D (**2**), and F(**3**)

	1 (CD_3OD)		2 (CD_3OD+tr. DMSO)		3 (CD_3OD)	
C#	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	—	88.53	—	96.50	—	88.34
2	—	181.37	—	206.11	—	184.92
3	—	101.87	—	57.77	—	101.42
4	2.36, 1.90	28.74	3.82	77.34	4.27	74.92
5	1.76	32.57	2.67	32.02	2.29	34.00
6	1.30, 0.58	40.86	1.50, 1.07	41.13	1.52, 0.82	41.23
7	1.22	32.60	1.60	28.39	1.13	28.34
8	1.13, 0.97	37.12	~1.20	36.83	1.01	37.88
9	1.36, 1.09	20.49	~1.62	21.70	1.36, 1.10	20.28
10	1.62, 1.17	39.38	1.44, 1.35	38.37	1.48, 1.27	39.55
11	—	40.98	—	41.85	—	41.04
12	4.98	132.81	5.09	131.47	4.98	132.83
13	—	135.20	—	137.39	—	135.46
14	2.04	42.39	2.19	41.61	2.06	42.18
15	2.47, 1.58	34.70	2.33, 2.14	31.95	2.49, 1.68	34.56
16	—	178.35	—	169.67	—	175.07
17	1.06	25.23	1.16	15.88	1.20	17.70
18	0.84	22.67	1.00	23.01	0.87	22.11
19	1.12	23.46	1.22	22.12	1.12	23.06
20	1.71	22.10	1.76	22.18	1.71	22.16
21	1.72, 1.54	26.11	1.72, 1.32	25.53	1.72, 1,58	26.10
22	0.92	13.61	0.95	13.46	0.93	13.53

methine at 75 ppm. No crystals of **3** could be prepared, but all evidence pointed to the presence of an alcohol at C4. The relative stereochemistry of the group was determined as shown by comparison of the coupling constant of H4 and H5 in both **2** and **3**. From the X-ray data on **2**, the dihedral angle of H4-C4-C5-H5 was -30° which would yield a theoretical coupling constant of 6-8Hz; a $J_{4,5}$ of 7 Hz was observed. Similarly, in **3**, $J_{4,5}$

of 7 Hz was seen, thus establishing the stereochemistry as shown where the configuration of the C-O bond is unchanged in A88696F as compared to the epoxide, A88696D.

A88696F, **3**, was found to be the most potent inhibitor with an IC_{50} = 0.5 μM, while the IC_{50}'s of **1** and **2** were 59 and >116 mM, respectively. These results are not surprising, given the relative lability of the C-4 OH. As is evident in the accompanying paper, we demonstrate some unusual chemistry of **3**.[9]

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7. Compound **4**: Tabular crystals in the orthorombic space group $P2_12_12_1$; unit cell a =6.467(5) A, b = 8.422(1) A, c = 22.522(5) A, D_X = 1.14 g/cm^{-3}

8. Compound **2**: Tabular crystals in the monoclinic space group $P2_1$; unit cell a = 11.037(2) A, b = 28.584(5) A, c = 6.596(1) A, b = 95.31(1)°, D_X = 1.155 g/cm^{-3}.

9. Bonjouklian, R., *Tetrahedron Lett.*, see accompanying paper.

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