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Chemistry of the Microbial Metabolite A88696F, a New 2-(α-Hydroxyalkyl) Tetronic Acid

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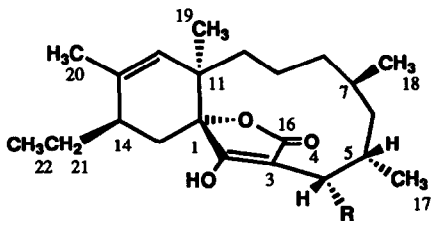
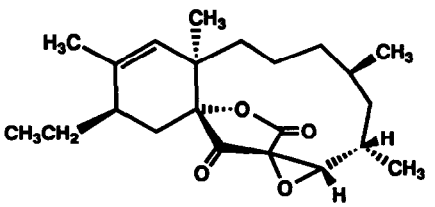
Abstract: Some unusual chemistry of the gastric ATP-ase inhibitor A88696F (**3**), a naturally-occurring 2-(α-hydroxyalkyl) tetronic acid, is described. It undergoes triethylamine-mediated reduction of the hydroxyl group to afford A88696C (**1**), presumably via the olefin **4**. This olefin was prepared from **3** in high yield under mild acidic conditions, and was stereoselectively rehydrated back to alcohol **3**, and epoxidized to A88696D (**2**).

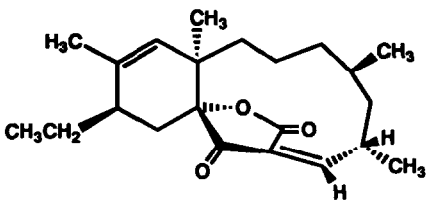
In the previous paper, the structures of the microbially-derived gastric ATP-ase inhibitors A88696C (**1**), D (**2**), and F (**3**) are reported.[1] A88696 F has an IC_{50} = 0.5 μM and is, by far, the most potent enzyme inhibitor of the three structurally related natural products. In this paper the chemical interconversions of **3** into **1** and **2** are presented which proceed via an olefinic intermediate **4**, whose facile formation may explain the observed biological activity. This example also provides some information regarding the chemical properties of 2-(α-hydroxyalkyl) tetronic acids, a moiety that is poorly described in the literature.[2]



1, R = H, A88696C
3, R = OH, A88696F



2, A88696D



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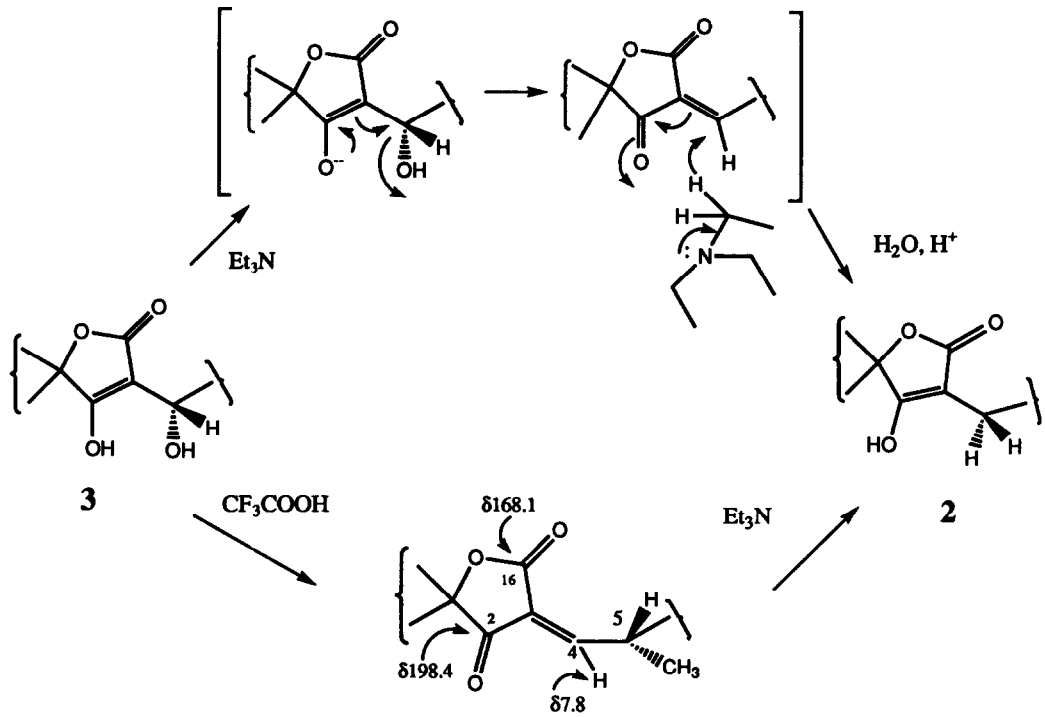
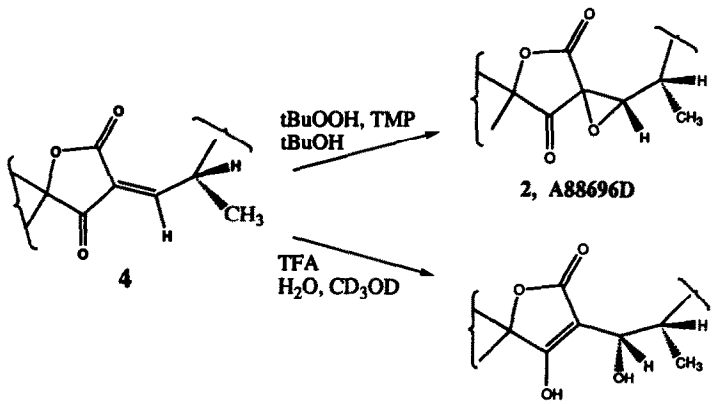
In an attempt to prepare a crystalline 4(S)-phenyloxazolidylacetate ester of A88696F (3), which had been successfully done with A88696 C (1)[3], low temperature (-78°) treatment of a CH_2Cl_2 solution of 3 with Et_3N and 4-S-phenyloxazolidylacetyl chloride unexpectedly led to the isolation of the C-4 dihydro tetronic acid, identical to the natural product, 1 in 50% yield. The yield of 1 was raised to 80% when Et_3N alone was used, thus pointing to its lesser known role as a reducing agent. A search of the literature provided limited examples where Et_3N was shown to reduce substrates such as trifluoroacetic anhydride[4] or triphenylcarbonium ion[5] via hydride transfer. In the case of A88696F, 3, however, the reduction may actually occur via the olefin 4, as shown below.



The olefin was prepared by treatment of 3 (3-4 mg) with 1-2 eq of CF_3COOH in $CDCl_3$, for example, in an NMR tube experiment, which provided direct evidence that the dehydration is rapid and quantitative. The new olefinic proton, H-4, is present at δ 7.8 with a $J_{4,5}$ = 9.2 Hz. H-5 is moved downfield to δ 2.81 along with its attached 17-CH_3 to δ 1.45. In the ^{13}C NMR, both C-2 and C-16 carbonyls are seen at 198.4 and 168.1 ppm, respectively, along with new olefinic signals at 136.0 (C-4) and 93.8 ppm (C-3). Following concentration of the 5 mg sample, a protonated molecular ion, $(M+H)^+$, at 345 was detected in the FAB-MS mode; high resolution MS generated a formula weight of 345.4232 for

$C_{22}H_{33}O_3$ (345.4230 calcd). When the olefin **4** was subjected to the same Et_3N/CH_2Cl_2 conditions as used for **3**, the reduction product, A88696C, **1**, was obtained in high yield.

To establish the geometry of the new double bond in **4**, and simultaneously perform a synthesis of A88696D (**2**), 6 mg of **4** in $CDCl_3$ was treated with a mixture of t-BuOH, tetramethylpiperidine (TMP, a base lacking α-hydrogens), and 90% t-BuOOH. After 2hr, epoxidation was complete and the natural product **2** was isolated as the sole product based on HPLC analysis, FABMS and NMR comparison with authentic material. Since the mechanism of epoxidation of olefins by t-BuOOH involves retention of double bond geometry[6], this result established the olefin geometry as "Z", which is seen in the epoxide **2** based on the X-ray crystallographic analysis as described in the preceding paper. Furthermore, the epoxidation occurs from the least hindered α face of the olefin to generate the naturally-occurring product.



Based on the successful epoxidation results, the reconversion of **4** into A88696F (**3**) was then attempted. A simple NMR tube experiment sufficed when a CD_3OD solution of 5 mg of **4** was treated with 10 ml of H_2O and a trace of TFA to rapidly afford **3**, as evidenced by NMR and HPLC. Again, attack of the nucleophile from the least-hindered face of the olefin led to the natural product.

Thus, this olefin, an α,β–unsaturated-α-dicarbonyl, may, in fact, be responsible for the observed enzyme inhibition activity as the bioassays are performed under acidic conditions. In addition, it might well be a possible biosynthetic precursor to the observed natural products perhaps by an aldol-type condensation of an aldehyde with the tetronic acid, rather than the well-known condensations with acyl species, or via a bio-reduction of a 2-acyl tetronic acid to the alcohol group The 2-acyl analogue of A88696F has not been detected in the microbial fermentation.

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3. See reference 1.

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