Studies on the Chemical Reactivity of Bicyclomycin: Acid Hydrolysis

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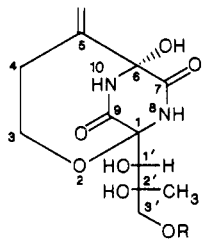
The hydrolysis of bicyclomycin (**1**) and the corresponding 3′-ethylcarbamate derivative **2** in acid has been examined. Treatment of each compound with aqueous H_2SO_4 (0.1–1.0 N, 100 °C, 90 min) led to a binary mixture of 2(5*H*)-furanone adducts. In the case of **1**, the major products formed were 3-hydroxy-4-(2-hydroxyethyl)-2-(5*H*)-furanone (**5**) and 3,4-dihydroxy-5-(hydroxymethyl)-5-methyl-2(5*H*)-furanone (**6**), while **2** gave **5** and (3,4-dihydroxy-5-methyl-2(5*H*)-oxofuran-5-yl)methyl *N*-ethylcarbamate (**10**). The probable mechanisms for the formation of these products are outlined and the implications of these findings for the various pathways for the chemical activation of bicyclomycin are discussed.

Bicyclomycin (**1**) is a structurally unique peptide[1] possessing an unusual profile of antibacterial activity.[2] Its emerging clinical importance has led to several elegant studies on the total synthesis[3-5] and chemical modification of the drug.[6-10] By comparison, little is known about the chemical reactivity of **1**,[6,11] and considerable speculation exists concerning the mode of action of bicyclomycin.[12] In this paper, we report on the acid hydrolysis of **1** and the corresponding 3′-ethylcarbamate derivative[6] **2** and the resulting implications of this study on the available chemical pathways for the activation of the antibiotic.



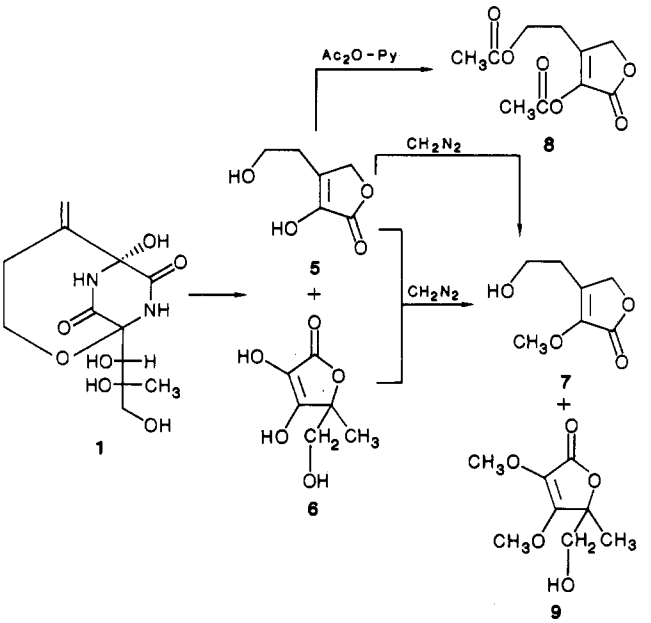
1: R = H
2: R = C(O)NHCH$_2$CH$_3$

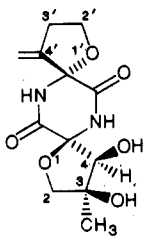
Results

The conditions employed in our study were patterned after those described by Maag and co-workers[11] in their investigation of the acid hydrolysis of **1**. These researchers demonstrated that dissolution of **1** in aqueous 0.1 N $HClO_4$ acid (100 °C, 15 min) gave a diastereomeric mixture of spiro diketopiperazines **3** and **4**. *No* modification of the carbon 5 *exo*-methylene group was observed under a variety of conditions (0.01–1.0 N acid, 100 °C). This result is significant, since previous suggestions have been made that the antibacterial activity of bicyclomycin is associated with the covalent binding of a nucleophile present on an inner-membrane protein (i.e., a sulfhydryl residue) to the exocyclic methylene group in **1**.[9,10,12,13]

In the present investigation, the hydrolysis was repeated under more vigorous conditions. Treatment of **1** with

Scheme I. Hydrolysis of 1 and Subsequent Derivatization Studies





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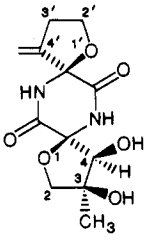

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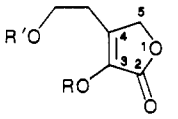
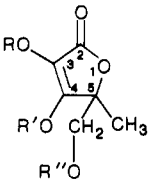
Table I. Select ^1H and ^{13}C NMR Spectral Properties for 3-Oxy-Substituted 2(5*H*)-Furanones



			^1H NMR		^{13}C NMR				
no.	R	R′	C_5-H_2	C_4-CH_2CH_2O	C-2	C-3	C-4	C-5	C_4-CH_2CH_2
5[a]	H	H	4.64 (s)	3.55 (t, 5.95)	173.80	140.49	130.18	71.18	60.70
7[b]	CH_3	H	4.72 (s)	3.82 (t, 5.9)	168.93	141.01	138.06	69.34	58.69
8[b]	C(O)CH_3	C(O)CH_3	4.84 (s)	4.25 (t, 6.0)	170.51	135.33	146.05	69.26	60.55

[a] ^1H NMR spectrum was taken in DMSO-d_6; ^{13}C NMR spectrum was taken in CD_3OD. [b] ^1H and ^{13}C NMR spectra were taken in $CDCl_3$.

Table II. Select ^1H and ^{13}C NMR Spectral Properties for 3,4-Bis(oxy-substituted) 2(5*H*)-Furanones



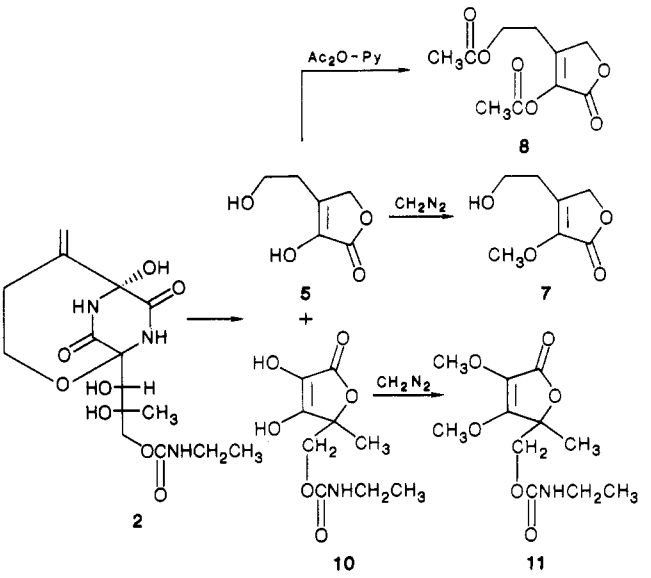
				^1H NMR:	^{13}C NMR				
no.	R	R′	R″	C_5-CH_2	C-2	C-3	C-4	C-5	C_5-CH_2
9[a]	CH_3	CH_3	H	3.60–3.68 (m)	168.66	122.29	160.55	81.33	65.26
10[b]	H	H	C(O)$NHCH_2CH_3$	4.13 (s)	176.29	114.92	174.74	82.82	67.72
11[a]	CH_3	CH_3	C(O)$NHCH_2CH_3$	4.16–4.22 (m)	168.18	122.22	155.31	79.35	65.16

[a] ^1H and ^{13}C NMR spectra were taken in $CDCl_3$. [b] ^1H and ^{13}C NMR spectra were taken in CD_3OD.

aqueous H_2SO_4 (0.1–1.0 N) acid (100 °C, 90 min) led to the formation of the *two 2(5H)-furanone derivatives* **5** and **6** (Scheme I). The more nonpolar adduct **5** was readily isolated by preparative thin-layer chromatography and identified by detailed spectral analysis and by conversion of **5** to select derivatives. The ^1H NMR of 3-hydroxy-4-(2-hydroxyethyl)-2(5*H*)-furanone (**5**) exhibited only three nonexchangeable sets of peaks (Table I). It is noteworthy that the two broad singlets (δ 5.11 and 5.40) for the carbon 5a olefinic protons in **1**[2b] appeared as a single sharp peak (δ 4.64) in **5** and have been assigned to the carbon 5 ring methylene moiety. The corresponding proton-decoupled ^{13}C NMR spectrum for furanone **5** displayed six lines. The carbons 3 and 4 resonances appeared at 140.49 and 130.18 ppm, respectively. These values were similar to those previously reported for substituted 2-hydroxy-2-cyclohexenones.[14] Treatment of **5** with excess diazomethane led to the formation of the carbon 3 monomethoxy derivative **7**, while acetic anhydride in pyridine provided the diacetoxy derivative **8**. The spectral properties of both adducts were consistent with the proposed structures (Table I). In particular, a large downfield shift (~16–17 ppm) was noted in the ^{13}C NMR spectrum for the carbon 4 signal in **8** versus the corresponding resonance in **5**. A comparable pattern has been observed in related systems.[14]

Identification of the remaining polar compound **6** was facilitated by treatment of the mixture of **5** and **6** with excess diazomethane to yield two products which were separated by careful preparative thin-layer chromatography. The more mobile component was identified as 3,4-dimethoxy-5-(hydroxymethyl)-5-methyl-2(5*H*)-furanone (**9**) and was isolated in 19% yield, while the second adduct proved to be **7** (26%). The carbons 3 and 4 methoxy protons in **9** appeared as singlets in the ^1H NMR spectrum at δ 3.82 and 4.18, respectively (Table II), while evidence for a five-membered ring lactone was obtained by the

Scheme II. Hydrolysis of 2 and Subsequent Derivatization Studies



detection of the carbonyl absorption at 1755 cm^{-1} in the infrared spectrum.[15]

A comparable study was conducted with the 3′-ethylcarbamate derivative **2**. Hydrolysis of this compound in aqueous H_2SO_4 (0.1–1.0 N) acid (100 °C, 90 min) proceeded slightly faster than **1** (TLC analysis) and gave two products which could be separated by preparative thin-layer chromatography (Scheme II). The more nonpolar adduct proved identical with **5** isolated from the hydrolysis of **1** on the basis of the observed spectral properties and by subsequent conversion of **5** to **7** and **8**. The second product was identified as (3,4-dihydroxy-5-methyl-2(5*H*)-oxo-

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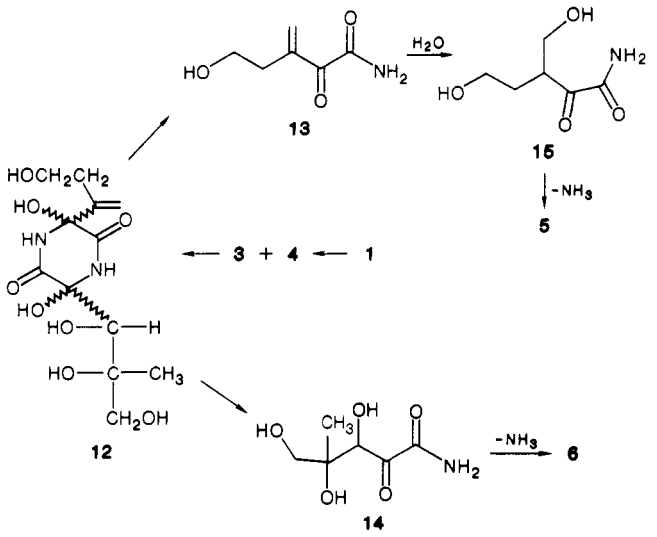
Scheme III. Proposed Pathway for the Hydrolysis of Bicyclomycin (1)



furan-5-yl)methyl *N*-ethylcarbamate (**10**). The ^{13}C NMR spectrum of **10** displayed two resonances which were pD-dependent. At pD 6.1 the signals for carbons 3 and 4 appeared at 113.77 and 177.77 ppm, respectively, while at pD 1.0 these resonances shifted to 117.98 and 158.45 ppm, respectively. A comparable pattern has been reported for ascorbic acid.[16] Compound **10** was converted to the dimethoxy derivative **11** by treatment with excess diazomethane. Support for the proposed five-membered ring lactone was obtained by the observation of the 1759-cm^{-1} band in the infrared spectrum for the carbonyl group.[15]

Several additional experiments concerning these transformations were performed and can be summarized briefly as follows.

1. In aqueous 1.0 N H_2SO_4 (100 °C, 90 min), hydrolysis of **1** gave only the furanone products **5** and **6** (TLC analysis). Reduction of the temperature of the reaction (1.0 N H_2SO_4, 55–60 °C, 9 h) yielded **5** and **6**, along with an unidentified minor compound, while use of solutions of lower acidity (0.01–0.1 N H_2SO_4 or 0.01 N $HClO_4$, 100 °C, 90 min) led to decreased amounts of **5** and **6**, the spiro diketopiperazines **3** and **4**, and two unidentified minor compounds. Finally, treatment of bicyclomycin with 1.0 N H_2SO_4 at 100 °C for short periods of time (3–5 min) furnished **3** and **4** as the only isolable compounds.

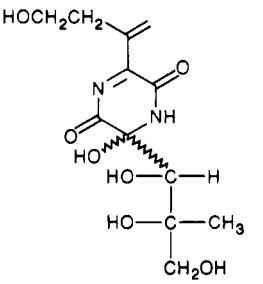
2. Spiro diketopiperazines **3** and **4** served as precursors to **5** and **6** (aqueous 1.0 N H_2SO_4, 100 °C, 90 min).

3. Hydrolysis of **2** proceeded under a variety of conditions. The reaction was complete within 90 min in aqueous 0.1–1.0 N H_2SO_4 or 1.0 N HCl acid (100 °C). Reduction of either the time (i.e., 1.0 N H_2SO_4, 100 °C, 5 min) or the temperature (i.e., 1.0 N H_2SO_4, 55 °C, 8 h) of the reaction led to decreased amounts of **5** and **10** and the observation of starting material **2** (TLC analysis).

4. Use of nonaqueous acid conditions for the solvolysis of **2** led to either no reaction (i.e., 1:1 tetrahydrofuran–formic aicd, 22 °C, 2 days; tetrahydrofuran–*p*-toluenesulfonic acid, 22 °C, 2 days) or to complex product mixtures (1:1 tetrahydrofuran–formic acid, 60 °C, 4 h; tetrahydrofuran–*p*-toluenesulfonic acid, 60 °C, 6 h).

Discussion

Hydrolysis of both bicyclomycin (**1**) and the 3′-ethylcarbamate derivative **2** led to the scission of the diketopiperazine ring and functionalization of the carbon 5 *exo*-methylene group. One mechanism consistent with the results obtained for **1** is depicted in Scheme III. Hydrolysis of bicyclomycin is envisioned to generate initially the spiro diketopiperazines **3** and **4** and the corresponding ring-opened bis α-hydroxy adducts **12**. Subsequent sequential cleavage of the hemiaminal groups in **12** (ring-chain tautomerism[17,18]) gives the two α-keto amides **13** and **14**. Formation of **13** permits the Michael addition of water across the carbon–carbon double bond to produce the non-Markovnikov alcohol **15**. Cyclization of **15** and **14** furnishes furanones **5** and **6**, respectively, along with the concomitant loss of ammonia. A similar pathway is envisioned for the hydrolysis of the 3′-ethylcarbamate **2**. In this case, however, the formation of the corresponding bis-spiro diketopiperazine adducts (i.e., **3** and **4**) is not possible. Alternative pathways for these transformations are conceivable that differ both in the sequence of chemical events and the mode of hydrolysis of the piperazinedione ring system. For example, dehydration of **12** to **16** followed by the addition of water to the olefinic system in **16** could have taken place prior to the rupture of the ring. Correspondingly, hydrolysis of the 2,5-piperazinedione ring in bicyclomycin may have proceeded by an acyl bond cleavage process,[19] followed by functionalization of the olefinic bond.



Conclusions

A new mode of reactivity has been observed for the antibiotic agent bicyclomycin. This transformation permits the Michael addition of water to the olefinic group in the drug under acidic conditions. The present investigation complements previous studies which were conducted with sulfur nucleophiles under basic conditions.[10,12a] The observed results have added significance in light of the recent proposals of Vasquez[13] and Williams.[10] These investigators have suggested that bicyclomycin undergoes proteolytic (i.e., amide hydrolysis) activation prior to the binding of the biological nucleophile to the *exo*-methylene group in **1**. Studies are currently in progress aimed at determining the facility of this transformation, the biological relevance of this reaction, and the mechanism of the corresponding base-mediated process.

Experimental Section

General Methods. Infrared spectra (IR) were run on either a Perkin-Elmer 283 or a Nicolet 10DX FT spectrometer and calibrated against the 1601-cm^{-1} band of polystyrene. Absorption values are expressed in wavenumbers (cm^{-1}). Proton (^1H NMR)

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and carbon (^{13}C NMR) nuclear magnetic resonance spectra were taken on Nicolet NT-300 and General Electric QE-300 NMR instruments. Chemical shifts (δ) are in parts per million (ppm) relative to Me_4Si and coupling constants (J values) are in hertz. Low-resolution mass spectral data (MS) were obtained at an ionizing voltage of 70 eV on a Bell and Howell 21-491 mass spectrometer at the University of Texas—Austin. High-resolution mass spectra were performed on a CEC 21-110B double-focusing magnetic-sector spectrometer at the University of Texas—Austin by Dr. John Chinn. Microanalysis were obtained from Spang Microanalytical Laboratory, Eagle Harbor, MI. pD measurements were determined on a pHM26 meter. The pD of the solution was obtained by using the following relationship: pD = pH meter reading + 0.4.[20]

All glassware was dried before use. The solvents and reactants were of the best commercial grade available and were used without further purification. Thin- and thick-layer chromatography were run on precoated silica gel GHLF microscope slides (2.5 × 10 cm; Analtech No. 21521) or silica gel GHLF (20 × 20 cm; Analtech No. 11187).

Hydrolysis of Bicyclomycin (1). A solution of **1** (50 mg, 0.166 mmol) in aqueous 1 N H_2SO_4 (5 mL) was heated at 100 °C for 3–5 min. The reaction was cooled to room temperature, extracted with ethyl acetate (5 × 10 mL), and dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was triturated with ethyl acetate (2 mL) and the remaining white crystalline material was filtered, washed with ethyl acetate (1 mL), and dried to give 13 mg (28% yield of **3** and **4**): mp 238–240 °C (lit.[11] mp 238–242 °C); R_f 0.50 (10% methanol–chloroform); ^1H NMR (DMSO-d_6) δ 1.20 (s, 3 H, CH_3), 2.65–2.72 (m, 2 H, C(3′)H_2), 3.62 and 3.64 (2 d, J = 9.0 Hz, 1 H, C(2)*H*H), 3.79 and 3.81 (2 d, J = 9.0 Hz, 1 H, C(2)H*H*), 3.92 and 3.94 (2 d, J = 7.1 Hz, 1 H, C(2′)*H*H), 4.00 and 4.04 (2 d, J = 7.1 Hz, 1 H, C(2′)H*H*), 4.25–4.30 (m, 1 H, C(4)H), 5.05 (s, 1 H, OH), 5.24, 5.25, and 5.30 (3 s, 2 H, C=C*H*(*H*)), 5.72–5.76 (m, 1 H, OH), 7.43 and 7.50 (2 s, 1 H, NH), 9.07 and 9.11 (2 s, 1 H, NH).

Hydrolysis of Bicyclomycin (1). A solution of **1** (30 mg, 0.099 mmol) in aqueous 1 N H_2SO_4 (5 mL) was heated at 100 °C for 1.5 h. The reaction was cooled to room temperature and extracted with ethyl acetate (3 × 10 mL). The ethyl acetate fractions were combined and concentrated in vacuo and dried (Na_2SO_4). The crude material (17 mg) was purified by preparative TLC with 25% methanol–chloroform as the eluent to give **5** as a liquid: yield 4.0 mg (28%); ^1H NMR (CD_3OD) δ 2.58 (t, J = 5.7 Hz, 2 H, $HOCH_2CH_2$), 3.75 (t, J = 5.7 Hz, 2 H, $HOCH_2CH_2$), 4.72 (s, 2 H, C(5)H_2).

Hydrolysis of Bicyclomycin (1) Followed by Treatment with Diazomethane. A solution of **1** (80 mg, 0.26 mmol) in aqueous 1 N H_2SO_4 (10 mL) was heated at 100 °C (1.5 h). The reaction mixture was cooled to room temperature, extracted with ethyl acetate (3 × 10 mL), and dried (Na_2SO_4), and the solvent was removed in vacuo. The crude material (48 mg) was dissolved in dry methanol (3 mL) and an excess of a distilled ethereal solution of diazomethane was added at 0 °C. The reaction was maintained at 0 °C for 16 h and then stirred at room temperature for an additional 3 h. The solvent was removed in vacuo and the crude material was purified by preparative TLC with 5% methanol–chloroform as the eluent to give **9** (9.5 mg, 19%) and **7** (11 mg, 26%).

3,4-Dimethoxy-5-(hydroxymethyl)-5-methyl-2(5*H*)-furanone (9): R_f 0.50 (5% methanol–chloroform); FTIR ($CHCl_3$) 3402, 1755, 1682 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 3 H, C(5)CH_3), 3.60–3.68 (m, 2 H, C(5)CH_2O), 3.82 (s, 3 H, C(3)OCH_3), 4.18 (s, 3 H, C(4)OCH_3); ^{13}C NMR ($CDCl_3$) 19.37 (C(5)CH_3), 59.45 (OCH_3), 60.52 (OCH_3), 65.26 (C(5)CH_2O), 81.33 (C(5)), 122.29 (C(3)), 160.55 (C(4)), 168.66 (C(2)) ppm; MS, m/e (relative intensity) 188 (20), 157 (100), 83 (26), 69 (28); M_r 188.0685 (calcd for $C_8H_{12}O_5$ 188.0685). Anal. Calcd for $C_8H_{12}O_5$: C, 51.06; H, 6.42. Found: C, 50.55; H, 6.61.

4-(2-Hydroxyethyl)-3-methoxy-2(5*H*)-furanone (7): R_f 0.45 (5% methanol–chloroform); FTIR ($CHCl_3$) 3417, 1759, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.61 (t, J = 5.9 Hz, 2 H, $HOCH_2CH_2$), 3.82 (t, J = 5.9 Hz, 2 H, $HOCH_2CH_2$), 3.96 (s, 3 H, OCH_3), 4.72 (s, 2 H, C(5)H_2); ^{13}C NMR ($CDCl_3$) 28.61 ($HOCH_2CH_2$), 58.69 ($HOCH_2CH_2$), 60.15 (C(3)OCH_3), 69.34 (C(5)), 138.06 (C(4)), 141.01 (C(3)), 168.93 (C(2)) ppm; MS, m/e (relative intensity) 158 (29), 140 (84), 97 (100), 69 (85), 59 (85), 55 (75); M_r 158.0582 (calcd for $C_7H_{10}O_4$, 158.0579). Anal. Calcd for $C_7H_{10}O_4$: C, 53.16; H, 6.37. Found: C, 52.78; H, 6.56.

Methylation of 3-Hydroxy-4-(2-hydroxyethyl)-2(5*H*)-furanone (5). Preparation of 4-(2-Hydroxyethyl)-3-methoxy-2(5*H*)-furanone (7). An excess amount of a distilled ethereal solution of diazomethane was added to a solution of **5** (4.0 mg, 0.027 mmol) in dry methanol (2 mL) and was stirred for 16 h at 0 °C and then at room temperature for 3 h. The solvent was removed in vacuo, and the residue was purified by preparative TLC with 5% methanol–chloroform as the eluent to give **7**: 3.0 mg (69%): R_f 0.45 (5% methanol–chloroform); ^1H NMR ($CDCl_3$) δ 2.61 (t, J = 6.0 Hz, 2 H, $HOCH_2CH_2$), 3.83 (t, J = 6.0 Hz, 2 H, $HOCH_2CH_2$), 3.97 (s, 3 H, OCH_3), 4.72 (s, 2 H, C(5)H_2).

Acetylation of 3-Hydroxy-4-(2-hydroxyethyl)-2(5*H*)-furanone (5). Preparation of 3-Acetoxy-4-(2-acetoxyethyl)-2(5*H*)-furanone. A solution of **5** (15 mg, 0.1 mmol) in dry pyridine (1 mL) and acetic anhydride (1 mL) was stirred at room temperature under nitrogen (16 h). The solvent was removed in vacuo and the crude material was purified by preparative TLC with chloroform as the eluent (three developments) to yield **8** (13 mg, 55%) as an oil: R_f 0.25 (chloroform); IR ($CHCl_3$) 2950, 2900, 1770, 1745, 1685, 1425, 1355, 1225, 1175 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.06 (s, 3 H, $COCH_3$), 2.31 (s, 3 H, $COCH_3$), 2.72 (t, J = 6.0 Hz, 2 H, OCH_2CH_2), 4.25 (t, J = 6.0 Hz, 2 H, OCH_2CH_2), 4.84 (s, 2 H, C(5)H_2); ^{13}C NMR ($CDCl_3$) 20.17 ($COCH_3$), 20.73 ($COCH_3$), 25.41 (OCH_2CH_2), 60.54 (OCH_2CH_2), 69.25 (C(5)), 135.33 (C(3)), 146.04 (C(4)), 166.98 ($COCH_3$), 167.04 ($COCH_3$), 170.50 (C(2)) ppm; M_r 229.0705 (calcd for $C_{10}H_{13}O_6$, 229.0712, M + 1), 186.0524 (calcd for $C_8H_{10}O_5$, 186.0528), 126.0314 (calcd for $C_6H_6O_3$, 126.0316). Anal. Calcd for $C_{10}H_{12}O_6$: C, 52.63; H, 5.30. Found: C, 52.80; H, 5.41.

Hydrolysis of Bicyclomycin 3′-Ethylcarbamate (2). A solution of **2** (80 mg, 0.214 mmol) in aqueous 1 N H_2SO_4 (5 mL) was heated at 100 °C with stirring (1.5 h). The reaction mixture was cooled to room temperature and extracted with ethyl acetate (3 × 10 mL) and dried (Na_2SO_4), and the solvent was removed in vacuo. The crude material (72 mg) was purified by preparative TLC with 25% methanol in chloroform as the eluent to obtain **5** (15 mg, 49%) as a liquid and **10** (22 mg, 44%) as a hygroscopic solid.

3-Hydroxy-4-(2-hydroxyethyl)-2(5*H*)-furanone (5): R_f 0.70 (25% methanol–chloforom); FTIR (neat) 3628, 3382, 1753, 1678, 1613, 1453, 1359, 1140 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.40 (t, J = 5.95 Hz, 2 H, $HOCH_2CH_2$), 3.55 (t, J = 5.95 Hz, $HOCH_2CH_2$), 4.64 (s, 2 H, C(5)H_2); ^{13}C NMR (CD_3OD) 29.19 ($HOCH_2CH_2$), 60.70 ($HOCH_2CH_2$), 71.18 (C(5)), 130.18 (C(4)), 140.49 (C(3)), 173.80 (C(2)) ppm; MS, m/e (relative intensity) 144 (24), 126 (20), 100 (46), 99 (51), 98 (46), 86 (100), 70 (53), 69 (72), 68 (84), 57 (39); M_r 144.041 85 (calcd for $C_6H_8O_4$, 144.042 26).

(3,4-Dihydroxy-5-methyl-2(5*H*)-oxofuran-5-yl)methyl *N*-ethylcarbamate (10): R_f 0.15 (25% methanol–chloroform); IR (KBr) 3380, 1745, 1720, 1680, 1580 cm^{-1}; ^1H NMR (CD_3OD) δ 1.06 (t, J = 7.5 Hz, 3 H, CH_2CH_3), 1.34 (s, 3 H, C(5)CH_3), 3.09 (q, J = 7.5 Hz, 2 H, CH_2CH_3), 4.13 (s, 2 H, C(5)CH_2O); ^1H NMR (DMSO-d_6–D_2O) δ 1.03 (t, J = 6.8 Hz, 3 H, CH_2CH_3), 1.27 (s, 3 H, C(5)CH_3), 3.02 (q, J = 6.8 Hz, 2 H, CH_2CH_3), 3.88 (d, J = 11.5 Hz, 1 H, OC(*H*)H), 4.11 (d, J = 11.5 Hz, 1 H, OC(H)*H*); ^{13}C NMR (CD_3OD) 15.23 (CH_2CH_3), 19.93 (C(5)CH_3), 36.58 (CH_2CH_3), 67.72 (C(5)CH_2O), 82.82 (C(5)), 114.92 (C(3)), 158.51 (OC(O)NH), 174.73 (C(4)), 176.29 (C(2)) ppm; ^{13}C NMR (D_2O, pD 6.1) 15.79 (CH_2CH_3), 20.15 (C(5)CH_3), 37.22 (CH_2CH_3), 68.31 (C(5)CH_2O), 84.26 (C(5)), 113.77 (C(3)), 159.62 (OC(O)NH), 177.76 (C(4)), 179.60 (C(2)) ppm; ^{13}C NMR (D_2O, pD 1.0) 14.94 (CH_2CH_3), 19.03 (C(5)CH_3), 36.47 (CH_2CH_3), 65.30 (C(5)CH_2O), 82.24 (C(5)), 117.98 (C(3)), 158.18 (OC(O)NH), 158.45 (C(4)), 172.87 (C(2)) ppm.

Methylation of (3,4-Dihydroxy-5-methyl-2(5*H*)-oxofuran-5-yl)methyl *N*-Ethylcarbamate (10). Preparation of (3,4-Dimethoxy-5-methyl-2(5*H*)-oxofuran-5-yl)methyl *N*-Ethylcarbamate (11). To a solution of **10** (17 mg, 0.0736 mmol) in dry methanol (1 mL) was added a large excess of a distilled ethereal solution of diazomethane at 0 °C. The mixture was kept at 0 °C for 16 h and then stirred for another 3 h at room tem-

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perature. The solvent was removed in vacuo and the crude product was purified by preparative TLC with chloroform as the eluent (three developments) to yield 11 (13 mg, 69%) as an oil: R_f 0.25 (chloroform): FTIR ($CHCl_3$) 3416, 1759, 1725, 1683 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13 (t, J = 7.20 Hz, 3 H, CH_2CH_3), 1.43 (s, 3 H, C(5)CH_3), 3.16–3.24 (m, 2 H, CH_2CH_3), 3.83 (s, 3 H, C(3)-OCH_3), 4.13 (s, 3 H, C(4)OCH_3), 4.16–4.21 (m, 2 H, C(5)CH_2O), 4.74–4.85 (m, 1 H, NH); ^{13}C NMR ($CDCl_3$) 15.08 (CH_2CH_3), 19.58 (C(5)CH_3), 35.90 (CH_2CH_3), 59.39 (OCH_3), 60.42 (OCH_3), 65.15 (C(5)CH_2O), 79.34 (C(5)), 122.22 (C(3)), 155.30 (C(4)), 159.71 (OC(O)NH), 168.18 (C(2)) ppm; MS, m/e (relative intensity) 259 (34), 158 (100), 143 (32), 115 (7), 83 (15), 72 (21), 69 (13); M_r 259.104 93 (calcd for $C_{11}H_{17}NO_6$, 259.105 59). Anal. Calcd for $C_{11}H_{17}NO_6$: C, 50.96; H, 6.60; N, 5.40. Found: C, 51.09; H, 6.55; N, 5.51.

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Regioselective Oxidation of 3-Alkylfurans to 3-Alkyl-4-hydroxybutenolides

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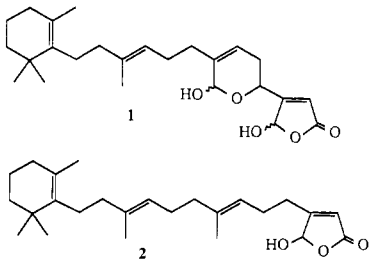
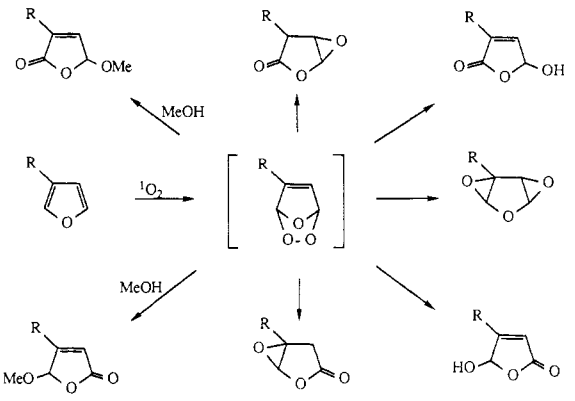
3-Alkyl-4-hydroxybutenolides are synthesized in good to high yields by the oxidation of 3-alkylfurans with singlet oxygen, generated from molecular oxygen with a polymer-bound rose bengal catalyst in dichloromethane solution at −78 °C, in the presence of a hindered base such as 2,2,6,6-tetramethylpiperidine or, preferably, diisopropylethylamine.

The antiinflammatory properties of manoalide (**1**)[1] and luffariellolide (**2**),[2] which are respectively irreversible and reversible inhibitors of the enzyme phospholipase A_2, have caused these and related compounds to become desirable synthetic targets. The common feature of these antiinflammatory agents is the terminal 3-alkyl-4-hydroxybutenolide moiety, which has also been found occasionally in other marine natural products.[3] In contrast with the relatively rare occurrence of the 3-alkyl-4-hydroxybutenolides, their presumed biosynthetic precursors, the 3-alkylfurans, are commonly found as major metabolites of marine sponges.[4] We therefore sought a general method for the regiospecific oxidation of 3-alkylfurans, such as ambliol A (**3**), to obtain the corresponding 3-alkyl-4-hydroxybutenolides (e.g., **6**) for screening as antiinflammatory agents.

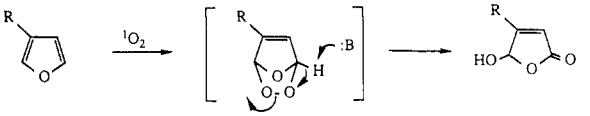


It was known that 3-alkylfurans that were substituted at the 1-position by trimethylsilyl, formyl, or carboxylic acid groups could be oxidized by singlet oxygen to obtain the corresponding 3-alkyl-4-hydroxybutenolides.[5,6] We therefore attempted to prepare a 1-(trimethylsilyl)furan from the sponge metabolite ambliol A (**3**)[7] by treatment with *n*-butyllithium at −78 °C followed by quenching of the lithium derivative with trimethylsilyl chloride but could at best obtain only a 3:1 mixture of the trimethylsilyl derivatives **4** and **5**. As expected, oxidation of the mixture of trimethylsilyl derivatives **4** and **5** with singlet oxygen gave a 3:1 mixture of the 3-alkyl-4-hydroxybutenolide **6** and the 2-alkyl-4-hydroxybutenolide **7**. Other reagents, such as *m*-chloroperbenzoic acid and pyridinium chlorochromate, that had been reported to oxidize furans to the corresponding hydroxybutenolides did not react cleanly with ambliol A(**3**).

Scheme I. Typical Products of $^1O_2$ Oxidation of 3-Alkylfurans



Scheme II. Oxidation of 3-Alkylfurans by $^1O_2$ in the Presence of a Hindered Base



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