Acid-Catalyzed Reactions of Homohalichondrin B, a Marine Sponge-Derived Antitumor Polyether Macrolide

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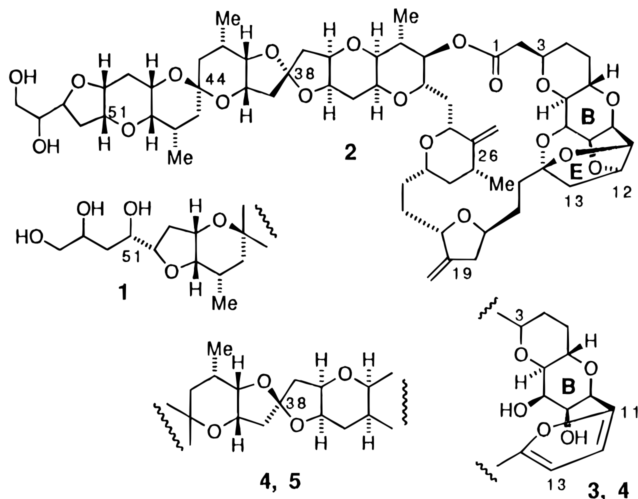
The halichondrins are a series of marine sponge-derived polyether macrolide compounds with a remarkable antitumor potency.[1−6] Halichondrin B (**1**) has been selected by the NCI (USA) for preclinical development as an effective agent against selected human cancer cells.[7] An immediate consequence of this status at the NCI is the pressing need for adequate supplies of this rare compound. This can be addressed from natural resources, or possibly synthesis. A knowledge of the acid stability of the halichondrin family is essential for any large-scale isolation, structure−activity studies on, and synthesis of this series. It has already been our experience, and that of others,[2,8] that the halichondrins are sensitive to exposure to acid conditions, such as can exist in the $CDCl_3$ used for NMR spectroscopy experiments. We now report on a systematic study of the behavior of the halichondrins under a variety of acidic conditions.



Results and Discussion

The investigation was carried out on the more readily available homohalichondrin B (**2**), but the conclusions can be extrapolated to the other naturally occurring halichondrins. Homohalichondrin B was found to be stable in CH_2Cl_2 at 20 °C for up to 48 h when treated with a 200-fold molar excess of *p*-toluenesulfonic acid, either as a two-phase system with water or as a homogeneous solution. However, treatment of the halichondrin with equimolar pyridinium *p*-toluenesulfonate in a $CDCl_3$ solution at 20 °C showed evidence of reaction after 77 h (indicated by a slight reduction of the doublet signal for CH_3-46 at 0.90 ppm in the ^1H NMR spectrum, relative to the signals for other methyl protons, and the appearance of new olefinic proton resonances). After 22 d the CH_3-46 signal had completely disappeared. In contrast, a more rapid reaction of homohalichondrin B was noted in a CD_3OD solution at 23 °C containing a 20-fold molar excess of trifluoroacetic acid (TFA). Changes in the ^1H NMR spectrum were observed immediately after addition of the TFA and were significant after 3 h.

To characterize the compounds formed on acid treatment, homohalichondrin B (8.8 mg) was treated with a 1.5-fold molar excess of TFA in CH_2Cl_2 solution at 20 °C for 0.5 h. After neutralization, the product mixture was separated by reverse phase (RP) HPLC (C18) using 70% acetonitrile/H_2O as eluent. The four peaks (eluting at 230, 300, 800, and 1000 s) were identified as homohalichondrin B (**2**) (2.0 mg), product **3** (2.0 mg), product **4** (1.8 mg), and product **5** (2.0 mg), respectively. Interestingly, all four compounds **2**−**5** had equivalent polarities when examined on diol or silica TLC, in contrast to their very divergent behavior on RPHPLC. HRFABMS showed that each of the products **3**−**5** was isobaric with **2**, having the formula $C_{61}H_{86}O_{19}$. The structural determination of the products was achieved through a combination of HMQC, HMBC, COSY, TOCSY, and NOE NMR experiments, in conjunction with a series of equilibration experiments.

A comparison of the NMR data (Table 1) for compound **5** with those of **2**, which has now been fully assigned, showed that significant changes were apparent only for atoms in the vicinity of C38. Given that all of the connectivities for **5** in this area were the same as for **2** (HMQC, HMBC, COSY, TOCSY), the most reasonable structure for **5** is the C38 epimer. Unfortunately, key changes to coupling constants which would have been expected in this area (e.g., $^3J_{HH}$ H36−H37,H37′ 9.0, 7.7 calculated for **2**, and 5.1, 1.3 Hz calculated for **5**) could not be extracted from any of the NMR spectra because of the complexity of these spectra in the regions of H36 and H37. However, additional support for the proposal for the C38 epimer came from the equilibration studies (*vide infra*).

For product **3**, the signficant changes, relative to **2**, were centered on rings B and E (see data in Table 1). In particular, resonances for positions 11−14 in ring E of **2** had been replaced by resonances characteristic of a 2,5-disubstituted furan. This suggested that the ether links between 8,14 and 9,12 had been cleaved, leaving a C11−C14 furan ring and two hydroxyl groups at C8 and C9. This was confirmed from an examination of the connectivities revealed in the COSY, TOCSY, HMQC, HMBC, and NOE spectra of **3**. A reaction analogous to this double elimination has previously been observed for a tricyclofragment, developed for a total synthesis of the halichondrins,[8] on exposure to acidic $CDCl_3$ in an NMR experiment.

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Table 1. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR Chemical Shift Data for Compounds 2, 3, 4, and 5[a]

	2		5		3		4	
position	C	H	C	H	C	H	C	H
1	171.2		171.0		172.0		171.0	
2	40.4	2.34, 2.58	40.3	2.35, 2.62	40.5	2.43, 2.53	40.2	2.45, 2.55
3	73.7	3.88	73.6	3.86	75.4	3.95	75.2	3.95
4	30.7	1.34, 1.72	*b*	1.35, 1.75	*b*	1.40, 1.70	*b*	1.42, 1.72
5	30.0	1.38, 2.08	*b*	1.38, 2.08	*b*	1.48, 2.08	*b*	1.48, 2.09
6	68.2	4.34	68.0	4.33	68.9	3.98	68.9	4.02
7	77.7	2.94	77.4	2.94	78.8	3.12	79.0	3.17
8	74.3	4.32	74.1	4.31	64.6	4.04	64.8	4.00
8-OH						3.57		3.56
9	73.8	4.04	73.7	4.04	69.0	3.90	69.0	3.90
10	76.5	4.20	76.3	4.18	71.0	4.90	71.2	4.90
11	82.1	4.58	81.8	4.59	151.0		151.0	
12	81.1	4.68	80.8	4.67	108.6	6.18	109.1	6.19
13	48.3	1.94, 2.16	48.2	1.93, 2.14	106.0	5.95	105.9	5.95
14	110.1		110.0		155.0		155.0	
15	34.4	1.60, 2.16	34.2	1.62, 2.14	24.2	2.60, 2.82	24.0	2.66, 2.82
16	28.1	1.40, 2.16	*b*	*b*, *b*	*b*	*b*, 1.75	34.0	1.80, 1.80
17	75.4	4.10	*b*	4.06	77.0	3.87	77.2	3.90
18	38.7	2.26, 2.79	38.6	2.25, 2.80	39.7	2.20, 2.82	39.6	2.20, 2.80
19	151.8		*b*		*b*		*b*	
19=CH_2	104.5	4.91, 4.98	104.8	4.91, 4.98	105.4	4.82, 5.10	105.2	4.86, 5.08
20	75.0	4.38	75.0	4.36	78.3	4.70	78.6	4.70
21	29.0	1.44, 1.88	*b*	*b*, 1.85	*b*	1.60	*b*	1.55, 1.65
22	32.0	1.60, 1.60	32.0	1.60, 1.60	30.2	1.40, 1.58	*b*	1.40, 1.55
23	74.8	3.52	74.5	3.50	77.1	3.60	77.2	3.62
24	43.4	1.05, 1.70	43.2	1.05, 1.68	43.2	0.94, 1.85	43.2	0.94, 1.87
25	35.9	2.20	35.9	2.20	36.0	2.20	36.0	2.15
25-CH_3	18.0	1.06	18.0	1.04	*b*	1.03	17.6	1.03
26	151.5		151.8		151.1		151.1	
26=CH_2	104.2	4.75, 4.80	104.0	4.75, 4.81	105.0	4.78, 4.81	104.5	4.78, 4.82
27	73.5	3.54	73.2	3.50	74.6	3.70	74.9	3.68
28	36.9	1.94, 2.02	*b*	1.96, 1.96	33.8	1.55, 2.25	33.8	1.60, 2.20
29	71.2	4.20	71.0	4.18	70.8	3.80	71.4	3.80
30	76.6	4.65	76.8	4.66	75.8	4.52	76.2	4.50
31	36.8	2.04	36.2	2.05	38.0	1.88	36.5	1.95
31-CH_3	15.0	0.99	14.7	0.99	*b*	1.03	14.6	1.03
32	77.5	3.17	77.1	3.12	76.4	3.35	76.2	3.32
33	66.5	3.78	67.0	3.76	67.2	3.82	68.0	3.78
34	29.4	1.80, 2.14	*b*	*b*, 2.14	*b*	1.90, *b*	*b*	1.92, 2.08
35	75.3	4.10	*b*	4.08	75.2	4.12	78.0	4.12
36	76.3	4.10	*b*	4.04	*b*	4.04	*b*	4.02
37	43.4	1.90, 2.35	43.9	2.22, 2.22	44.2	1.89, 2.35	44.2	2.19, 2.19
38	112.4		114.0		112.4		114.0	
39	42.5	2.21, 2.21	44.5	2.02, 2.28	42.8	2.18, 2.18	44.3	2.02, 2.26
40	70.8	3.92	71.3	3.92	70.7	3.90	71.4	3.94
41	79.4	3.60	78.6	3.82	79.1	3.60	78.8	3.85
42	25.8	2.32	25.3	2.26	25.8	2.32	25.8	2.26
42-CH_3	17.7	0.93	17.9	0.99	17.5	0.92	17.5	0.92
43	36.5	1.29, 1.45	37.0	1.25, 1.40	37.0	1.29, 1.45	36.8	1.30, *b*
44	96.6		96.8		96.5		96.0	
45	36.8	1.42, 1.42	37.0	1.29, 1.42	36.8	1.42, 1.42	36.8	1.28, 1.45
46	28.9	2.18	29.0	2.08	29.0	2.15	28.9	2.08
46-CH_3	17.1	0.90	17.3	0.94	17.2	0.90	17.0	0.92
47	72.8	3.06	72.5	3.06	72.6	3.06	72.7	3.07
48	63.6	3.53	63.4	3.50	63.4	3.53	63.4	3.52
49	31.4	1.79, 2.15	31.2	1.76, 2.15	31.2	1.79, 2.15	31.0	1.79, 2.15
50	74.7	3.90	*b*	3.90	74.5	3.90	74.6	3.92
51	76.3	4.04	*b*	4.01	76.0	4.04	76.3	4.04
52	37.2	2.02, 2.02	*b*	2.00, 2.00	36.8	2.02, 2.02	36.7	2.02, 2.02
53	79.8	4.25	79.4	4.25	79.5	4.25	79.7	4.25
54	72.0	3.57	71.8	3.55	71.8	3.55	72.1	3.55
55	65.6	3.69	65.3	3.69	65.3	3.69	65.5	3.69

[a] Values in ppm relative to C*H*Cl_3 (δ 7.25) and *C*DCl_3 (δ 77.0). [b] Values not detected.

Finally, the NMR spectra (Table 1) for product **4** showed features characteristic of both **3** and **5**, and **4** was thus assigned as the C38 epimer of the furan **3**.

There was evidence suggesting that one or more of the products from the acid treatment of homohalichondrin B were existing in equilibrium. This was explored by subjecting each of the products **3**−**5** to acid conditions (TFA:product (2:1) in CH_2Cl_2 at 20 °C) and following the time course of any changes by RPHPLC analysis. Analysis of the reaction of the furan product **3** showed that there was a conversion to the C38 epimer **4**, but no reversal of the furan formation to the tricyclosystem in **2** or **5**. A similar result was obtained when the C38 epimer **4** was treated with acid, thus showing that the epimerization at C38 is reversible. The equilibrium position was attained after 20 h and contained approximately equal amounts of the two epimers. Treatment of the C38 epimer **5** with acid showed an initial reversal to **2** (maximum amounts of **2** after 5 h), with a subsequent slower cleavage of the tricyclo ring system

to give the furans **3** and **4** as the eventual products. Extended reaction times (>50 h) led to a decrease in the amounts of the furan products and gave rise to other uncharacterized products.

There was no evidence from the NMR data for any changes having occurred at the remaining spiroketal center at C44. This absence of change may result from a difference in reactivity of spiroketals joining two six-membered or two five-membered rings, or may arise from the position of the equilibrium at C44 giving almost complete predominance to the configuration found in the natural product.

The biological activities of the products **3**−**5** were determined. The IC_{50} values against the murine leukemia cell line P388 were 1.8, 36.5, 74.7, and 133.5×10^{-10} M respectively for **2**, **3**, **4**, and **5**, while the GI_{50} values in the NCI 60 human tumor cell panel[9] were 1.58, 116, 478, and 475×10^{-10} M, respectively. These results suggest that the epimerization at C38 gives a greater reduction in activity than the furan formation. Modeling studies, together with the marked change in behavior on RPHPLC ($t_{R2} = 230$ s *vs* $t_{R5} = 1000$ s), suggest major changes in the overall conformation of the ring systems following epimerization at C38. This observation was supported by the COMPARE correlation coefficients extracted from the NCI's primary screen mean graph profiles.[9] The halichondrins are mitotic inhibitors and as such display characteristic GI_{50}-centered profiles.[5] The COMPARE correlation coefficients for compounds **2**−**5** were 0.95, 0.91, 0.69, and 0.86, respectively, against halichondrin B (**1**), which was used as the "seed" (compound with defined mode of action). The *epi* compounds **4** and **5** are the least correlated with halichondrin B (**1**), in keeping with the suspected conformational changes induced on epimerization at C38.

This acid lability of the C38 spiroketal and the tricyclo moeities of the halichondrins has prompted us to reexamine previous reports involving the chemistry of the halichondrins. For example, one of the reported syntheses of the halichondrins involves, as the very last step, acid-catalyzed closure of the C38 spiroketal from the corresponding C41-hydroxy C38-hemiketal.[10] As this report was in the form of a Communication, experimental detail was minimal and no indication was given of the concentration of the camphorsulfonic acid (CSA) used, or of the time of reaction. However, we have found that whereas a mixture of **2** and CSA in CH_2Cl_2 at 20 °C (CSA: **2**; 1:10) gave no reaction after 2 h, when the ratio CSA:**2** was increased to 1:3, the changes described above for **2** with TFA were found to occur on a similar time scale. Given the similarities in NMR profiles, and the identical chromatographic behavior on normal phase media, up to at least 5% of the C38 epimer could be present undetected in any synthetic halichondrin that has been exposed to moderate acid conditions in the terminal sequences of the synthetic route. The design of future total syntheses of the halichondrins, and any hemisynthetic conversions within this series, should take cognisance of the acid lability of the C38 spiroketal and the tricyclo moeities.

Experimental Section

All NMR spectra were recorded at 23 °C on a 300 MHz spectrometer fitted with a 3 mm indirect detection probe, using $CDCl_3$ with 0.1% C_5D_5N as solvent. FAB mass spectra were obtained using Xe as the reagent gas, with the ion gun operating at 8 kV and 2 mA current with a *m*-nitrobenzyl alcohol (NOBA) matrix. A Brownlee Labs analytical C18 column (dimensions 220 mm (l) × 4.6 mm (i.d.), 5 mm particle size) was used for analytical and semipreparative HPLC. The mobile phase was 70% acetonitrile/H_2O. The homohalichondrin B used in this work was isolated from a *Lissodendoryx* sp. sponge as described previously.[6]

Preparation of Acid-Derived Products from Homohalichondrin B. Homohalichondrin B (**2**, 4.0 mg) was dissolved in a TFA/CH_2Cl_2 solution (30.04 mM TFA, 200 μL) and stirred at 20 °C for 30 min. Water (1 mL) was then added, and the pH of the aqueous layer was adjusted to 7.5−8.0 with 0.1 M $NaOH_{(aq)}$. The CH_2Cl_2 was washed with water (4 × 1 mL). Removal of the solvent gave a mixture (4.0 mg) which was separated by RPHPLC. The mixture was dissolved in 200 μL of the mobile phase, and 5 × 40 μL RPHPLC injections were performed. The peaks eluting at *ca.* 230, 300, 800, and 1000 s were collected separately to yield **2** (0.9 mg), **3** (0.8 mg), **4** (1.0 mg), and **5** (1.1 mg), respectively. A second reaction and separation using 4.8 mg of **2** was carried out in an identical manner to that described above, to give combined yields of **2** (2.0 mg), **3** (2.0 mg), **4** (1.8 mg), and **5** (2.0 mg). Compounds **3**, **4**, and **5** were each clear, colorless oils. **3**: calcd for $C_{61}H_{87}O_{19}$ (MH^+) 1123.5841, found 1123.5859. **4**: calcd for $C_{61}H_{86}O_{19}Na$ (MNa^+) 1145.5661, found 1145.5641. **5**: calcd for $C_{61}H_{87}O_{19}$ (MH^+) 1123.5841, found 1123.5869.

Equilibration Studies. In order to obtain a quantitative measure of the relative amounts of the compounds **2**−**5** present during the acid-equilibration studies, the relative molar absorptivities of the furan compounds (**3**, **4**) *vs* the non-furan compounds (**2**, **5**) were determined. The ^1H NMR spectrum of a mixture of **2**−**5** was obtained. Comparison of the integrals of the furan resonances at δ_H 5.95 ppm and δ_H 6.18 ppm with that for H7 resonance at δ_H 3.06 ppm (unique for compounds **2** and **5**) showed that the furan compounds **3** and **4** constituted 24% of the mixture. Integration of the four peaks showing in the RPHPLC trace for this mixture gave a ratio of 65:35 for the combined integrals (at λ 199 nm) of **3** and **4** to those of **2** and **5**. Thus the ratio of the molar absorptivities at λ 199 nm for the furan compounds (**3**, **4**) *vs* the non-furan components (**2**, **5**) was calculated as 5.9:1 for ϵ_{furans}:$\epsilon_{non\text{-}furans}$. A typical equilibration study was made as follows: the 38-*epi* compound **5** (0.5 mg) was dissolved in CH_2Cl_2 (85 μL). The solvent was removed from an aliquot (*ca.* 2 μL) of this solution and then dissolved in the HPLC mobile phase (10 μL) for analysis by RPHPLC. To the remainder of the solution of **5** in CH_2Cl_2 was added a TFA/CH_2Cl_2 solution (15.02 mM TFA, 14.8 μL) to give a 1:2 molar ratio of TFA:**5**. Aliquots (*ca.* 2 μL) were removed after 15 min, 45 min, 1.17 h, 3 h, 3.5 h, 4.5 h, 7 h, 20.5 h, and 47 h of reaction. RPHPLC analyses were made immediately by removing the solvent from each aliquot and redissolving it in the HPLC mobile phase (10 μL).

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Supporting Information Available: ^1H NMR spectra of **3**, **4**, and **5** (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS. See any current masthead page for ordering information.

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