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AUTOOXIDATION STUDIES ON THE MARINE SESTERTERPENE TETRONIC ACID, VARIABILIN

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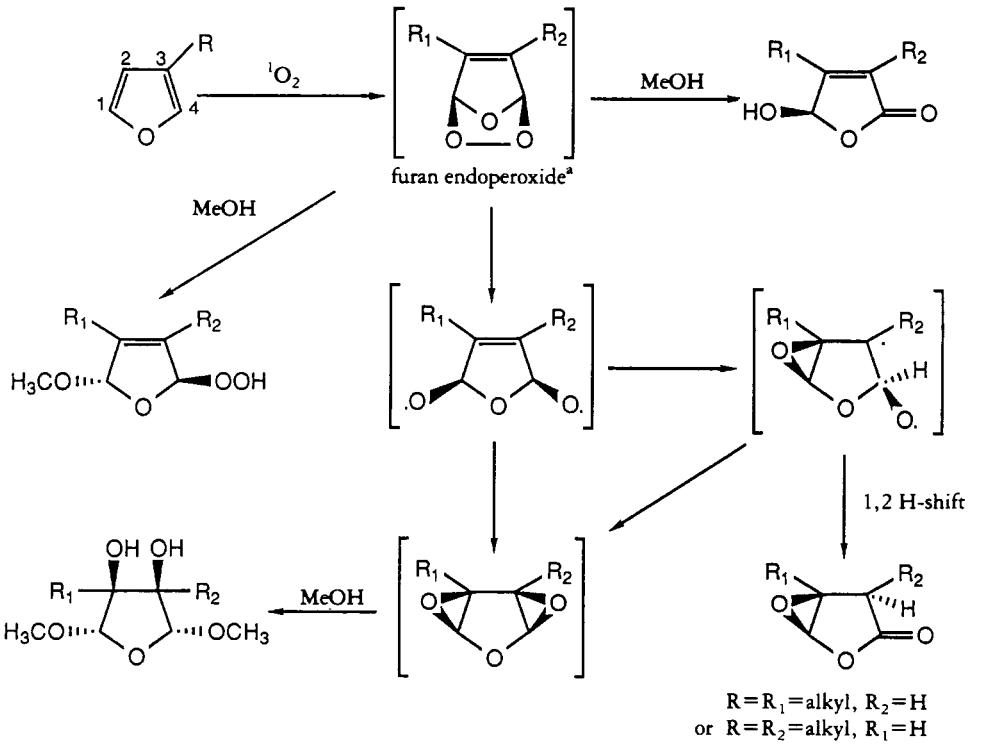
ABSTRACT.—Autooxidation studies on the sesterterpene variabilin [**1**] have established that the tetronic acid moiety acts as a sensitizer for the production of singlet oxygen, which then reacts with the furan moiety of variabilin to produce a range of compounds that have been characterized. A comparison of the bioactivity of variabilin with that obtained for some analogues, including the C_{21} furanoterpene **25** and some stable oxidation products of 22-*O*-methylvariabilin [**8**], has shown that neither the furan nor the tetronic acid moiety is essential for the in vitro antiviral activity of variabilin. Structural modifications of variabilin have not eliminated the high cytotoxicity shown by this compound, so that variabilin and related compounds are likely to be of limited use as antiviral agents.

During a search for antiviral and antitumor agents from New Zealand marine invertebrates, the furanosesterterpene tetronic acid variabilin [**1**] was isolated as the major bioactive component from a sponge of the genus *Sarcotragus* (1). Variabilin is a major component in all New Zealand collections of sponges of the genera *Ircinia, Psammocinia,* and *Sarcotragus* (2). Because of its ready availability and its initially promising antiviral activity (3), variabilin was examined for its potential as an antiviral agent. However, it soon became apparent that this potential would be limited because of the rapid decomposition of variabilin in the presence of light and air. Similar decomposition occurs for a number of other furanoterpene tetronic acids isolated from sponges in our collection (1,4). An investigation into the nature of the decomposition products of variabilin and their mode of formation has led to the production of variabilin analogues that exhibit biological activity similar to that shown by variabilin but that are stable in the presence of light and air.

RESULTS AND DISCUSSION

Variabilin [**1**] decomposed in the presence of light and air to a complex mixture of more polar products. Monitoring by hplc and tlc showed the rapid formation of a number of products that were then converted to a further suite of products, at a much slower rate, on further irradiation. A high resolution ^{1}H-nmr spectrum of the initially formed mixture revealed that the tetronic acid and central chain regions of variabilin [**1**] remained intact. The replacement of the resonances previously assigned to the furan group with a number of new resonances implied that the initial oxidation had occurred at the furan moiety. The reaction of singlet oxygen with 3-alkyl furans is known to result in a variety of products formed by the thermal rearrangement of the initially formed furan endoperoxides and also by polar processes (5,6). Products typical of such oxidations are shown in Scheme 1 and include hydroxybutenolides, 1,3-diepoxides, epoxylactones, hydroperoxides, and diols. Ignoring physical parameters such as temperature, the actual range of products formed is very sensitive to the solvent used in the oxidation and the nature of the substituents on the furan (5,6). In the singlet oxygen oxidation of variabilin [**1**] in MeOH, the products resulting from the thermal rearrangement of the initially formed furan endoperoxides (Scheme 1) were epoxylactones, and diols formed (presumably) by MeOH addition to 1,3-diepoxides. The other major pathways for breakdown of the furan endoperoxides were polar processes involving the solvent MeOH and resulted in a range of hydroxybutenolides and an hydroperoxide.

Separation of the initial oxidation mixture by hplc gave three fractions containing the unstable compounds identified tentatively, by ^{1}H and ^{13}C nmr (Tables 1 and 2), as



SCHEME 1. Pathway for the oxidation of 3-alkyl furans.

the diastereoisomeric mixtures of hydroxybutenolides **2** + **3**, **4** + **5**, and diols **6** + **7**. Difficulties arose in the interpretation of ^{1}H- and ^{13}C-nmr spectra of these compounds in $CDCl_3$ solution due to unexpected differences in chemical shifts between diastereoisomers. In particular, H-20 appeared as two well-separated doublets in the ^{1}H-nmr spectra. However, when the ^{1}H- and ^{13}C-nmr spectra were obtained in CD_3OD, the spectra were simplified, with the only evidence of the presence of diastereoisomers being at resonances from atoms close to the modified furan. This use of CD_3OD presumably reduced the extent of intramolecular hydrogen bonding between the polar groups at each end of the molecules, thus simplifying the spectra by reducing the interaction between the chiral center at C-18 and the new chiral centers formed during oxidation.

In an attempt to remove this hydrogen-bonding interaction in the products, 22-*O*-methylvariabilin [**8**] was treated under the same conditions as used for the autooxidation of variabilin [**1**]. However, **8** remained unaltered after 6 h exposure to a 200 W incandescent lamp. Addition of small quantities of the singlet oxygen sensitizer rose bengal allowed the furan moiety to be rapidly oxidized in a manner similar to that observed for both the autooxidation and the rose-bengal-sensitized oxidation of variabilin [**1**].

Confirmation that the products from the oxidation of 22-*O*-methylvariabilin [**8**] were identical to those from the oxidation of variabilin [**1**] (with the exception of the C-26 methyl group) was achieved by the CH_2N_2 methylation of the initial oxidation mixture of variabilin. The products obtained from this methylation were identical (by hplc and nmr spectroscopy) to those formed from the sensitized oxidation of **8**. The characterization of the now stable products from the rose-bengal-sensitized oxidation of **8**,

[a]Scheme shows pathways for one of two possible stereoisomeric furan endoperoxides.

TABLE 1. ^{1}H-Nmr Data for Compounds **2–7**.[a]

Position	Compound					
	2+3	**2**[b]**+3**	**4+5**	**4**[b]**+5**	**6+7**	**6**[b]**+7**
1	6.11 s	6.04 s			4.90 d (3.8)	4.85 d (4.5)
2	6.85 q (1.3)	6.94 d (1.4)	6.02 s	5.99 d (0.9)	3.96, 3.97[c] d (3.8)	3.80 dd (4.5, 1.1)
4			5.83 m	5.85 m	4.75 s	4.64 s
5	2.28 t (5.5)	2.26 s	2.42, 2.52 bm	2.41, 2.47 bm	1.70 bm	1.60 m
6	2.23 q (5.5)	2.26 m	2.32 t (6.8)	2.32 m	2.10 bm	2.10 bm
7	5.04 t (5.5)	5.12 t (5.5)	5.06 tq (6.5, 1.4)	5.14 tq (7.0, 1.3)	5.11 tq (6.5, 1.2)	5.11 tq (6.5, 1.2)
9	1.56 d (1.2)	1.60 d (1.2)	1.59 d (1.1)	1.63 d (1.3)	1.58 d (1.2)	1.60 d (1.0)
10	2.00 m	2.00 m	2.02 m	2.02 m	2.05 m	2.07 m
11	2.06 m	2.07 m	2.07 m	2.09 m	2.00 m	1.98 m
12	5.00 tq (5.5, 1.2)	5.08 tq (5.5, 1.2)	4.99 tq (6.8, 1.2)	5.08 tq (7.0, 1.3)	5.00 tq (6.5, 1.2)	5.09 tq (6.5, 1.2)
14	1.52 d (1.2)	1.55 d (1.2)	1.53 d (1.2)	1.55 d (1.3)	1.53 d (1.2)	1.55 d (1.2)
15	1.92 q (5.5)	1.96 m	1.91 m	1.95 m	1.91 m	1.95 m
16	1.33 m	1.37 m	1.33 m	1.37 m	1.33 m	1.37 m
17	1.33 m	1.37 m	1.33 m	1.37 m	1.33 m	1.37 m
18	2.67 bm	2.74 bm	2.77 bm	2.73 bm	2.77 bm	2.74 bm
19	1.03 d (6.6)	1.05 d (6.7)	1.04 d (6.8)	1.05 d (6.7)	1.03 d (6.7)	1.06 d (6.7)
20	5.30, 5.29 d (10.2)	5.27 d (10.1)	5.30, 5.29 d (10.0)	5.27 d (10.1)	5.25, 5.24 d (10.1)	5.28 d (10.1)
25	1.78 s	1.74 s	1.78 s	1.74 s	1.79 s	1.75 s
27					3.46 s	3.43 s
28					3.395, 3.39 s	3.35 s

[a]Values in ppm, relative to $\delta_H = 0.00$ for TMS in $CDCl_3$ solutions (coupling constants in Hz).
[b]CD_3OD solutions.
[c]Two values are quoted for doubled resonances.

TABLE 2. ^{13}C-Nmr Data for Compounds **2–14**.[a]

Carbon	Compound							
	2+3	**9+10**	**9+10**	**4+5**	**11+12**	**6+7**	**13[b]+14**	**13+14**
1	99.11	96.62, 96.51[f]	99.0–99.4	[g]	174.00[c]	111.68	110.42, 110.36	111.81
2	146.73	143.47, 143.29	146.5–147.0	118.08	118.25	81.02	79.79	81.06
3	137.93	137.70, 137.58	137–138	[g]	172.44[c]	81.95	80.80	81.95
4	174.20	171.65	174.47	101.29	101.46	110.06	108.79, 108.73	110.17
5	26.58	25.15	26.7–27.2	26.51	26.48	23.17	21.73	23.16
6	27.11	25.37, 25.32	27.75	29.04	29.02	34.85	32.63	34.90
7	124.23	122.56	124.40	124.20	124.34	125.81	123.65, 123.61	125.95
8	137.81	136.53, 136.45[c]	136.24[c]	137.97	136.33	135.96[c]	135.98, 135.88	136.10[c]
9	16.49	15.96, 15.93[d]	16.24[d]	16.52	16.49	16.25[d]	15.73[c]	16.26[d]
10	41.00	39.34[e]	41.00[e]	40.95	40.95	41.13	39.29	41.13
11	27.78	25.99, 25.93	27.13	27.64	27.66	27.92	25.39	27.89
12	125.70	124.00	125.85	125.58	125.73	125.93	124.20, 124.16	126.13
13	136.09	134.91[c]	137.94[c]	136.20	138.14	136.20[c]	134.84	136.29[c]
14	16.28	15.81, 15.78[d]	16.45[d]	16.28	16.25	16.35[d]	15.63[c]	16.35[d]
15	40.71	39.30[e]	40.71[e]	40.70	40.70	40.70	39.12	40.70
16	27.16	25.55	27.09	27.17	27.14	27.10	25.89, 25.84	27.08
17	37.90	36.43	37.85	37.91	37.85	37.86	36.46	37.83
18	32.19	30.55, 30.52	32.24	32.13	32.23	32.17	30.48	32.21
19	21.43	20.57	21.35	21.41	21.36	21.44	20.45	21.39
20	116.13	115.78, 115.64	116.61	115.97	116.63	116.17	115.25, 115.18	116.46
21	145.03	142.54	144.61	145.20	144.61	145.00	142.38	144.60
22	164.56	162.16, 162.07	164.10	[g]	164.10	164.48	161.77	164.02
23	99.27	98.87	100.21	98.95	100.23	99.30	98.69	100.22
24	173.56	171.37	173.41	[g]	173.40[c]	173.58	[g]	[g]
25	6.51	8.44	8.77	6.20	8.78	6.50	8.27	8.82
26		58.78	60.22		60.21		58.55	60.22
27						56.83	56.03	56.82
28						55.65	54.86	55.65

[a]Values in ppm in CD_3OD solutions.
[b]$CDCl_3$ solutions.
[c–e]Assignments in vertical columns may be interchanged.
[f]Two values are quoted for doubled resonances.
[g]Signals not observed due to inadequate intensity.

therefore, effectively permitted the full characterization of the unstable products formed from the oxidation of variabilin [**1**].

Separation of compounds in the initial product mixture from the sensitized oxidation of **8** was achieved by semi-preparative silica nplc. One fraction (23% by weight) contained the diastereoisomeric mixture of the hydroxybutenolides **9** and **10**, the structures of which followed from the high resolution negative ion chemical ionization mass spectrum and the ^{1}H- and ^{13}C-nmr spectra of the mixture (Tables 2 and 3). These spectra were consistent with the presence of the α-substituted-γ-hydroxybutenolide system (7,8). Reduction of the mixture with $NaBH_4$ gave the expected unsaturated-γ-lactone **15** (7) as a single isomer (Tables 4 and 5).

A similar diastereoisomeric mixture of the regioisomeric hydroxybutenolides **11** and **12**, isolated in 16% yield from the oxidation mixture of **8**, gave mass, ^{1}H- and ^{13}C-nmr spectra consistent with the presence of the β-substituted-γ-hydroxybutenolide moiety (8,9). Reduction of the **11**,**12** mixture with $NaBH_4$ gave the expected unsaturated-γ-lactone **16** as a single isomer.

The most polar fraction from semi-preparative nplc of the **8** oxidation mixture on silica contained the diastereoisomeric diols **13** and **14**. High resolution negative ion cims gave the molecular formula $C_{28}H_{44}O_8$. The presence of the diol moiety was suggested by ^{13}C-nmr resonances at 81.06 and 81.95 ppm, together with the ^{1}H-nmr resonance at 3.79 ppm, which appeared as two doublets separated by 0.8 Hz (in CD_3OD, Tables 2 and 3). Selective irradiations and a COSY experiment confirmed that this 0.8-Hz shift was not due to coupling. Two three-proton singlets at 3.35 and 3.43 ppm in the ^{1}H-nmr spectrum and ^{13}C-nmr resonances at 111.81 and 110.17 ppm were consistent with the presence of methoxy groups at C-1 and C-4. An nOe effect between a one-proton doublet at 4.85 ppm and the methoxyl singlet at 3.43 ppm confirmed that these groups were attached at C-1, while an nOe effect between the methoxyl singlet at 3.35 ppm and a one-proton singlet at 4.65 ppm placed these groups at C-4.

The diol structures of **13** and **14**, along with their suggested *cis* diol stereochemistries, were confirmed via reaction of the mixture with Me_2CO/perchloric acid to form acetonides **17** and **18** (Tables 4 and 5). An nOe effect from the acetonide methyl at 1.43 ppm (H-31) to both H-1 and H-4 distinguished the C-30 and C-31 methyl groups and confirmed that both the C-27 and C-28 methoxyl groups were *trans* to the acetonide functionality and, therefore, *trans* to the diol moiety in the diol precursors **13** and **14**. The acetonides **17** and **18** were not distinguishable by nmr spectroscopy, but both must have been present due to their formation from the diol stereoisomers **13** and **14**.

The stereochemistries of diols **13** and **14** are consistent with their formation via MeOH addition to two isomeric 1,3-diepoxides, although these intermediates were not observed. However, diepoxides of this type have previously been isolated from singlet oxygen addition to the furan moiety (10) and are known to add MeOH in the suggested fashion (11).

The two epoxides **19** and **20**, isolated separately in 13% yield each from the **8** oxidation mixture, showed identical uv, ir, nmr, and mass spectral properties, these being consistent with the presence of α-substituted-β,γ-epoxy-γ-lactones (Tables 4 and 5). Because compounds **19** and **20** were indistinguishable by nmr they must have the same relative configurations between C-1, C-2, and C-3. From mechanistic considerations, the stereochemistry of the epoxide functionality and H-3 must be *trans*, an observation borne out by the magnitude of the H-1,H-2 and H-2,H-3 coupling constants (~2.5 Hz). The most probable mechanism from the furan endoperoxides is the formation of a *cis* oxygen diradical, followed by epoxide formation and a 1,2 H-shift from C-4 to C-3 to give the *trans* product (Scheme 1). The two compounds were confirmed to be different from the magnitude of their respective ord curves, with the rotations at 589 nm

TABLE 3. ^{1}H-Nmr Data for Compounds **9–14**.[a]

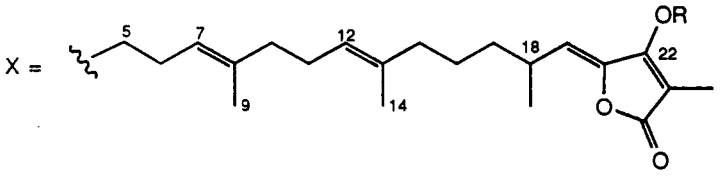
Position	Compound					
	9 + 10	**9[b] + 10**	**11 + 12**	**11[b] + 12**	**13 + 14**	**13[b] + 14**
1	6.07 bs	6.18 bs			4.89 d (3.9)	4.85 d (4.5)
2	6.82 q (1.4)	7.00 bs	6.01 m	6.00 s	3.97, 3.95[c] d (3.9)	3.795, 3.79 d (4.5)
4			5.83 bs	5.86 m	4.745, 4.74 s	4.65 s
5	2.31 t (5.5)	2.38 m	2.39, 2.51 bm	2.32, 2.44 bm	1.74 m	1.62 m
6	2.25 q (5.5)	2.36 m	2.31 m	2.33 t (6.5)	2.21 m	2.16 bm
7	5.06 m	5.23 tq (6.0, 1.3)	5.09 m	5.14 tq (7.0, 1.3)	5.16 tq (6.5, 1.2)	5.12 m
9	1.55 d (1.4)	1.69 d (1.4)	1.60 d (1.2)	1.63 d (1.3)	1.61 d (1.2)	1.61 d (1.0)
10	2.00 m	2.10 m	2.02 m	2.02 m	2.05 m	2.07 m
11	2.06 m	2.17 m	2.06 m	2.08 m	2.01 t (5.0)	1.98 m
12	5.03 m	5.18 tq (6.0, 1.8)	5.04 m	5.07 tq (7.0, 1.3)	5.05 tq (6.5, 1.2)	5.09 m
14	1.54 d (1.3)	1.64 d (1.3)	1.54 d (1.2)	1.55 d (1.3)	1.54 d (1.2)	1.55 d (1.2)
15	1.92 m	2.05 m	1.91 m	1.95 m	1.93 m	1.95 m
16	1.33 m	1.45 m	1.32 m	1.35 m	1.33 m	1.35 m
17	1.33 m	1.45 m	1.32 m	1.35 m	1.33 m	1.35 m
18	2.75 bm	2.81 bm	2.74 bm	2.71 bm	2.75 bm	2.71 bm
19	1.02 d (6.7)	1.13 d (6.8)	1.02 d (6.7)	1.03 d (6.7)	1.02 d (6.7)	1.04 d (6.7)
20	5.17, 5.16 d (10.2)	5.31 d (10.3)	5.18, 5.17 d (10.2)	5.21 d (10.2)	5.15 d (10.3)	5.21 d (10.0)
25	2.05 s	2.14 s	2.05 s	2.04 s	2.06 s	2.05 s
26	4.12 s	4.27 s	4.12 s	4.17 s	4.12 s	4.18 s
27					3.40 s	3.35 s
28					3.465, 3.46 s	3.43 s

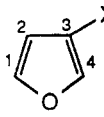
[a]Values in ppm, relative to $\delta_H = 0.00$ for TMS in $CDCl_3$ solutions (coupling constants in Hz).
[b]CD_3OD solutions.
[c]Two values are quoted for doubled resonances.

TABLE 4. ^{1}H-Nmr Data for Compounds **15–24**.[a]

Position	Compound					
	15	**16**	**17 + 18**	**19 + 20**	**21 + 22**	**23 + 24**
1	4.75 q (1.8)		4.93 s	5.57 d (2.4)		5.52 d (1.2)
2	7.09 qu[b] (1.8)	5.83 qu (1.8)	4.27 s	3.78 t (2.5)	2.81 dd (19.0, 1.0) 2.69 d (19.0)	5.75 t (1.2)
3				2.80 ddd (10.2, 4.9, 2.6)		
4		4.72 d (1.8)	4.92 s		5.41 t (1.0)	5.83 d (1.2)
5	2.32 m	2.44 bt (7.0)	1.69 m	1.79, 1.90 m	1.86 m	2.22 bm
6	2.26 m	2.29 bq (7.0)	2.12 m	2.26 m	2.13 q (6.0)	2.22 m
7	5.09 tq (6.0, 1.3)	5.07 tq (6.5, 1.3)	5.14 tq (6.5, 1.3)	5.13 tq (7.0, 1.3)	5.07 tq (7.0, 1.3)	5.10 tq (6.5, 1.3)
9	1.59 d (1.3)	1.60 d (1.3)	1.59 d (1.3)	1.63 d (1.3)	1.60 d (1.3)	1.60 d (1.2)
10	1.98 m	2.00 m	1.96 m	2.02 m	1.94 m	1.98 m
11	2.03 m	2.04 m	2.04 m	2.06 m	2.02 m	2.03 m
12	5.05 tq (6.0, 1.3)	5.04 tq (6.5, 1.4)	5.07 tq (6.5, 1.3)	5.05 tq (7.0, 1.3)	5.05 tq (7.0, 1.3)	5.06 tq (6.5, 1.3)
14	1.54 d (1.3)	1.54 d (1.3)	1.54 d (1.3)	1.54 d (1.3)	1.55 d (1.3)	1.54 tq (6.5, 1.3)
15	1.91 m	1.92 m	1.94 m	1.92 m	1.93 m	1.93 m
16	1.31 m	1.32 m	1.33 m	1.32 m	1.33m	1.34 m
17	1.31 m	1.32 m	1.33 m	1.32 m	1.33 m	1.34 m
18	2.74 bm	2.75 bm	2.75 bm	2.75 bm	2.75 bm	2.75 bm
19	1.01 d (6.7)	1.01 d (6.7)	1.01 d (6.8)	1.02 d (6.6)	1.02 d (6.7)	1.02 d (6.7)
20	5.13 d (10.2)	5.13 d (10.2)	5.14 d (10.1)	5.14 d (9.1)	5.13 d (10.3)	5.14 d (10.2)
25	2.05 s	2.05 s	2.03 s	2.05 s	2.06 s	2.06 s
26	4.11 s	4.11 s	4.12 s	4.11 s	4.11 s	4.11 s
27			3.40 s			3.48 s
28			3.43 s			8.44 (OH)
30			1.41 s			
31			1.43 s			

[a]Values in ppm, relative to $\delta_H = 0.00$ for TMS in $CDCl_3$ solutions (coupling constants in Hz).
[b]qu = quintet.





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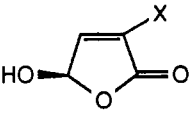

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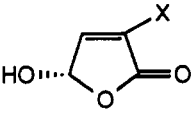

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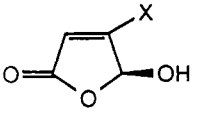
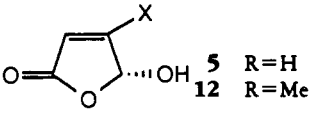
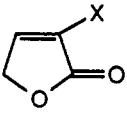


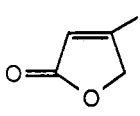
4 R=H
11 R=Me



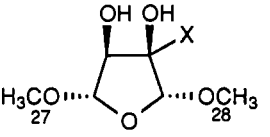
5 R=H
12 R=Me



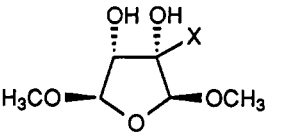
15 R=Me



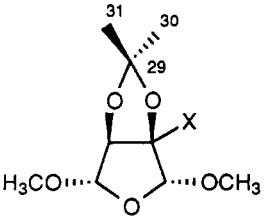
16 R=Me



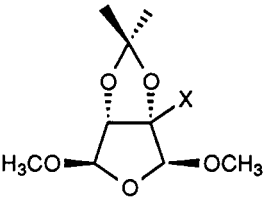
6 R=H
13 R=Me



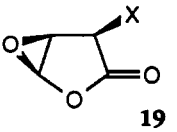
7 R=H
14 R=Me



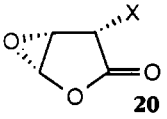
17 R=Me



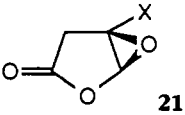
18 R=Me



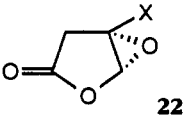
19 R=Me



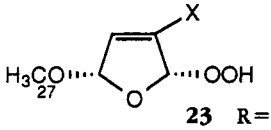
20 R=Me



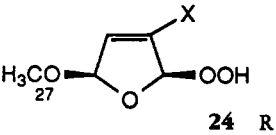
21 R=Me



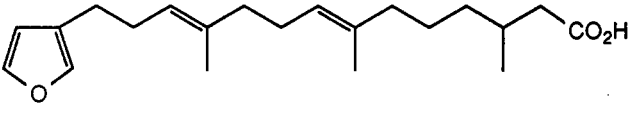
22 R=Me



23 R=Me



24 R=Me



25

being $-60°$ for **19** and $-10°$ for **20**. The actual configuration of each compound was assigned arbitrarily, as neither the absolute configuration of the new chiral centers nor that of C-18 was known.

The epoxides **21** and **22**, structurally isomeric with **19** and **20**, were isolated as the

TABLE 5. ^{13}C-Nmr Data for Compounds **15–24**.[a]

Carbon	Compound					
	15	**16**	**17+18**	**19+20**	**21+22**	**23+24**
1	70.12	174.15	111.40	77.62	173.45	107.41
2	144.34	115.60	89.59	53.69	36.12	115.30
3	133.96	170.25	93.35	43.07	74.01	142.06
4	174.40	73.17	110.33	175.70	82.95	110.61
5	25.45	25–26 (broad)	22.87	25.78	23.13	25.66
6	25.71	28.75	33.30	26.82	29.67	29.70
7	122.65	121.86	123.84	122.52	122.05	123.01
8	136.70	137.56	135.46	137.28	137.28	135.82
9	16.09	16.18	15.89	16.12	16.09	16.10
10	36.65[b]	39.51[b]	39.58[b]	39.54[b]	39.51[b]	39.55[b]
11	26.53	26.40	25.71	26.41	26.36	26.52
12	124.13	123.92	124.36	124.06	123.93	124.17
13	135.03	135.25	134.89	135.18	135.26	135.02
14	15.84	15.87	15.81	15.87	15.87	15.84
15	39.54	39.57[b]	39.73[b]	39.67[b]	39.58[b]	39.63[b]
16	25.71	25.73	26.67	25.73	25.72	25.72
17	39.62[b]	36.66	36.69	36.66	36.66	36.67
18	30.77	30.78	30.81	30.77	30.77	30.79
19	20.70	20.72	20.71	20.72	20.72	20.71
20	115.24	115.24	115.31	115.24	115.22	115.26
21	142.69	142.91	142.69	142.71	142.72	142.71
22	161.98	161.99	161.99	161.99	162.00	161.99
23	98.98	99.02	99.01	98.99	99.02	99.01
24	171.11	171.12	171.12	171.12	171.11	171.12
25	8.58	8.59	8.60	8.59	8.59	8.60
26	58.83	58.85	58.82	58.83	58.83	58.83
27			55.44			
28			55.85			
29			112.82			
30			27.87			
31			28.36			

[a]Values in ppm, relative to $\delta_c = 0.00$ for TMS in $CDCl_3$ solutions.
[b]Assignments in vertical columns may be interchanged.

diastereoisomeric mixture in 6% yield from the oxidation mixture of **8**. These diastereoisomers were indistinguishable by nmr spectroscopy (Tables 4 and 5), but on silica nplc [hexane-iPrOH (96:4)] two distinguishable peaks in a 1:1 ratio were observed. Diastereoisomers **21** and **22** were not separated due to a paucity of material, and spectroscopic evidence for the proposed β,γ-epoxy-γ-lactone structure was obtained from a mixture of the two (8).

The ^{1}H- and ^{13}C-nmr chemical shifts (Tables 4 and 5) of the final fraction, isolated as 3% of the **8** oxidation mixture, when considered with the molecular formula of $C_{27}H_{47}O_7$ inferred from hrms, were consistent only with a hydroperoxy-methylacetal functionality. The solvent-assisted opening of the furan endoperoxide (Scheme 1) would lead to both diastereoisomers **23** and **24** (12), although the ^{1}H-nmr spectrum displayed one set of peaks only, consistent with the observations on other compounds in this series in which the remoteness of the chiral center at C-18 would have prevented the appearance of diastereoisomeric signals. The high resolution negative ion chemical ionization mass spectrum gave an ion corresponding to $[M - H_2O]^-$, consistent with the ready loss of H_2O that is common in peroxidic systems of this type (10). The regio-

specificity of the solvent-assisted opening of the endoperoxide to give **23** and **24** was concluded from observation of an nOe effect from H-1 to H-27 and from H-1 to H-2. The relative orientation of the methoxyl group with respect to the hydroperoxyl remains undefined. On mechanistic grounds formation of *trans* isomers would appear to be favored for steric reasons (13). However, it has been shown that addition of MeOH to this type of endoperoxide is entirely stereospecific with the product formed being the *cis* isomer. This is consistent with other results where the stereochemistry between the -OMe and -OOH groups has been determined (10,12). This type of hydroperoxyl system is known to be stable in some circumstances and has been shown to be formed from furan oxidations in MeOH (14).

It was concluded that the mechanism for the autooxidation of variabilin [**1**] was the same as that for the rose-bengal-sensitized oxidation of 22-*O*-methylvariabilin [**8**], i.e., sensitization of ground state triplet oxygen to oxygen in the first excited singlet state followed by a Diels-Alder type oxygen addition to the furan moiety, with the formation of two stereoisomeric furan endoperoxides (see Scheme 1) (5,6). These endoperoxides can then undergo solvent-assisted thermal rearrangement and/or solvent addition to give the observed products (5,6,8,10).

One would expect variabilin [**1**] to behave as did its methylated derivative **8** and to have been unreactive to dissolved oxygen in the absence of added sensitizer. However, because the same products were formed during the autooxidation of **1** as were formed from the rose-bengal-sensitized reaction of **1**, and these products were identical (after CH_2N_2 methylation) with those obtained from the rose-bengal-sensitized oxidation of **8**, then **1** must be acting as its own sensitizer and promoting the formation of singlet oxygen. To confirm that **1** was promoting the formation of singlet oxygen, the known singlet oxygen quencher, 2,6-di-*tert*-butyl-4-methylphenol (BHT) was added to the variabilin [**1**]/MeOH autooxidation solution, and the rate of oxidation was monitored by hplc measurement of the disappearance of **1** with time. BHT, which has a quenching rate of 4.0 mol per liter per sec in MeOH, was used in preference to more efficient quenchers such as β-carotene [quenching rate 1.2×10^4 mol per liter per sec in C_6H_6 (15)] because of the similar solubility properties of BHT and variabilin [**1**]. On addition of BHT at a ratio of 10:1 BHT/**1**, the rate was substantially decreased (Figure 1).

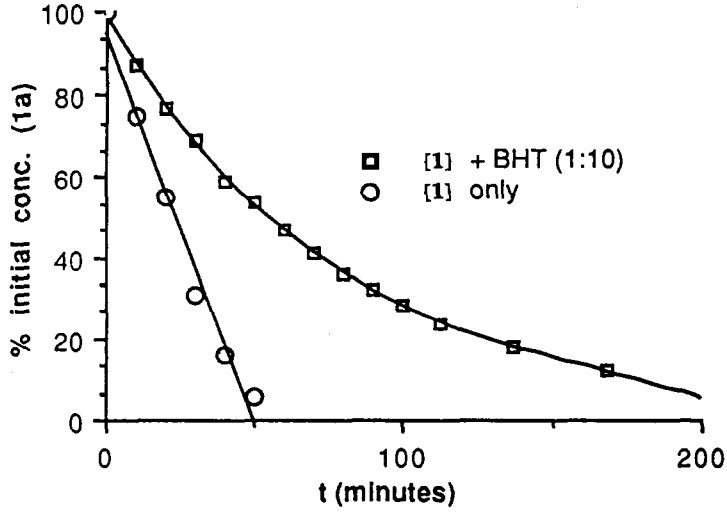


FIGURE 1. Autooxidation of variabilin [**1**] (3.0 mg) in presence and absence of added BHT in MeOH (0.6 ml).

This, along with a large rate increase on addition of rose bengal, confirmed the production of singlet oxygen and its participation in the oxidation of variabilin [**1**].

The ability of variabilin [**1**] and its oxidation products to act as sensitizers in the production of singlet oxygen was further confirmed by performing the autooxidation of a mixture of **1** and **8** in the absence of added sensitizer (Figure 2). The indistinguishable rates of oxidation for **1** and **8** in this reaction show that singlet oxygen, produced by photosensitization by **1**, has equal probability of reacting with the furan moiety of either **1** or **8**. The straight lines obtained for the plots of the concentrations of **1** and **8** vs. time show that the overall reaction rates are independent of the substrate concentrations and that the production of singlet oxygen is the rate determining step in the oxidation sequence.

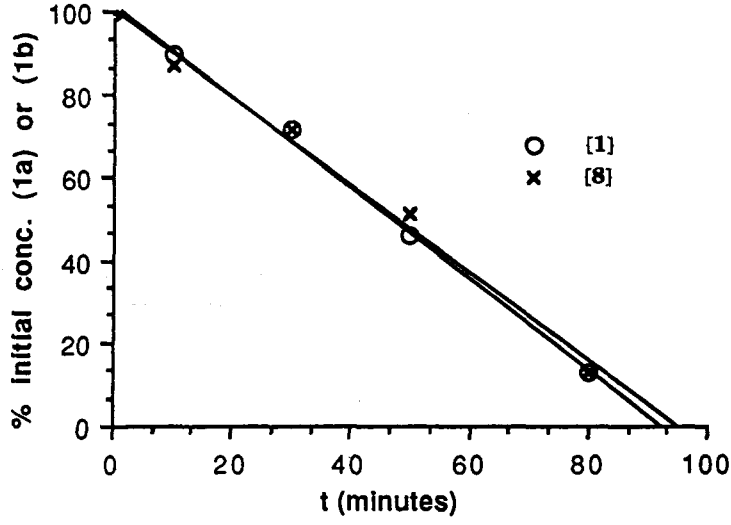


FIGURE 2. Autooxidation of a mixture of **1** (3.9 mg) and **8** (2.8 mg) in MeOH (1.3 ml).

The identical oxidation rates of variabilin [**1**] and its methylated derivative **8** in a mixture of **1** and **8** and in the absence of added sensitizer implies that electron transfer between the excited sensitizer **1** and ground state oxygen occurs away from the furan. This is consistent with the tetronic acid functionality being responsible for the production of singlet oxygen. This appears to be the first report of a tetronic acid moiety acting as a photosensitizer in the production of singlet oxygen. These findings emphasize the need for great care to be taken in the handling of tetronic-acid-containing natural products, so that oxidation products are not produced during isolation procedures.

Variabilin [**1**] and many of its derivatives were tested for antiviral activity using the BSC cell line growing in continuous culture and infected with either Herpes simplex Type 1 (HSV) or Polio Type 1 (PV1) viruses (3). This assay system gives a measure of the in vitro antiviral activity of test compounds or extracts, and the relative cytotoxicity of the test compounds is simultaneously evaluated (3). Variabilin [**1**] showed positive in vitro antiviral activity against both viruses (HSV and PV1), but this was accompanied by high cytotoxicity (Table 6). The biological activity of a C_{21} furanoterpene acid **25**, obtained as a natural product from the same *Sarcotragus* sponge (1), was comparable with that of variabilin [**1**] itself. In contrast, 22-*O*-methylvariabilin [**8**] was inactive at a 10-fold higher concentration. These results suggested that neither the furan nor the tetronic acid moieties per se were essential for activity in this series, leading to the conclusion that biological activity in this series is very dependent on the presence of

TABLE 6. Antiviral and Cytotoxic Activities of Variabilin [1] and Related Compounds.

Compound	PVI[a]	HSV[b]	Cytotoxicity[c]	Weight (μg/disk)
1	+++[d]	+++	+[d]	2
8	—	—	—	20
9,10	?[e]	?	+++	20
	++	?	++	2
11,12	++++	++++	++	20
	++	?	+	2
13,14	+++	+++	+	20
	—	—	—	2
15	—	—	—	20
16	—	—	—	20
25	++	?	++	5

[a]Polio vaccine virus type I.

[b]Herpes simplex virus type I.

[c]Cytotoxicity of the test compound against the BSC cell line.

[d]Zone of either inhibition of virus replication or of cytotoxicity: ++++, effect over whole well; +++, 4–5 mm excess radius from 6-mm disk in 16-mm well; ++, 2–4 mm; +, 1–2 mm.

[e]Antiviral effect not measurable because of BSC cell mortality.

terminal polar groupings such as hydroxyl or carboxyl functionalities. For example, the hydroxybutenolide pairs **9,10** and **11,12**, and the diol pair **13,14**, derived from the inactive 22-*O*-methylvariabilin [**8**], showed bioactivity indistinguishable from that shown by variabilin [**1**]. In contrast, the reduction products **15** and **16**, from the same hydroxybutenolides **9–12**, showed neither cytotoxicity nor antiviral activity in the BSC assay system (Table 6).

In all cases we have investigated, compounds with terminal polar groupings exhibited similar antiviral activity and cytotoxicity in the BSC assay. However, the antiviral activity of these compounds is very sensitive to assay conditions, and the cytotoxic effect produced often overwhelms any antiviral effect in both the in vitro and in vivo assay (3). The high cytotoxicity observed for **1** is not due to its sensitivity toward oxidation nor to its ability to produce singlet oxygen, as cytotoxicity is evident for both variabilin [**1**] and its oxygen-stable derivatives. This high cytotoxicity exhibited by variabilin [**1**] and the variabilin derivatives severely limits their potential usefulness as antiviral agents.

EXPERIMENTAL

GENERAL.—Ir and uv spectra were recorded on Shimadzu IR 27G and Varian DMS 100 spectrometers, respectively. All nmr spectra were recorded on a Varian XL300 instrument. Chemical ionization mass spectra were recorded on a Kratos MS 80 spectrometer. Nplc was performed on a Shimadzu LC4A series chromatograph using an Econosil silica column, while rplc was performed on a Varian 5000 liquid chromatograph using an Alltech C8 column, 250 × 10 mm. All solvents were spectral grade or distilled prior to use. The variabilin [**1**] used in this study was isolated as previously described (1,4).

AUTOOXIDATION OF VARIABILIN [**1**].—A solution of **1** (50 mg) in MeOH (1 ml), in an open Pyrex container, was irradiated using a 200 W incandescent lamp. The disappearance of **1** was monitored by rplc. When no starting material **1** remained, the reaction was halted by removal of the light source. Separation by semi-preparative rplc gave, as the major isolated fraction, the C-1 epimeric hydroxybutenolides **2** and **3** (10 mg, 20%): uv λ max (ε) (MeOH) 268 (24000), 261 nm (26000); ir ν max 3400, 2940, 1735, 1635, 1450, 1285, 1185, 1060, 1000, 920, 760 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2. The epimeric hydroxybutenolides **4** and **5** were isolated in slightly lower yield (7 mg, 15%): uv λ max (ε) (MeOH) 268 (22000), 261 nm (24000); ir ν max 3350, 2950, 1735, 1640, 1445, 1390, 1275, 1125, 950, 760 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2. A third fraction isolated was a mixture of the diols **6** and **7** (7 mg, 15%): uv λ max (ε) (MeOH) 268 (18000), 261 (19000); ir ν max 3400, 2930, 1640, 1440, 1285, 1180, 1080, 980, 760, 740 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2. The remaining 50% of the mixture

was resistant to separation, with decomposition occurring on both rplc and nplc. The compounds **2–7** also decomposed readily on standing.

The autooxidation of **1** was repeated using direct sunlight as the photon source. The time for complete disappearance of **1** varied from 30 min (midsummer) to 6 h (midwinter), correlating approximately with light intensity. The products were shown by rplc, nplc, and ^{1}H-nmr spectroscopy to be identical with those obtained above.

SENSITIZED OXIDATION OF 22-*O*-METHYLVARIABILIN [**8**].—A solution of 22-*O*-methylvariabilin [**8**] (66 mg) and rose bengal (1 mg) in MeOH (1 ml) was irradiated with a 200 W incandescent lamp, and the concentration of substrate was monitored as above. An initial separation of the oxidation mixture using semi-preparative silica nplc [hexane-iPrOH (90:10)] gave the isomeric diol mixture **13** and **14** as an oil (11 mg, 16%): uv λ max (ε) (MeOH) 286 (18000), 261 nm (19000); ir ν max 3450, 1130, 1090, 1060, 980, 760, 660 cm^{-1}; ^{13}C nmr see Table 2; ^{1}H nmr see Table 3; negative ion cims *m/z* [M]$^-$ 508.3062 ($C_{28}H_{44}O_8$ requires 508.3036), [M − Me]$^-$ 493.2833 ($C_{27}H_{41}O_8$ requires 493.2801).

The remaining mixture on semi-preparative silica nplc [hexane-iPrOH (96:4)] gave as the major component the C-1 epimeric hydroxybutenolides **9** and **10** as an oil (15.5 mg, 23%): uv λ max (ε) (MeOH) 268 (24000), 261 nm (26000); ir ν max 3380, 2940, 2880, 1750, 1630, 1455, 1400, 1360, 1300, 1220, 1140, 1080, 1010, 980, 920, 760, 650 cm^{-1}; ^{13}C nmr see Table 2; ^{1}H nmr see Table 3; negative ion cims *m/z* [M]$^-$ 444.2530 ($C_{26}H_{36}O_6$ requires 444.2512).

A second fraction contained the C-4 epimeric hydroxybutenolides **11** and **12** as an oil (10.5 mg, 16%); uv λ max (ε) (MeOH) 268 (22000), 261 nm (24000); ir ν max 3400, 2950, 2880, 1760, 1640, 1460, 1400, 1360, 1300, 1220, 1140, 1060, 980, 950, 760, 650 cm^{-1}; ^{13}C nmr see Table 2; ^{1}H nmr see Table 3; negative ion cims *m/z* [M]$^-$ 444.2524 ($C_{26}H_{36}O_6$ requires 444.2512), [M − Me]$^-$ 429.2252 ($C_{25}H_{33}O_6$ requires 429.2277).

Another compound isolated from this oxidation mixture was the epoxide **19** as an oil (8.5 mg, 13%): uv λ max (ε) (MeOH) 265 nm (19000); ir ν max 2950, 2880, 1800, 1760, 1460, 1390, 1360, 1300, 1220, 1135, 1060, 980, 850, 750, 680, 655 cm^{-1}; ^{13}C nmr see Table 2; ^{1}H nmr spectra see Table 3; [α]λ −60° (589 nm), −1360 (290), 0 (278), 3760 (253), 2720 (240) (*c* = 1.2, MeOH); negative ion cims *m/z* [M]$^-$ 444.2491 ($C_{26}H_{36}O_6$ requires 444.2512), [M − Me]$^-$ 429.2292 ($C_{25}H_{33}O_6$ requires 429.2277).

Isolated in a separate fraction was the diastereoisomeric epoxide **20** as an oil (8.5 mg, 13%): uv λ max (ε) (MeOH) 265 nm (18000); ir ν max 2950, 2880, 1800, 1760, 1640, 1460, 1390, 1360, 1300, 1220, 1135, 1060, 980, 850, 750, 680, 655 cm^{-1}; ^{1}H nmr see Table 4; ^{13}C nmr see Table 5; [α]λ −10° (589 nm), −7870 (290), −3200 (253) (*c* = 1.1, MeOH); negative ion cims *m/z* [M]$^-$ 444.2522 ($C_{26}H_{36}O_6$ requires 444.2512), [M − Me]$^-$ 429.2275 ($C_{25}H_{33}O_6$ requires 429.2277).

The epoxides **21** and **22** (4.0 mg, 6%) were isolated together and characterized as the diastereoisomeric mixture: uv λ max (ε) (MeOH) 268 nm (20000); ir ν max 2940, 2870, 1810, 1760, 1640, 1460, 1395, 1360, 1300, 1220, 1150, 1040, 975, 910, 850, 920, 750, 675, 650 cm^{-1}; ^{1}H nmr see Table 4; ^{13}C nmr see Table 5; negative ion cims *m/z* [M]$^-$ 444.2510 ($C_{26}H_{36}O_6$ requires 444.2512), [M − Me]$^-$ 429.2285 ($C_{25}H_{33}O_6$ requires 429.2277).

Also isolated were the hydroperoxides **23** and **24** as an oil (2.0 mg, 3%): uv λ max (ε) (MeOH) 262 nm (18000); ir ν max 3040, 2940, 2870, 1760, 1640, 1460, 1360, 1300, 1220, 1150, 1110, 1060, 1030, 980, 755, 675, 655 cm^{-1}; ^{1}H nmr see Table 4; ^{13}C nmr see Table 5; negative ion cims *m/z* [M − H_2O]$^-$ 458.2660 ($C_{27}H_{38}O_6$ requires 458.2669), [M − H_2O − Me]$^-$ 443.2310 ($C_{26}H_{35}O_6$ requires 443.2313).

METHYLATION OF THE VARIABILIN [**1**] AUTOOXIDATION MIXTURE.—The product mixture from the autooxidation of **1** (8 mg) was methylated with CH_2N_2 in Et_2O to give products identical with those from the rose-bengal-sensitized oxidation of 22-*O*-methylvariabilin [**8**], as shown by silica nplc and ^{1}H-nmr spectroscopy.

REDUCTION OF γ-HYDROXYBUTENOLIDES **9** AND **10**.—The mixture of γ-hydroxybutenolides **9** and **10** (10 mg) was reduced with excess $NaBH_4$ (15 mg) in MeOH (2 ml). The reaction was worked up as before to give the unsaturated-γ-lactone **15** as an oil (10 mg, 100%); uv λ max (ε) (MeOH) 268 (22000), 261 nm (21000); ir ν max 2940, 2870, 1750, 1640, 1450, 1395, 1360, 1300, 1220, 1125, 1060, 980, 830, 755, 650 cm^{-1}; ^{1}H nmr see Table 4; ^{13}C nmr see Table 5; negative ion cims *m/z* [M]$^-$ 428.2561 ($C_{26}H_{36}O_5$ requires 428.2563), [M − Me]$^-$ 413.2326 ($C_{25}H_{33}O_5$ requires 413.2329).

REDUCTION OF γ-HYDROXYBUTENOLIDES **11** AND **12**.—The mixture of γ-hydroxybutenolides **11** and **12** (4.5 mg) was reduced with excess NaBH (8 mg) in MeOH (1 ml), as above, to give the unsaturated-γ-lactone **16** as an oil (4 mg, 90%): uv λ max (ε) (MeOH) 268 (18000), 261 nm (19000); ir ν max 2940, 2870, 1750, 1635, 1455, 1395, 1360, 1300, 1220, 1170, 1140, 1060, 1035, 975, 880, 845,

750, 650 cm^{-1}; ^1H nmr see Table 4; ^{13}C nmr see Table 5; negative ion cims *m/z* $[M]^-$ 428.2571 ($C_{26}H_{36}O_5$ requires 428.2563), $[M-Me]^-$ 413.2333 ($C_{25}H_{33}O_5$ requires 413.2329).

ACETONIDE FORMATION FROM DIOLS **13** AND **14**.—Five drops of 1% perchloric acid/Me_2CO solution were added to a solution of the diol mixture **13** and **14** (5 mg) in Me_2CO (1 ml). After 10 minutes solid $NaHCO_3$ was added until the solution was basic to litmus. The solution was filtered and the solvent removed under reduced pressure. The residue was taken up in EtOAc and washed with $NaHCO_3$ (saturated) followed with H_2O. The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure to give acetonides **17** and **18** as an oil (4 mg, 80%): uv λ max (ε) (MeOH) 268 (18000), 262 nm (18000); ir ν max 2940, 2870, 1760, 1460, 1380, 1350, 1300, 1260, 1210, 1140, 1100, 1000, 975, 870, 800, 750, 730, 670, 650, 600, 510 cm^{-1}; ^1H nmr see Table 4; ^{13}C nmr see Table 5; negative ion cims *m/z* $[M]^-$ 548.3395 ($C_{31}H_{48}O_8$ requires 548.3349).

SENSITIZED OXIDATION OF VARIABILIN [**1**].—A solution of **1** (3.0 mg) and rose bengal (3.0 mg) in MeOH (0.6 ml) was irradiated with a 200 W incandescent lamp and the disappearance of **1** monitored by rplc. The oxidation of **1** was complete in approximately 3 min.

AUTOOXIDATION OF VARIABILIN [**1**] IN THE PRESENCE OF BHT.—A solution of **1** (3.0 mg) in MeOH (0.6 ml) was irradiated with a 200 W incandescent lamp in the presence of BHT (30 mg) [**1**-BHT (1:10 by weight)], and the reaction was monitored as above (Figure 1).

A solution of **1** (3.0 mg) in MeOH (0.6 ml) with no added BHT was irradiated and the substrate concentration monitored as above (Figure 1).

AUTOOXIDATION OF THE VARIABILIN [**1**]/22-*O*-METHYLVARIABILIN [**8**] MIXTURE.—A mixture of **8** (2.8 mg) and **1** (3.9 mg) in MeOH (1.3 ml) was irradiated with a 200 W incandescent lamp and substrate concentrations monitored as above (Figure 2).

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