Registry No. 7, 129757-53-5; 8, 129830-34-8; **9aa**, 127222-03-1; **9ba**, 127221-99-2; **9cb**, 129757-50-2; **9da**, 127222-04-2; **10bb**, 129757-51-3; **11aa**, 129757-52-4; **11ba**, 129757-54-6; **11bb**, 129757-56-8; **11cb**, 129757-55-7; (*R*)-**14a**, 129757-61-5; (*R*)-**14b**, 129757-59-1; (*S*)-**14b**, 129757-63-7; (*S*)-**14c**, 129757-62-6; **15**, 129757-57-9; **16**, 129757-58-0; SAMEMP hydrazine, 129757-60-4; $Ph(CH_2)_3Ph$, 1081-75-0.

New Acetylenic Alcohols from the Sponge *Cribrochalina vasculum*

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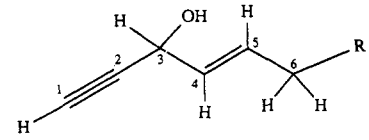
In our continuing search for biologically active compounds from marine organisms, we have isolated five new acetylenic alcohols from a sponge *Cribrochaline vasculum* collected in Belize. These terminal mono acetylenic compounds are simpler in structure and differ from the previously reported polyacetylenic compounds from sponges belonging to genera *Cribrochaline*,[1] *Siphonochaline*,[2] *Petrosia*,[3,4] and *Xestospongia*.[5] We report here their isolation and structure determination.

The methanol–toluene extract of the sponge was partitioned between ethyl acetate and water. The ethyl acetate soluble fraction on chromatography over SiO_2 gel followed by reverse-phase HPLC gave five compounds. These compounds, **1–5**, showed in vitro immunosuppressive activity on MLR assays[6,7] and in vitro antitumor activity against the mouse leukemia P388 cell lines.[8] The molecular formulas for these compounds were established by high-resolution mass spectrometry.

Compound **1**, $C_{20}H_{36}O$, showed infrared absorptions indicative of a terminal alkyne (3295 cm^{-1}) and a hydroxyl group (3590 cm^{-1}). LRFDMS indicated ready loss of 17 mass units (OH group) to give the base peak ion. Homonuclear decoupling experiments and a two-dimensional COSY NMR spectrum identified the spin system in the partial structure A. A long-range coupling of 2.0 Hz was observed between the acetylenic proton at δ 2.54 and the proton attached to the carbon bearing oxygen at δ 4.81. The latter proton was in turn coupled to the two olefinic protons observed at δ 5.59 and 5.89 with coupling constants of 6.1 and 1.4 Hz, respectively. The olefinic protons observed at δ 5.59 and 5.89 are coupled to each other with a coupling constant of 15.3 Hz and to the allylic methylene protons at δ 2.04 with coupling constants of 1.6 and 6.7 Hz, respectively. *E* geometry was assigned to the olefinic double bond based upon the 15.3-Hz vicinal coupling constant. This partial structure A had been reported previously in several metabolites isolated from other sponges.[1,3,5] The compound **1** has only one A unit in contrast to other known compounds which possess two or more of this unit.

The ^1H NMR spectrum further indicated a broad signal (δ 1.33–1.39) integrating to 13 or 14 methylene units and a methyl triplet (δ 0.85, J = 6.5 Hz) for the presense of an alkyl chain in the molecule. The presence of signals equivalent to 14 methylene carbons and a high-field signal for a methyl carbon in the inverse gated[9] ^{13}C spectrum confirmed the presence of a C_{15} alkyl chain. Combination of the above data established the structure **1** for the compound. The structure was confirmed by NMR and mass spectral comparison with a synthetic sample of (±)-3-hydroxyeicos-4(*E*)-en-1-yne.[10] A trace of the next higher homologue was detected in the HREI mass spectrum.

Compounds **2–5** were found to contain the same structural unit A (see the Experimental Section) and differ only in the nature of the alkane chain.



A

1, R = $(CH_2)_{13}$-CH_3

2, R = $(CH_2)_8$-^{15}CH=^{E}CH-$(CH_2)_5$-CH_3

3, R = $(CH_2)_9$-$^{16}CH(CH_3)$-$(CH_2)_3$-$^{20}CH_3$

4, R = $(CH_2)_{12}$-$^{19}CH(CH_3)_2$

5, R = $(CH_2)_8$-^{15}CH=^{E}CH-$(CH_2)_4$-$^{21}CH(CH_3)_2$

Compound **2** has molecular formula $C_{22}H_{38}O$. Comparison with **1** indicated the presence of an additional C_2H_2 unit in **2**. The ^1H NMR spectrum showed in the upper field region a methyl triplet at δ 0.85 and methylene signals around δ 1.3 integrating for 22 protons. Allylic signal integrating for four additional protons in comparison to these of **1** at δ 2.01 and an additional olefinic signal integrating for two protons at δ 5.32 confirmed the presence of an isolated double bond in the alkyl chain. Periodate–permanganate oxidation[11] of **2** at room temperature, followed by methylation of the mixture with CH_2N_2, gave a mixture of products. The mixture was separated, and the major product was identified by ^1H NMR as $(CH_2)_9(COOCH_3)_2$. The position of the isolated double bond was confirmed by the mass spectrum of the hydroxydimethylamino derivative[12] obtained by peroxidation of the double bond with *m*-chlorobenzoic acid followed by heating the epoxide solution in $(CH_3)_2NH$ in a sealed tube. The strongest fragment obtained in the mass spectrum, the ammonium ion $CH_3(CH_2)_5CH{=}N^+(CH_3)_2$ (m/z 142), determined unequivocally the position of the isolated double bond. The geometry of the isolated double bond could not be determined by ^1H NMR, as the two olefinic signals appeared at the same chemical shift value. The assignment of the trans configuration of the isolated double bond was based on the absence of any IR absorptions between 730 and 675 cm^{-1}.

Compound **3**, with the molecular formula $C_{21}H_{38}O$, is the next higher homologue of compound **1**. The ^1H NMR spectrum of **3** was identical with that of **1**, except that the higher field region indicated in addition to the terminal

(1) Wright, A. E.; McConnell, O. J.; Kohmoto, S.; Lui, M. S.; Thompson, W.; Snader, K. M. *Tetrahedron Lett.* **1987**, *28*, 1377–1380.
(2) Rotem, M.; Kashman, Y. *Tetrahedron Lett.* **1979**, 3193–3196.
(3) Cimino, G.; De Giulio, A.; De Rosa, S.; De Stefano, S.; Sodano, G.; *J. Nat. Prod.* **1985**, *48*, 22–27.
(4) Fusetani, N.; Kato, Y.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1983**, *24*, 2771–2774.
(5) Quinoa, E.; Crews, P. *Tetrahedron Lett.* **1989**, *29*, 2037–2040.
(6) Montgomery, D. W.; Zkoski, C. F. *Transplantation* **1985**, *40*, 49–56.
(7) Gunasekera, S. P.; Cranick, S.; Longley, R. E. *J. Nat. Prod.* **1989**, *52*, 757.
(8) Compounds **1–5** had in vitro activity against murine P388 IC_{50} 1.0, 1.3, 1.1, 0.2, and 0.1 μg/mL, respectively.
(9) Derome, A. E. *Modern NMR Techniques for Chemistry Research*; Pergamon: New York, 1987; p 169.
(10) Gunawardana, G. P.; Saucy, G. et al. Manuscript in preparation.
(11) Lemieux, R. U.; von Rudloff, E. *Can. J. Chem.* **1955**, *34*, 1413.
(12) Budzikiewicz, H.; Djerassi, C.; Williams, D. H. *Structure Elucidation of Natural Products by Mass Spectrometry*; Holden-Day: San Francisco, 1964; Vol. 2.

0022-3263/90/1955-6223$02.50/0

Table I. Immunosuppressive Activity[a]

compd	dose, μg/well	% MLR[b]	% LCV[c]	% suppression
1	0.1	0	61	100
	0.01	24	80	76
2	0.1	0	34	100
	0.01	33	67	67
3	0.1	0	42	100
	0.01	21	78	79
4	0.1	0	49	100
	0.01	21	82	79
5	0.1	0	45	100
	0.01	20	67	33

[a] For details see ref 7. [b] Percent of the positive (no drug) MLR control. [c] Percent of the positive (no drug) LCV control.

methyl triplet another methyl doublet at δ 0.81 for a secondary methyl group. ^{13}C NMR spectrum was similar to that of 1, and in the APT spectrum[13] one higher field signal at δ 32.72 appeared as a methine carbon, confirming the branching in the alkyl chain. The HREI mass spectrum showed the usual fragmentation pattern of a branched alkyl chain[14] and gave a strong peak at m/z 85 (85.1020, Δ 0.3 mmu for C_6C_{13}) followed by the base peak at m/z 84 (84.0935, Δ 0.4 mmu for C_6C_{12}), confirming the presence of a $CH(CH_3)(CH_2)_3CH_3$ fragment in the molecule.

Compound 4, with the molecular formula $C_{21}H_{38}O$, indicated that it is an isomer of compound 3. The ^1H NMR spectrum was similar to that of 1, but the upper field region gave a doublet (δ 0.84, J = 6.4 Hz) integrating for six protons, indicating the presence of a terminal isopropyl group. The ^{13}C NMR spectrum showed a signal at δ 22.65 for two methyl carbon atoms and a signal at δ 27.95 for a methine carbon, which confirmed the presence of the terminal isopropyl group in the alkyl chain.

Compound 5, with the molecular formula $C_{22}H_{40}O$ indicated that it is the higher homologue of compound 2. The ^1H NMR spectra of 5 and 2 were similar except in the upper field region. The ^1H NMR spectrum of 5 showed a doublet (δ 0.84, J = 6.3, Hz) integrating for six protons. The ^{13}C NMR spectrum showed a signal at δ 22.62 for two methyl carbons and a signal at δ 27.95 for a methine carbon. These data confirmed the presence of a terminal isopropyl group in the molecule. The position of the isolated double bond was determined by the mass spectrum of the hydroxydimethylamino derivative[12] prepared as for compound 2. The strongest fragment obtained in the mass spectrum, the ammonium ion $(CH_3)_2CH(CH_2)_4CH{=}N^+(CH_3)_2$ (HREIMS m/z 156.17483, Δ 0.4 mmu for $C_{10}H_{22}N$), determined unequivocally the position of the isolated double bond. The assignment of the trans configuration of the isolated double bond as in 2 was based on the absence of IR absorptions between 730 and 675 cm^{-1}.

This is the first report of aliphatic, branched acetylenic compounds isolated from a marine source. The immunosuppressive effect of the isolates 1–5 on the murine mixed lymphocyte response is given in Table I.

Experimental Section

Chemical shifts are reported relative to $CDCl_3$ (δ 7.24 for ^1H, 77.0 for ^{13}C). ^{13}C multiplicities were determined by APT experiments.

Extraction and Isolation Procedure. The marine sponge *Cribrochalina vasculum* was collected by scuba at Glover reef, Belize, at a depth of 13 m. The freshly thawed sponge (197 g, wet wt) was extracted twice with methanol–toluene (3:1), and the concentrated extract was partitioned between ethyl acetate and water. The ethyl acetate soluble fraction (128 mg) was chromatographed over SiO_2 gel and monitored by P388 assay. The biologically active fraction (33 mg) was further separated by RP-HPLC (C-18, 5 μm, 250 × 10 mm, 7% H_2O–MeOH) to yield five related compounds 1–5. These compounds appeared as a gum, and the UV spectra in MeOH gave λ_{max} 202 nm (ε 1400).

3-Hydroxyeicos-4(*E*)-en-1-yne (1): 0.8 mg; $[\alpha]^{25}_D$ 3.8° (c = 0.9, MeOH); IR ($CHCl_3$), 3590 and 3295 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (3 H, t, J = 6.5 Hz, 20-H), 1.33–1.39 (26 H, m, 7–19-H), 1.84 (1 H, d, J = 5.7 Hz, 3-OH), 2.04 (2 H, dt, J = 7.0, 6.7 Hz, 6-H), 2.54 (1 H, d, J = 2.0 Hz, 1-H), 4.81 (1 H, dddd, J = 6.1, 5.7, 2.0, 1.4 Hz, 3-H), 5.59 (1 H, ddt, J = 15.3, 6.1, 1.6 Hz, 4-H), 5.89 (1 H, ddt, J = 15.3, 1.4, 6.7 Hz, 5-H); ^{13}C NMR ($CDCl_3$) δ 14.10 (C, q), 22.68 (C, t), 28.81 (C, t), 29.17 (C, t), 29.36 (C, t), 29.45 (C, t), 29.57 (C, t), 29.67 (7C, t), 31.93 (C, t), 62.81 (C, d), 73.93 (C, d), 83.34 (C, s), 128.31 (C, d), 134.62 (C, d); HREIMS m/z 292.2757, Δ 0.9 mmu for $C_{23}H_{36}O$; LRFDMS m/z (relative intensity) 292 (60), 275 (100), 267 (33), 197 (5), 190 (6), 132 (8).

3-Hydroxydocosa-4(*E*),15(*E*)-dien-1-yne (2): 3.7 mg; $[\alpha]^{25}_D$ 4.9° (c = 4.5, MeOH); IR ($CHCl_3$) 3590 and 3290 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (3 H, t, J = 6.5 Hz, 22-H), 1.20–1.39 (22 H, CH_2 groups), 1.88 (1 H, d, J = 5.7 Hz, 3-OH), 2.01 (6 H, m, 6,14,17-H), 2.54 (1 H, d, J = 2.0 Hz, 1-H), 4.81 (1 H, ddd, J = 6.1, 5.7, 2.0 Hz, 3-H), 5.32 (2 H, m, 15,16-H), 5.59 (1 H, ddt, J = 15.3, 6.1, 1.6 Hz, 4-H), 5.89 (1 H, ddt, J = 15.3, 1.4, 6.7 Hz, 5-H); ^{13}C NMR ($CDCl_3$) δ 14.07 (C, q), 22.62 (C, t), 27.19, (2 C, t), 28.81 (C, t), 28.96 (C, t), 29.16 (C, t), 29.27 (C, t), 29.42 (C, t), 29.51 (2 C, t), 29.73 (2 C, t), 31.75 (C, t), 31.90 (C, t), 62.78 (C, d), 73.90 (C, d), 83.34 (C, s), 128.35 (C, d), 129.85 (C, d), 129.91 (C, d), 134.56 (C, d); HRFABMS (mb) m/z 319.3003, Δ 0.2 mmu for $C_{22}H_{39}O$ (M^+ + 1); LRFDMS m/z 318 (27), 301 (100), 300 (90), 292 (24), 200 (9), 150 (21).

3-Hydroxy-16-methyleicos-4(*E*)-en-1-yne (3): 3.3 mg; $[\alpha]^{25}_D$ 1.4° (c = 5.6, MeOH); IR ($CHCl_3$) 3575 and 3290 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81 (3 H, d, J = 6.4 Hz, 21-H), 0.86 (3 H, t, J = 6.5 Hz, 20-H), 1.04 (2 H, m, CH_2), 1.2–1.4 (23 H, m, CH and CH_2 groups), 1.84 (1 H, d, J = 4.9 Hz, 3-OH), 2.04 (2 H, ddt, J = 7.1, 1.6, 6.8 Hz, 6-H), 2.53 (1 H, d, J = 2.0 Hz, 1-H), 4.81 (1 H, dddd, J = 6.1, 4.9, 2.0, 1.4 Hz, 3-H), 5.58 (1 H, ddt, J = 15.3, 6.1, 1.6 Hz, 4-H), 5.89 (1 H, ddt, J = 15.3, 1.4, 6.8 Hz, 5-H); ^{13}C NMR ($CDCl_3$) δ 14.09 (C, q), 19.68 (C, q), 22.67 (C, t), 27.02 (C, t), 27.04 (C, t), 28.81 (C, t), 29.17 (C, t), 29.45 (C, t), 29.59 (C, t), 29.67 (C, t), 29.97 (C, t), 31.91 (C, t), 31.93 (C, t), 32.72 (C, d), 37.06 (C, t), 37.07 (C, t), 62.75 (C, d), 73.90 (C, d), 83.34 (C, s), 128.35 (C, d), 134.54 (C, d); HRFABMS (mb) m/z 307.3008, Δ 0.7 mmu for $C_{21}H_{39}O$, (M^+ + 1); LRFDMS m/z 306 (55), 289 (95), 287 (38), 281 (22), 280 (22), 153 (10), 85 (32), 84 (100).

3-Hydroxy-19-methyleicos-4(*E*)-en-1-yne (4): 2.2 mg; $[\alpha]^{25}_D$ 2.6° (c = 1.0, MeOH); IR (neat) 3270 and 2922 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84 (6 H, d, J = 6.7 Hz, 20,21-H), 1.10–1.34 (25 H, m, 7–19-H) 1.76 (1 H, d, J = 6.1 Hz, 3-OH), 2.04 (2 H, ddt, J = 7.1, 1.6, 6.8 Hz, 6-H), 2.54 (1 H, d, J = 2.0 Hz, 1-H), 4.81 (1 H, dddd, J = 6.1, 6.1, 2.0. 1.4 Hz, 3-H), 5.58 (1 H, ddt, J = 15.3, 6.1, 1.6 Hz, 4-H), 5.90 (1 H, ddt, J - 15.3, 1.4, 6.8 Hz, 5-H); ^{13}C NMR ($CDCl_3$) δ 22.65 (2 C, q), 27.09 (C, t), 27.39 (C, t), 27.95 (C, d), 28.81 (C, t), 29.17 (C, t), 29.45 (C, t), 29.57 (C, t), 29.66 (3 C, t), 29.92 (C, t), 30.00 (C, t), 31.90 (C, t), 39.04 (C, t), 62.78 (C, d), 73.90 (C, d), 83.34 (C, s), 128.34 (C, d), 134.62 (C, d); HREIMS m/z 306.2914, Δ 0.8 mmu for $C_{21}H_{38}O$; LRFDMS m/z 306 (100), 291 (27), 288 (22), 280 (30), 277 (32), 263 (65).

3-Hydroxy-21-methyldocosa-4(*E*),15(*E*)-dien-1-yne (5): 1.7 mg; $[\alpha]^{25}_D$ 2.0° (c = 0.01, MeOH); IR (neat) 3318 and 2930 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84 (6 H, d, J = 6.3 Hz, 22,23-H), 1.14–1.39 (21 H, m, 7–13- and 18–21-H) 1.82 (1 H, d, J = 5.3 Hz, 3–OH), 2.03 (6 H, m, 6,14,17-H), 2.54 (1 H, d, J = 2.0 Hz, 1-H), 4.81 (1 H, dddd, J = 6.1, 5.3, 2.0, 1.4 Hz, 3-H), 5.32 (2 H, m, 15,16-H), 5.58 (1 H, ddt, J = 15.3, 6.1, 1.6 Hz, 4-H), 5.89 (1 H, ddt, J = 15.3, 1.4, 6.7 Hz, 5-H); ^{13}C NMR ($CDCl_3$) δ 22.62 (2 C, t), 27.06 (C, t), 27.19 (C, t), 27.95 (C, d), 28.80 (C, t), 29.27 (C, t), 29.44 (2 C, t), 29.51 (C, t), 29.66 (C, t), 29.75 (C, t), 30.00 (C, t), 31.91 (C, t), 38.88 (C, t), 62.79 (C, d), 73.92 (C, d), 83.24 (C, s), 128.34 (C, d), 129.87 (2 C, d), 134.59 (C, d); HREIMS m/z 332.3071, Δ 0.8 mmu for $C_{23}H_{40}O$; LRFDMS m/z 332 (44), 315 (100), 314 (84), 307 (9), 306 (13).

(13) Patt, S. L.; Shoolery, J. N. *J. Magn. Reson.* **1982**, *46*, 535–539.
(14) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 4th ed.; John Wiley and Sons: New York, 1981; p 17.

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Registry No. **1**, 129364-93-8; **2**, 129364-94-9; **3**, 129364-95-0; **4**, 129364-96-1; **5**, 129364-97-2.

Supplementary Material Available: ^{13}C NMR spectra of compounds **1–5** and APT spectrum of compound **3** (6 pages). Ordering information is given on any current masthead page.

Naphthyl Dioxetane Phosphates: Synthesis of Novel Substrates for Enzymatic Chemiluminescent Assays

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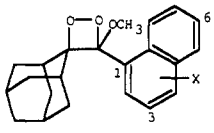
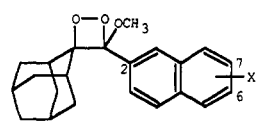
We wish to report the first synthesis of enzyme-cleavable naphthyl 1,2-dioxetane phosphates **2a–d** designed for use in bioassay systems. Although several literature accounts



2a: X=3-OPO3Na2
2b: X=6-OPO3Na2

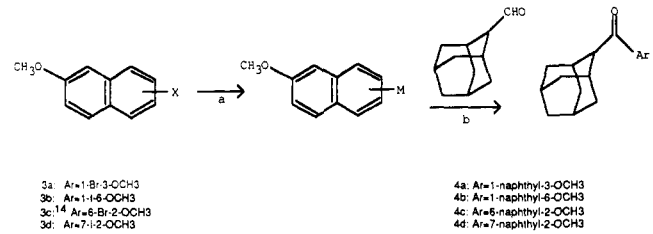


2c: X=6-OPO3Na2
2d: X=7-OPO3Na2

give chemiluminescent properties of related phenyl and xanthenyl dioxetane phosphates, at this time no corresponding syntheses have been described.[1,2] These compounds, unlike luminol, acridinium esters, and other common chemiluminescent systems,[3] operate as a direct light source in the presence of alkaline phosphatase, requiring no additional reagents for generating chemiluminescence. Recent work in our laboratory, using a similar phenyl 1,2-dioxetane phosphate (disodium 3-(4-methoxyspiro-[1,2-dioxetane-3,2′-tricyclo[3.3.1.1^{3,7}]decan]-4-yl)phenyl phosphate, AMPPD), demonstrates that incorporating these substrates into enzyme-linked immunoassays or DNA probe protocols generates a chemiluminescent signal proportional to the concentration of an alkaline phosphatase label. Amplification of the signal by efficient enzyme turnover provides very high detection sensitivity.[4] Such sensitivity offers an attractive alternative to radioisotopic methods.

A general synthesis for aryl 1,2-dioxetane phosphates must accommodate limitations in synthetic methods imposed by the inherent instability of a peroxidic bond confined to a small, four-membered ring. Consequently, it is desirable to introduce the labile dioxetane function late in the synthesis, preferably as the last synthetic step. The key intermediate, from which the dioxetane will be generated, depends on the choice of dioxetane synthesis. Dioxetanes are commonly prepared by one of two routes. The Kopecky method, in which β-bromo hydroperoxides are cyclized,[5] is limited to small-scale synthesis since the use of concentrated hydrogen peroxide is required and decomposition of the unstable hydroperoxide intermediates may occur during purification even when low temperatures are used. An alternative method depends on oxygenation of electron-rich olefins with singlet oxygen produced by photosensitization or by nonphotochemical reactions.[6] One disadvantage of the singlet oxygen approach is that other oxidative processes may compete, such as ene reactions or 2 + 4 cycloadditions. For our synthesis, we chose to photooxygenate electron-rich enol ether

Scheme I[a]

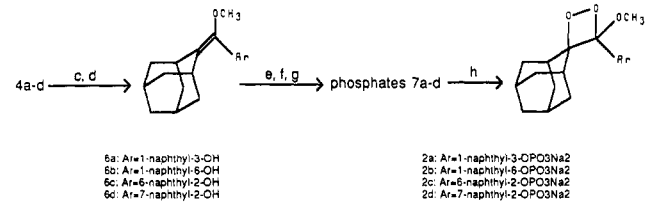


3a: Ar=1-Br-3-OCH3
3b: Ar=1-I-6-OCH3
3c:[14] Ar=6-Br-2-OCH3
3d: Ar=7-I-2-OCH3

4a: Ar=1-naphthyl-3-OCH3
4b: Ar=1-naphthyl-6-OCH3
4c: Ar=6-naphthyl-2-OCH3
4d: Ar=7-naphthyl-2-OCH3

[a] (a) nBuLi, Et_2O; (b) H^+ workup, Jones oxidation.

Scheme II[a]



5a: Ar=1-naphthyl-3-OH
5b: Ar=1-naphthyl-6-OH
5c: Ar=6-naphthyl-2-OH
5d: Ar=7-naphthyl-2-OH

2a: Ar=1-naphthyl-3-OPO3Na2
2b: Ar=1-naphthyl-6-OPO3Na2
2c: Ar=6-naphthyl-2-OPO3Na2
2d: Ar=7-naphthyl-2-OPO3Na2

[a] (c) tBuOK, $(MeO)_2SO_2$, DMSO; (d) NaSEt, DMF, reflux; (e) NaH, 2-chloro-2-oxo-1,3,2-dioxaphospholane, DMF; (f) NaCN, DMF; (g) 7 M NH_4OH, Na_2CO_3; (h) $^1O_2$, TPP, $CHCl_3$, 10 °C.

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(1) Bronstein, I.; Edwards, B.; Voyta, J. *J. Biolumin. Chemilumin.* **1988**, *2*, 186. Voyta, J.; Edwards, B.; Bronstein, I. *Clin. Chem.* **1988**, *34*, 1157. Bronstein, I.; Edwards, B.; Voyta, J. *Proc. Int. Symp. Biolumin. Chemilumin., 5th* **1989**, 99–111. Schaap, A. P. *J. Biolumin. Chemilumin.* **1988**, *2*, 253.

(2) A. P. Schaap has reported photooxygenation of an aryl enol ether acetate to the corresponding 1,2-dioxetane acetate (see compound **1c**, 6-acetoxy(methoxytricyclo[3.3.1.1^{3,7}]dec-2-ylidenemethyl)-2-naphthalene in *Tetrahedron Lett.* **1987**, *28*, 935–938). Schaap also reported a similar photooxygenation of an aryl alkene phosphate to the related 1,2-dioxetane phosphate (see compound **1**, pyridinium 3-(tricyclo[3.3.1.1^{3,7}]dec-2-ylidenemethyl)-9,9′-xanthenyl phosphate in *Tetrahedron Lett.* **1987**, *28*, 1159–1162). After an extensive search, we have not found any literature examples of photooxygenation of aryl enol ether phosphates to 1,2-dioxetane phosphates or syntheses of the aryl enol ether acetate and aryl alkene phosphate.

(3) McCapra, F.; Gundermann, K.-D. *Chemiluminescence in Organic Chemistry*; Hafner, K., et al., Eds.: Springer-Verlag: New York, 1987; Vol. 23. *Bioluminescence and Chemiluminescence: Basic Chemistry and Analytical Applications*; DeLuca, M. A., McElroy, W. D., Eds.; Academic Press: New York, 1981. DeLuca, M. A.; McElroy, W. D. *Bioluminescence and Chemiluminescence, Methods in Enzymology*; Colowick, S. P., Kaplan, N. O., Eds., Academic Press: New York, 1986; Vol. 133, pp 331–569. Kricka, L. J. *Ligand Binder Assays*; Marcel Dekker: New York, 1985, Chapter 7, pp 199–216.

(4) Bronstein, I.; Voyta, J.; Thorpe, G.; Kricka, L. *Clin. Chem.* **1989**, *35*, 1441–1446. Bronstein, I.; Voyta, J. *Ibid.* **1989**, *35*, 1856–1857. Thorpe, G. H. G.; Bronstein, I.; Kricka, L. J.; Edwards, B.; Voyta, J. C. *Ibid.* **1989**, *35*, 2319–2321. Bronstein, I.; Voyta, J.; Edwards, B. *Anal. Biochem.* **1989**, *180*, 95. Clyne, J. M.; Running, J. A.; Stempien, M.; Stephens, R. S.; Akhavan-Tafti, H.; Schaap, A. P.; Urdea, M. S. *Proc. Int. Symp. Biolumin. Chemilumin., 5th* **1989**, 357–366. Schaap, A. P.; Pollard-Knight, D.; Simmons, A. *J. Cell Biochem.* **1989**, *130*, 34. Schaap, A. P.; Akhavan-Tafti, H.; Desilva, R. *Clin. Chem.* **1989**, *35*, 1201.

(5) Kopecky, K. R.; Mumford, C. *Can. J. Chem.* **1969**, *47*, 709. Kopecky, K. R.; Filby, J. E.; Mumford, C.; Lockwood, P. A.; Ding, J. Y. *Can. J. Chem.* **1975**, *53*, 1103.

(6) Bartlett, P. D.; Schaap, A. P. *J. Am. Chem. Soc.* **1970**, *92*, 3223. Bartlett, P. D.; Schaap, A. P. *Ibid.* **1970**, *92*, 6055. Adam, W. "Four-membered Ring Peroxides: 1,2-Dioxetanes and α-Peroxylactones" In The Chemistry of Functional Groups, Peroxides; Patai, S., Ed.; John Wiley & Son: New York, 1983; pp 849–850.

0022-3263/90/1955-6225$02.50/0