

A NEW OXIDATIVE REARRANGEMENT OF A PHORBOL DERIVATIVE

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ABSTRACT.—Alkaline degradation of phorbol 20-trityl ether in the presence of oxygen gave the *A-seco*-3,9-lactone **2**, presumably the result of the fragmentation and rearrangement of an intermediate 2-hydroperoxide.

Phorbol esters are valuable probes for the investigation of cellular and biochemical events associated with the activation of protein kinase C (1). Furthermore, certain derivatives of phorbol bind to the vanilloid receptor (2), and 12-deoxyphorbol-13-acetate (prostratin) is a potential anti-HIV lead (3). Phorbol derivatives have also attracted much attention in the chemical community. Seminal contributions to its chemistry have been reported (4), and the total synthesis of phorbol has been achieved (5,6). However, some aspects of the chemistry of phorbol still await clarification. Indeed, it has been known for a long time that phorbol derivatives are sensitive to air oxidation, especially in basic media (4). Nothing is known, however, about the structure of the product(s) formed during this degradation or whether this reaction can be exploited to obtain more oxygenated derivatives in synthetically useful yields. We present here the structure of the major product formed during the atmospheric oxidation of alkaline solutions of phorbol 20-trityl (=triphenylmethyl) ether, a reaction that can serve as a model for the degradation of phorbol itself.

As part of an investigation on diterpenoid ligands for the vanilloid receptor (7), we needed large amounts of 4 α -phorbol 20-trityl ether [**1a**], and planned to prepare it from phorbol 20-trityl ether [**1b**] (8). Compound **1b** is soluble in organic solvents, and is easier

to handle and more stable than the H₂O-soluble parent compound. The retroaldol isomerization of phorbol is shifted toward the formation of the 4 α -isomer (9), but the 20-trityl ether gave instead an equilibrium mixture containing comparable amounts of both epimers. Furthermore, the reaction was plagued by the formation of variable amounts (traces→10%) of the less polar compound **2**. The mass spectrum of **2** showed that this compound was not one of the minor compounds formed during the base-induced epimerization of phorbol (4), because the mol wt was 14 mass units higher than the starting material (*m/z* 620 vs. *m/z* 606). This suggested the presence of an extra oxygen atom and of a further unsaturation. Compound **2** was obtained as the major reaction product (yield 30%) when the isomerization was carried out under an oxygen atmosphere, confirming that **2** is an oxidation product of **1b**.

Comparison of the ¹H-nmr spectra of **1b** and **2** showed the absence of the H-10 signal in **2**, and an identical multiplicity pattern for the remaining resonances. Remarkable differences in the chemical shifts of H-1 (δ 6.49 in **2** vs. 7.54 in **1b**) and the C-2 methyl (δ 2.29 in **2** vs. 1.78 in **1b**) were observed. The ¹³C-nmr spectrum of **2** showed changes of all the ring A carbons; an enone system was still evident (δ 197.7 s, 121.2 d, 154.1 s), but the appearance of a novel carbonyl resonance at δ 176.2 required a different

TABLE 1. ^1H - and ^{13}C -Nmr Data of **2** (400 and 100 MHz, respectively, CDCl_3 , TMS as reference).

Position	[mult., J (Hz)]	Long-range correlations (HMBC spectrum)	Position	δ (mult.)
H-1	6.49 s	C-4, C-10, C-2, C-9	C-1	121.2 d
			C-2	197.7 s
H-5a	2.70 br d (16.6)	C-4, C-6, C-7, C-10	C-3	176.2 s
H-5b	2.44 br d (16.6)	C-4, C-6, C-7, C-3	C-4	75.0 s
			C-5	45.7 t
H-7	5.35 br s		C-6	127.3 s
H-8	3.00 br s	C-6, C-7	C-7	127.1 s
			C-8	40.0 d
H-11	2.55 d q (9.5, 6.2)	C-12	C-9	92.4 s
H-12	4.13 d (9.5)	C-18, C-15, C-11, C-13	C-10	154.1 s
			C-11	37.9 d
H-14	0.73 d (4.4)	C-16, C-7	C-12	78.7 d
			C-13	63.2 s
H-16	1.31 s	C-17, C-15, C-14, C-13	C-14	36.8 d
H-17	1.42 s	C-16, C-15, C-14, C-13	C-15	26.6 s
H-18	0.89 d (6.2)	C-11, C-12, C-9	C-16	23.6 q
H-19	2.29 s	C-2	C-17	16.5 q
H-20a,b	3.52 br s	C-6, C-7	C-18	12.8 q
Trityl			C-19	31.5 q
			C-20	70.9 t
ortho	7.40 d (7.4)		Trityl	87.0 s
meta	7.29 t (7.4)		ipso	143.8 s
para	7.23 t (7.4)		ortho	128.6 d
			meta	127.9 d
			para	127.1 d

carbon connectivity and the cleavage of ring A. Furthermore, the doublet of C-10 was missing, and the signals of C-19 and C-9 had moved downfield [δ 31.5 (q), and 92.4 (s), respectively]. The further degree of unsaturation required by the molecular formula dictated the presence of an additional ring, identified as a γ -lactone ring from the presence of a diagnostic ir absorption at 1770 cm^{-1} . These data could be combined to give the *A-seco*-phorbol structure **2**, which was confirmed by a detailed connectivity analysis via long-range (2J and 3J) proton-carbon correlations (Table 1). The *E*-configuration of the enone double bond was indicated by the absence of nOe effects between H-1 and H-11 and CH_3 -18. In the *Z*-configuration, these groups would instead be close spatially, and nOe effects expected.

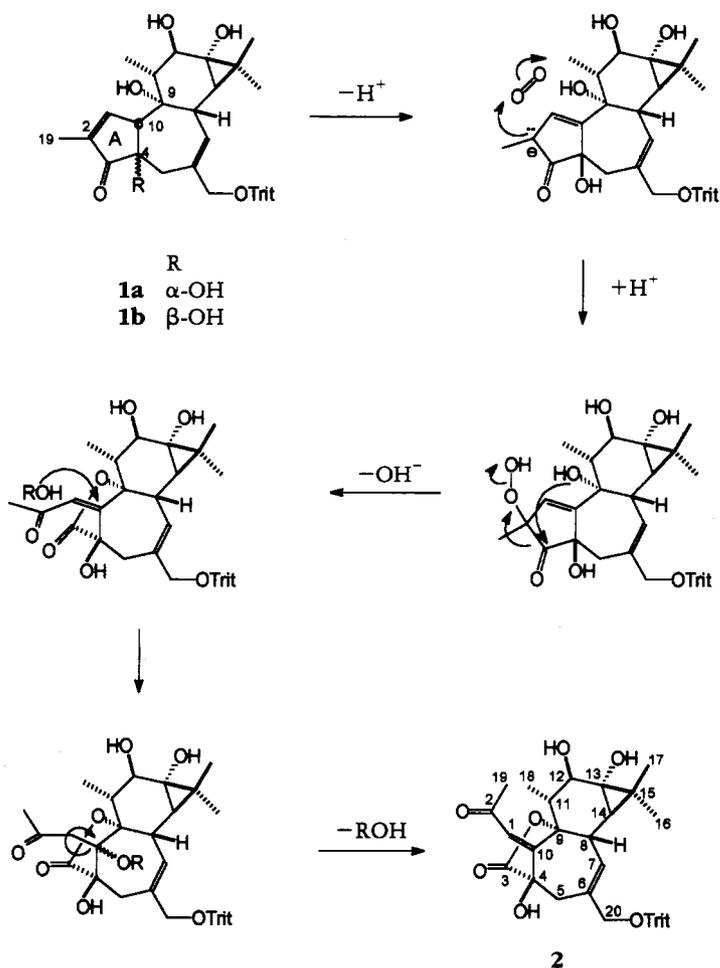
A plausible mechanism for the formation of **2** is presented in Scheme 1, and

involves the regioselective reaction of the dienolate of **1b** with oxygen, followed by the 9-OH-assisted fragmentation of the resulting hydroperoxide. This generates the *Z* isomer of **2**, which is then isomerized to the *E* isomer, presumably via the reversible Michael addition of an oxygen nucleophile.

Many fascinating rearrangements of phorbol are known (4), but the formation of **2** from **1b** is the first one involving changes in the carbon connectivity of ring A.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured with a Perkin Elmer 141 automatic polarimeter. Uv and ir spectra were taken on Beckman DB-GT and Perkin-Elmer model 237 spectrophotometers, respectively. ^1H - (400 MHz) and ^{13}C -nmr (100 MHz) spectra were recorded on a Bruker AM 400 spectrometer, using TMS as internal reference. Ms were taken on a VG EQ-70/70 apparatus. Cc was carried out on Si gel (Merck, 70–230 mesh). A



SCHEME 1. Possible mechanism for the oxidative rearrangement of **1b** to **2**.

Waters microPorasil column (0.8×30 cm) was used for hplc, with detection by a Waters differential refractometer 3401.

SYNTHESIS OF 2.—Phorbol 20-trityl ether (**1b**, 200 mg) was dissolved in 10 ml of freshly prepared NaOMe in MeOH (0.2 M). The reaction mixture turned yellow-orange, and was stirred under O_2 (balloon), followed by tlc (hexane-EtOAc, 1:9; R_f [**1a**]: 0.12; R_f [**1b**]: 0.25; R_f [**2**]: 0.50). After two weeks the reaction mixture was worked up by neutralization with aqueous NH_4Cl and was extracted with EtOAc. After removal of the solvent, the residue was purified by cc (Si gel, hexane-EtOAc, 3:7) to give 62 mg of crude **2** as a dark-orange powder. An analytical sample was obtained after hplc (hexane-EtOAc, 3:7) as an amorphous, colorless foam, $[\alpha]^{25}_{\text{D}} + 20^\circ$ ($c=0.60$, MeOH); uv (EtOH) λ_{max} 245 nm (ϵ 5100); ir ν_{max} (KBr) 3440, 1770, 1740, 1700, 1645, 1450, 1370, 1240, 1180, 1050 cm^{-1} ; cims (NH_3) $[\text{M}+\text{NH}_4]^+$ 638 ($\text{C}_{35}\text{H}_{40}\text{O}_7+\text{NH}_4^+$) (35), 620 (25), 592 (15),

602 (25), 293 (100); hreims m/z 602.26803 ($\text{M}^+-\text{H}_2\text{O}$, $\text{C}_{35}\text{H}_{35}\text{O}_6$ requires 602.26684) (2); ^1H - and ^{13}C -nmr data, see Table 1).

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