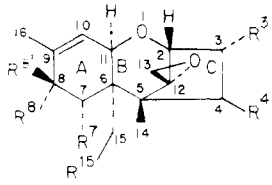
Synthesis of 8-Methoxy-15,16-dinor-6,8,10-trichothecatriene 12,13α-Epoxide and 12,13β-Epoxide as Potential Antineoplastic Agents

Wayne K. Anderson* and George E. Lee

Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14260.
Received August 20, 1979

8-Methoxy-15,16-dinor-6,8,10-trichothecatriene 12,13α-epoxide (**5**) and 12,13β-epoxide (**3**) were prepared; the stereochemistry of the epoxides was assigned on the basis of ^{13}C NMR. The epoxide **5** was active against 9KB in vitro and P388 in vivo, while the isomeric epoxide **3** was inactive in both test systems.

The trichothecanes are a group of related sesquiterpenes that possess the general structure 1. Compounds in this



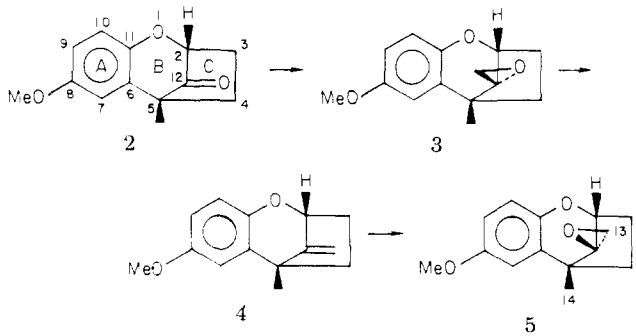
1

class range in complexity from scirpene (1, $R^3 = R^4 = R^7 = R^8 = R^{8'} = R^{15} = H$) to more heavily oxidized compounds such as nivalenol (1, $R^3 = R^4 = R^7 = R^{15} = OH$; R^8 and $R^{8'} = O$); macrocyclic lactones, bridging C-4 and C-15, are also known and one such lactone derivative with a 9,10-epoxide moiety has been reported. Members of this class exhibit a broad range of biological properties, including activity against fungi, protozoa, viruses, plant and animal cell cultures, and animal tumors. Most, if not all, of the naturally occurring trichothecanes are dermatotoxic and most show rather pronounced acute and chronic toxic effects. It is significant that the potency and toxicity of various members of this class vary quite markedly with structure and that relatively little is known about the structural requirements for activity.[1,2]

We have reported a synthesis for A-ring aromatic trichothecanes as part of a continuing project to develop efficient and versatile syntheses of trichothecanes and trichothecane analogues.[3] We now report the synthesis and antileukemic activity of both 12,13-spiroepoxide isomers of 8-methoxy-15,16-dinor-6,8,10-trichothecatriene.

Chemistry. Our earlier paper delineated the synthesis of **2** and its subsequent conversion to a spiroepoxide (stereochemistry unspecified) by treatment with dimethylsulfonium methylide (Scheme I).[3] Treatment of **2** with methyltriphenylphosphorane (potassium *tert*-butoxide/*tert*-butyl alcohol) failed to yield **4** even after the mixture was heated under reflux for 24 h. We were able to convert **2** to **4** in 88% yield using an alternate method of ylide preparation (sodium methylsulfinylmethide/Me_2SO) and forcing conditions (120 °C for 8 h). The olefin **4** was also prepared from the spiroepoxide **3** by a reductive deoxygenation procedure using a zinc–copper couple.[4]

Scheme I



The epoxidation of **4** under a number of standard conditions failed to give **5** in yields greater than 5%. Ultimately, **5** was prepared by using 99.9% *m*-chloroperbenzoic acid[5] in 1,2-dichloroethane heated under reflux with added disodium phosphate (which was found to be superior to other heterogeneous bases such as sodium carbonate, sodium bicarbonate, and sodium acetate) and a free-radical inhibitor to suppress the thermal decomposition of the peracid.[6]

Examination of molecular models of **2** and **4** reveals that approach to the C-12 ketone in **2** and the C-12 olefin in **4** is less hindered from the A-ring side. The hindrance of the C-ring side is due to the 3β and 4β protons and the C-14 methyl group. Consequently, attack of the sulfur ylide on **2** and of the peracid on **4** would be expected to yield **3** and **5**, respectively. These stereochemical predictions are in contrast with the stereochemistry of spiroepoxide formation in the synthesis of (±)-trichodermin; in this latter case, preferred attack of the C-12 ketone or olefin occurred from the C-ring side.[7] This apparent difference between the aromatic and aliphatic A-ring trichothecanes is not too surprising: the puckered aliphatic A ring sterically hinders attack on C-12 from the direction of the A ring, and the C-7 methylene is particularly prominent in this regard.

Support for these stereochemical proposals may be drawn from the ^{13}C NMR spectra of **3** and **5** (Table I). The principal steric interaction in **5** is between the C-13 methylene and the C-14 methyl groups; no such interaction

(1) (a) Bamburg, J. R. *Clin. Toxicol.* **1972**, *5*, 495; (b) Tamm, C. *Fortschr. Chem. Org. Naturst.* **1974**, *31*, 64; (c) Purchase, I. F. H., Ed. "Mycotoxins", American Elsevier: New York, 1974; (d) Kupchan, S. M.; Jarvis, B. B.; Dailey, R. G. Jr.; Bright, W.; Bryan, R. F.; Shizuri, Y. *J. Am. Chem. Soc.* **1976**, *98*, 7092.

(2) Baccharin has a β-epoxide in place of the 9,10 double bond (ref 1d).

(3) Anderson, W. K.; LaVoie, E. J.; Lee, G. E. *J. Org. Chem.* **1977**, *42*, 1045.

(4) Lambert, J. B.; Keong, F. R.; Hamersma, J. W. *J. Org. Chem.* **1971**, *36*, 2941.

(5) (a) 99.9% *m*-chloroperbenzoic acid (as determined by iodometric titration) was prepared by washing technical grade *m*-chloroperbenzoic acid with aqueous disodium phosphate adjusted to pH 7.5 (ref 5b); (b) Schwartz, N. N.; Blumbergs, J. H. *J. Org. Chem.* **1964**, *29*, 1976.

(6) (a) Kishi, Y.; Aratani, M.; Tanino, H.; Goto, T. *J. Chem. Soc., Chem. Commun.* **1972**, *64*; (b) Kishi, Y.; Aratani, M.; Fukuyama, T.; Nakatsubo, F. *J. Am. Chem. Soc.* **1972**, *94*, 9217.

(7) Colvin, E. W.; Malchenko, S.; Raphael, R. A. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1989.

(8) Smaller scale reactions (ca. 200 mg of **4**) were more consistently successful. The reaction was ended when, by TLC evaluation, **5** was being destroyed faster than it was being formed.

0022-2623/80/1823-0096$01.00/0 © 1979 American Chemical Society

Table I. ^{13}C NMR Spectra of 3-5[a]

compd	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	OCH_3
3	78.89	28.45	40.09	40.09	132.16	110.87	153.64	112.77	116.63	146.42	65.61	49.79	15.26	55.75
4	80.17	30.96	42.32	43.94	134.42	109.66	153.41	112.26	116.01	146.33	152.68	103.37	17.53	55.58
5	80.98	29.66	41.08	41.96	133.12	110.56	153.73	112.61	116.28	146.40	66.76	47.53	13.11	55.64
Δδ	2.11	1.21	0.99	1.87	−0.04	−0.31	0.09	−0.16	−0.35	−0.02	1.25	−2.26	−2.15	−0.11

[a] Proton noise-decoupled, proton-coupled, and single frequency off-resonance decoupled spectra were determined for each compound. [b] Δδ refers to the difference in chemical shift between 5 and 3, the two spiroepoxide isomers.

occurs in **3**. The sterically induced δ-gauche effect observed in the ^{13}C NMR spectrum of **5**, compared to **3**, for C-13 and C-14 is in accord with the structural assignments.

Biological Results and Discussions. Spiroepoxides **3** and **5** were evaluated for in vitro cytotoxicity (9KB) and in vivo antileukemic activity (P388). The epoxide **3** showed weak (insignificant) cytotoxic potency, ED_{50} = 26 μg/mL, and no activity against P388 at 12.5, 25, and 50 mg/kg dose levels. In contrast, epoxide **5** showed significant reproducible cytoxicity in the 9KB assay, ED_{50} = 4.5 μg/mL, and antileukemic activity in the P388 assay: at 80 mg/kg, **5** had a % T/C = 134 with no toxicity deaths or weight loss.

While the antineoplastic activity of **5** is statistically significant, the activity of the spiroepoxide must be considered marginal. Further work related to structure–activity relationships in this series of compounds is in progress and will be reported at a later time.

Experimental Section

Melting points (uncorrected) were determined in an open capillary with a Thomas-Hoover Unimelt apparatus. IR spectra were determined with a Perkin-Elmer 237 or 227B spectrophotometer. NMR spectra (^1H and ^{13}C) were determined for $CDCl_3$ solutions (unless otherwise specified) containing 1% (v/v) tetramethylsilane as an internal standard with a Varian T-60 or FT-80 spectrometer. Low-resolution mass spectra were determined with a Perkin-Elmer RMU-6 mass spectrometer at an ionizing voltage of 70 eV. UV spectra were determined with a Beckman DB-G spectrophotometer using 1-cm matched cells. Elemental analyses were performed by Atlantic Microlabs, Inc., Atlanta, Ga.

Preparation of Methylenetriphenylphosphorane in Me_2SO. A stirred suspension of dry sodium hydride (98%, 1.0 g, 41.67 mmol) in Me_2SO (30 mL, freshly distilled over CaH_2) was heated under a nitrogen atmosphere at 71 °C for ca. 4 h or until the evolution of hydrogen ceased. The resulting solution of sodium methylsulfinylmethide (41.67 mmol) was cooled in an ice–water bath, and methyltriphenylphosphonium bromide (14.88 g, 41.67 mmol, dried under high vacuum over P_2O_5) dissolved in warm anhydrous Me_2SO (15 mL) was added (via syringe through a rubber injection septum). The resulting dark-yellow solution of the ylide was stirred at room temperature for 10 min before use.

8-Methoxy-15,16-dinor-6,8,10,12-trichothecatetraene (4). Method A. A mixture of 8-methoxy-15,16-dinor-12,13β-epoxy-6,8,10-trichothecatriene (**3**; 0.03 g, 0.129 mmol), zinc–copper couple freshly prepared from zinc dust (0.166 g, 1.3 mmol), and 95% ethanol (5 mL) was heated under reflux for 24 h. The mixture was filtered and the filtrate was concentrated in vacuo to give a semicrystalline residue, which by NMR was shown to be exclusively unreacted starting material (**3**). The above procedure was repeated a second time; this time the mixture was distilled to dryness during the last 5 h of the reaction, causing the mixture to be heated at the oil bath temperature of 120 °C. The residue was taken up in 95% ethanol (5 mL), filtered through analytical Celite, and concentrated in vacuo to give a light amber oil, which was purified by column chromatography (silica gel, methylene chloride) to yield 0.021 g (75%) of 8-methoxy-15,16-dinor-6,8,10,12-trichothecatetraene (**4**) as a light yellow oil: IR (CCl_4) 3003, 2976, 1686, 1618, 1587, 1429, 886 cm^{-1}; UV λ_{max} 298 nm (ε 3700), 232 (7310), 208 (10 480); ^1H NMR (CCl_4) δ 1.45 (s, 3), 1.60–2.33 (m, 4), 3.62 (s, 3), 4.60 (br s, 1), 4.82 (s, 1), 5.00 (s, 1), 6.47 (m, 3). Anal. Calcd for $C_{14}H_{16}O_2$: C, 77.75; H, 7.46. Found: C, 77.96; H, 7.39.

8-Methoxy-15,16-dinor-6,8,10,12-trichothecatetraene (4). Method B. 8-Methoxy-13,15,16-trinortrichotheca-6,8,10-trien-12-one (**2**; 8.19 g, 37.5 mmol) dissolved in anhydrous Me_2SO (25 mL) was added via syringe through a rubber injection septum to a solution of methylenetriphenylphosphorane (41.67 mmol) in anhydrous Me_2SO (45 mL). The reaction mixture was heated under a nitrogen atmosphere for 6 h at 120 °C. The dark-red reaction mixture was cooled in an ice–water bath and diluted with water (2 L) to give a light-yellow turbid solution, which was extracted with ether (3 × 250 mL). The ethereal solution was washed with saturated NaCl solution (100 mL), dried (anhydrous sodium sulfate), and concentrated in vacuo. The residue was purified by column chromatography (silica gel–methylene chloride) to give 7.1 g (88%) of 8-methoxy-15,16-dinor-6,8,10,12-trichothecatetraene (**4**) as a light-yellow oil identical in all respects with the material prepared according to method A.

8-Methoxy-12,13α-epoxy-15,16-dinor-6,8,10-trichothecatriene (5). A stirred mixture of 8-methoxy-15,16-dinor-6,8,10,12-trichothecatetraene (**4**; 2.0 g, 9.25 mmol), 3-*tert*-butyl-4-hydroxy-5-methoxyphenyl sulfide (1.65 g, 4.6 mmol), disodium phosphate (1.3 g, 9.25 mmol), and 1,2-dichloroethane (20 mL) was heated under reflux and treated with *m*-chloroperbenzoic acid (1.6 g, 9.25 mmol, 99+% pure) which was added in 0.2-g portions every 5 min. After the addition of 1 equiv of peracid, the reaction mixture was evaluated by TLC (silica gel, methylene chloride); the ratio of **4** to **5** was found to be approximately 60:40. Further addition of disodium phosphate (0.65 g, 4.6 mmol) and *m*-chloroperbenzoic acid (0.8 g, 4.6 mmol in 0.2-g portions) was made. Complete consumption of the last portion of peracid occurred within ca. 10 min following its addition (as determined by a negative starch–iodide test). The epoxidation mixture was immediately cooled to ambient temperature (by immersion in an ice–water bath), diluted with methylene chloride (250 mL, to facilitate the precipitation of *m*-chlorobenzoic acid), and filtered through analytical Celite, and the solid residue was washed with methylene chloride (50 mL). The combined methylene chloride fractions were washed with saturated sodium carbonate solution (150-mL portions until the washings were clear), water (150 mL), and saturated NaCl solution (150 mL) and concentrated in vacuo until all of the 1,2-dichloroethane had been removed. (It was impossible to remove the last traces of *m*-chlorobenzoic acid by base extraction in the presence of 1,2-dichloroethane.) The residue was diluted with methylene chloride (250 mL), washed with saturated sodium carbonate solution (50 mL) until the washings were clear and saturated NaCl solution (50 mL), and concentrated in vacuo to give an amber oil which was shown by NMR to contain starting material, epoxide, and less than 1% of *m*-chlorobenzoic acid. Column chromatography (silica gel, methylene chloride) afforded 0.78 g (39%) of 8-methoxy-15,16-dinor-6,8,10,12-trichothecatetraene (**4**) and a yellow oil which was further purified by column chromatography (basic alumina activity grade III, methylene chloride) to give 0.924 g (43%) of 8-methoxy-12,13α-epoxy-15,16-dinor-6,8,10-trichothecatriene [**5**; the yield of **5** based on recovered **4** was 82%]: IR ($CHCl_3$) 1490, 1464, 1377, 1264, 1218, 1202, 1052, 1037, 982 cm^{-1}; UV λ_{max} 295 nm (ε 3880), 232 (5410); ^1H NMR ($CDCl_3$) δ 1.17 (s, 3), 1.92–2.32 (m, 4), 2.88 (d, J_{AB} = 4 Hz, 1), 3.10 (d, J_{AB} = 4 Hz, 1), 3.73 (s, 3), 4.10 (br s, 1), 6.65 (m, 3); MS *m/e* (relative abundance) M^+ + 1233 (15), M^+ 232 (87), 203 (9), 202 (32), 201 (100), 200 (14), 199 (11), 198 (13), 187 (14), 186 (13), 185 (10), 173 (16), 175 (36), 159 (15), 158 (24), 141 (12), 128 (16), 115 (20), 91 (12), 77 (18), 43 (29). Anal. Calcd for $C_{14}H_{16}O_3$: C, 72.39; H, 6.94. Found: C, 72.25; H, 6.95.

Acknowledgment. This investigation was supported by Grant CA-11880, awarded by the National Cancer Institute, DHEW.