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Simple Carbocyclic Trichothecene Analogues

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ABSTRACT

Three spiro-oxiranes (11, 14 and 16), carbocyclic trichothecene analogues were prepared from the corresponding ketones with dimethyloxosulfonium methylide in a good yield. These compounds were evaluated with Ehrlich ascites carcinoma and p-388 lymphocytic leukemia in mice. Compound 14 was shown to have high activity in the ascites screen (inhibition 99.6%). None of the compounds tested is active against p-388 screen in vivo. Although epoxypyran could retain some antitumor activity. However, the better activity of the carbocyclic analogues suggests that the pyran oxygen does not seem to be essential for activity. No direct relationship between lipophilicity and activity could be made from the present study.

INTRODUCTION

The trichothecenes, illustrated by trichodermol (**1**) are a large group of sesquiterpene fungal metabolites which show a wide variety of biological activities[4]. These compounds possess an "epoxy-pyran" structure which give them a characteristic chemical properties and the unique moeity has been believed to be responsible for their activities. Previously, several simple analogues, 1,5-dioxaspiro [2.5] octanes (**2-8**) were prepared and tested in the mouse Ehrlich ascites screen and mouse P-388 lymphocytic leukemia screen[6]. Although these analogues are shown less active than the natural trichothecenes. However, the preliminary result supports that the epoxy-pyran system appears to be an absolute necessity for their biological activity. But is the pyran oxygen a necessity? If not, carbocyclic analogues could be made with much less effort than making pyran derivatives. Synthesis of the pyran derivatives is very difficult. In contrast, hundreds of ketone carbocyclic compounds are commercially available, and in a single step they could be converted to epoxyspiranes. The first carbocyclic analogue prepared in the previous study[6] was **9** - - - - the epoxyspirane of cyclohexanone. Its moderate antitumor activity combined with its greatly different partition coefficient promoted us to investi-

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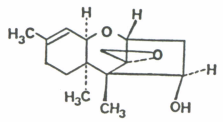

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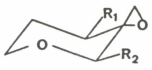
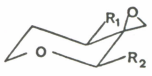


$R_1 = R_2 = H$ (2)

$R_1 = H$; $R_2 = CH_3$ (3)

$R_1 = CH_3$; $R_2 = H$ (4)

$R_1 = R_2 = CH_3$ (5)



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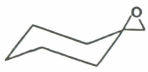

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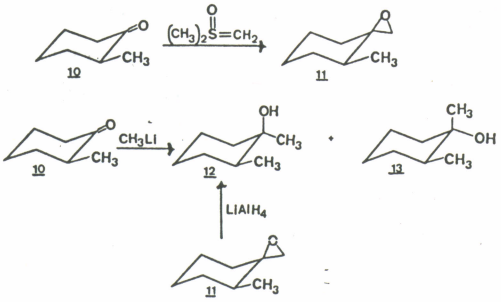
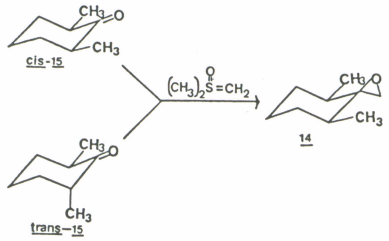
gate further. It was therefore our intention to prepare a few carbocyclic epoxyspiranes in the present research.

RESULTS AND DISCUSSION

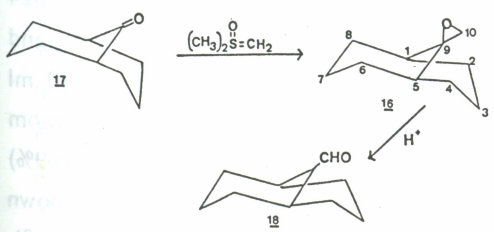
Carbocyclic analogues were prepared from easily accessible ketones with dimethyloxosulfonium methylide[3]. 2-Methylcyclohexanone (**10**) reacted with dimethyloxosulfonium methylide in DMSO at room temperature for 1 hour gave a 68% yield of 4-methyl-1-oxaspiro[2.5]octane (**11**) as a volatile liquid. Vapor phase chromatography showed a single component in the product. The two methylene protons of the epoxide formed an AB quartet at 2.7 δ and 2.43 δ (J=4.5 Hz), and the methyl was a doublet at 0.82 δ (J=6 Hz). It has previously been demonstrated that dimethyloxosulfonium methylide addition to a carbonyl is favored from the equatorial direction in cyclohexanones[1,8]. Spirane **11** was presumably therefore an equatorial addition product - - - - the methyl *cis* to oxirane. Direct determination of the stereochemistry at C_3 was performed using chemical studies. Lithium aluminum hydride reduction of **11** gave a tertiary alcohol which was identical by vpc to the major methyllithium addition product **12** obtained from **10**.



4,8-Dimethyl-1-oxaspiro[2.5]octane (**14**) was prepared from a mixture of *cis* and *trans*-2,6-dimethylcyclohexanone (**15**) by reacting with dimethyloxosulfonium methylide at room temperature for 1 hour. The reaction mixture was found to be 85% of **14** and 15% of unreacted ketone **15**. The carbonyl group of **15** is more hindered than **10**, and complete conversion of **15** to **14** required additional 5 hr at an elevated temperature (60°). The nmr spectrum of **14** showed that the methyls formed a single doublet at 0.72 δ (J=6 Hz) and the oxirane methylene was a sharp singlet at 2.63 δ. From the nmr spectrum **14** is therefore a symmetrical molecule and presumed to be the isomer with the methyls *cis* to each other. The less stable *trans* ketone in **15** would probably be isomerized to the more stable *cis*-ketone in the reaction basic condition before reaction with oxosulfonium methylide. Thus only the *cis* oxirane **14** was obtained.



The synthesis of 9,10-epoxybicyclo[3.3.1] nonane (**16**) was started with commercially available bicyclo[3.3.1]nonan-9-one (**17**). This bicyclo compound was reacted with dimethyloxosulfonium methylide in dry DMSO. The carbonyl of **17** is in a relatively sterically hindered position. Reaction of **17** with methylide was slow at room temperature and required an elevated temperature (60°) and a longer reaction period (24 hr) for completion. Nonane **16** was very unstable even in mild acid. When the benzene solution of **16** was chromatographed on silical gel, the first fraction contained a rearranged product identified as bicyclo aldehyde **18**. Aldehyde **18** has the typical aldehyde ir absorption at 2700 and 1705 cm^{-1} and the nrm has the usual aldehyde singlet at 9.73 δ. However, the pure oxirane **16** could be purified and isolated by neutral alumina column chromatography as a crystalline solid, mp 125-126°. The ir spectrum of **16** showed a characteristic



oxirane band at 815-950 cm^{-1}. The two germinal methylene protons of the epoxide were a singlet at 2.58 δ, due to the symmetrical structure of **16**.

All carbocyclic analogues (**11**, **14** and **16**) were evaluated in the Ehrlich ascites and p-388 lymphocytic leukemia screen. The Ehrlich ascites antitumor test was performed by Dr. Iris H. Hall, University of North Carolina, following the published method of Piantadosi and coworkers[9]. The p-388 antitumor screen was also completed by Dr. Hall following the NIH Protocol 1200[7]. The results of the Ehrlich ascites screen data and the compounds' partition coefficients[5] are shown in Table 1. The p-388 data are shown in Table 2.

The present studies reveal some interesting aspects of trichothecenes analogue structure-activity relationships, but leaves additional questions unanswered as the objects of future research. It is clear in Table 1 that only compound **14** is very active against Ehrlich ascite tumor cells at the testing dose. The striking activity of **14** supports that a sterically hindered epoxide might have greater activity than unhindered epoxide analogues[2,6]. We thought that good activity of **14** might be due to the optimal lipid solubility. As can be seen in Table 2, the previously discussed structure-activity relationship with Ehrlich ascites screen did not follow in the p-388 tumor test. None of the compounds tested is considered active against the p-388 cell line *in vivo*.

In conclusion, it is apparent from these studies that simple carbocyclic epoxy-spirane analogues are not as active tumor inhibition as the trichothecenes and that the increased

Table 1. Ehrlich Ascites Screen DF_1 Male Mice

Compound	mg/kg/day[g]	N	Survival at 8th Day	Vol[a] (ml)	Ascrit. (%)[b]	% inhibition[c]	P.C.[d]
11	33.3	6	6/6	1.08	35.0	64.0	165
14	33.3	6	6/6	0.20	20.0	99.6	360
16	33.3	6	6/6	1.25	36.5	56.9	910
6-MP[f]	20	6	6/6	0.07	0.3	99.6	–
Control[e]	–	6	6/6	3.00	36	–	–

a) The volume of ascitic fluid. b) The percentage of packed cell by volume (ascitocrit). c) Over 75% inhibition is considered active. d) 1-octanol/water partition coefficients were determined by the method of Hansch and coworkers[5]. e) The control group received 0.05% Tween 80 in saline. f) 6-MP: 6-mercaptopurine. g) Compounds were administered for 7 days.

Table 2. Lymphocytic Leukemia p-388 Screen BDF_1 Male Mice

Compounds	N	Dose mg/kg/day	Survival time day (T/C)	T/C (%)[a]
11	6	25	11.1/11.2	99
14	6	25	10.8/11.2	96
16	6	25	11.8/11.2	105
5-FU[b]	7	18	22.4/9.6	233

a) Compounds are considered active by the NIH Protocol if T/C is $\geq$ 125%; T: treated group, C: control group. b) 5-FU: 5-fluorouracil.

activity of the latter is not simple due to relatively high lipid solubility. The pyran oxygen does not seem to be essential for activity, which will greatly simplify future research in this area.

EXPERIMENTAL

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Garden City, MI., and agreed with theoretical value to within ± 0.4%. Ir spectra were obtained neat or KBr with a Perkin-Elmer 237 Grating Infrared Spectrophotometer. Nmr spectra were taken in $CDCl_3$ with a Varian Associate Model A-60D or T-60A NMR Spectrometer using TMS as internal standard and expressed in δ units. Mass spectra were performed by the Mass Spectroscopy Laboratory Service, University of Minnesota. Using a Hitachi Perkin-Elmer RMU-6D Mass Spectrometer. Analytical gas chromatographies were obtained with a Perkin-Elmer 900 Gas Chromatograph.

4-Methyl-1-oxaspiro[2.5] octane (11)

Following the previous procedure[6], 2.24 g (0.02 mol) of 2-methyl-cyclohexanone (**10**) was reacted with dimethyloxosulfonium methylide prepared from 5.28 g (0.024 mol) of trimethyloxosulfonium iodide and 1 g (0.024 mol) of NaH (57%) in 40 ml of DMSO under nitrogen for 1 hr at room temperature. Work-up gave 1.73 g (69%) of **11** as a colorless liquid which was shown to be a homogenous in vpc (3% OV-1, 75°).

The analytical sample was obtained by vacuum distillation, bp. 67-68° (10 mm); ir (neat) cm^{-1}; 825-900 (oxirane); nmr ($CDCl_3$) δ: 2.70, 2.43 (2H, AB quartet, methylene of epoxide, J_{AB} = 4.5 Hz), 1.60 (9H, methylene and methine), 0.82 (3H, d, methyl, J=6 Hz).

Anal. Calcd for $C_8H_{14}O$: C, 76.20; H, 11.10. Found: C, 76.05; H, 11.37.

Reduction of 4-methyl-1-oxaspiro[2.5]octane (11)

Following the reduction procedure[6], 60 mg (0.47 mmol) of **11** was reduced with 40 mg (1 mmol) of lithium aluminum hydride in 20 ml of anhydrous ether. Work-up gave 53 mg (88%) of the alcohol **12** as colorless liquid; ir (neat) cm^{-1}: 3500 (-OH); nmr ($CDCl_3$) δ: 1.42 (9H, broad, m, methine and methylene), 1.17 (3H, s, methyl at C_1), 0.90 (3H, d, methyl at C_2, J=5 Hz). Vpc analysis (5% FFAP at 90°) showed the presence of one component at the retetion time of 8.3 min.

Anal. Calcd for $C_8H_{16}O$: m/e 128.1200. Found: m/e 128.1202.

Reaction of 2-methylcyclohexanone (10) with methyllithium

2-Methylcyclohexanone **(10)** (0.2 g, 1.78 mmol) was refluxed with 4 ml (7.2 mmol) of methyllithium (1.8 M) in 30 ml of anhydrous ether for 1.5 hr under nitrogen. Work-up gave 0.2 g (88%) of the isomeric mixture of **12** and **13** (90.10% by VPC and nmr analysis). The major methyllithium adduct **12** was identical to the lithium aluminum hydride reduction product from **11** by vpc analysis.

4,8-Dimethyl-1-oxaspiro[2.5]octane (14)

Following the procedure for **11**, 2.52 g (0.02 mol) of *cis*-and *trans*-2,6-dimethylcyclohexanone **(15)** was reacted with dimethyloxosulfonium methylide prepared from 1 g (0.024 mol) of NaH (57%) and 5.28 g (0.024 mol) of trimethyloxosulfonium iodide in 40 ml of DMSO. The mixture was stirred at room temperature for 1 hr and then raised the temperature to 60° for another 5 hr. Work-up gave 2.3 g (83%) of **14** as a colorless liquid which by vpc had at least 95% purity. The analytical sample of **14** was obtained by vacuum distillation, bp. 79-80° (15 mm); ir (neat) cm^{-1}: 800, 900 (epoxy); nmr ($CDCl_3$) δ: 2.63 (2H, s, methylene of oxirane), 1.50 (8H, m, methylenes and methines), 0.72 (6H, d, methyls, J=6 Hz).

Anal. Calcd for $C_9H_{16}O$: C, 77.15; H, 11.42. Found: C, 77.03; H, 11.42.

9,10-Epoxybicyclo[3.3.1]nonane (16)

Following the procedure used for **11**, 1.68 g (0.012 mol) of bicyclo[3.3.1]nonan-9-one **(17)** was reacted with dimethyloxosulfonium methylide generated from 0.6 g (0.0144 mol) of NaH (57%) and 3.12 g (0.0144 mol) of trimethyloxosulfonium iodide in 60 ml of DMSO under nitrogen at 60° for 24 hr. Work-up gave a solid material of crude oxirane **16** (1.6 g). The crude oxirane was purified by column chromatography on neutral alumina (Woelm, activity I, 100 g) and the benzene eluted fraction gave 1.565 g (85%) of pure oxirane **16** as white crystals, mp 125-126°; ir ($CHCl_3$): 815, 870, 830, 950 (epoxide); nmr ($CDCl_3$)

δ; 2.58 (2H, s, methylene of oxirane), 1.82 (14 H, m, ring-H).

Anal. Calcd. for $C_{10}H_{16}O$: C, 78.96; H, 10.51. Found: C, 78.99; H, 10.46.

The crude oxirane **16** (2.1 g) was chromatographed on silical gel (Baker, SiO_2, 60-200 mesh, 50 g), eluted with benzene. Each fraction was collected 50 ml of eluent. The second fraction gave 0.31 g of pure aldehyde **18** as an odorous white crystals, mp 84-86°; ir ($CHCl_3$) cm^{-1}: 2700, 1705 (aldehyde); nmr ($CDCl_3$) δ: 9.73 (1H, s, aldehyde-H); 2.67-1.33 (15 H, m, ring-H). The following 5 fractions yielded 1.3 g of the mixture of **16** and **18.**

Anal. Calcd for $C_{10}H_{16}O$: C, 78.96; H, 10.51. Found: C, 79.06; H, 10.35.

Determination of partition coefficient[5]

1-Octanol (saturated with distilled water) and distilled water (saturated with 1-octanol) were used to partition the compounds stuied. The relative concentrations in the 1-octanol and water layer were quantitated by gas chromatography. Each compound (1.0-2.0 mg) was shaken with 0.5 ml of water and 0.5 ml of 1-octanol for 5 min at room temperature. A precise aliquot of each layer was injected into a gas chromatograph using 3% OV-1 column at the temperature of 70°. The area under each peak was then measured.

ACKNOWLEDGEMENT

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簡單 Trichothecene 含碳之類似化合物

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摘 要

Trichothecenes 抗生素具多種活性，前報曾證實其分子中之「Epoxypyran」爲其作用所必備之結構。今次欲探求 Pyran 環上的氧分子的必要性，故以三種酮類物質爲原料經Dimethyloxosulfonium methylide 反應得三種 Spiro-oxiranes[11,14,16] 爲簡單含碳之 trichothecene 類似物，然後以老鼠Ehrlich 腹水癌以及P－388淋巴血癌測其抗癌藥效，結果以Compound(*14*)對腹水癌抑制效果最高（99.6％抑制作用），但此三種物質對p-388癌細胞並無作用。由於Compound(*14*)對Ehrlich 腹水癌具高效果來看，Pyran 環中之氧原子似乎不是作用所須的。本實驗亦發現三物之抗癌效能與其各別的油脂性並無直接關係存在。

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民國七十三年八月二十八日受理