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## Trichothecene Analogues. 1. 1,5-Dioxaspiro[2.5]octanes

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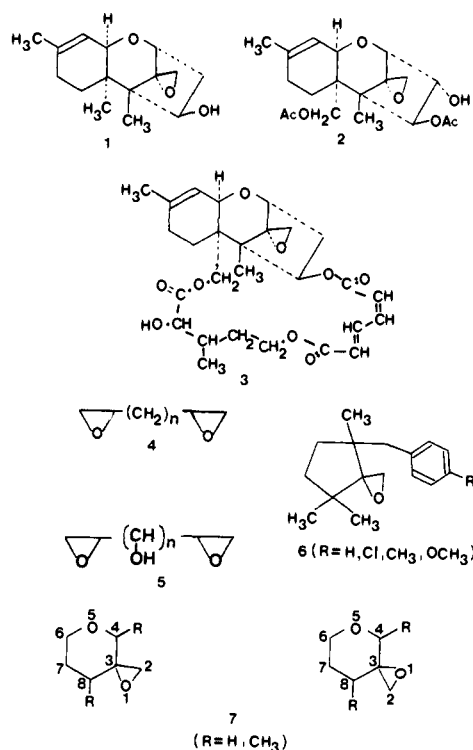
Seven 1,5-dioxaspiro[2.5]octanes were synthesized and tested in the mouse P388 lymphocytic leukemia screen and the mouse Ehrlich ascites screen. These compounds possess the "epoxy pyran" structure which has been believed to be the active portion of the trichothecene class of sesquiterpene tumor inhibitors. Three of the compounds were found to have marginal to moderate activity in the Ehrlich ascites screen (inhibition 74.1–86.3%) and low activity in the P388 screen (T/C = 126–131). A carbocyclic analogue, 1-oxaspiro[2.5]octane (**9**), was moderately active in both screens (inhibition 78.8%, T/C = 140). In the Ehrlich ascites screen, T-2 toxin (**2**) was about 25 times more potent than **9**. None of the spirooctanes studied caused any skin irritation in 10-mg doses on the skin of rabbits, whereas **2** caused extensive necrosis at 0.1-mg doses.

The trichothecenes, illustrated by trichodermol (**1**), T-2 toxin (**2**), and the related macrocyclic esters, the verrucarins and roridins, e.g., verrucarin A (**3**), are a large group of fungal metabolites which have a wide variety of biological activities (see Chart I).<sup>1–5</sup> For example, at 1 ng/ml verrucarin A (**3**) and other trichothecenes cause a 50% inhibition in mouse tumor cell (p815) growth, making them among the most active cytostatic agents known.<sup>1,2,6</sup> Some trichothecenes, e.g., T-2 toxin, are potent skin irritants and can cause extensive dermal necrosis.<sup>1,2,7</sup> Many are potent mycotoxins of significant agricultural-economic importance, being implicated in moldy corn toxicosis, fescue foot diseases, and alimentary toxic aleukia in farm animals.<sup>1,2,8</sup> Several trichothecenes have been found to have antifungal activity in vitro, and a few have been used as agricultural fungicides.<sup>2,6</sup> Insecticidal and larvicidal activities have even been reported.<sup>9,10</sup>

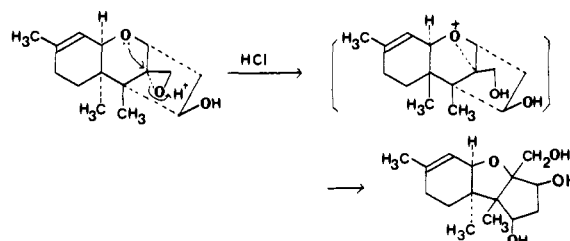
The chemistry and biological activities of the trichothecenes have recently been reviewed by Bamberg,<sup>1,2</sup> Ciegler,<sup>3</sup> Tamm,<sup>4</sup> and Wogan.<sup>5</sup> Two syntheses of trichothecenes have been reported<sup>11,12</sup> and other papers have reported the syntheses of possible synthetic intermediates.<sup>13–16</sup> However, little is known about the structure-activity relationships of the trichothecenes. Although not all compounds of this structure have been tested, and only selected activities have been evaluated, some preliminary generalizations have been made.<sup>2,17</sup> The epoxy pyran system appears to be the "active center" of the trichothecenes, although other structural features may contribute to maximal activity. Acid-catalyzed rearrangement to the apotrichothecene structure (Scheme I) also abolishes activity.<sup>17,18</sup> Furthermore, alcohol derivatives of the trichothecene esters are not as active as the esters themselves. Hydrogenation of the double bond reduces activity.<sup>17,18</sup> It should also be noted that bisepoxides, for example, **4** and **5**, have been found to have moderate to good antitumor activity, at least when "n" is small (0–2).<sup>19–21</sup>

The significant cytostatic antitumor activity of the trichothecenes, combined with their other varied activities, made us interested in studying spirane structure-activity relationship in greater detail. This interest has been shared

Chart I



Scheme I



by McChesney and Corbin at the University of Kansas who have recently synthesized the spirane epoxides of general

Table I. Ehrlich Ascites Screen<sup>a</sup> in CF<sub>1</sub> Male Mice

Compd <sup>b</sup>	N	Survival at 8th day	Ascrit	Vol	% inhib <sup>b</sup>	Partition coeff <sup>c</sup>
19 (0.50 mg/day)	6	6/6	34.0	1.08	54.7	3.2
20 (0.50 mg/day)	6	6/6	15.0	1.37	74.7	<i>d</i>
17 (0.28 mg/day)	6	6/6	21.8	0.96	74.1	<i>d</i>
16 (0.50 mg/day)	6	5/6	27.5	1.20	59.3	3.8
12 (0.50 mg/day)	6	6/6	44.3	0.25	86.3	4.3
13 (0.50 mg/day)	6	6/6	36.0	1.23	45.2	4.4
9 (0.14 mg/day)	6	6/6	36.7	0.47	78.8	33.5
8 (0.50 mg/day)	6	6/6	32.5	0.80	67.9	0.8
2 (4 μg/day)	6	5/6	40.0	0.3	89.2	250
6-Mercaptopurine (0.50 mg/day)	6	6/6	0.30	0.07	99.6	
Control (0.05% Tween 80-saline)	6	6/6	30.0	2.70		

<sup>a</sup> The screening procedure followed the method of Piantadosi and co-workers.<sup>33</sup> <sup>b</sup> The compounds were injected daily ip in 0.05% Tween 80 in normal saline 24 h after injection of 10<sup>6</sup> cancer cells into 25-g CF<sub>1</sub> male mice. Injections continued for 7 days. On the eighth day the mice were sacrificed and the total volume of the ascites tumor and packed cell volume (ascrit) were measured to calculate the percent inhibition of tumor growth. Over 75% inhibition is considered active.

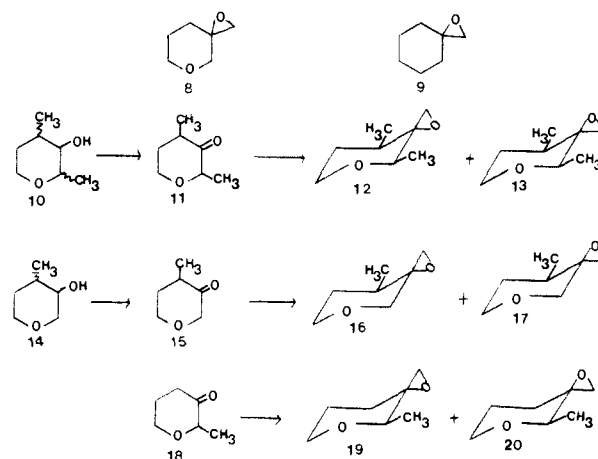
<sup>c</sup> Octanol-water partition coefficients were determined by the method of Hansch<sup>35</sup> and co-workers, using analytical gas chromatography to quantitate the relative concentrations in the octanol and water layers. <sup>d</sup> Satisfactory gas chromatography conditions could not be found to separate the compound from octanol.

structure 6<sup>22</sup> and by Anderson and co-workers at SUNY—Buffalo in unpublished studies.<sup>23</sup> We now report the first of our own studies—the synthesis and biological activity of 1,5-dioxaspiro[2.5]octanes 7, compounds which retain the “active” spiroepoxy pyran structure of the trichothecenes. We have also prepared a carbocyclic analogue 9 to make a preliminary evaluation of the importance of the pyran oxygen in biological activity.

**Chemistry.** The general method used to prepare the 1,5-dioxaspiro[2.5]octanes 8, 12, 13, 16, 17, 19, and 20 began with the appropriately substituted dihydropyrans (Scheme II). With the exception of commercially available dihydropyran used to prepare 8, the substituted dihydropyrans were synthesized as racemic mixtures following previously reported methods.<sup>24–26</sup> The dihydropyrans were hydroborated<sup>27,28</sup> to the corresponding 3-ols, oxidized with Jones reagent<sup>28,29</sup> to the corresponding 3-ones, and finally treated with dimethylloxosulfonium methylide<sup>30</sup> to give racemic spirooctane products. With the substituted oxiranes, two major products were obtained, i.e., one with the oxirane oxygen on the same side as the methyl(s) (13, 17, and 20) and one with the oxirane oxygen and methyl(s) on opposite sides (12, 16, and 19). These isomeric oxiranes were separated into their individual racemic mixtures and collected by preparative gas-liquid chromatography (5 ft × 0.25 in., 5% OV-17, 80 °C). In the case of 12 and 13, traces of additional products were seen by GLC but not collected. These trace products were presumed to be the less stable axial-equatorial dimethyl isomers formed by some base-catalyzed isomerization of 11 during addition of the methylide reagent.

The relative stereochemistry at C-3 of the oxirane rings in the isomeric pairs 12 and 13, 16 and 17, and 19 and 20 was determined by spectral and chemical studies. The chemical studies used to differentiate 12 and 13, and 19 and 20, are shown in Scheme III. Addition of methyl-lithium to the dimethyl ketone 11 gave primarily 21, also obtained by lithium aluminum hydride reduction of 13. Since 21 is the product of the favored equatorial attack<sup>31,32</sup> on 11, it follows that 13 has the oxirane oxygen *cis* relative to the methyls. (The ratio of 21 to 22 in the methyl-lithium addition to 11 was 95:5 by GLC.) The minor product 22 was also formed by lithium aluminum hydride reduction of 12. In a completely analogous way, as shown on Scheme III, 20 was shown to be the isomer with the methyl and oxirane oxygen in relative *cis* configurations. The lithium aluminum hydride reduction product 24 was formed from

Scheme II



19; and 23 was the only methyl-lithium addition product from 18. Isomers 16 and 17 were differentiated by examination of the C-4 protons in the NMR. In 17, each C-4 proton is in nearly an identical environment with respect to the two oxygens, equally close to one oxygen (either the oxirane or pyran oxygen) and equally far from the other. However, in 16, one of the C-4 protons becomes flanked by two oxygens, giving it a significantly different NMR environment. Thus the compound with an AB quartet ( $J = 13$  Hz) for the C-4 proton is 16, and the compound with a singlet for the C-4 protons is 17.

**Biology.** The spirooctanes prepared in this study were tested in the Ehrlich ascites carcinoma in mice following the procedure of Piantadosi and co-workers,<sup>33</sup> and the results are shown in Table I. Only the dimethyl 12 and the carbocyclic analogue 9 were significantly active, with the monomethyl compounds 20 and 17 marginally active. The most active compound, 9, was nevertheless about 25 times less potent than T-2 toxin (2). It is interesting to note that 2 is much more lipid soluble than the other compounds; and we have, therefore, begun to study more lipid-soluble spiranes. The greater activity of the dimethyl “protected” spirane 12 relative to the monomethyl 16 and 19 supports the premise of McChesney and Corbin<sup>22</sup> that hindered epoxides would likely have the best activity. The significant role of the spirane stereochemistry in biological activity is also illustrated by the significant difference in activity between 12 and 13.

## Scheme III

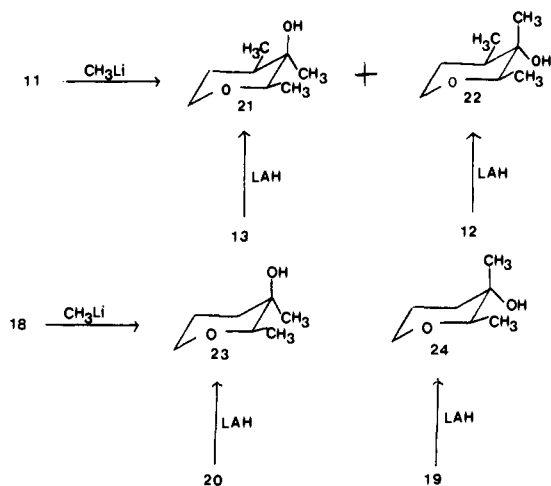


Table II. Lymphocytic Leukemia P388 Screen<sup>a</sup> in BDF<sub>1</sub> Male Mice

Compd	N	Dose, <sup>b</sup> mg/kg/ day	Survival time, <sup>c</sup> days (T/C)	T/C, <sup>d</sup> %
20	8	26	10.4/11.1	94
	7	15	12.1/9.6	126
17	7	32	12.3/9.6	127
	7	14	12.6/9.6	131
12	8	28	10.6/11.1	96
	7	14	10.8/9.6	123
9	7	37	12.1/9.6	126
	8	29	12.8/11.1	115
5-Flurouracil	6	14	13.5/9.6	140
	7	18	22.4/9.6	233

<sup>a</sup> The screening procedure followed the NIH protocol 1.200 published by Geran and co-workers. <sup>b</sup> The compounds were injected ip as suspensions in 0.05% Tween 80 in normal saline, daily with the first injection 24 h after injection of  $10^6$  cancer cells into 20-g BDF<sub>1</sub> (C57BL/6 × DBA/2) male mice. Injections continued daily for 9 days. <sup>c</sup> Calculated by the NIH protocol 11 published by Geran and co-workers.<sup>34</sup> Two experiments were completed, one with a control group average survival time of 9.6 days and the other with a control group average of 11.1 days. <sup>d</sup> Compounds are considered active by the NIH protocol if T/C is  $\geq 125\%$ .

The most active spirooctanes, i.e., 12, 9, 17, and 20, were then tested in the lymphocytic leukemia P388 screen in mice following the NIH protocol of Geran and co-workers.<sup>34</sup> As shown in Table II, all four compounds were marginally active. Activity generally increased in the dose range tested as the dose was decreased. The carbocyclic analogue 9 was the most active of all, clearly suggesting that the pyran structure of the other trichothecene spirooctane analogues is not essential for maximal activity. Further studies on carbocyclic analogues are in progress.

All the spirooctanes prepared in this study were evaluated by Professor C. J. Mirocha and co-workers for skin irritant properties using New Zealand white rabbits, following their previously published methods.<sup>36</sup> Whereas 0.1 mg of T-2 toxin (2) was sufficient to cause dermal necrosis and hemorrhaging, the spirooctanes were inactive even at 10-mg topical doses.

### Experimental Section

Elemental analyses were performed by MHW Laboratories, Garden City, Mich. Where analyses are indicated only by symbols, they are  $\pm 0.4\%$  of the theoretical values. Infrared spectra were obtained neat or in potassium bromide with a Perkin-Elmer 237B. NMR spectra were obtained in  $\text{CDCl}_3$  with a Varian A-60D at

ambient temperature as approximately 10% solutions. Melting points were determined with a Thomas-Hoover melting point apparatus and are corrected.

Preparative vapor-phase chromatographics were done on a Varian Aerograph Model 700 chromatograph using 5% OV-17 on Chromosorb W (45–60 mesh) (5 ft × 0.25 in.): column temperature, 80 °C; helium carrier gas flow rate, 60 ml/min; sample size for each injection, 25  $\mu\text{l}$ . Analytical gas chromatographies were obtained with a Perkin-Elmer 900 gas chromatograph using 5% FFAP (6 ft × 1/8 in.), 15% DEGS (10 ft × 1/8 in.), or 3% OV-1 (6 ft × 1/8 in.).

Organic solvents were removed at 35 °C in vacuo using a Büchi rotary evaporator.

**1,5-Dioxaspiro[2.5]octane (8).** Dihydro-2H-pyran-3(4H)-one was prepared by hydroboration of dihydropyran followed by Jones oxidation as previously reported.<sup>27,28</sup> Sodium hydride–mineral oil dispersion (Alfa, 1.5 g, 57%) (equivalent to 0.036 mol of sodium hydride) was washed under nitrogen three times with 5-ml portions of anhydrous ether which were removed from the dense sodium hydride powder with a pipet. The washed sodium hydride, still wet with ether, was added under nitrogen to 7.92 g (0.036 mol) of trimethyloxosulfonium iodide and immediately 60 ml of anhydrous dimethyl sulfoxide (distilled from calcium hydride) was added. The milky colored suspension was allowed to stir under nitrogen for 10 min at room temperature, and then a solution of 3 g (0.03 mol) of dihydro-2H-pyran-3(4H)-one in 10 ml of dry  $\text{Me}_2\text{SO}$  was added at room temperature, the reaction was stirred for 1 h, and then 15 ml of ice-water was added. The mixture was extracted with 50 ml of ether three times and the total ether solution was washed twice with 20 ml of water, dried over anhydrous sodium sulfate, and evaporated. The residue was distilled over a Vigreux column and 0.5 g (44%) of 8 was collected at 57–59 °C (15 mm): ir (neat) 1075 (COC), 800–950  $\text{cm}^{-1}$  (oxirane); NMR ( $\text{CDCl}_3$ )  $\delta$  3.57, 3.35 [AB quartet, 2 H,  $\text{OCH}_2\text{C}(\text{O})\text{C}$ ,  $J_{AB} = 11.5$  Hz], 2.62 (2 H, s,  $\text{CH}_2$  of oxirane), 1.78 (4 H, broad,  $\text{CH}_2$ ), 3.54 (2 H, m,  $-\text{OCH}_2\text{C}$ ). Anal. ( $\text{C}_6\text{H}_{10}\text{O}_2$ ) C, H.

**1-Oxaspiro[2.5]octane (9).** Cyclohexanone (2.94 g, 0.03 mol) was allowed to react with dimethyloxosulfonium methylide prepared from 0.036 mol of NaH, 7.92 g (0.036 mol) of trimethyloxosulfonium iodide, and 60 ml of  $\text{Me}_2\text{SO}$  (as described for 8) and worked up to give 3.3 g of crude oxirane. The crude product was distilled under vacuum over a Vigreux column to give 2.1 g (60%) of 9 as a colorless liquid: bp 50–51 °C (20 mm) [lit.<sup>37</sup> bp 61–62 °C (39 mm)]; ir (neat) 900  $\text{cm}^{-1}$  (oxirane); NMR ( $\text{CDCl}_3$ )  $\delta$  1.58 (10 H, s, methylene), 2.53 (2 H, s,  $\text{CH}_2$  of oxirane).

**3-Hydroxy-2,4-dimethyltetrahydropyran (10).** 2,4-Dimethyl-5,6-dihydro-2H-pyran was synthesized from 2-methyl-1,3-pentadiene and powdered paraformaldehyde with hydroquinone in a stainless steel bomb at 190 °C as previously reported.<sup>24</sup> To 14.7 g (0.131 mol) of this dimethylpyran in 100 ml of dry tetrahydrofuran was added 0.437 mol of 1 M borane–THF complex (Aldrich) under nitrogen at 0–5 °C. After the reaction mixture had stirred in an ice bath for 3 h, the temperature was raised to 25 °C and the mixture was stirred for an additional hour. To it was added 30 ml of 3 N NaOH solution, followed by dropwise addition of 25 ml of 30%  $\text{H}_2\text{O}_2$ . The reaction was stirred for 1 h, 20 g of NaCl was added, and the mixture was extracted three times with 100-ml portions of ether. The combined ether extracts were washed once with 20 ml of saturated NaCl and dried over  $\text{MgSO}_4$ . The ether solution was fractionally distilled over a Vigreux column to give 8.1 g (46%) of 10 as a colorless liquid: bp 72–76 °C (5 mm); ir (neat) 3400 ( $-\text{OH}$ ), 1050, 1100  $\text{cm}^{-1}$  (COC); NMR ( $\text{CDCl}_3$ )  $\delta$  1.06 (3 H, d, methyl at  $\text{C}_2$ ,  $J = 5$  Hz), 1.28 (3 H, d, methyl at  $\text{C}_4$ ,  $J = 7$  Hz), 1.57 (2 H, broad, methylene at  $\text{C}_5$ ). Anal. ( $\text{C}_7\text{H}_{14}\text{O}_2$ ) C, H.

**2,4-cis-Dimethyldihydro-2H-pyran-3(4H)-one (11).** Jones reagent<sup>28,29</sup> was added dropwise to a rapidly stirred solution of 2.5 g (0.019 mol) of 10 in 450 ml of reagent acetone at room temperature until the solution remained red-orange in color. The excess chromic acid was destroyed by adding isopropyl alcohol (changing the solution to dark green) and the mixture was stirred with 2 g of anhydrous potassium carbonate for 1 h. The solution was evaporated at room temperature in vacuo and the resulting residue was extracted with 100 ml of anhydrous ether and dried over anhydrous  $\text{MgSO}_4$ . Evaporation gave 2.1 g (85%) of the ketone 11: bp 54–56 °C (15 mm); ir 1720  $\text{cm}^{-1}$  ( $\text{C}=\text{O}$ ); NMR

(CDCl<sub>3</sub>)  $\delta$  1.08 (3 H, d, methyl at C<sub>2</sub>,  $J$  = 6 Hz), 1.23 (3 H, d, methyl at C<sub>4</sub>,  $J$  = 6 Hz), 3.63–5.65 (3 H, m, methylene at C<sub>6</sub> and methine at C<sub>2</sub>). Anal. (C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>) C, H.

**(3RS,4RS,8RS)-4,8-Dimethyl-1,5-dioxaspiro[2.5]octane (12)** and **(3SR,4RS,8RS)-4,8-Dimethyl-1,5-dioxaspiro[2.5]octane (13)**. Following the procedure used for 8, 0.25 g (0.006 mol) of NaH dispersion, 1.32 g (0.006 mol) of trimethyloxosulfonium iodide, and 10 ml of anhydrous dimethyl sulfoxide were allowed to react. To this suspension was allowed to react 0.64 g (0.005 mol) of ketone 11 as for 8. Work-up gave 0.6 g (83%) of 12 and 13 (ratio 51:49 by GLC). The isomeric epoxides were separated by preparative GLC (see introduction to Experimental Section for general conditions). The first fraction, 12, was collected at the retention time of 11.2 min as a colorless liquid: ir (neat) 1100 (COC), 950, 920, 850, 825 cm<sup>-1</sup> (epoxide); NMR (CDCl<sub>3</sub>)  $\delta$  4.20–3.38 (3 H, m, methylene at C<sub>6</sub> and methine at C<sub>4</sub>), 2.75, 2.58 (2 H, AB quartet, methylene of oxirane,  $J_{AB}$  = 4.5 Hz), 2.32–1.37 (3 H, m, methylene at C<sub>7</sub> and methine at C<sub>8</sub>), 1.04 (3 H, d, methyl at C<sub>4</sub>,  $J$  = 6.5 Hz), 0.82 (3 H, d, methyl at C<sub>8</sub>,  $J$  = 7 Hz). Anal. (C<sub>8</sub>H<sub>14</sub>O<sub>2</sub>) C, H.

The second fraction, 13, was collected at the retention time of 20.5 min as a colorless liquid: ir (neat) 1150, 1100 (COC), 900 cm<sup>-1</sup> (oxirane); NMR (CDCl<sub>3</sub>)  $\delta$  3.33–2.48 (3 H, m, methylene at C<sub>6</sub> and methine at C<sub>4</sub>), 2.80, 2.73 (2 H, AB quartet, methylene of oxirane,  $J_{AB}$  = 4.5 Hz), 2.33–1.50 (3 H, m, methylene at C<sub>7</sub> and methine at C<sub>8</sub>,  $J$  = 7 Hz). Anal. (C<sub>8</sub>H<sub>14</sub>O<sub>2</sub>) C, H.

Relative assignment of the structures of 12 and 13 was based in part upon their lithium aluminum hydride reduction products as compared with the methylolithium adduct products of 11. The experimental details are discussed in the subsequent sections.

**Reduction of (3RS,4RS,8RS)-4,8-Dimethyl-1,5-dioxaspiro[2.5]octane (12)**. A solution of 85 mg (0.6 mmol) of 12 in 10 ml of anhydrous ether was added dropwise to a rapidly stirring suspension of 45 mg (1.2 mmol) of lithium aluminum hydride in 40 ml of anhydrous ether. The mixture was refluxed for 1 h and the excess hydride was destroyed by carefully adding microdrops of water (1 ml total). To the resulting suspension was added 5 ml of 5% aqueous KOH. The mixture was extracted three times with 20 ml of ether. The ether extract was washed once with 5 ml of water, dried over anhydrous MgSO<sub>4</sub>, and evaporated to give 64 mg (75%) of the alcohol 22 as a colorless liquid: ir (neat) 3450 cm<sup>-1</sup> (–OH); NMR (CDCl<sub>3</sub>)  $\delta$  4.07–3.40 (2 H, br m, methylene at C<sub>6</sub>), 3.17 (1 H, q, methine at C<sub>2</sub>,  $J$  = 13 Hz), 1.17 (6 H, d, methyl at C<sub>2</sub> and C<sub>4</sub>,  $J$  = 6 Hz), 1.05 (3 H, s, methyl at C<sub>3</sub>). GLC analysis (5% FFAP, 80 °C) showed one peak at the retention time of 5.5 min, identical with the major product of methylolithium addition to 11.

**Reduction of (3SR,4RS,8RS)-4,8-Dimethyl-1,5-dioxaspiro[2.5]octane (13)**. Following the reduction procedure for 12, the oxirane 13 (50 mg, 0.35 mmol) was reduced with 25 mg (0.67 mmol) of LiAlH<sub>4</sub> and worked up to yield 41 mg (80%) of the alcohol 21 as a colorless liquid: ir (neat) 3450 cm<sup>-1</sup> (–OH); NMR (CDCl<sub>3</sub>)  $\delta$  4.08–3.40 (2 H, m, methylene at C<sub>6</sub>), 3.27 (1 H, q, methine at C<sub>2</sub>,  $J$  = 13 Hz), 2.02 (1 H, –OH), 1.17 (6 H, d, methyl at C<sub>2</sub> and C<sub>4</sub>,  $J$  = 6.5 Hz), 1.08 (3 H, s, methyl at C<sub>3</sub>). GLC (5% FFAP, 80 °C) showed the presence of one peak at the retention time of 2.2 min.

**Reaction of cis-2,4-Dimethyldihydro-2H-pyran-3(4H)-one (11) with Methylolithium**. A solution of 0.15 g (1.17 mmol) of 11 in 5 ml of ether was added dropwise to a rapidly stirring ether solution of 2.7 mmol of methylolithium (1.8 M, Alfa). The mixture was refluxed with stirring for 1 h and, after cooling, 2 ml of water was very carefully added dropwise to destroy excess methylolithium. The mixture was extracted three times with 15 ml of ether, the ether extracts were washed with water and dried over anhydrous MgSO<sub>4</sub>, and the ether was evaporated to give 0.16 g (95%) of 21 and 22 (ratio 95:5) with identical GLC and spectral characteristics with the 21 and 22 obtained by LiAlH<sub>4</sub> reduction of 13 and 12 (described in the previous experiments).

**3-Hydroxy-4-methyltetrahydropyran (14)**. 4-Methyl-3,4-dihydro-2H-pyran was prepared as previously described<sup>25,38</sup> by the Diels–Alder addition of crotonaldehyde and isobutyl vinyl ether, followed by Raney nickel hydrogenation and treatment with P<sub>2</sub>O<sub>5</sub>. Following the procedure for 8, 11 g (0.1122 mol) of this dihydropyran was hydroborated with 0.055 mol of 1 M borane–THF complex and oxidized with 30 ml of 30% H<sub>2</sub>O<sub>2</sub> and

30 ml of 3 N NaOH. Distillation yielded 5.54 g (42%) of 14, bp 70–72 °C (1.2 mm) as a colorless liquid: ir (neat) 3400 (–OH), 1100 cm<sup>-1</sup> (COC); NMR (CDCl<sub>3</sub>)  $\delta$  4.03–3.02 (6 H, m, methylene at C<sub>2</sub> and C<sub>6</sub>, methine at C<sub>3</sub> and –OH), 1.57 (3 H, m, methylene at C<sub>5</sub> and methine at C<sub>4</sub>), 1.01, 10.8 (3 H, 2 doublets, methyl at C<sub>4</sub> cis and trans to –OH,  $J$  = 6 and 5.5 Hz). Anal. (C<sub>6</sub>H<sub>12</sub>O<sub>2</sub>) C, H.

**4-Methyldihydro-2H-pyran-3(4H)-one (15)**. Following the procedure for 11, oxidation of the alcohol 14 (4.4 g, 0.0379 mol) with excess Jones reagent in acetone gave 3.6 g (83%) of 15 as a colorless liquid: bp 53–55 °C (15 mm); ir (neat) 1720 (C=O), 1100 cm<sup>-1</sup> (COC–); NMR (CDCl<sub>3</sub>)  $\delta$  4.01 (2 H, s, methylene at C<sub>2</sub>), 3.95–3.67 (2 H, m, methylene at C<sub>6</sub>), 2.78–1.43 (3 H, m, methine and methylene), 1.14 (2 H, d, methyl,  $J$  = 6.5 Hz). Anal. (C<sub>6</sub>H<sub>10</sub>O<sub>2</sub>) C, H.

**(3RS,8SR)-8-Methyl-1,5-dioxaspiro[2.5]octane (17)** and **(3SR,8SR)-8-Methyl-1,5-dioxaspiro[2.5]octane (16)**. Dimethyloxosulfonium methylide prepared from 0.006 mol of NaH, 1.32 g (0.006 mol) of trimethyloxosulfonium iodide, and 10 ml of Me<sub>2</sub>SO (as described for 8) was added to 0.57 g (0.005 mol) of 15. After work-up, a mixture of oxiranes (0.55 g, 86% yield) was obtained which showed two components in the ratio of 62:38 by GLC analysis. The two isomeric oxiranes were separated by preparative GLC. The first fraction, 17, retention time of 13.8 min, was a colorless liquid: ir (neat) 1100 (COC), 960, 940, 880 cm<sup>-1</sup> (oxirane); NMR (CDCl<sub>3</sub>)  $\delta$  3.87 (2 H, m, methylene at C<sub>6</sub>), 3.57 (2 H, s, methylene at C<sub>4</sub>), 2.82, 2.62 (2 H, AB quartet, methylene of oxirane,  $J_{AB}$  = 13 Hz), 2.20–1.33 (3 H, m, methylene at C<sub>7</sub> and methine at C<sub>8</sub>), 0.91 (3 H, d, methyl,  $J$  = 7 Hz). Anal. (C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>) C, H.

The second fraction, 16, retention time of 22.3 min, was a colorless liquid: ir (neat) 1050 (COC), 935, 890, 865 cm<sup>-1</sup> (oxirane); NMR (CDCl<sub>3</sub>)  $\delta$  3.78, 3.44 (2 H, AB quartet, methylene at C<sub>4</sub>,  $J_{AB}$  = 13 Hz), 3.88 (2 H, m, methylene at C<sub>6</sub>), 2.86, 2.56 (2 H, AB quartet, methylene of oxirane,  $J_{AB}$  = 4.5 Hz), 2.07 (1 H, m, methine), 1.70 (2 H, m, methylene at C<sub>7</sub>), 0.87 (3 H, d, methyl,  $J$  = 7 Hz). Anal. (C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>) C, H.

**(3RS,4RS)-4-Methyl-1,5-dioxaspiro[2.5]octane (19)** and **(3SR,4RS)-4-Methyl-1,5-dioxaspiro[2.5]octane (20)**. 2-Methyldihydro-2H-pyran-3(4H)-one (18) was made as previously reported by reaction of sodium ethyl acetoacetate and 1,3-dibromopropane, hydrolysis of the resulting dihydropyran ethyl ester, and thermal decarboxylation. Following the procedure used for 8, dimethyloxosulfonium methylide [prepared from 0.006 mol of NaH, 1.32 g (0.006 mol) of trimethyloxosulfonium iodide, and 10 ml of anhydrous dimethyl sulfoxide] was allowed to react with 0.57 g (0.005 mol) of 18. After work-up, a mixture of oxiranes (0.50 g, 75% yield) was obtained which was shown to contain two products in the ratio of 42:58 by GLC analysis. The two isomeric oxiranes were separated by preparative gas–liquid chromatography. The first fraction, retention time of 9.2 min, was 19, a colorless liquid: ir (neat) 1100 (COC), 900, 860 cm<sup>-1</sup> (oxirane); NMR (CDCl<sub>3</sub>)  $\delta$  4.15–3.22 (3 H, m, methine at C<sub>4</sub> and methylene at C<sub>6</sub>), 2.90, 2.54 (2 H, AB quartet, methylene of oxirane,  $J_{AB}$  = 4.5 Hz), 1.85 (4 H, m, methylene at C<sub>7</sub> and C<sub>8</sub>), 1.07 (3 H, d, methyl,  $J$  = 6.5 Hz). Anal. (C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>) C, H.

The second fraction, retention time of 15.3 min, was 20, a colorless liquid: ir (neat) 1100 (COC), 950, 900, 870, 850 cm<sup>-1</sup> (oxirane); NMR (CDCl<sub>3</sub>)  $\delta$  4.22–3.33 (3 H, m, methylene at C<sub>6</sub> and methine at C<sub>4</sub>), 2.73, 2.53 (2 H, AB quartet, methylene of oxirane,  $J_{AB}$  = 4.5 Hz), 2.18–1.27 (4 H, m, methylene at C<sub>7</sub> and C<sub>8</sub>), 1.03 (3 H, d, methyl,  $J$  = 6.5 Hz). Anal. (C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>) C, H.

Relative assignment of the structure of 19 and 20 was based in part on their lithium aluminum hydride reduction products as compared with the methylolithium adduct products of 18. The experimental details are presented in the subsequent sections.

**Reaction of 2-Methyldihydro-2H-pyran-3(4H)-one (18) with Methylolithium**. Following the procedure for the reaction of 11, 0.1 g (0.87 mmol) of 18 was allowed to react with 2.7 mmol of methylolithium. Work-up gave 50 mg (46%) of the alcohol 23 as a colorless liquid: ir (neat) 3450 cm<sup>-1</sup> (–OH); NMR (CDCl<sub>3</sub>)  $\delta$  4.15–3.45 (2 H, m, methylene at C<sub>6</sub>), 6.72 (1 H, q, methine at C<sub>4</sub>,  $J$  = 13 Hz), 2.20 (1 H, broad, –OH), 1.17 (3 H, d, methyl at C<sub>4</sub>,  $J$  = 5.5 Hz), 1.11 (3 H, s, methyl at C<sub>2</sub>). The spectral and GLC characteristics of 23 were identical with those of 23 obtained by LiAlH<sub>4</sub> reduction of 20.

**Reduction of (3RS,4RS)-4-Methyl-1,5-dioxaspiro[2.5]octane (19).** Following the procedure for 12, 31 mg (0.24 mmol) of 19 was reduced with 15 mg (0.4 mmol) of  $\text{LiAlH}_4$ . Work-up gave 25 mg (80%) of the alcohol 24 as a colorless liquid: ir (neat)  $3450\text{ cm}^{-1}$  ( $-\text{OH}$ ); NMR ( $\text{CDCl}_3$ )  $\delta$  4.07–3.45 (2 H, m, methylene at  $\text{C}_6$ ), 3.28 (1 H, q, methine at  $\text{C}_3$ ,  $J = 13\text{ Hz}$ ), 1.70 (4 H, m, methylene at  $\text{C}_4$  and  $\text{C}_5$ ), 1.21 (3 H, s, methyl at  $\text{C}_3$ ), 1.17 (3 H, d, methyl at  $\text{C}_2$ ,  $J = 5.5\text{ Hz}$ ). GLC analysis (5% FFAP,  $80^\circ\text{C}$ ) showed one peak at the retention time of 6.2 min.

**Reduction of (3SR,4SR)-4-Methyl-1,5-dioxaspiro[2.5]octane (20).** Following the procedure for 12, 30 mg (0.24 mmol) of 20 was reduced with 15 mg (0.4 mmol) of  $\text{LiAlH}_4$ . Work-up gave 20 mg (76%) of the alcohol 23 as a colorless liquid: ir (neat)  $3450\text{ cm}^{-1}$  ( $-\text{OH}$ ); NMR ( $\text{CDCl}_3$ )  $\delta$  4.15–3.43 (2 H, m, methylene at  $\text{C}_6$ ), 6.72 (1 H, q, methine at  $\text{C}_2$ ,  $J = 13\text{ Hz}$ ), 2.20 (1 H, broad,  $-\text{OH}$ ), 1.17 (3 H, d, methyl at  $\text{C}_2$ ,  $J = 5.5\text{ Hz}$ ), 1.11 (3 H, s, methyl at  $\text{C}_3$ ). GLC (5% FFAP,  $80^\circ\text{C}$ ) showed the presence of one component at the retention time of 2.7 min, identical with the major product of methylolithium addition to 18.

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## Steroidal 3,5-Dienes

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A series of  $\Delta^{3,5}$ -androstadienes, estradienes, and gonadienes was prepared and evaluated as claudogenic agents. Claudogenic activity was limited to relatively few members of the series, but three compounds—12, 14, and 15—were very potent.

The function of the carbonyl moiety at C-3 of steroid hormones in eliciting biological responses has been the subject of some study in this<sup>1</sup> and other laboratories.<sup>2,3</sup> In the course of our search for claudogenic steroids, we needed to prepare estr-4-ene-3 $\alpha$ ,17 $\beta$ -diol by reduction of 19-nortestosterone with  $\text{LiAlH}_4$ . Purification of the crude product gave estr-4-ene-3 $\beta$ ,17 $\beta$ -diol and estra-3,5-dien-

17 $\beta$ -ol (1). Routine screening of the latter compound, which presumably comes from dehydration of the 3 $\alpha$ -alcohol, showed marked claudogenic activity.

Searching the literature, we were impressed by a related family of steroidal 3-aryl- $\Delta^{3,5}$ -dienes 2 which were purported to "... share with estrone the capacity to inhibit uterine response to progesterone."<sup>4</sup> Based on the reported