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SHORT COMMUNICATION

SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF CYCLODIMERS OF STYRENE OXIDES

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ABSTRACT. A simple synthetic procedure for preparation of 1,4-dioxanes or 1,3-dioxolanes from styrene oxides is described. Electron-donating groups on the aromatic ring of the styrene oxides were found to favour formation of 1,4-dioxanes while electron-withdrawing groups favoured formation of 1,3-dioxolanes. Antibacterial activities of the prepared cyclodimers are reported.

KEY WORDS: Dioxanes, Dioxolanes, Styrene, Epoxidation, Cyclodimerization, Antibacterial activities

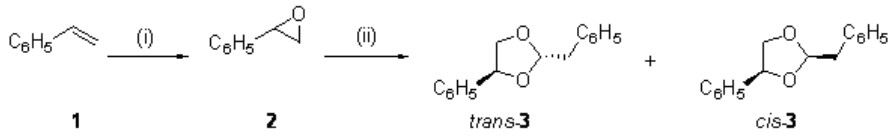
INTRODUCTION

Epoxides, readily available and inexpensive materials, have been used extensively in organic syntheses and generally react stereospecifically with several nucleophiles producing highly-functionalized molecules which may further be transformed. Nucleophilic ring-opening reactions of epoxides can be catalysed under either acidic or basic conditions. Among the more efficient nucleophiles are water, alcohols, thiols, amines, halides and pseudohalides [1, 2].

In connection with our study of the usefulness of epoxides in organic synthesis, we earlier reported on the cyclodimerization of styrene oxide and its chloro derivatives into 2,4-disubstituted 1,3-dioxolanes [3] which are widely used as protecting groups for ketones, aldehydes and 1,2-diols in organic synthesis [4]. In this paper, we report on the effects of electron-donating groups (methoxy and methyl) as opposed to a mildly electron withdrawing-group (chloro) on the outcome of the cyclodimerization reaction of styrene oxides and the biological activities of the resulting dimers.

RESULTS AND DISCUSSION

Styrene oxide **2** was prepared in good yield by the reaction of styrene **1** with *m*-chloroperbenzoic acid (*m*-CPBA) in dichloromethane. Subsequent treatment of **2** with phosphoric acid (H_3PO_4) afforded two dioxolane isomers viz., *trans*-**3** and *cis*-**3** with the *trans*-isomer being the major (Scheme 1). The reaction is thought to proceed through a critical 1,2-hydride shift [3].



1 **2** *trans*-**3** *cis*-**3**

Scheme 1. Reagents and conditions: (i) *m*-CPBA, CH_2Cl_2, $NaHCO_3$, 25 °C, 88%; (ii) H_3PO_4, 25 °C, 61% (*trans*-3:*cis*-3, 75:25).

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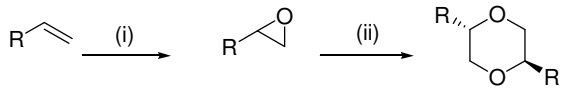
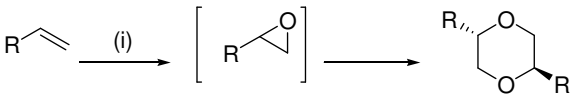
p-Methylstyrene, **4a** was subjected to the epoxidation conditions and the resulting epoxide **5a** was treated with H_3PO_4 as described earlier. Interestingly, the six-membered dimer, 1,4-dioxane **6a** was isolated in 83 % yield as the only isomer (Scheme 2). *m*-Methylstyrene oxide **5b** also afforded the corresponding 1,4-dioxane **6b** signifying that the position of the electron-donating methyl group on the aromatic ring does not affect the outcome of the reaction.



4a. R = *p*-$CH_3C_6H_4$ **5a** **6a**
4b. R = *m*-$CH_3C_6H_4$ **5b** **6b**

Scheme 2. Reagents and conditions: (i) *m*-CPBA, CH_2Cl_2, $NaHCO_3$, 25 ^oC, 82%; (ii) H_3PO_4, 25 ^oC, 6a 83%, 6b 76%.

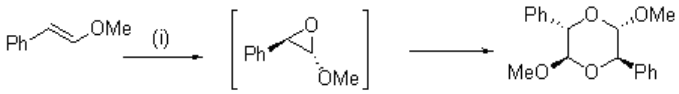
In addition, two 1,4-dioxanes **6c** and **6d** with electron-donanting methoxy groups on the aromatic ring were prepared from the corresponding methoxystyrenes in high yields using the epoxidation procedure described above. It is worth noting that for the methoxy-substituted styrene substrates, it was not possible to isolate the epoxides **5c** and **5d** as the reaction proceeded directly to give the 1,4-dioxanes under the epoxidation conditions [3] (Scheme 3).



4c. R = *p*-$CH_3OC_6H_4$ **5c** **6c**

Scheme 3. Reagents and conditions: (i) *m*-CPBA, CH_2Cl_2, $NaHCO_3$, 25 ^oC, 6c 85%, 6d 96%.

Placement of electron-donating methoxy group on the double bond instead of the aromatic ring also gave 1,4-dioxane. Thus, treatment of the β-methoxystyrene **7** with *m*-CPBA led to the isolation of dioxane **9** as the only product (Scheme 4). The absence of NOESY correlations between H-2(axial) and H-3(axial) and the coupling constants (5.4 Hz) of the two hydrogens were used to suggest the *trans* relative configuration. It is worthy mentioning that dioxane **9** has been reported in literature as a product of the acid-catalysed dimerization of hydroxyphenylethanal in methanol [5]. However, no spectral data was reported. NOESY correlations were observed between H-2 and H-6 and that confirmed the structure of 9.

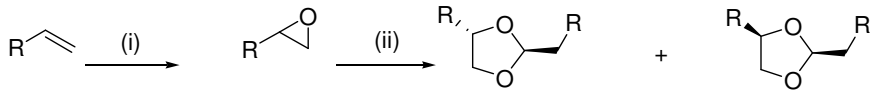


7 **8** **9**

Scheme 4. Reagents and conditions: (i) *m*-CPBA, CH_2Cl_2, $NaHCO_3$, 25 ^oC, 96%.

Alternatively, when styrene substrates containing the electron-withdrawing chloro group were subjected to the epoxidation conditions and the resulting epoxides treated with phosphoric acid, cyclodimerization occurred to give 1,3-dioxolanes. Thus, treatment of *o*-chlorostyrene **10a** with *m*-CPBA in CH_2Cl_2 afforded epoxide **11a** (73%) which when subjected to phosphoric acid treatment produced the 1,3-dioxolanes **12a** and **13a** in a 75:25 ratio, respectively and a yield of

72%. The procedure was repeated for *m*-chlorostyrene **10b** and *p*-chlorostyrene **10c** which afforded 1,3-dioxolanes **12b** and **12c**, respectively, as the major cyclodimers [3] (Scheme 5). The relative stereochemistry of the 1,3-dioxolanes was based on the NOESY spectral correlations. Thus the *trans* relationship for **12** between H-2 and H-4 protons is suggested due to the absence of any NOE correlation between these two protons whereas it was observed for the cis-isomers **13**.



10a. R = *o*-ClC_6H_4 **11a** **12a** **13a**
10b. R = *m*-ClC_6H_4 **11b** **12b** **13b**
10c. R = p-ClC_6H_4 **11c** **12c** **13c**

Scheme 5. Reagents and conditions: (i) *m*-CPBA, CH_2Cl_2, $NaHCO_3$, 25 °C, 73-75%; (ii) H_3PO_4, 25 °C, 72% (12a:13a, 75:25), 62% (12b:13b, 75:25), 84% (12c:13c, 100:0).

The antibacterial activities of the prepared major cyclodimers were evaluated on two *Gram*-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two *Gram*-negative bacteria (*Escherichia coli* and *Pseudonomas aeruginosa*) using the agar-overlay bioautography method. Results are summarized in Table 1. Antimicrobial activities of the prepared compounds were compared, using chloramphenicol as the reference antibacterial agent. All the compounds showed activities against both *Gram*-negative and *Gram*-positive bacteria with a minimum inhibitory concentration (MIC) between 0.5 and 100 mg/mL. *Bacillus subtilis* was most sensitive to dioxanes **6a**, **6c** and **6d** with a MIC of 0.5 mg/mL. The same MIC was observed for the activity of dioxolane **12c** on *Escherichia coli*. Both the prepared dioxanes and dioxolanes are relatively non-polar compounds, therefore, their biological activity can be attributed to the lipophilicity of the dimers of styrene oxides [6, 7].

Table 1. Antibacterial activities of the prepared cyclodimers of styrene oxides.

Compounds	Bacteria and minimum inhibitory quantity (MIQ) in µg			
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
Trans-**3**	50	50	10	10
6a	10	0.5	10	10
6b	10	10	50	100
6c	10	0.5	10	10
6d	10	0.5	10	10
9	10	10	10	10
12a	10	10	10	10
12b	50	10	10	50
12c	50	10	0.5	10
Chloramphenicol	0.001	0.001	0.001	0.001

Results are means of three replicates measurements.

We have demostrated that the acid-mediated cyclodimerizations of styrene oxides can be directed to either dioxanes or dioxolanes by the nature of the substituent(s) on the aromatic ring. The resulting cyclodimers exhibited antibacterial activities against both *Gram*-positive and *Gram*-negative bacteria. Further work is on-going in our laboratory on the cyclodimerizations of epoxides of 1,2-diphenylethene derivatives, vinylfurans and vinylpyridines.

EXPERIMENTAL

General procedure for the reaction of m-CPBA and derivatives of styrene. *m*-CPBA (2 mmol) in dichloromethane (50 mL) was added slowly over a period of 10 min to a stirred and cooled (0 ^{o}C) mixture of the styrene substrate (1 mmol) and $NaHCO_3$ (3 mmol) in dichloromethane (50 mL). The reaction mixture was stirred at room temperature for 2 hours and then saturated $NaHCO_3$ (60 mL) was added. The organic layer was removed. The aqueous phase was extracted with dichloromethane (3 x 30 mL) and the organic fractions were combined, dried ($MgSO_4$) and concentrated.

5a. Clear oil, 0.73 g, 82%; δ_H (300 MHz, $CDCl_3$): 2.38 (3H, *s*, 4-Me), 2.83 (1H, *dd*, *J* = 2.4, 5.7 Hz, H-8a), 3.17 (1H, *dd*, *J* = 4.2, 5.4 Hz, H-8b), 3.88 (1H, *dd*, *J* = 2.7, 3.9 Hz, H-7), 7.21 (4H, *br s*, H-2, 3, 5 and 6). δ_C (75 MHz, $CDCl_3$): 51.1 (C-8), 52.4 (C-7), 125.5 (C-2 and 6), 129.2 (C-3 and 5), 134.5 (C-1) and 137.9 (C-4). HR-TOF EIMS found M^+, 134.0737. $C_9H_{10}O$ requires 134.0732.

5b. Clear oil, 0.41 g, 82%; δ_H (300 MHz, $CDCl_3$): 2.84 (1H, *dd*, *J* = 2.4, 5.7 Hz, H-8a), 3.17 (1H, *dd*, *J* = 3.9, 6.3 Hz, H-8b), 3.87 (1H, *dd*, *J* = 2.4, 3.9 Hz, H-7), 7.16 (2H, *br s*, H-4 and 6), 7.27 (2H, *m*, H-2 and 3). δ_C: 51.1 (C-8), 52.4 (C-7), 122.7 (C-2), 126.1 (C-6), 128.4 (C-4), 128.9 (C-3), 137.5 (C-1) and 138.2 (C-5). HR-TOF EIMS found M^+, 134.0737. $C_9H_{10}O$ requires 134.0732.

Typical procedure for the acid-mediated cyclodimerization reaction. H_3PO_4 (0.20 mL, 0.86 mol) was added drop-wise to styrene epoxide **5a** (0.71 g, 4.27 mmol) and the mixture was stirred at room temperature for 120 minutes. Saturated $NaHCO_3$ (20 mL) was added and the mixture was extracted with dichloromethane (2 x 15 mL) and the organic fractions were combined, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was subjected to flash chromatography eluting with petroleum ether/ethyl acetate (PE; 4:1) to give a clear gum **6a**.

6a. Colourless gum, 0.59 g, 83%; ν_{max} (KBr) 2922, 2855, 1605, 1280, 1251, 1115, 1022, 831, 744 cm^{-1}; δ_H: 2.26 (6H, *s*, Me-4", 4'), 3.83 (2H, *dd*, *J* = 4.2, 7.8 Hz, H-3a, 6a), 3.96 (2H, *dd*, *J* = 4.2, 7.8 Hz, H-3b, 6b), 5.99 (2H, *dd*, *J* = 4.2, 7.5 Hz, H-2, 5), 7.12 (2H, *d*, *J* = 7.8 Hz, H-2", 6", 2', 6') and 7.26 (6H, *d*, H-3", 5", 3', 5'). δ_C: 21.1 (Me-4´,4´´), 65.9 (C-3, 6), 77.8 (C-2, 5), 126.6 (C-2", 6", 2', 6'), 129.4 (C-3", 5", 3', 5'), 133.7 (C-1", 1'), 138.5 (C-4", 4'). HR-TOF EIMS found M^+, 268.0529. $C_{18}H_{20}O_2$ requires 268.1463.

6b. Colourless gum, 0.29 g, 76%; ν_{max} (KBr): 2922, 2855, 1605, 1280, 1251, 1115, 1022, 831, 744 cm^{-1}.δ_H: 2.41 (6H, *s*, Me-3", 3'), 4.46 (2H, *dd*, *J* = 3.6, 8.1 Hz, H-3a, 6a), 4.55 (2H, *dd*, *J* = 3.6, 8.1 Hz, H-3b, 6b), 5.11 (2H, *dd*, *J* = 3.6, 8.1 Hz, H-2, 5), 7.16 (2H, *d*, *J* = 6.9 Hz, H-2', 2") and 7.29 (6H, *br s*, H-4", 5", 6", 4', 5', 6'). δ_C: 21.4 (Me-3´,3´´), 70.1 (C-3, 6), 72.6 (C-2, 5), 123.2 (C-6", 6'), 126.8 (C-5", 5'), 128.5 (C-4", 4'), 129.1(C-2", 2'), 138.4 (C-1", 1'), 139.7 (C-3", 3'). HR-TOF EIMS found M^+, 268.0529. $C_{18}H_{20}O_2$ requires 268.1468.

The epoxidation procedure directly led to formation of dioxanes **6c**, **6d** and **9** without the isolation of epoxides **5c**, **5d** and **8**.

6c. Yellow gum, 0.34 g, 85%; ν_{max} (KBr): 2923, 2856, 1606, 1290, 1251, 1118, 1029 cm^{-1}; δ_H (300 MHz, $CDCl_3$): 3.71 (6H, *s*, 2 x MeO), 3.81 (2H, *dd*, *J* = 12.1 Hz, 3.9 Hz, H-3a, 6a), 3.94 (2H, *dd*, *J* = 12.1 Hz, 7.8 Hz, H-3b, 6b), 6.05 (2H, *dd*, *J* = 7.8 Hz, 3.9 Hz, H-2, 5), 6.83 (4H, *d*, *J* = 8.7 Hz, H-2', 6', 2'', 6''), 7.33 (4H, *d*, *J* = 8.7 Hz, H-3', 5', 3'', 5''); δ_C (75 MHz, $CDCl_3$): 55.2 (2 x MeO), 65.5 (C-3, 6), 77.6 (C-2, 5), 114.1 (C-2', 6', 2'', 6''), 128.2 (3', 5', 3'', 5''),

129.1 (1', 1''), 159.7 (4', 4''). HR-TOF EIMS found M^+, 300.1435. $C_{18}H_{20}O_4$ requires 300.1362.

6d. Brown gum, 0.53 g, 96%; ν_{max} (KBr): 2914, 2829, 1593, 1456, 1247, 1134, 1020, 806, 746 cm^{-1}; δ_H (300 MHz, $CDCl_3$): 3.82 (6H, *s*, 2 x MeO), 3.85 (6H, *s*, 2 x MeO), 3.87 (2H, *s*, H-3a, 6a), 4.00 (2H, *dd*, *J* = 12.1 Hz, 8.1 Hz, H-3b, 6b), 6.01 (2H, *dd*, *J* = 8.1 Hz, 3.9 Hz, H-2, 5), 6.81 (2H, *d*, *J* = 8.1 Hz, H-5', 5''), 6.94 (2H, *d*, *J* = 1.8 Hz, H-2', 2''), 6.96 (2H, *dd*, *J* = 8.1 Hz, 1.8 Hz, H-6', 6''); δ_C (75 MHz, $CDCl_3$): 55.8 (2 x MeO-4'), 55.9 (2 x MeO-3'), 65.6 (C-3, 6), 77.8 (C-2, 5), 110.1 (C-2', 2''), 111.2 (C-5', 5'), 119.2 (C-6', 6''), 129.4 (1', 1''), 148.9 (3', 3''), 149.1 (4', 4''). HR-TOF EIMS found M^+, 360.1559. $C_{20}H_{24}O_6$ requires 360.1573.

9. Colourless gum, 0.79 g, 96%; ν_{max} (KBr): 3076, 2933, 2829, 1573, 1290, 1257, 1199, 1082, 935, 750 cm^{-1}; δ_H (300 Hz, $CDCl_3$): 3.40 (6H, *s*, MeO-2, 5), 4.88 (2H, *d*, *J* = 5.4 Hz, H-3, 6), 6.11 (2H, *d*, *J* = 5.4 Hz, H-2, 5), 7.29-7.37 (6H, *m* H-3",4", 5", 3', 4', 5') and 7.44-7.52 (4H, *m*, H-2",6", 2' 6'). δ_C (75 MHz, $CDCl_3$): 57.8 (MeO-2, 5), 74.4 (C-3, 6), 100.8 (C-2, 5), 127.1 (C-2", 6", 2', 6'), 128.0 (C-4", 4'), (C-6", 6'), 128.3 (3", 5", 3', 5'), 138.5 (1", 1'). HR-TOF EIMS found M^+, 300.1179. $C_{18}H_{20}O_4$ requires 300.1362).

Procedure for antimicrobial testing. The antimicrobial activities were evaluated using agar-overlay bioautography method. Stock solutions of 10 mg/mL of the test sample were prepared and serially-diluted to obtain concentrations of 5.0, 1.0, 0.05, 0.001 mg/mL. An aliquot of 10 µL of each concentration was spotted on Merck pre-coated silica gel 60 HF_{254} TLC plates (0.25 mm thickness; 10 cm x 10 cm), corresponding to loading quantities of 100, 50, 10, 0.5 and 0.01 µg. The spots were of the same size and the solvent was allowed to evaporate in the fume hood. The following microorganisms obtained for the Department of Biological Sciences, University of Botswana were used: *Staphylococcus aureus* (ATCC 9144), *Escherichia coli* (ATCC 11229), *Bacillus subtilis* (ATCC 6633) and *Pseudonomas aeruginosa* (NCTC 10332). 3.4.0. The seed layers were prepared by inoculating 10 mL aliquot of culture into 100 mL agar solution. Using a sterile Pasteur pipette the TLC plates were overlaid with the agar. Plates were run in duplicates. After solidification of the medium the TLC plates were incubated at 37 ^oC (*S. aureus*, *E. coli*) and 25 ^oC (*B. subtilis*, *P. aeruginosa*) for 24 h. The bio-autograms were sprayed with an aqueous solution of thiazoyl blue (methylthiazolyltetrazolium bromide; 200 mg in 100 mL distilled water) and further incubated for 4 hours after which results were scored. White spots against purple background indicated inhibition zones. The minimum inhibitory quantity (MIQ in µg) was taken as the lowest loading quantity to exhibit an inhibition zone.

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