Asian Journal of Chemistry Vol. 18, No. 3 (2006), 2285–2298

Novel Synthesis and Antimicrobial Activity of 3,7-Dimethyl phenoxathiin Nucleus and Some Related Analogues

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The synthesis of important precursor to biologically active compounds, 3,7-dimethyl phenoxathiin, is described using a new approach involving aromatic dihydroxy ditolyl sulphone intermediate. Synthesis of the same target in addition to some mono and dimethyl substituted phenoxathiin derivatives by alternative methods is also reported. The antibacterial and antifungal properties of all prepared substituted methyl phenoxathiins and their oxidized derivatives have been evaluated against assortment of micro-organisms. The oxidized derivatives of the methyl and the dimethyl phenoxathiins showed weak *in-vivo* antifungal activity, whereas the non-oxidized derivatives showed negligible antimicrobial activity.

Key Words: Synthesis, 3,7-Dimethyl phenoxathiin, 3,7-Dimethyl phenoxathiin-10-oxide, 3,7-Dimethyl phenoxathiin-10,10-dioxide, Dihydroxyditolyl sulphones, Antimicrobial activity.

INTRODUCTION

Synthesis of phenoxathiin nucleus and substituted derivatives is of current interest to many researchers because they exhibit a broad spectrum of biological activity and applications. Many have been developed as fungicides or plant growth regulators upon side chain modification[1-3]; some have antibacterial properties[4]; others exhibit antitumour activity[5] and yet others may be useful as antidepressants[6]. Phenoxathiins may also be used as catalysts to conduct chemical reactions[7] and may be used to form new types of polymers with pharmaceutical or industrial applications[8]. Upon reviewing the literature to investigate dimethyl substituted phenoxathiin, it has been noticed that most of the biologically targeted modifications were carried out on positions 1, 2, 8 and 9, either through sulfurization of methyl substituted diphenyl ethers or lithiation of non-substituted phenoxathiin. Only few hits have indicated methyl groups at position 3 or/and 7. This was due to the difficulty faced when mono or dimethyl substituents were prepared using the above approaches. Therefore, and since we are interested in

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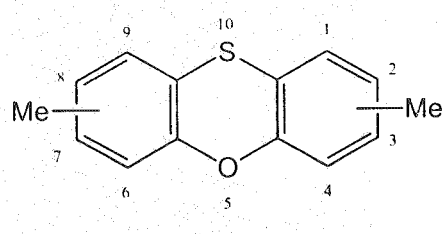
preparing natural alkaloids using dimethyl groups on the phenoxathiin nucleus, a new approach was developed in this work to prepare 3,7-dimethyl and monomethyl phenoxathiin. Alternative approaches to prepare the target nucleus were attempted, in addition to the preparation of other dimethyl substituents for biological screening purposes.



Phenoxathiin nucleus

EXPERIMENTAL

4-Bromo-3-methylanisole, cuprous oxide, dimethyl acetamide, anhydrous aluminium chloride and sulphur were purchased from Acros. *m*-Cresol, *m*-bromotoluene, DIBAl-H, dioxane and carbon disulphide were purchased from Aldrich. Other chemicals and reagents were obtained through local agents.

Melting points were determined using capillary tubes on SMP1 Stuart Scientific melting point apparatus and are expressed in degrees centigrade (°C), (uncorrected). Infrared spectra (IR) were determined on Avatar 370 FT-IR Thermo Nicolet spectrophotometer. High resolution mass spectra (MS) were determined on a Jeol DX303 spectrometer for electron impact (+ EI) analysis and fast atom bombardment (FAB) (Strathclyde University, UK). Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were determined on Bruker WM 250 (250 MHz), Jeol EX-270 (270 MHz), and Bruker AMX-400 (400 MHz) spectrometers (University of Strathclyde, UK). Other samples were recorded on a Bruker DPX-300 spectrometer at the University of Jordan, Amman. Trimethyl silane (TMS) was used as internal reference and all solvents used were deuterated. Most samples were run using $CDCl_3$, otherwise as indicated. Microanalyses were performed using Euro EA Elementak Analyzer for Euro Vector Instruments and Software, at the Faculty of Pharmacy, Jordan University.

Approach A: 3,7-Dimethyl phenoxathiin nucleus

3,3′-Dimethyl diphenyl ether (*m*-tolyl ether, 1): The phenoxide was prepared from *m*-tolyl phenol (100 g, 0.924 mol) and sodium hydroxide (37 g, 0.924 mol). The *m*-cresol was dissolved in methanol (50 mL), then added to a solution of NaOH (85% methanol/water 200 mL). The solvent was then removed at 60°C under reduced pressure. Toluene was added and the solvent was removed *in vacuo* at 60°C. This process was repeated several times and then the solid material was dried *in vacuo* at room temperature for three days to give the sodium salt in 95% yield after drying.

(A) The dried product (15.6 g, 0.1198 mol), *m*-bromo toluene (20.5 g, 0.1198 mol), copper bronze powder (0.15–0.2 g) and *m*-cresol (17 g) were slowly heated in a dried flask fitted with a reflux condenser. The reaction commenced at 200°C

with the separation of sodium bromide. The heating was continued overnight at 180–190°C. The dark product was cooled, then made alkaline with aqueous NaOH and purified using fractional distillation. Unchanged bromo toluene passed over, followed by a colourless oil which was collected. The oil was further purified on a flash silica gel column using *n*-hexane as mobile phase. The remaining bromo toluene passed first, then the diphenyl ether was collected as a colourless oil (11.4 g, 48%).

(B) Cuprous oxide Cu_2O (27.47 g, 0.192 mol), *m*-bromo toluene (65 g, 0.380 mol) and the dried sodium phenoxide (25 g, 0.192 mol) were dissolved in dimethyl acetamide (25 mL), then heated under reflux for 72 h in a nitrogen atmosphere. The reaction was commenced under nitrogen with stirring. Diluted hydrochloric acid (20%, v/v, 200 mL) was added and the precipitated product was isolated by ethyl acetate extraction. The crude product was further purified on a silica gel column. The first fraction contained *m*-bromo toluene, whereas the second contained *m*-tolyl ether which solidified upon standing (12.5 g, 33%). Using excess phenoxide (1.1 M) improved the yield to 62%. ^{1}H NMR 250MHz δ ($CDCl_3$): 2.38 (6H, s, —CH_3), 6.86–6.89 (4H, m, Ar-H), 6.95 (2H, d, d, J = 8.1, 1.5 Hz, Ar-H), 7.24 (2H, d, d, J = 8.3, 8.2 Hz, Ar-H). MS EI m/z (%): 198.10442 (M^+, 100). Calcd. 198.10447, $C_{14}H_{14}O$. IR (NaCl, cm^{-1}): 3031, w, ν(aromatic C—H *str.*), 2921, w, ν(aliphatic C—H *str.*), 1486, s, ν(aromatic C=C *str.*), 1585, s, ν(aromatic C=C *str.*), 1259, s, ν(diaryl ether C—O—C *str.*), 777, s, ν(aromatic C—H *def.*), 688, s, ν(aromatic, meta substituted, C—H *def.*). TLC *n*-hexane: R_f = 0.36.

3,7-Dimethyl phenoxathiin (2): *m*-Tolyl ether (4.75 g, 0.024 mol) was added to ground aluminum chloride (2.35 g, 0.0176 mol) and sulphur (0.81 g, 0.0253 mol). The mixture was heated to 100°C using an oil bath and reflux condenser. Stirring was continued overnight. The crude solution was poured into iced hydrochloric acid solution (30%, v/v, 200 mL), stirred vigorously to dissolve the product and then extracted with ether (3 × 250 mL). The organic layer was dried over anhydrous magnesium sulphate, filtered and reduced *in vacuo* to give green oil. The oil was purified using Kogelrohr distillation. *m*-Tolyl ether distilled between 110–130°C at 0.3–0.4 mm Hg, while the product distilled as yellow oil between 140–160°C at 0.3–0.4 mm Hg (2.03 g, 37%). The yellow oil obtained was crystallized from methanol to give pure white crystals of 3,7-dimethyl phenoxathiin (0.58 g, 11%). ^{1}H NMR 400 MHz δ ($CDCl_3$): 2.29 (6H, s, —CH_3), 6.81–6.83 (4H, m, Ar-H), 6.96 (2H, d, J = 7.6, Ar-H).^{13}C NMR 400 MHz δ ($CDCl_3$): 152.3, 138.1, 126.5, 125.3, 118.6, 116.9, 21.2. MS EI m/z (%): 228.0606 (M^+, 100). Calcd. 228.0608, $C_{14}H_{12}OS$. EI m/z (%): 228 (M^+, 100), 213 (6), 195 (40). MA Found: C 73.31, H 5.24, S 13.35; Calcd.: C 73.65, H 5.29, S 14.04. IR (KBr, cm^{-1}): 3043, w, ν(aromatic C—H *str.*), 2913, w, ν(aliphatic C—H *str.*), 1477, s, ν(aromatic C=C *str.*), 1250, s, ν(diaryl ether C—O—C *str.*), 803 and 740, s, ν(aromatic C—H *def.*). TLC *n*-hexane, R_f = 0.40, toluene: R_f = 0.81; m.p. 102–104°C

Oxidation of the product was noticed through changes in the colour of the crude mixture upon heating. Two oxidized products were isolated by flash chromatography on silica gel, Kieselgel 60, Merck, 0.040–0.063 using toluene as

mobile phase, followed by ethyl acetate. The first fraction was identified as 3,7-dimethyl phenoxathiin-10-oxide (**3**). The second product was identified as 3,7-dimethyl phenoxathiin-10,10-dioxide (**4**).

3,7-Dimethyl phenoxathiin-10-oxide (3): 3,7-Dimethyl phenoxathiin (2.80 g, 12.3 mmol) was dissolved in a mixture of ethanol (100 mL) and hydrogen peroxide (30%, 50 mL). The reaction mixture was heated under reflux overnight. An additional amount of H_2O_2 (20 mL) was added and the reflux continued for further 3 h. The reaction mixture was left to cool to room temperature; then the solvent was reduced *in vacuo* until dryness. The white solid obtained was dissolved in toluene and applied to a silica gel column using toluene as eluent. Fraction (1) had R_f 0.17 and was identified as sulphone (yield 0.300 g). Ethyl acetate was then used to elute the sulphoxide, which was obtained as white crystalline material (1.334 g, 44.5%). ^{1}H NMR 250 MHz δ ($CDCl_3$): 2.33 (6H, s, —CH_3), 6.98 (2H, d, J = 1.9 Hz, Ar-H), 7.10 (2H, d,d, J = 1.9, 8.0 Hz, Ar-H), 7.54 (2H, d, J = 8.0Hz, Ar-H). MS EI m/z (%): 244.05473 (M^+, 100). Calcd. 244.05580, $C_{14}H_{12}O_2S$. IR (KBr, cm^{-1}): 3015, br, ν(aromatic C—H *str.*), 2920, w, ν(aliphatic C—H *str.*), 1458, s, ν(aromatic C=C *str.*), 1261, s, ν(Ar—O—Ar *str.*), 1131, s, ν(S=O *str.*). TLC toluene, R_f = 0.0; m.p. 140–142°C.

3,7-Dimethyl phenoxathiin-10,10-dioxide (4): 3,7-Dimethyl phenoxathiin (8.0 g, 0.035 mol) was suspended in glacial acetic acid (60 mL) and aqueous hydrogen peroxide (30%, 60 mL). The suspension was heated to reflux and left stirring for 48 h. An extra 20 mL of hydrogen peroxide solution was added to the reaction mixture and the solution was heated for a further 3 h. The solution was diluted with iced water (30 mL) to give a white precipitate. The mixture was filtered to yield sulphone as a white solid (8.3 g, 91%). ^{1}H NMR 250 MHz δ ($CDCl_3$): 2.35 (6H, s, —CH_3), 6.95 (2H, d, J = 1.8 Hz, Ar-H), 7.19 (2H, d, d, J = 1.8, 8.0 Hz, Ar-H), 7.75 (2H, d, J = 8.0 Hz, Ar-H). MS EI m/z (%): 260.05183 (M^+, 41). Calcd. 260.05072, $C_{14}H_{12}O_3S$. IR (KBr, cm^{-1}): 3063, w, ν(aromatic C—H *str.*), 2923, w, ν(aliphatic C—H *str.*), 1483, s, ν(aromatic C=C *str.*), 1265, s, ν(Ar—O—Ar *str.*), $1366_{antisym}$, s, ν(S=O str.), 1147_{sym}, s, ν(S=O *str.*). TLC Toluene: R_f = 0.17; m.p. 187–189°C.

Approach B

5-Bromo-2-methoxy-4-methyl benzene sulfonyl chloride (6): 4-Bromo-3-methyl anisole (**5**) (12 g, 0.0597 mol) was placed in a 250 mL round bottom flask and stirred at –5°C. Chlorosulfonic acid ($ClSO_3H$, 34.77 g, 0.298 mol) was added to the flask dropwise. The reaction mixture was stirred for 1 h at 0.0°C, then poured on to crushed ice to produce a white solid. The product was filtered and collected as a white solid (15.50 g, 87%). The solid was then recrystallized from ethanol to give the pure white product (14.42 g, 81%). The yield increased upon increasing the time and using fresh chlorosulfonic acid up to 90%. ^{1}H NMR 250 MHz δ ($CDCl_3$): 2.47 (3H, s, —CH_3), 4.04 (3H, s, —OCH_3), 7.0 (1H, s, Ar-H), 8.08 (1H, s, Ar-H). MS EI m/z (%), M^+: 301.9051 (27.5). Calcd. 301.90165, $C_8H_8O_3^{37}Cl^{81}$ br-s. 299.9065 (100). Calcd. 299.90366, $C_8H_8O_3^{37}Cl^{79}$ br-s. 299.9065 (100). Calcd. 299.90461, $C_8H_8O_3^{35}Cl^{81}$br-s. 297.9097 (71). Calcd. 297.90662, $C_8H_8O_3^{35}Cl^{79}BrS$. IR (KBr, cm^{-1}): 3102, w, ν(aromatic C—H *str.*), 2944, w,

ν(aliphatic C—H *str.*), 1471, s, ν(aromatic C=C *str.*), 1261, s, ν(alkyl aryl ether C—O—C *str.*), $1375_{antisym}$ and 1150_{sym}, s, ν(—SO_2—, *str.*); TLC toluene: $R_f = 0.72$; m.p. 126–128°C.

2,2′-Dimethoxy-4,4′-dimethyl-5-5′-dibromodiphenyl sulfone (7): 1-Methoxy-3-methyl-4-bromo benzene (**5**), (4 g, 0.0198 mol) and 5-bromo-2-methoxy-4-methyl benzene sulfonyl chloride (**6**) (4.5 g, 0.015 mol) were dissolved in 25 g of carbon disulphide (25 g, 0.328 mol). Then several portions of aluminium chloride (3 g, 0.022 mol) were added. The reaction mixture was left at 60–70°C and refluxed overnight. The reaction mixture was heated for 2 h and the CS_2 was evaporated under vacuum. The crude product was added to a mixture of ice and sodium chloride, and the precipitate was filtered using a Büchner funnel (7.36 g). The product was washed with water, 50 mL ether and crystallized from ethanol to give a white solid upon filtration and drying (6.45 g, 93%). ^{1}H NMR 250 MHz δ ($CDCl_3$): 2.43 (6H, s, —CH_3), 3.72 (6H, s, —OCH_3), 6.76 (2H, s, Ar-H), 8.26 (2H, s, Ar-H). MS EI m/z (%), M^+: 461.9125 (33). Calcd. 461.91363, $C_{16}H_{16}O_4^{79}Br^{79}$ br-s. 463.9085 (66). Calcd. 463.91162, $C_{16}H_{16}O_4^{79}Br^{81}$ br-s. 465.9095 (36). Calcd. 465.90962, $C_{16}H_{16}O_4^{81}Br^{81}$BrS. IR (KBr, cm^{-1}): 1465, s, ν(aromatic C=C *str.*), 1245, s, ν(alkyl aryl ether C—O—C *str.*), 1370_{antsym} and 1140_{sym}, s, ν(sulfonyl halide, —SO_2—, *str.*). TLC ethyl acetate: $R_f = 0.86$; m.p. 300–304°C.

2,2′-Dimethoxy-4,4′-dimethyl diphenyl sulfone (8): 2,2′-Dimethoxy-4,4′-dimethyl-5-5′-dibromo diphenyl sulfone (2.09 g, 0.0045 mol) was added to 50 mL of an alcoholic solution of potassium hydroxide (20%, w/v), in a 3-necked round bottomed flask. Anhydrous zinc powder (0.88 g, 0.014 mol, 3.1 M excess) was added and the mixture was refluxed for 10 h at 100–110°C. The product was filtered off and diluted with water to furnish white crystals after several hours. The white solid was filtered off and recrystallized from 50% ethanol (0.41 g, 30%). Although the yield was very poor, it was improved by using zinc in excess, nickel bromide as a catalyst and increasing the reflux time for 3 d (1.28 g, 93%). ^{1}H NMR 250 MHz δ ($CDCl_3$): 2.40 (6H, s, —CH_3), 3.73 (6H, s, —OCH_3), 6.76–6.79 (4H, m, Ar-H), 8.28 (2H, d, J = 7.8, Ar-H). MS EI m/z (%): 306.09284 (M^+, 100). Calcd. 306.09258, $C_{16}H_{18}O_4S$. IR (KBr, cm^{-1}): 1455, s, ν(aromatic C=C *str.*), 1240, s, ν(alkyl aryl ether C—O—C *str.*), 1370_{antsym} and 1140_{sym}, s, ν(—SO_2—, *str.*). TLC ethyl acetate: $R_f = 0.56$; m.p. 198–199°C.

2,2′-Dihydroxy-4,4′-dimethyl diphenyl sulfone (9):

(A) A mixture of 2,2′-dimethoxy-4,4′-dimethyl diphenyl sulfone (**8**) (4.0 g, 0.0131 mol), concentrated hydrochloric acid solution (20 mL) and absolute ethanol (30 mL) was heated at 80°C overnight in a 3-necked round bottom flask. The mixture was kept stirring overnight under nitrogen atmosphere. Water (20 mL) was added carefully and ethanol was evaporated under reduced pressure. 50 mL of 10% solution of NaOH was added and the residue was extracted with chloroform. The organic layer was collected, boiled with charcoal and filtered. The filtrate was then dried using anhydrous magnesium sulphate, filtered and reduced *in vacuo* to give a yellow semi-solid gum (3.5 g, 96%). Crystallization from methanol gave the product as white solid crystals (3.1 g, 85%).

(B) In a round bottom flask, a mixture of compound (**8**) (4.0 g, 0.0131 mol),

50 mL of xylene and of $AlCl_3$ (5.5 g, 0.041 mol, 3.1 M excess) was placed. After refluxing the mixture for 5 h, concentrated HCl (20 mL) was added, dissolved in crushed ice and the mixture was stirred overnight. The xylene layer was taken while hot, filtered to remove any impurities, then left to cool down at room temperature. Part of the product crystallized while cooling as yellow precipitate. The organic layer was then treated with a solution of NaOH, petroleum ether and charcoal. Then it was boiled, filtered, cooled and treated with HCl, then the product was collected upon crystallization. Recrystallization of the product from methanol gave the compound as white crystalline solid in 55% (2.0 g) yield. ^1H NMR 250 MHz δ ($CDCl_3$): 2.38 (6H, s, —CH_3), 5.17 (2H, br-s, OH, D_2O exch.), 6.70–6.81 (4H, m, Ar-H), 8.15 (2H, d, J = 7.8, Ar-H). MS EI m/z (%): 278.06251 (M^+, 75). Calcd. 278.06128, $C_{14}H_{14}O_4S$. IR (KBr, cm^{-1}): 3291, broad, ν(O—H *str.*), 3063, w, ν(aromatic C—H *str.*), 2935, 2848, m, ν(aliphatic C—H *str.*), 1455, s, ν(aromatic C=C *str.*), 1370_{antsym} and 1140_{sym}, s, ν(—SO_2—, *str.*). TLC DCM: R_f = 0.13, hexane: R_f = 0.05; m.p. 196–198°C.

3,7-Dimethyl phenoxathiin-10,10-dioxide (sulphone 4): In a round bottom flask, 2,2′-dihydroxy-4,4′-dimethyl diphenyl sulfone (5.0 g, 0.0179 mol) was triturated with cold concentrated sulphuric acid and the mixture was left stirring at room temperature for 5 h. Next, it was carefully poured into cold iced water and the muddy precipitate formed was collected and washed thoroughly with 100 mL of 20% NaOH solution and water to remove unreacted sulphuric acid. The solid product was then dissolved in chloroform and dried with magnesium sulphate, filtered and collected. NMR sample of the crude product indicated that the product contains mainly the sulphone (**4**), with minor amounts of the sulphoxide (**3**) and the sulphide phenoxathiin (**2**). This was deduced based on the chemical shifts and ratios for proton numbers 1 and 9. Finally, it was crystallized from dilute methanol (85%) to give the sulphone (**4**) as white solid (4.1 g, 88%).

3,7-Dimethyl phenoxathiin (sulphide 2)

(A) 3,7-Dimethyl phenoxathiin-10,10-dioxide as crude from last experiment (4.1 g, 0.0158 mol) was placed in a three-necked round bottom flask, fitted with guard column and condenser. 50 mL of glacial acetic acid and zinc powder (4 g, 0.061 mol, 3.87 M) were added to the flask and the mixture was left stirring at reflux for 10 h. Excess zinc was removed by filtration and the filtrate was then triturated with 100 mL of water. The oily material formed was extracted with dichloro methane (2 × 100 mL) and the organic layer was dried, filtered and reduced to obtain a colourless oil which solidified at room temperature. This material was then crystallized from methanol to obtain white solid crystals (2.2 g, 61%).

(B) Dioxane (100 mL, dry, distilled) was placed in a three-necked round bottomed flask. Diisobutyl aluminium hydride (DIBAL-H, 1.0M in toluene, 130 mL, 0.1294 mol, 15 M) was added dropwise over 1 h under nitrogen flow passing through a dririte apparatus. 3,7-Dimethyl phenoxathiin-10,10-dioxide (2.25 g, 8.64 mmol, dissolved in dioxane (20 mL)) was then added to the stirring mixture dropwise over 30 min. The solution was heated to reflux (130°C) for 5 d. The reaction mixture was left to cool to room temperature and water was added

dropwise under nitrogen until the effervescence ceased. The toluene/dioxane organic layer was collected, reduced, diluted with aqueous NaOH solution, then extracted with $CHCl_3$ (3×200 mL). The aqueous layer was basified with concentrated NaOH, then extracted with $CHCl_3$ (2×150 mL). The chloroform extracts from both layers were combined, dried over $MgSO_4$, filtered and reduced *in vacuo* to give a pale yellow oily material (2.2 g). The oil was crystallized from a small amount of methanol overnight to give the product after filtration as white crystals (1.85 g, 94%). NMR data showed a very pure spectrum with no peaks for other derivatives. TLC indicated one spot only with R_f value of 0.81 from toluene.

Reduction of 2,2′-dihydroxy-4,4′-dimethyl diphenyl sulfone (9) to give the sulphide (2). The reduction procedure employed was similar to procedure B above utilizing dioxane, DIBAL-H, 1.0M in toluene. This procedure gave the crude product which was cyclized directly to give the sulphide **2**, but in low yields.

2,8-Dimethyl phenoxathiin nucleus

4,4′-Dimethyl diphenyl ether (*p*-tolyl ether, 10): This compound was prepared according to the reported literature method[12–14, 29].

2,8-Dimethyl phenoxathiin (11): This compound was prepared from (**10**) according to the reported literature method[12–14, 28–29].

2,8-Dimethyl phenoxathiin-10-oxide (12): This compound was prepared from (**11**) according to the reported literature method[12, 28, 29].

2,8-Dimethyl phenoxathiin-10,10-dioxide (13): This compound was prepared from (**11**) according to the reported literature method[14, 29].

2-Methyl phenoxathiin (14): This compound was prepared following the similar procedure used for (**10**) and (**11**), starting from *p*-cresol and benzene.

3-Methyl phenoxathiin (15): This compound was prepared following the procedure used for (**10**) and (**11**), starting from *m*-cresol and benzene. The yield was very poor and isomeric side products were isolated.

Antimicrobial activity tests

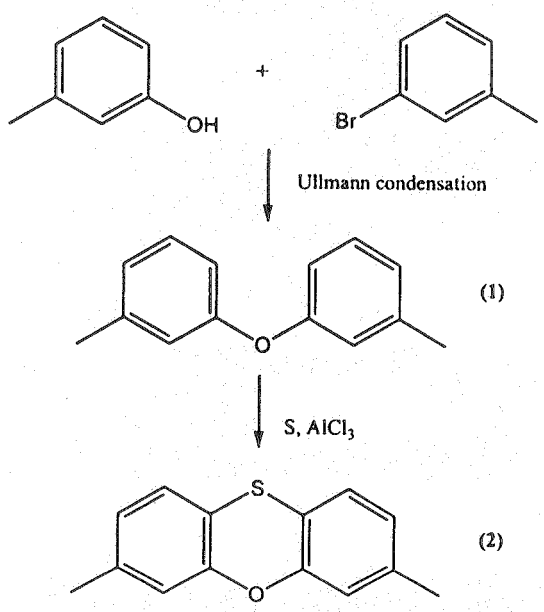
The MICs were determined by the conventional broth dilution method using two serial dilution technique. The standardization of bacterial test suspension was carried out according to McFarland standard method. Stock solutions of the test compounds were prepared using DMSO. Serial dilutions were prepared to obtain test concentrations ranging from 156–0.3 $\mu g/cm^3$. Each tube was then inoculated with 0.1 cm^3 of the cultured bacteria or fungi (containing *ca.* 1 to 2×10^8 CFU/cm^3), mixed and incubated at 37°C for 24 h. Growth inhibition with concentrations at 156 $\mu g/cm^3$ or lower were carried out in duplicate. All test tubes showing positive/negative growth were confirmed by the agar plate method. The results were recorded according to the presence and absence of growth. The MICs were calculated as the average concentration of the test agent in the broth tubes showing consecutive positive and negative growth.

RESULTS AND DISCUSSION

Dimethyl phenoxathiin nucleus is prepared through few known approaches. The most common approach involves Ullmann condensation reaction of suitably methyl substituted cresol and suitably methyl substituted bromo toluene to form diphenyl ethers[9–11]. Substituted dimethyl ether can be then heated with sulphur in presence of $AlCl_3$ to give the methyl substituted phenoxathiin[12–14]. The problems associated with this approach involve initially poor yields from Ullmann condensation, although some reported methods claimed 70 to 90% yield upon improving the conditions[4, 10, 11], production of the dimethyl phenoxathiin with very low yields upon cyclization and oxidation of the product at the sulphur atom furnishing the mono and dioxide side products in the crude mixture[14]. A second approach entails side chain substitution on previously prepared unsubstituted phenoxathiin utilizing Friedel-Craft's acylation[15] or lithiation[16] at the aromatic rings. Both methods encounter problems upon electrophilic aromatic substitution and orientation at the targeted site, in addition to very low yields. The last approach investigated was direct synthesis of methyl substituted phenoxathiins through diazotization and the Mauthner pathway[17, 18]. Like the aforementioned methods, long steps were required with very low yields. Moreover, none of these methods were reported, to the best of our knowledge, to prepare our target 3,7-derivative. Therefore, the aim of this work is to synthesis 3,7-dimethyl phenoxathiin as a precursor with better yields, since the difficulty in synthesis and low yields obtained made researchers lose interest in such intermediates. To the best of our knowledge, only few hits discuss their attempts to prepare this nucleus[19–22], with disappointing results.

Approach A

The first step in the preparation of the tricyclic 3,7-dimethyl phenoxathiin (**Scheme-1**) involves the preparation of *m*-tolyl ether (**1**). Synthesis of *m*-tolyl ether



Scheme-1. Preparation of 3,7-dimethyl phenoxathiin (Approach 1)

was achieved successfully by Ullmann condensation of *m*-cresol with 3-bromo toluene to give the *m*-tolyl ether (48%) as a colourless oil. This initial step produced very poor yields (20–30%) and the oily product was very difficult to purify. Many attempts were made to increase the yield. A series of reactions were performed using various copper species in different solvents to evaluate the most suitable conditions. Although many copper species (CuO, Cu, Cu_2O, CuCl, CuBr, $CuCl_2$) have been used, high yields were noticed for CuO, Cu_2O and Cu. Low yields were observed for CuBr, CuCl and $CuCl_2$. The best yield was obtained when using a combination of cuprous oxide (Cu_2O) and dimethyl acetamide (62%), and this figure was not reproducible. Although different solvents were employed in our experimental work, such as pyridine, collidine and phenols, the pronounced effect of dimethyl acetamide as a medium for the phenoxide reactions was noticed. This might be due to its ability to dissolve these salts. The best yields obtained when 5 Å molecular sieves was used or when azeotropic water removal was employed, **(Scheme-1).**

The next step in the preparation of 3,7-dimethyl phenoxathiin **(2)** involved heating excess *m*-tolyl ether, sulphur and aluminium chloride at 110°C. The reaction was expected to produce 3 major isomers in the ratio of 1 : 1 : 1 (Fig. 1). However, recrystallization of the distilled product from methanol gave only the 3,7-dimethyl phenoxathiin isomer in 10% yield. Oxidation of the crude product was

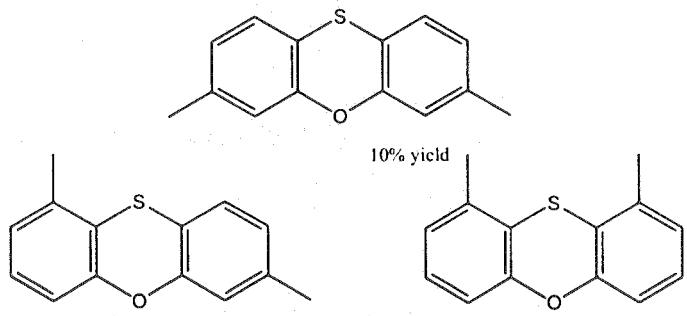
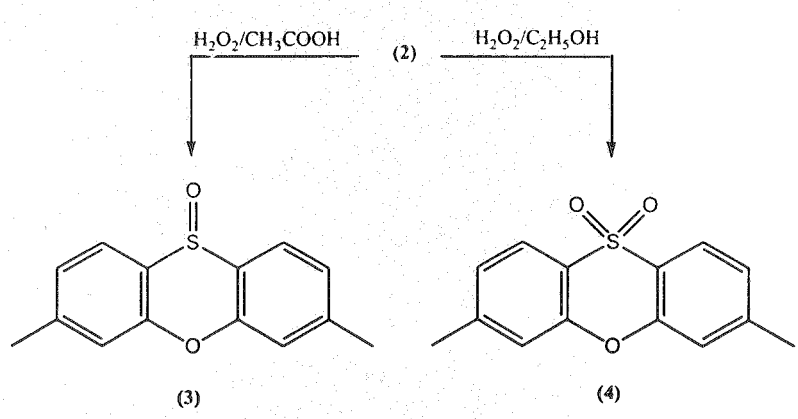


Fig. 1. Possible isomeric structures produced upon cyclization of *m*-tolyl ether

observed through changes in the colour of the crude mixture from green to orange upon distillation at high temperature. TLC (toluene) showed 2 new spots with R_f values of 0.17 and 0.0 respectively, in addition to the product **(2)**, which had an R_f value of 0.81. MS showed 2 peaks for m/z 244 and 260 (M + 16). This indicates that these compounds were 3,7-dimethyl phenoxathiin-10-oxide **(3)** and 3,7-dimethyl phenoxathiin-10,10-dioxide **(4)**. NMR of the crude mixture showed a mixture of compounds with a distinctive aromatic splitting for each oxidized product. These oxidative products were prepared, separated and identified as shown in the experimental part **(Scheme-2).**

Approach B

Very recently, it was brought to our attention that if there were an approach to prepare the dihydroxy mono or ditolyl sulphones instead of dimethyl diphenyl ether, it would be possible to dehydrate the product yielding the required methyl substituted phenoxathiin. Reviewing the literature for an alternative solution, a modification of an existing route for related *p*-cresol-*m*-sulphoxides was consi-

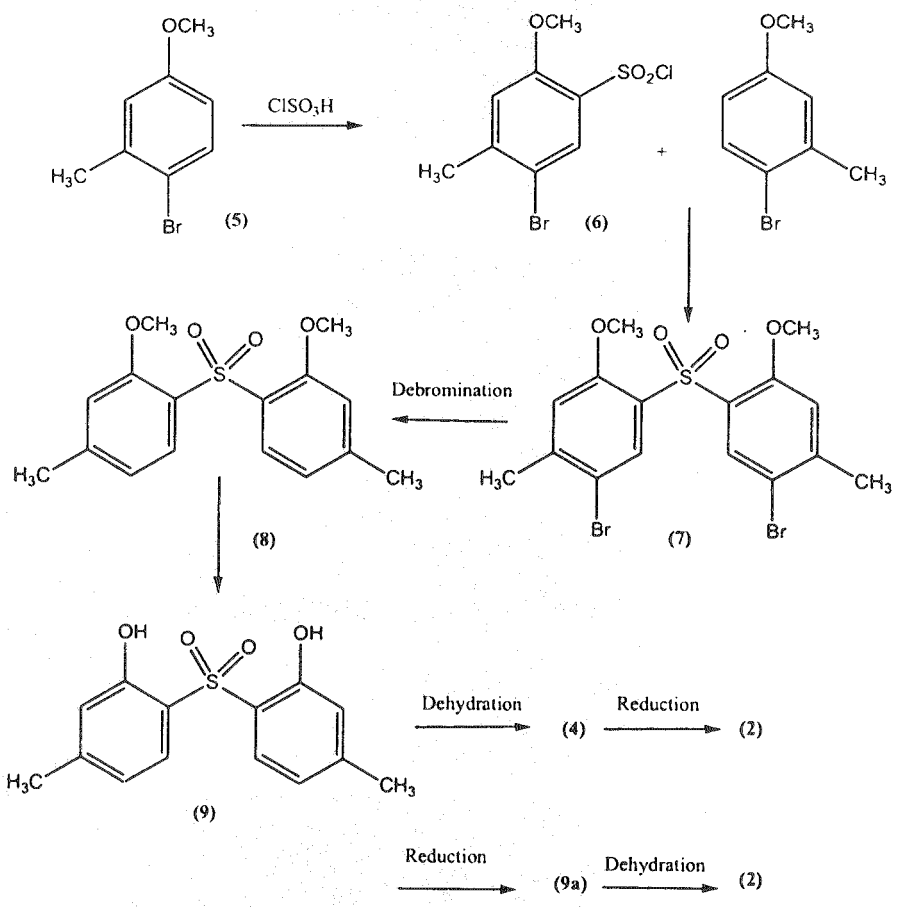


Scheme-2. Oxidation of 3,7-dimethyl phenoxathiin

dered[23, 24]. The intermediate bis(hydroxy tolyl)sulphone can be used to synthesize the 2,8-dimethyl phenoxathiin. Accordingly, it was postulated that if the intermediate 2,2′-dihydroxy-4,4′-dimethyl diphenyl sulphone (**9**) could be prepared, then the synthesis of 3,7-dimethyl phenoxathin could also be achieved successfully. This approach needs to start with protected sites on the starting material and therefore compound (**5**) was chosen as starting material (**Scheme-3**). This approach would avoid structural isomeric formation and it would also reduce the number of isomers produced from oxidation of the sulphur atom. However, reduction of the sulphone group would be required at a later stage.

This approach starts with the relatively cheap starting material: 4-bromo-3-methyl anisole (**5**). Compound (**5**) contains a methoxy group which is *ortho* and *para* directing group. The *para* position is blocked, while position 2 is sterically hindered. Therefore, the only free position is position 6, where the concentration of electron density leads to considerable reactivity in electrophilic substitution reactions.

Chloro sulfonation of (**5**) has produced compound (**6**) in high yield and at the desired position. This step was followed by treating the reactive product (**6**) with another unit of (**5**) to produce the sulphone (**7**) and in very high yield (93%). The following debromination step produced initially no product. Under the conditions reported, it produces (**8**) with very poor yield and only in one experiment. The results were not reproducible and do contradict the literature reports[23] regarding this compound. However, using excess zinc, nickel bromide as a catalyst and refluxing for longer period gave the product in very high yields (93%). Different conditions were attempted for removal of bromine, such as using sodium amalgam in methanol, $LiAlH_4$ and Grignard reaction, but the highest yield obtained was upon implementation of the above modified method. The demethylation step involved using different mineral acids: HCl, HI, HBr in absolute solvents. Only HCl gave the pure product in high yield. All other acids used gave a mixture of the product and other side products which were assumed to be the phenoxathiins (**2, 3, 4**). The dihydroxy sulphone was then treated with conc. H_2SO_4 to give mainly the phenoxathiin dioxide (**4**) in high yield (88%). Traces of the sulphoxide (**3**) and the sulphide (**2**) were present in the crude sample. At this stage, it was necessary to reduce the sulphone to the sulphide so that the yield would be further improved and one product would be obtained.



Scheme-3. New approach for preparation of 3,7-dimethyl phenoxathiin (Approach B)

It is widely recognized that reduction of sulphones is a difficult process. They usually require an excess of a powerful reducing agent such as $LiAlH_4$ in ethyl *t*-butyl ether, DIBAL-H in mineral oil, DIBAL-H in dioxane or DIBAL-H in toluene[25, 26]. A high temperature must also be employed.

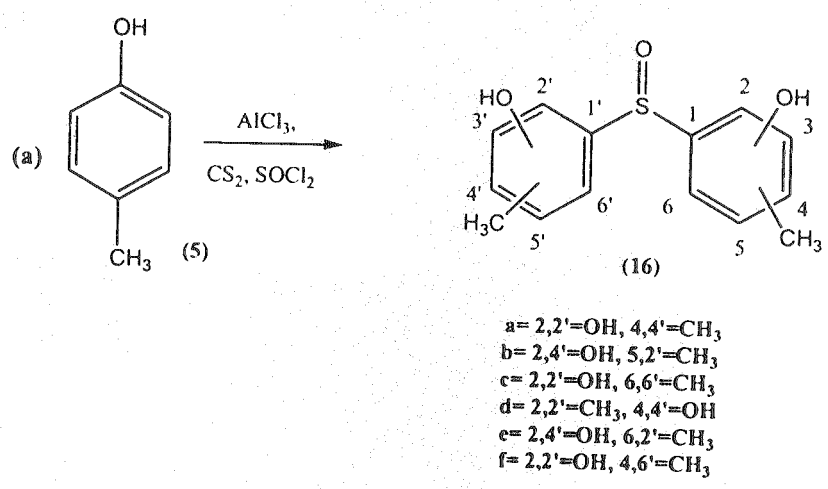
Researchers[26] have shown that sulphones can be reduced to sulphides if DIBAL-H is used as reducing agent. However, conducting such a reaction under harsh conditions (110°C) gave the sulphide as a minor product with side chain cleavage in which a C—O bond was broken. The best method found was the use of a large excess of DIBAL-H in refluxing dioxane. Gardner and co-workers[25] used 9 molar equivalents of DIBAL-H to the sulphone at reflux for 22 h. They claimed that the large excess of the reducing agent contributed to the high yield obtained, although only 2 equivalents of DIBAL-H were required in theory. Our attempts to reduce the sulphone (4) using the conditions reported did not achieve similar results and the sulphide (2) was detected in the crude mixture in a very low percentage. This might be due to either incomplete reduction or side chain cleavage. Many reactions were set up to improve the reduction conditions. It was observed that increasing the number of DIBAL-H equivalents and increasing the time of the reaction improved the yield significantly although it was feared that cleavage of the side chain may occur. When 15 molar excess of DIBAL-H was used to reduce the sulphone (4) in

refluxing dioxane, a gray colour was observed after 2–3 days. This colour was a good indication for sulphone reduction. Although the reaction was left up to an extra 2 days for completion, no significant difference in yield figures was noticed with extra time after the colour changed. This procedure succeeded in producing the sulphide (2) in 94%.

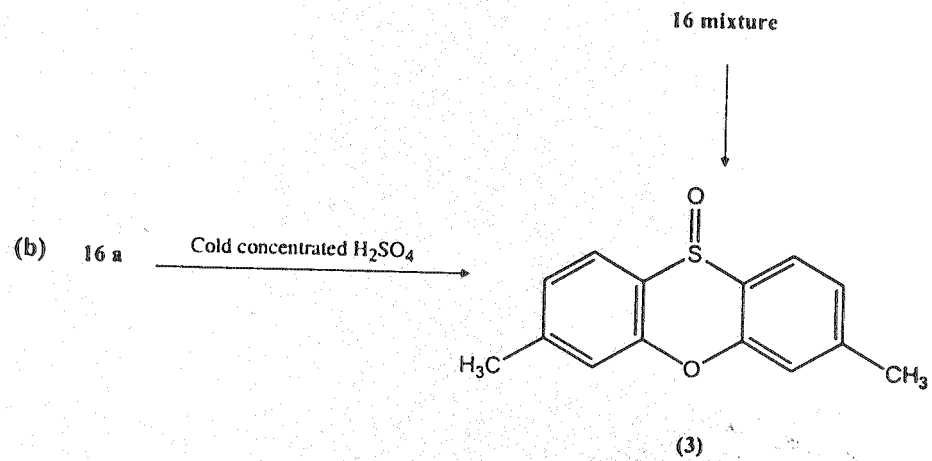
In conclusion, this approach was successful in producing the required 3,7-dimethyl phenoxathiin in high yields. It did avoid the structural isomeric formation and oxidative side products.

Approach C

While conducting this research, it was cited in literature that a very old method was reported to prepare aromatic dihydroxy sulphoxide and 2,8-phenoxathiin disulfonic acid[14, 24, 27]. The use of *p*-cresol as the starting material and aluminium chloride, carbon disulfide and thionyl chloride as reagents to produce *p*-cresol-*m*-sulphoxide is reported, which could be easily dehydrated to produce 2,8-dimethyl phenoxathiin. This procedure was employed utilizining *m*-cresol as the starting material. The experimental attempt furnished many isomeric structures which were

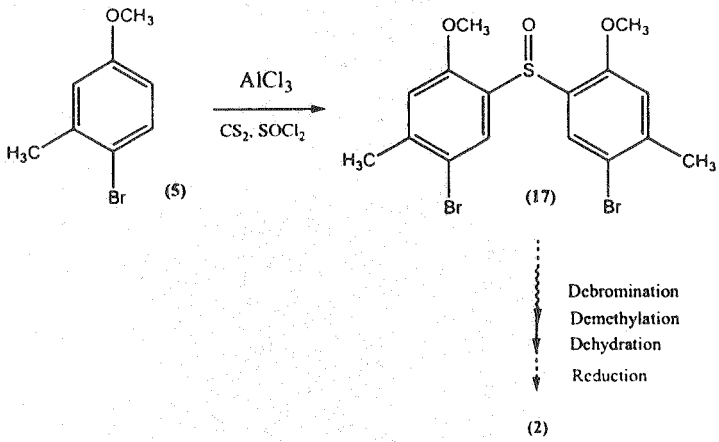


a= 2,2'=OH, 4,4'=CH_3
b= 2,4'=OH, 5,2'=CH_3
c= 2,2'=OH, 6,6'=CH_3
d= 2,2'=CH_3, 4,4'=OH
e= 2,4'=OH, 6,2'=CH_3
f= 2,2'=OH, 4,6'=CH_3



Scheme-4. Preparation of 3,7-dimethyl phenoxathiine (Approach C)

very difficult to separate. The crude mixture was then treated with cold concentrated sulphuric acid for 4 h. Assuming that this would produce few cyclized isomers, the experiment produced even more side products which could not be separated. Oxidation of the products was a major problem that made separation and identification very difficult processes. This work indicated that this approach must be started with protected compound to limit the number of possible isomers, as shown in **Scheme-5**. Compound **17** was isolated and identified with very low yields from a pink viscous mixture. Such disappointing results prohibited the progress in this pathway, but this approach is under investigation at the moment.



Scheme-5

Antimicrobial activity

As indicated in literature, phenoxathiin derivatives do exhibit antimicrobial activity. This fact prompted us to screen these derivatives in addition to others **(10–15)** prepared by similar procedure for antifungal and antibacterial activity. The results are summarized in Table-1.

TABLE-1
IN VITRO ANTIBACTERIAL ACTIVITY (MIC VALUES, μg/mL) OF METHYL SUBSTITUTED PHENOXATHIIN DERIVATIVES AND OF AMOXYCILLIN (A) AND TETRACYCLINE (T) AS REFERENCE AGENTS

	A	T	3	4	7	9	12	13
Staphylococcus aureus ATCC 6538p	1.14	1.83	> 156	> 156	14.6	58.6	> 156	> 156
Escherichia coli ATCC 8739	2.34	1.83	> 156	> 156	29.3	> 156	> 156	> 156
Candida albicans ATCC 10231			58.6	117.1	117.1	29.3	58.6	117.1

For comparative study, compounds **1, 2, 3, 4, 7, 9, 10, 11, 12, 13, 14, 15** were prepared and screened. The oxidized phenoxathiin derivatives **3, 4, 12** and **13** exhibited weak antifungal activity and negligable antibacterial properties. However, intermediates **7** and **9** exhibited some antibacterial activity against both *S. aureus* (gram positive) and *E. coli* (gram negative bacteria). Compound **7** was the most active against gram posive *S. aureus* with activity of 14.6 μg/mL. None of the methyl substituted phenoxathiin have shown any interesting activity.

ACKNOWLEDGEMENTS

Thanks are due to the Deanship of Academic Research and the Faculty of Pharmacy at the University of Jordan for supporting this work. The authors are also indebted to Dr. J. Zahra for NMR analyses and to Dar Al-Dawa Pharmaceutical Company, Na'our for the antimicrobial screening data.

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(*Received*: 9 November 2005; *Accepted*: 3 March 206) AJC-4714