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Synthesis of the antifungal 1-benzoxepin pterulone

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Abstract—The chlorinated 1-benzoxepin derivative pterulone (**1a**), a potent antifungal metabolite isolated from a *Pterula* species, was synthesised from 4,5-dihydro-2*H*-benzoxepin-3-one **6** by the oxidation of **6** to 3(2*H*)-oxepinone **7**, a Wittig reaction that transformed the keto functionality of **7** to a chlorovinyl group, and a Friedel Craft's acetylation. The mixture of *E* and *Z* isomers (**1a** and **1b**) obtained could transformed to pure pterulone (**1a**) by photochemical isomerisation and separation by chromatography. The yield was 23% starting from **6**.

1. Introduction

The chlorinated fungal metabolites pterulone (**1a**),[1] pterulone B (**2**)[2] and pterulinic acid (**3**)[1] were recently reported from fermentations of a *Pterula* species on both natural[3] and artificial[2] substrates. They all possess antifungal activity, the most potent being pterulone (**1a**) which in the plate diffusion assay exhibits significant activity at 1 μg/disc. As the pterulones are only weakly cytotoxic towards mammalian cells[3] and do not contain any functionalities immediately associated with reactivity or toxicity, their antifungal activity is potentially useful. Other structurally related fungal 1-benzoxepins are the metabolites **4** and **5**, reported from the fungus *Mycena galopus* although no details were given about their biological activities.[4] Compounds **1a**, **2**, and **3** have been shown to be inhibitors of eucaryotic respiration, interfering with the NADH/ubiquinone oxidoreductase (complex I), and pterulone (**1a**) inhibits the respiration rate of bovine heart mitochondria using NADH as substrate with an IC_{50} value of 36 μM.[3] The potency and selectivity of pterulone (**1a**), together with the fact that it represents a structural type not previously associated with antifungal activity or inhibition of respiration, motivated a synthetic study with the aim of obtaining larger amounts for biological studies as well as to prepare derivatives and analogues for structure–activity relationship studies. This paper describes the synthesis of pterulone (**1a**) and its exocylic double bond isomer **1b**.

2. Results and discussion

A number of synthetic routes to pterulone (**1a**) were studied, and the most successful in our hands is based on 4,5-dihydro-2*H*-benzoxepin-3-one **6** prepared according to Lachapelle and St-Jaques[5] (see Scheme 1). Three synthetic

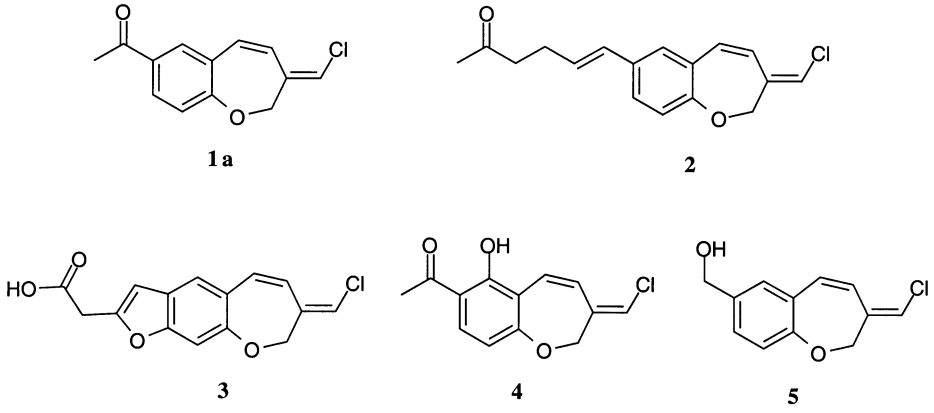

Figure 1. Pterulone **1a**, pterulone B **2**, pterulinic acid **3** and the related metabolites **4** and **5**.

Keywords: pterulone; 1-benzoxepin; *Pterula*; antifungal; complex I.
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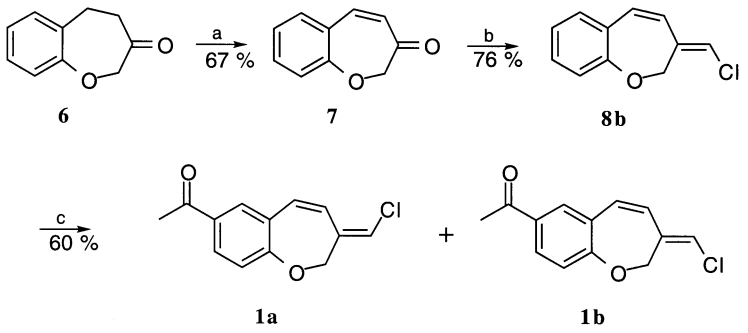


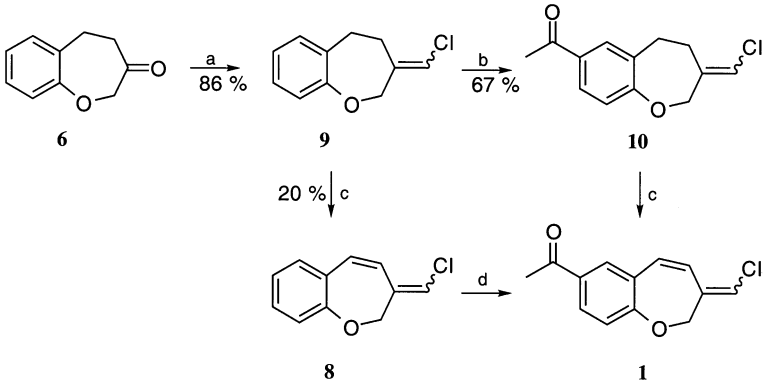
Scheme 1. (a) NBS, Bz_2O_2, CCl4, reflux; (b) $Ph_3P^+CH_2Cl_2^-$, KOtBu, THF, −78°C; (c) AcCl, AgTf, CH_2Cl_2, −78°C; (d) I_2, $h\nu$.

transformations would convert **6** to the target compound, the introduction of a double bond in the seven-membered ring, the acetylation of the aromatic part and the transformation of the keto function into a chlorovinyl function. Although the order of these transformations to some extent could be transposed, as discussed below, the highest yields were obtained when an initial oxidation of the 4,5 bond was followed by the introduction of the chlorovinyl group, and the acetylation was performed as the last step (Fig. 1).

Initially, the introduction of the unsaturation in **6** to obtain 3(2*H*)-oxepinone **7** was attempted by oxidative selenylation of **6** followed by elimination. Various combinations of selenylating agents such as *N*-phenylselenophtalimide,[6] phenylselenyl bromide and phenylselenyl chloride[6] and different bases such as KHMDS, LiHMDS, NaH and LDA were tested. However, somewhat surprisingly the selenylations were found to be non-selective and only low yields of the desired product **7** were obtained. Instead, the double bond was introduced via a radical bromination with NBS[7] followed by elimination of HBr to give the 3(2*H*)-oxepinone **7** in reasonable yield (67%). The transformation of the keto functionality to a chlorovinyl group was carried out by a Wittig reaction with chloromethyltriphenylphosphonium chloride and KOtBu in dry THF.[8] Only the *Z* isomer **8b** was obtained, in 76% yield. This is expected since conjugated ketones, as well as α-alkoxy substituted ketones are known to predominantly give the product corresponding to **8b** in Wittig reactions.[9] The following acetylation turned out to be difficult as too harsh conditions degraded the product and diminished the yields. Finally, a successful Friedel Craft's acetylation was performed according to Lindner et al., with silver triflate and acetyl chloride in dry methylene chloride,[10] yielding a 1:3 mixture of pterulone (**1a**) and its *Z* isomer **1b** in 60% overall yield. Prolonged reaction times increased the relative amounts of **1a** but decreased the yields. The fact that the exocyclic double bond was partly isomerised during the acetylation conditions indicated that it should be possible to isomerise **1b** at least partly under milder conditions, for example with iodine and light, and this turned out to be true. In CCl_4 and in the presence of catalytic amounts of I_2, pure **1b** was converted to a 3:1 mixture of **1a/1b** when irradiated with blue light (300 nm) for 4 h at room temperature. As the two isomers could be separated by chromatography, pure pterulone **1a**, identical in all details with the natural product, was obtained in 23% total yield starting from **6**.

When the same Wittig reaction was carried out directly with **6**, a 1.75:1 *Z*/*E* mixture of the corresponding chlorovinylated product **9** was obtained in 86% yield. The acetylation of this using the same conditions as discussed above gave the acetylated product **10** as an approximately 1:1 *E*/*Z* mixture in 67% overall yield. However, the remaining introduction of the (2*H*)-benzoxepine double bond did not work well, only low yields were obtained with NBS and other techniques proved unsuccessful. It is obvious that the chlorovinyl group interferes with the oxidation, as attempts to oxidise prior to acetylation failed as well. The less electron-withdrawing properties of the chlorovinyl group compared with the ketone could explain these difficulties, as the corresponding α-proton should be less acidic. At most 20% deacetylpterulone **8** (as a 1.7:1 *E*/*Z* mixture) was obtained (Scheme 2).

Only few (2*H*)-benzoxepine derivatives related to the



Scheme 2. (a) $Ph_3P^+CH_2Cl_2^-$, KOtBu, THF, −78°C; (b) AcCl, AgTf, CH_2Cl_2, −78°C; (c) NBS, Bz_2O_2, CCl_4, reflux; (d) $ZnCl_2$, Ac_2O, benzene, rt. **a**=*E*, **b**=*Z*.

pterulones have been reported from natural sources, and as far as we know only the pterulones have been assayed for antifungal activity. The basic structure of pterulone (**1a**) is not present in any of the classical antifungal agents used today, the only pharmaceutical agent with a (2*H*)-benzoxepine moiety is doxepine which is used as an antidepressant.[11] The increasing demand for novel antimicrobial agents to treat infections by resistant strains makes it important to explore the properties of apparently selective and non-toxic agents to identify new suitable lead structures. The synthesis of pterulone (**1a**) presented here facilitates the various biological tests that need to be carried out in order to determine the potential of the compound, and has also made it possible to prepare derivatives and analogues for QSAR studies.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded at room temperature with Bruker DRX400 or Bruker ARX500 spectrometers in $CDCl_3$, and the solvent signals (7.27 and 77.23 ppm, respectively) were used as reference. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001). Mass spectra (EI) were recorded with a Jeol SX102 spectrometer. Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F_{254} plates (Merck), while preparative TLC was performed on precoated PLC plates, Silica Gel 60F-254, 2 mm. Column chromatography was performed on SiO_2 (Matrex LC-gel: 60A, 35-70 MY, Grace). Melting points (uncorrected) were determined with a Reichert microscope.

3.1.1. Preparation of 1-benzoxepin-3(2*H*)-one 7. NBS (1.11 g, 6.23 mmol) and Bz_2O_2 (75 mg, 0.312 mmol) was added to a solution of 3,4-dihydro-2*H*-1-benzoxepin-3-one (1.01 g, 6.23 mmol) in CCl_4 (50 ml) in a 100 ml round bottomed flask. The mixture was refluxed under nitrogen for 20 h. After the reaction vessel had cooled down, the solution was filtered through an alumina column and eluted with CH_2Cl_2. The product was purified by chromatography on SiO_2 (heptane/ethyl acetate 15:1) and obtained as a yellowish oil in 67 % yield. ν_{max}(liquid film) 3060, 3035, 1675, 1620, 1485, 1305, 1050, 785 cm^{-1}; ^{1}H NMR ($CDCl_3$) 4.55 (s, 2H), 6.36 (d, 1H, *J*=12.1 Hz), 7.15–7.21 (m, 2H), 7.20 (d, 1H, *J*=12.1 Hz), 7.35–7.39 (m, 2H); ^{13}C NMR ($CDCl_3$) 78.2, 121.2, 124.8, 127.8, 129.5, 132.7, 133.9, 142.7, 159.4, 196.8. MS (EI, *m/z*): 160.0529 (100%, M^+, $C_{10}H_8O_2$ requires 160.0524), 132 (47%), 131 (62%), 121 (14%), 91 (13%), 77 (8%).

3.1.2. Preparation of (3*Z*)-3-(chloromethylene)-2,3-dihydro-1-benzoxepine 8b. KOtBu (0.604 g, 5.38 mmol) was added to (chloromethyl)triphenylphosphonium chloride (1.868 g, 5.38 mmol) in dry THF (50 ml) under inert atmosphere (nitrogen) at −78°C. The mixture was allowed to stand for 1 h before **7** (0.575 g, 3.59 mmol) was added. The solution darkened, after 40 min the reaction mixture was poured on ice, neutralised with 2 M aq. HCl, extracted with ether and washed with brine. The organic phase was dried with $MgSO_4$ and evaporated. The product was purified by chromatography on SiO_2 (heptane/ethyl acetate 6:1) and obtained as a colourless oil. The isolated yield was 76%. ν_{max} (liquid film) 3065, 1560, 1480, 1455, 1270, 1215, 1100, 1005, 770, 755 cm^{-1}; ^{1}H NMR ($CDCl_3$) 4.90 (s, 2H), 6.33 (s, 1H), 6.34 (d, 1H, *J*=11.9 Hz), 6.40 (d, 1H, *J*=11.9 Hz), 7.01–7.09 (m, 2H), 7.21 (ddd, 1H, *J*=7.9, 7.8, 1.8 Hz), 7.25 (d, 1H, *J*=7.8 Hz); ^{13}C NMR ($CDCl_3$) 68.3, 120.0, 120.3, 123.3, 127.7, 127.9, 128.3, 128.9, 132.4, 134.1, 138.6. MS (EI, *m/z*): 194 (28%), 192.0339 (91%, M^+, $C_{11}H_9OCl$ requires 192.0342), 157 (100%), 129 (52%), 128 (42%), 127 (20%), 118 (27%).

3.1.3. Preparation of pterulone 1a and 1-[(3*Z*)-3-(chloromethylene)-2,3-dihydro-1-benzoxepin-7-yl]ethanone 1b. AgTf (200 mg, 0.779 mmol) was added to 10 ml of freshly distilled AcCl in a 25 ml round bottomed flask under inert atmosphere (N_2) kept at −78°C. The mixture was allowed to stand for 1 h before **8b** (30 mg, 0.156 mmol) was added. After 40 min at the same temperature NaCl-saturated methanol was added slowly (exoterm reaction) to the reaction mixture until it became transparent. The reaction was extracted with CH_2Cl_2 and washed and neutralised with 10% aq. Na_2CO_3. The organic phase was dried with $MgSO_4$ and evaporated, and chromatography on SiO_2 (toluene) gave a 1:3 mixture of *E* and *Z* in 60% yield. Pure **1b** was obtained from this mixture by chromatography on SiO_2 (heptane/toluene 1:1) as colourless crystals with mp 80–83°C; ν_{max} (KBr) 3060, 1680, 1600, 1495, 1485, 1265, 1235, 1125, 1005, 815, 665 cm^{-1}; ^{1}H NMR ($CDCl_3$) 2.59 (s, 3H), 4.91 (s, 2H), 6.40 (s, 1H), 6.41 (s, 2H), 7.09 (d, 1H, *J*=8.4 Hz), 7.79 (dd, 1H, *J*=8.4, 2.2 Hz), 7.88 (d, 1H, *J*=2.2 Hz); ^{13}C NMR ($CDCl_3$) 26.9, 68.5, 120.9, 121.8, 125.3, 128.1, 129.2, 129.6, 131.1, 133.8, 138.1, 163.2, 197.2. MS (EI, *m/z*): 236 (33%), 234.0445 (100%, M^+, $C_{13}H_{11}O_2Cl$ requires 234.0448), 221 (15%), 199 (62%), 156 (17%), 128 (18%), 92 (8%).

When a catalytic amount of iodine was added to either the 1:3 mixture of **1a/1b** or pure **1b** obtained as described above in CCl_4 (typically 10 mg in 2.5 ml) in a round bottomed flask which was put in a Rayonette light reactor (300 nm, 100 W) for 5 h, a 3:1 mixture of **1a/1b** (according to ^{1}H NMR integrals) was obtained. Pure pterulone (**1a**), identical in all respects with the natural product,[1] was obtained in 75% yield from this mixture by chromatography on SiO_2 (heptane/toluene 1:1).

3.1.4. 3-(Chloromethylene)-2,3,4,5-tetrahydro-1-benzoxepine 9. The compound was prepared from **6** according to the procedure described for **7** leading to **8a**. The crude product was purified by chromatography on SiO_2 (heptane/ethyl acetate 60:1) and **9** was obtained as a colourless oil in 86% yield (as a 1:1.75 inseparable mixture of *E* and *Z*). ν_{max} (liquid film, of the mixture) 2940, 1485, 1455, 1270, 1225, 1100, 985, 825, 760 cm^{-1}; (**9a**): ^{1}H NMR ($CDCl_3$) 2.69–2.72 (m, 2H), 2.91–2.94 (m, 2H), 4.46 (d, 2H, *J*=0.9 Hz), 6.22 (s, 1H), 7.01–7.07 (m, 2H), 7.17–7.21 (m, 2H); ^{13}C NMR ($CDCl_3$) 28.9, 32.1, 75.5, 117.3, 121.3, 124.0, 128.2, 131.1, 133.5, 140.5, 159.3. (**9b**): ^{1}H NMR ($CDCl_3$) 2.55–2.59 (m, 2H), 2.92–2.95 (m, 2H), 4.74 (d, 2H, *J*=1.1 Hz), 6.07 (p, 1H, *J*=1.1 Hz), 7.01–7.06 (m, 2H), 7.14–7.20 (m, 2H); ^{13}C NMR ($CDCl_3$) 33.3, 33.4,

71.3, 114.2, 121.7, 124.2, 128.1, 131.0, 133.3, 140.5, 159.9. MS of the mixture (EI, *m/z*): 196 (22%), 194.0492 (68%, M^+, $C_{11}H_{11}OCl$ requires 194.0498), 159 (100%), 144 (10%), 131 (32%), 129 (11%), 115 (10%), 91 (25%).

3.1.5. 1-[3-(Chloromethylene)-2,3,4,5-tetrahydro-1-benzoxepin-7-yl]ethanone 10. Silver triflate (0.820 g, 3.19 mmol) was added to a 50 ml round bottomed flask. The flask was put in a cooling bath, −78°C, under inert conditions (N_2), and dry CH_2Cl_2 (20 ml) was added to the flask followed by AcCl (227 μl, 3.19 mmol) 10 min later. After 1.5 h **10** (0.414 mg, 2.13 mmol) in dry CH_2Cl_2 (2 ml) was added to the flask. After 5 h NaCl-saturated methanol (2 ml) was added to the reaction vessel and after an additional 2 h brine was added and the mixture was neutralised with NaOH aq. (33%). The mixture was extracted with CH_2Cl_2, the organic phase was washed with brine, dried with $MgSO_4$ and evaporated. The crude product was purified by chromatography on SiO_2 (toluene) and **10** was obtained as a colourless oil in 67% yield (as an approximately 1:1 inseparable mixture of the *E* and *Z* isomers). ν_{max} (liquid film, of the mixture) 2940, 1680, 1610, 1495, 1370, 1260, 1225, 1120, 980, 830, 735 cm^{-1}; (**10a**): ^1H NMR ($CDCl_3$) 2.57 (s, 3H), 2.73–2.76 (m, 2H), 2.97–3.02 (m, 2H), 4.57 (d, 2H, *J*=0.9 Hz), 6.26 (s, 1H), 6.99 (dd, 1H, *J*=8.3, 0.3 Hz), 7.76–7.79 (m, 2H); ^{13}C NMR ($CDCl_3$) 28.8, 31.6, 74.5, 118.3, 121.1, 129.1, 129.1, 132.2, 133.1, 132.7, 139.3, 163.4, 197.6. (**10b**): ^1H NMR ($CDCl_3$) 2.55 (s, 3H), 2.57–2.60 (m, 2H), 2.95–2.98 (m, 2H), 4.78 (d, 2H, *J*=1.1 Hz), 6.06 (p, 1H, *J*=1.2 Hz), 7.04 (dd, 1H, *J*=8.8, 1.7 Hz), 7.74–7.76 (m, 2H); ^{13}C NMR ($CDCl_3$) 26.9, 33.0, 71.0, 114.8, 121.7, 128.9, 128.9, 131.8, 132.5, 133.0, 139.5, 163.8, 197.5. MS of the mixture (EI, *m/z*): 238 (33%), 236.0592 (100%, M^+, $C_{13}H_{13}O_2Cl$ requires 236.0604), 223 (22%), 221 (60%), 201 (72%), 193 (43%), 157 (17%), 129 (20%), 128 (18%), 107 (15%).

3.1.6. 3-(Chloromethylene)-2,3-dihydro-1-benzoxepine 8. NBS (183 mg, 1.03 mmol) and Bz_2O_2 (12 mg, 0.051 mmol) was added to a solution of **9** (200 mg, 1.03 mmol) in dry CCl_4 (25 ml) in a 50 ml round bottomed flask. The mixture was refluxed under nitrogen for 20 h. After the reaction vessel had cooled down, the solution was filtered through an alumina column and eluted with CH_2Cl_2. The product was purified by chromatography on SiO_2 (heptane/ethyl acetate 20:1) and isolated as a colourless oil (1.7:1 inseparable mixture of *E* and *Z*) in 20% yield. (**8a**): ν_{max} (liquid film) 3065, 1560, 1480, 1455, 1270, 1215, 1100, 1005, 770, 755 cm^{-1}; ^1H NMR ($CDCl_3$) 4.58 (s, 2H), 6.12 (s, 1H), 6.57 (d, 1H, *J*=11.9 Hz), 6.83 (d, 1H, *J*=11.9 Hz), 7.00–7.03 (m, 1H), 7.07 (dd, 1H, *J*=7.5, 1.3 Hz), 7.18–7.25 (m, 2H); ^{13}C NMR ($CDCl_3$) 73.0, 119.4, 120.7, 123.8, 124.1, 128.2, 128.7, 129.8, 131.7, 133.7, 137.0. MS of the mixture (EI, *m/z*): 194 (30%), 192.0328 (94%, M^+, $C_{11}H_9OCl$ requires 192.0342), 157 (100%), 129 (54%), 128 (56%), 127 (29%), 118 (32%).

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