

Synthesis of Patulin and Its Cyclohexane Analogue from Furan Derivatives

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Patulin, a mycotoxin from fungi of *Penicillium* and *Aspergillus* species, and its cyclohexane analogue were synthesized concisely via oxidation of furan derivatives, followed by cyclization to give a ylidenebutenolide ring.

Keywords patulin; total synthesis; furan oxidation; ylidenebutenolide; γ -lactone

Patulin **1** is a widely distributed mycotoxin which is produced by various species of *Penicillium* and *Aspergillus* fungi.¹⁾ There appears to be a risk that this mycotoxin may contaminate foods.²⁾ Patulin **1** shows mutagenic, carcinogenic, and antibiotic activities³⁾ by inhibiting the syntheses of DNA, RNA and proteins.⁴⁾ The unique structure of patulin **1**, with ylidenebutenolide (2,4-diene-1,4-olide) and acetal rings, is presumably responsible for such biological activity. Patulin is reported to react with cysteine, but the products have not been characterized.⁵⁾ The same ylidenebutenolide structure is observed in antibiotic plant constituents, such as protoanemonin **2**⁶⁾ and chloranthalactone A **3**.⁷⁾ In order to study the relationship between the biological activity and the reactivity of ylidenebutenolide compounds, we planned to synthesize^{8,9)} patulin and its cyclohexane analogue **4** via a concise route such that isotopes could be introduced easily. We report here a synthesis of patulin and its cyclohexane analogue from furan derivatives.

The diester **5** having the requisite carbons for patulin synthesis was prepared by condensation of acetonedicarboxylic acid dimethyl ester with chloroacetaldehyde in pyridine at 50 °C for 24 h (78%). It was then reduced with LiAlH₄ to give **6** (80%) and the hydroxyl group of **6** adjacent to the furan ring was oxidized by activated MnO₂ to afford an aldehyde **7** (56%). Treatment of the aldehyde with pyridinium *p*-toluenesulfonate (PPTS) in refluxing methanol–benzene (1 : 2) for 1.5 h with Dean–Stark water trap gave a methyl acetal **8** (91%). Oxidation of the furan

ring of **8** with 2 eq of *m*-chloroperbenzoic acid (MCPBA) in CH₂Cl₂ for 2 h followed by methylation with CH₂N₂ afforded a keto-ester **9** (67%).¹⁰⁾ This was isomerized to the *E* isomer **10** by heat or light. In order to obtain the ylidenebutenolide ring, **9** was treated with various Lewis acids. Finally, treatment of **9** with Ca(OH)₂ as a catalyst in refluxing benzene using a Dean–Stark water trap for 30 min in the dark was found to cause efficient cyclization to give methylpatulin **11** (41%). Demethylation of **11** was performed with trifluoroacetic acid (TFA) in water at 50 °C for 1 h to give patulin **1** (78%).⁹⁾ The synthesized **1** was identical (IR, ¹H-NMR, ¹³C-NMR and MS spectra) with natural patulin.

A cyclohexane analogue **4** of patulin was then synthesized in order to study the reactivity of the 2,4-diene-1,4-olide moiety. Condensation of cyclohexane-1,3-dione with chloroacetaldehyde in pyridine at 50 °C for 24 h gave a furan derivative **12** (56%). Reduction of **12** with NaBH₄ in methanol afforded quantitatively an alcohol **13**, which was oxidized with 2 eq of MCPBA in CH₂Cl₂ and then methylated with CH₂N₂ to give a keto-ester **14** (16%). Cyclization of **14** with Ca(OH)₂ in refluxing benzene for 30 min in the dark gave the patulin analogue **4** (60%).

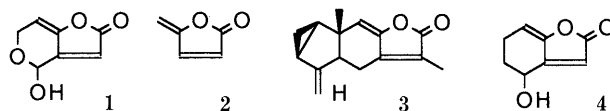


Fig. 1

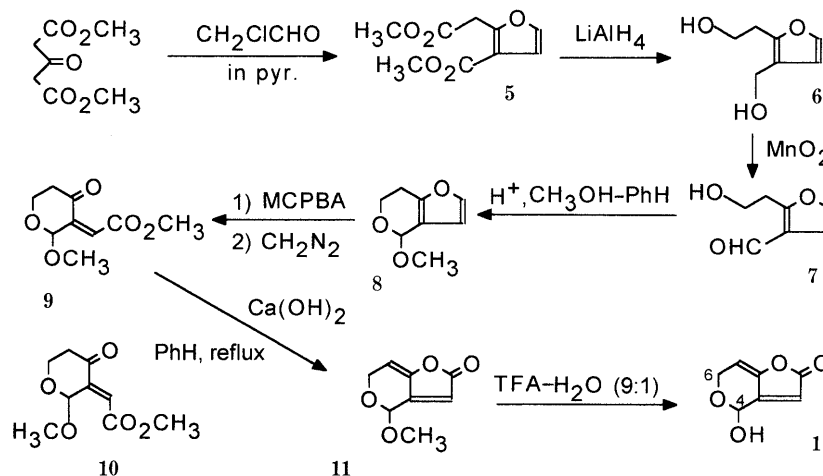


Fig. 2

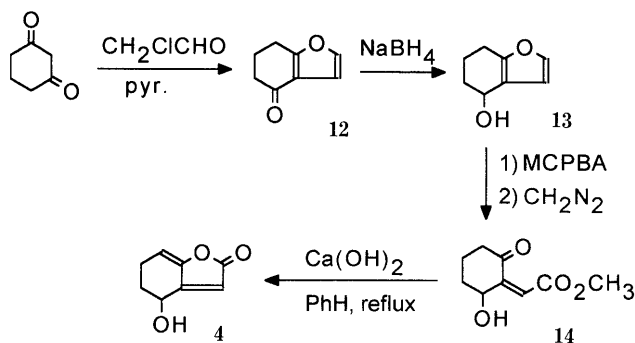


Fig. 3

In conclusion, ylidenebutenolide compounds can be synthesized concisely *via* a route involving oxidation of the furan ring, followed by enol-lactonization.

Experimental

NMR spectra were measured on a JEOL GX-270 spectrometer at 270 (^1H) and 67.89 MHz (^{13}C) for samples in CDCl_3 containing tetramethylsilane as an internal standard. IR and UV spectra were measured on a JASCO IR-810 IR spectrometer and JASCO UVDEC-460 spectrophotometer, respectively. MS were recorded on a JEOL JMS-DX-300 spectrometer. TLC was carried out on Kiesel-gel GF $_{254}$ (0.25 mm thickness). Wakogel C-200 was used for column chromatography. HPLC was performed on a JASCO BIP-1 instrument (refractive index (RI) and UV detectors) with a column (10 $\times$ 250 mm) of LiChroprep Si 60 (Merck) (hexane–EtOAc).

Methyl 3-Methoxycarbonyl-2-furylacetate (5) A solution of chloroacetaldehyde (40%, 29 ml) in water was added dropwise to a solution of acetone dicarboxylic acid dimethyl ester (25 g) in pyridine (50 ml) with stirring. Stirring was continued for 24 h at 50 $^\circ\text{C}$ under Ar, then the reaction mixture was extracted with water and ethyl ether. The organic layer was washed successively with 2 M HCl, 5% NaHCO_3 , 10% NaOH and brine, and dried over anhydrous MgSO_4 . After removal of the solvent, the residue was subjected to column chromatography (SiO $_2$; hexane–EtOAc) to give **5** (22.2 g, 78%) as a yellow oil. IR (neat): 1740, 1710, 1605, 1507, 1435, 1310, 1200, 1170, 1150, 1060 cm^{-1} . MS m/z : 198 (M^+ , 17), 166 (100), 139 (82), 109 (60), 83 (30), 71 (22), 53 (37). ^1H -NMR δ : 7.35 (1H, d, J = 2.0 Hz), 6.69 (1H, d, J = 2.0 Hz), 4.08 (2H, s), 3.81 (3H, s), 3.71 (3H, s). ^{13}C -NMR δ : 168.7, 136.5, 154.0, 141.6, 115.2, 110.5, 52.0, 51.2, 33.1.

3-Hydroxymethyl-2-furyl ethanol (6) A solution of **5** (22.0 g) in dry ethyl ether (100 ml), cooled in an ice bath, was treated with LiAlH_4 (12.7 g) with stirring. The reaction mixture was stirred for 3 h and then an excess of EtOAc was added. The mixture was acidified with 0.1 M HCl and extracted with EtOAc. The extract was washed with brine and dried over MgSO_4 . After removal of the solvent under reduced pressure, the residue was purified by chromatography on a silica gel column with EtOAc–hexane to afford **6** (12.6 g, 80%) as an oil. IR (neat): 3340, 2880, 1040, 1000, 980 cm^{-1} . MS m/z : 141 (M^+ – 1), 124 (15), 110 (15), 94 (85), 71 (55). ^1H -NMR δ : 7.24 (1H, d, J = 1.6 Hz), 6.31 (1H, d, J = 1.6 Hz), 4.51 (2H, brs), 4.31 (2H, s), 3.68 (2H, t, J = 6.1 Hz), 2.78 (2H, t, J = 6.1 Hz). ^{13}C -NMR δ : 150.2, 140.1, 120.7, 111.2, 60.0, 55.5, 29.4.

2-(2-Hydroxyethyl)-3-furancarbaldehyde (7) The diol **6** (12.0 g) was dissolved in CH_2Cl_2 (50 ml) and treated with activated MnO_2 (180 g) for 1.5 h under Ar. The solid (MnO_2 and products) was collected by filtration and dissolved in 2 M HCl. This solution was extracted with EtOAc. The EtOAc layer and the CH_2Cl_2 filtrate were combined, washed with brine, dried and evaporated. The residue was chromatographed on a silica gel column with hexane–EtOAc to give **7** (8.05 g, 68%) as an oil. IR (neat): 3430, 2750, 1675, 1520, 1420 cm^{-1} . MS m/z : 140 (M^+), 139 (10), 138 (23), 122 (90), 110 (100), 94 (85), 80 (50). ^1H -NMR δ : 9.91 (1H, s), 7.35 (1H, d, J = 2.5 Hz), 6.70 (1H, d, J = 2.5 Hz), 3.94 (2H, t, J = 6.3 Hz), 3.30 (2H, t, J = 6.3 Hz), 2.83 (1H, brs). ^{13}C -NMR δ : 185.6, 162.6, 142.5, 123.7, 108.3, 60.4, 30.6.

4-Methoxy-6,7-dihydro-4H-furo[3,2-c]pyran (8) A mixture of **7** (4.0 g), benzene (50 ml), methanol (25 ml) and PPTS (300 mg) was refluxed for 1.5 h with a Dean–Stark water-trap (3A molecular sieves) under Ar.

The solvent was evaporated *in vacuo* and the residue was chromatographed on a silica gel column (EtOAc) to give **8** (4.1 g, 91%) as an oil. IR (neat): 1637, 1503, 1319, 1189, 1114, 1081, 1034 cm^{-1} . ^1H -NMR δ : 7.27 (1H, d, J = 2.2 Hz), 6.30 (1H, d, J = 2.2 Hz), 5.40 (1H, d, J = 2.2 Hz), 5.40 (1H, s), 4.07 (1H, ddd, J = 11.3, 11.1, 5.9 Hz), 3.93 (1H, dd, J = 11.3, 5.9 Hz), 3.48 (3H, s), 2.85 (1H, ddd, J = 163.3, 11.1, 5.9 Hz), 2.50 (1H, dd, J = 16.3, 4.0 Hz). ^{13}C -NMR δ : 150.3, 141.1, 116.5, 107.6, 96.0, 57.5, 55.0, 24.0.

(Z)-2-Methoxy-3-methoxycarbonylmethylene-2,3,5,6-tetrahydro-4-pyranone (9) A solution of the acetal **8** (79 mg, 0.513 mmol) and 70% MCPBA (310 mg, 1.26 mmol) in CH_2Cl_2 (4 ml) was stirred with citrate buffer solution (1.5 ml) at pH 8 for 2 h in the dark. The reaction mixture was treated with Me_2S to reduce the unreacted MCPBA and then extracted with EtOAc. The organic layer was dried over MgSO_4 and evaporated. To a solution of the residue in MeOH, an ethyl ether solution of diazomethane was added little by little with monitoring by TLC to avoid 1,3-dipolar addition to the carbon–carbon double bond. After evaporation of the solvents, the residue was chromatographed on a silica gel column (hexane–EtOAc) to give **9** (69 mg, 67%) as an oil. IR (neat): 2950, 1717, 1702, 1618 cm^{-1} . MS m/z : 200 (M^+ , 1.5), 185 (6.0), 169 (14), 139 (43). ^1H -NMR δ : 6.02 (1H, s), 5.21 (1H, s), 4.18 (1H, dt, J = 11.3, 3.5 Hz), 3.97 (1H, ddd, J = 11.3, 7.1, 2.8 Hz), 3.75 (3H, s), 3.45 (3H, s), 2.80 (1H, ddd, J = 15.1, 11.3, 7.1 Hz), 2.60 (1H, ddd, J = 15.1, 13.5, 2.8 Hz). ^{13}C -NMR δ : 196.0, 165.9, 143.3, 125.1, 102.2, 59.0, 55.2, 52.9, 42.4. The reaction of **9** was performed quickly in the dark to avoid isomerization.

4-Methoxy-4H-furo[3,2-c]pyran-2(6H)-one (11) A mixture of dry benzene (10 ml), the methyl ester **9** (30 mg) and $\text{Ca}(\text{OH})_2$ (3 g) was refluxed with a Dean–Stark water trap-5A molecular sieves for 30 min in the dark. After evaporation of the solvent, the residue was chromatographed on a silica gel column (hexane–EtOAc) to give methylpatulin **11** (10 mg, 41%) as a waxy solid. IR (neat): 2920, 1770, 1740 cm^{-1} . MS m/z : 168 (M^+ , 28), 137 (16), 126 (15), 112 (20). ^1H -NMR δ : 5.97 (1H, brs), 5.92 (1H, m), 5.61 (1H, s), 4.58 (1H, brd, J = 16.7 Hz), 4.38 (1H, dd, J = 16.7, 4.7 Hz), 3.57 (3H, s). ^{13}C -NMR δ : 168.5, 148.6, 146.2, 111.2, 107.4, 94.5, 58.9, 56.2.

Patulin 1 A solution of methylpatulin **11** (10 mg) in 1 ml of TFA–water (9:1) was stirred at 50 $^\circ\text{C}$ for 1 h. After evaporation of the solvent, the residue was dissolved in EtOAc and the solution was washed with saturated NaHCO_3 and brine, and evaporated. The residue was chromatographed on a silica gel column (hexane–EtOAc) to give patulin **1** (7.2 mg, 78%) as a waxy solid. IR (neat): 2920, 1780, 1740 cm^{-1} . MS m/z : 154 (M^+ , 15), 136 (17), 126 (27), 110 (54). ^1H -NMR δ : 6.06 (1H, s), 6.03 (1H, brd, J = 1.0 Hz), 5.96 (1H, m), 4.73 (1H, ddd, J = 17.3, 3.1, 1.0 Hz), 4.42 (1H, ddd, J = 17.3, 4.2 Hz). ^{13}C -NMR δ : 168.8, 150.1, 146.2, 111.1, 107.7, 88.8, 59.5.

6,7-Dihydrobenzofuran-4(5H)-one (12) A 40% chloroacetaldehyde solution (30 ml) in water was added dropwise to a solution of cyclohexane-1,3-dione (3.0 g) in pyridine (25 ml), over a period of 30 min. The mixture was stirred at 50 $^\circ\text{C}$ for 24 h under Ar. Then 30 ml of water was added and the whole was extracted with ethyl ether. The organic layer was washed with 2 M HCl, 5% NaHCO_3 , 10% NaOH and brine, dried and evaporated. The residue was chromatographed on a silica gel column (hexane–EtOAc) to give **12** (2.04 g, 56%) as a waxy solid, mp. 29.5 $^\circ\text{C}$. IR (neat): 2950, 1670, 1590, 1455, 1445, 1410, 1290, 1240 cm^{-1} . MS m/z : 136 (M^+ , 67), 108 (100), 80 (80). ^1H -NMR δ : 7.34 (1H, d, J = 2.0 Hz), 6.66 (1H, dd, J = 2.0, 1.0 Hz), 2.89 (2H, dt, J = 6.2, 1.0 Hz), 2.49 (2H, dt, J = 6.2, 1.5 Hz), 2.18 (2H, dqui, J = 6.2, 1.5 Hz). ^{13}C -NMR δ : 194.3, 166.8, 142.5, 120.9, 106.2, 37.6, 23.0, 22.3.

4-Hydroxy-4,5,6,7-tetrahydrobenzofuran (13) Sodium borohydride (570 mg) was added to a solution of **12** (1.86 g) in methanol (30 ml) and the mixture was stirred at room temperature for 30 min, then extracted with water and EtOAc. The organic layer was dried over MgSO_4 and evaporated. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **13** (1.89 g, quantitative yield) as an oil. IR (neat): 3350, 2940 cm^{-1} . MS m/z : 138 (M^+ , 23), 120 (60), 118 (51), 110 (60). ^1H -NMR δ : 7.24 (1H, d, J = 2.0 Hz), 6.37 (1H, d, J = 2.0 Hz), 4.67 (1H, dd, J = 5.0, 3.8 Hz), 3.18 (1H, brs), 2.53 (3H, m), 1.82 (3H, m). ^{13}C -NMR δ : 152.1, 140.6, 119.9, 109.1, 63.6, 32.3, 22.7, 18.8.

Oxidation of the Alcohol 13 A solution of the alcohol **13** (482 mg) in CH_2Cl_2 (28 ml) was treated with 70% MCPBA (1.72 g), and the mixture was stirred for 2 h at room temperature in the dark. The unreacted MCPBA was reduced with Me_2S , and then CH_2N_2 solution in ethyl ether was added carefully to avoid 1,3-dipolar addition, as in the case

of **9**. After evaporation of the solvents, the residue was chromatographed on a silica gel column (hexane–EtOAc) to give **14** as an oil. IR (neat): 3430, 2950, 1720, 1710, 1695, 1435, 1240 cm^{-1} . MS m/z : 184 (M^+ , 1.4), 169 (1.3), 153 (12), 124 (82), 113 (17). ^1H -NMR δ : 5.98 (1H, d, $J=2.5$ Hz), 4.44 (1H, ddd, $J=7.9, 5.4, 2.5$ Hz), 3.84 (1H, br s), 3.70 (3H, s), 2.57 (2H, dd, $J=9.1, 7.9$ Hz), 2.15 (2H, m), 1.80 (2H, m). ^{13}C -NMR δ : 204.0, 166.5, 157.5, 116.6, 73.4, 52.0, 42.5, 34.8, 20.3.

Patulin Analogue 4 A mixture of **14** (10 mg), dry benzene (1 ml) and $\text{Ca}(\text{OH})_2$ was refluxed with a Dean–Stark water trap-5A molecular sieves for 30 min in the dark. The solvent was evaporated and the residue was chromatographed on a silica gel column (hexane–EtOAc) to give **4** (60%) as a waxy solid. IR (neat): 3440, 2920, 1770, 1740 cm^{-1} . MS m/z : 152 (M^+ , 96), 134 (100), 106 (67). ^1H -NMR δ : 6.05 (1H, br s), 5.94 (1H, m), 4.73 (1H, ddd, $J=12.0, 4.5, 2.0$ Hz), 2.50 (2H, m), 2.16 (1H, m), 1.85 (1H, m). ^{13}C -NMR δ : 170.3, 159.6, 149.0, 111.1, 110.0, 65.1, 32.1, 21.8.

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