Synthesis of Patulin and Its Cyclohexane Analogue from Furan Derivatives

Masahiro TADA,* Kazuhisa OHTSU, and Kazuhiro CHIBA

Laboratory of Bio-organic Chemistry, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan.
Received April 28, 1994; accepted June 8, 1994

Patulin, a mycotoxin from fungi of *Penicillium* and *Aspergillis* 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 activities[3] 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 $LiAlH_4$ to give **6** (80%) and the hydroxyl group of **6** adjacent to the furan ring was oxidized by activated MnO_2 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 CH_2Cl_2 for 2 h followed by methylation with CH_2N_2 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, ^{1}H-NMR, ^{13}C-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 $NaBH_4$ in methanol afforded quantitatively an alcohol **13**, which was oxidized with 2 eq of MCPBA in CH_2Cl_2 and then methylated with CH_2N_2 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%).

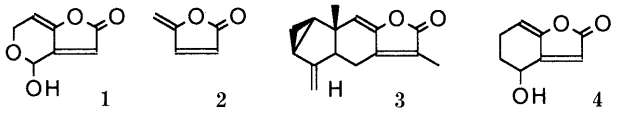

Fig. 1

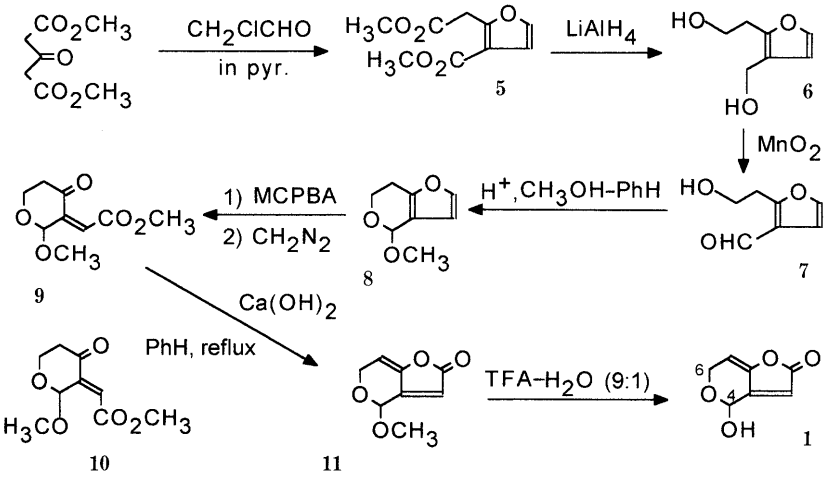

Fig. 2

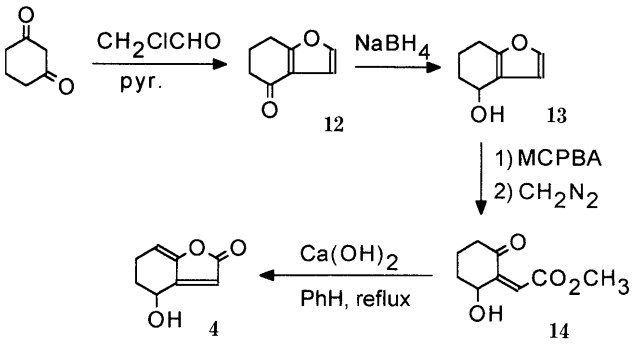

Fig. 3

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 (^{1}H) 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 × 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 °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). ^{1}H-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-furylethanol (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). ^{1}H-NMR δ: 7.24 (1H, d, J=1.6 Hz), 6.31 (1H, d, J=1.6 Hz), 4.51 (2H, br s), 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). ^{1}H-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, br s). ^{13}C-NMR δ: 185.6, 162.6, 142.5, 123.7, 108.3, 60.4, 30.6.

4-Methoxy-6,7-dihydro-4*H*-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}. ^{1}H-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). ^{1}H-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-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one (11) A mixture of dry benzene (10 ml), the methyl ester **9** (30 mg) and $Ca(OH)_2$ (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). ^{1}H-NMR δ: 5.97 (1H, br s), 5.92 (1H, m), 5.61 (1H, s), 4.58 (1H, br d, 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 °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). ^{1}H-NMR δ: 6.06 (1H, s), 6.03 (1H, br d, 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(5*H*)-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 °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 °C. IR (neat): 2950, 1670, 1590, 1455, 1445, 1410, 1290, 1240 cm^{-1}. MS *m/z*: 136 (M^+, 67), 108 (100), 80 (80). ^{1}H-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). ^{1}H-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, br s), 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). ^{1}H-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 $Ca(OH)_2$ 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). ^{1}H-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.

References

1) J. H. Birkinshaw, A. Bracken, S. E. Michael, H. Raistrick, *Lancet*, **245**, 625 (1943a).
2) P. M. Scott, B. P. C. Kennedy, *J. Ass. Off. Anal. Chem.*, **56**, 813 (1973).
3) A. Ciegler, R. W. Detroy, F. B. Lillehoj, "Microbial Toxins," Vol. 6 , Academic Press, New York, 1971, p. 409.
4) F. Hatey, Y. Moule, *Ann. Nutr. Aliment.*, **31**, 867 (1977); *idem*, *Toxicology*, **13**, 223 (1979); J. G. Morton, F. H. Jeffery, J. K. Miller, *FEMS. Microbiol. Lett.*, **10**, 179 (1981).
5) A. Ciegler, A. C. Beckwith, L. K. Jackson, *Applied Environmental Microbiology*, **31**, 664 (1976); R. Cooray, K. Kiessling, K. Lindahl-Kiessling, *Fd. Chem. Toxic.*, **20**, 893 (1982).
6) R. Hill, R. Heyningen, *Biochem. J.*, **49**, 332 (1951).
7) M. Uchida, G. Kusano, Y. Kondo, S. Nozoe, T. Takemoto, *Heterocycles*, **9**, 139 (1978).
8) R. B. Woodward, G. B. Singh, *J. Am. Chem. Soc.*, **72**, 5351 (1950).
9) G. B. Gill, G. Pattenden, A. Stapleton, *Tetrahedron Lett.*, **29**, 2875 (1988); J. A. Seijas, M. P. V. Tato, R. Estevez, L. Castedo, R. Riguera, *Heterocycles*, **29**, 181 (1989).
10) M. Tada, *Chem. Lett.*, **1982**, 441; M. Tada, H. Yamada, A. Kanamori, K. Chiba, *J. Chem. Soc., Perkin Trans. 1*, **1993**, 239.