BIOGENETIC SYNTHESIS OF BIFLAVONOIDS, LOPHIRONES B AND C, FROM *LOPHIRA LANCEOLATA*

Tomoyuki Shimamura, Yuka Arakawa, Kiyomi Hikita, and Masatake Niwa*

Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468, Japan

Abstract – A biogenetic synthesis of biflavonoids, lophirone B and lophirone C, was achieved by enzymatic oxidation of the corresponding chalcone.

Biflavonoids, lophirone B (**1**) and lophirone C (**2**), were reported as the constituents of an African medicinal plant, *Lophira lanceolata* (Ochnaceae), and their biogeneses were also discussed as shown in Figure 1.[1]

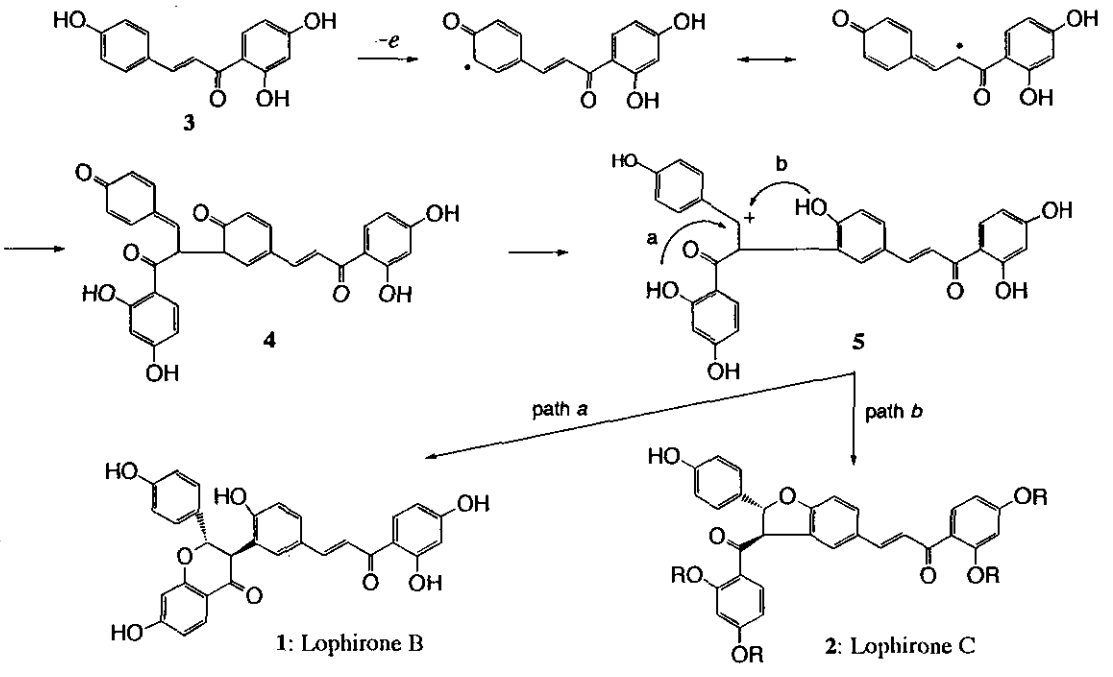
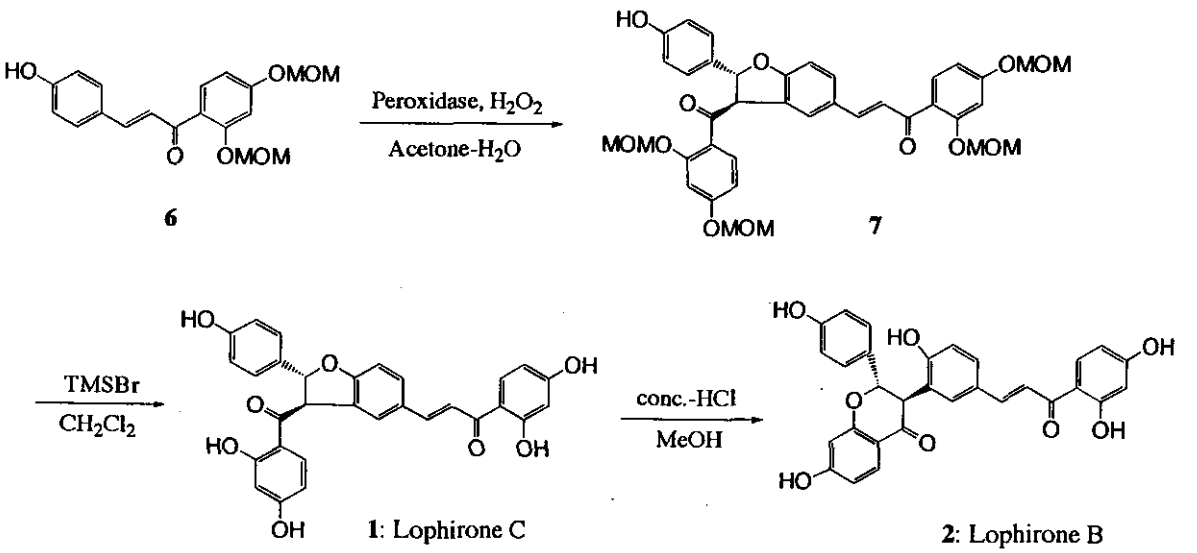


Figure 1. Biogeneses of lophirone C (**1**) and lophirone B (**2**)

Both biflavonoids (**1**) and (**2**) apparently are derived from a common dimeric chalcone intermediate (**5**). One electron-oxidation of a chalcone (**3**) and subsequent regioselective dimerization give a dienone (**4**). The conversion of **4** to its enol form gives the common intermediate (**5**), which can cyclise in either path *a* to give a chromanone ring as in **1** or path *b* to give a dihydrofuran ring as in **2**. According to these plausible pathways, we attempted synthesis of lophirone B (**1**) and lophirone C (**2**). In this paper, we describe the biogenetic synthesis of lophirone B (**1**) and lophirone C (**2**) on the basis of oxidative dimerization of the chalcone with horseraddish peroxidase and hydrogen peroxide and of recyclization of a dihydrofuran ring to a chromanone ring.

4,2',4'-Trihydroxychalcone protected partially as methoxymethyl (MOM) ether (**6**) was treated with horseraddish peroxidase and hydrogen peroxide[2,3] in a mixture of acetone and water (1 : 1) at 20 °C to give only one dimeric compound cyclised in path *a* in 32 % yield, which was characterized as a lophirone C derivative (**7**) on the basis of spectral data. The resulting compound (**7**) was subjected to deprotection by trimethylsilyl bromide (TMSBr)[4] in dichloromethane at -30 °C for 10 min to give lophirone C (**2**) in 80 % yield. The synthetic lophirone C was optically inactive, although the natural product was optically active ($[\alpha]_D$ -16.3 °C).[1] The cation produced by cleavage of the dihydrofuran ring of lophirone C (**2**) corresponds to the intermediate (**5**) in Figure 1, which can cyclise in path *b* to give a chromanone ring. We therefore tried to transform lophirone C (**2**) to lophirone B (**1**) by an acid-catalyzed reaction.



Scheme 1.

The treatment of lophirone C (**2**) with concentrated hydrochloric acid in methanol at 70 °C for 60 min gave lophirone B (**1**) in 40 % yield.

These results demonstrate the validity of the biogeneses proposed by R. G. Tih *et al.* as shown in Figure 1.[1]

EXPERIMENTAL

Optical rotations were measured with a JASCO DIP-181 automatic polarimeter. Ir spectra were taken on a JASCO FT/IR-5000 infrared spectrophotometer. Uv spectrum was recorded on a JASCO UVIDEC-610 spectrophotometer. ^{1}H and ^{13}C nmr spectra were recorded on a JEOL A-600 (600 and 125 MHz, respectively) spectrometer. Chemical shifts are presented in terms of δ_H and δ_C (ppm) with chloroform (7.24 and 77.0 ppm, respectively) or acetone (2.04 and 29.8 ppm, respectively) in the deuterated solvents as an internal standard. Mass spectra were recorded on JEOL HX-110 and Hitachi M-80 spectrometers.

Preparation of 2,4-bismethoxymethoxyacetophenone

A mixture of 2,4-dihydroxyacetophenone (3.04 g, 20 mmol), *N,N*-diisopropylethylamine (10.34 g, 80 mmol), and chloromethyl methyl ether (6.44 g, 80 mmol) in dried DMF (60 ml) was stirred at room temperature for 5 h. The mixture was poured into water (50 ml), and extracted with ethyl acetate (60 ml x 3). The combined extracts were washed with 5% NaOH (10 ml x 2), saturated NaCl (10 ml x 2), dried over Na_2SO_4, then concentrated *in vacuo* to give 2,4-bismethoxymethoxyacetophenone (4.23 g, 90%) as a colorless liquid. The crude product was used without further purification. The product shows the following spectral data : HREIms *m/z* 240.1030 [M^+] ($C_{12}H_{16}O_5$ requires: 240.0997); ir (film) ν 1670 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 2.61 (3H, s), 3.48 (3H, s), 3.52 (3H, s), 5.20 (2H, s), 5.27 (2H, s), 6.72 (1H, dd, *J*=8.74, 2.20 Hz), 6.82 (1H, d, *J*=2.20 Hz), 7.79 (1H, d, *J*=8.74 Hz).

Preparation of 2',4'-bismethoxymethoxy-4-hydroxychalcone

A solution of 2,4-dihydroxyacetophenone (6.45 g, 26.9 mmol) and 4-hydroxybenzaldehyde (3.33 g, 27.3 mmol) in methnol (15 ml) was added into 60% aqueous KOH (15 ml) and the reaction mixture was stirred at room temperature overnight. After neutralization with 1*N* HCl, the mixture was extracted with ethyl acetate (50 ml x 3). The combined extracts were washed with saturated NaCl (15 ml x 2), dried over

Na_2SO_4, then concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, chloroform/ethyl acetate) to give 2',4'-bismethoxymethoxy-4-hydroxychalcone (5.31 g, 58%) as a yellow liquid: HREIms *m/z* 344.1247 [M^+] ($C_{19}H_{20}O_6$ requires: 344.1258); ir (film) ν 3300 br, 1680 cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.48 (3H, s), 3.49 (3H, s), 5.21 (2H, s), 5.23 (2H, s), 6.77 (1H, dd, *J*=8.79, 2.20 Hz), 6.85 (1H, d, *J*=2.20 Hz), 6.87 (2H, d, *J*=8.43 Hz), 7.31 (1H, d, *J*=15.75 Hz), 7.47 (2H, d, *J*=8.43 Hz), 7.61 (1H, d, *J*=15.75 Hz), 7.65 (1H, d, *J*=8.79 Hz).

Reaction of 2',4'-bismethoxymethoxy-4-hydroxychalcone with peroxidase

A mixture of 2',4'-bismethoxymethoxy-4-hydroxychalcone (250 mg, 0.73 mmol) and horseradish (Wako Pure Chemical Industries Co. Ltd., Osaka, Japan) (0.5 mg) in 50% aqueous acetone (50 ml) was stirred at 20 °C for 15 min. To the mixture, hydrogen peroxide (100 μl, 0.88 mmol) was added. After stirring for 15 min, the mixture was extracted with ethyl acetate (30 ml x 3). The combined extracts were washed with saturated NaCl (10 ml x 2), dried over Na_2SO_4, then concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexane / ethyl acetate = 1 : 1) followed by preparative thin layer chromatography (silica gel, thexane / ethyl acetate = 1 : 1) to give tetrakismethoxymethoxylophirone C (60 mg, 24 %) as a yellow viscous liquid: HRFABms *m/z* 687.2434 [$M+H^+$] ($C_{38}H_{39}O_{12}$ requires: 687.2441); $[\alpha]_D$ 0 ° (*c* 0.3, acetone, cell length 100 mm); ir (film) ν 3350, 1655, 1605 cm^{-1}; ^1H nmr (acetone-d_6) δ 3.29 (3H, s), 3.38 (3H, s), 3.44 (6H, s), 5.18 (2H, s), 5.24 (4H, s), 5.27 (2H, s), 5.61 (1H, d, *J*=6.23 Hz), 6.16 (1H, d, *J*=6.23 Hz), 6.76 (1H, dd, *J*=8.42, 2.20 Hz), 6.81 (1H, dd, *J*=8.79, 2.20 Hz), 6.85 (2H, d, *J*=8.43 Hz), 6.86 (1H, d, *J*=2.20 Hz), 6.90 (1H, d, *J*=8.43 Hz), 6.91 (1H, d, *J*=2.20 Hz), 7.26 (2H, d, *J*=8.43 Hz), 7.28 (1H, d, *J*=15.75 Hz), 7.38 (1H, d, *J*=2.20 Hz), 7.48 (1H, d, *J*=15.75 Hz), 7.56 (1H, d, *J*=8.42 Hz), 7.58 (1H, dd, *J*=8.43, 2.20 Hz), 7.68 (1H, d, *J*=8.79 Hz).

Reaction of tetrakismethoxymethoxylophirone C with trimethylsilyl bromide

To a solution of tetrakismethoxymethoxylophirone C (28 mg, 41 μmol) in dichloromethane (2 ml), trimethylsilyl bromide (100 mg, 650 μmol) was added at -30 °C. The temperature was kept for 10 min and then elevated gradually to 0 °C. After dilution with dichloromethane (15 ml), the solution was washed with aqueous NaHCO3 (5 ml x 2), saturated NaCl (5 ml x 2), dried over Na_2SO_4, then concentrated *in vacuo*. The residue was purified by preparative thin layer chromatography (silica gel, chloroform / methanol = 10 :

1) to give lophirone C (17 mg, 80%) as a yellow viscous liquid: HRFABms *m/z* 511.1398 [M+H$^+$] ($C_{30}H_{23}O_8$ requires: 511.1393); $[\alpha]_D$ 0 ° (*c* 0.3, acetone, cell length 100 mm); ^{1}H nmr, δ_H (acetone-d_6) 5.47 (1H, d, *J*=6.59 Hz), 6.18 (1H, d, *J*=6.59 Hz), 6.34 (1H, d, *J*=2.56 Hz), 6.40 (1H, d, *J*=2.56 Hz), 6.42 (1H, dd, *J*=8.79, 2.56 Hz), 6.54 (1H, dd, *J*=8.79, 2.56 Hz), 6.85 (2H, d, *J*=8.42 Hz), 6.98 (1H, d, *J*=8.43 Hz), 7.31 (2H, d, *J*=8.42 Hz), 7.61 (1H, d, *J*=1.47 Hz), 7.69 (1H, d, *J*=15.39 Hz), 7.79 (1H, d, *J*=15.39 Hz), 7.81 (1H, dd, *J*=8.43, 1.47 Hz), 7.97 (1H, d, *J*=8.79 Hz), 7.98 (1H, d, *J*=8.79 Hz).

Reaction of lophirone C with hydrochloric acid

A mixture of lophirone C (10 mg) and concentrated hydrochloric acid (1 drop) in MeOH (1 ml) was heated at 60 °C for 1h. The reaction mixture was cooled, diluted with water (3 ml), and neutralized with aqueous $NaHCO_3$. The mixture was extracted with ethyl acetate (5 ml x 3). The combined extracts were washed with saturated NaCl (5 ml x 2), dried over Na_2SO_4, then concentrated *in vacuo*. The residue was purified by preparative thin layer chromatography (silica gel, chloroform / methanol = 10 : 1)) to recover the starting material (5 mg) and to give lophirone B (2 mg, corrected yield 40 %) as a yellow viscous liquid: HRFABms *m/z* 511.1398 [M+H$^+$] ($C_{30}H_{23}O_8$ requires: 511.1393); $[\alpha]_D$ 0 ° (*c* 0.3, acetone, cell length 100 mm); ^{1}H nmr, δ_H (acetone-d_6) 4.53 (1H, d, *J*=12.09 Hz), 5.92 (1H, d, *J*=12.09 Hz), 6.34 (1H, d, *J*=2.20 Hz), 6.42 (1H, dd, *J*=8.42, 2.20 Hz), 6.44 (1H, d, *J*=2.56 Hz), 6.62 (1H, dd, *J*=8.79, 2.56 Hz), 6.71 (2H, d, *J*=8.80 Hz), 6.82 (1H, d, *J*=8.43 Hz), 7.29 (2H, d, *J*=8.80 Hz), 7.50 (1H, dd, *J*=8.43, 2.56 Hz), 7.52 (1H, d, *J*=2.56 Hz), 7.63 (1H, d, *J*=15.39 Hz), 7.71 (1H, d, *J*=15.39 Hz), 7.79 (1H, d, *J*=8.42 Hz), 8.00 (1H, d, *J*=8.79 Hz).

ACKNOWLEDGEMENT

We are grateful to the Meijo University Grants Committee for financial support.

REFERENCES AND NOTES

1. R. Ghogomu Tih, B. L. Sondengam, M. T. Martin, and B. Bodo, *Phytochemistry*, 1989, **28**, 1557.
2. P. Langcake and R. J. Pryce, *J. Chem. Soc., Chem. Commun.*, 1977, 208.
3. Horseradish peroxidase was available from Wako Pure Chemical Industries. Ltd. (Osaka, Japan).
4. S. Hanessian, D. Delorme, and Y. Dufresne, *Tetrahedron Lett.*, 1984, **25**, 2515.

Received, 19th June, 1996