Synthesis of (±)-Epoxydon and Related Natural Compounds†

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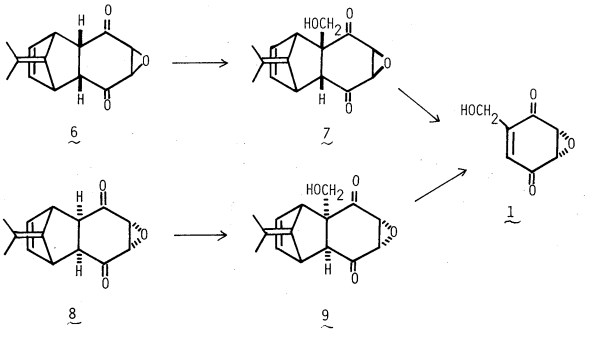
Synthesis of (±)-phyllostine, (±)-epoxydon, (±)-epiepoxydon, (±)-epoformin and (±)-epiepoformin by the retro-Diels–Alder reaction was described.

Phyllostine (**1**) and epoxydon (**2**) are phytotoxic metabolites isolated from the culture filtrate of *Phyllosticta sp*, a pathogenic fungus of red clover, as the principal toxic compounds causing wilting and simultaneous dark discoloration of leafy stem cuttings of the host plant.[1] Epoxydon was also isolated from *Phoma sp.* as a cytotoxic compound.[2] Epiepoxydon (**3**) was initially obtained as a synthetic artifact[3] together with epoxydon, and later characterized as a phytotoxin along with epiepoformin (**5**) from an unidentified fungus, separated from diseased leaves of crapemyrtle (*Lagerstroemia indica* L.),[4] and then identified as an intermediate in the patulin pathway in *Penicillium urticae*.[5] Epoformin (**4**) is an antibiotic and cytotoxic compound.[6]

All of these bioactive compounds are categorized into naturally occurring highly oxygenated cyclohexane derivatives,[7] and the syntheses of most of them have been reported.[8] Our previous synthesis of (±)-phyllostine and (±)-epoxydon started from rather expensive gentisic acid and involved non-stereoselective multi-step reactions.[9] In this report, we wish to describe the simpler and efficient synthesis of these bioactive compounds through the retro-Diels–Alder reaction starting from readily available *p*-benzoquinone.[3]

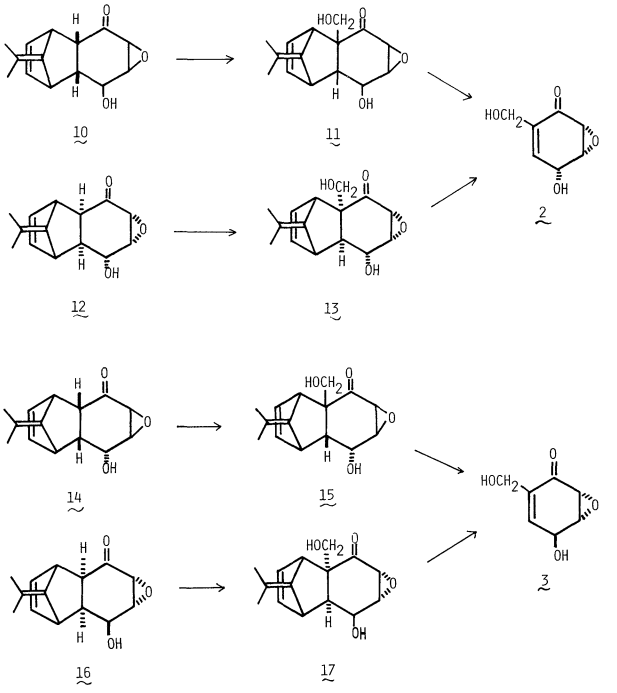
The starting materials, **6** and **8**, for the synthesis of (±)-phyllostine (**1**) were easily prepared by epoxidation of the Diels–Alder adduct of *p*-benzoquinone and dimethylfulvene according to the known procedure described in the previous paper.[10] The treatment of **6** with formaldehyde in the presence of diazabicycloundecene (DBU) in THF under ice-cooling gave compound **7** in a 65% yield.



† Synthetic Studies of Highly Oxygenated Cyclohexane Derivatives. Part XV. For Part XIV, A. Ichihara, M. Ubukata and S. Sakamura, *Tetrahedron*, **36**, 1547 (1980).

TABLE I. RETRO-DIELS–ALDER REACTION OF THE ALKYLATED COMPOUNDS

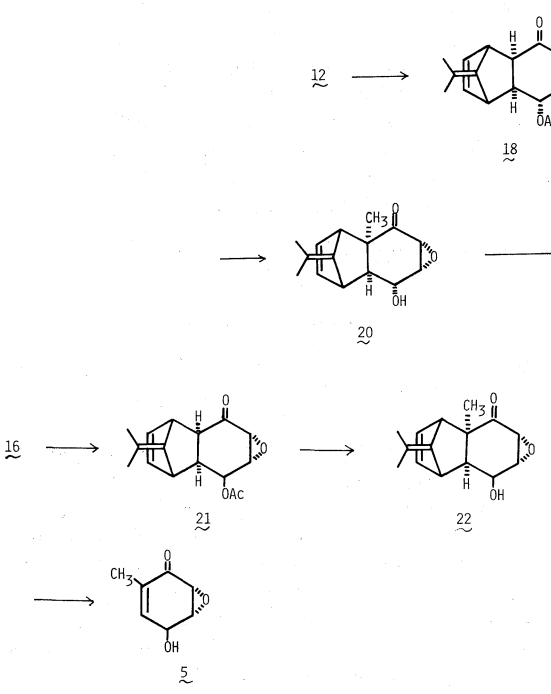
Starting material	Solvent	Temperature (°C)	Reaction time (min)	Product	Yield (%)
7	THF	140～150	30	**1**	70
9	THF	140～150	40	**1**	62
11	AcOEt	140～150	30	**2**	65
13	AcOEt	160～170	50	**2**	60
15	THF	110～120	30	**3**	76
17	THF	140～150	30	**3**	70
20	AcOEt	170～175	30	**4**	Quant
22	AcOEt	140	20	**5**	Quant



The retro-Diels–Alder reaction (Table I) of **7** at 140°C for 30 min in a sealed tube afforded (±)-phyllostine (**1**) whose spectroscopic data were identical with those of the authentic sample in all respects. Similar treatment of **8** afforded also (±)-phyllostine (**1**) through the retro-Diels–Alder reaction of **9** (Table I).

The syntheses of (±)-epoxydon (**2**) and (±)-epiepoxydon (**3**) were accomplished starting from known compounds,[10] **10**, **12**, **14** and **16**, which were obtained by the reduction of **6** and **8**. Each of **10** and **12** was treated with formaldehyde by the same procedure as used for the synthesis of **1** and converted to **11** and **13**, respectively. The retro-Diels–Alder reaction (Table I) of both **11** and **13** yielded (±)-epoxydon (**2**), mp 64～65°C, whose melting point was not depressed by mixing with an authentic sample and the spectral data were identical with those of the stock sample. The present synthetic route for (±)-epoxydon much improved the overall yield (10% from benzoquinone) as compared with the previous one (1% from gentisyl alcohol).

The same treatment of **14** and **16** gave a stereoisomer, (±)-epiepoxydon (**3**), mp 78.5～79°C. The stereochemistry of **3**, in which the epoxy and hydroxyl groups are oriented *trans* to each other, was confirmed by the NMR spectrum which exhibited a long range-



coupling ($J=1$ Hz) between 2-H and 4-H due to W-arrangement.[11]

The syntheses of (±)-epoformin **4** and (±)-epiepoformin **5** were completed starting from **12** and **16**, respectively. Since the stereochemistry of these starting materials is unambiguous, this synthesis established the relative configurations of epoformin (**4**) and epiepoformin (**5**). Since direct alkylation of **12** involved *O*-alkylation of the hydroxyl group to give the methyl ether of **12**, the acetate **18** was treated with methyl iodide in the presence of potassium *tert*-butoxide in DMF under ice-cooling to yield a mixture of **19** and methyl ether in a ratio of 2:1. Purification of the mixture was difficult, but a small amount of pure **19** was possible to separate by silica gel column chromatography. Then, the mixture was treated with aqueous KOH (10%) and the products were separated by silica gel chromatography to give the alcohol **20**. The retro-Diels–Alder reaction of **20** gave quantitatively (±)-epoformin (**4**), whose spectroscopic data are identical with those of natural epoformin.

Similarly (±)-epiepoformin (**5**) was prepared from **16**. The treatment of **16** with acetic anhydride in pyridine afforded the acetate **21**. Through alkylation with methyl iodide and subsequent hydrolysis, the acetate **21** was converted to **22**. The retro-Diels–Alder reaction (Table I) of **22** proceeded smoothly to give (±)-epiepoformin (**5**), whose NMR data are identical with those of (±)-epiepoformin.

EXPERIMENTAL

Melting points were determined on a hot-stage microscope, Yanako Micromelting Point Apparatus MP-3D, and were uncorrected. NMR spectra were obtained with a Hitachi 90 MHz High Resolution Spectrometer Model R-22. IR spectra were recorded on a Hitachi IR Spectrophotometer, Model 285. Mass spectra were determined on a Hitachi RMU-4 spectrometer.

The starting materials, **6**, **8**, **10**, **12**, **14** and **16**, were prepared by the same procedure as described previously.[11]

General procedure of hydroxymethylation of **6**, **8**, **10**, **12**, **14** *and* **16**. To a solution of 200 mg of the starting material in 4 ml of tetrahydrofuran was added 0.2 ml of diazabicycloundecene and 0.2 ml of formaldehyde (40% solution). After disappearance (1~5 hr) of the starting material on TLC, the reaction mixture was extracted with chloroform (**7**, **9**) or ethyl acetate (**11**, **13**, **15**, **17**) and the extracts were dried over Na_2SO_4 and evaporated *in vacuo* to give a residue. The residue was chromatographed on silica gel to yield the products (**7**, **9**, **11**, **13**, **15** and **17**).

7, recrystallized from benzene–ethyl ether, yield 65%, mp 122~123°C. IR ν_{max}^{KBr} cm^{-1}: 3450, 1710; NMR $\delta_{TMS}^{CDCl_3}$; 1.60 (6H, s, $=\!\!\diagdown_{CH_3}$), 2.74 (1H, d, $J=4$ Hz, –CH–), 3.60, 4.10 (2H, ABq, $J=11$ Hz, $-CH_2O$), 3.55 (2H, s, $\overset{H}{\diagdown}\!\!\diagup\!\!\diagdown\!\!\diagup\overset{O\ H}{}$), 3.75 (2H, m, –CH–), 6.15 (2H, m, $=\!\!\diagup^{H}$). MS *m/z*: 260 (M^+). Found: C, 68.84; H, 6.18. Calcd. for $C_{15}H_{16}O_4$: C, 69.21; H, 6.21%.

9, recrystallized from benzene–ethyl ether, yield 73%, mp 130~131°C; IR ν_{max}^{KBr} cm^{-1}: 3490, 3400, 1705; NMR

$\delta_{TMS}^{CDCl_3}$: 1.48 (6H, s, CH_3), 2.10 (1H, s, –CH–), 3.45, 4.25 (2H, ABq, J=11 Hz, $-CH_2O-$), 3.75 (2H, s, H O H), 4.05 (2H, m, –CH), 6.40 (2H, m, =CH). MS m/z: 260 (M^+). Found: C, 68.66; H, 6.18. Calcd. for $C_{15}H_{16}O_4$: C, 69.21; H, 6.21%.

11, recrystallized from chloroform–ethyl acetate, yield 86%, mp 144~145°C. IR ν_{max}^{KBr} cm^{-1}: 3450, 3360, 1700, NMR $\delta_{TMS}^{acetone\text{-}d_6}$: 1.60 (6H, s, CH_3), 1.85 (1H, dd, J=4 Hz, 8 Hz, –CH–), 3.20 (1H, d, J=4 Hz, O H), 3.35, 4.35 (2H, ABq, J=11 Hz, $-CH_2O-$), 3.45~3.90 (4H, m), 6.22 (2H, m, =CH). MS m/z: 262 (M^+). Found: C, 68.86; H, 6.89. Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92%.

13, recrystallized from chloroform–ethyl acetate, yield 90%, mp 171~173°C, IR ν_{max}^{KBr} cm^{-1}: 3350, 1700, 1680. NMR $\delta_{TMS}^{acetone\text{-}d_6}$: 1.25 (1H, d, J=8 Hz, –CH–), 1.50 (6H, s, CH_3), 3.20, 4.10 (2H, ABq, j=11 Hz, CH_2O-), 3.30~3.40 (2H, m), 3.55~3.70 (2H, m), 3.80 (1H, m, –CH–), 6.25 (2H, m, =CH). MS m/z: 262 (M^+). Found: C, 68.42; H, 6.93. Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92%.

15, recrystallized from chloroform–ethyl acetate, yield 91%, mp 114~117°C. IR ν_{max}^{KBr} cm^{-1}: 3450, 3300, 1700. NMR $\delta_{TMS}^{acetone\text{-}d_6}$: 1.55 (6H, s, CH_3), 3.15 (1H, d, J=4 Hz, –CH–), 3.30, 4.20 (2H, ABq, J=11 Hz, CH_2–O–), 3.40~3.70 (4H, m), 4.60 (1H, m), 6.00, 6.20 (each 1H, m, =CH).

17, recrystallized from chloroform–ethyl acetate, yield 92%, mp 132~133°C, IR ν_{max}^{KBr} cm^{-1}: 3450, 1695. NMR $\delta_{TMS}^{acetone\text{-}d_6}$: 1.42, 1.46 (each 3H, s, CH_3), 1.50 (1H, d, J= 6 Hz, –CH–), 3.20, 4.12 (2H, ABq, J=11 Hz, $-CH_2-O-$), 3.25 (1H, d, J=4 Hz, –CH–), 3.35 (1H, m, –CH–), 3.59 (1H, dd, J=4 Hz, 3 Hz, –CH–), 3.84 (1H, m, –CH–), 4.60 (1H, dd, J=6 Hz, 3 Hz, CHOH), 6.30 (2H, m, =CH). MS m/z: 262 (M^+). Found: C, 66.32; H, 6.90. Calcd. for $C_{15}H_{18}O_4$, H_2O: C, 66.40; H, 7.06%.

General procedure of the retro-Diels–Alder reaction of hydroxymethylated products, **7**, **9**, **11**, **13**, **15** *and* **17** *(Table I).* A solution of 100 mg of the sample in 4 ml of tetrahydrofuran (ethyl acetate in the cases of **11** and **13**) was heated for 30~50 min at 110~170°C in a sealed tube. The reaction mixture was concentrated *in vacuo* to give a residue, which was chromatographed on a silica gel column to yield products **1**, **2** and **3**. Recrystallization of each sample from chloroform–ethyl acetate yielded phyllostine **1**, mp 47~48°C (lit.[9] 47.5~48°), epoxydon **2**, mp 64~65°C (lit. 60.1~61.7°C), and epiepoxydon **3**, mp 78.5~79°C, IR ν_{max}^{KBr} cm^{-1}: 3350, 1680: NMR $\delta_{TMS}^{acetone\text{-}d_6}$: 3.40 (1H, dd, J=3.5 Hz, 1 Hz, –CH–), 3.78 (1H, m, –CH–), 4.20 (2H, s, CH_2–O), 4.65 (1H, br. dd, J=5 Hz, 1H, –CHO), 6.70 (1H, dd, J=5 Hz, 2.5 Hz, =CH). Found: C, 53.40; H, 4.96, Calcd. for $C_7H_8O_4$: C, 53.84; H, 5.16%.

Acetylation of **12**. A solution of 650 mg of the ketol **12** and 3 ml of acetic anhydride in 7 ml of pyridine was allowed to stand overnight at room temperature. The reaction mixture was poured onto ice-water, and extracted with benzene. Combined extracts were dried over Na_2SO_4 and evaporated *in vacuo* to give a crystalline material quantitatively. The product was recrystallized from benzene to yield acetate **18**, mp 145.5~145.9°C, IR ν_{max}^{nujol} cm^{-1}: 1750, 1705. NMR $\delta_{TMS}^{CDCl_3}$ 1.53, 1.60 (each 3H, s, CH_3), 2.22 (3H, s, $COCH_3$), 2.25 (2H, m, –CH–), 3.15 (1H, m, –CH–), 3.39 (1H, d, J=4 Hz, –CHO–), 3.59 (1H, dd, J=4 Hz, 2 Hz, –CH–O), 3.83 (1H, m, –CH–), 4.84 (1H, dd, J=7 Hz, 2 Hz, –CHOAc), 6.24 (2H, m, =CH). MS m/z: 274 (M^+).

Methylation of **18**. A mixture of 480 mg of potassium and 9.6 ml of *tert*-BuOH was refluxed for 3 hr, and then cooled with ice-water. To the mixture was added 800 mg of the acetate **18** in 8 ml of dimethylformamide and 1.3 ml of methyl iodide under stirring and ice cooling. The mixture was stirred for 10 min, poured into ice-water and then extracted with benzene. The combined extracts were washed with brine, dried over Na_2SO_4 and evaporated *in vacuo* to yield a residue which was chromatographed on a silica gel column. The column was eluted with benzene–ethyl acetate (19 : 1 v/v) to yield a small amount of **19** and a mixture (745 mg) of **19** and **20** in a ratio of 2 : 1. **19** IR ν_{max}^{nujol} 1745, 1715 cm^{-1}; NMR $\delta_{TMS}^{CDCl_3}$ 1.18 (3H, s, CH_3), 1.55 (6H, s, =CH_3), 1.70 (1H, d, J=8 Hz, –CH), 2.22 (3H, s, CH_3), 3.12 (1H, br. s, –CH), 3.42, 3.62 (2H, ABq, J=5 Hz, O, CH–CH), 3.71 (1H, d, J=2 Hz, CH), 4.89 (1H, d, J=8 Hz, >CHOAc), 6.25 (2H, d, J=2 Hz, =CH); MS m/z: 288 (M^+).

*Hydrolysis of the mixture (***19**, **20***).* A mixture (745 mg) of **19** and **20** was dissolved into 13 ml of THF and to the solution was added 5 ml of KOH aq. solution (10%) at room temperature, and the mixture was stirred for 8 hr. The reaction mixture was poured into 100 ml of water, and extracted with ethyl acetate. The extracts were dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed on silica gel using benzene–ethyl acetate as an eluent to yield 360 mg of a white solid which was recrystallized from benzene to yield **20**, mp 156~157°C, IR ν_{max}^{nujol} 3400, 1695 cm; NMR $\delta_{TMS}^{CDCl_3}$ 1.15 (3H, s, CH_3), 1.55 (6H, s, =CH_3), 3.36 (1H, m, CH), O, 3.45, 3.65 (2H, ABq, J=4 Hz, –CH–CH–), 3.68 (2H, m, CH). MS m/z: 246 (M^+). Found: C, 71.79; H, 7.23. Calcd. for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37%.

(±)-Epoformin **4**. A solution of 200 mg of **20** in 200 ml of ethyl acetate was heated at 170°C for 15 min in a sealed

tube. The reaction mixture was concentrated *in vacuo* and chromatographed on silica gel using benzene–ethyl acetate as eluents to give quantitatively a solid, which was recrystallized from benzene to yield (±)-epoformin **4**, mp 61.5~61.9°C. IR $\nu_{TMS}^{CDCl_3}$ 3560, 1680 cm^{-1}. NMR $\delta_{TMS}^{CDCl_3}$ 1.85 (3H, d, J=2 Hz, CH_3), 3.50 (1H, d, J=4 Hz, CH), 3.85 (1H, m, CH), 4.70 (1H, br s, CH), 6.30 (1H, m, $=\!\!\diagup^{H}$). MS m/z: 140 (M^+). Found: C, 59.99; H, 5.75. Calcd. for $C_7H_8O_3$; C, 59.99; H, 5.75%.

Acetylation of **16**. A solution of 1.5 g of **16** in 14 ml of pyridine and 6 ml of acetic anhydride was allowed to stand overnight. The reaction mixture was extracted with benzene and the extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield **21** quantitatively. Recrystallization from benzene gave pure **21**, mp 140.5~141.5°C, IR ν_{max}^{nujol} 1735, 1700 cm; NMR $\delta_{TMS}^{CDCl_3}$ 1.52, 1.64 (each 3H, s, $=\!\!\diagup^{CH_3}$), 2.05 (3H, s, CH_3), 2.45 (2H, m, CH), 3.16 (1H, m, CH), 3.34 (1H, d, J=5 Hz, CH), 3.78 (1H, dd, 5 Hz, 3 Hz, CH), 3.79 (1H, m, CH), 5.72 (1H, m, CHOAc), 6.30 (2H, m, $=\!\!\diagup^{H}$).

Preparation of **22**. A mixture of 300 mg of potassium in 6 ml of dried *tert*-BuOH was refluxed for 3 hr. To the mixture was added a solution of 470 mg of **21** and 1.1 ml of methyl iodide in 5 ml of DMF under ice cooling, and the mixture was stirred for 10 min. The reaction mixture was poured into ice-water and extracted with benzene. The extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel using benzene–ethyl acetate (19 : 1 v/v) as eluent to give a mixture (320 mg) of **21** and **22** in a ratio of 2 : 1. A solution of 320 mg of the mixture was treated with 3 ml of aq. KOH (10%) under stirring for 10 hr. The reaction mixture was poured into water and extracted with ethyl acetate. The extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on a silica gel column using benzene–ethyl acetate (7 : 3 v/v) to give 186 mg of **22**, which was recrystallized from benzene, mp 105~106°C, IR ν_{max}^{nujol} 3400, 1685 cm^{-1}; NMR $\delta_{TMS}^{CDCl_3}$ 1.20 (3H, s, CH_3), 1.59, 1.61 (each 3H, s, $=\!\!\diagup^{CH_3}$), 1.85 (1H, d, J=7 Hz, CH), 3.36 (2H, m, CH), 3.65 (2H, m, CH), 4.65 (1H, m, CH), 6.25 (2H, m, $=\!\!\diagup^{H}$). MS m/z: 246 (M^+). Found: C, 72.93; H, 7.44. Calcd. for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37%.

(±)-Epiepoformin **5**. A solution of 90 mg of **22** in 90 ml of ethyl acetate was heated at 140°C for 20 min in a sealed tube. The reaction mixture was concentrated *in vacuo* to give a residue which was chromatographed on silica gel using ethyl acetate (7 : 3 v/v) to yield 45 mg of oily **5**. IR $\nu_{max}^{CHCl_3}$ 3575, 1680 cm^{-1}. NMR $\delta_{TMS}^{CDCl_3}$ 1.87 (3H, d, J=1 Hz, CH_3), 3.46 (1H, dd, J=4 Hz, 1 Hz, CH), 3.80 (1H, m, CH), 4.63 (1H, br s, CH), 6.47 (1H, br m, $=\!\!\diagup^{H}$). MS m/z: 140 (M^+).

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