
Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb20

# Structural Confirmation of 15-Norlubiminol and 15-Norepilubiminol, Isolated from Solanum aethiopicum, by Chemical Conversion from Lubimin and Epilubimin, and their Antifungal Activity

Ayako WATANABE[ab], Hiroaki TOSHIMA[a], Hiroshi NAGASE[a], Toshinori NAGAOKA[c] & Teruhiko YOSHIHARA[ab]

[a] Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University

[b] CREST, Japan Science and Technology Corporation

[c] Faculty of Applied Biological Science, Hiroshima University

Published online: 22 May 2014.

To cite this article: Ayako WATANABE, Hiroaki TOSHIMA, Hiroshi NAGASE, Toshinori NAGAOKA & Teruhiko YOSHIHARA (2001) Structural Confirmation of 15-Norlubiminol and 15-Norepilubiminol, Isolated from Solanum aethiopicum, by Chemical Conversion from Lubimin and Epilubimin, and their Antifungal Activity, Bioscience, Biotechnology, and Biochemistry, 65:8, 1805-1811, DOI: 10.1271/bbb.65.1805

To link to this article: http://dx.doi.org/10.1271/bbb.65.1805

*Biosci. Biotechnol. Biochem.*, **65** (8), 1805–1811, 2001



# Structural Confirmation of 15-Norlubiminol and 15-Norepilubiminol, Isolated from *Solanum aethiopicum*, by Chemical Conversion from Lubimin and Epilubimin, and their Antifungal Activity

Ayako WATANABE,[1,2] Hiroaki TOSHIMA,[1] Hiroshi NAGASE,[1] Toshinori NAGAOKA,[3] and Teruhiko YOSHIHARA[1,2,†]

[1]*Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan*
[2]*CREST, Japan Science and Technology Corporation, Honcho 4-1-8, Kawaguchi 332-0012, Japan*
[3]*Faculty of Applied Biological Science, Hiroshima University, Kagamiyama 1-4-4, Higashi-Hiroshima 739-8528, Japan*

Received February 22, 2001; Accepted April 17, 2001



**15-Norlubiminol and 15-norepilubiminol were obtained from *Solanum aethiopicum* as an inseparable 1:1 mixture in a relatively poor yield to that of the major phytoalexins, lubimin and epilubimin. Their structures were confirmed by chemical conversion starting from lubimin and epilubimin. Baeyer-Villiger oxidation of the protected lubimins with *m*-chloroperoxybenzoic acid provided the desired formates. Deoxygenation with triphenylphosphine selenide and subsequent methanolysis provided 15-norlubiminols, whose $^1$H-NMR spectra were respectively identical with that of the corresponding isomer in the natural 15-norlubiminol mixture. The antifungal activity of 15-norlubiminols would be weaker than that of lubimins.**

**Key words:** lubimin; epilubimin; sesquiterpene; 15-norlubiminol; 15-norepilubiminol

In our series of chemical studies on the resistance of wild-type crops,[1,2,3] solavetivone and the lubimin class of sesquiterpenes (**1–4**, **8**, **9**) and norsesquiterpenes (**5–7**, **10**)[4] have been isolated from the roots of *Solanum aethiopicum* (a wild relative of the eggplant),[4] which exhibited strong resistance against some soil-borne diseases caused by *Fusarium oxysporum* f. sp. *melongenae*, *Verticillium dahliae*, and *Ralstonia solanacearum* (formerly *Pseudomonas solanacearum*).[5] Solavetivone (**1**),[6] lubimin (**2**),[7] and epilubimin (**8**)[8] have been isolated as phytoalexins from potato tubers (*Solanum tuberosum*) infected with some fungi. It is therefore required to examine the antifungal or other biological activities of such a class of sesquiterpenes. Among newly isolated compounds (**3**, **5–7**, **9**, **10**), there are four $C_1$ degraded sesquiterpenes (**5–7**, **10**). 15-Norlubiminol (**6**) and 15-norepilubiminol (**10**) were obtained as an inseparable 1:1 mixture (0.3 mg) in a poor yield in contrast to that of major phytoalexins **1**, **2** and **8**. The structures of **6** and **10** have been estimated from the limited spectral data of their mixture, and aethione (**7**) was reduced to **6** in a low yield.[4] The structure of **5** possessing no oxygen functional group at the C-10 position was also estimated from the limited spectral data (Nagase *et al.*, unpublished result). To reveal the structure and antifungal activity of **6** and **10**, and to supply such minor constituents, chemical conversion starting from major constituents **1** and **2** was attempted. We are also interested in the structure-activity relationship and biogenesis of the lubimin class of sesquiterpenes and norsesquiterpenes.

## Materials and Methods

*General methods.* $^1$H- and $^{13}$C-NMR spectra were recorded with a Jeol JNM-EX-270 spectrometer ($^1$H at 270 MHz; $^{13}$C at 67.8 MHz) or a Bruker AM-500 spectrometer ($^1$H at 500 MHz; $^{13}$C at 125 MHz). Chemical shifts in the $^1$H-NMR spectra are reported as $\delta$ (ppm) values relative to the residual proton ($\delta$ 7.26 ppm) of $CDCl_3$, and in the $^{13}$C-NMR spectra as $\delta$ (ppm) values relative to the carbon signal ($\delta$ 77.0 ppm) of $CDCl_3$. Partial assignments for **6**, **10–13**, and **15–19** are described by the common numbering shown in Fig. 1, and for **14** and **20** by the spirocyclic numbering shown in Scheme 2. IR spectra were measured with a Perkin Elmer 2000 FT-IR spectrometer, and mass spectra were recorded with a Jeol JMS-AX500 or Jeol JMS-SX102A spectrometer. Specific rotation values were measured with a Jasco DIP-370 digital polarimeter. Analytical and prepara-

† To whom correspondence should be addressed. Fax: +81-11-706-2505; E-mail: yosihara@chem.agr.hokudai.ac.jp
*Abbreviations*: *m*-CPBA, *m*-chloroperoxybenzoic acid; TFA, trifluoroacetic acid

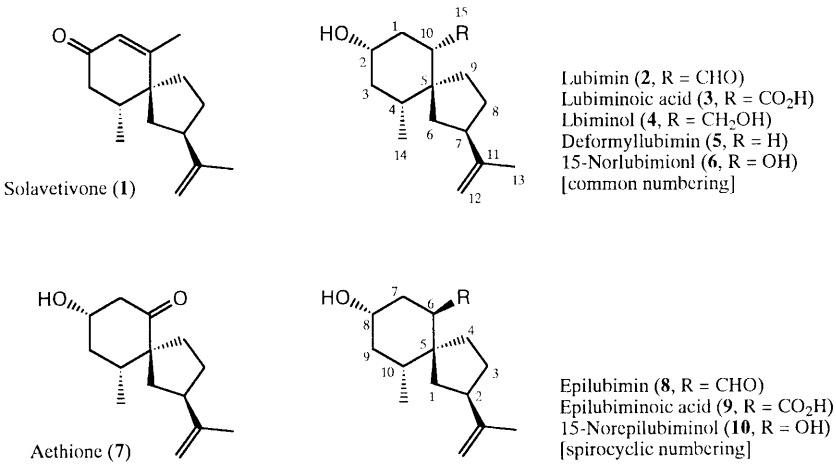



**Fig. 1.** Structures of Solavetivone and the Lubimin-class Sesqui- and Norsesquiterpenes.



tive TLC was performed on precoated silica gel 60 $F_{254}$ plates of Merck Art. 5715 and Art. 5744, respectively.

*(2R,5S,6S,8S,10R)-8-Benzoyloxy-6-formyl-2-isopropenyl-10-methylspiro[4.5]decane [2-O-Benzoyl-lubimin (11)].* To a solution of lubimin **2** (34.0 mg, 144 $\mu$mol) in $CH_2Cl_2$ (0.8 ml) were added benzoyl chloride (5 drops) and pyridine (5 drops) with Pasteur pipettes at room temperature. After being stirred for 5 h, the mixture was directly purified by preparative TLC (hexane:$Et_2O$ = 3:7) to give **11** (42.0 mg, 86%) as a colorless oil. $[\alpha]_D^{24}$ +50.4° (*c* 1.15, $CHCl_3$); EIMS *m/z*: 340 (1.59, $M^+$), 218 (94.4, $M^+ - C_6H_5CO_2H$), 105 (100), 77 (31.7); HRMS *m/z* ($M^+$): calcd. for $C_{22}H_{28}O_3$, 340.2038; found, 340.2031; $^1$H-NMR (270 MHz, $CDCl_3$) $\delta$: 0.98 (3H, d, *J* = 6.6 Hz, H-14), 1.72 (3H, br. s, H-13), 1.20–1.95 (10H, m), 2.13 (1H, m, H-1a), 2.42 (2H, m, H-7, H-10), 4.69 (2H, br.s, H-12), 5.02 (1H, m, H-2), 7.43 (2H, br. t, *J* = 8.0 Hz, Ar-*m*-$H_2$), 7.55 (1H, br. t, *J* = 8.0 Hz, Ar-*p*-*H*), 8.02 (2H, br. d, *J* = 8.0 Hz, Ar-*o*-$H_2$), 9.87 (1H, d, *J* = 2.0 Hz, H-15); $^{13}$C-NMR (67.8 MHz, $CDCl_3$) $\delta$: 16.3, 21.2, 25.8, 29.5, 32.4, 36.4, 40.9, 41.6, 47.1, 47.3, 57.9, 72.1, 108.9, 128.3, 129.5, 130.5, 132.9, 147.2, 166.0, 204.0; IR $\nu_{max}$ (film) $cm^{-1}$: 3070, 2950, 2875, 1717, 1647, 1559, 1451, 1315, 1275, 1113, 1070, 1026, 888, 713.

*(2R,5S,6R,8S,10R)-8-Benzoyloxy-6-formyl-2-isopropenyl-10-methylspiro[4.5]decane [2-O-Benzoyl-epilubimin (16)].* According to the same method as that described for **11**, epilubimin **8** (13.4 mg, 57.0 $\mu$mol) was converted into **16** (16.5 mg, 85%) as a colorless oil. $[\alpha]_D^{24}$ −3.92° (*c* 1.18, $CHCl_3$); EIMS *m/z*: 340 (0.54, $M^+$), 218 (87.5, $M^+$-$C_6H_5CO_2H$), 105 (100), 77 (41.2); HRMS *m/z* ($M^+$): calcd. for $C_{22}H_{28}O_3$, 340.2038; found, 340.2056; $^1$H-NMR (270 MHz, $CDCl_3$) $\delta$: 1.00 (3H, d, *J* = 6.9 Hz, H-14), 1.70 (3H, br. s, H-13), 1.20–2.10 (10H, m), 2.25–2.50 (2H, m, H-1, H-7), 2.59 (1H, t, *J* = 4.3 Hz, H-10), 4.70 (2H, br.s, H-12), 5.10 (1H, m, H-2), 7.43 (2H, br. t, *J* = 8.0 Hz, Ar-*m*-$H_2$), 7.55 (1H, br. t, *J* = 8.0 Hz, Ar-*p*-*H*), 8.02 (2H, br. d, *J* = 8.0 Hz, Ar-*o*-$H_2$), 9.91 (1H, br. s, H-15); $^{13}$C-NMR (67.8 MHz, $CDCl_3$) $\delta$: 16.9, 21.4, 28.9, 31.0, 31.6, 35.7, 36.9, 41.8, 46.1, 47.8, 57.0, 69.9, 108.7, 128.3, 129.5, 130.1, 132.8, 147.5, 165.8, 204.8; IR $\nu_{max}$ (film) $cm^{-1}$: 3070, 2954, 2871, 1717, 1646, 1559, 1452, 1316, 1277, 1113, 1070, 1023, 888, 713.

*Baeyer-Villiger oxidation of 11 with m-CPBA.* To a stirred solution of **11** (20.5 mg, 60.3 $\mu$mol) in $CH_2Cl_2$ (0.8 ml) were added $NaHCO_3$ (12.7 mg, 151 $\mu$mol) and *m*-CPBA (32.6 mg, 151 $\mu$mol and 80% purity) at room temperature. After 3.5 h, to the reaction mixture were added sat. aq. $Na_2S_2O_3$ and sat. aq. $NaHCO_3$. The resulting mixture was extracted with $CHCl_3$. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by preparative TLC (*n*-hexane:$Et_2O$ = 3:7) to give **13** (2.9 mg, 13%), **12** (8.1 mg, 36%) and **14** (8.4 mg, 39%) as colorless oils, respectively.

*(2R,5S,6S,8S,10R)-8-Benzoyloxy-2-[(2RS)-1,2-epoxy-2-propyl]-6-formyloxy-10-methylspiro[4.5]decane [2-O-Benzoyl-11,12-epoxy-lubiminoic acid (13)].* $[\alpha]_D^{25}$ +27.2° (*c* 0.5, $CHCl_3$); EIMS *m/z*: 372 (2.31, $M^+$), 326 (17.7, $M^+$-$HCO_2H$), 250 (5.08, $M^+ - C_6H_5CO_2H$), 204 (50.9), 105 (100), 77 (43.6); HRMS *m/z* ($M^+$): calcd. for $C_{22}H_{28}O_5$, 372.1937; found, 372.1926; $^1$H-NMR (270 MHz, $CDCl_3$) $\delta$: 0.96 (3H, d, *J* = 6.9 Hz, H-14), 1.29 (3H, br. s, H-13), 1.16–2.10 (11H, m), 2.32–2.69 (3H, m), 4.88 (1H, dd, *J* = 11.5, 3.6 Hz, H-10), 5.02 (1H, m, H-2), 7.42 (2H, br. t, *J* = 7.3 Hz, Ar-*m*-$H_2$), 7.55 (1H, br. t, *J* = 7.3 Hz, Ar-*p*-*H*), 8.00 (2H, br. d, *J* = 7.3 Hz, Ar-*o*-$H_2$), 8.12 (1H, s, H-15); $^{13}$C-NMR (67.8 MHz,

$CDCl_3$) $\delta$: 16.1, 20.2, 25.3, 28.9, 33.2, 34.4, 36.1, 36.87, 36.90, 37.2, 45.2, 48.4, 52.8, 57.47, 57.54, 57.6, 69.9, 76.9, 128.2, 129.4, 130.2, 132.8, 160.4, 165.5; IR $\nu_{max}$ (film) $cm^{-1}$: 2958, 1722, 1647, 1557, 1451, 1315, 1275, 1175, 1113, 1071, 1027, 976, 755, 713.

*(2R,5S,6S,8S,10R)-8-Benzoyloxy-6-carboxy-2-[(2RS)-1,2-epoxy-2-propyl]-10-methylspiro[4.5]decane [2-O-Benzoyl-11,12-epoxy-10-O-formyl-15-norlubiminol (12)].* $[\alpha]_D^{25}$ +22.2° (*c* 1.29, $CHCl_3$); EIMS *m/z*: 372 (2.46, $M^+$), 250 (17.4, $M^+$-$C_6H_5CO_2H$), 204 (8.13), 105 (100), 77 (37.2); HRMS *m/z* ($M^+$): calcd. for $C_{22}H_{28}O_5$, 372.1937; found, 372.1911; $^1$H-NMR (270 MHz, $CDCl_3$) $\delta$: 0.94 (3H, d, *J*=6.9 Hz, H-14), 1.27 (3H, br. s, H-13), 1.20–2.10 (12H, m), 2.21 (2H, m), 2.45 (2H, m), 4.96 (1H, m, H-2), 7.42 (2H, br. t, *J*=7.3 Hz, Ar-*m*-$H_2$), 7.54 (1H, br. t, *J*=7.3 Hz, Ar-*p*-*H*), 8.01 (2H, br. d, *J*=7.3 Hz, Ar-*o*-$H_2$), the proton signal of the carboxyl group could not be assigned due to broadening; $^{13}$C-NMR (67.8 MHz, $CDCl_3$) $\delta$: 16.5, 19.95, 20.04, 25.0, 25.1, 29.0, 29.5, 32.25, 32.30, 36.4, 37.7, 41.12, 41.14, 45.3, 46.9, 47.2, 50.76, 50.80, 52.6, 57.85, 57.90, 71.7, 128.2, 129.4, 130.3, 132.8, 165.8, 178.6; IR $\nu_{max}$ (film) $cm^{-1}$: 3400–2500 (br.), 2960, 2871, 1716, 1602, 1451, 1382, 1315, 1277, 1177, 1114, 1071, 1027, 976, 755, 714.

*(2R,5S,9S,11R)-9-Benzoyloxy-2-[(2RS)-1,2-epoxy-2-propyl]-11-methyl-6-oxaspiro[4.6]undecan-7-one (14).* $[\alpha]_D^{25}$ +14.3° (*c* 0.56, $CHCl_3$); EIMS *m/z*: 358 (0.27, $M^+$), 341 (0.6, $M^+$-OH), 236 (1.67, $M^+$-$C_6H_5CO_2H$), 105 (29.0), 77 (21.8), 41 (100); FDMS *m/z*: 359 (100, $MH^+$); HRMS *m/z* ($MH^+$): calcd. for $C_{21}H_{27}O_5$, 359.1859; found, 359.1850; $^1$H-NMR (270 MHz, $CDCl_3$) $\delta$: 1.24 (3H, d, *J*=7.2 Hz, H-12), 1.33 and 1.35 (each 1.5H, s, total 3H, H-15), 1.62–2.38 (10H, m), 2.58–2.69 (2H, m, H-13), 2.99 (1H, dd, *J*=14.8, 1.9 Hz, H-8a), 3.20 (1H, dd, *J*=14.8, 6.9 Hz, H-8b), 5.45 (1H, m, H-9), 7.43 (2H, br. t, *J*=7.3 Hz, Ar-*m*-$H_2$), 7.56 (1H, br. t, *J*=7.3 Hz, Ar-*p*-*H*), 8.01 (2H, br. d, *J*=7.3 Hz, Ar-*o*-$H_2$); $^{13}$C-NMR (67.8 MHz, $CDCl_3$) $\delta$: 16.8, 19.4, 20.8, 27.1, 27.5, 36.3, 39.2, 39.56, 39.64, 40.6, 40.7, 41.6, 44.6, 45.0, 52.0, 53.0, 57.3, 57.6, 67.8, 93.4, 93.5, 128.3, 128.4, 129.7, 133.2, 165.3, 170.7; IR $\nu_{max}$ (film) $cm^{-1}$: 2970, 2950, 2870, 1716, 1647, 1452, 1315, 1271, 1165, 1099, 1070, 1026, 977, 713.

*Baeyer-Villiger oxidation of **16** with m-CPBA.* According to the same method for Baeyer-Villiger oxidation of **11**, **16** (21.0 mg, 61.8 μmol) was converted into **18** (12.4 mg, 54%), **17** (5.5 mg, 24%) and **14** (1.0 mg, 4.5%) as colorless oils, respectively.

*(2R,5S,6R,8S,10R)-8-Benzoyloxy-2-[(2RS)-1,2-epoxy-2-propyl]-6-formyloxy-10-methylspiro[4.5]decane [2-O-Benzoyl-11,12-epoxy-epilubiminoic acid (18)].* $[\alpha]_D^{24}$ −2.8° (*c* 1.25, $CHCl_3$); EIMS *m/z*: 372 (1.70, $M^+$), 326 (8.47, $M^+$-$HCO_2H$), 250 (3.79, $M^+$ − $C_6H_5CO_2H$), 204 (47.3), 105 (100), 77 (51.2); HRMS *m/z* ($M^+$): calcd. for $C_{22}H_{28}O_5$, 372.1937; found, 372.1965; $^1$H-NMR (270 MHz, $CDCl_3$) $\delta$: 0.94 (3H, d, *J*=6.6 Hz, H-14), 1.29 (3H, br. s, H-13), 1.20–2.18 (11H, m), 2.28 (1H, m), 2.52 (1H, br. d, *J*=4.6 Hz, H-12a), 2.62 and 2.64 (each 0.5 H, br. d, *J*=4.6 Hz, total 1H, H-12b), 5.03 (1H, m, H-10), 5.20 (1H, m, H-2), 7.42 (2H, br. t, *J*=7.3 Hz, Ar-*m*-$H_2$), 7.55 (1H, br. t, *J*=7.3 Hz, Ar-*p*-*H*), 8.00 (2H, br. d, *J*=7.3 Hz, Ar-*o*-$H_2$), 8.14 (1H, s, H-15); $^{13}$C-NMR (67.8 MHz, $CDCl_3$) $\delta$: 16.4, 20.3, 20.4, 28.1, 28.7, 33.2, 33.9, 36.1, 37.4, 38.0, 45.5, 45.6, 47.6, 52.6, 52.7, 57.5, 69.6, 77.7, 78.7, 128.2, 129.4, 130.3, 132.8, 160.5, 165.7; IR $\nu_{max}$ (film) $cm^{-1}$: 2958, 2871, 1717, 1603, 1451, 1382, 1315, 1277, 1177, 1113, 1071, 1026, 982, 905, 845, 714.

*(2R,5S,6R,8S,10R)-8-Benzoyloxy-6-carboxy-2-[(2RS)-1,2-epoxy-2-propyl]-10-methylspiro[4.5]decane [2-O-Benzoyl-11,12-epoxy-10-O-formyl-15-norepilubiminol (17)].* $[\alpha]_D^{24}$ +7.9° (*c* 0.56, $CHCl_3$); EIMS *m/z*: 372 (1.17, $M^+$), 354 (2.38, $M^+$-$H_2O$), 250 (33.5, $M^+$-$C_6H_5CO_2H$), 204 (17.1), 105 (100), 77 (37.9); HRMS *m/z* ($M^+$): calcd. for $C_{22}H_{28}O_5$, 372.1937; found, 372.1946; $^1$H-NMR (270 MHz, $CDCl_3$) $\delta$: 0.96 (3H, d, *J*=6.9 Hz, H-14), 1.32 (3H, br. s, H-13), 1.17–2.10 (10H, m), 2.22–2.37 (2H, m), 2.55 (1H, br. d, *J*=4.6 Hz, H-12a), 2.66 and 2.68 (each 0.5 H, br. d, *J*=4.6 Hz, total 1H, H-12b), 2.74 (1H, m, H-10), 5.32 (1H, m, H-2), 7.43 (2H, br. t, *J*=7.3 Hz, Ar-*m*-$H_2$), 7.55 (1H, br. t, *J*=7.3 Hz, Ar-*p*-*H*), 8.02 (2H, br. d, *J*=7.3 Hz, Ar-*o*-$H_2$), the proton signal of the carboxyl group could not be assigned due to broadening; $^{13}$C-NMR (67.8 MHz, $CDCl_3$) $\delta$: 16.9, 20.2, 20.7, 27.6, 28.2, 31.9, 35.8, 38.5, 45.3, 45.6, 45.9, 46.1, 52.7, 53.0, 57.8, 57.9, 70.2, 128.2, 129.4, 130.5, 132.7, 165.7, 178.3; IR $\nu_{max}$ (film) $cm^{-1}$: 3400–2500 (br.), 2958, 2871, 1716, 1602, 1452, 1382, 1316, 1277, 1112, 1070, 1026, 967, 755, 713.

*(2R,5S,6S,8S,10R)-8-Benzoyloxy-6-formyloxy-2-isopropenyl-10-methylspiro[4.5]decane [2-O-Benzoyl-10-O-formyl-15-norlubiminol (15)].* To a solution of **13** (20.0 mg, 53.8 μmol) and TFA (4.14 μl, 53.8 μmol) in $CH_2Cl_2$ (1.0 ml) was added triphenylphosphine selenide (45.8 mg, 135 μmol) at 4°C. After being stirred for 3 h at room temperatue, the mixture was concentrated under reduced pressure. The residue was dissolved in a small amount of EtOAc, and the EtOAc-soluble portion was purified by preparative TLC (*n*-hexane:$Et_2O$=1:1) to give **15** (17.1 mg, 89%) as a colorless oil. $[\alpha]_D^{22}$ +22.0° (*c* 1.71, $CHCl_3$); FDMS *m/z*: 357 (40.1, $MH^+$), 356 (100, $M^+$); HRMS *m/z* ($M^+$): calcd. for $C_{22}H_{28}O_4$,

356.1987; found, 356.1997; $^1$H-NMR (270 MHz, $CDCl_3$) $\delta$: 1.00 (3H, d, $J$=6.9 Hz, H-14), 1.20–1.35 (2H, m), 1.43–1.95 (8H, m), 1.71 (3H, br. s, H-13), 2.32–2.47 (2H, m), 4.68 (2H, br.s, H-12), 4.89 (1H, dd, $J$=11.9, 4.6 Hz, H-10), 5.04 (1H, m, H-2), 7.43 (2H, br. t, $J$=8.3 Hz, Ar-*m*-$H_2$), 7.55 (1H, br. t, $J$=8.3 Hz, Ar-*p*-*H*), 8.02 (2H, br. d, $J$=8.3 Hz, Ar-*o*-$H_2$), 8.10 (1H, s, H-15); $^{13}$C-NMR (67.8 MHz, $CDCl_3$) $\delta$: 16.2, 21.3, 25.7, 32.5, 34.4, 36.1, 36.9, 39.9, 47.2, 48.5, 70.0, 76.9, 108.5, 128.2, 129.4, 130.2, 132.8, 147.6, 160.4, 165.5; IR $\nu_{max}$ (film) cm$^{-1}$: 3070, 2957, 2875, 1725, 1645, 1451, 1377, 1315, 1275, 1175, 1114, 1070, 1027, 985, 932, 888, 713.

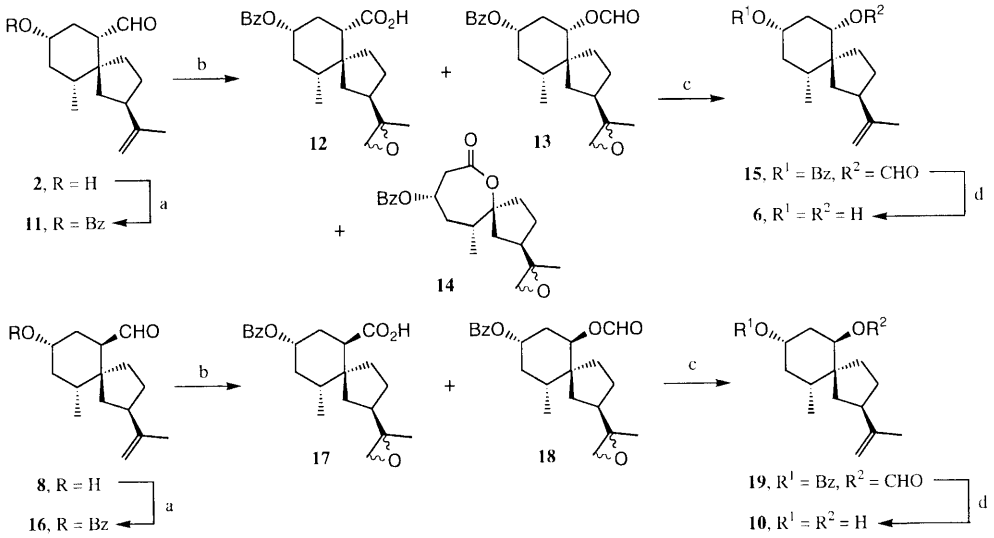
*(2R,5S,6R,8S,10R)-8-Benzoyloxy-6-formyloxy-2-isopropenyl-10-methylspiro[4.5]decane [2-O-Benzoyl-10-O-formyl-15-norepilubiminol (19)].* According to the same method as that described for **15**, **18** (7.9 mg, 21.2 $\mu$mol) was converted into **19** (4.6 mg, 61%) as a colorless oil. $[\alpha]_D^{23}$ +3.25° (*c* 1.17, $CHCl_3$); FDMS *m/z*: 357 (41.6, $MH^+$), 356 (100, $M^+$), 311 (35.2, $M^+$-$CHO_2$); HRMS *m/z* ($M^+$): calcd. for $C_{22}H_{28}O_4$, 356.1987; found, 356.1974; $^1$H-NMR (270 MHz, $CDCl_3$) $\delta$: 0.97 (3H, d, $J$=6.9 Hz, H-14), 1.23–1.60 (4H, m), 1.64–2.00 (5H, m), 1.71 (3H, br. s, H-13), 2.13 (1H, m), 2.28 (1H, m), 2.43 (1H, m), 4.68 (2H, br. s, H-12), 5.06 (1H, br.s, H-10), 5.22 (1H, m, H-2), 7.43 (2H, br. t, $J$=8.3 Hz, Ar-*m*-$H_2$), 7.55 (1H, br. t, $J$=8.3 Hz, Ar-*p*-*H*), 8.01 (2H, br. d, $J$=8.3 Hz, Ar-*o*-$H_2$), 8.14 (1H, s, H-15); $^{13}$C-NMR (67.8 MHz, $CDCl_3$) $\delta$: 16.4, 21.3, 28.9, 31.4, 33.3, 34.0, 36.2, 41.1, 47.65, 47.68, 69.7, 77.9, 108.6, 128.2, 129.4, 130.4, 132.8, 147.4, 160.6, 165.8; IR $\nu_{max}$ (film) cm$^{-1}$: 3070, 2954, 2875, 1722, 1603, 1450, 1377, 1315, 1276, 1176, 1113, 1070, 1027, 979, 890, 712.

*(2R,5S,9S,11R)-9-Benzoyloxy-2-isopropenyl)-11-methyl-6-oxaspiro[4.6]undecan-7-one (20).* According to the same method as that described for **15**, **14** (35.5 mg, 99.2 $\mu$mol) was converted into **20** (11.2 mg, 33%) as a colorless oil. $[\alpha]_D^{22}$ +11.1° (*c* 1.12, $CHCl_3$); EIMS *m/z*: 343 (0.14, $MH_+$), 342 ($M^+$, 0.36), 220 (35.7, $M^+$-$C_7H_6O_2$), 105 (100), 77 (32.1); HRMS *m/z* ($M^+$): calcd. for $C_{21}H_{26}O_4$, 342.1831; found, 342.1837; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 1.25 (3H, d, $J$=7.3 Hz, H-12), 1.74 (1H, m, H-4a), 1.75 (3H, br. s, H-15), 1.82 (2H, m, H-3), 1.93 (1H, m, H-1a), 2.07 (1H, m, H-11), 2.22 (1H, m, H-10a), 2.29 (1H, m, H-10b), 2.31 (1H, m, H-4b), 2.48 (1H, m, H-1b), 2.53 (1H, m, H-2), 2.99 (1H, dd, $J$=14.8, 1.9 Hz, H-8a), 3.19 (1H, ddd, $J$=14.8, 6.9, 1.3 Hz, H-8b), 4.76 (2H, br.s, H-13), 5.44 (1H, m, H-9), 7.42 (2H, br. t, $J$=7.6 Hz, Ar-*m*-$H_2$), 7.55 (1H, br. t, $J$=7.6 Hz, Ar-*p*-*H*), 8.02 (2H, br. d, $J$=7.6, Hz, Ar-*o*-$H_2$); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$: 16.6 (C-12), 20.8 (C-15), 29.9 (C-3), 36.4 (C-10), 39.7 (C-11), 41.0 (C-4), 41.5 (C-8), 43.1 (C-1), 47.5 (C-2), 67.8 (C-9), 93.7 (C-5), 109.8 (C-13), 128.3 (Ar-C3′), 129.6 (Ar-C1′), 129.7 (Ar-C2′), 133.1 (Ar-C4′), 146.2 (C-14), 165.3 (Ar-CO), 170.9 (C-7); IR $\nu_{max}$ (film) cm$^{-1}$: 3070, 2954, 2943, 2875, 1717, 1645, 1601, 1452, 1378, 1314, 1270, 1209, 1164, 1097, 1070, 1026, 975, 893, 713.

*(2R,5S,6S,8S,10R)-2-Isopropenyl-10-methylspiro[4.5]decane-6,8-diol [15-Norlubiminol (6)].* A mixture of **15** (2.9 mg, 8.15 $\mu$mol) and $K_2CO_3$ (2.81 mg, 20.4 $\mu$mol) in MeOH (0.5 ml) was stirred at room temperature for 19 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in a small amount of EtOAc, and the EtOAc-soluble portion was purified by preparative TLC (*n*-hexane:$Et_2O$=3:7, 3-times developed) to give **6** (1.8 mg, 99%) as a colorless oil. $[\alpha]_D^{25}$ +10.5° (*c* 0.21, $CHCl_3$); EIMS: *m/z*: 224 (9.83, $M^+$), 206 (13.7, $M^+$-$H_2O$), 188 (33.9, $M^+$-$H_4O_2$), 107 (100); HRMS *m/z* ($M^+$): calcd. for $C_{14}H_{24}O_2$, 224.1777; found, 224.1730; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 0.96 (3H, d, $J$=6.9 Hz, H-14), 1.04 (1H, q, $J$=11.2 Hz), 1.25 (1H, m), 1.35–1.65 (8H, m), 1.74 (3H, br. s, H-13), 1.80 (2H, m), 2.18 (1H, m, H-1eq.), 2.40 (1H, m, H-7), 3.40 (1H, dd, $J$=11.9, 4.3, H-10), 3.66 (1H, m, H-2), 4.69 (1H, br.s, H-12a), 4.70 (1H, br.s, H-12b); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$: 16.3 (C-14), 21.3 (C-13), 24.7 (C-9), 33.1 (C-8), 37.1 (C-4), 40.0 ($CH_2$), 40.4 ($CH_2$), 41.5 ($CH_2$), 47.2 (C-7), 49.9 (C-5), 68.1 (C-2), 75.3 (C-10), 108.3 (C-12), 148.4 (C-11); IR $\nu_{max}$ (film) cm$^{-1}$: 3333, 3070, 2936, 2865, 1646, 1456, 1363, 1261, 1179, 1102, 1019, 967, 884, 802, 769.

*(2R,5S,6R,8S,10R)-2-Isopropenyl-10-methylspiro[4.5]decane-6,8-diol [15-Norepilubiminol (10)].* According to the same method as that described for **6**, **19** (6.0 mg, 16.9 $\mu$mol) was converted into **10** (3.6 mg, 95%) as a colorless oil. $[\alpha]_D^{24}$ −20.9° (*c* 0.22, $CHCl_3$); EI-MS *m/z*: 224 (22.2, $M^+$), 206 (30.8, $M^+$-$H_2O$), 188 (22.3, $M^+$-$H_4O_2$), 107 (100); HRMS *m/z* ($M^+$): calcd. for $C_{14}H_{24}O_2$, 224.1777; found, 224.1752; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 0.91 (3H, d, $J$=6.9 Hz, H-14), 1.17 (1H, q, $J$=11.2 Hz), 1.22–1.76 (9H, m), 1.73 (3H, br. s, H-13), 1.80 (1H, m, H-8a), 1.98 (1H, m, H-4a), 2.06 (1H, m, H-1eq.), 2.41 (1H, m, H-7), 3.67 (1H, br. t, $J$=3.0 Hz, H-10), 4.0 (1H, m, H-2), 4.68 (1H, br.s, H-12a), 4.69 (1H, br.s, H-12b); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$: 16.6 (C-14), 21.6 (C-13), 29.1 (C-9), 31.7 (C-8), 32.7 (C-4), 39.6 ($CH_2$), 40.5 ($CH_2$), 41.3 ($CH_2$), 47.9 (C-7), 48.9 (C-5), 66.2 (C-2), 76.8 (C-10), 108.2 (C-12), 148.0 (C-11); IR $\nu_{max}$ (film) cm$^{-1}$: 3355, 3070, 2949, 2871, 1646, 1456, 1375, 1135, 1042, 1015, 929, 885, 767.

*Antifungal activity.* The inhibitory effect on spore germination of the natural and synthetic compounds against *F. oxysporum* f. sp. *melongenae* and *V. dah-*



**Scheme 1.** (a) BzCl, pyridine/$CH_2Cl_2$, **11** (86%); **16** (85%); (b) *m*-CPBA, $NaHCO_3/CH_2Cl_2$, **12** (36%), **13** (13%), **14** (39%); **17** (24%), **18** (54%), **14** (4.5%); (c) $Ph_3P=Se$, TFA/$CH_2Cl_2$, **15** (89%), **19** (61%); (d) $K_2CO_3$/MeOH, **6** (99%); **10** (95%).

*liae* was examined according to the reported method.[11] Spores were incubated in each test solution (0.4% glucose in $H_2O$:EtOH = 9:1) at 25°C for 20 h. The germinated spores in an arbitrary group of 200 spores were counted under a microscope. The relative germination rate of the control solution is defined as 100%.

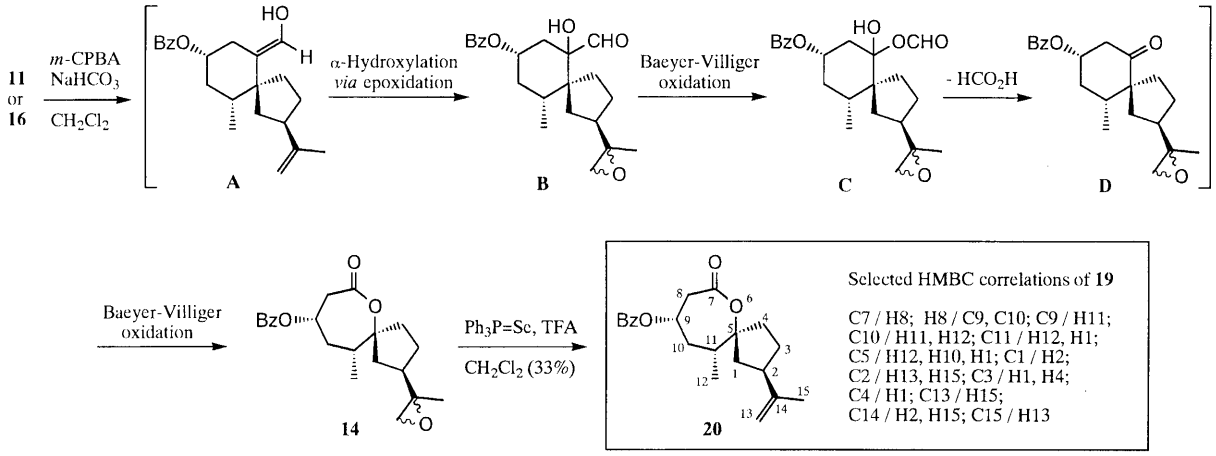
## Results and Discussion

One carbon degradation and stereoselective introduction of a hydroxyl group were required to obtain **6** and **10** from **2** and **8**. Baeyer-Villiger oxidation with a peroxyacid would fulfil both requirements,[9] except for epoxidation of the double bond of the isopropenyl group. However, a commercially available triphenylphosphine selenide has been reported to be a useful reagent for deoxygenating epoxides.[10] Therefore, oxidation with *m*-chloroperoxybenzoic acid (*m*-CPBA) was used as the key step in the conversion of lubimins into 15-norlubiminols.

In order to suppress its volatility, **2** was treated with benzoyl chloride and pyridine to give benzoate **11** in an 86% yield (Scheme 1). Oxidation of **11** with *m*-CPBA gave desired formate **13** in a 13% yield, together with undesired carboxylic acid **12** in a 36% yield and unexpected lactone **14** in a 39% yield. In spite of several attempts with varied reaction temperature and stoichiometry of *m*-CPBA, the yield of **12** could not be increased. While the Baeyer-Villiger oxidation proceeded stereoselectively, epoxidation of the double bond of the isopropenyl group proceeded non-stereoselectively to give each of the three products as a 1:1 mixture of diastereomers. The signals of proton and/or carbon adjacent to the epoxide were only observed as duplicate signals in their $^1$H- and $^{13}$C-NMR spectra. In the $^1$H-NMR spectrum of **13**, the H-10 signal exhibited a coupling constant ($J$ = 11.5 Hz) based on *trans*-diaxial orientation. This result means that the stereochemistry at the C-10 position was retained in the Baeyer-Villiger oxidation process. The structure of carboxylic acid **12**, corresponding to lubiminoic acid (**3**), was determined from the presence of the carboxyl group in the IR ($\nu_{max}$ 1722 cm$^{-1}$) and $^{13}$C-NMR ($\delta$ 160.4 ppm) spectra.

In the same manner, **8** was converted into benzoate **16** in an 85% yield. Oxidation of **16** with *m*-CPBA gave desired formate **17** in a moderate (54%) yield, together with undesired carboxylic acid **18** in a 24% yield. In this case, a trace amount of lactone **14** was also detected by the TLC analysis. The ratio of products in the Baeyer-Villiger oxidation of **11** and **16** was rather different. The selectivity would have depended upon the stereochemistry at the C-10 position. Both **18** and **17** were also obtained as a 1:1 mixture of diastereomers from their $^1$H- and $^{13}$C-NMR spectra. The $^1$H-NMR spectrum of **18** was distinct from that of **13**. In particular, the H-10 signal (m, $W_{1/2}$ = 4.0 Hz) had no large coupling constant based on *trans*-diaxial orientation. The stereochemistry at the C-10 position was also retained in the Baeyer-Villiger oxidation process. The structure of carboxylic acid **17**, corresponding to epilubiminoic acid (**9**), was also determined from the presence of the carboxyl group in the IR ($\nu_{max}$ 1716 cm$^{-1}$) and $^{13}$C-NMR ($\delta$ 178.3 ppm) spectra.

The structure of unexpected lactone **14** was estimated from the $^1$H-, $^{13}$C-NMR, and HRMS spectra. A characteristic pair of double doublets due to the C-8 methylene group attached to both the lactone carbonyl and benzoyloxymethine group was observed at $\delta$ 2.99 ppm (1H, dd, $J$ = 14.8, 1.9 Hz) and 3.20 ppm (1H, dd, $J$ = 14.8, 6.9 Hz) in the $^1$H-NMR




**Scheme 2.** Possible Reaction Mechanism for Lactone Formation.



spectrum. Another carbonyl carbon was observed at $\delta$ 170.7 ppm, in addition to the benzoyl ester carbon ($\delta$ 165.3 ppm), in the $^{13}$C-NMR spectrum. The molecular formula, $C_{21}H_{26}O_5$, determined from the HRMS spectrum suggested that one carbon degradation had occurred. The structure is reasonable from consideration of the possible reaction mechanism (Scheme 2), and was characterized again after conversion to deoxygenated product **20**. An enol intermediate [**A**] resulting from **11** and **16** would have undergone epoxidation to provide an $\alpha$-hydroxylated intermediate [**B**]. Since the common intermediate would have been obtained at the first step from both **11** and **16**, this reaction would provide the same product **14**. The Baeyer-Villiger oxidation of [**B**] provided a rearranged ketal intermediate [**C**] which released formic acid to provide a ketone intermediate [**D**]. The final Baeyer-Villiger oxidation of [**D**] provided lactone **14** *via* selective rearrangement of the tertiary alkyl group. Deoxygenation of **14** with triphenylphospine selenide and trifluoroacetic acid (TFA)[10] provided **20** in a 33% yield. The structure of **20** is reasonably well supported by all of the spectral data, including those of HMBC correlations.

The ease of enolization of **11** and **16** would reflect the ratio of products in the Baeyer-Villiger oxidation. Since enolization of the axial H-10 of **11** would occur more readily than that of the equatorial H-10 of **16**, **11** produced a significant amount of lactone **14**, while **16** produced only a trace amount of **14**. While most of **16** did not undergo enolization under the conditions used and predominantly provided desired formate **18**, **11** provided desired formate **13** as a minor product. From the viewpoint of biogenesis of the lubimin class of sesquiterpenes, the $C_1$ degradation process from **2**/**8** to **6**/**10** might have been associated with the Baeyer-Villiger rearrangement. The existence of a possible intermediate [**D**] might also be explicable for the $C_1$ degradation process to **7**.

Deoxygenation of **13** with triphenylphospine selenide and TFA[10] regenarated the isopropenyl group to give **15** as a single stereoisomer in an 89% yield. Methanolysis of **15** with potassium carbonate gave 15-norlubiminol (**6**) in a quantitative yield. In the $^1$H-NMR spectrum of **6**, the *axial* proton signal at the C-10 position was observed at $\delta$ 3.40 ppm (dd, $J$=11.9, 4.3 Hz). The $^1$H-NMR spectrum of synthetic **6** was identical with that of **6** in the natural 15-norlubiminol mixture. By the same method as that used for the preparation of **6**, **18** was converted into **10** in a 95% yield. In the $^1$H-NMR spectrum of **10**, the *equatorial* proton signal at the C-10 position was observed at $\delta$ 3.67 ppm (1H, br. t, $J$=3.0 Hz). The $^1$H-NMR spectrum of synthetic **10** was also identical with that of **10** in the natural 15-norlubiminol mixture.

In conclusion, the structures of 15-norlubiminol (**6**) and 15-norepilubiminol (**10**) were confirmed by chemical conversion starting from lubimin (**2**) and epilubimin (**8**) in four steps by using Baeyer-Villiger oxidation as the key step. These norsesquiterpenes are promising intermediates for producing **5** and **7**. Both **6** and **10** were respectively obtained as a single product. This made it possible to evaluate the antifungal activity of **6** and **10**. In a preliminary result from the inhibitory effect on spore germination,[11] **6** and **10** did not exhibit antifungal activity or exhibited only very weak antifungal activity, in contrast to the strong antifungal activity of **2** and **8**. When each test solution (100 ppm concentration) was applied against *F. oxysporum* f. sp. *Melongenae*, the relative germination rates were as follows: **2** (5%), **8** (30%), **6** (114%), **10** (113%). Against *V. dahliae*, the relative germination rates were as follows: **2** (42%), **8** (54%), **6** (52%), **10** (57%). It seems likely that the oxidative metabolism of **2** and **8** reduced the antifungal activity. Examining the reproducibility of the antifungal activity of **6**, **10** and related compounds is now in progress.

## Acknowledgments

We are grateful to Mr. K. Watanabe and Dr. E. Fukushi in our faculty for measuring the MS data.

## References

1) Yoshihara, T., Hagihara, Y., Nagaoka, T., Chiba, S., and Sakamura, S., Fungitoxic compounds from the roots of the eggplant stock. *Ann. Phytopath. Soc. Jpn.*, **54**, 453–459 (1988).
2) Nagaoka, T., Yoshihara, T., Ohra, J., and Sakamura, S., Steroidal alkaloids from roots of tomato stock. *Phytochemistry*, **34**, 1153–1157 (1993).
3) Nagaoka, T., Ohra, J., Yoshihara, T., and Sakamura, S., Fungitoxic compounds from the roots of tomata stock. *Ann. Phytopath. Soc. Jpn.*, **61**, 103–108 (1995).
4) Nagase, H., Nagaoka, T., Watanabe, A., Sakata, Y., and Yoshihara, T., Sesquiterpenoids from the roots of *Solanum aethiopicum*. *Z. Naturforsch.*, **56c**, 181–187 (2001).
5) Ano, G., Hebert, Y., Prior, P., and Messiaen, C. M., A new source of resistance to bacterial wilt of eggplants obtained from a cross: *Solanum aethiopicum* L x *Solanum melongena* L. *Agronomies*, **11**, 555–560 (1991).
6) Coxon, D. T., Price, K. R., Howard, B., Osman, S. F., Kalan, E. B., and Zacharius. R. M., Two new vetispirane derivatives: stress metabolites from potato (*Solanum tuberosum*) tubers. *Tetrahedron Lett.*, 2921–2924 (1974).
7) Katsui, N., Matsunaga, A. and Masamune, T., The structure of lubimin and oxylubimin, antifungal metabolites from diseased potato tubers. *Tetrahedron Lett.*, 4483–4486 (1974).
8) Katsui, N., Yagihashi, F., Murai, A., and Masamune, T., Structure of epilubimin, epioxylubimin, and isolubimin, spirovetivane stress metabolites in diseased potato. *Bull. Chem. Soc. Jpn.*, **55**, 2424–2427 (1982).
9) Mislow, K., and Brenner, J., Stereospecificity of the Baeyer-Villiger rearrangement. *J. Am. Chem. Soc*, **75**, 2318–2322 (1953).
10) Clive, D. L. J., and Denyer, C., New method for converting epoxides into olefins: use of triphenylphosphine selenide and trifluoroacetic acid. *J. Chem. Soc. Chem. Commun.*, 253 (1973).
11) Uesugi, Y., Spore germination method. In "Methods in Pesticide Science, Vol. 2," eds. Fukami, J., Uesugi, Y., Ishizuka, K., and Tomizawa, C., Soft Science, Inc., Tokyo, pp. 39–43 (1981).