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Research Article

Asymmetric epoxidation of digeranyl by cultured cells of *Nicotiana tabacum*

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Summary

Asymmetric epoxidation of digeranyl, which is a squalene analog, with cultured cells of *Nicotiana tabacum* was investigated. Feeding of [8-^{3}H]-digeranyl into the cultured cells of *N. tabacum* resulted in the formation of (3*S*)-2,3-epoxydigeranyl and 6,7-epoxydigeranyl. It was found that the epoxidation of digeranyl with *N. tabacum* was highly stereoselective.

Key Words: Asymmetric epoxidation; Biotransformation; Digeranyl; Cultured cells of *Nicotiana tabacum*

Introduction

The epoxy group plays an important role in organic synthesis,[1–4] with the development of asymmetric epoxidation being particularly noteworthy.[5–8] However, many asymmetric epoxidation reactions can only be performed under restricted conditions, e.g. by using organometallic reagents, acting under rigorous exclusion of air, and act only when specific substituents are present in the substrates.

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Recently, biological transformations has enabled the accomplishment of asymmetric epoxidations.[9] Such studies revealed that several bacteria, such as *Pseudomonas* and *Corynebacterium,* transformed stereoselectively the unsaturated hydrocarbons into the corresponding epoxides.[10–14] We also found that plant cultured cells, such as *Nicotiana tabacum* and *Catharanthus roseus,* have the ability to introduce enantioselectively an epoxy group into monoterpenes.[15–17] Asymmetric epoxidation of squalene into (3*S*)-2,3-epoxysqualene is one of the most fascinating reactions, because epoxysqualene is a key precursor for the syntheses of steroids and triterpenoids.[18] If such a reaction can be adapted to squalene analogs, it may be extremely useful from the viewpoint of formation of many cyclic compounds.

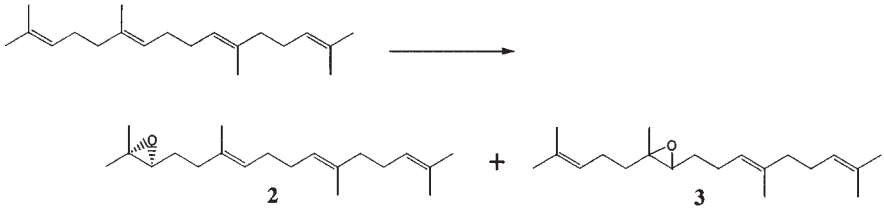
Here we have investigated the epoxidation of digeranyl (**1**), which is a C_{20} squalene analog, with the cultured cells of *N. tabacum.*

Results and discussion

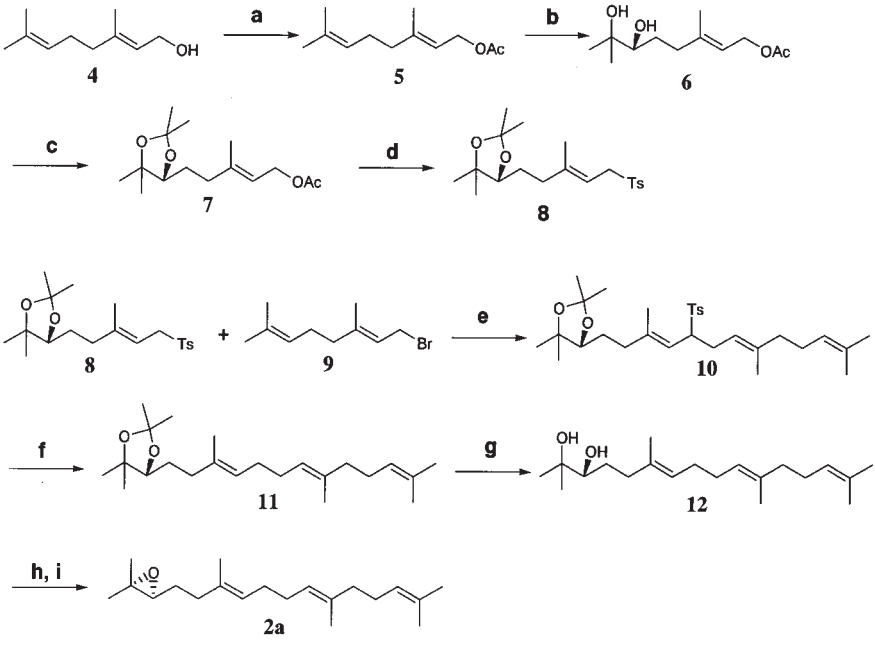
Biotransformation of digeranyl with cultured cells of N. tabacum

[8-^{3}H]-Digeranyl (**1**) was administered to the cultured suspension cells of *N. tabacum,* and the cultures were incubated at 25°C for 2 days. Two products were found by **TLC** autoradiography and **HPLC** analyses of the ether extract of the reaction mixture in 15.4 and 13.6% yields. These products were identified as 2,3-epoxydigeranyl (**2**) and 6,7-epoxydigeranyl (**3**), respectively, by comparison of their TLC and GLC with the synthetic specimens. Identification of these products was confirmed by GC-MS analyses of the products in the incubation of non-labeled digeranyl with the cultured cells of *N. tabacum.* The absolute configuration and enantiomeric purity of the 2,3-epoxydigeranyl obtained by the biotransformation were determined by comparison of the chiral GLC with those of synthetic (*3S*)-2,3-epoxydigeranyl and (*RS*)-2,3-epoxydigeranyl. The stereochemistry of the 2,3-epoxydigeranyl was shown to be S in $>99\%$ ee (Scheme 1).

Thus, it was found that the cultured cells of *N. tabacum* are capable of transforming digeranyl into 2,3-epoxy- and 6,7-epoxydigeranyls stereoselectively.



Scheme 1. Biotransformation of digeranyl by cultured cells of *N. tabacum*



Scheme 2. Reagents and conditions: (a) AC_2O, Pyridine, DMAP, rt; (b) PYDZ Ligand, K_2OsO_4, $K_3Fe(CN)_6$, K_2CO_3, Methanesulfonamide, *t*-BuOH:H_2O, 4 °C; (c) 2,2-dimethoxypropane, PPTS,CH_2Cl_2, rt ; (d) $Pd(PPh_3)_4$, TsNa-4H_2O, THF, rt; (e) *n*-BuLi, THF, 0 °C; (f) $PdCl_2$(dppp), $LiHBEt_3$, THF, 0 °C; (g) TsOH, MeOH, rt; (h) MsCl, TEA, CH_2Cl_2 −40 °C; (i) K_2CO_3, MeOH, rt

Synthesis of authentic (3S)-2,3-epoxydigeranyl

(3*S*)-2,3-Epoxydigeranyl (**2a**) was synthesized from geraniol (**4**) as shown in Scheme 2. The asymmetric dihydroxylation of geranyl acetate (**5**) using PYDZ ligand,[19] K_2OsO_4, K_2CO_3 and $K_3Fe(CN)_6$ in 1:l *t*-BuOH/H_2O gave a diol **6** in 70% yield. Protection of the diol **6** followed by the sulfonylation of the resultant **7** with $Pd(PPh_3)_4$, TsNa-4H_2O in THF: MeOH furnished geranyl *p*-tolylsulfone (**8**). The product

8 was lithiated, followed by addition of geranyl bromide (**9**), which was prepared using *N*-bromosuccinimide and dimethyl sulfide in CH_2Cl_2, to give **10** in 60% yield. **10** was reduced with $PdCl_2$(dppp) and $LiHBEt_3$, in THF to give **11** in 79% yield. **11** was deprotected to give a diol **12** with TsOH in MeOH. The resulting diol **12** was converted to (3*S*)-2,3-epoxydigeranyl (**2a**) by sulfonylation of the secondary alcohol with methanesulfonyl chloride and pyridine followed by ring closure with K_2CO_3 in MeOH in 25% yield over two steps.

Thus, the synthesis of (3*S*)-2,3-epoxydigeranyl (**2a**) from geraniol (**4**) was achieved by asymmetric dihydroxylation, coupling of geranyl bromide (**9**) with geranyl *p*-tolylsulfone derivative (**8**), and reductive desulfonylation, as key steps.

Experimental

General: Analytical TLC was performed on precoated TLC plates (Merck 60 F_{254} Column chromatography was conducted using silica-gel (Merck Silica gel 60, and Wakogel C-300 HG). ^1H and ^{13}C NMR spectra were obtained using a JEOL JNM-LA500 spectrometer using tetramethylsilane as an internal standard. Mass spectra (MS) were obtained on JEOL JMS-SX102A and Hewlett Packard MSD-5971A mass spectrometers.

Substrates: The preparation of [8-^{3}H]-digeranyl (**1**) was assayed by the synthetic method of [8-^{2}H]-digeranyl reported in our previous paper.[20] To a solution of 8-*p*-tolylsulfonated digeranyl (500 mg, 0.583 mmol), prepared from geranyl *p*-tolylsulfone and geranyl chloride, and 5 mol% 1,3-bis(diphenylphosphino)propane palladium(II) chloride complex [$PdCl_2$(dppp)] in THF (40 ml), LiB^3HEt_3 (1.17 mmol in 2.3 ml THF; 1.48 GBq) was added at 0°C under a N_2 atmosphere. After stirring at 0°C for 1 h, 3 M NaOH and KCN (40 mg) was added to the reaction mixture, and then the mixture was extracted with hexane to give the crude product. The latter was subjected to column chromatography on silica gel and eluted with hexane to give [8-^{3}H]-digeranyl (**1**) (145 mg, 670 MBq, 90%).

Feeding of [8-^{3}H]-digeranyl (**1**) with the cultured cells of *N. tabacum*: The suspension cells of *N. tabacum* were prepared as reported previously.[21] To the flask containing the suspension cells (about 40 g in 100 ml of Murashige and Skoog's medium[21]), [8-^{3}H]-digeranyl (1) (58 μg; 131 kBq) was administered and incubated at 25°C for 2 days on a

rotary shaker. After incubation, the cells were collected by filtration and extracted with ether. The ether layer was evaporated and then subjected to short-column chromatography on silica gel with ether to give a crude product (36 kBq)

The crude product was traced by TLC autoradiography. A charged TLC was developed with hexane:EtOAc = 5:1 (v/v) as eluent and then analyzed using Fuji.BAS2000 imaging analyzer. The R_f values of the radioactive spots were 0.51, 0.57, and 0.65, which were identical with those of authentic 2,3-epoxydigeranyl, 6,7-epoxydigeranyl, and digeranyl, respectively. A part of the crude product was subjected to preparative HPLC (Inertsil SIL 5pm; 4.6×250 mm) using hexane:2-propanol = l000:l (v/v) as eluent and the radioactivities of the isolated fractions were measured by liquid scintillation counting. Three radioactive fractions eluted at R_t 10 (12 Bq; 71%), 16 (2.6 Bq; 15.4%), and 19 min (2.3 Bq; 13.6%) were identical with those of authentic digeranyl, 6,7-epoxydigeranyl, and 2,3-epoxydigeranyl, respectively.

*Feeding of digeranyl (**1**) with the cultured cells of N. tabacum:* To the flask containing the suspension cells (about 40 g in 100 ml of MS medium), non-labeled digeranyl (25 mg) was administered and the mixture was incubated at 25°C for 2 days on a rotary shaker. After incubation, the cells were collected by filtration and extracted with ether. The ether layer was evaporated and then subjected to short-column chromatography on silica gel with ether to give a crude product (2 mg). Two products were identified by the comparison of their GLC [OV-17 on Chromosorb WAW-DMCS; 80–100 mesh; column temperature., 100–250°C (10°C/min, initial. time 5 min); inject temparature., 250°C] and GC-MS analysis with those of authentic 2,3- and 6,7-epoxydigeranyl. Retention times for synthetic 2,3- and 6,7-epoxydigeranyl in the GLC were 18.4 and 18.9 min, respectively. GC-MS analyses of the peaks with retention times 18.4 and 18.9 min in the administered sample with *N. tabacum* showed identical fragmentation patterns to synthetic 2,3- and 6,7-epoxydigeranyl, respectively.

The absolute configuration and enantiomeric purity of the 2,3-epoxy product were determined by comparison of their chiral GLC (CP cyclodextrin β236M-19 column; column temperature, 200°C; inject temperature, 220°C) of incubated products with those of synthetic samples, (3*S*)-2,3-epoxydigeranyl and racemic 2,3-epoxydigeranyl [R_t values of (3*S*)- and (3*R*)-epoxydigeranyl: 17.3 and 17.5 min, respectively].

Syntheses of racemic 2,3- and 6,7-epoxydigeranyl: Digeranyl (500 mg, 1.82 mmol) was dissolved in 52 ml of dry CH_2C1_2. *m*-Chloroperbenzoic acid (377 mg, 2.18 mmol) was added to the stirred reaction mixture at 0°C. After stirring at room temperature for 12 h, aqueous $Na_2S_20_3$ and $NaHCO_3$ were added at 0°C. The reaction mixture was extracted with ether to give a crude product, which was purified by column chromatography on silica-gel with hexane-ether (95:5) to give 2,3-epoxydigeranyl (**2**) (4.1 mg, 7%) [EI-MS *m*/*z* 290 (M^+), 275, 166, 153, 135, 109, 95, 69; ^{1}H NMR ($CDC1_3$) $\delta = 5.14$ (m, 3H), 2.70 (t, lH, $J = 6.2$ Hz), 2.07 (m, 10H), 1.68 (s, 3H), 1.64 (m, 2H), 1.62 (s, 3H), 1.60 (s, 6H), 1.30 (s, 3H) and 1.26 (s, 3H); ^{13}C NMR ($CDC1_3$) $\delta = 135.2$, 134.2, 131.3, 125.0, 124.4, 124.1, 64.2, 58.3, 39.7, 36.3, 28.3, 28.2, 27.5, 26.8, 25.7, 24.9, 18.7, 17.7 and 16.0] and 6,7-epoxydigeranyl (**3**) (9.4 mg, 16%) [EI-MS *m*/*z* 290 (M^+); ^{1}H NMR ($CDC1_3$) $\delta = 5.14$ (m, 3H), 2.71 (t, lH, $J = 6.3$ Hz), 2.07 (m, 8H), 1.68 (s, 6H), 1.64 (m, 2H), 1.61 (s, 9H), 1.51 (m, lH), 1.42 (m, 1H) and 1.25 (s, 3H); ^{13}C NMR ($CDC1_3$) $\delta = 135.9$, 131.8, 131.4, 124.2, 123.7, 123.3, 63.3, 60.7, 39.7, 38.9, 29.0, 26.7, 25.7, 24.8, 23.9, 17.6, 16.5, 16.0 and 15.3].

*Synthesis of (3S)-2,3-epoxydigeranyl. (6R)-6,7-Dihydroxygeranyl acetate (**6**):* Following the method described,[23] a mixture of DHQD-PYDZ ligand[19] (190 mg, 0.26 mmol), K_2OsO_4 (20 mg, 0.05 mmol), $K_2Fe(CN)_6$ (25 g, 76.5 mmol), K_2CO_3 (10.6 g, 76.5 mmol), methanesulfonamide (2.42 g, 25.5 mmol), geranyl acetate (5 g, 25.5 mmol) and 300 ml of 1:l *t*-BuOH-H_2O was stirred for 12 h at 4°C. Sodium sulfite (30 g) was added, and the mixture was concentrated under reduced pressure. The mixture was extracted with CH_2Cl_2, washed with 2 M KOH and dried over Na_2SO_4. The crude product was purified by column chromatography on silica gel using ethyl acetate–hexane (2:1) as eluent to give a product **6** (2.6 g, 70%): EI-MS *m*/*z* 231 (MH^+); ^{1}H-NMR ($CDCl_3$) $\delta = 5.33$ (t, lH, $J = 7.0$ Hz), 4.53 (d, 2H, $J = 7.0$ Hz), 3.27 (dd, lH, $J = 10.5$, 2.0 Hz), 2.27 (m, lH), 2.04 (m, 1H), 2.00 (s, 3H), 1.66 (s, 3H), 1.55 (m, 1H), 1.39 (m, 1H), 1.15 (s, 3H) and 1.10 (s, 3H); ^{13}C-NMR ($CDCl_3$) $\delta = 171.2$, 142.0, 118.5, 77.9, 72.9, 61.3, 36.5, 29.4, 26.3, 23.1, 20.9 and 16.3.

*(5R)-3-Methyl-5(2,2,5,5-tetramethyl-[1,3]dioxolan-4-yl)-pent-2-enyl acetate (**7**):* To the suspension of **6** (4.8 g, 20.8 mmol) in 20 ml of CH_2C1_2, 2,2-dimethoxypropane (4.8 g, 41.7 mmol) and pyridinium *p*-toluenesulfonate (10.3 g, 41.7 mmol) were added at 0°C under a N_2 atmosphere. After stirring at room temperature for 1 h, the reaction mixture was treated with Et_3N and concentrated. The crude product

was purified by column chromatography on silica-gel with hexane-ethyl acetate (2 : 1) to give a product **7** (5.3 g, 94%): EI-MS *m/z* 270 (M^+); ^{1}H-NMR ($CDCl_3$) $\delta = 5.39$ (bt, lH), 4.59 (d, 2H), 3.65 (dd, lH, $J = 9.7$, 3.4 Hz), 2.28 (m, lH), 2.10 (m, lH), 2.04 (s, 3H), 1.73 (s, 3H), 1.64 (m, 3H), 1.51 (m, 3H), 1.40 (s, 3H), 1.31 (s, 3H), 1.24 (s, 3H) and 1.09 (s, 3H); ^{13}C-NMR ($CDCl_3$) $\delta = 170.4$, 141.0, 118.6, 106.2, 82.4, 79.7, 60.9, 36.3, 28.3, 27.1, 26.5, 25.7, 22.7, 20.6 and 16.2.

*(5R)-2,2,4,4-Tetramethyl-5-[3-methyl-5-(toluene-4-sulfonyl)-pent-3-enyl-[1, 3]dioxolane (**8**):* To the solution of sodium *p*-tolylsulfonate tetrahydrate (5.8 g, 23.2 mmol) in THF (43 ml) and MeOH (14 ml) was added acetate **7** (5.7 g, 21.1 mmol) and 5 mol% $Pd(PPh_3)_4$ in THF (10 ml) under a N_2 atmosphere. After stirring at room temperature overnight, aqueous KCN was added dropwise at 0°C to deactivate the palladium catalyst. The reaction mixture was extracted with ether to give a crude product, which was purified by column chromatography on silica gel using hexane-ethyl acetate (4:1) as eluent to give a product **8** (5.3 g, 68%): FAB-MS *m/z* 367.8 (MH^+); ^{1}H-NMR ($CDCl_3$) $\delta = 7.73$ (d, 2H, $J = 8.2$ Hz), 7.32 (d, 2H, $J = 8.2$ Hz), 5.24 (t, lH, $J = 7.9$ Hz), 3.80 (d, 2H, $J = 7.9$ Hz), 3.63 (dd, lH, $J = 9.7$, 3.0 Hz), 2.44 (s, 3H), 2.25 (m, lH), 2.06 (m, lH), 1.55 (m, lH), 1.41 (s,3H), 1.40 (m, lH), 1.39 (s, 3H), 1.32 (s, 3H), 1.24 (s, 3H) and 1.08 (s, 3H); ^{13}C-NMR ($CDC1_3$) $\delta = 145.0$, 143.9, 135.6, 129.1, 127.9, 110.5, 106.0, 82.1, 79.5, 55.5, 36.3, 28.1, 27.0, 26.4, 25.6, 22.5, 21.1 and 15.8.

*2,2,4,4-Tetramethyl-5-[3,8,12-trimethyl-5-(toluene-4-sulfonyl)-trideca-3,7, 11-trienyl-[1,3]dioxolane (**10**):* To the suspension of **8** (5.3 g, 14.5 mmol) in 20 ml of THF, 1.5 M *n*-BuLi (9.1 ml, 17.4 mmol) in THF and geranyl bromide (**9**) (3.0 g, 17.4 mmol) in THF (10 ml) were added dropwise at 0°C under a N_2 atmosphere. After stirring at 0°C for 10 min, the reaction mixture was treated with 20 ml of 10% NH_4C1 and extracted with ether to give a crude product, which was purified by column chromatography on silica-gel with hexane-ethyl acetate (4:1) to give a product **10** (4.3 g, 60%).

*2,2,4,4-Tetramethyl-5-[3,8,12-trimethyltrideca-3,7,11-trienyl-[1,3]dioxolane (**11**):* To the solution of **10** (2.6 g, 5.2 mmol) and 5 mol% PdC_{12}(dppp) in THF (173 ml), $LiHBEt_3$, (1.2 ml, 10.2 mmol) was added at 0°C under N_2 atmosphere. After stirring at 0°C for 12 h, 3 M NaOH and KCN (50 mg) was added to the reaction mixture, and then the mixture was extracted with ether to give a crude product. The crude product was subjected to column chromatography on silica-gel and eluted with hexane-ethyl acetate (5:l) to give **11** (1.4 g, 79%): EI-MS *m/z*

348 (M^+); ^{1}H NMR ($CDC1_3$) $\delta = 5.13$ (m, 3H), 3.67 (dd, lH, $J = 9.3$, 3.5 Hz), 2.12 (m, lH), 1.68 (s, 3H), 1.62 (s, 3H), 1.60 (s, 6H), 1.48 (m, 2H), 1.42 (s, 3H), 1.33 (s, 3H), 1.24 (s, 3H) and 1.10 (s, 3H).

*(3R)-Dihydroxydigeranyl (**12**):* To the solution of **11** (23 mg, 66 μmol) in MeOH (2 ml) was added TsOH (500 mg, 79.2 μmol). After stirring at room temperature for 3 h, 20 ml of 5% $NaHCO_3$ was added, and washed with brine. The reaction mixture was extracted with ether to give a crude product, which was purified by column chromatography on silica gel using hexane–ethyl acetate (3 : 1) as eluent to give a product **12** (25 mg, quant): EI-MS *m/z* 308 (M^+); ^{1}H-NMR ($CDC1_3$) $\delta = 5.13$ (m, 3H), 3.34 (dd, lH, $J = 10.5$, 1.7 Hz), 2.25 (m, lH), 2.02 (m, 9H), 1.68 (s, 3H), 1.62 (s, 3H), 1.60 (s, 6H), 1.55 (m, lH), 1.41 (m, lH), 1.19 (s, 3H) and 1.15 (s, 3H); ^{13}C-NMR ($CDCl_3$) $\delta = 135.1$, 134.9, 131.1, 124.9, 124.3, 124.1, 78.2, 73.0, 39.7, 36.8, 29.8, 28.2, 28.1, 26.7, 26.3, 25.6, 23.1, 17.6, 16.0 and 15.9.

*(3S)-2,3-epoxydigeranyl (**2a**):* A solution of **12** (63 mg, 0.20 mmol) and pyridine (0.03 ml, 0.40 mmol) in 3 ml of CH_2Cl_2 was treated at −40°C with methanesulfonyl chloride (34 mg, 0.30 mmol). After 30 min, the mixture was warmed to room temperature and stirred for 3 h. An additional 0.3 ml pyridine was added, and the mixture was stirred for 8 h. The mixture was poured into a suspension of 1 g of K_2CO_3 in 5 ml of MeOH and stirred for 6 h at room temperature. The mixture was concentrated, diluted with water, extracted with ether, washed with 10% $CuSO_4$ and brine. The crude product was purified by column chromatography on silica gel using hexane–ether (95:5) as eluent to give a product **2a** (14.8 mg, 25%): EI-MS *m/z* 290 (M^+), 275, 166, 153, 135, 109, 95, 69; ^{1}H NMR ($CDC1_3$) $\delta = 5.14$ (m, 3H), 2.70 (t, lH, J = 6.2 Hz), 2.07 (m, 10H), 1.68 (s, 3H), 1.64 (m, 2H), 1.62 (s, 3H), 1.60 (s, 6H), 1.30 (s, 3H) and 1.26 (s, 3H); ^{13}C NMR ($CDCl_3$) $\delta = 135.2$, 134.2, 131.3, 125.0, 124.4, 124.1, 64.2, 58.3, 39.7, 36.3, 28.3, 28.2, 27.5, 26.8, 25.7, 24.9, 18.7, 17.7 and 16.0.

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