

Fixation of Natural Furanoeremophilane by Diels–Alder Reaction

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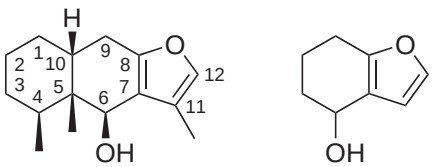
Diels–Alder reaction of furanoeremophilan-6β-ol (petasalbin) and its synthetic analogue, 4-hydroxy-4,5,6,7-tetrahydrobenzofuran, was studied using *N*-ethyl- and *N*-phenylmaleimides as the dienophiles, affording corresponding adducts as mixtures of stereoisomers. The adduct of petasalbin and maleimide was also obtained when the latter compound was added to a crude extracted solution of *Petasites japonicus* var. *giganteus*. By using this method, it was estimated that the contents of unstable furanoeremophilan-10β-ol in *L. cymbulifera* is more than 32% of the extract.

Furanoeremophilane compounds are found in nature in some plants of Compositae tribe Senecioneae, such as *Ligularia*,[1] *Petasites*,[2] *Farfugium*,[3] and *Syneilesis*.[4] Although many compounds have been isolated in pure form and their structures have been determined, a problem is present in the handling of these compounds because some of them are unstable. For example, on the course of our investigation on the diversity in *Ligularia* species in the Hengduan Mountains area,[5] we have found the presence of extremely unstable compound in *L. tongolensis*, but the compound has not been isolated.[5a] Some unstable furanoeremophilanes decompose even in $CDCl_3$ solution, and therefore, many NMR data have been acquired in C_6D_6.[1c,e,3,4] The instability of these compounds are due to the presence of an electron-rich trisubstituted furan ring, which reacts easily with electron deficient reagents, such as proton. For the structure determination of these unstable compounds, the problem is to obtain the targeted molecules in pure form.

A possible solution to this problem is to "fix" the furan ring by conversion to some stable functional group. To this purpose, we planned to use a Diels–Alder reaction for the transformation of the furan ring into stable adduct, because furan is a good diene,[6] and especially, the furan ring of natural furanoeremophilanes is electron-rich and therefore the Diels–Alder reaction is expected to proceed smoothly. A natural intramolecular Diels–Alder adduct of 1-acyloxyfuranoeremophilane derivative has been reported, indicating that the reaction proceeds without heating.[7] Here, we report Diels–Alder reaction of natural petasalbin and synthetic analogue with *N*-phenyl- and *N*-ethyl-maleimide.

Results and Discussion

In the present study, two substrates, furanoeremophilan-6β-ol (=ligularol or petasalbin, **1**)[8,9] and a synthetic analogue **2** were used. Compound **1** was isolated from the rhizome of the cultivated plant *Petasites japonicus* var. *giganteus* (Compositae),[10] which was collected in Nagano Prefecture, Japan. The synthetic analogue **2** was prepared by the Feist–Benary method[11] from 1,3-cyclohexanedione and chloroacetaldehyde followed by $LiAlH_4$ reduction, as described previously (Chart 1).[8]



1 **2**

Chart 1.

At first, the reaction of model compound **2** was examined with *N*-ethyl- and *N*-phenylmaleimide as the dienophiles (Scheme 1). The reaction was carried out in $EtOH/H_2O$ 9:1 as the solvent at room temperature, which is a model condition of extraction of natural products, since the extracted solutions of natural products sometimes contain water and higher reaction temperature must be avoided for the treatment of unstable compounds. When **2** was treated with 3 molar amounts of *N*-ethylmaleimide (**3**) under these conditions, the adduct was afforded in good yield as a mixture of three diastereomers. The isomers of the products were partly separated by silica-gel column chromatography giving a mixture of exo-adducts **4a** and **4b** (57% yield, ratio 7:3), and an isomer of endo-adduct **4c** (41% yield). The fourth possible stereoisomer was not detected. The stereochemistry of the products was determined from ^{1}H NMR spectra. Namely, **4a** and **4b** were determined to be exo-adducts based on the J value between H_a and H_b ($J = 0$ Hz), which indicated that H_b is endo to 7-oxabicyclo-[2.2.1]heptane ring system. The orientation of the hydroxy group was axial for **4a** and equatorial for **4b**, respectively, judged from the coupling constants of the proton attached to the hydroxy-bearing carbon. However, since two conformers were possible for the cyclohexane ring, it was difficult to determine the relative stereochemistry of **4a** and **4b**. Compound **4c** was judged to be endo-adduct based on a relatively large J value (5.5 Hz) between H_a and H_b.

The reaction of **2** with *N*-phenylmaleimide (**5**) was also carried out similarly. Formation of adduct **6** was observed on TLC; however, in contrast to **4a**–**4c**, it was difficult to obtain each products in pure form, because a retro-Diels–Alder reaction occurred during the isolation process. Some other dieno-

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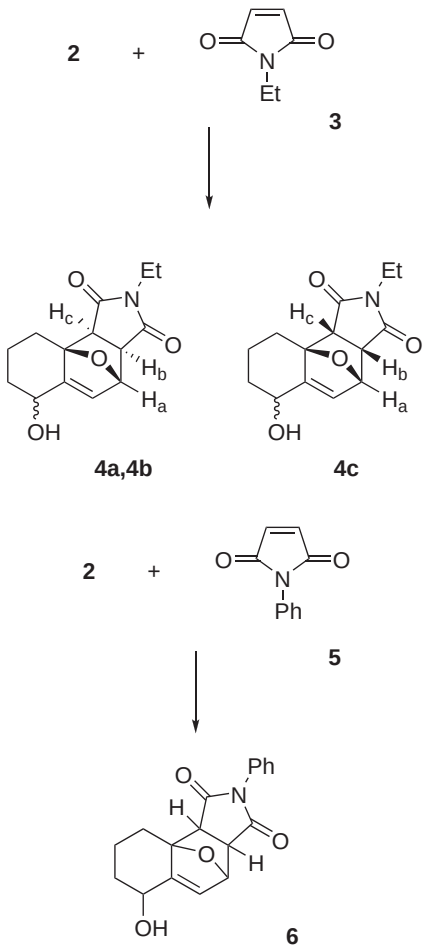
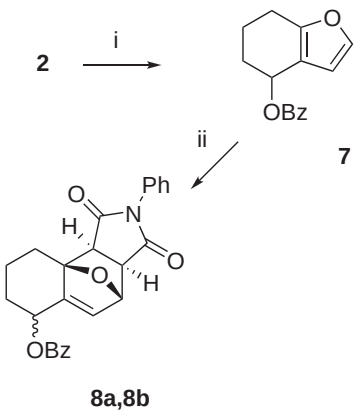


Scheme 1. Reaction conditions: in $EtOH/H_2O$ (9:1), r.t., 1 day.



Scheme 2. Reagents and conditions: i) benzoyl chloride, pyridine, CH_2Cl_2, r.t., 2 h. ii) see Table 1.

philes, such as benzoquinone, ethyl acrylate, diethyl fumarate, maleic anhydride, and diethyl acetylenedicarboxylate, were tested other than maleimide. However, none of them afforded the corresponding adducts in better yields than *N*-ethylmaleimide.

In order to show the utility of *N*-phenylmaleimide, the reaction was further studied using benzoate **7** as the substrate, which was prepared by benzoylation of **2** (Scheme 2). The adducts were obtained as a mixture of two stereoisomers, and the each diastereomer was stable enough for the isolation procedure to obtain **8a** (51%) and **8b** (14%) after purification on silica gel. Both products were determined to be exo-adducts as described above.

Table 1. Reaction of **7** and **5** under Various Conditions[a)]

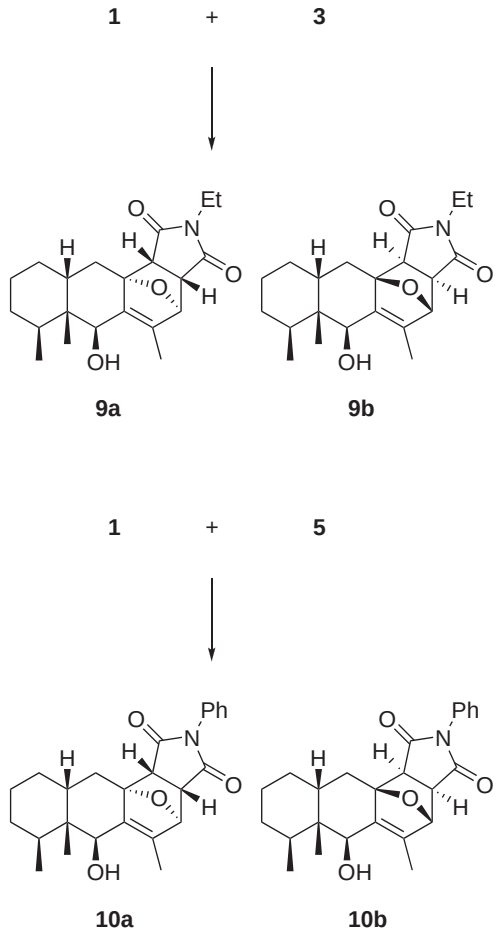
Entry	Equiv. of **5**	Time/d	Yield/%
1	1	1	55
2	1	3	78
3	2	1	69
4	2	3	80
5	3	1	84
6	3	3	80

a) All reactions were carried out in $EtOH/H_2O$ (9:1) with 0.1 mol L^{-1} concentration of diene at room temperature.

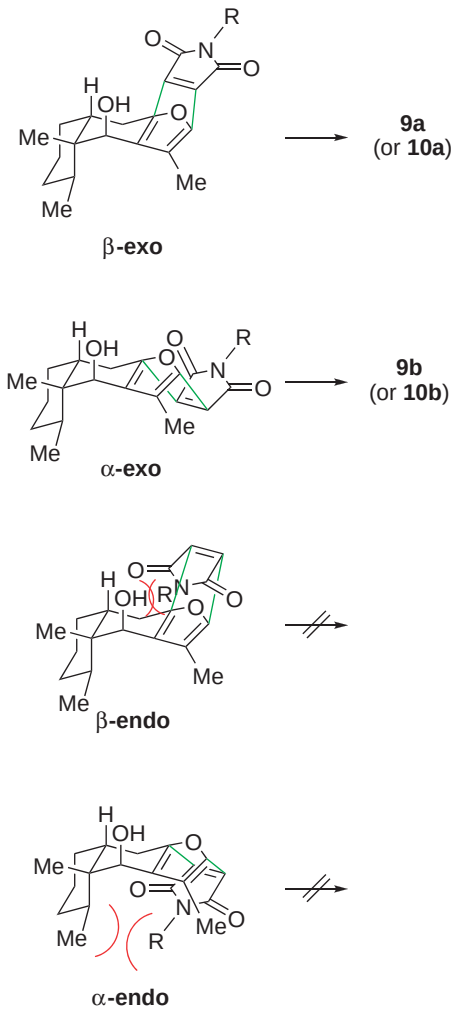
Optimization of the reaction condition was made for the reaction of **7** to **8**. The results are shown in Table 1. The use of a stoichiometric amount of maleimide **5** was found to be enough if the reaction was carried out for a longer time (Entry 2). However, in the present study, we chose to use 3 equivalent of maleimide (Entry 5) in order to use natural product more effectively.

Following the succession with the model compound, the Diels–Alder reaction of natural furanoeremophilane was studied. When compound **1** was treated with **3** in $EtOH/H_2O$ (9:1) at room temperature, a pair of diastereomers **9a** and **9b** were obtained in 39 and 16% yields, respectively (Scheme 3). In contrast to the case of model compound **2**, the adducts with *N*-phenylmaleimide (**5**) were obtained in better yield (74%) without the occurrence of the retro-Diels–Alder reaction. The ratio of the two diastereomers **10a** and **10b** was 4:1. In both cases, stereoisomers of the two products were obtained, and each isomer could be isolated by silica-gel column chromatography. The stereochemistry of the all four products were determined to be exo-adducts based on ^1H NMR spectra as described for the model compounds. The major isomer (**9a**/**10a**) and the minor isomer (**9b**/**10b**) were determined to be the β-exo and the α-exo adducts, respectively, based on the presence or absence of long-range coupling between olefinic methyl group and the proton attached to the hydroxy-bearing carbon.[12] These protons were almost planar with the C=C double bond for **9a**/**10a** ($J = 0$ Hz) but not for **9b**/**10b** ($J = 2.2$ Hz).

The stereoselective formation of the *exo*-isomers can be rationalized as follows. Although four approaching directions of dienophile **3** (or **5**) to **1** are possible, as shown in Scheme 4, β-endo and α-endo approaches are unfavorable because of steric congestion. Namely, the R group on the maleimide comes close to the C-6 substituent in the β-endo approach. In the α-endo approach, maleimide molecule must come inside the "umbrella" shape of the *cis*-decaline system of the furanoeremophilane. Among two exo-approaches, β-exo is more favorable than α-exo because steric interaction with decaline ring is minimum. The conformation of furanoeremophilane illustrated in Scheme 4 is "steroidal," and there is another conformer, "non-steroidal."[13] The story described here must be the same for the non-steroidal conformation, except that steric interac-



Scheme 3. Reaction conditions: in EtOH/H_2O (9:1), r.t., 1 day.

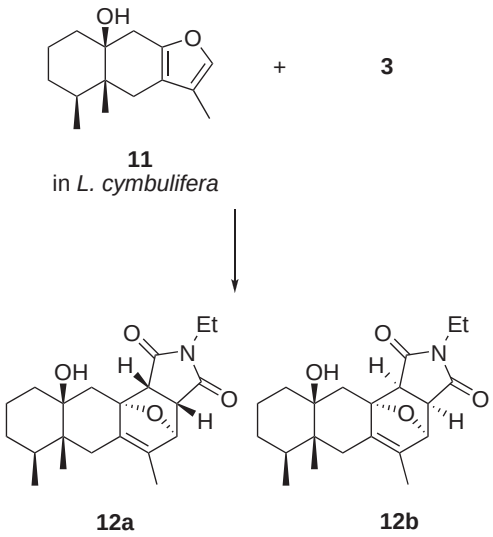


Scheme 4. Stereochemistry of the reaction of **1** and **3** (or **5**).

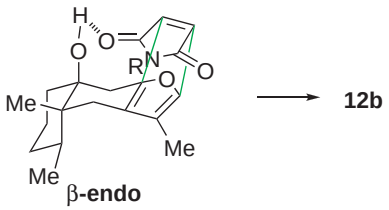
tion between C-5 methyl and dienophile must be considered for the β-endo approach. For the reaction of the model compound, related explanation can be applied for the formation of **4a**, **4b**, **8a**, and **8b**. The formation of α-endo product (**4c**) can be explained by the lack of interaction with the fused cyclohexane ring.

The present Diels–Alder reaction was applied to transform natural furanoeremophilane into stable adduct before the isolation procedure. At first, the utility of the present "fixation" method was estimated by comparison with the standard isolation using the same extracted solution. When 12 cm^3 of AcOEt extracted solution of half-dried roots of *P. japonicus* var. *giganteus* was treated with *N*-ethylmaleimide (**3**), 6.6 mg of the adducts **9a** and **9b** (correspond to 4.3 mg of **1**) were isolated. The same treatment with *N*-phenylmaleimide (**5**) afforded 8.4 mg (correspond to 4.8 mg of **1**) of **10a** and **10b**. In contrast, only 1.5 mg of **1** could be isolated by direct column chromatography from the same amount of the extracted AcOEt solution, although acidic conditions were avoided by using neutral silica gel. In order to reduce handling time after harvesting, the method was also applied to "fix" compound **1** in fresh root. When **3** was added to a crude extracted ethanol solution of the fresh root, a spot of the adduct **9** immediately appeared on TLC; however, it was difficult to judge the disappearance of the spot of **1**. From 12 g of the fresh root, **9a** (54.5 mg) and **9b** (19.4 mg) were afforded as crystals.

We have recently studied chemical constituents of *L. cymbulifera*, and four furanoeremophilanes have been isolated from the plant.[5a] Among them, furanoeremophilan-10β-ol (**11**) has been detected as the major component on TLC of the crude extracted solution, but the compound is too labile. From 1.5 g of the extract, only 176.3 mg (12%) of the compound has been obtained. Although we have not stated in the report, the compound decomposes when the solvent is evaporated after purification. Ehrlich's reaction of **11** was faster than the other furanoeremophilanes, indicating that the furan ring in **11** is highly reactive. Then, the present Diels–Alder method was applied to "fix" the unstable molecule. When *N*-ethylmaleimide was added to a crude extracted solution of *L. cymbulifera*, the reaction immediately occurred, and the spot of **11** on TLC disappeared. The other Ehrlich-positive spot did not disappear in a short reaction time, indicating that only **11** had been reacted. Form 10 cm^3 of the extracted solution, the adduct (119.1 mg) was obtained as a mixture of two diastereomers **12a** and **12b** (Scheme 5). Since 2 cm^3 of the extracted solution gave 48.7 mg of the residual oil, the result implies that the content of **11** is more than 32% of the extract. Interestingly, minor isomer **12b** was not α-exo-adduct, but β-endo-adduct, which was determined from the *J* value



Scheme 5. Diels–Alder reaction of furanoeremophilan-10β-ol in *L. cymbulifera*.



Scheme 6. Stereochemistry of the formation of **12b**.

(5.5 Hz) between the proton attached to the ethereal oxygen-bearing carbon and the adjacent proton, as in the case of **4c**. This unexpected stereoselectivity can be rationalized by the formation of hydrogen-bond between hydroxy group at C-10 and the carbonyl oxygen of the maleimide for the β-endo approach (Scheme 6).

In conclusion, the Diels–Alder reaction of natural furanoeremophilane compound with *N*-ethyl- and *N*-phenyl-maleimide was found to proceed smoothly at room temperature; however, *N*-ethylmaleimide was found to be better for the model compound. Although the occurrence of retro-Diels–Alder reaction in some case indicates limited usefulness, the method was shown to be useful for fixation of unstable natural furanoeremophilanes.

Experimental

General Procedure. Melting points were measured on a Laboratory Devices Mel-Temp apparatus. IR spectra were recorded on a Jasco FT/IR-230 spectrometer. Both ^{1}H and ^{13}C NMR spectra were measured on a Jeol GSX-400 (400 MHz for ^{1}H; 100 MHz for ^{13}C) spectrometer in $CDCl_3$ as the solvent, unless otherwise noted. Chemical shifts were recorded on the δ scale (ppm) with tetramethylsilane as an internal standard. For ^{13}C NMR, the signal of the solvent ($CDCl_3$: 77.0 ppm) was used as the reference. Both low-resolution mass spectra (MS) and high-resolution mass spectra (HRMS) were obtained on a Jeol SX-102A, CMATE II, JMS AX-500, or Shimadzu GCMS-QP5050 mass spectrometer. Analytical TLC was done on precoated TLC plates (Kieselgel 60 F254, layer thickness 0.2 mm). Wakogel C-200 was used for column chromatography unless otherwise noted.

Materials. Compound **1** was isolated from roots of *Petasites japonicus* var. *giganteus* (Compositae) collected in Nagano Prefecture, Japan. See our previous report[8] for the isolation procedure. Compound **2** was prepared via the known Feist–Benary procedure.[8,11]

4-Benzoyloxy-4,5,6,7-tetrahydrobenzofuran (7). In a 50 cm^3 round-bottomed flask attached with $CaCl_2$ drying tube, a stirred solution of **2** (1.90 g, 13.8 mmol) in CH_2Cl_2 (50 cm^3) was prepared. To this was added pyridine (5.8 cm^3) and benzoyl chloride (5.00 cm^3, 61.8 mmol) successively, and the stirring was continued at room temperature for 2 h. Water (40 cm^3) was added, and the mixture was extracted with CH_2Cl_2 and dried over anhydrous $MgSO_4$. Evaporation of the solvent, followed by silica-gel column chromatography using hexane/AcOEt (99:1) as eluent, afforded **7** (3.19 g, 96%) as an oil; IR (neat) 2949, 1714, 1271, 1109, and 712 cm^{-1}; ^{1}H NMR (C_6D_6) δ 1.30–1.40 (1H, m), 1.49–1.58 (1H, m), 1.69–1.84 (2H, m), 2.12–2.21 (1H, m), 2.33–2.41 (1H, m), 6.06 (1H, t, J = 3.8 Hz), 6.44 (1H, d, J = 1.5 Hz), 6.95–7.13 (4H, m), and 8.14–8.18 (2H, m); ^{13}C NMR ($CDCl_3$) δ 19.01, 22.76, 28.97, 66.71, 110.08, 116.42, 128.09 (2C), 129.41 (2C), 130.46, 132.62, 140.77, 153.84, and 166.09; MS (EI) m/z 137 (M^+ − PhCO), 120 (base), 105, 91, and 77; HRMS (EI) Found: m/z 242.0943 (M^+); Calcd for $C_{15}H_{14}O_3$: M, 242.0943.

Diels–Alder Reaction of 2 and 3. Compound **2** (137.1 mg, 0.993 mmol) was dissolved in EtOH/H_2O (10 cm^3, ratio 9:1), and to this was added a solution of *N*-ethylmaleimide (**3**, 378.9 mg, 3.03 mmol) in EtOH/H_2O of the same ratio (10 cm^3) at room temperature with stirring, which was continued for 1 day. Without extraction, water was eliminated from the mixture by adding Na_2SO_4, followed by filtration. Evaporation of the solvent afforded an oily residue, which was chromatographed on silica gel (35 g) using hexane/AcOEt (8:2 to 2:8) as the eluent to afford a mixture of **4a** and **4b** (148.5 mg, 57%) and **4c** (106.3 mg, 41%).

A mixture of **4a** and **4b**: Mp 138–140 °C; IR (KBr) 3377 (OH), 1695 (C=O), 1408, 1344, 1232, and 1142 cm^{-1}; ^{1}H NMR δ 1.14 (3H, t, J = 7.2 Hz, NCH_2CH_3), 1.40–2.21 (6H, m), 2.51–2.63 (1H, m), 2.72 (1H × 3/10, d, J = 6.5 Hz, H_c of **4b**), 2.98 (1H × 7/10, d, J = 6.5 Hz, H_c of **4a**), 3.00 (1H × 3/10, d, J = 6.5 Hz, H_b of **4b**), 3.11 (1H × 7/10, d, J = 6.5 Hz, H_b of **4a**), 3.51 (2H, q, J = 7.2 Hz, NCH_2CH_3), 4.20 (1H × 3/10, ddd, J = 2.5, 5.5, 11.6 Hz, C*H*OH of **4b**), 4.71 (1H × 7/10, t, J = 2.5 Hz, C*H*OH of **4a**), 5.13 (1H × 7/10, d, J = 1.6 Hz, H_a of **4a**), 5.14 (1H × 3/10, d, J = 1.6 Hz, H_a of **4b**), 6.30 (1H × 3/10, br s, C=CH of **4b**), and 6.32 (1H × 7/10, s, C=CH of **4a**); ^{13}C NMR assigned for **4a**: δ 12.91, 16.50, 26.21, 32.45, 33.74, 47.63, 50.93, 64.26, 79.59, 87.80, 131.36, 149.99, 174.87, and 176.18; assigned for **4b**: δ 12.94, 20.41, 25.74, 33.81, 34.73, 46.76, 51.80, 67.98, 79.88, 89.58, 127.47, 153.30, 174.70, and 175.91; MS (CI) m/z 264 (M^+ + H), 246, 166, 138, and 121 (base); HRMS (CI) Found: m/z 264.1235 (M^+ + H); Calcd for $C_{14}H_{18}NO_4$: M, 264.1236.

4c: Mp 111–112 °C; IR (KBr) 3438 (OH), 1697 (C=O), 1402, 1342, 1225, 1066, and 895 cm^{-1}; ^{1}H NMR δ 1.05 (3H, t, J = 7.2 Hz, NCH_2CH_3), 1.34–2.43 (7H, m), 3.20 (1H, d, J = 7.4 Hz, H_c), 3.39 (2H, q, J = 7.2 Hz, NCH_2CH_3), 3.68 (1H, dd, J = 5.5, 7.4 Hz, H_b), 3.99 (1H, br, $W_{1/2}$ = 23 Hz, C*H*OH), 5.17 (1H, dd, J = 1.6, 5.5 Hz, H_a), and 6.19 (1H, br s, C=CH); ^{13}C NMR δ 12.59, 19.76, 30.66, 33.33, 34.70, 50.02, 50.98, 68.27, 78.01,

89.77, 125.32, 151.38, 174.90, and 174.97; MS (CI) m/z 263 (M^+), 246, 166, 118, 126, and 121 (base); HRMS (CI) Found: m/z 264.1237 ($M^+ + H$); Calcd for $C_{14}H_{18}NO_4$: M, 264.1236.

Diels–Alder Reaction of 7 and 5. By the same procedure described above, compound **7** (50.5 mg, 0.209 mmol) and **5** (36.2 mg, 0.209 mmol) in EtOH/H_2O afforded **8a** (44.5 mg, 51%) and **8b** (12.4 mg, 14%) after silica-gel chromatography using hexane/AcOEt (2:1) as the eluent.

8a: An oil; IR (neat) 1712 (C=O), 1385, 1265, 1192, and 737 cm^{-1}; ^1H NMR δ 1.74–2.23 (5H, m), 2.73 (1H, br d, J = 13 Hz), 2.97 (1H, d, J = 6.5 Hz, CHC=O), 3.07 (1H, d, J = 6.5 Hz, CHC=O), 5.23 (1H, d, J = 1.6 Hz, CH–O), 5.97 (1H, br s, C*H*OBz), 6.55 (1H, br s, C=CH), 7.21–7.56 and 7.95–7.99 (10H, m, Ph × 2); ^{13}C NMR δ 17.56, 25.98, 29.97, 47.49, 50.43, 66.74, 80.20, 88.11, 126.44 (2C), 128.56 (2C), 128.60, 129.00 (2C), 129.36 (2C), 129.85, 131.61, 133.26, 135.23, 145.91, 165.02, 173.79, and 174.88; MS (FAB) m/z 438 (M^+ + Na), 416 ($M^+ + H$), 413, 329, 307, 154 (base), and 136; HRMS (FAB) Found: m/z 416.1485 ($M^+ + H$); Calcd for $C_{25}H_{22}NO_5$: M, 416.1499.

8b: Mp 169–170 °C; IR (neat) 1712 (C=O), 1385, 1265, and 737 cm^{-1}; ^1H NMR δ 1.67–1.95 (3H, m), 2.08–2.16 (1H, m), 2.29–2.36 (1H, m), 2.69 (1H, br d, J = 13 Hz), 3.01 (1H, d, J = 6.5 Hz, CHC=O), 3.19 (1H, d, J = 6.5 Hz, CHC=O), 5.28 (1H, d, J = 1.5 Hz, CH–O), 5.65 (1H, ddd, J = 2.6, 5.9, 11.2 Hz, C*H*OBz), 6.26 (1H, br s, C=CH), 7.25–7.64 and 8.05–8.09 (10H, m, Ph × 2); ^{13}C NMR δ 20.33, 25.76, 30.76, 46.93, 51.61, 69.25, 80.48, 90.19, 126.51 (2C), 128.51 (2C), 128.58, 128.72, 129.09 (2C), 129.57, 129.71 (2C), 131.65, 133.41, 148.96, 165.72, 173.72, and 175.04; MS (FAB) m/z 438 (M^+ + Na), 416 ($M^+ + H$), 413, 391, 294, 154 (base), and 121; HRMS (FAB) Found: m/z 416.1482 ($M^+ + H$); Calcd for $C_{25}H_{22}NO_5$: M, 416.1499.

Diels–Alder Reaction of Furanoeremophilan-6β-ol (1). In a 10 cm^3 round-bottomed flask, a solution of compound **1** (13.1 mg, 0.056 mmol) in EtOH/H_2O (0.3 cm^3, ratio 9:1) was prepared, and to this was added a solution of **5** (30.3 mg, 0.175 mmol) in the same solvent (0.3 cm^3) with stirring at room temperature. After being stirred for 24 h, water was removed by adding anhydrous Na_2SO_4 followed by filtration. The solvent EtOH was evaporated, and the resultant residue was chromatographed on silica gel (3 g) using hexane/AcOEt (4:1 to 0:1) as the eluent to afford a mixture of **10a** and **10b** (16.8 mg, 74%). Similarly, the reaction of **1** (12.5 mg, 0.0533 mmol) and **3** (23.0 mg, 0.184 mmol) in EtOH/H_2O (0.5 cm^3) afforded **9a** (7.7 mg, 39%) and **9b** (3.2 mg, 16%) after silica-gel (2 g) column chromatography using hexane/AcOEt (7:3 to 4:6) as eluent.

9a: Mp 70–72 °C; IR (KBr) 3448, 2927, 1693, 1406, and 999 cm^{-1}; ^1H NMR δ 0.69 (3H, d, J = 6.5 Hz, Me), 1.08 (3H, s, Me), 1.14 (3H, t, J = 7.2 Hz, NCH_2CH_3), 1.23–1.94 (8H, m), 1.86 (3H, s, C=CMe), 2.22–2.29 (3H, m), 2.91 (1H, d, J = 6.5 Hz, CHC=O), 3.13 (1H, d, J = 6.5 Hz, CHC=O), 3.51 (2H, q, J = 7.2 Hz, NCH_2CH_3), 4.39 (1H, br s, C*H*OH), and 4.89 (1H, s, CHOC); ^{13}C NMR δ 9.92, 12.97, 15.76, 16.53, 19.44, 25.50, 25.68, 29.50, 30.29, 32.69, 33.71, 40.09, 49.83, 50.29, 68.77, 83.39, 88.79, 140.20, 141.67, 175.37, and 176.60; MS (CI) m/z 360 ($M^+ + H$), 342, 234, 217 (base), 126, and 124; HRMS (CI) Found: m/z 360.2172 ($M^+ + H$); Calcd for $C_{21}H_{30}NO_4$: M, 360.2176.

9b: Mp 151–152 °C; IR (KBr) 3448, 2925, 1697, and 1400 cm^{-1}; ^1H NMR δ 0.91 (3H, s, Me), 1.11 (3H, d, J = 7.1 Hz, Me), 1.13 (3H, t, J = 7.2 Hz, NCH_2CH_3), 1.19–2.10 (10H, m), 2.01 (3H, d, J = 2.2 Hz, C=CMe), 2.25 (1H, dd, J = 2.2, 13.8 Hz), 2.75 (1H, d, J = 6.5 Hz, CHC=O), 2.92 (1H, d, J = 6.5 Hz, CHC=O), 3.50 (2H, q, J = 7.2 Hz, NCH_2CH_3), 4.78 (1H, br, $W_{1/2}$ = 14 Hz, C*H*OH), and 4.78 (1H, s, CHOC); ^{13}C NMR δ 11.05, 12.97, 14.86, 18.94, 20.78, 27.05, 28.56, 29.32, 31.89, 33.78, 37.24, 41.50, 49.98, 51.22, 70.34, 83.88, 89.77, 140.21, 142.21, 174.98, and 176.33; MS (CI) m/z 360 ($M^+ + H$), 342, 235, 217, 124 (base); Analysis Found: C, 70.24; H, 8.44; N, 3.61%; Calcd for $C_{21}H_{29}NO_4$: C, 70.17; H, 8.13; N, 3.90%.

10a: Mp 163–164 °C; IR (KBr) 3498, 2925, 1703, 1389, and 1190 cm^{-1}; ^1H NMR δ 0.70 (3H, d, J = 6.8 Hz, Me), 1.09 (3H, s, Me), 1.24–2.34 (11H, m), 1.90 (3H, s, C=CMe), 3.08 (1H, d, J = 6.5 Hz, CHC=O), 3.31 (1H, d, J = 6.5 Hz, CHC=O), 4.42 (1H, s, C*H*OH), 5.02 (1H, s, CHOC), and 7.26–7.48 (5H, m, Ph); ^{13}C NMR δ 10.04, 15.80, 16.56, 19.42, 25.47, 25.73, 29.50, 30.28, 32.67, 40.09, 49.92, 50.36, 68.80, 83.87, 89.29, 126.58 (2C), 128.59, 129.06 (2C), 131.80, 141.33, 141.89, 174.56, and 175.91; MS (FAB) m/z 430 (M^+ + Na), 413, 408 ($M^+ + H$), 390, 217 (base), and 124; HRMS (FAB) Found: m/z 408.2155 ($M^+ + H$); Calcd for $C_{25}H_{30}NO_4$: M, 408.2176.

10b: Mp 111–113 °C; IR (KBr) 3450, 2929, 1705, 1387, and 1190 cm^{-1}; ^1H NMR δ 0.93 (3H, s, Me), 1.02 (3H, d, J = 7.2 Hz, Me), 1.18–2.33 (11H, m), 2.05 (3H, d, J = 2.2 Hz, C=CMe), 2.92 (1H, d, J = 6.5 Hz, CHC=O), 3.10 (1H, d, J = 6.5 Hz, CHC=O), 4.82 (1H, br dq, J = 8.7, 1.9 Hz, C*H*OH), 4.92 (1H, s, CHOC), and 7.24–7.48 (5H, m, Ph); ^{13}C NMR δ 11.15, 14.87, 18.98, 20.76, 27.11, 28.56, 29.28, 31.89, 32.23, 41.51, 50.04, 51.34, 70.37, 84.36, 90.27, 126.53 (2C), 128.65, 129.09 (2C), 131.72, 140.43, 142.41, 174.17, and 175.64; MS (FAB) m/z 430 (M^+ + Na), 413, 408 ($M^+ + H$), 390, 329, 307, 154 (base), and 136; HRMS (FAB) Found: m/z 408.2151 ($M^+ + H$); Calcd for $C_{25}H_{30}NO_4$: M, 408.2176.

Diels–Alder Reaction of Unpurified Extract of *Petasites japonicus* var. *giganteus*. Comparison of the Diels–Alder Method and the Conventional Isolation: Half-dried roots (569 g) were extracted with AcOEt at room temperature as usual. The extracted solution (36 cm^3) was obtained by decantation and was divided into three portions. To the first 12 cm^3 portion was added a solution of *N*-ethylmaleimide (126.8 mg) in AcOEt (5 cm^5), and the mixture was stirred at room temperature for 1 week. Evaporation of the solvent followed by silica-gel (2 g) column chromatography using hexane/AcOEt (9:1) as the eluent afforded a mixture of **9a** and **9b** (6.6 mg). The second 12 cm^3 portion of the extracted solution was similarly treated with a solution of *N*-phenylmaleimide (175.7 mg) in AcOEt (5 cm^3) giving **10a** and **10b** (8.4 mg). The third 12 cm^3 portion was evaporated to an oily residue (52.4 mg), which was chromatographed on neutral silica gel (Silica Gel 60 N) (5 g) using hexane/AcOEt (9:1) as the eluent to afford **1** (1.5 mg).

Application to the Extraction of Fresh Root: Fresh roots (12 g) were extracted with ethanol (13 cm^3) for 1 week at room temperature. The plant material was filtered off, and to the filtrate was added *N*-ethylmaleimide (108.7 mg). The reaction mixture was stirred at room temperature for 1 week, followed by evaporation of the solvent. The resultant residue was chromatographed as described above to afford **9a** (54.5 mg) and **9b** (19.4 mg).

Diels–Alder Reaction of Unpurified Extract of *Ligularia cymbulifera*. Dried roots, collected near Zhongdian city, Yunnan, China, were extracted with ethyl acetate for 1 week at room temperature. An extracted solution (10 cm^3) was obtained by decantation, and a solution of *N*-ethylmaleimide (2.8 g) in AcOEt (4 cm^3) was added. After keeping the mixture for 30 min at room

temperature, the solvent was evaporated off, and the residual oil was separated by silica-gel (8 g) column chromatography using hexane–AcOEt (9:1 to 3:1) as the eluent to afford a mixture of **12a** and **12b** (119.1 mg, ratio 3:2). The following spectral data were obtained after further separation.

At the same time, in order to calculate the content of **11**, the extracted solution of *L. cymbulifera* (2 cm^3) was simply evaporated to yield an oily residue (48.7 mg).

12a: Mp 138–139 °C; IR (KBr) 3517 and 1682 cm^{-1}; ^{1}H NMR δ 0.77 (3H, d, $J = 6.5$ Hz, Me), 0.97 (3H, s, Me), 1.15 (3H, t, $J = 7.1$ Hz, NCH_2CH_3), 1.25–1.97 (8H, m), 1.74 (3H, d, $J = 2.5$ Hz, C=CMe), 2.09 (1H, d, $J = 14.6$ Hz), 2.10 (1H, dq, $J = 16.6$, 2.5 Hz), 2.33 (1H, d, $J = 16.6$ Hz), 2.63 (1H, d, $J = 14.6$ Hz), 2.86 (1H, d, $J = 6.4$ Hz, CHC=O), 2.90 (1H, d, $J = 6.4$ Hz, CHC=O), 3.51 (2H, q, $J = 7.1$ Hz, NCH_2CH_3), and 4.88 (1H, s, CHOC); ^{13}C NMR δ 9.87, 12.98, 14.35, 15.71, 22.61, 28.62, 29.57, 33.33, 33.45, 33.72, 36.50, 41.36, 49.82, 50.93, 74.52, 80.07, 88.63, 135.89, 139.60, 176.30, and 176.62; MS (CI) m/z 360 ($M^+ + H$), 342, 291, 235, 217 (base), 126, and 89; HRMS (CI) Found: m/z 360.2172 ($M^+ + H$); Calcd for $C_{21}H_{30}NO_4$: M, 360.2176.

12b: Mp 129–131 °C; IR (KBr) 3479 and 1682 cm^{-1}; ^{1}H NMR δ 0.72 (3H, d, $J = 6.5$ Hz, Me), 0.97 (3H, s, Me), 1.02 (3H, t, $J = 7.1$ Hz, NCH_2CH_3), 1.11–1.90 (8H, m), 1.69 (3H, s, C=CMe), 2.14 (1H, d, $J = 15.4$ Hz), 2.30 (1H, br d, $J = 16.5$ Hz), 2.87 (1H, d, $J = 15.4$ Hz), 3.27 (1H, d, $J = 7.3$ Hz, CHC=O), 3.41 (2H, q, $J = 7.1$ Hz, NCH_2CH_3), 3.69 (1H, dd, $J = 5.5$, 7.3 Hz, CHC=O), 4.53 (1H, br, OH), and 4.95 (1H, d, $J = 5.5$ Hz, CHOC); ^{13}C NMR δ 11.09, 13.13, 14.58, 15.60, 22.49, 29.34, 29.70, 33.48, 33.53, 33.88, 38.56, 42.89, 49.55, 52.49, 73.87, 81.62, 88.79, 134.93, 137.23, 174.31, and 177.37; MS (CI) m/z 360 ($M^+ + H$), 342, 291, 235, 217 (base), and 126; HRMS (CI) Found: m/z 360.2184 ($M^+ + H$); Calcd for $C_{21}H_{30}NO_4$: M, 360.2176.

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