Chemical and Genetic Study of *Ligularia cyathiceps* in Yunnan Province of China

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Dedicated to Professor Emeritus *Takeyoshi Takahashi* on the occasion of his 83rd birthday

Root chemicals and evolutionarily neutral DNA regions in *L. cyathiceps* samples collected in the Zhongdian (Shangrila) County of Yunnan, P. R. China, were examined. Twenty compounds were isolated, including three new ones, 1β,10β-epoxy-6β-(propionyloxy)furanoeremophilan-9-one (**6**), 1β,10β-epoxy-8α-ethoxyeremophila-6,11-diene (**14**), and 11αH-6β-isobutyryloxy-1β,10β,7β,8β-diepoxy-eremophilan-12,8α-olide (**15**). The chemical diversity was found to be limited, with cacalol (**1**) and 6-(acyloxy)furanoeremophilan-9-ones (**4** and/or **5**) being major components in all the samples. The nuclear ribosomal RNA gene was also found to harbor little variation, although two distinct sequence types were found for the plastid *atpB-rbcL* intergenic region.

Introduction. – Diversification and evolution of plant chemicals is a fundamental subject in natural-product science. The genus *Ligularia* (Asteraceae) in the Hengduan Mountains area provides us with interesting materials for studies of this subject, since evolution of many species of *Ligularia* is considered to be continuing in this area [1][2]. We have been studying the chemical diversity of *Ligularia* by combining two different approaches. One is to analyze chemical constituents in the root and the other, to determine nucleotide sequences of evolutionarily neutral DNA regions. To date, we have found that there are several different modes of intraspecific diversity in *Ligularia*,

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implying that the mechanism(s) of generation of chemical diversity is complex. For example, both *L. tsangchanensis* (FRANCH.) HAND.-MAZZ. [3] and *L. pleurocaulis* (FRANCH.) HAND.-MAZZ. [4] were separated into two distinct groups in accordance with geographic distribution. *L. virgaurea* (MAXIM.) MATTF. was also grouped into two, however, the two groups were not geographically separated [5]. The chemical spectrum in *L. subspicata* (BUREAU & FRANCH.) HAND.-MAZZ. was continuous [6], while those in *L. dictyoneura* (FRANCH.) HAND.-MAZZ. [7] and in *L. kanaitzensis* (FRANCH.) HAND.-MAZZ. [8] were complex. At the other extreme, *L. cymbulifera* (W. W. SMITH) HAND.-MAZZ. [9] was chemically uniform.

In this report, we describe results of chemical and genetic analyses of *L. cyathiceps* HAND.-MAZZ., chemical constituents of which have not been reported. The plant grows in a variety of habitats including stream banks, valleys, and grassy slopes in the northwestern Yunnan Province, P. R. China [1]. We isolated three new eremophilanes in addition to several known compounds, and found that the plant has a limited diversity both in the chemical composition and in evolutionarily neutral DNA sequences.

Results. – Eleven samples were collected in the Zhongdian (Shangrila) County in northwestern Yunnan (*Table* and *Fig. 1*). For a rough examination of the composition of root chemicals in each sample, extraction with EtOH was carried out without drying and compounds therein were analyzed by TLC. *Ehrlich*'s test [4][9] detected two weak

Table. *Collection Locality and Nucleotide Sequences of* L. cyathiceps *Samples*

Sample[a])	Location	Elevation [m]	Nucleotide sequences					
			ribosomal RNA gene[b])[c])					*atpB-rbcL*[d])
			ITS1		5.8S	ITS2		
			6	13	152	18	216	
1	Qianhushan	3600	A	G	Y	C	Y	T8 A10 409A
2	Qianhushan	3500	A	S	C	C	Y	T8 A10 409A
3	Dabaoshan	3300	R	G	C	C	Y	T10 A9 409T
4	Dabaoshan	3400	A	S	C	C	Y	T10 A9 409T
5	Dabaoshan	3700	A	G	C	C	Y	T10 A9 409T
6	Xiaozhongdian	3400	A	G	C	C	C	T8 A10 409A
7	Tianchi	3700	A	G	C	C	C	T10 A9 409T
8	Tianchi	3900	A	G	C	C	C	T10 A9 409T
9	Tianchi	3500	A	G	C	C	C	T8 A10 409A
10	Hongpi	3400	A	G	C	Y	Y	T10 A9 409T
11	Hongshan (Geza)	4000	A	G	C	C	C	T10 A9 409T
Ref.[c])			A	G	C	C	C	

[a]) Samples *1*, *3*, *5*, and *9* were collected in 2004; samples *4*, *6*, and *10* were collected in 2006; samples *2*, *7*, *8*, and *11* were collected in 2008. [b]) Y = C + T; R = A + G; S = G + C. [c]) Only the nucleotide sites that where different from the reference sequence (DQ272328) are shown. [d]) The numbers of Ts in a stretch around the 390th base position and of As around the 510th positions are shown, followed by the base at the 409th position. The base numbering is based on the published *L. tongolensis* sequence [9]. The base sequences were otherwise the same as that of the *L. tongolensis* sequence, except for the 28th base being a G in the present *L. cyathiceps* samples.

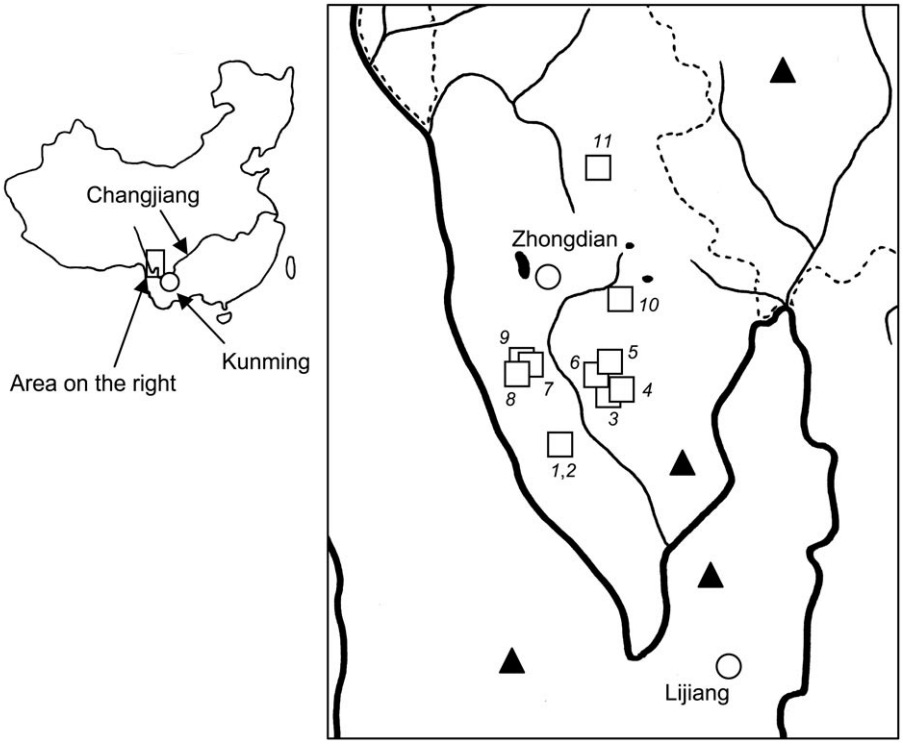
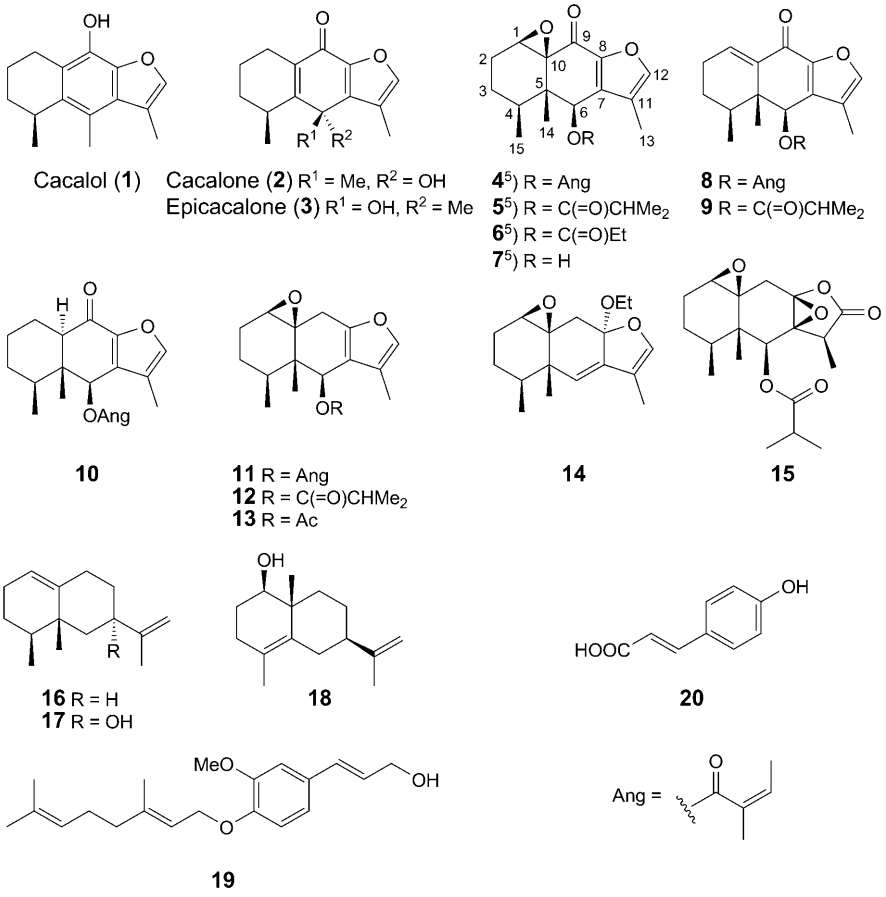


Fig. 1. *Locations where samples of* L. cyathiceps *species* (open squares) *were collected.* Locations *1* and *2* were close to each other. Circles and filled triangles indicate major cities and major peaks, respectively.

spots at R_f 0.77 and 0.61 (hexane/AcOEt 7:3), indicating the composition of furanoeremophilanes and/or related compounds was similar among the samples. *Ehrlich*-negative spots (the most major at R_f 0.33) were also observed, when compounds were detected with $Ce(SO_4)_2/H_2SO_4$.

For analysis of the chemical constituents, roots of each sample were dried and extracted with EtOH or AcOEt. Compounds were separated by silica-gel column chromatography and HPLC. Cacalol (**1**) [10–12], cacalone (**2**), and epicacalone (**3**) [3][12][13], eremophilanes **4** [14], **5** [15], **6**, **7** [16], **8** [17], **9** [18], **10** [18], **11** [19], **12** [20], **13** [21], **14**, **15**, **16** [22], and **17** [23], an eudesmane **18** [24], a coniferyl alcohol derivative **19** [25], and *trans*-4-hydroxycynnamic acid (**20**) [26] were isolated. Compounds **6**, **14**, and **15** were new. Although compound **17** was briefly described in 1979 [23], physicochemical data pertaining to its configuration have not been reported. Therefore, we describe below the structure determination of **17** as well as of **6**, **14**, and **15**.

The CI mass spectrum of **6** showed a *quasi*-molecular-ion peak at *m*/*z* 319, and the molecular formula was deduced as $C_{18}H_{22}O_5$ by HR-CI-MS. The ^{1}H-NMR spectrum indicated the presence of a *singlet* Me (δ(H) 1.16), a *doublet* Me (δ(H) 0.97, $J = 7.6$), a *triplet*-like Me (δ(H) 0.91, $J = 7.6$), and a *doublet* Me (δ(H) 1.55, $J = 1.2$) group. A H-



atom at δ(H) 6.69 (*q*, $J = 1.2$), characteristic of H–C(12)[5]) of the furan moiety in furanoeremophilanes, and a H-atom at δ(H) 6.41 (*s*, H–C(6)) were observed. Analysis of 2D-NMR spectra (*Fig. 2*) suggested a furanoeremophilane with an epoxide at C(1) and C(10) and a propanoyloxy group at the C(6) position. The position of a C=O group, detected by IR at 1690 cm^{-1}, was deduced from the chemical shifts of C=O (δ(C) 180.1) and C(7) (δ(C) 135.8), as the former was low-field shifted and the latter high-field shifted. Therefore, the planar structure in the formula **6** was established. The configuration of **6** was determined by NOESY. NOE was detected between H_α–C(2) and H–C(1), and between H_β–C(2) and Me(15). These and other NOE signals established the structure as 1β,10β-epoxy-6β-(propanoyloxy)furanoeremophilan-9-one.

The mass spectrum of compound **14** showed a molecular-ion peak at *m*/*z* 276, and its formula was deduced to be $C_{17}H_{24}O_3$ by HR-MS. The ^{1}H-NMR spectrum showed a

[5]) Eremophilane numbering. For systematic names, see *Exper. Part.*

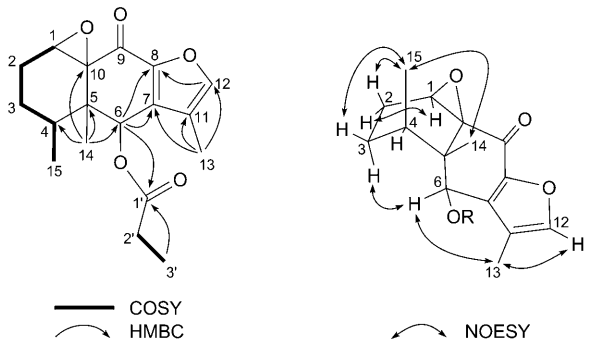


Fig. 2. *Selected COSY, HMBC, and NOESY correlations of compound* **6**[5])

singlet for Me (δ(H) 1.06), a *doublet* for Me (δ(H) 0.77, $J = 6.9$), a *doublet* for Me (δ(H) 1.46, $J = 1.3$), and a *triplet*-like signal for Me (δ(H) 1.07, $J = 7.1$) group. These signals as well as the H-atom signals of two *doublet* of *quartets* (δ(H) 3.50, 3.31) indicated the presence of an EtO group in the molecule. The HSQC spectrum showed that low-field H-atoms at δ(H) 6.22 and 5.51 were attributable to two olefinic H-atoms attached to the C-atoms at δ(C) 146.7 (C(12)[5])) and 125.1 (C(6)), respectively. The COSY and HMBC spectra exhibited the correlations shown in *Fig. 3*. These indicated the eremophilane skeleton with an EtO group at the C(8) position although direct correlations to the EtO group were not observed. The chemical shifts of C(1) and C(10) indicated the presence of an epoxide group at these positions. The acetal C-atom (δ(C) 107.6) was found to be substituted by the EtO group (*Fig. 3*).

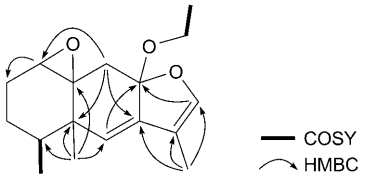


Fig. 3. *Selected COSY and HMBC correlations of compound* **14**

The configuration of **14** was determined by NOESY spectrum. NOE Correlations were detected between H–C(1) and H_α–C(9), and between H_β–C(9) and Me(14) (*Fig. 4*). Therefore, Me(14) and H_β–C(9) should be in β-orientation, and H–C(1) and H_α–C(9) on the α-side. However, no significant NOE was detected between the EtO group and any H-atom on the skeletal C-atoms. Two diastereoisomers **14** and **14a** were theoretically possible (*Fig. 4*). The α-orientation for the EtO group was compatible with the observed NOE correlations, whereas the β-orientation was incompatible with the observed NOE correlations for Me(14). Thus, the configuration in the formula **14** was established. This is the first compound bearing a diene moiety at the C(6) and C(11) positions in the eremophilane skeleton although it might be an artifact due to extraction with EtOH.

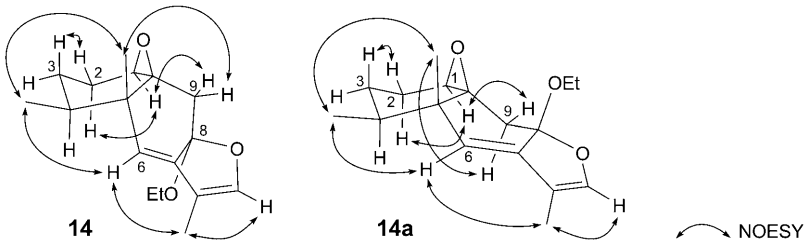


Fig. 4. *Selected NOESY correlations of compound* **14**[5])

Compound **15** exhibited a *quasi*-molecular-ion peak at *m*/*z* 351, and its molecular formula was determined to be $C_{19}H_{26}O_6$. The IR spectrum indicated the presence of an epoxy-lactone or an enol-lactone by the absorption at 1800 cm^{-1} as well as an ester (1730 cm^{-1}) group. The ^{1}H-NMR spectrum exhibited four *doublet*s and one *singlet* for Me groups, and it indicated the presence of two O-bearing CH groups. The HMBC spectrum showed correlations between Me(14)[5]) and C(4), C(5), C(6), and C(10), between Me(15) and C(3), between Me(13) and C(7), C(11), and C(12), and between CH_2(9) and C(1), C(10), C(5), and C(8) (*Fig. 5*). These correlations indicated the eremophilane skeleton with O-functions at the C(1), C(6), and C(10) positions. The ^{13}C chemical shifts of C(7) and C(8) were δ(C) 64.5 and δ(C) 85.7, respectively. Comparison of the chemical shifts with those of previously reported compounds [6][27–29] indicated the presence of an epoxide, not an enol, at the C(7) and C(8) positions with a *doublet* Me at C(11). The isobutyryloxy group was determined to be at C(6) because an HMBC between H–C(6) and C(1′) was observed. The configuration was determined by NOESY. NOE Correlations between H–C(11) and H–C(6), H–C(6) and H_α–C(3), H–C(6) and H_α–C(4), Me(14) and H_β–C(9), and Me(15) and H_β–C(2) were observed. These correlations indicated the β-orientation for Me(13), Me(14), and Me(15), and for the isobutyryloxy group. The epoxide at C(1) and C(10) was judged to be in the β-orientation because the detected NOE correlations would, otherwise, be incompatible. The epoxide at C(7) and C(8) should be in the β-configuration according to the biosynthetic pathway discussed previously [27]. Hence, the structure of **15** was established to be 11α*H*-6β-isobutyryloxy-1β,10β,7β,8β-di-epoxyeremophilan-12,8α-olide.

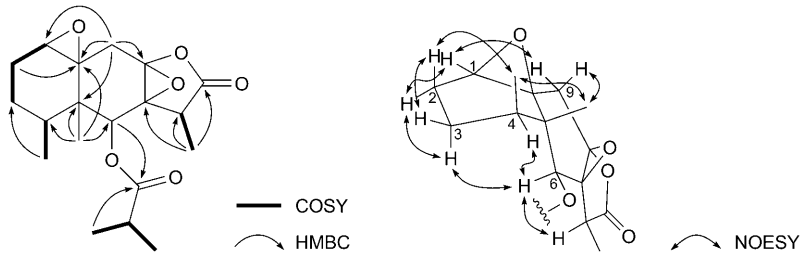


Fig. 5. *Selected COSY, HMBC, and NOESY correlations of compound* **15**[5])

Compound **17** showed a *quasi*-molecular-ion peak at *m*/*z* 221, and the molecular formula was deduced to be $C_{15}H_{24}O$ by HR-MS. The IR spectrum indicated the

presence of an OH group (3300 cm^{-1}). ^1H- and ^{13}C-NMR spectra exhibited of a *doublet* for Me (δ(H) 0.81, $J = 6.6$), a *singlet* for Me (δ(H) 0.91), and a *doublet* for Me (δ(H) 1.83, $J = 0.5$), as well as signals for two C=C bonds, suggesting an eremophilane skeleton, which was supported by 2D-NMR (*Fig. 6*). One of the C=C bonds was part of an isopropenyl group and the other was assigned to be between C(1)[5]) and C(10) by HMBC. HMBC Correlations from the isopropenyl group indicated the presence of a quaternary C-atom bearing an OH group which was assigned at C(7). The configuration of **17** was deduced by NOE between H_β–C(9) and Me(14), between H_β–C(9) and H–C(12), between H_β–C(6) and Me(13), and between H_α–C(9) and H–C(1). Therefore, the structure of compound **17** was established to be eremophila-1(10),11-dien-7α-ol.

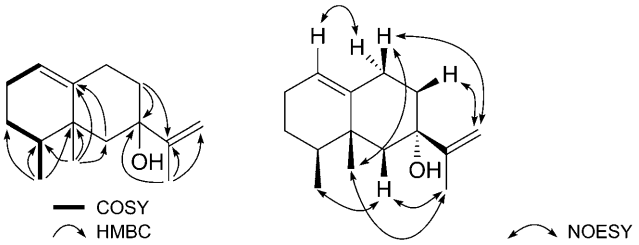


Fig. 6. *Selected COSY, HMBC, and NOESY correlations of compound* **17**

DNA Sequencing was carried out for a continuous region in the nuclear ribosomal RNA (rRNA) gene, consisting of internal transcribed spacer 1 (ITS1), 5.8S rRNA, and ITS2, and also for the *atpB-rbcL* intergenic region of the plastid genome. These regions are nonfunctional, and consequently most mutations therein should be neutral [4][9]. The results are summarized in the *Table*. The sequence of the ITS1-5.8S-ITS2 region was very similar among the samples. The listed numbers of Ts and As and the 409th base in the *atpB-rbcL* region have often been found to vary within *Ligularia* species [3–9]. The 28th base, which is found to vary frequently, was G in all the present samples.

Discussion. – Cacalol (**1**) and 6-(acyloxy)furanoeremophilan-9-ones (**4** and/or **5**) were the major components in all the samples. Related compounds, including cacalone (**2**), epicacalone (**3**) and 6-(acyloxy)furanoeremophilan-9-one derivatives **4**–**10**, were also found in many samples. Compounds **1**–**10** are presumed to be highly related in their structure, as biosynthetic relation between cacalol and a 6-(acyloxy)-9-oxofuranoeremophilane derivative has been suggested [10]. Coincidental isolation of cacalol and 9-oxygenated furanoeremophilane(s) has been reported for some species of *Cacalia* [30] and *Senecio* [19][31][32].

Intra-specific variation was limited to the composition of other components, as seen in the relative abundance of **12** in sample *10*. Variation was found to be limited also in the DNA sequences. Although two distinct sequence types were found for the *atpB-rbcL* region, the ITS1-5.8S-ITS2 sequences were essentially the same. These similarities in the chemical composition and in the DNA sequences among the samples may be a

result of limitation in the collection locality. However, in the case of *L. dictyoneura*, higher diversity has been observed within the area of the present sample collection [7].

Thus far, we have examined the chemical composition of 16 species of *Ligularia* (see [3–9][33] and the references cited therein). Among them, *L. tsangchanensis* is the only *Ligularia* species that produces cacalol as a major component [3]. Although *L. tsangchanensis* and *L. cyathiceps* belong to the same Section *Ligularia*, the former belongs to Series *Racemiferae*, whereas the latter to Series *Ligularia* [1]. Thus, the two species are not closely related according to taxonomy based on morphology. However, the occurrence of cacalol in the two species may not be a mere coincidence since reticulate evolution within and among *Ligularia* and related genera has been strongly suggested on the basis of DNA sequences [34].

Conclusions. – Twenty compounds were isolated from *L. cyathiceps*, and the structures of the three new eremophilanes were established. The chemical composition of root extracts of *L. cyathiceps* was found to be similar in that cacalol and 9-oxofuranoeremophilane derivatives were major components. Although two types of base sequence were found for the *atpB-rbcL* region, the nuclear ribosomal RNA gene sequence indicated that the plant was quite homogeneous. The occurrence of cacalol in both *L. cyathiceps* and *L. tsangchanensis* may support a reticulate evolution proposed for the *Ligularia-Cremanthodium-Parasenecio* (*Cacalia*) [34].

The authors wish to thank Mrs. *Guowen Hu* of the Kunming Institute of Botany for research coordination and Dr. *Masami Tanaka* of Tokushima Bunri University for 600 MHz NMR measurements. This work was partly supported by a Grant-in-Aid for Scientific Research from JSPS (No. 21404009).

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; *Fuji Sylisia* (70–230 mesh), *Merck Kieselgel 60*, or *Kanto 60 N*). Anal. TLC: silica gel (*Merck Kieselgel 60 F_{254}*; layer thickness, 0.25 mm). HPLC: *JASCO* pump system, *RI-930* detector, and *Chemcopak Nucleosil 50-5* (4.6 × 250 mm) SiO_2 column. Specific rotations and CD: *JASCO DIP-1000*, *JASCO J-725*, or *JASCO DPI-181* polarimeter. IR: *JASCO FT/IR-5300* or *Shimadzu FTIR-8700* spectrometer. ^{1}H- and ^{13}C-NMR Spectra: *Varian Unity 600* (600 and 150 MHz, resp.), *JEOL ECP 400* or *JEOL AL 400* (400 and 100 MHz, resp.) spectrometers with $CDCl_3$ or C_6D_6 as the solvent and TMS as an internal standard. EI-MS: including HR-EI-MS: *JEOL JMS-700 MStation*. DNA Sequencing: *BigDye Terminator Ver3.1 Kit* (*Applied Biosystems*) and *3130xl Genetic Analyzer* (*Applied Biosystems*). For *Ehrlich*'s test on TLC, see [4][9].

Plant Materials. Samples of *L. cyathiceps* were collected in August 2004, 2006, and 2008 at eleven locations (*Table* and *Fig. 1*). Each plant was identified by *X. G.*

Extraction and Purification. The roots of each plant (2–10 g) were harvested. For *Ehrlich*'s test, extraction with EtOH was started immediately without drying. Solid plant material was removed after several d, and the soln. was subjected to TLC without concentration. For structure determination, the roots were dried for *ca.* one week, and extracted with EtOH or AcOEt at r.t. Oily extracts were obtained by a standard method.

Chemical Analysis of Sample 1. The EtOH extract (98.5 mg) was subjected to CC (hexane/AcOEt 20:1) to give less polar fractions (17.6 mg), cacalol (**1**) (2.8 mg; $[\alpha]_D^{32} = +9.3$ ($c = 0.14$, $CHCl_3$); [10]: $[\alpha]_D^{24} = +6$ ($c = 1.0$, $CHCl_3$)), and more polar fractions (41.8 mg). The less polar fractions were subjected to CC (hexane/AcOEt 40:1) to give an oily product (6 mg), which was then subjected to CC together with an oily product (5.1 mg) obtained from re-extraction (40.8 mg), to give compound **12** (1.8 mg). The more polar fractions were subjected to CC (hexane/AcOEt 10:1) to give **4** (4.2 mg), **5** (2.6 mg), and a mixture of **4** and **5** (20.6 mg).

Chemical Analysis of Sample 2. The AcOEt extract (2.13 g) was subjected to CC (hexane/AcOEt, gradient), followed by HPLC (*Nucleosil 50-5*, hexane/AcOEt 5–20%), to afford **1** (182.6 mg), **2** (4.3 mg), **3** (4.3 mg), **4** (243.1 mg), **5** (806.0 mg), **7** (7.6 mg), **8** (9.4 mg), **9** (5.8 mg), **10** (5.4 mg), **11** (5.8 mg), **12** (38.7 mg), **13** (6.5 mg), and **18** (5.4 mg).

Chemical Analysis of Sample 3. The EtOH extract (531.2 mg) was subjected to CC (hexane/AcOEt, gradient), followed by HPLC (*Nucleosil 50-5*, hexane/AcOEt 15–30%), to afford **1** (20.7 mg), **4** (6.2 mg), **5** (91.9 mg), **6** (1.5 mg), **8** (1.9 mg), **9** (1.5 mg), **12** (2.2 mg), **14** (2.5 mg), **19** (1.0 mg), and **20** (2.0 mg).

Chemical Analysis of Sample 4. The AcOEt extract (468.5 mg) was subjected to CC (hexane/AcOEt, gradient), followed by HPLC (*Nucleosil 50-5*, hexane/AcOEt 5–20%), to afford **1** (16.5 mg), **4** (32.3 mg), **5** (142 mg), **6** (6.3 mg), **8** (21.9 mg), **9** (4.5 mg), and **12** (3.3 mg).

Chemical Analysis of Sample 5. The AcOEt extract (780.7 mg) was subjected to CC (hexane/AcOEt, gradient), followed by HPLC (*Nucleosil 50-5*, hexane/AcOEt 3%), to afford **1** (31.4 mg), **5** (161.6 mg), **12** (9.9 mg), and **16** (11.7 mg).

Chemical Analysis of Sample 6. The AcOEt extract (727.7 mg) was subjected to CC (hexane/AcOEt, gradient), followed by HPLC (*Nucleosil 50-5*, hexane/AcOEt 5%), to afford **1** (26.9 mg) and **5** (292.4 mg).

Chemical Analysis of Sample 7. The AcOEt extract (196.9 mg) was subjected to CC (hexane/AcOEt, gradient), followed by HPLC (*Nucleosil 50-5*, hexane/AcOEt 5–20%), to afford **1** (17.6 mg), **2** (0.8 mg), **3** (1.3 mg), **4** (44 mg), **5** (87.4 mg), **8** (1.0 mg), **9** (1.8 mg), and **15** (0.5 mg).

Chemical Analysis of Sample 8. The AcOEt extract (2.52 g) was subjected to CC (hexane/AcOEt, gradient), followed by HPLC (*Nucleosil 50-5*, hexane/AcOEt 5–20%), to afford **1** (44.5 mg), **4** (280.1 mg), **5** (1.02 g), and **12** (1.1 mg).

Chemical Analysis of Sample 9. The AcOEt extract (1.95 g) was subjected to CC (hexane/AcOEt, gradient), followed by HPLC (*Nucleosil 50-5*, hexane/AcOEt 10–20%), to afford **1** (28.7 mg), **4** (16.8 mg), **5** (95.7 mg), **6** (3.6 mg), and **17** (0.5 mg).

Chemical Analysis of Sample 10. The AcOEt extract (816 mg) was subjected to CC (hexane/AcOEt, gradient) followed by HPLC (*Nucleosil 50-5*, hexane/AcOEt 10–30%) to afford **1** (53.9 mg), **2** (0.9 mg), **3** (1.7 mg), **4** (60.9 mg), **5** (226.7 mg), **8** (4.1 mg), **9** (2.9 mg), and **12** (99.3 mg).

Chemical Analysis of Sample 11. The AcOEt extract (3.07 g) was subjected to CC (hexane/AcOEt, gradient), followed by HPLC (*Nucleosil 50-5*, hexane/AcOEt 5–20%), to afford **1** (202.4 mg), **2** (10.6 mg), **3** (9.9 mg), **4** (231.2 mg), **5** (759.9 mg), **6** (33.7 mg), **8** (6.7 mg), **9** (4.4 mg), **11** (15.5 mg), **12** (45.8 mg), **13** (12.4 mg), and **15** (1.2 mg).

*(1a*R*,4*S*,4a*S*,5*S*,9a*S*)-2,3,4,4a,5,9-Hexahydro-4,4a,6-trimethyl-9-oxo-1a*H*-oxireno[8,8a]naphtho[2,3-*b*]furan-5-yl Propanoate* (**6**). $[\alpha]_D^{23} = -12.3$ ($c = 0.11$, EtOH). CD (EtOH): +3900 (320.9), +6800 (286.1), −18100 (202.5), −17300 (200.2). FT-IR: 1740, 1690. ^{1}H-NMR (400 MHz, C_6D_6): 6.69 (*q*, J = 1.2, H−C(12)); 6.41 (*s*, H−C(6)); 3.12 (*d*, J = 4.8, H−C(1)); 2.01 (*dq*, J = 16.8, 7.6, H_a−C(2′)); 1.97 (*dq*, J = 16.8, 7.6, H_b−C(2′)); 1.72–1.63 (*m*, H_β−C(2)); 1.55 (*d*, J = 1.2, Me(13)); 1.54–1.44 (*m*, H_α−C(3), H−C(4)); 1.30–1.22 (*m*, H_α−C(2)); 1.16 (*s*, Me(14)); 1.17–1.08 (*m*, H_β−C(3)); 0.97 (*d*, J = 7.6, Me(15)); 0.91 (*dd*, J = 7.6, 7.6, Me(3′)). ^{13}C-NMR (100 MHz, C_6D_6): 180.1 (C(9)); 173.5 (C(1′)); 147.4 (C(8)); 146.0 (C(12)); 135.8 (C(7)); 121.2 (C(11)); 69.3 (C(6)); 65.4 (C(10)); 62.1 (C(1)); 45.0 (C(5)); 32.4 (C(4)); 27.3 (C(2′)); 24.9 (C(3)); 19.3 (C(2)); 16.1 (C(14)); 15.7 (C(15)); 9.0 (C(3′)); 8.1 (C(13)). CI-MS: 319 ($[M+H]^+$), 262, 245 (100), 227, 217, 178, 85, 57. HR-CI-MS: 319.1546 ($[M+H]^+$, $C_{18}H_{23}O_5^+$; calc. 319.1545).

*(1a*R*,4*S*,4a*S*,8a*R*,9a*S*)-8a-Ethoxy-2,3,4,4a,8a,9-hexahydro-4,4a,6-trimethyl-1a*H*-oxireno[8,8a]naphtho[2,3-*b*]furan* (**14**). $[\alpha]_D^{19.5} = -70.8$ ($c = 0.306$, EtOH). CD (EtOH): +1472 (322), −157 (262), +1162 (242), −115 (230), +1595 (220), −8481 (207). ^{1}H-NMR (400 MHz, C_6D_6): 6.22 (*q*, J = 1.3, H−C(12)); 5.51 (*s*, H−C(6)); 3.50 (*dq*, J = 8.8, 7.1, H_a−C(1′)); 3.31 (*dq*, J = 8.8, 7.1, H_b−C(1′)); 3.13 (br. *s*, H−C(1)); 2.48 (*d*, J = 11.8, H_β−C(9)); 1.96 (*d*, J = 11.8, H_α−C(9)); 1.98–1.91 (*m*, H_β−C(2)); 1.64 (*dddd*, J = 14.5, 12.5, 5.0, 1.7, H_α−C(2)); 1.48 (*qd*, J = 12.5, 4.3, H_β−C(3)); 1.46 (*d*, J = 1.3, Me(13)); 1.34–1.24 (*m*, H−C(4)); 1.07 (*dd*, J = 7.1, 7.1, Me(2′)); 1.06 (*s*, Me(14)); 0.98–0.91 (*m*, H_α−C(3)); 0.77 (*d*, J = 6.9, Me(15)). ^{13}C-NMR (100 MHz, C_6D_6): 146.7 (C(12)); 140.2 (C(7)); 125.1 (C(6)); 110.9 (C(11)); 107.6 (C(8)); 63.9 (C(1)); 62.3 (C(10)); 58.8 (C(1′)); 41.8 (C(9)); 39.7 (C(4)); 39.4 (C(5)); 26.6

(C(2)); 25.6 (C(3)); 17.0 (C(14)); 15.9 (C(2′)); 15.8 (C(15)); 7.1 (C(13)). EI-MS: 276 (M^+), 231 (100), 230. HR-EI-MS: 276.1720 (M^+, $C_{17}H_{24}O_3^+$; calc. 276.1726).

*(1a*R*,4*S*,4a*S*,5*S*,5a*S*,6*S*,8a*S*,9a*S*)-Octahydro-4,4a,6-trimethyl-7-oxo-5a,8a-epoxyoxireno[8,8a]naphtho[2,3-*b*]furan-5(9*H*)-yl 2-Methylpropanoate* (**15**). $[\alpha]_D^{18.8} = -58.7$ ($c = 0.07$, EtOH). FT-IR: 1800, 1730. ^{1}H-NMR (600 MHz, C_6D_6): 0.94 (*d*, $J = 7.0$, H–C(3′)); 0.95 (*d*, $J = 7.0$, H–C(4′)); 0.97 (*d*, $J = 7.2$, Me(15)); 1.02–1.08 (*m*, H_b–C(3)); 1.20 (*d*, $J = 7.0$, Me(13)); 1.31 (*s*, Me(14)); 1.31–1.39 (*m*, H–C(4)); 1.35–1.42 (*m*, H_b–C(2)); 1.54–1.62 (*m*, H_a–C(3)); 1.55 (*d*, $J = 15.4$, H_b–C(9)); 1.70 (*ddd*, $J = 14.7$, 12.0, 6.4, H_a–C(2)); 2.24 (*sept*, $J = 7.0$, H–C(2′)); 2.43 (*q*, $J = 7.0$, H–C(11)); 2.48 (*d*, $J = 4.9$, H–C(1)); 2.70 (*d*, $J = 15.4$, H_a–C(9)); 5.60 (*s*, H–C(6)). ^{13}C-NMR (150 MHz, C_6D_6): 11.5 (C(13)); 15.2 (C(15)); 15.4 (C(14)); 18.7 (C(3′)); 19.0 (C(4′)); 19.8 (C(2)); 23.6 (C(3)); 30.8 (C(9)); 33.3 (C(4)); 34.1 (C(2′)); 40.3 (C(5)); 42.7 (C(11)); 60.8 (C(10)); 62.9 (C(1)); 64.5 (C(7)); 70.6 (C(6)); 85.7 (C(8)); 175.1 (C(12)); 176.3 (C(1′)). CI-MS: 351 ($[M+H]^+$), 307, 281, 263, 235 (100), 207, 71. HR-CI-MS: 351.1803 ($[M+H]^+$, $C_{19}H_{27}O_6^+$; calc. 351.1807).

*(2*S*,8*S*,8a*R*)-1,2,3,4,6,7,8,8a-Octahydro-8,8a-dimethyl-2-(prop-1-en-2-yl)naphthalen-2-ol* (**17**). $[\alpha]_D^{23} = -52.3$ ($c = 0.06$, EtOH). FT-IR: 3300, 1650. ^{1}H-NMR (600 MHz, C_6D_6): 5.33 (*dd*, $J = 4.9$, 2.5, H–C(1)); 4.97 (*s*, H–C(12)); 4.81 (*t*-like, $J = 1.1$, H–C(12)); 2.40 (br. *t*, $J = 14$, H_β–C(9)); 2.33 (*dd*, $J = 13.5$, 2.5, H_β–C(6)); 2.12–2.08 (*m*, H_β–C(8)); 2.02–1.97 (*m*, H_α–C(9)); 1.99–1.95 (*m*, H–C(2)); 1.89–1.84 (*m*, H–C(2)); 1.83 (*d*, $J = 0.5$, Me(13)); 1.53 (*ddd*, $J = 13.5$, 13.5, 4.9, H_α–C(8)); 1.42–1.38 (*m*, H_α–C(4)); 1.38–1.33 (*m*, H–C(3)); 1.31 (*d*, $J = 13.5$, H_α–C(6)); 1.27–1.24 (*m*, H–C(3)); 0.91 (*s*, Me(14)); 0.81 (*d*, $J = 6.6$, Me(15)). ^{13}C-NMR (150 MHz, C_6D_6): 149.3 (C(11)); 143.0 (C(10)); 120.4 (C(1)); 110.9 (C(12)); 73.1 (C(7)); 47.8 (C(6)); 41.6 (C(4)); 38.3 (C(8)); 38.2 (C(5)); 31.1 (C(9)); 26.9 (C(3)); 26.1 (C(2)); 19.4 (C(13)); 18.0 (C(14)); 15.9 (C(15)). CI-MS: 221 ($[M+H]^+$), 219, 203 (100). HR-CI-MS: 221.1900 ($[M+H]^+$, $C_{15}H_{25}O^+$; calc. 221.1905).

DNA Analysis. See our previous reports [7][9].

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Received May 29, 2009