Chemical and Genetic Diversity of *Ligularia latihastata* and *Ligularia villosa* in Yunnan Province of China

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The chemical constituents of the root extracts and the nucleotide sequences of the *atpB-rbcL* intergenic region of *Ligularia latihastata* and *L. villosa*, collected in northwestern Yunnan Province, were studied. In the twelve collected samples of *L. latihastata*, two major benzofurans, 5,6-dimethoxy-2-(1-methylethenyl)-1-benzofuran (**1**) and euparin (**2**) were detected as major components. The minor compound (2*R**,3*S**)-5-acetyl-2,3-dihydro-6-hydroxy-2-(1-methylethenyl)-1-benzofuran-3-yl (2*Z*)-2-[(acetoxy)methyl]but-2-enoate (**4**) was found to be susceptible to artifact formation upon extraction with EtOH. The intra-specific diversity in chemical composition of the samples was small, but the diversity in the *atpB-rbcL* sequence was fairly large. Compounds **1** and **2** were also found in the three collected samples of *L. villosa*, indicating that the two species are chemically close to each other, in agreement with morphological taxonomy.

Introduction. – *Ligularia* CASS. (Asteraceae) in China is a highly diversified genus, with a rich chemical biodiversity. In addition, some *Ligularia* species are known to exhibit biological activities [1][2]. *L. latihastata* (W. W. SMITH) HAND.-MAZZ. and *L. villosa* (HAND.-MAZZ.) S. W. LIU, both belonging to the section Ligularia, series Speciosae, inhabit the Hengduan Mountains in northwestern Yunnan Province [3][4]. The former grows in grasslands and forest understories of 2500–4000 m in altitude, and the latter grows on alpine meadows at *ca.* 3000 m. The dried roots of *L. latihastata* have been used as a Chinese herbal medicine known as '*Shanziyuan*' ('*San-shion*' in Japanese), which is used against cough and asthma [4]. Despite their importance in traditional medicine, the terpenoid constituents of the roots of *L. latihastata* and *L. villosa* have not been reported yet.

We have been studying the intra-specific diversity in *Ligularia* species by the combination of chemical, genetic, and morphological approaches [5–8]. So far, we found that *L. tongolensis* (FRANCH.) HAND.-MAZZ. [5], *L. pleurocaulis* (FRANCH.) HAND.-MAZZ. [6], *L. virgaurea* (MAXIM.) MATTF. [7], and *L. tsangchanensis* (FRANCH.) HAND.-MAZZ. [8] are diverse, while *L. cymbulifera* (W. W. SMITH) HAND.-MAZZ. is uniform [5]. In these studies, we have chosen the furanoeremophilane

[1]) Corresponding author for general information and chemical aspects of the work. The genetic and taxonomic works were performed under the supervisions of *R. H.* and *X. G.*, resp.

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composition in the root as a chemical index, since these compounds can be easily detected by *Ehrlich*'s test on thin-layer chromatography (TLC) plates [9]. As a genetic index, DNA base sequences of the *atpB-rbcL* region in the plastid genome and/or the ribosomal internal transcribed spacers (ITSs) have been used. These sequences are non-coding, and variations therein are thought to be neutral to evolution, and useful in reconstructing phylogeny [10].

One aim of the present study was to examine the chemical composition of the root extracts as well as the chemical and genetic diversity in two related species, *L. latihastata* and *L. villosa*, to glean more on the diversity within this genus. Another aim was to examine the relation of *L. latihastata* to a '*San-shion*' sample studied some 30 years ago. One of us (*H. H.*) and co-workers had previously isolated several guaiane sesquiterpenes such as 5,11-expoxyguaiane from the dried roots of a putative '*San-shion*' sample imported from China to Japan as a herbal medicine [2][11]. However, the identity of the plant had been unknown at that time, because the material consisted solely of roots. Besides, not only *L. latihastata*, but also some other *Ligularia* species – including *L. veitchiana* (HEMSL.) GREENM. [4][12], *L. franchetiana* (LEVL.) HAND.-MAZZ. [4], *L. sibirica* (L.) CASS., and *L. hodgsonii* HOOK. [1][2] – are registered as '*Shanziyuan*' or '*San-shion*', and the roots of these plants all look alike [13].

Here, we report that *L. latihastata* and *L. villosa* are close to each other in terms of chemical composition and that the intra-specific diversity in *L. latihastata* is fairly large in the *atpB-rbcL* region, but limited in the chemical constituents.

Results. – Twelve samples (*1–12*) of *L. latihastata* (*Figure* and *Table 1*) were collected in the Lijiang and the Zhongdian areas, in northwestern Yunnan, in August of each of 2003–2006. Three samples of *L. villosa* were also collected (*13–15*), two of which were obtained in the Ninglang area. *L. latihastata* was abundant in the south of Zhongdian (Shangrila) city (*6–10*), and also in the Lijiang area, especially in Sandawan (*1* and *2*).

The roots of each sample were extracted with EtOH, and the compounds therein were subjected to TLC analysis. All the 15 samples of *L. latihastata* and *L. villosa* were negative to *Ehrlich*'s test, which suggested that these two species do not produce furanoeremophilanes. When a mixture of *para*-methoxybenzaldehyde, AcOH, and H_2SO_4 was used as coloring agent, two major spots were detected on TLC for all the samples, with R_f values (on silica-gel plates) of 0.63 and 0.67 in hexane/AcOEt 7:3 as solvent system.

The compounds were isolated, and their structures were determined. Two compounds, 5,6-dimethoxy-2-(1-methylethenyl)-1-benzofuran (**1**; R_f 0.63) [14] and euparin (**2**; R_f 0.67) [14][15] were isolated from the EtOH extract of sample *14*. From the EtOH extract of sample *2*, collected in 2004, a minor component was also isolated, and its structure was deduced to be 1-[(2*R**,3*S**)-3-ethoxy-2,3-dihydro-6-hydroxy-2-(1-methylethenyl)-1-benzofuran-5-yl]ethanone (**3**) according to ^{1}H-, ^{13}C-, and 2D-NMR (COSY, HBMC) spectroscopic analyses. The 2,3-*trans* arrangement on the dihydrofuran ring of **3** was inferred from a *J* value of 2 Hz between the corresponding methine H-atoms [16]. Because some EtO-substituted derivatives had previously been isolated from EtOH extracts as artifacts, the plant was re-harvested at the same place in 2005, and the corresponding AcOEt extract was analyzed. Now, a different minor compound

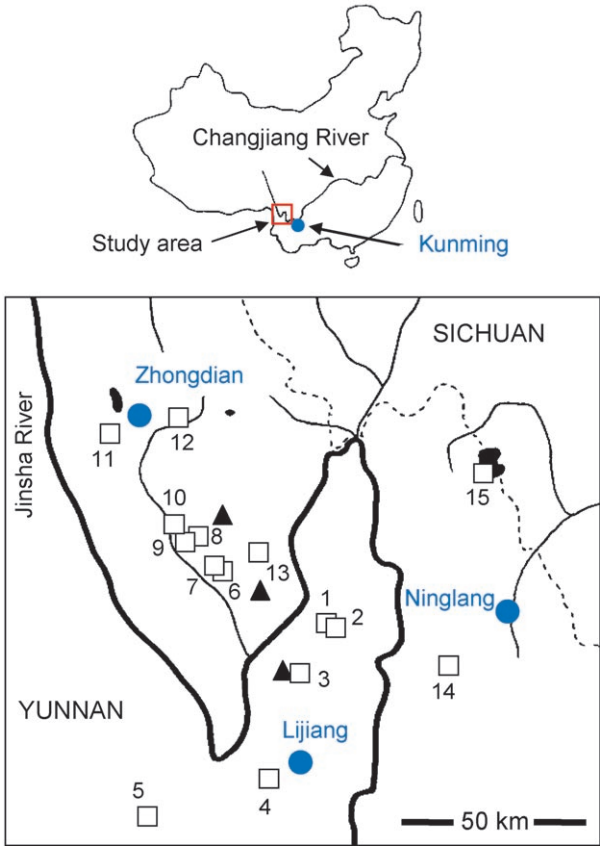


Figure. *Map of sampling site in northwestern Yunnan Province*. Sample locations of *L. latihastata* and *L. villosa* are numbered and indicated as open squares (□); filled triangles (▲) indicate major peaks. For details, see *Table 1*.

was isolated, a known constituent (2*R**,3*S**)-5-acetyl-2,3-dihydro-6-hydroxy-2-(1-methylethenyl)-1-benzofuran-3-yl (2*Z*)-2-[(acetoxy)methyl]but-2-enoate (**4**) [16], instead of the ethyl ether **3**, together with the two major components **1** and **2**. Hence, compound **3** was an artifact, presumably generated from **4** during extraction with EtOH.

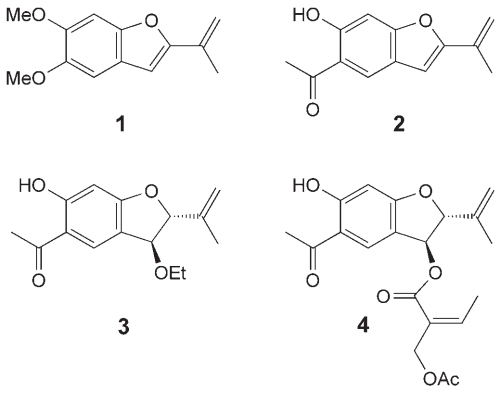
We also attempted to isolate some guaiane derivatives from the root extracts. Since 5,11-expoxyguaiane and related compounds are highly volatile, precautions were taken to prevent their evaporation during isolation. However, no guaiane compounds were found in the samples of the present study.

The intra-specific diversity in chemical composition was examined by GC/MS analysis. The two major components **1** and **2** were detected in all the *L. latihastata* and *L. villosa* samples. The compound with the shorter retention time showed the M^+ peak at *m*/*z* 218 (**1**), and the other at *m*/*z* 216 (**2**). Their ratio was found to vary considerably (see *Table 1*). However, there appears to be no correlation between this ratio and the sampling location.

Table 1. *Collection Details and Ratio of Major Compounds from Samples of* L. latihastata *and* L. villosa

Sample No.	Collection year[a])	Location[b])	Altitude [m]	**1**/**2**[c])
L. latihastata:				
1	2004	Sandawan	3300	56 : 44
2	2004, 2005	Sandawan	3300	55 : 45
3	2004	Yulongxueshan	3200	51 : 49
4	2006	Lijiang	2500	6 : 94
5	2003	Jianchuan	2300	30 : 70
6	2004	Tuguancun	3000	9 : 91
7	2006	Tuguancun	3000	23 : 77
8	2004	Dabaoshan	3300	42 : 58
9	2006	Heping	3200	18 : 82
10	2006	Xiaozhongdian	3200	15 : 85
11	2004	Shikashan	3400	18 : 82
12	2006	Tianshengqiao	3300	32 : 68
L. villosa:				
13	2005	Baishuitai	2500	11 : 89
14	2004	Ninglang	2900	17 : 83
15	2004	Luguhu	2700	19 : 81

[a]) All samples were collected in August. [b]) See *Figure.* [c]) Compound ratio, as determined by GC/MS.



The nucleotide sequence of the *atpB-rbcL* region was next determined, and the results are summarized in *Table 2*. Variation was found at seven places. The samples appear to be grouped into two. Most of them (*1–3*, *5*, *7*, *9*, *11,* and *12*) had T residues at positions 245 and 301, as well as a C residue at position 469. The other samples (*4*, *6*, *8*, and *10*) had G, C, and A moieties at the respective positions. However, there appears to be no correlation among the ratio of the major compounds, the sequence, and the sampling location.

Discussion. – The extracts of *L. latihastata* and *L. villosa* were negative to *Ehrlich*'s test, and the two benzofurans **1** and **2** were isolated as the major components. These compounds have been isolated from *L. stenocephala* MATSUM. et KOIDZ. collected in

Table 2. atpB-rbcL *Sequence of Samples of* L. latihastata *and* L. villosa

Species	Sample	Base position[a])							
		28	245	301	409	469	N_T[b])	N_A[c])	Other
L. latihastata	*1*	G	T	T	A	C	11	9	
	2	G	T	T	A	C	11	9	
	3	G	T	T	A	C	11	9	
	4	G	G	C	T	A	9	9	
	5	G	T	T	A	C	11	9	
	6	G	G	C	G	A	9	9	
	7	G	T	T	A	C	11	9	
	8	G	G	C	T	A	9	9	
	9	G	T	T	A	C	11	9	
	10	G	G	C	T	A	9	9	
	11	G	T	T	A	C	10	10	G[d])
	12	G	T	T	A	C	11	9	
L. villosa	*13*	G	T	T	A	C	10	10	G[d])
	14	G	T	T	A	C	10	9	
	15	G	T	T	A	C	10	9	
Reference[e])		A/G	G	C	A	A	9	9–12	

[a]) Base numbering according to the published sequence of *L. tongolensis* [5]; the sequence at the other positions was the same as that reported for *L. tongolensis.* [b]) Number of thymines at a stretch around the 390th residue. [c]) Number of adenines at a stretch around the 510th residue. [d]) Insertion of a guanine between the 515th (A) and the 516th (T) residues. [e]) *L. cymbulifera*, *L. tongolensis*, *L. atroviolacea*, and *L. pleurocaulis* [5–7].

Japan [14][17] as well as in China [18]. Compound **2** has also been isolated from *L. intermedia* NAKAI [19] and *L. speciosa* FISCH. et MEY. [20], the latter corresponding to *L. fischeri* TURCZ. [3]. Related benzofuran derivatives, such as the acetyl-substituted analogue of **1** in which the isopropenyl group is replaced, have been isolated from *L. przewalskii* (MAXIM.) DIELS [21]. These species belong to the section Ligularia, series Speciosae, to which both *L. latihastata* and *L. villosa* belong to. Thus, the terpenoid composition indicates that the mentioned six Speciosae species are chemically close to one another, in agreement with their taxonomy based on morphology [3].

The intra-specific diversity in *L. latihastata* was found to be large in the *atpB-rbcL* sequence, when compared with the *Ligularia* species we have reported on. In them, variation has been limited to the base at position 28 and the number of A residues in a stretch (*Table 2*) [5–7]. Because the variation in *L. latihastata* is fairly large and because no intermediate between the two types of the sequence is found, the diversity is likely to result from hybridization. Actually, the majority sequence type, with 245T, 301T, 409T, and 469C, has been found in other *Ligularia* species in the section Ligularia, and in *L. dictyoneura* [22]. Further molecular, cytological, and morphological studies are necessary to determine whether *L. latihastata* is a product of recent hybridization speciation or if the multiple *atpB-rbcL* types have resulted from introgression [23].

These variations in the *atpB-rbcL* region are likely to be functionally neutral. It has been shown that three promoters in the region and the leader sequence of the *rbcL* gene are well-conserved among dicotyledons [24]. Inspection of the *Ligularia atpB-*

rbcL sequence confirmed the conservation of the proximal *atpB* promoter, the *rbcL* promoter, and the *rbcL* leader sequence. However, the cpt1 part of the distal *atpB* promoter was missing, although its cpt2 part and adjacent eleven base pairs upstream were conserved. This phenomenon appears to be common in Asteraceae, since all the other complete *atpB-rbcL* sequences of Asteraceae species in the current data base [*Jacobaea uniflora* (Asteroidae; Senecianae), *Helianthus annuus* (Asteroidae; Heliantheae), *Lactuca sativa* (Cichorioideae; Cichorieae)] also lacked the cpt1 element. Thus, some alteration of the functioning of the distal *atpB* promoter is likely in Asteraceae. In any case, none of the variations listed in *Table 2* fell in crucially conserved sequences, indicating that they are likely to be functionally neutral.

In contrast to the large diversity in the *atpB-rbcL* sequence, the diversity in chemical composition was observed only in the ratio of the two major constituents **1** and **2**, which are similar to each other. Namely, root components of all *L. latihastata* samples are roughly the same. The conservation of the chemicals, face-to-face with the diversity in the DNA sequence, suggests that the compounds have selective advantage. Although the precise biochemical role(s) of these compounds has yet to be established, the wide occurrence of **1** and **2**, as well as of related benzofuran derivatives in Speciosae species, seems to indicate that these compounds are biologically important.

The '*San-shion*' sample collected and analyzed some 30 years ago, as mentioned above, contained guaiane derivatives and furanoligularinone, the latter of which is positive to *Ehrlich*'s test [6][11]. However, neither guaiane nor any *Ehrlich*-positive compounds were found in the samples of the present study. Therefore, the previously analyzed sample was not *L. latihastata* from Yunnan Province.

Conclusions. – Two benzofuran derivatives were found to be present as the major components in the root extract in twelve samples of *L. latihastata*, and in three samples of *L. villosa*, collected in northwestern Yunnan Province. The intra-specific diversity in chemical composition of *L. latihastata* was found to be small. In contrast, the diversity in the plastid *atpB-rbcL* sequence was large enough to suggest hybridization. *L. villosa* was found to be close to *L. latihastata* both chemically and genetically.

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Experimental Part

General. Column chromatography (CC): silica gel (*Merck Kieselgel 60* or *Wakogel C-200*). TLC: silica gel (*Merck Kieselgel 60* F_{254}, layer thickness 0.2 mm). HPLC: *JASCO Gulliver* system, with *PU-1580* pump and *UV-975* UV/VIS detector, and silica-gel *YMC Pack SIL A-023* (10 × 250 mm) column. The known compounds **1**, **2**, and **4** were identified by comparison of the corresponding physico-chemical and spectroscopic data with those reported in the literature [14–16]. *Ehrlich*'s TLC test and the determination of DNA sequences were performed as published previously [5–7]. Melting points (m.p.) were determined on a *Yanaco MP-J3* apparatus; uncorrected. UV Spectra were recorded on a *Pharmacia*

Biotech Ultospec-3000 apparatus; λ_{max} (log ε) in nm. Optical rotations were determined on a *Horiba SEPA-300* digital polarimeter. IR Spectra were recorded on a *Horiba FT-720* spectrophotometer; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra were recorded on *JEOL GSX-400* (400/100 MHz, resp.) or *Bruker DRX-500* (500/125 MHz, resp.) spectrometers; in $CDCl_3$ soln.; δ in ppm rel. to residual $CHCl_3$ (δ(H) 7.24) or $CDCl_3$ (δ(C) 77.0); *J* in Hz. Both low- and high-resolution (HR) FAB mass spectra were obtained on a *JEOL JMS-GCmate II (MS-BU25)* apparatus; in *m*/*z*. GC/MS Analyses were recorded on a *Shimadzu GCMS-QP5050* mass spectrometer operated in EI mode, using a *DBI* cap. column (0.25 mm × 30 m, 0.25 μm).

Plant Material. Samples of *L. latihastata* and *L. villosa* were collected in 2003–2006, always in August, as described in *Table 1* and the *Figure*. The plants were identified by *X. G.*

Extraction, Purification, and GC/MS Analysis. The root samples of *L. latihastata* and *L. villosa* were dried and then extracted at r.t. with EtOH (samples collected in 2003 and 2004) or with AcOEt (2005 and 2006). Solid materials were removed by filtration, and the solvent was evaporated to afford an oily residue. The residue was taken up in AcOEt, and the resulting mixture was pre-purified by filtration over silica gel, followed by GC/MS analysis (He as carrier gas at a flow rate of 1.7 ml/min; temp. program: 50° (2 min) to 200° (15 min)).

5,6-Dimethoxy-2-(1-methylethenyl)-1-benzofuran (**1**) *and Euparin* (*=1-[6-Hydroxy-2-(1-methylethenyl)-1-benzofuran-5-yl]ethanone*; **2**). The root extract of sample *17* (2.318 g) was dissolved in AcOEt, and the insoluble materials were removed by filtration. The solvent was evaporated, and the residual oil was subjected to CC (30 g SiO_2; hexane/AcOEt gradient). Two major components were eluted with hexane/AcOEt 9:1, and further separated by repeated CC to afford **2** (less polar; 49.7 mg) and **1** (more polar; 185.1 mg).

*1-[(2*R**,3*S**)-3-Ethoxy-2,3-dihydro-6-hydroxy-2-(1-methylethenyl)-1-benzofuran-5-yl]ethanone* (**3**; artifact). The EtOH extract of sample *2* collected in 2004 (160.2 mg) was separated by CC (30 g SiO_2; benzene/Et_2O gradient). The fraction eluted with benzene/Et_2O 9:1 was further purified by CC to afford **3** (6.5 mg). Colorless needles. M.p. 62–65° (MeOH). UV (MeOH): 219 (4.3), 237 (4.1), 277 (4.1), 318 (3.8). $[\alpha]_D^{24} = -20.1$ ($c = 0.95$, $CHCl_3$). IR (film): 1640, 1625, 1487, 1372, 1331, 1262, 1137, 1085. ^1H-NMR ($CDCl_3$): 1.27 (*t*, *J* = 7.0, 3 H); 1.71 (br. *s*, 3 H); 2.58 (*s*, 3 H); 3.62 (*q*, *J* = 7.0, 2 H); 4.77 (*d*, *J* = 2.0, 1 H); 4.94 (br. *s*, 1 H); 5.05 (br. *s*, 1 H); 5.09 (br. *d*, *J* ≈ 2, 1 H); 6.44 (*s*, 1 H); 7.73 (*s*, 1 H); 13.01 (*s*, 1 H). ^{13}C-NMR ($CDCl_3$): 15.35; 17.47; 26.27; 63.62; 81.86; 92.73; 98.58; 113.22; 114.36; 118.35; 129.16; 141.25; 167.01; 167.09; 202.31. FAB-MS (pos.; glycerol): 263 ($[M+H]^+$), 217 ($[M - EtO]^+$). HR-FAB-MS (pos.; glycerol): 263.1286 ($[M+H]^+$, $C_{15}H_{19}O_4^+$; calc. 263.1283).

*(2*R**,3*S**)-5-Acetyl-2,3-dihydro-6-hydroxy-2-(1-methylethenyl)-1-benzofuran-3-yl (2*Z*)-2-[(Acetoxy)methyl]but-2-enoate* (**4**). The AcOEt extract of sample *2* collected in 2005 (270.0 mg) was separated by CC (15 g SiO_2; hexane/AcOEt gradient) to give seven fractions (Fr.). *Fr. 7* was further subjected to HPLC (hexane/AcOEt 7:3, 2 ml/min) to afford **4** (6.5 mg). Similarly, HPLC separation of *Fr. 4* and *Fr. 5* afforded **1** (8.7 mg) and **2** (6.7 mg), resp.

REFERENCES

[1] Institute of Materia Medica, Chinese Academy of Medical Sciences, 'Zhongyaozhi', People's Medical Publishing House, Beijing, 1961, Part 1, pp. 485–489.
[2] M. Okazaki, Y. Ishizaki, Y. Tanahashi, H. Hirota, T. Takahashi, *Yakugaku Zasshi* **1974**, *94*, 881.
[3] S.-W. Liu, 'Flora Reipublicae Popularis Sinicae', Science Press, Beijing, 1989, Vol. 77.
[4] T.-L. Min, 'Flora Yunnanica', Science Press, Beijing, 2004, Vol. 13.
[5] R. Hanai, X. Gong, M. Tori, S. Kondo, K. Otose, Y. Okamoto, T. Nishihama, A. Murota, Y. Shen, S. Wu, C. Kuroda, *Bull. Chem. Soc. Jpn.* **2005**, *78*, 1302.
[6] H. Nagano, Y. Iwazaki, X. Gong, Y. Shen, C. Kuroda, R. Hanai, *Bull. Chem. Soc. Jpn.* **2006**, *79*, 300.
[7] M. Tori, K. Honda, H. Nakamizo, Y. Okamoto, M. Sakaoku, S. Takaoka, X. Gong, Y. Shen, C. Kuroda, R. Hanai, *Tetrahedron* **2006**, *62*, 4988.
[8] A. Torihata, R. Hanai, X. Gong, Y. Shen, C. Kuroda, *Chem. Biodiv.* **2007**, *4*, 500.

[9] C. Kuroda, T. Ueshino, H. Nagano, *Bull. Chem. Soc. Jpn.* **2004**, *77*, 1737; C. Kuroda, E. Nishio, *Nat. Prod. Commun.* **1997**, *2*, 581.
[10] V. Savolainen, J.-F. Manen, E. Douzery, R. Spichiger, *Mol. Phylogenet. Evol.* **1994**, *3*, 27; D. E. Soltis, P. S. Soltis, in 'Molecular Systematics of Plants. II. DNA Sequencing,' Eds. P. S. Soltis, D. E. Soltis, J. J. Doyle, Kluwer Academic Publishers, Boston, 1998, pp. 1–42.
[11] H. Hirota, Y. Tanahashi, T. Takahashi, *Tetrahedron Lett.* **1975**, 4579; H. Hirota, Y. Tanahashi, T. Takahashi, *Bull. Chem. Soc. Jpn.* **1980**, *53*, 785.
[12] 'Colored Icons of Chinese Medicine', Ed. H.-S. Lo, World Publishing, Guangzhou, 2002, Vol. 6.
[13] F. Patil, G. Ourisson, Y. Tanahashi, M. Wada, T. Takahashi, *Bull. Soc. Chim. Fr.* **1968**, 1047.
[14] T. Murae, Y. Tanahashi, T. Takahashi, *Tetrahedron* **1967**, *24*, 2177.
[15] B. Kamthong, A. Robertson, *J. Chem. Soc.* **1939**, 925; B. Kamthong, A. Robertson, *J. Chem. Soc.* **1939**, 933.
[16] F. Bohlmann, C. Zdero, M. Grenz, *Chem. Ber.* **1977**, *110*, 1034.
[17] K. Toyoda, Y. Yaoita, M. Kikuchi, *Chem. Pharm. Bull.* **2005**, *53*, 1555.
[18] F.-L. Yan, A.-X. Wang, Z.-J. Jia, *Pharmazie* **2005**, *60*, 155.
[19] B. Ma, K. Gao, Y.-P. Shi, Z.-J. Jia, *Phytochemistry* **1997**, *46*, 915.
[20] F. Bohlmann, U. Fritz, *Phytochemistry* **1980**, *19*, 2471.
[21] Z. Jia, Y. Zhao, *J. Nat. Prod.* **1994**, *57*, 146.
[22] H. Nagano, Y. Iwazaki, M. Matsushima, M. Sato, X. Gong, Y. Shen, H. Hirota, C. Kuroda, R. Hanai, *Chem. Biodiv.* **2007**, in press; H. Nagano, Y. Iwazaki, M. Matsushima, R. Hanai, C. Kuroda, Y. Shen, X. Gong, 'Proceedings of the 50th Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics', Yokohama, Japan, 2006, pp. 346–348.
[23] L. H. Rieseberg, D. E. Soltis, *Evol. Trends Plants* **1992**, *5*, 65; M. J. Hegarty, S. J. Hiscock, *New Cytologist* **2005**, *165*, 411.
[24] J.-F. Manen, V. Savolainen, P. Simon, *J. Mol. Evol.* **1994**, *38*, 577.

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