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Two New Furanoeremophilane Sesquiterpenoids from *Ligularia oligonema*

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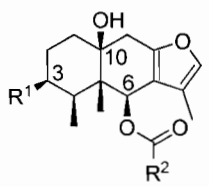
From the roots of *Ligularia oligonema* and an unidentified sample of *Ligularia* collected in Yunnan Province of China, two new sesquiterpenoids, 3β-acetoxy-6β-(2-methylbutyryloxy)furanoeremophilan-10β-ol and 3β-acetoxy-6β-isobutyryloxy-furanoeremophilan-10β-ol, were isolated. DNA sequencing showed that the unidentified sample was likely to be *L. oligonema*. In addition, the DNA data suggested that the chemical evolution among *L. oligonema* and related *Ligularia* species is rather complex.

Keywords: *Ligularia oligonema*, Asteraceae, Sesquiterpenoid, Furanoeremophilane, Isolation, Structure.

Ligularia (Asteraceae) is a genus consisting of over 100 species. The genus is highly diversified in the Hengduan Mountains of China [1-3], especially in Yunnan and Sichuan Provinces. We have been studying the chemical evolution in the genus by examining inter- and intra-specific diversity in root chemicals and nucleotide sequences of evolutionarily neutral DNA regions. To date, we have revealed intra-specific diversity in a number of species in Yunnan and Sichuan Provinces. For example, *L. pleurocaulis* [4], *L. virgaurea* [5], *L. vellerea* [6] and *L. tongolensis* [7] were moderately diverse, *L. dictyoneura* [8], *L. kanaitzensis* [9] and *L. subspicata* [10] were highly diverse, and *L. cymbulifera* [7] and *L. cyathiceps* [11] were uniform.

As part of our effort to elucidate the mechanism(s) of chemical diversification in *Ligularia* in the Hengduan Mountains of China, we carried out chemical analysis of *L. oligonema*, which was collected in Laojunshan (altitude: 2900 m), Yunnan Province, China in 2008. To the best of our knowledge, there has been no report of the chemical constituents of the species.



1 R^1 = OAc, R^2 = CHMeEt-(*S*)
1' R^1 = OAc, R^2 = CHMeEt-(*R*)
2 R^1 = OAc, R^2 = $CH(Me)_2$
3 R^1 = OAc, R^2 = CMe=CHMe-(*Z*)
4 R^1 = H, R^2 = CHMeEt-(*S*)
4' R^1 = H, R^2 = CHMeEt-(*R*)

Figure 1: Structures of **1**-**4**, **1'** and **4'**.

The EtOH extract of fresh root of the *L. oligonema* sample showed a very intense Ehrlich-positive spot on TLC (R_f: 0.4 (*n*-hexane-EtOAc 7:3)), as well as some minor Ehrlich-positive spots. A mixture of new furanoeremophilane derivatives (**1** and **2**) was obtained from the EtOH extract of dried root in 25% yield (**1**:**2** = 62:38) (Figure 1). The mixture was partly separated by careful and repeated column chromatography (vide infra). Attempts to separate the minor components were unsuccessful due to their complexity and paucity.

The structures of **1** and **2** were determined on the basis of their MS, ^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY and NOESY spectra and through preparation of **1** from a known furanoeremophilane derivative (**3**) [8] by catalytic hydrogenation. The molecular formula of compound **1**, an oil, was determined to be $C_{22}H_{32}O_6$ by high-resolution EIMS (*m/z* 392.2200). ^{1}H NMR [δ 0.88 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 1.16 (3H, d, *J* = 7.3 Hz, $CHCH_3$), 2.08 (3H, s, Ac), 2.36 (1H, m, CH), 4.97 (1H, br s, H-3), 6.16 (1H, s, H-6)], and ^{13}C NMR (δ 74.9, 72.6, 69.5) spectra indicated the presence of a hydroxy, an acetoxy and a 2-methylbutyryloxy group on the furanoeremophilane skeleton [12]. The presence of the 2-methylbutyryl-oxy group at C-6 was supported by the observation of a retro-Diels-Alder fragment (*m/z* 208) in the mass spectrum of **1** (Figure 2) [13]. A pair of doublet signals of 9-H at δ 2.75 (1H, d, *J* = 18.1 Hz) and 3.13 (1H, br d, *J* = 18.1 Hz) indicated the presence of a tertiary hydroxy group at C-10 (δ 74.9). These results suggest that compound **1** is likely to be 3β-acetoxy-6β-(2-methylbutyryloxy)furanoeremophilan-10β-ol. The spectral data of compound **1** were identical with those of 3β-acetoxy-6β-angeloyloxyfuranoeremophilan-10β-ol (**3**), which has been isolated from *L. dictyoneura* collected in Baishuitai, Yunnan Province, China [8], except for the signals arising from the side chain attached to C-6.

The structure of compound **1** was confirmed by comparing its NMR spectrum with that of transformation products of compound **3**. Compound **3** was hydrogenated over Pd-C to yield a 1:1 diastereomeric mixture of (2'*S*)- and (2'*R*)-3β-acetoxy-6β-(2-methylbutyryloxy)furanoeremophilan-10β-ols (**1** and **1'**). One of the stereoisomers showed an ^{1}H NMR spectrum identical with that of **1** isolated from *L. oligonema*. Tori and co-workers recorded the ^{1}H NMR spectrum of 6β-(2'*S*)-2-(methylbutyryl-oxy)furanoeremophilane-10β-ol (**4**) and (2'*R*)-epimer **4'** in C_6D_6 and found that the methyl group at C-2' in **4** resonated at lower field, compared with that of **4'** [9]. The doublet signal of the methyl group at C-2' in **1** in C_6D_6 resonated at lower field in comparison with that of **1'** (δ 1.00 for **1** and δ 0.96 for **1'**). Thus, the absolute configuration of 2-methylbutyryl group in **1** was putatively judged to be *S*.

The molecular formula of compound **2**, an oil, was determined to be $C_{21}H_{30}O_6$ by high-resolution EIMS (*m/z* 378.2026). The ^{1}H NMR spectral data of **2** were identical with those of **1** except for the signals arising from the side chain attached to C-6. It showed the presence of an isobutyryloxy group [δ 2.56 (1H, septet, *J*=6.8 Hz), 1.19 (3H, d, *J*=6.8 Hz), 1.16 (3H, d, *J*=7.3 Hz)] [14] instead of the 2-methylbutyryloxy group

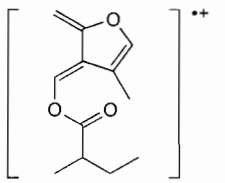


Figure 2: retro-Diels-Alder fragment of **1**

present in **1**. The mass spectrum also showed a fragment peak at *m/z* 71 arising from an isobutyryl group. COSY and NOESY spectra were also in agreement with the structure of **2**.

Compounds **1** and **2** were also isolated from another *Ligularia* sample collected at a higher elevation in Laojunshan (altitude: 3500 m) in 2005. Careful and repeated column chromatography of its EtOH extract gave compounds **1** and **2** in 0.5 and 1.4% yields, respectively (vide supra). Because the plant was still too small to carry any flowers, its identity was unknown. However, the plant was very likely to be *L. oligonema*, because the DNA sequence of the ITS1-5.8S-ITS2 region of the ribosomal RNA gene, which is quite variable and has been used for phylogenetic analyses [15], was essentially the same in the *L. oligonema* sample and the unidentified sample. The difference was only at the 2nd base site in ITS1, which was C in *L. oligonema* and a Y(=C+T) in the unidentified sample. In addition, the sequence of the plastid *atpB-rbcL* intergenic region was exactly the same.

L. oligonema belongs to the section Corymbosae series Retusae [1]. The plants in this series for which chemical analysis has been carried out include *L. duciformis* [16], *L. nelumbifolia* [17], and *L. franchetiana* [18]. *L. franchetiana* was found to produce furanoeremohilanes, while the other two did not. In this regard, *L. oligonema* is more similar to *L. franchetiana*. However, when the ITS1-5.8S-ITS2 sequences of *L. oligonema*, *L. franchetiana* (**AB375311**), *L. duciformis* (**AY458827**) and *L. nelumbifolia* (**DQ272344**) were analyzed with the program suite PHYLIP [19], using *Farfugium japonicum* (**DQ272323**) as an outgroup, *F. japonicum* and *L. oligonema* were found to be separated from the others with a bootstrap value of 93%: *L. oligonema* is somewhat separated from the other three *Ligularia* species. These results suggest that chemical evolution in this series is complex.

Experimental

Column chromatography was carried out on silica gel (Kanto silica gel 60 N (spherical neutral)). Merck Kieselgel 60 F_{254} (layer thickness 0.25 mm) was used for analytical TLC. ^{1}H and ^{13}C NMR spectra were measured on a JEOL AL 400 spectrometer with $CDCl_3$ as the solvent and TMS as an internal standard. Mass

spectra (MS) (EI^+), including high-resolution ones, were obtained on a JEOL JMS-700 MStation.

Plant material: Samples of *L. oligonema* and the unidentified species were collected in August 2008 and 2005, respectively. Each plant was identified by Xun Gong, one of the authors, and voucher specimens, No. 0849 (*L. oligonema*) and 0511 (unknown species), were deposited in the Herbarium of Kunming Institute of Botany.

Extraction: The roots of *L. oligonema* and the unidentified species were harvested. For Ehrlich's test, extraction with EtOH was started immediately without drying. Solid plant material was removed after several days, and the solution was subjected to TLC without concentration. For structure determination, the roots were dried (*L. oligonema*: 10 g, unidentified species: 2 g) and extracted with EtOH at room temperature. Oily extracts were obtained by a standard method.

Isolation: Half of the EtOH extract of the roots of *L. oligonema* (114 mg) was subjected to silica gel column chromatography [SiO_2 (6 g); *n*-hexane-EtOAc 12:1] to give a mixture of **1** and **2** (29 mg; **1**:**2** = 62:38). Part of the EtOH extract of the roots of the unidentified plant (82 mg) was subjected to column chromatography [SiO_2 (3 g); *n*-hexane-EtOAc 10:1] to give **1** (less than 1 mg) and a mixture containing **1** and **2** (2.0 mg). The remaining EtOH extract of the unidentified species (129 mg) was subjected to column chromatography [SiO_2 (4 g); *n*-hexane-EtOAc 10:1] to give **1** (0.4 mg) and a mixture containing **1** and **2** (3.2 mg). The mixtures of **1** and **2** were combined and again chromatographed [SiO_2 (1 g); *n*-hexane-EtOAc 10:1] to give a mixture containing **1** (1.3 mg) and **2** (2.3 mg).

3β-Acetoxy-6β-(2-methylbutyryloxy)furanoeremophilan-10β-ol (1)

$[\alpha]_D^{25}$: -21 (*c* 1.4, $CHCl_3$).
^1H NMR ($CDCl_3$, 400 MHz): 0.88 (3H, t, *J*=7.5 Hz, CH_3), 0.98 (3H, d, *J*=6.8 Hz, H-15), 1.16 (3H, d, *J*=7.3 Hz, CH_3), 1.27 (3H, s, H-14), 1.44 (1H, m, CH), 1.67 (1H, m, CH), 1.90 (3H, s, H-13), 2.08 (3H, s, OAc), 2.36 (1H, m, C(=O)CH), 2.75 (1H, d, *J*=18.1 Hz, H-9), 3.13 (1H, br d, *J*=18.1 Hz, H-9), 4.96 (1H, br s, H-3), 5.36 (1H, s, OH), 6.16 (1H, s, H-6), 7.11 (1H, s, H-12).
^{13}C NMR ($CDCl_3$, 100 MHz): 8.1 (C-13), 11.7 (CH_3), 12.0 (br, C-14 or C-15), 12.1 (br, C-15 or C-14), 16.9 (CH_3), 21.4 (CH_3C(=O)), 26.7 (CH_2), 29.0 (C-2), 29.5 (br, C-1), 33.6 (br, C-9), 37.1 (C-4), 41.4 (CH), 45.1 (C-5), 69.5 (C-6), 72.6 (C-3), 74.9 (C-10), 114.9 (C-7), 119.1 (C-11), 138.9 (C-12), 150.8 (br, C-8), 170.3 (C=O), 175.4 (C=O).
MS (EI, 70 eV): *m/z* (%) = 392 (M^+, 25), 290 (91), 212 (87), 208 (29), 197 (100), 85 (82), 43 (34).
HRMS-EI: *m/z* $[M]^+$ calcd for $C_{22}H_{32}O_6$: 392.2199; found: 392.2200.

3β-Acetoxy-6β-isobutyryloxyfuranoeremophilan-10β-ol (2)

$[\alpha]_D^{25}$: -30 (*c* 0.71, $CHCl_3$).
^1H NMR ($CDCl_3$, 400 MHz): 0.99 (3H, d, *J*=7.3 Hz, H-15), 1.16 (3H, d, *J*=7.3 Hz, CH_3), 1.19 (3H, d, *J*=6.8 Hz, CH_3), 1.26 (3H, s, H-14), 1.89 (3H, s, H-13), 2.08 (3H, s, OAc), 2.56 (1H, septet, *J*=6.8 Hz, C(=O)CH), 2.75 (1H, d, *J*=18.1 Hz, H-9), 3.13 (1H, br d, *J*=18.1 Hz, H-9), 4.97 (1H, br s, H-3), 6.16 (1H, s, H-6), 7.11 (1H, br s, H-12).
^{13}C NMR ($CDCl_3$, 100 MHz): 8.1 (C-13), 11.7 (br, C-14 or C-15), 12.0 (br, C-15 or C-14), 19.0 (CH_3), 19.2 (CH_3), 21.4 (CH_3C(=O)), 29.4 (br, C-1), 29.7 (C-2), 34.0 (br, C-9), 34.4 (CH), 37.0 (C-4), 45.2 (C-5), 69.5 (C-6), 72.6 (C-3), 74.9 (C-10), 114.8 (C-7), 119.2 (C-11), 138.9 (C-12), 150.8 (br, C-8), 170.3 (C=O), 175.8 (C=O).
MS(EI, 70 eV): *m/z* (%) = 378 (M^+, 18), 290 (57), 212 (76), 197 (91), 124 (72), 71 (42), 43 (100).
HRMS-EI: *m/z* $[M]^+$ calcd for $C_{21}H_{30}O_6$: 378.2026; found: 378.2026.

Catalytic hydrogenation of 3β-acetoxy-6β-angeloyloxyfuranoeremophilan-10β-ol (3): Compound **3** (6.5 mg) was stirred with Pd-C (6.9 mg) in EtOH (3 mL) under H_2 at r. t. for 8 h. The mixture was filtered and the filtrate concentrated under reduced pressure to give an inseparable mixture of **1** and **1'** quantitatively.

DNA analysis: Purification of DNA and amplification and sequence determination were carried out as described [9], with the following modifications. The ITS1-5.8S-ITS2 region was amplified with LC5 (5' GAGTCATCAGCTCGCGTTGA 3') and LC6 (5' GTCCGTCGCTGAGGACGCTT 3') and with an annealing temperature of 56°C. Sequencing of the region was carried out with LC1 (5' CCGGTGAAGTGTTAGGATCG 3'), LC2 (5' GACTCTCGGCAACGGATATC 3'), LC3 (5' GGGCGCAACTTGCGTTCAAA 3') and LC4 (5' CTTCTCCAGACTACAATTCG 3'). The *atpB-rbcL* intergenic region was amplified with La (5' AGCTACATCTAGTACCGGACC 3') and ar (5' GGTTGAGGAGTTACTCGAAATGCTGCCAAGAT ATC 3') and with an annealing temperature of 56°C. Sequencing of the region was carried out with La, ar, and aa2 (5' ACCAATGATTTGGGCGATACGCCCCAG 3').

The sequences of the two regions of a *L. oligonema* sample, which was collected at the same place as the

voucher 0849 in 2005, were deposited in the database (accession **AB523364** and **AB523847**). The sequences of both regions were the same in the two samples, except for the 119th site of ITS2 being C in the 0849 sample instead of Y in the database sequence.

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References

[1] Liu SW. (**1989**) *Flora Republicae Popularis Sinicae*. Science Press, Beijing, Vol. *77*.

[2] Min TL. (**2004**) *Flora Yunnanic*a. Science Press, Beijing, Vol. *13*.

[3] Liu SW, Deng DS, Liu JQ. (**1994**) The origin, evolution and distribution of *Ligularia* Cass. (Compositae). *Acta Phytotaxonomica Sinica*, ***32***, 514-524.

[4] Nagano H, Iwazaki Y, Gong X, Shen Y, Kuroda C, Hanai R. (**2006**) Chemical and genetic differentiation of *Ligularia pleurocaulis* in northwestern Yunnan and southwestern Sichuan Provinces of China. *Bulletin of the Chemical Society of Japan*, ***79***, 300-304.

[5] Tori M, Honda K, Nakamizo H, Okamoto Y, Sakaoku M, Takaoka S, Gong X, Shen Y, Kuroda C, Hanai R. (**2006**) Chemical constituents of *Ligularia virgaurea* and its diversity in southwestern Sichuan of China. *Tetrahedron*, ***62***, 4988-4995.

[6] Tori M, Nakamizo H, Mihara K, Sato M, Okamoto Y, Nakashima K, Tanaka M, Saito Y, Sono M, Gong X, Shen Y, Hanai R, Kuroda C. (**2008**) Chemical and genetic diversity of *Ligularia vellerea* in Yunnan, China. *Phytochemistry*, ***69***, 1158-1165.

[7] Hanai R, Gong X, Tori M, Kondo S, Otose K, Okamoto Y, Nishihama T, Murota A, Shen Y, Wu S, Kuroda C. (**2005**) Chemical and genetic study of *Ligularia tongolensis*, *Ligularia cymbulifera*, and *Ligularia atroviolacea* in the Hengduan Mountains of China. *Bulletin of the Chemical Society of Japan*, ***78***, 1302-1308.

[8] Nagano H, Iwazaki Y, Matsushima M, Sato M, Gong X, Shen Y, Hirota H, Kuroda C, Hanai R. (**2007**) High diversity of *Ligularia dictyoneura* in chemical composition and DNA sequence. *Chemistry and Biodiversity*, ***4***, 2874-2888.

[9] Tori M, Watanabe A, Matsuo S, Okamoto Y, Tachikawa K, Takaoka S, Gong X, Kuroda C, Hanai R. (**2008**) Diversity of *Ligularia kanaitzensis* in sesquiterpenoid composition and neutral DNA sequences. *Tetrahedron*, ***64***, 4486-4495.

[10] Tori M, Okamoto Y, Tachikawa K, Mihara K, Watanabe A, Sakaoku M, Takaoka S, Tanaka M, Gong X, Kuroda C, Hattori M, Hanai R. (**2008**) Isolation of new eremophilane-type sesquiterpenoids, subspicatins A–D and subspicatolide from *Ligularia subspicata*, and chemical and genetic diversity of the species. *Tetrahedron*, ***64***, 9136-9142.

[11] Nagano H, Torihata A, Matsushima M, Hanai R, Saito Y, Baba M, Tanio Y, Okamoto Y, Takashima Y, Ichihara M, Gong X, Kuroda C, Tori M. (**2009**) Chemical and genetic study of *Ligularia cyathiceps* in Yunnan Province of China. *Helvetica Chimica Acta*, ***92***, 2071-2081.

[12] Torres P, Ayala J, Grande C, Macías M J, Grande M. (**1998**) Furanoeremophilanes and a bakkenolide from *Senecio auricula* var. *major*. *Phytochemistry*, ***47***, 57-61.

[13] Nagano H, Kuroda C, Moriyama Y, Tsuyuki T, Takahashi T. (**1982**) Mass spectra of furanoeremophilane derivatives. *Bulletin of the Chemical Society of Japan*, ***55***, 1221-1227.

[14] Wang Y, Yuan C-S, Han Y-F, Jia Z-J. (**2003**) Sesquiterpenes from roots of *Ligularia veitchiana*. *Die Pharmazie*, ***58***, 349-352.

[15] Soltis DE, Soltis PS. (**1998**) Choosing an approach and an appropriate gene for phylogenetic analysis. In *Molecular systematic of plants. II. DNA sequencing*. Soltis PS, Soltis DE (Eds). Kluwer Academic Publishers, Boston, Massachusetts, 1-42.

[16] (a) Gao K, Yang L, Jia ZJ. (**1999**) Sesquiterpenes from the roots of *Ligularia duciformis*. *Journal of the Chinese Chemical Society (Taipei)*, ***46***, 619-622; (b) Gao K, Wang WS, Jia ZJ. (**1998**) Coniferyl and sinapyl alcohol derivatives from *Ligularia duciformis*. *Phytochemistry*, ***47***, 269-272.

[17] (a) Zhao Y, Jia Z, Yang L. (**1994**) Sinapyl alcohol derivatives and other constituents from *Ligularia nelumbifolia*. *Phytochemistry*, ***37***, 1149-1152; (b) Peng HR, Shi YP, Jia ZJ. (**1997**) New sesquiterpenes from two *Ligularia* species. *Planta Medica*, ***63***, 335-338.

[18] Tori M, Tanio Y, Okamoto Y, Saito Y, Gong X, Kuroda C, Hanai R. (**2008**) Chemical constituents of *Ligularia franchetiana* collected in Yunnan Province of China. *Heterocycles*, ***75***, 2029-2034.

[19] Felsenstein, J. (**2004**) PHYLIP (Phylogeny Inference Package) version 3.6. *Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, Washington.*