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THREE NEW BISABOLANE-TYPE SESQUITERPENOIDS FROM *CREMANTHODIUM RHODOCEPHALUM* (ASTERACEAE)

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Dedicated to Professor Dr. Ei-ichi Negishi on the occasion of his 77th birthday.

Abstract – From *Cremanthodium rhodocephalum* (Asteraceae) collected in Yunnan Province of China, four bisabolane-type (three of them were new) and one oplopane-type sesquiterpenoids were isolated. Two new hydroperoxides were the first example with the bisabolane-type skeleton. This is the first report about the study on *C. rhodocephalum*.

INTRODUCTION

Plants belonging to the genus *Cremanthodium* grow on high mountains (usually more than 4000 m in elevation) of Tibet and adjacent areas.¹ Because their physical size is too small to collect large amounts,^{2,3} chemical studies on these plants have been limited so far.⁴⁻⁷ Several papers have appeared since 1996,⁴⁻⁹ but those studies were limited to *C. ellisii*,⁴ *C. discoideum*,⁵ *C. potaninii*,⁶ and *C. pleurocaule*.⁷ We reported the chemical constituents of *C. helianthus*⁸ and *C. stenactinium*⁹ in the course of our investigation on *Ligularia* and related plants in the Hengduan Mountains area for more than 10 years.^{10,11} In 2007 we had a chance to collect one sample of *C. rhodocephalum* in Yunnan Province, China. From 146 mg of the EtOAc extract obtained from 6.8 g of dried roots, we isolated five compounds, three of which were new. Here we describe the details of our work.

RESULTS AND DISCUSSION

The EtOAc extract of *C. rhodocephalum* was separated by silica gel column chromatography and HPLC to afford three new compounds **1-3** and two known compounds **4**¹² and **5**.¹³ (Figure 1).

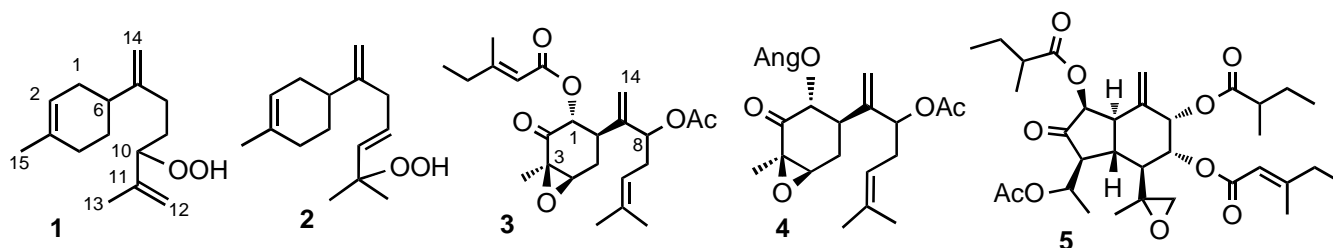


Figure 1. New compounds, **1-3**, and known compounds, **4** and **5**, isolated in this work.

Compound **1** showed a quasi-molecular ion peak at m/z 259 ($M+Na$)⁺ in the FABMS spectrum and the molecular formula was determined to be $C_{15}H_{24}O_2$ by HRMS. This compound indicated a positive reaction with starch-potassium iodide, suggesting the presence of a hydroperoxy group. This was supported by a signal of -OOH at δ 7.10 (1H, s) in ¹H NMR, as well as IR absorption at 3350 cm^{-1} . The ¹H NMR spectrum showed the presence of two doublet methyls (δ 1.606 and 1.609, each $J = 1.6$ Hz), two sets of exomethylenes (δ 4.94, 4.87; 4.85, 4.81), and an oxymethine proton (δ 4.26, br t, $J = 6.6$ Hz). The exomethylene protons (δ 4.85, 4.81) correlated with a methylene at C-8 (HMBC), which connected with a methylene H₂-9. The C-9 was correlated with a methine at δ 4.26 (H-10) (HMBC), which also coupled with H₂-9 (COSY) (Figure 2). The H₃-13 correlated with C-10, C-11, and C-12 (HMBC). Thus an eight-carbon side chain unit was revealed. The exomethylene H-14 also correlated with C-6 (HMBC), and H-6 was found in the proton connectivity H-2/H₂-1/H-6/H₂-5/H₂-4 by the COSY spectrum. The H₃-15 correlated with C-2, C-3, and C-4 (HMBC), and hence a six-carbon unit was connected to a cyclohexane ring connected with the aforementioned eight-carbon unit at C-6 and C-7. Therefore, this compound has a bisabolane-type skeleton with three double bonds and a hydroperoxy group (Figure 2). The structure of **1** was established to be 10-hydroperoxybisabola-2,7(14),11-triene. The relative configuration for compound **1** was not determined.

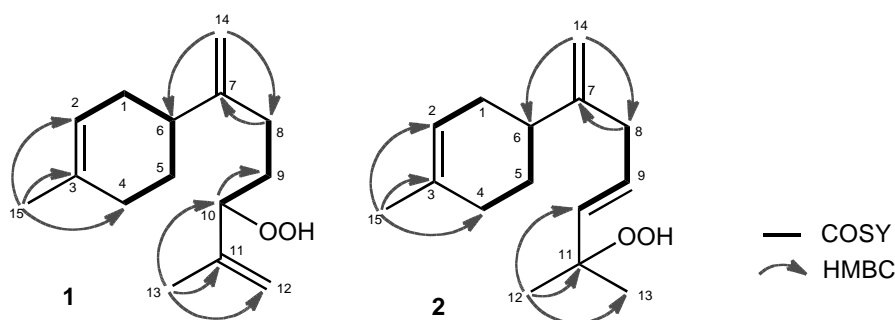


Figure 2. Selected COSY and HMBC correlations of compounds **1** and **2**.

The molecular formula of compound **2** was determined to be $C_{15}H_{24}O_2$ by HRFABMS. The ¹H and ¹³C NMR spectra indicated the presence of three double bonds (one exomethylene, one disubstituted, and one trisubstituted), three singlet methyl groups, and a quaternary carbon bearing an oxygen function. This

compound also indicated a positive reaction with starch-potassium iodide, and the presence of a hydroperoxy group was also suggested by a signal of -OOH at δ 6.73 (1H, s) in ^1H NMR, as well as IR absorption (3400 cm^{-1}). The bisabolane-type skeleton was indicated by COSY and HMBC spectra as shown in Figure 2. The position of the hydroperoxy group was determined by the HMBC correlation between H-12 and C-11 (δ 81.6). The geometry of the double bond at C-9 and 10 was determined to be *E* by the coupling constant ($J = 15.6\text{ Hz}$). Thus, compound **2** was established to be 11-hydroperoxybisabola-2,7(14),9*E*-triene.

Compound **3** showed a quasi-molecular ion peak at m/z 405 and the molecular formula was determined to be $\text{C}_{23}\text{H}_{32}\text{O}_6$ by HRMS. The IR spectrum indicated the presence of several carbonyl groups (1733 and 1716 cm^{-1}). The ^{13}C NMR and HSQC spectra exhibited the presence of six methyl, four methylene, six methine, and seven quaternary carbons. Nine sp^2 carbons including three carbonyl groups were observed. The degree of unsaturation was eight, and hence this compound should be bicyclic. The ^1H NMR spectrum showed the presence of an acetyl methyl (δ 1.73), a triplet methyl, a singlet methyl attached to the carbon bearing an oxygen function, three methyl groups attached to olefinic carbons, an exomethylene, and three oxymethine protons. The COSY spectrum indicated the presence of an ethyl group, and two partial proton connectivity (Figure 3). The HMBC spectrum showed correlations between H-15 and C-2, 3, and 4, between H-14 and C-6, 7, and 8, between H-5 and C-3 and 4, and between H-12 (or 13) and C-10, 11, and 13 (or 12). The bisabolane skeleton with the carbonyl (C-2) and two acyloxy groups (C-1 and 8) was established from these data. The presence of an epoxide ring was determined by the chemical shifts of C-3 (δ 61.5) and C-4 (δ 64.0) as well as bicyclic nature of this compound. The position of an acetate was C-8, because the HMBC correlation between H-8 and the acetyl carbonyl group (δ 169.5) was observed. The other acyl group was determined to be (*E*)-3-methylpent-2-enoate from the HMBC correlations between H-6' and C-2', 3', and 4' and the COSY correlation (Figure 3). The geometry of this acyl group was determined by the NOE between H-2' and H-4' and the position was also determined by the long-range correlation between H-1 (δ 6.16) and C-1' (δ 165.3). The planar structure was established to be 8-acetoxy-3,4-epoxy-1-((*E*)-3-methylpent-2-enyloxy)bisabola-7(14),10-dien-2-one.

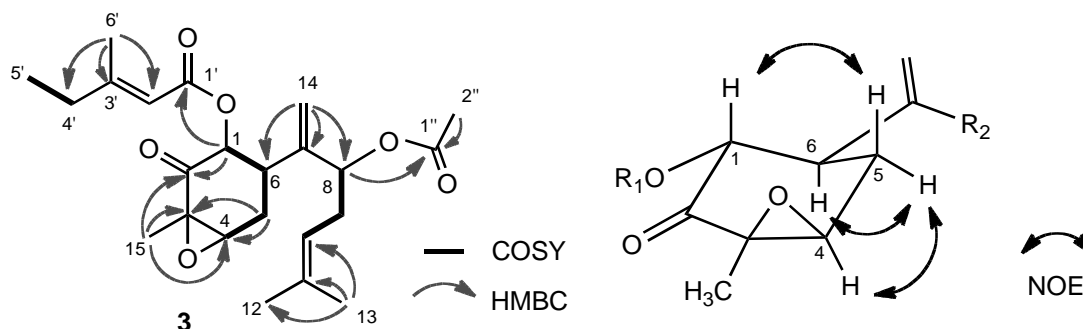


Figure 3. Selected COSY and HMBC correlations of compound **3**.

The relative configuration of the cyclohexane ring in compound **3** was deduced from the J_{HH} values and the NOEs (Figure 3). When H-6 is assumed to be in α -orientation, H-1 must be β -quasi-axial due to the large J value ($J = 13.0$ Hz). The other two couplings for H-6 ($J = 11.0, 7.8$ Hz) are due to two protons at C-5. The quasi-diaxial nature of H-1 and H-5 β was revealed by the NOESY spectrum. The H-5 α proton had an NOE with H-4 and the $J_{4,5\beta}$ value was nearly 0 Hz, hence, the epoxide ring should be in β -orientation. The configuration at C-8 was not determined. The CD spectrum exhibited a positive Cotton effect at 297 nm and hence the absolute configuration of the cyclohexane ring was established as shown in the formula.¹⁴

We isolated three new and two known sesquiterpenoids from *C. rhodocephalum*. Four of them were bisabolane-type and one was an oplopane-type. Compounds **1** and **2** were the first examples of hydroperoxides with a bisabolane skeleton. Compound **4** was reported in 1981 by Bohlmann as a mixture with the corresponding senecionate.¹² We have isolated **4** in pure form, and it is worth recording the spectral data in Experimental. Compound **5** was an oplopane-type sesquiterpenoid previously isolated from *Senecio implexus* by Bohlmann.¹³

Bisabolane sesquiterpenoids have been isolated from *C. ellisii*⁴ and *C. discoideum*,⁵ and oplopane sesquiterpenoids from *C. ellisii*.⁴ We previously isolated eremophilane sesquiterpenoids from *C. helianthus*⁸ and *C. stenactinium*.⁹ The genus *Cremanthodium* is taxonomically very close to *Ligularia*.³ Our research on *Ligularia* in the Hengduan Mountains area clarified that most of the *Ligularia* species produce sesquiterpenes such as eremophilanes, bisabolanes, and oplopanes.¹¹ Thus, the present and the preceding results on the *Cremanthodium* species indicate that the two genera, *Ligularia* and *Cremanthodium*, are closely related not only morphologically but also chemically. We continue to search for more *Cremanthodium* plants in Hengduan Mountains area.

EXPERIMENTAL

General Specific rotations and CD spectra were measured on a JASCO P-1030 and a JASCO J-725 auto recording polarimeter; IR spectra, on a Shimadzu FT/IR-8400S spectrophotometer; ¹H and ¹³C NMR spectra (400 MHz and 100 MHz, respectively), on a Varian 400-MR spectrometer. Mass spectra, including high-resolution ones, were recorded on a JEOL JMS-700 MStation. A Chemcopak Nucleosil 50-5 column (4.6 × 250 mm) and a hexane-ethyl acetate solvent system was used for HPLC (JASCO pump system). Silica gel BW127ZH (100–270 mesh, Fuji Silysia) was used for column chromatography. Silica gel 60 F₂₅₄ plates (Merck) were used for TLC.

Plant Material The plant was collected at Daxueshan in Yunnan Province of China (28°34'48"N, 99°49'47"E) at 4300 m altitude and identified by Xun Gong, one of the authors (voucher specimen No. 2007118 was deposited in the herbarium at Kunming Institute of Botany).

Extraction and Isolation The washed and dried roots (6.8 g) were cut into pieces and were extracted with EtOAc at rt to give a residue (145.6 mg). The extract was separated by silica gel column chromatography (hexane-EtOAc, gradient) followed by HPLC (hexane-EtOAc) to afforded compounds **1** (1.5 mg), **2** (0.9 mg), **3** (0.6 mg), **4** (0.4 mg), and **5** (4.2 mg).

10-Hydroperoxybisabola-2,7(14),11-triene (**1**): $[\alpha]_D^{21} -33.6$ (*c* 0.15, EtOH); FT-IR (KBr) 3350, 1643 cm^{-1} ; ^1H NMR (C_6D_6) δ 7.10 (1H, s, OOH), 5.42-5.38 (1H, m, H-2), 4.95-4.93 (1H, m, H-12a), 4.87 (1H, quint, $J = 1.6$ Hz, H-12b), 4.85 (1H, br s, H-14a), 4.81 (1H, br s, H-14b), 4.26 (1H, br t, $J = 6.6$ Hz, H-10), 2.14 (1H, ddd, $J = 15.6, 9.8, 5.6$ Hz, H-8a), 2.14-2.00 (2H, m, H-8b, 1a), 2.10-2.02 (1H, m, H-6), 1.98-1.90 (1H, m, H-1b), 1.92-1.79 (2H, m, H-4a, 4b), 1.81-1.75 (1H, m, H-9a), 1.77-1.70 (1H, m, H-5a), 1.609 (3H, d, $J = 1.6$ Hz, H-13 or 15), 1.606 (3H, d, $J = 1.6$ Hz, H-15 or 13), 1.62-1.56 (1H, m, H-9b), 1.44 (1H, dtd, $J = 12.5, 11.2, 5.7$ Hz, H-5b); ^{13}C NMR (C_6D_6) δ 153.7 (C-7), 144.3 (C-11), 133.5 (C-3), 121.2 (C-2), 113.9 (C-12), 108.0 (C-14), 89.0 (C-10), 40.1 (C-6), 31.8 (C-1), 31.1 (C-8), 30.9 (C-4), 29.9 (C-9), 28.6 (C-5), 23.6 (C-15), 17.2 (C-13); MS (FAB) m/z 259 $[\text{M}+\text{Na}]^+$ (base); HRMS (FAB) obs m/z 259.1675 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{15}\text{H}_{24}\text{O}_2\text{Na}$ 259.1674).

11-Hydroperoxybisabola-2,7(14),9*E*-triene (**2**): $[\alpha]_D^{19} -55.1$ (*c* 0.10, EtOH); FT-IR (KBr) 3400, 1637 cm^{-1} ; ^1H NMR (C_6D_6) δ 6.73 (1H, s, OOH), 5.65 (1H, dt, $J = 15.6, 6.6$ Hz, H-9), 5.55 (1H, dt, $J = 15.6, 1.2$ Hz, H-10), 5.43-5.40 (1H, m, H-2), 4.88 (1H, br s, H-14a), 4.86 (1H, q, $J = 1.4$ Hz, H-14b), 2.71 (1H, br dd, $J = 15.6, 6.6$ Hz, H-8a), 2.68 (1H, br dd, $J = 15.6, 6.6$ Hz, H-8b), 2.16-2.10 (1H, m, H-1a), 2.15-2.08 (1H, m, H-6), 1.99-1.92 (1H, m, H-1b), 1.92-1.85 (1H, m, H-4a), 1.87-1.80 (1H, m, H-4b), 1.79-1.73 (1H, m, H-5a), 1.62 (3H, br s, H-15), 1.50-1.40 (1H, m, H-5b), 1.24 (6H, s, H-12, 13); ^{13}C NMR (C_6D_6) δ 152.8 (C-7), 136.1 (C-10), 133.5 (C-3), 129.0 (C-9), 121.1 (C-2), 109.1 (C-14), 81.6 (C-11), 40.1 (C-6), 38.4 (C-8), 31.7 (C-1), 30.8 (C-4), 28.6 (C-5), 24.6 (C-12), 24.6 (C-13), 23.6 (C-15); MS (FAB) m/z 259 $[\text{M}+\text{Na}]^+$ (base); HRMS (FAB) obs m/z 259.1687 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{15}\text{H}_{24}\text{O}_2\text{Na}$ 259.1674).

8-Acetoxy-3,4-epoxy-1-((*E*)-3-methylpent-2-enyloxy)bisabola-7(14),10-dien-2-one (**3**): $[\alpha]_D^{22} -152.3$ (*c* 0.04, CHCl_3); CD: +4000 (297 nm) (EtOH); FT-IR (KBr) 1733, 1716 cm^{-1} ; ^1H NMR (C_6D_6) δ 6.16 (1H, d, $J = 13.0$ Hz, H-1), 5.87 (1H, sept, $J = 1.2$ Hz, H-2'), 5.24-5.20 (1H, m, H-10), 5.19 (1H, dd, $J = 7.3, 5.1$ Hz, H-8), 5.11 (1H, br s, H-14a), 4.95 (1H, br s, H-14b), 2.65 (1H, d, $J = 3.9$ Hz, H-4), 2.55 (1H, ddd, $J = 13.0, 11.0, 7.8$ Hz, H-6), 2.43-2.33 (2H, m, H-9a, 9b), 2.09 (3H, d, $J = 1.2$ Hz, H-6'), 1.97 (1H, ddd, $J = 15.7, 7.8, 3.9$ Hz, H-5a), 1.91 (1H, dd, $J = 15.7, 11.0$ Hz, H-5b), 1.73 (3H, s, H-2''), 1.71 (2H, qd, $J = 7.4, 1.2$ Hz, H-4'), 1.65 (3H, br s, H-12), 1.56 (3H, br s, H-13), 1.21 (3H, s, H-15), 0.70 (3H, t, $J = 7.4$ Hz, H-5'); ^{13}C NMR (C_6D_6) δ 201.5 (C-2), 169.5 (C-1'), 165.3 (C-1'), 162.8 (C-3'), 148.5 (C-7), 134.2 (C-11), 120.0 (C-10),

114.1 (C-2'), 112.3 (C-14), 75.6 (C-8), 74.3 (C-1), 64.0 (C-4), 61.5 (C-3), 44.8 (C-6), 33.6 (C-4'), 32.6 (C-9), 31.8 (C-5), 25.8 (C-12), 20.7 (C-2''), 19.0 (C-6'), 18.0 (C-13), 15.0 (C-15), 11.6 (C-5'); MS (CI) m/z 405 [M+H]⁺, 345, 231 (base), 203, 97; HRMS (CI) obs m/z 405.2278 [M+H]⁺ (calcd for C₂₃H₃₃O₆ 405.2277). 8-Acetoxy-3,4-epoxy-1-angeloyloxybisabol-7(14),10-dien-2-one (**4**): $[\alpha]_D^{23}$ -193.0 (*c* 0.02, CHCl₃); ¹H NMR (C₆D₆) δ 6.18 (1H, d, *J* = 13.0 Hz, H-1), 5.69 (1H, qq, *J* = 7.4, 1.5 Hz, H-2'), 5.21-5.17 (1H, m, H-10), 5.16 (1H, dd, *J* = 7.8, 4.9 Hz, H-8), 5.08 (1H, br s, H-14a), 4.89 (1H, br s, H-14b), 2.63 (1H, d, *J* = 4.2 Hz, H-4), 2.46 (1H, ddd, *J* = 13.0, 10.6, 7.6 Hz, H-6), 2.39-2.30 (2H, m, H-9a, 9b), 2.00 (3H, dq, *J* = 7.4, 1.5 Hz, H-4'), 1.99 (1H, ddd, *J* = 15.6, 7.6, 4.2 Hz, H-5a), 1.94 (3H, quint, *J* = 1.5 Hz, H-5'), 1.90 (1H, dd, *J* = 15.6, 10.6 Hz, H-5b), 1.68 (3H, s, H-2''), 1.63 (3H, br s, H-12), 1.54 (3H, br s, H-13), 1.19 (3H, s, H-15); ¹³C NMR (C₆D₆) δ 201.3 (C-2), 169.5 (C-1''), 166.5 (C-1'), 148.4 (C-7), 138.4 (C-3'), 134.4 (C-11), 127.8 (C-2'), 119.9 (C-10), 111.9 (C-14), 75.0 (C-8), 74.4 (C-1), 63.9 (C-4), 61.5 (C-3), 45.4 (C-6), 32.8 (C-9), 31.4 (C-5), 25.8 (C-12), 20.8 (C-5'), 20.7 (C-2''), 18.0 (C-13), 15.9 (C-4'), 14.9 (C-15).

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