SHORT REPORT

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Flavonoids and a New Calamenene-type Sesquiterpene from Rhizomes of *Alpinia oxymitra* K. Schum. (Zingiberaceae)

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Abstract: Chemical constituents of rhizomes of *Alpinia oxymitra* (Zingiberaceae) were investigated. Using chromatographic techniques, two known flavonoids, epicatechin and galloepicatechin, were isolated together with a new calamenene sesquiterpene, (–)-(1*R*,4*S*)-8-hydroxy-13-calamenenoic acid. Structure elucidation of the isolated compounds was accomplished by means of spectroscopic techniques, especially NMR spectroscopy and mass spectrometry.

Keywords: *Alpinia oxymitra; Cenolophon oxymitrum;* Zingiberaceae; flavonoids; calamenene sesquiterpene.

1. Plant Source

The Zingiberaceae (ginger family) is one of the most well-known monocotyledon plant families. This family is characterized by the occurrence of diarylheptanoids, gingerols, flavonoids, various terpenoids and many other secondary metabolites. *Alpinia oxymitra* K. Schum. (synonym *Cenolophon oxymitrum* (Schum.) Holttum.) is a perennial herb native to Thailand and Malaysia [1,2]. In Thailand, *A. oxymitra* is eaten as a vegetable and used in traditional medicine as a carminative and an anti-diarrhetic. For the present phytochemical investigation, *A. oxymitra* was grown in the greenhouse of the MPI for Chemical Ecology. Freshly harvested rhizomes were studied for their major chemical constituents.

2. Previous Studies

There has been no phytochemical investigation of *Alpinia oxymitra* reported previously.

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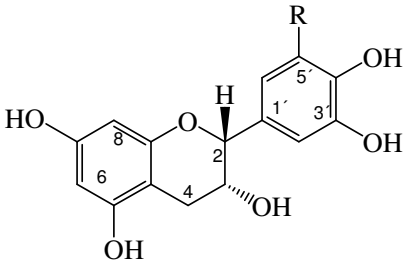
3. Present Study

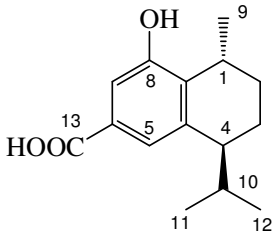
Plants of *Alpinia oxymitra* K. Schum. were obtained from the Phu Keaw Cliff, Khao Yai National Park, Nakorn Rachasima Province, Thailand, and grown in the greenhouse of the MPI for Chemical Ecology. A voucher specimen of the plant material is deposited at the Herbarium Hausknecht (JE), University of Jena, Germany. Rhizome material (78.5 g, fresh weight) was harvested, thoroughly washed, cut into small pieces, macerated and exhaustively extracted with MeOH. The MeOH extract was evaporated to dryness with a rotary evaporator (<50° C). The residue was suspended in 50% aqueous MeOH and partitioned with *n*-hexane, $CHCl_3$ and EtOAc, respectively. The fractions were analyzed by thin-layer chromatography for UV-absorbing spots (UV 254 nm). The EtOAc fraction (1 g) and $CHCl_3$ fraction (240 mg) of rhizomes were further separated and purified.

The EtOAc fraction was fractionated by gel filtration chromatography (Sephadex LH 20) using MeOH as an eluent. Fractions of 10 mL each were collected. Fractions 15 to 20 were pooled and evaporated to dryness, and the residue was again subjected to Sephadex LH 20 column using 50% CH_2Cl_2 in MeOH as an eluent. Fractions of 5 mL each were collected. Fractions 22-28 were pooled and further purified by preparative HPLC (Merck-Hitachi LiChrograph HPLC system; column: LiChrospher RP18, 10 µm, 250×10 mm). A linear gradient of MeCN in H_2O containing 0.1% trifluoroacetic acid (TFA) was used as a mobile phase (0 min: 10% MeCN, 40 min: 30% MeCN, 45 min: 100% MeCN, 50 min: 10% MeCN) at a flow rate of 3.5 mL min^{-1} and UV detection at 254 nm. Compound **1** (0.9 mg) was eluted at R_t 19.5 min. Fractions 29-37 were pooled and further purified by preparative HPLC using isocratic elution with 10% MeCN in H_2O containing 0.1% TFA as a mobile phase. Other HPLC conditions were the same as for compound **1**. Compound **2** (1 mg) eluted at R_t 19.1 min. Compounds **1** (HR ESIMS: *m/z* 289.0713, calc. for $C_{15}H_{13}O_6$: *m/z* 289.0712 $[M-H]^+$) and **2** (HR ESIMS: *m/z* 305.0663, calc. for $C_{15}H_{13}O_7$: *m/z* 305.0661 $[M-H]^+$) were identified as epicatechin and galloepicatechin, respectively, by their analytical data. Mass spectra (ESI) and accurate masses were recorded in the positive ionization mode on a Micromass Quattro II tandem quadrupole mass spectrometer. ^1H NMR, $^1H,^1H$ COSY, HSQC, HMBC, TOCSY, ROESY spectra were recorded on a Bruker AVANCE 500 NMR spectrometer equipped with a 5 mm TXI cryoprobe. The spectra matched those of authentic compounds. Compounds **1** and **2** are not restricted to Zingiberaceae but widely distributed in higher plants.

The $CHCl_3$ extract was chromatographed by gel filtration (Sephadex LH 20). The fractions were eluted with mixtures (20 mL each) of CH_2Cl_2 in 50%, 60%, 70%, 80% MeOH and 100% MeOH. Fractions of 5 mL each were collected. Fractions 21-28 were pooled, again applied to gel filtration chromatography (Sephadex LH 20) and eluted with 100% MeOH. Compound **3** (12.6 mg) was obtained in a pure form.



1 R = H
2 R = OH



3

Figure 1. Chemical structures of the compounds isolated from *Alpinia oxymitra*. **1**: Epicatechin, **2**: Galloepicatechin, **3**: (–)-(1*R*,4*S*)-8-Hydroxy-13-calamenenoic acid.

The high-resolution mass spectrum of compound **3** showed an $[M]^+$ ion at *m/z* 248.1415 indicating a molecular formula of $C_{15}H_{20}O_3$ (calc. 248.1412) of a sesquiterpene. The ^1H NMR spectrum of compound **3** ($CDCl_3$, unless otherwise noted) showed three doublet methyl signals at δ 0.82 (H-12), 0.98 (H-11), and 1.21 (H-9). ^1H NMR signals of two methylene groups appeared at δ 1.82 (H-3), δ 1.55 (H-2b) and 1.97 (H-2a). Three signals of aliphatic methine protons at δ 2.02 (H-10), 2.57 (H-4), and 3.19 (H-1) and two signals of aromatic methines at δ 7.53 (H-5) and 7.28 (H-7) completed the ^1H NMR spectrum. $^1H,^1H$ COSY and TOCSY spectra indicated that all aliphatic signals belonged to one spin system, and the signals at δ 7.53 and 7.28 are due to an aromatic AX spin system. HSQC and HMBC correlations of the two AX protons established the aromatic ring, the position of the carboxyl and hydroxyl group. The HMBC spectrum displayed cross peaks of H-5 with C-7 (δ 113.2), C-8a (δ 136.1), the carboxyl group (δ 170.8), and a methine carbon at δ 43.1 assignable to C-4 of the aliphatic part of the molecule. Long-range correlations of H-7 through three bonds were observed with C-5 (δ 124.4), C-8a and the carboxyl group. A correlation of H-7 through two bonds with δ 153.1 assigned this low-field carbon to the hydroxylated C-8. HMBC correlations of the hydroxyl proton (measured in DMSO-d_6) with C-7, C-8, and C-8a confirmed the position of the hydroxyl group at C-8. The eight carbon atoms of the aliphatic part of the molecule were also assigned from their HMBC, HSQC and $^1H,^1H$ COSY correlations. The methyl proton signal at δ 1.21 coupled with C-8a and showed a strong unresolved cross signal with C-1 (δ 27.1) and C-2 (δ 26.5). The two other methyl signals (H-12, δ 0.82; H-11, δ 0.98), mutually correlated by HMBC, showed further cross peaks with C-10 (δ 33.2) and C-4 (δ 43.1) and therefore were attributed to the isopropyl side chain attached to C-4. The methine proton H-4 (δ 2.57) coupled not only with the isopropyl carbons C-10 (δ 33.2), C-11 (δ 21.9) and C-12 (δ 19.6) but also with C-4a (δ 141.6), C-5, and C-8a of the aromatic ring and methylene carbons C-2 (δ 26.5) and C-3 (δ 18.8). From these data, the constitution of compound **3** was determined as 1,2,3,4-tetrahydro-8-hydroxy-4-isopropyl-1-methylnaphthalene-6-carboxylic acid (8-hydroxy-13-calamenenoic acid), a new natural product of the calamenene type. Since, due to missing ROESY correlation between the geminal methyl groups of the isopropyl moiety and the secondary methyl group (C-9, δ 20.7), *cis* orientation is unlikely, (1*R*,4*R*)- and (1*S*,4*S*) configuration can be excluded. The absolute stereochemistry of **3** was assessed based on data reported from closely related calamenenes. The optical rotation was measured on a Jasco P-1030 polarimeter. The $[\alpha]_D^{22}$ = -10.95 (c 0.00065, $CHCl_3$) of compound **3** was in good agreement with that of synthetic (1*R*,4*S*)-8-hydroxycalamenene [α]D = -24° (c 1.06, $CHCl_3$) [3] but clearly different from the positive optical rotation values $[\alpha]_D$ = +38° of (1*S*,4*R*)-hydroxycalamenene and $[\alpha]_D$ = +40° of (1*R*,4*R*)-8-hydroxycalamenene [4]. Moreover, 6-substituted (1*S*,4*R*)-8-hydroxy calamenenes from *Tarenna madagascariensis* displayed positive optical rotation values [5]. Hence, compound **3** is (–)-(1*R*,4*S*)-8-hydroxy-13-calamenenoic acid.

The plant was treated with jasmonic acid (JA) to induce the production of natural products and enhance the concentration of minor components, but no significant change in its natural product profile was observed (data not shown). In this study, diarylheptanoids and gingerols were not found in *A. oxymitra*.

^1H NMR (500 MHz, $CDCl_3$): δ 7.53 (1H, br. s, H-5), 7.28 (1H, br. s, H-7), 3.19 (1H, m, H-1), 2.57 (1H, br. s, H-4), 2.02 (1H, m, H-10), 1.97 (1H, m, H-2a), 1.82 (2H, m, H-3), 1.55 (1H, br. d, *J* = 12.6 Hz, H-2b), 1.21 (3H, d, *J* = 6.9 Hz, H-9), 0.98 (3H, d, *J* = 6.6 Hz, H-11), 0.82 (3H, d, *J* = 6.9 Hz, H-12).

^1H NMR (500 MHz, DMSO-d_6): δ 12.45 (1H, br. s, 13-COOH), 9.51 (1H, s, 8-OH), 7.21 (1H, d, *J* = 1.6 Hz, H-5), 7.18 (1H, d, *J* = 1.6 Hz, H-7), 3.09 (1H, m, H-1), 2.47 (1H, br. s, H-4), 1.91 (1H, m, H-10), 1.85 (1H, m, H-2a), 1.76 (1H, m, H-3a), 1.72 (1H, m, H-3b), 1.44 (1H, br. d, *J* = 13.2 Hz, H-2b), 1.10 (3H, d, *J* = 7.0 Hz, H-9), 0.92 (3H, d, *J* = 6.6 Hz, H-11), 0.76 (3H, d, *J* = 6.9 Hz, H-12).

^{13}C NMR (125 MHz, $CDCl_3$, chemical shifts extracted from HSQC and HMBC): δ 170.8 (C-13), 153.1 (C-8), 141.6 (C-4a), 136.1 (C-8a), 126.3 (C-6), 124.4 (C-5), 113.2 (C-7), 43.1 (C-4), 33.2 (C-10), 27.1 (C-1), 26.5 (C-2), 21.9 (C-11), 20.7 (C-9), 19.6 (C-12), 18.8 (C-3).

UV (MeCN/H_2O): λ max (log ε) 212 (2446.28), 254 (1023.04), 304 (396.40).

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