(+)-Bornyl Piperate, a New Monoterpene Ester from *Piper* aff. *pedicellatum* Roots

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A new monoterpene ester, (+)-bornyl piperate was isolated from the underground roots of *Piper* aff. *pedicellatum* and its structure was elucidated on the basis of spectroscopic evidence and confirmed by X-ray analysis. The compound crystallizes in the triclinic space group *P*1 with $a=7.3232(4)$ Å, $b=11.4705(7)$ Å, $c=23.2520(14)$ Å, $V=1943.6(2)$ Å^3. This compound showed an antituberculosis activity against *Mycobacterium tuberculosis* (H_{37}Ra strain) with the minimum inhibitor concentration (MIC) of 25 μg/ml.

Key words *Piper* aff. *pedicellatum*; Piperaceae; antituberculosis; *Mycobacterium tuberculosis*

Piper aff. *pedicellatum* C. DC. (Piperaceae) is distributed mainly in Northern Thailand. Its roots and stems are used as a carminative in Thai folk medicine. Although there are many reports on the phytochemistry of the genus *Piper*,[1] no previous record on the chemical constituents of this plant species has been found in the literature. As part of our ongoing project on bioactive compounds from Thai medicinal plants for the treatment of tropical diseases, we have investigated this plant species. We now describe the isolation and structure elucidation of a new monoterpene ester, (+)-bornyl piperate (**1**) together with eight known compounds (**2**—**9**) from the hexane and the methanol extracts of the underground roots of *P.* aff. *pedicellatum*.

The powdered roots of *P.* aff. *pedicellatum* were extracted successively with *n*-hexane and MeOH in a Soxhlet apparatus. The hexane extract on chromatography over silica gel gave one new compound (**1**) and six known compounds (**2**—**7**), while chromatography of the methanol extract afforded two more known compounds (**8**—**9**). The known compounds were identified as a mixture of β-sitosterol (**2**)[2] and stigmasterol (**3**),[2] pellitorine (**4**),[3] guineensine (**5**),[4] pipernonaline (**6**),[5] piperine (**7**)[6] and a mixture of β-sitosteryl-3-*O*-β-glucopyranoside (**8**)[7] and stigmasteryl-3-*O*-β-glucopyranoside (**9**)[8] by comparison of their physical and spectroscopic data with those reported in the literature.

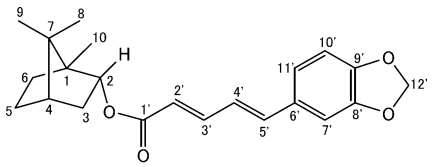

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Chart 1

Compound **1** was obtained as colorless needles and its molecular formula was determined to be $C_{22}H_{26}O_4$ by HR-FAB-MS (*m*/*z* 355.1905, $[M+H]^+$). Its IR spectrum displayed an absorption band at 1710 cm^{-1}, indicating the presence of a conjugated ester group. The ^{1}H- and ^{13}C-NMR spectra exhibited the presence of the 2*E*,4*E*-piperoyl moiety.[6] The remaining ^{1}H- and ^{13}C-NMR signals of **1** were very similar to those reported for the bornyl group of bornyl *p*-coumarate isolated from *P. ribesioides*.[9] In the HMBC spectrum, a long range correlation was observed between the piperoyl carbonyl carbon C-1′ (δ 167.5) and the H-2 proton (δ 4.96) of the bornyl moiety. The stereochemistry at C-2 was determined by means of a NOE experiment. Upon irradiation of H-2, a NOE enhancement was observed for H-3β, 8-CH_3 and 10-CH_3, showing that the piperoyl group is located at the *endo* side of the bornyl moiety. Moreover, the X-ray structure (Fig. 1) confirmed the relative stereochemistry of **1**. It should be noted that the X-ray crystal structure of (+)-bornyl *p*-coumarate isolated from *P. caninum*[10] has recently been established. Therefore, the structure of **1** was established as 2-*endo*-bornyl piperate.

Compound **1** exhibited an antituberculosis activity against *Mycobacterium tuberculosis* (H_{37}Ra strain)[11] with the MIC of 25 μg/ml.

Experimental

General Procedures Melting points were determined on an Electrothermal apparatus and are uncorrected. UV spectra were measured with a Perkin Elmer Lamda 20 spectrophotometer. IR spectra were obtained with a Perkin

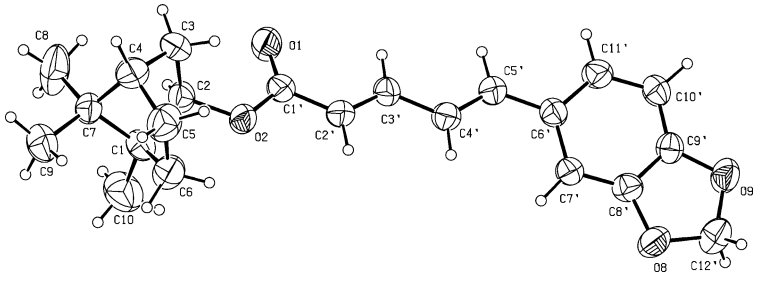

Fig. 1. ORTEP Perspective Drawing of **1**

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Elmer Spectrum 2000 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AVANCE 400 NMR spectrometer, operating at 400 and 100 MHz, respectively. Mass spectra were recorded with a Finnigan MAT 90 instrument. Column chromatography and TLC were carried out using Merck silica gel 60 (>230 mesh) and precoated silica gel 60 F_{254} plates, respectively. Spots on TLC were visualized under UV light and by spraying with anisaldehyde-H_2SO_4 reagent followed by heating.

Plant Material The underground roots of *P.* aff. *pedicellatum* were collected from Pasang district, Lamphun province, Thailand in December, 1999. A voucher specimen (No. BKF 93307) has been deposited at the herbarium of the Royal Forest Department, Ministry of Agriculture and Cooperatives, Bangkok.

Extraction and Isolation The air-dried, powdered roots of *P.* aff. *pedicellatum* (314.5 g) were extracted successively with *n*-hexane and MeOH using a Soxhlet apparatus. The hexane and MeOH extracts were evaporated to dryness *in vacuo*. The hexane extract (7.44 g) was subjected to a silica gel column (150 g) using a gradient solvent system of hexane, hexane–EtOAc and EtOAc (5% increment of the polar solvent for each 250 ml of mobile phase) to give 20 main fractions. Fr. 5 (193 mg) was rechromatographed on a silica gel column (40 g) using EtOAc–hexane (1.5 : 98.5) (1.5 l) to give 5 subfractions. Fr. 5.3 yielded **1** (12 mg). Fr. 9 (209 mg) was fractionated into 4 subfractions on a siliga gel column (8 g) using EtOAc–hexane (8 : 92) (300 ml) as eluent. Subsequent filtration of fr. 9.3 afforded a mixture of **2**[2] and **3**[2] (63 mg). Repetitive CC (silica gel, 25 g) of fr. 12 (293 mg) using EtOAc–hexane (80 : 20) (1.5 l) as eluent gave 4 subfractions. Fr. 12.3 yielded **4**[3] (44 mg). Fr. 14 (211 mg) was further purified on a silica gel column (10 g) eluting with EtOAc–hexane (80 : 20) (300 ml) to give 7 subfractions. Compound **5**[4] (13 mg) was isolated from fr. 14.4. Fr. 16 (110 mg) was rechromatographed on a silica gel column (7 g) using EtOAc–hexane (30 : 70) (300 ml) as eluent to give 6 subfractions. Fr. 16.4 afforded **6**[5] (50 mg) while filtration of fr. 18 (123 mg) furnished **7**[6] (71 mg). The MeOH extract (7.37 g) was subjected to CC (silica gel, 150 g) eluting with a gradient of $CHCl_3$, $CHCl_3$–MeOH and MeOH in increasing proportions of the polar solvent (each 200 ml) to give 9 main fractions. Fr. 7 (540 mg) was further purified on a silica gel column (30 g) eluting with MeOH–$CHCl_3$ (2 : 98) (2 l) to give 15 subfractions. Fr. 7.12 (136 mg) was filtered to give a mixture of **8**[7] and **9**[8] (56 mg).

Bornyl Piperate (**1**): Colorless needles; mp 93—95 °C (hexane); $[\alpha]_D^{27}$ +7.80° (c=0.1, $CHCl_3$). UV λ_{max} (MeOH) nm: 261, 316, 353. IR (KBr) cm^{-1}: 3024, 2951, 1710, 1624, 1502, 1489, 1447, 1375, 1325, 1253, 1196, 1175, 1139, 1037, 995, 931, 880, 848, 799, 754, 724. EI-MS *m*/*z* (rel. int.): 354 (M^+, 30), 218 (38), 202 (14), 201 (100), 173 (18), 143 (11), 137 (27), 115 (13), 81 (26), 69 (11). HR-FAB-MS *m*/*z*: 355.1905 (Calcd for $C_{22}H_{26}O_4$ $[M+H]^+$: 355.1909). ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, s, 10-CH_3), 0.87 (3H, s, 9-CH_3), 0.91 (3H, s, 8-CH_3), 1.01 (1H, dd, J=13.1, 4.3 Hz, H-3α), 1.24 (1H, m, H-5α), 1.33 (1H, m, H-6β), 1.68 (1H, br t, J=4.3 Hz, H-4), 1.74 (1H, m, H-5β), 2.00 (1H, m, H-6α), 2.38 (1H, m, H-3β), 4.96 (1H, br ddd, J=13.1, 2.6, 2.6 Hz, H-2), 5.96 (1H, d, J=15.3 Hz, H-2′), 5.97 (2H, s, H-12′), 6.69 (1H, dd, J=15.7, 10.8 Hz, H-4′), 6.77 (1H, d, J=8.2 Hz, H-10′), 6.80 (1H, d, J=15.7 Hz, H-5′), 6.90 (1H, dd, J=8.2, 1.5 Hz, H-11′), 6.98 (1H, d, J=1.5 Hz, H-7′), 7.38 (1H, dd, J=15.3, 10.8 Hz, H-3′). ^{13}C-NMR ($CDCl_3$) δ: 13.5 (C-10), 18.9 (C-8), 19.7 (C-9), 27.2 (C-6), 28.1 (C-5), 36.9 (C-3), 45.1 (C-4), 47.8 (C-1), 48.9 (C-7), 79.7 (C-2), 101.4 (C-12′), 105.9 (C-7′), 108.5 (C-10′), 121.1 (C-2′), 122.8 (C-11′), 124.6 (C-4′), 130.7 (C-6′), 139.9 (C-5′), 144.3 (C-3′), 148.3 (C-9′)*, 148.5 (C-8′)*; 167.5 (C-1′). (Values with an asterisk are interchangeable.)

Crystal Data of (+)-Bornyl Piperate (**1**): $C_{22}H_{26}O_4$, MW 354.45, Triclinic, $P1$, a=7.3232(4) Å, b=11.4705(7) Å, c=23.2520(14) Å, V=1943.6(2) Å^3. A total of 7259 unique reflections (6,528 observed, $|F_o|>4\sigma|F_o|$) were measured at room temperature from a 0.30×0.15×0.05 mm^3 colorless crystal using graphite monochromated MoKα radiation (λ=0.71073 Å) on a Bruker-Nonius kappa CCD diffractometer. With Z=4, the asymmetric unit contains four molecules of bornyl piperate with the calculated density of 1.211 g cm^{-3}. The crystal structure was solved by direct methods using SIR-97, and then all atoms except hydrogen atoms were refined anisotropically on F^2 using SHELXL-97 to give a final R-factor of 0.0561 (R_w=0.1470) with a data-to-parameter ratio of 7.74 : 1. Atomic coordinates, bond lengths, bond angles, and thermal parameters have been deposited with the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2 1EZ, ENGLAND (CCDC 214540).

Bioassay Procedure The antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H_{37}Ra strain using the Microplate Alamar Blue Assay (MABA).[11] The MIC values of the standard drugs isoniazid and kanamycin sulfate are 0.050 and 2.5 μg/ml, respectively.

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