Parnapimarol and Nepetaparnone from *Nepeta parnassica*

Giorgos Gkinis,[†] Efstathia Ioannou,[†] Antonio Quesada,[‡] Constantinos Vagias,[†] Olga Tzakou,*[,†] and Vassilios Roussis[†]

Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, Panepistimiopolis Zografou, Athens 15771, Greece, and Department of Didactics of Sciences, University of Jaén, PO 23071, Jaén, Spain

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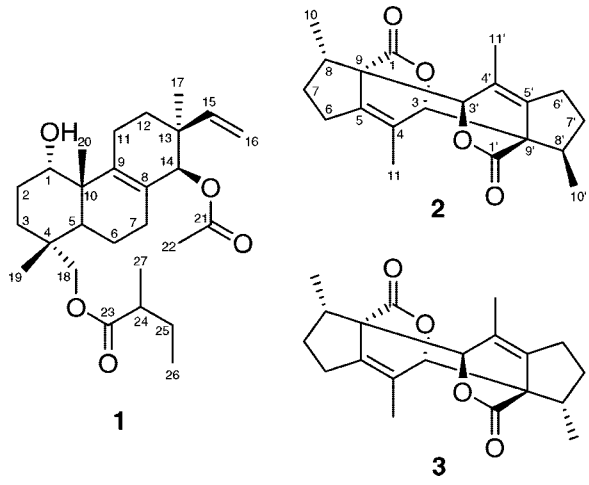
Parnapimarol (**1**), a new pimarane diterpene, along with nepetaparnone (**2**) and nepetanudone (**3**), one new and one previously reported nepetalactone dimer, respectively, were isolated from the dichloromethane extract of the aerial parts of *Nepeta parnassica*, collected on Mt. Parnassos, Greece. The structures and relative configurations of **1**–**3** were determined on the basis of their spectroscopic characteristics (1D and 2D NMR, IR, MS). The structure of **2** was confirmed by single-crystal X-ray diffraction analysis. The insecticidal activity of **1**−**3** against ants and mosquito larvae was also evaluated.

The genus *Nepeta* comprises approximately 250 species of annual or perennial herbs distributed in temperate Europe, Asia, and Africa.[1] Nepetalactones, iridoids, and their glucosides, diterpenes, and triterpenes are reported in the literature as major constituents of *Nepeta* species.[2–6] Their extracts and essential oils have exhibited analgesic, anti-inflammatory, antiviral, antioxidant, and antimicrobial activities.[7–11]

Nepeta parnassica Heldr. & Sart. (Lamiaceae) is an aromatic perennial herb, endemic to Greece and South Albania.[12] The essential oil of *N. parnassica* has been the subject of previous studies.[13,14] However, there are no reports dealing with the isolation of natural products from the organic extract of this species. In the present report, we describe the isolation and structure elucidation of one pimarane diterpene (**1**) and two nepetalactone dimers (**2**, **3**).



Parnapimarol (**1**), isolated as a colorless oil, displayed an ion peak at *m/z* 387.2905 (HRFABMS), corresponding to $C_{25}H_{39}O_3$ and consistent for $[M - OAc]^+$. The ^{13}C NMR spectrum and DEPT experiments revealed 27 signals, corresponding to six methyl, nine methylene, five methine, and seven quaternary carbon atoms. Two ester carbonyls (δ 171.5 and 176.8), three oxygenated carbons (one methylene and two methines resonating at δ 70.6, 72.4, and 78.3), two quaternary sp^2 carbons (δ 129.4 and 139.0), and a monosubstituted double bond (δ 113.1 and 142.4) were evident. The above evidence indicated a molecular formula of $C_{27}H_{42}O_5$. Identifiable in the ^1H NMR spectrum were four methyl groups on quaternary carbons (δ 0.87, 0.95, 1.05, and 2.01), two methyl groups on a tertiary and a secondary carbon each (δ 1.13 and 0.88, respectively), three olefinic protons of a monosubstituted double bond (δ 5.03, 5.04, and 5.89), two oxygenated methines (δ 3.85 and 5.14), and two oxygenated methylene protons (δ 3.65 and 3.93). The above information, in combination with correlations provided by HSQC, HMBC, and COSY experiments, suggested a pimarane skeleton with a tetrasubstituted double bond between C-8 and C-9 and three oxygenation sites (C-1, C-14, and C-18). The chemical shifts of the methine at δ 5.14 and the methylene protons at δ 3.65 and 3.93, in conjunction with the two carbonyl carbons, indicated esterification with an acetoxy and a second five-carbon alkoxy group. The latter was identified on the basis of the homonuclear and heteronuclear correlations as 2-methylbutoxy, whose presence is uncommon in natural products. The heteronuclear correlations between H_3-20 and C-1, in combination with those of H-1 with C-3 and C-5, placed the free hydroxy group at C-1. The acetoxy group was placed at C-14 on the basis of HMBC correlations of C-21 with H-14 and H_3-22, whereas the 2-methylbutoxy group was placed at C-18 due to the C-4 and C-23 interactions with H_2-18.

The relative configuration of **1** was assigned on the basis of NOE enhancements and coupling constants of certain proton signals. Specifically, the small coupling constant of H-1 (2.3 Hz) implying equatorial orientation, in combination with the correlations of the same proton with H_3-20, H-5 with H-18a, and H_3-19 with H_3-20, as well as the absence of cross-peaks between H-5 and H-1, H_3-19, and H_3-20 determined the relative structure of ring A and its *trans* fusion with ring B. On ring C, the correlations of H-14 with H-12α and H_3-17 suggested diaxial orientation for the first two and equatorial orientation for the latter. This was supported by the lack of interactions between H_3-17 and H_2-11, which both had correlations with H-1. Thus, the relative configuration of **1** was assigned as 1*S**,4*R**,5*S**,10*S**,13*S**,14*R**.

Nepetaparnone (**2**), isolated as colorless crystals, displayed an ion peak for $[M + H]^+$ at *m/z* 329.1759 (HRFABMS), corresponding to $C_{20}H_{25}O_4$. The ^{13}C NMR spectrum exhibited only 10 carbon signals, indicating that **2** was a symmetrical dimeric compound. Signals of an oxygenated methine (δ 4.08), a vinylic methyl group (δ 1.81), and an aliphatic secondary methyl group (δ 1.02) were evident in the ^1H NMR spectrum. An IR absorption band at 1741 cm^{-1} was indicative for a δ-lactone carbonyl in the molecule. The spectroscopic characteristics observed closely resembled those of nepetalactones.[3] The above, in conjunction with the base peak at *m/z* 165 (EIMS), suggested that **2** was a symmetrical nepetalactone dimer. Analysis of HSQC, HMBC, and COSY experiments confirmed the monomeric structure. HMBC correlations of C-9 with the methine resonating at δ 4.08 and of C-3 with the methine at δ

* To whom correspondence should be addressed. Tel/Fax: +30-210-7274591. E-mail: tzakou@pharm.uoa.gr.
[†] University of Athens.
[‡] University of Jaén.

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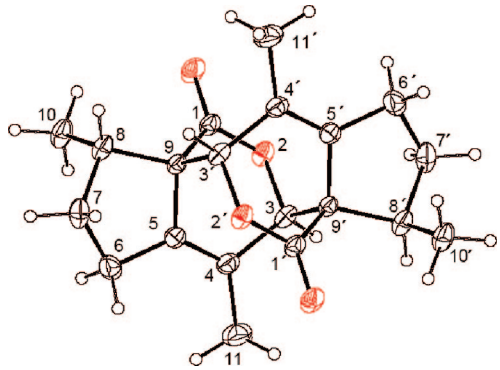

Figure 1. ORTEP drawing of compound **2**.

2.35, unexpected for nepetalactones, could only be justified in the case of bond formation between C-9 and C-3′, as well as between C-3 and C-9′. Single-crystal X-ray diffraction analysis confirmed the structure and relative configuration of **2** (Figure 1).[15]

Compound **3** had the same molecular weight as nepetaparnone (**2**), but exhibited distinct signals for the two different monomeric units in both the ^{1}H and ^{13}C NMR spectra, thus indicating lack of a symmetry element in **3**. Compound **3** was identified as nepetanudone, isolated previously from *Nepeta tuberosa* ssp. *tuberosa*, by comparison of its spectroscopic and physical characteristics with those reported in the literature.[16–18] Extensive analysis of its NMR data allowed the correction of several ^{1}H and ^{13}C NMR assignments of **3**.

The insecticidal activity of the isolated compounds, induced by contact, was evaluated against *Leptothorax* sp. ants and *Culex pipiens* biotype *molestus* mosquito larvae. The metabolites displayed moderate to marginal toxicity against ants. The mortality induced by parnapimarol (**1**), nepetaparnone (**2**), and nepetanudone (**3**) was 31%, 55%, and 45%, respectively, higher than that observed in the control vials. At the tested concentrations, **1**–**3** did not show any mosquito larvicidal activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 341 polarimeter with a 10 cm cell. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer. IR spectra were obtained on a Paragon 500 Perkin-Elmer spectrometer. NMR spectra were recorded on Bruker AC 200 and Bruker DRX 400 spectrometers. Chemical shifts are given on the δ (ppm) scale using TMS as internal standard. The 2D experiments (HMQC, HMBC, COSY, NOESY) were performed using standard Bruker microprograms. High-resolution mass spectra were provided by the University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, IN. Low-resolution mass spectra were recorded either on a Thermo Electron Corporation DSQ mass spectrometer or on a Hewlett-Packard 5973 mass spectrometer. Column chromatography was performed using Kieselgel 60 (Merck). HPLC separations were conducted using a CECIL 1100 Series liquid chromatography pump equipped with a GBC LC-1240 refractive index detector, using a Supelcosil SPLC-Si (25 cm × 10 mm) column. TLC was performed using Kieselgel 60 F_{254} (Merck aluminum support plates), and spots were detected after spraying with 15% H_2SO_4 in MeOH reagent and heating at 100 °C for 1 min.

Plant Material. Aerial parts of wild populations of *N. parnassica* were collected from Mt. Parnassos, prefecture Viotia, Greece, at an altitude of 1600 m, during the flowering stage in July 2003. The plant was identified by Dr. Th. Constandinidis (Laboratory of Systematic Botany, Agricultural University of Athens), and a voucher specimen (OT-14) has been deposited at the Herbarium of the University of Athens (ATHU).

Extraction and Isolation. The air-dried, powdered aerial parts of the plant (1.1 kg) were exhaustively extracted with CH_2Cl_2/MeOH (3:1) at room temperature. Evaporation of the solvents *in vacuo* afforded a residue (157.5 g), which was initially defatted with *c*-hexane and then partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 extract was concentrated under vacuum to give a residue (27.9 g), which was subjected to vacuum column chromatography (VCC) on silica gel, using *c*-hexane with increasing amounts of EtOAc, followed by EtOAc with increasing amounts of MeOH as the mobile phase, to afford fractions I−IX. Fraction V (50% EtOAc, 8.7 g) was fractionated by VCC on silica gel, using *c*-hexane with increasing amounts of CH_2Cl_2, followed by CH_2Cl_2 with increasing amounts of MeOH as the mobile phase, to yield fractions V1−V11. Fraction V2 (70% CH_2Cl_2, 862 mg) was separated by VCC on silica gel, using mixtures of *c*-hexane/CH_2Cl_2 of increasing polarity, into fractions V2a−V3 h. Fraction V2d (80% CH_2Cl_2, 101 mg) was subjected to normal-phase HPLC, using *c*-hexane/EtOAc (90/10) as eluent, to yield compounds **2** (9.7 mg) and **3** (6.4 mg). Fraction V3 (80% CH_2Cl_2, 947 mg) was separated by VCC on silica gel, using mixtures of *c*-hexane/CH_2Cl_2 of increasing polarity, into fractions V3a−V3i. Fraction V3g (75% CH_2Cl_2, 600 mg) was subjected to two successive gravity column chromatographic separations, using *c*-hexane/EtOAc (9:1), to yield compound **1** (5.3 mg).

Parnapimarol (1): colorless oil; $[\alpha]_D^{20}$ +9.0 (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 244.0 (3.15) nm; IR (thin film) ν_{max} 3428, 2952, 1728, 1633, 1457 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.89 (1H, dd, J = 18.1, 10.8 Hz, H-15), 5.14 (1H, s, H-14), 5.04 (1H, dd, J = 18.1, 1.5 Hz, H-16a), 5.03 (1H, dd, J = 10.8, 1.5 Hz, H-16b), 3.93 (1H, d, J = 10.9 Hz, H-18a), 3.85 (1H, brd, J = 2.3 Hz, H-1), 3.65 (1H, d, J = 10.9 Hz, H-18b), 2.38 (1H, m, H-24), 2.22 (1H, m, H-11α), 2.06 (1H, m, H-11β), 2.02 (1H, m, H-7β), 2.01 (3H, s, H-22), 1.88 (1H, m, H-3α), 1.84 (1H, m, H-2a), 1.82 (1H, m, H-12β), 1.81 (1H, m, H-7α), 1.80 (1H, m, H-5), 1.66 (1H, m, H-25a), 1.64 (1H, m, H-2b), 1.60 (1H, m, H-6a), 1.58 (1H, m, H-6b), 1.52 (1H, m, H-12α), 1.48 (1H, m, H-25b), 1.18 (1H, m, H-3β), 1.13 (3H, d, J = 7.0 Hz, H-27), 1.05 (3H, s, H-20), 0.95 (3H, s, H-17), 0.88 (3H, t, J = 7.6 Hz, H-26), 0.87 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 176.8 (C, C-23), 171.5 (C, C-21), 142.4 (CH, C-15), 139.0 (C, C-9), 129.4 (C, C-8), 113.1 (CH_2, C-16), 78.3 (CH, C-14), 72.4 (CH_2, C-18), 70.6 (CH, C-1), 43.2 (C, C-10), 41.4 (CH, C-24), 39.2 (C, C-13), 38.5 (CH, C-5), 36.4 (C, C-4), 30.7 (CH_2, C-12), 28.5 (CH_2, C-3, C-7), 26.7 (CH_2, C-25), 24.1 (CH_2, C-2), 23.8 (CH_3, C-17), 21.0 (CH_3, C-22), 20.7 (CH_3, C-20), 20.2 (CH_2, C-11), 17.9 (CH_2, C-6), 17.4 (CH_3, C-19), 16.8 (CH_3, C-27), 11.7 (CH_3, C-26); EIMS 70 eV *m*/*z* (rel int %) 386 (32), 371 (88), 266 (33), 253 (100), 251 (98), 237 (20), 187 (39), 185 (20), 145 (16), 143 (19), 131 (21), 105 (16), 85 (15), 57 (32); HRFABMS *m*/*z* 387.2905 [M − OAc]$^+$ (calcd for $C_{25}H_{39}O_3$, 387.2899).

Nepetaparnone (2): colorless crystals; $[\alpha]_D^{20}$ –3.0 (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ): 246.3 (2.76) nm; IR (thin film) ν_{max} 2963, 2929, 1741, 1450, 1375, 1183 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.08 (2H, s, H-3, H-3′), 2.35 (2H, m, H-8, H-8′), 2.29 (4H, m, H-6, H-6′), 1.87 (2H, m, H-7a, H-7a′), 1.81 (6H, s, H-11, H-11′), 1.56 (2H, m, H-7b, H-7b′), 1.02 (6H, d, J = 6.9 Hz, H-10, H-10′); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 172.4 (C, C-1, C-1′), 140.8 (C, C-4, C-4′), 133.0 (C, C-5, C-5′), 87.6 (CH, C-3, C-3′), 67.7 (C, C-9, C-9′), 43.5 (CH, C-8, C-8′), 30.6 (CH_2, C-7, C-7′), 25.6 (CH_2, C-6, C-6′), 17.0 (CH_3, C-11, C-11′), 16.8 (CH_3, C-10, C-10′); EIMS 70 eV *m*/*z* (rel int %) 165 (100), 149 (86), 136 (22), 121 (8), 107 (5), 91 (15), 77 (12); HRFABMS *m*/*z* 329.1759 [M + H]$^+$ (calcd for $C_{20}H_{25}O_4$, 329.1753).

Nepetanudone (3): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.43 (1H, s, H-3), 4.09 (1H, s, H-3′), 2.36 (1H, m, H-8), 2.34 (1H, m, H-6a′), 2.30 (2H, m, H-6), 2.29 (1H, m, H-8′), 2.12 (1H, m, H-6b′), 1.88 (1H, m, H-7a), 1.86 (1H, m, H-7a′), 1.78 (6H, s, H-11, H-11′), 1.57 (1H, m, H-7b), 1.39 (1H, m, H-7b′), 1.30 (3H, d, J = 6.7 Hz, H-10′), 1.04 (3H, d, J = 6.9 Hz, H-10); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 174.2 (C, C-1′), 171.9 (C, C-1), 142.5 (C, C-4′), 140.8 (C, C-4), 132.2 (C, C-5, C-5′), 87.7 (CH, C-3′), 79.1 (CH, C-3), 67.3 (C, C-9), 65.0 (C, C-9′), 43.5 (CH, C-8), 41.3 (CH, C-8′), 31.4 (CH_2, C-7′), 30.6 (CH_2, C-7), 26.7 (CH_2, C-6′), 25.6 (CH_2, C-6), 16.8 (CH_3, C-10), 16.6 (CH_3, C-11′), 16.0 (CH_3, C-11), 14.0 (CH_3, C-10′).

Bioassays. For the ant toxicity bioassay, 0.5 mg of metabolites **1**−**3** dissolved in 50 μL of EtOH each were placed in 20 mL glass vials, and solvent was evaporated under a stream of N_2. Subsequently, eight *Leptothorax* sp. ants were placed inside each vial without any food and kept for 24 h. Control vials were prepared in a similar way, adding only 50 μL of EtOH. The number of dead ants was recorded after 6, 12, and 24 h.[19] The mortality was calculated as [(number of dead ants in the treatment vial − number of dead ants in the control vial)/number of ants in each vial] × 100%. Assays were run in triplicate. The mosquito larvicidal bioassay was carried out according to a larval susceptibility test method suggested by the World Health Organiza-

tion.[20] Specifically, 10 fourth-instar larvae of *Culex pipiens* biotype *molestus* were placed in glass beakers containing 10 mL of H_2O. Metabolites **1**−**3** dissolved in EtOH were added in proper volumes, so that the final concentrations of the solutions would be 10, 20, and 50 mg/L. Control glass beakers were similarly prepared. Mortality of the mosquito larvae was recorded after 6, 12, and 24 h. Assays were run in triplicate.

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Supporting Information Available: CIF data for the crystal structure of **2** are available free of charge via the Internet at http://pubs.acs.org.

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(15) Crystallographic data (excluding structure factors) for **2** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 670571. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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