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Tropane Aromatic Ester Alkaloids Obtained from a Large-Scale Recollection of *Erythroxylum pervillei* Stem Bark Collected in Madagascar[#]

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Abstract

Fractionation by pH zone-refining countercurrent chromatography of an extract of the stem bark of *Erythroxylum pervillei*, obtained on a kilogram scale in southern Madagascar, led to the isolation and characterization of four tropane aromatic ester alkaloids as minor constituents, namely, pervilleines G (**5**) and H (**6**), and *cis*-pervilleines B (**7**) and F (**8**). Their structures were determined by spectroscopic data interpretation.

The plant genus *Erythroxylum* (Erythroxylaceae) is constituted by around 200 species and found in tropical regions of South America, Africa, and the island of Madagascar.^{1–3} *Erythroxylum* species are best known for the production of the tropane diester alkaloid, cocaine, but only a few members of this genus accumulate this alkaloid in quantity.³ Besides tropane and other alkaloid derivatives,³ diterpenoids,^{4–6} flavonoids,^{7,8} tannins,^{9,10} and triterpenoids⁸ have also been found in species of the genus *Erythroxylum*. In modern medicine, tropane alkaloid esters from plants in the family Solanaceae are an important group due to their analgesic, anesthetic, anticholinergic, antiemetic, antihypertensive, and parasympholytic effects, with atropine and scopolamine, in particular, being of considerable medicinal value.^{1,2}

In previous work, bioactivity-guided fractionation of a chloroform extract of the roots of *Erythroxylum pervillei* Baillon, collected in southern Madagascar in 1992, led to the isolation of nine tropane alkaloid esters, inclusive of seven new compounds, pervilleines A–F and pervilleine A N-oxide, all bearing a trimethoxycinnamate group at C-6.¹¹ Of these substances, pervilleines A–F were found in a small tumor panel to reverse multidrug resistance (MDR) for the KB-V1 vinblastine-resistant oral epidermoid carcinoma cell line in the presence of vinblastine, while being much less cytotoxic for normal KB cells and other cancer cell lines.^{11,12} The parent alkaloid, pervilleine A [3α -(3,4,5-trimethoxybenzoyloxy)-6 β -(*E*)-(3,4,5-trimethoxycinnamoyloxy)-7 β -tropane] (**1**) was also found to restore the vinblastine sensitivity

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of CEM/VLB₁₀₀ (multidrug-resistant human leukemic lymphoblast CEM) cells as well as the chemosensitivity to colchicine of the KB-8-5 cell line.¹² Pervilleine A (**1**) was shown to be effective as an MDR inhibitory agent in an *in vivo* hollow fiber assay using KB-V1 cells when co-administered with vinblastine, with this tropane alkaloid postulated to act mechanistically by inhibiting P-glycoprotein mediated drug efflux.^{12,13} The MDR inhibitory activities of pervilleines B (**2**), C (**3**), and F (**4**) were confirmed in the *in vivo* hollow fiber assay in an analogous manner for pervilleine A (**1**).^{14,15} Pervilleines A-C (**1–3**) and F (**4**) were of approximately the same yield (0.0042, 0.0035, 0.0043, and 0.0038% w/w, respectively), and were among the major tropane aromatic ester alkaloid constituents from the initial plant collection of *E. pervillei* roots.¹¹

Owing to the promising MDR-inhibitory activities of pervilleines A-C (**1–3**) and F (**4**), which were comparable in potency to the standard MDR inhibitor, verapamil, these compounds were selected for further development through the RAID (Rapid Access to Invention Development) program of the U.S. National Cancer Institute.¹⁶ The primary purpose of this award was to prioritize either pervilleine A (**1**), B (**2**), C (**3**), or F (**4**) for preclinical development as an MDR inhibitor. Accordingly, arrangements were made for a recollection of 50 kg each of the roots and stem bark of *E. pervillei* to be recollected in January, 2003, from the original site of collection in Southern Madagascar, so that gram quantities of the tropane alkaloids of interest could be purified and prioritized biologically. After additional biological testing was conducted, the parent compound, pervilleine A (**1**), was eventually chosen as the best tropane aromatic ester constituent from *E. pervillei* for further development as a potential MDR inhibitor. In the present study, we describe the isolation by pH zone-refining countercurrent chromatography^{17,18} of four minor tropane alkaloid esters from this recollected *E. pervillei* stem bark, namely, pervilleines G (**5**) and H (**6**), and *cis*-pervilleines B (**7**) and F (**8**).

Compound **5** was obtained as an amorphous solid and in its HRESIMS exhibited a sodiated molecular ion at m/z 400.1743, consistent with a molecular formula of C₂₀H₂₇NO₆Na. The ¹H NMR spectrum of **5** revealed signals for two oxymethines at δ 5.14 (H-3 β) and 4.70 (H-6 α), two methines at δ 3.41 and 3.18, and three methylenes at δ 2.71, 2.23–2.32, 2.05, and 1.76, and an *N*-methyl at δ 2.61, assignable to a 3,6-disubstituted tropane alkaloid skeleton.^{11,18} The remaining proton peaks at δ 7.56 (1H, d, J = 15.9 Hz, H-7'), 6.76 (2H, s, H-2'; and 6'), 6.30 (1H, d, J = 15.9 Hz, H-8'), and 3.87–3.91 (3 $\times$ OMe), were indicative of a 3,4,5-trimethoxycinnamoyl (Tmc) group with *trans* geometry. The location of the Tmc group in **5** was positioned at C-3 through an ester linkage by the observed HMBC correlation between δ 5.14 (H-3 β) and δ 166.0 (C-9'). The configuration of **5** was established relative to the nitrogen-containing bridge. A broad H-3 triplet with a coupling constant (4.5 Hz) was consistent with an α -orientation of the acyl moiety substituted at C-3.^{19,20} The H-6 resonance exhibited only two couplings (J = 7.2, 2.4 Hz) with the two protons attached to C-7 in the ¹H-¹H COSY NMR spectrum, and no coupling with H-5. This observation suggested that the dihedral angle of H-5 and H-6 is almost 90 degrees, and hence it was inferred that OH at C-6 is β -oriented.²⁰ Thus, the structure of compound **5** was elucidated as 3 α -(*E*)-(3,4,5-trimethoxycinnamoyloxy)-6 β -hydroxytropane, and has been named as pervilleine G according to a previous convention.¹¹

The molecular formula of **6** was determined as C₂₀H₂₇NO₇, based on the sodiated molecular ion at m/z 416.1668 in the HRESIMS. The ¹H NMR spectrum of **6** displayed distinctive signals at δ 5.14 (1H, t-like brs, H-3 β), 4.60 (2H, s, H-6 α and H-7 α), 3.23 (2H, s, H-1 and H-5), 2.60 (3H, N-Me), 2.32 (2H, brd, J = 15.8 Hz, H-2 α x and H-4 α x), and 1.71 (2H, brd, J = 15.8 Hz, H-2 α eq and H-4 α eq), which together accounted for a 3,6,7-trisubstituted tropane moiety.^{19,21} Other ¹H NMR signals suggested that a Tmc was present in **6**, as was the case for **5**. A long-range correlation between δ_H 5.14 (H-3) and δ_C 165.8 (C-9') in the HMBC spectrum was used to locate the Tmc unit at C-3 of the tropane moiety in **6**. The relative configuration of **6** was established from the splitting pattern of H-3 and the lack of coupling between H-6 and H-5 as

well as between H-7 and H-1. Based on all these data, compound **6** was assigned as [3 α -(*E*)-(3,4,5-trimethoxycinnamoyloxy)-6 β ,7 β -dihydroxytropene], and named pervilleine H.

The sodiated molecular ion of compound **7** was observed in the HRESIMS at m/z 594.2315, corresponding to the molecular formula of C₃₀H₃₇NO₁₀Na⁺. The ¹H NMR spectrum of **7** exhibited the presence of a 3,6-disubstituted tropane skeleton as well as two acyl moieties, namely, a Tmc unit with *cis* configuration [δ 6.83 (1H, d, J = 12.9 Hz, H-7'') and 5.91 (1H, d, J = 12.9 Hz, H-8'')] and a 3,4,5-trimethoxybenzoyl (Tmb) unit with ortho aromatic proton signals at δ 7.36 (2H, s, H-2', and H-6') and three methoxy groups resonating at δ 3.84–3.95.²² The HMBC NMR experiment led to the placement of the Tmc unit at C-6 and the Tmb unit at C-3. Therefore, compound **7** was identified as 3 α -(3,4,5-trimethoxybenzoyloxy)-6 β -(*Z*)-(3,4,5-trimethoxycinnamoyloxy) tropane, or (*cis*)-pervilleine B.

The NMR data of **8** suggested the presence of a phenylacetyl ester group with the characteristic ¹H NMR signals at δ 7.25–7.29 (5H, m, H-2', 3', 4', 5' and 6') and 3.64 (2H, s, H-7'), and their corresponding ¹³C NMR signals at δ 170.5 (C-8'), 133.8 (C-1'), 130.3 (C-3' and 5'), 128.7 (C-2' and 6'), 127.1 (C-4'), and 42.2 (C-7'), in addition to a Tmc unit with *cis*-configuration and 3,6-disubstituted tropane skeleton.^{11,23} The 2D-NMR (¹H-¹H COSY, HMQC and HMBC) analysis of **8** made it possible to determine its structure as (*cis*)-pervilleine F [3 α -phenylacetoxo-6 β -(*Z*)-(3,4,5-trimethoxycinnamoyloxy)tropane].

Compounds **5**–**8** were not tested for their cytotoxic activities in the present study, since they were only obtained as minor constituents from chromatographic column cuts during the preparative isolation of pervilleines A–C (**1**–**3**) and F (**4**). However, from previous structure-cytotoxicity studies of this class of tropane alkaloid esters, it can be predicted that pervilleines G (**5**) and H (**6**) would be inactive as MDR inhibitors, since they lack a *trans*-3,4,5-trimethoxycinnamoyl group at C-6.^{11–15} On the other hand, compounds **7** and **8** with a C-6 *cis*-3,4,5-trimethoxycinnamoyl functionality would be expected to be active when tested against the KB-V1 drug-resistant KB cell line, since the occurrence of *cis* or *trans* stereochemistry within this ester unit has been shown not to affect the resultant cytotoxic activity.²⁴

Experimental Section

General Experimental Procedures

Optical rotations were measured using a Perkin-Elmer 241 automatic polarimeter. UV spectra were obtained with a Beckman DU-7 spectrometer. IR spectra were run on an ATI Mattson Genesis Series FT-IR spectrophotometer. NMR spectroscopic data were recorded at room temperature on Bruker Avance DPX-300 and DRX-400 spectrometers with tetramethylsilane (TMS) as internal standard. Electrospray ionization (ESI) mass spectrometric analyses were performed with a 3-Tesla Finnigan FTMS-2000 Fourier Transform mass spectrometer, and electron-impact (EI) mass spectra were obtained with a Kratos MS-25 mass spectrometer, using 70 eV ionization conditions. High-speed countercurrent chromatography (HSCCC) was carried out using a commercial apparatus (P.C. Inc., Potomac, MD) with an Ito multilayer-coil separation column. Analysis of all fractions was accomplished using HPLC-MS, consisting of a Waters delta 600 pump, a Micromass ZQ electrospray mass spectrometer (cone voltage = 30), a Waters 996 photodiode array spectrometer, and a Sedex 75 evaporative laser light scattering detector. Millennium and FractionLynx software systems were used for data acquisition and processing. Column chromatography was carried out on Purasil[®] (230–400 mesh, Whatman, Clifton, NJ). Analytical thin-layer chromatography (TLC) was performed on precoated 250 μ m thickness Partisil[®] K6F (Whatman) glass plates, while preparative TLC was conducted on precoated 20 $\times$ 20 cm, 500 μ m Partisil[®] K6F (Whatman) glass plates. All solvents used for chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ).

Plant Material

The stem bark of *E. pervillei* was collected in Andranamy, Betioky region, Madagascar (GPS: 23°45'10"S 44°03'27"E, altitude: 400 m) in January 2003. The plant was identified by Armand Rakotozafy, and a voucher specimen (No. IMRA/PR-005/2003) has been deposited at the Institut Malgache de Recherches Appliquées.

Extraction and Isolation

The stem bark of *E. pervillei* (25 kg) was sequentially extracted with CHCl₃, CHCl₃-MeOH, and MeOH. The combined extract (3.0 kg) was washed with hexane, and then partitioned with CHCl₃-MeOH-H₂O, to afford an organic-soluble fraction (370 g). This fraction was subjected to flash column chromatography (silica gel, 5.1 kg) using solvent systems of increasing polarity (CHCl₃-acetone-28% NH₄OAc, 20:10:0.1, 10:15:0.5, 5:20:1, and MeOH), and pooled into three fractions (F1-F3). The alkaloid-containing fraction, F2, was further processed using pH-zone refining HSCCC.^{16,17} For this separation, a biphasic solvent system (methyl *t*-butyl ether-water, 1:1) was applied. The solvent mixture was equilibrated in a separatory funnel at room temperature overnight. The upper (organic) layer was prepared as the stationary phase by adding 2 mL of triethylamine (1.1 L, pH 10). The lower (aqueous) layer for the mobile phase was made acidic by adding 1 mL of 37% hydrochloric acid (0.9 L, pH 2). The Ito coil was filled with the organic stationary phase, then rotated at 750 rpm. The fractions were dissolved in the basic organic stationary phase and injected onto the HSCCC apparatus. The aqueous mobile phase was pumped in at a flow rate of 3 mL/min. Alkaloid fractions were combined into one pool and then fractionated into two alkaloidal fractions, F2A (mainly pervilleine A) and F2B (other pervilleines), via HPLC-MS (Waters Xterra MSC₁₈ column, 7 μm, 19 × 300 mm, MeCN-20 mM NH₄OAc pH 8.5 = 45:55, isocratic, 10 mL/min).

Fraction F2B (8.9 g) was chromatographed over a silica gel column (90 × 300 mm), using a gradient of increasing polarity with CHCl₃ and MeOH as solvents, and afforded six sub-fractions (F2B01-F2B06). Fraction F2B02 (1.2 g) was subjected to silica gel column chromatography (40 × 350 mm, *n*-hexane-EtOAc-diethylamine = 7:3:0.5) and pooled into eight sub-fractions (FB0201-FB0208). From F2B0204, *cis*-pervilleine B (**7**, 2.9 mg, 0.0000116%) was obtained during the purification using silica gel column chromatography (*n*-hexane-EtOAc-diethylamine, 8:2:0.5). Fraction F2B03 (6.8 g) was passed over a silica gel column (40 × 350 mm, *n*-hexane-EtOAc-diethylamine, 7:3:0.5) and pooled into seven sub-fractions (F2B0301-F2B0307). Sub-fraction F2B0302 was fractionated into ten further sub-fractions using silica gel column chromatography (26 × 350 mm, *n*-hexane-EtOAc-diethylamine, 8:2:0.5). Sub-fraction F2B030209 was purified by preparative TLC (500 μm thickness layer, *n*-hexane-EtOAc-diethylamine, 6:4:0.5, *R_f* 0.38) and yielded pervilleine G (**5**, 1.9 mg, 0.0000076%). Sub-fraction F2B030210 was purified by preparative TLC (500 μm thickness layer, *n*-hexane-EtOAc-diethylamine, 6:4:0.5, *R_f* 0.15) and yielded pervilleine H (**6**, 10 mg, 0.00004%). *cis*-Pervilleine F (**8**, 6.0 mg, 0.000024%) was isolated from F2B0303 using silica gel column chromatography (36 × 250 mm, *n*-hexane-EtOAc-diethylamine, 8:2:0.5).

Pervilleine G (5)

Amorphous solid: [α]_D +24.5° (*c* 0.19, CHCl₃); UV (CHCl₃) $\lambda_{\max}$ (log ϵ) 311 (4.42) nm; IR (dried film) $\nu_{\max}$ 3446, 2936, 1715, 1635, 1577, 1506, 1457, 1276, 1246, 1126 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 7.56 (1H, d, *J* = 15.9 Hz, H-7'), 6.76 (2H, s, H-2' and H-6'), 6.30 (1H, d, *J* = 15.9 Hz, H-8'), 5.14 (1H, brt, *J* = 4.5 Hz, H-3 β), 4.70 (1H, dd, *J* = 7.2, 2.4 Hz, H-6 α), 3.87–3.91 (3 × OMe), 3.41 (1H, brd, *J* = 7.2 Hz, H-1), 3.18 (1H, brs, H-5), 2.71 (1H, dd, *J* = 13.8, 7.5 Hz, H-7 α), 2.61 (3H, N-Me), 2.23–2.32 (3H, m, H-2ax and H-4ax), 2.05 (1H, m, H-7 β), 1.76 (1H, brd, *J* = 16.2 Hz, H-4eq), 1.60 (1H, brd, *J* = 16.2 Hz, H-2 eq); ¹³C NMR (CDCl₃, 75 MHz) δ 166.0 (C-9'), 153.5 (C-1', C-4' and C-5'), 145.0 (C-7'), 129.7 (C-1'), 117.4 (C-8'), 105.3 (C-2' and C-6'), 75.6 (C-6), 67.0 (C-3), 66.9 (C-5), 61.0 (OMe-4'), 58.3 (C-1),

56.3 (OMe-3' OMe-5'), 40.5 (C-7), 35.8 (N-Me), 29.9 (C-2), 28.5 (C-4); HRESIMS m/z 400.1743 (calcd for $C_{20}H_{27}NO_6Na^+$, 400.1731).

Pervilleine H (6)

Amorphous solid: $[\alpha]_D^{20}$ (c 0.40, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 312 (4.15) nm; IR (dried film) ν_{max} 3420, 2938, 1704, 1652, 1583, 1506, 1456, 1276, 1249, 1126 cm^{-1} ; 1H NMR ($CDCl_3$, 300 MHz) δ 7.56 (1H, d, $J = 15.9$ Hz, H-7'), 6.77 (2H, s, H-2, and 6'), 6.30 (1H, d, $J = 15.9$ Hz, H-8'), 5.14 (1H, *t*-like brs, H-3 β), 4.60 (2H, s, H-6 α and H-7 α), 3.87–3.92 (3 $\times$ OMe), 3.23 (2H, s, H-1 and 5), 2.60 (3H, N-Me), 2.32 (2H, brd, $J = 15.8$ Hz, H-2ax and 4ax), 1.71 (2H, brd, $J = 15.8$ Hz, H-2eq and 4eq); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.8 (C-9'), 153.4 (C-3', C-4' and C-5'), 145.4 (C-7'), 129.5 (C-1'), 117.0 (C-8'), 105.4 (C-2' and C-6'), 74.1 (C-6 and C-7), 66.4 (C-3), 65.8 (C-1 and C-5), 60.9 (OMe-4'), 56.2 (OMe-3', OMe-5'), 34.4 (N-Me), 26.5 (C-2 and C-4); HRESIMS m/z 416.1668 (calcd for $C_{20}H_{27}NO_7Na^+$, 416.1680).

(cis)-Pervilleine B (7)

Amorphous solid: $[\alpha]_D^{25.2}$ (c 0.28, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 299 (4.16) nm; IR (dried film) ν_{max} 2938, 1711, 1582, 1505, 1456, 1415, 1329, 1220, 1127 cm^{-1} ; 1H NMR ($CDCl_3$, 300 MHz) δ 7.36 (2H, s, H-2' and 6'), 7.00 (2H, s, H-2'' and 6''), 6.83 (1H, d, $J = 12.9$ Hz, H-7''), 5.91 (1H, d, $J = 12.9$ Hz, H-8''), 5.66 (1H, dd, $J = 7.5, 3.0$ Hz, H-6 α), 5.31 (1H, brt, $J = 5.4$ Hz, H-3 β), 3.84–3.95 (6 $\times$ OMe), 3.37 (1H, brd, $J = 4.2$ Hz, H-1), 3.22 (1H, brs, H-5), 2.72 (1H, dd, $J = 13.5, 7.2$ Hz, H-7 α), 2.49 (3H, N-Me), 2.18–2.33 (3H, m, H-2 ax, H-4ax and H-7 β), 1.90 (1H, brd, $J = 15.0$ Hz, H-4eq), 1.72 (1H, brd, $J = 15.0$ Hz, H-2eq); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.7 (C-9''), 165.3 (C-7'), 153.1 (C-3', C-4' and C-5'), 152.8 (C-3'', C-4'' and C-5''), 143.5 (C-7''), 130.2 (C-1''), 125.3 (C-1'), 119.1 (C-8''), 107.9 (C-2' and C-6'), 106.6 (C-2'' and C-6''), 78.4 (C-6), 67.6 (C-3), 64.9 (C-5), 60.9 (OMe-4', OMe-4''), 59.3 (C-1), 56.3* (OMe-3', OMe-5'), 56.2* (OMe-3'', OMe-5''), 38.7 (N-Me), 37.1 (C-7), 32.6 (C-2), 31.2 (C-4) * (assignments are exchangeable); HRESIMS m/z 594.2315 (calcd for $C_{30}H_{37}NO_{10}Na^+$, 594.2310).

(cis)-Pervilleine F (8)

Amorphous solid: $[\alpha]_D^{+4.0}$ (c 0.23, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 308 (4.18) nm; IR (dried film) ν_{max} 2938, 1716, 1577, 1506, 1456, 1243, 1128 cm^{-1} ; 1H NMR ($CDCl_3$, 300 MHz) δ 7.25–7.29 (5H, m, H-2', H-3', H-4', H-5' and H-6'), 7.08 (2H, s, H-2'' and H-6''), 6.83 (1H, d, $J = 12.9$ Hz, H-7''), 5.91 (1H, d, $J = 12.9$ Hz, H-8''), 5.31 (1H, dd, $J = 7.5, 3.3$ Hz, H-6 α), 5.02 (1H, brt, $J = 5.4$ Hz, H-3 β), 3.88–3.91 (3 $\times$ OMe), 3.64 (2H, s, H-7'), 3.20 (1H, brd, $J = 7.5$ Hz, H-1), 3.08 (1H, brs, H-5), 2.42 (1H, s, N-Me), 2.11–2.21 (4H, m, H-2ax, H-4ax, and H-7), 1.75 (1H, brd, $J = 14.7$ Hz, H-4eq), 1.45 (1H, brd, $J = 14.7$ Hz, H-2eq); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.5 (C-8'), 165.9 (C-9''), 152.7 (C-3'', C-4'' and C-5''), 143.4 (C-7''), 133.8 (C-1'), 130.3 (C-3' and C-5'), 129.3 (C-1''), 128.7 (C-2' and C-6'), 127.1 (C-4'), 119.3 (C-8''), 107.9 (C-2'' and C-6''), 79.0 (C-6), 67.5 (C-3), 65.0 (C-1), 60.9 (OMe-4''), 59.0 (C-5), 56.2 (OMe-3'', OMe-5''), 42.2 (C-7'), 38.6 (N-Me), 35.9 (C-7), 32.8 (C-2), 31.3 (C-4); HREIMS m/z 495.2244 (calcd for $C_{28}H_{33}NO_7^+$, 495.2252).

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References and Notes

1. Fodor, G. Rodd's Chemistry of Carbon Compounds. 2. Sainsbury, M., editor. 4. Elsevier Science; New York: 1997. p. 251-276.
2. Christen, P. Studies in Natural Products Chemistry, Volume 22, Bioactive Natural Products (Part C). Atta-ur-Rahman, editor. Elsevier Science; New York: 2000. p. 717-749.
3. Griffin WJ, Lin GD. *Phytochemistry* 2000;55:623-637. [PubMed: 10746874]
4. Ansell SM, Pegel KH, Taylor DAH. *Phytochemistry* 1993;32:937-943.
5. Ansell SM, Pegel KH, Taylor DAH. *Phytochemistry* 1993;32:945-952.
6. Ansell SM, Pegel KH, Taylor DAH. *Phytochemistry* 1993;32:953-959.
7. Johnson EL, Schmidt WF, Emche SD, Mossoba MM, Musser SM. *Biochem System Ecol* 2003;31:59-67.
8. Chávez JP, Dos Santos ID, Gruz FG, David JM. *Phytochemistry* 1996;41:941-943.
9. Kolodziej H, Bonefeld M, Burger JFW, Brandt EV, Ferreira D. *Phytochemistry* 1991;30:1255-1258.
10. Bonefeld M, Friedrich H, Kolodziej H. *Phytochemistry* 1986;25:1205-1207.
11. Silva GL, Cui B, Chávez D, You M, Chai H, Rasoaiaivo P, Lynn SM, O'Neill MJ, Lewis JA, Besterman JM, Monks A, Farnsworth NR, Cordell GA, Pezzuto JM, Kinghorn ADJ. *Nat Prod* 2001;64:1514-1520.
12. Mi Q, Cui B, Lantvit D, Lim E, Chai H, You M, Hollingshead MG, Mayo JG, Kinghorn AD, Pezzuto JM. *Cancer Res* 2001;61:4030-4037. [PubMed: 11358822]
13. Mi Q, Cui B, Chávez A, Chai B, Zhu H, Cordell GA, Hedayat S, Kinghorn AD, Pezzuto JM. *AnticancerRes* 2002;22:1385-1398.
14. Mi Q, Cui B, Silva GL, Lantvit D, Lim E, Chai H, Hollingshead MG, Mayo JG, Kinghorn AD, Pezzuto JM. *Cancer Lett* 2002;184:13-20. [PubMed: 12104043]
15. Mi Q, Cui D, Lantvit E, Reyes-Lim H, Chai H, Pezzuto JM, Kinghorn AD, Swanson S. M. *AnticancerRes* 2003;23:3607-3616.
16. http://dtp.nci.nih.gov/docs/raid/raid_index.html
17. Weiz A, Andrzejewski D, Ito Y. *Chromatogr A* 1994;678:77-84.
18. Chadwick LR, Wu CD, Kinghorn ADJ. *Liq Chromatogr Rel Technol* 2001;24:2445-2453.
19. Al-Said MS, Evans WC, Grout RJ. *Phytochemistry* 1989;11:3211-3215.
20. Zanolari B, Guilet D, Marston A, Queiroz EF, Paulo MQ, Hostettmann KJ. *Nat Prod* 2003;66:497-502.
21. Al-Said MS, Evans WC, Grout RJJ. *Chem Soc Perkin Trans I* 1986:957-959.
22. Payo-Hill AL, Saruy-Dominguez R, Suarez MO, Batista-Baez M, Velez-Castro HT, Rastrelli L, Aquino R. *Phytochemistry* 2000;54:927-932. [PubMed: 11014291]
23. Khattak KF, Atta-ur-Rahman Choudhary ML, Hemalal KD, Tillekeratne LMJ. *Nat Prod* 2002;65:929-931.
24. Chávez D, Cui B, Chai HB, García R, Mejía M, Farnsworth NR, Cordell GA, Pezzuto JM, Kinghorn ADJ. *Nat Prod* 2002;65:606-610.

		R ₁	R ₂
1	Pervilleine A	Tmb	OH
2	Pervilleine B	Tmb	H
3	Pervilleine C	Tmc	H
4	Pervilleine F	PhCH ₂ CO	H

1. .

5

6

7

8

2. .