Dihydroagarofuran Sesquiterpene Alkaloids from *Maytenus putterlickoides*

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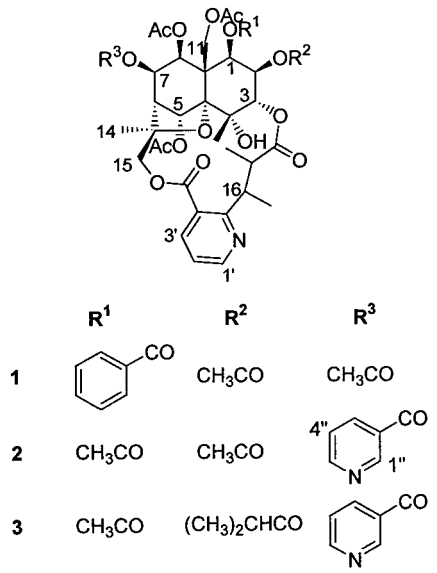
Two new dihydroagarofuran sesquiterpene alkaloids, putterines A (**2**) and B (**3**), and a known alkaloid, mayteine (**1**), were isolated from the root of *Maytenus putterlickoides*. The structures of these compounds were elucidated by various spectroscopic techniques, including ^{1}H and ^{13}C NMR, COSY, ROESY, HMBC, and high-resolution FABMS.

The Celastraceae family contains many plants that have been studied extensively due to their use in folkloric medicine. In particular, plants of the genus *Maytenus*, a widely distributed member of the Celastraceae family, have been used as a treatment for cancer in Africa, as an insecticide in Asia, and by shepherds on the Canary Islands to battle fatigue.[1−3] A diverse group of secondary metabolites, maytansinoids, quinone-methide triterpenoids, and dihydroagarofuran sesquiterpene alkaloids, are responsible for the various biological activities of plants in this genus.[1,4,5] Ethanolic extracts of the roots of *Maytenus putterlickoides* (Celastraceae) had previously demonstrated significant activity against the P388 lymphocytic leukemia in vivo and the 9KB cell culture system, but were not actively fractionated. More recently, extracts of the roots of *M. putterlickoides* were found to demonstrate inhibition of protein kinase C activity, as well as lethality against brine shrimp. These activities prompted reexamination of the extracts in order to isolate the compounds responsible for the activity.

A number of sesquiterpene alkaloids, derived from the dihydroagarofuran nucleus, have been isolated from the Celastraceae family. One group of these sesquiterpene alkaloids is characterized by a pyridine dicarboxylic acid macrocyclic bridge (e.g., evoninic acid, wilfordic acid, hydroxywilfordic acid, etc.) linked via two ester moieties at the C-3 and C-15 positions.[4] The isolation of dihydroagarofuran sesquiterpene alkaloids, mayteine (**1**) and 6-benzoyl-6-deacetylmayteine from *Maytenus krukovii*[6] and 5-benzoyl-5-deacetylwilfordine from *Maytenus buchananii*,[7] prompted specific examination of the extract of the root material from *M. putterlickoides* for similar alkaloids. From this work, mayteine (**1**) and two new dihydroagarofuran sesquiterpene alkaloids, putterine A (**2**) and putterine B (**3**), have been isolated and characterized.

A CH_2Cl_2-soluble fraction from the ethanolic Soxhlet extract of the roots of *M. putterlickoides* was separated initially by silica gel column chromatography. Further separation was carried out by PTLC to give the dihydroagarofuran sesquiterpene alkaloids **1**, **2**, and **3**.

The basic structure of **1** was determined from comparison of the ^{1}H and ^{13}C NMR data with data of known alkaloids. The molecular formula $C_{43}H_{49}NO_{18}$ was determined from the parent ion at *m*/*z* 868.3023 (M^+ + H) in the high-resolution FABMS. From these data, **1** was determined to be mayteine, previously isolated from *M. guianensis* and *M. krukovii*.[6,8] The identification was confirmed by comparison to an authentic sample.



Putterine A (**2**) (7β-nicotinoyl-7-deacetyleuonymine) was shown to have the molecular formula $C_{42}H_{48}N_2O_{18}$ by analysis of the FAB mass spectrum (parent ion at *m*/*z* 869.2996 (M^+ + H)). The ^{1}H NMR spectrum showed the presence of five acetyl groups (δ 1.84, 1.99, 2.09, 2.16, and 2.23) and one nicotinoyl ester moiety (δ 7.46 (dd), 8.40 (ddd), 8.85 (dd), and 9.36 (d)). There were also resonances for two methyl groups on quaternary carbons, two oxymethylene groups, and seven methine protons, all of which could be attributed to a dihydroagarofuran nucleus. The ^{1}H−^{1}H COSY spectra of **2** revealed that six of the methine protons existed as two sets of three coupling partners at δ 5.59 (d), 5.25 (dd), and 4.74 (d) and at δ 2.55 (d), 5.70 (dd), and 5.48 (d). These resonances were assigned to the C-1, C-2, and C-3 protons and the C-6, C-7, and C-8 protons on the dihydroagarofuran nucleus, respectively, based upon comparison to mayteine (**1**) and other examples. The remaining methine proton (δ 7.15, s) was assigned to C-5. The diacid moiety of **2** was determined to be evoninic acid. Two methyl doublets at δ 1.21 and 1.40 (C-18 and C-19, respectively) were coupled to methine protons at δ 2.59 (C-17H, q) and 4.65 (C-16H, q), respectively. The three aromatic protons of the pyridine moiety of evoninic acid were found at δ 7.28 (dd), 8.08 (dd), and 8.70 (dd).

The ^{1}H−^{13}C HMBC spectra of **2**, in combination with data from the literature, were used to determine placement of the five acetyl groups and the one nicotinoyl group. From the chemical shifts of the C-1, C-2, C-5, C-7, C-8, and C-11 protons, all of these sites must bear esters. The HMBC spectrum showed clear correlations between the protons

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on C-1, C-2, C-5, and C-8 and acetyl carbonyl resonances, indicating that these carbons bore acetyl moieties. The deshielding of the C-7 proton (δ 5.70) in the spectrum of **2**, as compared to the C-7 proton of euonymine (δ 5.51), indicated that the nicotinoyl ester must be located at the C-7 position.[9] (Euonymine has all acetyl moieties as the ester substituents on the dihydroagarofuran nucleus.) This conclusion was supported by similar deshielding effects on the C-6 and C-8 protons of **2**, again as compared to the C-6 and C-8 protons of euonymine. Thus, the remaining acetyl moiety in **2** was sited at C-11.

Coupling constants and ROESY experiments served to determine the relative stereochemistry around the dihydroagarofuran moiety. The coupling constant between the C-7 proton and the C-6 proton, which is β as a requirement of the structure, is 3.9 Hz, suggesting that both protons are equatorial. The protons at C-7 and C-8 show a coupling constant of 5.9 Hz, indicating a *syn* relationship, and both the C-7 and C-8 protons correlate through space to the C-14 methyl protons. Thus, both the C-7 and C-8 protons must be α. The C-6 proton shows a ROESY correlation to the C-5 proton, but no vicinal coupling, indicating that these two protons must have a *syn* relationship. The C-5 proton also shows a ROESY correlation to the C-4 methyl group, indicating that the C-4 methyl must also be β. The C-3 proton must also be β as a requirement of the structure, and this resonance shows a ROESY correlation to the C-4 methyl resonance. The coupling constant of 2.4 Hz between the C-3 and C-2 protons indicates a diequatorial relationship, and the coupling constant of 3.9 Hz between the C-2 and C-1 protons indicates a *syn* relationship between these protons. These data are consistent with the data for mayteine (**1**), which has the same stereochemistry around the dihydroagarofuran moiety, and verify the structure of putterine A (**2**).

Putterine B (**3**) (2-isobutyroyl-7β-nicotinoyl-2,7-deacetyleuonymine) was shown to have the molecular formula $C_{44}H_{52}N_2O_{18}$ by analysis of the FAB mass spectrum (parent ion at *m/z* 897.3293 ($M^+ + H$)). The ^{1}H NMR and COSY spectra again indicated the presence of a dihydroagarofuran sesquiterpene alkaloid with the diacid segment derived from evoninic acid (two methyl doublets at δ 1.21 and 1.40 (C-18 and C-19, respectively), correlated to two methine protons at δ 2.56 (q, C-17) and 4.64 (q, C-16)). Six ester moieties were present as four acetyl groups (δ 1.84, 1.99, 2.16, and 2.24), one nicotinoyl group (ring protons at δ 9.34 (d), 8.85 (dd), 8.42 (dd), and 7.46 (dd)), and one isobutyrate group (two methyl groups at δ 0.72 and 1.14 correlated to a methine proton at δ 2.55). The remaining resonances could be assigned to protons on the dihydroagarofuran moiety by COSY and ROESY experiments, and the relative stereochemistry was determined from coupling constant data to be identical to that in **1** and **2**.

HMBC experiments at 300 MHz placed acetyl groups at the C-1, C-5, and C-8 positions, and the nicotinoyl group was assigned to the C-7 position due to deshielding of the C-6, C-7, and C-8 protons. As with the spectra of **2**, shielding of the C-1 or C-2 protons did not occur. Final placement of the last acetyl and the isobutyrate groups at C-2 or C-11 was determined from an HMBC experiment at 500 MHz. In this experiment, correlation of the carbonyl carbon of the isobutyrate ester moiety with the C-2 proton was observed. From these data, the isobutyrate group was assigned to C-2 and the remaining acetate to C-11. Thus, the structure of putterine B was confirmed as **3**.

Putterines A (**2**) and B (**3**) are two further variations of the many sesquiterpene nicotinoyl alkaloids routinely isolated from plants in the Celastraceae family. They are not responsible for the PKC inhibition activity found in extracts of *M. putterlickoides*, but do have marginal brine shrimp toxicity.

Experimental Section

General Experimental Procedure. IR spectra were obtained on a Perkin-Elmer 1600 FTIR as KBr pellets. NMR spectra were recorded at Virginia Commonwealth University on a Varian Mercury spectrometer at 300 MHz or a Varian Unity 500 MHz spectrometer in $CDCl_3$. Low- and high-resolution FAB mass spectra were obtained at the Virginia Commonwealth University Mass Spectrometry Center on a JEOL HX110 double focusing sector mass spectrometer in an *n*-butyl acetate matrix. Column chromatography employed 60−200 mesh silica gel (J. T. Baker), and preparative TLC was carried out on Baker Si250F TLC plate-silica gel plates. The compounds were visualized on TLC plates by short (λ = 254 nm) and long (λ = 366 nm) wave ultraviolet light. Solvents were reagent grade and used as purchased.

Plant Material. Roots of *Maytenus putterlickoides* (Exell and Mendonca) (PR-36417) were collected in Kenya in 1973 and were supplied by the Medicinal Plant Resources Laboratory, USDA, Beltsville, MD, where voucher specimens are preserved.

Extraction and Isolation. Dried, ground roots (8.0 kg) of *M. putterlickoides* were extracted twice with 8 L of EtOH in a Soxhlet apparatus for 24 h. The EtOH was removed in vacuo to give approximately 130 g of a dark red, tar-like solid. The ethanolic concentrate was then subjected to a 1:1 CH_2Cl_2−$_2$O (700:700 mL) partition. The H_2O layer was extracted three more times with 700 mL of CH_2Cl_2. The CH_2Cl_2 layers were combined and concentrated in vacuo to give 30 g of material as a dark brown solid. An aliquot of the CH_2Cl_2 concentrate (12.0 g) was then subjected to gradient CC over silica gel in CH_2Cl_2 followed by increasing amounts of MeOH in CH_2Cl_2. A fraction eluted with 1% MeOH in CH_2Cl_2 (926.8 mg) was determined to contain alkaloidal material. An aliquot of this fraction (80 mg) was subjected to preparative TLC over silica gel eluted with EtOAc to give the alkaloidal material concentrated in a single band (12.4 mg). This band was then subjected to preparative TLC over silica gel developed with 2% MeOH in CH_2Cl_2 to give mayteine (**1**, 3.0 mg), putterine A (**2**, 1.5 mg), and putterine B (**3**, 2.0 mg).

Mayteine (1): white amorphous solid (3.0 mg); identical to sample obtained from *M. krukovii*.[6]

Putterine A (2): white amorphous solid (1.5 mg); IR (KBr) ν_{max} 3462, 2938, 1752, 1370, 740; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.20 (3H, d, J = 7.2 Hz, CH_3-18), 1.40 (3H, d, J = 7.2 Hz, CH_3-19), 1.52 (3H, s, CH_3-12), 1.74 (3H, s, CH_3-14), 1.84 (3H, s, OAc), 1.99 (3H, s, OAc), 2.09 (3H, s, OAc), 2.16 (3H, s, OAc), 2.23 (3H, s, OAc), 2.55 (1H, d, J = 3.9 Hz, H-6), 2.59 (1H, q, J = 7.2 Hz, H-17), 3.74 (1H, $^1/_2$ ABq, J = 12.0 Hz, H-15), 4.34 (1H, $^1/_2$ ABq, J = 13.5 Hz, H-11), 4.65 (1H, q, J = 6.9 Hz, H-16), 4.80 (1H, d, J = 2.4 Hz, H-3), 5.25 (1H, dd, J = 2.4,3.9 Hz, H-2), 5.34 (1H, $^1/_2$ ABq, J = 13.5 Hz, H-11), 5.48 (1H, d, J = 5.9 Hz, H-8), 5.59 (1H, d, J = 3.9 Hz, H-1), 5.70 (1H, dd, J = 3.9, 5.9 Hz, H-7), 5.96 (1H, $^1/_2$ ABq, J = 12.0 Hz, H-15), 7.15 (1H, s, H-5), 7.28 (1H, dd, J = 4.8, 7.8 Hz, H-2′), 7.48 (1H, dd J = 4.8, 7.8 Hz, H-4″), 8.08 (1H, dd J = 1.8, 7.8 Hz, H-3′), 8.40 (1H, ddd, J = 1.8, 1.8, 7.8 Hz, H-3″), 8.70 (1H, dd, J = 1.8, 4.8 Hz, H-1′), 8.85 (1H, dd, J = 1.8, 4.8 Hz, H-5″), 9.36 (1H, d, J = 1.8 Hz, H-1″); HRFABMS *m/z* 869.2996 ($M^+ + H$) (calcd for $C_{42}H_{49}N_2O_{18}$, 869.2980).

Putterine B (3): white amorphous solid (2.0 mg); IR (KBr) ν_{max} 3504, 2923, 1751, 1370, 744; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.72 (3H, d, J = 6.9 Hz, CH_3CHCO), 1.14 (3H, d, J = 6.9 Hz, CH_3CHCO), 1.21 (3H, d, J = 7.2 Hz, CH_3-18), 1.40 (3H, d, J = 6.9 Hz, CH_3-19), 1.55 (3H, s, CH_3-12), 1.72 (3H, s, CH_3-14), 1.84 (3H, s, OAc), 1.99 (3H, s, OAc), 2.16 (3H, s, OAc), 2.24 (3H, s, OAc), 2.55 (1H, m, J = 6.9 Hz, CH_3CHCO), 2.56 (1H, q, J = 6.9 Hz, H-17), 2.57 (1H, d, J = 3.9 Hz, H-6), 3.74 (1H, $^1/_2$ ABq, J = 11.4 Hz, H-15), 4.35 (1H, $^1/_2$ ABq, J = 14.1 Hz,

H-11), 4.64 (1H, q, $J = 7.2$ Hz, H-16), 4.75 (1H, d, $J = 2.5$ Hz, H-3), 5.24 (1H, dd, $J = 2.5$,3.9 Hz, H-2), 5.22 (1H, $^1/_2$ ABq, $J = 14.1$ Hz, H-11), 5.40 (1H, d, $J = 6.3$ Hz, H-8), 5.62 (1H, d, $J = 3.9$ Hz, H-1), 5.69 (1H, dd, $J = 3.9$, 6.3 Hz, H-7), 5.91 (1H, $^1/_2$ ABq, $J = 11.4$ Hz, H-15), 7.02 (1H, s, H-5), 7.28 (1H, dd, $J = 4.8$, 7.8 Hz, H-2′), 7.46 (1H, dd $J = 4.8$, 7.8 Hz, H-4″), 8.08 (1H, dd $J = 1.8$, 7.8 Hz, H-3′), 8.42 (1H, ddd, $J = 1.8$, 1.8, 7.8 Hz, H-3″), 8.70 (1H, dd, $J = 1.8$, 4.8 Hz, H-1′), 8.85 (1H, dd, $J = 1.8$, 4.8 Hz, H-5″), 9.34 (1H, d, $J = 1.8$ Hz, H-1″); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 10.01 (C-19), 12.25 (C-18), 18.93 (C-14), 18.94 (^{i}Bu CH_3), 19.50 (^{i}Bu CH_3), 20.71 (C-1 OAc), 20.87 (C-8 OAc), 21.23 (C-5 OAc), 21.29 (C-11 OAc), 23.29 (C-12), 33.91 (^{i}Bu CH), 36.73 (C-16), 45.10 (C-17), 51.31 (C-6), 52.52 (C-9), 60.94 (C-11), 69.00 (C-2), 69.95 (C-15), 70.76 (C-4), 71.15 (C-1), 73.46 (C-8), 73.47 (C-7), 74.52 (C-5), 75.87 (C-3), 84.10 (C-13), 93.85 (C-10), 121.39 (C-2′), 123.74 (C-2″), 125.30 (C-4′), 126.01 (C-4″), 137.65 (C-3″), 138.05 (C-3′), 151.17 (C-5″), 151.71 (C-1′), 154.02 (C-1″), 165.21 (C-7 CO), 165.40 (C-5′), 168.63 (C-21), 168.90 (C-11 CO), 169.35 (C-1 CO), 169.38 (C-8 CO), 169.65 (C-11 CO), 170.09 (C-5 CO), 174.15 (C-20), 177.62 (C-2 CO); HRFABMS *m*/*z* 897.3293 ($M^+ + H$) (calcd for $C_{44}H_{53}N_2O_{18}$, 897.3290).

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