Pyridine Alkaloids from a *Parthenium* Hybrid

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Parthenium hybrid, Asteraceae, Pyridine Alkaloids

Two pyridine alkaloids were isolated from the derubberized resin of the hybrid *Parthenium argentatum* × *P. tomentosa*. These alkaloids are (±)-N-[4-(1-aminoethyl) phenyl]-4-[3-methylbutenylidine]-1, 4-dihydropyridine (guayulamine A) and (±)-N-[4-(1-aminoethyl) phenyl]-4-[4-methylpentenylidine]-1, 4-dihydropyridine (guayulamine B). The structures were established by one- and two-dimensional NMR spectroscopy and mass spectrometry.

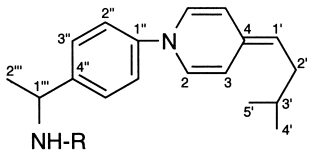
Introduction

Parthenium argentatum (guayule), Asteraceae is being developed for its natural rubber (Whiteworth and Whitehead, 1991). As an ongoing part of our investigation of secondary metabolites from guayule resin, we detected the presence of a homologous mixture of two alkaloids in the hybrid *Parthenium argentatum* × *P. tomentosa*. The literature contains only a few reports on the occurrence of alkaloids in the Asteraceae family. The most common alkaloids are pyridine, piperidine, pyrrolidine, pyrrolizidine, quinoline, quinolizidine, tropane and diterpene alkaloids (Gibbis, 1974; Heywood *et al*., 1977). This report describes the isolation and the structural elucidation of two new pyridine alkaloids, guayulamine A and B.

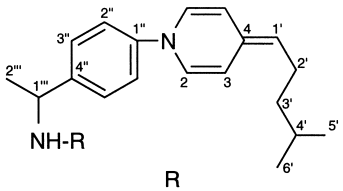
Results and Discussion

During our investigation of guayule resin for antifungal agents (Maatooq *et al*., 1996), a bright red spot was observed on TLC after spraying with cerium sulfate spray reagent. Acetylation of this material resulted in a TLC spot with a lower R_f and the color reaction with Ce^{IV} changed from red to greenish-blue. Flash column reversed phase chromatography and preparative HPLC afforded the separation of each spot (before and after acetylation) into two compounds. All compounds were soluble in aqueous HCl and precipitated upon the addition of NaOH. Furthermore, they gave a pale reddish-brown color with Dragendorff's spray reagent. In the EIMS, the parent ion peak in both compounds was even. These data indicated the possible presence of an alkaloids with even numbers of nitrogen atoms.

The analysis of the spectral data of **1** indicated its structure is (±)-N-[4-(1-aminoethyl) phenyl]-4-[3-methylbutenylidine]-1, 4-dihydropyridine. The EIMS of **1** gave a parent ion peak at *m/z* of 268 (48%), while its acetate **1a** gave *m/z* 310 (100%). The HREIMS of **1a** gave *m/z* 310.2042 which indicated an empirical formula of $C_{20}H_{26}N_2O$ (calcd., 310.2046) and conclude that **1** should be $C_{18}H_{24}N_2$.



	R
1	H
1a	Ac



	R
1	H
1a	Ac

The *m/z* 269 (10%) and 270 (1%) in **1** MS-spectrum and the *m/z* 311 (22%) and 312 (2%), in **1a** MS-spectrum, were assigned to the corresponding M+1 and M+2, respectively. These observations indicated that M+1 and M+2 are representing 20% and 2% of the parent ion peaks, respectively, in both **1** and **1a**. This finding is supporting the likely presence of two nitrogen atoms in the molecule, since the calculated% of M+1 and M+2 are 20.52% and 1.91% of the parent ion peak, respectively, in both **1** and **1a** (Silverstien *et al*., 1991). The ^{13}C-NMR data of **1** and **1a** (Table I) indicated the likely absence of any oxygenation including acetylatable hydroxyl groups. The conversion of **1** to **1a** under acetylation conditions indicated the likely presence of a free NH_2 group in **1** to give the corresponding acetamide, **1a**. The ^{13}C-NMR spectrum of **1** displayed thirteen carbon signals. The DEPT experiment discriminated them into fourteen different signals, represented by three methyl groups at 21.06, 22.54 and 22.98 ppm, one methylene group at 46.9 ppm, five protonated olefinics at 113.92, 114.64, 118.52, 124.06 and 129.12 ppm, two aliphatic methin groups at 25.05 and 46.90 ppm and three non-protonated olefinics at 131.95, 144.04 and 146.29 ppm. The olefinic methin signals at 113.92, 114.64, 124.06 and 129.12 ppm each was accounted for two symmetrical carbon atoms. This conclusion based on their high intensity and the ^{1}H-NMR profile. The ^{1}H-NMR of **1** demonstrated the presence of nine olefinic protons, represented by one pair of doublets at 6.58 and 6.98 ppm (two protons each), four overlapping doublets at 6.81, 6.82, 7.16 and 7.18 ppm (one proton each) and one triplet at 6.75 ppm (for one proton). The COSY correlation indicated that the doublet at 6.58 ppm is coupled to the doublet at 6.98 ppm, which were correlated to (HETCOR) the carbon signals at 129.12 and 113.92 ppm, respectively, and were assigned to (2, 6) and (3, 5)-positions of the 1, 4-dihydropyridine ring, respectively. The doublets at 6.81 and 6.82 ppm were correlated to (HETCOR) the carbon signal at 114.64 and were interacting with (COSY) the doublets at 7.16 and 7.18 ppm, which was correlated to (HETCOR) the carbon signal at 124.06 ppm, were assigned to (6′′, 2′′) and (3′′, 5′′)-positions of the 1, 4-disubstituted benzene ring. The non-protonated olefinic carbon signal at 146.29 ppm was assigned to the nitrogen bonded 1′′-position, while that at 131.95 ppm was assigned to 4′′-position, likely connected to a side chain. The 144.04 ppm signal has to be assigned to 4-position of the pyridine ring formng an exocyclic double bond. This was confirmed by the presence of only one more olefinic carbon signal at 118.52 ppm. This carbon signal was correlated to (HETCOR) the proton triplet at 6.75 ppm and was assigned to 1′-position. The appearance of this proton signal as a triplet indicated that its likely attachment to the methylene carbon signal at 46.90 ppm which is correlated to (HETCOR) the proton multiplets at 1.26 and 1.47 ppm. This was confirmed by COSY interactions of these positions (Fig. 1).

Table I. ^{13}C-NMR data for compounds **1**, **la**, **2** and **2a***.

C#	**1**	**1a**	**2**	**2a**
2	129.12 d	130.75 d	129.15 d	130.83 d
3, 5	113.92 d	116.42 d	113.97 d	116.84 d
4	144.04 s	142.20 s	144.04 s	142.20 s
6	129.12 d	131.02 d	129.15 d	131.10 d
1′	118.52 d	122.02 d	118.55 d	121.92 d
2′	46.90 t	43.92 t	35.34 t	35.88 t
3′	25.05 d	25.10 d	35.02 t	32.94 t
4′	22.98 q	22.88 q	28.14 d	28.22 d
5′	22.54 q	22.52 q	22.66 q	22.82 q
6′	–	–	22.57 q	22.62 q
1″	146.29 s	143.55 s	146.34 s	143.55 s
2″, 6″	114.64 d	118.82 d	114.64 d	118.81 d
3″, 5″	124.06 d	129.22 d	124.08 d	129.44 d
4″	131.95 s	131.15 s	132.01 s	131.63 s
1‴	46.90 d	47.88 d	49.29 d	50.33 d
2‴	21.06 q	19.52 q	20.83 q	19.30 q
Ac	–	170.65 s	–	170.98 s
	–	23.61 q	–	23.50 q

At 125 MHz, using $CDCl_3$ as a solvent, TMS is the internal standard and the chemical shifts (δ) are expressed in ppm, multiplicities (assigned by DEPT) s = C, d = CH, t = CH_2, q = CH_3.

The ^{1}H-NMR spectrum of **1** indicated the presence of three methyl groups doublets at 1.15, 0.94 and 0.92 ppm which were correlated to the methyl carbon signals (DEPT) at 21.06, 22.98 and 22.54 ppm, respectively. The two methin carbon signals (DEPT) at 46.90 and 25.05 ppm were correlated to (HETCOR) the protons signals at 3.48 and 1.76 ppm, respectively. This indicated the likely presence of an isopropyl group and an aminoethyl residue. The methyl proton doublet at 1.15 ppm was coupled to (COSY) the proton signal at 3.48 ppm assigned to the aminomethin proton of 1‴-postion. The COSY correlation (Fig. 1)

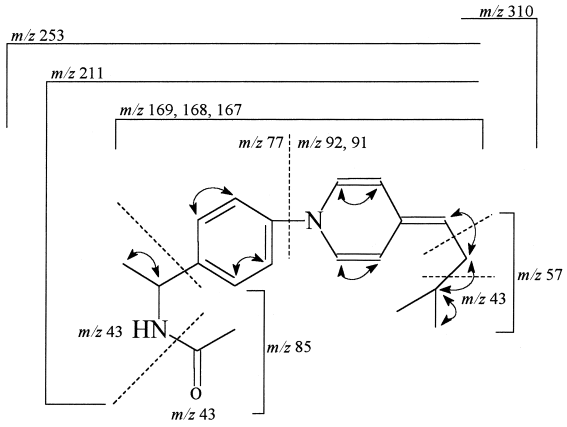


Fig. 1. EIMS fragmentation pattern and COSY results (↷) for compound **1a**.

indicated that the methyl doublets at 0.94 and 0.92 ppm were coupled to the proton signal at 1.76 ppm which is also coupled to the methylene protons signals at 1.26 and 1.47 ppm. This indicated that the isopropyl group is attaching to the methylene group to form the 3-methylbutenylidine residue attached to 4-position. This made it possible to confirm that the 1-aminoethyl residue is attached to 4′′-position of the benzene ring. Further structural confirmations came through the intensive use of the EIMS fragmentation pattern (Fig. 1). The *m/z* 211 $[M\text{-}Me_2CHCH_2]^+$ confirm the presence of the 3-methylbutenylidine side chain. The *m/z* 167 $[M\text{-}Me_2CHCH_2\text{-}MeCHNH_2]^+$, *m/z* 91 $[C_6H_5N]^+$ and *m/z* 77 $[C_6H_5]^+$ are representing good evidences and provide a partial structure of **1**. Furthermore, the new ion peak at m/z 85 $[MeCON{=}CHMe]^+$ observed in **1a** MS-spectrum confirming the presence of an aminoethyl side chain. Compounds **1** and **1a** are optically inactive which indicated their diastereoisomeric nature. This indicated that **1** is (±)-N-[4-(1-aminoethyl) phenyl]-4-[3-methylbutenylidine]-1, 4-dihydropyridine (guayulamine A).

The spectroscopic data of **2** and **2a** are very similar to those of **1** and **1a** with a few differences. The EIMS of **2** gave a parent ion peak at 282 and **2a** gave *m/z* 324, while the HREIMS of **2a** gave 324.2198 for $C_{21}H_{28}N_2O$ (calcd., 324.2203) and conclude $C_{19}H_{26}N_2$ for **2**. This indicated a difference of 14 mass units between **1** and **2**. This difference could be attributed to an additional methyl or methylene group. The ^{13}C-NMR (Table I) and DEPT spectra supported this, where two methylene carbon signals (35.02 and 35.34 ppm) were observed for **2** (**2a**: 32.94 and 35.88 ppm), while only one (46.90 ppm) was observed for **1** (**1a**: 43.92 ppm). The location of this new methylene group was found to be adjacent to the methylene group of **1** to form 4-methylpentenylidine side chain in **2** rather than the 3-methylbutenylidine residue in **1**. These two methylene carbon signals at 32.94 and 35.88 ppm were correlated to the protons multiplets at (1.24, 1.28 ppm) and (1.22,

Table II. ^{1}H-NMR data for compounds **1, 1a, 2** and **2a***.

H#	**1**	**1a**	**2**	**2a**
2, 6	6.58 d, *J* = 8.8 Hz	6.92 d, *J* = 8.8 Hz	6.56 d, *J* = 8.8 Hz	6.92 d, *J* = 8.7 Hz
3, 5	6.98, d, *J* = 8.8 Hz	7.06 d, *J* = 8.8 Hz	6.99 d, *J* = 8.8 Hz	7.15 d, *J* = 8.7 Hz
1′	6.75 t, *J* = 7.5 Hz	6.97 t, *J* = 7.4 Hz	6.76 t, *J* = 7.5 Hz	6.98 t, *J* = 7.4 Hz
2′	1.26 m, 1.47 m	1.06 m, 1.33 m	1.29 m, 1.52 m	1.22 m, 1.41 m
3′	1.76 m	1.61 m	1.24 m, 1.42 m	1.24 m, 1.28 m
4′	0.94 d, *J* = 6.7 Hz	0.96 d, *J* = 6.6 Hz	1.56 m	1.54 m
5′	0.92 d, *J* = 6.7 Hz	0.92 d, *J* = 6.6 Hz	0.90 d, *J* = 6.8 Hz	0.89 d, *J* = 6.8 Hz
6′	–	–	0.87 d, *J* = 6.8 Hz	0.86 d, *J* = 6.8 Hz
2″	6.82 d, *J* = 7.5 Hz	7.15 d, *J* = 7.5 Hz	6.82 d, *J* = 7.5 Hz	7.08 d, *J* = 7.5 Hz
3″	7.16 d, *J* = 7.5 Hz	7.31 d, *J* 7.5 Hz	7.16 d, *J* =7.5 Hz	7.31 d, *J* = 7.5 Hz
5″	7.18 d, *J* = 7.5 Hz	7.30 d, *J* = 7.5 Hz	7.18 d, *J* =7.5 Hz	7.29 d, *J* = 7.5 Hz
6″	6.81 d, *J* = 7.5 Hz	7.14 d, *J* = 7.5 Hz	6.82 d, *J* = 7.5 Hz	7.08 d,.7= 7.5 Hz
1″	3.48 m	5.02 m	3.44 m	4.85 m
2‴	1.16 d, *J* = 7.7 Hz	1.02 d, *J* = 7.8 Hz	1.16 d, *J* =7.8 Hz	1.03 d, *J* = 7.8 Hz
Ac	–	1.79 s	–	1.82 s

* At 500 MHz, using $CDCl_3$ as a solvent, TMS is the internal standard and the chemical shifts (δ) are expressed in ppm, *J* = coupling constant, d = doublet, m = multipler, s = singlet and t = triplet.

1.41 ppm), respectively, which were coupled to each other (COSY), confirming their direct connection. The EIMS provided more structural confirmation, since, the base peak for both **1** and **2** is *m/z* 211, which indicated that the difference must be located in one of the cleaved fragments for each compound. For **1** this corresponds to *m/z* 57 $[M\text{-}C_4H_9]^+$, which is consistent with an isobutyl residue, and for **2** *m/z* 71 $[M\text{-}C_5H_{11}]^+$, for an isopentyl fragment. While the rest of the fragments are very similar in both **1** and **2**. The ^{13}C and ^1H-NMR data of **2** and **2a** are listed in Tables I and II. Compounds **2** and **2a** are optically inactive which indicated their diastereoisomeric nature. This indicated that **2** is (**+**)-N-[4-(1-aminoethyl) phenyl]-4-[4-methylpentenylidine]-1, 4-dihydropyridine (guayulamine B).

Experimental

For instrumentation, plant information, source of resin and preliminary fractionation, see Maatooq *et al. (*1996*)* then: Frs 7–11 (23.5 gm), were column chromatographed on silica gel (900 g, 60–230 μ, 6 cm × 60 cm). Elution started with C_6H_{14} then 5% increments of Me_2CO for each liter. Frs eluted with 5% and 10% Me_2CO/C_6H_{14} (3.4 g), demonstrated a major spot at R_f 0.63 (25% Me_2CO/C_6H_{14}) and 0.54 (5% *iso*-PrOH/C_6H_{14}) on silica gel GF_{254}. It gave a red color upon spraying with Ce^{IV} and became darker on heating. It was subjected to column chromatography (flash silica gel, 40 µm, 300 g, 2.5 cm × 55 cm) using 5% Me_2CO/C_6H_{14} as a solvent. This afforded 150 mg of yellow oil, which gave a clean single spot. The rest of the impure mixture (2.1 g) was subjected to acetylation, by dissolving in 25 ml C_6H_{14} and 25 ml pyridine, then an equal amount of Ac_2O was added. The reaction mixture was left overnight at room temperature with stirring. ETOAc was added (400 ml), then 200 ml of H_2O, with stirring. The organic phase was separated, and washed with 6 N HCl, then with water and brine several times. It was observed that the acetylation product gave a greenish-blue color after spraying with Ce^{IV}, and moved to a lower R_f value (0.25, 5% *iso*-PrOH/C_6H_{14}). The acetylation product (2.5 g) was purified on silica gel column (63–200 µ, 300 g, 2.5 cm × 55 cm), using 2% Me_2CO/C_6H_{14} as a solvent. Both the pure spot and the acetylation product proved to be a mixture by NMR spectroscopy.

The unacetylated pure spot (150 mg) was resolved into two compounds **1** (60 mg) and **2** (50 mg), by prep HPLC (Hitachi, L4500 diode array detector, AS-4000 autosampler, D-6000 interface, L 6200A pump, with 486 IBM PC compatible computer programmed with model D-6500 DAD system manager; the analytical column is Rainin Microsorb-MV, 10 cm L × 0.46 cm i.d., packed with C18, with pore size 100 Å. The preparative column is Alltech econosil, 50 cm Lx 2.25 cm i.d., packed with C18, 10 µ. The Elution was isocratic using 60% MeCN/H_2O).

The acetylated fr (800 mg) was column chromatographed on RP-C18 silica (15 µ, 50 g, 1.5 cm × 40 cm). Elution was isocratic with 75% MeCN/H_2O, and 25 ml frs were collected. This afforded 65 mg of compound **1a**, and 345 mg of compound **2**a. Both are slightly pink solid gum. Compounds **1a** and **2a** displayed an R_f of 0.46 and 0.41, respectively (RP-C18 plates, 75% MeCN/H_2O).

(±)-N-[4-(1-aminoethyl) phenyl]-4-[3-methylbutenylidine]-1, 4-dihydropyridine, guayulamine A, **1**: Oil, $[\alpha]_D^{25}$ 0.0 ($CHCl_3$; c 0.475), UV λ_{max} 285.7 nm. IR $\nu_{max}^{cm\text{-}1}$, 3400, 2990, 1600, 1520, 1305, 1265, 1170, 810, 740, 690. EIMS, *m/z* (rel. int.): 270 $[M+2]^+$ (1), 269 $[M+1]^+$ (10), 268 $[M]^+$ (48), 253 $[M\ -CH_3]^+$ (12), 225 $[M\text{-}Me_2CH]^+$ (1), 224 $[M\text{-}\ MeCHNH_2]^+$ (2), 211 $[M\text{-}Me_2CHCH_2]^+$ (100), 196 $[M\text{-}MeCHCH_2\text{-}Me]^+$(3), 183 (16), 169 $[M\text{-}MeCHNH_2\text{-}Me_2CHCH_2{+}2]^+$ (10), 168 $[M\text{-}MeCHNH_2\text{-}Me_2CHCH_2{+}1]^+$ (7), 167 $[M\text{-}MeCHNH_2\text{-}Me_2CHCH_2]^+$ (12), 154 (3), 128 (3), 119 $[phCNHMe]^+$ (6), 105 (12), 92 $[C_6H_6N]^+$ (3), 91 $[C_6H_5N]^+$ (3), 77 $[C_6H_5]^+$ (6), 65 (3), 51 (3), 43 (7) and 41 (6).

(±)-N-[4-(1-aminoethyl) phenyl]-4-[3-methylbutenylidine]-1, 4-dihydropyridine acetamide, guayulamine A acetamide,**1a** : Solid gum, $[\alpha]_D^{25}$ 0.0 ($CHCl_3$; c 1.5), UV λ_{max} 285.7 nm. IR $\nu_{max}^{cm\text{-}1}$, 3320, 2960, 1650, 1600, 1510, 1400, 1310, 1240, 1170, 840, 745, 690. HREIMS, *m/z* 310.2042, for $C_{20}H_{26}N_2O$, (calcd, 310.2046). EIMS, *m/z* (rel. int.): 312 $[M+2]^+$ (2), 311 $[M+1]^+$ (22), 310 $[M]^+$ (100), 295 $[M\ -CH_3]^+$ (2), 267 $[M\text{-}Ac]^+$ (5), 253 $[M\text{-}Me_2CHCH_2]^+$ (13), 226 (46), 211$[M\text{-}Me_2CHCH_2\text{-}Ac]^+$ (73), 185 (38), 184 (5), 183 (21), 169 [M-Ac-MeCHNH$_2$-Me2CHCH$_2$+2]$^+$ (6), 168 [M-Ac-MeCHNH$_2$-Me$_2$CHCH$_2$+1]$^+$ (6), 167 [M-Ac-

$MeCHNH_2$-$Me_2CHCH_2]^+$ (18), 156 (3), 126 (3), 107 (2), 105 (2), 85 [MeCON=CHMe]+ (39), 77 $[C_6H_5]^+$ (5), 65 (3), 43 (39) and 41 (9).

(±)-N-[4-(1-aminoethyl) phenyl]-4-[4-methylpentenylidine]-1, 4-dihydropyridine, g*uayulamine B*, **2**: Oil, $[\alpha]_D^{25}$ 0.0 ($CHCl_3$; c 1.0), UV λ_{max} 288.7 nm. IR ν_{max}^{cm-1}, 3400, 2980, 1600, 1515, 1310, 1260, 1175, 805, 745, 690. EIMS, *m/z* (rel. int.): 284 $[M+2]^+$ (1), 283 $[M+1]^+$ (9), 282 $[M]^+$(44), 267 $[M-Me]^+$ (8), 211 $[M-Me_2CHCH_2CH_2]^+$ (100), 184 (6), 183 (12), 169 $[M-MeCHNH_2-Me_2CHCH_2CH_2+2]^+$ (6), 168 $[M-MeCHNH_2-Me_2CHCH_2CH_2+1]^+$ (5), 167 $[M-MeCHNH_2-Me_2CHCH_2CH_2]^+$ (7), 154 (2), 128 (3), 119 $[phCNHMe]^+$ (4), 118 (5), 105 (8), 92 $[C_6H_6N]^+$ (3), 91 $[C_6H_5N]^+$ (3), 77 $[C_6H_5]^+$ (5), 57 (3), 55 (4),43 (6) and 41 (8).

(±)-N-[4-(1-aminoethyl) phenyl]-4-[4-methylpentenylidine]-1, 4-dihydropyridine acetamide, g*uayulamine B acetamide*, **2a** : Solid gum, $[\alpha]_D^{25}$ 0.0 ($CHCl_3$; c 1.0), UV λ_{max} 288.7 nm. IR ν_{max}^{cm-1}, 3330, 2980, 1640, 1600, 1520, 1405, 1330, 1240, 1170, 845, 745, 695. HREIMS, *m/z* 324.2198, for $C_{21}H_{28}N_2O$, (calcd, 324.2203). EIMS, *m/z* (rel. int.): 326 $[M+2]^+$ (2), 325 $[M+1]^+$ (23), 324 $[M]^+$ (100), 305 $[M-Me]^+$ (2), 281 $[M-Ac]^+$ (4), 267 (6), 253 $[M-Me_2CHCH_2CH_2]^+$ (6), 226 (42), 211 $[M-Ac-Me_2CHCH_2CH_2]^+$ (72), 185 (38), 184 (40), 183 (18), 169 $[M-Ac-MeCHNH_2-Me_2CHCH_2CH_2+2]^+$ (4), 168 $[M-Ac-MeCHNH_2-Me_2CHCH_2CH_2+1]^+$ (11), 167 $[M-Ac-MeCHNH_2-Me_2CHCH_2CH_2]^+$ (4), 156 (3), 140 (3), 99 (18), 92 $[C_6H_6N]^+$ (2), 91 $[C_6H_5N]^+$ (2), 77 $[C_6H_5]^+$ (5), 57 (22), 43 (28) and 41 (8).

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