

Two New Pyridine Monoterpene Alkaloids by Chemical Conversion of a Commercial Extract of *Harpagophytum procumbens*

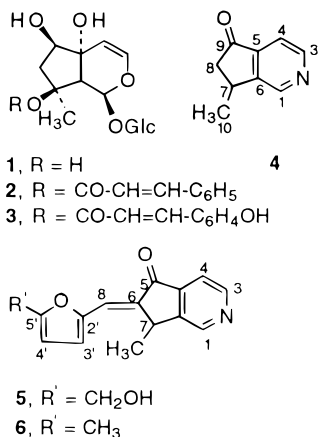
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The treatment of harpagide (**1**), harpagoside (**2**), or 8-*O*-*p*-coumaroylharpagide (**3**), the main iridoids of *Harpagophytum procumbens* and *Harpagophytum zeyheri*, with NH₃ and HCl led to aucubinine B (**4**), a pyridine monoterpene alkaloid (PMTA). A similar procedure applied to a commercial extract of *H. procumbens* yielded **4** and two new PMTAs named beatrine A (**5**) and beatrine B (**6**). The structures of these new PMTAs were established using ESIMS and 2D NMR. Their semisynthesis was analyzed in terms of reaction mechanisms.

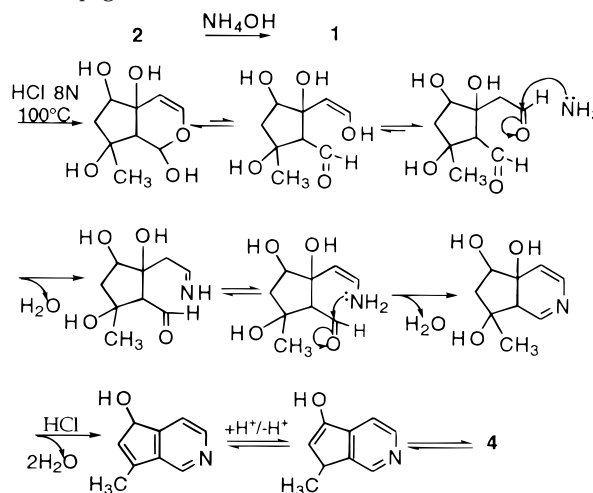
The formation of pyridine monoterpene alkaloids (PMTAs) from iridoids such as antirrhinoside,¹ and from secoiridoids such as gentiopicoside² and swertiamarin,² by treatment with ammonia and acid has been reported. The conversion of iridoids into PMTAs has also been reported using human intestinal bacteria^{3,4} or by enzymatic reaction with β -glucosidase on aucubin,³ geniposide,^{5,6} gardenoside,⁴ 8-epiloganin,⁶ cornin,⁶ and antirrhinoside.⁶ In this paper, we report the transformation of harpagide (**1**), harpagoside (**2**), or 8-*O*-*p*-coumaroylharpagide (**3**), the main iridoids of the genus *Harpagophytum*, to PMTAs using ammonia and hydrochloric acid. *H. procumbens* (Pedaliaceae) is an herbaceous plant growing in the Kalahari desert and in the Namibian steppes. The secondary tuberized roots of this plant, which contain iridoids, are used for their antiinflammatory and analgesic effects.⁷ Conversion of harpagoside (**2**) into a PMTA could account for the observed discrepancies between pharmacological data obtained with harpagoside and with *H. procumbens* preparations.⁸



Results and Discussion

Treatment of individual pure compounds **1**, **2**, or **3** first with ammonia and then hydrochloric acid yielded a product

Scheme 1. Proposed Mode of Formation of Aucubinine B (**4**) from Harpagoside (**2**).



mixture that was purified by preparative liquid chromatography on Sephadex LH-20 to give aucubinine B (**4**) as the primary product. The ESIMS of **4** showed [MH]⁺ at *m/z* 148, corresponding to a molecular formula of C₉H₉ON, and other prominent fragmentation ions at *m/z* 133 [MH - CH₃]⁺, 130 [MH - H₂O]⁺, 120 [MH - CO]⁺, and 106 [MH - CH₂CO]⁺. The ¹H 400 MHz NMR spectrum of **4** had signals characteristic of a disubstituted pyridine 3,4-ring⁹ at δ 8.94 (sbr), 8.67 (d, *J* = 5.0 Hz), and 7.52 (dd, *J* = 4.9, 1.0 Hz). Close examination of the remaining resonances indicated a partial structure -CH(CH₃)CH₂- (see the Experimental Section for data). The ¹³C NMR spectrum confirmed these results and further indicated the presence of nine carbons including a carbonyl group and five aromatic carbons. At this point, **4** was identified as aucubinine B on the basis of the above arguments and general similarity of its ¹H NMR spectrum with that of previously reported aucubinine B.³ Complete assignments of the ¹³C NMR signals of **4** was realized from the concerted application of HMQC¹⁰ and HMBC¹¹ experiments.

Aucubinine B (**4**) was thus obtained for the first time from these iridoids by a controlled chemical transformation. Scheme 1 gives a plausible mechanism for the conversion of harpagoside (**2**) into aucubinine B (**4**).

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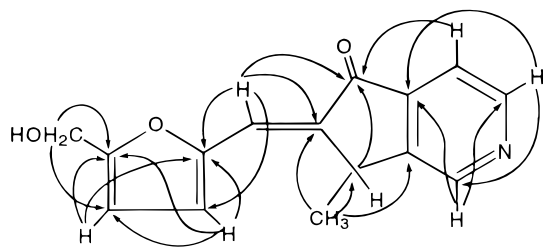


Figure 1. Proton-carbon connectivities found in the HMBC spectrum of beatrine A (**5**).

Compounds **1–3** are the main iridoids present in *H. procumbens* and *H. zeyheri*.⁸ A commercial extract of *H. procumbens* treated first with ammonia and then with hydrochloric acid gave, in low yield (near 1.0%), a brown viscous residue. From this residue, three PMTAs, aucubinine B (**4**), beatrine A (**5**), and beatrine B (**6**), were isolated by preparative liquid chromatography. The ESIMS of beatrine A (**5**) showed the molecular ion $[MH]^+$ at m/z 256 in agreement with a molecular formula of $C_{15}H_{13}O_3N$.

The 1H NMR spectrum of **5** revealed signals at δ 8.98, 8.72 (d, $J = 4.4$ Hz) and 7.65 (d, $J = 4.8$ Hz), which characterized the disubstituted pyridine ring. Further analysis of the 1H NMR data indicated a secondary methyl group at δ 1.58 (d, $J = 7.0$ Hz), a methylene bearing an oxygen at δ 4.74, and a deshielded resonance at δ 7.43 (d, $J = 1.7$ Hz) showing long-range allylic coupling between the methine signal and the methyl group. Finally, the presence of a 2',5'-disubstituted furan ring in **5** was suggested by a pair of low-field doublets at δ 6.81 (d, $J = 3.4$ Hz) and 6.49 (d, $J = 3.4$ Hz).

The occurrence of a conjugated carbonyl function was supported by the signal at δ 193.6 in the ^{13}C NMR spectrum of **5**. Multiplicities of the individual ^{13}C peaks were determined using the DEPT pulse sequence¹² and the results suggested a tricyclic structure for **5**.

The molecular framework and the complete 1H and ^{13}C chemical shift assignments of beatrine A (**5**) were deduced from the combination of both direct (HMQC) and long-range (HMBC) correlation experiments. Consideration of the various connectivities (Figure 1) in conjunction with the inferences drawn from the 1D NMR data establish that **5** is 6-[(5'-hydroxymethylfuran-2'-yl)methylene]-7-methyl-6,7-dihydro[2]pyridin-5-one.

The stereochemistry at C-10 was unequivocally established using a phase-sensitive NOESY experiment¹³ in which H-12 NOE cross-peaks were observed with H-16 and H-7. The molecular formula for beatrine B (**6**) was established as $C_{15}H_{13}O_2N$ (ESIMS $[MH]^+$ m/z 240). The structural similarity between beatrine A (**5**) and beatrine B (**6**) was shown by the close similarity of the 1H and ^{13}C chemical shifts, the striking difference being the presence of a methyl group instead of the hydroxymethyl function at C-14. Beatrine A and B (**5** and **6**) are new compounds.

The furan ring in **5** and **6** may be due to carbohydrates present in the *H. procumbens* extract. To confirm this hypothesis, we have applied the experimental procedure previously described to **2** in the presence of added sugars (stachyose, fructose, galactose, or glucose); these compounds are the main sugars of *H. procumbens*. Under these conditions the reaction yielded **5**, especially in the presence of fructose. Aucubinine B (**4**) may undergo a keto-enol tautomerism, and under acidic conditions, the enol form can react with the aldehydic group of 5-(hydroxymethyl)-furfural obtained by dehydration of the corresponding carbohydrate, yielding **5**. The formation of **6** could be explained by the presence of 6-deoxyhexose in the extract.

Studies of the conversion of these iridoids by an enzymatic pathway, β -glucosidase, and by microbiological pathways with intestinal bacteria are in progress in order to determine if these compounds may be produced in vivo.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in $CDCl_3$ on a Bruker AMX400 spectrometer using tetramethylsilane (TMS) as internal reference in 1H and ^{13}C measurements and standard Bruker pulse sequences for two-dimensional experiments. ESIMS were obtained using a Nermag R-10-10 H mass spectrometer. Melting points were determined on a Büchi apparatus and are uncorrected. UV spectra were recorded with a Kontron 930 spectrophotometer and IR spectra (film) with a Perkin-Elmer 1600 spectrometer. Optical rotations $[\alpha]$ were measured on a Perkin-Elmer 341 Orot polarimeter. Elemental analyses were performed with a Technicon auto-analyzer. Column chromatography was carried out on Sephadex LH-20 (Pharmacia) and Lichroprep C18 (Merck).

Plant Materials. *H. procumbens* and *H. zeyheri* were collected, identified, and provided by G. J. R. Betti in Namibia. Voucher specimens (*H. procumbens* 4-93 and *H. zeyheri* 4-93) are kept in the department of Pharmacognosy, Faculty of Pharmacy, Marseilles, France. Harpagide (**1**) and harpagoside (**2**) were purified from *H. procumbens* roots by preparative liquid chromatography (Jobin-Yvon, France), on RP-18 (Lichroprep C18, Merck, 200 g), elution with aqueous MeOH solutions. 8-*O*-p-Coumaroylharpagide (**3**) was purified from *H. zeyheri* roots as previously described.⁸ The commercial extract of *H. procumbens* was from Indena (Milano, Italy). This aqueous extract contained 2.5% of **1**, 10.2% of **2**, and 4.3% of **3**.⁸

Conversion of Harpagide (1), Harpagoside (2), or 8-O-p-Coumaroylharpagide (3) to Aucubinine B (4). Each individual iridoid (1.0 g) was dissolved in 200 mL 25% aqueous NH_4OH -MeOH (50:50) and stirred for 1 h at room temperature. The reaction mixture was concentrated to 100 mL under vacuum, and 50 mL of 8 N HCl was added. After reflux for 15 min, the mixture was basified with NH_4OH (pH = 8–9) and extracted with $CHCl_3$. The organic layer was extracted with 3 N HCl, and then the aqueous layer was rebasified and extracted with $CHCl_3$. Chromatography of the residue on Sephadex LH-20 (MeOH) yielded 5 mg of **4**: mp 110–113 °C; $[\alpha] -10^\circ$; UV (MeOH) λ_{max} 225, 280 nm; IR (MeOH) ν_{max} 1720 cm^{-1} ; 1H NMR ($CDCl_3$, 400 MHz) δ 8.94 (1H, sbr, H-1), 8.67 (1H, d, $J = 5.0$ Hz, H-3), 7.52 (1H, dd, $J = 1.0, 4.9$ Hz, H-4), 3.55 (1H, m, H-7), 2.30 (1H, dd, $J = 3.5, 19.4$ Hz, H-8a), 2.95 (1H, dd, $J = 7.5, 19.4$ Hz, H-8b), 1.45 (3H, d, $J = 7.1$ Hz, H-10); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 149.22 (C-1), 148.56 (C-3), 116.24 (C-4), 142.39 (C-5), 152.61 (C-6), 31.63 (C-7), 45.32 (C-8), 206.07 (C-9), 21.29 (C-10); ESIMS m/z 148 $[MH]^+$.

Conversion of a Commercial Extract of Harpagophytum procumbens (5 and 6). The procedure previously described for the pure compounds was applied to 10 g of commercially dried extract. A portion of the residue obtained (200 mg) was purified on a column of Sephadex LH-20 (elution with CH_2Cl_2) yielding 4 mg of **4** and 10 mg of beatrine A (**5**): mp 95–98 °C; $[\alpha] -36^\circ$ (MeOH); UV (MeOH) λ_{max} 250, 380 nm; IR (MeOH) ν_{max} 1750, 1615, 1570 cm^{-1} ; 1H NMR ($CDCl_3$, 400 MHz) δ 8.98 (1H, sbr, H-1), 8.72 (1H, dbr, $J = 4.4$ Hz, H-3), 7.65 (1H, dbr, $J = 4.8$ Hz, H-4), 4.42 (1H, dq, $J = 1.7, 7.0$ Hz, H-7), 7.43 (1H, d, $J = 1.7$ Hz, H-8), 6.81 (1H, d, $J = 3.4$ Hz, H-3'), 6.49 (1H, d, $J = 3.4$ Hz, H-4'), 4.74 (2H, s, CH_2 -OH), 1.58 (3H, d, $J = 7$ Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.9 (C-1), 148.7 (C-3), 116.7 (C-4), 149.1 (C-4a), 193.6 (C-5), 137.1 (C-6), 36.3 (C-7), 148.8 (C-7a), 20.6 (CH_3), 121.7 (C-8), 151.0 (C-2), 120.0 (C-3'), 111.0 (C-4'), 158.3 (C-5'), 57.9 (CH_2OH); ESIMS m/z 256 $[MH]^+$; anal. C 70.26%, H 5.80%, N 5.37%, calcd for $C_{15}H_{13}O_3N$, C 70.58%, H 5.13%, N 5.49%.

The remaining residue (300 mg) was separated by chromatography on Lichroprep C18 (50% MeOH \rightarrow MeOH) leading to 6 mg of beatrine B (**6**): mp 80–82 °C; $[\alpha] -51^\circ$ (MeOH);

UV (MeOH) λ_{\max} 252, 386 nm; IR (MeOH) ν_{\max} 1750, 1615, 1570 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 8.96 (1H, sbr, H-1), 8.70 (1H, dbr, $J = 4.9$ Hz, H-3), 7.64 (1H, dbr, $J = 4.9$ Hz, H-4), 4.38 (1H, dq, $J = 1.3, 7.0$ Hz, H-7), 7.39 (1H, d, $J = 1.3$ Hz, H-8), 6.77 (1H, d, $J = 3.3$ Hz, H-3'), 6.20 (1H, d, $J = 2.8$ Hz, H-4'), 2.44 (3H, s, H- CH_3), 1.57 (3H, d, $J = 7.0$ Hz, CH_3); ^{13}C NMR (CDCl_3 , 100 MHz) δ 148.7 (C-1), 148.5 (C-3), 116.7 (C-4), 143.4 (C-4a), 193.7 (C-5), 135.4 (C-6), 36.3 (C-7), 148.9 (C-7a), 20.4 (CH_3), 122.0 (C-8), 149.8 (C-2'), 121.3 (C-3'), 109.9 (C-4'), 157.6 (C-5'), 14.3 (CH_3); ESIMS m/z 240 $[\text{MH}]^+$; anal. C 74.98%, H 5.15%, N 5.64%, calcd for $\text{C}_{15}\text{H}_{13}\text{O}_2\text{N}$, C 75.31%, H 5.44%, N 5.86%.

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