10-Hydroxydarlingine, a New Tropane Alkaloid from the Australian Proteaceous Plant *Triunia erythrocarpa*

Mark S. Butler,[†] Peter L. Katavic,[†] Rohan A. Davis,[†] Paul I. Forster,[‡] Gordon P. Guymer,[‡] and Ronald J. Quinn *,[†]

Queensland Pharmaceutical Research Institute, Griffith University, Brisbane, Queensland, 4111, Australia, and Queensland Herbarium, Brisbane Botanic Gardens, Toowong, Queensland, 4066, Australia

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Triunia erythrocarpa was identified as containing alkaloids during chemical screening of Queensland Proteaceae using Dragendorff's reagent. A new tropane, 10-hydroxydarlingine (**1**), and the known tropane, darlingine (**2**), were isolated from the leaves of *T. erythrocarpa*. The absolute stereochemistry of 10-hydroxydarlingine (**1**) was assigned using the advanced Mosher method. *T. erythrocarpa* is only the seventh member of the Proteaceae to have been shown to produce alkaloids.

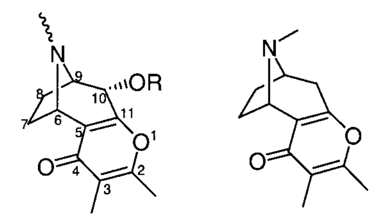
Although there are more than 1350 species of Proteaceae worldwide, only six have been found to produce alkaloids.[1,2] These six species, *Agastachys odorata* and *Bellendena montana* from Tasmania, *Darlingia darlingiana* and *Darlingia ferruginea* from North Queensland, and *Knightia deplanchei* and *Knightia stobilina* from New Caledonia, contain a variety of novel tropane and pyrrolidine alkaloids.[1,2] Recently, a new tropane, darlingine *N*-oxide, was reported from *D. darlingiana*.[3]

In an attempt to identify new alkaloid-containing proteaceous plants, various plant parts from 60 Queensland Proteaceae were tested for alkaloids using Dragendorff's reagent. A positive alkaloid test was obtained from wood, bark, and leaf samples of *Triunia erythrocarpa* Foreman. In this note, we report the isolation of a new tropane alkaloid, 10-hydroxydarlingine (**1**), and darlingine (**2**) from the leaves of *T. erythrocarpa*. Alkaloids **1** and **2** were also present in the wood and bark. There have been no previous reports on the chemistry of any *Triunia* species.

An alkaloid extract from the leaves of *T. erythrocarpa* was separated by countercurrent chromatography using $CHCl_3$−MeOH−H_2O (13:7:8) in ascending mode to give 10-hydroxydarlingine (**1**). An earlier eluting fraction was purified using Sephadex LH-20 (MeOH) to give (+)-darlingine (**2**), which was identical in all respects to that previously reported.[3−5] The absolute stereochemistry of **2** has been previously established by a synthesis of (−)-darlingine.[5]

A molecular formula of $C_{13}H_{17}NO_3$ was assigned to alkaloid **1** on the basis of a (+)-HRESIMS of the $[M + H]^+$ mass ion peak. The UV spectrum of **1** displayed λ_{max} at 216 and 258 nm, which was characteristic of the γ-pyrano ring in darlingine (**2**). The NMR data of **1** was also similar to darlingine (**2**) except for the presence of a hydroxymethine resonance at δ 5.06 (d, J = 5.4 Hz) and the absence the methylene group at C-10. Placement of the hydroxymethine at C-10 was confirmed by the observation of a COSY correlation to H-9 (δ 3.52, dd, J = 5.4, 5.4 Hz). Examination of the COSY, HSQC, and HMBC NMR data (Table 1) confirmed the structure as 10-hydroxydarlingine. An α-orientation of the hydroxyl group was assigned on the basis of a 5.4 Hz $^1H-^1H$ coupling constant between H-9 and H-10. Selective refocusing of the *N*-methyl resonance of **1** in a 1D NOESY experiment[6] showed enhancements to both sides of the sides of the tropane ring (H-7β, H-8β, and H-10), which suggested that there was no preferred conformation of the *N*-methyl group in **1**. This was in contrast to darlingine (**2**), where it has been shown that the *N*-methyl group is oriented exclusively toward the pyran ring.[3]

The absolute stereochemistry of 10-hydroxydarlingine was determined using the advanced Mosher method.[7] Esterification of **1** yielded the diastereoisomeric esters, (*S*)-MTPA (**3**) and (*R*)-MTPA (**4**). Diagnostic ^1H NMR chemical shift differences between the MTPA esters [$\delta\Delta = \delta_S - \delta_R$; H-7α (+0.05 ppm), H-7$\beta$ (+0.05 ppm), H-8α (+0.52 ppm), H-8β (+0.17 ppm), 2-CH_3 (−0.14 ppm), 3-CH_3 (−0.03 ppm)] revealed the absolute stereochemistry at C-10 to be *R*.



1 R = H
3 R = (*S*)-MTPA
4 R = (*R*)-MTPA

2

Experimental Section

General Experimental Procedures. General experimental procedures have been reported elsewhere.[8] A Sanki centrifugal partition chromatograph model LLB-M was used in countercurrent separations. Anhydrous pyridine (Sigma-Aldrich) and (*S*)- and (*R*)-MTPA-Cl (Fluka) and were used for the preparing the Mosher esters, paying particular attention that the (*S*)-MTPA-Cl gives the (*R*)-MTPA ester and vice versa.

Plant Material. Wood, bark, and leaves of *T. erythrocarpa* Foreman (Proteaceae) were collected during June 1996, from Longlands Gap, State Forest 353, in Queensland, Australia. A voucher specimen (PIF 19227) has been lodged at the Queensland Herbarium.

Extraction and Isolation. Dried and ground leaves (50 g) of *T. erythrocarpa* were extracted with 2 M H_2SO_4−MeOH (9:1). The MeOH was removed in vacuo and the residue partitioned with $CHCl_3$. The acidic layer was basified to pH 10.5 with 28% NH_4OH and extracted with $CHCl_3$ to give a crude alkaloid fraction (90 mg). The alkaloid fraction was separated by countercurrent chromatography using a solvent

* To whom correspondence should be addressed. Tel.: 61 7 3875 6000. Fax: 61 7 3875 6001. E-mail: R.Quinn@qpri.gu.edu.au.
[†] Griffith University.
[‡] Queensland Herbarium.

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Table 1. NMR Data of 10-Hydroxydarlingine (**1**)[a]

position	$^{13}C(\delta)$	^{1}H (δ, mult., J in Hz)	COSY	HMBC
2	161.1			
3	120.7			
4	176.3			
5	125.3			
6	57.8	4.17 (d, 5.4)	H 7β	C-4, -5, -7, -8, -9, -11, N-CH_3
7α	29.5	1.76 (ddd, 3.0, 9.0, 12.0)	H 7β, 8α,β	C-5, -6, -8, -9
7β		2.23 (m)	H 6, 7β, 8β	C-5, -6, -8, -9
8α	19.7	2.16 (m)	H 7α, 8β	C-6, -7, -9, -10
8β		1.98 (m)	H 7α,β, 9	C-6, -7, -9, -10
9	65.2	3.52 (dd, 5.4, 5.4)	H 8β, 10	C-6, -7, -8, -10, -11, N-CH_3
10	68.6	5.06 (d, 5.4)	H 9	C-5, -8, -9, -11
11	158.9			
CH_3−2	17.8	2.28 (s)		C-2, -3
CH_3−3	9.9	1.99 (s)		C-2, -3, -4
N-CH_3	39.8	2.43 (s)		C-6, -9

[a] Spectra recorded in $CDCl_3$ at 30 °C.

system $CHCl_3$−MeOH−H_2O (13:7:8) in ascending mode to give fraction 1 and 10-hydroxydarlingine (**1**, 7.6 mg, 0.015% dry wt). Fraction 1 was separated using Sephadex LH-20 (eluent MeOH) to give darlingine (**2**, 17 mg, 0.034% dry wt).

10-Hydroxydarlingine (1): white solid; $[\alpha]^{27}_{D}$ + 12° (c 0.30, $CHCl_3$); UV λ_{max} (log ϵ) 216 (4.0), 258 nm (4.0); IR (film) 1656 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; (+)-ESIMS m/z 236 [M + H]$^+$; (+)-HRESIMS m/z 236.1281 (calcd for $C_{13}H_{17}NO_3$, 236.1281).

Darlingine (2): $[\alpha]^{27}_{D}$ + 45° (c 0.46, $CHCl_3$); identical in all respects to that previously reported.[3−5]

Preparation of MTPA Esters, 3, and 4. (S)- or (R)-MTPA-Cl (4 μL, 0.02 mmol) was added to 10-hydroxydarlingine (**1**, 0.5 mg, 0.002 mmol) in anhydrous pyridine (100 μL), and the resulting mixture was allowed to stand at room temperature for 4 h. Upper and lower phases (2 mL each) of the solvent system 13:7:8 ($CHCl_3$−MeOH−H_2O/NH_3 pH 10) were added to the reaction mixture, and the lower phase was removed and evaporated to dryness to yield the Mosher ester (**3** or **4**). ^{1}H and gCOSY NMR studies were then performed on the Mosher esters (**3** and **4**) to obtain the δ_S and δ_R values, which were used to determine the absolute stereochemistry, at C-10.

(*S*)-MTPA ester of 10-hydroxydarlingine (3): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.57 (2H, m, MTPA-ArH), 7.44 (3H, m, MTPA-ArH), 6.30 (1H, d, J = 5.4 Hz, H-10), 4.15 (1H, d, J = 6.6 Hz, H-6), 3.64 (1H, dd, J = 5.4, 5.4 Hz, H-9), 3.56 (3H, s, MTPA-OCH_3), 2.44 (3H, s, N−CH_3), 2.22 (1H, m, H-7β), 2.22 (1H, m, H-8α), 2.09 (3H, s, 2-CH_3), 1.98 (1H, m, H-8β), 1.89 (3H, s, 3-CH_3), 1.75 (1H, m, H-7α).

(*R*)-MTPA ester of 10-hydroxydarlingine (4): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.57 (2H, m, MTPA-ArH), 7.42 (3H, m, MTPA-ArH), 6.28 (1H, d, J = 5.4 Hz, H-10), 4.15 (1H, d, J = 6.6 Hz, H-6), 3.63 (1H, dd, J = 5.4, 5.4 Hz, H-9), 3.62 (3H, s, MTPA-OCH_3), 2.43 (3H, s, N-CH_3), 2.23 (3H, s, 2-CH_3), 2.17 (1H, m, H-7β), 1.92 (3H, s, 3-CH_3), 1.81 (1H, m, H-8β), 1.70 (1H, m, H-7α), 1.70 (1H, m, H-8α).

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