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Tropane alkaloids from *Darlingia darlingiana*

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Abstract

A new γ-pyranotropane, darlingine *N*-oxide, was isolated from the bark and leaves of *Darlingia darlingiana*, along with the known compound, darlingine. 1D-NOESY NMR experiments indicated that the *N*-methyl groups of both alkaloids were orientated towards the pyran ring. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

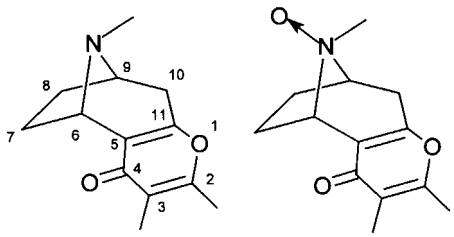
The only proteaceous plants known to produce alkaloids are *Agastachys odorata*, *Bellendena montana*, *Darlingia darlingiana*, *D. ferruginea*, *Knightia deplanchei* and *K. strobilina* (Lounasmaa & Tamminen, 1993). A small group of these alkaloids, bellendine (Bick, Bremner & Gillard, 1971; Motherwell et al., 1971), isobellendine (Bick, Gillard & Leow, 1979a), darlingine (**1**) (Bick, Gillard & Leow, 1979b) and strobiline (Lounasmaa, Pusset & Sévenet, 1980), contain a unique γ-pyranotropane skeleton. In this report, we describe the isolation of a new γ-pyranotropane alkaloid, darlingine *N*-oxide (**2**), found in addition to darlingine (**1**) in the bark and leaves of *D. darlingiana* (F. Muell.) L.A.S. Johnson.

2. Results and discussion

An alkaloid extract from the bark (100 g) of *D. darlingiana* was separated by counter current chromatography [solvent system $CHCl_3$–MeOH–H_2O (13:7:8)] to give darlingine (**1**) (74 mg) and darlingine *N*-oxide (**2**) (17 mg). The leaves contained **1** and **2** in a similar ratio to the bark.

The major alkaloid **1** was identified as the known compound (+)-darlingine (**1**). Darlingine (**1**) has been previously isolated from *D. darlingiana* (Bick et al., 1979b), as well as *D. ferruginea* (Bick, Gillard & Leow, 1979c) and *B. montana* (Bick et al., 1979a). In 1995 Majewski and Lazny established the absolute stereochemistry of **1** by an enantiomeric synthesis of (−)-darlingine. Some of the ^1H and ^{13}C NMR resonances of **1** reported by Bick et al. (1979c) have been reassigned using 2D NMR experiments and are given in the experimental section.

The spectroscopic data of the minor alkaloid **2** was similar to that of **1**, which suggested these compounds to be closely related. A molecular formula of



(1) **(2)**

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Table 1
NMR data of darlingine *N*-oxide (**2**) (DMSO-d_6)

Position	^{13}C	^{1}H	gCOSY	gHMBC
2	161.6			
3	119.2			
4	174.6			
5	121.4			
6	69.0	4.18 d (6.0)	H-7β	C-4, C-5, C-7, C-8, C-9, C-11, N-CH_3
7α	32.9	1.65 m	H-7β, H-8α, β	C-5, C-6, C-8, C-9
7β		2.75 m	H-6, H-7α, H-8α, β	C-5, C-6, C-8, C-9
8α	28.5	1.71 m	H-7α, β, H-8β	C-6, C-7, C-9, C-10
8β		2.52 m	H-7α, β, H-8α, H-9	C-6, C-7, C-9, C-10
9	71.6	3.65 dd (5.4, 6.0)	H-8β, H-10β	C-6, C-7, C-8, C-10, C-11, N-CH_3
10α	35.3	2.82 d (18.6)	H-10β	C-5, C-8, C-9, C-11
10β		3.24 dd (5.4, 18.6)	H-9, H-10α	C-5, C-8, C-9, C-11
11	158.1			
2-CH_3	17.5	2.26 s		C-2, C-3
3-CH_3	9.4	1.82 s		C-2, C-3, C-4
N–CH_3	50.1	3.07 s		C-6, C-9

$C_{13}H_{17}NO_3$, determined by accurate mass measurement of the $[M+H]^+$ mass ion peak (*m*/*z* 236.1276) in the positive ESI mass spectrum, indicated that **2** had an extra oxygen atom compared to darlingine (**1**). Examination of the 1D and 2D NMR data (Table 1) established that **2** had the same gross structure as **1** and the extra oxygen atom in **2** could only be present as an *N*-oxide. This was confirmed by a zinc mediated reduction of **2** to give (+)-darlingine.

1D-NOESY NMR experiments were used to establish the orientation of the *N*-methyl groups in darlingine *N*-oxide (**2**) and darlingine (**1**). Selective refocusing of the *N*-methyl group (δ 3.07) of darlingine *N*-oxide (**2**) gave enhancements to H-6 (δ 4.18), H-9 (δ 3.65) and H-10β (δ 3.24). No enhancements were observed to either H-7β or H-8β, which indicated that the *N*-methyl group was orientated towards the pyran ring. Enhancements to H-6 (δ 4.14), H-9 (δ 3.42) and H-10β (δ 2.96) and none to H-7β or H-8β upon selective refocusing of the *N*-methyl group (δ 2.30) of darlingine (**1**) indicated the *N*-methyl group was also orientated towards the pyran ring. This orientation is the opposite to the way the *N*-methyl group is depicted in literature for the other γ-pyranotropanes, apart from a paper on the X-ray crystal structure of bellendine (Motherwell et al., 1971).

3. Experimental

Optical rotations: JASCO P-1020 polarimeter. NMR: Varian INOVA ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz): $CDCl_3$ and DMSO-d_6 were used as solvents with chemical shifts calculated relative to the solvent peaks ^{1}H δ 7.26, ^{13}C δ 77.0 and ^{1}H δ 2.49, ^{13}C δ 39.5, respectively. 2D NMR data (gradient COSY, HSQC and HMBC) were recorded using standard pulse sequences. 1D-NOESY as described by Stott, Stonehouse, Keeler, Hwang and Shaka (1995). Positive ESI-MS: Fisons VG Platform II. High resolution positive ESI-MS: Bruker BioAPEX 47e. Counter current: Sanki Centrifugal Partition Chromatograph Model LLB-M.

3.1. Plant material

Bark and leaves of *D. darlingiana* [(F. Muell.) L.A.S. Johnson] were collected in November 1995 from Timber Reserve 165 in North Queensland, Australia. *D. darlingiana* is a ditypic genus endemic to the 'wet tropics' and grows as a canopy tree in complex notophyll vineforest on basalt or granite derived soils. A voucher specimen (PIF 18157) of this species is lodged at the Queensland Herbarium.

3.2. Extraction and isolation

The dried and ground bark (100 g) of *D. darlingiana* was extracted sequentially with CH_2Cl_2, MeOH and H_2O. The CH_2Cl_2 and MeOH extracts were combined and partitioned between 2 M H_2SO_4 and $CHCl_3$. The acid layer was basified to pH 10 with 25% NH_4OH and partitioned with $CHCl_3$. The $CHCl_3$ layer (110 mg) was separated by counter current chromatography using the solvent system $CHCl_3$–MeOH–H_2O (13:7:8) with the MeOH–H_2O layer as the stationary phase to give darlingine (**1**) and a 1:15 mixture of **1** and **2**. The counter current separation was repeated on the mixture to give darlingine (**1**) (overall yield 75 mg, 0.075% dry weight) and darlingine *N*-oxide (**2**) (17 mg, 0.017% dry weight). An identical scheme was used to isolate alkaloids **1** and **2** from the leaves.

3.3. Darlingine (**1**)

$[\alpha]_D^{26}$ +51.7° ($CHCl_3$; *c* 1.00); $[\alpha]_D^{26}$ +46.4° (MeOH; *c* 1.00) [lit. (Majewski & Lazny, 1995) +51.5° (MeOH; *c* 1.02)]; positive ESI-MS *m/z* 220 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 1.48 (1H, m, H-8β), 1.78 (1H, m, H-7α), 1.90 (3H, s, 3-CH_3), 2.07 (1H, d, J = 18.6 Hz, H-10α), 2.17 (1H, m, H-7β), 2.19 (1H, m, H-8α), 2.21 (3H, s, 2-CH_3), 2.30 (3H, s, N-CH_3), 2.96 (1H, dd, J = 5.4, 18.6 Hz, H-10β), 3.42 (1H, dd, J = 5.4, 6.0 Hz, H-9), 4.14 (1H, d, J = 6.0 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 9.7 (q, 3-CH_3), 17.6 (q, 2-CH_3), 29.2 (t, C-8), 33.1 (t, C-10), 33.4 (t, C-7), 36.7 (q, N-CH_3), 55.5 (d, C-6), 57.8 (d, C-9), 120.2 (s, C-3), 123.5 (s, C-5), 159.3 (s, C-11), 160.6 (s, C-2), 177.1 (s, C-4); COSY and HMBC correlations: identical to **2**.

3.4. Darlingine N-oxide (**2**)

$[\alpha]_D^{27}$ +16.8° (MeOH; *c* 0.67); UV_{max}^{MeOH} nm (log ε) 216 (4.0), 258 (4.0); IR ν_{max}^{NaCl} cm^{-1}: 1652; positive ESI-MS *m/z* 236 $[M+H]^+$; HRMS positive ESI *m/z* 236.1276 ($[M+H]^+$, $C_{13}H_{18}NO_3$ requires 236.1281). ^1H and ^{13}C NMR (DMSO-d_6): Table 1.

3.5. Reduction of darlingine N-oxide (**2**)

Darlingine *N*-oxide (4.0 mg) was dissolved in 1 M HCl (2 ml), a small amount of Zn was added and the resulting mixture stirred at room temperature (3 h). The reaction mixture was filtered and the filtrate made alkaline with 25% NH_4OH and extracted with CH_2Cl_2. The CH_2Cl_2-soluble material was further purified by C18 column chromatography to give darlingine (**1**) (2.5 mg), which was identical in all respects to the natural product.

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