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Phytochemistry 64 (2003) 609–615
PHYTOCHEMISTRY
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Alkaloids and limonoids from *Bouchardatia neurococca*: systematic significance

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Received 26 December 2002; received in revised form 4 March 2003

Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

Five alkaloids, four β-indoloquinazoline and one furoquinoline, and four terpenoids, three limonoids and one modified sesquiterpene, have been obtained from the aerial parts of *Bouchardatia neurococca* (Rutaceae). Two of the alkaloids, 1,2-dihydroxyrutaecarpine and 2-(2-[3-formylindolyl])-(3H)-quinazolin-4-one (bouchardatine), and two of the limonoids, 23-oxo-21ξ-hydroxy-21,23-dihydroveprisone (veprisonic acid) and 21-oxo-23ξ-hydroxy-21,23-dihydroveprisone (isoveprisonic acid) are new. The pattern of secondary metabolites isolated is rather unusual in the Rutaceae and is reminiscent of *Tetradium*, a genus with which *Bouchardatia* has not previously been associated.

Keywords: *Bouchardatia neurococca*; Rutaceae; Alkaloids; β-Indoloquinazolines; Limonoids; Veprisone; Chemosystematics

1. Introduction

Bouchardatia Baill. (Rutaceae) is a monotypic genus in the Tribe Zanthoxyleae, subfamily Rutoideae of the Rutaceae. The single species *B. neurococca* (F. Muell.) Baill. (common name "Union Nut") forms a small tree up to 15 m tall and is endemic to the subtropical dry rainforests of the coastal areas of northeastern New South Wales (Richards, 1991) and southeastern Queensland, Australia (Forster, 2002). To the best of our knowledge no medicinal or culinary uses have been reported for any part of the tree.

Previously, an analysis of essential oil from the leaves of *B. neurococca* by GC/MS found that the major constituents were the sesquiterpenes β-caryophyllene, caryophyllene oxide, α-humulene and bicyclogermacrene (Brophy et al., 1994).

2. Results and discussion

Nine compounds were isolated from *B. neurococca* using a mixture of vacuum liquid chromatography and then preparative HPLC. Compounds **1**–**4** were identified as β-indoloquinazoline alkaloids, **5** as a furoquinoline, **6**–**8** as limonoids and **9** as a β-ionone.

Compound **1** was identified as the β-indoloquinazoline alkaloid rutaecarpine, which has been isolated from a number of sources in the Rutaceae (Mester, 1983), including the ripe fruit of *Tetradium rutaecarpum* (Tschesche and Werner, 1967). Similarly **2** was characterized as 1-hydroxyrutaecarpine, previously isolated from *Euxylophora paraensis* (Danieli et al., 1974).

Alkaloid **3** was obtained as a yellow amorphous solid. The API–ES mass spectrum (positive mode) showed a pseudomolecular ion $[M+H]^+$ at m/z 320, suggesting the molecular formula $C_{18}H_{13}N_3O_3$, 32 amu more than **1**. The UV and IR spectra supported the argument that this was a further β-indoloquinazoline alkaloid.

The ^{1}H NMR spectrum (Table 1) was similar to **1**, exhibiting two series of aromatic signals (4H and 2H, respectively) and two methylene groups. Analysis of the

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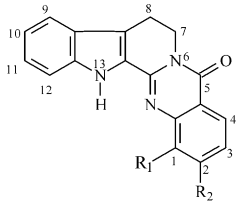
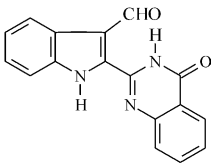
doi:10.1016/S0031-9422(03)00205-X



1 $R_1 = R_2 = H$

2 $R_1 = OH$, $R_2 = H$

3 $R_1 = R_2 = OH$



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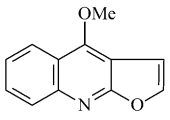

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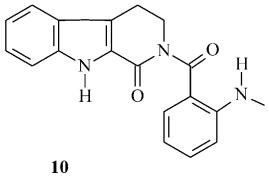

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COSY spectrum indicated that signals at δ 7.69, 7.14, 7.31 and 7.54 formed a spin system that, by comparison with **1** and **2**, could be assigned to H-9, H-10, H-11 and H-12 of ring-A.

The second aromatic spin system was made up of two *ortho*-coupled protons at δ 7.00 and 7.59, which were assigned as ring-E protons. Placement of these protons was achieved by analysis of an HMBC experiment (carbon values are approximate as insufficient material was available for a C-detected experiment). The proton at δ 7.59 revealed ^3J correlations with carbons resonating at $\sim\delta$ 160.6 (C-5), $\sim\delta$ 148.0 (C-14a) and $\sim\delta$ 137.1 (C-2) while that at δ 7.00 had ^3J correlation with carbons resonating at $\sim\delta$ 114.0 (C-4a) and $\sim\delta$ 138.1 (C-1). Thus, these protons could be assigned as H-4 and H-3, respectively. The two substituents on ring-E, at C-1 and C-2, must be hydroxyl groups.

The mass spectrum showed fragments at *m*/*z* 169, 168, 167, 155 and 115, the same as **1**, confirming that there was no substitution on ring A. Alkaloid **3** was, therefore, characterized as 1,2-dihydroxyrutaecarpine, which is a novel compound.

Alkaloid **4** was obtained as yellow amorphous powder. The API–ES (negative mode) showed an $[M-H]^-$ ion at *m*/*z* 288, suggesting the molecular formula $C_{17}H_{11}N_3O_2$. The UV spectrum showed absorption at λ_{max} 254, 351, 367 and 391 nm, indicating the presence of a highly conjugated system, similar to **1**–**3**. The IR spectrum showed carbonyl stretching for a secondary amide, an aldehyde carbonyl at 1686 cm^{-1} and N–H stretching at 3429 cm^{-1}.

Table 1
^{1}H NMR spectral assignments of **1**, **2** and **3**, run in acetone-d_6

H	1	2	3
1	7.64 (*brd*, 8.2)	8.60 (*brs*, OH)	–
2	7.77 (*ddd*, 8.2, 7.0, 1.2)	7.20 (*brd*, 7.8)	–
3	7.46 (*brdd*, 8.0, 7.0)	7.31 (overlap)	7.00 (*d*, 8.6)
4	8.22 (*dd*, 8.0, 1.2)	7.53 (*brd*, 8.2)	7.59 (*d*, 8.6)
7	4.56 (*t*, 7.0)	4.55 (*t*, 7.0)	4.51 (*t*, 6.9)
8	3.28 (*t*, 7.0)	3.30 (*t*, 7.0)	3.27 (*t*, 6.9)
9	7.70 (*brd*, 8.0)	7.70 (*brd*, 8.0)	7.69 (*brd*, 8.0)
10	7.15 (*brdd*, 8.0, 7.0)	7.15 (*brd*, 7.8, 7.2)	7.14 (*brdd*, 8.0, 7.1)
11	7.32 (*brdd*, 8.2, 7.0)	7.31 (overlap)	7.31 (*brdd*, 8.3, 7.1)
12	7.62 (*brd*, 8.2)	7.65 (*brd*, 7.0)	7.54 (*brd*, 8.3)
NH	11.05 (s)	11.07 (s)	11.10 (s)

The ^{1}H NMR in pyridene-d_5 (Table 2) showed two spin systems for 1,2-disubstituted aromatic rings, comparable to **1** (Table 1). A highly deshielded proton at δ 14.60, could be assigned to an N–H proton in a cyclic amide environment. Unlike **1**–**3** the ^{1}H NMR spectrum did not reveal two methylene groups, but it did show a signal of an aldehyde proton at δ 10.69.

Analysis of the HMBC experiment (Table 2) indicated this compound was an oxidized derivative of **1**. Correlation between the proton resonating at δ 8.57 (H-4) and a ^{13}C resonance at δ 162.3 (C-5), confirming the existence of the amide carbonyl as in the β-indoloquinazolines. The API–ES (negative mode) showed fragments for loss of an aldehyde from the molecular ion and another attributable to the quinazoline moiety at *m*/*z* 145. Analysis of the HMBC correlations associated with the second aromatic moiety (Table 2) indicated an indole type substructure with the aldehyde substituted at C-3 of the indole.

On this basis structure **4** can be assigned, and this has been given the trivial name bouchardatine. Bouchardatine **4** is probably a catabolic product of rutaecarpine (**1**) derived through oxidative fission of the 7/8 bond in 7,8-dehydrorutaecarpine. In this respect it is analogous to rhetsinine (**10**), which has been isolated from *Tetra-*

Table 2
^{1}H and protonated ^{13}C NMR spectral assignments for **4**, run in pyridine-d_5

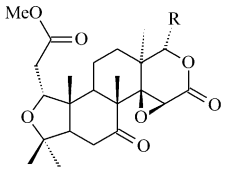
H/C	H	C	HMBC
1	7.65 (*dd*, 8.1, 0.7)	128.3	123.4[a] (C-4a), 127.7 C-3)
2	7.71 (*ddd*, 8.2, 7.1, 1.6)	135.1	127.3 (C-4), 149.6[a] (C-14a)
3	7.47 (overlap)	127.7	123.4[a] (C-4a), 128.3 (C-1)
4	8.57 (*dd*, 7,9, 1.5)	127.3	135.1 (C-2), 149.6[a] (C-14a), 162.3[a] (C-5)
9	8.38 (*brd*, 7.3)	120.3	126.4 (C-11), 137.3[a] (C-12a)
10	7.47 (overlap)	124.2	113.9 (C-12), 129.9[a] (C-8b)
11	7.50 (*ddd*, 8.4, 7.2, 1.3)	126.4	120.3 (C-9), 137.3[a] (C-12a)
12	7.80 (*brd*, 7.3)	113.9	124.2 (C-10), 129.9[a] (C-8b)
N-H	14.60 (*brs*)		
CHO	10.69 (s)	187.5	117.0[a] (C-8a), 137.0[a] (C-13a), 129.9 (C-8b)[a]

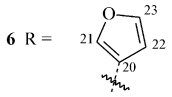
[a] Inferred from HMBC spectrum.

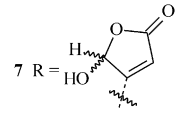
dium (Evodia) rutaecarpum and other Rutaceae and which involves fission of rutaecarpine through C13b-N14 and N14-C14a (Waterman, 1973).

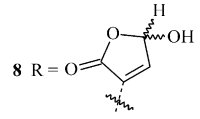
One further alkaloid was obtained and identified as the furoquinoline dictamnine (**5**), one of the commonest alkaloids of the Rutaceae.

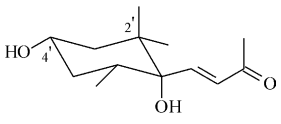
The API–ES (positive mode) MS of compound **6** gave a pseudomolecular ion $[M+H]^+$ at *m/z* 487, compatible with the molecular formula $C_{27}H_{34}O_8$. The IR spectrum showed ketone absorption at 1710 cm^{-1} and ester and lactone carbonyl absorption at 1745 cm^{-1}. The ^1H NMR spectrum in $CDCl_3$ (Table 3) suggested a limonoid, with signals that could be assigned to a β-substituted furan, five methyls (all quaternary), three oxymethines (one an epoxide) and a methoxyl. The ^{13}C NMR spectrum (Table 3) in $CDCl_3$ showed all 27 carbon signals. The most down field signal was a ketone-carbonyl, resonating at δ 208.3. The ^{13}C NMR spectrum also showed ester or lactone carbonyls at δ 171.4 and 167.4.











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It is common of limonoids isolated from Rutaceae to have a ketone oxygen at 7 position and a δ-lactone in ring D (Dreyer, 1983). This presumption was confirmed by an HMBC experiment (Table 3) that showed ^3J correlation between methyl protons at δ 1.21 (H-30) and the ketone-carbonyl at δ 208.3 (C-7) and oxymethine proton at δ 4.15 (H-15) showed ^2J correlation to ester carbon at δ 167.4 (C-16). The placement of the second ester carbonyl was again accomplished through the HMBC experiment. The HMBC experiment also showed the methoxyl δ 3.70 and methylene protons at δ 2.43 and 2.23 with correlation to the ester carbon at δ 171.4. Furthermore, an H–H COSY spectrum revealed these methylene protons coupled with the proton at δ 4.24, and this proton, through HMBC correlations could be placed as H-1. This required that that the methyl ester was substituted on C-2 of an obacunoic acid type limonoid (Dreyer, 1983). The configuration of the furan at C-17 is presumed to be α, as is the case in all limonoids of the Rutaceae, Meliaceae and Cneoraceae (Dreyer, 1983). Stereochemistry at C-1 is more problematical (H-1α = isoobacunoic acid, H-1β = epiisoobacunoic acid). The proton H-1 shows a clear double doublet (11.1, 3.4 Hz) in contrast to the α-H of iso-obacunoic acid which is reported as an ill-defined multiplet (Govindachari et al., 1964) and of the related 6-hydroxy epiobacunoic acid where it is recorded as a multiplet (Herman et al., 1987). Compound **6** showed weak levorotatory activity comparable to veprisone and was therefore characterized as this rare limonoid (reported $[\alpha]_D -18°$), first isolated from *Vepris bilocularis* Engler (Rutaceae) (Govindachari et al., 1964). Full NMR spectroscopic data does not appear to have been reported for veprisone and consequently is presented here (Table 3).

The API–ES (positive mode) mass spectrum of compound **7** showed a pseudomolecular ion $[M+H]^+$ at *m/z* 519 suggesting another limonoid, molecular formula $C_{27}H_{34}O_{10}$. The ^1H and ^{13}C NMR spectra in $CDCl_3$ (Table 3) were comparable to those of veprisone except with respect to the furan ring, which gave resonances for an α-hydroxy dihydrofuran lactone (derived from an oxidized furan), with signal splitting showing that the hydroxy was existing as a mixture of both epimers.

The major epimer showed two furan protons resonating at δ 6.30 (*d*, 1.0) and 6.04 (*brs*) and bonded to carbons resonating at δ 123.6 and 97.9, respectively, the latter being typical for an acetal. The carbon C-20 resonated at δ 162.8, which, because it is highly deshielded, must be β to the lactone carbonyl requiring that the lactone carbonyl and acetal carbons be assigned to C-23 and C-21, respectively. Compound **7** can thus be defined as 23-oxo-21ξ-hydroxy-21,23-dihydroveprisone or veprisonic acid.

Compound **8** gave spectral characteristics very similar to **7**, and again exhibited split signals suggesting two α-hydroxylactone epimers. In this case the ^1H and ^{13}C resonances for the methine protons of the major furan ring epimer (Table 3) were observed at δ 7.33 (δ 150.5) and δ 6.20 (δ 97.8) and for the two quaternary carbons at δ 169.4 (lactone carbonyl) and 133.7 (C-20). The shielded resonance for C-20 places it α to the carbonyl and allows compound **8** to be identified as 21-oxo-

Table 3
^{1}H, ^{13}C NMR assignments (run in $CDCl_3$) for the limonoids **6**, **7** and **8** (with HMBC correlations observed)

	6		**7**		**8**		Observed HMBC correlations[a]
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	
1	4.24 (*dd*, 11.1, 3.4)	84.0	4.25 (*dd* 11.0,3.3)	84.0	4.23 (*dd*, 11.0, 3.5)	83.9	C-4, C-5, C-19
2	2.43 (*dd*, 14.1, 11.1)	37.1	2.46 (*dd* 14.0,11)	37.3	2.44 (*m*)	37.3/37.2	C-1, C-3
	2.23 (*dd*, 14.1, 3.4)		2.27 *dd* (14.0,3.2)		2.30 (*m*)		C–3
3	–	171.4	–	171.5	–	171.7/171.4	
4	–	81.1	–	81.0	–	81.0	
5	2.10 (*dd*, 15.3, 3.4)	56.2	2.10 (*dd* 15.0,4.3)	56.6	2.09 (*m*)	55.9/55.8	C-4, C-6, C-10, C-19, C-28, C-29
6	2.82 (*dd*, 15.3, 14.0)	36.9	2.85 (*dd* 15.0,14.0)	36.9	2.79 (*m*)	36.9	C-5, C-7
	2.36 (*dd*, 14.0, 3.4)		2.37 *dd* (14.0,3.0)		2.34 (*m*)		C–5, C–7, C–10
7	–	208.3	–	208.1	–	208.4	
8	–	51.6	–	52.1	–	51.3/51.2	
9	2.26 (*dd*, 12.2, 2.7)	43.7	2.22 (*m*)	43.9	2.23 (*m*)	43.4/43.3	C-8, C-10, C-11, C-12, C-19, C-30
10	–	47.3	–	47.3	–	47.3	
11	1.84–1.93 (*m*)	18.4	1.90 (*m*)	18.6	1.97 (*m*)	18.0/17.9	
	1.54 (*m*)		1.62 (*m*)		1.54 (*m*)		C–8
12	1.77 (*m*)	30.8	1.99 (*m*)	31.0	2.16 (*m*)	28.5/28.3	C-17
	1.41 (*m*)	1.59 (*m*)	1.27 (*m*)				
13	–	38.5	–	38.7	–	39.4	
14	–	66.7	–	65.9	–	67.2/67.1	
15	4.15 (*s*)	54.4	3.95/3.93 (*s*)	53.5	4.29/4.25 (*s*)	54.6/54.4	C-14, C-16
16	–	167.4	–	166.0	–	167.5/167.0	
17	5.50 (*s*)	78.2	5.39/5.40 (*d* 1.1)	78.8	5.46 (*brd*)	75.9/75.6	C-13, C-14, C-18, C-20, C-21, C-23
18	1.19 (*s*)	21.2	1.19 (*s*)	21.7	1.19/1.17 (*s*)	20.5/20.4	C-12, C-13, C-14, C-17
19	1.41 (*s*)	19.9	1.42 (*s*)	20.2	1.40 (*brd*)	19.7/19.6	C-1, C-5, C-9, C-10
20	–	120.4	–	162.8	–	133.9/133.7	
21	7.42 (*dd*, 1.5, 0.7)	141.4	6.04/6.08 (*br s*)	97.9	–	169.8/169.4	C-20
22	6.34 (*dd*, 1.8, 0.7)	110.0	6.30/6.27 *d* (1.0)	123.6	7.35/7.33 (*s*)	150.7/150.5	C-20, C-21, C-23
23	7.40 (*dd*, 1.8, 1.6)	143.4	–	168.9	6.20/6.17 (*s*)	97.8/97.5	C-20, C-21, C-22
28	1.30 (*s*)[b]	32.0[b]	1.30 (*s*)[b]	32.0[b]	1.30 (*s*)[b]	32.1[b]	C-4, C-5, C-29
29	1.18 (*s*)[b]	23.9[b]	1.18 (*s*)[b]	23.8[b]	1.17 (*s*)[b]	24.0/23.9[b]	C-4, C-5, C-28
30	1.21 (*s*)	18.0	1.17/1.19 (*s*)	17.5	1.23/1.27 (*s*)	18.4/18.3	C-7, C-8, C-9
OCH_3	3.70 (*s*)	52.2	3.73 (*s*)	52.4	3.72 (*s*)	52.4/52.3	C-3

[a] Not all recorded correlations were necessarily observed for each limonoid.
[b] Assignments within a column are interchangeable. In **7** and **8**, particularly the ^{13}C NMR spectrum of **8**, doubling of signals was seen due to the presence of both hydroxy epimers. Both signals are recorded, separated by a (/), with the predominant resonance recorded first.

23ξ-hydroxy-21,23-dihydroveprisone or isoverprisonic acid.

The ^{1}H NMR spectrum of **9** in $CDCl_3$ showed signals of two *trans* olefinic protons, an oxymethine and four methyl groups at δ 2.29 (*s*), 1.05 (*s*), 0.89 (*s*) and 0.82 (*d*, 6.8). Five further signals (each 1H) occurred as discrete resonances. A COSY experiment indicated an R–CH_2–CH(O)–CH_2–C(Me)H–R spin system. The ^{13}C NMR spectrum in $CDCl_3$ showed three quaternary carbons, a carbonyl at δ 197.8 and two sp^3 carbons as well as carbons associated with each of the proton resonances. This suggested an empirical formula $C_{13}H_{22}O_3$ although APCI–MS (positive mode) indicated *m*/*z* 209 ($M+H-H_2O$). On the basis of the HMBC experiment and a comparison of chemical shift data with that published (Busch et al., 1998) **9** was characterized as boscialin, first isolated from *Boscia salicifolia* (Capparidaceae) (Pauli et al., 1990) and subsequently synthesized by Busch et al. (1998). The ^{13}C NMR spectrum was in close agreement with that of (−)-boscialin (Bausch et al., 1998). Additional information was obtained on assignments of H-3′ to H-6′ in the ^{1}H NMR spectrum and these are listed in the experimental. Compound **9** was shown to be levorotatory but attempts to obtain an accurate $[\alpha]_D$ were unsuccessful because of impurities.

With the exception of boscialin **9** all of the compounds isolated from *B. neurococca* are typical of the Rutaceae. Previous reports of β-indoloquinazolines are restricted to the Rutaceae and include the genera *Euxylophora*, *Tetradium*, *Zanthoxylum*, *Araliopsis*, *Hortia, Phellodendron* and *Vepris* (Mester, 1983; Hegnauer, 1990). Veprisone is a rare limonoid, originally found in *Vepris bilocularis*, a species occurring in Madagascar and India.

There are two particular convergences that can be seen between the chemistry of *B. neurococca* and other Rutaceae.

Convergence with Tetradium. *Tetradium* is an interesting genus found in east Asia from Indonesia to Japan and which is thought to have affinities to genera such as *Zanthoxylum, Fagaropsis* and *Phellodendron* (Hartley, 1981) which are the only sources of 1-benzylisoquinoline alkaloids in the family (Ng et al., 1987a). *Tetradium* species produce both β-indoloquinazoline alkaloids and limonoids, the latter sharing the characteristic of occurring as dihydrofurans, as in **7** and **8** (Ng et al., 1987a, b; Quader et al., 1990).

Convergence with Vepris *and allied taxa*. *Vepris* is a largely African and Madagascan genus with one species, *V. bilocularis*, also being found on the Indian subcontinent. Limonoids are known from two species, notably veprisone, while β-indoloquinazolines occur as well, although they are not the dominant class of alkaloid (Dagne et al., 1988).

According to Hartley (T. G. Hartley, personal communication) *Bouchardatia neurococca* has no obvious close affinities among the Australian Rutaceae, although he has recently placed it in close proximity to the genera *Acradenia* Kippist and *Bosistoa* F. Muell. ex Benth. (Hartley, 2001). In attempting to identify the relationships of this species it would seem appropriate to consider both *Tetradium* and *Vepris*, particularly *Tetradium*, on the basis of their close metabolic affinities.

3. Experimental

3.1. General

The ^{1}H and ^{13}C NMR spectra were acquired on a Bruker AVANCE DRX-500 spectrometer operating at 500.13 MHz and 125.77 MHz, respectively. Mass spectra were obtained using a Agilent 1100 series LC/MSD in APCI or API-ES mode. Analytical HPLC-UV analysis and semi-prep. separations were performed on HP 1100 series HPLC system (Hewlett Packard) with an UV–vis detector and a Hypersil ODS RP C_{18} column (125×4 mm, 5 μm) (Hewlett-Packard). Prep. HPLC (Gilson) was performed with an UV–vis detector and a Altima RP C_{18} column (150×22 mm, 5 μm) (Alltech, USA). Vacuum liquid chromatography was carried out using silica gel 60 G HF-254 (mean particle size = 15 μm). CC was carried out using silica gel (60–120 mesh). TLC was performed using Merck precoated silica gel (60 F_{254}). Spots were observed under UV light after spraying with vanillin–H_2SO_4 reagent, followed by heating.

3.2. Plant material

Leaf, bark and twig of *B. neurococca* were collected from Colloseum Creek, State Forest 695 Polmaily, Queensland, 24° 23′ S, 151 27′ E. A voucher (Forster PIF25477) has been deposited at the Queensland Herbarium (BRI).

3.3. Extraction

The air-dried stem bark, leaves, and twigs of *Bouchardatia neurococca* were each separately ground and macerated with hexane to remove the chlorophyll and non-polar compounds. The residuals from the hexane extractions were macerated with MeOH. The MeOH extracts were concentrated using a rotary evaporator.

The bark (58.7 g) yielded a MeOH extract (3.1 g) of which 3.0 g was subjected to vacuum chromatography (VLC) over silica gel eluting with hexane containing increasing amounts of EtOAc and then EtOAc containing increasing amounts of MeOH. The fraction obtained with 15% EtOAc in hexane was subjected to

semi-prep. HPLC (Supelcosil LC-18-DB, 5μm, 250 mm×10 mm) with a flow rate of 1.5 ml min^{-1}. Solvent system MeOH:H_2O–45% MeOH for 10 min, 45–80% MeOH in 15 min, 80–95% MeOH in 15 min, 95–45% MeOH in 5 min. Detection—UV at 210 nm. The following compounds were obtained: **7** (elution 29.2–30.2 min, 6.0 mg), **8** (30.8–31.8 min, 8.4 mg) and **4** (38.6–39.3 min, 1.8 mg). Between 41.0 and 42.0 min a mixture was obtained that was subsequently separated by semi-prep. HPLC using the same column, flow rate and detection with an isocratic solvent system AcCN:H_2O(+ 0.05% TFA) ratio 60:40. Compounds **2** (19.8–20.5 min, 1.1 mg) and **1** (20.7–21.5 min, 2.6 mg) were separated.

The VLC fraction obtained with 35-70% EtOAc in hexane was purified by semi-preparative HPLC (same column, flow rate and detection) with an MeCN:H_2O (+0.05% TFA) solvent, changing as follows: 30% MeCN for 10 min, 30–90% AcCN over 30 min. A single compound (**3**) was obtained (32.8–33.5 min, 1.0 mg).

The ground twigs (209 g) yielded an extract (8.2 g) of which an aliquot (8.0 g) was subjected to VLC as before. Elution of with 40-50% EtOAc in MeOH gave a fraction that was fractionated by semi-prep. HPLC (Zorbax SB-C_{18}, 5 μm, 250 mm×9.4 mm) with a flow rate of 1.5 ml min^{-1}. Solvent system MeOH:H_2O–45% MeOH for 10 min, 45–80% MeOH in 15 min, 80–95% MeOH in 15 min, 95–45% MeOH in 5 min. Detection—UV at 210 nm. The eluant from 32.6–33.4 min yielded **5** (4.8 mg).

The ground leaf (200 g) yielded 19.5 g of extract of which an aliquot (10.0 g) was subjected to VLC as earlier. From the eluate with 60% EtOAc in hexane a mixture was obtained that was purified by prep. HPLC (Altima C_{18}, 5μm, 150 mm×22 mm) with a flow rate of 25 ml min^{-1}. Solvent system MeCN (+0.1% TFA):H_2O (+0.1% TFA) 5–90% MeCN in 30 min. The fractions obtained between 21.0 and 21.5 min yielded **6** (9.5 mg). A further eluate, obtained with 80–90% EtOAc in hexane followed by the same HPLC procedure gave, between 9.50 and 9.75 min, **9** (2.3 mg).

3.3.1. *Rutaecarpine (**1**)*

Pale yellow amorphous solid. MS–API–ES (positive mode): *m/z* (rel. int.) $[C_{18}H_{13}N_3O + H]^+$ 288 (45), 258 (22), 243 (22), 169 (48), 168 (37), 167 (22), 155 (40), 144 (61), 143 (76), 142 (79), 116 (59), 115 (100), 105 (44). UV λ_{max} nm : 277, 288, 331, 344, 361, 389. ^{1}H NMR see Table 1. ^{13}C NMR in close agreement with literature data (Ikuta et al., 1998).

3.3.2. *1-Hydroxyrutaecarpine (**2**)*

Pale yellow amorphous solid. MS–API–ES (positive mode): *m/z* (rel. int.) $[C_{18}H_{13}N_3O_2 + H]^+$ 304 (37), 260 (22), 231 (17), 169 (100), 168 (27), 167 (18), 155 (25), 143 (58), 142 (50), 115 (94), 105 (36). ^{1}H NMR see Table 1. UV and IR data in close agreement with that reported for 1-hydroxyrutaecarpine isolated from *Tetradium glabrifolium* (Ng, 1986; Ng et al., 1987b).

3.3.3. *1,2-Dihydroxyrutaecarpine (**3**)*

Yellow amorphous solid. MS–API–ES (positive mode): *m/z* (rel. int.) $[C_{18}H_{13}N_3O_3 + H]^+$ 320 (9), 245 (19), 218 (18), 169 (11), 167 (62), 166 (49), 140 (100), 129 (26), 115 (43), 114 (30). UV λ_{max} (log ε) nm: 256 (3.6), 343 (3.5), 360 (3.5), 377 (*sh*). IR ν_{max} cm^{-1} (KBr disc): 3433, 2925, 2853, 1631, 1460, 1402, 1261, 1155, 1029. ^{1}H NMR see Table 1.

3.3.4. *Bouchardatine (**4**)*

Yellow amorphous powder. MS–API–ES (negative mode): *m/z* (rel. int.) $[C_{17}H_{11}N_3O_2-H]^-$ 288 (34), 260 (53), 209 (14), 141 (25), 116 (8). UV λ_{max} (log ε) nm: 254 (3.3), 351 (3.2), 367 (sh), 391 (*sh*). IR ν_{max} cm^{-1} (KBr disc): 3429, 2925, 2855, 1686, 1607, 1592, 1468, 1442, 1390, 1328, 1215. ^{1}H and ^{13}C NMR see Table 2.

3.3.5. *Dictamnine (**5**)*

Amorphous powder. MS–APCI (positive mode): *m/z* (rel. int.) $[C_{12}H_9O_2N]^+$ 200 (17), 185 (86), 161 (16), 156 (36), 129 (100), 102 (33). UV, IR and ^{1}H and ^{13}C NMR spectrum in agreement with those of an authentic sample isolated from *Tetradium trichotomum* (Quader et al., 1990).

3.3.6. *Veprisone (**6**)*

White amorphous solid. $[\alpha]_D-11^\circ$ (*c*, 0.22, $CHCl_3$). MS–API–ES (positive mode): *m/z* (rel. int.) $[C_{27}H_{34}O_8 + H]^+$ 487 (100), 459 (21). MS–API–ES (negative mode): *m/z* (rel. int.) $[C_{27}H_{34}O_8-H]^-$ 485 (6), 429 (24), 363 (28), 291 (31), 249 (45). IR ν_{max} cm^{-1} (KBr disc): 2963, 2926, 1745, 1710, 1443, 1392, 1168, 1026. ^{1}H and ^{13}C NMR spectra, see Table 3.

3.3.7. *23-Oxo-21ξ-hydroxy-21,23-dihydroveprisone (**7**)*

White amorphous solid. MS–API–ES (positive mode): *m/z* (rel. int.) $[C_{27}H_{34}O_{10} + H]^+$ 519 (53), 501 (60), 483 (11), 473 (14), 445 (31), 427 (15), 417 (16), 399 (31), 371 (14). IR ν_{max} cm^{-1} (KBr disc): 3434, 2961, 2929, 1764, 1744, 1709, 1443, 1276, 1180, 1129. ^{1}H and ^{13}C NMR spectra, see Table 3.

3.3.8. *21-Oxo-23ξ-hydroxy-21,23-dihydroveprisone (**8**)*

White amorphous solid. API-ES (positive mode): *m/z* (rel. int.) $[C_{27}H_{34}O_{10} + H]^+$ 519 (60), 501 (14), 473 (7), 445 (39), 427 (14), 417 (6), 399 (6), 371 (6). IR ν_{max} cm^{-1} (KBr disc): 2924, 2854, 1751, 1709, 1457, 1261, 1202, 1023. ^{1}H and ^{13}C NMR spectra, see in Table 3.

3.3.9. *(−)-Boscialin (**9**)*

Colourless amorphous solid. $[\alpha]_D$ levorotatory, $<8^\circ$, ($CHCl_3$). APCI (positive mode): *m/z* (rel. int.) $[MOH]^+$

209 (100), 191 (23), 167 (5), 153 (24), 109 (65). UV λ_{max} nm: 230. IR ν_{max} cm^{-1} (KBr disc): 3442, 2964, 2934, 1673, 1626, 1462, 1364, 1266, 1171, 1041, 995. ^1H NMR ($CDCl_3$): δ 6.77 (1H, *d*, $J=15.9$ Hz, H-3), 6.38 (1H, *d*, $J=15.9$ Hz, H-3), 3.92 (1H, *m*, H-4′), 2.29 (3H, *s*, Me-1), 2.09 (1H, *dq*, $J=12.6$, 6.8 Hz, H-6′), 1.85 (1H, *m*, H-5′eq), 1.63 (1H, *t*, $J=12.2$ Hz, H-3′ax), 1.56 (1H, *ddd*, $J=12.6$, 4.6, 2.0 Hz, H-3′eq), 1.36 (1H, *ddd*, $J=12.6$, 12.6, 12.6 Hz, H-5′ax), 1.05 (3H, *s*, 2′-Me), 0.89 (3H, *s*, 2′-Me), 0.82 (3H, *d*, $J=6.8$ Hz, 6′-Me). ^{13}C NMR spectra in agreement with published data for boscialin (Busch et al., 1998).

Acknowledgements

C.W. extends his grateful thanks to the Royal Thai Government for the award of a scholarship. P.I.F. wishes to thank R. Booth for assistance with the collection of material and the Department of Natural Resources for permits to collect in State Forests and Timber Reserves in Queensland.

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