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TERPENOIDS, ALKALOIDS AND COUMARINS FROM *BORONIA INORNATA* AND *BORONIA GRACILIPES*

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Key Word Index—*Boronia inornata*; *Boronia gracilipes*; Rutaceae; furoquinoline alkaloids; prenylcoumarins; sesquiterpenes; triterpenes; protolimonoids; chemical systematics.

Abstract—Aerial parts and roots of *Boronia inornata* yielded the sesquiterpenes spathulenol and 1(10),5-germacradien-4-ol, the triterpenes moronic acid, moronic aldehyde (novel), betulonic acid and lupeol, the alkaloids dictamnine, evolitrine, isodictamnine and hordenine, and 8-(3,7-dimethyl-2,6-octadienyl)-7-hydroxycoumarin. A second sample of *B. inornata* gave the protolimonoids niloticin and piscidinol-A in addition to all those compounds noted above. A sample *B. gracilipes* yielded spathulenol, rutin and the triterpenes betulonic acid, betulonic aldehyde, oleanonic acid,3-epioleanonic acid and oleanonic aldehyde. Results are now available for investigations of species belonging to all three of the sections of *Boronia* and the distribution of compounds is discussed.

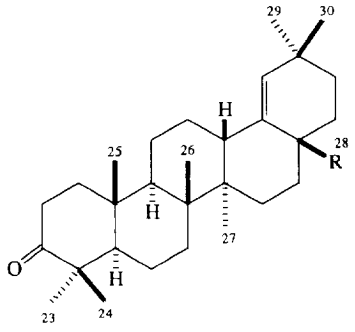
INTRODUCTION

In this paper we report the results of phytochemical investigations of two further species of the genus *Boronia*. *Boronia inornata* Turcz. has a disjunct distribution occurring in south-western and south-eastern Australia, while *B. gracilipes* F. Muell. is restricted to the karri forests of south-western Australia [1]. Both species belong to the section Boronia [1] and with their investigation information is now available on taxa belonging to all three of the sections of the genus delineated by Weston *et al.* [1]. In this paper the opportunity is taken to review what is known of the distribution of secondary metabolites in relation to the classification of Weston *et al.* [1].

RESULTS AND DISCUSSION

Ground samples of plant material were extracted by Soxhlet with petrol, then ethyl acetate and finally methanol. Extracts were concentrated and then compounds separated by VLC followed by either PTLC, CC or gel filtration, or a combination thereof.

The petrol extract of the sample of *B. gracilipes* yielded only known compounds, the sesquiterpene spathulenol and a series of oleanane and lupane triterpenes. The methanol extract gave the quercetin glycoside rutin. All compounds were identified by direct comparison with authentic samples or by spectroscopic analysis and comparison with literature data (see Experimental).



1 R = COOH

2 R = CHO

The petrol extract of *B. inornata* roots and stems yielded moronic acid (**1**) as the major compound. A full NMR analysis of **1** was undertaken using the HCCOBI [2] and HMBC [3] techniques to establish $^{1}H-^{13}C$ connectivities. These data are presented in Table 1 for comparative purposes with those of a minor component of the same extract which was characterized as moronic aldehyde (**2**), which appears to be novel. Two sesquiterpenes were obtained from this extract, spathulenol and 1(10),5-germacradien-4-ol, this being the first time the latter has been reported from the Rutaceae. A separate extraction of the leaves of this sample yielded 8-(3,7-dimethyl-2,6-octadienyl)-7-hydroxycoumarin. A second

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Table 1. ^1H and ^{13}C NMR data for moronic acid (1) and moronic aldehyde (2)

C/H	δ_H 1	δ_H 2	δ_C 1	δ_C 2
1			40.0	40.1
2	2.48 *m*	2.48 *m*	34.2	34.3
3			218.5	218.3
4			47.5	47.5
5			55.1	55.1
6			19.8	19.8
7			33.7	34.0
8			40.7	40.8
9			50.6	50.3
10			37.1	37.1
11			21.6	21.6
12			26.2	26.3
13			41.6	41.5
14			42.8	42.9
15			29.5	29.6
16			34.0	29.9*
17			48.1	51.7
18			136.7	135.6
19	5.15 *br s*	5.31 *br s*	133.4	134.9
20			32.2	32.4
21			33.6*	33.3
22			33.5*	29.0*
23	1.07 *s*	1.08 *s*	27.0	27.1
24	1.02 *s*	1.03 *s*	21.1	21.2
25	0.94 *s*	0.96 *s*	16.7	16.7
26	1.01 *s*	1.00 *s*	16.0	16.1
27	0.78 *s*	0.80 *s*	15.0	15.0
28		9.39 *s*	182.8	205.2
29	0.99 *s*	1.00 *s*	30.5	30.8
30	0.97 *s*	1.00 *s*	29.3	29.4

Spectra run at 400 MHz (^1H) or 100 MHz (^{13}C) in $CDCl_3$

*In same column indicate signals interchangeable.

collection of *B. inornata* yielded the same compounds plus small amounts of the known protolimonoids nilotiticin and piscidinol-A.

In the course of her studies on *Boronia* one of us (M.A.) has accumulated information on the secondary metabolites of eight species [4–10]. These have demonstrated the presence of quinoline and acridone alkaloids, coumarins, flavonoids, acetophenones, triterpenes and limonoids. The distribution of compounds isolated in these studies plus that previously available on *B. ternata* [11] is shown in Table 2.

The information in Table 2 is arranged according to the sectional classification suggested by Weston *et al.* [1] based on a cladistic analysis using a total of 32 anatomical and growth characters. There is an encouraging degree of convergence between the system of Weston *et al.* [1] and the results of the metabolic profiles available so far. Most striking is the section Cyanothamnus where all three species investigated have been characterized by the production of O- and/or C-prenylated flavonoids, a type of metabolite that has yet to be found in any species of the other two sub-genera. Furthermore, the two south-western species of this section, *B. coerulescens* and *B. ramosa*, are further distinguished by the presence of prenylated acetophenones.

Equally significant from a systematic viewpoint is the concentration of 4-quinolinone and acridone alkaloids in species of the section Valvatae. These are the major constituents of two species investigated by us and had previously been found in *B. ternata* by other workers. The presence of 8-geranylcoumarins is a further feature of *B. lanceolata* and *B. bowmanii*, but these also occur in *B. inornata* of section Boronia, so they are not entirely characteristic of the Valvatae. The one atypical species in the Valvatae is *B. alata* which has yielded only limonoids and protolimonoids. *Boronia alata* appears in the analysis of Weston *et al.* [1] as a separate basal clade somewhat

Table 2. Distribution of compounds among the species of *Boronia* investigated to date

Subgenus species	Alkaloids a	Alkaloids b	Alkaloids c	Coumarins	Flavonoids a	Flavonoids b	Acetophenones	Triterpenes	Limonoids
Cyanothamnus									
B. coerulescens F. Muell. [4]	+				+		+		
B. inconspicua Benth. [5]					+	+			
B. ramosa Benth. [6]					+		+		
Valvatae									
B. alata Sm [7]									+
B. bowmanii F. Muell. [8]	+	+	+	+					
B. lanceolata F. Muell. [9, 10]		+	+	+		+			
B. ternata Endl. [11]		+							
Boronia									
B. gracilipes F. Muell.								+	
B. inornata Turcz	+			+		+		+	+

Alkaloids: a = furoquinolones and furoquinolones; b = 4-quinolones; c = acridones.
Flavonoids: a = aglycones (usually C- or O- prenylated); b = glycosides.

distant from the *lanceolata/bowmanii* group and this distinction appears to be supported by the secondary metabolism.

In contrast to the metabolic diversity of the Valvatae and Cyanothamnus the two species of section Boronia on which information is available (this paper) appear rather uniform. In both, the major metabolites appear to be the triterpenes of the lupane and oleanane types. *Boronia inornata* is the more diverse yielding both an 8-C-geranylcoumarin and two protolimonoids, and in this respect may be indicative of an association with the Valvatae, or at least with the aberrant *B. alata*.

Two other groups of metabolites seem to occur sporadically. These are the furoquinoline alkaloids which can be regarded as characteristic of the family [12] and flavonol glycosides, which are again common throughout the family [13]. We are currently undertaking studies on selected species of *Boronia* in order to test the taxonomic robustness of the secondary metabolite data.

EXPERIMENTAL

UV: MeOH, IR: KBr disks. NMR spectra were run in $CDCl_3$ at 400 MHz for ^1H spectra and 100.6 MHz for ^{13}C spectra. 2D experiments were run using the standard Bruker microprograms. Petrol refers to petroleum ether (bp 60–80°).

Plant material. Samples of *B inornata* subsp. *leptophylla* were collected near Kumarl, close to Peak Charles National Park, and near Gora Hill in Cape Arid National Park and voucher specimens, PERTH 01156748 and PERTH 01156758, respectively, have been deposited in the Western Australian Herbarium, Perth. A sample of *B. gracilipes* was collected in karri forest near Walpole, *ca* 450 km south of Perth and a voucher PERTH 01157787 has been deposited in the Western Australian Herbarium, Perth.

Isolation of compounds from B. inornata *root and stem* (*Perth* 01156748). Ground roots and stems (105 g) were extracted sequentially with petrol, then EtOAc and finally MeOH. On concn the petrol extract gave a ppt. of **1** (200 mg). The supernatant was subjected to VLC over silica gel eluting with petrol containing increasing amounts of EtOAc. PTLC (silica gel, toluene–EtOAc, 24:1) of the 5–8% EtOAc eluate gave 2 (4 mg), 1(10),5-germacradien-4-ol (20 mg) and spathulenol (2.5 mg). The 20% EtOAc eluate yielded betulonic acid (4 mg). A similar VLC sepn of the original EtOAc extract gave, on elution with 30–50% EtOAc a mixt. of alkaloids which were sepd by PTLC (silica gel, toluene–EtOAc–HOAc, 40:9:1) to yield dictamnine (0.8 mg) and evolitrine (2.2 mg). Elution with 60–90% EtOAc gave isodictamnine (1.5 mg). Concn of the MeOH extract and PTLC (silica gel, $CHCl_3$–MeOH, 4:1) gave hordenine (21 mg).

Isolation of compounds from B. inornata *leaves* (*Perth* 01156748). Ground leaves (110 g) were extracted using the same regime. From the VLC of the petrol extract elution with 12–15% EtOAc gave lupeol (20 mg) and 20–30% EtOAc gave 8-(3,7-dimethyl-2,6-octadienyl)-7-hydroxycoumarin (30 mg).

Isolation of compounds from B. inornata *root* (*Perth* 01156758). Ground root (35 g) was extracted as before. TLC analysis showed the same series of compounds to be present as in sample Perth 01156748, but with two additional major spots in the EtOAc extract. These were sepd by VLC followed by PTLC (silica gel, petrol–EtOAc) to give niloticin (10 mg) and piscidinol-A (5 mg).

Isolation of compounds from B. gracilipes. Ground aerial parts (400 g) were extracted sequentially with petrol, EtOAc and MeOH. VLC (silica gel) of the concd petrol extract, eluting with petrol containing increasing amounts of EtOAc gave, with 5–8% EtOAc a mixt. which was sepd by PTLC (silica gel, toluene–EtOAc, 46:1) into betulonic aldehyde (10 mg) and oleanonic aldehyde (5 mg). The 10–15% EtOAc eluate yielded spathulenol (30 mg). The 25–30% eluate yielded a mixt. which was sepd by PTLC (silica gel, toluene–EtOAc, 19:1) to give betulonic acid (12.5 mg), 3-epioleanonic acid (7 mg) and oleanonic acid (12 mg); the MeOH extract yielded rutin (40 mg).

Rutin (commercial), dictamnine [14], spathulenol [15], betulonic acid [16], betulonic aldehyde [16], lupeol [16], oleanonic acid [16], niloticin [17], piscidinol-A [17], 8-(3,7-dimethyl-2,6-octadienyl)-7-hydroxycoumarin [18] and hordenine [19] were all identified by direct comparison with material previously isolated in the Strathclyde laboratories.

Evolitrine. Needles from EtOAc, mp 114° (lit. [20] 114–115°). Found: $[M]^+$ 229.0714; $C_{13}H_{11}NO_3$ requires 229.0739. UV, IR, NMR in agreement with that anticipated [20, 21].

Isodictamnine. Needles from EtOAc, mp 178–180° (lit. [22] 185–188°). Found: $[M]^+$ 199.0609; $C_{12}H_9NO_2$ requires 199.0633. UV, IR, NMR in agreement with that anticipated [21, 22].

1(10),5-Germacradien-4-ol. Oil $[\alpha]_D$ − 64° ($CHCl_3$; *c* 1.0) (lit. [23] − 109°). Found: $[M]^+$ 222.1982; $C_{15}H_{26}O$ requires 222.1983. IR, NMR in agreement with that published [23].

Oleanonic aldehyde. Needles from petrol, mp 141° (lit. [24] 138–140°), $[\alpha]_D$ + 52° ($CHCl_3$; *c* 0.5) (lit. [24] + 89°). Found: $[M]^+$ 438.3493; $C_{30}H_{46}O_2$ requires 438.3498. IR, NMR, EIMS in agreement with that published [24].

3-Epioleanonic acid. Needles from petrol, mp 298–300° (lit. [25] 297–299°), $[\alpha]_D$ + 37° ($CHCl_3$; *c*. 0.5) 0.5 (lit. [25] + 61°). Found: $[M]^+$ 456.3596; $C_{30}H_{48}O_3$ requires 456.3603. IR, NMR, EIMS in agreement with that published [25].

Moronic acid (**1**). Needles from EtOAc, mp 223° (lit. [26] 222°), $[\alpha]_D$ + 22° ($CHCl_3$; *c* 1.0) (lit. [26] + 29°). Found: $[M]^+$ 454.3453; $C_{30}H_{46}O_3$ requires 454.3447. IR, EIMS in agreement with that published [26]. ^1H and ^{13}C NMR: Table 1.

Moronic aldehyde (**2**). Needles from $CHCl_3$–petrol, mp 145–148°, $[\alpha]_D$ + 46° ($CHCl_3$; *c* 0.5). Found: $[M]^+$

438.3516; $C_{30}H_{46}O_2$ requires 438.3498. IR ν_{max} cm^{-1}: 2940, 2920, 1720, 1710, 1700, 1470, 1450, 1385, 1370, 1280, 1145, 1120, 850. ^1H, and ^{13}C NMR: see Table 1. EIMS *m/z* (rel. int): 438 (38), 409 (100), 232 (15), 219 (44), 203 (42), 191 (58), 189 (40), 175 (16), 153 (19).

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REFERENCES

1. Weston, P. H., Carolin, R. C. and Armstrong, J. A. (1984) *Aust. J. Botany* **32**, 187.
2. Bax, A. (1983) *J. Mag. Res.* **53**, 517.
3. Bax, A. and Summers, M. F. (1984) *J. Am. Chem. Soc.* **108**, 2093.
4. Ahsan, M., Armstrong, J. A., Gray, A. I. and Waterman, P. G. (1994) *Phytochemistry* **37**, 259.
5. Ahsan, M., Armstrong, J. A. and Waterman, P. G. (1994) *Phytochemistry* **36**, 799.
6. Ahsan, M., Armstrong, J. A., Gray, A. I. and Waterman, P. G. (1994) *J. Nat. Prod.* **57**, 673.
7. Ahsan, M., Armstrong, J. A., Gray, A. I. and Waterman, P. G. (1994) *Aust. J. Chem.* **47**, 1783.
8. Ahsan, M., Armstrong, J. A., Gray, A. I. and Waterman, P. G. (1994) *J. Nat. Prod.* **57**, 670.
9. Ahsan, M., Gray, A. I., Leech, G. and Waterman, P. G. (1993) *Phytochemsitry* **33**, 1507.
10. Ahsan, M., Gray, A. I., Leech, G. and Waterman, P. G. (1994) *Phytochemistry* **36**, 777.
11. Duffield, A. M. and Jefferies, P. R. (1963) *Aust. J. Chem.* **16**, 292.
12. Mester, I. (1983) in *Chemistry and Chemical Taxonomy of the Rutales* (Waterman, P. G. and Grundon, M. F., eds), p. 31. Academic Press, London.
13. Harborne, J. B. (1983) in *Chemistry and Chemical Taxonomy of the Rutales* (Waterman, P. G. and Grundon, M. F., eds), p. 147. Academic Press, London.
14. Grundon, M. F. and McCorkindale, N. J. (1957) *J. Chem. Soc.* 2177.
15. Surburg, H. and Mondon, A. (1981) *Chem. Ber.* **114**, 118.
16. Zakaria, M., Jeffreys, J. A. D., Waterman, P. G. and Zhong, S. M. (1994) *Phytochemistry* **23**, 1481.
17. Gray, A. I., Bhandari, P. and Waterman, P. G. (1988) *Phytochemistry* **27**, 1805.
18. Rashid, M. A., Armstrong, J. A., Gray, A. I. and Waterman, P. G. (1992) *Z. Naturforsch.* **47b**, 284.
19. El-Feraly, F. S. and Turner, C. E. (1975) *Phytochemistry* **14**, 2304.
20. Cooke, R. J. and Haynes, H. F. (1954) *Aust. J. Chem.* **71**, 273.
21. Gray, A. I. (1993) in *Methods in Plant Biochemistry* (Waterman, P. G. ed.), Vol. 8, p. 271. Academic Press, London.
22. Collins, J. F., Gray, G. A., Grundon, M. F., Harrison, D. M. and Spyropoulos, C. G. (1973) *J. Chem. Soc. Perkin Trans. I* 94.
23. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1984) *Phytochemistry* **23**, 1798.
24. Monaco, P., Caputo, R., Palumbo, G. and Mangoni, L. (1973) *Phytochemistry* **12**, 939.
25. Konishi, T. and Shoji, J. (1981) *Chem. Pharm. Bull. (Tokyo)* **29**, 2807.
26. Majumder, P. L., Maiti, R. N., Panda, S. K., Mal, D., Raju, M. S. and Wenkert, E. (1989) *J. Org. Chem.* **44**, 2811.