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AN ALKALOID, COUMARINS AND A TRITERPENE FROM *BORONIA ALGIDA*

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Key Word Index—*Boronia algida*; Rutaceae; coumarins; 3′-hydroxyxanthyletin; 5-deoxyprotobruceols; 4-quinolone alkaloid; dammarane triterpene; chemotaxonomy.

Abstract—Examination of the aerial parts of *Boronia algida* has led to the isolation of six coumarins, an alkaloid and a triterpene. The coumarins were identified as (+)-marmesin, 7-demethylsuberosin, 5-deoxyprotobruceol-II hydroperoxide, a mixture of the diastereomers of 5-deoxyprotobruceol-III 3′ξ-hydroperoxide and 3′-hydroxyxanthyletin; the alkaloid as 1-methyl-2-pentadecyl-4(1*H*)-quinolone and the dammarane triterpene as (*E*)-25-hydroperoxy-3β-hydroxydammar-20,23-diene. The latter compound and 3′-hydroxyxanthyletin are novel natural products. The chemotaxonomic significance of these isolates in the genus *Boronia* is discussed.

INTRODUCTION

Boronia algida F. Muell. (Rutaceae), a dichotomously branching shrub found extensively in New South Wales, Eastern Australia [1], has not previously been studied. As part of an on-going chemotaxonomic survey of the genus *Boronia* [2–7] we have undertaken a thorough phytochemical investigation on this species. In this paper we report on the major isolates and discuss their chemotaxonomic significance.

RESULTS AND DISCUSSION

From a petrol extract of the aerial parts of *Boronia algida* six coumarins, one 4-quinolone alkaloid and a dammarane type triterpene were isolated by a combination of VLC, CC, PTLC and HPLC. Five coumarins, (+)-marmesin [8–10], 7-demethylsuberosin [3], 5-deoxyprotobruceol-II hydroperoxide [3], a diastereomeric mixture of 5-deoxyprotobruceol-III 3′ξ-hydroperoxide [3] and the 4-quinolone alkaloid, 1-methyl-2-pentadecyl-4(1*H*)-quinolone [11, 12] were characterized by direct comparison of their physical and spectroscopic characteristics with those published in the literature for the respective compounds. The other two isolated compounds were characterized by spectroscopic means.

Compound **1** was visualized on TLC as a bluish-white fluorescent spot under UV light (366 nm). The UV, IR and ^{1}H NMR data led to the identification of this compound as a coumarin [13]. The HREI-mass spectrum revealed the empirical formula $C_{14}H_{12}O_4$, with the base peak for $[M - Me]^+$ which is characteristic for pyranocoumarins [13, 14]. In the ^{1}H NMR spectrum (Table 1) the absence of any oxygenation at C-5 was evident from the relatively shielded chemical shift value for H-4 [13]. In addition to the signals for H-3, H-4, and the *gem*-dimethyl groups, the ^{1}H NMR spectrum displayed three singlets assignable to two aromatic protons H-5 and H-8, and to a single aromatic/olefinic proton. This suggested the compound was a linear pyranocoumarin in which, either C-3′ or C-4′ of the pyran ring was oxygenated. Due to the paucity of sample, conclusive ^{13}C NMR (Table 1) and HMBC spectra [15] could not be obtained. However, in the HMBC spectrum the *gem*-dimethyls (δ1.70) showed mutual ^3J coupling between themselves, a ^2J coupling to C-2′ (δ71.0) and a ^3J correlation with C-3′ (δ164.0). On this basis this coumarin was identified as 3′-hydroxyxanthyletin (**1**).

Compound **2** was visualized on TLC as a purple spot after being sprayed with vanillin–H_2SO_4 and heated at 100° for 15 min. The HREI-mass spectrum revealed the empirical formula $C_{30}H_{50}O_3$ with a major fragment ion at *m/z* 317 for $[M - C_8H_{13}O_2]^+$, which strongly suggested the presence of the C-8 side chain found in tetracyclic triterpenes [16]. The ^{1}H and ^{13}C NMR spectra (Table 2) were typical of the dammarane group of triterpenes [17–20]. In the ^{1}H NMR spectrum (Table 2) notable features were the occurence of seven methyl singlets at δ0.79–1.36, a doublet (J = 16 Hz) and a doublet of triplets (J = 7, 16 Hz) due to the *trans* olefinic protons, a 2H doublet for the methylene group adjacent to H-23, two singlets for an exocyclic methylene, and a 1H doublet of doublets at δ3.20 (J = 10.6 Hz) characteristic for H-3α.

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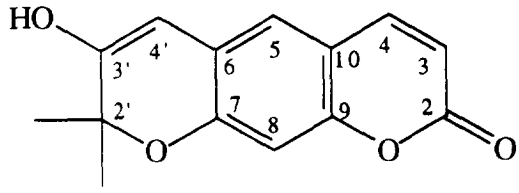

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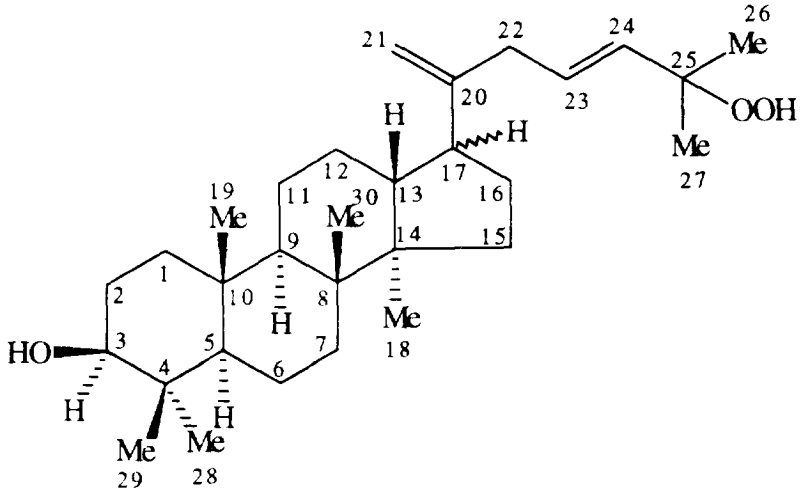

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Table 1. ^1H and ^{13}C spectral NMR data of compound 1

Position	δ_H*	δ_C†	Position	δ_H*	δ_C†
2		‡	8	7.45 1H, *s*	100.0
3	6.38 1H, *d* (J = 9.6 Hz)	114.8	9		‡
4	7.79 1H, *d* (J = 9.6 Hz)	144.3	10		‡
5	7.61 1H *s*	100.2	2′		71.0
6		‡	3′		164.0
7		‡	4′	6.64 *s*	119.7
2 × Me-2′	1.70 *s*	28.9			

*Solution in $CDCl_3$ referenced to $CHCl_3$ at δ7.27 ppm; 400 MHz.
†Solution in $CDCl_3$ referenced to $CHCl_3$ at δ77.23 ppm; 100 MHz.
‡Not observed.

The presence of an $-O_2H$ group could be assumed from a broad singlet at δ7.25. The ^1H NMR spectrum was similar to that data for isofouquierol [16] with the exception of signals that related to the side chain in **2**, e.g. the presence of an exocyclic methylene (C-21) instead of a methyl, and the $-O_2H$ (attached to C-25) instead of -OH. A ^{13}C BB dec spectrum displayed signals for exocyclic methylene and quaternary carbon, two quaternary carbons bearing oxygen one of which was more deshielded than usual, supporting the presence of $-O_2H$ group attached to it and the signals for other methine, methylene, methyl and quaternary carbons. Presence of $-O_2H$ group was further confirmed from the positive chemical test for hydroperoxy group [21, 22].

Due to the paucity of sample, all the possible ^1H–^{13}C long range correlations were not observed in the HMBC spectrum (Table 3), however some key correlations were well observed. The methyls (δ0.99 and 0.79) were *geminal* as they showed mutual ^3J coupling between themselves, ^2J correlation to a quaternary carbon (C-4) and ^3J couplings to the oxymethine (C-3) and to the methine (C-5). Another *gem*-dimethyl system (C-26, C-27) showed equivalent chemical shifts in the ^1H NMR spectrum and displayed ^3J coupling to each other and a ^2J coupling

Table 2. ^1H and ^{13}C NMR spectral data for compound **2**

Position	δ_H*	δ_H*	Position	δ_H*	δ_C*
1	1.40 1H, *m*; 1.46 1H, *m*	37.5	16	0.70–2.20 2H, *m*	29.1
2	1.58 1H, *m*; 1.62 1H, *m*	27.7	17	0.70–2.20 1H, *m*	47.9
3α	3.20 1H, *dd* (J = 10, 6 Hz)	79.2	18	0.88 3H, *s*	16.4
4		392.	19	0.86 3H, *s*	15.9
5α	0.70–2.20 1H, *m*	56.1	20		151.4
6	0.70–2.20 2H *m*	21.7	21	4.71 1H, *s*; 4.79 1H, *s*	109.8
7	0.70–2.20 2H, *m*	36.1	22	2.74 2H, *d* (J = 7 Hz)	37.5
8		49.7	23	5.72 1H, *dt* (J = 16, 7 Hz)	130.6
9α	0.70–2.20 1H, *m*	51.0	24	5.58 1H, *d* (J = 16 Hz)	134.9
10		39.3	25		82.5
11	0.70–2.20 2H, *m*	24.5	26	1.36 3H, *s*	25.1
12	0.70–2.20 2H, *m*	24.6	27	1.36 3H, *s*	25.1
13β	0.70–2.20 1H, *m*	45.4	28	0.79 3H, *s*	15.6
14		40.7	29	0.99 3H, *s*	28.3
15	0.70–2.20 2H, *m*	31.6	30	0.98 3H, *s*	16.1

*Solution in $CDCl_3$ referenced to $CHCl_3$ at δ7.27 ppm (400 MHz).
†Solution in $CDCl_3$ referenced to $CHCl_3$ at δ77.23 ppm (100 MHz).

Table 3. Major H–C–C–C HMBC correlations in compound **2**

Protons	^2J ^{13}C	^3J
3H-18	40.7 (C-14)	31.6 (C-15), 45.4 (C-13), 49.7 (C-8)
3H-19	39.3 (C-10)	37.5 (C-1), 51.0 (C-9), 56.1 (C-5)
2H-21		47.9 (C-17)
2H-22	151.4 (C-20), 130.6 (C-23)	109.8 (C-21), 134.9 (C-24)
3H-26	82.5 (C-25)	25.1 (C-27), 134.9 (C-24)
3H-27	82.5 (C-25)	25.1 (C-26), 134.9 (C-24)
3H-28	39.2 (C-4)	28.3 (C-29), 56.1 (C-5), 79.2 (C-3)
3H-29	39.2 (C-4)	15.6 (C-28), 56.1 (C-5), 79.2 (C-3)
3H-30	49.7 (C-8)	36.1 (C-7), 40.7 (C-14), 51.0 (C-9)

with a quaternary carbon bearing oxygen (C-25) and an olefinic methine (^3J). The methylene protons (δ2.75) showed ^3J couplings to C-21 and C-24, and ^2J correlations to C-20 and C-23. The exocyclic methylene (2H-21) showed ^3J coupling to the methine (C-17). The methyl protons (δ0.88) showed ^2J couplings to C-14 and ^3J correlations to C-13, C-15 and C-8. Similarly methyls at δ0.86 showed $^2J/^3J$ correlations to C-5, C-9, C-10 and C-1, and that at δ0.98 showed $^2J/^3J$ correlations to C-7, C-8, C-14 and C-9. The rest of the carbons were assigned by direct comparison with the published ^{13}C values of dammarane triterpenes [16]. Thus the structure of this compound was assigned as **2**.

The 4-quinolone alkaloid, 1-methyl-2-pentadecyl-4(1*H*)-quinolone, is the major compound in *Boronia algida*. This type of 4-quinolone has also been reported from two other species: *B. bowmanii* [5] and *B. lanceolata* [2, 3]. Most of the coumarins found in this study also co-occur in *B. lanceolata* [3]. According to the phylogenetic relationships in *Boronia* suggested by Weston *et al.* [23] all these three species are closely related and belong to *Boronia* section *Valvatae*. The co-existence of the same type of secondary metabolites in these species strongly supports this relationship.

EXPERIMENTAL

UV spectra were run in EtOH and IR spectra as KBr discs. NMR spectra were obtained on Bruker AMX-400 instrument using standard Bruker microprograms. For HMBC experiments ^2J and ^3J coupling was set for approximately 7 Hz. HREI-MS were obtained by direct probe insertion at elevated temp. (120–140°) and at 70 eV. Silica gel 60 H (Merck 7736), silica gel 60-PF254 (Merck 7749) and silica gel (Merck 7734), respectively, for VLC, TLC and CC were used. Sephadex LH20 (Sigma 82H0368) was used for gel-filtration. HPLC separation was performed in a Waters HPLC apparatus comprising two Model-501 HPLC pumps, a Model-680 gradient controller, a Model-7125, 200 μl injector (Rheodyne) and a photodiode array detector (Model-991) coupled with an APC-H4313 advanced personal computer (NEC) for recording chromatogram and UV-Vis spectra and using a semiprep. silica column (10 × 250 mm, 5 μ Spherisorb, S5W). Petrol stands for petroleum ether (40–60°) throughout this text.

Plant material. The aerial parts of *Boronia algida* (voucher T. G. Hartley 15150, at the National Herbarium) were collected from the Braidwood–Nowra Road at

Sassafras, in S.E. New South Wales (150° 15'E, 35°05'S).

Extraction of plant material. Powdered plant material (300 g) was extracted in a Soxhlet with, successively, petrol, $CHCl_3$ and MeOH and the extracts were concd using a rotary evaporator at a maximum temperature of 40°.

Isolation of compounds. VLC of concd petrol extract (9.5 g) eluting with solvents of increasing polarity starting from petrol (100%) via EtOAc to MeOH (100%) yielded 30 fractions.

Prep. TLC ($CHCl_3$–EtOAc, 4:1) of the VLC fr. 5–8 (5% EtOAc in petrol) yielded 7-demethylsuberosin (2.8 mg). Fr. 14–15 (95% EtOAc in petrol) was subjected to CC and eluted with $CHCl_3$–EtOAc mixtures of increasing polarity. Prep. TLC ($CHCl_3$–EtOAc, 4:1) of column fraction (2% EtOAc in $CHCl_3$) produced (+)-marmesin (2.3 mg) and **1** (1.9 mg). Prep. TLC ($CHCl_3$–EtOAc 5:1) of CC fraction (100% $CHCl_3$) yielded two bands containing 5-deoxyprotobruceol-II hydroperoxide (1.5 mg), diastereomeric mixture of 5-deoxyprotobruceol-III 3'ξ-hydroperoxide (1.9 mg) and **2** (2.3 mg) which were further purified by HPLC (isocratic elution with *n*-hexane–EtOAc, 4:1). 1-Methyl-2-pentadecyl-4(1*H*)-quinolone (15 mg) was isolated from the VLC fraction (100% EtOAc) by prep. TLC ($CHCl_3$–EtOAc, 4:1).

3'-Hydroxyxanthyletin (**1**). Amorphous. UV λ_{max} nm: 268, 306, 348. IR ν_{max} cm^{-1}: 3420, 2960, 2930, 1724, 1710, 1690, 1690, 1620, 1550, 1490, 1370, 1286, 1132, 920, 820. ^1H NMR ($CDCl_3$): see Table 1. ^{13}C NMR ($CDCl_3$): see Table 1. Found: $[M]^+$ 244.0742; $C_{14}H_{12}O_4$ requires 244.0736. HREI-MS *m/z*: (rel. int.): 244 $[M]^+$ (38), 229 $[M - Me]^+$ (100), 201 (6).

(E)-25-Hydroperoxy-3β-hydroxydammar-20,23-diene (**2**). Gum. $[\alpha]_D$ + 25 ($CHCl_3$; *c* 0.1). IR ν_{max} cm^{-1}: 3455, 2950, 1460, 1095, 1010, 890. ^1H NMR ($CDCl_3$): see Table 3. ^{13}C NMR ($CDCl_3$): see Table 3. Found: $[M]^+$ 458.3762; $C_{30}H_{50}O_3$ requires 458.3760;. HREI-MS *m/z* (rel. int.): 458 $[M]^+$ (1), 444 (2), 442 $[M - O]^+$ (17), 440 $[M - H_2O]^+$ (12), 428 (7), 427 (9), 426 (8), 424 (16), 345 (11), 344 (6), 317 $[M - C_8H_{13}O_2]^+$ (13), 316 (8), 315 (11), 299 (19), 247 (24), 245 (8), 229 (11), 221 (7), 220 (10), 217 (6), 208 (27), 207 (88), 206 (12), 205 (14), 203 (26), 202 (10), 201 (9), 191 (43), 190 (41), 189 (48), 188 (7), 187 (22), 179 (8), 177 (11), 175 (24), 173 (12), 163 (18), 162 (11), 161 (25), 159 (18), 157 (9), 153 (13), 152 (10), 151 (9), 149 (23), 148 (14), 147 (31), 145 (17), 143 (6), 141 (10), 139 (13), 136 (21), 135 (60), 134 (24), 133 (31), 125 (17), 123 (31), 121 (49), 119 (39), 109 (57), 108 (33), 107 (957), 105 (32), 95 (93), 93 (58), 81 (87), 69 (75), 67 (56), 55 (100).

(+)-Marmesin. Amorphous. UV, IR, ^1H, ^{13} NMR and HREI-MS data as reported [8–10].

7-Demethylsuberosin. Amorphous. UV, IR, ^1H, ^{13}C NMR and HREI-MS data as reported [3].

5-Deoxyprotobruceol-II hydroperoxide. Amorphous. UV, IR, ^1H, ^{13}C NMR and HREI-MS data as reported [3].

Diastereomeric mixture of 5-deoxyprotobruceol-III 3'ξ-hydroperoxides. Amorphous. UV, IR, ^1H, ^{13}C NMR and HREI-MS data as reported [3].

1-Methyl-2-pentadecyl-4(1H)-quinolone. Solid. Mp 78° (80° in the literature [12]), UV, IR, ^1H, ^{13}C NMR and HREI-MS data as reported [11, 12].

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