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Phytochemistry and Pharmacology of *Boronia pinnata* Sm.

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Boronia pinnata Sm. (Rutaceae) is a plant that is widespread in New South Wales (Australia). Although there are no reports about the use of this species in the local ethnomedical traditions, recent investigations led to the characterization of several secondary metabolites, most belonging to the class of prenyloxyphenylpropanoids. Some of the compounds extracted from *B. pinnata* showed valuable biological properties, such as anti-inflammatory activity and *in vitro* inhibition of growth of *Helicobacter pylori.* The aim of this review is to cover what has been reported so far in the literature on the title plant from a phytochemical and pharmacological point of view.

Keywords: Anti-inflammatory activity, *Boronia pinnata*, *Helicobacter pylori*, prenyloxyphenylpropanoids.

Prenylation is a chemical or enzymatic addition of an hydrophobic side chain to an accepting molecule (another terpenoid molecule, an aromatic compound, a protein, etc.). In particular, prenylation of aromatic secondary metabolites plays a critical role in the biosynthesis of a wide range of molecules exerting valuable pharmacological effects across phylogenetically different classes of living organisms, from bacteria to mammals and plants. Frequently, the addition of an isoprenoid chain renders the molecule more effective than the parent compound from a pharmacological point of view. These ''hybrid'' natural products represent nowadays a new frontier for the development of novel drugs, in particular as antimicrobial, anti-oxidant, anti-inflammatory and anti-cancer agents. Oxyprenylated natural products are compounds of mixed biosynthetic origin for which the final step of the biosynthetic process is the prenylation of either an alkaloid or a phenylpropanoid core using prenyl diphosphate as alkylating agent [1], the latter coming in turn from either the mevalonate [2] or 1-DOXP pathways [3]. Oxyprenylated secondary metabolites have been considered for decades merely as biosynthetic intermediates of *C*-prenylated compounds and only in the last ten years have been characterized as phytochemicals exerting interesting and valuable biological activities [4]. Considering the length of the carbon chain, three types of prenyloxy skeletons can be identified: C5 (isopentenyl), C10 (geranyl) and C15 (farnesyl). Isopentenyloxy and geranyloxy chains are quite abundant in nature, while farnesyloxy ones are less common. The skeleton may consist only of carbon and hydrogen or may contain oxygen atoms, usually in the form of alcohols, ethers or ketones. Several species have been identified up to now as producing prenyloxyphenylpropanoids. Among these *Boronia pinnata* Sm. (Rutaceae) has been characterized as one of the species to biosynthesize a wide variety of the above cited secondary metabolites.

While several plants of the genus *Boronia* have been reported to be used in local ethnomedical traditions [5-10], *B. pinnata* has never been cited in the literature in this regard. However, recent investigations led to a detailed phytochemical profile of the main secondary metabolites of this plant and have revealed that some of these compounds exert valuable anti-cancer and anti-inflammatory effects,

mainly targeting the lipoxygenase system, and an inhibitory activity *in vitro* against *Helicobacter pylori*. The aim of this review is to cover what has been reported so far in the literature on the title plant from a phytochemical and pharmacological point of view.

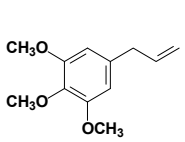
Botany of *Boronia pinnata*

B. pinnata is a species belonging to the family Rutaceae that typically grows in a very restricted area of Australia, namely New South Wales. The name of the genus comes from Francesco Borone, an Italian botanist (1769-1794), while "pinnata" alludes to the paired leafets that are an anatomical feature of this plant [10]. *B. pinnata* grows in sandstone country up to lower mountain levels in dry sclerophyll forests and in well-drained sandy heaths, mainly along coasts. It is a beautiful waxy-flowered shrub, 0.5-1.5 m high. Branchlets are glabrous and slightly angled to prominently 4-angled. The foliage is ornamental and ferny, light to mid-green. Leaves are up to 25 mm long, opposite and with several pairs of widely spaced leaflets. Flowers are grouped in inflorescences that are subterminal to axillary, collected in corymbose cymes, each comprising from 3 to 8 flowers. Petals are in group of 4, imbricate, 5-10 mm long, colored bright to purplish pink. The flowering stage occurs from June to February [11].

Phytochemistry of *B. pinnata*

The first studies describing the phytochemical composition of *B. pinnata* refer to the analysis of the essential oil obtained from the aerial parts of the plant, which were reported in two manuscripts published in 1919 and 1922 [12,13]. The essential oil was obtained with a yield of 0.1% and showed a very high quantity of elemicin (**1**) (> 75%) that was also claimed as a distinctive feature of *B. pinnata* in comparison with other *Boronia* species. At the same time, Morrison reported the isolation of rutin (**2**) from the aqueous extract of the leaves [14].

Studies on the title plant were then abandoned for about 80 years. In 1999 and 2000 Itoigawa et al reported the isolation and structural characterization of 23 novel and already known secondary metabolitesfrom the roots of *B. pinnata* [15,16]. In particular, they isolated six cinnamic aldehyde derivatives, boropinals A (**3**), B (**4**) and C (**5**), together with 3',4'-dimethoxycinnamic aldehyde (**7**) and 3',4',5'-trimethoxycinnamic aldehyde (**8**), five cinnamoyl alcohol derivatives, boropinol A (**9**),



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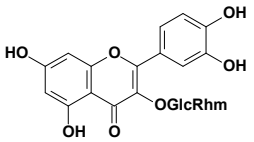

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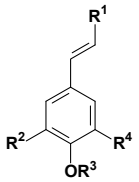

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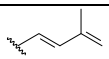
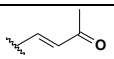
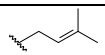
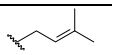
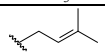
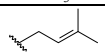
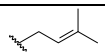
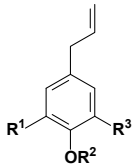
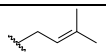
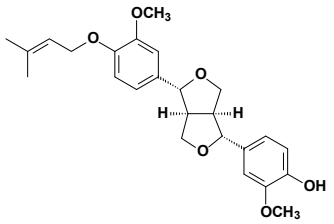
3	CHO		H	H
4	CHO		H	H
5	CHO	OCH_3		H
6	CHO	H	H	
7	CHO	OCH_3	CH_3	H
8	CHO	OCH_3	CH_3	OCH_3
9	CH_2OH	OCH_3		H
10	CH_2OCH_3	OCH_3	CH_3	OCH_3
11	CH_2OH	OCH_3		OCH_3
12	CH_2OH	OCH_3	CH_3	H
13	CH_2OH	OCH_3	CH_3	OCH_3
14	COOH	H	H	H
15	COOH	OCH_3		H



	R^1	R^2	R^3
16	H		OCH_3
17	OCH_3	CH_3	H



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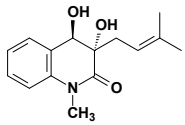

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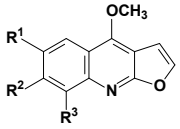
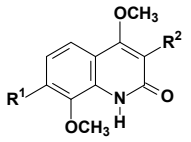
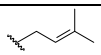
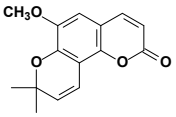


	R^1	R^2	R^3
20	H	H	H
21	H	OCH_3	H



	R^1	R^2
22	OCH_3	
23	H	H



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methoxyboropinol B (**10**), boropinol C (**11**), 3',4'-dimethoxycinnamyl alcohol (**12**) and 3',4',5'-trimethoxycinnamyl alcohol (**13**), two cinnamic acids, *p*-hydroxycinnamic (**14**) and boropinic acid (**15**), three phenylpropenes, 3-(3'-methoxy-4'-prenyloxy)phenyl-1-propene (**16**), methyleugenol (**17**) and elemicin (**1**), one lignan, boropinan (**18**), five alkaloids, pinolinone (**19**), dictamnine (**20**), evolitrine (**21**), preskimmianine (**22**) and folimine (**23**), and finally one coumarin, braylin (**24**).

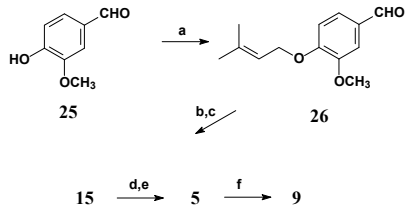
Some of the novel secondary metabolites extracted from the roots of *B. pinnata* have been also obtained by chemical synthesis.

The first synthesis in this regard was reported by Hou and coworkers in 2003 [9]. They synthesized boropinol A (**9**), boropinal C (**5**) and boropinic acid (**15**) using commercially available vanillin (**25**) as starting material (Scheme 1).

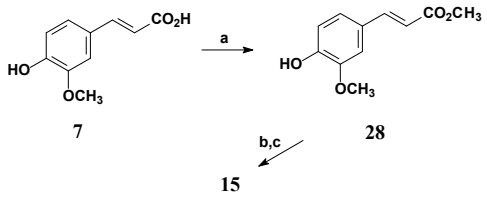
Condensation of the latter with α-carbethoxy-methyl-phosphorane in DME furnished the corresponding ester, followed by alkaline hydrolysis to give boropinic acid (**15**) in 91% yield. Reduction of **15** with $LiAlH_4$ in Et_2O yielded boropinol A (**9**), although not in a sufficiently pure form. So the mixture was oxidized with $K_2Cr_2O_7$ in DMSO to give boropinal C (**5**) in 61% yield. Finally, reduction of **5** with $NaBH_4$ in MeOH yielded pure boropinol A (**9**) (70%) [17].

The reaction of **25** with isopentenyl bromide in DMF gave the *O*-prenylated adduct **26** in 84% yield.

Three years later Curini and coworkers reported a short and high-yielding synthesis of boropinic acid (**15**) starting from readily commercially available ferulic acid (**7**) (Scheme 2) [18].



Scheme 1: Reagents and conditions: (a) isopentenyl bromide, DMF; (b) $Ph_3P{=}CH\text{-}CO_2Et$, DME; (c) KOH (aq); (d) LAH, dry Et_2O; (e) $K_2Cr_2O_7$, DMSO; (f) $NaBH_4$, MeOH.



Scheme 2: Reagents and conditions: (a) MeOH, conc. H_2SO_4, reflux; (b) isopentenyl bromide, K_2CO_3, acetone, reflux; (c) NaOH 70°C.

Ferulic acid (**7**) was first converted to the corresponding methyl esters **28** in 99% yield by reaction in refluxing MeOH under the catalysis of conc. H_2SO_4; compound **28** was submitted to prenylation using isopentenyl bromide as alkylating agent and dry K_2CO_3 as base in refluxing acetone, followed by alkaline hydrolysis in the same reaction vessel to obtain boropinic acid (**16**) in 96% yield.

So, compound **16** has been easily synthesized from widely available and non-toxic starting materials by a high-yielding, environment friendly, and cheap synthetic route.

Pharmacological properties of active principles from *B. pinnata*

The fact that *B. pinnata* was not part of local ethnomedical traditions, mostly due to its restricted habitat, has not attracted, for many decades, the attention of researchers to carry out pharmacological studies of extracts or single secondary metabolites of this plant. The first study, aimed at investigating the anticancer properties of selected compounds extracted from roots of *B. pinnata*, was reported by Itoigawa and coworkers in 1999 [15]. Twelve phenylpropanoids, namely compounds **1**, **3**, **5**, **7-12**, **16** and **17** were tested *in vitro* as inhibitors of Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells (EBV genome-carrying human lymphoblastoid cells; EBV non –producer type). One of the extracted compounds, 3-(3'-methoxy-4'-isopentenyloxy)phenyl-1-propene (**16**), was also tested *in vivo* as an inhibitor of effects on skin tumor

Table 1: Inhibitory effects of selected phenylpropanoids from roots of *B. pinnata* on TPA-induced EBV-EA activation.

Compd.	EBV-EA positive cells (% viability)		
	Compd. concentration (mol ratio/32 pmol TPA)		
	500	100	10
1	29.2 ± 1.6	71.2 ± 2.2	90.1 ± 1.3
3	26.7 ± 1.3	69.7 ± 2.1	86.2 ± 2.2
5	23.2 ± 1.1	67.7 ± 2.0	84.5 ± 2.1
7	33.5 ± 1.3	75.5 ± 2.1	91.3 ± 1.7
8	30.6 ± 1.1	71.4 ± 2.2	89.3 ± 1.8
9	29.8 ± 1.1	69.4 ± 2.0	87.6 ± 2.1
10	37.2 ± 1.4	77.3 ± 2.3	92.4 ± 1.5
11	26.4 ± 1.4	69.5 ± 1.9	87.3 ± 1.3
12	36.2 ± 1.4	77.0 ± 2.3	92.3 ± 1.8
13	32.5 ± 1.3	75.8 ± 2.1	90.6 ± 1.2
16	26.2 ± 1.3	69.5 ± 1.9	87.3 ± 1.1
17	31.5 ± 1.5	74.2 ± 2.3	92.4 ± 1.6

promotion by means of a two-stage carcinogenesis test of mouse skin papilloma using dimethylbenz[*a*]anthracene (DMBA) as initiator and TPA as promoter. Results of the test on Raji cells are reported in Table 1.

Although not reported in Table 1, all compounds showed a 100% inhibitory effect at the concentration value of 1000, expressed as mol ratio/32 pmol TPA. Among the tested aldehydes, boropinal C (**5**) exhibited the most significant inhibitory activity on EBV-EA activation. Among the cinnamyl alcohols, boropinol A (**9**), boropinol C (**11**) and compound (**16**) showed similar activities to that of boropinal C (**5**). It's interesting to note that secondary metabolites lacking a prenyloxy side chain like compounds **7**, **8**, **10**, **12** and **13** are less effective as inhibitors of EBV-EA activation. This suggests that the presence of a prenyloxy side chain in position 4' on 3-phenyl-2-propenal and –propenol cores enhance the inhibitory effects. The same observation was made for the activity of several other prenyloxyphenylpropanoids [4].

Results of the *in vivo* two-stage carcinogenesis test of mouse skin papillomas induced by DMBA and promoted by TPA using compound **15** in comparison with a positive control that developed papillomas after only 10 weeks of promotion**,** showed that when applied before TPA treatment, **15** delayed the formation of papillomas. In each group of animals treated with compound **16** only about 20% of mice bore papillomas at 10 weeks after promotion and even after 20 weeks of promotion 80% of the mice bore tumors. Moreover, **16** reduced the incidence of papillomas (average number of tumors per mouse): less than 5 papillomas were formed per mouse after 11 weeks of promotion and only about 3.8 papillomas per mouse even after 20 weeks of promotion. The same in *vitro* test on EBV-EA activation was later carried out on 4'-hydroxy-3'-prenylcinnamaldehyde (**6**) and boropinic acid (**15**), which had been isolated in a second step of the ongoing studies of Itoigawa and coworkers [16]. Results are reported in Table 2.

Table 2: Inhibitory effects of compounds **3** and **14** on TPA-induced EBV-EA activation.

Compd.	EBV-EA positive cells (% viability)		
	Compd. concentration (mol ratio/32 pmol TPA)		
	500	100	10
6	27.2 ± 1.8	65.5 ± 1.0	88.5 ± 0.4
15	23.1 ± 1.1	62.2 ± 1.5	84.0 ± 0.3

Table 3: Inhibition of 5-LOX mediated PUFAs peroxidation by boropinic acid (**15**).

Compds.	IC_{50} (μmol/mL)[a]
15	$2.89 \times 10^{-5} \pm 2.62 \times 10^{-6}$
Ascorbic acid	0.105 ± 0.0072
BHT	0.023 ± 0.0052
Trolox	0.047 ± 0.0048

[a]$p<0.05$ at Student's *t* test

Both compounds showed a 100% inhibitory activity at a concentration value of 1000, expressed as mol ratio/32 pmol TPA, as had the previous compounds, and a good inhibitory activity on TPA-induced EBV-EA activation, also at lower doses. All the other secondary metabolites that had been extracted from the roots of *B. pinnata* were not active in the same test.

In the frame of an ongoing study devoted to the synthesis and characterization of pharmacological properties of prenyloxyphenylpropanoids, Curini and coworkers first studied the *in vitro* anti-inflammatory and anti-bacterial properties of boropinic acid (**15**).

These authors found first that compound **15** did not exhibit significant antioxidant properties when submitted to the DPPH radical scavenging assay. To enforce this finding they also performed the assay for inhibition of polyunsaturated fatty acids (PUFAs) peroxidation mediated by soybean 5-lipoxygenase (5-LOX), using ascorbic acid, butyl hydroxytoluene (BHT) and Trolox as positive controls [18]. Results of the latter test are reported in Table 3.

Contrasting results obtained by means of chemical and enzymatic assays suggested that boropinic acid (**15**) acted as an effective 5-LOX inhibitor. It is noteworthy that other prenyloxyphenylpropanoids, like cinnamic acid bearing longer *O*-chains or coumarins, were not active in both tests.

Table 4: MIC values for inhibition of growth against *H. pylori* by boropinic acid (**15**).

Compd	MIC (μg/mL)[a]
15	1.62
Metronidazole	> 200
Amoxicillin	0.781
Tetracycline	4.00
Clarithromycin	1.25

[a]Values are means of three experiments.

To rationalize tentatively the inhibitory mechanism observed for boropinic acid and the lack of efficacy of some other prenyloxyphenylpropanoids, Curini and coworkers inferred a possible 5-LOX/ligand docking by comparative modelling. As a result of this analysis, a peculiar feature of the modelled 5 LOX/boropinic acid complex is the possibility for the hydrophobic side chain represented by the isopentenyloxy moiety to be oriented and to enter in van der Waal's contact with a cluster of hydrophobic amino acids. This interaction is enforced by polar interactions at the same site of the carboxylic group with Ile 857 and the amide side chain Gln 514 of the enzyme. Since these additional interactions might contribute to the enhancement of the complex stability, it may be hypothesized that the loss of activity of 5-LOX in the presence of **15** could be the result of enzyme inhibition as a consequence of stable ligand docking in the active site.

The same research group studied the anti-bacterial properties of boropinic acid (**15**). After having screened several Gram positive and Gram negative bacterial strains, they found that **15** is an effective inhibitor *in vitro* of the growth of *Helicobacter pylori* [19]. Results of the test, performed by the agar dilution method with metronidazole, amoxicillin, tetracycline and clarithromycin as reference drugs, are reported in Table 4, expressed as minimum inhibitory concentration (MIC).

Although from the data reported in Table 4 it is evident that the strain of *H. pylori* (namely DSMZ 4867 obtained from human gastric samples) used to perform the test is clearly resistant to metronidazole, the activity of boropinic acid (**15**) as an inhibitory agent of the growth of *H. pylori* is comparable to that of most common antibiotics currently used in therapy to eradicate bacterial infections. Based on these preliminary results, boropinic acid (**15**) could be viewed as a potential lead compound for a novel class of *H. pylori* inhibitors. However, studies aimed to clearly depict the mechanism of action of this secondary metabolite, and *in vivo* tests using a suitable animal model, and *in vitro* and *in vivo* tests to evaluate the activity of **15** against strains of *H. pylori* isolated from clinical patients have to be carried out in the near future.

Conclusions and future perspectives

In this review we have reported what is known so far in the literature about the Australian shrub *B. pinnata*. Twenty-four secondary metabolites have been isolated in low concentrations and structurally characterized from different parts of this plant and the major part of these natural compounds belong to the class of prenyloxyphenylpropanoids. With the aim of obtaining these compounds in sufficient quantities to determine a detailed pharmacological profile, bypassing difficulties linked to the low quantities obtainable from natural sources, a few of the compounds have been obtained by chemical synthesis by means of environmentally sound, friendly and high yielding methodologies. In particular, boropinic acid has been obtained in nearly quantitative yield. Preliminary pharmacological studies on simple and oxyprenylated phenyl-propanoids from *B. pinnata* have shown that compounds like boropinal C and boropinic acid show valuable biological properties, such as anti-cancer, anti-inflammatory and anti-ulcer activities. In the search for novel therapeutic remedies from nature, the data reported in this review will certainly prompt further studies on this plant to better define the profile of its secondary metabolites and their pharmacological properties, in particular by means of *in vivo* studies employing suitable animal models.

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