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Variation of the alkaloid content of *Peumus boldus* (boldo)

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ABSTRACT

Eighteen alkaloids were detected in the bark, leaves, wood and roots of *Peumus boldus*, including traces of secoboldine, *N*-methylsecoboldine (boldine methine), glaucine and norreticuline, not reported previously as constituents of this species. Using appropriate standards, we quantified thirteen of them by UHPLC-MS/MS. Boldine was dominant in the bark, and laurolitsine in wood and roots. The alkaloid composition of the leaves, determined for 130 individually identified trees, classified by age and sex, was highly variable, where *N*-methyllaurotetanine, laurotetanine, coclaurine and in some cases isocorydine predominated, but not boldine.

1. Introduction

Peumus boldus Mol. (*Monimiaceae*; 'boldo' in the vernacular and internationally) is an endemic tree of central Chile, where it dominates the landscape of many parts of the Mediterranean climate zone [1,2]. Boldo has developed physiological mechanisms that allow it to tolerate drought, high temperatures and strong solar irradiation [3,4] and the ability to regrow from the roots after felling or burning, making it appropriate to colonize land that has suffered desertification processes [5]. Archeological findings show that the aromatic boldo leaves were chewed for unknown purposes by some of the earliest inhabitants of South America 14,600 years ago [6], and its bark was smoked, apparently in a ritual context, 1000–1500 years ago [7]. Traditional medicinal applications include earache, headache, rheumatism, nasal congestion and, prominently, digestive and biliary disorders [8]. Boldo leaves are arguably one of the most valuable non-timber forest products in central Chile, currently attracting an export income of around five million US dollars per year [9]. Their widespread use outside Chile, mainly for dyspepsia and mild digestive spasms, for its hepatoprotective, choleretic and cholagogic properties, and also as a mild sedative, has led to their inclusion in the European Pharmacopoeia (*boldi folium*) [10] and their recent assessment by the European Medicines Agency (EMA) [11]. While clinical studies are lacking, the traditional use of boldo leaf infusions as an aid to digestion seems justified [12]. While many studies attribute the beneficial effects of boldo to its content of boldine [8,12,13], considered its most characteristic alkaloid, the remarkable content of polyphenols in this species [14], especially catechin and related compounds [15,16], plus relevant concentrations of other phenolic alkaloids [8], suggest a more complex interpretation. In this regard it is interesting to note that the documented antiinflammatory activity of boldine [17], is potentiated when this alkaloid is co-administered with reticuline, also present in boldo [18].

The alkaloid footprint of boldo leaves has been studied for decades, initially adding the aporphines isocorydine, *N*-methyllaurotetanine and norisocorydine [19] to boldine, which had been found almost a century before [20]. Some years later the 1-benzyl-1,2,3,4-tetrahydroisoquinoline reticuline, and the aporphines isoboldine, laurotetanine, laurolitsine and isocorydine *N*-oxide were identified [21–23]. Early use of HPLC led to the quantification of some of these (only tentatively identified from their retention times) in boldo leaves and extracts purchased from European suppliers, where boldine was usually found to be a relatively minor alkaloid [24,25]. This basically unchanged methodology is dictated by the European Pharmacopoeia, where boldine is still considered a reference [10]. It is also surprising that the EMA should state that "boldine is usually the major alkaloid" [11]. Quite recently the benzylisoquinoline *N*-methylcoclaurine and the noraporphine norglaucine [26], and in a particularly thorough investigation, the benzylisoquinolines *N*-methylcoclaurine, reticuline, the aporphine norisocorydine, isocorydine, *N*-methyllaurotetanine, boldine and laurotetanine, the proaporphines glaziovine and pronuciferine, and

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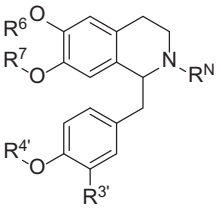
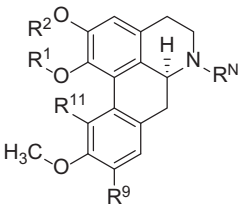
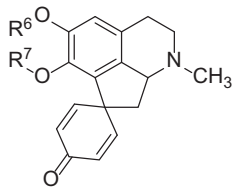
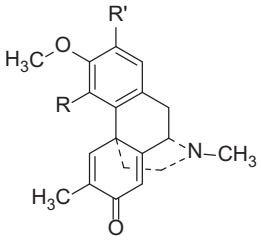


R^6	R^7	R^N	$R^{3'}$	$R^{4'}$	
H	H	H	H	H	Higenamine
CH_3	H	H	H	H	Coclaurine
CH_3	H	CH_3	H	H	*N*-Methylcoclaurine
CH_3	H	CH_3	OH	CH_3	Reticuline
CH_3	H	H	H	CH_3	Norreticuline

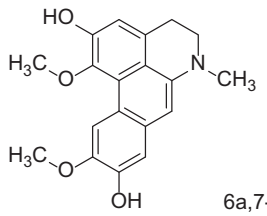


R^1	R^2	R^N	R^9	R^{11}	
H	CH_3	CH_3	OH	H	Isoboldine
CH_3	H	H	OH	H	Laurolitsine
CH_3	H	CH_3	OH	H	Boldine
CH_3	CH_3	H	OH	H	Laurotetanine
CH_3	CH_3	CH_3	OH	H	*N*-Methyllaurotetanine
CH_3	CH_3	H	OCH_3	H	Norglaucine
CH_3	CH_3	CH_3	OCH_3	H	Glaucine
CH_3	CH_3	CH_3	H	OH	Isocorydine
CH_3	CH_3	H	OCH_3	OH	Norisocorydine

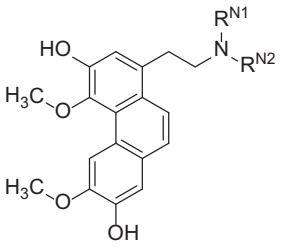




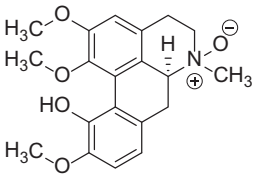
R^6	R^7		R	R'	
CH_3	H	Glaziovine	OH	H	Sinoacutine
CH_3	CH_3	Pronuciferine	H	OH	Pallidine



6a,7-dehydroboldine



R^{N1}	R^{N2}	
H	CH_3	Secoboldine
CH_3	CH_3	*N*-methylsecoboldine



R^1	R^2	R^N	R^9	R^{11}	
CH_3	CH_3	CH_3	H	OH	Isocorydine N-oxide

Fig. 1. Alkaloids identified from *Peumus boldus*.

the morphinandienones pallidine and sinoacutine were isolated from the leaves [27]. See Fig. 1.

Boldo bark has been known for decades to be a good commercial source of boldine [8], which is clearly the dominant alkaloid in this tissue. A more detailed study revealed the presence of *N*-methyllaurotetanine, isocorydine and norisocorydine plus pronuciferine, sinoacutine, and 6a,7-dehydroboldine [28,29]. Both coclaurine enantiomers were subsequently identified in the bark [30]. Boldo wood was examined for alkaloids in two undergraduate theses, which concluded that the noraporphine laurolitsine (norboldine) was the major component together with smaller amounts of boldine, laurotetanine and several unidentified bases [31,32].

The HPLC analyses of several samples of boldo leaves and extracts found laurotetanine or *N*-methyllaurotetanine as the major alkaloids, often followed by isocorydine [25,26]. However, an analysis of leaves collected in central-southern Chile and of boldo tea bags indicated isocorydine, with less *N*-methyllaurotetanine, as the major alkaloids [33], while a ^{13}C NMR profile of a crude boldo leaf extract suggested that norisocorydine was the most abundant, also followed by *N*-methyllaurotetanine [34]. Taken together, these literature results suggest that the alkaloid profile of *Peumus boldus* is highly variable. As the medicinal properties of boldo leaves are presumably affected by such differences, we decided to address the variability of the alkaloidal composition of the leaves, extending our quantitative analysis to the alkaloids present in the bark, wood and roots of boldo trees.