



BOLDO AND BOLDINE: AN EMERGING CASE OF NATURAL DRUG DEVELOPMENT

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SUMMARY

Boldo (*Peumus boldus* Mol.), a Chilean tree traditionally employed in folk medicine and recognized as a herbal remedy in a number of pharmacopoeias, mainly for the treatment of liver ailments, has recently been the subject of increasing attention. Boldine, in particular, the major and most characteristic alkaloidal constituent of this plant species, now emerges as its most interesting active principle from the pharmacological viewpoint. The recent demonstration that boldine is an effective antioxidant in both biological and non-biological systems has opened up the perspective of a broad range of uses in medicine and industry. Given the toxicological data on this alkaloid, its antioxidative properties situate it as a potentially useful substance in many disease states featuring free-radical related oxidative injury. This review attempts to cover and discuss the studies conducted over the last four decades on the chemical and pharmacological properties of boldo and its main constituent.

KEY WORDS: Boldo, boldine, natural antioxidant, free radicals.

INTRODUCTION

The medicinal use of plants or substances contained in diverse plant species has usually been initiated by the cultural recognition or the simple belief that exposure of humans or domestic animals to such materials is associated with beneficial effects to their health. The continued use of a given herbal remedy has presumably resulted from the popular perception of the therapeutic efficacy of the particular species. On the other hand, the abandonment of plant-based preparations may be more a consequence of the recognition of unacceptable side effects than of a lack of evidence for their claimed therapeutic benefits.

According to recent estimates [1], around 75% of the world population still uses or relies on medicinal plants, or plant-based galenicals, as tools for the prevention and/or treatment of disease. In Latin America, in spite of widespread and

increasing access of urban populations to modern allopathic medicine and various alternative therapies, the use of medicinal herbs and native plants is still massive and strongly rooted in local cultures.

Although the use of plant species for medicinal purposes dates back thousands of years, it is only in this century that systematic scientific efforts have been made to evaluate the actual extent of therapeutic benefit associated with their use. Although this has signified a renewed interest in natural medicine, there are many plants which, while enjoying widespread acceptance, still lack the scientific support needed to justify their continued use or, at least, the therapeutic claims generally associated with them [2].

During this century, natural products research, including the pharmacological screening of plants as potential sources of pharmaceuticals, has proven to be an effective and relatively inexpensive approach for the introduction of 'new substances' as prescription drugs. In fact, we can easily recognize over 120 clinically useful prescription drugs derived from higher plants which are in current use worldwide. About three-quarters of them came to the attention of pharmaceutical houses because of their present and/or past use in traditional medicine [1].

BOLDO

Boldo (*Peumus boldus* Mol., Monimiaceae) represents a case of a medicinal plant whose use is deeply rooted in traditional medicine and which lately has been the subject of considerable attention from the pharmacological viewpoint. At least two factors have contributed to a recent resurgence of interest in this species: the accumulation of a broad base of chemical knowledge of its alkaloidal constituents (isolation, identification and quantification), and its widespread cultural perception as an effective medicinal plant for the treatment of digestive and hepatobiliary disorders.

Boldo is an abundant and widespread native tree from central and southern Chile. First described botanically by the Abbé Molina two centuries ago, *P. boldus* grows spontaneously in Chile between 33 and 39 degrees South latitude, from the warm and semi-arid Fourth (Limari) Region to the cool and rainy Tenth (Osorno) Region. Mature boldo trees are usually between 6 and 12 m tall, although some individuals may attain heights of up to 20 m [15]. However, after the trunk has been cut down, boldo occurs as a bush with several main stems. The name 'boldo' or 'boldu' is presumably derived from the Mapuche verbs 'weltún' (to sprout again) or 'volitún' (to put out new roots) [3], which may refer to this feature. Boldo leaves are perennial, leathery, ovate-elliptical, with a dark green, densely glandular upper side, and a light greyish green lower side rich in fascicular pili. The structure of boldo leaves is described in a number of textbooks of pharmacognosy [e.g. 4], and the anatomy and histology of the bark have also been studied [5]. As boldo only grows in abundance in Chile, this country has been for decades the sole original source of the leaves and bark, currently exporting about 800 tons of dried boldo leaves per annum, mainly to Argentina, Brazil, Italy, France, and Germany [6].

As one of the most commonly used medicinal plants of Chile, boldo is employed in the form of infusions, tinctures and extracts (*vide infra*). In the practice of traditional medicine, boldo preparations are generally indicated for the treatment of a variety of conditions amongst which digestive and hepatobiliary disorders have been most commonly mentioned [7]. A fairly recent publication [8] states erroneously that boldo 'was first used in Chile when the local population discovered that sheep with usually fatal liver infections survived when they ate boldo leaves continuously'. This is a misinterpretation of Murillo's account [9] of a fortuitous cure of sheep infested with liver flukes, which 20 years earlier had drawn attention in the local press to the medicinal properties of this plant and led to widespread belief in its virtues as a liver remedy. In fact, it is hard to believe that the indigenous peoples, settled in central Chile long before the Spanish conquest, were unaware of the uses of this abundant plant. The most ancient pharmacological indication of boldo is probably not for the treatment of liver disorders, as the Mapuche people apparently used boldo in the treatment of rheumatism [10]. Fossilized boldo leaves bearing the imprint of human molars have been found somewhat south of the current range of the tree, in an archeological site dated to about 13000 years before present [11] but it can only be guessed whether the early Americans inhabiting that region used the leaves as medicine or merely for their pleasant, refreshing taste. The indications for the use of boldo are extremely broad in range, and just as unsubstantiated. According to treatises dealing with medicinal plants, boldo has been used for headache, earache, nasal congestion, rheumatism, 'nervous weakness', dropsy, dyspepsia, flatulence, menstrual pain, syphilis and gonorrhoea [7, 9, 12–14] and is also claimed to be a sedative and mild hypnotic [15].

Beyond its merely vernacular use, however, boldo-based preparations have been described in several official pharmacognostic texts including the French Pharmacopoeia (8th and 9th edition), the Martindale Extra Pharmacopoeia (25th edition) and the official Pharmacopoeias of Brazil, Chile, Germany, Portugal, Roumania, Spain and Switzerland. At present there are over 60 pharmaceuticals registered in different countries which include boldo or its constituents, usually as minor ingredients, in their formulation [16]. Most of these boldo-containing preparations are indicated for the treatment of digestive and/or hepatobiliary disorders. Boldo is also currently used in homeopathic medicine (HAB 2).

CHEMISTRY

Studies on the chemical constituents of boldo, beginning in the second half of last century, continue to appear. Boldo leaves reportedly contain 1.2% of tannins and 2–3% of essential oils (up to 45% ascaridole and 30% cineole, and at least 22 other identified constituents, mainly terpenoid) [17–20]. The seasonal variation of the essential oil composition has been studied [21]. Boldo essential oil was until recently employed in medicine for its recognized antihelminthic properties due to the presence of ascaridole. Flavonoids (e.g., quercetin) are present, as usual, and five flavonol glycosides [22, 23] have been identified: peumoside (rhamnetin-3-arabinoside-3'-rhamnoside), boldoside (isorhamnetin 3-glucoside-7-rhamnoside),

fragroside (an isorhamnetin dirhamnoside), kaempferol-3-glucoside-7-rhamnoside, and isorhamnetin-3-arabinoside-7-rhamnoside.

Alkaloids were early recognized as active components in boldo. Of these, boldine was the first to be isolated, more than 120 years ago, by Bourgoin and Verne [24]. This substance was prepared in a pure state in 1922 in Merck's laboratory [25], and its structure was proven a few years later, by Späth and Tharrer [26] and independently by Schlittler [27], to be (*S*)-2,9-dihydroxy-1,10-dimethoxy-aporphine [(I), Fig. 1]. In addition to boldine, at least six other aporphine alkaloids (isoboldine II; isocorydine III; norisocorydine IV; isocorydine *N*-oxide V; *N*-methyllaurotetanine VI; laurotetanine VII; and laurolitsinc VIII) have been identified in *P. boldus* [28, 29] (for complete chemical reviews on aporphine alkaloids see [30–34]). There is evidence, however, for the occurrence of at least 17 different alkaloids in boldo [29]. A minor aporphinoid apparently present in boldo bark, but which may well be an artefact of storage or isolation, is 6a,7-dehydroboldine (IX, unsaturated between carbon atoms 6a and 7) [35]. Amongst the non-aporphinoid alkaloids of this plant, the benzyltetrahydroisoquinoline reticuline (Fig. 2, X) [29] is a common biosynthetic precursor of 1,2,9,10- and 1,2,10,11-tetraoxygenated aporphines [36]. More recently, we have isolated a mixture of (*R*)- and (*S*)-coclaurines (Fig. 2, XI) from boldo bark [37], the latter of which, aside from being the key precursor of reticuline, undergoes a broad variety of biotransformations to originate most other isoquinoline alkaloids [38–40]. Two structurally divergent coclaurine-derived

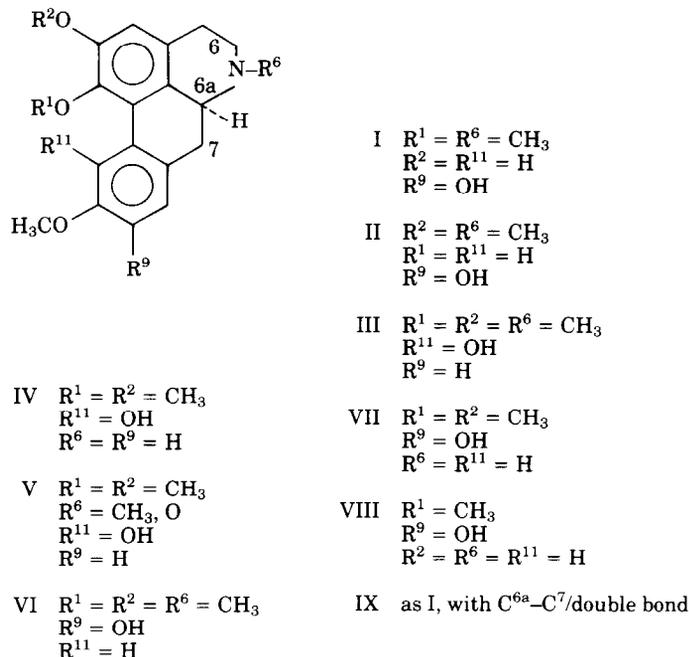


Fig. 1.

alkaloids isolated from boldo bark are the proaporphine (*R*)-pronuciferine (Fig. 3, XII) and the morphinandienone sinoacutine (Fig. 4, XIII) [41].

Boldine has been reported to occur as a minor constituent in more than a dozen other plant species belonging to the Lauraceae, Magnoliaceae and Monimiaceae, along with many other aporphinoids [31–34]. Dried leaves of *Peumus boldus* have been reported to contain alkaloids in the 0.25–0.54% [42] or 0.4–0.5% [28] range, of which approximately 12–19% is boldine [43]. Boldo bark is an unusually rich source of alkaloids, of which boldine makes up about 75% [41].

The analytical detection and quantification of boldine have relied on paper electrophoresis [44], colorimetric methods [45, 46], TLC [47], and GLC [48]. Currently, the assay of boldine and related alkaloids contained in crude boldo samples, in extracts, or in galenical and pharmaceutical preparations is based on the initial separation of the alkaloids by high performance liquid chromatography followed by its UV spectroscopic quantification, as boldine exhibits two absorption maxima at about 282 and 303 nm [48a–51]. We have recently described an HPLC method which is appropriate for boldine assay in plasma and other biological fluids [52], which should make pharmacokinetic studies possible.

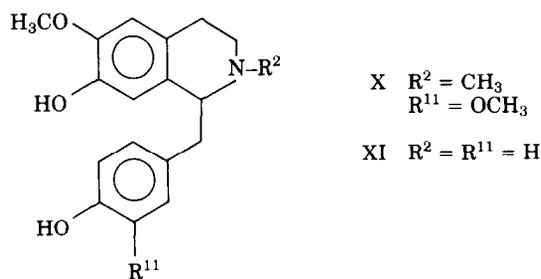
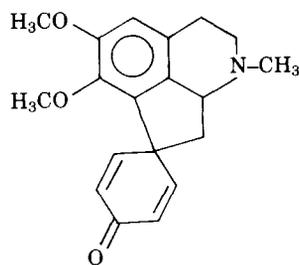
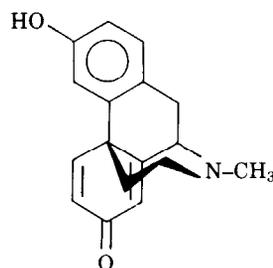


Fig. 2.



XII

Fig. 3.



XIII

Fig. 4.

PHARMACOLOGY OF BOLDINE

From the pharmacological viewpoint, boldine is the active principle that has attracted most attention amongst the many identified secondary metabolites of boldo. Among the earlier pharmacological studies on boldo and its constituents are those conducted by Kreitmair [53], in which the assessment of several actions of boldine led to the finding that the alkaloid exerts choleric and cholagogic effects in several experimental animals. Such effects were subsequently confirmed by other researchers [54–57]. Although the flavonoid glycosides of boldo have been found to be devoid of choleric activity by themselves [22], these compounds have been claimed to enhance the effect of the alkaloids [58]. In addition, it has been reported that, in large doses, boldine may exert a diuretic action [59]. The latter, however, could not be observed when this substance was administered as part of the total boldo alkaloid mixture [53].

Besides its hepatobiliary actions, boldine was shown early on to inhibit peristaltic contractions of the small intestine in the anaesthetized cat preparation [60], even after subcutaneous administration of carbachol [53]. In line with the latter observations, we have recently shown that, at concentrations between 10^{-5} and 10^{-4} M, boldine exerts *in vitro* a smooth muscle-relaxing effect on rat ileum which is at least partly mediated through anticholinergic actions [61]. On the other hand, although it has been reported that boldine appears to lack direct alpha-adrenergic activity in rat vas deferens [62], a very recent study has shown that this alkaloid behaves as a cardiovascular alpha-adrenergic blocker and calcium antagonist in the rat [63]. Administered parenterally, boldine, like other aporphine derivatives, exerts some inhibitory central actions which are probably mediated through the blocking of dopamine D2 receptors [see 64].

BOLDINE AS AN ANTIOXIDANT

Recently, our studies [65] led to the finding that boldine behaves as a very potent antioxidative substance in biological systems undergoing peroxidative free radical-mediated damage. The addition of low concentrations ($CI_{50}=5-15 \times 10^{-6}$ M) of boldine to such experimental systems completely prevents the chemically-induced peroxidation of red blood cell plasma membranes, as well as the spontaneous peroxidation of membranes in brain homogenates. Interestingly, in the latter experimental system, boldine displays a 10–100-fold greater antioxidative activity than that of silybin ($CI_{50}=4 \times 10^{-4}$ M), a mixture of lignanoflavonoids currently available for therapeutic use as an antioxidant in humans [66]. In addition, boldine protects enzymes susceptible to peroxidative inactivation (lysozyme, [65]; mono-oxygenases, [67]), preventing the loss of catalytic activity. In studies directed to elucidate the mechanism of the antioxidative action of boldine, we have recently established that the boldine molecule acts as an efficient hydroxyl ($HO\cdot$) radical scavenger [67]. The latter radicals, recognized as the most reactive oxygen species generated by biological systems [68], are trapped by boldine with even greater efficiency than that exhibited by dimethyl sulphoxide, a compound used experimentally as a

paradigmatic HO· radical scavenger. Boldine ($IC_{50}=10^{-5}$ M) prevents *in vitro* the damage to the cytochrome P-450 systems and to microsomal membranes exposed to different peroxidative conditions, inhibiting both chemically and enzymatically catalysed microsomal lipid peroxidation. Moreover, this antioxidant was proven to be very effective in the protection of microsomal membranes against damage induced by toxic agents such as carbon tetrachloride and *tert*-butyl-hydroperoxide [67]. Laboratory studies have shown that 10^{-4} M boldine effectively protects isolated rat hepatocytes against the peroxidative and cytotoxic effects of *tert*-butyl-hydroperoxide [69]. More important yet, a recent study by Lanhers *et al.* [70] showed the usefulness of boldine administered intraperitoneally (i.p.) as an *in vivo* preventive of hepatitis induced in mice by carbon tetrachloride. Nevertheless, the actual correspondence between plasma boldine levels reached after parenteral administration [70] and the concentrations of boldine proven to be antioxidative and cytoprotective *in vitro* [69] remains to be established. It has also been shown that a boldo leaf extract containing no more than 0.4% of total alkaloids, of which boldine comprises less than 0.12%, can mimic *in vivo* the hepatoprotective effect of boldine against CCl_4 [70]. Considering the method used by the authors to prepare the dried hydro-alcoholic extract, it is likely that the flavonoids expected to be abundant in this preparation make a major contribution to its hepatoprotective activity. On the other hand, it is also possible that the cytoprotective effect reflects the presence of a number of alkaloids co-occurring with boldine which may also exhibit antioxidative activity. In fact, recent studies addressing structure-activity relationships for benzyloquinolines suggest that all the known boldo alkaloids are likely to exhibit at least some antioxidative activity [71, 72]. Interestingly, in the case of aporphine alkaloids, the presence of phenolic groups is clearly not essential for these molecules to display their activity. In the case of boldine, while *O*-methylation of the two phenol functions to afford glaucine was not associated with a loss of potency, subsequent *N*-methylation led to a derivative which was shown to be virtually devoid of antioxidative activity [72]. Thus, the available evidence suggests that in the case of aporphine alkaloids, in addition to the expected contribution of phenolic functions to their radical-scavenging ability, the hydrogen atom bonded to the benzylic carbon (C-6a) next to the basic nitrogen atom may be a key to the antioxidative activity displayed by the non-phenolic boldo alkaloids.

Many substances are known to be effective in preventing membrane peroxidation. Nevertheless, some features distinguish boldine mechanistically from other currently used antioxidants. Some substances which can act as effective antioxidants, such as propyl gallate [73] or phytic acid [74], can also behave as iron chelators, thus enhancing the auto-oxidation of Fe^{2+} to Fe^{3+} . Boldine does not cause this effect, and inhibits iron-mediated peroxidation at concentrations 10 times lower than that of the metal catalyst [67]. Some synthetic antioxidants such as butylated hydroxyanisole (BHA), ethoxyquin, propyl gallate, and the natural 3-cyanidanol have been shown to increase liver microsomal H_2O_2 production [75–77]. Similarly, butylated hydroxytoluene (BHT), BHA, ethoxyquin and propyl gallate decrease cytochrome P-450 content and depress mixed-function oxidase activity of microsomes [78–80]. The antioxidative activity associated with these substances appears to enhance the oxidase function of

cytochrome P-450. Boldine, in contrast, does not interfere with the production of H_2O_2 by microsomes nor does it alter NADPH-P450 reductase or P450 oxidase activities. Also, the presence of boldine has been shown not to modify microsomal biotransformation of several prototypical xenobiotics (ethanol, dimethyl-nitrosamine, etc.). This latter represents a highly desirable condition in the perspective of developing and using this natural product in therapeutics [67]. Antioxidants such as propyl gallate, BHA, BHT and vanillin, can stimulate the production of $HO\cdot$ radicals by Fenton-type systems [81, 82], while boldine behaves as a potent scavenger of these reactive species [67]. Furthermore, in the abiotic experimental model of fish oil free radical-mediated peroxidation, with or without heavy metal catalysis, boldine exhibits an antioxidative efficacy three to four times greater than those observed for vitamin E, BHA or BHT [83]. Although the oil stabilizing activity of boldine resembles that of the natural flavonoid quercetin, the demonstration that the latter compound is mutagenic *in vitro* [see 84] is likely to curtail its technological development as a food antioxidant.

TOXICOLOGY OF BOLDINE

Beyond the precedent of the prolonged tradition of pharmaceutical use of boldine and of preparations based on boldo, the low toxicity of this substance was shown early on by the high doses needed to induce death in several mammalian species (e.g., 15 g of boldine, administered orally, were necessary to kill a 12 kg dog [85]; by the same route, 500 and 1000 mg kg^{-1} of body weight were required to kill mice and guinea pigs, respectively [53]). No signs of toxicity could be observed after administering up to 3000 mg kg^{-1} p.o. of a boldine-containing soft hydro-alcoholic blood leaf extract to rats [8].

Recently, boldine was demonstrated to be non-mutagenic in the SOS chromotest, and in several Ames tester strains, with or without prior metabolic activation [86]. Furthermore, recent studies conducted in our laboratory have shown that boldine, administered i.p. at sublethal doses, induces no signs of genotoxicity in mouse bone marrow as assessed by the micronucleus test [87]. The cytogenetic analysis associated with the micronucleus test provides a highly reliable measure of the *in vivo* structural or numerical chromosomal aberrations induced by a chemical. The application of this method, which represents an official criterion recommended by the US Environmental Protection Agency Gen-Tox Program [88], should become a routine procedure in the toxicological assessment of novel candidate compounds for development as drugs or food additives.

FINAL REMARKS

The increasing recognition of the participation of free radical-mediated oxidative events in both physiological and pathological processes is giving rise to the use of antioxidative substances in preventive and therapeutic medicine. Consequently, at this point, the major prospects of pharmacological application of boldine seem to

stem from its recently demonstrated antioxidative activity. The latter pharmacological property situates this alkaloid as a potentially useful substance in the prevention and treatment of a broad range of pathological conditions in which free radicals have been implicated as either aetiogenic initiators or promoters. Atherosclerosis, ischemia-reperfusion damage, drug-induced hepatotoxicity, autoimmune and inflammatory diseases, certain forms of cancer and some neurodegenerative diseases, are among the aforementioned conditions which could become targets for intervention by means of antioxidants such as boldine.

Although the effectiveness of boldine as an antioxidant and its relative innocuousness encourage the pursuit of research directed to explore its future as a pharmacological agent, basic studies regarding its pharmacokinetics and biotransformation, among others, will be necessary before its therapeutic value can be assessed in human beings. On the other hand, while further studies on the development of boldine as an antioxidative food additive appear warranted, additional toxicological research linked to its uses in food technology will be required.

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