Journal of Chromatography, 511 (1990) 373–378
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 483

Note

Chromatographic evaluation of boldine and associated alkaloids in Boldo

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(Received January 8th, 1990)

In 1988, Pietta *et al.*[1] recorded high-performance liquid chromatographic (HPLC) assays of some alkaloids, and thin-layer chromatographic (TLC) studies of Boldo extracts from *Peumus boldus* Molina leaves, a herb from Chile with a reputation for urinary and liver treatment. They devised a reversed-phase HPLC method using an amine-phosphate buffer reported as containing about 85% acetonitrile as mobile phase, which after a "clean-up" procedure gave at 270 nm a chromatogram (illustrated) with six peaks, only three of which (not including the major one!) they named. Their thin-layer chromatography (TLC) method used 25% diethylamine in chloroform on silica gel F_{254}.

In the course of some studies of several commercial samples of Boldo, I reviewed and modified the procedures of Pietta *et al.*[1], and my observations and results are given below.

Alkaloids which have been recorded from Boldo are boldine, isocorydine and nor-isocorydine, N-methyllaurotetanine[2] and laurotetanine, and isoboldine[3] (see Fig. 1). Boldine occurs in two other plant genera along with some of these above-mentioned aporphine alkaloids[4,5].

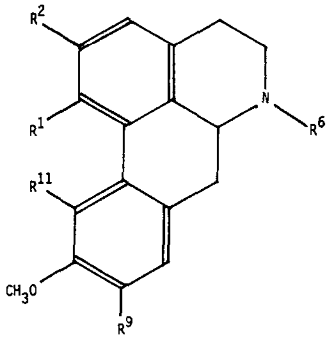
EXPERIMENTAL

Apparatus

A Laboratory Data Control HPLC 7800 system with Constametric pumps, UV Spectromonitor III 1204A, accessory control module, computer chromatography control module, and recorder/printer was applied.

Materials

Boldo herb (mostly leaf) and Boldo extract commercial samples from various European manufacturers were used. Boldine alkaloid (crystallised with a molecule of chloroform) was obtained from Sigma.



R^1	R^2	R^6	R^9	R^{11}		
CH_3O	HO	CH_3	HO	H	boldine	diphenols
HO	CH_3O	CH_3	HO	H	isoboldine	
CH_3O	CH_3O	CH_3	HO	H	N-methyllaurotetanine	
CH_3O	CH_3O	H	HO	H	laurotetanine	
CH_3O	CH_3O	CH_3	H	HO	isocorydine	
CH_3O	CH_3O	H	H	HO	nor-isocorydine	

Fig. 1. Structures.

Methods

HPLC. Reversed-phase Spherisorb S5 ODS S1 columns 4.9 or 4.6 mm I.D. were used at 30°C. A 20-μl injection loop was applied. The detector was set at 304 nm.

Solvent system 1, for quick review of an extract, with some peak overlapping, was isocratic, with 77.4% acetonitrile in water, containing 0.05% diethylamine, and the pH brought below 4 by the addition of dilute phosphoric acid; flow-rate 0.8 ml/min. Six peaks appear between 5 and 10 min (Fig. 2b).

Solvent System 2, for accurate boldine analysis, was initially isocratic, with 16.3% acetonitrile in water, containing 0.2% diethylamine, and the pH below 4, as above. After 8 min at a flow-rate of 2.1 ml/min the acetonitrile content was increased linearly to 28.0% at 11 min elapsed time, then left isocratically until 18 min. After 5 min a series of six peaks appeared, designated U to Z, with V corresponding to reference boldine (Fig. 2a).

Evaluation of alkaloids. Boldine was evaluated by HPLC peak area against a solution of about 6 mg% reference boldine in acetonitrile (73.28% of the weight taken due to chloroform of crystallisation). This was stable over several weeks if stored away from light. Other peaks were evaluated as though they were boldine, which is of course, somewhat inaccurate. Results are given in Table I.

Extraction of Boldo herb or extract. Exactly 1 g is percolated (leaf, in filter

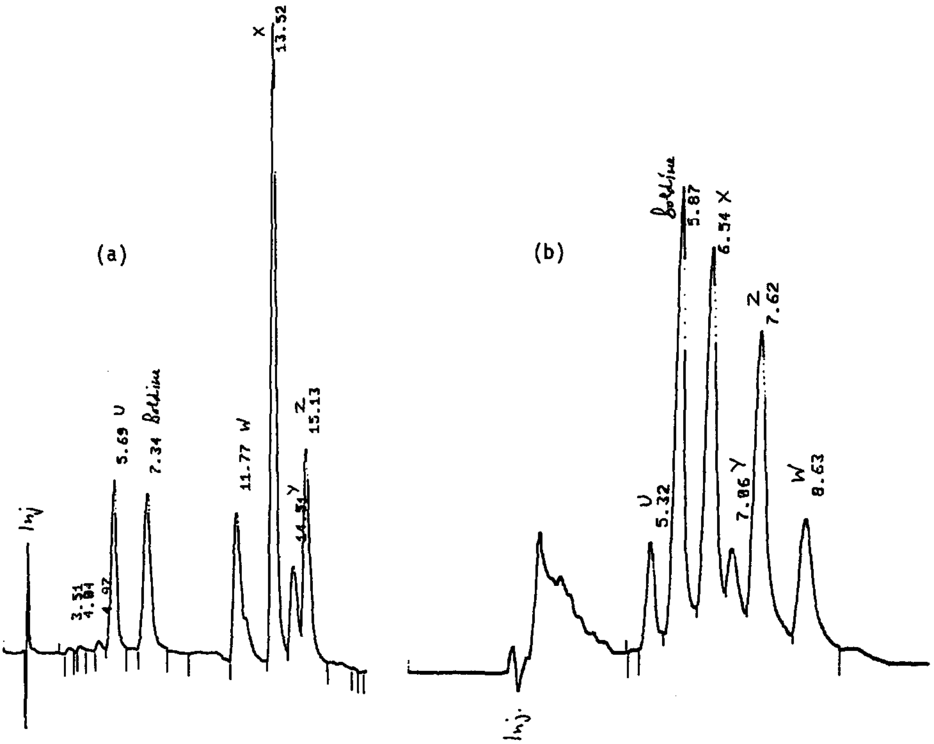


Fig. 2. HPLC traces of processed Boldo. (a) Solvent system 2 (programmed from 16.3 to 28% acetonitrile in water) used on Boldo leaf B. (b) Solvent system 1 (isocratic with 77% acetonitrile in water) used on Boldo extract H. The deduced identity of peaks is given in Table I. The numbers at the peaks indicate retention times in min.

paper) or rubbed with a spatula (commercial extract) in nearly boiling dilute hydrochloric acid. After cooling, this is exhausted of interfering pigments by careful shaking with a solvent mix of equal parts of ethyl acetate and carbon tetrachloride (discarded). The residual aqueous extract is made alkaline with ammonia and carefully extracted with fresh solvent mix, which is evaporated to dryness. The residue is taken up in acetonitrile (exactly 10 ml) for HPLC assay. For TLC study the residue is used in concentrated form.

TLC. A two-dimensional system was devised, using silica gel plates with fluorescent (254 nm) indicator. The first-direction solvent consisted of equal parts of methanol and ethyl acetate which elutes pigment to about R_F 0.8, with a complex main alkaloidal spot R_F 0.5–0.6, followed by traces of minor spots. The second-direction solvent was 5% diethylamine in ethyl acetate (which avoids amine interaction with halogenated solvents). Boldine is most polar and so slow moving, R_F 0.20 or less. Two other strong spots ahead of boldine at R_F 0.35 and 0.70 should be N-methyllaurotetanine and isocorydine (-N-oxide? — due to the large surface area of the TLC plate), respectively, according to other studies[1,6] using diethylamine systems.

TABLE I

mg% BOLDINE AND OTHER BOLDO ALKALOIDS (CALCULATED AS BOLDINE FROM PEAK AREAS AT 304 nm) IN BOLDO SAMPLES

Letters U–Z refer to chromatographic peaks in Fig. 1. They are provisionally identified by their chromatographic responses. Letters A-H refer to different samples. Numbers 1 and 2 refer to solvent systems (see text).

	Boldo leaf "whole" samples												*Commercial extracts*			
	A		*B*		*C*		*D*		*E*		*F*		*G*		*H*	
	2	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*
Boldine	4	6	6	6	8	8	9	9	9	9	13	14	27	27	8	10
U Isoboldine?	3	3	5	5	4	4	6	6	5	5	13	13	5	5	1	2
X Laurotetanine?	16	16	16	18	14	14	22	22	27	26	62	57	23	23	8	10
Z N-Methyllaurotetanine?	14	15	7	8	7	8	20	22	17	17	27	27	22	21	9	10
Y Isocorydine?	6	6	3	4	4	6	13	12	15	15	9	10	9	8	3	3
W Isocorydine-N=O?	4	11	7	3	6	6	13	13	14	17	14	14	6	12	2	4
Total	47		44		43		83		87		138		92		31	
% Boldine	8.5		13.6		18.6		10.8		10.3		9.4		29.3		25.8	
% "Laurotetanines"	64		52		49		51		51		64		49		55	
% "Isocorydines"	21		23		23		31		33		17		16		16	

Minor spots scattered over the plate nearer the start point should be relatively polar other diphenolic substances and nor-alkaloids. Visualisation is by fluorescence quenching of the plate background viewed in UV at 254 nm, and subsequent darkening of the spots to slaty-brown on standing in daylight or UV light. A reaction to Dragendorff's or iodoplatinate reagents can be obtained, but this is not as sucessful as the slow darkening in daylight; the plate can best be viewed the following day. The phenolic reaction sequence of ferric chloride solution followed by potassium ferricyanide spray is a better method of detection.

The relatively high polarity of boldine compared to the other alkaloids was confirmed by reversed phase TLC using Whatman KC_{18} F plates, with solvent methanol–acetonitrile–water–diethylamine (55:5:40:0.2) (with pH brought below 4 with dilute phosphoric acid). Boldine has an R_F just below 0.30, followed by other alkaloidal spots, these being strongest at R_F about 0.15.

DISCUSSION

The only possible way to obtain a reversed-phase HPLC result like that illustrated by Pietta *et al.*[1] is to use a solvent mix with about 16% acetonitrile instead of the 85% they quote. In other words, their quoted proportions of acetonitrile to watery component need reversing. The gap between their boldine peak 1 and peak 2 only occurs with low percentages of acetonitrile in the solvent, as also for their later peaks with long retention times. Their mysterious major peak just after their peak 2, which they make no reference to at all, is clearly an artefact introduced by their use of Sep-Pak C_{18} cartridges for "clean-up", as it is absent from their unpurified Boldo extract chromatogram. If an HPLC solvent mix close to that given by Pietta *et al.*[1] is used, and the flow-rate adjusted to give the retention time for boldine that they record (about 6 min) then all the later peaks are eluted and the chromatogram finished in about 11 min, not the 25 min shown by them.

Pietta *et al.*[1] note that the Boldo alkaloids "exhibit different UV absorption maxima, and (their) determination was carried out at 270 nm which represents the best compromise". This wavelength in fact only suits the 11-hydroxy alkaloids such as isocorydine (see Fig. 1) and not the others like boldine, which have absorption maxima at about 283 and 303 nm. Using 270-nm detection has deflated the results for boldine and those alkaloids without an 11-hydroxy group, which hardly seems desirable.

My work was performed at 304 nm, where there is a slightly lower intensity peak for boldine than at 283 nm[7], but it is less likely to interference from various other constituents, including ascaridole[8] from the volatile oil. By using detection at both 304 and 269 nm it was possible to recognise the 11-hydroxy alkaloids by their higher peaks shown at the shorter wavelength. This applied to peaks designated W and Y by me, which were thus noted as members of the isocorydine group. W revealed behaviour unlike any other peak from Boldo extracts, in that it shifted position in the sequence of peaks to overlapping X and Y at solvent concentrations of about 40–60% acetonitrile, and to coming after Z in 77% acetonitrile. Isocorydine is recorded as susceptible to oxidation, and so I suspected that it was isocorydine-N-oxide, leaving Y as isocorydine itself. If this is correct, peak 2 of Pietta *et al.*[1] which they called "isocorydine" is actually its N-oxide. They "isolated" this substance (and N-methyllaurotetanine) "by preparative TLC" without any identification, and the large surface area of a TLC adsorbent can readily catalyse oxidation.

Thus a diphenolic alkaloidal boldine early peak V was identified using authentic alkaloid, and a late peak Y characterised as monophenolic isocorydine. This is an appropriate sequence for a reversed-phase system, with the less polar monophenolic being retained longest on the non-polar column. Thus the small peak(s) U ahead of boldine should also be diphenolic(s) like isoboldine and later peaks X and Z should be monophenolics of the (non-11-hydroxy)laurotetanine type. N-Methyllaurotetanine will be less polar than the nor-alkaloid and so should be retained longer by the non-polar column (Z), leaving X as laurotetanine itself. This does not correspond to the peak 3 designated by Pietta *et al.*[1] as "N methyllaurotetanine" (I believe it to be iso-corydine) but their reliance on UV detection at 270 nm hints that their peak 3 (and also 2) are 11-hydroxy alkaloids.

Boldine, from Table I (and Fig. 1) is clearly a minor alkaloid in both the Boldo herb samples and commercial extracts. It forms 8.5 to 18.6% of the total leaf alkaloids, and about 25–30% of the extracts. It is interesting that commercial processing (probably by hot-water extraction) increases the proportion of boldine. However, put in this manner, the proportion of boldine really depends on the total amount of other alkaloids there. Total alkaloids calculated as boldine form 43–138 mg% of the leaf samples and 31–92 mg% of the extracts. Extract H, at least, has thus undergone a preferential loss of non-boldine constituents, which is perhaps desirable, as the other alkaloids may not assist the physiological effects of boldine. For three commercial extracts, Pietta *et al.*[1] found 8, 14 and 16 mg% boldine. In 1983, by titrimetry, total alkaloids of 53 and 68 mg% were recorded, with boldine forming 13 and 19% of the total. These values agree with my results.

From my limited observations, there may be at least two chemical forms of Boldo herb, one with total alkaloids of 40–50 mg%, and the other with double or treble this. It seems desirable to look for Boldo leaf with at least 8 mg% boldine, which forms at least one-tenth of the total alkaloids (to limit excessive amounts of these other substances). Commercial extracts should show an increased boldine content, which forms greater than a quarter of the total alkaloids. This is best monitored by using HPLC solvent system 1 (isocratic, and quick) supported by two dimensional TLC.

ACKNOWLEDGEMENT

This work was performed in the Technical Services Laboratory of William Ransom and Son, PLC, Hitchin, Hertfordshire, U.K. I am grateful for the opportunity to undertake research there during 1989, as part of an Outside Study Programme.

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