Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, Nos 10–12, pp. 1083–1087, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00

Characterization of *Sanguinaria canadensis* L. fluid extract by FAB mass spectrometry*

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Abstract: Positive-ion fast atom bombardment mass spectrometry was used for the rapid characterization of commercial *Sanguinaria canadensis* L. fluid extracts. Quaternary and non-quaternary benzophenanthridine alkaloids afford persistent peaks due to $[M]^+$ and $[M + H]^+$ ionic species, respectively, and their relative abundances are in good agreement with previously reported per cent analytical data. The procedure allowed sanguinarine, chelerythrine, chelirubine, sanguilutine, protopine, allocryptopine and the isomers sanguirubine and/or chelilutine to be effectively detected by means of persistent and intense peaks in all the samples examined.

Keywords: *FAB mass spectrometry;* Sanguinaria canadensis *L. fluid extract; benzophenanthridine alkaloids.*

Introduction

The extract from *Sanguinaria canadensis* L. (sanguinaria) rhizome has been reported to be effective in controlling plaque and gingivitis through a combination of antimicrobial action and retention of sanguinarine, its most abundant quaternary benzophenanthridine alkaloid [1–3]. Subsequent clinical trials on the plaque-inhibitory effect have had conflicting results [4–8]. However, the sanguinaria extract forms the active principle of some anti-plaque oral rinses and toothpastes sold in various countries.

Sanguinaria extract is substantially a mixture of benzophenanthridine alkaloids (Fig. 1) [9, 10], of which sanguinarine and chelerythrine have been evaluated as the main components, while there are smaller amounts of sanguilutine, sanguirubine, chelirubine and chelilutine [11]. Sanguinarine and chelerythrine were actually found to be about 70% of the total alkaloid content [11, 12]. Since good antimicrobial effect and antiinflammatory activity were reported for both sanguinarine and chelerythrine [10, 13], it has been hypothesized that the content of the two quaternary benzophenanthridine alkaloids is responsible for the anti-plaque effectiveness of sanguinaria extract-based pharmaceutical preparations [14]. Therefore the search for a rapid analytical method which can directly and simultaneously identify the benzophenanthridine alkaloids in commercial samples of the extract would seem to be very useful for its characterization and for a preliminary evaluation of its potential efficacy. Positive-ion fast atom bombardment mass spectrometry (FAB-MS) of the cationic moieties of the extract appeared to be particularly suitable, in view of the fact that success was recently achieved in applying this method to the assay of cationic surfactants in antiseptic formulations [15, 16]. Therefore, FAB-MS was employed in the present work for the characterization of different samples of commercially-available sanguinaria fluid extract without any previous extraction procedure.

Experimental

Materials

Samples of sanguinaria fluid extract, prepared in the usual manner [17], were acquired from the Italian manufacturers Lifepharma (Milan), Curt Georgi Imes (Milan) and Indena (Milan).

* Presented at the "Third International Symposium on Pharmaceutical and Biomedical Analysis", April 1991, Boston, MA, USA.

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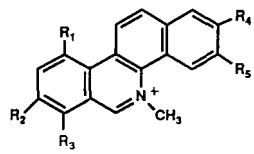
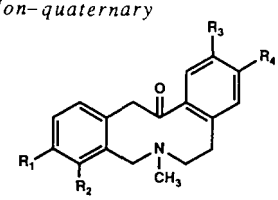
Quaternary



	MW	
Sanguinarine (SA)	332	$R_1 = H$; $R_2 + R_3$, $R_4 + R_5 = OCH_2O$
Chelerythrine (CH)	348	$R_1 = H$; R_2, $R_3 = OCH_3$; $R_4 + R_5 = OCH_2O$
Chelirubine (CR)	362	$R_1 = OCH_3$; $R_2 + R_3$, $R_4 + R_5 = OCH_2O$
Sanguirubine (SR)	378	$R_1 = OCH_3$; $R_2 + R_3 = OCH_2O$; R_4, $R_5 = OCH_3$
Chelilutine (CL)	378	R_1, R_2, $R_3 = OCH_3$; $R_4 + R_5 = OCH_2O$
Sanguilutine (SL)	394	R_1, R_2, R_3, R_4, $R_5 = OCH_3$

Non-quaternary



Protopine (PR)	353	$R_1 + R_2$, $R_3 + R_4 = OCH_2O$
Allocryptopine (AL)	369	R_1, $R_2 = OCH_3$; $R_3 + R_4 = OCH_2O$

Figure 1
Structures of alkaloids.

Apparatus

Mass spectra were obtained with a VG Analytical 7070 EQ double-focusing instrument operating at 6 kV accelerating voltage and fitted with a Digital PDP8/A computer system. The FAB ion source employed xenon atoms of 7 keV kinetic energy.

Procedure

Glycerol, thioglycerol and *m*-nitrobenzyl alcohol were comparatively tested in order to optimize the FAB liquid matrix in relation to the samples to be examined. Glycerol appeared to be the most effective in obtaining FAB mass spectra in the positive ion mode. Each sample (about 1 μl) was added by microsyringe to a layer of glycerol on the FAB target and mass spectra were taken by repetitive scanning in the mass range 90–800 *m/z*, at 1500 resolution with a scan speed of 5 s decade^{-1}. In order to equalize the small ion current variations of the individual ionic species, which usually occurred in each scan, it was thought opportune to obtain the average of a few of the best spectra in the run and consider it as the sample spectrum. The spectra to be averaged were those after the initial ion current stabilization and before the ion current decrease due to sample consumption. In this case averaging from the fifth to the 10th scan gave the best run-to-run reproducibility. The entire procedure required no more than 10 min.

Results and Discussion

The FAB mass spectra of three different brands of sanguinaria commercial fluid extract are reported in Fig. 2. This kind of extract is largely constituted of quaternary benzophenanthridine alkaloids and intense peaks, due to the intact cationic moieties $[M]^+$, were prominent as expected [15]. In particular, the peaks at *m/z* 332 ($C_{20}H_{14}NO_4^+$), 348 ($C_{21}H_{18}NO_4^+$), 362 ($C_{20}H_{16}NO_5^+$) and 394 ($C_{23}H_{24}NO_5^+$) can be unequivocally related to sanguinarine, chelerythrine, chelirubine and sanguilutine

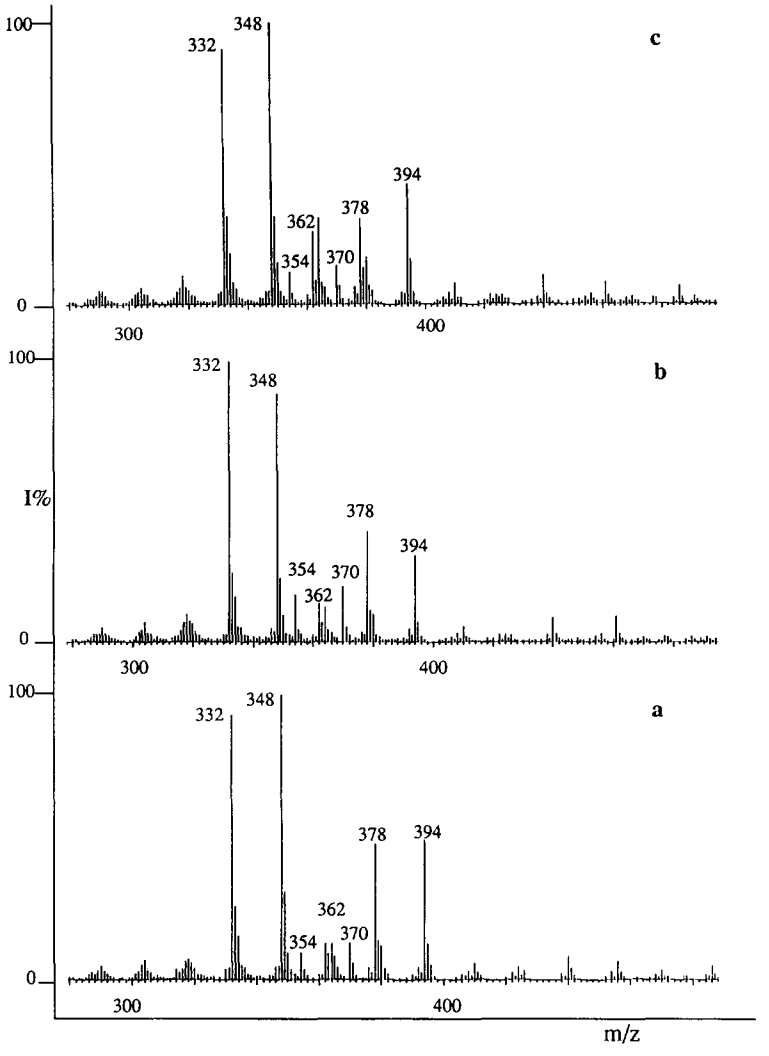


Figure 2
Positive-ion FAB mass spectra of commercially available sanguinaria fluid extracts manufactured by: (a) Lifepharma, (b) Indena, (c) Curt Georgi Imes.

cations, respectively [9]. The sole exception is the peak at *m/z* 378 corresponding to the $C_{22}H_{20}NO_5^+$ cationic species which can be attributed to sanguirubine and/or chelilutine, two isomeric alkaloids which are well known as components of sanguinaria extract [9, 12].

In the first exhaustive phytochemical study on sanguinaria roots [12], the presence of a group of non-quaternary alkaloids amounting altogether to about 8% of the total alkaloid content, was also reported. A more recent paper [11], based on an HPLC method of analysis, did not mention those components. These are mainly protopine (MW = 353) and allocryptopine (MW = 369), which under FAB conditions should give rise, as tertiary bases, to $[M + H]^+$ ionic species at *m/z* values of 354 and 370, respectively. These ions are in fact observed in the spectra of Fig. 2. More recently sanguidimerine, a dimeric dihydrobenzophenanthridine ($C_{43}H_{32}N_2O_9$; MW = 720), has been identified by Tin-Wa *et al.* [18, 19] as one of the major alkaloids deriving from sanguinaria roots. However, in the spectra of our samples no peaks were detected above 550 daltons, thus corroborating the hypothesis of the above author that the presence of this compound is strongly dependent on the extraction procedure employed.

Moreover, two more peaks at *m/z* 318 and 364 were present in all the extracts considered, in very similar amounts. These peaks might be $[M]^+$ or $[M + H]^+$ ionic species arising from quaternary or non-quaternary alkaloids, re-

spectively. Consequently, it may be hypothesized that *m/z* 318 and 364 peaks might be formally related to $[C_{19}H_{12}NO_4]^+$ or $[C_{19}H_{11}NO_4 + H]^+$ and $[C_{21}H_{18}NO_5]^+$ or $[C_{21}H_{17}NO_5 + H]^+$ ionic species, respectively, and belong to alkaloids which have not as yet been detected in sanguinaria roots.

Finally, it may be noted that a series of peaks (Fig. 2) due to glycerol adducts with the above mentioned ionic species were present at 410 (318 + gly), 424 (332 + gly), 440 (348 + gly), 454 (362 + gly), 456 (364 + gly), 470 (378 + gly) and 486 (394 + gly) *m/z*, thus confirming the peaks at 318, 332, 348, 362, 364, 378 and 394 as 'true' molecular species [20].

It is known that the quantitative response of the FAB ionization method is highly dependent on the operative conditions and even more highly on the nature of the molecules involved. Consequently, it can be expected that very similar molecules with only minor physico-chemical differences might behave very differently in producing molecular ions under FAB mass spectrometric conditions [20]. Nevertheless, in this case the relative abundances of the above-mentioned peaks were in good agreement with the per cent analytical data reported in the paper of Slavik and Slavikova [12]. Thus it could be claimed that under the operative conditions of this study, the peak heights can be correlated with the relative per cent content of the corresponding alkaloids. A synoptic view of these findings is reported in Fig. 3 based on the equation:

$$\text{Per cent alkaloid}_k = \frac{h_k}{\sum_{i=1}^{n} h_i} \times 100$$

where h_k is the peak height of the alkaloid k, and the argument for the denominator is the sum of the heights of the n considered peaks.

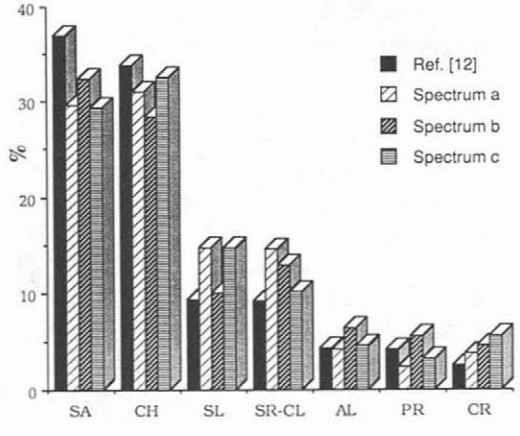


Figure 3
Relative percentages of the benzophenantridine alkaloids (SA, sanguinarine; CH, chelerythrine; SL, sanguilutine; SR–CL, sanguirubine and/or chelilutine; AL, allocryptopine; PR, protopine; CR, chelirubine) identified in the FAB mass spectra (a), (b) and (c) of Fig. 2. The results were obtained by means of the algorithm reported in the text and compared with the per cent analytical data of Slavik and Slavikova [12].

Conclusion

This study highlights the ability of FAB-MS to characterize extracts of sanguinaria. Rapidity of execution and specificity are the main features of the method which allows the individual recognition and the relative abundance evaluation of the alkaloids contained in commercial extracts in only a few minutes, by simple comparison with a certified reference extract.

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[Received for review 29 April 1991; revised manuscript received 11 June 1991]