Pergamon

0731-7085(94)00066-2

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 10, pp. 1283–1287, 1994
Elsevier Science Ltd
Printed in Great Britain
0731-7085/94 $7.00 + 0.00

Isolation and biological activity of aspidospermine and quebrachamine from an Aspidosperma tree source

HAROLD F. DEUTSCH, MERLE A. EVENSON,† PETER DRESCHER,‡ CHRISTOPH SPARWASSER‡ and PAUL O. MADSEN*‡§

Department of Biomolecular Chemistry
† Department of Pathology-Laboratory Medicine
‡ Dept of Surgery, University of Wisconsin School of Medicine, Madison, WI 53705, USA

Abstract: The indolealkaloids aspidospermine and quebrachamine have been isolated in crystalline form by a relatively rapid fractionation from the extract of a powdered material designated "Quebracho" derived from an Aspidosperma tree species. We present a novel isocratic LC method that provides baseline resolution of these two compounds and of the structurally related yohimbine in less than 15 min. Gas chromatography–mass spectrometry was employed to identify these compounds as well as several minor derivatives of aspidospermine during and after the purification process. Aspidospermine and quebrachamine like yohimbine have been found to possess adrenergic blocking activities for a variety of urogenital tissues.

Keywords: *Aspidospermine; quebrachamine; indoleolkaloids; liquid chromatography.*

Introduction

Various extracts of natural products have been or are used extensively in attempts to alleviate the symptoms of benign prostatic hyperplasia (BPH) and to treat erectile impotence. One of these designated "Quebracho" derived from the bark of an Aspidosperma tree species has been used to isolate components related to yohimbine, an α-adrenergic blocking agent. Extracts of a powder produced from the bark of such trees has been found to inhibit smooth muscle contractions in tissues such as human prostate strips, rabbit corpus spongiosum and cavernosum and guinea pig vas deferens. Therefore, it was of interest to determine whether such inhibitory activity could be related to specific components of the "Quebracho" preparation employed. Two compounds, both of which were found to possess this activity were isolated from this source material and physically characterized to establish their identities as aspidospermine and quebrachamine.

Experimental

Fractionation procedure

It was necessary to employ reagents that could be readily and completely removed by evaporation procedures since various organic solvent residues in the products could effect the biological assay. After numerous preliminary experiments, it was found that the biological activity of the "Quebracho" powder employed could best be extracted with hot methanol (MeOH). The usual fractionation utilized 3 g of the "Quebracho" powder. This amount in 60 ml of MeOH was brought to the point of incipient boiling (65°C) and stirred for 5 min. The suspension was then rapidly cooled to room temperature and centrifuged at 0°C for 20 min at 9000*g*. The 0°C supernatant was decanted and the MeOH concentration lowered to 20% by the addition of water at room temperature with rapid stirring. This suspension was heated with stirring at 65°C for 5 min, rapidly cooled to room temperature and the insoluble material removed by centri-

* Author to whom correspondence should be addressed.
§ Present address: VA Hospital, 2500 Overlook Terrace, Madison, WI 53705, USA.

fugation at 0°C for 20 min at 9000*g*. The 0°C supernatant was subjected to rotary evaporation at 40–50°C. The residue was suspended in 25 ml of water, the pH adjusted to 7.2 (±0.2) and the suspension warmed to 80°C with stirring. After cooling to 25°C the solution was centrifuged at 0°C for 20 min at 9000*g*. The supernatant was brought to room temperature and extracted twice with two volumes of $CHCl_3$ and the solvent then removed by rotary evaporation. The residue was usually taken up in dimethyl sulphoxide (DMSO) since such solutions could be used directly for biological assay. Upon standing at room temperature the DMSO solutions often gave rise to crystals. The amounts and rapidity of crystal formation could be increased by diluting the DMSO solutions to 70% with water, heating the resultant turbid solutions to effect clarity and allowing it to cool slowly. The most usual shaped crystals first forming were sheaths of needles. They were removed by centrifugation, dissolved in DMSO and recrystallized by dilution to 70% aqueous DMSO.

The $CHCl_3$ soluble extract residues or crystals forming in DMSO could also be crystallized by dissolving them in MeOH or ethanol (EtoH) and then diluting the solution with water until turbidity appeared. A variety of different shaped crystals formed in various experiments. This appeared to depend on the rapidity of crystallization, the concentration of the material being crystallized, the concentration of DMSO, MeOH or EtOH, etc. The crystals formed in aqueous solutions of DMSO were usually more uniform and of better yield than those from MeOH and EtoH. The recrystallization procedures could be repeated as often as desired. From 20 to 25 mg of aspidospermine, crystallized three times from DMSO, could be isolated from 3 g of the "Quebracho". Spectral, LC and gas chromatography–mass spectrophotometric (GC–MS) analyses of the crystals first forming in 70% DMSO established that the major portion of it was aspidospermine.

The supernatant to the crystals first formed in 70% DMSO was diluted with water to 40% DMSO. Upon standing overnight at 4°C or at room temperature for several days, considerable amounts of irregular rectangular plate crystalline material formed. The amounts of these crystals increased when such solutions were held for several days at 4°C. This material as in the case of aspidospermine could be readily recrystallized by dissolving it in DMSO, MeOH or EtOH and diluting such solutions until turbidity appeared. Mass spectrometric, spectral and LC analyses of these crystals indicated that they were quebrachamine. From 10 to 15 mg of three times crystallized quebrachmine could be isolated from 3 g of the "Quebracho". DMSO was employed as the solvent for assays of biological activity and MeOH for various physical–chemical evaluations.

Spectrophotometry

A Cary Model 118 apparatus was employed. Samples were dissolved in MeOH, and the ultraviolet (UV) extinctions given for aspidospermine and quebrachamine [1] were used to calculate concentrations following their identification.

Chromatography

Following multiple crystallizations, the two isolates were subjected to LC. The apparatus employed included a Waters UK-6 injector, a Gilson Medical Electronics Model 116 flow monitor spectrophotometer at 254 nm, and a Houston Instruments Model B5217-1A1 Omniscribe recorder. A 0.45 × 25 cm Dupont Zorbax Cyanopropyl adsorption column was used in a reversed-phase-ion pair mode. The isocratic mobile phase was 70% 0.5 M acetic acid, 30% acetonitrile and 0.01% *t*-butylamine. The flow rate was 2.5 ml min^{-1}. Assay samples in 1–25 μl of MeOH were utilized.

GC–MS

Aliquots of crystallized isolates and components resolved by LC experiments were subjected to GC–MS analyses. A J & W Scientific 0.18 × 10 mm DB-17 GC-capillary column of fused silica bonded with 50% phenyl-50% methyl silicone in line with a Hewlett–Packard Model 5890-59872E/F GC–MS data system was employed. The GC separation was initiated at 50°C with a linearly programmed temperature increase of 20°C min^{-1} up to 200°C. The jet separation entrance to the MS was maintained at 250°C.

Biological assays

The effects of various fractions of the "Quebracho" powder employed and of purified components were measured for their adrenergic blocking activities as previously described [2] using human prostatic tissue

strips obtained from transurethral resections or by use of rabbit corpus spongiosum or cavernosum or guinea pig vas deferens. Some of the fractions assayed were taken up in pH 7.4 phosphate buffered saline (PBS) or in Krebs–Ringer buffer, but components with low solubilities in these solutions were dissolved in DMSO. The *in vitro* assay employed is designed to determine the agonist or antagonistic activity of materials on either the electrically or adrenergic induced contraction of the tissue employed. It is presumed that the inhibition of contraction represents blocking of adrenergic receptors.

Results

Spectrophotometry

The UV spectra of aspidospermine and quebrachamine in the 240–340 nm range are presented in Fig. 1 and are in keeping with those reported in the literature [1, 3]. Even though the structures of these two alkaloids are quite similar, it is apparent that their absorption spectra differ markedly. Aspidospermine has its λ_{max} (MeOH) at 255 nm with a minor one at about 285 nm while quebrachamine has closely related λ_{max} (MeOH) at 283 and 291 nm. The extinction coefficients given for these two compounds [1] were employed to determine their concentrations when needed.

Chromatography

The LC profiles for crystallized aspidospermine and quebrachamine are shown in Figs 2(A) and (B), respectively. Small amounts of materials eluting earlier than the main component are seen in the case of quebrachamine. This may relate to the slow generation of such components since ageing of quebrachamine in MeOH at room temperature leads to the formation of such material. The LC result for a solution of crystallized quebrachamine aged for about 4 weeks in MeOH at room temperature is shown in Fig. 2(C). No changes in the LC properties of quebrachamine or of aspidospermine occurred when they were stored in DMSO.

Since the alkaloid yohimbine which is structurally related to the above compounds is also stated to be present in "Quebracho", it was of interest to determine its elution properties in the LC system employed. It can be seen from the chromatogram presented as Fig. 2(D) that yohimbine (Sigma) resolves completely from

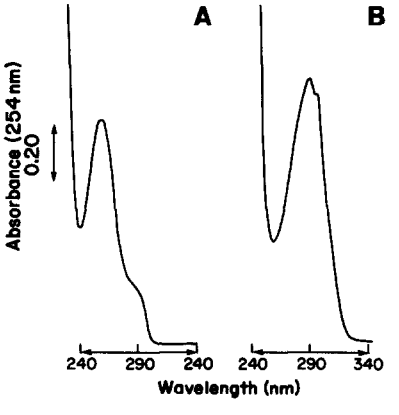

Figure 1
The ultraviolet absorption spectra in methanol of: (A) aspidospermine; (B) quebrachamine.

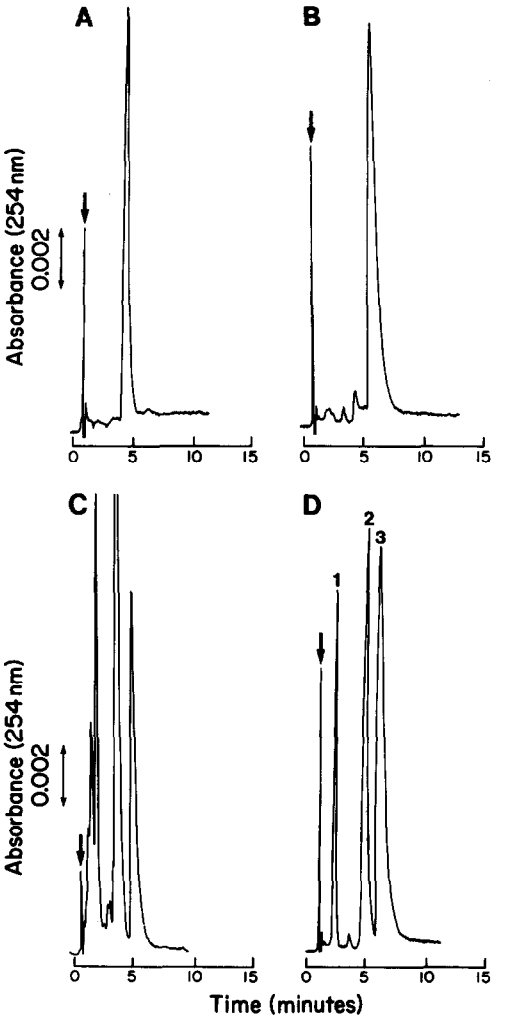

Figure 2
The results for the LC analyses of: (A) crystallized aspidospermine; (B) crystallized quebrachamine; (C) quebrachamine aged in methanol; (D) yohimbine (1), aspidospermine (2) and quebrachamine (3) combined.

aspidospermine and quebrachamine when chromatographed in a mixture with them. This result also indicates that no significant amounts of yohimbine were present in our aspidospermine and quebrachamine isolates.

GC–MS

The initial analysis was carried out on material crystallized once from DMSO. The result, given in Fig. 3, indicates that it was composed primarily of a mixture of aspidospermine and quebrachamine. The GC-elution profile shown in Fig. 3(A) indicates that these compounds eluted at 16.1 and 18.2 min and comprised 41% and 47% of the total, respectively. The small amounts of other components seen in Fig. 3(A) were identified by mass spectrometry as follows: The one eluting at 13.6 min which comprised 3% of the total was 1,2-didehydro-aspidospermidine, the one at 15.7 min comprised 8% and was 17-methoxy-aspidospermidine, the one at 19.1 min comprised only 1% and was 16-methoxy,17-hydroxy-1-(1-oxopropyl)-aspidospermidin.

The mass spectrograms of quebrachamine and aspidospermine are shown in Fig. 3(B) and (C), respectively. GC–MS analyses of recrystallized material forming in 70 and 40% DMSO as well as of analogous fractions recrystallized from MeOH solutions were found to be composed of only aspidospermine and quebrachamine, respectively. These latter homogeneous preparations were used for the biological assays.

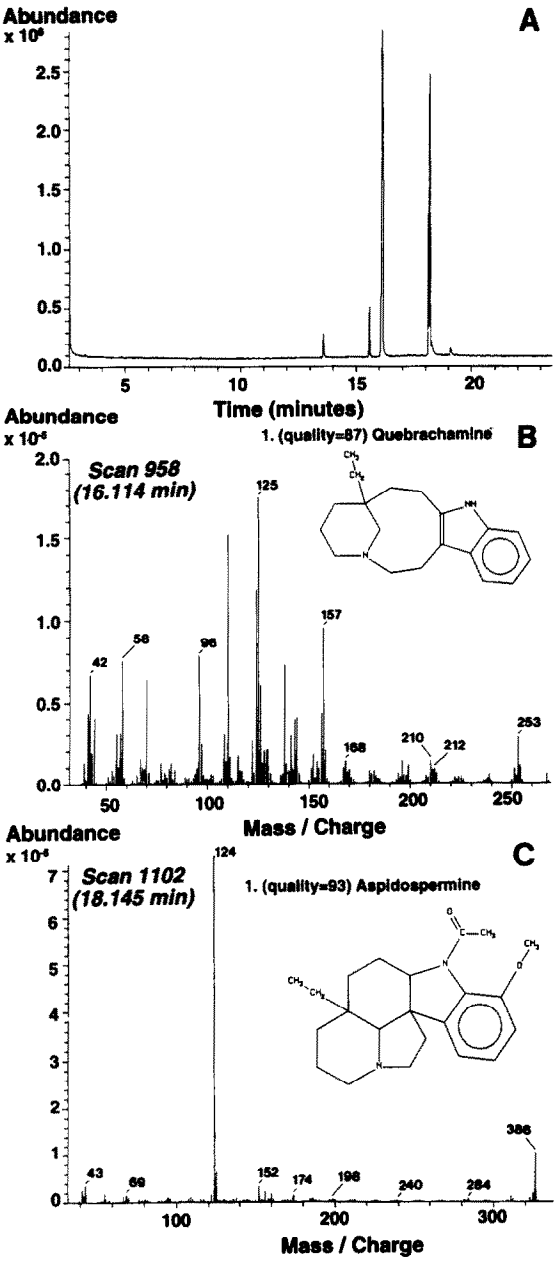

Figure 3
Results of gas chromatography–mass spectrometry analyses: (A) GC-profile of the first crystals formed in 70% DMSO (B) the MS-result for quebrachamine (C) the MS-result for aspidospermine.

Biological activity

Solutions of both aspidospermine and quebrachamine in DMSO were found to block the electrically or phenylephrine induced contractions of human prostatic tissue, rabbit corpus spongiosum and cavernosum and guinea pig vas deferens. An example of the effects of these compounds of the structurally related yohimbine and of the "Quebracho" powder extract on the inhibition of the phenylephrine induced contraction of rabbit corpus spongiosum is presented in Fig. 4. It can be seen that aspidospermine has about the same activity of quebrachamine in this assay. Yohimbine is a much stronger inhibitor and has from 50 to 100 times the potency of the

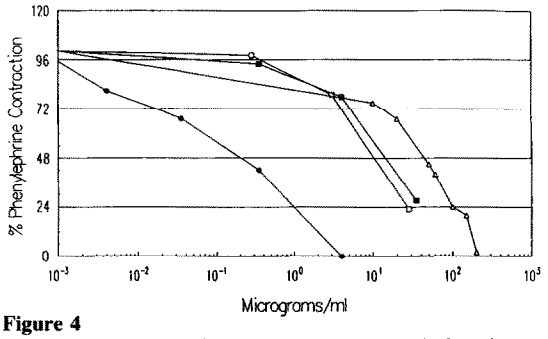

Figure 4
Results for the inhibition of phenylephrine induced contractions of rabbit corpus spongiosum by yohimbine (–●–), quebrachamine (–□–), aspidospermine (–■–) and "Quebrachamine extract" (–△–).

latter two compounds. The α-adrenergic blocking activity of "Quebracho" extract is likely related to the presence of aspidospermine, quebrachamine and yohimbine as well as other such inhibitors. For this reason, quantitative assay results for the starting material cannot be interpreted in terms of its content of α-adrenergic blocking materials and of the relative recoveries of aspidospermine and quebrachamine.

Discussion

Our experiments have shown that various plant extracts in common use as agents for alleviation of symptomatic BPH may contain components that have adrenergic blocking activities. The one employed in the present investigations is called "Quebracho" in keeping with it being an extract of the bark of the Aspidosperma quebracho-blanco Schlechtend tree. Such extracts were formerly employed for the relief of muscle spasm, as an anti-pyretic agent and as a respiratory stimulant. At present, its main use appears to be for the preparation of an aphrodisiac known as Afrodor 2000 (Farco–Pharma GmbH, Cologne, Germany).

Following our isolation and identification of aspidospermine and quebrachamine from extracts of "Quebracho" and finding that they contained adrenergic blocking activity, we became aware of the classical literature relating to these indolalkaloids. Aspidospermine was first isolated over a century ago by Fraude [4, 5] and by Hesse [6]. Later isolations and more detailed descriptions of the properties of this compound were reported by Hartmann and Schlittler [7], Deylofeu *et al.* [8], Witkop [3] and Holker *et al.* [9]. Quebrachamine was first isolated by Hesse [6] and later by Field [10] from the same source material. It was also prepared from the bark of *Aspidosperma chakensis* by Orazi *et al.* [11] and from Gonioma kamassi by Schlittler and Gellert [12]. The latter workers named their isolates kamassin which was later shown by Gellert and Witkop [13] to be identical to quebrachamine.

Previous isolations of aspidospermine and quebrachamine employed relatively large amounts of the starting plant materials and utilized rather large and tedious fractionation conditions. Our method which has usually started with 1–3 g of the bark extract permits isolation of crystalline material within 1 day.

Aspidospermine and quebrachamine bear strong structural relationships to yohimbine, a known adrenergic blocking agent. Since the latter compound has also been found in "Quebracho" type extracts, it was of interest to compare its LC properties with those of aspidospermine and quebrachamine. The result shown in Fig. 2(D) indicates that it is well resolved from these compounds. The LC results also indicate that yohimbine was not a component of our preparations of aspidospermine and quebrachamine, and that the adrenergic blocking activity of the latter two compounds was an inherent biological property. The UV spectrum of yohimbine is so closely related to quebrachamine that detection of its presence in the latter compound by this method would not be possible.

The results of our investigations present a rapid method for the relatively easy isolation of two previously known compounds and also indicate that they possess pharmacological activities which may explain the alleged beneficial effect of the compounds in the treatment of symptoms of BPH. We also developed an LC method that quickly and quantitatively identifies these very similar indolalkaloids found in natural products.

Acknowledgements — The "Quebracho" material utilized in these studies was supplied by Farco-Pharma GmbH of Cologne, Germany. This work was partly supported by the Veterans Administration Research Service.

References

[1] *The Merck Index*, 11th Ed. Merck & Co., Inc., Rahway, NJ, USA (1989).
[2] P.O. Madsen, H.F. Deutsch, J. Aagaard and P. Drescher, in *Urology* (L. Guliani and P. Puppo, Eds), pp. 205–208. Monduzzi, S.P.A., Bologna, Italy (1992).
[3] B. Witkop, *Am. Chem. Soc.* **70**, 3712–3716 (1948).
[4] G. Fraude, *Ber.* **11**, 2189–2191 (1887).
[5] G. Fraude, *Ber.* **12**, 1560–1563 (1879).
[6] O. Hesse, *Ann.* **211**, 249–284 (1882).
[7] M. Hartmann and E. Schlittler, *Helv. Chem. Acta* **22**, 547–549 (1939).
[8] V. Deylofeu, J. De Langhe, R. Labriola and V. Carcamo, *J. Chem. Soc.* 1051–1052 (1940).
[9] J.S.E. Holker, M. Cais, E.A. Hochstein and C. Djerassi, *J. Org. Chem.* **24**, 314–316 (1959).
[10] E.J. Field, *J. Chem. Soc.* **125**, 1444–1450 (1924).
[11] O.O. Orazi, R.A. Corral, C.S.E. Holker and C. Djerassi, *J. Org. Chem.* **21**, 979–984 (1956).
[12] E. Schlittler and E. Gellert, *Helv. Chim. Acta* **34**, 920–923 (1952).
[13] E. Gellert and B. Witkop, *Helv. Chem. Acta* **35**, 114–115 (1952).

[*Received for review 2 December 1993; revised manuscript received 16 May 1994*]