

Secondly, since diazepam is a well-known sedative and trihexyphenidyl is an antiparkinsonism agent, more than one anti-epileptic mechanism may be involved. Other techniques are needed for the further investigation of this topic.

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Note added at proof

(1) Cannabidiol has recently been demonstrated to possess anti-epileptic properties in man (Mechoulam, R. & Carlini, E. A. (1978). *Naturwissenschaften* **65**, 174-179).

(2) The structure of the antiepileptic diphenylsilanediol has recently been determined (Fawcett, J. K., Camerman, N. & Camerman, A. (1977). *Can. J. Chem.*, **55**, 3631-3635). The molecule possesses two hydrophobic rings (Ph) and two electron donor groups (-OH), and a space-filling model of it closely resembles diphenylhydantoin. The authors therefore state that this supports their postulate that the two rings and two electron donor groups are necessary for anticonvulsant activity. The fixed O...O separation of 2.66 Å is,

however, the smallest donor-donor distance so far observed for an anti-epileptic; the ring to donor atom distances (all close to 4.1 Å) also represents one new limit (see Tables 1 & 2). This lends further support to our view that the wide range of observed values must lead to questioning of the stereochemical postulates for antiepileptic activity.

(3) The structure of the antiepileptic oxazepam (Fig. 1, iii; R₁ = H, R₂ = OH, X = Cl, Y = H) has recently been determined (Gilli, G., Bertolasi, V., Sacerdoti, M. & Borea, P. A. (1978). *Acta Cryst.* **B34**, 2826-2829). The authors state that "no correlation between molecular geometry and activity can be established within this class of drugs".

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Rauwolfia schuели as a potential source of ajmaline

V. S. MARTINO, A. L. BANDONI, O. HNATYSZYN, R. V. D. RONDINA, J. D. COUSSIO*, *Departamento de Bioquímica Vegetal, Cátedra de Farmacognosia, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 Buenos Aires, Argentina*

Ajmaline, first isolated by Siddiqui & Siddiqui (1931), is an alkaloid having antiarrhythmic activity, which has been reported to be present in a high yield in several African species of *Rauwolfia*, *R. mombasiana*, *R. vomitoria*, *R. caffra*, etc. (Court, Evans & Trease, 1958; Madati, Kayani & others, 1977).

Rauwolfia schuели Sp. (= *Rauwolfia boliviana* Mg.) (*Apocynaceae*), is one of the species growing in Argentina together with *R. sellowii* and *R. mollis* (Xifreda, 1975). It is a small tree, with folk medicinal properties (Schulz, 1976), common name 'lecherón del monte' or 'lecherón negro', widespread in the northwestern region of Argentina (Provinces of Tucumán, Salta and Jujuy) and in the Andine regions of Bolivia (local name 'lecherón amarillo' or 'tinajero') in open woods and sandy hills.

In a previous work Iacobucci & Deulofeu (1958) reported its root bark to contain the alkaloids aricine, reserpiline, isoreserpiline, reserpine and ajmaline.

In the present paper we report the ajmaline content of root and stem samples of *R. schuели* collected in Argentina and discuss several extraction methods.

Samples of plant material were collected at Departamento de General Güemes, Province of Salta, between September and November 1976. Root barks, roots without bark and stem bases of different diameters and height were separately analysed. Each sample was dried at 60° to constant weight and milled to a fine powder.

Several extraction procedures were tested, using quantitative operations.

Extraction procedure A. The sample (10.0 g) was macerated overnight with 100 ml methanol and refluxed for 3 h. The extract was filtered. The operation was re-

* Correspondence.

peated twice. The extracts were mixed, concentrated in a vacuum and taken to 100 ml.

Extraction procedure B. The sample (10.0 g) was moistened with 13 ml of 5% aqueous Na_2CO_3 and extracted by maceration with 50 ml acetone over 12 h. The extraction was repeated five times, each time after 3 h maceration. Adjustment of pH to 8–9 was made if necessary. The extractives were filtered, and treated as in A.

Extraction procedure C. The sample was treated as in B, but using methanol, and re-extracted three times.

Extraction procedure D. The sample as in B was refluxed with 50 ml of methanol for 3 h. The operation was repeated three times and treated as in A.

Quantitative determination of ajmaline. A sample of each of the extracts was applied to Silica gel plates (20 × 20 cm; 0.5 mm) Merck HF254 and developed with methanol–methyl ethyl ketone–n-heptane–concentrated ammonia (10:30:50:1). Solutions were spotted in 2.5 cm bands. The plates were developed to 15 cm, dried at 110° for 1 h and viewed under 254 nm light. The bands were scraped off the plate and eluted with 5 ml of 3% concentrated hydrochloric acid in methanol. The absorbances of the bands were immediately determined at 291 nm. The absorptivity of ajmaline was previously calculated from standard solutions made by diluting a 0.2% ajmaline methanolic solution with 3% acid methanol to final concentrations between 0 and 8.0 mg/100 ml over which range the absorbance/concentration relation was linear. As the recovery of ajmaline after t.l.c. was close to 100%, the absorptivity value was used to calculate the concentration of ajmaline in the final solution by using the mean of the absorbance of the bands. The ajmaline content of each sample was calculated from the knowledge that the volume of the extract spotted corresponded to 10 mg of dried material. The results in g of ajmaline/100 g dried material, using extraction A, were; root bark 3.1, root without bark 0.34; stems 5 cm diam. first 20 cm above ground 1.4,

remaining 50 cm 1.8 and stems 3 cm diam. first 20 cm above ground 2.1 (n = 3).

Isolation of ajmaline. Each of the extraction procedures was applied to a 50 g sample of dried plant material. The extractives were filtered and concentrated to 50 ml. A 5% aqueous phosphoric acid solution was added and the whole heated to 35–40° until the syrup residue was totally dissolved. The resulting aqueous acid solution was washed with chloroform (3 × 20 ml) and the chloroform solution discarded. The aqueous phase was taken to pH 8–9 by addition of concentrated ammonia and extracted with chloroform (6 × 30 ml). The chloroform extract was dehydrated with anhydrous sodium sulphate and taken to dryness under vacuum at 35–40°. The residue was dissolved in a small quantity of hot methanol and left at 2° for 24–48 h. Ajmaline crystallized from this fraction. The identity of the product was determined by t.l.c. and m.p. (Pharmacopoeia of Japan, 1971), ultraviolet and infrared by comparison with an authentic sample. The mother liquors were submitted to column chromatography on alumina grade II–III (Merck standardized) to recover part of the remaining ajmaline. The alkaloid crystallized from the fractions eluted with chloroform–methanol. The yield of ajmaline from each extract was calculated by weighing the crystallized ajmaline. The results of the extractions in g ajmaline/100 g dried material (n = 3) were: Extraction A 3.1, Extraction B 4.8, Extraction C 4.7 and Extraction D 3.5. Thus methods B and C gave much higher recoveries than A and D.

According to the ajmaline yields of other *Rauwolfia* species (Ghosh, 1958; Court & others, 1958; Los & Court, 1969; Habib & Court, 1971, 1974; Majumdar, Poisson & Poitier, 1973; Madati & others, 1977) *R. schueli* is a species yielding one of the highest amounts of this alkaloid.

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