Journal of Chromatography A, 662 (1993) 251–254
Elsevier Science B.V., Amsterdam

CHROM. 25 551

Determination of ajmaline stereoisomers by combined high-performance liquid and thin-layer chromatography

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(First received May 6th, 1993; revised manuscript received September 8th, 1993)

ABSTRACT

A procedure for the determination of ajmaline and its stereoisomers using a combined TLC–HPLC approach is described. TLC permitted the discrimination of ajmaline, isoajmaline and sandwicine or isosandwicine, whereas HPLC separated every pair of the alkaloids except for the normal series of bases (ajmaline–sandwicine). The analysis of several semipurified alkaloidal mixtures obtained from various *Rauwolfia* species is discussed.

INTRODUCTION

Ajmaline (**1**) is an indolic alkaloid first isolated from *Rauwolfia serpentina* Benth [1], that is currently used, together with several semi-synthetically derived drugs, for the treatment of cardiac arrhythmias [2]. This importance prompted several studies on the separation and determination of ajmaline in *Rauwolfia* extracts and official drugs containing this alkaloid, either by TLC [3–6] or HPLC techniques [7–13]. To our knowledge, none of these studies involved the determination of ajmaline in the presence of its stereoisomers, namely, isoajmaline (**2**), sandwicine (**3**) and isosandwicine (**4**) (Fig. 1), in spite of their co-occurrence in the roots of several *Rauwolfia* species.

In the course of our studies on *Rauwolfia*, looking for commercially and therapeutically important alkaloids, we have found that the above techniques were unsuccessful in resolving satisfactorily ajmaline and its stereosisomers. Some of them gave poorly reproducible experimental results. This paper describes our results on the application of TLC and HPLC

	R	R_1	R_2	R_3	R_4	R_5
[1] ajmaline	H	OH	OH	H	H	Et
[2] isoajmaline	H	OH	H	OH	Et	H
[3] sandwicine	OH	H	OH	H	H	Et
[4] isosandwicine	OH	H	H	OH	Et	H

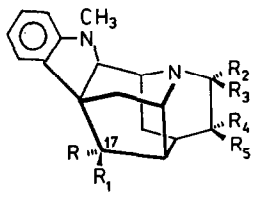

Fig. 1. Ajmaline stereoisomers.

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0021-9673/94/$07.00
SSDI 0021-9673(93)E0974-Y

techniques for the separation and determination of the above alkaloids present in artificial and natural mixtures.

EXPERIMENTAL

Chemicals

Ajmaline was purchased from INFA (Milan, Italy). The stereoisomers **2–4** were prepared semi-synthetically using published procedures [14]. The products obtained were unequivocally characterized by physico-chemical and spectroscopic analysis.

Semi-purified alkaloidal mixtures, obtained from several available *Rauwolfia* species by previously reported procedures [15], were included in the analysis.

The products were dissolved in analytical-reagent grade methanol (Merck, Darmstadt, Germany) at concentrations of *ca.* 1 mg/ml. In each instance 25 μg of the samples were applied.

Chromatography

TLC was performed on thin-layer plates of silica gel G (Merck, Type 60, layer thickness 0.25 mm) previously activated at 110°C for 1 h. Among the solvent systems tried, the best separation was recorded with acetone–light petroleum (b.p. 40–60°C)–diethylamine (2:7:1). The chromogenic reagent used was a 1% solution of ammonium cerium(IV) sulphate in concentrated orthophosphoric acid [16].

HPLC was carried out on a Knauer (Berlin, Germany) system consisting of a variable-wavelength monitor, a high-pressure mixing chamber, two reciprocating pumps, a helium degasser and a cartridge column. For data processing a Facit S212-10 computer with specialized software was used. A Rheodyne Model 7125 valve with a 20-μl loop was employed for the introduction of the samples. The column (100 × 4 mm I.D.) was packed with Nucleosil SA (Knauer) of 5-μm particle size. The mobile phase was 0.1 mol/l dibasic ammonium phosphate (pH 7)–acetonitrile–2-propanol (80:20:7) at a flow-rate of 1 ml/min. Samples were injected in the range 0.2–5.0 μg. The detection wavelength was 290 nm.

RESULTS AND DISCUSSION

Under the above TLC experimental conditions we achieved the resolution of three of the four ajmaline stereoisomers (Fig. 2). However, none of the experiments developed, using a wide range of solvent mixtures, was successful in the separation of sandwicine and isosandwicine.

In the course of TLC analysis we observed that the C-17 (*S*) stereoisomers, **3** and **4**, yielded a more stable reddish pink colour with the chromogenic reagent employed for the detection of the alkaloidal spots. Instead, ajmaline and isoajmaline [the C-17 (*R*) series of stereisomers] showed a faint pink colour; the difference was useful in the analysis of alkaloidal mixtures.

As shown in Fig. 2, ajmaline was the only component detected in the root extracts of *Rauwolfia viridis* Roem. et Schult., ajmaline and sandwicine or isosandwicine being encountered in the root of the other species (*R. cubana* A. DC., *R. salicifolia* Griseb. and *R. linearifolia* Britt. et Wilson). None of these taxa gave spots corresponding to the isoajmaline reference sample by this method (see below, however).

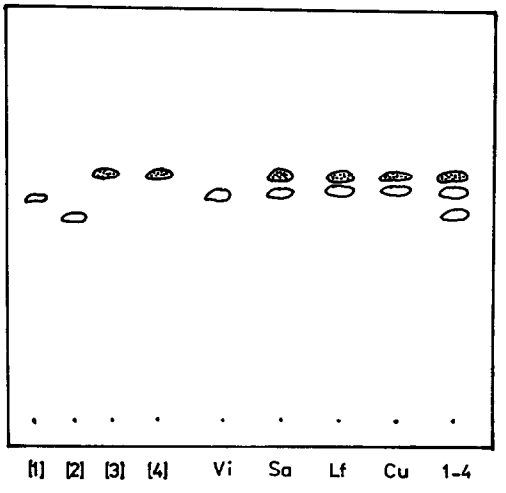

Fig. 2. TLC of ajmaline stereoisomers and several *Rauwolfia* root extracts. [1]–[4] = ajmaline stereoisomers **1–4**; Vi = *Rauwolfia viridis*; Sa = *R. salicifolia*; Lf = *R. linearifolia*; Cu = *R. cubana*. In each instance 25 μg of the samples were applied. Dotted spots indicate reddish pink colour with the chromogenic reagent employed; open spots indicate a faint pink colour with the same reagent (see text).

In the HPLC experiments, we did not obtain good results when employing the conditions reported by Flanagan *et al.* [11], who used non-aqueous developing systems with silica gel columns in separations of a wide variety of basic compounds, including ajmaline. In the course of the experiment, we found it difficult to stabilize the equipment under Flanagan *et al.*'s conditions, probably owing to the large amount of methanol present in the mobile phase and to difficulties usually encountered in conditioning silica gel columns [17]. We also tried several modifications of the conditions employed by Flanagan *et al.* [11], modifying the components (methanol–*n*-hexane, methanol–ethyl acetate and methanol–acetic acid) and the concentration of methanol in the mobile phase (17:3 to 7:3) and the length of the column (125 and 250 mm), but none of these approaches achieved the desired results in terms of resolution and reproducibility.

Jane *et al.* [12] reported analogous difficulties with the use of similar conditions of Flanagan *et al.*'s, owing to the asymmetry of the peaks and long retention times on silica gel columns.

It was therefore decided to follow the recomendations of Rogers [13], who reported good results for ajmaline resolution in *Rauwolfia* alkaloidal mixtures using long ion-exchange columns (1000 × 2.6 mm I.D.) with phosphate buffer–methanol as the mobile phase. After several adaptations of the experimental conditions in terms of pH, column dimensions, ionic strength and methanol content of the mobile phase, as described previously, we were able to obtain the results shown in Fig. 3. Thus, with the exception of the ajmaline–sandwicine pair, which always co-eluted, it is possible to separate and determine the stereoisomers.

Combined analysis using both TLC and HPLC allows better information to be obtained regarding the composition of these alkaloidal mixtures. For example, although in *R. viridis* crude root alkaloid mixture the HPLC results do not permit a distinction between ajmaline and sandwicine, TLC convincingly demonstrates the absence of **3**. For *R. linearifolia*, TLC results established the existence of ajmaline and one or both C-17 (*S*) stereoisomers (**3** or **4**); HPLC revealed the three components and, in trace amounts, the presence of isoajmaline (Fig. 3).

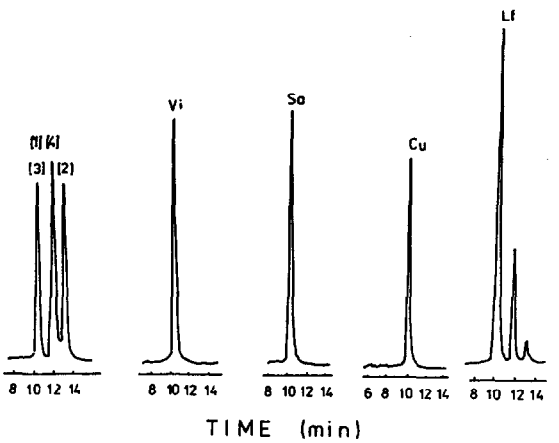

Fig. 3. HPLC of ajmaline stereoisomers and several *Rauwolfia* root extracts. Amount injected: **1–4**, 2 μg; *Rauwolfia* extracts, 5 μg. numbers and abbreviations as in Fig. 2.

In conclusion, in spite of the above-described limitations of each method for the separation of all natural ajmaline stereoisomers, the combination of the two techniques permits us obtain more information about the composition of alkaloidal mixtures containing these bases. Work is in progress on the application of HPLC for the complete resolution of ajmaline stereoisomers.

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