Acta Farm. Bonaerense **23** (4): 503-5 (2004)
Recibido el 07 de enero de 2004
Aceptado el 07 de junio de 2004

Optimization in the Separation of Bioactive Compounds from *Mandevilla illustris* Vel. Woodson (Apocynaceae)

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SUMMARY. This work presents a comparative study of the efficacy of different chromatographic supports in the isolation of the illustrol, a bioactive nor-pregnane compound obtained from an ethyl acetate extract of *Mandevilla illustris* rhizomes. When chromatographed over magnesium oxides, the speed, yield and purity grade, for this compound, was higher than for a traditional support such as silica gel 60.

RESUMEN. "Optimización en la Separación de Compuestos Bioactivos de la *Mandevilla illustris* Vel. Woodson (Apocynaceae)". Este trabajo presenta un estudio comparativo de la eficacia de diferentes adsorbentes cromatográficos en el aislamiento del illustrol, un compuesto bioactivo con estructura de tipo nor-pregnano, a partir del extracto obtenido con acetato de etilo de rizomas de *Mandevilla illustris.* La cromatografía en óxidos de alúmina o magnesio permitió demostrar que la velocidad, rendimiento y el grado del pureza para el componente de interés resultaron ser mayores que para un soporte tradicional como sílica gel 60.

INTRODUCTION

One of the main problems in pharmaceutical research searching for new plants as a source of New Chemical Entities (NCE), is the availability of rapid isolation strategies, particularly for those NCE present in slow amounts and with poor resolution on separation [1].

Mandevilla illustris (Apocynaceae), popularly known as "flor-de-barbado" or "rosa- do-campo", is a native Brazilian plant used in folk medicine, in the treatment of different diseases, including inflammation and snake bites [2,3]. Previous studies from our research group have shown that both the hydroalcoholic extract and different compounds obtained of the ethyl acetate extract of this plants antagonize, in a quite selective manner, bradykinin-induced responses when assessed in different *in vivo* and *in vitro* pharmacological models in several animal species [4-6]. Recently, we have reported the isolation of two new derivatives of illustrol, whose structures were defined by spectroscopic data and X-ray diffraction [7,8]. Since their molecular structures are very similar, chromatographic separation using silica gel as the stationary phase proved to be very laborious, resulting in small amounts of each compound, and subsequently giving a poor yield. These observations led us to determine other possible supports which could be used as alternative methods.

The aim of the present investigation was to study the performance of aluminum and magnesium oxides as stationary phases in the separation by chromatographic column of the nor-pregnan compounds present in the chloroform extract of *M. illustris.*

MATERIAL AND METHODS

Plant material

Rhizomes of *M. illustris* (Vell.) Woodson were collected from flowering plants in Coro-

KEY WORDS: Chromatographic supports, Illustrol, *Mandevilla illustris.*
PALABRAS CLAVE: Illustrol, *Mandevilla illustris*, Soportes cromatográficos.

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mandel, State of Minas Gerais, Brasil, in January 1996. The identification was carried out by Dr. Ademir Reis (Department of Botany, Federal University of Santa Catarina). A voucher specimen was deposited at Herbarium FLOR (UFSC) under number 17871.

Extraction and separation

The fresh rhizomes (10 kg) were reduced to moderately coarse powder and macerated with ethyl acetate 99% for ten days at room temperature. After solvent evaporation at reduced pressure until dry, the yield was 72 g of a brown syrup. An aliquot (40 g) of this extract was then filtered using a glass column on silica gel (0.063 - 0.20 mm, 400 g) using hexane, chloroform and acetone (3 L each) as eluent. Removal of solvents under vacuum gave 0.9 g, 14.0 g and 7.5 g of each fraction, respectively.

Aliquots of the chloroform fraction were used for comparative studies of the adsorption. 300 mg were chromatographied on a chromatographic column (CC) (2.0 x 30 cm) using 3.5 g of different adsorbents such as: silica gel, Aluminum oxide(neutral Alumina) and Magnesium oxide, and eluted with hexane/acetone gradient. Fractions of 5 mL were collected and then monitored by thin layer chromatography (TLC) using the same solvent system. The fractions, showing similar Rf, which showed positive reaction with sulfuric acid-anisaldehyde reactive (1 mL p-anisaldehyde and 1 mL H_2SO_2 in 18 mL ethanol) were combined and illustrol was identified by comparison of its Rf on TLC with authentic samples previously isolated.

RESULTS AND DISCUSSION

To evaluate the separation performance of the different stationary phases, the experimental conditions, such as weight of the bed and diameter of the column, elution flow-rate and polarity of the mobile phase were maintained constant throughout the chromatographic experiment. The illustrol (Figure 1), was eluted in all the experiments with hexane-acetone (95:5) as mobile phase. The compound was one-step purified and the results of the chromatographic separation and yields are compared in Table 1.

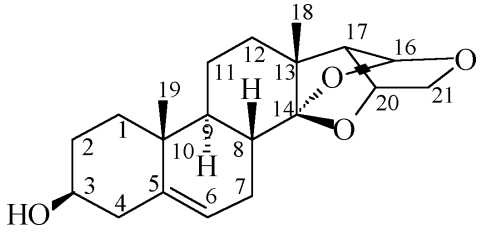

Figure 1. Molecular structure of Illustrol.

support	mg [a]	%
Sílica gel	7.5 ± 0.3	1.9
Alumina	25.0 ± 1.8	6.2
Magnesium oxide	25.0 ± 0.9	6.2

Table 1. Efficiency of different supports studied through the separation of illustrol from *M. Illustris* (350 mg) chloroform extract. a Means and standard deviation for three determinations.

When silica gel was used as stationary phase, an increase in the polarity of the eluent caused the co-elution of a resinous substance with the illustrol, so reducing the amount of pure compound. On the other hand, when aluminum and magnesium oxides were used as stationary phases, the resinous substance was retained in the top of the column. The difference in the resolution and yield of the illustrol can be attributed to the different active centers on each stationary phase. In the silica gel, the main active centers are the free silanol groups (Si-OH). While Alumina presents several active centers, such as acidic centers (OH groups) and Al^{3+} ions (Lewis acids) basic centers (Al-O-Al groups, O^{2-}). Alumina is prepared from aluminum hydroxide by calcination at moderate temperatures followed by washing, could form three types of alumina: acidic, basic and neutral. The amount of water greatly affects the chromatographic behavior of alumina. For control, the solid may be heated at specific temperatures before uses. The magnesium oxide present Mg^{2+} ions (electron acceptors) as active centers and strongly basic center O^{-2} ions [10,11]. Thus, the order of polarity of the stationary phases is as follows: Alumina > Magnesium oxide > silica-gel.

CONCLUSIONS

Our results showed that Magnesium and Aluminum oxides are more efficient in the separation of the illustrol than silica gel. These stationary phases are being tested in the separation of other compounds present in *M. illustris* and *Mandevilla vellutina* [12].

Acknowledgments. The authors are grateful to Prof. Dr. A. Reis for the botanical identification and to CNPq, CAPES, and UNIVALI/Brazil for its financial support.

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