

Separation of C-glycoside Flavonoids from *Aleurites moluccana* Using Chitin and Full N-acetylated Chitin

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Z. Naturforsch **57c**, 957–959 (2002);
received April 24/July 10, 2002

Chitin, Swertisin and 2"-O-rhamnosylswertisin

This paper describes a comparative study using different chromatographic supports (fully N-acetylated chitin, chitin and silica gel) to separate the flavonoids swertisin and 2"-O-rhamnosylswertisin from *Aleurites moluccana*. The results show that the flavonoids have apparently been separated by the hydrogen bond between the stationary phase (chitin and chitin-100) and flavonoids under the conditions studied.

Introduction

Aleurites moluccana L (Willd) (Euphorbiaceae), known in Brazil as "Nogueira-da-Índia", is frequently used in folk medicine to treat fever, headaches, tumors, diarrhea and asthma (Duke, 1991; Meyre-Silva *et al.*, 1997; Meyre-Silva *et al.*, 1998).

Chitin, which is perhaps the second most important natural polysaccharide, is a straight copolymer composed of β (1,4)-linked GlcNAc units (85%) and β (1,4)-2-amino-2-deoxy-D-glucose units (15%) with a three-dimensional α -helical configuration stabilized by intramolecular hydrogen bonding. Chitin has a chemical structure similar to that of cellulose, because of the great variety of the functional groups present (-OH, -NH₂ and -NHCOCH₃), (Rózylo *et al.*, 1988). Chitin is widely used as chromatographic support for the separation of flavonoids, biflavonoids, amino acids, nucleic acids, phenols saccharides (Rodrigues *et al.*, 2000; Nahlik *et al.*, 1985; Rodrigues *et al.*, 1998; Rózylo *et al.*, 1989). On the other hand, phenolic compounds can be kept in chitin column due to the strong interaction between

-NH₂ free (15%) and OH phenolic (Rodrigues *et al.*, 2000).

This paper describes the preparation of fully N-acetylated chitin (chitin-100), in order to assess the influence of the remaining -NH₂ groups on the yield of the flavonoid compound separation. The new sorbent was applied to chromatographic separation of flavonoids swertisin (**1**) and 2"-O-rhamnosylswertisin (**2**) from *A. moluccana* extract.

Material and Methods

Plant material

Leaves of *A. moluccana* were collected in Itajaí, in the state of Santa Catarina, Brazil, in February 1998, and identified by Dr. Ademir Reis. A voucher specimen was deposited at Barbosa Rodrigues Herbarium (Itajaí) under number VC Filho 001.

The methanolic extract was obtained after maceration with methanol (5 l) at room temperature for 10 days (812 g dried leaves). The extract was concentrated and then successively partitioned with hexane, dichloromethane, and ethyl acetate, respectively. The ethyl acetate fraction, which yielded the flavonoids, verified by TLC, was dried at room temperature to give a brown residue (1.6 g).

Preparation of the stationary phase

Chitin flakes (85% N-acetylation) were obtained in NIQFAR laboratories according to the method of Rinaudo *et al.* (1993). The material was ground and sieved and fractions with sizes of between 43–100 μ m were used the chromatographic column.

Chitosan (76% N-desacetylation) was obtained through basic hydrolysis of chitin according as previously described Rinaudo *et al.* (1993). The material was ground and sieved and fractions with sizes of between 43–100 μ m were used for the preparation of the chromatographic column.

The N-acetylation of chitosan lead to fully N-acetylated chitin. Complete N-acetylation was achieved under heterogeneous conditions using a binary mixture of methanol/formamide and acetic anhydride. 20 g of chitosan (0.12 mol) was main-

tained by stirring with acetic anhydride 25 g (0.25 mol) in 200 ml of methanol/formamide 50:50 for 4 h. The resulting solid was exhaustively and successively washed with methanol and acetone to remove the excess of the acetic anhydride, and dried under vacuum at room temperature. The material obtained was characterized by infrared spectrum and potentiometric titration (Brugnerotto *et al.*, 2001). The N-acetylation degree of the chitosan, after the acetylation process, was 99.2% (determined by potentiometric titration). The infrared spectra were obtained as KBr disk on an IR-Bomen spectrophotometer model MB-100. The potentiometric titration was conducted on ORION-pHmeter model A920.

Chromatographic separation

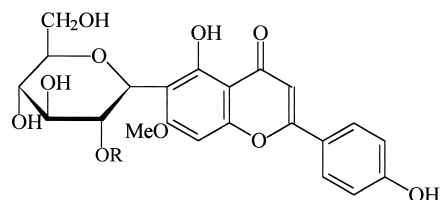
The ethyl acetate fraction (150 mg) was chromatographed on chitin-100 (3 g) packed into a glass column (1.0 × 30 cm) with the bed of 15 cm height. The elution was performed with a CHCl₃:MeOH gradient and fractions of 5 ml were collected. After being monitored by thin layer chromatography (TLC), the fractions eluted with CHCl₃:MeOH 70:30 v/v which showed a positive reaction with FeCl₃ were combined.

Similarly, 150 mg of the ethyl acetate fraction was chromatographed over silica gel (6 g) and chitosan (3 g).

The purity of all isolated substances was examined by TLC precoated with a 0.25 mm layer of silica gel 60 HF₂₅₄ from Merck and eluted with CHCl₃:MeOH 85:15 v/v. The compounds were detected by spraying with a FeCl₃ (2% in ethanol) solution or visualized under UV light (254 nm). The compounds were identified by direct comparison with authentic samples previously isolated from *A. moluccana* (Meyre-Silva *et al.*, 1999).

Results and Discussion

We have utilized extensively chitin as a chromatographic support in column chromatographic for the separation of biflavonoids and diterpenes (Rodrigues *et al.*, 1999, 2000). The chromatographic performance seems to be very influenced by the hydrogen bond formed between the -OH-group in phenolic compounds and the CH₃CONH-group in chitin.



1- R = H

2- R = Rhamnosyl

Fig. 1: Molecular structures of flavonoids isolated in *A. moluccana*: 1- swertisin, 2- 2''-O-rhamnosylswertisin.

Table I: Efficiency of different support studied in the separation of flavonoids of *A. Moluccana* (150) mg of ethyl acetate extract.

	Swertisin		2''-O-rhamnosylswertisin	
support	mg ^a	%	mg	%
Chitin	11.2	8.0	10.9	7.2
Chitin-100	12.8	13.5	15.8	10.5
Silica gel	3.0	2.3	9.2	6.1

^a Means for two experiment.

The results of chromatographic separation of flavonoids from *A. moluccana* denoted, swertisin (1) and 2''-O-rhamnosylswertisin (2), Fig. 1 onto chitin, chitin-100 and silica gel are compared in Table I.

Natural chitin, obtained from shrimp shells, contain about 10–15% of deacetylated form (NH₂) free group. The presence of the amino group favors the formation of a strong hydrogen bond between OH phenolic and NH₂ present in chitin. In this case the yield decreases once a fraction of the compounds is kept in the column.

On the other hand, full n-acetylation of the chitosan significantly reduces the amount of the groups free NH₂ from the sorbent, leaving the chitin-100 with polyamide characteristic (Shmakov *et al.*, 1996). The interaction among the groups OH of swertisin and 2''-O-rhamnosylswertisin with the acetamide groups of chitin-100 is less intense than with the NH₂ groups of the chitin, therefore the yield of the compounds is larger when chitin is used as sorbent, as shown in the Table I.

Another interesting result is the low yield of the compositions when the silica gel was used as sor-

bent. In this case, the interaction between group OH of the silica and OH of the compositions is very strong, resulting in the retention of the phenolic compounds in the column. The unexpected results when we compared the yields of the two flavonoids can be explained, since the 2''-O-rhamnosylswertisin is more abundant in *A. moluccana* (Meyre *et al.*, 1999).

Our results show that the flavonoids swertisin and 2''-O-rhamnosylswertisin have apparently been separated by the hydrogen bond between the

stationary phase (chitin and chitin-100) and flavonoids under the conditions studied. The results should be informative for improving the yield of the other phenolic compounds present in medicinal plant extracts.

Acknowledgements

This work was supported by grants from PROBIC/ProPPEX/UNIVALI and CNPq (Brasil). The authors are grateful to Dr. Ademir Reis for the plant classification.

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