

# Lignanes from the Brazilian *Melia azedarach*, and their Activity in *Rhodnius prolixus* (Hemiptera, Reduviidae)

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*A study of the phagoinhibitor and anti-moulting activities of the Brazilian Melia azedarach, collected in the State of Rio de Janeiro, Brazil, allowed the isolation of four lignanes identified as pinoresinol (1), bis-epi-pinoresinol (2), the hemicetal (3) and the diacid (4). These substances are devoid of anti-moulting activity.*

Key words : lignanes - Meliaceae - *Melia azedarach* - insects - *Rhodnius prolixus*

Chagas disease is widely spread in South America. Dissemination of the illness involves haematophagous insects, such as *Rhodnius prolixus* and some other related ones. It is long known that human contamination occurs through the feces that triatomid infested insects invariably deposit on feeding (Garcia & Azambuja 1991). Obviously, control of the disease depends heavily upon the efficient control of its vectors. A number of insecticides are commonly used for that purpose. They offer a series of disadvantages since they are usually both highly toxic and non-specific. In addition, most of them are not biodegradable and must thus be considered as polluting agents (van Beek & de Groot 1986). Safer alternatives may be found among natural sources, mainly higher plants that afford a number of repellent and toxic secondary metabolites (Subrahmanyam 1990).

Abundant literature describes plants of the family Meliaceae that produce meliacins (or limonoids), some of which are endowed with potent phagorepellent properties (Nakanishi 1976). For example, azadirachtin-A, a highly oxygenated tetra-*nor*-triterpenoid, has been isolated from the Indian *Azadirachta indica* (Butterworth & Morgan 1968) and from the Kenyan *Melia azedarach* (Morgan & Thornton 1973). This compound is a remarkable phagoinhibitor (40 µg/ml) with anti-moulting (DE<sub>50</sub> 4.10<sup>-4</sup> µg/ml) activities on *R. prolixus* (Garcia et al. 1984), and has turned out to be a potential useful drug for Chagas disease control. However, production of azadirachtins might suffer from strong biogeographic

dependance since it has been reported recently that crude extracts of *M. azedarach* from Paraguay were devoid of any anti-moulting activity (Arias & Hirschmann 1988). Only fatty acids, some classical phytosterols and two coumarins, scoparone and isofraxidin, could be isolated (Arias & Hirschmann 1988). Thus, it seemed of great interest to start a study of the Brazilian *M. azedarach*, in order to look for the presence of anti-moulting principles. This exotic plant, of African origin, is widely cultivated in Brazil where it is known as "cinamomo". As far as we are aware, there seems to be no report on the isolation of anti-ecdysis compounds from the Brazilian plant. This paper reports on the structure identification and bioassays of four lignanes isolated from a bioactive fraction of cinamomo seeds.

## MATERIALS AND METHODS

*Extraction and fractionation* - Seeds of *M. azedarach* (1,300 g), collected in the neighbourhood of Niterói (State of Rio de Janeiro, Brazil), were extracted exhaustively with MeOH (2 x 5 l). Filtration and evaporation of the solvent under reduced pressure furnished a gummy residue (245g). Partition between hexane and 5% aq.MeOH, followed by evaporation of the methanolic phase and treatment with EtOAc afforded an active fraction "A" (71 g). Bioassay guided fractionation of a 1g aliquot of "A", by conventional chromatographic processes using silica gel and mixtures of CHCl<sub>3</sub>-MeOH, furnished the active fraction, F-58 (21 mg) that contained phytosterols, four lignanes (1-4) and one triterpene whose structure elucidation will be reported elsewhere. Preparative scale purification of "A" was carried out as follows. Fraction "A" (49 g) was coated on silica gel, placed in a Büchner filter and submitted, under reduced pressure, to selective

This work was supported by CNPq, FINEP, CAPES, DAAD and Volkswagen Foundation

Received 14 March 1995

Accepted 12 July 1995

desorption using successively hexane,  $\text{CHCl}_3$  and acetone. Fractions 1-3 were combined, affording "B" (11 g) that was submitted to quick filtration on silica gel (eluent : step gradient of acetone 2-5-10-20-50-80 and 100% in  $\text{CHCl}_3$ ). Fractions 6-7 ("M", 2 g) from the latter filtration contained lignanes 1 and 2, phytosterols and one triterpene. Fractions 9-11 ("E", 300 mg) contained the more polar lignanes 3 and 4. Fraction "M" was submitted to conventional silica gel column chromatography (eluent : gradient of AcOEt in hexane from 2 to 50%). Lignanes 1 (50 mg) and 2 (30 mg) were obtained both better than 90% pure. Finally, fraction "E" was submitted to preparative HPLC purification on a RP-8 column ( $\phi$  50mm), eluted with MeOH- $\text{H}_2\text{O}$  45:55, at a pressure of 32 bars, under continuous flow of 100ml/min and UV detection at 215nm. This allowed the isolation of lignane 4 (fractions E1-E2, 10.5 mg, 91% pure) and lignane 3 (fraction E8, 4.5 mg, 98% pure).

**Bioassays** - Fourth instar nymphs of *R. prolixus* were used. Test material was dissolved in EtOH-saline (1:4). Aliquots were added to blood in order to obtain desired final concentrations (10 to 100  $\mu\text{g/ml}$ ). Test blood was placed in special designed feeders (Garcia & Rembold 1984), and the insects were allowed to feed. After feeding, the insects were weighted, incubated at 28°C and observed every two days over a 1 month period. Only fully fed insects were used ; partially fed ones were discarded. Death and ecdysis were counted.

## RESULTS

We observed both phagoinhibitor and anti-moulting activities in the methanolic crude extract of the seeds of *M. azedarach*. Bioassay-guided frac-

tionation of this crude extract, by solvent-solvent partition, furnished an EtOAc phase that contained almost all the activity. Repetitive silica gel column chromatographies yielded the active fraction F-58 that inhibited 100% moulting of *R. prolixus* at 25  $\mu\text{g/ml}$  blood (Cabral et al. in preparation). HPLC analysis, on a RP-18 column, indicated the presence, in the crude extract, of traces of a constituent whose retention time was identical to an authentic sample of azadirachtin-A (Cabral et al. in preparation). Purification of this active fraction by reversed phase HPLC yielded classical phytosterols (cholesterol, campesterol and stigmasterol), one triterpene and four lignanes (1-4, Fig. 1).

Compound 1 was unequivocally identified as the well known pinoresinol, on the basis of its  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (Tables I, II) identical with literature data (Fonseca et al. 1979, Marcos et al. 1990, Cuenca & Catalan 1991), and also from direct comparison with the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of an authentic sample.

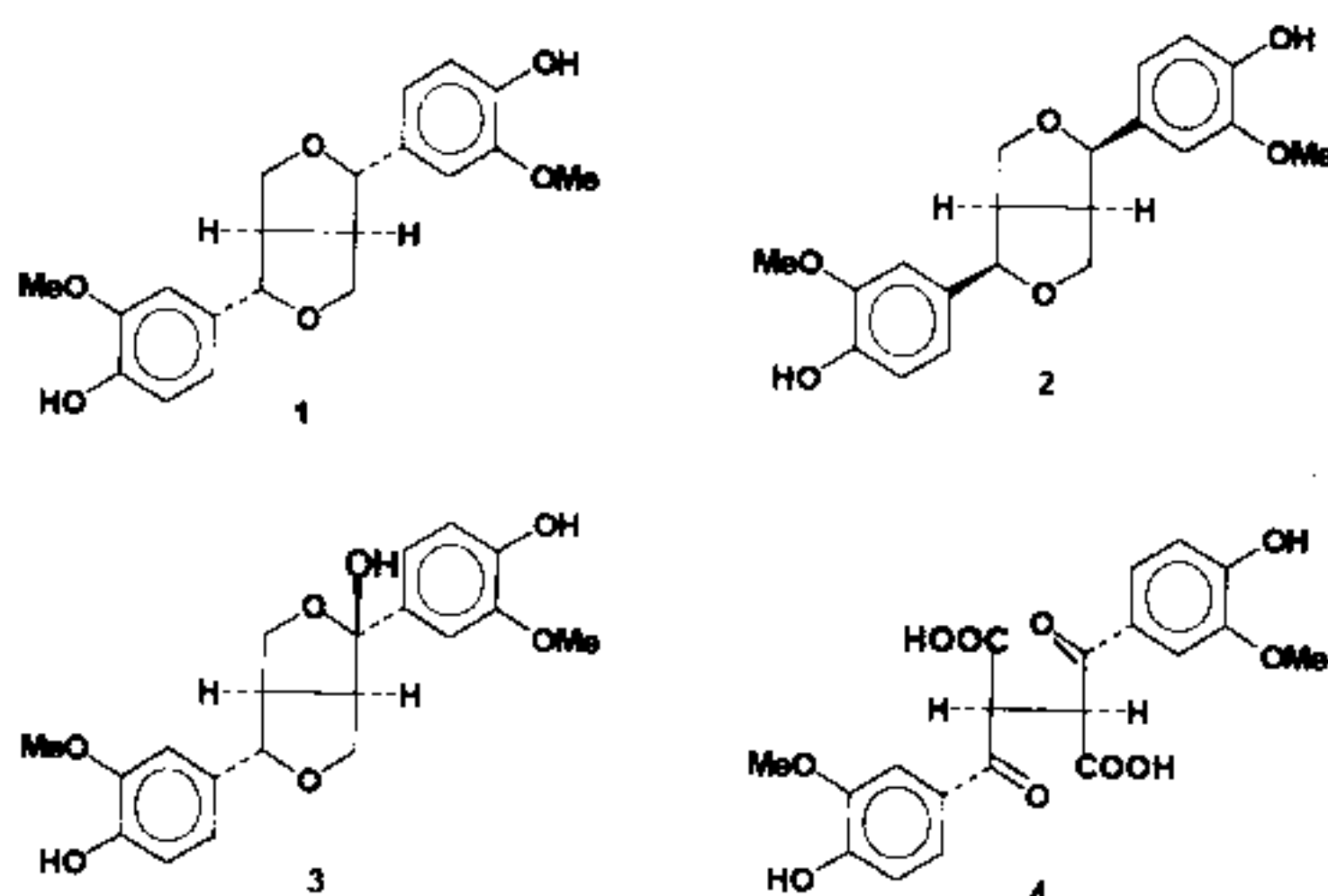


Fig. 1 : structures of the lignanes from *Melia azedarach*.

TABLE I

$^1\text{H}$  NMR data for compounds 1-4

H	1	1 <sup>a</sup>	2	3	4
H-2,2'	6.87 d 1.5	6.89 d	6.86 d 1.8	6.94 d 1.6	7.57 bs
H-5,5'	6.88 d 8.5	6.88 d 8.0	6.82 d 8.4	6.76 d 8.1	6.80 d 8.5
H-6,6'	6.79 dd 8.5; 1.5	6.82 dd	6.75 dd 8.4; 1.8	6.81 dd 8.1; 1.6	7.52 bd 8.5
H-7,7'	4.71 d 4.6	4.73 d 4.3	4.69 d 4.7	4.70 d	4.4 -
H-8,8'	3.08 m	3.10 m	3.05 m	3.14 m (?)	3.65 s
H-9,9' eq	4.22 dd 6.9; 9.0	4.27 dd 6.9; 9.1	4.19 dd 7.0; 9.3	4.23 dd 6.9; 9.0	-
H-9,9' ax	3.84 dd 3.9; 9.0	3.87 dd 3.8; 9.2	3.85 dd 3.7; 9.3	3.84 dd 3.6; 9.0	-
OMe	3.89 s	3.91 s	3.81 s	3.85 s	3.88 s
OH	5.58 m	5.89 m	n.o.	n.o.	n.o.

a: Cuenca & Catalan (1991); n.o.= not observed; 1 and 2 in  $\text{CDCl}_3$  at 200 MHz; 3 and 4 in  $\text{CD}_3\text{OD}$  at 250 MHz

TABLE II

<sup>13</sup>C NMR data of 1 (CDCl<sub>3</sub>) compared with literature data (Fonseca et al. 1979)

C	1	pinoresinol
C-1,1'	132.85 s	132.0
C-2,2'	118.93 d	118.5
C-3,3'	114.23 d	114.4
C-4,4'	145.18 s	145.2
C-5,5'	146.66 s	146.8
C-6,6'	108.57 d	108.8
C-7,7'	85.86 d	85.7
C-8,8'	54.11 d	53.7
C-9,9'	71.63 t	71.3
OMe	55.93 s	55.6

Compound 2 had a structure very close to that of pinoresinol (1), showing almost the same <sup>1</sup>H NMR spectrum (Table I) with only slight differences in the pattern of the aromatic protons. Since epimerization at one of the benzylic positions should change the pattern of H-7,7' from a 2H doublet at δ 4.71 into two 1H doublets at δ 4.71 and 4.55 (Pelter et al. 1976, Marcos et al. 1990), and since the <sup>1</sup>H NMR spectrum showed that 2 maintained the symmetry of 1, we propose for 2 the structure bis-epi-pinoresinol. This structure needs further confirmation.

Compound 3 again showed, in <sup>1</sup>H NMR, the same aromatic moiety as 1 and 2, and the presence of signals corresponding to the bis-perhydrofuran system. However, integration of the benzylic signal indicated the presence of only one such proton (H-7); carbon-7' is thus quaternary. We propose for this metabolite the hemiacetal structure 3 derived from pinoresinol. This hypothesis, although reasonable, could not be corroborated by <sup>13</sup>C NMR spectroscopy due to the very low amount of available pure sample and must be considered as provisional.

Finally, the highly polar compound 4 again showed, in <sup>1</sup>H NMR, a symmetrical structure having a tri-substituted aromatic ring and an OMe group. However, major differences were observed between the <sup>1</sup>H NMR spectra of 4 and 1-3. Thus, the low field positions of H-2,2' and H-6,6' indicated the presence of a deshielded group at the C-7,7' positions, probably a carbonyl function. This hypothesis was in agreement with the absence of the 2H doublet attributable to the H-7,7' signals. In addition, the 4H AB system of H-9,9'eq and H-9,9'ax is also absent and the H-

8,8' signal, a multiplet at δ 3.10 in 1, appears now as a sharp singlet at δ 3.65. All this pointed to structure 4, that may be either an isolation artifact or a metabolite obtained by oxidative degradation as depicted in Fig. 2.

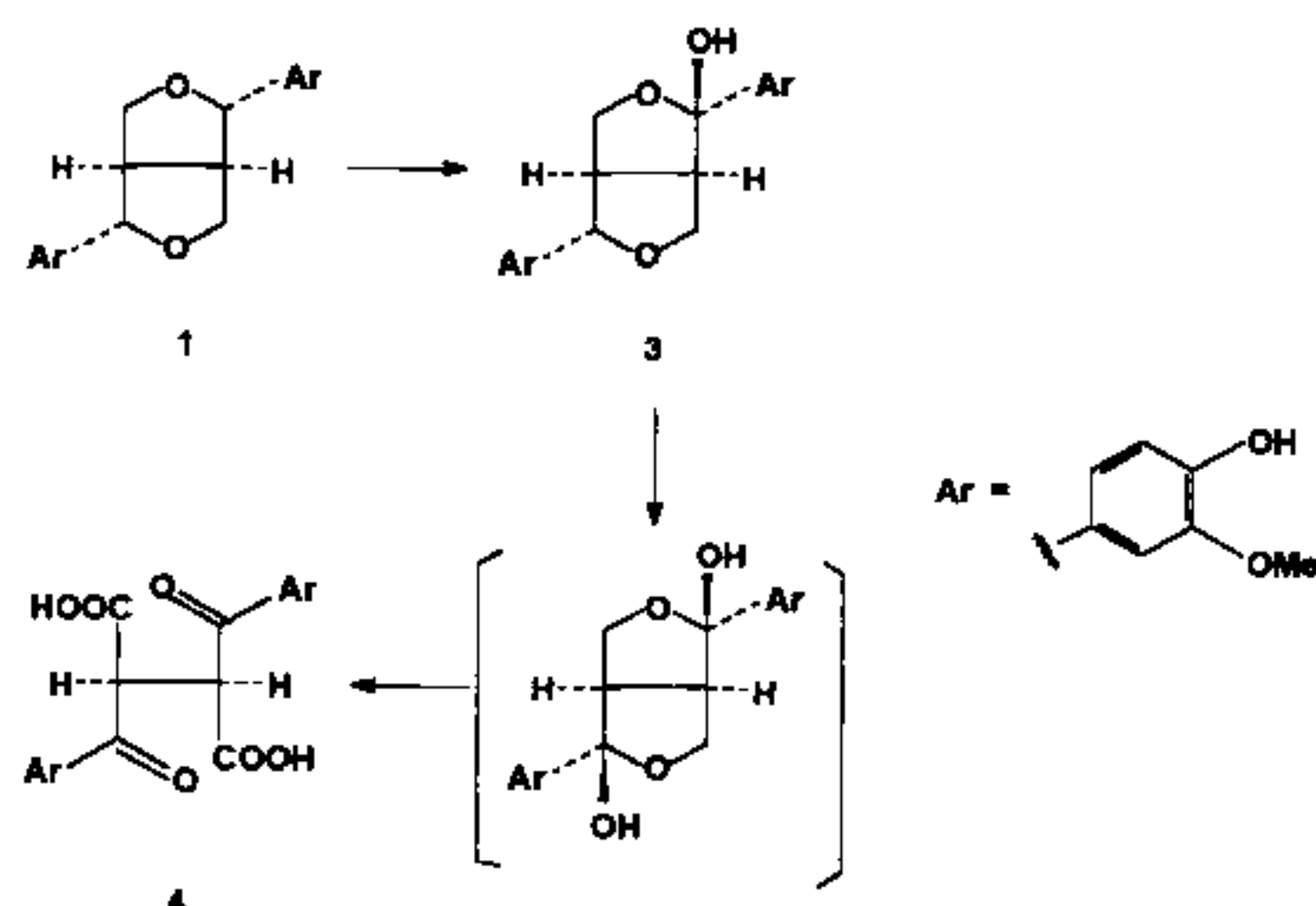


Fig. 2 : catabolic pathway from 1 to 4.

These results are remarkable since, although lignanes were known from the order Rutales, this is the first report of this class of metabolites in the family Meliaceae, and also in the very well studied genus *Melia* (Gottlieb & Yoshida 1989).

Abundant literature reports on the biological activities of lignanes. They were found to be antineoplastic (Brewer et al. 1979, Dewick & Jacson 1981), antimitotic (Gensler et al. 1977), antibacterial (Nakatani et al. 1988), and anti-hypertensive agents (Sih et al. 1976). They also inhibit the enzyme c-AMP phosphodiesterase (Mac Rae & Towers 1984). On insects, lignanes act as antifeedant (Harmatha & Nawrot 1984) and as larval growth inhibitors (Mac Rae & Towers 1984). In addition, juvenile hormone activity has been reported (Bowers 1968). On the other hand, a number of lignanes act synergistically with a variety of insecticides, increasing their toxicity (Haller et al. 1941, 1942, Fales et al. 1970, Matsui & Munakata 1975, Mac Rae & Towers 1984). Despite this broad spectrum of activities, our bioassays (Fig. 3a,b) showed that lignanes 1-3 are not responsible, by themselves, for the anti-moulting activity observed in the crude extract (Cabral et al. in preparation). Synergistic effects are however not excluded and are now under study in our laboratories. In the absence of botanical confusion of the Paraguayan plant commented above (Arias & Hirschmann 1988), our results suggest that production by *M. azedarach* of anti-moulting principles and other secondary metabolites may be very latitude-dependent.

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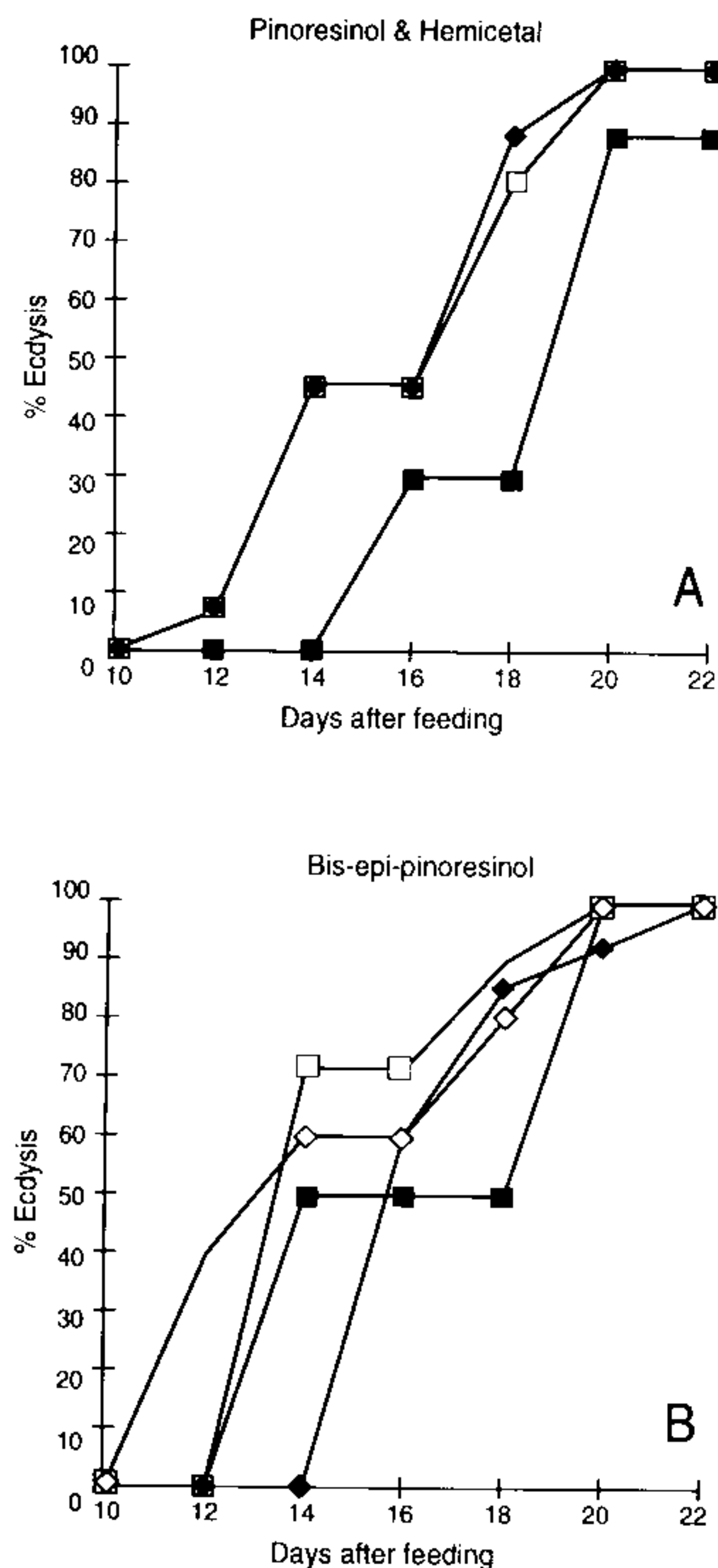


Fig. 3 : anti-moulting tests on compounds 1-3. A: effects of pinoresinol (10 µg/ml) and the hemicetal (10 µg/ml) on ecdysis in fourth-instar larvae of *Rhodnius prolixus*. Controls (◆), pinoresinol (■), and hemicetal (□). Groups of 15-20 nymphs. B: effects of bis-epi-pinoresinol on ecdysis in fourth-instar larvae of *R. prolixus*. Controls (◇), treated with 1 µg/ml (◆), 10 µg/ml (□) and 50 µg/ml (■) bis-epi-pinoresinol/ml blood. Groups of 15-20 nymphs.

## ACKNOWLEDGEMENTS

MMOC is grateful to CNPq and FAPERJ for fellowships and to Dr OR Gottlieb for fruitful discussions. AK thanks Dr H Rembold (Max-Planck Institut für Biochemie, München, Germany) for technical support, Dr R Riguera (Dept. of Chemistry, University of Zaragoza, Spain) for original NMR spectra of pinoresinol and related lignanes, Dr R Ottinger (CIREM, Free University of Brussels, Belgium) and Dr Aurélio B Buarque Ferreira (Dept. of Chemistry, Universidade Federal Rural do Rio de Janeiro, Brazil) for the NMR spectra.

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