



## ARDISIACRISPIN B, A PROSTAGLANDIN LIKE EFFECT SAPONIN, ISOLATED FROM *ARDISIA CRISPA*

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**Résumé:** la présente étude a pour but de caractériser l'activité pharmacologique de l'Ardisiacrispin B. Des études ont été réalisées sur des préparations *in vitro* de muscles lisses d'utérus, de petit intestin, et d'aorte thoracique (muscle lisse vasculaire) issus de rates en œstrus. Des courbes dose-réponse (Courbes DR) ont été obtenues pour l'Ardisiacrispin B, un dérivé de la Prostaglandine E<sub>2</sub> (Nalador), l'Oxytocine et le chlorure d'acétylcholine. L'implication possible de la synthèse de prostaglandine dans l'activité contractile de l'utérus de l'Ardisiacrispin B a été étudiée par analyse de la courbe DR de l'Ardisiacrispin B en présence d'indométhacine 10<sup>-6</sup>M, inhibiteur de la cyclo-oxygénase.

L'Ardisiacrispin B provoque une contraction dose dépendante du muscle lisse d'utérus, d'intestin et d'aorte thoracique de façon similaire au dérivé d PGE<sub>2</sub>. L'Oxytocine provoque aussi la contraction de la lanière d'utérus mais n'a aucun effet sur l'intestin. L'Acétylcholine provoque la contraction de l'utérus et de l'intestin, mais différemment de l'Ardisiacrispin B. La présence d'Indométhacine n'affecte pas la courbe DR de l'Ardisiacrispin B sur le muscle lisse d'utérus. Ces résultats indiquent que l'Ardisiacrispin B agit de la même façon que la prostaglandine E<sub>2</sub>, mais sur le récepteur de la prostaglandine E<sub>2</sub> lui-même, et non pas par stimulation de la synthèse de prostaglandines.

**Abstract :** the present study aimed to characterize the pharmacologic action of the Ardisiacrispin B. Studies were performed *in vitro* preparation, using uterine smooth muscle, small intestine and thoracic aorta (vascular smooth muscle) obtained from female rats in estrus. Dose response-curve (DR-curve) to Ardisiacrispin B, Prostaglandin E<sub>2</sub> derivative (Nalador), Oxytocin and Acetylcholine chloride were obtained. The possible involvement of prostaglandin synthesis in the utero-contracting activity of Ardisiacrispin B was also explored by investigation of the DR-curve to Ardisiacrispin B in the presence of 10<sup>-6</sup> M indomethacin, a cyclo-oxygenase inhibitor.

Ardisiacrispin B caused contraction in dose-dependent fashion of uterine smooth muscle, small intestine and thoracic aortae in a similar pattern to PGE<sub>2</sub> derivative. Oxytocin also caused uterine strip contraction but had no effect on small intestine. Acetylcholine caused uterine and small intestine contraction in a different manner from that obtained with Ardisiacrispin B. However, the presence of indomethacin did not alter the DR-curve to Ardisiacrispin B of uterine smooth muscle. These results indicate that Ardisiacrispin B exerts a prostaglandin E<sub>2</sub>-like effect which may act at the prostaglandin E<sub>2</sub>-receptor but not by stimulation or enhancement of prostaglandin synthesis.

### Introduction

Prostaglandin E<sub>2</sub> is a potent agent for pregnancy termination (Conrad & Umland, 1979; Rayburn, 1989). The clinical usefulness is based on its ability to stimulate the uterus to contract. However, the limitation of the compound are its instability, incidence of side effects and high price. In 1987, Jansakul *et al.* reported that ardisiacrispin B, one of the two major saponins isolated from *Ardisia crispa* showed a pronounced contraction on rat uterine smooth muscle in isolated preparation. Thus, it is possible that ardisiacrispin B, which is a stable compound, may be useful as an alternative drug for pregnancy termination.

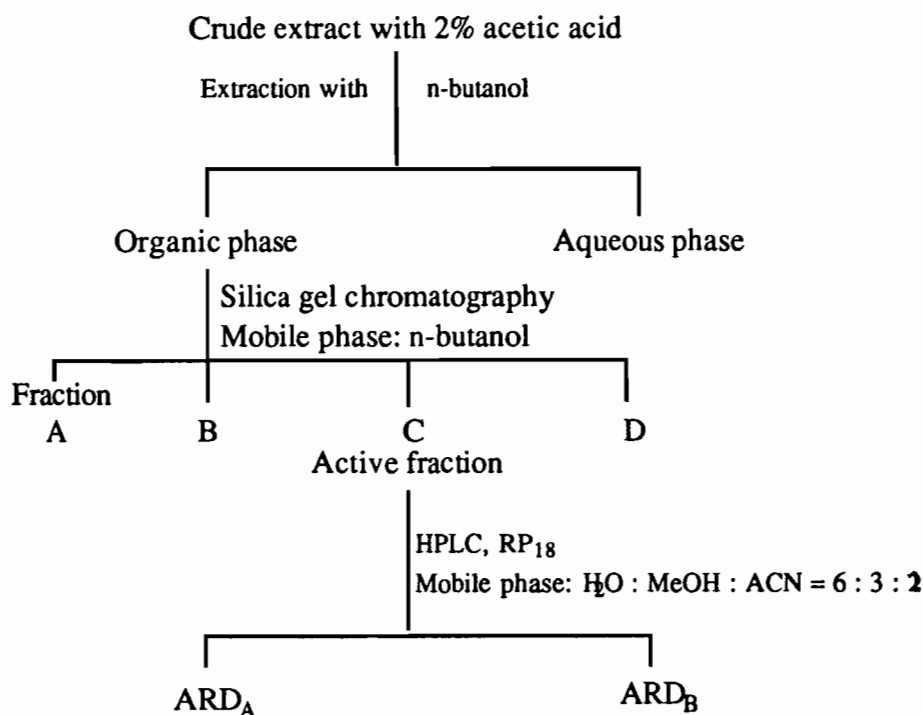
The present study was designed to characterize the pharmacologic action of ardisiacrispin B. Studies were performed *in vitro* using uterine smooth muscle, small intestine



and thoracic aorta (vascular smooth muscle) obtained from female rats in estrus. Dose-response relationship to ardisiacrispin B, prostaglandin E<sub>2</sub> derivative (Nalador), oxytocin and acetylcholine chloride were determined.

### Materials and methods

Isolation of ardisiacrispin B followed Jansakul's method (Jansakul *et al.* 1987) as in the following diagram



Animal tissue isolated preparation using female Wistar rat in œstrus. The animal was killed by cervical dislocation. A uterine sheet about 4mm long was cut at the anterior end, or a 5 mm length of jejunum removed from the small intestine. Each piece of tissue was mounted in a 20 ml organ bath. One end was fixed at the bottom and the other end was connected to a force transducer.

For thoracic aorta, a ring approximately 7 mm long was cut and placed into a 20 ml organ bath using two vertically placed stainless steel stirrup hooks passed through its lumen, the lower being fixed, and the other connected to an isometric force transducer (Grass FT03C). Contractions were continuously recorded with polygraph (7D WU). The organ bath contained Krebs's Henseleit solution, maintained at 37° C, and continuously bubbled with 95% O<sub>2</sub> and 5%CO<sub>2</sub>.

Prior to addition of drugs, the tissues were equilibrated for 60 min under a resting tension of 0.5 g for uterus and small intestine and 1.0 g for thoracic aorta. The Krebs's solution was replaced every 10 min.

After equilibration, the ability of contraction of the uterine and small intestine tissue were tested using 10<sup>-6</sup> M acetylcholine. The presence of functional endothelium of the thoracic aorta was tested as follows. The aortic ring was precontracted with 3x10<sup>-7</sup> M noradrenaline for 5-8 min (by which time the response had plateaued), and dilator responses to 10<sup>-6</sup> M acetylcholine recorded. Eighty to ninety per cent vasodilatation to acetylcholine occurred with endothelium-intact rings.

**Contractile responses on uterine sheet or small intestine of ardisiacrispin B, PGE<sub>2</sub>, oxytocin and acetylcholine:**

After testing for the ability of tissue contraction, tissues were incubated for 20 min in Krebs-Henseleit solution. A discrete dose-response relationship was performed (allowing 20-25 min interval between dose) to the agonist. To avoid drug-interaction, each tissue was exposed to only one agonist.

**Contractile responses of ardisiacrispin B and PGE<sub>2</sub> on thoracic aorta:**

After testing for the presence of functional endothelium as described above, tissues were incubated for 45 min in Krebs-Henseleit solution. A cumulative dose-response relationship to PGE<sub>2</sub> was performed. Following multiple washing to remove PGE<sub>2</sub>, the tissues were then incubated for 50 min. A cumulative dose-response relationship to ardisiacrispin B was obtained.

**Effects of indomethacin on contractile response of ardisiacrispin B on the uterine sheet:**

After testing for the ability of the contractile activity of the uterine sheet, tissues were incubated for 40 min in the presence of 10<sup>-6</sup> M indomethacin. A discrete DR-curve to ardisiacrispin B was obtained.

The following drugs were used: Prostaglandin E<sub>2</sub> (Nalador, Schering Ltd., Germany), oxytocin (sigma, U.S.A.), Acetylcholine (sigma, U.S.A.) and indomethacin (sigma, U.S.A.). Drugs were dissolved in distilled water except for indomethacin, which was dissolved in 0.1% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) solution.

**Results:**

Results are shown in Fig.1 - Fig.6. Fig.1 shows typical contractile responses of the uterine smooth muscle to ardisiacrispin B, PGE<sub>2</sub> derivative, oxytocin and acetylcholine, from 4 experiments with each agonist. The contractile responses of these 4 agonists are dose-dependent.

The contractile responses to ardisiacrispin B of rat uterine smooth muscle are quite similar to those obtained with oxytocin. Small doses of each of these two agonists caused an increase in both amplitude and frequency of phasic contraction compared to basal spontaneous rhythmic contraction. When higher doses of each of these two agonists were applied, a mixed type contraction with both phasic and tonic components was obtained. However, in the case of the oxytocin, the contractile responses disappeared in a few minutes after washing, but in the case of ardisiacrispin B persisted for at least 30 min.

PGE<sub>2</sub> derivative, Nalador, caused an increase in both amplitude and rhythmicity. All concentrations of PGE<sub>2</sub> provoked rhythmic contraction, and each phasic contraction was prolonged for a few seconds by a tonic component. The frequency of phasic contractions was dose-dependent, and the contractile response persisted after washing (40-60 min for high doses).

Low doses of acetylcholine caused an increase in frequency of phasic contraction, moderate doses caused an increase in both phasic and tonic contraction, while high doses of the drug provoked a complete tetanic contraction. However, all of these effects disappeared after washing.

Fig. 2 shows typical contractile responses of small intestine to ardisiacrispin B, PGE<sub>2</sub>, oxytocin and acetylcholine. The effects of the ardisiacrispin B, PGE<sub>2</sub> and acetylcholine are dose-dependent. Oxytocin had no effect on small intestine in any doses studied (3.2 x 10<sup>-4</sup> - 3.2 x 10<sup>-2</sup> I.U./ml). The contractile responses to ardisiacrispin B of small intestine are similar to those obtained from PGE<sub>2</sub>. The contractile response to acetylcholine was rapid and



disappeared immediately after washing.

The DR-curve to ardisiacrispin B and PGE<sub>2</sub> of small intestine are shown in Fig.3. The maximum contractile responses were not different between these two agonists. However, ardisiacrispin B is about 200 times more potent than PGE<sub>2</sub>. The patterns of contraction to ardisiacrispin B and PGE<sub>2</sub> on thoracic aorta with intact endothelium (n=4) (Fig.4) were also similar, as are the DR-curves (Fig.5). Again, ardisiacrispin B is about 200 times more potent than PGE<sub>2</sub>.

The presence of 10<sup>-6</sup> M indomethacin, a cyclo-oxygenase inhibitor, did not alter the contractile responses to ardisiacrispin B of rat uterine smooth muscle (Fig.6)

## Discussion

Furchgott and Zawadzki (1980) have shown that acetylcholine has no effects on thoracic aortae with intact endothelium obtained from rats or rabbits, but caused vasodilatation in rings precontracted with noadrenaline. In the present study, ardisiacrispin B caused vasoconstriction of endothelium-intact thoracic aortae of the rat. Moreover, the contractile responses on uterine smooth muscle or small intestine to ardisiacrispin B are different from those effected by acetylcholine. Thus, an acetylcholine-like pharmacologic action of ardisiacrispin B can be ruled out.

The contractile pattern of ardisiacrispin B on rat uterine muscle is quite similar to that provoked by oxytocin (see Fig.1). However, the contractile response to oxytocin on uterine muscle was abolished immediately after washing while those caused by ardisiacrispin B persisted for a long period. Moreover, ardisiacrispin B also caused contraction of small intestine while this effect was not found when using oxytocin as an agonist. Thus, it is unlikely that ardisiacrispin B exerts its effects as an oxytocin-like activity.

Although the pattern of contractile activity of ardisiacrispin B on rat uterine smooth muscle is not similar to those provoked by PGE<sub>2</sub> (see Fig.1), both agonists had a long lasting effect on the muscle after washing which was not found in the case of oxytocin or acetylcholine. The pattern of contraction of different kinds of smooth muscle eg. small intestine or thoracic aorta, to ardisiacrispin B and PGE<sub>2</sub> were similar. Together with the finding that 10<sup>-6</sup> M indomethacin, a cyclo-oxygenase inhibitor, did not alter the contractile effect of ardisiacrispin B on the uterine muscle, these results suggest that ardisiacrispin B exerts a prostaglandin E<sub>2</sub>-like effect which may act at the prostaglandin E<sub>2</sub>-receptor but not by stimulation or enhancement of prostaglandin synthesis.

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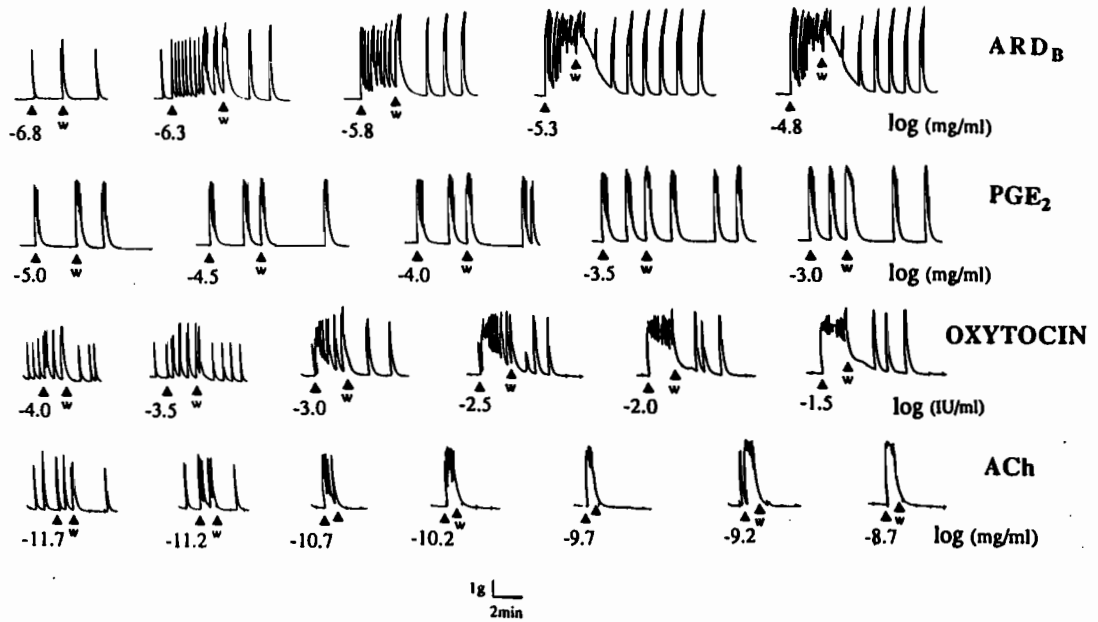


Fig. 1 Typical contractile responses to ardisiacrispin B (ARD<sub>B</sub>), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), oxytocin and acetylcholine (ACh) of uterine smooth muscle, w indicates washing.

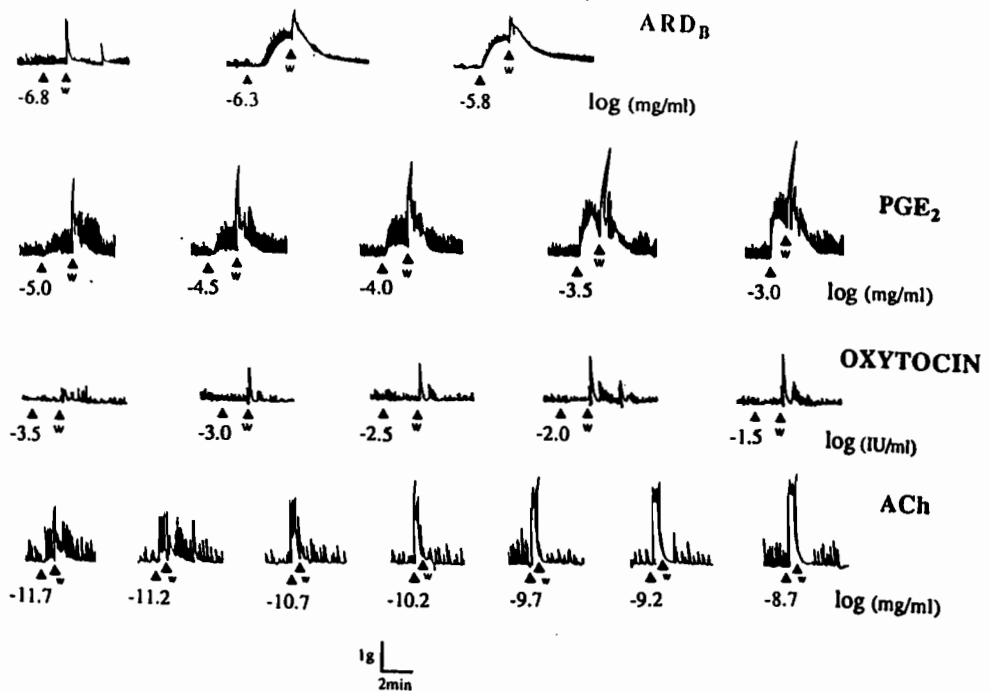


Fig. 2 : Typical contractile responses to ardisiacrispin B (ARD<sub>B</sub>), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), oxytocin and acetylcholine (ACh) of small intestine, w indicates washing.

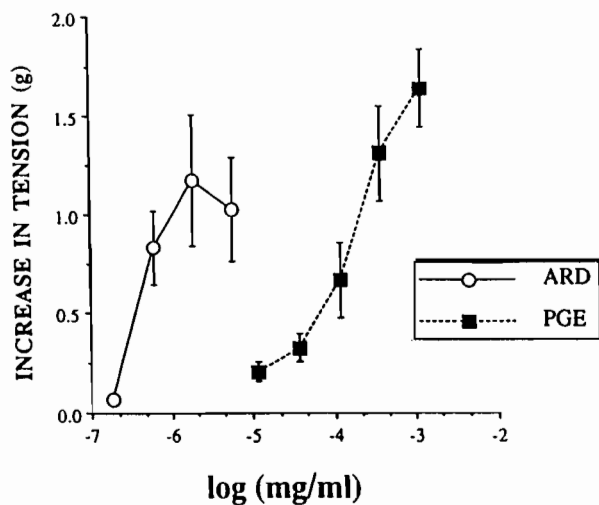


Fig. 3 : Mean (x+s.e, n=4) dose-response curve of ardisiacrispinB (ARD) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) on small intestine.

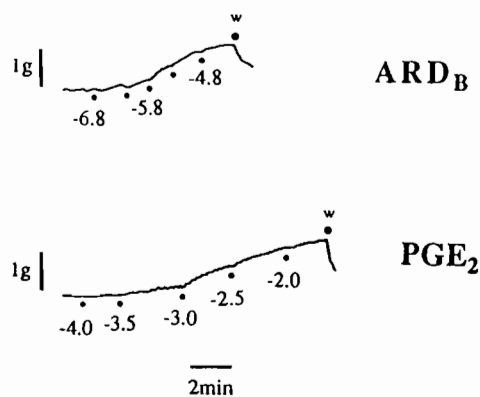


Fig. 4 : Typical contractile responses to ardisiacrispinB (ARD<sub>B</sub>) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) of thoracic aorta, w indicates washing.

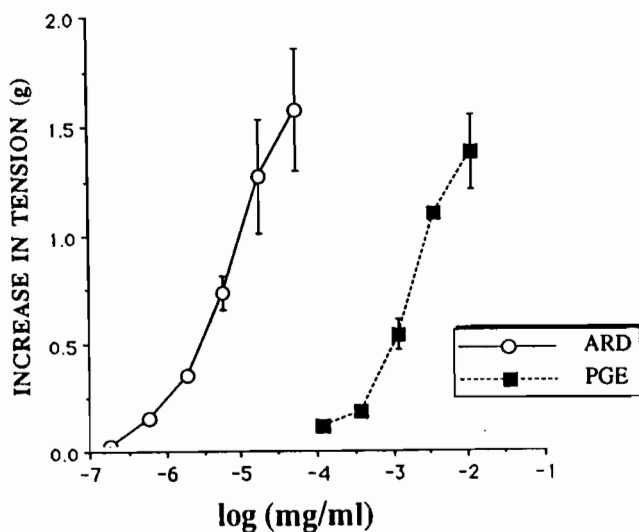
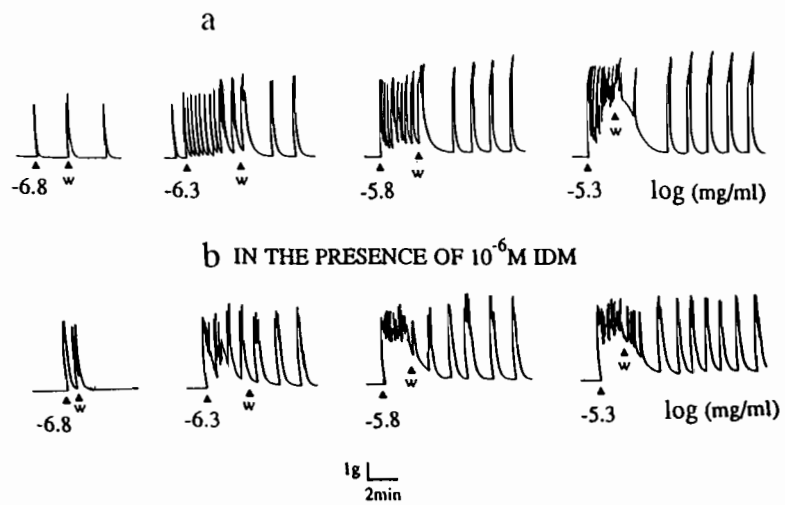


Fig. 5 : Mean (x+s.e, n=4) dose-response curve of ardisiacrispin B (ARD) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) on thoracic aorta.

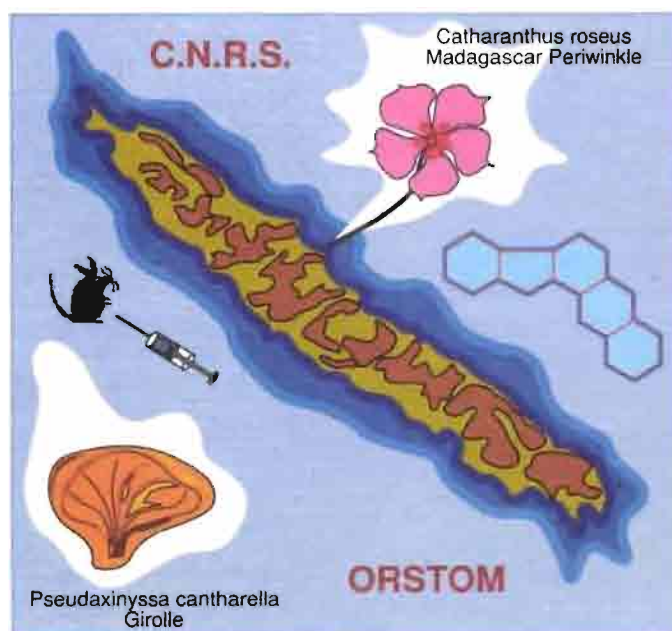


**Fig. 6 : Contractile responses to ardisiacrispin B of uterine smooth muscle in the absence (a) or presence (b) of  $10^{-6}$  M indomethacin, w indicates washing.**

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