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# Synthesis and Preliminary Pharmacological Evaluation of Coumestans with Different Patterns of Oxygenation

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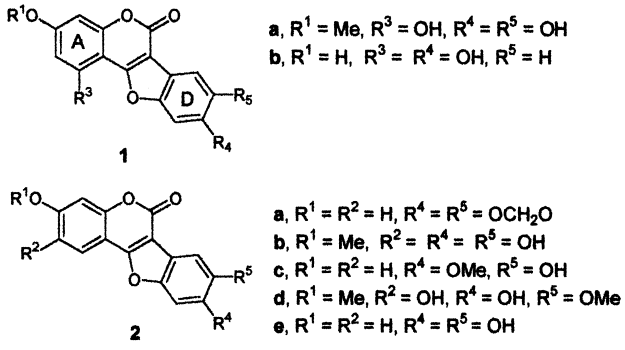
**Abstract**—Five coumestans with different patterns of oxygenation in rings A and D were synthesized from resorcinol and aromatic aldehydes, and screened for their antimyotoxic activity. The most potent compound (**2b**, $IC_{50} = 1\ \mu M$) was selected for study of its pharmacological profile. © 2001 Elsevier Science Ltd. All rights reserved.

Naturally occurring coumestans, used in folk medicine as snake antivenom, mainly isolated from plants of the family *Fabaceae*, have interesting pharmacological properties.[1] Mors et al.[2] and Melo et al.[3] reported that **1a**, a constituent of the plant *Eclipta prostrata* L. (*Asteraceae*), antagonized the muscle damage, the haemorrhagic effect, and the proteolytic and phospholipase activity of different Brazilian and North American snake venoms and their isolated toxins, in vitro and in vivo. Wagner and Fessler[4] found a potent and selective 5-lipoxygenase-inhibitor action for wedelolactone (**1a**) ($IC_{50} = 2.5\ \mu M$) while coumestrol (**1b**) was 40 times less potent. Wagner et al.[5] also reported an antihepatotoxic activity for **1a** and synthetic derivatives such as **2e** in assays employing CCL4, GaIN, and phalloidin-cytotoxicity in rat hepatocytes. As part of a program aimed at synthesizing biologically active flavonoids,[6] some coumestans bearing different patterns of oxygenation at rings A and D (**2a**–**e**) were chosen as target molecules. Coupound **2a** is natural and **2e** was previously prepared by Wagner et al.[5] On the other hand, **2b**–**d** are described for the first time in this letter.

The action of **2a**–**e** as antimyotoxic was evaluated and compared with that observed for wedelolactone (**1a**). The pharmacological profile of a selected coumestan (**2b**) was also further examined.




## Chemistry

Pterocarpans **7a**–**e** (Scheme 1) were considered as interesting precursors to prepare the target coumestans through oxidation followed by debenzylation.[7] These compounds could be obtained by a Heck type reaction between appropriately substituted chromenes (**5a**,**b**) and organomercurials (**6a**–**d**). The different patterns of oxygenation present in these intermediates was assured by the use of easily available benzaldehyde derivatives (**3a**–**c**), resorcinol derivatives (**4e**), and sesamol (**4d**), as starting

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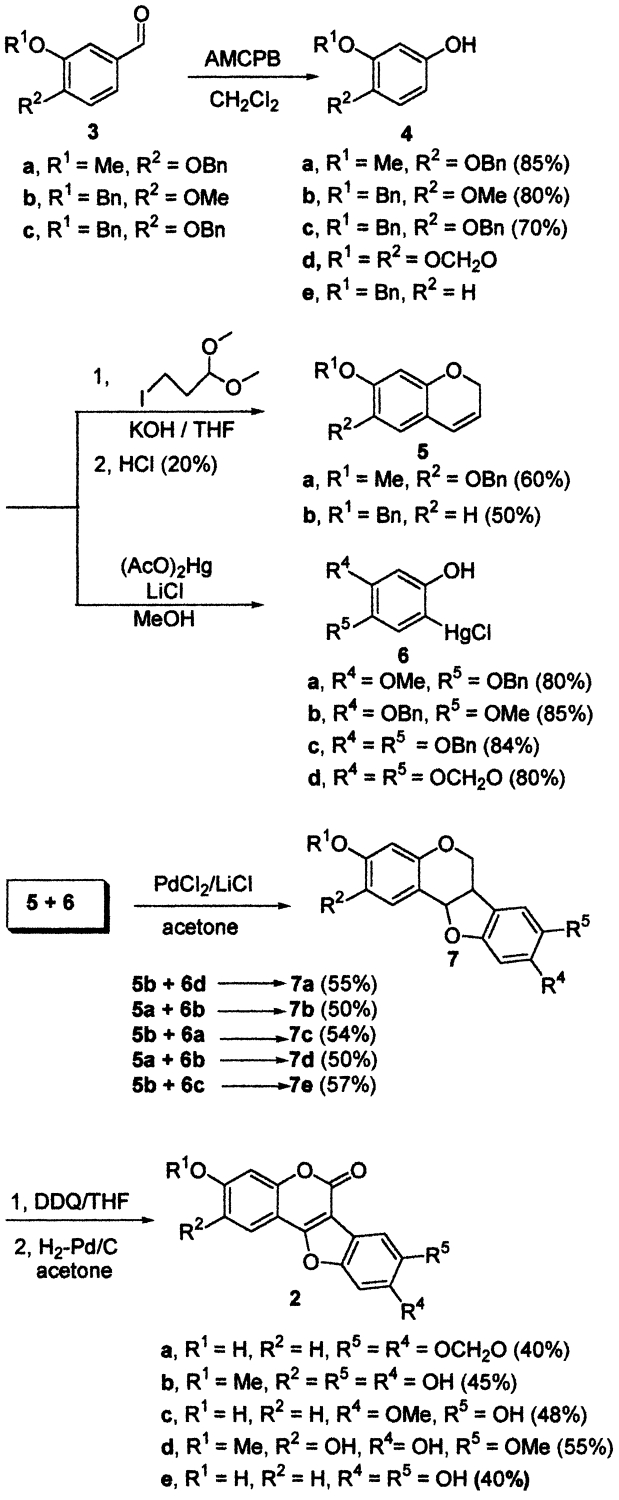


**Scheme 1.**

materials. The syntheses of the necessary chromenes and organomercurials were accomplished as shown in Scheme 1.

Baeyer–Villiger oxidation of aldehydes **3a–c** furnished the corresponding phenols **4a–c**,[8] while **4d–e** (resorcinol and sesamol, respectively) are commercially available. Reaction of some of these phenols with $(AcO)_2Hg$ led to the corresponding organomercurials **6a–d**, while alkylation with 3-iodo propanal dimethylacetal followed by cyclization in acid medium led to the obtention of chromenes **5a**,**b**. As expected, the coupling reaction between **5a–e** and **6a–d** furnished the corresponding pterocarpans **7a–e**. Coumestans **2a–e** were prepared by oxidation of pterocarpans **7a–e** with DDQ in THF at room temperature,[9] followed by hydrogenolysis of the protecting benzil groups.

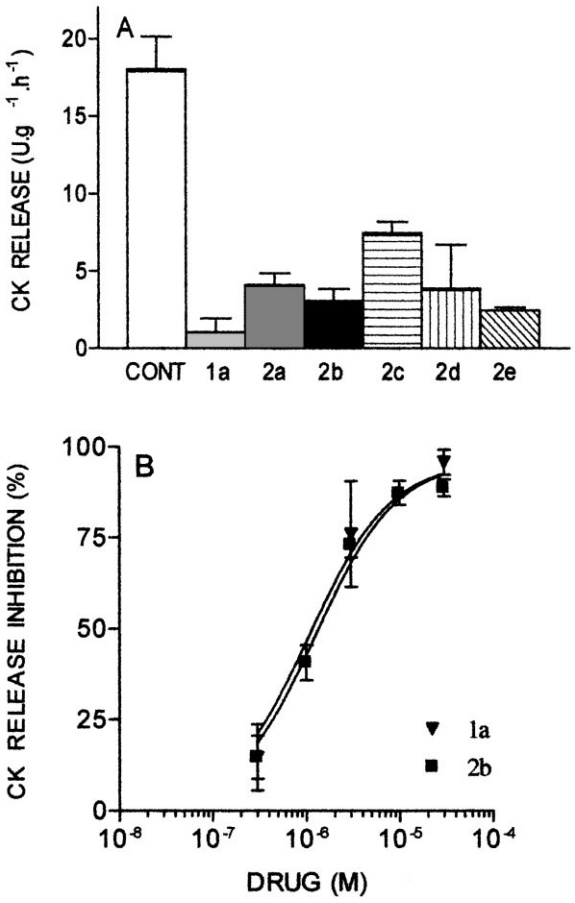


**Figure 1.** Effect of some coumestan derivatives on CK release induced by *Bothrops jararacussu* venom. (A) Mouse EDL muscles were exposed during 60 min to physiological saline solution (PSS) or *B. jararacussu* venom (25 μg/mL) either alone or in the presence of 30 μM of **1a** (wedelolacetone) or one of its analogues (compounds **2a–e**). The basal rate of CK release in control conditions was $0.35\pm0.07$ U $g^{-1}$ $h^{-1}$ ($n=29$). (B) Concentration–effect curve for compounds **1a** and **2b**. Each point represents the mean±SE ($n=3$–6).

## Biological Results and Discussion

At 30 μM, all five wedelolactone analogues antagonized the creatine kinase (CK) release[10] induced by *Bothrops jararacussu* venom (Fig. 1A).

Compound **2b** inhibited the venom myotoxic activity in a concentration-dependent manner, with an $IC_{50}$ (1 μM) similar to the one of compound **1a** (wedelolactone, Fig. 1B).

Similarly to compound **1a**, **2b** inhibited the proteolytic and phospholipase activities of Bothrops venom (data not shown).[11] Compound **2b** was further submitted to

radioreceptor and enzymatic assays in order to screen for different potential molecular targets. As shown in Figure 2A, both **1a** and **2b** were relatively potent ($IC_{50} = 0.7$ and 0.5 μM, respectively) for inhibiting rat kidney $Na^+$, $K^+$-ATPase,[12] the enzymatic target for the therapeutic and toxic effects of cardiac glycosides.[13] At about the same concentration ($IC_{50} = 2\,\mu M$), **1a** also inhibited [$^3$H]-flunitrazepam binding to rat brain synaptosomes,[14] indicating a potential modulatory effect on the $GABA_A$/chloride ion channel involved in inhibitory transmission in the central nervous system.[15] On the other hand, **2b** was practically without effect in this assay since its $IC_{50}$ was higher than 100 μM (Fig. 2B).

The newly synthesized **2b** is equipotent to wedelolactone for its antimyotoxic action and inhibition of $Na^+$, $K^+$-ATPase. In addition, compound **2b** is less potent for binding to benzodiazepine receptors, a finding which makes compound **2b** less susceptible to produce adverse effects in the central nervous system. Furthermore, we can hypothesize that some of the other derivatives synthesized could differ in their selectivity for the three main effects described here, due to probable differences in the structural requirements for binding to the respective molecular targets. Further structure–activity studies are needed for selecting profiles of interest.

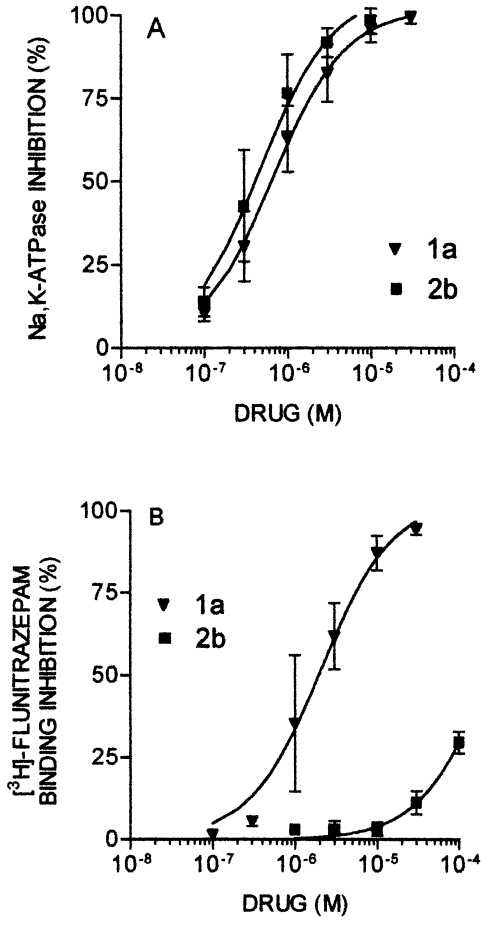


**Figure 2.** (A) Effect of compounds **1a** (wedelolactone) and **2b** on rat kidney $Na^+$, $K^+$-ATPase and (B) [$^3$H]-flunitrazepam binding to rat synaptosomes.


## Acknowledgements

This work has been done in honour of the 80th birthday of Professor Walter B. Mors, who stimulated and initiated the study of wedelolactone as a snake antivenom. Our research was supported by grants from PRONEX (No. 41.96.0888.00), FAPERJ, FUJB-UFRJ and CAPES. A.J.M.S is a postdoctoral fellow of FAPERJ (No. 26/151.081/97). F.N. and P.R.R.C. are fellows of CNPq. F.V.B., C.D.B., D.V.S. and V.P.R. are recipients of CNPq (PIBIC) fellowships. E.S.C.P. is a recipient of the FAPERJ fellowship. We thank ROCHE S/A for the supply of flunitrazepam.


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9. To a solution of **7a–e** (1 mM) in THF (38 mL) was added DDQ (2 mM). The resulting mixture was stirred at room temperature for 2 h. The intermediate *O*-benzylated coumestan precipitated out of solution and it was collected by filtration and washed with cold hexane. The crude product was allowed to react with hydrogen (2 atm in acetone for 3 h). The resulting debenzylated coumestanes **2a–e** were purified by flash chromatography (hexane/AcOEt, 1/1, v/v).
10. In vitro myotoxicity was evaluated at room temperature as previously described.[3a] Briefly, extensor digitonum longos muscles were blotted, weighed rapidly and then transferred to sample collecting units of 2.5 mL capacity, where they were superfused continuously at a rate of 3 mL/min with physiological saline solution (PSS) equilibrated with 95% $O_2$/5% $CO_2$. At 40 min intervals, the solutions perfusing the muscles were collected and replaced with fresh media. The collected samples were stored at 4 °C until their CK activity was determined using a diagnostic kit purchased from Sigma Chemical Co. The rate of CK release from the isolated muscles is expressed as international enzyme units released into the medium per gram of muscle per hour of collection ($U\,g^{-1}\,h^{-1}$). The basal release rate refers to the enzyme loss from the muscles into the PSS at the beginning of the experiment, after the preparations had been mounted in the sample collecting units for at least 1 h.

The composition of the PSS was (mM) NaCl, 135; KCl, 5 $CaCl_2$, 2; $MgCl_2$, 1; $NaHCO_3$, 15; $NaH_2PO_4$, 1; glucose 11. The pH of this solution after equilibration with 95% $O_2$/5%

$CO_2$ was 7.4±0.02. *Bothrops jararacussu* venom alone or associated to the different coumestans was added to the nutrient solution which superfused the isolated muscles.

The experiments with (CK) release were performed in multiple (at least four) independent experiments.

11. Phospholipase $A_2$ activity of *B. jararacussu* venom (1–30 μg/mL) was evaluated by the indirect hemolysis of washed rabbit red blood cell with egg yolk as a substrate, as described earlier.[3]

12. Procedures for preparation of partially purified microsomes from rat kidney and determination of $Na^+$, $K^+$-ATPase inhibition ('Fiske and Subbarrow assay' but with 1.2 mM, instead of 3 mM, ATP) were previously described in: Noël, F.; Godfraind, T. *Biochem. Pharmacol.* **1984**, *33*, 47.

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14. Crude synaptosomes (200 μg protein) prepared from rat brain (without cerebellum and brainstem) were incubated at 4 °C for 90 min in a buffered Krebs solution containing 0.2 nM [$^3$H]-flunitrazepam.

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