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Preliminary Communications

Structural determination *Vitex cymosa* Bertero active principle: Diastereoselective synthesis of (±)-*trans*-4-hydroxy-6-propyl-1-oxocyclohexan-2-one and its antinociceptive activity

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ABSTRACT

The diastereoselective synthesis of (±)-*trans*-4-hydroxy-6-propyl-1-oxocyclohexan-2-one, as a mixture *trans:cis* (3:1), was accomplished using a protocol that combine the Prins cyclization and RuO_4 oxidation. The synthesis this lactone allowed the elucidation of the correct structure of the substance isolated from the barks of *Vitex cymosa*. The δ-lactones mixture showed significant antinociceptive properties in preliminary tests using the tail flick model assay.

1. Introduction

Vitex cymosa (Verbenaceae) is a tree found in Central and Amazonic regions of Brazil, where it is popularly used as antirheumatic [1,2]. Extracts from this plant's barks, collected at State of Maranhão, Brazil, were tested in antinociceptive models, such as the tail flick, in order to assess central analgesia and a significant antinociceptive activity was observed for the dichloromethane extract. The silica gel column chromatography fractioning of this extract led to the isolation of a substance, which structure was assumed to be (6-ethyltetrahydropyran-2-yl)-formic acid, **1** [1,2]. Recently, our group developed a synthetic route to **1** [3–5] (Fig. 1) and the spectroscopic data from a synthetic sample did not match the spectroscopic data reported for the natural product, but this acid presented potent antinociceptive activity in the racemic form [5]. The published spectroscopic data reported for the natural product [1,2] were re-evaluated and the isomeric lactone **2** emerged as the mayoritary structure for the isolated substance (Fig. 1). The spectroscopic data for both diastereoisomers of **2** are available in the literature [6]. Analysis of these data indicated the *trans* isomer as the most likely.

2. Results and discussion

The synthesis of the *cis*/*trans*-lactones **2** was then pursued for evaluation of its antinociceptive activity and for unequivocal structure confirmation. Numerous reports regarding the synthesis of δ-lactones with skeletons analogous to **2** can be found in the literature [6–8], since this moiety is constituent of the statines which are important compounds with known hypocholesteremic activity [9–11]. In our pursuit to synthesize (±)-**2** we have hypothesized a new strategy based on the use of a protocol that combine the Prins cyclization and RuO_4 oxidation as key steps which turned out to be a new, versatile and highly concise strategy to obtain δ-lactones such as **2**. The retroanalysis to **2** is outlined in Scheme 1.

In this strategy, the *trans*-δ-lactone **2** could be obtained through configuration inversion of C4 in the acetyl group of **3**. A regioselective ruthenium tetroxide mediated oxidation of **4** would furnish the *cis*-lactone **3**, which, when hydrolysed, would lead to *cis*-**2**. A

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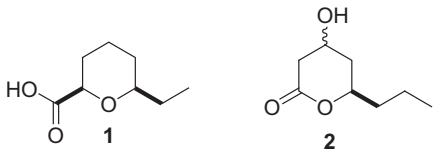


Fig. 1. Proposed structures to natural product from *Vitex cymosa.*

Prins cyclization reaction between the homoallylic alcohol **5** and butyraldehyde **6**, would furnish the *cis*-substituted tetrahydropyran **4**. The lactones **2** and **3** thus obtained could allow the structural confirmation of the isolated natural product.

The Prins cyclization between homoallylic alcohols and aldehydes under strongly protic or Lewis acidic conditions is now emerging as an efficient methodology for the preparation of substituted tetrahydropyrans [12–18,8]. In general, all equatorial substituted 2,4,6-*cis*-tetrahydropyrans are obtained in high diastereoselectivity. Many reports on the literature shows the susceptibility of tetrahydropyran and tetrahydrufuran rings to RuO_4 mediated oxidation, leading to corresponding lactones or anhydrides depending on the substitution pattern of the ether substrate. Such oxidation of tetrahydrofuranes has successfully been used in total synthesis of natural products [19,20]. However, reports about the use of tetrahydropyrans are scarce [21,22].

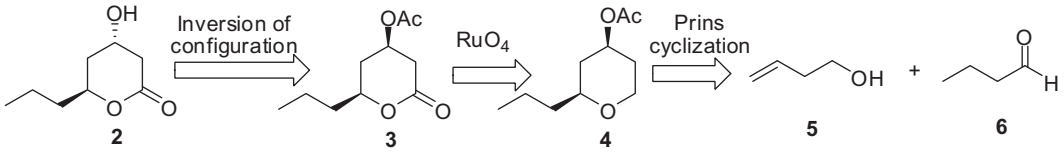
The tetrahydropyran **4** was easily prepared in 85% isolated yield from the reaction between the butyraldehyde **6** and the homoallylic alcohol 3-buten-1-ol **5**, mediated by boron trifluoride etherate in the presence of acetic acid and using cyclohexane as solvent. The diastereoselectivity of this reaction was measured by NMR spectroscopy, where a coupling constant of 2.2 and 4.7 Hz between H_{3eq}–H_{2ax} and H_{3eq}–H_{4ax}, respectively indicated the formation of the 2,4-*cis*-diastereoisomer in 90% diastereoisomeric excess [23]. Interestingly, the use of formaldehyde and homoallylic alcohol **7** in this reaction has only furnished the symmetric product **8** in 51% isolated yield and 91% d.e., for the 2,4,6-*cis*-diastereoisomer (Scheme 2).

The pyran **4** was submitted to the Sharpless protocol [24] for the ruthenium tetroxide catalyzed oxidation. The expected lactone **3** could not be detected and the acid **9** was isolated in 85% yield as the only product (Scheme 3).

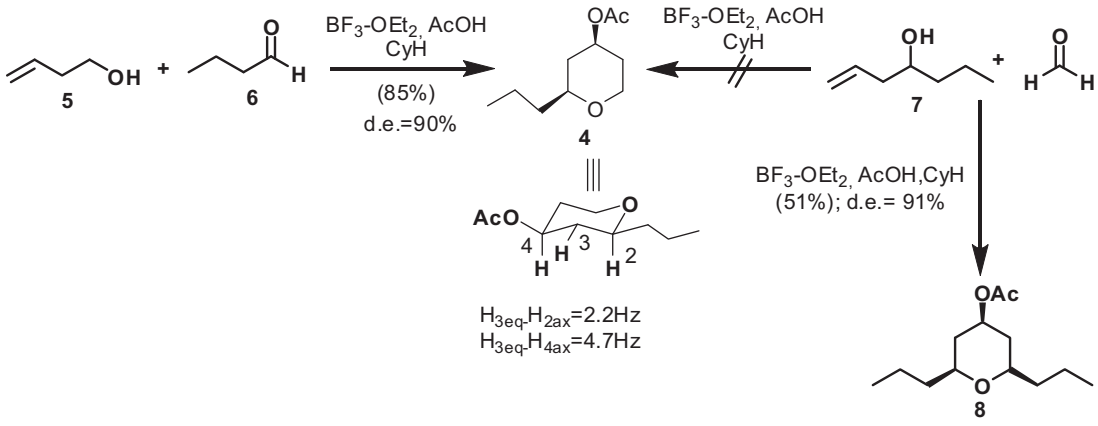
In an attempt to understand the outcome of the oxidation reaction, two possible paths for the formation of the acid **9** are suggested based on reactivity differences between C2 and C6 of **4** (Scheme 4). The first path (path A) implies the oxidation of the methylene at C6 position of **4** tetrahydropyran ring, followed by acidic hydrolysis of the δ-lactone ring of **3** and oxidation of the secondary alcohol present in the *seco*-acid **12**. A second possibility would be the oxidation of the lactone **3**, leading to intermediary **13** which can furnish the acid **9** via a tautomeric equilibrium. The second path (path B) is related to the C2 oxidation of the tetrahydropyran, forming the hemiketal **10** which is in equilibrium with the hydroxyketone form **11**. This ketone is then oxidized by the oxidant leading to acid **9**.

In an attempt to elucidate the regioselectivity issue of C2 and C6 oxidation in the ruthenium tetroxide-mediated reaction (path A versus path B) a competition experiment was conducted. Tetrahydropyrans **4** and the symmetric **8** were simultaneously subjected to oxidation under the same conditions as in the formation of **9** (Scheme 5). Based in this experiment, it is believed that the methyne group in C2 of **4** and the one in **8** presents similar reactivity in the oxidation reaction. In this case, oxidation of **8** leading to diketone **14**, would be observed if path B was to be operative under the reaction conditions.

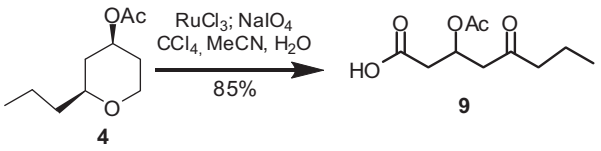
After 24 h at room temperature the products were isolated. Analysis of the crude reaction products (^{1}H NMR and GC/MS) did not show the presence of the diketone **14**, only the acid **9** and the symmetric THP **8** could be detected. The products were separated in a silica gel column, yielding 83% of acid **9** and 86% of recovery of **8**. These results indicate a mechanism through the lactone **3** (Path A) as the most likely pathway leading to acid **9**, where a regioselectivity for the oxidation at C6 methylene position of **4** is observed. This regioselectivity was previously suggested by Bakke and *col* [25]. However, at this point a mechanism through *seco*-acid **12** or acylketal **13** remains unclear.



Scheme 1. Retrosynthesis to obtain **2** via a protocol that combine the Prins cyclization and RuO_4 oxidation.



Scheme 2. Synthesis of **4** and **8** from Prins cyclization reaction mediated by BF_3-OEt.



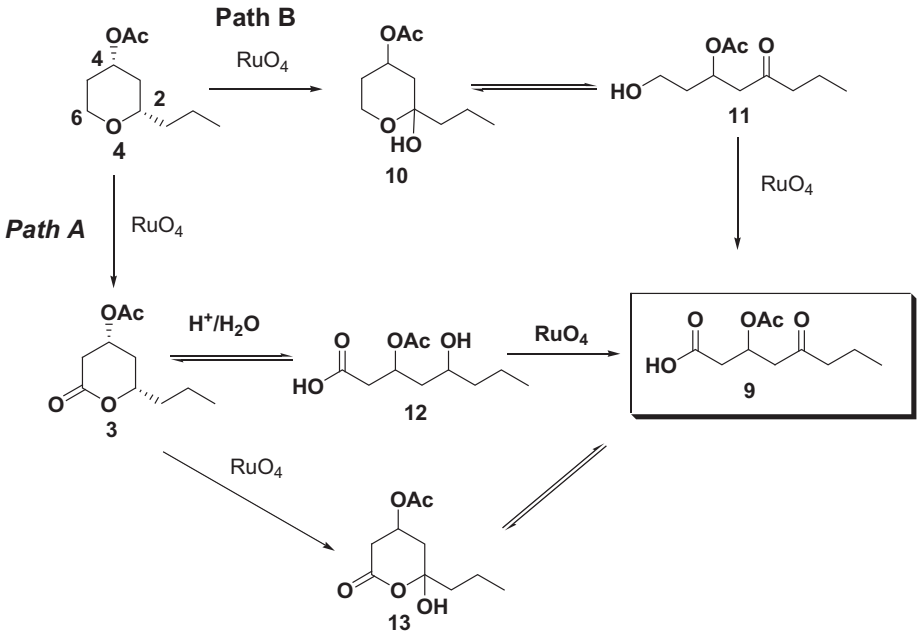
Scheme 3. Oxidation **4**–**9** mediated by ruthenium tetroxide.

The results presented in Scheme 5 indicates the overoxidation of the lactone **3** as the major drawback in the synthesis of *trans*-lactone **2**. During the reaction, a decrease in the pH of the solution is observed and the high susceptibility of δ-lactones to acid hydrolysis is believed to be the cause of the overoxidation of **3** [26]. Attempts to avoid such overoxidation were essayed varying the reaction conditions, as depicted in table 1.

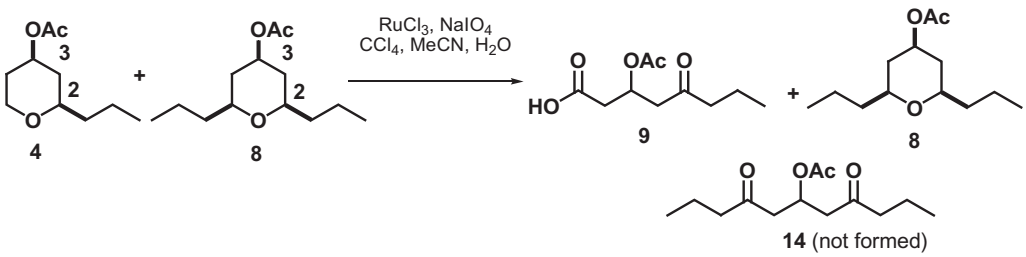
As presented in Table 1, the addition of sodium bicarbonate (entries 2, 4 and 5) during the course of the reaction or the use of buffered solutions (entries 6 and 7), in an attempt to avoid the acidification of the medium and consequently the acid hydrolysis of lactone **3**, inhibited the reaction completely and led only to the recovery of the starting reactants even after 72 h. This result is probably due to the pH dependence of the ruthenium tetroxide–ruthenate–perruthenate equilibrium [27,28]. At a pH above 7, this equilibrium favors the ruthenate specie which is a much milder oxidant that ruthenium tetroxide being unable to achieve the oxidation in question. Concerning the conditions employed in entries 2 and 5, despite the addition of the base, a decrease in the pH was observed during the course of the reaction. This result points to a limitation of the ruthenium tetroxide oxidation of ethers with acid sensitive substrates. This limitation has no precedents in the literature. While other RuO_4 mediated oxidation is in general a very fast reaction, in the presented case the time necessary for complete consumption of the substrate allows the ruthenium mediated oxidation of water as a side reaction which is responsible for the acidification of the reaction media [29]. It was also noted that water is necessary for the reaction, since experiments conducted under anhydrous conditions did not furnished any product.

While unable to overcome the overoxidation of the lactone **3**, during the ruthenium tetroxide mediated oxidation of **4**, the acid **9** could still lead to the desired lactone **2**. In fact, the *trans*-lactone **2** was obtained through a one-pot reduction with sodium borohydride in methanol of the keto and ester groups of **9** followed by lactonization in acidic medium. This reduction led to the lactone **2** in 68% yield as a *trans:cis* diastereoisomeric ratio 3:1 [6]. The comparison of spectral data with the data of natural product [1,2] indicates the *trans*-**2** lactone as the isolated product from *Vitex cymosa*. It is important to note that to the best of our knowledge there are no reports of lactone **2** presence in natural sources.

Preliminary results concerning the antinociceptive activity of lactone **2** were conducted with the diastereoisomeric mixture thus

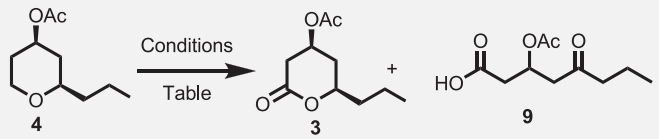


Scheme 4. Proposed paths to obtainment of **9** via oxidation of **4** mediated by ruthenium tetroxide.



Scheme 5. Competition experiments in the oxidation of **4**.

Table 1
Experiments aiming the overcome the overoxidation of **4**–**9**.



Entry	Reaction conditions	Time (h)	Yield (%)[a] 4:3:9
1[c]	$RuCl_3$[b], $NaIO_4$, MeCN, CCl_4, H_2O	24	0:0:85
2	$RuCl_3$[b], Oxone, $NaHCO_3$, H_2O, MeCN, CCl_4	3	0:0:83
3	$RuCl_3$[b], H_2O_2, MeCN, EtOAc	48	77:0:0
4	$RuCl_3$[b], $NaIO_4$, MeCN, CCl_4, H_2O, $NaHCO_3$	72	80:0:0
5	$RuCl_3$[b], Oxone, $NaHCO_3$, EtOAc, H_2O	24	28:0:32
6	$RuCl_3$[b], CH_2Cl_2, NaClO (pH = 7.5)	24	73:0:0
7	$RuCl_3$[b], CH_2Cl_2, $NaBrO_3$, $NaHCO_3$, (pH = 8)	72	77:0:0

[a] After purification by flash chromatography.
[b] 0.02% mol.
[c] Ref. [9].

obtained, once these diastereoisomers proved difficult to separate through silica gel column chromatography.

2.1. Biological studies

Fig. 2 shows the results obtained, in the tail flick model of antinociceptive activity, after oral administration of **2** at the doses of 6, 300, or 600 μmol/kg. This substance developed a maximal effect increasing the antinociceptive [30] activity in 150% after 1 h and decreasing thereafter. When **2** was compared with the analgesic drug morphine at the dose corresponding to its IC_{50} values (in our model) we observed that the curve of **2** (at 6 μmol/kg) was almost the same as that of morphine. Results indicate that substance **2** develops antinociceptive activity more rapidly than morphine and reached higher levels of antinociception.

The significant effect of **2** on tail flick responses in the thermal test further confirmed that this substance had a central antinociceptive effect since the tail flick test is predominantly a spinal reflex [31]. Furthermore, the thermal painful stimuli are known to be selective to centrally but not peripherally acting analgesic drugs [32]. Detailed pharmacological and toxicological essays are under investigation.

3. Conclusion

In conclusion, the correct structure of the substance isolated from the barks of *Vitex cymosa* is reported. The mixture of *cis:trans*-lactones **2** presented significant antinociceptive properties in the preliminary tests by the tail flick model, which supports the traditional use of this plant. In addition, a better understanding of the RuO_4 mediated oxidation of THP rings is also reported, where during the course of the synthesis of **2** the regiochemical issue in the ruthenium tetroxide mediated oxidation of THP rings is disclosed which favors the methylene ring as previously suggested [25].

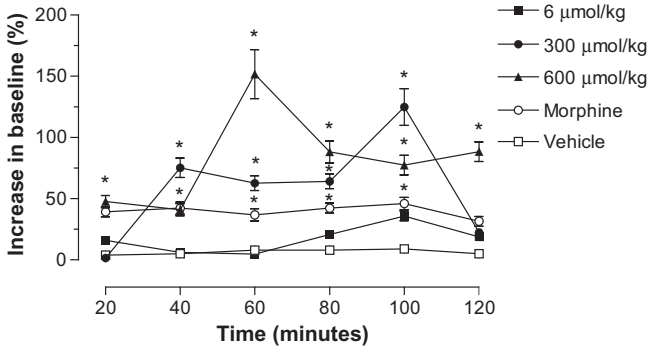


Fig. 2. Effects of the **2** in the tail flick test. The animals were pre-treated with **2**, morphine (10 μmol/kg), or vehicle. The results are presented as the mean ± SD (n = 7–10). Statistical significance was calculated by ANOVA followed by Bonferroni's test. $^*P < 0.05$ was calculated when comparing the morphine- or **2**-treated group with the vehicle-treated group. Where no error bars are shown, it is because they are smaller than the symbol.

4. Experimental

4.1. Materials and methods

All organic and inorganic reagents are commercially available (Aldrich, Across or Merck) and were used as purchased. ^{1}H NMR and ^{13}C NMR spectra were recorded on Gemini-200 (200 MHz) Varian Instruments with TMS as internal reference. The coupling constant (J) is in Hertz (Hz). All solvents were distilled before use. The IR spectra were recorded on a Nicolet Magna-IR-760 spectrometer and only the main bands are reported.

4.2. General procedure for the Prins cyclization

A round bottom flask equipped with a magnetic stir bar was charged with 0.81 mL of acetic acid, 7 mL of cyclohexane, 4.5 mmol of homoallylic alcohol and 9 mmol of aldehyde. The mixture was cooled to 0 °C and after slow addition of 1.2 mL of boron trifluoride etherate was stirred in this temperature for 3 h. To the reaction media was added $NaHCO_3$ sat (10 mL) followed extraction with EtOAc (3 × 10 mL). The combined organic phase are washed with brine (3 × 10 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography on silica gel, (Hexane/EtOAc, 10:1) furnishing **4** (85%) and **8** (51%), both as a pale yellow liquid. Spectroscopy data for **4**: ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 4.87 (dt, J = 11.21, 4.56, 1H); 4.02 (dd, J = 11.81, 4.91, 1H); 3.46 (dd, J = 11.5, 1.9, 1H); 3.36 (m, 1H); 2.04 (s, 3H); 1.94 (m, 1H); 1.9 (m, 1H); 1.71–1.2 (m, 6H); 0.91 (t, J = 6.98, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 170.38; 75.69, 70.40; 65.53; 38.09; 37.51; 31.87; 21,17; 8.44; 13.89. Spectroscopy data for **8**: ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 4.89 (dt, 1H, J = 11.21, 4.79); 3.30 (m, 2H); 2.02 (s, 3H); 1.95 (dd, J = 11.88, 4.79, 2H); 1.71–1.2 (m, 5H); 0.91 (t, J = 6.98, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 170.50; 74.98; 70.74; 38.10; 37.42; 21.24; 18.72; 13.89.

4.3. Synthesis of 3-acetoxy-5-oxooctanoic acid (**9**)

To a solution of **4** (1.01 g, 5.43 mmol) in $CCl_4/CH_3CN/H_2O$ (5/5/10 mL) was added 7.5 g of $NaIO_4$ (35.1 mmol). After 30 min under

vigorous stirring 2.3 mg (0.01 mmol) of $RuCl_3$ were added. The mixture was stirred at room temperature for 24 h, and then diluted with 15 mL of brine. The aqueous phase was extracted with dichloromethane (3 × 10 mL), the combined organic phases were dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography on silica gel eluted with EtOAc yielding **9** (0.99 g, 85%) as colorless oil. IR (neat, υ_{max} cm^{-1}): 3417; 3226; 2965; 2937; 2887; 1738; 1716; 1376; 1242; 1030. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm): 5.58–5.44 (qt, J = 6.2, 1H), 2.85 (t, J = 6.45, 2H), 2.74 (dd, J = 6.02, 4.52, 2H), 2.42 (t, J = 7.32, 2H), 2.00 (s, 3H); 1.60 (qt, J = 7.3, 2H); 0.92 (t, J = 7.37, 3H). ^{13}C RMN (50 MHz, $CDCl_3$) δ (ppm): 207.59; 174.46; 170.08; 66.30; 46.39; 44.88; 37.75; 20.66; 16.72; 13.31.

*4.4. Synthesis of (±)-trans-4-hydroxy-6-propyl-1-oxocyclohexan-2-one (**2**)*

To a solution of **9** (2.0 g, 12.65 mmol) em EtOH (20 mL) under magnetic stirring, at room temperature was added, in small portions, $NaBH_4$ (1.42 g, 37.53 mmol). The suspension was stirred under reflux for 3 h. The reaction media was cooled to room temperature, HCl 10% was added until pH 2 (~10 mL) and the stirring continued for 1 h. The reaction solvent was removed to reduced pressure and EtOAc (50 mL) was added. The organic phase was washed with brine (3 × 40 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography on silica gel (Hex/EtOAc, 7:3) yielding **2** (1.07 g, 60%) as a colorless oil constituted by a mixture of diastereoisomeric δ-lactones (*trans:cis*, 3:1). *Trans*-**2**: ^{13}C RMN (50 MHz, $CDCl_3$) δ (ppm): 171.69; 75.90; 61.89; 38.1; 37.3; 35.2; 17.8; 13.6. *Cis*-**2**: ^{13}C RMN (50 MHz, $CDCl_3$) δ (ppm): 171.9; 77.20; 63.1; 39.1; 37.2; 35.2; 17.8; 13.5.

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[30] Mice were tested according to reference 22. The animals tail were placed on a water bath set at 55 ± 1 °C and the reaction time was recorded when the animals withdraw their tail. The reaction time (s) was measured 40 and 20 min before and 20, 40, 80, 100, and 120 min after oral treatment with 6, 300, or 600 μmol/kg of TD. In order to compare with commercially available analgesic drug, one group was composed by animals that received oral administration 10 μmol/kg of morphine.
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