SYNTHESIS AND PRELIMINARY BIOLOGICAL EVALUATION OF TWO NEW CACALOL ESTERS OF NAPROXEN AND IBUPROFEN

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ABSTRACT

The successful synthesis of two propionates Naproxen and Ibuprofen of the tricyclic terpenic phenol cacalol (Naproxen-cacalol and Ibuprofen-cacalol) is reported. Their NMR data, preliminary anti-inflammatory and analgesic evaluations, and molecular modelling are described. *www.relaquim.com*

Keywords: Anti-inflammatory propionates, Cacalol Esters, Naproxen and Ibuprofen derivatives.

RESUMEN

Se realizó la síntesis de dos propionatos del fenol triciclico terpenico (Naproxen-cacalol and Ibuprofen-cacalol). Se reportan sus datos de RMN, modelaje molecular y evaluación preliminar de sus propiedades antiinflamatorias y analgésicas. *www.relaquim.com*

INTRODUCTION

The pharmacological treatment of inflammatory process was greatly developed in the last two decades. Most of compounds bearing anti-inflammatory properties with quite different structures were synthesized during this period, and several of their mechanisms of action were elucidated but not without controversies, which however lead to better understanding of their activity. Probably, since the first anti-inflammatory agents (AIA), the salicilates, the linkage between analgesic, anti-inflammatory, and antipyretic properties was studied. The arrival of nonsteroidal anti-inflammatory drugs (NSAID), and progressive abandon of salicilates, and antiinflammatory steroidal (SAID) agents, the retreat of VIO-XX from the market, enabled to advance studies in order to achieve a better use, preparation and application of this class of drugs (Jankowski *et al.*, 2004). Two generally used NSAlD are: Naproxen (Nap,

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2-(6-methoxy-2-naphtyl) propionic acid), and Ibuprofen (Ibu, 2-(4-Isobutylphenyl) propionic acid), commonly prescribed for their anti-inflammatory, or analgesic properties. The presence of carboxylic group of propionic acid in both of them is however responsible for their gastrointestinal side effects, which is the most common problem for the acidic drugs of this group, which however could be avoided by esterification. The choice of ester is dependent on the use, and solubility; it could also lead to the substitution by the alcohol residue bearing some physiological properties by itself.

For example, in our previous works, we prepared a series of diastereomeric esters of **Naproxen** and **Ibuprofen** after reduction to their corresponding alcohols (Jankowski *et al.*, 2004). Under hydrolytic conditions, the esters will release the propionic acid during passage through the gastric tract. The corresponding alcohol should produce additional quantities of active acid after the oxidation within the system. In the present paper we synthesized two new esters of Naproxen and Ibuprofen with Cacalol a natural product isolated from *Psacalium decompositum* A. Gray, a popular plant in Mexican traditional medicine and known as "matarique" (Martínez, 1959).

This species, also known as *Senecio grayanus,* Hemsley. *Cacalia decompositae* A. Gray, and *Mesedenia decomposita,* belongs to the Asteraceae, and is widely distributed in the States of Sonora, and Chihuahua, Mexico. It is recognized for various medicinal applications, for instance, the roots tincture is used to calm rheumatic pains, neuralgia, and to improve healing of wounds and ulcers; the root decoction is used for the treatment of diabetes, diarrhea, liver congestion, also used as antiseptic, astringent and cathartic (Díaz, 1976). Romo *et al* (1964) first isolated cacalol, a phenolic compound, as a major constituent from the hexane extract of the roots, and partially characterized it. The structure of compound as a methylated tricyclic cyclohexene furanophenol derivative was finally established by Yuste & Walls (1976) and Soriano *et al* (1987). More recently, pharmacological studies revealed the antibacterial (Jiménez-Estrada *et al.*, 1992) and anti-inflammatory (Jiménez-Estrada *et al.*, 2006) properties of this compound. Pursuing the studies on the anti-inflammatory and anesthetic propionic acid derivatives in **Naproxen** and **Ibuprofen** series, the synthesis of both propionates of cacalol was completed (**1a** and **1b**) (Fig. 1).

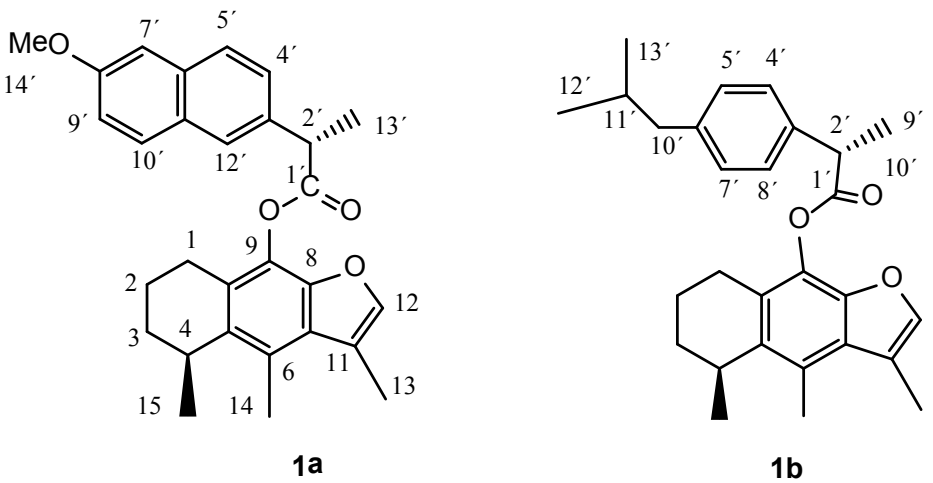

Fig. 1. Cacalol Esters

It is expected that such phenolic esters will maintain the physiological properties of both constituents, and reduce the gastrointestinal effects. The optimization of esterification conditions is also of some practical interest. The blocking of the carboxylic group with easily removable and quite bulky phenol moiety should prevent both the gastrointestinal irritation, and assure easy release of the anti-inflammatory or analgesic agent into the system.

MATERIAL AND METHODS

Reactions and chromatographic fractionation of plant extract were monitored by TLC (Silica Gel, ALUGRAM/SIL G/UV 254), observed under UV light 254, and 365 nm and after spraying the plates with 1.0 % $Ce(S0_4)_2$ solution in H_2S0_4 2N. Prep. TLC was carried out on chromathoplates (ALUMGRAM SIL 100 G/ uv 254). ^{1}H and $^{13}CNMR$ routine spectra were recorded on a 200, and 300 MHz Varian-Gemini, and a 300 MHz JEOL spectrometer with deuterated solvents, and TMS δ=0.0 as internal reference. IR spectra were recorded in a Perkin-Elmer 283B and Nicolet Magna IR TM 750. EI and CI-MS was carried out on a JEOL JMS-AX 5505 HA spectrometer.

Roots and rhizomes of *P. decompositum* were acquired at the Sonora Herbal Market at Mexico City. A specimen is deposited at IMSSM Herbarium (voucher 11489). (S)-Naproxen and (S)-Ibuprofen were bought from Aldrich Chemicals. Their purity was checked by NMR, and their spectroscopic data were in agreement with data previously reported.

High resolution NMR Spectroscopy

NMR 1D experiments were performed using Varian INOVA spectrometer, 300 MHz for proton and 75 MHz for carbon-13, and for 2D experiments Bruker AMX, 600 MHz for proton and 150 MHz for carbon-13 respectively. In all cases the samples recorded at 295 K were prepared in $CDCl_3$ or in dimethylsulfoxide-d6 ca. 10 % solution at 25 °C. Chemical shifts were refereed to the TMS δ= 0.0 ppm and referenced to a residual signal of chloroform at 7.24 ppm.

Selected 2D experiments were run using standard Varian package of INOVA programs for COSY and NOESY. For HSQC experiments the INOVA program for short range coupling (less than 140 Hz for ^{1}JCH) was used. The long range proton-carbon experiments (HMBC) were recorded with Varian INOVA pulse program, optimized for long range couplings (^{3}JCH=9.0 Hz). DQF-COSY spectrum (Rance *et al.*, 1983) was collected into 800x1024 data matrix and the TOCSY spectrum was collected with a mixing time of 80 ms (Rance, 1987).

In all experiments, the carrier frequency was set at the centre of the spectrum.

The (1H, 13C)-HSQC experiments (Bodenhausen G, Ruben D. 1980) were recorded with a delay of 3.5 ms ($_{1}J_{CH}$=143 Hz) and the (1H, 13C)-HSQC-TOCSY experiment with a mixing time for proton-proton transfer of 80 ms in order to identify the network of one bond and several proton-carbon connectivity, respectively. All data was processed with the XWINNMR software and one zero filling and π/3 phase-shifted sine bell window function were applied in both dimensions before Fourier transform.

General Procedure: Esterification of Naproxen and Ibuprofen by cacalol. Naproxen (115.6 mg: 0.502 mmol) or Ibuprofen (208.2 mg: 1.05 mmol) were placed in a 50 mL round flask (1) and dissolved with thionyl chloride (1.0 mL) and kept under reflux (1.5 h, 50 °C) under anhydrous conditions ($CaCl_2$ trap), agitation provided by magnetic stirrer. Reaction mixture was allowed to cool to room temperature, and solvent was reduced to minimum by distillation under reduced pressure.

Cacalol (115.8 mg: 0.503 mmol) was placed in other 50 mL round flask (2) with pyridine (6.0 mL). After this period, this solution (round flask, 2) was added to round

flask (1), and maintained at reflux during one hour under magnetic stirring. After this time, water was added to the reaction mixture, and which was subsequently extracted with EtOAc. The organic fraction was washed with water until achieving neutral pH, and furtherly dried over Na_2S0_4. The solvent was distilled under reduced pressure leading a brown solid. The products mixture was purified by preparative TLC (Silica Gel; Hex-AcOEt 8:2 v/v), obtaining 95.1 mg (42.83 %) of compound **1a**, or 198.7 mg (50.76 %) of compound **1b**. Compound **1a**, brown solid, Rf= 0.63 (Hexane-EtOAc 8:2, v/v). 1a IR ($CHCl_3$, cm^{-1}): 3008 (C-H aromatic), 2935, 2871, 1454, 1393 (C-H aliphatic), 1753 (C=O), 1606 (C=C aromatic), 1237, 1173 (C-O-C). EI-MS (CH_4) (70 eV) m/*z*: 442 $[M]^+$ (11), 230 $[MC_{14}H_{12}O_2]^+$ (38), 215 $[M\text{-}C_{15}H_{15}O_2]^+$ (24), 212 $[M\text{-}C_{15}H_{18}O_2]^+$ (100), 185 $[M\text{-}C_{16}H_{17}O_3]^+$ (100). ^{1}HNMR ($CDCl_3$, 400 MHz), δ (ppm): 7.77 (s, 1H, H-C12´), 7.65-7.69 (m, 2H, HC5´ and H-C10´), 7.49 (dd, J=8.5, 1.8 Hz, 1H, H-C4´), 7.18 (s, 1H, H-C12), 7.09-7.12 (m, 1H, H-C9´), 7.07 (s, 1H, H-C7´), 4.15 (q, J=7.1 Hz, 1H, H-C2´), 3.86 (s, 3H, H-C14´), 3.09-3.13 (m, 1H, H-C4), 2.46 (s, 3H, H-C14), 2.28 (d, J=1.3 Hz, 3H, H-C13), 2.13-2.17 (m, 1H, H-C1), 1.68 (d, J=7.2 Hz, 3H, H-C13´), 1.04 (d, J=7.0 Hz, 3H, H-C15). ^{13}CNMR ($CDCl_3$, 100 MHz), δ (ppm): 172.13 (C=O), 157.68 (C8´), 145.21 (C8), 141.40 (C12), 135.38 (C10), 135.32 (C3´), 131.40 (C9), 129.33 (C10´), 129.01 (C11´), 127.03 (C5), 126.66 (C7), 126.52 (C5´), 126.38 (C4´, C12´), 125.06 (C6), 118.94 (C9´), 116.59 (C11), 105.67 (C7´), 55.31 (C14´), 45.44 (C2´), 29.86 (C3), 28.85 (C4), 23.19 (C1), 21.35 (C15), 18.79 (C13´), 16.46 (C2), 14.22 (C14), 11.27 (C13).

Compound **1b**, beige solid amorphous, 1b Rf= 0.55 (Hexane-EtOAc 8:2, v/v). IR ($CHCl_3$ cm^{-1}): 2953, 2929, 2868, 1454 (C-H aliphatic), 1760 (C-O-ester), 1575 (C=C aromatic). EI-MS (70 ev) m/*z*: 418 M^+, 230 (100), 215 (40). ^{1}HNMR ($CDCI_3$, 300 MHz), δ (ppm): 7.66 and 7.36 (2bs, 2H, 2CH, C-4´and C-8´), 7.19 (bs, 1H, CH, C-11), 7.14 and 7.17 (2s, 2H, 2CH, C-5´and C-7´), 4.06 (q, J= 7.6 Hz, 1H, CH, C-2´), 3.19 (m, 1H, CH, C-4), 2.52 (s, 3H, CH_3, C-14), 2.48 (d, J=7.2 Hz, 2H, CH_2, C-10'), 2.35 (d, J=1.0 Hz, 3H, CH_3, C-13), 2.21 and 2.44 (m, 2H, CH_2, C-1), 1.86 (m, 1H, CH, C-11´), 1.69 and 1.67 (2m, 2H, CH_2, C-3), 1.69 and 1.52 (m, 2H, CH_2, C-2), 1.67 (d, J= 6.9 Hz, 3H, CH_3, C-9'), 1.12 (d, J=7.3 Hz, 3H, CH_3, C-15), 0.91 (d, J=6.45 Hz, 6H, $2CH_3$, C-12' and C-13'); ^{13}CNMR ($CDCl_3$, 75 MHz) δ (ppm): 171.90 (C-1´), 146.03 (C-8), 141.38 (C-11), 139.6 (C-6´), 137.04 (C-9), 135.03 (C-5), 131.05 (C-3´), 129.34 (C-5´), 129.30 (C-7´), 127.40 (C- 4´ and C-8´), 126.5 (C-7), 125.08 (C-6),122.01 (C-12), 116.08 (C-10), 44.8 (C-10´), 44.05 (C-2´), 30.10 (C-11´), 29.86 (C-3), 28.84 (C-4), 23.06 (C-1), 22.23 (C-12´), 21.33 (C-15), 18.33 (C-13´), 16.41 (C-2), 16.01 (C-9´), 14.21 (C-14), 11.28 (C-13).

Anti-inflammatory activity

The anti-inflammatory activity of naproxen-cacalol (**1a**) and ibuprofen-cacalol (**1b**) esters was examined with the TPA induced ear edema in mice as previously described (Jiménez-Estrada *et al.*, 2006), using as positive control ketoprophene.

Analgesic activity

The analgesic activity of both esters naproxen-cacalol (**1a**) and ibuprofen-cacalol (**1b**) were tested using the writhing test in mice as previously developed (Martínez-Vazquez, 1994).

This experimental model is based on measuring the number of writhes in response to intraperitoneal irritation and pain induced by injection of acetic acid. Both compounds **1a** and **1b**, were administered orally (100 mg/Kg) one hour previously to the i.p. administration of 0.1 mL/10 g mice weight of 0.5% acetic acid solution. Immediately afterwards each animal was placed in a acrylic cylinder and the number of writhes were recorded during 20

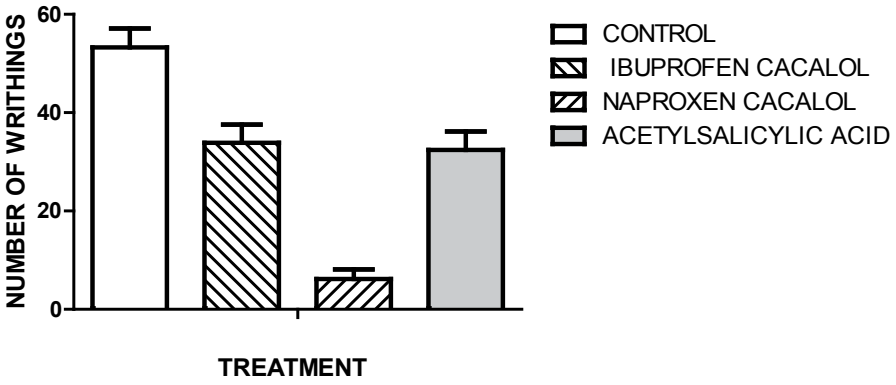

Fig. 2. Analgesic effect of naproxen cacalol (**1a**) and ibuprofen cacalol (**1b**) on mice. Each value is the mean + standard error, n = 10.

minutes. Acetylsalicylic acid (250 mg/Kg) was used as the positive control. Reported values are the mean of ten replicates + standard error.

RESULTS AND DISCUSSION

The synthesis of both propionate esters of **ketoprophen** was successfully accomplished. The yield of synthesis reactions of Nap ester **1a** and Ibu ester **1b**, vary from 42 to 50 % of purified esters and showed the expected spectroscopic properties, in particular the NMR. Both of the esters showed analgesic effects when exposed to the writhing test with mice. Naproxen-cacalol (**1a**) and ibuprofen-cacalol (**1b**) esters, as well as, acetylsalicylic acid inhibited the frequency of writhes as compared with vehicle in 77.1, 36.4 and 39.2 %, respectively.

However, both of the synthesized esters applied topically did not induced a significant reduction in inflammation as compared with the vehicle control, therefore are devoid of activity at the concentrations (0.1, 0.5, 1.0 mg/ear) tested.

In search of an explanation for the lack of anti-inflammatory activity, the molecular modeling of cacalol Nap and Ibu esters **1a** and **1b** complexes with COX-2 type protein was performed using the substrate access channels defined by the MolAxis program as in our previous works performed with different simple anti-inflammatory agents (Jankowski *et al*, 2004). We concluded that the presence of the large cacalol moiety do not allow the ester to enter the heme cavity, and that the substrate is not reaching even the necessary for binding Arg 121 or 521. In this case then it seems that within the physiological tests framework, as the esters do not undergo the hydrolysis they lack antiinflammatory activity, as would occur in the stomach that allow the release of free Nap or Ibu. As far as the cacalol and both propionate derivatives **naproxen** and **ibuprofen** activity alone is concerned, when we tried to model their passage through these channels, and they reached the heme pocket without difficulty. The weak cacalol antiinflammatory activity is then justified, as well as, the already proven activity of both propionic acids **naproxen** and **ibuprofen**. As a hypothesis, it seems that the absence of more pronounced anti-inflammatory activity is certainly related to the fact that products have some difficulty to reach the heme cavity when having the bulky cacalol moiety attached.

CONCLUSION

In this study we tried to optimize the anti-inflammatory and analgesic properties of new compounds by coupling them together through ester bonding. The cacalol molecule is accessible from natural sources, both propionate residues are coming from the popular synthetic drugs naproxen and ibuprofen of known stereochemistry. The esterification, which follows the nucleophilic substitution mechanisms cannot modify the absolute configuration of propionate part of such esters in order to modify bioactivity. Although preliminary, the biological tests are promising, as far as the analgesic properties are concerned, probably the longer exposure to the hydrolytically acidic conditions is necessary to release both anti-inflammatory parts of the esters into the system.

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