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The Triterpenes of *Kageneckia oblonga*

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Abstract

Three known triterpenes were isolated from leaf extracts of *Kageneckia oblonga* by conventional chromatographic methods: ursolic acid, benthamic acid and a third called Kc-III. The structure of Kc-III was determined by RMN spectroscopy, FT-IT and HR-MS. The compound was identified as fern-7-en-3β-ol (motiol), not previously reported in *Kageneckia*.

Keywords: *Kageneckia oblonga*; motiol; triterpene derivative; hopane.

INTRODUCTION

Kageneckia oblonga Ruiz & Pav (known as "Bollén") is an evergreen tree native to Chile. It is found from the northern part of Coquimbo to Malleco in north-central and central Chile, in dry or semi-humid soils below 1800 m elevation in the lower part of the Andes range. Its wood is hard and resistant, due to which the natives used it to construct tools.Gay, 2010) . Leaves and roots of *K. oblonga* are used in ethnomedicine; infusions or powders are used to treat fevers, renal and hepatic lesions and digestive problems. These medicines should be used in moderation, since an excess may cause poisoning. Its use as a medicinal plant has decreased due to this property. Its seeds are sometimes used to treat the "evil eye" (Berguecio, Nicolás García. Pagliotti, 2008; Gay, 2010; Mélica. Muñoz & Barrera, 1981).

Previous studies of *K. oblonga* have reported triterpenes and cyanogenic glucosides, ursolic acid and benthamic acid (Cassels et al., 1973); cucurbitacins and a new cucurbitane triterpene (23,24 dihidrocucurbitacin F). Biological and toxicological trials of cucurbitacins isolated from different extracts of the tree showed high toxicity in dichloromethane and methanol extracts. Biological activity trials to test anti-inflammatory, antipyretic and analgesic effects showed that the cucurbitacins found are partly responsible for these biological effects (Delporte et al., 2002; O. Muñoz et al., 2002). A screening of 31 Chilean medicinal plants evaluated for anti-*Trypanosoma cruzi* activity found that the methanol extract of showed significant inhibition in the MTT trial: MTT IC_{50} = 35.7 ± 40 ug/ml. These trials were performed with *T. cruzi* trypomastigotes (O. Muñoz et al., 2013).

The compound fern-7-en-3β-ol was previously isolated from several plant species, including *Ainsliaea yunnanensis*, *Hibiscus cannabinus*, *Rhododendron macrocepalum*, *Rhodendron linearifolium* and *Scorzonera latifolia*, among others (Acikara et al., 2014; Ageta & Ageta., 1984; Nakamura et al., 1965; Seca et al., 2000). The compound Kc-III has some important pharmacological properties. It has been reported that it has anti-cancer agonistic properties against the cell line THP-1 (IC_{50} of 1.75 µM) used to study leukemia (Li et al., 2016). The presence of this triterpene in *K. oblonga* reinforces the biological effects of this tree.

MATERIAL AND METHODS

Experimental Section

Samples of *K. oblonga* were collected in April, 2017 near the Las Trancas bridge in the Quebrada del Cepillo sector, road G-546, 8 km from the Laguna Aculeo, province of Maipo, Metropolitan Region, Chile (33°50'02.3"S 71°01'17.2"W). The plants (4.2 Kg) were identified by Dr. José San Martin (U. of Talca). A herbarium specimen was stored in the Natural Products Laboratory of the Science Faculty of the University of Chile, identified as N°0017-017. The leaves (used for extraction) were removed from the branches and dried for three weeks in the shade. Then they were ground in an electric grinder with a 1.5 mm grill. The powdered material (2.6 Kg) was placed in 5L glass recipients and degreased with hexane (3*4L), after which a sufficient

volume of DCM was added to cover the plant material (3*3 L). The mixture was percolated with sporadic agitation for 72 hours. The extract was then filtered and concentrated by distilling to dryness under reduced pressure. This procedure was repeated three times, obtaining a total of 18g of DCM extract.

Extraction and Isolation

The total H-DCM extract was placed on a silica gel 60 chromatographic column (Merck). Dichloromethane was used as the mobile phase; 35 fractions were obtained. Chromatographic analysis of the fractions revealed a mixture of the triterpenes ursolic acid and benthamic acid. These compounds were identified by TLC, using commercial standards and estimating their fusion points. The remaining fractions were concentrated in a rotatory evaporator, dried with anhydrous Na_2SO_4, filtered and concentrated to a yellow residue. This fraction was purified by crystallization, dissolving the sample in methanol and then cooling to 0 °C. The result was 0.14g of white Kc-III crystals.

Analysis of the sample

Some of the separation and purification steps were performed in the Department of Phytochemistry and Bioactive Natural Products University of Geneva UNIGE(Suiza). lUHPLC was performed on an Acquity I-Class Plus UPLC system hyphenated with an Acquity photodiode array (PDA) detector (Waters). The separation was performed in an Acquity BEH C18 UPLC column (50 mm × 2.1 mm i.d.; 1.7 µm, Waters), using a generic gradient (MeCN and H_2O both containing 0.1% formic acid) of 5% to 98% MeCN in 4 min, followed by a washing step with 98% MeCN for 2 min. After the washing step, the column was equilibrated with 5% MeCN for 2 min before the next injection. The flow rate was set to 0.6 mL/min, the temperature to 40 °C, and the injection volume was 1 µL. HRMS: HRMS spectra were obtained on a Q Exactive Focus Hybrid quadripole-orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA) using electrospray in positive or negative mode. The spray voltage was set at 3.5 kV or 2.5 kV; the sheath gas flow rate (N_2), 50 units; the capillary temperature, 320 °C; the S lens RF level, 50 and the probe heater temperature, 425 °C. Spectroscopy and complementary spectroscopic analyses of 1H-RMN, ^{13}C-RMN, COSY, HMQC and HMBC were performed in a Bruker AVANCE 400 MHz nuclear magnetic resonance spectrometry at 25 ºC. The Kc-III sample was dissolved in deuterated chloroform. Tetramethylsilane was used as internal standard. The spectra were processed using the Mestre nova 9.0 program. The FT-IR spectrum was obtained from a Bruker FT-IR Perkin-Elmer 1310 spectrometer in KBr disks recorded from 500-4000cm^{-1}. These last RMN and FT-IR analyses were performed in the Instrumentation Unit of the Pontificia Universidad Católica de Chile.

RESULTS

Compound Kc-III was identified as fern7-en-3β-ol (motiol) by spectroscopic comparison and MP: 210-213 °C. (Ageta & Ageta., 1984; Nakamura et al., 1965; Seca et al., 2000).

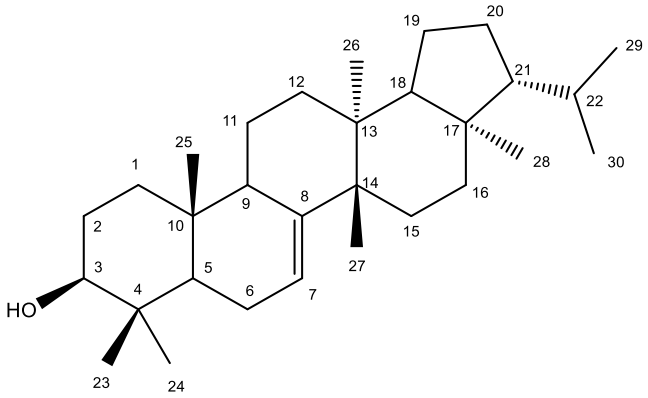

Figure 1. Kc-III structure, fern7-en-3β-ol (motiol).

fern-7-en-3β-ol (motiol): Translucent yellow crystals, apparently rectangular in shape. Yield: 0.14g, 0.004% of dried leaf. MP: 210-213 °C. **IR**: 3500 cm-1, 2941 cm-1,2852 cm-1,2361 cm-1,1469 cm-1,1386 cm-1. **ESIMS**: 514.25 m/z, [M+CH3CN+ HCOOH] +; 498.26 m/z, [M-CH3 + CH3CN + HCOOH] +; 404.20 m/z, [M -OH-CH3CN] +; 227.10 m/z y 167.01 m/z [fragmentation D ring] +. **^{1}H-RMN**: δ 5.37 (d, J = 3.6Hz, 1H, H-7), 3.23 (1H, H-3), 2.34 (m, 1H, H-9), 2.17 (m, 1H, H-6a), 2.00 (m, 1H, H-6b), 1.82 (m, 1H, H-20), 1.73 (m, 1H,H-16a), 1.69 (m, 4H, H-1,2), 1.58 (m, 1H, H-11a), 1.54 (m, 1H, H-11b), 1.50 (m, 1H, H-16), 1.48 (m, 1H, H-18), 1.46 (m, 1H, H-15), 1.43(m, 1H, H-22), 1.40(m, 1H, H-19a), 1.35 (m, 1H, H-12a), 1.33 (m, 1H, H-5), 1.32 (m, 1H, H-12b), 1.25 (m, H, H-19b) 1.24 (m, H, H-20), 1.06 (m, 1H,H-2), 0.99 (s, 3H, H-26), 0.96 (s, 4H, H-24,H-21), 0.91 (s, 3H, H-30), 0.89 (s, 3H, H-27), 0.85 (s, 3H, H-23), 0.83 (d, J = 6.7 Hz3H, H-29), 0.74 (s, 3H, H-25), 0.73 (s, 3H, H-28).**^{13}C-RMN**: δ 145.29 (C-8), 116.28 (C-7), 79.41 (C-3), 59.71 (C-21), 54.26 (C-18), 50.89 (C-5), 48.04 (C-9), 42.99 (C-17), 41.66 (C-14), 39.10 (C-4), 36.95 (C-2); 36.41 (C-16), 36.21 (C-13), 35.48 (C-10), 32.47 (C-12), 30.82 (C-22), 30.44 (C-15), 28.37 (C-20), 27.85 (C-1), 27.67 (C-24), 24.29 (C-6), 24.15 (C-26), 23.14 (C-29), 22.26 (C-30), 21.20 (C-27), 20.15 (C-19), 16.22 (C-11), 14.76 (C-23), 14.19 (C-28), 13.01 (C-25).

DISCUSSION

The IR spectrum of compound Kc-III allowed the rapid assignment of the signal at 3500 cm^{-1}, assigned to an alcohol. The absorbances between 2350 and 2450 cm^{-1} were assigned to an alicyclic skeleton, with possible presence of a double bond, and the proton of neighboring hydrogens to OH. Complementary analysis with reported spectroscopic data for this kind of structure allowed deducing that Kc-III should have a hopane skeleton.

The mass spectrum signals showed that ion 514.25 m/z could be assigned to the sum of the masses of the sample, acetonitrile and formic acid [M+ CH_3CN+ HCOOH]$^+$. The 498.26 m/z and 496.24 m/z fragments correspond to the mentioned adduct, but with a loss of 15 uma, typical of the loss of a methyl group [M- CH_3+ CH_3CN+ HCOOH]$^+$ for 498.26m/z. By the 18 m/z difference from 514.25m/z it may be inferred that the signal at 496.24 m/z is due to dehydration, [M+ CH_3CN+ HCOOH- H_2O]$^+$. Ion 404.20 m/z was assigned to the mass of the molecule, with loss of the hydroxyl at C-3 and isopropyl in C-21 forming two new double bonds in their respective carbons that underwent the elimination and addition of acetonitrile [M-OH-58]$^+$.

In consequence, compound Kc-III has the molecular formula $C_{30}H_{50}O$. The C-NMR showed 30 signals, including eight methyl groups, nine methylene groups, seven methine groups and four quaternary carbons, all deduced from the DEP 135 experiments. The high-field signals of the H-NMR spectrum showed six singletons, assignable to tertiary methyl groups and two secondary methyl groups, assigned as doublets. The δ 5.37ppm signal (1H, q, J = 3.3 Hz) is typical of double bonds, ratifying the IR signal at 3050 cm^{-1} and 1639 cm^{-1}, while the H-3 axial singleton at δ 3.23 ppm is displaced to the low field due to the presence of the 3 β-OH group in C-3.

The analysis of the bi-dimensional spectra and the conclusions of the previous paragraphs allow assigning the double bond to the B ring, and the connectivity shown in Figure 2. The interactions of the hydrogens (COSY) show the correlations of the H-22 with the hydrogens of the H-29 and H-30 methyl groups; the interaction of H-22 with H-21. Both interactions describe the union of the isopropanol group in the C-21 carbon, which coincides with the fractioning of the ms of 167 m/z, corresponding to a retro Diels-Alder ion of the E ring after losing a methyl group.

The HMBC spectrum (S12) shows evidence of the union of the A-B, B-C, C-D and D-E rings by the interactions of the C-3 and H-5 atoms; H-5 with C-9, H-9 with C-12; H-12 with C-18 and H-18 with C-21, respectively. The position of the geminal group formed by C-23 and C-24 in the A ring is inferred by the interactions with C-5 and H-3 with the methyl substituents in C-4. The position of C-24 between the union of the A-B rings is deduced from the interactions of C-5 and C-9. C-26 and C-27 in the union of the C-D rings is interpreted by the interaction of its hydrogens with C-13, C-14, C-15 and C-18. The C-28 methyl is assigned as the substituent in C-17 by the interactions of H-28 with the four carbons that surround C-17. The interaction of C-29 and C-30 and the interaction of its CH_3 hydrogens with C-21 reaffirm the isopropyl structure in the molecule.

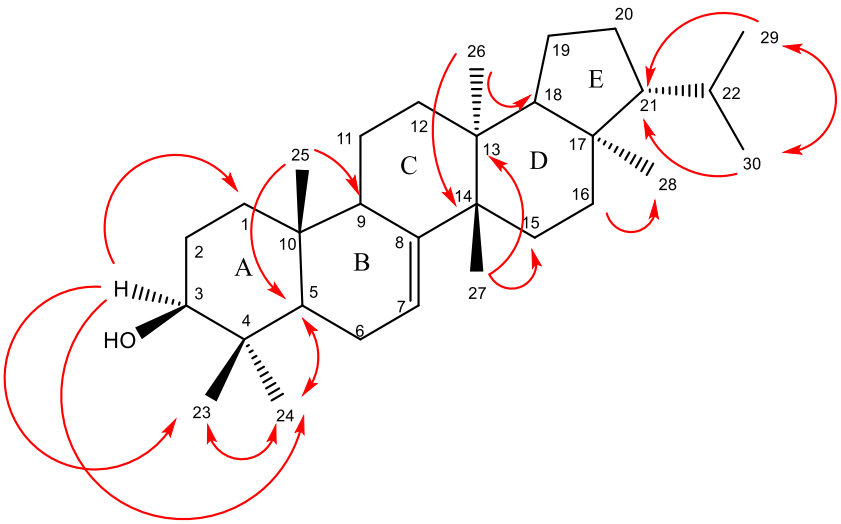

Figure 2. HMBC coupling of the Kc-III structure.

CONCLUSION

The triterpene fern-7-en-3β-ol (motiol) is reported for the first time in the genus *Kageneckia*, and thus in *K. oblonga*. The presence of motiol in the leaves of *K. oblonga* along with the presence of other substituted triterpenes and cucurbitacins would explain some of the medicinal properties of this tree. The toxicity of its

extracts recommends against its medicinal use. However, this native Chilean tree has potential uses as an antiparasite, anti-inflammatory and anti-carcinogen. Thus pharmacological *in vitro*, *in vivo*, pre-clinical and clinical trials should be continued, to produce the bases for a specific and sure phytopharmaceutical for the population, which self-medicates with crude extracts.

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Competing Interests: The authors declare that there are no competing interests.

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