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Artigo

Isolation, Bioactivity and Structural Studies of 6-acetyl-7-hydroxy-2,2-dimethylchoromene from *Calea hispida*

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Isolamento, Bioatividade e Estudos Estruturais do 6-acetil-7-hidroxi-2,2-dimetilcromeno de *Calea hispida*

Resumo: Os voláteis obtidos de folhas de *Calea hispida* foram analisados por GC-MS-FID e apresentaram (*E*)-nerolidol, β-cubebeno, espatulenol, α-cadinol e 6-acetil-7-hidroxi-2,2-dimetilcromeno como componentes principais, sendo este último o de maior quantidade (54,76%), que foi cristalizado e analisado por difração de raios X. Ensaios microbiológicos indicaram atividade deste cromeno contra *Helicobacter pylori* (ATCC 26695), o que estimula uma investigação mais aprofundada a este respeito. O estudo da estrutura cristalina não mostrou nenhuma diferença em relação à descrita antes para este composto, enquanto otimização por mecânica quântica indicou que a estrutura do cristal está próxima de uma conformação de energia mínima, o que inclui a posição relativa do grupo hidroxila ao grupo acetila. De fato, mapeamento de energia potencial e dinâmica molecular mostraram que a ligação de hidrogênio intramolecular entre estes dois grupos é bastante estável, tanto em fase gasosa quanto em solução.

Palavras-chave: 6-acetil-7-hidroxi-2,2-dimetilcromeno; *Calea hispida*; dinâmica molecular; *Helicobacter pylori*.

Abstract

The volatiles from Calea hispida leaves were analyzed by GC-MS-FID and presented (*E*)-nerolidol, β-cubebene, spathulenol, α-cadinol and 6-acetyl-7-hydroxy-2,2-dimethylchromene as main components, the latter being the major one (54.76%), which was crystallized and analyzed by X ray diffraction. Microbiological assays indicated activity of this chromene against *Helicobacter pylori* (ATCC 26695), which prompts a deeper investigation. The crystal structure study showed no difference from that reported before for this compound, while quantum mechanics optimization indicated that the crystal structure is close to a minimum energy conformation, which includes the relative position of the hydroxyl to the acetyl moiety. In fact, potential energy scan and molecular dynamics showed that the intramolecular hydrogen bond within these two moieties is quite stable, both in gas phase and in solution.

Keywords: 6-acetyl-7-hydroxy-2,2-dimethylchromene; *Calea hispida*; molecular dynamics; *Helicobacter pylori* activity.

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Isolamento, Bioatividade e Estudos Estruturais do 6-acetil-7-hidroxi-2,2-dimetilcromeno de *Calea hispida*

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1. Introduction

Most species of the genus *Calea* (Asteraceae) are found in subtropical regions of the Americas. This genus encompasses about 110 species, with a large number of occurrences in Central America and South America, including Brazil.[1]

The volatile compounds obtained from

Calea species by hydrodistillation include monoterpenes, sesquiterpenes and aromatic substances, as observed for *Calea pinnatifida*,[2] *Calea clematidea*[3] and *Calea serrata*; the latter, particularly, presented the chromene precocene II as its major volatile component[4]. Precocene II is known for its interference in the secretion of juvenile hormones in insects and has great potential as acaricide.[5] In a previous study on the *Calea hispida* chemistry, two petroleum ether extracts led to the isolation of several sesquiterpenes, sesquiterpene lactones and hydroxyacetophenones from the leaves, and thymol and four chromene derivatives from the roots in low yields, including 6-acetyl-7-hydroxy-2,2-dimethylchromene.[6] That first study, however, was conducted in Germany with plant material collected in northeastern Brazil, but the location and time of collection were not exactly specified. In this study, on the volatile components from the leaves of *C. hispida* collected in southern Brazil, the chromene was found as the main component and it was easily obtained in pure crystalline form.

Chromenes in general have important biological properties, such as estrogenic;[7] anticancer;[8] antivascular;[9] antimicrobial;[10-11] antifungal;[12] antioxidant;[13-14] anticonvulsant;[15] antiviral;[16] TNF-α inhibitor;[17] anti-HIV;[18] antitubercular[19] and anti-inflammatory activity.[20-21]

In this study, we present the extraction, purification and spectroscopic identification of 6-acetyl-7-hydroxy-2,2-dimethylchromene from the leaves of *C. hispida*. For the first time, this compound has been obtained in large amounts and, in pure form, assayed for bactericidal activity towards *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 11778), *Helicobacter pylori* (ATCC 26695), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Bacillus subtilis* (ATCC 23857). We also carried out a number of theoretical and experimental studies regarding its three dimensional structure and conformations, which might foster future activity optimizations.

2. Experimental procedures

2.1. Plant collection

The botanical material was collected near Highway PR-513, Ponta Grossa, Paraná, Brazil, on 3 December 2012, at 9:00 o'clock under climatic conditions of high humidity and temperature of 17 °C, at coordinates 25°06'25'' south and 50°02'14'' west, altitude 1,030 m, growing in a soil with good natural fertility. The vouchers were identified as *Calea hispida* BAKER, which is a botanical heterotypic synonym of *Calea triantha* (Vell.) Pruski, and deposited under number 19062 at the State University of Ponta Grossa Herbarium (HUPG).

2.2. Extraction and chromatographic analyses of the volatiles

Fresh leaves (121 g) were separated immediately after collection and subjected to hydrodistillation for 3 hours in a 2 L flask fitted with a glass apparatus[22] containing 1 L of distilled water. The volatiles were taken up in diethyl ether, the water was decanted and the solvent evaporated at room temperature giving a 0.21% yield (w/w). The oil was analyzed by TLC on silica gel plates using neat $CHCl_3$ or $CHCl_3$/hexane (2:1) as mobile phases. The chromatograms were observed under UV light of 254 and 366 nm and then revealed with $FeCl_3$ (5% in MeOH) and H_2SO_4/MeOH (1:1), followed by heating. The composition of the oil was qualitatively analyzed in a Shimadzu® GCMS-QP2010 Plus chromatograph using the software GC-Solution® Workstation 5:51 under the following conditions: samples diluted in ethyl ether; injections of 1 μL in split mode with a ratio of 1:20; RTX-5MS® column (30 m × 0.25 mm × 0.25 μm); helium as carrier gas at a flow rate of 1 mL/min; operation in scanning mode (40-550 u); electron impact of 70 eV; temperature of 250 °C in the injector and ion source; interface temperature of 280 °C; heating program: oven at 60 °C for 5 minutes followed by elevation of 3 °C/min up to 240 °C, remaining there for 5 min. The components were identified by their mass spectra associated with the respective relative retention indices (IRR), calculated on the basis of the retention times (Rt) of a series of n-alkanes (C9-C25). The determination of the oil relative composition was conducted using a GCFID Shimadzu® 2010 gas chromatograph equipped with an OV-5® column (30 m × 0.25 mm × 0.25 μm), hydrogen as carrier gas at a flow rate of 1 mL/min and other conditions as described above.

2.3. Bactericidal activity assay

Commercial strains of the pathogenic bacteria *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 11778), *Helicobacter pylori* (ATCC 26695), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Bacillus subtilis* (ATCC 23857) were previously grown in liquid LB culture medium until the optical density ($OD_{600\ nm}$) reached between 0.5-0.7. Then, they were transferred, in a laminar flow hood, to ELISA 96-well plates, so that each well was prepared with 198 μL of bacterial culture and 2 μL of the compound solution in DMSO, to give a final compound concentration in the well of 100 μg/mL. For each tested bacterial strain, experiments were carried out with two controls. In the positive control, the compound solution was replaced by an equal volume of culture medium; in the (DMSO) interference control, the replacement was made with pure DMSO, to give a final concentration of 1% in the well. All wells were prepared in triplicate. After assemblage, the plate was taken to a micro-plate spectrophotometer (Epoch spectrophotometer microplate-BioTek Synergy®, Winooski, VT, USA) and absorbances were read at $OD_{600\ nm}$, which was considered the initial reading. The plate was then left at 37 °C in a shaker at 50 rpm for 24 hours and the final reading was performed. For each bacterium, inhibition percentages were calculated based upon the effect of the compound on bacteria growth subtracted from eventual sole DMSO effect. Therefore, inoculum growth without the presence of DMSO and compound was considered complete growth without inhibition, and the inoculum growth reduction in the presence of compound subtracted of the DMSO effect gave the inhibition percentage.

2.4. Crystallization of 6-acetyl-7-hydroxy-2,2-dimethylchromene

The volatiles were taken up in ethyl ether, the solvent was evaporated at room temperature and then crystals were observed, which were separated and washed with cold hexane for purification, providing about 150 mg of pure crystalline compound.

2.5. X ray crystallography structure determination

Crystals were mounted on a mitegen loop with the help of polybutene and led to a goniometer under a nitrogen stream at 100 K. Diffraction images were collected with a Bruker® Venture V8 diffractometer at Federal University of Paraná with a microfocus copper tube equipped with a kappa goniometer and processed with the apex2® software.[23] The structure was solved by direct methods and refined with the shelx suite of programs (Sheldrick, 2008).

2.6. Structure minimization and conformation by quantum chemistry

The gamess package was used for structure optimization with the Restricted Hartree-Fock method and the 31G basis set with 6 gaussians.[24] Then, a potential energy surface was calculated in which the torsional angles of the hydroxyl and acetyl groups were frozen from 0 to 345° at a 15° step, in all combinations. The structures were optimized and their energies calculated in the same way as that for the free structure optimization.

2.7. Ring puckering coordinate calculations and structure superpositions

For both the crystallographic and the quantum mechanics optimized structures, the Cremer and Pople ring puckering coordinates were calculated with the program conforma.[25-26] Structure superpositions were performed with program lsqkab from the CCP4 suite.[27-28]

2.8. Molecular dynamics studies

The gaff force field was used to parameterize the chromene molecule and the TIP3 model was used for water molecules.[29-30] RESP (restrained electrostatic potential) charges for the chromene were estimated using the REDServer with 64 different conformations in which the quantum mechanics optimized structure had its hydroxyl and acetyl group torsions changed from 0 to 315°,[31] at a 45° step. The sander module of Amber was used for energy minimizations and dynamic calculations.[32] Initially, the chromene molecule was immersed in a water box so that there was a minimum distance of 12 Å to any border. Then, the following steps were pursued: i) system energy minimisation with strong restraints on the chromene, until convergence; ii) complete system minimization until convergence; iii) system heating from 0 to 300 K during 50 ps with weak restraints on the chromene, iv) short dynamics with weak restraints on the chromene for 50 ps, v) exploration dynamics for 2 ns. In all cases, a cut-off of 12 Å was applied for the non-bonded interactions. Snapshots of each trajectory at equal intervals (0.5 ps to give 4000 snapshots) were recorded and used for distance/angle analyses. The whole procedure was repeated 10 times with different initial random seeds and the results were averaged.

3. Results and Discussion

In the CG-MS-FID analysis, the main component of the sample (54.76%) was identified as 6-acetyl-7-hydroxy-2,2-dimethylchromene (1) (Figure 1). This component presented an RRI of 1768.67±2.65 (6 injections) with the use of apolar capillary columns (RTX-5MS® and OV-5®), which is not included in the data banks NIST107, NIST21 and WILEY8 and appears here for the first time in the literature. The mass spectrum showed $M^{+\cdot}$ = m/z 218 ($C_{13}H_{14}O_3$) and as principal mass fragments m/z 203 (100%) and m/z 185 (20%). Other spectroscopic analysis results for the oil follow: FT-IR (KBr, cm^{-1}): 3650, 3052, 2970, 1647, 1577, 1439, 1266. UV (MeOH, $\lambda_{máx}$, nm): 256.3, 349.6; (MeOH+NaOH): 259.0, 357.6; (MeOH+NaOH+HCl): 257.7, 348.8. ^1H RMN ($CDCl_3$, δ): 5.57 d, 1H, 10.0Hz; 6.28 d, 1H, 10.0Hz; 7.30 s, 1H; 6.32 s, 1H; 2.53 s, 3H; 1.44 s, 6H; 12.70 s, 1H. ^{13}C RMN ($CDCl_3$, ppm): 202.32 (C), 165.10 (C), 160.45 (C), 128.88 (CH), 128.57 (CH), 120.99 (CH), 113.93 (C), 113.55 (C), 104.48 (CH), 77.89 (C), 28.54 ($2xCH_3$), 26.19 (CH_3).

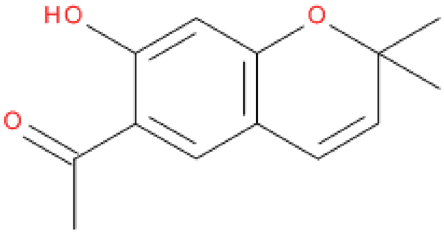

Figure 1. Chemical structure of 6-acetyl-7-hydroxy-2,2-dimethylchromene (1)

The compounds identified in the essential oil are shown in Table 1, where it can be observed that only two sesquiterpenes are just above the 5% proportion, whereas the main component, 6-acetyl-7-hydroxy-2,2-dimethylchromene, accounts for almost 55%.

Table 1. Relative composition (%) of the essential oil from leaves of *C. hispida* as analyzed by GC-MS-FID

Components	**(%)**	**RRI_{cal}***	**RRI_{lit}****
β-cubebene	5.19	1389	1390
germacrene D	0.52	1480	1480
bicyclogermacrene	0.81	1496	1494
(*S*)-(*Z*)-α-bisabolene	1.18	1504	1504
δ-cadinene	0.16	1523	1524
2,6-dibutyl-4-methyphenol	1.44	1512	1512
(*E*)-nerolidol	5.29	1563	1564
spathulenol	2.43	1578	1576
β-oplopenone	1.94	1584	1579
viridiflorol	0.45	1592	1590
cis-isolongifolanone	0.58	1603	1606
α-cadinol	2.29	1657	1653
cis-β-santalol	1.09	1716	1720
(**1**)	54.76	1769	-
Total identified (%)	**78.13**		

*RRI_{cal} = calculated relative retention indices; **RRI_{lit} = published relative retention indices.[33]

In previous studies, compound 6-acetyl-7-hydroxy-2,2-dimethylchromene was isolated from *C. hispida* and from other species belonging to several genera of the Asteraceae family,[34-36] including *Ageratina arsenei*, which allowed its first X ray diffraction structure determination.[36] In the present study, this chromene was identified initially in the volatile extract from *C. hispida* leaves by spectroscopic techniques and obtained in pure form by successive recrystallizations.

The analysis of the crude oil by TLC revealed the presence of the main component under UV light, which showed positive reaction for phenols. The presence of the phenol compound was confirmed by the observation of a bathochromic effect in the oil UV spectrum in alkaline medium. Its massive presence was demonstrated by the GC-MS analysis and its structure could be established by analyses of the FTIR, ^{1}H and ^{13}C NMR spectra, which are in very good agreement with published data.[35]

3.1. Chromene purification

Crystals of 6-acetyl-7-hydroxy-2,2-dimethylchromene have been previously obtained from a hexane extract of the plant *Ageratina arsenei* and analyzed by X ray diffraction, after purification by chromatography using Hexane-EtOAc to increase the polarity.[36] In this study, crystals could be observed even in the crude volatile oil; they were collected after evaporation and washed in hexane, which indicates the compound high crystallizability.

3.2. Biological assays

The assays revealed that the compound inhibited 23.4% on average, with 2.3% standard deviation, of *H. pylori* growth (ATCC 26695), notably even with the rather low concentration used in the assay. On the other hand, bacteria *S. typhimurium* (ATCC 14028), *S. aureus* (ATCC 6538), *B. cereus* (ATCC 11778), *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922) and *B. subtilis* (ATCC 23857) were not affected by the compound.

Although the plants are promising sources for discovering new antibiotics, the antimicrobial activity displayed by the compound 6-acetyl-7-hydroxy-2,2-dimethylchromene, against *H. pylori* bacteria is relevant, especially when one considers that the chromene class of compounds features a diverse number of activities that include antimicrobial activity.[10,38] When comparing the structure of this compound class with the structure and properties of the main categories of penicillins that are in clinical use, [38] one observes them to be quite distinctive.

The gram-negative bacterium *H. pylori* is found in the stomach causing chronic gastritis and ulcer. Currently, patients infected by this bacterium are treated with omeprazole, amoxicillin and clarithromycin. Yet it is a common bacterium, there have been reports of resistance to currently used drugs,[39-40] which reinforces the need for new medicines against it. Biological assays of 2-oxo-2*H*-chromene-3-carboxamide derivatives have already shown them to be active against *H. pylori*.[10] We emphasize that the compound 6-acetyl-7-hydroxy-2,2-dimethylchromene showed activity against this bacterium in its native form in the essential oil and is present in large amounts in *C. hispida* leaves; such feature has not been reported before for chromenes. The search for new natural or synthetic molecules with bactericidal or bacteriostatic activity is due to the urgent need for new antibiotic agents that can combat efficiently infectious diseases, the second leading cause of mortality worldwide.[41] According to the World Health Organization (WHO)[42] resistance to commercially available antibiotics can be considered a serious threat to global public health and this adds to the fact that the total number of new antimicrobial agents approved by the Food and Drug Administration (FDA)[43] has been decreasing.[42] It is, therefore, desirable to find new molecules that act upon bacteria through different mechanisms.

3.3. Crystallographic analyses

The structure was refined to final R1 and wR2 values of 6.70% and 16.39%, respectively, space group P-1 with two molecules in the asymmetric unit. It is essentially identical to the structure[37] available at the Cambridge Structural Database[44] under deposition code number 910196. An asymmetric unit superposition showed that the rmsd is 0.014 Å and the largest deviation is 0.059 Å on one of the methyl carbons, which attests the identity between the two crystal structures. We found in our refined structure that the phenyl rings present as Cremer and Pople Q coordinate the values 0.015 and 0.005 Å, therefore, they are basically planar as expected, while the pyran rings present Q = 0.250 and 0.168 Å, θ = 65.5 and 63.8°, ϕ = 36.1 and 35.9°, therefore, they are in a hybrid conformation between half-chair and twisted boat, in which the oxygens and the sp^3 carbons are the most out-of-plane atoms. These compare quite well to the values Q = 0.254 and 0.169 Å, θ =65.0 and 65.3°, ϕ = 35.8 and 36.1° in the previously cited similar structure solved.

3.4. Quantum chemistry minimization and surface potential for the intramolecular hydrogen bond

The quantum chemistry optimized structure shows a favorable intramolecular hydrogen bond interaction, in which the H-A (hydrogen-hydrogen acceptor) distance is 1.83 Å and the D-H-A (hydrogen donor-hydrogen-hydrogen acceptor) angle is 136.5°. When one superposes this to the two crystallographic structures in the asymmetric unit, the less similar structure leads to an overall rmsd of 0.106 Å and the largest deviation is 0.240 Å, between one of the methyl carbons. Yet, Cromer and Pople ring puckering parameters for the minimized structure are Q = 0.003 Å for the phenyl ring and Q = 0.161 Å, θ = 65.9°, ϕ = 35.0° for the pyran ring, therefore, again quite similar to the crystallographic structures.

To investigate further the stability conferred to the molecule by the intramolecular hydrogen bond, a potential energy surface scan was produced (Figure 2). One can observe a peak (negative energy) showing the stabilization property of the intramolecular hydrogen bond at and around 0° for the torsional angles (convention, angle value for both groups in which the hydrogen bond is favored), with a difference between this and a conformer in which these torsions are at 180° of circa 28,7 kJ/mol.

3.5. Molecular dynamics

The input structure for molecular dynamics analyses comprehended both hydroxyl and acetyl torsional angles so that they were in opposition for intramolecular hydrogen bonding, i.e., 180° as the convention used. According to the simulations, on average at about 0.073 ns, these two moieties come close to form a hydrogen bond (using a 120° D-H-A angle and a 3.5 Å H-A distance cut off criteria). Thereafter, during the remaining dynamics time, one observes that on average 82.2% of the time, the hydrogen bond is kept in water solution, according to the cited cut off criteria. These results indicate the stability this internal interaction presents, even in aqueous environment.

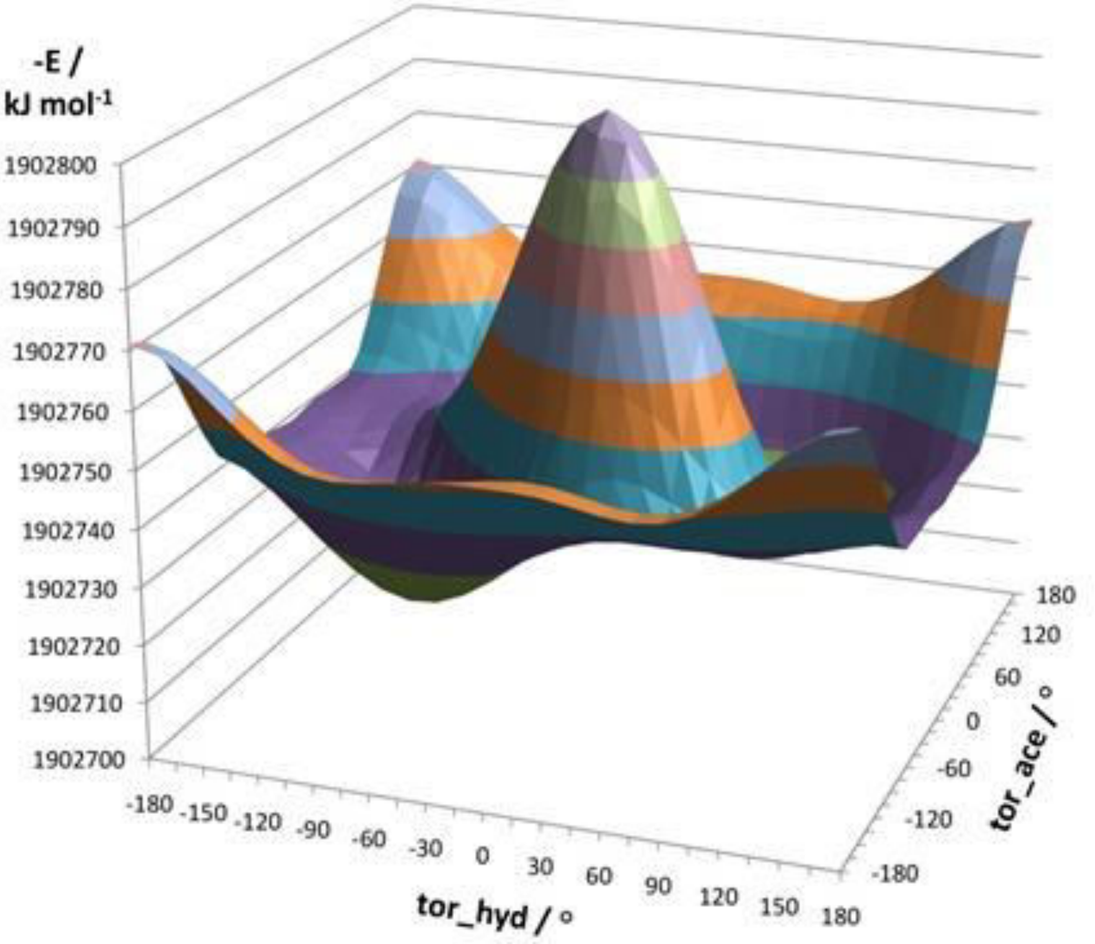

Figure 2. Potential energy surface. Hydroxyl and acetyl group torsional angle scans

4. Conclusions

In this work, 6-acetyl-7-hydroxy-2,2-dimethylchromene was isolated in high yield from the leaves of *C. hispida* using hydrodistillation and crystallization, which allowed the determination of its relative retention index (RRI) as 1768.67±2.26 in two apolar capillary columns (RTX-5MS® and OV-5®) for the first time. Hydrodistillation proved to be advantageous in the isolation of similar chromenes when compared to the use of organic solvents. The isolated compound presented activity towards *H. pylori*, showing a potential starting material to develop new compounds with antimicrobial activity. During the structural studies, we confirmed the compound to be the same chromene that was previously isolated from *C. hispida*, however, the yield obtained in this study was much higher and the compound was separated directly from the volatiles.[6] Its crystal structure was basically the same reported in other previous work.[36] Quantum mechanics structure optimization revealed that the overall structure, the pyran ring and the torsional angles for the hydroxyl and acetyl moieties are basically in a low energy state in the crystal. Also, potential energy scan and molecular dynamics showed that the intramolecular hydrogen bond is quite stable, both in the gas phase and in solution.

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References

[1] Nascimento, A. M.; Oliveira, D. C. R. A 5-deoxyflavone glycoside from *Calea uniflora* L. (Asteraceae). *Biochemical Systematics and Ecology* **2004**, *32*, 1079. [CrossRef]

[2] Kato, E. T. M.; Akisue, M. K.; Matos, F. J. A.; Craveiro, A. A.; Alencar, J. M. Constituents of *Calea pinnatifida*. *Fitoterapia* **1994**, *65*, 377. [Link]

[3] Flach, A.; Gregel, B.; Simionatto, E.; Silva, U. F.; Zanatta, N.; Morel, A. F.; Linares, C. E. B.; Alves, S. H.; Chemical analysis and antifungal activity of the essential oil of *Calea clematidea*. *Planta Medica* **2002**, *68*, 836. [CrossRef] [PubMed]

[4] Ribeiro, V. L. S.; dos Santos, J. C.; Martins, J. R.; Schripsema, J.; Siqueira, I. R.; von Poser, J. L.; Apel, M. A. Acaricidal properties of the essential oil and precocene II obtained from *Calea serrata* (Asteraceae) on the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). *Veterinary Parasitology* **2011**, *179*, 195. [CrossRef]

[5] Ribeiro, V. L. S.; Vanzella, C.; Moisés, F. S.; dos Santos, J. C.; Martins, J. R. S.; Siqueira, I. R.; Poser, J. L. Effect of *Calea serrata* Less. *n*-hexane extract on acetylcholinesterase of larvae ticks and brain of Wistar rats. *Veterinary Parasitology* **2012**, *189*, 322. [CrossRef]

[6] Bohlmann, F.; Gupta, R. K.; Jakupovic, J.; King, R. M.; Robinson, H. Furanoheliangolides and farnesol derivatives from *Calea hispida*. *Phytochemistry* **1980**, *21*, 2899. [CrossRef]

[7] Jain, N.; Xu, J.; Kanojia, R. M.; Du, F.; Jian-Zhong, G.; Pacia, E.; Lai, M. T.; Musto, A.; Allan, G.; Reuman, M.; Li, Xi.; Hahn, D.; Cousineau, M.; Peng, S.; Ritchie, D.; Russell, R.; Lundeen, S.; Sui, Z. J. Identification and structure–activity relationships of chromene-derived selective estrogen receptor modulators for treatment of postmenopausal symptoms. *Medicinal Chemistry* **2009**, *52*, 7544. [CrossRef] [PubMed]

[8] Aliaa, M. K.; Manal, M. K.; Eman, K. A. E.; Alshemy, H. A. H. Design and synthesis of substituted chromenes as potential anticancer agents. *International Journal of Pharmaceutical Research and Development* **2012**, *4*, 310. [Link]

[9] Gourdeau, H.; Leblond, L.; Hamelin, B.; Desputeau, C.; Dong, K.; Kianicka, I.; Custeau, D.; Boudreau, C.; Geerts, L.; Cai, S.-X.; Drewe, J.; Labrecque, D.; Kasibhatla, S.; Tseng, B. Antivascular and antitumor evaluation of 2-amino-4-(3-bromo-4,5-dimethoxy-phenyl)-3-cyano-4H-chromenes, a novel series of anticancer agents. *Molecular Cancer Therapeutics* **2004**, *3*, 1375. [PubMed]

[10] Chimentia, F.; Bizzarria, B.; Bolascoa, A.; Seccia D.; Chimentia, P.; Carradoria, S.; Granesea, A.; Rivanerab, D.; Lillib, D.; Zicaric, A.; Scaltritod, M.M.; Sistod, F. A novel class of selective anti-*Helicobacter pylori* agents 2-oxo-2H-chromene-3-carboxamide derivatives. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, 3065. [CrossRef] [PubMed]

[11] Sangani, C. B.; Shah, N. M.; Patel, M. P.; Patel, R. G. Microwave assisted synthesis of novel 4h-chromene derivatives bearing phenoxypyrazole and their antimicrobial activity assessment. *Journal of the Serbian Chemical Society* **2012**, *77*, 1165. [CrossRef]

[12] Thareja, S.; Verma, A.; Kalra, A.; Gosain, S.; Rewatkar, P. V.; Kokil, G. R. Novel Chromeneimidazole derivatives as antifungal compounds: synthesis and in vitro evaluation. *Acta Poloniae Pharmaceutica* **2010**, *67*, 423. [PubMed]

[13] Johnson, A. J.; Kumar, R. A.; Rasheed, S. A.; Chandrika, S. P.; Chandrasekhar, A.; Baby, S.; Subramoniam, A. Antipyretic, analgesic, anti-inflammatory and antioxidant activities of

two major chromenes from *Melicope lunu-ankenda*. *Journal of Ethnopharmacology* **2010**, *130*, 267. [CrossRef]

[14] Mldenovic, M.; Mihailovic, M.; Bogojevic, D.; Matic, S.; Niciforovic, N.; Mihailovic, V.; Vukovic, N.; Sukdolak, S.; Solujic, S. *In Vitro* Antioxidant Activity of Selected 4-Hydroxy-chromene-2-one derivatives - SAR, QSAR and DFT studies. *International Journal of Molecular Sciences* **2011**, *12*, 2822. [CrossRef]

[15] Bhat, M. A.; Siddiqui, N.; Khan, S. A. Synthesis of novel 3-(4-acetyl- 5H/methyl-5-substituted phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2H-chromen-2-ones as potential anticonvulsant agents. *Acta Poloniae Pharmaceutica* **2008**, *65*, 235. [PubMed]

[16] Mori, J.; Iwashima, M.; Takeuchi, M.; Saito, H. A synthetic study on antiviral and antioxidative chromene derivative. *Chemical & Pharmaceutical Bulletin* **2006**, *54*, 391. [CrossRef]

[17] Cheng, J. F.; Ishikawa, A.; Ono, Y.; Arrhenius, T.; Nadzan, A. Novel chromene derivatives as TNF-α inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2003**, *13*, 3647. [CrossRef]

[18] Karia, D. C.; Pandya, H. K; Godvani, N. K. G. Synthesis, characterization & anti-HIV activity of 4-hydroxy-3-(5-methylisoxazol-3-yl)pyrano(3,2-C)chromene-2,5-dione. *Asian Journal of Biochemical and Pharmaceutical Research* **2012**, *2*, 126. [Link]

[19] Kamdar, N. R.; Haveliwala, D. D.; Mistry, P. T.; Patel, S. K. Synthesis and evaluation of in vitro antitubercular activity and antimicrobial activity of some novel 4H-chromeno[2,3-d]pyrimidine via 2-amino-4-phenyl-4H-chromene-3-carbonitriles. *Medicinal Chemistry* **2011**, *20*, 854. [CrossRef]

[20] Johnson, A.J.; Kumar, R.A.; Rasheed, S.A.; Chandrika, S.P.; Chandrasekhar, A.; Baby, S.; Subramoniam, A. Antipyretic, analgesic, anti-inflammatory and antioxidant activities of two major chromenes from *Melicope lunuankenda*. *Journal of Ethnopharmacology*. **2010**, *130*, 267. [CrossRef] [PubMed]

[21] Nitin, K.; Sushil, K.; Himanshu, G.; Sharma, P. 3-Hydroxy-2- (substituted phenyl) -4H-chromen-4-one derivatives- synthesis, spectral characterization and pharmacological screening. *World Research Journal of Biochemistry* **2012**, *1*, 1. [Link]

[22] Stahl, E.; Schild, W. *Pharmazeutische Biologie, 4: Drogenanalyse II – Inhaltsstoffe und Isolierungen.* Gustav Fischer Verlag: Stuttgart:, 1981. [CrossRef]

[23] Bruker, APEX2, SAINT and SADABS. Bruker AXS Inc., Madison, Wisconsin, USA **2009**. [Link]

[24] Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. H.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S. J.; Windus, T. L.; Dupuis, M.; Montgomery, J. A. General atomic and molecular electronic structure system. *Journal of Computational Chemistry* **1993**, *14*, 1347. [CrossRef]

[25] Cremer, D.; Pople, J. A. A General definition of ring puckering coordinates. *Journal of the American Chemical Society* **1975**, *97*, 1354. [CrossRef]

[26] Iulek, J.; Zukerman-Schpector, J. Conforma: a program for determining ring conformations and puckering coordinates. *Química Nova* **1997**, *20*, 433. [CrossRef]

[27] Kabsch, W. A solution for the best rotation to relate two sets of vectors. *Acta Crystallographica, Section A: Foundations of Crystallography* **1976**, *32,* 922. [CrossRef]

[28] Winn, M. D.; Ballard, C. C.; Cowtan, K. D.; Dodson, E. J.; Emsley, P.; Evans, P. R.; Keegan, R. M.; Krissinel, E. B.; Leslie, A. G. W.; McCoy, A.; McNicholas, S. J.; Murshudov, G. N.; Pannu, N. S.; Potterton, E. A.; Powell, H. R.; Read, R. J.; Vagin, A.; Wilson, K. S. Overview of the CCP4 suite and current developments. *Acta Crystallographica, Section D: Biological Crystallography* **2011**, *67*, 235. [CrossRef] [PubMed]

[29] Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of simple potential functions for simulating liquid water. *Journal of Chemical Physics* **1983**, *79*, 926. [CrossRef]

[30] Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. Development and testing of a general amber force field. *Journal of Computational Chemistry* **2004**, *25*, 1157. [CrossRef]

[31] Vanquelef, E.; Simon, S.; Marquant, G.; Garcia, E.; Klimerak, G.; Delepine, J. C.; Cieplak, P.; Dupradeau, F. Y. Server: a web

service for deriving RESP and ESP charges and building force field libraries for new molecules and molecular fragments. *Nucleic Acids Research* **2011**, *39*, 511. [CrossRef] [PubMed]

[32] Case, D. A; Darden, T. A.; Cheatham, T. E.; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Walker, R. C.; Zhang, W.; Merz, K. M.; Roberts, B.; Hayik, S.; Roitberg, A.; Seabra, G.; Swails, J. A.; Goetz, W.; Kolossvai, I.; Wong, K. F.; Paesani, F.; Vanicek, J.; Wolf, R. M.; Liu, J.; Wu, X.; Brozell, S. R.; Steinbrecher, T.; Gohlke, H.; Cai, Q.; Ye, X.; Wang, J.; Hsieh, M.-J.; Cui, G.; Roe, D. R.; Mathews, D. H.; Seetin, M. G.; Salomon-Ferrer, R.; Sagui, C.; Babin, V.; Luchko, T.; Gusarov, S. A. Kovalenko and P. A. Kollman, AMBER 12; Program for Molecular Dynamics; University of California, San Francisco **2012**. [Link]

[33] Adams, R. P. *Identification of essential oil components by Gas Chromatography – Mass Spectroscopy.* Allured: Carol Stream, 1995. [Link]

[34] Bohlmann, F.; Jakupovic, J. Über neue Chromene und andere Inhaltsstoffe von Lagascea rigida. *Phytochemistry* **1978**, *17*, 1677. [CrossRef]

[35] Bohlmann, F.; Grenz, M. Neue Isopentenyl-acetophenon-derivate aus *Helianthella uniflora*. *Chemische Berichte* **1970**, *103*, 90. [CrossRef]

[36] Zhai, H. L.; Zhao, G. J.; Yang, G. J.; Sun, H.; Yi, B.; Sun, L. N.; Chen, W. S.; Zheng, S. Q. A new chromene glycoside from *Tithonia diversifolia*. *Chemistry of Natural Compounds* **2010**, *46*, 198. [Link]

[37] Gómez-Hurtado, M. A.; Aviña-Verduzco, J.; González-Campos, B.; López-Castro, Y.; Rodríguez-Garcia, G.; Cerda-García-Rojas, C. M.; Del Rio, R. E. X-Ray diffraction and NMR studies of two chromenes from the roots of *Ageratina arsenei*. *Revista Latinoamericana de Química* **2012**, *40,* 199. [Link]

[38] Siqueira, J. S.; Lima, P. S. S.; Barreto, A. S.; Quintans-Junior, L. J. Aspectos gerais nas infecções por *Helicobacter pylori* revisão. *Revista Brasileira de Analises Clinicas* **2007**, *39,* 9. [Link]

[39] Picoli, S. U.; Mazzoleni, L. E.; Fernández, H.; Bona, L. R.; Neuhauss, E.; Longo, L.; Prolla, J. C. Resistence to amoxicillin, clarithromycin and ciprofloxacin of *Helicobacter pylori* isolated from southern Brazil patients. *Revista do Instituto de Medicina Tropical de São Paulo* **2014**, *56,* 197. [CrossRef] [PubMed]

[40] Guimarães, D. O.; Momesso, L. S.; Pupo, M. T. Antibióticos: importância terapêutica e perspectivas para a descoberta e desenvolvimento de novos agentes. *Química Nova* **2010**, *33,* 667. [CrossRef]

[41] Tang, Y.; Wei, J.; Zhong, W.; Liu, X. Synthesis and characterization of chroman-containing compounds and their preliminary assessment of cytotoxicity toward two human cancer cell lines. *Heteroatom Chemistry* **2010**, *21,* 423. [CrossRef]

[42] World Health Organization. Available in: <http://www.who.int/>. Access on: 5 January 2016.

[43] U.S. Food and Drug Administration. Available in: <http://www.fda.gov/Drugs/>. Access on: 5 January 2016.

[44] Allen, F. H. The Cambridge Structural Database: a quarter of a million crystal structures and rising. *Acta Crystallographica, Section B: Structural Science* **2002**, *58,* 380. [CrossRef] [PubMed]