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NMR and X-ray studies of apetalic acid isolated from *Calophyllum brasiliense* and of its chiral amides

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Abstract: The tropical tree *Calophyllum brasiliense* (Clusiaceae) grows in the rain forests from Brazil to Mexico. Its leaves, as well as those of other *Calophyllum* species, are rich sources of chromanone acids, such as apetalic acid, isoapetalic acid, and their derivatives. Apetalic acid has shown significant antimycobacterial activity. The biological activity of apetalic acid has been related to the configuration of three asymmetric centers and the stereochemistry of the molecule; however, the C-19 configuration in the acidic side chain has not been fully resolved. For this reason, the unequivocal determination of the absolute configuration by means of X-ray crystallography in a sample of unique homogeneous apetalic acid stereoisomer was the most important point to start this study. We prepared some chiral amides using the carboxyl group. We determined the C-19 stereochemistry of apetalic acid, and its specific chiral derivatives, using NMR, X-ray diffraction methods, and molecular mechanics. Finally, we observed that steric hindrance in the side chain of apetalic acid leads to restriction of rotation around the pivotal link C-10 and C-19 establishing chiral centers at C2(*R*), C3(*S*), and C19(*R*). We were able to separate derivatives of these two high-rotatory-barrier conformers of apetalic acid by forming diastereoisomeric amides with phenylglycine methyl ester having a chiral center at C-2'. Our results allowed the conclusion of the existence of atropisomerism in the apetalic acid molecule.

Key words: NMR of apetalic acid chiral amides, absolute configuration and conformation, X-ray studies versus NMR, stable conformers of apetalic acid, high-rotatory-barrier conformers of apetalic acid.

Résumé : L'arbre *Calophyllum brasiliense* (Clusiaceae) pousse dans les forêts tropicales du Brésil jusqu'au Mexique. Ses feuilles, ainsi que celles d'autres espèces de *Calophyllum*, sont de riches sources de chromanone acides, tels que l'acide apétalique, l'acide isoapétalique et leurs dérivés.

L'acide apétalique a montré une activité antimycobactérienne importante. L'activité biologique de l'acide apétalique a été liée à la configuration de trois centres asymétriques et la stéréochimie de la molécule; cependant, la configuration en C-19 dans la chaîne latérale acide n'a pas été entièrement résolue. Pour cette raison, la détermination sans équivoque de la configuration absolue au moyen de la cristallographie aux rayons X sur un échantillon d'acide apétalique de stéréoisomère unique homogène a été le point le plus important pour commencer cette étude. Nous avons préparé des amides chiraux en utilisant le groupement carboxyle. Nous avons déterminé la stéréochimie de l'acide apétalique en C-19 et ses dérivés chiraux spécifiques, en utilisant la RMN, les méthodes de diffraction des rayons X et la mécanique moléculaire. Enfin, nous avons observé qu'un encombrement stérique dans la chaîne latérale de l'acide apétalique entraîne une restriction de rotation autour du lien pivot C-10 et C-19 établissant des centres chiraux en C2(*R*), C3(*S*) et C19(*R*). Nous avons pu séparer des dérivés de ces deux conformères à haute barrière de rotation de l'acide apétalique en formant des amides diastéréoisomériques avec l'ester méthylique de la phénylglycine possédant un centre chiral en C-2'. Nos résultats permettent de conclure à l'existence d'atropisomérisme dans la molécule d'acide apétalique.

Mots-clés: RMN d'amides chiraux de l'acide apétalique, configuration absolue et conformation, études par rayons X versus RMN, rotamères stables de l'acide apétalique, conformères à barrière de rotation élevée de l'acide apétalique.

Introduction

The *Calophyllum* genus belonging to the Clusiaceae is composed of about 200 tree species with pantropical distribution.¹⁻² Most of them thrive within the Indo-Pacific zone, particularly from the Malay Peninsula to New Guinea. Only eight species are found in the American Continent, where *C. brasiliense* is the most distributed species, growing in the tropical rain forests from Brazil to Mexico.³ Several *Calophyllum* species are known to be rich sources of chromanone acids, such as apetalic acid (**1**). This compound was first isolated from *C. apetalum*⁴ and afterwards from several other species, such as: *C. brasiliense*,⁵⁻⁶ *C. caledonicum*,⁵ *C. blancoi*,⁷ *C. pinetorum*,⁸ *C. polyanthum*,⁹ *C. decipiens*,¹⁰ *C. rivulare*,² *C. antillanum*,¹¹ and *C. ferrugineum*.¹²

The leaves, bark, and latex of *Calophyllum* species display constant presence of apetalic acid (**1**), full name 3-(5-hydroxy-2,3,8,8-tetramethyl-4-oxo-2,3,4,8-tetrahydropyrano[3,2-g]chromen-10-yl)hexanoic acid), but also of isoapetalic acid (**2**), and their derivatives. The main difference between **1** and **2** is the relative configuration of two methyls (C-15 and C-16) on the C-2 and C-3 carbons of the γ -pyrane moiety of the molecule, which is *cis* in apetalic acid (**1**) and *trans* in isoapetalic acid (**2**) (Fig. 1).

Apetalic acid (**1**) showed good activity against Gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, and *Mycobacterium tuberculosis*; however, it did not show any activity against Gram-negative bacteria.¹² It also showed high antioxidant activity measured by DPPH test.¹⁰ On the other hand, this compound showed cytotoxicity activity against A-549 (human breast cancer) and MCF-7 (human lung carcinoma) cell lines: apetalic acid and isoapetalic acid display similar CC₅₀ values against A-549; in contrast, apetalic acid was more toxic than isoapetalic acid against MCF-7.¹² Besides, apetalic acid is cytotoxic in KB (human oral epidermoid carcinoma) and Hela (human cervical epithelioid carcinoma) cell lines. Nevertheless,

the methyl ester and 5-*O*-acetate of apetalic acid are even more potent against these cell lines.⁷ Apetalic acid and isoapetalic acid tested *in vitro* at 1 mM were shown to inhibit in 2.0 and 20.6% HIV reverse transcriptase.⁶

Apetalic acid (**1**), as most chromanone acids, possesses three asymmetric centers, two on the C-2 and C-3 of the 2,3-dimethylchromanone ring, and one on the methine carbon C-19 of the hexanoic acid side chain.¹³ The configuration of the three asymmetric centres of apetalic acid (**1**) was the object of several reports but generated also several ambiguities.^{4-5, 7, 13-17} In the quite abundant literature on this subject, the names of apetalic and isoapetalic acids (**1** and **2**) were sometimes used without taking care of details of their stereochemistry. It is possible that the right application of Cahn-Ingold-Prelog rules together with the plant origin and isolation procedure were one source of differences in assignments. Most of the assignments concerned the two skeleton asymmetric carbons (C-2, C-3), the more difficult side-chain carbon C-19 remained undefined. Some existing low-resolution X-rays done without determination of the absolute configuration, NMR, IR, and the optical rotation as well as limited degradations served the conclusions to the determination of such partial configuration of apetalic acid (**1**) as being 2*R*,3*S* or its diastereomer 2*S*,3*R*.^{4-5, 7, 13-17} However, the configuration of C-19 in the acidic side chain has not been completely resolved; it can be *R*, as suggested by the isolation of optically active (*R*)-(+)-pentylsuccinic acid from the oxidative degradation of blancoic acid.¹⁸ The determination of the absolute configuration at C-19 reported in the literature is often *R*.¹⁹ In some cases, however, it could be the result of an incorrect assignment. Also, the possible epimerization at C-19 during sample isolation cannot be completely turned down. This asymmetric carbon was also supposed to have the *S* configuration by X-ray crystallography in the related caloteysmannic acid form *Calophyllum teysmannii*.²⁰ For this reason, the unambiguous determination of the absolute

configuration by X-ray crystallography on a homogeneous single stereoisomer sample of apetalic acid (**1**), and in particular the absolute configuration at C-19, was the most important point to start this study.

We prepared some chiral amides using the carboxyl group, and address a quite peculiar observation that the steric hindrance at the side chain of **1** is leading to the restriction of rotation around pivotal C-10–C-19 bond. In this respect, the optical purity of **1** at C-19 is of crucial importance.

In order to answer these questions, the cautioned determination of the optical purity at C-19 was performed this time using different NMR methodology. The X-ray recording among the determination of the absolute configurations at C-19 will complete our existing data and help to study the phenomena of coexistence in solid state of two stable conformers at this rotor site. Finally, we would like to answer a question if the hindered rotation is responsible for the existence of two stable conformers of **1**.

We were also interested in determining the possible existence and population of rotamers of **1** in solution using high-resolution NMR, and to compare these results with solid state. The determination of the C-19 stereochemistry of apetalic acid (**1**), and of its specific chiral derivatives, by using NMR, X-ray diffraction methods, and molecular mechanics is reported, and discussed in this paper.

Experimental

Extraction, isolation, and purification of apetalic acid

Calophyllum brasiliense was collected in San Andrés Tuxtla, state of Veracruz, Mexico. A voucher was deposited with the number 14425 at Herbarium of Mexican Institute for Social

Security (IMSSM) in Mexico City. The leaves were dried at room temperature and powdered (945.5 g). The leaves were extracted with hexane, and further concentrated in a rotary evaporator. A mixture of the triterpenoids friedelin and canophyllol precipitated spontaneously during solvent evaporation. Part of the extract (16 g) was fractionated by silica gel (70-230 mesh) column chromatography (CC) eluting with a gradient of hexane–ethyl acetate gradually enriched. Similar fractions in TLC were combined to give 27 pooled fractions. Fraction 18 eluted with a 95:5 mixture was subjected to chromatography column (silica gel C-18). Apetalic acid (**1**) (520 mg) was obtained in subfractions eluted with acetone–water (7:3). Crystallized from hexane–diethyl ether, yellow cubes, mp = 120–121 °C. $[\alpha]^{25}_D = +29.41^\circ$ (c 1.7, CHCl₃); reported mp = 117 °C, $[\alpha]^{25}_D = +28.43^\circ$ (c 2.56, CHCl₃).

Chemicals

(*S*)-1,1'-binaphthalene-2,2'-diol [(*S*)-BINOL], (*R*)- and (*S*)-phenylglycine methyl ester (PGME), dimethylformamide (DMF), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), 1-hydroxybenzotriazole (HOBT), and *N*-methylmorpholine were purchased from Sigma–Aldrich (St. Louis, MO).

(*S*)-BINOL experiment

10 mg of apetalic acid (**1**) was dissolved in 0.7 mL of CDCl₃, then up to four portions of 3.6 mg each of (*S*)-BINOL, total of 14.4 mg, corresponding to the molar ratio 1:4, was added as solid powder to this solution. The solutions were stabilized at room temperature for 5 min, and spectra recorded on 400 MHz NMR spectrometer (Bruker Advance III, Instituto de Química, UNAM).

Preparation of (*R*)- and (*S*)-PGME amides of apetalic acid

To a stirred solution of apetalic acid (**1**) (25 mg, 0.064 mmol) and (*R*)- or (*S*)-PGME (17 mg, 0.084 mmol) in 0.34 ml of dry DMF were added PyBOP (44 mg, 0.085 mmol), HOBT (13 mg, 0.085 mmol), and *N*-methylmorpholine (45 μ l) at 0 °C. The mixture was stirred at room temperature, while the reaction was monitored by TLC on silica gel using hexane–ethyl acetate 3:1 as eluent until apetalic acid had quantitatively reacted (3 h), then 5 ml of ethyl acetate was added, and the reaction mixture was washed with one volume of 5% HCl, saturated NaHCO₃, and brine. The organic layer was dried with Na₂SO₄, filtered, and concentrated. The reaction mixture was purified on a 1 g silica-based Sep-Pak (Waters, Milford, MA) using hexane–ethyl acetate 12:1 to 8:1 as eluent to afford the PGME amide.

X-ray crystallography

A yellow crystal of apetalic acid (**1**), of dimension 0.36 $\times$ 0.32 $\times$ 0.22 mm, was mounted on a fiber glass and placed on a Bruker D8 venture diffractometer with Cu-target micro-focus X-ray source (1.54178 Å). The detector was placed at 50 mm from the crystal. Frames were collected with a scan width of 0.3° in the ω scan and the exposure time of 10 s/frame at 150 K. Frames were integrated with the Bruker SAINT software package using a narrow-frame integration algorithm. Systematic absences and intensity statistics were used in system monoclinic, space group P2(1). The structure was solved using direct methods using SHELXS-2014/7 program.²¹ The remaining atoms were located via a few cycles of least squares refinements and difference Fourier maps. Hydrogen atoms were input at calculated positions and allowed to ride on the atoms to which they are attached. Thermal parameters were refined for hydrogen atoms on the phenyl groups using a $U_{eq} = 1.2$ Å and a $U_{eq} = 1.5$ Å for methyl groups to precedent atom in all cases. The final cycle of

refinement was carried out on all non-zero data using SHELXL-2014/7.²¹ Absorption correction was applied using SADABS program.

NMR spectroscopy

The compounds were dissolved in CDCl₃, and TMS was used as an internal standard. High-resolution ¹H (300.3 MHz) and ¹³C (75.5 MHz) NMR spectra were acquired between 20 and 40 °C on a Chemagnetics Infinity 300 spectrometer using Nalorac 5 mm probes: z-gradient inverse broadband ¹H{¹⁵N–³¹P} or dual ¹³C/¹H. Connectivities between protons were obtained using 2D gradient-selected COSY.²² The 2D phase-sensitive NOESY²³ experiment was performed with a mixing time of 500 ms. The 1D ¹³C NMR spectra were recorded with or without WALTZ proton decoupling.²⁴ The multiplicity of the carbon signals was established using the 1D APT experiment²⁵ with an evolution time of 7 ms or the DEPT experiment²⁶ with reading pulses of 90 and 135° and an evolution time of 3.5 ms. The 2D phase-sensitive HSQC spectra²⁷ were acquired with a 1.75 ms evolution time and MPF9 carbon decoupling²⁸ during acquisition. A higher precision in the proton dimension was obtained from the CHORTLE experiment²⁹ run with four increments for cosine and sine subspectra: 0.4, 1.0, 2.4 and 3.2 ms. The 2D gradient-selected magnitude HMBC experiment³⁰ was run without carbon decoupling with one-bond and long-range evolution times of 3.5 and 62.5 ms, respectively. The EXSIDE experiment³¹ was run with a long-range evolution time of 86.2 ms and a *J* scaling factor of 15. Accurate ¹H chemical shifts and coupling constants were obtained by spectral simulation using the program gNMR from IvorySoft.

Molecular modeling

Energy calculation and geometry optimization to 0.1 kcal mol⁻¹ Å⁻¹ of (*R*)- and (*S*)-PGME derivatives of apetalic acid were performed with the program HyperChem 8.0.10 from Hypercube

using the force field Hyper MM+ starting from the two crystallographic structures of apetalic acid (**1**) to which (*R*)- or (*S*)-PGME was attached in its ideal planar conformation.

Results and discussion

In this section, we describe the X-ray diffraction pattern of apetalic acid (**1**); to our best knowledge reported here for the first time. We also report the unambiguous assignments of NMR signals for C and H of apetalic acid (**1**), and used the newer methods of optical purity determination by BINOL complexes. As been said before, the C-19 highly crowded environment by the propyl group and carboxylic acid moieties lying next to the tricyclic main skeleton of the molecule could lead to the presence of two rotamers in solid state, as well as in solution where the exchange between two rotamers could be expected. This point should be checked by derivatization of the products of known configuration, such as derivatives of chiral amino acids. This hypothesis is very appealing because of the eventual separation of two conformers of apetalic acid (**1**) with help of diastereomers formed in this way. It could be assumed reasonably that the presence of two rotamers of **1** would lead to obtainment of 4 diastereomeric amides (2 by 2 in each series) differing in NMR properties because of the increased hindrance in rotation among the already discussed bond. The validity of this hypothesis was tested by molecular modeling of apetalic acid PGME derivatives.

X-ray crystallography

The unit cell contains two crystallographically independent molecules of apetalic acid (**1**) (Figs. 2 and 3). The chromanone frame is almost planar; the six-atom ring has a screw boat conformation. However, in molecule 1 in the ring designed by O1–C2–C3–C4–C12–C11, the pseudoaxial H(2) atom on C2 is on the same side as the propyl chain; in contrast the H(2a) atom on C2a in molecule 2 is on the same side as the carboxylic group. The absolute configuration was

determined using a Flack parameter, and the chiral centers were established as C2(*R*), C3(*S*), and C19(*R*) for molecule 1 and C2a(*R*), C3a(*S*), and C19a(*R*) for molecule 2.

NMR of apetalic acid

Table 1 lists the NMR data obtained for apetalic acid (**1**). ¹H chemical shifts were in relatively good agreement with previously published values,^{7, 14-16} but more spin systems could be resolved. On the other hand, HSQC (Fig. S1) and HMBC (Table 1) experiments helped in correcting major errors in previously published ¹³C NMR assignments.¹⁴ C-11 and C-14 signals were broad at 20 °C, but increasing the temperature to 40 °C was not sufficient to reach the fast exchange regime.

Nuclear Overhauser effects (NOEs) between protons in the ring and side-chain parts of apetalic acid (**1**) would help in determining the conformation at C-19. Only one such NOE was observed on the NOESY spectrum, between H-15 and H-23a (Table 1), but since the latter could not be stereochemically assigned, the question was not resolved.

The long-range coupling constant (³*J*) between C-11 or C-14 in the aromatic moiety and H-19 in the aliphatic side chain would give a definitive proof of the absolute configuration at C-19.³² The EXSIDE experiment was used to precisely measure these coupling constants. Only one peak with a *ca.* 5 Hz coupling was observed on the 2D spectrum at δ 160, but the resolution was insufficient to unambiguously identify whether it corresponds to C-11, C-14, or both.

Determination of enantiomeric purity of apetalic acid at C*-19 asymmetric center

We were interested in an additional verification of the absolute configuration of C*-19 via some newer methods, such as NMR and the molecular tweezers using in particular (*S*)-BINOL.³³⁻³⁵ Successful experiments with this reagent were reported for a variety of chiral natural products, drugs, or compounds coming from total synthesis.^{33, 35-36 33, 35-36} Usually, after the addition of (*S*)-

BINOL, the split of signals corresponding to even small, partial racemization, if any, could be observed.

As it is reported, when molar excess of (*S*)-BINOL³⁷ is added to substrate, a shift of the equilibrium toward the associate species occurs and consequently, the increase of signal separation is observed. In the presence of H-bonding, π,π -interactions, or van der Waals forces, the formation of host–guest complex in solution takes place. In such a manner, the use of (*S*)-BINOL as a chiral solvating agent, enabled to study the enantiodiscrimination, and larger application in the chirality, as in particular of natural products, some synthetic drugs studies, etc.³³⁻³⁵

In the case of apetalic acid (**1**), in the presence of up to four equivalents of (*S*)-BINOL, in CDCl₃ solution, most of the resonances did not undergo any split at room temperature. This result alone is evidence of the absence of two diastereomers at C-19 carbon host–guest complexes in solution. The details are provided in Supplementary data.

The experiment with chiral camphorate shift reagent was also performed, but the H-19 signal was not moved, probably because of the fact that two weak bases in presence, OH and COOH, cannot form stable complexes with lanthanide.

The conclusion of this experiment is that the apetalic acid sample we worked with is optically homogenous, and not even partial racemization at this C* was observed. Most of the (*S*)-BINOL proton signals also showed small upfield shifts when forming the complex, an effect which could be attributed to the magnetic anisotropy from heteroaromatic groups of the guest molecule, which also confirmed the presence of complex with apetalic acid.

NMR and molecular modeling of (*R*)- and (*S*)-PGME amides of apetalic acid

Because NOEs and long-range carbon–proton coupling constants were unsuccessful in determining the absolute configuration of apetalic acid (**1**) at C-19, two derivatives were prepared

with both (*R*)- and (*S*)-PGME. In each case, two compounds were obtained, which were separated on Sep-Pak. TLC on silica gel with hexane–ethyl acetate 3:1 gave R_f values of 0.39 and 0.33 for (*R*)-PGME derivatives and 0.36 and 0.31 for (*S*)-PGME derivatives. These compounds will be referred to as **Ra**, **Rb**, **Sa**, and **Sb**, respectively. It is hypothesized that in each case the two compounds are conformers corresponding to the two crystallographic structures, i.e. one where H-19 is on C-11 side (conformer A) and the other on C-14 side (conformer B). The energy barrier between the two conformers of each derivative would be too high for exchange to take place at room temperature.

All ^1H and ^{13}C NMR spectra of the four PGME derivatives of apetalic acid showed broadened peaks indicative of conformational dynamics occurring on the NMR timescale (Table 2). Compared to the original acid, the amides showed α and β substituents effects of *ca.* -8.4 and $+2.4$ ppm, respectively, on their ^{13}C chemical shifts. Additionally, both *R* and *S* derivatives having the largest R_f values (**Ra** and **Sa** conformers) also showed important downfield shifts of *ca.* $+2.8$ and $+3.1$ ppm at C-2 and C-15, respectively. Similarly, the proton that experienced the most important shift (*ca.* -0.44 ppm) was H-2 in the same derivatives, suggesting a shielding effect due to the proximity of the phenyl ring in these conformers.

Another possibility for explaining the formation of two compounds for each of the (*R*)- and (*S*)-PGME amide derivatives of apetalic acid, namely a mixture of *trans*- and *cis*-amide rotational isomers, was ruled out by the absence of NOE across the amide bond for all derivatives.³⁸

The method of Yabuuchi and Kusumi (2000)³⁹ is based on the postulate that the stable conformers, [G] and [H], for the dihedral angle $\text{CO}-\text{CH}_2-\text{C}^*-\text{H}$, where C^* is the chiral carbon bearing substituents R_1 and R_2 for which the absolute configuration is to be determined, could be represented by an average conformation [J] (Fig. S2). Depending on the sign of the chemical shift

differences ($\Delta\delta = \delta_R - \delta_S$) between protons in the right and left part of the molecule, an *R* or *S* absolute configuration could be assigned to the chiral carbon in the original acid. In our particular case, chemical shift differences were calculated for all possible combinations of *R* and *S* compounds taken pairwise ($\delta_{Ra} - \delta_{Sa}$; $\delta_{Ra} - \delta_{Sb}$; $\delta_{Rb} - \delta_{Sa}$; $\delta_{Rb} - \delta_{Sb}$), but in all cases there was no definite positive or negative sign for protons in each side (aromatic ring or aliphatic chain) of the molecule. Clearly, the method did not work in this case ($\Delta\delta$ should be positive on one side, negative on the other side, and zero in the plane), probably due to steric hindrance arising from the bulkiness of the original acid. For the method to be applicable, the populations for the two conformers ([G] and [H]) must be similar.

The validity of this hypothesis was tested by molecular modeling. In a first experiment, the energy of conformers A[G], A[H], A[I], B[G], B[H], and B[I] was calculated (Table 3, Fig. 4A). Considering the starting structures only, conformers [I] were not stable. In a second experiment, the structure was minimized starting from the same conformers, and the dihedral angles of the minimum-energy conformer were measured (Table 4, Fig. 4B). Energy minimization resulted in stable conformers sometimes far from the starting structures, mainly due to interactions between the aromatic rings of apetalic acid and PGME. In the case of *S* derivatives, structures [H]_m were the most stable, whereas structures [I]_m unexpectedly were more stable for *R* derivatives. Of course, during minimization, the planar structure of PGME was lost for the most stable conformers, but in all cases, the amide bonds remained in the *trans* conformation.³⁸

Experimentally, two different compounds were obtained for each derivatization reaction with (*R*)- and (*S*)-PGME. They were labelled **a** and **b** according to their *R_f* on TLC. Obviously, the energy barrier for rotation between the **a** and **b** conformers was too high for them to exchange at room temperature. In both *R* and *S* cases, **b** derivatives were more abundant than **a** derivatives and

are thus assumed to be more stable. According to ^1H NMR, **a** derivatives are those in which the proton in position 2 experienced shielding, presumably due to proximity of an aromatic ring. Looking at the minimized structures, in both $\text{AR}[\text{I}]_{\text{m}}$ and $\text{AS}[\text{H}]_{\text{m}}$, the most stable conformers for both *R* and *S* derivatives, the aromatic ring of PGME would be on the top of the molecule opposite to H-2, whereas in $\text{BR}[\text{I}]_{\text{m}}$ and $\text{BS}[\text{H}]_{\text{m}}$, the second most stable conformers, it would be located on the bottom side in close proximity to H-2. In conclusion, the correspondence between the calculated conformers and the purified compounds is the following: **Ra**, where H-2 and H-3 were shielded, and **Rb**, where no H was shielded, correspond to the calculated conformers $\text{BR}[\text{I}]_{\text{m}}$ and $\text{AR}[\text{I}]_{\text{m}}$, respectively; on the other hand, **Sa**, where H-2 was shielded, and **Sb**, where H-15 and H-16 were shielded, correspond respectively to $\text{BS}[\text{H}]_{\text{m}}$ and $\text{AS}[\text{H}]_{\text{m}}$ conformers (Fig. 5).

Conclusion

First of all, from the crystallographic study reported here, the absolute configuration of the asymmetric centres of apetalic acid (**1**) was unambiguously established as C2(*R*), C3(*S*), and C19(*R*), in agreement with some solution evidences, mostly from 2D NMR spectra. This configuration was used as a base to a series of chemical derivatization, spectrometric, and molecular modeling experiments.

As could be concluded from the ensemble of experiments presented on the enantiopure sample of **1**, of known C-19 absolute configuration, the coexistence of two stable, high-rotatory-barrier conformers at the C-10–C-19 pivotal bond in solid state was proven. Particularly, when this complex chromanone acid with three asymmetric centres was subjected to derivatization by (*R*)- or (*S*)-PGME, a pair of amides for each rotamer were formed and isolated. The isolation of these four diastereomeric amides enabled to record and analyse their NMR spectra and proceed to

the complete analysis of energies and angular parameters (dihedral angles) of PGME derivatives via molecular modeling. The population of these conformers was not evaluated; such data are always approximate. In both series of PGME derivatives, the slight predominance of population of one conformer, named **b**, and its stability was observed. The structure of these **b** conformers was established with the help of molecular modeling and is in total agreement with their NMR spectra.

Cases of experiments on conformers with restricted rotation due to high rotational barrier reported in the literature are both challenging and rare. Facing a problem of lack of NOE or unambiguous long-range proton–carbon coupling data implicating the key signal H-19 and a lack of clear-cut results from the Yabuuchi-Kusumi method, the use of derivatization was an interesting approach to study the behaviour of apetalic acid conformer population in solution, especially that the isolation of resulting amides was successfully achieved.

Variable-temperature NMR experiments in solution showed that the exchange between two rotamers was observed as expected between 20 and 40 °C. For this reason, imitating an asymmetric induction determination using optically active derivatization methodology, the hydrolysis of PGME derivatives of apetalic acid to get two different single conformers of free apetalic acid sample was not attempted. The hydrolysis conditions alone will facilitate the jump through the rotational energy barrier on the transition state level of this reaction leading to averaging of conformer populations.

Supplementary data

Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjc->.

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Draft

1 **Table 1.** 300 MHz ¹H and ¹³C NMR data of apetalic acid (**1**).

DEPT							
C	δ _C (ppm)	Mult	δ _H (ppm) ^a	Mult	J (Hz) ^b	HMBC	NOESY ^c
2	76.14	CH	4.504	qd	6.5, 3.3	15(² J), 16(³ J)	3, 15
3	44.28	CH	2.546	qd	7.2, 3.3	15(³ J), 16(² J)	2, 15, 16
4	201.21	C	—			2(³ J), 3(² J), 16(³ J), OH5(⁴ J)	
5	157.38	C	—			6(³ J), OH5(² J)	
6	115.71	CH	6.598	d	10.0	—	7, OH5
7	125.75	CH	5.458	d	10.0	17(³ J), 18(³ J)	6, 17
8	78.28	C	—			6(³ J), 7(² J), 17(² J), 18(² J)	
10	108.90	C	—			19(² J), 20a(³ J), 20b(³ J), 22b(³ J)	
11	159.91	C	—			2(³ J), 19(³ J) ^d	
12	101.33	C	—			OH5(³ J)	
13	102.65	C	—			6(² J), 7(³ J), OH5(³ J)	
14	160.07	C	—			6(³ J), 7(⁴ J), 19(³ J) ^d	
15	16.33	CH ₃	1.352	d	6.5	2(² J)	2, 3, 23a

16	9.40	CH ₃	1.135	d	7.2	3(² <i>J</i>)	(2), 3
17	28.59	CH ₃	1.445	s		18(³ <i>J</i>)	7
18	28.17	CH ₃	1.371	s		17(³ <i>J</i>)	(7)
19	30.55	CH	3.686	m	10.2, 8.7, 6.7, 5.4	20a(² <i>J</i>), 20b(² <i>J</i>), 22a(² <i>J</i>), 22b(² <i>J</i>), 23(³ <i>J</i>)	20a, 20b, 22b
20	38.70	CH ₂	2.827	dd	−15.1, 8.7	19(² <i>J</i>)	19, 20b
			2.662	dd	−15.1, 6.7		19, 20a, 22b
21	179.78	C	—			20a(² <i>J</i>), 20b(² <i>J</i>)	
22	35.53	CH ₂	1.824	m	−13.0, 10.2, 8, 6	19(² <i>J</i>), 20a(³ <i>J</i>), 23(² <i>J</i>), 24(³ <i>J</i>)	22b, 23a
			1.516	m	−13.0, 9, 7, 5.4		19, 20b, 22a, 23b
23	20.87	CH ₂	1.2	m		22a(² <i>J</i>), 22b(² <i>J</i>), 24(² <i>J</i>)	15, 22a, 24
			1.1	m			22b
24	14.09	CH ₃	0.853	t	7	23(² <i>J</i>)	23a
OH5	—		12.391	s			6
COOH	—		11.361	br s			—

^aThree decimals are given when simulation of a proton in a spin system was possible, one otherwise.

^bOne decimal is given when simulation of both coupled protons in a spin system was possible, none otherwise.

^cOne-way correlations in parentheses.

5 ^dEither or both.

Draft

6 **Table 2.** 300 MHz ¹H and ¹³C NMR data of PGME derivatives of apetalic acid.

C	Ra				Rb				Sa				Sb			
	δ _C	δ _H	Mult ^a	<i>J</i>	δ _C	δ _H	Mult	<i>J</i>	δ _C	δ _H	Mult	<i>J</i>	δ _C	δ _H	Mult	<i>J</i>
	(ppm) ^a	(ppm) ^b		(Hz) ^c	(ppm) ^a	(ppm) ^b		(Hz) ^c	(ppm) ^a	(ppm) ^b		(Hz) ^c	(ppm) ^a	(ppm) ^b		(Hz) ^c
2	79.1	4.034	m	11, 6.3	76.28	4.478	m	6.5, 3.2	78.84	4.1	br s		76.24	4.4	br s	
3	45.5	2.3	br s		44.24	2.525	m	7.2, 3.2	45.44	2.4	br s		44.14	2.5	br	
4	199.36	–			201	–			199	–			199	–		
5	157.13	–			157	–			157.12	–			157.29	–		
6	115.70	6.619	d	10.0	115.73	6.628	d	10.0	115.70	6.611	d	10	115.66	6.612	d	10
7	125.81	5.466	d	10.0	125.8	5.467	d	10.0	125.6	5.5	br		125	5.5	br d	
8	78.34	–			78.41	–			78.32	–			78.35	–		
10	108	–			108.92	–			108	–			108	–		
11	159	–			159	–			160	–			159.63	–		
12	101	–			101	–			101.92	–			101.52	–		
13	102	–			102	–			102.87	–			102.81	–		
14	159	–			159	–			160	–			159.63	–		
15	19.5	1.445	d	6.3	16.29	1.362	d	6.5	19.3	1.3	br s		15.81	1.2	br	
16	10.0	1.127	d	7	9.29	1.084	br d	7.2	10.54	1.146	d	7	9.21	0.966	d	7
17	28.3	1.419	s		28.4	1.427	br s		29	1.5	br		29	1.6	br	

18	28.2	1.404	s		28.2	1.407	s		27.8	1.3	br		27.76	1.4	br	
19	30.5	3.636	m	10.5, 10.0, 5.8, 5.2	30.90	3.7	m		31.30	3.7	m		31.51	3.698	m	10.6, 10.3, 5.9, 5.5
20	40.94	2.923	dd	−15.4, 10.5	41.03	2.853	dd	−14.8, 10	41.14	2.865	dd	−13.5, 10	41.17	2.835	dd	−13.2, 10.6
		2.620	dd	−15.4, 5.8		2.610	dd	−14.8, 7		2.514	dd	−13.5, 6		2.507	dd	−13.2, 5.9
21	171.29 ^d	–			171.64	–			171.40 ^d	–			171.33 ^d	–		
22	35.9	1.829	m	−13.0, 10.0, 7, 7	35.992	1.800	m	−13.1, 10, 10, 5	36.22	1.8	m		36.24	1.771	m	−13.0, 10.3, 9, 5
		1.487	m	−13.0, 8, 8, 5.2		1.496	m	−13.1, 10, 6, 5		1.5	m			1.512	m	−13.0, 9, 7, 5.5
23	20.72	1.2	m		20.75	1.1	m		20.67	1.2	m		20.70	1.2	m	
		1.1	m			1.1	m			1.1	m			1.1	m	
24	14.0	0.849	t	7	14.1	0.846	t	7	13.97	0.838	t	7	14.05	0.840	t	7
OH5	–	12.478	s		–	12.416	s		–	12.471	s		–	12.391	s	
OCH ₃	52.58	3.683	s		52.63	3.656	s		52.64	3.656	s		52.68	3.662	s	
1'	171.56 ^d	–			171.64	–			171.48 ^d	–			171.50 ^d	–		
2'	56.14	5.511	d	7	56.16	5.491	d	7.1	55.96	5.475	d	8	55.92	5.449	d	7
i	136.67	–			136.64	–			136.74	–			136.72	–		
o	127.04	7.1	m		127.02	7.2	m		126.85	7.0	m		126.71	7.0	m	
m	128.75	7.2	m		128.79	7.3	m		128.63	7.2	m		128.63	7.2	m	
p	128.25	7.2	m		128.28	7.3	m		128.12	7.2	m		128.07	7.2	m	
NH	–	6.6	br s		–	6.459	br d	7.1	–	6.5	br s		–	6.5	br s	

^aMultiplicity: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad.

- 8 ^bTwo decimals are given for narrow peaks, one for large peaks, none when obtained from 2D spectra.
- 9 ^cThree decimals are given when simulation of a proton in a spin system was possible, one otherwise.
- 10 ^dOne decimal is given when simulation of both coupled protons in a spin system was possible, none otherwise.
- 11 ^eAssignments in the same column could be interchanged.

Draft

12 **Table 3.** Energy and RMS gradient of conformers of PGME derivatives of apetalic acid.

Conformer	Torsion angles (°)		Total energy	RMS gradient
			(kcal mol ⁻¹)	(kcal mol ⁻¹ Å ⁻¹)
	11–10–19–20	10–19–20–21		
AR[G]	120	180	11.0	1.1
AR[H]	120	–60	7.1	0.7
AR[I]	120	60	537.3	71.3
BR[G]	–60	180	11.1	1.2
BR[H]	–60	–60	7.5	0.5
BR[I]	–60	60	970.8	111.3
AS[G]	120	180	10.9	0.9
AS[H]	120	–60	5.9	0.8
AS[I]	120	60	426.4	56.6
BS[G]	–60	180	11.1	1.1
BS[H]	–60	–60	7.5	1.0
BS[I]	–60	60	908.6	125.5

13

14 **Table 4.** Energy and selected dihedral angles of conformers of PGME derivatives of apetalic acid after energy minimization.

Conformer	Total energy									
	(kcal mol ⁻¹)	Torsion angles (°)								
		11–10–19–20	10–19–20–21	19–20–21–N	20–21–N–2'	21–N–2'–1'	N–2'–1'–O	2'–1'–O–Me	20–19–22–23	19–22–23–24
AR[G] _m	8.0	119.7	–170.3	–164.6	173.3	164.6	–179.6	–176.8	–177.9	177.8
AR[H] _m	6.5	120.2	–55.8	–176.9	177.7	175.4	179.1	–177.9	–173.9	177.9
AR[I] _m	2.5	92.1	50.8	–77.9	179.3	85.5	–6.6	–179.3	–174.5	174.3
BR[G] _m	8.3	–61.6	–170.4	–166.2	174.1	165.7	–179.9	–176.4	–175.0	178.0
BR[H] _m	6.8	–59.9	–54.0	–171.5	176.6	171.6	179.9	–178.2	–175.1	176.6
BR[I] _m	2.8	–95.0	50.4	–80.7	–175.1	91.7	–150.5	–179.7	–178.2	–178.2
AS[G] _m	8.1	113.2	–167.1	–178.8	180.0	–171.5	175.2	179.7	178.0	176.9
AS[H] _m	1.0	114.7	–51.1	78.6	–179.7	–93.2	161.9	178.7	–178.7	177.5
AS[I] _m	10.5	108.1	85.5	–154.6	–177.1	–175.2	178.7	178.7	–170.0	–179.4
BS[G] _m	8.5	–67.1	–168.0	–177.8	179.8	–173.9	178.8	178.3	178.8	178.4
BS[H] _m	1.5	–66.1	–53.5	74.6	174.6	–91.7	152.6	179.0	–179.5	179.4
BS[I] _m	11.5	–74.3	92.7	–171.3	–176.4	176.6	–178.0	177.6	–166.0	–179.9

15

16 **Figure captions**

17 **Fig. 1.** Structure and numbering of apetalic acid (**1**) and isoapetalic acid (**2**).

18 **Fig. 2.** Molecule 1.

19 **Fig. 3.** Molecule 2.

20 **Fig. 4.** Conformers of PGME derivatives of apetalic acid displaying starting (**A**) and energy-
21 minimized (**B**) structures. In each case, the left and right halves correspond to the two conformers
22 observed in the crystal structure (180° rotation around C-10–C-19). The top and bottom rows
23 correspond to (*R*)- and (*S*)-PGME derivatives, respectively. In each quadrant, the three structures
24 represent conformations [G], [H], and [I] of Fig. 2 in Yabuuchi and Kusumi (2000)³⁹ resulting
25 from rotation around C-19–C-20, where the PGME plane was initially in its ideal planar
26 conformation. The energy is given in kcal mol⁻¹ for each structure.

27 **Fig. 5.** Stable conformers of PGME derivatives of apetalic acid and their corresponding purified
28 compound showing shielding of protons in ring 1–2–3–4–12–11.

1 **2**

Fig. 1. Structure and numbering of apetalic acid (1) and isoapetalic acid (2).

84x140mm (300 x 300 DPI)

A

B