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Crystal Structure Analysis of 6,7-di-*O*-Methyl-Quercetagetin-3-*O*-*β*-D-Glucopyranoside dihydrate Isolated from *Urena sinuata* L

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Resumen:

En este trabajo, el análisis estructural de la 3-*O*-*β*-D-glucopiranosil-6,7-di-*O*-metil-quercetagetina dihidratada (**I**), aislada de *Urena sinuata* L. colectada en Táchira-Venezuela, fue realizado por difracción de rayos X en monocristal. El compuesto **I** cristaliza en el sistema monoclínico con un grupo espacial C2 (No. 5) y parámetros de celda de *a* = 29.289(3) Å; *b* = 6.6352(7) Å; *c* = 14.6533(13) Å; β = 113.636(6)°; V = 2608.8(5) Å^3; Z = 4. El refinamiento de la estructura convergió a los valores de *R* = 0.0421, *wR*2 = 0.1195, S = 1.02. Este es el primer reporte de rayos-X de este compuesto obtenido de *U. sinuata* L..

Palabras clave: análisis estructural; *Urena sinuata* L; difracción de rayos X.

Abstract

In the present work, the structural analysis of 6,7-di-*O*-methyl-quercetagetin-3-*O*-*β*-D-glucopyranoside dihydrate (**I**), which was isolated from *Urena sinuata* L. (dog wart) collected in Táchira-Venezuela, was achieved by single crystal X-ray diffraction. Compound **I** crystallizes in the monoclinic system, space group C2 (No. 5) with unit cell parameters *a* = 29.289(3) Å; *b* = 6.6352(7) Å; *c* = 14.6533(13) Å; β = 113.636(6)°; V = 2608.8(5) Å^3; Z = 4. The structure refinement converged to *R* = 0.0421, *wR*2 = 0.1195, S = 1.02. This is the first X-ray report of this compound obtained from *U. sinuata* L.

Keywords: structural analysis; *Urena sinuata* L; crystal X-ray diffraction.

Introduction

Urena L. (Malvaceae family) is a genus that comprises two species named *Urena lobata* L. and *Urena sinuata* L., although some botanists suggest that *U. sinuata* L. is a subspecies of *U. lobata*[1]. *Urena* plant species have been used in the Venezuelan folk medicine for their pharmacological properties as antidiarrheal[2], antiparasitic[3], antibacterial[4], anti-inflammatory and analgesic[5]. Phytochemical studies carried out on *Urena* species have reported a variety of compounds, such as xanthones[6], steroids (i.e., *β*-sitosterol)[7] and flavonoids such as quercetin, kaempferol, hypolaetin, gossypetin, luteolin, apigenin and chrysoeriol[5]. However, fatty acids have been the only metabolites reported for the *U. sinuata* species[5].

In the present investigation, the structural analysis of 6,7-di-*O*-methyl-quercetagetin-3-*O*-*β*-D-glucopyranoside dihy-drate (**I**), which was isolated from *Urena sinuata* L. (dog wart) collected in Táchira-Venezuela, was established by single crystal X-ray diffraction. It is interesting to note that neither this compound nor metabolites with similar skeleton have been reported previously from this plant. A search of the Cambridge Structural Database (CSD)[8] did not indicate any report of the compound **I.**

Experimental

Plant material

Urena sinuata L. (Malvaceae) was collected at San Cristóbal suburbs (Táchira State-Venezuela). Voucher specimens were stored at the MERC Herbarium, Sciences Faculty, Universidad de los Andes-Venezuela. The compound was extracted from the dried leaves of *Urena sinuata* using standard procedures previously described, followed by characterization relying on spectroscopic methods[5] (figure 1).

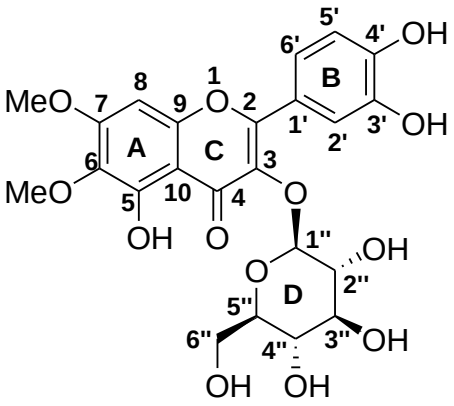

Figure 1: Molecular diagram of compound I.

Extraction and Isolation

1 Kg of fresh plant was extracted successively in a Soxhlet with *n*-hexane, dichloromethane, acetone and methanol. The acetone extract (8.9 g) was percolated on a Sephadex LH-20® column with methanol. The methanol eluate was dried to give a residue (525 mg), which was further purified by PTLC, eluting 8 times with *n*-hexane/acetone (1:8), to furnish 6,7-di-*O*-methyl-quercetagetin-3-*O*-*β*-D-glucopyranoside (**I**, 45 mg).

X-ray Data Collection and Structure Determination

All the data were collected with a R-Axis Rapid Rigaku MSC diffractometer using the CuKα radiation and a graphite mono-chromator. All reflections were used for unit cell refinement. The structures were solved by direct methods and refined using SHELX 97[9] suites of programs and the positions of the H atoms of the compound were deduced from coordinates of the non-H atoms and confirmed by Fourier synthesis. The non-H atoms were refined with anisotropic temperature parameters. Two (2) ordered water molecules were refined. All the H atoms were included for structure factor calculations but not refined. The program PLATON was used for structure analysis[10-13] (table 1). Experimental and crystal data are given in Table 1.

Table 1: Crystal data and details of the structure determination of compound I

Crystal Data	
Formula	$C_{23}H_{28}O_{15}$, $2(H_2O)$
Formula Weight	544.45
Crystal System	Monoclinic
Space group	C2 (No. 5)
a, b, c [Å]	29.289(3), 6.6352(7), 14.6533(13)
β [°]	113.636(6)
V [Å^3]	2608.8(5)
Z	4
D(calc) [g/cm^3]	1.386
Mu(CuKα) [/mm]	1.019
F(000)	1144
Crystal size [mm]	0.08 x0.08 x0.18
Data collection	
Temperature (K)	213
Radiation [Å] CuKα	1.54180
θ Min-Max [°]	6.6, 71.8
Data set	-35: 36 ;-7:6 ; -18: 18
Tot., Uniq. Data, R(int)	18381, 4530, 0.027
Observed data [I > 2.0 σ(I)]	4220
Refinement	
Nref, Npar	4530, 365
R, wR2, S	0.0421, 0.1195, 1.02
Flack x	-0.01(17)

Compound **I** crystallized in the monoclinic system, the space group C2 and cell parameters ***a***=29.289(3), ***b***=6.6352(7) and ***c***=14.6533(16) Å, β=113.636(6)° and V=2608.8(4) Å^3. Non–hydrogen atoms were anisotropically refined. The final indices were R=0.0421, wR2= 0.1195 and S= 1.02. The absolute configuration was established by anomalous scattering [Flack parameter -0.01(17)].

Results and Discussion

The details of crystal data and refinement are given in Table 1. The compound exhibits four (4) six-membered rings labeled A to D. Figure 2 shows the molecular structures with the atom numbering scheme[14,15]. The rings A, B and C exhibit planar conformation and asymmetry parameters[9] [$\Delta C_2(C_1\text{-}C_2)_{max}$= 1.3(4), $\Delta C_2(C_3\text{-}C_4)_{min}$= 0.4(4), $\Delta C_S(C_2)_{max}$= 1.0(3), $\Delta C_S(C_3)_{min}$= 0.2(3)] for the A ring formed by atoms C1-C2-C3-C4-C5-C6; [$\Delta C_2(C_{17}\text{-}C_{18})_{max}$= 2.3(5), $\Delta C_2(C_{19}\text{-}C_{20})_{min}$= 0.3(4), $\Delta C_S(C_{18})_{max}$= 2.0(3), $\Delta C_S(C_{17})_{min}$= 1.2(3)] for the B ring, which is formed by atoms C17-C18-C19-C20-C21-C22; [$\Delta C_2(C_6\text{-}C_{15})_{max}$= 12.0(4), $\Delta C_2(C_{15}\text{-}C_{14})_{min}$= 2.5(4), $\Delta C_S(C_6)_{max}$= 10.2(3), $\Delta C_S(C_{14})_{min}$= 3.4(3)] for the C ring formed by atoms O12-C5-C6-C15-C14-C13; [$\Delta C_2(C_{31}\text{-}C_{30})_{max}$= 8.9(2), $\Delta C_2(C_{26}\text{-}C_{31})_{min}$=2.8(2), $\Delta C_S(C_{30})_{max}$= 6.3(2), $\Delta C_S(C_{26})_{min}$= 2.2(2)] for the D ring, which exhibits a chair conformation and is formed by atoms O27-C26-C31-C30-C29-C28.

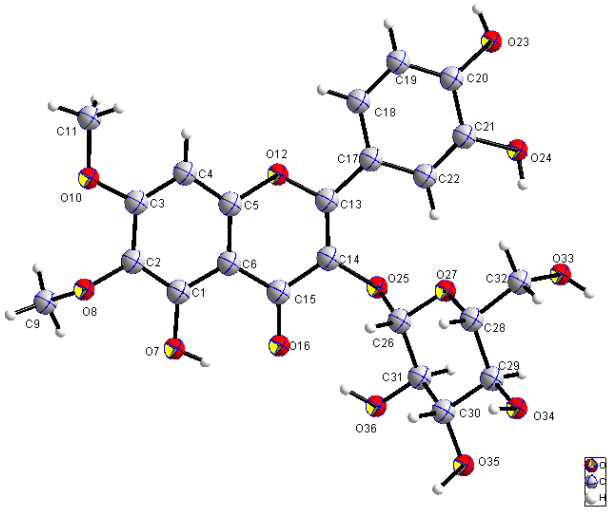

Figure 2: Asymmetric unit of compound I.

There are twelve (12) hydrogen bonds, three (3) of them are conventional intra-molecular hydrogen bonds, and four (4) are intramolecular non-conventional interactions (table 2). The intramolecular hydrogen bonds involve the atoms O7-H7···O16, C22-H22···O25 and C26-H26···O16, which can be described by the graph set symbol[16] S(6), while the hydrogen bonds patterns that involve the atoms O36-H36···O25, C18-H18···O12 and C29-H29···O33, are represented by the graph set symbol[16] S(5), and finally O36-H36···O16 is described by the graph set symbol S(8).

It is worth noting that O16 acts as a trifurcated acceptor. The hydroxyl substituent O7 on the C1 center of the flavonoid skeleton and the hydroxyl O36 located on C31 from the glucose, form a ring described by the second order graph set symbol $R^1_2(12)$. Moreover, O16 also participates in a non-conventional interaction with the methine hydrogen of C26 from the glucose.

Two water molecules are involved in intermolecular hydrogen bonds. Nine (9) conventional intermolecular hydrogen bonds are observed. One of them involves the atoms O7-H7···O7*i* [2.46 Å, 105°] where *i* indicates the O7 of a molecule with coordinates 1-*x*, *y*, 1-*z* forming a dimer described by the graph set symbol $R^2_2(4)$.

On the other hand, the hydroxyl group O35, located in the equatorial position of C30 with an angle of 72.07° to the plane of the D ring, which corresponds to the glucose ring, forms a hydrogen bond O35-H35···O41 [1.85 Å, 164°] with a water molecule with coordinates *x*, 1+ *y*, *z*.

In turn, atom O41 interacts via a hydrogen bond with the O8 of the ether substituent which is attached to C2 of the flavonoid skeleton forming the bond O41-H41A···O8 [1.80 Å, 161°].

The atom O40 of another water molecule with coordinates ½-*x*, ½+*y*, 1-*z*, forms a hydrogen bond with the hydroxyl group O24 located at C21of the B ring in the flavonoid skeleton. The geometric parameters are O24-H24···O40 [1.79 Å, 172°].

Table 2: Hydrogen bonds in the structure of compound I

Bond	D-H	H-A	D-A	D-H-A	Symmetry	Graph set symbol
O7 -- H7···O16	0.8300	1.8800	2.612(2)	146.00		S(6)
O7 -- H7···O7	0.8300	2.4600	2.789(3)	105.00	*1-x, y,1-z*	R^2_2 (4)
O24 -- H24···O40	0.8300	1.7900	2.610(3)	172.00	*1/2-x,1/2+y,1-z*	D(3)
O33 -- H33···O35	0.8300	1.9300	2.742(3)	168.00	*1/2-x,1/2+y,2-z*	C(8)
O34 -- H34···O36	0.8300	2.0900	2.857(3)	150.00	*x,1+y, z*	C(7)
O35 -- H35···O41	0.8300	1.8500	2.662(4)	164.00	*1/2-x,1/2+y,1-z*	D(3)
O36 -- H36···O16	0.8300	1.9300	2.757(2)	173.00		S(8)
O36 -- H36···O25	0.8300	2.5200	2.817(2)	102.00		S(5)
O40 -- H40A···O33	0.94(3)	1.90(2)	2.817(3)	164(4)	*1/2-x,1/2+y,1-z*	D(3)
O40 -- H40B···O34	0.92(3)	2.34(4)	2.991(3)	127(3)	*x,-1+y,-1+z*	D(3)
O40 -- H40B···O35	0.92(3)	2.07(3)	2.915(3)	153(3)	*x,-1+y, -1+z*	D(3) $R^2_1(5)$
O41 -- H41A···O8	0.98(4)	1.80(4)	2.751(4)	161(4)	*1/2+x,-1/2+y, z*	D(3)
C18 -- H18···O12	0.9400	2.3000	2.653(3)	101.00		S(5)
C22 -- H22···O25	0.9400	2.2200	2.887(3)	127.00		S(6)
C26 -- H26···O16	0.9900	2.5800	2.981(3)	104.00		S(6)
C29-- H29···O33	0.9900	2.5800	2.954(3)	103.00		S(5)

The hydroxyl group O33 also forms a hydrogen bond with this water molecule, H40A-O40···O33 [1.90 Å, 164°]. Each of these hydrogen bonds can be described with a graph set symbol corresponding to finite D hydrogen bonding patterns, but the two of them form a ring which is described by $R^2_2(15)$[16].

This water molecule formed by H40B-O40-H40A forms two additional hydrogen bonds O40-H40B-O34 [2.34 Å, 127°] and O40-H40B-O35 [2.07 Å, 153°], where H40B acts as a bifurcated donor and the hydrogen bonding pattern is described by the second order symbol $R^2_1(5)$.

Additionally, compound **I** shows five (5) chiral centers whose configuration, obtained by anomalous dispersion, is represented by C26-S, C28-R, C29-S, C30-S, C31-R[12,13].

The hydrogen bonds described above and the van der Waals interactions produce a lattice existing in layers parallel to the *bc* plane with a percentage of space occupied of 65.3%.

The occluded water molecules occupy 10.5% on the inside of the packing arrangement (272.8 Å³), thus leaving voids in the structure corresponding to a volume of 631.3 Å³, and a percentage of 24.2% (figure 3).

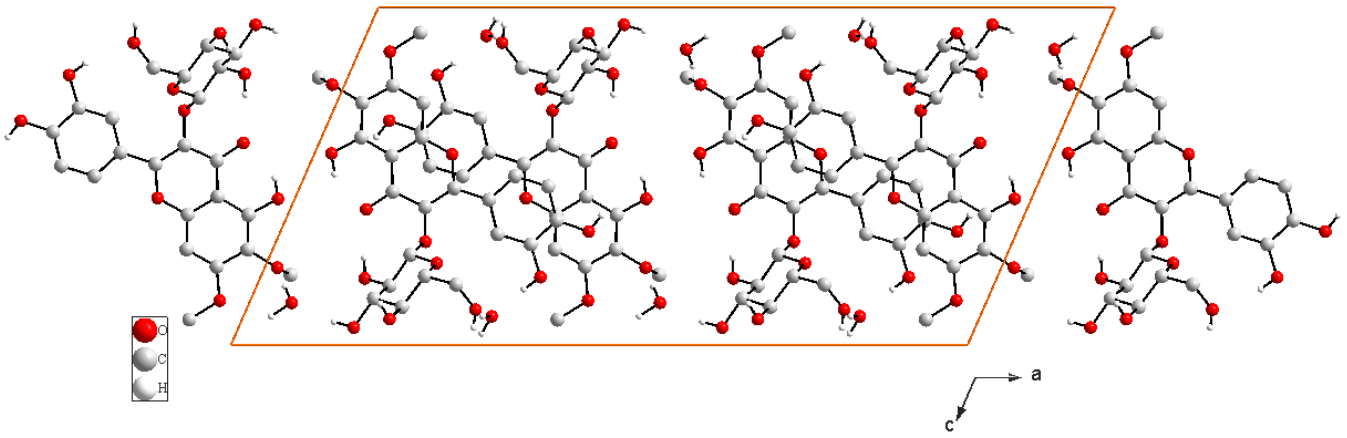

Figure 3: Packing arrangement along the *b* axis in compound I.

Conclusion

The structural analysis of 6,7-di-*O*-methyl-quercetagetin-3-*O*-*β*-D-glucopyranoside dihydrate (**I**) isolated from *Urena sinuata* L. indicated that the structure consists of three 6-membered rings for the aglycone fragment and one 6-membered ring from the glycoside unit, which exhibits a chair conformation. The packing arrangement is governed by twelve (12) conventional hydrogen bonds, three (3) of them are conventional intra-molecular hydrogen bonds and nine (9) conventional intermolecular hydrogen bonds. In addition, four (4) intra-molecular non-conventional interactions are clearly displayed.

Supporting Information Available: X-ray crystallographic data for the structure **I** have been deposited at the Cambridge Crystallographic Data Center under code CCDC 826368

Acknowledegments

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