Research Article

Synthesis, Characterization, and Crystal Structure of Negundoside (2'-p-Hydroxybenzoyl Mussaenosidic Acid)

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The structure of title compound Negundoside (2'-p-hydroxybenzoyl mussaenosidic acid) was established by spectral and X-ray diffraction studies. The compound crystallizes in the monoclinic crystal system with space group $P2_1$ having unit cell parameters: $a = 11.6201(5) \text{ \AA}$, $b = 9.2500(4) \text{ \AA}$, $c = 12.2516(5) \text{ \AA}$, $\beta = 97.793(4)^\circ$, and $Z = 2$. The crystal structure was solved by direct method using single crystal X-ray diffraction data collected at room temperature and refined by full-matrix least-squares procedures to a final R value of 0.0520 for 3389 observed reflections.

1. Introduction

Vitex negundo Linn (family: Verbenaceae) is widely used in the indigenous system of medicine in India. The roots and leaves of the plant are used as expectorant, febrifuge, vermifuge tonic, and aromatic [1]. The plant is reported to have anti-inflammatory and antiarthritic properties [2]. A variety of constituents have been reported from this plant. From the leaves Ghosh and Krishna isolated glucononitol, p-hydroxybenzoic acid, 5-hydroxyisophthalic acid, and 3,4-dihydroxybenzoic acid along with two glucosides and an amorphous alkaloid [3]. In the essential oil of leaves α -pinene, camphene, citral, and β -caryophyllene have been reported [4].

A large number of flavonoids, namely, casticin, orientin, isoorientin, luteolin, luteolin-7-O-glucoside, corymbosin, gardenins A and B, 3-O-desmethyl-artemetin, 5-O-desmethyl-nobiletin, 3',4',5,5',6,7,8-heptamethoxyflavone, 3',5-dihydroxy-4',7,8-trimethoxy flavanone, and 3',5-dihydroxy-4',6,7-trimethoxy-flavanone, have also been reported from this plant [5–8]. However the major chemical constituents of the plant are the iridoid glycosides. Five iridoid glycosides have been reported from the leaves of *V. negundo*; these are aucubin, agnuside [9], negundoside, and nishindaside [10]. Negundoside have been found to have significant hepatoprotective activity [11]. A number of pharmacological properties have been attributed to *V. negundo* including snake venom

neutralization [12], hepatoprotective [13], tyrosinase inhibition [14], antiandrogenic [15], antifeeding [16], antifungal [17], analgesic, anti-inflammatory [18], central nervous system activity [19], mosquito repellent [20], nitric oxide scavenging [21], antiradical, antilipoperoxidative [22], antibacterial [23], and larvicidal [24].

2. Experimental

2.1. Synthesis

2.1.1. Extraction of Plant Material. The shade dried and powdered leaves (1 kg) of *V. negundo* were soaked in ethanol (5 L) and kept overnight. The percolate was filtered and concentrated under reduced pressure at below 50°C . The extraction procedure was repeated three times more using three litres of ethanol each time. The combined ethanolic extract was concentrated at 50°C to get 160 g of dry extract.

2.1.2. Isolation of Negundoside. The ethanolic extract (50 g) was adsorbed over silica gel (100 g) to make it slurry which was packed over a column of silica gel (1 kg) packed in chloroform. Elution was done with chloroform followed by mixture of chloroform and methanol. Elution with 10% methanol in chloroform gave agnuside followed by mixture of agnuside

SCHEME 1: Chemical structure of the compound 2'-p-hydroxybenzoyl mussaenosidic acid.

and negundoside and then negundoside. The pure compounds were characterized on the basis of ^1H NMR, ^{13}C NMR Mass spectral data.

2.1.3. Crystallization. White block shaped single crystals of the title compound were grown at room temperature by slow evaporation technique.

2.1.4. Characterisation of Negundoside. Negundoside was obtained as crystalline compound from methanol, m.p. 163–64°C M^+ : 496, ^1H NMR (200 MHz, CD_3OD) δ 5.48 (J, 3, H-1) 7.09 (s, H-3) 2.95 (m-H-5) 2.19 (m, H-9) 1.25 (s, H-10), 6.80 (dd, 2, 7, H-3'', H-5''), 7.83 (d, 2, 7, H-2'', 6'') 4.99 (d, 7, H-1') 4.69 (d, J, 7, H-2'') ^{13}C NMR, 122.99 (C-1), 133.67, (C-2'', 6'') 116.92 (C-3'', 5''), 164.11 (C-4'') 168.08 (CO) (95.83 (C-1), 151.98 (C-3), 116.92 (C-4), 30.96 (C-5) 31.98 (C-6) 42.04 (C-7), 80.64 (C-8), 53.19 (C-9) 25.15 (C-10), 170.71 (C-11) 98.64 (C-1'), 76.85 (C-2') 75.70 (C-3'), and 72.55 (C-4') 79.30 (C-5') 63.54 (C-6').

For crystallographic studies the compound was recrystallized from water to get needles.

2.1.5. X-Ray Intensity Data Collection. X-ray intensity data of 13067 reflections (of which 5060 unique) were collected at 293(2) K on a CCD area-detector diffractometer (*Xcalibur system—Oxford diffraction make, UK*) equipped with graphite monochromated $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$). The crystal used for data collection was of dimensions 0.30 mm $\times$ 0.20 mm $\times$ 0.20 mm. The cell dimensions were determined by least-squares fit of angular settings of 6074 reflections in the θ range from 3.3499° to 28.9448°. The intensities were measured by ω scan mode for θ range from 3.41° to 26.00° with hkl values ($-14 \leq h \leq 14$, $-11 \leq k \leq 11$, $-15 \leq l \leq 15$). 3389 reflections were treated as observed ($I > 2\sigma(I)$). Data were corrected for Lorentz, absorption, and polarisation factors.

2.1.6. Structure Solution and Refinement. The crystal structure was solved by direct methods using *SHELXS-97* software [25]. The values of $R_{\text{int}} = 0.0440$ and $R_{\text{sigma}} = 0.0747$ show the quality of data satisfactory. A total of 256 phase

sets were refined with the correct phase set having an absolute figure of merit, $M(\text{abs}) = 1.026$, and combined figure of merit $\text{CFOM} = 0.068$. Multisolution tangent refinement was carried out using 845 E -values with $E > 1.2$. Two water molecules were also observed. An E -map drawn with the correct set of phases revealed all the nonhydrogen atoms of the molecule. The R -factor based on the 845 E -values was $R_E = 0.196$.

Full-matrix least-squares refinement was carried out using *SHELXL-97* software. During an anisotropic refinement of all the nonhydrogen atoms, one of the water molecules, O2W, was found disordered to another position with refinable occupancy. The refined occupancy converged to 0.56(1):0.44(1). All the hydrogen atoms (except water hydrogen atoms) were geometrically fixed and allowed to ride on the corresponding nonhydrogen atoms with $\text{C-H} = 0.93 \text{ \AA}$ – $0.98 \text{ \AA}$, and $U_{\text{iso}} = 1.5U_{\text{eq}}$ of the attached C-atom for methyl H atoms and $1.2U_{\text{eq}}$ for other H atoms. Water hydrogen atoms could not be located due to disorder. The final refinement cycles converged to $R = 0.0520$ and $wR(F^2) = 0.1111$. Final cycles of refinement resulted in a residual electron density in the range $-0.219 < \Delta\rho < 0.306 \text{ e\AA}^{-3}$. Chemical structure of the molecule is shown in Scheme 1.

3. Results and Discussion

The molecular structure of 2'-p-hydroxybenzoyl mussaenosidic acid is depicted in Figure 1 and crystallographic data are given in Table 1. The molecule consists of four rings in which two are condensed rings. The bond angles around the carbon atom C7 (i.e., $\text{O3-C7-C8} = 111.5 (3)^\circ$, $\text{O3-C7-O4} = 123.3 (3)^\circ$, and $\text{O4-C7-C8} = 125.2 (3)^\circ$) and carbon atom C23 (i.e., $\text{C21-C23-O9} = 119.4 (3)^\circ$, $\text{C21-C23-O10} = 115.7 (3)^\circ$, and $\text{O9-C23-O10} = 124.8 (3)^\circ$) are close to 120° which shows that these two carbon atoms are in sp^2 hybridized state. The bond length C7=O4 is $1.199 (3) \text{ \AA}$ which is very close to average C=O bond length in esters, that is, $1.196 \text{ \AA}$. The six C-C bond lengths in phenyl ring-A lie in the range from $1.359 (4) \text{ \AA}$ to $1.389 (4) \text{ \AA}$ as expected for a fully delocalized benzyl system. The other important bond lengths and bond angles of the molecule are listed in Table 2. These bond lengths and angles are comparable with the expected values.

TABLE 1: Crystal and experimental data.

Crystal description	White block
Empirical formula	$C_{23}H_{28}O_{12} \cdot 2H_2O$
Formula weight	528.5
Radiation, wavelength	MoK α , 0.71073 Å
Unit cell volume	1304.71(10) Å ³
Number of molecules per unit cell, Z	2
Temperature	293(2) K
Absorption coefficient	0.113 mm ⁻¹
$F(000)$	556
Scan mode	ω scan
θ range for entire data collection	3.41° < θ < 26.00°
Reflections collected/unique	13068/5060
Reflections observed ($I > 2\sigma(I)$)	3389
R_{int}	0.0440
R_{sigma}	0.0747
Structure determination	Direct methods
Refinement	Full-matrix least-squares on F^2
Number of parameters refined	344
Weight	$1/[\sigma^2(F_o^2) + (0.0600P)^2 + 0.0000P]$ where $P = [F_o^2 + 2F_c^2]/3$
Goodness-of-fit	0.949

FIGURE 1: ORTEP view of the molecule with displacement ellipsoids drawn at 40% probability level. H atoms are shown as small spheres of arbitrary radii.

The phenyl ring-A is almost planar with maximum deviation from planarity observed for the atom C11 by -0.0099 (36) Å. The oxygen atom O12 deviated from the least square plane of ring-A by 0.0554 (29) Å. In the title molecule, ring-B is in chair conformation with best mirror plane passing through O1 and C1 (asymmetric parameter $\Delta C_s = 2.92$) and the best twofold rotation axis bisecting the bonds O1-C3 and C1-C5 (asymmetric parameter $\Delta C_2 = 1$). Torsion angles for nonhydrogen atoms are listed in Table 3.

Six membered ring-C adopts a sofa conformation with best mirror plane passing through C14 and C21 (asymmetric parameter $\Delta C_s = 1.905$). The carboxylic group attached to ring-C is found to be coplanar with the least square plane of ring-C as reflected from the small values of torsion angles $C22-C21-C23-O9 = 0.4$ (5)° and $C20-C21-C23-O10 = -0.1$ (4)°, respectively (Table 3). The five membered ring-D adopts half chair conformation with best twofold axis passing through atom C20 and bisecting the bond C16-C18 (asymmetry parameter $\Delta C_2 = 7.16$).

TABLE 2: Bond lengths (Å) and bond angles (°) for nonhydrogen atoms (e.s.d.'s are given in parentheses).

Bond lengths			
O1–C3	1.412(3)	O1–C4	1.431(4)
C5–O6	1.424(4)	C5–C1	1.499(4)
C5–C4	1.517(4)	O3–C7	1.357(4)
O3–C2	1.441(3)	O5–C1	1.424(4)
O2–C3	1.392(3)	O2–C14	1.417(3)
C3–C2	1.511(4)	C4–C6	1.513(4)
C2–C1	1.517(4)	O8–C22	1.365(4)
O8–C14	1.438(4)	O11–C16	1.438(4)
C7–O4	1.199(3)	C7–C8	1.481(4)
C8–C9	1.387(4)	C8–C13	1.389(4)
C15–C14	1.496(4)	C15–C16	1.528(4)
C15–C20	1.555(4)	C6–O7	1.416(4)
C13–C12	1.359(4)	C11–O12	1.366(4)
C11–C12	1.384(5)	C11–C10	1.386(4)
C21–C22	1.319(4)	C21–C23	1.465(4)
C21–C20	1.487(5)	C23–O9	1.262(4)
C23–O10	1.270(4)	C9–C10	1.380(4)
C20–C19	1.562(4)	C16–C17	1.496(5)
C16–C18	1.524(5)	C18–C19	1.515(5)
Bond angles			
C3–O1–C4	113.4(2)	O6–C5–C1	112.1(2)
O6–C5–C4	109.1(3)	C1–C5–C4	110.5(2)
C7–O3–C2	118.0(2)	C3–O2–C14	113.7(2)
O2–C3–O1	106.9(2)	O2–C3–C2	109.6(2)
O1–C3–C2	109.1(2)	O1–C4–C6	105.6(3)
O1–C4–C5	109.7(2)	C6–C4–C5	113.4(2)
O3–C2–C3	107.0(2)	O3–C2–C1	109.5(2)
C3–C2–C1	108.9(2)	C22–O8–C14	114.6(2)
O5–C1–C5	109.3(2)	O5–C1–C2	112.1(3)
C5–C1–C2	109.9(2)	O4–C7–O3	123.3(3)
O4–C7–C8	125.2(3)	O3–C7–C8	111.5(3)
C9–C8–C13	118.5(3)	C9–C8–C7	118.4(3)
C13–C8–C7	123.1(3)	C14–C15–C16	116.5(3)
C14–C15–C20	114.2(3)	C16–C15–C20	106.0(2)
O7–C6–C4	109.9(3)	C12–C13–C8	121.3(3)
O12–C11–C12	121.0(3)	O12–C11–C10	118.8(3)
C12–C11–C10	120.2(3)	C22–C21–C23	117.8(3)
C22–C21–C20	122.9(3)	C23–C21–C20	119.3(3)
O9–C23–O10	124.8(3)	O9–C23–C21	119.4(3)
O10–C23–C21	115.7(3)	C10–C9–C8	120.9(3)
C9–C10–C11	119.3(3)	C21–C22–O8	125.6(3)
C21–C20–C15	111.7(3)	C21–C20–C19	114.4(3)
C15–C20–C19	103.7(3)	O2–C14–O8	108.2(2)
O2–C14–C15	108.1(2)	O8–C14–C15	114.1(2)
O11–C16–C17	106.0(3)	O11–C16–C18	112.0(3)
C17–C16–C18	112.1(3)	O11–C16–C15	110.5(2)
C17–C16–C15	114.9(3)	C18–C16–C15	101.5(3)
C13–C12–C11	119.9(3)	C19–C18–C16	103.8(3)
C18–C19–C20	106.1(3)		

TABLE 3: Torsion angles (°) for nonhydrogen atoms (e.s.d.'s are given in parentheses).

C14–O2–C3–O1	–78.3(3)	C14–O2–C3–C2	163.5(2)
C4–O1–C3–O2	178.7(2)	C4–O1–C3–C2	–62.8(3)
C3–O1–C4–C6	–177.4(2)	C3–O1–C4–C5	60.0(3)
O6–C5–C4–O1	–178.4(2)	C1–C5–C4–O1	–54.7(3)
O6–C5–C4–C6	63.8(3)	C1–C5–C4–C6	–172.5(3)
C7–O3–C2–C3	135.0(3)	C7–O3–C2–C1	–107.1(3)
O2–C3–C2–O3	–65.6(3)	O1–C3–C2–O3	177.7(2)
O2–C3–C2–C1	176.2(2)	O1–C3–C2–C1	59.4(3)
O6–C5–C1–O5	–60.2(3)	C4–C5–C1–O5	177.9(3)
O6–C5–C1–C2	176.4(2)	C4–C5–C1–C2	54.4(3)
O3–C2–C1–O5	65.1(3)	C3–C2–C1–O5	–178.2(2)
O3–C2–C1–C5	–173.1(2)	C3–C2–C1–C5	–56.4(3)
C2–O3–C7–O4	4.3(4)	C2–O3–C7–C8	–175.2(2)
O4–C7–C8–C9	12.8(5)	O3–C7–C8–C9	–167.7(3)
O4–C7–C8–C13	–168.1(3)	O3–C7–C8–C13	11.4(4)
O1–C4–C6–O7	–61.4(3)	C5–C4–C6–O7	58.7(4)
C9–C8–C13–C12	–0.1(5)	C7–C8–C13–C12	–179.2(3)
C22–C21–C23–O9	0.4(5)	C20–C21–C23–O9	178.2(3)
C22–C21–C23–O10	–177.8(3)	C20–C21–C23–O10	–0.1(4)
C13–C8–C9–C10	–0.6(5)	C7–C8–C9–C10	178.5(3)
C8–C9–C10–C11	0.0(5)	O12–C11–C10–C9	–177.9(3)
C12–C11–C10–C9	1.4(5)	C23–C21–C22–O8	176.2(3)
C20–C21–C22–O8	–1.5(5)	C14–O8–C22–C21	–21.2(4)
C22–C21–C20–C15	–0.4(4)	C23–C21–C20–C15	–178.0(3)
C22–C21–C20–C19	–117.9(4)	C23–C21–C20–C19	64.5(4)
C14–C15–C20–C21	23.7(4)	C16–C15–C20–C21	–105.8(3)
C14–C15–C20–C19	147.4(3)	C16–C15–C20–C19	17.9(3)
C3–O2–C14–O8	–72.8(3)	C3–O2–C14–C15	163.3(2)
C22–O8–C14–O2	–75.7(3)	C22–O8–C14–C15	44.6(4)
C16–C15–C14–O2	–161.8(3)	C20–C15–C14–O2	74.0(3)
C16–C15–C14–O8	77.9(3)	C20–C15–C14–O8	–46.3(4)
C14–C15–C16–O11	74.9(4)	C20–C15–C16–O11	–156.9(3)
C14–C15–C16–C17	–44.9(4)	C20–C15–C16–C17	83.3(4)
C14–C15–C16–C18	–166.1(3)	C20–C15–C16–C18	–37.9(3)
C8–C13–C12–C11	1.4(5)	O12–C11–C12–C13	177.2(3)
C10–C11–C12–C13	–2.1(5)	O11–C16–C18–C19	161.4(3)
C17–C16–C18–C19	–79.5(4)	C15–C16–C18–C19	43.5(4)
C16–C18–C19–C20	–33.0(4)	C21–C20–C19–C18	131.2(4)
C15–C20–C19–C18	9.2(4)		

A packing view of the molecules in the unit cell viewed down the *b*-axis is shown in Figure 2. A stereo view of unit cell is shown in Figure 3 which shows how the molecules are stacked over each other parallel to [010] direction. An examination of nonbonded contacts reveals five O–H···O intermolecular hydrogen bonds which are responsible for the stability of molecules within the unit cell. A summary of intra- and intermolecular hydrogen bonds is given in Table 4.

TABLE 4: Geometry of intra- and intermolecular hydrogen bonds.

D-H...A	D-H (Å)	D...A (Å)	H...A (Å)	D-H...A (°)
O11-H11...O2W	0.82	2.72	1.94	158.6(3)
O7-H7...O9 ⁱ	0.82	2.65	1.84	170.1(2)
O5-H5A...O10 ⁱⁱ	0.82	1.89	1.90	155.8(2)
O6-H6...O7 ⁱⁱⁱ	0.82	2.87	2.10	156.4(2)
O10-H10A...O5 ^{iv}	0.82	2.66	1.90	157.6(2)
O12-H12...O6 ^v	0.82	2.77	2.02	152.4(2)

ⁱ $x, y, z + 1$.ⁱⁱ $-x, y + 1/2, -z + 1$.ⁱⁱⁱ $-x, y + 1/2, -z + 2$.^{iv} $-x, y - 1/2, -z + 1$.^v $x, y, z - 1$.

FIGURE 2: Packing of the molecules down b-axis.

FIGURE 3: Stereo plot of the unit cell viewed down the b-axis with c horizontal and a vertical.

Supplementary Data

CCDC no. 955069 contain the supplementary crystallographic data for the compound 2'-p-hydroxybenzoyl musaenosidic acid. The data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif by e-mailing request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallography Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK. Fax: +44(0) 1223-336033.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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