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Venkata Sai Prakash Chaturvedula
Natural Ingredient Development, Blue California, 30111 Tomas, Rancho Santa Margarita, CA 92688, USA.

Ruo Huang
Natural Ingredient Development, Blue California, 30111 Tomas, Rancho Santa Margarita, CA 92688, USA.

Correspondence:
Venkata Sai Prakash Chaturvedula
Natural Ingredient Development, Blue California, 30111 Tomas, Rancho Santa Margarita, CA 92688, USA
Email: saipc@bluecal-ingredients.com
Tel: +1-949-257-7351

Isolation and NMR Spectral Studies of Dihydromyricetin

Venkata Sai Prakash Chaturvedula, Ruo Huang

ABSTRACT

From the alcoholic extract of the leaves of *Hovenia dulcis*, a pentahydroxyl flavanonol has been isolated as a major compound, whose structure has been characterized as dihydromyricetin (DHM) on the basis of extensive 1D and 2D Nuclear Magnetic Resonance (NMR) as well as High Resolution Mass Spectral (HRMS) data. Also, a comparative study of the ^{1}H and ^{13}C NMR spectral data of dihydromyricetin has been studied in three different solvent systems namely C_5D_5N (d5-pyridine) or CD_3OD (d4-methanol) or C_2D_6SO (d6-DMSO).

Keywords: *Hovenia dulcis*, Flavanonol, Dihydromyricetin, 1D and 2D NMR spectral data, Structure characterization, NMR study

1. Introduction

Hovenia dulcis Thunb, also known as Japanese raisin tree, is commonly found in East Asia over the eastern China and Korea to the Himalayas. *H. dulcis* belongs to the family of Rhamnaceae has a long history as a food supplement and traditional medicine in Japan, China and Korea, but is little known and used in Western countries so far [1]. Extracts from *H. dulcis* accelerate possess various medicinal properties including hepatoprotective, antioxidative, antimicrobial and antidiabetic as well as detoxification of ethanol. Although the underlying molecular mechanisms of *H. dulcis* are not fully understood, free radical scavenging and enhancement of ethanol catabolism have been reported in the literature. *H. dulcis* is a glabrous tree with lenticular branches, and grows up to 10 m, which cultivated in plantations in China, invasive in South American rainforests and Tanzania. The tree has been introduced as an ornamental tree to several countries, and the fruit is also edible. The taste of the fleshy peduncles is like a combination of raisin, clove, cinnamon and sugar. The peduncles contain high levels of sugar, while leaves of *H. dulcis* contain several dammarane-type of terpene sweetness inhibitors. Earlier phytochemical analysis of *H. ducis* resulted in the isolation of several terpenes, phenolic and fatty compounds [2-4].

In our continuing research to discover bioactive natural compounds, we have been on various plant species obtained across the globe. Recently, we have reported several minor diterpene glycosides from the commercial extracts of *Stevia rebaudiana* Bertoni and their structures have been characterized based on the 1D and 2D NMR spectral data as well as chemical studies [5-6]. In this article, we are describing the isolation of a pentahydroxyl flavanonol namely dihydromyricetin (1) from the leaves of *H. dulcis*. Further, we are reporting the ^{1}H and ^{13}C NMR spectral data studies of 1 in the deuterated solvents namely d5-pyridine, d4-methanol, and d6-DMSO.

2. Materials and Methods

2.1. General Instrumentation

The 1D and 2D NMR spectral data were acquired on Bruker Avance DRX 500 MHz or Varian INOVA 600 MHz instrument instruments using standard pulse sequences. The NMR spectra were performed in C_5D_5N (d5-pyridine) or CD_3OD (d4-methanol) or C_2D_6SO (d6-DMSO); chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. MS and MS/MS data were generated with a Thermo LTQ-FTMS mass spectrometer (100,000 resolutions) equipped with a Nano spray ionization source.

Samples were diluted with methanol and introduced via infusion using the onboard syringe pump.

2.2. Isolation and purification of dihydromyricetin (1)

The leaves of *H. dulcis* were extracted with ethanol and concentrated under vacuum at low temperature. The residue obtained has been centrifuged and then finally crystallized to yield a pure compound which has been identified as dihydromyricetin (**1**).

2.3. Identification and spectroscopic data of dihydromyricetin (5, 7, 3′, 4′, 5′-pentahydroxyl flavanonol, 1)

Off-White powder; ^{1}H-NMR (600 MHz, d5-pyridine/d4-methanol/d6-DMSO, δ ppm) and ^{13}C-NMR (150 MHz, d5-pyridine/d4-methanol/d6-DMSO, δ ppm) spectroscopic data see Table 1; HRMS (M+Na) $^{+}$ m/z 343.0426 (calcd. for $C_{15}H_{12}O_8Na$: 343.0424).

3. Results and Discussion

Compound **1** was isolated as an off-white powder, and its molecular formula has been was deduced as $C_{15}H_{12}O_8$ from the adduct ion corresponding to $[M+Na]^+$ ion observed at *m/z* 343.0426; this composition was further supported by the ^{13}C NMR spectral data. . The UV spectrum of **1** showed λ max at 261, 319, and 348 nm suggested a flavonoid structure [7-9]. The ^{1}H NMR spectra data of **1** has been acquired in all three solvents namely d5-pyridine, d4-methanol and d6-DMSO. The ^{1}H NMR spectra data of **1** showed two doublet signals between δ 4.48 and 5.47 in d5-pyridine, and d4-methanol, whereas a doublet and doublet of doublets at δ 4.42 and 4.91 in d6-DMSO corresponding to a proton each suggested the 3-hydroxyflavanone or 2, 3-dihydroflavonol skeleton in the molecular structure of **1**. The presence of 2, 3-dihydroflavonol was further supported by the ^{13}C NMR spectral data which showed the presence of oxymethine groups resonating between δ 71.7 and 85.8. In addition, the ^{1}H NMR spectra data of **1** also showed the presence of two meta-coupled aromatic protons as doublets between δ 5.86 and 6.50, and an additional two meta-coupled aromatic protons δ 6.40 and 7.24 as singlets corresponds to a pentahydroxyl flavanonol scaffold. The ^{1}H and ^{13}C NMR values for all the protons and carbons for the compound **1** were assigned on the basis of COSY, HMQC and HMBC correlations and are given in Table 1. The key HMBC correlations confirmed the placement of all the five hydroxyl groups at 5, 7, 3′, 4′, 5′ positions as shown in Figure 1. On the basis of above 1D and 2D NMR spectroscopic data, the structure of **1** was determined unambiguously as dihydromyricetin (5, 7, 3′, 4′, 5′-pentahydroxyl flavanonol) [10].

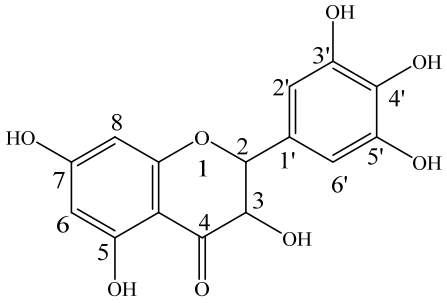

Fig 1: Structure of dihydromyricetin (**1**)

Table 1. ^{1}H and ^{13}C NMR spectral data (chemical shifts and coupling constants) for dihydromyricetin (1) [a-c].

Position	NMR Data in d5-pyridine		NMR Data in d4-methanol		NMR Data in d6-DMSO	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	5.47 (1H, d, 11.6)	85.8	4.85 (1H, d, 12.1)	85.4	4.91 (1H, d, 12.6)	83.3
3	5.08 (1H, d, 11.8)	73.9	4.48 (1H, d, 12.4)	73.8	4.42 (1H, dd, 12.8, 6.4)	71.7
4		199.3		198.4		197.7
5		165.6		165.4		163.4
6	6.37 (1H, d, 2.4)	97.9	5.88 (1H, d, 2.1)	97.4	5.86 (1H, d, 2.4)	95.9
7		169.3		168.9		166.8
8	6.50 (1H, d, 2.4)	96.8	5.92 (1H, d, 2.2)	96.4	5.91 (1H, d, 2.1)	95.0
9		164.5		164.5		162.6
10		102.3		101.9		100.5
1′		129.6		129.2		127.2
2′, 6′	7.24 (2H, s)	109.0	6.63 (2H, s)	108.2	6.40 (2H, s)	106.9
3′, 5′		148.7		146.9		145.7
4′		136.7		135.0		133.5
3-OH					5.76 (1H, 6.2)	

[a] assignments made on the basis of COSY, HMQC and HMBC correlations; [b] Chemical shift values are in δ (ppm); [c] Coupling constants are in Hz.

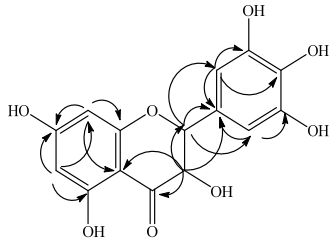

Fig 2: Key HMBC correlations of dihydromyricetin (**1**)

4. Conclusion

We are herewith reporting the isolation of the main constituent of the alcoholic extract of the leaves of *Hovenia dulcis*, whose structure has been characterized as dihydromyricetin (**1**) on the basis of extensive 1D (^{1}H and ^{13}C) and 2D (COSY, HMQC and HMBC) NMR as well as High Resolution Mass Spectral (HRMS) data. Also, we are herewith reporting for the first time the ^{1}H and ^{13}C NMR spectral data of dihydromyricetin in three different solvent systems namely d5-pyridine/d4-methanol/d6-DMSO.

5. Acknowledgements
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