

IR Spectral Analysis of Diterpene Glycosides Isolated from *Stevia rebaudiana*

Venkata Sai Prakash Chaturvedula^{1*}, Christopher Mubarak², Indra Prakash¹

¹Organic Chemistry Department, The Coca-Cola Company, Global Research and Development, One Coca-Cola Plaza, Atlanta, USA;

²Analytical Chemistry Department, The Coca-Cola Company, Global Research and Development, One Coca-Cola Plaza, Atlanta, USA.

Email: *vchaturvedula@coca-cola.com

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ABSTRACT

The objective is to identify the Infra-Red (IR) spectral analysis of the diterpene glycosides present in the commercial extracts of *Stevia rebaudiana* was achieved by PerkinElmer Spectrum 400 Fourier Transform (FT) spectrometer employing a PerkinElmer Universal Attenuated Total Reflection (ATR) accessory. Using this technique the IR spectral pattern of 15 steviol glycosides which belongs to three different classes of *ent*-kaurane diterpene glycosides namely *ent*-13-hydroxykaur-16-en-19-oic acid, *ent*-13-hydroxykaur-15-en-19-oic acid, and 13-methyl-16-oxo-17-nor-*ent*-kauran-19-oic acid were identified. From the wave numbers found for all 15 steviol glycosides, it was observed that that though there are differences in the number of sugar units, nature of sugar units, and their attachments; there are not any notable differences in the IR values.

Keywords: *Stevia rebaudiana*; Diterpene Glycosides; IR Spectral Data

1. Introduction

Stevia rebaudiana (Bertoni) is a perennial shrub belonging to the family of Asteraceae (Compositae) native to Brazil and Paraguay, but now grown commercially in a number of countries, particularly in Japan, Taiwan, Korea, Thailand and Indonesia [1,2]. Extracts of the leaves of *S. rebaudiana* have been used for decades to sweeten food and beverages in Japan, South America and China. The major constituents in the leaves of *S. rebaudiana* are the potently sweet diterpenoid glycosides namely stevioside, and rebaudioside A; which are glycosides of the diterpene steviol, *ent*-13-hydroxykaur-16-en-19-oic acid [3]. These compounds are also known as Stevia sweeteners; rebaudioside F is about 200 times sweeter compared to sucrose and is non-caloric.

In our continuing research to discover natural sweeteners, we have collected commercial extracts of *S. rebaudiana* from various suppliers all over the World and isolated several novel diterpene glycosides [4-10]. Apart from isolating novel compounds from *S. rebaudiana* and utilizing them as possible natural sweeteners or sweetness enhancers, we are also engaged in understanding the stability of the steviol glycosides in various systems of interest and identification of degradation products using various spectroscopic techniques [11-13] as well as syn-

thesis using naturally occurring starting materials [14]. In this article we are providing the IR spectral analysis achieved by PerkinElmer Spectrum 400 Fourier Transform (FT) spectrometer employing a PerkinElmer Universal Attenuated Total Reflection (ATR) accessory of 15 naturally occurring steviol glycosides identified from *S. rebaudiana* namely rebaudioside A (1), stevioside (2), dulcoside A (3), steviolbioside (4), rebaudioside F (5), rebaudioside D (6), 13-[(2-*O*-(3-*O*- α -D-glucopyranosyl)- β -D-glucopyranosyl-3-*O*- β -D-glucopyranosyl- β -D-glucopyranosyl)oxy] *ent*-kauran-19-oic acid β -D-glucopyranosyl ester (7), 13-[(2-*O*- β -D-glucopyranosyl-3-*O*-(4-*O*- α -D-glucopyranosyl)- β -D-glucopyranosyl- β -D-glucopyranosyl)oxy] *ent*-kauran-19-oic acid β -D-glucopyranosyl ester (8), 13-[(3-*O*- β -D-glucopyranosyl- β -D-glucopyranosyl)oxy] *ent*-kauran-19-oic acid β -D-glucopyranosyl ester (9), 13-hydroxy-*ent*-kauran-19-oic acid β -D-glucopyranosyl ester (10), rubusoside (11), 13-[(2-*O*- β -D-glucopyranosyl-3-*O*- β -D-glucopyranosyl- β -D-glucopyranosyl)oxy]-15-ene-*ent*-kauran-19-oic acid β -D-glucopyranosyl ester (12), 13-[(2-*O*- β -D-glucopyranosyl- β -D-glucopyranosyl)oxy] *ent*-kaur-15-en-19-oic acid β -D-glucopyranosyl ester (13), 13-[(2-*O*- β -D-glucopyranosyl-3-*O*- β -D-glucopyranosyl- β -D-glucopyranosyl)oxy]-17-hydroxy-*ent*-kaur-15-en-19-oic acid β -D-glucopyranosyl ester (14), and 13-methyl-16-oxo-17-nor-*ent*-kauran-19-oic acid- β -D-glucopyranosyl ester

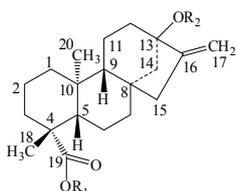
*Corresponding author.

(15). Further, the steviol glycosides studied belongs to three different classes of *ent*-kaurane diterpene glycosides: *ent*-13-hydroxykaur-16-en-19-oic acid (**1** - **11**, **Figure 1**), *ent*-13-hydroxykaur-15-en-19-oic acid (**12** - **14**, **Figure 2**), and 13-methyl-16-oxo-17-nor-*ent*-kauran-19-oic acid (**15**, **Figure 3**).

2. Materials and Methods

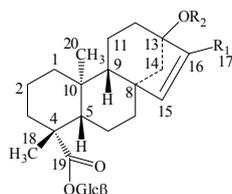
2.1. IR Spectral Analysis

Infrared measurements were performed on a PerkinElmer



Compound	R ₁	R ₂
1	Glcβ1	Glcβ1-2-(Glcβ1-3)Glcβ1-
2	Glcβ1	Glcβ1-2Glcβ1-
3	Glcβ1	Rhaα1-2Glcβ1-
4	H	Glcβ1-2Glcβ1-
5	Glcβ1	Xylβ1-2-(Glcβ1-3)Glcβ1-
6	Glcβ1-2Glcβ1-	Glcβ1-2-(Glcβ1-3)Glcβ1-
7	Glcβ1-	Glcβ1-2Glcα1-3-(Glcβ1-3)Glcβ1-
8	Glcβ1-	Glcβ1-2-(Glcβ1-3Glcα1-4)Glcβ1-
9	Glcβ1	Glcβ1-3Glcβ1-
10	Glcβ1	H
11	Glcβ1	Glcβ1-

Figure 1. Structures of *ent*-13-hydroxykaur-16-en-19-oic acid glycosides.



Compound	R ₁	R ₂
12	CH ₃	Glcβ1-2-(Glcβ1-3)Glcβ1-
13	CH ₃	Glcβ1-2Glcβ1-
14	CH ₂ OH	Glcβ1-2Glcβ1-

Figure 2. Structures of *ent*-13-hydroxykaur-15-en-19-oic acid glycosides.

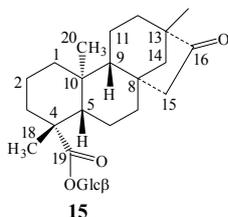


Figure 3: Structure of 13-methyl-16-oxo-17-nor-*ent*-kauran-19-oic acid glycoside. Glcβ: β-D-glucopyranosyl; Glcα: α-D-glucopyranosyl; Rhaα: α-L-rhamnopyranosyl; Xylβ: β-D-Xylopyranosyl; 6-Deoxyglcβ: 6-Deoxy-β-D-gluco-Pyranosyl.

Spectrum 400 FT-IR spectrometer employing a PerkinElmer Universal Attenuated Total Reflection (ATR) accessory. The spectrometer is fitted with potassium bromide (KBr) optics and a medium band MCT detector. The ATR accessory contains a single reflection Diamond/ZnSe crystal top-plate. All spectra were acquired over a scan range of 4000 - 650 cm⁻¹ at a resolution of 4 cm⁻¹ and a scan speed of 1 cm/s. Before acquisition, the sample was placed on the ATR diamond top-plate and using the interactive pressure control, approximately 150 N of force was applied to ensure optimum contact is made between the sample and the diamond. Atmospheric vapour compensation function was enabled in the PerkinElmer Spectrum software to reduce the effects of unwanted atmospheric absorptions in sample spectra. Thirty-two scans were collected and averaged in each acquisition.

2.2. Steviol Glycosides 1-15

The steviol glycosides, rebaudioside A (**1**), stevioside (**2**), dulcoside A (**3**), steviolbioside (**4**), rebaudioside F (**5**), rebaudioside D (**6**), and rubusoside (**11**), were isolated by AMRI (Bothell, WA) or obtained from Chromadex (Irvine, CA). The other steviol glycosides **7** - **10**, and **12** - **15** were isolated by The Coca-Cola Company as reported in the literature [4-14].

3. Results and Discussion

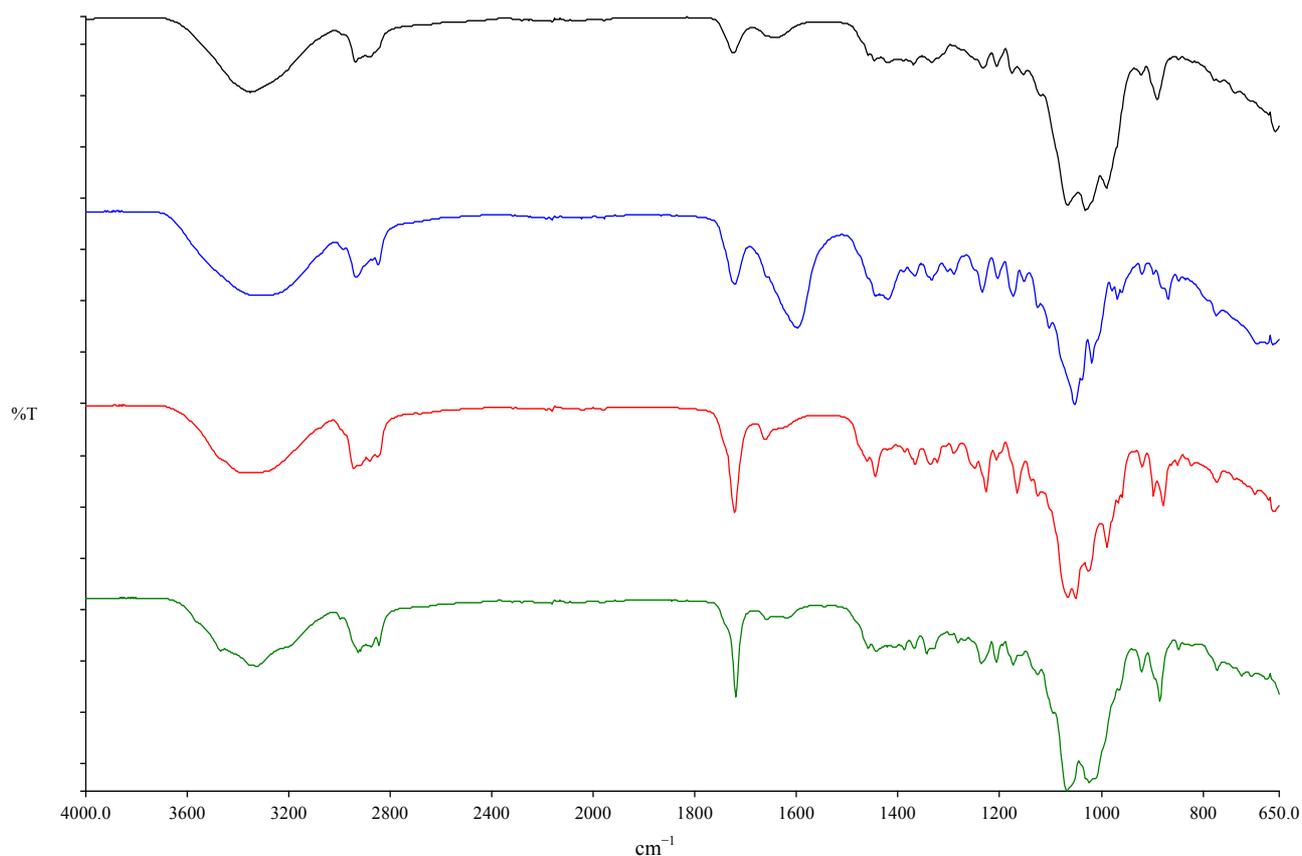
The primary objective of this study is to utilize Infrared (IR) spectroscopy to find variations in wavenumbers of the steviol glycosides **1-15** of *S. rebaudiana*.

The IR spectral data for all the 15 steviol glycosides which belongs to *ent*-kaurane diterpene glycosides: *ent*-13-hydroxykaur-16-en-19-oic acid (**1-11**), *ent*-13-hydroxykaur-15-en-19-oic acid (**12-14**), and 13-methyl-16-oxo-17-nor-*ent*-kauran-19-oic acid (**15**) was performed under the same condition and the wavenumbers for the stretching of hydroxyl (-OH), alkane (sp³-CH), carbonyl (-C=O), alkene (-C=C-), ether (-C-O-C-) groups; and alkene (-C=C-) bending vibrations were identified by employing the method described above which were given in **Table 1**.

From the wave numbers observed in **Table 1**, it was evident that though there are differences in the number of sugar units, nature of sugar units, and their attachments; there are not any notable differences in the IR values of **1-11**, and **12-14**. The hydroxyl stretching for compounds **1-11**, and **12-14** was observed between the wavenumbers 3319 - 3379 cm⁻¹; alkane stretching was observed between 2918 - 2947 cm⁻¹; carbonyl groups between 1719 - 1728 cm⁻¹; alkene stretching between 1641 - 1662 cm⁻¹; ether groups between 1054 - 1070 cm⁻¹; and alkene bending between 870 - 895 cm⁻¹. Compound **15** did not

Table 1. IR spectral data for the steviol glycosides 1-15.

Compound Number	OH Stretching (cm ⁻¹)	Alkane CH Stretching (cm ⁻¹)	C=O Stretching (cm ⁻¹)	C=C Stretching (cm ⁻¹)	C-O-C Stretching (cm ⁻¹)	C=C Bending (cm ⁻¹)
1	3352	2938	1725	1643	1067	891
2	3338	2924	1724	1643	1066	893
3	3350	2929	1725	1645	1058	891
4	3319	2940	1723	1649	1054	870
5	3343	2920	1724	1641	1067	894
6	3345	2946	1735	1659	1066	893
7	3321	2940	1723	1660	1064	896
8	3329	2924	1725	1646	1068	895
9	3345	2928	1725	1641	1068	893
10	3379	2947	1723	1662	1067	879
11	3330	2930	1720	1659	1070	886
12	3343	2937	1722	1644	1068	888
13	3344	2934	1725	1643	1068	894
14	3347	2937	1728	1643	1067	891
15	3365	2918	1741, 1719		1067	

**Figure 4. IR spectral data for compounds 1 (black), 4 (blue), 10 (red), and 11 (green).**

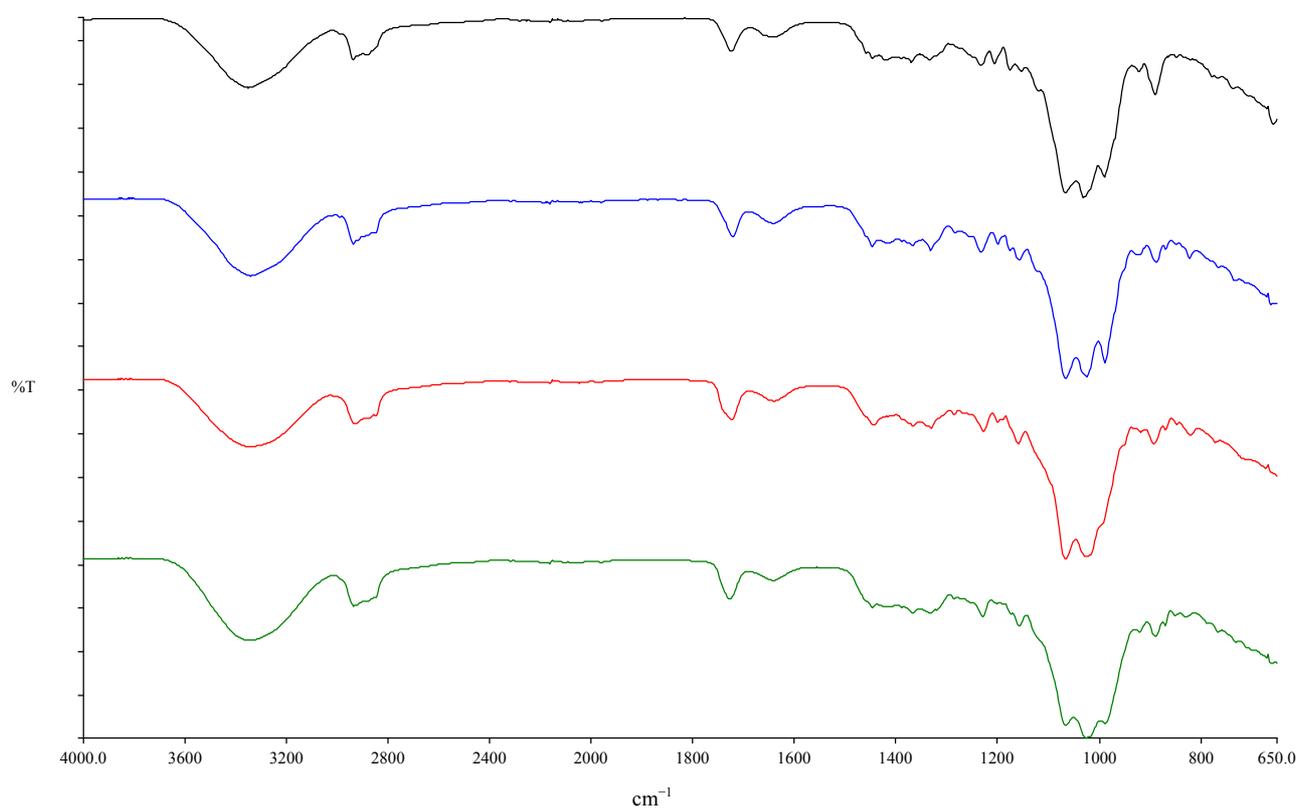


Figure 5. IR spectral data for compounds 1 (black), 12 (blue), 13 (red), and 14 (green).

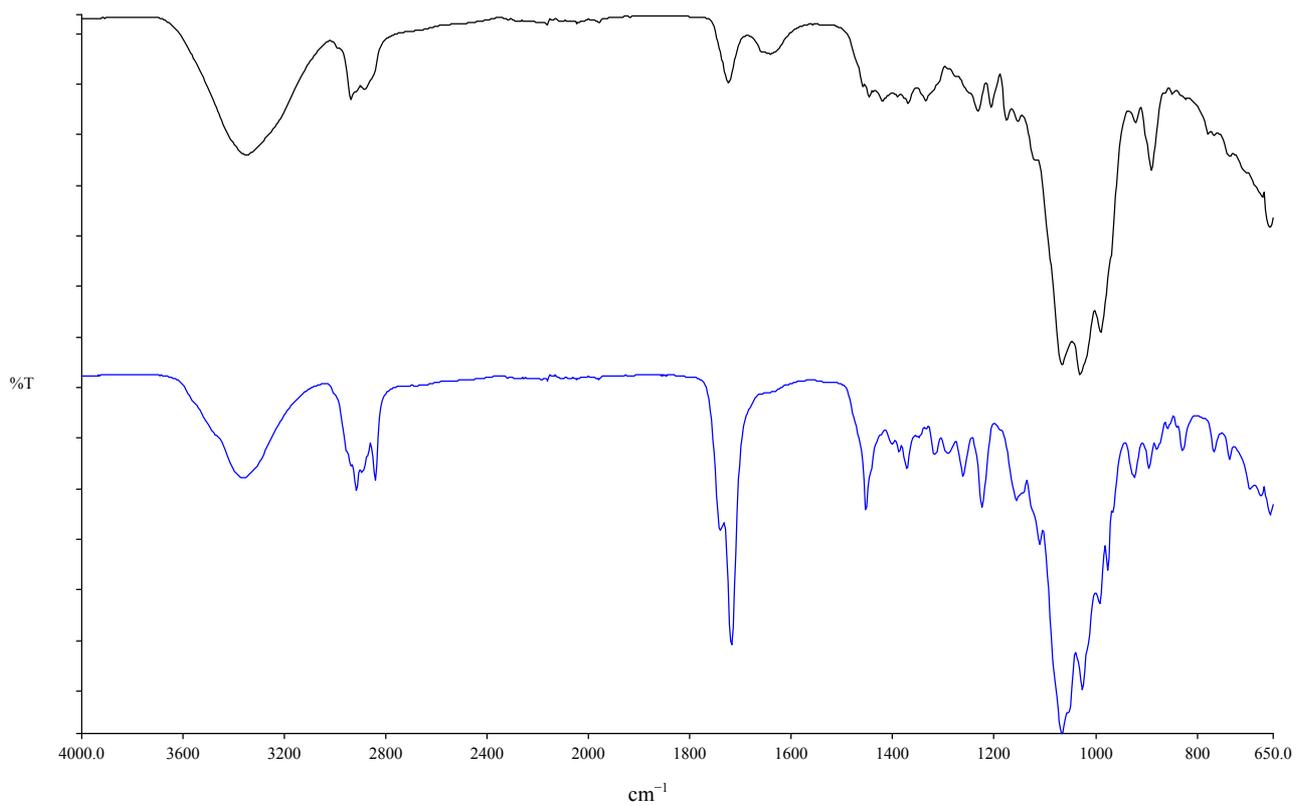


Figure 6. IR spectral data for compounds 1 (black), and 15 (blue).

show any vibrations corresponding to alkene stretching and bending, supported the IR pattern of steviol glycosides without having any alkene functionality. Also, it was observed that compound **15** showed an additional carbonyl group at 1741 cm^{-1} corresponding to the saturated carbonyl group at C-16 position of steviol glycoside. The IR spectral data for the steviol glycosides of the four classes of *ent*-kaurane diterpene glycosides: *ent*-13-hydroxykaur-16-en-19-oic acid (**4**, **10-11**), *ent*-13-hydroxykaur-15-en-19-oic acid (**12-14**), and 13-methyl-16-oxo-17-nor-*ent*-kauran-19-oic acid (**15**) overlapped with rebaudioside A (**1**) were given in **Figures 4-6**.

4. Conclusion

Based on the IR analysis experiments utilized in this study, the spectral pattern of aglycone moieties from steviol glycosides were identified which belongs to three different classes of *ent*-kaurane diterpene glycosides namely *ent*-13-hydroxykaur-16-en-19-oic acid, *ent*-13-hydroxykaur-15-en-19-oic acid, and 13-methyl-16-oxo-17-nor-*ent*-kauran-19-oic acid.

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