Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(5):2666-2670

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

NMR Spectral Assignments of Steviol and Steviol Monoacetate

Venkata Sai Prakash Chaturvedula[1]*, Komal P Kinger[2], Mary R Campbell[2], and Indra Prakash[1]

[1]Organic Chemistry Department, The Coca-Cola Company, Global Research and Development, One Coca-Cola Plaza, Atlanta, GA 30313, USA
[2]Product Development Department, The Coca-Cola Company, Coca-Cola North America Division, One Coca-Cola Plaza, Atlanta, GA 30313, USA

ABSTRACT

The complete NMR spectral assignments for the two diterpenes, steviol obtained from the hydrolysis of stevioside that was isolated from Stevia rebaudiana and its monoacetylated product were achieved by the extensive 1D (^1H and ^{13}C) and 2D NMR (COSY, HMQC, and HMBC) as well as mass spectral data.

Keywords: Stevioside, Steviol, Steviol monoacetate, 1D and 2D NMR spectral data, HRMS data, Acid and enzymatic hydrolysis.

INTRODUCTION

Stevia rebaudiana (Bertoni) is a perennial shrub belonging to the family of Asteraceae (Compositae) which is native to Brazil and Paraguay; but now is 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]; also known as Stevia sweeteners. Stevioside tastes about 150-250 times sweeter than sucrose and rebaudioside A tastes about 200-300 times sweeter than sucrose; both are non-caloric.

In our continuing research to discover natural sweeteners, we have isolated several novel diterpene glycosides [4-9] from the commercial extracts of *S. rebaudiana* obtained from various suppliers all over the World. Apart from isolating novel compounds 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 characterization of degradation products using various spectroscopic analysis, and their synthesis using naturally occurring starting materials [10-13]. In this article, we are describing the complete ^1H and ^{13}C NMR spectral assignments for the two diterpenes steviol (**1**) and its monoacetylated product steviol monoacetate (**2**) that were achieved by the extensive NMR (^1H and ^{13}C, COSY, HSQC, and HMBC) and mass spectral data.

EXPERIMENTAL SECTION

General Methods

NMR spectra were acquired on a Varian Unity Plus 600 MHz instrument using standard pulse sequences at ambient temperature. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. HRMS and MS/MS data were generated with a Waters Premier Quadrupole Time-of-Flight (Q-TOF) mass spectrometer equipped with an electrospray ionization source operated in the positive-ion mode and Thermo Fisher Discovery OrbiTrap in the

electrospray positive mode. Samples were diluted with water: acetonitrile (1:1) containing 0.1% formic acid and introduced via infusion using the on-board syringe pump.

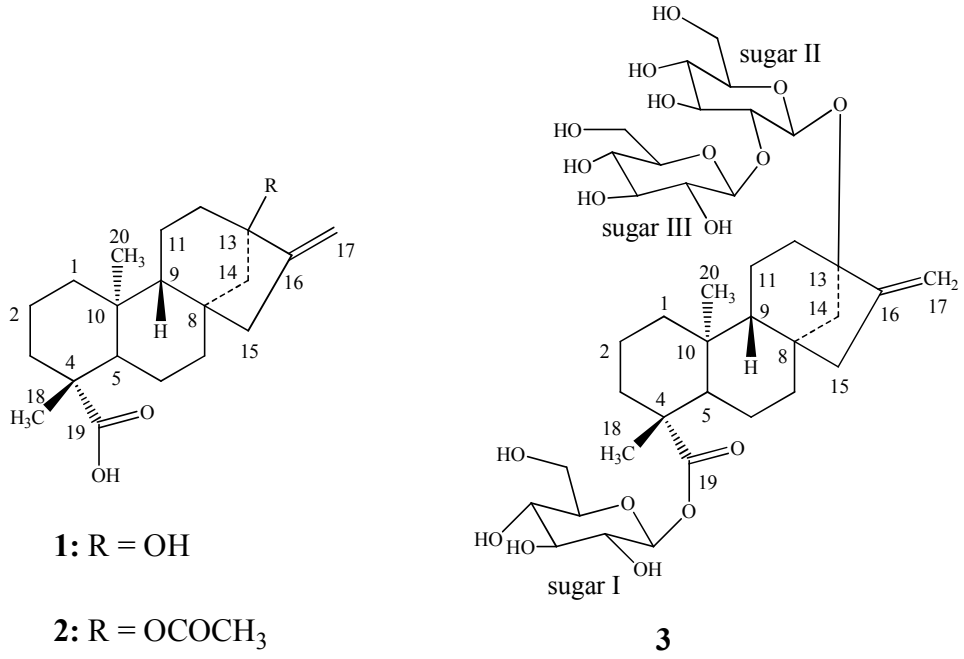

Figure 1. Structures of steviol (**1**), steviol monoacetate (**2**) and stevioside (**3**)

Materials

SG95, the commercial aqueous extract consisting of a mixture of diterpenoid glycosides of the leaves of *S. rebaudiana* was obtained from the Pure Circle (Kuala Lumpur, Malaysia). The authenticity of the crude extract was confirmed by performing its retention time (t_R) comparison with the internal standard compounds of known steviol glycosides namely rebaudioside A-D, and rubusoside isolated from *S. rebaudiana* using the preparative HPLC method as reported earlier [9-10]. A voucher specimen is deposited at The Coca-Cola Company, No. VSPC-3166-002. Stevioside (**3**) was purified by using an Agilent HPLC 1200 system equipped with a Phenomenex Synergi-Hydro column (250 mm x 4.6 mm, 4 µm) equipped with a Phenomenex Security guard C_{18} cartridge. Using the HPLC method (Table 1), collected the peak eluting at t_R 19.06 min; and dried the solution under nitrogen yielded **3**.

Table 1. RP-HPLC method for the separation of stevioside (3)

Time (min)	% of Mobile Phase A	% of Mobile Phase B	% of Mobile Phase C
0.0	75	25	0
8.5	75	25	0
10.0	71	29	0
16.5	70	30	0
18.5	0	34	66
24.5	0	34	66
26.5	0	52	48
29.0	0	52	48
31.0	0	70	30
37.0	0	70	30
37.1	0	90	10
40.0	0	90	10
40.1	75	25	0
43.0	75	25	0

Isolation and Purification of Compounds 1 and 2

*Enzymatic hydrolysis of stevioside (**3**)*

Stevioside (**3**, 10 g) was dissolved in 25 ml of 0.1 M sodium acetate buffer, pH 4.5 and crude pectinase from *Aspergillus niger* (50 mL, Sigma-Aldrich, P2736) was added. The mixture was stirred at 45-50^o C for 72 hr. The product precipitated out during the reaction and was filtered and then crystallized. The resulting product obtained

from the hydrolysis of **3** was identified as steviol (**1**) by comparison of its co-TLC with standard compound and NMR spectral data [15].

*Acetylation of steviol (**1**)*
Steviol (**1**, 100 mg) was suspended in 10 ml of pyridine and added 20 ml of acetic anhydride. The mixture was stirred at room temperature for 96 hr. The reaction mixture after evaporation and subsequent chromatography over Silica gel 60 (60 g) with 25% EtOAc in *n*-hexane furnished a compound which was identified as steviol monoacetate (**2**) by comparison of its ^{1}H NMR spectral data [15, 16].

*Steviol (**1**):* White powder; mp 208-210 °C; IR ν_{max}: 3305, 2945, 1690, 1050, 900 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm) and ^{13}C-NMR (150 MHz, $CDCl_3$, δ ppm) spectroscopic data see Table 2; HRMS $(M+NH_4)^+$ *m/z* 336.2535 (calcd. for $C_{20}H_{34}O_3N$: 336.2539); $(M+Na)^+$ *m/z* 341.2098 (calcd. for $C_{20}H_{30}O_3Na$: 341.2093).

*Steviol monoacetate (**2**):* White powder; mp 193-196 °C; IR ν_{max}: 2940, 1730, 1695, 1045, 890 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm) and ^{13}C-NMR (150 MHz, $CDCl_3$, δ ppm) spectroscopic data see Table 2; HRMS $(M+NH_4)^+$ *m/z* 378.3641 (calcd. for $C_{22}H_{36}O_4N$: 378.2644); $(M+Na)^+$ *m/z* 383.2192 (calcd. for $C_{22}H_{32}O_4Na$: 383.2198).

RESULTS AND DISCUSSION

Compound **1** was obtained as white powder from the enzymatic hydrolysis of stevioside (**3**) and its molecular formula has been deduced as $C_{20}H_{30}O_3$ on the basis of its HRMS data which showed the presence of an $[M+NH_4]^+$ ion at *m/z* 336.2535 together with $[M+Na]^+$ adduct at *m/z* 341.2098, this composition was supported by the ^{13}C NMR spectral data. The ^{1}H NMR spectrum of **1** showed the presence of two methyl singlets at δ 0.96 and 1.24, two olefinic protons as singlets at δ 4.82 and 4.99 of an exocyclic double bond, nine methylene and two methine protons between δ 0.86-2.21 characteristic for the diterpenes belongs to the class of *ent*-kaurenes isolated earlier from the genus *Stevia* [4-9].

Table 2. ^{1}H and ^{13}C NMR chemical shift values for 1–2 recorded in $CDCl_3$ [a-c]

Position	1		2	
	^{1}H	^{13}C	^{1}H	^{13}C
1	0.83 (m, 1H), 1.87 (m, 1H)	41.4	0.81 (m, 1H), 1.85 (m, 1H)	41.6
2	1.41 (m, 1H), 1.92 (m, 1H)	19.2	1.40 (m, 1H), 1.90 (m, 1H)	19.4
3	1.02 (m, 1H), 2.21 (m, 1H)	38.0	1.00 (m, 1H), 2.20 (m, 1H)	38.2
4		43.8		43.6
5	1.12 (m, 1H)	57.0	1.10 (m, 1H)	57.2
6	1.85 (m, 1H), 2.04 (m, 1H)	22.0	1.83 (m, 1H), 2.02 (m, 1H)	22.1
7	1.43 (m, 1H), 1.55 (m, 1H)	41.9	1.42 (m, 1H), 1.55 (m, 1H)	41.7
8		43.7		43.8
9	0.98 (m, 1H)	54.0	0.96 (m, 1H)	54.2
10		39.7		39.8
11	1.45 (m, 1H), 1.80 (m, 1H)	20.7	1.43 (m, 1H), 1.82 (m, 1H)	20.6
12	1.56 (m, 1H), 1.97 (m, 1H)	40.7	1.54 (m, 1H), 1.95 (m, 1H)	38.9
13		80.5		86.4
14	1.55 (m, 1H), 2.18 (m, 1H)	47.5	1.56 (m, 1H), 2.16 (m, 1H)	42.6
15	2.04 (m, 1H), 2.14 (m, 1H)	51.0	2.02 (m, 1H), 2.16 (m, 1H)	51.1
16		155.9		155.6
17	4.82 (s, 1H) 4.99 (s, 1H)	103.3	4.84 (s, 1H) 4.96 (s, 1H)	103.1
18	1.24 (s, 3H)	29.0	1.21 (s, 3H)	29.2
19		183.2		183.0
20	0.96 (s, 3H)	15.6	0.98 (s, 3H)	15.7
OCO*CH*$_3$			2.03	21.1
O*CO*CH$_3$				170.2

[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.

The basic skeleton of *ent*-kaurene diterpenoids was supported by the COSY (H-1/H-2; H-2/H-3; H-5/H-6; H-6/H-7; H-9/H-11; H-11/H-12) and HMBC (H-1/C-2, C-10; H-3/C-1, C-2, C-4, C-5, C-18, C-19; H-5/C-4, C-6, C-7, C-9, C-10, C-18, C-19, C-20; H-9/C-8, C-10, C-11, C-12, C-14, C-15; H-14/C-8, C-9, C-13, C-15, C-16 and H-17/C-13, C-15, C-16) correlations. The ^{1}H- and ^{13}C-NMR values for all the protons and carbons were assigned on the basis of COSY, HMQC and HMBC correlations (Table 2). Thus, based on the above spectral data, structure of **1** was assigned as steviol consistent to the reported literature values [14].

Compound **2** was obtained on the acetylation of steviol (**1**) with acetic anhydride and pyridine. The molecular formula of **2** was established as $C_{22}H_{32}O_4$ from its HRMS spectral data which showed $[M+Na]^+$ ion at *m/z* 383.2192. The ^{1}H NMR spectrum of **2** also showed the presence of two methyl singlets at δ 0.98, and 1.21, nine methylene and two methine protons, similar to **1** (Table 2). The ^{1}H NMR showed the presence of an additional methyl group at δ 2.03 corresponding to the acetyl functional group which was supported by the additional carbonyl and methyl groups at δ 170.2 and 21.1 respectively. The ^{1}H and ^{13}C NMR values for all the carbons were assigned on the basis of HMQC and HMBC correlations and are given in Table 2. The above spectral data supported the monoacetylation of steviol (**1**) at C-13 position, which was evident by the presence of the acetyl group at δ 170.2 and 21.1 corresponding to the carbonyl and methyl groups respectively. Thus, structure of **2** was assigned as steviol acetate consistent to the reported literature values [14-15]. The structure was further supported by the COSY and HMBC correlations (Figure 2) [16-18].

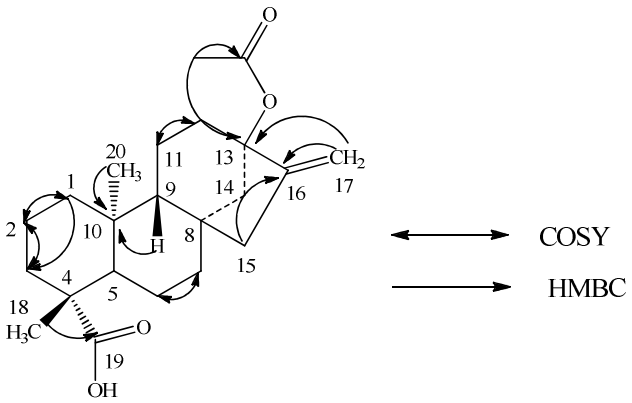

Figure 2: Key COSY and HMBC correlations of **1**

CONCLUSION

We are herewith reporting the complete ^{1}H and ^{13}C NMR spectral assignments for the two *ent*-kaurane diterpenoids steviol and its monoacetate derivative that were assigned on the basis of extensive 1D and 2D NMR as well as high resolution mass spectral data.

REFERENCES

[1] E Mosettig; WR Nes, *J. Org. Chem*., **1955**, 20, 884-899.
[2] E Mosettig; U Beglinger; F Dolder; H Lichiti; P Quitt; JA Waters, *J. Am. Chem. Soc.*, **1963**, 85, 2305.
[3] JE Brandle; AN Starrratt; M Gijen, *Can. J. Plant Sci.*, **1998**, 78, 527-536.
[4] VSP Chaturvedula; U Mani; I Prakash, *Molecules*, **2011**, 16, 3552-3562.
[5] VSP Chaturvedula; I Prakash, *Molecules*, **2011**, 16, 2937-2943.
[6] VSP Chaturvedula; I Prakash, *Carbohydr. Res*., **2011**, 346, 1057-1060.
[7] VSP Chaturvedula; J Rhea; D Milanowski; U Mocek; I Prakash, *Nat. Prod. Commun*., **2011**, 6, 175-178.
[8] VSP Chaturvedula; I Prakash, *Nat. Prod. Commun*., **2011**, 6, 1059-1062.
[9] VSP Chaturvedula; I Prakash, *J. Chem. Pharm. Res*., **2011**, 3, 799-804.
[10] VSP Chaturvedula; JF Clos; I Prakash, *Int. J. Pharm. Pharm. Sci.*, **2011**, 3, 316-323.
[11] VSP Chaturvedula; JF Clos; I Prakash, *Int. J. Pharm. Pharm. Sci.*, **2011**, 3 (suppl 5), 421-425.
[12] VSP Chaturvedula; J Klucik; U Mani; I Prakash, *Molecules*, **2011**, 16, 8402-8409.
[13] VSP Chaturvedula; JF Clos; I Prakash, *Food Res. Intl.*, **2012**, 48, 65-75.
[14] K Ohtani; Y Aikawa; R Kasai; W Chou; K Yamasaki; O Tanaka, *Phytochemistry*, **1992**, 31, 1553-1559.
[15] T Gianfagna; JAD Zeevaart; WJ Lusk, *Phytochemistry*, **1983**, 22, 427-430.
[16] S Arora; S Vijay; D Kumar, *J. Chem. Pharm. Res*., **2011**, 3, 145-150.

[17] DE Okwu; FU Nnamdi, *J. Chem. Pharm. Res*., **2011**, 3, 1-10.
[18] VH Barve; SN Hiremath; SR Pattan; SC Pal, *J. Chem. Pharm. Res*., **2011**, 2, 200-209.