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Research Article

STABILITY OF STEVIOL GLYCOSIDES IN MOCK BEVERAGES UNDER ACIDIC CONDITIONS

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ABSTRACT

Stability of a mixture of steviol glycosides was studied under a variety of conditions covering a typical pH range and various temperatures that simulated both relevant and extreme beverage storage conditions. Thus, steviol glycosides was evaluated in mock beverage solutions by simulating formulations used in commercial cola soft drinks (pH 2.8 and pH 3.2), lemon-lime soft drinks (pH 3.8), and root beer soft drinks (pH 4.2) but lacking the flavor components. The mock beverage samples, formulated at ~500 mg/l, were subjected to various temperature conditions (5, 20, 30, and 40 °C) for 26-weeks at each pH. Samples were analyzed at scheduled intervals throughout the 26-week period for steviol glycosides, known impurities, known degradation products, and unidentified compounds greater than or equal to 0.100% of the starting concentration of steviol glycosides Experimental results indicated that steviol glycoside mixture did not undergo any major decomposition but yielded two minor compounds which were identified as 13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-gluopyranosyl-β-D-glucopyranosyl)oxy] *ent*-kaur-15-en-19-oic acid β-D-glucopyranosyl ester, and 13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-16β-hydroxy-*ent*-kauran-19-oic acid β-D-glucopyranosyl ester, on the basis of ^{1}H & ^{13}C NMR, COSY, HSQC, HMBC, MS, MS/MS spectral data as well as hydrolysis studies.

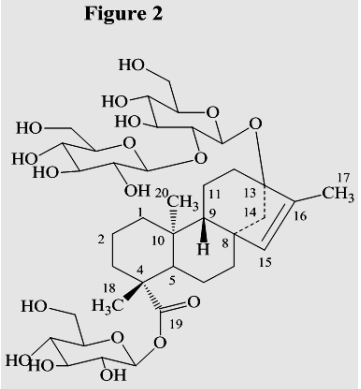
Keywords: Steviol glycosides, Stability, pH, Temperature, Degradation products, Spectroscopic data.

INTRODUCTION

Many soft drink manufacturers recently have driven their focus towards natural high-potency sweeteners to reduce calories by introduction of non-caloric sweeteners into their beverage systems. One such example in recent years is *Stevia rebaudiana*. Extracts of *Stevia rebaudiana* (Bertoni), a perennial shrub of the Asteraceae (Compositae) family native to certain regions of South America (Paraguay and Brazil) [1-2] resulted in the isolation of several potently sweet diterpenoid glycosides namely rebaudiosides A and D, stevioside, and dulcoside A which are known as stevia sweeteners [3]. These compounds are all glycosides of the diterpene *ent*-13-hydroxykaur-16-en-19-oic acid known as steviol [4]. Due to its continuing demand, there has been intense interest on *S. rebaudiana* and that is why it is grown commercially in a number of countries, particularly in Japan, Taiwan, Korea, Thailand and Indonesia.

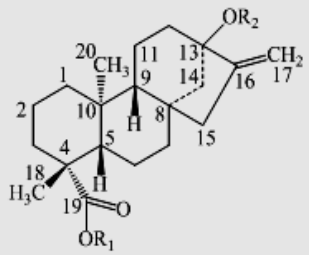
As a part of our continuing research to discover natural sweeteners, we have recently isolated several novel diterpene glycosides from the commercial extracts of the leaves of *S. rebaudiana* obtained from various suppliers around the world [5-9]. Apart from isolating novel compounds from *S. rebaudiana* and utilizing them as possible natural sweeteners or sweetness enhancers, we are also engaged in understanding the physicochemical profiles of steviol glycosides in various systems of interest. This article describes the stability of a mixture of steviol glycosides in mock beverages at different pH levels under various temperatures. The composition of the steviol glycoside mixture studied in this experimentation is mainly the nine compounds namely stevioside (**1**), rebaudioside A (**2**), rebaudioside B (**3**), steviolbioside (**4**), rubusoside (**5**), dulcoside A (**6**), rebaudioside C (**7**), rebaudioside D (**8**), rebaudioside F (**9**), as well as the other nine compounds which were appeared at relatively low abundance, 13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] *ent*-kaur-16-en-19-oic acid-(2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl) ester (**10**), 13-[(2-*O*-(6-*O*-β-D-glucopyranosyl)-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] *ent*-kaur-16-en-19-oic acid β-D-glucopyranosyl ester (**11**), 13-[(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] *ent*-kaur-16-en-19-oic acid (6-*O*-β-D-xylopyranosyl-β-D-glucopyranosyl) ester (**12**), 13-[(2-*O*-β-D-glucopyranosyl-3-*O*-(4-*O*-α-D-glucopyranosyl)-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] *ent*-kaur-16-en-19-oic acid β-D-glucopyranosyl ester (**13**), 13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-xylopyranosyl-β-D-glucopyranosyl)oxy] *ent*-kaur-16-en-19-oic acid β-D-glucopyranosyl ester (**14**), 13-[(2-*O*-β-D-xylopyranosyl-β-D-glucopyranosyl)oxy] *ent*-kaur-16-en-19-oic acid β-D-glucopyranosyl ester (**15**), 13-[(3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] *ent*-kaur-16-en-19-oic acid β-D-glucopyranosyl ester (**16**), 13-[(2-*O*-6-deoxy-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] *ent*-kaur-16-en-19-oic acid β-D-glucopyranosyl ester (**17**), and 13-[(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] *ent*-kaur-15-en-19-oic acid β-D-glucopyranosyl ester (**18**). The above twenty steviol glycosides belongs to two different classes of *ent*-kaurane diterpene glycosides: *ent*-13-hydroxykaur-16-en-19-oic acid (**1**-**17**) (Figure 1), and *ent*-13-hydroxykaur-15-en-19-oic acid (**18**) (Figure 2).

Figure 2



Compound 18

Figure 1



Compound	R_1	R_2
1	Glcβ1	Glcβ1-2Glcβ1-
2	Glcβ1	Glcβ1-2(Glcβ1-3)Glcβ1-
3	H	Glcβ1-2(Glcβ1-3)Glcβ1-
4	H	Glcβ1-2Glcβ1-
5	Glcβ1	Glcβ1-
6	Glcβ1	Rhaα1-2Glcβ1-
7	Glcβ1	Rhaα1-2(Glcβ1-3)Glcβ1-
8	Glcβ1-2Glcβ1-	Glcβ1-2(Glcβ1-3)Glcβ1-
9	Glcβ1	Xylβ1-2(Glcβ1-3)Glcβ1-
10	Rhaα1-2Glcβ1-	Glcβ1-2(Glcβ1-3)Glcβ1-
11	Glcβ1-	Glcβ1-6(Glcβ1-3)Glcβ1-2Glcβ1-
12	Xylβ1-6Glcβ1-	Glcβ1-2Glcβ1-
13	Glcβ1	Glcβ1-2Glcα1-4(Glcβ1-3)Glcβ1-
14	Glcβ1	Glcβ1-2(Xylβ1-3)Glcβ1-
15	Glcβ1	Xylβ1-2Glcβ1-
16	Glcβ1	Glcβ1-3Glcβ1-
17	Glcβ1	6-DeoxyGlcβ1–2(Glcβ1-3)Glcβ1-

Glcβ: β–D-glucopyranosyl; DeoxyGlcβ: 6–Deoxy–β–D-glucopyranosyl;
Glcα: α–D-glucopyranosyl; Rhaα: α–L-rhamnopyranosyl; Xylβ: β–D-Xylopyranosyl

MATERIAL AND METHODS

Major Computer Systems, Reagents and Chemicals,

The major computer systems, as well as the reagents and chemicals used for this study were identical to the reported in the literature [10].

Mobile Phase, Dilution Buffer and Standard Compounds

The preparation of dilution buffer, mobile phase for HPLC and the standard compounds was performed as per the procedure reported earlier [11].

Reference Standards and Other Compounds

All reference standards were isolated by AMRI (Bothell, WA) or prepared by The Coca-Cola Company and were certified by Chromadex (Irvine, CA).

Preparation of Mock Beverage Samples, Carbonation and Bottling

Bottles of mock beverages containing deionized water, potassium benzoate, tri-sodium citrate (dihydrate), citric acid (anhydrous), potassium chloride, sodium chloride, magnesium chloride, and calcium sulfate (anhydrous) were prepared at pH 2.8, 3.2, 3.8 and 4.2 using phosphoric acid. Each product was placed into a Zahm & Nagel carbonator unit. The air was purged from the tank by sparging the product with CO_2 and bleeding off the headspace gas. The carbonator tank was then sealed, placed into an ice water bath and pressurized with CO_2. The product was carbonated by adjusting the temperature and CO_2 pressure to levels that corresponded to 3.8 +0.2 volumes of carbonation. Carbonation level was tested using a Zahm DT piercing device. When the product met the desired carbonation range, the product was bottled, sealed with a crimp-on crown closures, and then placed into refrigerated storage. All the products were prepared by The National Food Laboratory, Livermore, CA (The NFL) per The NFL Project Number PR7784-B and were stored refrigerated by Covance in a desiccator at 5 ±3° C.

Instrumentation and Conditions

An Agilent (Wilmington, DE) 1200 HPLC, including a quaternary pump, a temperature controlled column compartment with additional 6-port switching valve, an auto sampler and a UV absorbance detector, was used for the analysis. A Charged Aerosol Detector (CAD), ESA, Inc. (Chelmsford, MA), was also used for the analysis. The scale on the CAD was 100 pA and the filter was set to medium. The switching valve diverted the first 5.5 minutes of each injection away from the CAD detector to prevent fouling of the detector. The system was controlled using Waters (Milford, MA) Empower software. For Karl Fischer moisture analysis, titration was performed using a Metrohm 784 KFP Titrino titrator. The RP-HPLC employed a Phenomenex (Torrance, CA) Synergi-Hydro column (250 mm x 4.6 mm, 4 µm) with a Phenomenex Security guard C_{18} cartridge and a tertiary solvent mobile phase (A: 0.040% NH_4OAc/AcOH buffer, B: MeCN and C: 0.040% AcOH). The column was at a temperature of 55°C and the flow rate was 1.0 ml/minute. The injection volume of each sample was 100 µl, which were kept at ambient temperature while in the auto sampler. Ultraviolet (UV) detection at 215 nm was used for analysis of both **1** and **2,** and 210 nm was used for **2** when analyzing for it as an impurity. In all cases for UV detection, a 4 nm bandwidth was used with a reference wavelength of 260 nm (100 nm band width). CAD was used for the

analysis of all steviol glycosides with a total run time of 43 min. NMR spectra were acquired on Bruker Avance DRX 500 MHz and Varian Unity Plus 600 MHz instruments using standard pulse sequences. 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 a 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 onboard syringe pump.

Analysis Procedure

For the RP-HPLC method, the column was flushed with 50 ml of 90% MeCN to waste before use and the samples were bracketed with standards by injecting them at the beginning and at the end of a run for accuracy of their retention times. The details of the solvents used for the RP-HPLC gradient method are described earlier [10].

Quantitation of Analytes. Each analyte was identified by retention time matching with reference standards. The area response of each analyte was determined for the samples and standards. Full fit 1/x weighted linear regression standard curves for the UV detector data were prepared by plotting analyte concentrations in mg/l. In a similar manner, the CAD detector data were fitted to a 1/x weighted quadratic standard curve line. The Empower data acquisition software was used to prepare the calibration curves and to calculate concentrations of analytes.

Identification and spectroscopic data for the degradation products

*13-[(2-O-β-D-glucopyranosyl-3-O-β-D-gluopyranosyl-β-D-glucopyranosyl)oxy] ent-kaur-15-en-19-oic acid β-D-glucopyranosyl ester (**19**)*: colorless oil; ^{1}H NMR (500 MHz, CD_3OD) δ 0.96 (s, 3H, C_{20}-CH_3), 1.20 (s, 3H, C_{18}-CH_3), 1.70 (s, 3H, C_{17}-CH_3), 4.62 (d, *J*=8.4 Hz,1H), 4.64 (d, *J*=7.6 Hz,1H), 4.78 (d, *J*=7.4 Hz,1H), 5.10 (s, 1H, C_{15}-H), 5.38 (d, *J*=7.8 Hz,1H); MS (ESI): $[M+H]^+$ 967; $[M+Na]^+$ 989.

*13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-16β-hydroxy ent-kauran-19-oic acid β-D-glucopyranosyl ester (**20**)*: white powder; ^{1}H NMR (500 MHz, CD_3OD) δ 0.97 (s, 3H, C_{20}-CH_3), 1.21 (s, 3H, C_{18}-CH_3), 1.26 (s, 3H, C_{17}-CH_3), 4.65 (d, *J*=7.6 Hz,1H), 4.69 (d, *J*=7.8 Hz,1H), 4.86 (d, *J*=8.1 Hz,1H), 5.39 (d, *J*=8.1 Hz,1H); MS (ESI): $[M+H]^+$ 985; $[M-H]^-$ 983.

*General procedure for acid hydrolysis and determination of sugar configuration in **19-20:*** Each compound (500 µg) was hydrolyzed with 0.5 M HCl (0.5 mL) for 1.5 h. After cooling, the mixture was passed through an Amberlite IRA400 column and the eluate was lyophilized. The residue was dissolved in pyridine (0.25 mL) and heated with L-cysteine methyl ester HCl (2.5 mg) at 60ºC for 1.5 h, and then *O*-tolyl isothiocyanate (12.5 uL) was added to the mixture and heated at 60ºC for an additional 1.5 h. The reaction mixture was analyzed by HPLC: column Phenomenex Luna C18, 150 x 4.6 mm (5 u); 25% acetonitrile-0.2% TFA water, 1 mL/min; UV detection at 250 nm. The sugars were identified as D-glucose (*t*R, 12.24 and *t*R, 12.21 min) in compound **19** and **20** (*t*R, 12.30 min) [authentic samples, D-glucose (*t*R, 12.38) and L-glucose (*t*R, 11.12 min)] [12].

*Enzymatic hydrolysis of **19** and **20**:* Each compound (250 µg) was dissolved in 2.5 ml of 0.1 M sodium acetate buffer, pH 4.5 and crude pectinase from *Aspergillus niger* (50 ul, Sigma-Aldrich, P2736) was added. The mixture was stirred at 50° C for 48 hr. The product precipitated out during the reaction and was filtered and then crystallized. The resulting products obtained from the hydrolysis of **19** and **20** were identified as *ent*-13-hydroxykaur-15-en-19-oic acid, and *ent*-13, 16β-dihydroxykauran-19-oic acid respectively by comparison of their ^{1}H NMR spectral data [13].

RESULTS AND DISCUSSION

The primary objective of this study was to assess the stability of steviol glycosides under a variety of conditions covering a typical pH range at various temperatures that simulated both relevant and extreme beverage storage conditions. The stability of steviol glycosides was evaluated in mock beverage solutions by simulating formulations used in commercial cola soft drinks (pH 2.8 and pH 3.2), lemon-lime soft drinks (pH 3.8), and root beer soft drinks (pH 4.2) but lacking the flavour components.

Also, we are herewith reporting the mass (mole) balances of the mixture of steviol glycosides and its major degradation products obtained during the course of study.

Eight different formulations were prepared. The formulations represented 2 products each at pH 2.8, 3.2, 3.8 and 4.2 one with steviol glycosides at a concentration of about 500 mg/l and one without steviol glycosides. These products were mixed, carbonated and bottled as described above. The stability of a steviol glycoside mixture was evaluated in mock beverage solutions by simulating formulations at the above four pH at various temperatures 5 ±3° C, 20 ±2° C, 30 ±2° C, and 40 ±2° C, but lacking the flavour components. Two sets of mock beverages at each pH under each temperature were prepared as described above. Samples were analysed in duplicate using the HPLC method as stated above for steviol glycosides, their known impurities and degradation products, as well as unidentified compounds that are greater than or equal to 0.100% from the starting concentration of steviol glycosides at scheduled intervals (0, 1, 2, 4, 6, 8, 10, 12, 16, 18 weeks) throughout the 26-week period. All samples were treated identically during analysis to minimize assay bias.

HPLC analysis indicated that the stability of steviol glycosides in mock beverage solutions was pH-, temperature-, and time-dependent. The rate of degradation product formation was increased at lower pH levels and at higher temperatures. The majority of degradation product formation occurred after extended storage.

For example, after 12-weeks of storage, concentrations of **1** in samples at pH 2.8 relative to the initial concentration, ranged from 99.2% at 5 °C to 63.1% at 40 °C. Similarly, the concentration of **1** at pH 3.2 changed from 100.0% at 5 °C to 80.6% at 40 °C. At pH 3.8, concentration of **1** ranged from 99.2% at 5 and 20 °C to 93.6% at 40 °C, whereas at pH 4.2 its concentrations ranged from 99.2% at 5 °C to 96.0% at 40 °C. Likewise, HPLC analysis after 26-weeks of storage suggested that, concentrations of **1** at pH 2.8 were changed from 100.3% at 5 °C to 38.8% at 40 °C; pH 3.2 from 100.5% at 5 °C to 64.2% at 40 °C; pH 3.8 from 100.3% at 5 and 20 °C to 87.7% at 40 °C; and pH 4.2, from 100.0% at 5 °C to 92.8% at 40 °C.

Patterns of other steviol glycoside degradation were similar at each of the conditions tested although the extent and rate of degradation product formation were pH-, temperature-, and time-dependent. Compounds **3**, **4**, **11**, **16**, and **18** were the major degradation products detected during the course of the study and were detected at increased concentration levels at every pH at temperatures 30 and 40 °C. Lower pH levels caused greater formation of both **11** and **18** than higher pH levels.

Samples prepared at pH 3.2 and stored for 12-weeks at 20 °C were taken as the representative of conditions for the evaluation of non-nutritive sweeteners stability in carbonated soft drinks. After 12-weeks of storage at 20 °C, the sum of the seven steviol glycosides **1**-**7** in the pH 3.2 samples was quantitated at 98.8% of initial level. In addition to the seven known steviol glycosides (**1**-**7**), all other minor steviol glycosides **8**-**18** were detected at levels greater than or equal to 0.500 mg/l (0.100%). From the HPLC results, it was observed that compound **19** was present after 12 weeks in addition to the known degradation products. Further, after 12 weeks at 20 °C under pH 3.2; compounds **11**, **16**, and **18** showed increase in concentrations from 0.768 to 1.84 mg/L, 1.43 to 4.91 mg/L, and 0.825 to 1.31 mg/L, respectively compared to their initial 0-week levels. No other known degradation products or impurities increased by more than fifteen percent of their initial 0-week levels under standard conditions.

After 26-weeks of storage at 20 °C, the sum of the seven steviol glycosides **1**-**7** in the pH 3.2 samples was quantitated at 97.8% of initial level. The sum of the known and unknown compounds (**1**-**18**) was quantitated at 99.2% of initial level with the minor glycosides **8**-**18** were detected at levels greater than or equal to 0.500 mg/L (0.100%). Also, from the HPLC results it was found that an

additional compound **20** was identified apart from **19** which were observed after 12-weeks. Further, like in 12 week data, after 26-weeks at 20 °C under pH 3.2, compounds **11**, **16**, and **18** showed increase in concentrations from 0.768 to 2.90 mg/l, 1.43 to 8.43 mg/l, and 0.825 to 1.76 mg/l, respectively. No other known degradation products or impurities increased by more than thirty percent of their initial 0-week levels under each condition.

The mass balance for each analyte at every test period was calculated based on their respective concentrations found in test solutions and molecular weights. Thus, the pH 3.2 samples stored at 20 °C for 12-weeks had a mass balance of 99.5 mole percent. The mass balance for all the other pH and storage conditions found after 12- weeks ranged from 98.5 to 100.3 mole percent. The pH 3.2 samples stored at 20 °C for 26-weeks had a mass balance of 99.3 mole percent. The mass balance for all the other pH and storage conditions found after 26-weeks of storage ranged from 97.5 mole percent to 100.5 mole percent. The high values found for molar recoveries in this study make it unlikely that any appreciable amount of an undetected degradation product was formed under the conditions of the study.

The structures of the two additional compounds **19** and **20** identified during the course of this study were characterized on the basis of extensive spectroscopic data (^{1}H & ^{13}C NMR, COSY, HSQC, HMBC, MS, MS/MS) and the details are given below.

Compound **19** was obtained as white powder, and its molecular formula was assigned as $C_{44}H_{70}O_{23}$ from the positive ESI spectrum which showed $[M+H]^+$ ion at *m/z* 967 and was further supported with an $[M+Na]^+$ adduct at *m/z* 989. The ^{1}H NMR spectrum of **19** showed the presence of three methyl singlets at δ 0.96, 1.20 and 1.70, eight methylene and two methine protons between δ 0.86-2.28, a trisubstituted olefinic proton as a singlet at δ 5.10. The ^{1}H NMR of **19** showed the presence of four anomeric protons as doublets at δ 4.62 (*J*=8.4 Hz, 1H), 4.64 (*J*=7.6 Hz, 1H), 4.78 (*J*=7.4 Hz, 1H), and 5.38 (*J*=7.8 Hz, 1H), suggesting the presence four sugar units in its structure. Enzymatic hydrolysis of **19** furnished a compound which was found identical to steviol on the basis of NMR spectral data [13]. Acid hydrolysis of **19** afforded D-glucose that was identified by preparing its corresponding thiocarbamoyl-thiazolidine carboxylate derivative with L-cysteine methyl ester and *O*-tolyl isothiocyanate, and in comparison of its retention time with the standard sugars as described in the literature comparison [12]. The large coupling constants observed for the four anomeric protons suggested their β-orientation similar to the reported steviol glycosides.

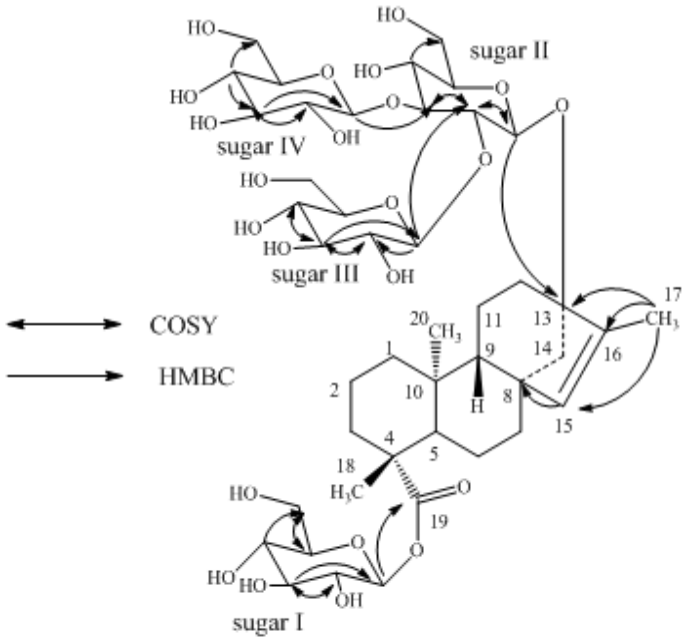


Figure 4: Key COSY and HMBC correlations of **19**

A close comparison of the ^{1}H and ^{13}C NMR values of **19** with rebaudioside A (**2**) [14] suggested the presence of the additional glucose moiety at C-3′′ position of sugar II as a 2,3-branched β-D-glucotriosyl substituent at C-13 and another glucosyl unit at C-19 with a trisubstituted double bond between C-15 and C-16 as shown in Figure 3; which was supported by the key COSY and HMBC correlations. Thus, the structure of **19** was deduced as 13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D glucopyranosyl-β-D-glucopyranosyl)oxy] *ent*-kaur-15-en-19-oic acid β-D-glucopyranosyl ester [11,15].

Compound **20** was obtained as white powder, and its molecular formula was assigned as $C_{44}H_{72}O_{24}$ from the ESI mass spectrum which showed $(M+H)^+$ ion at *m/z* 985 in its positive mode and *m/z* 983 in its negative mode. The ^{1}H NMR spectrum of **20** showed the presence of three methyl singlets at δ 0.97, 1.21, and 1.26, nine methylene and two methine protons between δ 0.85-2.26, and four anomeric protons that were observed as doublets at δ 4.65 (*J*=7.6 Hz, 1H), 4.69 (*J*=7.8 Hz, 1H), 4.86 (*J*=8.1 Hz, 1H), and 5.39 (*J*=8.1 Hz, 1H). Acid hydrolysis of **20** afforded D-glucose similar to **19**. Enzymatic hydrolysis of **20** furnished an aglycone that was identified as *ent*-13, 16β-dihydroxykauran -19-oic acid on the basis of NMR spectral data comparisons with the literature values [13].

Based on the COSY, HSQC, HMBC and 1D-TOCSY correlations, it was suggested that **20** has a 2,3-branched β-D-glucotriosyl moiety unit at C-13 and a β-D-glucosyl substituent unit as an ester at C-19 on the aglycone moiety, *ent*-13, 16β-dihydroxykauran-19-oic acid. Based on the above spectral and hydrolysis studies the structure of **20** was deduced as 13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-16β-hydroxy *ent*-kauran-19-oic acid β-D-glucopyranosyl ester (Figure 4) consistent with literature reported values [10, 15].

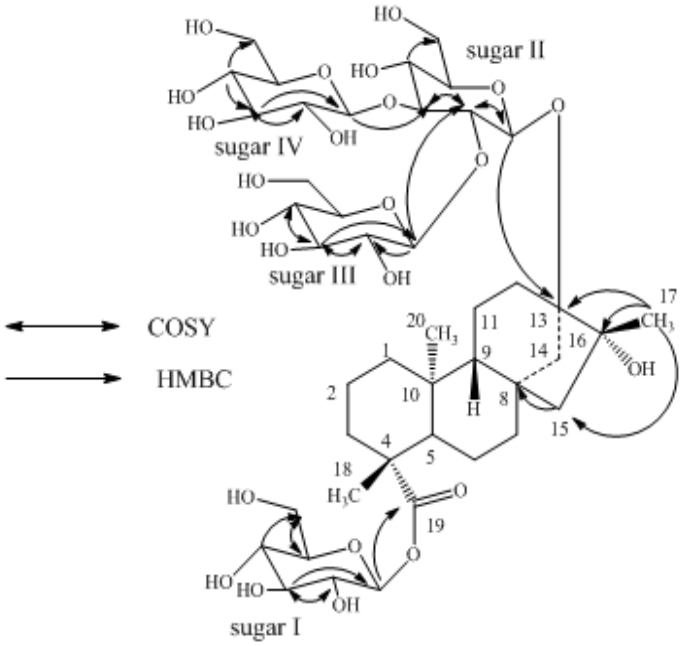


Figure 5: Key COSY and HMBC correlations of **20**

Further, the possible explanation for the formation of the degradation products **19** and **20** could be the migration of the exocyclic double bond between C-16/C-17 to C-15/C-16, and the addition of water to the exocyclic double bond at C-16/C-17 in **2** respectively.

CONCLUSION

The stability of steviol glycosides in mock beverage solutions is pH, temperature-, and time dependent. The rate and extent of degradation product formation is increased under acidic conditions (lower pH) and at higher temperatures with the majority of degradation product formation occurring after extended period of storage. All degradation was accounted for by the formation of known degradation products and excellent mass balance was achieved at all conditions. Thus, steviol glycosides in mock beverages under relevant conditions of intended use are considered stable.

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