

Fluorescent Light Exposure of Rebaudioside A in Mock Beverages under International Conference on Harmonization (ICH) Guidelines

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Abstract

Photostability of rebaudioside A, a sweet constituent of *Stevia rebaudiana* was studied under fluorescent light exposure at 25° C in mock beverages at pH 3.8 using International Conference on Harmonization (ICH) technical requirements covering the stability testing of new drug substances and products. Experimental results indicated that rebaudioside A did not undergo any major decomposition with fluorescent light exposure for 2 weeks. Identification of the degradation products furnished two minor compounds which were identified as 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 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 extensive spectroscopic data and chemical studies. Also, the mass balance for rebaudioside A was calculated against their control samples which were found 99.1%, suggesting any appreciable amount of undetected degradation products were formed under the conditions of the study.

Keywords: Rebaudioside A, fluorescent light exposure, stability, degradation products, structure elucidation, spectral data, chemical studies

1. Introduction

Due to the increasing awareness of obesity problem, many soft drink manufacturers are trying to reduce calories by introducing synthetic and natural non-caloric sweeteners into their systems. The recent interest of organic foods has driven the focus towards natural high-potency sweeteners. One such example is *Stevia rebaudiana* (Bertoni), a perennial shrub of the Asteraceae (Compositae) family native to certain regions of South America (Paraguay & Brazil) which is often referred to as “the sweet herb of Paraguay” (Mosettig et al., 1955, 1963). The major constituents from *S. rebaudiana* are the potently sweet diterpenoid glycosides rebaudiosides A and D, stevioside, and dulcoside A; known as stevia sweeteners of which rebaudioside A is the sweetest of all the components (Wayne et al., 2009). These compounds are all glycosides of the diterpene ent-13-hydroxykaur-16-en-19-oic acid known as steviol (Brandle et al., 1998). Due to their continuing demand, there has been intense interest on *S. rebaudiana* and 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 isolated several novel diterpene glycosides from the commercial extracts of the leaves of *S. rebaudiana* obtained from various suppliers around the world (Chaturvedula et al., 2011a-h). Apart from isolating novel compounds from *S. rebaudiana* and utilizing them as possible sweeteners or sweetness enhancers, we are also engaged in understanding the stability of the steviol glycosides into various systems of our interest and their physicochemical profiles as well as synthesis (Chaturvedula et al., 2011i-k; Prakash et al., 2012). The rebaudioside A used in this study is a mixture containing mainly rebaudioside A (**1**) along with minor quantities of other compounds namely rebaudioside B (**2**), rebaudioside F (**3**), 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 (**4**), and 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 (**5**). In this article, we are describing the photostability of rebaudioside A mixture under fluorescent light exposure at 25°C in mock beverages at pH 3.8 using ICH technical requirements at 60% relative humidity.

The compounds present in the rebaudioside-A mixture in this study belongs to two different classes of *ent*-kaurane diterpene glycosides: *ent*-13-hydroxykaur-16-en-19-oic acid (**1-4**) (Figure 1), and *ent*-13-hydroxykaur-15-en-19-oic acid (**5**) (Figure 2).

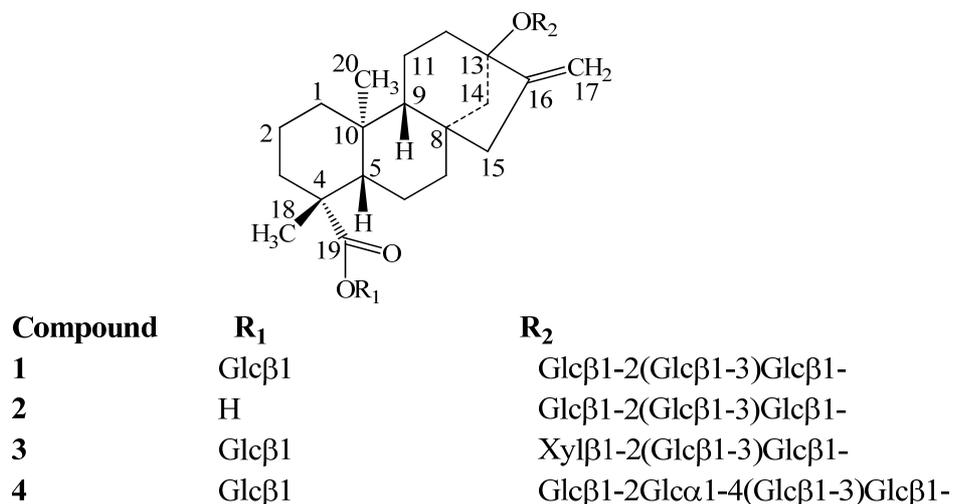


Figure 1. Structure of *ent*-13-hydroxykaur-16-en-19-oic acid compounds

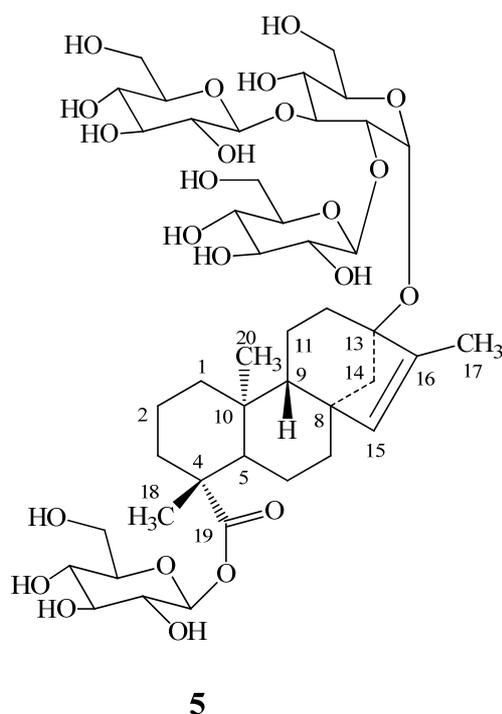


Figure 2. Structure of *ent*-13-hydroxykaur-15-en-19-oic acid compound

2. Experimental

2.1 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 (Chaturvedula et al., 2011i-j).

2.2 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 (Chaturvedula et al., 2011i-j).

2.3 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). The compound numbers, empirical formulae and molecular weights (adjusted to two decimal places) are provided in Table 1.

Table 1. Molecular weights and formulae of steviol glycosides present in rebaudioside A

Compound	Molecular Formula	Molecular Weight
1	C ₄₄ H ₇₀ O ₂₃	967.01
2	C ₃₈ H ₆₀ O ₁₈	804.88
3	C ₄₄ H ₇₀ O ₂₂	951.02
4	C ₅₀ H ₈₀ O ₂₈	1129.15
5	C ₄₄ H ₇₀ O ₂₃	967.01

2.4 Preparation of Mock Beverage Samples

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 3.8 by The National Food Laboratory, Livermore, CA (The NFL) and were stored refrigerated by Covance in a desiccator at 5 ± 3°C.

2.5 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 autosampler 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 C₁₈ cartridge and a tertiary solvent mobile phase (A: 0.040% NH₄OAc/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 autosampler. Ultraviolet (UV) detection at 215 nm was used for analysis of 1. In all cases for UV detection, a 4 nm bandwidth was used with a reference wavelength of 260 nm (100 nm bandwidth). 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. Samples were diluted with water: acetonitrile (1:1) containing 0.1% formic acid and introduced via infusion using the onboard syringe pump.

2.6 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 RP-HPLC gradient method utilized to analyze standard and degradation products were similar to the reported procedure (Clos et al., 2008) and the details were given in Table 2.

Table 2. RP-HPLC method for the separation of steviol glycosides

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

2.7 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.

2.8 Rebaudioside A (1): $^1\text{H NMR}$ (500 MHz, CD_3OD) δ 0.95 (s, 3H, $\text{C}_{20}\text{-CH}_3$), 1.23 (s, 3H, $\text{C}_{18}\text{-CH}_3$), 4.88 (s, 1H, $\text{C}_{17}\text{-H}$), 5.22 (s, 1H, $\text{C}_{17}\text{-H}$), 4.61 (d, $J=7.9$ Hz, 1H), 4.68 (d, $J=7.9$ Hz, 1H), 4.84 (d, $J=7.9$ Hz, 1H), 5.38 (d, $J=8.2$ Hz, 1H) (Kohda et al., 1976, Prakash et al., 2009).

2.9 Rebaudioside B (2): $^1\text{H NMR}$ (500 MHz, CD_3OD) δ 0.95 (s, 3H, $\text{C}_{20}\text{-CH}_3$), 1.23 (s, 3H, $\text{C}_{18}\text{-CH}_3$), 4.88 (s, 1H, $\text{C}_{17}\text{-H}$), 5.22 (s, 1H, $\text{C}_{17}\text{-H}$), 4.63 (d, $J=7.9$ Hz, 1H), 4.72 (d, $J=7.9$ Hz, 1H), 4.86 (d, $J=7.9$ Hz, 1H) (Kohda et al., 1976, Prakash et al., 2009).

2.10 Rebaudioside F (3): $^1\text{H NMR}$ (500 MHz, CD_3OD) δ 0.98 (s, 3H, $\text{C}_{20}\text{-CH}_3$), 1.21 (s, 3H, $\text{C}_{18}\text{-CH}_3$), 4.84 (s, 1H, $\text{C}_{17}\text{-H}$), 5.18 (s, 1H, $\text{C}_{17}\text{-H}$), 4.57 (d, $J=8.2$ Hz, 1H), 4.63 (d, $J=7.8$ Hz, 1H), 4.67 (d, $J=7.8$ Hz, 1H), 5.38 (d, $J=8.2$ Hz, 1H) (Starratt et al., 2002).

2.11 13-[(2-O- β -D-glucopyranosyl-3-O-(4-O- α -D-glucopyranosyl)- β -D-glucopyranosyl)-oxy]

ent-kaur-16-en-19-oic acid β -D-glucopyranosyl ester (4): $^1\text{H NMR}$ (500 MHz, CD_3OD) δ 0.97 (s, 3H, $\text{C}_{20}\text{-CH}_3$), 1.22 (s, 3H, $\text{C}_{18}\text{-CH}_3$), 4.87 (s, 1H, $\text{C}_{17}\text{-H}$), 5.24 (s, 1H, $\text{C}_{17}\text{-H}$), 4.59 (d, $J=7.8$ Hz, 1H), 4.71 (d, $J=7.8$ Hz, 1H), 4.83 (d, $J=7.8$ Hz, 1H), 5.15 (d, $J=3.5$ Hz, 1H), 5.38 (d, $J=8.2$ Hz, 1H) (Prakash et al., 2009).

2.12 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 (5):* $^1\text{H NMR}$ (500 MHz, CD_3OD) δ 0.99 (s, 3H, $\text{C}_{20}\text{-CH}_3$), 1.20 (s, 3H, $\text{C}_{18}\text{-CH}_3$), 1.71 (s, 3H, $\text{C}_{17}\text{-H}$), 4.55 (d, $J=7.8$ Hz, 1H), 4.63 (d, $J=8.5$ Hz, 1H), 5.10 (s, 1H, $\text{C}_{15}\text{-H}$), 5.37 (d, $J=8.2$ Hz, 1H) (Chaturvedula et al., 2011a).

2.13 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 (6): MP: 177-181 $^\circ\text{C}$; IR (KBr) ν_{max} : 3348, 1725, 1020, 965 cm^{-1} ; $^1\text{H NMR}$ (500 MHz, CD_3OD) δ 0.99 (s, 3H, $\text{C}_{20}\text{-CH}_3$), 1.21 (s, 3H, $\text{C}_{18}\text{-CH}_3$), 4.11 (d, $J=14.0$ Hz, 1H, $\text{C}_{17}\text{-H}$), 4.29 (d, $J=14.0$ Hz, 1H, $\text{C}_{17}\text{-H}$), 4.66 (d, $J=7.6$ Hz, 1H), 4.69 (d, $J=7.8$ Hz, 1H), 4.84 (d, $J=7.8$ Hz, 1H), 5.37 (d, $J=8.4$ Hz, 1H), 5.38 (s, 1H, $\text{C}_{15}\text{-H}$); MS (ESI): $[\text{M}+\text{H}]^+$ 983.5; $[\text{M}-\text{H}]^-$ 981.4 (Prakash et al., 2009; 2012).

2.14 13-[(2-O- β -D-glucopyranosyl-3-O- β -D-glucopyranosyl)- β -D-glucopyranosyl)-oxy]-16 β -hydroxy ent-kauran-19-oic acid β -D-glucopyranosyl ester (7): MP: 177-181 $^\circ\text{C}$; IR (KBr) ν_{max} : 3345, 1723, 1025, 958 cm^{-1} ; $^1\text{H NMR}$

(500 MHz, CD₃OD) δ 0.98 (s, 3H, C₂₀-CH₃), 1.20 (s, 3H, C₁₈-CH₃), 1.25 (s, 3H, C₁₇-CH₃), 4.67 (d, $J=7.8$ Hz, 1H, C₃₃-H), 4.70 (d, $J=8.2$ Hz, 1H), 4.88 (d, $J=7.8$ Hz, 1H), 5.37 (d, $J=8.2$ Hz, 1H); MS (ESI): [M+H]⁺ 985.5; [M-H]⁻ 983.6 (Prakash et al., 2009; 2012).

2.15 General procedure for acid hydrolysis and determination of sugar configuration in 6-7: Each compound (500 μ g) 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 60°C for 1.5 h, and then *O*-tolyl isothiocyanate (12.5 μ L) was added to the mixture and heated at 60°C for an additional 1.5 h. The reaction mixture was analyzed by HPLC: column Phenomenex Luna C18, 150 x 4.6 mm (5 μ); 25% acetonitrile-0.2% TFA water, 1 mL/min; UV detection at 250 nm. The sugar was identified as D-glucose in each experiment (*t*R, 12.17 to 12.32 min) [authentic samples, D-glucose (*t*R, 12.35) and L-glucose (*t*R, 11.12 min)] (Tanaka et al., 2007).

2.16 Enzymatic hydrolysis of 6-7: Each compound (250 μ g) was dissolved in 2.5 ml of 0.1 M sodium acetate buffer, pH 4.5 and crude pectinase from *Aspergillus niger* (50 μ L, 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 product obtained from the hydrolysis of **6** and **7** were identified as *ent*-13, 17-dihydroxykaur-15-en-19-oic acid and *ent*-13,16 β -dihydroxykauran-19-oic acid (Ohtani et al., 1992) respectively by comparison of their ¹H NMR spectral data.

3. Results and Discussion

The primary objective of this study was to assess the stability of rebaudioside A under fluorescent light exposure at 25°C at relative humidity [RH] of 60% in mock beverage storage conditions. The pH used for this study was 3.8 which is similar to lemon-lime beverage system. Also, we are herewith reporting the mass (mole) balances of the mixture rebaudioside A and its major degradation products obtained during the course of study as well as the structural characterization of the degradation products based on their spectral (1D and 2D NMR, MS) and chemical studies.

The stability of rebaudioside A mixture was evaluated in mock beverage solutions by simulating formulations used in commercial lemon-lime soft drinks (pH 3.8), but lacking the flavour components. Three sets of mock beverages at pH 3.8 were prepared as described above and rebaudioside A mixture at a concentration of about 500 mg/l was added which were covered with plastic wrap. Additional three sets of rebaudioside A mixture in mock beverage bottles at pH 3.8 at similar concentration as above were covered with plastic wrap as well as an aluminium foil and were studied as control samples. Bottles were placed side-by-side with a validated chemical actinometrical system to ensure that the specified light exposure is obtained and exposed to a minimum of 1.2 million lux hours and not less than 200 watt hours/m² exposure to near ultra-violet light at 25°C as defined in the ICH guidelines (Chaturvedula et al., 2011i). Samples were analysed using the HPLC method as stated above for rebaudioside A, its known impurities and degradation products that are greater than or equal to 0.100% from the starting concentration of rebaudioside A mixture. All samples were treated identically during analysis to minimize assay bias. From the results shown in Table 3, it was found that there was almost no change in the concentration of the compounds **1-5** in both the original rebaudioside A mixture and fluorescent light treated samples covered with plastic wrap and aluminium foil (control samples) whereas two additional compounds **6** and **7** were observed in fluorescent light treated samples wrapped with plastic wrap only. The characterization of compounds **6** and **7** was performed on the basis of extensive spectroscopic and chemical studies and is given below.

Compound **6** was obtained as white powder, and its molecular formula was assigned as C₄₄H₇₀O₂₄ from the ESI mass spectrum which showed (M+H)⁺ ion at *m/z* 983.5 in its positive mode and (M-H)⁻ ion at *m/z* 981.4 in its negative mode. The ¹H NMR spectrum of **6** showed the presence of two methyl singlets at δ 0.99 and 1.21, an oxymethylene group as doublets at δ 4.11 (d, $J=14.0$ Hz, 1H), 4.29 (d, $J=14.0$ Hz, 1H), and a trisubstituted olefinic proton as a singlet at δ 5.38. In addition, the ¹H NMR spectrum of **6** also showed the presence of four anomeric protons that were observed as doublets at δ 4.66 ($J=7.6$ Hz, 1H), 4.69 ($J=7.8$ Hz, 1H), 4.84 ($J=7.8$ Hz, 1H), and 5.37 ($J=8.4$ Hz, 1H). Acid hydrolysis of **6** 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 (Tanaka et al., 2007). Enzymatic and acid hydrolysis of **6** furnished an aglycone which was identified as *ent*-13, 17-dihydroxykaur-15-en-19-oic acid (Ohtani et al., 1992). Based on the above spectral and hydrolysis studies and in comparison with the literature values reported in the literature (Prakash et al., 2009; 2012), the structure of **6** was established as 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, which was supported by the key COSY and HMBC correlations as shown in Figure 3.

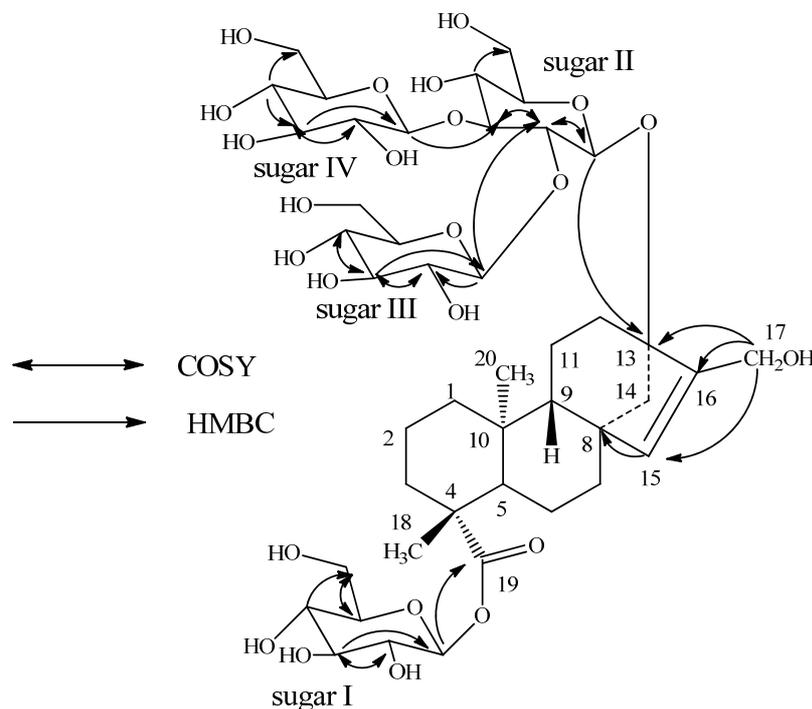


Figure 3. Key COSY and HMBC correlations of **6**

Compound **7** was also 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 985.5 in its positive mode and $(M-H)^+$ ion at m/z 985.6 in its negative mode. The 1H NMR spectrum of **7** showed the presence of three methyl singlets at δ 0.98, 1.20, and 1.25; four anomeric protons that were observed as doublets at δ 4.67 ($J=7.8$ Hz, 1H), 4.70 ($J=8.2$ Hz, 1H), 4.88 ($J=7.8$ Hz, 1H), and 5.37 ($J=8.2$ Hz, 1H). The absence of oxymethylene protons together with the presence of an additional methyl singlet in the downfield shift at δ 1.25 and 2 amu higher molecular mass of **7** compared to **6** suggested the presence of a methyl group connected to a tertiary hydroxyl group at C-16 position in its structure. Acid hydrolysis of **7** afforded D-glucose that was identified by preparing its corresponding thiocarbonyl-thiazolidine carboxylate derivative with L-cysteine methyl ester and *O*-tolyl isothiocyanate, as mentioned for compound **6**. Enzymatic hydrolysis of **7** furnished an aglycone which was identified as *ent*-13, 16 β -dihydroxykauran-19-oic acid on the basis of NMR spectral data comparisons (Ohtani et al., 1992). Based on the above spectral and hydrolysis studies as well as in comparison with the literature values reported (Clos et al., 2008; Prakash et al., 2012), the structure of **7** was deduced as 13-[(2-*O*- β -D-glucopyranosyl-3-*O*- β -D-glucopyranosyl- β -D-glucopyranosyl)oxy]-16 β -hydroxy *ent*-kauran-19-oic acid β -D-glucopyranosyl ester. The structure was further supported by the COSY and HMBC correlations that were shown in Figure 4.

Though the fluorescent light exposure experiments were performed on rebaudioside A earlier in cola (pH 2.4) and lemon-lime (pH 2.6) model beverage samples (Clos et al., 2008), this is the first report to identify the degradation products for rebaudioside A in mock beverages at pH 3.8 under 60% RH at 25 $^\circ$ C using ICH guidelines. The two degradation products formed during the study **6** and **7** are the analogues of *ent*-13, 17-dihydroxykaur-15-en-19-oic acid and *ent*-13,16 β -dihydroxykauran-19-oic acid respectively. Since both degradation compounds were observed in the light exposed samples, suggesting that they are acid independent and fluorescent light promoted products. Further, the possible explanation for the formation of the degradation products **6** and **7** could be the migration of the exocyclic double bond between C-16/C-17 in **1** to C-15/C-16 and hydroxylation of the methyl group at C-17 position, and addition of water to the exocyclic double bond at C-16/C-17 in **1** respectively. The retention times of the reference and degradation compounds (**1**-**7**) were given in Table 3.

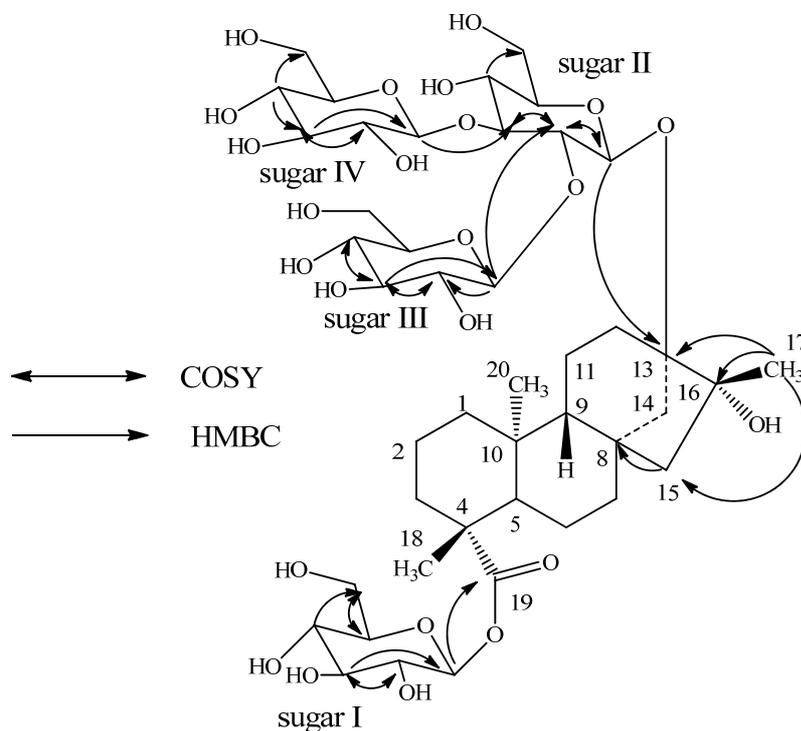


Figure 4. Key COSY and HMBC correlations of 7

At the end of 2 week time period, HPLC analysis was performed on the fluorescent treated rebaudioside A suggested that the two degradation compounds **6** and **7** were identified at 1.12 and 0.654 mg/l concentrations respectively (Table 4). The mass balance was calculated based on the molecular weights of the analytes present in the fluorescent treated rebaudioside A mixture sample and their concentration values obtained from HPLC analysis and were shown in Table 5.

Table 3. Retention times (t_R) of reference and degradation compounds **1-7**

Compound	Retention Time (t_R , min)
1	18.453
2	26.879
3	21.060
4	17.336
5	19.700
6	14.979
7	10.855

Table 4. Summary of light treated rebaudioside A at pH 3.8 (mg/l)^a

Compound	Rebaudioside A (Original)	Control Samples (Aluminum Foil Covered)	Fluorescent Light Treated
1	507	505	498
2	5.62	5.45	5.54
3	1.19	1.08	1.12
4	0.601	0.559	0.763
5	1.61	1.76	1.63
6	-	-	1.12
7	-	-	0.654

^a Results are mean of three sample preparations

Table 5. Mass balance of light treated rebaudioside A at pH 3.8 ($\mu\text{mol/l}$)

Compound	Control Samples (Aluminum Foil Covered)	Fluorescent Light Treated
1	522	515
2	6.77	6.88
3	1.15	1.20
4	0.495	0.676
5	1.82	1.69
6	-	1.24
7	-	0.664
Total	532	527
Fluorescent light treated vs Control samples		99.1%

4. Conclusion

This is the first report of the identification of degradation products for rebaudioside A in mock beverages at pH 3.8 under 60% RH under fluorescent light treatment at 25°C. From the HPLC analysis, it was found that there was minimal degradation at 25°C in mock beverages at pH 3.8 under 60% RH of rebaudioside A mixture. Further, the high mass balance values found for the molar recoveries in the fluorescent light exposure suggested that any appreciable amount of undetected degradation products were formed under the conditions of the study. This suggested that rebaudioside A is considered stable using the conditions of ICH guidelines in this study when exposed to fluorescent light.

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