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Profiling of α-Dicarbonyl Content of Commercial Honeys from Different Botanical Origins: Identification of 3,4-Dideoxyglucoson-3-ene (3,4-DGE) and Related Compounds

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The α-dicarbonyl contents of commercial honey samples from different botanical origins were analyzed as their quinoxaline derivatives using HPLC-DAD, HPLC-MS, HPLC-MS/MS, and HPLC-TOF-MS. A total of nine such compounds were detected, of which five were previously reported in honey (glucosone, 3-deoxyglucosone, glyoxal, methylglyoxal, and 2,3-butanedione) and three were reported only from sources other than honey [3-deoxypentulose, 1,4-dideoxyhexulose, and 3,4-dideoxyglucoson-3-ene (3,4-DGE)]. An unknown α-dicarbonyl compound was also tentatively identified as an oxidation product of 3,4-DGE and was termed 3,4-dideoxyglucosone-3,5-diene (3,4-DGD). Only glyoxal (0.3−1.3 mg/kg), methylglyoxal (0.8−33 mg/kg), and 2,3-butanedione (0−4.3 mg/kg) were quantified in all honey samples. Furthermore, analysis of the α-dicarbonyl profile of various honey samples indicated that certain α-dicarbonyl compounds are found in specific honey samples in much higher proportions relative to the average amounts. The free radical scavenging activity as measured by DPPH method has also indicated that the darker honey samples such as buckwheat, manuka, blueberry, and eucalyptus had higher antioxidant properties compared to lighter-colored samples.

KEYWORDS: Honey; α-dicarbonyl profile; antioxidant properties; 3; 4-dideoxyglucosone-3-ene; methylglyoxal

INTRODUCTION

Reactive α-dicarbonyl compounds can originate from various sources including sugar−amino acid model systems (*1*−*3*), peritoneal dialysis fluids (*4*, *5*), honey (*6*−*9*), carbonated beverages (*10*), beer (*11*), and wine (*12*) among others. A total of 18 α-dicarbonyl compounds have been identified so far from different matrices (see Table **1**). Their importance stems from the fact that they are associated with both beneficial and harmful physicochemical properties. They are responsible through glycation reactions for the formation of many advanced glycation end-products (AGEs) and other glycotoxins (*13*). On the other hand, some of the important Maillard reaction products such as flavors, aromas, and colors are also generated through their interaction with various amino acids (*13*). The formation of α-dicarbonyl compounds in honey has not been studied in detail; so far, only five α-dicarbonyl compounds have been identified (*6*), and the concentrations of three of them have been reported (see Table **2**). Most of the literature focuses on the concentration of methylglyoxal in manuka honey as it is responsible for its antibacterial activity (*7*, *9*). A recent study (*9*) has shown that methylglyoxal in manuka honey originates from dihydroxyacetone present in the nectar of manuka flowers. Amadori compounds have been identified in many honey samples; they accumulate over time and are considered, along with reducing sugars, to be the main precursors of α-dicarbonyl compounds (*6*, *14*). The objective of this study was to identify the profile of α-dicabonyl compounds and correlate their relative concentrations to the composition of precursors in a variety of floral honey samples.

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EXPERIMENTAL PROCEDURES

All chemicals and reagents were purchased from Aldrich Chemical Co. (Oakville, ON, Canada). 3-Deoxyglucosone was purchased from Toronto Chemicals (Ontario, Canada). A total of 15 commercial honey samples (see Table **3**) claimed to be from different botanical origins were purchased from local producers and different grocery stores. The samples were stored in the dark at 4−8 °C until analysis. The temperature of the incubator was electronically controlled and verified against a calibrated thermometer.

pH Measurements. The literature procedure was followed without modifications (*15*). Honey samples (2 g) were diluted with deionized water (5 mL). The pH of the solution was then directly measured with a pH-meter (Accumet 950 pH/ion meter) at room temperature.

Analysis of HMF. The HMF content of honey was determined by HPLC with UV detection at 280 nm according to a published procedure (*16*).

Preparation of Standard Stock Solution (*250 μg/mL*). HMF (25 mg) was dissolved in methanol (10 mL), and the solution was diluted to volume with water.

Calibration Curve and Working Solutions (*0.25*−*12.5 μg/mL*). HMF stock solutions of 50, 100, 200, 300, and 500 μL were pipetted into 10 mL volumetric flasks and diluted to volume with 10% methanol.

Sample Preparation and Analysis. Honey samples (2 g) were weighed into 15 mL polypropylene tubes. Methanol (2.5 mL) was added to

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Table 1. Currently Identified α-Dicarbonyl Compounds

α-dicarbonyl compound	matrix[a]
glyoxal (G)	carbonated beverages, honey, model systems
methylglyoxal (MG)	beer, carbonated beverages, honey
hydroxypyruvaldehyde (HPA)	model systems
2,3-butanedione (2,3-BD)	model systems, beer
erythrosone (ES)	model systems
3-deoxyerythrosone (3-DES)	model systems
ribosone (RS)	model systems
1-deoxypentosulose (1-DP)	beer
3-deoxypentosulose (3-DP)	beer
1,4-dideoxypentosulose (1,4-DDP)	beer
3,4-dideoxypentosulose (3,4-DDP)	model systems
glucosone (GS)	honey
1-deoxyglucosone (1-DG)	beer
3-deoxyglucosone (3-DG)	beer, carbonated beverages, honey
1,4-dideoxyglucosone (1,4-DDG)	beer
3,4-dideoxyglucoson-3-ene (3,4-DGE)	peritoneal dialysis fluids
2,3-dihydro-5-hydroxy-6-methyl-4*H*-pyran-4-one	beer
1,5-dideoxy-4-glucopyranosyl-2,3-hexodiulos-4-ene	beer

[a] Selected examples.

Table 2. Concentrations[a] of α-Dicarbonyl Compounds in Honey

α-dicarbonyl	range (mg/kg)	median (mg/kg)
glyoxal	0.2−2.7	1.7
methylglyoxal	0.4−5.4	2.4
3-deoxyglucosone	79−1266	180

[a] Weigel et al. (*6*).

Table 3. Characteristics of Honey Samples Studied

honey sample	country of origin	color[a]	pH	HMF content[b] (mg/kg)	SD
blend 1	Canada/Argentina	white	3.65	24.1	0.2
blend 2	Canada/Argentina	golden	3.75	37.2	0.1
alfalfa 1	Canada	white	3.79	22.5	0.2
alfalfa 2	Canada	white	3.92	35.5	0.1
manuka	Australia	dark	4.17	102.2	1.1
buckwheat 1	Canada	dark	3.91	51.2	1.5
buckwheat 2[c]	Canada	no claim[d]	3.74	54.2	0.3
blueberry 1	Canada	golden	4.2	77.1	0.6
blueberry 2	Canada	no claim[d]	3.95	31.2	0.4
clover 1	Canada	white	3.96	10.0	0.1
clover 2	Canada	no claim[d]	3.64	41.7	0.1
goldenrod	Canada	golden	3.85	25.5	0.1
sunflower	Canada	no claim[d]	3.69	84.2	1.9
acacia blossom	Hungary	golden	3.95	40.0	0.2
eucalyptus	Australia	amber	4.33	33.0	0.3

[a] Claimed on the label. [b] See Experimental Procedures. [c] Contaminated with clover honey. [d] Artisanal honey.

the sample, and the tube was shaken without heating until complete dissolution. Water (10 mL) was then added. The extract was transferred to a 25 mL volumetric flask and completed to volume with water. The extracts were filtered through a disposable 0.45 μm filter and analyzed by HPLC-DAD using an Agilent 1100 series HPLC coupled to a 1200 series diode array detector (Palo Alto, Ca). A Zorbax SB-C-18 4.6 × 100 mm, 3.5 μm (Agilent part 861953-902), column was used at a temperature of 30 °C. Fifteen microliters of the final extract was injected into the system. HMF was separated using an isocratic mobile phase of 10% methanol in water at a flow rate of 1 mL/min. The analyte was monitored at 280 nm, and the UV spectrum was recorded from 200 to 400 nm.

Analysis of Dicarbonyl Compounds in Honey. The α-dicarbonyl compounds were analyzed according to published methods (*6*). The α-dicarbonyl compounds contained in the aqueous honey extracts were derivatized as quinoxalines with *o*-phenylenediamine (OPD) prior to the HPLC-DAD and HPLC-MS analyses.

Preparation of Standard Stock Solutions. The α-dicarbonyl compounds (glyoxal, methylglyoxal, and 2,3-butanedione) were dissolved in water to achieve a final concentration of 400 μg/mL. The solutions (2 mL) were then derivatized overnight at room temperature with a 2% OPD solution (0.6 mL).

Calibration Curves. From derivatized stock solutions, four concentrations were prepared ranging from 3 to 300 μg/mL by dilution with water.

Sample Preparation. Honey samples (0.6 g) were diluted with water to a final volume of 2 mL. A 0.2% solution of OPD (0.6 mL) was added to the extract and mixed thoroughly. The solution was left overnight in the dark at room temperature and filtered through a 0.45 μm filter before analysis by HPLC-DAD and HPLC-MS.

HPLC-DAD Conditions. An Agilent 1100 series HPLC coupled with an 1100 series diode array detector (Palo Alto, Ca) was used for the determination of α-dicarbonyl compounds. A Zorbax SB-C-18 (4.6 × 150 mm, 5 μm Agilent part 880975-902) column was used at a temperature of 30 °C. The derivatized extract (20 μL) was injected on the system. The quinoxaline derivatives were separated using an isocratic mobile phase of 55% methanol and 45% water containing 0.1% formic acid at a flow rate of 0.7 mL/min (see Figure **1**). The analytes were monitored at 312 nm (slit width of 2 nm), and the UV spectrum was recorded from 200 to 400 nm. Peaks 1−9 exhibited the typical UV absorption patterns of quinoxaline derivatives.

HPLC-MS and MS/MS Conditions. An Agilent 1100 series HPLC coupled to an Agilent 1100 series LC/MSD Trap SL mass spectrometer (Palo Alto, Ca) was used to determine the mass of the derivatized α-dicarbonyl compounds and their collision-induced dissociation (CID) spectra. The HPLC conditions were the same as described above. The ion trap mass detector was operated in positive ion mode with ESI ionization. The nebulizer pressure was 50 psi, and the dry gas was set to 10 L/min at 325 °C. The ion charge control (ICC) and capillary voltage were set to 30000 and 3500 V, respectively. The scan range of the mass spectrometer was from m/z 100 to 420. In MS/MS mode, the detector isolated the selected precursor ion of the quinoxalines using the "Smart Frag" option to generate CID spectra.

HPLC-TOF/MS. An Agilent 1100 series HPLC coupled with an Agilent G1969A time of flight (TOF) mass spectrometer (Palo Alto, Ca) was operated using electrospray ionization in positive ion mode. The compounds hexakis(1*H*,1*H*,3*H*-tetrafluoropropoxy)phosphazine (CAS Registry No. 58943-98-9) ($[M + H]^+$ m/z 922.009798) and purine (CAS Registry No. 120-73-0) ($[M + H]^+$ m/z 121.050875) were used as references. The sample solution (50 μL) was injected onto the HPLC-TOF system. A Zorbax SB-C18 4.6 × 30 mm, 1.8 μm, column (Agilent) was used for separation. The mobile phase used was water + 0.05% TFA (solvent A) and ACN + 0.05% TFA (solvent B), and the gradient was 5−95% B in 4.5 min with a 0.5 min washing step (95% B) at the end. The mass to charge ratio and the isotopic ratio were compared to the theoretical mass using Mass Hunter software (Agilent) to confirm the molecular formulas.

DPPH Radical-Scavenging Activity. The DPPH radical-scavenging activity of honey samples was determined according to the procedure described by Vela et al. (*17*).

Calibration Curve. Ascorbic acid solutions were prepared by dissolving ascorbic acid in water to cover the concentration range from 0 to 0.2 μmol/mL. Ascorbic acid solutions were prepared to obtain a calibration curve that covered the complete range of radical-scavenging activity (0−100%). The calibration curve was linear (R^2 = 0.9868) across the range of inhibition, but a closer look revealed two distinct ranges of linearity. The first ranges from 0 to 0.075 μmol/mL (R^2 = 0.9948) and the second from 75 to 175 nmol/mL (R^2 = 0.9984).

Protocol. Honey samples (1 g) were dissolved in deionized water (40 mL). The samples or standard solutions (1.25 mL) were mixed with DPPH solution (1.5 mL of 9 mg/100 mL in methanol). The absorbance at 517 nm was read after 5 min of incubation against a 50% methanol/water blank. The antioxidant activity of honey was expressed in terms of DPPH radical depletion as percentage equivalents of ascorbic acid.

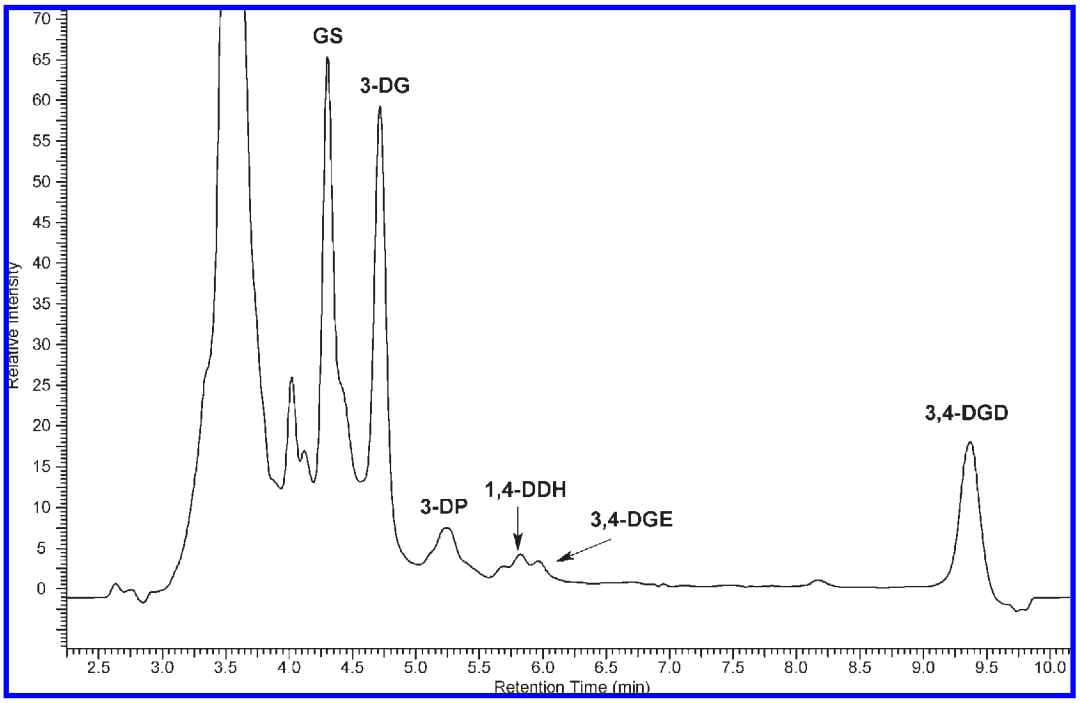

Figure 1. HPLC-DAD chromatogram of the quinoxaline derivatives of buckwheat honey on a Zorbax SB-C-18 column (mobile phase of 55% methanol in water). GS, glucosone; 3-DG, 3-deoxyglucosone; 3-DP, 3-deoxypentosulose; 1,4-DDH, 1,4-dideoxypentosulose; 3,4-DGE, 3,4-dideoxyglucoson-3-ene; 3,4-DGD, 3,4-dideoxyglucosone-3,5-diene.

Table 4. Retention Times, Target Masses, and CID Spectra of the Quinoxaline Derivatives of the Proposed α-Dicarbonyl Compounds Detected in Honey Samples

peak	proposed structure[a]	retention time (min)	mass $[M + H]^+$ (*m/z*)	CID spectrum
1	glucosone	4.2	251	233, 215, 203,197,187, 173, 161, **157**, **145**
2	3-deoxyglucosone	4.7 (4.7)[b]	235	217, 199, 187, 181, **171**, **157, 145**
3	3-deoxypentosone	5.3	205	187, **169**, **157**, **145**
4	1,4-dideoxypentosulose	5.8	219	201, 183, 173, **157**, **145**
5	3,4-dideoxyglucoson-3-ene	6.1	217	199, 181, **171**, **169**, **157**, **145**
6	glyoxal	7.8 (7.8)[b]	131	no fragments observed[c]
7	methylglyoxal	9.2 (9.2)[b]	145	no fragments observed[c]
8	($C_6H_6O_4$)[d]	9.4	215.0814[d]	197, 185, **169**, 159
9	2,3-butanedione	11.0 (11.0)[b]	159	no fragments observed[c]

[a] Based on molecular weight, DAD spectra, CID pattern, and comparison with standards when applicable. [b] Standard. [c] Except quasi-molecular ion $[M + H]^+$. [d] Based on MS-TOF analysis.

Statistical Treatment. The Dixon test was used to identify and reject outlier values. To determine if two groups of values were statistically different, the Student test (*t* test) and the *F* test were employed for the mean and the standard deviation of the two populations. For each statistical test, the calculated value was compared to the tabulated (critical) values at 95% confidence level.

RESULTS AND DISCUSSION

Analysis of the α-dicarbonyl content of honey samples of different botanical origins as their OPD derivatives yielded nine potential candidates (see Table **4**). Control experiments have indicated that the peaks generated after derivatization are not artifacts arising from the reagent and that the nine peaks are due to the honey components able to react with OPD. Four of nine peaks matched the molecular weights, retention times, and UV and CID spectra of commercially available standards of 3-deoxyglucosone (peak 2), glyoxal (peak 6), methylglyoxal (peak 7), and 2,3-butanedione (peak 9) as shown in **Table 4**. Peak 1 matched the molecular weight of glucoson and the elution order relative to 3-DG on reverse phase columns as reported by Weigel et al. (*6*). All of the above-mentioned five α-dicarbonyls have been previously identified in honey, and some were quantified (*6*) as shown in **Table 2**.

Tentatively Identified α-Dicarbonyl Compounds in Honey. The remaining four peaks were tentatively assigned structures (see **Table 4**) on the basis of their UV and CID spectra and by matching their molecular weights to known α-dicarbonyl compounds reported (*5*, *11*, *18*) except peak 8, for which the molecular formula ($C_6H_6O_4$) was calculated from the high-resolution mass spectrum; none of the reported α-dicarbonyl compounds matched this formula. This peak was most prominent in buckwheat honey extracts (see **Figure 1**). To ensure that the peak was indeed an α-dicarbonyl compound, the buckwheat honey extract was analyzed without derivatization with OPD. On the basis of the absence of a peak in both UV and MS detection modes at the observed retention time, it was concluded that the new compound was a quinoxaline derivative. On the other hand, the molecular weights and CID spectra of peaks 3, 4, and 5 were consistent with the structures of 3-deoxypentulose (3-DP), 1,4-dideoxyhexulose (1,4-DDH), and 3,4-dideoxyglucoson-3-ene (3,4-DGE), respectively. Peak 8 eluted at a retention time of 9.4 min with the cluster of glyoxal, methylglyoxal, and 2,3-butanedione. The compound exhibited the characteristic two absorption maxima of the quinoxaline derivatives, one at around 250 nm and the second

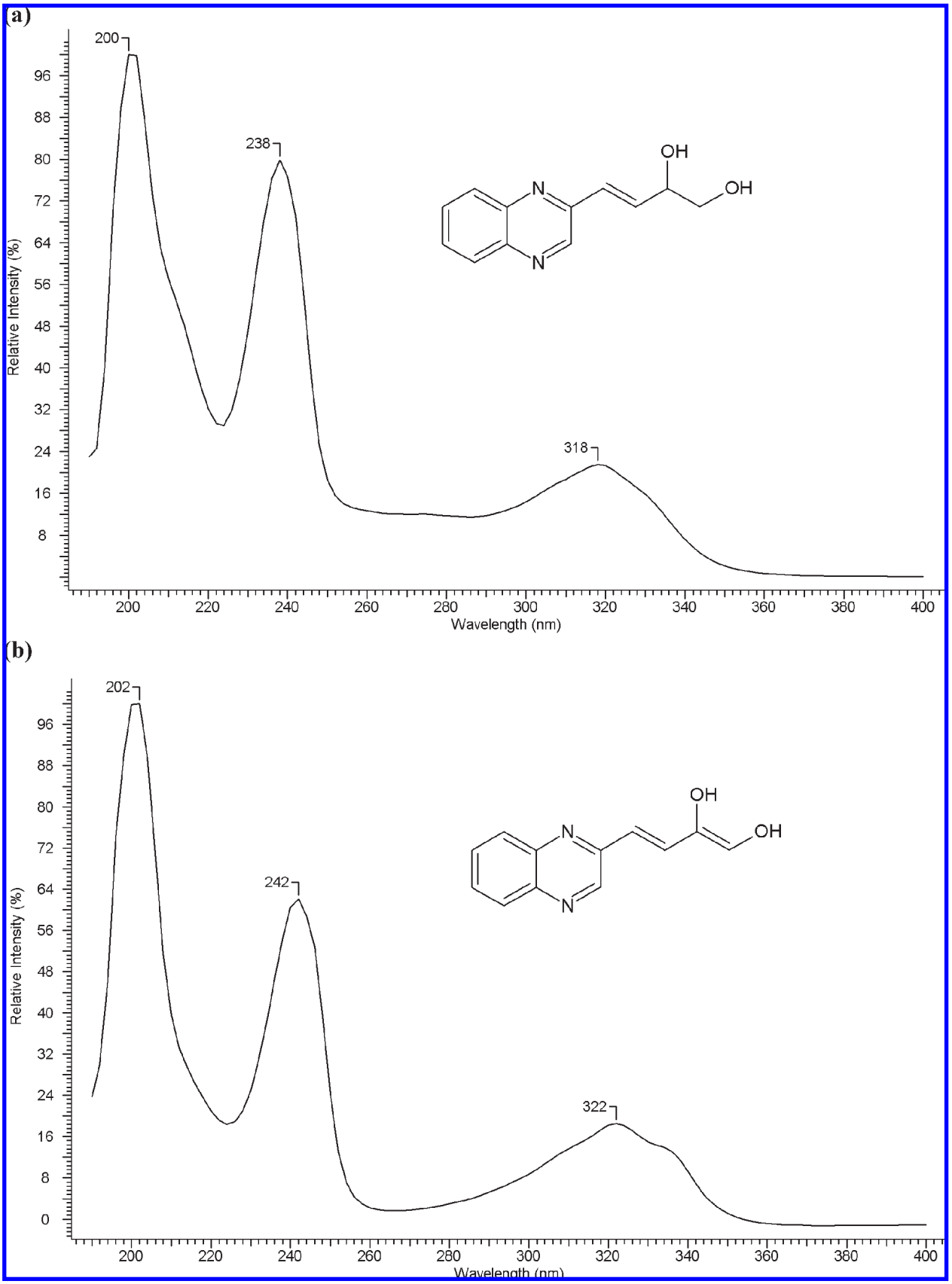

Figure 2. UV spectra of (**a**) peak 5 and (**b**) peak 8.

around 330 nm in the UV spectrum (Figure **2b**). The peak was collected after repeated injections into the LC for further characterization by a high-resolution mass spectrometer (TOF). The injection of the isolated material from peak 8 on a TOF mass spectrometer gave an accurate mass of m/z of 215.0814 for the $[M + H]^+$ peak. This mass corresponds to a molecular formula of $C_{12}H_{11}N_2O_2$ or $C_{12}H_{10}N_2O_2$ for M^+. The difference between theoretical mass and experimental

mass was −0.1041 mDa. The isotopic ratio of the masses at m/z 215 and 216 also confirmed the molecular formula.

Evidence for the Formation of 3,4-DGE in Honey (Peak 5). 3,4-DGE was first identified as a sugar degradation product by Anet (*19*). Kato et al. (*20*) were the first to isolate this compound from seaweed and demonstrate its toxicity and immunosuppressive effects on different cell lines. Subsequently, Linden et al. (*4*) confirmed its formation and cytotoxicity in peritoneal dialysis fluids. Recently, Frischmann et al. (*18*) developed an HPLC-based method for the quantification of 3,4-DGE and reported the UV, MS (ESI), and ^{1}H NMR spectra of its quinoxaline derivative. Peak 5 tentatively identified above as 3,4-DGE showed UV and CID spectra (see **Figure 2a** and Table **5**) consistent with values reported in the literature (*18*). In the present study the UV spectra were acquired in water/methanol, whereas those recorded by Frischmann et al. (*18*) were acquired in ammonium formate buffer and acetonitrile; this solvent change can account for the observed shifts in absorption maxima reported in **Table 5**.

Table 5. Comparison of UV and CID Spectra of Peak 5 with Literature Values for 3,4-Dideoxyglucoson-3-ene (3,4-DGE)

	UV λ_{max} (nm)	CID (m/z)
3,4-DGE quinoxaline derivative[a]	212, 257, 336	145, 157, 169, 171, 181, 199
peak 5[b]	200, 238, 318	145, 157, 169, 171, 181, 199

[a] Frischmann et al. (*18*). UV spectrum acquired in ammonium formate buffer/acetonitrile; CID spectrum generated in a quadrupole MS system (20 eV collision energy). [b] This study. UV spectrum acquired in water/methanol; CID spectrum generated in an ion trap system under conditions given under Experimental Procedures.

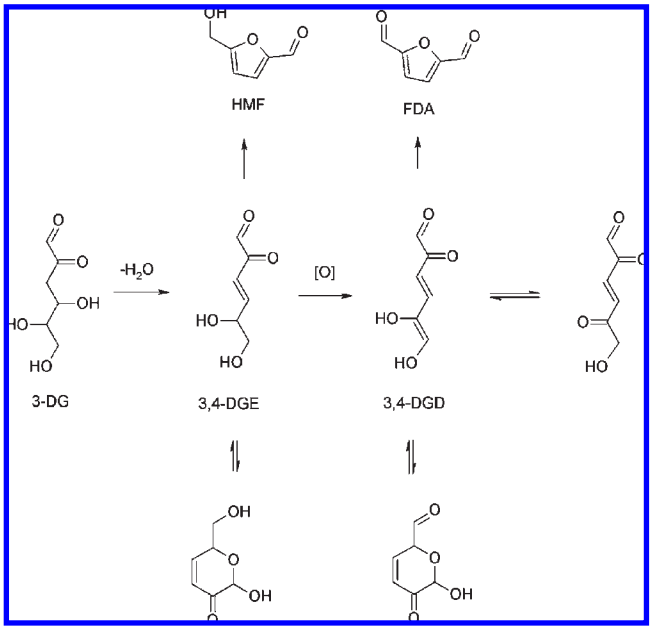

Figure 3. Proposed pathway of formation of 3,4-dideoxyglucosone-3,5-diene (3,4-DGD) from 3-DG and its transformations. HMF, 5-hydroxymethylfurfural; FDA, 2,5-furandialdehyde; 3-DG, 3-deoxyglucosone; 3,4-DGE, 3,4-dideoxyglucoson-3-ene; 3,4-DGD, 3,4-dideoxyglucosone-3,5-diene.

Proposed Origin and Structure of the Unknown Quinoxaline Peak 8. The molecular formula of peak 8 indicated that it can be derived from 3-deoxyglucosone (3-DG) through dehydration followed by oxidation (see Figure **3**). Both 3-DG and its dehydration product 3,4-DGE were detected in the honey samples (**Table 4**) as described above. Consequently, spiking assays were performed to confirm the proposed precursor. Buckwheat honey was spiked with commercially available 3-DG (the final concentration after spiking was 3.2-fold higher) a non spiked honey sample was included in the assay as well as a sample spiked with maltose as controls. The honey samples were incubated for 6 days at 80 °C in a sealed vial. This temperature was chosen to accelerate the reaction. The samples were analyzed for the target compound before and after incubation (Figure **4** shows the peak heights after incubation). The sample spiked with maltose did not show any increase in the intensity of the peak (t test = 1.283 vs t critical = 4.303). However, addition of 3-DG caused an increase in the peak height by 59.3% (t test = 21.609 vs t critical = 4.303). This result may indicate that 3-DG is involved in the formation of peak 8. Furthermore, the addition of 3-DG also enhanced the peak height of methylglyoxal by 34.3% and that of 3,4-DGE by 25.9%. Taking into account the accurate molecular formula, the UV spectrum (**Figure 2b**), and the spiking experiments, we propose 3,4-dideoxyglucosone-3,5-diene (3,4-DGD) as a possible structure for peak 8. As the cyclization of 3,4-DGE generates the well-known 5-hydroxymethylfurfural (HMF) structure (see **Figure 3**), similarly the cyclization of 3,4-DGD can generate 2,5-furandialdehyde (FDA) reported to be formed in honey under warm storage conditions (*21*).

α-Dicarbonyl Profile of Honey Samples. The relative amounts of nine α-dicarbonyl compounds in honey samples were analyzed to determine their profile (see Table **6**). According to **Table 6**, the two types of buckwheat honey not only had the highest concentrations of glucosone but also the highest ratio of glucosone to

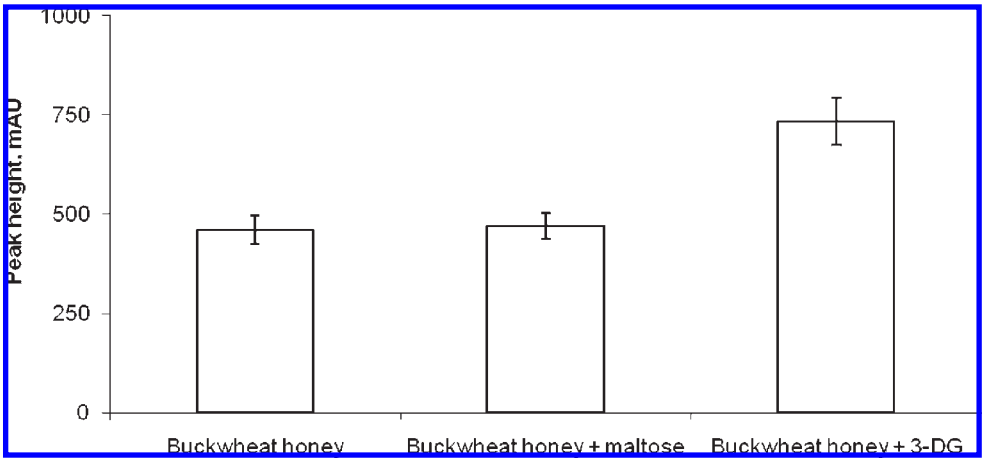

Figure 4. Intensities of peak 8 in buckwheat honey after incubation with 3-deoxyglucosone (3-DG).

Table 6. Distribution[a] of α-Dicarbonyl Compounds in Honey Samples

honey sample	GS mAU	3-DG mAU	peak 3 mAU	peak 4 mAU	3,4-DGE mAU	G mAU	MG mAU	peak 8 mAU	2,3-BD mAU
blend 1	138	342	4.5	11.5	6.2	4.5	3.9	3.8	0.7
blend 2	127	493	4.6	11.0	11.1	4.0	5.3	4.5	0.6
alfalfa 1	144	516	nd	8.3	8.5	3.2	4.3	4.1	nd
alfalfa 2	187	351	4.9	11.7	7.7	4.3	4.1	4.6	0.6
manuka	143	1671	nd	36.4	64.1	3.3	86.8	nd	3.6
buckwheat 1	1171	689	35.2	24.5	15.5	1.8	5.8	94.2	nd
buckwheat 2	629	784	17.2	19.8	15.7	1.5	7.8	58.1	nd
blueberry 1	86	1102	nd	10.6	41.4	1.4	5.1	5.9	nd
blueberry 2	71	543	3.7	5.7	12.1	1.1	2.6	3.2	nd
clover 1	104	217	nd	7.1	4.3	2.6	2.2	1.1	0.6
clover 2	48	365	3.2	5.9	7.2	1.2	2.0	1.9	0.7
goldenrod	141	403	4.4	10.9	8.7	3.1	3.5	5.4	0.7
sunflower	98	866	6.8	15.3	14.4	2.9	5.8	5.0	0.8
acacia blossom	72	654	nd	5.3	10.1	1.5	3.0	1.4	0.7
eucalyptus	83	1106	nd	12.2	39.0	2.2	9.4	3.0	7.8
mean	216	673	9.4	14.2	17.4	2.6	10.6	15.9	1.0
SD	298	386	10.6	8.9	17.6	1.2	23.0	29.2	1.0

[a] Values are expressed in peak heights. GS, glucosone; 3-DG, 3-deoxyglucosone; 3-DP, 3-deoxypentosulose (peak 3); 1,4-DDH, 1,4-dideoxypentosulose (peak 4); 3,4-DGE, 3,4-dideoxyglucoson-3-ene; G, glyoxal; MG, methylglyoxal; 2,3-BD, 2,3-butanedione; nd, not detected.

3-DG closer to or higher than 1. Although 3-DG is the precursor of the proposed 3,4-DGD as indicated by the spiking experiments, its presence in honey at high concentrations did not always indicate increased 3,4-DGD content due to its ability to undergo simultaneously other reactions such as formation of HMF and MG and, most importantly, due to the specific environmental factors present in a particular honey that can promote or retard the presumed redox reactions required for the conversion of 3-DG to 3,4-DGD. In fact, only the buckwheat honey produced a large amount of 3,4-DGD (see **Table 6**). 3-DG is considered to be a major precursor of methylglyoxal (MG), although in manuka honey both are present in relatively large amounts; however, only recently it has been shown (*9*) that dihydroxyacetone is the major precursor of MG and not 3-DG. Manuka honey is recognized for its high concentration of methylglyoxal compared with honeys from other botanical origins (*7*). Dark honeys such has manuka, buckwheat, blueberry, and eucalyptus had relatively higher α-dicarbonyl content than lighter colored honeys (see **Table 6**). Manuka honey had the highest concentrations of 3-DG, 1,4-DDH, 3,4-DGE, and MG. Buckwheat honey had more glucosone, 3-DP, and 3,4-DGD. Blueberry and eucalyptus honeys had high concentrations of 3-DG and 3,4-DGE, whereas eucalyptus honey had the highest concentration of 2,3-butanedione. In general, honey types of the same origin produced similar α-dicarbonyl profiles. The differences in the concentration between the buckwheat honey samples can be explained by the fact that buckwheat honey 2 contained some clover honey (known to have reduced amounts of α-dicarbonyls). The differences between blueberry honey samples could be explained by a more pronounced heat exposure of blueberry honey sample 1 consistent with their HMF content (see **Table 3**). Selected α-dicarbonyl compounds were quantified (see Table **7**) and compared with the data available in the literature (*6*, *7*). The concentrations obtained were in good agreement with literature values (see **Table 2**), the only difference being the maximum concentration of methylglyoxal due to the inclusion of manuka honey in the current study.

Correlations among the α-dicarbonyl compounds were hard to establish as the honey composition has an impact on the nature and the rate of production of α-dicarbonyls. A good correlation between glucosone and 3,4-DGD contents was found only for buchwheat honey samples. Both samples had similar glucosone to 3,4-DGD ratios (see **Table 6**) despite their different concentrations. This might be explained by the fact that both α-dicarbonyls require an oxidation step to be generated. A direct relationship (R^2 = 0.9097) was established between 3-deoxyglucosone and 3,4-dideoxyglucoson-3-ene content, the latter being the dehydration product of 3-DG. Honey samples that had high concentrations of glucosone influenced more the value of coefficient of correlation. Even when these samples were removed from the calculations, the correlation remained relatively good (R^2 = 0.8405). In addition, a modest correlation (R^2 = 0.7788) was found between the level of glucosone and that of glyoxal with the exception of buckwheat honey samples, which produced high levels of glucosone. The correlation studies between α-dicarbonyl levels in different samples have indicated that the physicochemical parameters and storage conditions had similar influence on some of them, whereas others responded differently to these parameters, depending upon the honey composition. The effect of storage conditions was also investigated. Unfortunately, it was not possible to identify a single factor that influenced their formation. This study provides further evidence on the influence of the botanical origin of honey on the ratio of α-dicarbonyl compounds and their concentration.

Table 7. Quantitation of α-Dicarbonyl Compounds in Honey Samples

α-dicarbonyl	range (mg/kg)	median (mg/kg)
glyoxal	0.3−1.3	0.8
methylglyoxal	0.8−33	2.0
2,3-butanedione	0−4.3	0.3
3-deoxyglucosone[a]	143−1099	153

[a] Calculated with glyoxal response factor.

Influence of Time and Temperature on α-Dicarbonyl Compounds. The blend honey sample 1 (see **Table 3**) was selected for a limited investigation of the effect of storage and temperature on the concentration of selected α-dicarbonyl compounds. The compounds were first monitored by UV detection, and derivatization with OPD was performed after the incubation once the samples were cooled at room temperature. 3-DP and 3,4-DGD were the most affected by temperature. On the other hand, glyoxal and 2,3-butanedione were affected neither by the temperature nor by the storage conditions. This was in agreement with literature reports (*6*). The 3-DG concentrations increased at

Table 8. Radical-Scavenging Properties of Honey Solutions (DPPH Assay)

	scavenging activity (%)		
honey type	mean[a]	SD	ascorbic acid equivalent (nmol/mL)
blend 1	6.8 ^{HI}	0.9	20.9
blend 2	8.4 ^{GH}	0.4	25.1
alfalfa 1	7.4 ^{HI}	0.3	22.4
alfalfa 2	8.1 ^{GH}	0.4	37.2
manuka	22.6 ^{B}	0.1	62.1
buckwheat 1	35.1 ^{A}	0.5	83.6
buckwheat 2	21.2 ^{B}	0.6	59.6
blueberry 1	13.4 ^{CD}	0.6	46.2
blueberry 2	9.6 ^{EFG}	0.5	39.8
clover 1	5.4 ^{I}	0.3	32.5
clover 2	7.6 ^{GHI}	0.9	36.3
golden rod	9.9 ^{EF}	0.4	40.3
sunflower	11.0 ^{DE}	0.9	42.2
acacia blossom	6.0 ^{I}	0.4	33.6
eucalyptus	14.4 ^{C}	0.7	48.0
mean	12.5	8.1	42.0
sugar control	1.1 ^{J}	0.3	6.1

[a] Different letters indicate statistical significance.

80 °C and after 3 days started to drop, indicating thermal degradation and/or chemical interaction.

Free Radical Scavenging Properties of Honey Samples. The free radical scavenging properties of honey were determined using the DPPH method according to the procedure described under Experimental Procedures (*17*). Fifteen honey solutions (0.025 g/L) were tested for their free radical scavenging properties. A sugar model (38% fructose, 35% glucose, 5% maltose, 1.5% sucrose, and 20.5% water) was also added as a control to investigate the activity of the sugars, if any. The sugar control had the least free radical scavenging activity of all the samples tested (see Table **8**) and was close to a scavenging activity of zero. The trend in antioxidant activity obtained by using the DPPH method was comparable to the trend found using other methods reported in the literature. That is, the darker honey samples, such as buckwheat, manuka, blueberry, and eucalyptus, had higher antioxidant properties compared to lighter colored samples (*22*). The differences between radical-scavenging properties among the similar pairs of alfalfa, blend, blueberry, and clover honey samples (**Table 8**) can be explained by their differences in heat exposure as confirmed by their measured HMF contents (see **Table 3**) with the exception of buckwheat honey samples that had very similar HMF levels but differing radical-scavenging properties. Both buckwheat honey samples having the highest levels of 3,4-DGD compared with honey from other botanical origins also exhibited the highest radical-scavenging properties. Interestingly, buckwheat honey sample 1, which exhibited higher radical-scavenging properties compared with buckwheat honey sample 2 (1.6-fold higher), also had higher levels of 3,4-DGD (1.6-fold), leading to the conclusion that perhaps similar to the structure of vitamin C, the presence of a relatively stable enediol moiety in 3,4-DGD may also contribute to the free radical scavenging properties. In general, it was difficult to correlate α-dicarbonyl content with radical-scavenging properties,; however, when the total α-dicarbonyl content expressed as the sum of all the peak heights was used, a moderately good correlation (R^2 = 0.8566) was established between the total α-dicarbonyl content and the radical-scavenging properties of honey.

Profiling of α-dicarbonyl content of honey from various botanical origins has indicated that each type of honey can generate a specific profile based on their intrinsic composition and the presence of different precursors and catalysts and that this profile may change during storage and as a result of exposure to temperature. This specific distribution pattern of different α-dicarbonyl compounds in honey, similar to its phenolic profile (*23*), may serve as a basis for developing a method for authentication of honey origin.

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