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Identification of a Novel Glycoside, Leptosin, as a Chemical Marker of Manuka Honey

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ABSTRACT: As a preliminary study, we have found that honey from manuka (*Leptospermum scoparium*) in New Zealand inhibits myeloperoxidase (MPO) activity. In this study, using a chromatographic technique, we isolated two active compounds for MPO-inhibition from manuka honey. One is methyl syringate (MSYR), and the other was identified as a novel glycoside of MSYR, methyl syringate 4-*O*-*β*-D-gentiobiose, which has been named "leptosin" after the genus *Leptospermum*. The amount of the glycoside ranged from 0.2 to 1.2 μmol/g honey. Leptosin was only found in honeys from the Oceania region, and abundantly in manuka honey including jelly bush honey from *Leptospermum polygalifolium* in Australia. Therefore, leptosin may be a good chemical marker for manuka honey. Interestingly, the concentration of leptosin in manuka honey was positively correlated with the unique manuka factor (UMF) value, which is expressed as phenol equivalents of its bactericidal activity.

KEYWORDS: *manuka honey, chemical marker, glycoside, leptosin, methyl syringate*

INTRODUCTION

Honey has been used as a medicine for thousands of years.[1] Among the various types of honeys, honey from manuka (*Leptospermum scoparium* or *Leptospermum polygalifolium*) shows strong bactericidal activity. The antibacterial activity of manuka honey is standardized as equivalents of phenol concentration, which is expressed as the unique manuka factor (UMF) value. There has been much research on the active antibacterial compounds in manuka honey, and methylglyoxal (MGO) has been identified as one of the major contributors to its activity.[2] Recent reports have also shown that MGO is partially responsible for the antibiofilm activity of manuka honey.[3,4] MGO is widely found in food and is one of the metabolites of the glycolytic pathway. Nonenzymatic glycation also generates MGO,[5] which further reacts with other biological molecules to form advanced glycation end products.[6] In a commercial context, the MGO content is expressed as "MGO", similar to "UMF".

Honey has various medicinal and bioactive properties. It is used to treat upper gastrointestinal dyspepsia, for wound healing, and so on. Although manuka honey has antibacterial activity against *Helicobacter pylori*,[7] it cannot eradicate it completely.[8] Ingestion of leafy branches of manuka (*Leptospermum scoparium*) by parakeets changes their pattern of grooming behavior, suggesting that some constituent of manuka leaves may act as an insecticide.[9] A recent study showed that manuka honey reduced the formation of dental plaque, a biofilm that develops on the surface of teeth, significantly better than did xylitol chewing gum.[10] However, the mechanisms underlying the biological actions of manuka and its honey are still unclear.

Because manuka honey is an expensive and medicinal honey, a specific chemical marker would be useful for identification and quality assessment. In this Article, we describe the isolation and identification of a novel glycoside of MSYR in manuka honey. The novel glycoside was found almost exclusively in manuka honey; therefore, it may be a good chemical marker. Moreover, the glycoside may account for some medicinal activity of manuka honey.

MATERIALS AND METHODS

Reagents. Myeloperoxidase was purchased from Planta Natural Products (Vienna, Austria). Methyl syringate (MSYR) was purchased from Alfa Aesar, Johnson Matthey Co. (Ward Hill, MA).

Honey Samples. Honeys from manuka (*Leptospermum scoparium* and *Leptospermum polygalifolium*), honeys from other plants, and honeydew honey were obtained from retail stores in Japan, Italy, and New Zealand.

Fractionation of Honey Samples (Small-Scale). Manuka honey (50 mg) was dissolved in 2 mL of water and applied to a solid-phase extraction column (Supelco Discovery DSC-18, 500 mg, Sigma-Aldrich, Japan, Tokyo), which had been preconditioned with 2 mL of methanol and 6 mL of water. Water (500 μL) was applied to the column, and then mixtures of 500 μL of water/methanol (8/2, 6/4, 4/6, 2/8) and methanol 100% were serially applied to the column. Eluates were collected and then dried with a centrifugal evaporator. The dried samples were dissolved in 100 μL of 0.1 M phosphate buffer (pH 7.4), assayed for MPO-inhibition activity as described below, and then further fractionated by high performance liquid chromatography (HPLC) (Prominence LC-20AT, Shimadzu Co., Kyoto, Japan). The column was a Develosil ODS-HG-5 (5 μm particle, 8 × 250 mm, Nomura Chemical Co., Ltd., Aichi, Japan), and the solvent system was 0.1% acetic acid in water/CH_3CN (86/14) delivered at a flow rate of 2 mL/min. The eluate was monitored at 262 nm by a photodiode array detector (PDA, Shimadzu SPD-M20A) and separated by a fraction collector every 1 min into glass tubes. The fractions were dried using a

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centrifugal evaporator and dissolved in 0.1 M phosphate buffer (pH 7.4) before use in the MPO-inhibition assay as described below.

Isolation of Glycoside (Large-Scale). Manuka honey (60 g) was dissolved in 200 mL of hot water (approximately 70 °C). The sample was applied to an open column containing 300 mL of swelled Diaion Sepabeads HP20 (Mitsubishi Chemical Co., Tokyo, Japan). Next, 500 mL of each of the following solvents was added to the column: water, water/methanol (8/2), water/methanol (6/4), water/methanol (4/6), water/methanol (2/8), and methanol. Each eluate was collected, and an aliquot was analyzed by HPLC to check for the presence of the glycoside. The 6/4 and 4/6 water/methanol fractions from the HP20 column were concentrated and further purified by HPLC (Shimadzu Prominence LC-20AT) on a Develosil Combi-RP column (5 μm particle size, 20 × 100 mm, Nomura Chemical Co., Ltd.) with a solvent system consisting of 0.1% aqueous acetic acid/CH_3CN (85/15) with monitoring at 262 nm. The HPLC purification was repeated twice, yielding approximately 30 mg of the glycoside. The yield of the glycoside from the wet manuka honey sample was approximately 0.05%.

Spectroscopy for Identification of Glycoside. NMR spectra were obtained on a Varian Unity 500 instrument (Varian Inc., Palo Alto, CA) at 500 MHz (^{1}H) and 125 MHz (^{13}C) in CD_3OD and referenced to the residual solvent resonance (CD_3OD at 3.30 ppm for ^{1}H and 49.0 ppm for ^{13}C NMR). Optical rotation was measured using a Jasco P-1030 automatic digital polarimeter (Jasco Co., Tokyo, Japan). The IR spectrum was recorded using a Jasco FT/IR-410 instrument (Jasco Co., Tokyo, Japan).

Quantification of Glycoside and MSYR by HPLC. The glycoside and MSYR were separated and analyzed as follows: Honey (0.1 g) was dissolved in 1 mL of water. A 5 μL aliquot of the sample or standards was injected into the HPLC (Shimadzu Prominence LC-20AT and SPD-M20A) and separated by gradient elution on a Develosil ODS-HG-5 column (5 μm particle size, 4.6 × 150 mm, Nomura Chemical Co., Ltd.) at a flow rate of 0.8 mL/min with monitoring at 262 nm. Solvent A was 0.1% acetic acid in water, and solvent B was CH_3CN. The gradient program was as follows: initial (A 100%), 30 min (A 70%), 35 min (A 100%), and 50 min (A 100%). The quantification was performed by comparison with isolated leptosin or commercially obtained MSYR. Under the above conditions, the limits of detection (LOD) were 5 nM for leptosin and 25 nM for MSYR, and the limits of quantitation (LOQ) were 25 nM for leptosin and 125 nM for MSYR.

Chemical Identification of Glycosides by LC−MS/MS. Each sample was injected into an HPLC (Agilent 1100) connected to a quadrupole tandem mass spectrometer, API3000 (AB Sciex Instruments, Foster City, CA), using electrospray ionization (ESI) in negative mode. The electrospray voltage was 4500 V, and the temperature was 450 °C. Nebulizer, curtain, and collision-activated dissociation gases (nitrogen) were 8, 10, and 4 instrument units, respectively. The sample was separated on a Develosil ODS-HG-3 column (3 μm particle, 2 × 150 mm), with solvent delivered at a flow rate of 0.2 mL/min. The eluate was introduced into the mass spectrometer. The components were identified by a mass scan (Q1 multiple ion scan of 535.1 for the novel glycoside (leptosin) and 373.1 for monoglycoside of MSYR), and multiple-reaction monitoring of the transition from 535.1 to 221.1 (leptosin) or from 373.1 to 221.1 (monoglycoside of MSYR). Solvent A was 0.1% acetic acid in water, and solvent B was CH_3CN. The gradient program was as follows: initial (A 100%), 18 min (A 70%), 18.4 min (A 100%), and 30 min (A 100%).

Measurement of Antimyeloperoxidase Activity. The activity of myeloperoxidase (MPO) was evaluated by measuring generation of dityrosine from tyrosine in the presence of hydrogen peroxide as previously described with some modifications.[11] MPO (1.1 units/ml), hydrogen peroxide (0.2 mM), and L-tyrosine (0.2 mM) were incubated in 0.1 M phosphate buffer (pH 7.4) containing 0.1 mM diethylenediaminetetraacetic acid in the absence or presence of samples. The reaction mixture was incubated at 37 °C for 1 h. To decompose residual hydrogen peroxide, catalase (25 μg/mL) was added, and the reaction mixture was further incubated for 10 min. The solution was transferred to a centrifugal filtration tube (Amicon Ultra 10K, Millipore) and centrifuged at 14 000g for 10 min at 4 °C. The filtrate was injected into the HPLC (Shimadzu Prominence LC-20AT) connected to a fluorescence detector (Shimadzu RF-535). The separation was performed using an ODS-HG-5 (4.6 × 150 mm) column, which was equilibrated with 0.5% acetic acid/methanol (29/1, v/v), at a flow rate of 0.8 mL/min. Dityrosine was detected by measuring the fluorescence intensity (excitation, 300 nm; emission, 400 nm).

Measurement of Antibacterial Activity. A methicillin-resistant *Staphylococcus aureus* (MRSA) strain isolated from a patient in a hospital at Wakayama[12] was kindly provided by Dr. Tanaka (Sakai City Institute of Public Health, Osaka). The strain (10^5 cell/0.5 mL in 0.9% NaCl solution) was streaked onto the surface of agar (Nissui Tube Sulfide Indole Motility Medium, Nippon Suisan Kaisha, Ltd., Tokyo, Japan). A paper disk (size 8 mm, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) was placed onto the agar, and 30 μL of sample (25 mM) was applied to the paper. After incubation at 37 °C overnight, the growth of MRSA was evaluated.

Statistical Analysis. Spearman's rank correlation was used to evaluate the relationship between the two biomarkers.

RESULTS

Isolation of Compounds with Antimyeloperoxidase Activity. Previously, we reported on the antimyeloperoxidase (MPO) activity of plant-derived phenolics such as quercetin and curcumin.[11,13] Prior to this study, we found that manuka (*L. scoparium*) honey showed the strongest inhibitory activity against myeloperoxidase (MPO) among the honeys examined. We separated the active compounds in manuka honey using a solid-phase extraction C18 column. As shown in Figure 1, two water/methanol fractions (6/4 and 4/6) showed strong inhibitory activity against MPO. Both fractions were further fractionated by reversed-phase HPLC, and the activities were reanalyzed. We identified methyl 4-hydroxy-3,5-dimethoxyben-

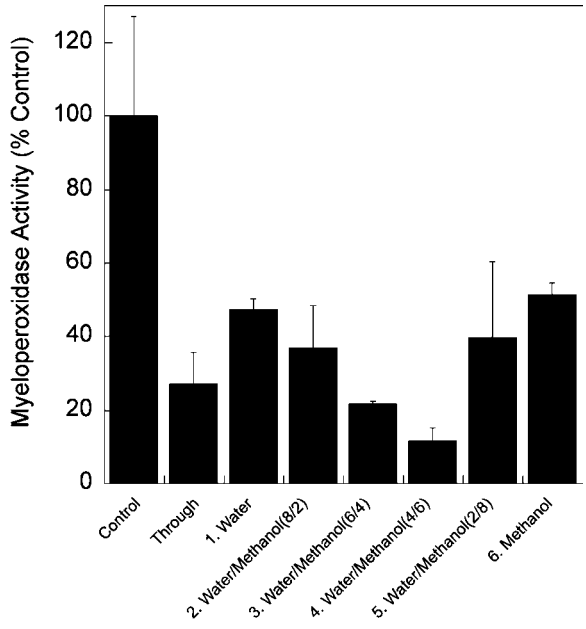

Figure 1. Fractionation and antimyeloperoxidase activity of manuka honey. Compounds in manuka honey were separated on a C18-solid phase extraction column. "Through" indicates nonbinding eluate when sample was applied. Bound compounds were eluted with water and methanol mixtures as solvents (nos. 1−6). Each fraction was assayed for antimyeloperoxidase activity as described in the Materials and Methods. Enzyme activity is expressed as a percentage of the control.

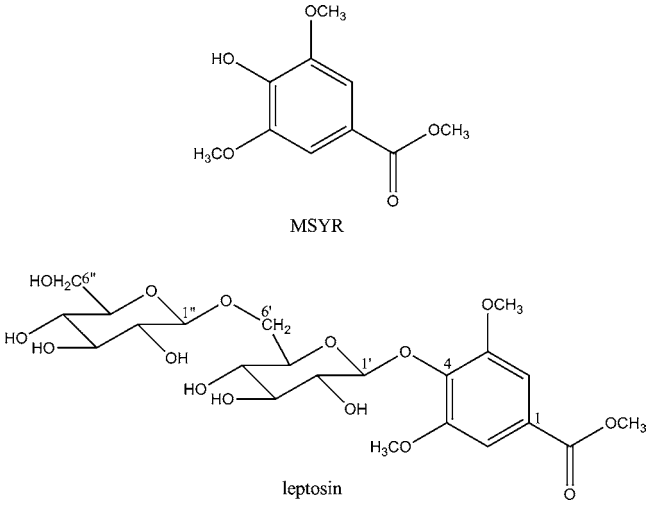

Figure 2. Chemical structures of methyl syringate (MSYR) and isolated novel glycoside, methyl syringate 4-*O*-*β*-D-gentiobiose (leptosin).

zoate, known as methyl syringate (MSYR), as the active compound from the 4/6 fraction by comparison with authentic MSYR (Figure 2). The HPLC analysis of the 6/4 fraction showed a single peak on the HPLC chromatogram. To isolate and identify the compound, 60 g of manuka honey was then fractionated by open column chromatography on Diaion HP20 as described in the Materials and Methods. From 60 g (wet) of manuka honey, we obtained approximately 30 mg of purified compound (yield 0.05%) as a white powder.

Chemical Identification of Isolated Compounds. The compound exhibited an optical rotation of $[\alpha]^{16}_{D} - 30.1°$ (*c* 0.69, CH_3OH) and an $[M-H]^-$ peak at *m*/*z* 535.1 by LC–MS analysis in the negative mode. The fragmentation of the compound by collision-induced dissociation generated a fragment ion peak at *m*/*z* 211, suggesting that the aglycone would be MSYR (MW 212). The IR spectrum revealed a broad strong absorption band at 3383 cm^{-1} due to alcoholic functions, and characteristic absorption bands of a conjugated

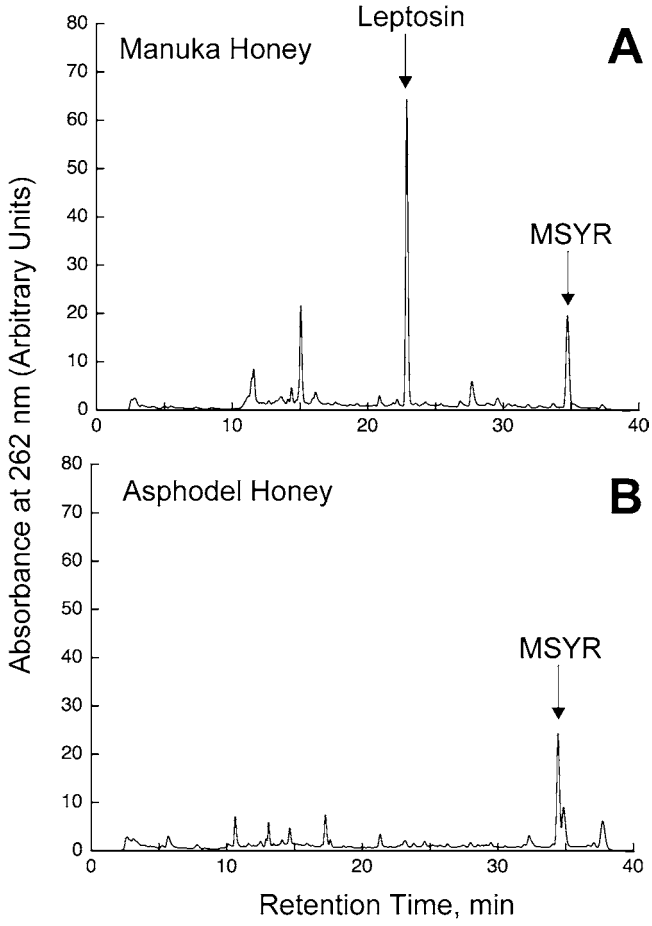

Figure 3. HPLC profile of honey. Honey sample was dissolved in water and then separated on an HPLC connected to a photodiode array detector. Arrows indicate novel glycoside (leptosin) or MSYR, respectively. (A) Manuka honey, (B) asphodel honey.

ester (1713 cm^{-1}) and an aromatic ring (1600, 1503, and 1462 cm^{-1}). In the ^{13}C NMR spectrum, four aromatic carbons were observed at δ_C 108.3, 127.2, 140.0, and 154.3, indicating the

Table 1. NMR Analyses of Isolated Glycoside

carbon no.	δ_C (ppm) 125 MHz	δ_H [ppm, multiplicity, *J*(Hz)] 500 MHz	HMBC (δ_H)
1	127.2		7.34
2,6	108.3	7.34 (2H, s)	
3,5	154.3		7.34; 3.887
4	140		7.34; 5.07
3,5-OCH_3	57	3.887 (6H, s)	
$\underline{C}OOCH_3$	168.1		7.34; 3.885
$COO\underline{C}H_3$	52.8	3.885 (3H, s)	
1′	104.1	5.07 (d, 7.8)	3.5
2′	75.6	3.50 (dd, 7.8, 9.3)	3.4
3′	77.7	3.40 (m)	5.07; 3.50
4′	71.3	3.40 (m)	4.01; 3.40
5′	78	3.40 (m)	5.07; 4.01; 3.77
6′	69.1	3.77 (dd, 4.9, 12.0) 4.01 (brd, 12.0)	4.20; 3.40
1″	104.3	4.20(d, 7.8)	4.01; 3.77; 3.10
2″	75.1	3.10 (dd, 7.8, 9.3)	3.2
3″	77.8	3.20 (m)	4.20; 3.10
4″	71.5	3.20 (m)	3.80; 3.60; 3.20; 3.05
5″	77.8	3.05 (ddd, 2.2, 5.9, 9.0)	4.20; 3.60; 3.20
6″	62.7	3.60 (dd, 5.9, 12.0) 3.80 (dd, 2.2, 12.0)	3.2

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Table 2. Quantification of Leptosin and Methyl Syringate in Various Floral Honeys and Honeydew Honeys[a]

bland, source	UMF *1	location, site	leptosin *2	MSYR *2
New Zealand Manuka Honey (*Leptospermum scoparium*)				
manuka, bland A	15	northwest	0.779	0.343
manuka, bland B	N.A.	not shown	0.458	0.802
manuka, bland C	10	northwest, east cape	0.554	0.877
manuka, bland D	13	not shown	1.078	0.466
manuka, bland E	5	not shown	0.433	0.879
manuka, bland E	10	not shown	0.604	0.435
manuka, bland E	15	not shown	0.614	0.392
manuka, bland E	20	not shown	0.951	0.469
manuka, bland F	5	far north of New Zealand	0.363	0.850
manuka, bland F	8	not shown	0.236	0.744
manuka, bland F	16	not shown	0.989	0.046
manuka, bland F	20	not shown	1.195	0.586
New Zealand Other Honeys and Honeydew Honey				
light amber honey *3	N.A.	not shown	0.720	0.457
white clover	N.A.	South island	–	–
viper bugloss (*Echium vulgare*)	N.A.	Marlborough/Otago provinces in South island	–	–
southan rata (*Metrosideros* sp.)	N.A.	west coast of South island	–	–
kamahi (*Weinmannia racemosa*)	N.A.	not shown	–	–
rewarewa (*Knightia excelsa*)	N.A.	not shown	<LOQ	<LOQ
tawari (*Ixerba brexioides*)	N.A.	not shown	<LOQ	<LOQ
blue borage (*Borago officinalis*)	N.A.	not shown	–	–
beech forest (honeydew honey)	N.A.	South island	0.275	0.166
Australian Honey				
berringa honey (*Leptospermum polygalifolium*) *4	N.A.	north coast of New South Wales, Southern Queensland	0.758	0.407
jarrah (*Eucalyptus marginata*)	N.A.	West Australia	–	–
leather wood (*Eucryphia lucida*)	N.A.	Tasmania	<LOQ	–
Honey from Other Countries				
acacia	N.A.	west coast of Lake Balaton, Hungary	–	–
asphodel (bland G)	N.A.	Sardinia, Italy	–	0.718
asphodel (bland H, lot 1)	N.A.	Sardinia, Italy	–	1.010
asphodel (bland H, lot 2)	N.A.	Sardinia, Italy	–	1.030
agrumi (citrus)	N.A.	Sardinia, Italy	–	0.449
lavender (*Lavandula stoechas*)	N.A.	Sardinia, Italy	–	0.590
millefiori (tuscan honey) *3	N.A.	Sardinia, Italy	–	–
eucalyptus	N.A.	Sardinia, Italy	–	–
cardoon (*Calactites tomentosa*)	N.A.	Sardinia, Italy	–	–
borragine (*Borago officinalis*)	N.A.	Campania, Italy	–	–
orange	N.A.	Calabria, Italy	–	–
eucalyptus	N.A.	Andalucia, Spain	–	–
lemon	N.A.	Murcia, Spain	–	–
lavender (*Lavandula stoechas*)	N.A.	Provence, France	–	–
sunflower (*Helianthus annuus*)	N.A.	Bourgogne, France	–	–
oak (honeydew honey)	N.A.	Burgos, Spain	–	–
fir (*Abies*) (honeydew honey)	N.A.	Franche-Comte, France	–	–
honey *3	N.A.	Japan	–	–
Nelumbo nucifera	N.A.	China	–	–

[a]*1: N.A. not available. *2: Numbers are expressed as μmol/g honey. "–" means "not detected". "<LOQ" indicates below limit of quantitation (LOQ). *3: Multifloral honey. *4: The honey was certified as "MGO 550".

presence of a symmetrical phenyl group. In the ^{1}H NMR spectrum (Table 1), a two-proton singlet at δ_H 7.34 and a six-proton singlet at δ_H 3.887 were assignable to two equivalent aromatic protons and two equivalent methoxyl protons, respectively. There was a nuclear Overhauser exchange spectroscopy (NOESY) correlation between the aromatic and methoxyl protons, indicating that they were in the *ortho* position with respect to each other. In addition, a three-proton singlet of another methoxyl group was observed at δ_H 3.885 in the ^{1}H NMR spectrum, which correlated with a carboxyl carbon (δ_C 168.1) in the heteronuclear multiple bond correlation (HMBC) spectrum. Furthermore, the aromatic protons (δ_H 7.34) had HMBC correlations with the carboxyl carbon and the oxygenated aromatic carbon at δ_C 140.0. These findings confirmed that a methyl 4-hydroxy-3,5-dimethoxybenzoate moiety was present in the molecule. Furthermore, two acetal carbons (δ_C 104.3 and 104.1), eight oxymethine carbons (δ_C 78.0, 77.8 (2C), 77.7, 75.6, 75.1, 71.5, and 71.3), and two oxymethylene carbons (δ_C 69.1 and 62.7) were attributable to two glucopyranose moieties. Two proton signals at δ_H 5.07 (d,

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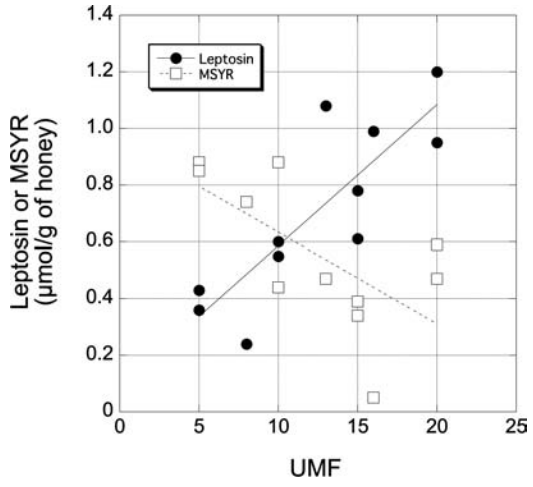

Figure 4. Correlation between unique manuka factor (UMF) and leptosin or MSYR.

7.8 Hz) and 4.20 (d, 7.8 Hz) assignable to the two anomeric protons indicated β-configurations. The HMBC correlations between the anomeric proton (δ_H 4.20) and the oxymethylene carbon (δ_C 69.1) resulted in a β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl moiety (β-gentiobiose moiety) in the molecule. The observation of the HMBC correlation between the anomeric proton (δ_H 5.07) and the aromatic carbon (δ_C 140.0) confirmed that the β-gentiobiose moiety was linked to C-4 through glycosidation. Thus, the compound was determined to be methyl 4-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-3,5-dimethoxybenzoate, a novel glycoside of MSYR with gentiobiose (Figure 2). The novel glycoside was named "leptosin" after the genus of *Leptospermum*, to which manuka belongs.

Analyses of MSYR and Novel Glycoside in Honeys. Next, we analyzed the contents of MSYR and the novel glycoside, leptosin, in honeys by HPLC-PDA detection (Figure 3A, Table 2). Honeys were directly dissolved in water (0.1 g/mL) and then injected into the HPLC. There were three major peaks on the chromatogram of manuka honey, and the latter two corresponded to leptosin (23 min) and MSYR (34 min). The unidentified former peak (15 min) was found in almost all honeys examined. Leptosin was detected in all manuka honeys examined including honey from Australian jelly bush (*Leptospermum polygalifolium*). The amount of leptosin in manuka honey ranged from 0.2 to 1.2 μmol/g honey. Other honeys from New Zealand show no or trace amounts of leptosin, suggesting that there can be some contamination of other honeys, even those labeled as monofloral, with manuka honey. Jarrah honey from *Eucalyptus marginata* (Australia) also has strong antibacterial activity,[14] but we could not detect leptosin or MSYR in this honey. MSYR is one of the characteristic compounds in manuka honey (Figure 2) and also shows superoxide scavenging activity.[15] However, MSYR is widely found among honeys, plants, and in the stomach of bees.[16] It is also considered to be a chemical marker for asphodel (*Asphodelus microcarpus* Salzm. et Viv.) monofloral honey.[17] In our study, MSYR was found in asphodel, lavender, and citrus honeys from Sardinia, Italy, but the novel glycoside leptosin was under the detection limit in those honeys (Figure 3B, asphodel honey).

Manuka trees are often infected with the scale insect *Eriococcus orariensis*, which produces honeydew.[18] To check for the presence of the glycoside in honeydew honeys, we analyzed three honeydew honeys, sapin (*Abies*) from Franche-Comté in France, encina (*Quercus*) from Bugos in Spain, and beech (*Nothofagus*) forest honeydew from New Zealand. Only the New Zealand beech forest honeydew honey contained small amounts of leptosin, which may have originated from manuka trees flowering around the area of the beech forest.

Interestingly, we found that the concentration of leptosin in manuka honey (n = 11) was positively correlated with the UMF value (Figure 4, r = 0.843, p = 0.001). On the other hand, the concentration of the aglycone (MSYR) tended to be negatively correlated with the UMF value (r = −0.655, p = 0.029). Because we have only examined a limited number of samples, additional measurements of more manuka honey samples should be carried out to confirm these correlations.

DISCUSSION

Honey has been used for various medicinal purposes, including wound healing, reduction of inflammation and gastroenteritis, for its antioxidant activity, and as a panacea for eye diseases.[20] However, the mechanisms underlying these biological activities are still unclear. Manuka honey has strong bactericidal activity, which is often expressed as the "unique manuka factor" (UMF). Here, we identified a novel glycoside of MSYR, methyl syringate 4-*O*-β-D-gentiobiose, named leptosin, from manuka honey. A monoglycoside of MSYR, methyl syringate 4-*O*-β-D-glycopyranoside, has been identified in fruit of anise (*Pimpinella anisum* L., Umbelliferae).[19] We were able to detect the monoglycoside of MSYR in anise, but not the novel diglycoside, leptosin (unpublished result).

Leptosin was characteristically detected in manuka honey as far as we examined, except that some nonmanuka honeys from the Oceania region showed trace amounts of leptosin (Table 2). There are several possible explanations for the leptosin contamination of some nonmanuka monofloral honeys from Oceania. First, even though the honey is labeled as a monofloral honey, honeybees collect pollen and nectar not only from a single (homogeneous) plant species but also from other flowering trees in the area. Second, the honey factory may use the same processing line to produce manuka honey and other honeys.

Our results of a correlation between leptosin and UMF (Figure 4) suggested that leptosin has antibacterial activity or enhances the antibacterial effects of a bactericide. We also analyzed antibacterial activity using *S. aureus*, a well-known pathogen that is often used for bactericidal assays of honey.[2,14,21] Only very high concentrations (25 mM) of the glycoside or MSYR inhibited the bacteria (data not shown). It was reported that phenolic compounds in honey are not the major active compounds for antibacterial activity.[21,22] Instead, methylglyoxal (MGO) seems to be the predominant antibacterial constituent of manuka honey.[2] However, non-aldehyde-type active compounds may contribute to bactericidal activity,[18] and bactericidal activity is also associated with the carbohydrate fraction of the honey.[21] Further studies are required to clarify the relationship between antibacterial activity and leptosin content.

In summary, we have identified a novel glycoside, leptosin, as a characteristic compound of manuka honey. There was a positive relationship between leptosin content and UMF value. Although the biological activities and biosynthetic pathway/origin of the glycoside are unknown at present, it may be a good chemical marker for purity of manuka honey. Further

examination of the biosynthetic pathway of the glycoside will be useful to clarify the ripening process of honey and may help to explain some of its medicinal effects.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

MPO, myeloperoxidase; MSYR, methyl syringate; HPLC, high performance liquid chromatography; MGO, methylglyoxal; UMF, unique manuka factor; PDA, photodiode array; LC−MS/MS, liquid chromatography tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantitation; HMBC, heteronuclear multiple bond correlation

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