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NEW LUTEOLIN 3'-*O*-ACYLATED RHAMNOSIDES FROM LEAVES OF *BURSERA GRAVEOLENS*

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Abstract-Three new luteolin 3'-*O*-rhamnopyranosides with an acetyl and/or a *p*-coumaroyl groups were isolated from leaves of *Bursera graveolens* along with five known flavonol glycosides, β-sitosterol 3-*O*-β-glucopyranoside and α-amyrin. The structures of the isolated compounds were determined by spectroscopic analysis. Their inhibitory activities for the Maillard reaction were also investigated.

The genus *Bursera* (Burseraceae) comprises about 40 species distributed in tropical zone of Latin and South America. Some species of them are used as perfumes and folk medicines.[1] *Bursera graveolens* HBK distributed from Mexico to Peru has been used for a remedy of stomachache and sudorific *etc*.[2] A methanolic extract of leaves of *B. graveolens* collected at Mexico was found to exhibit a strong inhibitory activity (68 %) for the Maillard reaction at 40 μg/mL. In this report, we describe the isolation and structural determination of flavonoid glycosides in the extract. The inhibitory activities of the respective compounds for the Maillard reaction were also estimated.

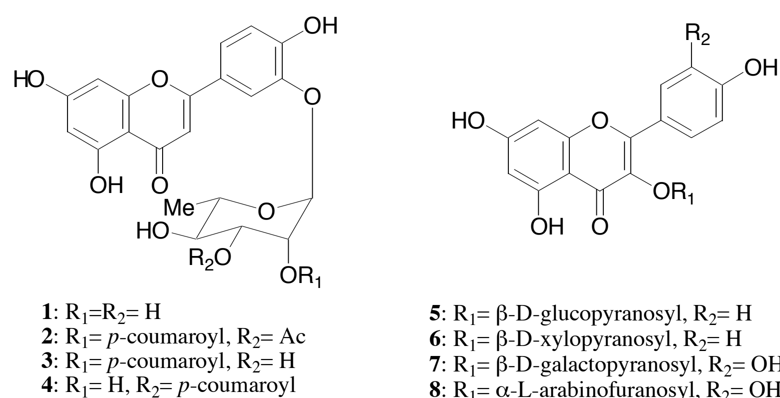

Figure 1

The methanol extract was partitioned as described in EXPERIMENTAL part. The constituents in a *n*-butanol soluble part were separated by silica gel column chromatography, followed by octadecyl silica gel (ODS) column chromatography, preparative TLC and repeated HPLC separation to afford three new flavone rhamnosides (**2**-**4**), along with five known flavonoid glycosides (**1**, **5**-**8**), β-sitosterol 3-*O*-β-D-glucopyranoside and α-amyrin.

Compound (**1**), an amorphous pale yellow solid, $[\alpha]_D$ –72.4°, showed the $[M-H]^-$ at *m/z* 431 in negative ion FAB-MS. High resolution (HR) FAB-MS in the same mode revealed the molecular formula $C_{21}H_{20}O_{10}$. Absorption bands at 268 and 335 nm in the UV spectrum showed that **1** had a flavone skeleton. The presence of an aromatic proton (δ 6.65 s, H-3), a 1,2,3,5-tetrasubstituted benzene ring [δ 6.26 (d, *J*= 2.2 Hz, H-6) and 6.53 (d, *J*= 2.2 Hz, H-8)] and a 1,2,4-trisubstituted benzene ring [δ 7.06 (d, *J*= 9.0 Hz, H-5'), 7.65 (dd, *J*= 9.0, 2.4 Hz, H-6') and 7.88 (d, *J*= 2.4 Hz, H-2')] in the flavone was

Table 1 ^{1}H NMR spectral data of compounds (**1**-**4**)

No.	**1**	**2**	**3**	**4**
aglycone				
3	6.65 s	6.65 s	6.64 s	6.63 s
6	6.26 d (2.2)	6.27 d (2.0)	6.25d (2.3)	6.25 d (2.2)
8	6.53 d (2.2)	6.55 d (2.0)	6.54 d (2.3)	6.53 d (2.2)
2'	7.87 d (2.4)	7.94 d (2.0)	7.93 d (2.0)	7.91 d (2.0)
5	7.06 d (9.0)	7.10 d (8.5)	7.07 d (8.5)	7.07 d (8.5)
6'	7.65 dd (9.0, 2.4)	7.66 dd (8.5,2.0)	7.65 dd (8.5,2.0)	7.65 dd (8.5,2.0)
rhamnosyl				
1"	5.63 d (1.6)	5.79 d (1.5)	5.74 d (1.5)	5.65 d (1.5)
2"	4.19 dd (3.6,1.6)	5.72 dd (3.5,1.5)	5.51 dd (3.5,1.5)	4.46 dd (3.5,1.5)
3"	3.97 dd (10.0,3.6)	5.44 dd (9.5,3.5)	4.25 dd (9.5,3.5)	5.34 dd (9.5,3.5)
4"	3.54 t (10.0)	3.83 t (9.5)	3.67 t (9.5)	3.88 t (9.5)
5"	3.79 dq (10.0,6.3)	4.15 dq (9.5, 6.5)	3.92 dq (9.5,6.5)	4.04 dq (9.5,6.2)
6"	1.27 d (6.3)	1.40 d (6.5)	1.34 d (6.5)	1.34 d (6.2)
p -coumaroyl				
2"',6"'		7.60 d (8.3)	7.57 d (8.5)	7.55 d (8.5)
3"',5"'		6.93 d (8.3)	6.92 d (8.5)	6.91 d (8.5)
7"'		7.73 d (16.0)	7.70 d (16.0)	7.69 d (16.0)
8"'		6.47 d (16.0)	6.43 d (16.0)	6.39 d (16.0)
acetyl				
1 "' (Me)		2.02 s		

No.	**1**	**2**	**3**	**4**
aglycone				
2	164.9	164.6	164.8	164.7
3	104.5	104.4	104.5	104.4
4	183.1	182.9	183.0	182.9
5	163.4	163.0	163.4	163.4
6	99.8	99.9	99.9	100.0
7	165.2	165.5	165.4	166.0
8	94.8	94.9	94.9	95.0
9	158.8	158.8	158.8	158.9
10	105.3	105.1	105.2	105.0
1'	123.7	123.6	123.7	123.5
2'	115.6	116.5	115.3	116.8
3'	145.8	145.2	145.6	145.8
4'	152.1	152.4	151.9	152.8
5'	117.5	117.9	117.8	117.9
6'	122.7	123.3	122.9	123.1
rhamnosyl				
1"	101.0	98.5	98.4	101.3
2"	71.5	70.2	72.8	69.6
3"	72.1	72.7	70.4	75.3
4"	73.6	71.0	74.0	71.0
5"	70.6	70.7	70.8	70.9
6"	18.2	18.2	18.3	18.3
p -coumaroyl				
1"'		126.6	126.9	127.0
2"',6"'		131.2	131.1	131.0
3"',5"'		116.8	116.6	116.8
4"'		161.0	160.8	160.8
7"'		146.8	146.3	145.6
8"'		114.5	115.3	115.9
9"'		166.9	167.3	167.5
acetyl				
1"" (Me)		21.0		
2"" (CO)		171.0		

Table 2 ^{13}C NMR spectral data of compounds (**1**-**4**)

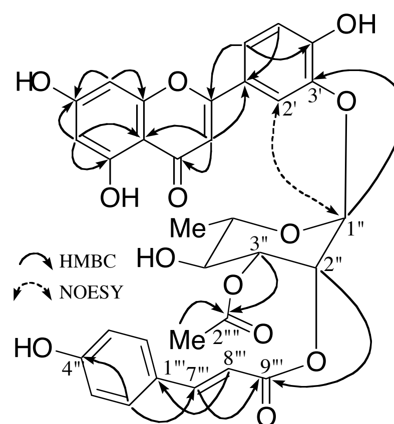

Figure 2 Selected NOESY and HMBC correlation of **2**

confirmed by the the ^{1}H NMR spectrum. A fragment ion due to the aglycone was observed at *m/z* 285 in the FABMS. Thus the aglycone was attributed to luteolin (5,7,3',4'-tetrahydroxyflavone).

Furthermore, the ^{1}H and ^{13}C NMR spectrum (Tables 1 and 2) indicated that the presence of a rhamnosyl moiety.

The negative signed rotations, combined with $J_{1,2}$ (1.6 Hz) of the anomeric proton[3] and $J_{C1\text{-}H1}$ (174 Hz) of the anomeric carbon[4] reasonably suggested **1** to be α-L-rhamnoside of luteolin. Furthermore, the presence of a HMBC correlation between the anomeric proton (H-1") and C-3' of luteolin, and a NOESY cross peak between H-1"/H-2' indicated that the rhamnosyl moiety was bonded to the hydroxyl group at C-3' of luteolin through a glycosidic linkage. Thus the structure of **1** was determined to luteolin-3'-O-α-L-rhamnopyranoside.[5]

Compound (**2**), an amorphous pale yellow powder, showed the $[M-H]^-$ at *m/z* 619.1443 in negative ion FAB-MS, indicating the molecular formula to be $C_{32}H_{28}O_{13}$. The alkaline hydrolysis of **2** gave **1**. In the ^{1}H NMR spectrum of **2**, H-2" and H-3" of a rhamnosyl moiety were shifted in lower field (1.53 and 1.47 ppm, respectively) than those of 1 (Table 1). In the ^{13}C NMR spectrum, C-1" and C-4" of the rhamnosyl moiety were also shifted to upfield by 2.5 and 2.6 ppm (Table 2). On the other hand, the presence of an acetyl [δ_H 2.02 s; δ_C 21.0 (Me), 171.0 (C=O)] and an *E-p*-coumaroyl [δ_H 6.93, 7.60 (2H each d, *J*= 8.3

Hz), 6.47, 7.73 (1H each, d, J= 16.0 Hz, *trans* olefinic protons); δ_C 166.9 (C=O)] groups was confirmed by the ^{1}H and ^{13}C NMR spectrum. Then the acetyl and the *p*-coumaroyl groups were attached at H-2" and H-3" of the rhamnosyl moiety in **2**. In the HMBC (Figure 2) spectrum, cross peaks between H-2"/C-9"' and H-3"/C-2"" indicated that the sugar part was a 3-acetyl-2-*E*-*p*-coumaroylrhamnopyranose. The structure of **2** was then characterized as luteolin 3'-*O*-α-L-(3"-acetyl-2"-*E*-*p*-coumaroyl)rhamonopyranoside.

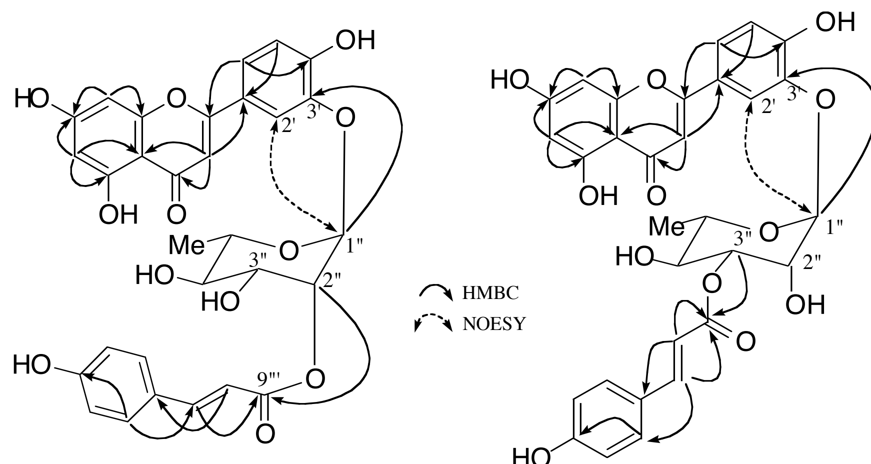

Figure 3 Selected NOESY and HMBC correlations of **3** and **4**

Compounds (**3**) and (**4**) were obtained as pale yellow solids and the same molecular formula $C_{30}H_{26}O_{12}$ were proposed by the negative ion HR-FABMS. These NMR spectra (Tables 1 and 2) indicated that **3** and **4** had commonly an *E*-*p*-coumaroyl moiety in **1**. Therefore **3** and **4** are isomeric compounds due to the different positions of the *E*-*p*-coumaroyl group esterified. On comparison with **1**, the upfield shifts of carbon in the rhamnosyl moiety were observed by 2.6 ppm (C-1") and 1.7 ppm (C-3") in **3**, and 1.9 ppm (C-2") and 2.6 ppm (C-4") in **4**. The significant correlations were observed between H-2"/C-9"' (**3**) and H-3"/C-9"' (**4**) in the HMBC spectrum (Figure 3). The configuration of rhamonose was supposed to be L by the consideration of biosynthesis. Thus the structures of **3** and **4** were determined to luteolin 3'-*O*-α-L-(2"-*E*-*p*-coumaroyl)rhamnopyranoside and luteolin 3'-*O*-α-L-(3"-*E*-*p*-coumaroyl)rhamnopyranosides.

Table 3. Inihibory activity of isolated compounds in the Maillard reaction

compound	IC_{50} (μM)
1	1.7
2	*
3	*
4	*
5	13.4
6	21.5
7	*
8	2.9
aminoguanidine	100

*IC_{50} >40 μg/mL

Although a number of acylated flavonoid glycosides have been isolated from plants, the flavonoid glycosides that possess an acylated sugar on B-ring, in particular, at C-3' are very rare in our best knowledge.

The structures of **5**-**8** were identified as kaempferol 3-*O*-β-*D*-glucopyranoside (astragalin, **5**),[6] kaempferol 3-*O*-β-D-xylopyranoside (**6**),[7,8] quercetin 3-*O*-β-D-galactopyranoside (**7**)[9,10] and quercetin 3-

O-α-L-arabinofuranoside (avicularin, **8**),[7,8] respectively by the spectroscopic analysis and the comparison with authentic data.

It is reported that protein glycation (Maillard reaction) is one of the causes of diabetic complications and aging of the skin. Among the isolated compounds, **1**, **5**, **6** and **8** showed the potent inhibitory activity for the Maillard reaction[11] as listed in Table 3.

EXPERIMENTAL

General Method

^{1}H and ^{13}C NMR spectra were measured on a GE-Omega 600 (^{1}H at 600 and ^{13}C at 150 MHz) spectrometer with acetone-d_6 as a solvent. Chemical shifts were given in δ values (ppm) relative to TMS as an internal reference. Negative FAB-MS and HR-FAB-MS spectra were recorded on JEOL JMS-700T spectrometer. UV spectra were recorded on UV-2200 spectrophotometer (Shimadzu) and optical rotations were measured on JASCO DIP-140 polarimeter. For column chromatography, Silica gel 60 (70-230 mesh, Merck), Chromatorex ODS DM102T (Fuji Silysia) were used. Kiesel gel 60 F_{254} (Merck) was used for analytical and preparative TLC. Preparative HPLC was performed on a JAI LC-908 instrument (column: JAIGEL GS-310).

Plant Material

Leaves of *Bursera graveolens* were obtained in July, 1998 at Chiapas, Mexico and the voucher specimen has been deposited in the Hervarium, Botanical Gardens, the University of Tokyo (TI), Japan.

Extraction and Isolation

The dried leaves (190 g) of *Bursera graveolens* were extracted methanol (2.1 L x weekly x 3) at rt. The methanol extract (26.6 g) was partitioned with *n*-hexane and methanol. The resulting methanol soluble part (18.7 g) was further partitioned with *n*-butanol and water. The *n*-butanol soluble part (11.4 g) was subjected to chromatography on silica gel (386 g) eluted with $CHCl_3$ (17 fractions: solvent A), $CHCl_3$–MeOH (20:1) (18 fractions: solvent B), $CHCl_3$–MeOH (10:1) (9 fractions: solvent C), $CHCl_3$–MeOH–H_2O (11:3:1: lower layer) [9 fractions: solvent D] and $CHCl_3$–MeOH–H_2O (9:3:1: lower layer) [12 fractions: solvent E], respectively. Fractions 6,7 (150 mg) by solvent A afforded α-amyrin (12 mg). Fractions 15,16 (280 mg) by solvent B was further purified by preparative TLC with EtOAc, and finally subjected to HPLC (MeOH) to afford **2** (108 mg). Fraction 18 by the same solvent system was purified by preparative TLC [$CHCl_3$-MeOH (5 : 1)] and crystallized from MeOH–H_2O (2:1) to give β-sitosterol 3-*O*-β-glucopyranoside (11 mg). Fraction 8 (solvent C) was repeatedly purified by preparative TLC developed with $CHCl_3$–MeOH–H_2O (7:3:1: lower layer), EtOAc–MeOH (50:1) and $CHCl_3$-MeOH (5:1), followed by further purification with HPLC (MeOH) to afford **3** (29 mg) and **4** (9 mg), respectively. Fractions 5-8 (540 mg) by solvent E was separated on ODS column chromatography eluted

with MeOH–H_2O (3:2) and finally purified by crystallization from MeOH to give **7** (140 mg). Fraction 3 (62 mg) by solvent D was fractionated by HPLC (MeOH) to give nine sub-fractions. Compounds (**5**) (29 mg) and (**8**) (13 mg) were obtained from the subfractions 7 and 8, respectively. The fractions 1-2 (240 mg) (solvent D) were re-chromatographed on ODS column (12 g) eluted with MeOH–H_2O (1 : 1). Compounds (**6**) (18 mg) and (**1**) (10 mg) were obtained from sub-fractions 11-13 and 24-28, respectively.

Luteolin 3'-*O*-α–L-rhamnopyranoside (1). A pale yellow amorphous solid; $[\alpha]_D$= -72.4° (*c*= 0.45, MeOH); HR negative ion FAB-MS: Negative ion FAB-MS *m/z*: 431.0977 (Calcd 431.0978 for $C_{21}H_{19}O_{10}$); Negative ion FAB-MS: *m/z*: 431 $[M-H]^-$, 285 $[aglycone-H]^-$; UV λmax (nm, MeOH) (log ε): 208 (4.5), 268 (4.2), 335 (4.1). The ^{1}H and ^{13}C NMR spectral data are listed in Tables 1 and 2.

Luteolin 3'-*O*-α-L-(3"-acetyl -2"-*E*-*p*-coumaroyl)rhamnopyranoside (**2**). A pale yellow amorphous solid; $[\alpha]_D$ +26.9° (*c*= 1.0, MeOH); HR negative ion FABMS *m/z*: 619.1443 $[M-H]^-$ (Calcd 619.1451 for $C_{32}H_{27}O_{13}$); negative ion FAB-MS *m/z*: 619 $[M-H]^-$, 577 $[M-acetyl]^-$, 473, 431, 285 $[aglycone-H]^-$; HR-EI-MS *m/z*: 286.0477 (aglycone) (Calcd 286.0477 for $C_{15}H_{10}O_6$); EI-MS *m/z* (rel. int.): 474 (4), 432 (36), 286 (100); UV λmax (nm, MeOH) (log ε): 211 (4.7), 271 (4.4), 319 (4.6). The ^{1}H and ^{13}C NMR spectral data are listed in Tables 1 and 2.

Alkaline hydrolysis of 2. The solution of 20% KOH-MeOH (7 mL) containing **2** (20 mg) was refluxed for 2 h. After cooling, the reaction mixture was poured into 4% HCl (70 mL) and ice, and then extracted with *n*-butanol–EtOAc (1:1) (20 mL x 3 times). The organic layer was washed with 5% aqueous $NaHCO_3$ (20 mL x 3 times), concentrated *in vacuo* and the residue was purified by HPLC to give **1** (7 mg).

Luteolin 3'-O-α-L-(2"-*E*-*p*-coumaroyl)rhamonopyranoside (**3**). A pale yellow amorphous powder; $[\alpha]_D$ –39.3° (*c*= 1.1, MeOH); HR negative ion FAB-MS *m/z*: 577.1354 $[M-H]^-$ (Calcd 577.1346 for $C_{30}H_{25}O_{12}$), Negative ion FABMS *m/z*: 577 $[M-H]^-$, 431$[M-coumaroyl]^-$, 285 $[aglycone-H]^-$; UV λmax (nm, MeOH) (log ε): 207 (4.5), 271 (4.2), 317 (4.39). The ^{1}H and ^{13}C NMR spectral data are listed in Tables 1 and 2.

Luteolin 3'-O-α-L-(3"-*E*-*p*-coumaroyl)rhamonopyranoside (**4**). A pale yellow amorphous powder; $[\alpha]_D$ –73.8° (*c*= 0.48, MeOH); HR-negative ion FABMS *m/z*: 577.1351 $[M-H]^-$ (Calcd 577.1346 for $C_{30}H_{25}O_{12}$); Negative ion FAB-MS *m/z*: 577 $[M-H]^-$, 431$[M-coumaroyl]^-$, 285 $[aglycone-H]^-$; UV λmax (nm, MeOH) (log ε): 208 (4.6), 271 (4.3), 318 (4.1). The ^{1}H and ^{13}C NMR spectral data are listed in Tables 1 and 2.

Maillard reaction. Inhibitory activity of isolated compounds for Maillard reaction was examined by the

method described in the literture.[11] Aminoguanidine was used as a positive control.

ACKNOWLEDGMENT

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