Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 5415–5419

Two novel carbonic acid esters conjugated with oligophenyl glucosides from *Rhamnus nakaharai*

Tzy-Ming Lu*,†

Department of Pharmacy, Tajen University of Technology, 20, Wei-Shin Rd, Shin-Ell Tsun, Yanpuu Hsiang, Pintung 907, Taiwan, ROC

Received 15 May 2007; revised 31 May 2007; accepted 4 June 2007
Available online 9 June 2007

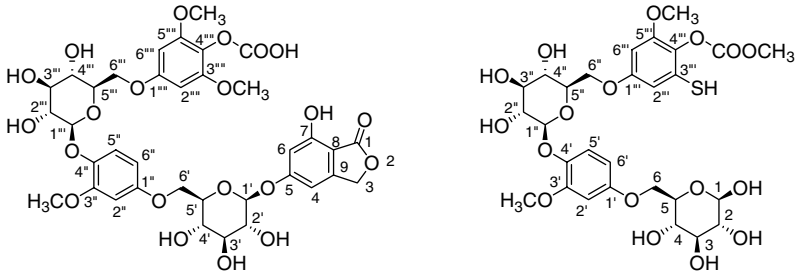
Abstract—Two novel carbonic acid esters conjugated with oligomeric phenyl glycosides have been isolated and characterized from the wood of *Rhamnus nakaharai*. The structures are characterized as 5,7-dihydroxyphthalide 5-*O*-β-[6-*O*-{3″-methoxy-4″-*O*-β-[6‴-*O*-(4⁗-*O*-carboxy-3⁗,5⁗-dimethoxy)phenyl]glucopyranosyl}phenyl]glucopyranoside (**1**) and 6-*O*-{3′-methoxy-4′-*O*-β-[6″-*O*-(3‴-mercapto-5‴-methoxy-4‴-*O*-methylcarboxy)phenyl]glucopyranosyl}phenyl β-glucopyranose (**2**), namely, rhamnakoside A (**1**) and B (**2**), all by NMR and other spectral methods, respectively. They could be a novel case of phase II detoxification products and biogenetic diversity in plant kingdom.

Rhamnus nakaharai Hayata (Rhamnaceae) one of the traditional folk medicines in Taiwan has been used for treating constipation, inflammation, tumors, and asthma.[1] The studies on this plant have proved 3-*O*-methyquercetin as an anti-inflammatory agent[2] and bronchodilator,[3] isotorachrysone as dual inhibitor of platelet aggregation[4] and antioxidant,[5] etc. Recently, the wood of the same plant which is rich in 6-methoxysorigenin glycosides, has been isolated and also proved as an excellent metal chelating antioxidant.[6] Rhamnaceous plants have long been used as laxatives,[7] however, this Formosan Rhamnaceous medicinal plant showed diverse bioactivities as described.[1–6] For continual search on this plant, two carbonic acid esters conjugated with phenyl glycoside oligomers, namely rhamnakoside A (**1**) and B (**2**) (Chart 1), have been further isolated from the hydrophilic portion of methanolic extract of wood. These two compounds could be a rather rare case of carbonic acid conjugates of natural products and also as a rare glycoside with oligomeric phenyl conjugates as known. Furthermore, rhamnakoside B (**2**) containing an aromatic thiol moiety suggested it could be a phase II glutathione conjugation metabolite[8a] of quinone by the plant as there was no known biogenetic source about aromatic thiol. These two compounds could be a novel example of the phase II conjugation metabolites and biogenetic diversity in plant chemistry. The structure elucidation of these two carbonic acid conjugates and their proper biosynthetic mechanism will be discussed herein.

Rhamnakoside A (**1**) appeared as colorless needles with an mp of 254 °C and major IR (KBr) absorption bands at ν_{max} 3497 (sharp, CO*OH*), 3380 (OH), 2899, 1744 (γ-lactonic C=O), 1703 (carbonic ester C=O), and 1612 (aromatic C=C) cm^{-1}. In the ^1H NMR (pyridine-d_5, 400 MHz) of **1**, three sets of aromatic signals could be differentiated at δ 7.63 (2H, s) as a symmetrically substituted benzene, an ABX type coupled benzene at δ 7.74 (1H, d, $J = 2$ Hz), 7.91 (1H, dd, $J = 2$, 8.4 Hz), and 7.66 (1H, d, $J = 8.4$ Hz), and a *meta*-coupled aromatic system at δ 6.82 and 6.97 (each 1H, d, $J = 1.6$ Hz). There were also three methoxy signals at δ 3.55 (3H, s) and 3.71 (6H, s), a pair of γ-lactonic methylene signals at δ 4.95 and 4.98 (each 1H, AB q, $J = 15.2$ Hz). The above aromatic systems were identified as 2,6-dimethoxyhydroquinonyl, 2-O-substituted hydroquinonyl,[9] and 5,7-dioxygenated phthalide moieties, respectively. In the meantime, there were two glucosyl anomeric signals at δ 5.72 and 5.76 (each 1H, d, $J = 7.6$ Hz) and both their C-6 methylene signals showed lower field shift at δ 5.21 (dd, $J = 12$, 2.0 Hz), 4.92 (t, $J = 11.6$ Hz) and 5.42

Keywords: Carbonic acid ester; Aromatic thiol; Oligomeric phenyl glycoside; Rhamnakoside; *Rhamnus nakaharai*.
* Tel.: +886 8 7624002x320; fax: +886 8 7625308; e-mail: cmlu@mail.tajen.edu.tw
† The author has previously published under the name Chai-Ming Lu.

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.06.017



1 **2**

Chart 1. Structures of **1** and **2**.

(dd, $J = 12$, 2.0 Hz), 5.02 (t, $J = 12.0$ Hz) in the ^{1}H NMR spectrum of **1**. The ^{13}C NMR spectrum (pyridine-d_5, 100 MHz) of **1** (Table 1) supported the structure of **1** in the feature of three aromatics connected by two glucopyranosides on head and tail by comparing with the ^{13}C NMR data of C-6 acylated glucoside,[10a] 1-*O*-methyl β-glucopyranoside, and 6-*O*-methyl β-glucopyranose.[10b] HMQC and HMBC spectra of **1** confirmed the connection of these sub-structures of **1** in the sequence of 2,6-dimethoxyhydroquinonyl (4→6) glucopyranoside (1→1) 2-methoxyhydroquinonyl (4→6) glucopyranoside (1→5) 5,7-dihydroxyphthalide (Fig. 1). A quaternary carbon signal at δ 143.2 in the ^{13}C NMR spectrum of **1** was elucidated as carbonic acid monoester, since IR spectrum showed additional carbonyl absorption at ν_{max} 1703 cm^{-1}. The carbonate ester linkage was assigned at C-1 of the 2,6-dimethoxyhydroquinonyl moiety by comparing the reported ^{13}C NMR data.[9] Based on the above

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2**

No. C	**1** (Pyridine-d_5)		**2** (Pyridine-d_5)	
	^{1}H (mult; J = Hz)	^{13}C[a]	^{1}H (mult; J = Hz)	^{13}C[a]
1		170.0	6.15 (d; 7.2)	103.5
2			4.21 (dt; 2.4, 9.6)	75.5
3	4.98, 4.95 (ABq; 15.2)	69.3	4.34 (t; 8.8)	79.1
4	6.82 (d; 1.6)	101.5	3.99 (t; 9.6)	73.2
5		165.3	4.54 (t; 10.8)	75.7
6	6.97 (d; 1.6)	105.1	5.17 (dd; 11.2, 2.4), 4.54 (t; 10.8)	66.6
7		159.7		
8		107.4		
9		151.6		
1′	5.72 (d; 7.6)	102.1		166.5
2′	4.32–4.42 (m)[b]	74.9	7.67 (d; 2.0)	113.3
3′	4.32–4.42 (m)[b]	78.5		153.6
4′	4.24 (t; 9.6)	71.7		151.7
5′	4.32–4.42 (m)[b]	76.0	7.53 (d; 8.4)	115.6
6′	5.21 (dd; 12, 2.0), 4.92 (t; 11.6)	65.3	7.43 (dd; 2.0, 8.4)	124.6
1″		166.5	5.64 (d; 7.2)	101.8
2″	7.74 (d; 2.0)	114.1	4.36 (t; 8.4)	74.9
3″		150.1	4.38 (t; 8.8)	79.0
4″		152.3	4.05 (t; 9.2)	72.8
5″	7.66 (d; 8.4)	115.4	4.42 (t; 8.8)	76.0
6″	7.91 (dd; 8.4, 2.0)	124.7	5.31 (dd; 11.6, 2.0), 5.09 (t; 10.8)	65.8
1‴	5.76 (d; 7.2)	102.0		166.3
2‴	4.32–4.42 (m)[b]	75.0	7.88 (d; 1.6)	108.8
3‴	4.32–4.42 (m)[b]	78.6		126.4
4‴	4.21 (t; 9.6)	72.0		139.8
5‴	4.51 (t; 9.6)	76.1		149.8
6‴	5.42 (dd; 12.0, 2.0), 5.02 (t; 12.0)	65.6	7.52 (d; 1.6)	108.0
1⁗		167.1	(*C*OOMe)	155.0
2⁗	7.63 (s)	108.6	(COO*Me*) 3.48 (s)	56.4
3⁗		149.0		
4⁗		120.6		
5⁗		149.0		
6⁗	7.63 (s)	108.6		
CO_2H		143.2		
OMe	3.55 (s), 3.71 (s, 2x)	56.2, 56.7	3.56 (s), 3.92 (s)	55.9, 56.9

[a] Signals obtained are verified by HMQC, HMBC, and DEPT techniques.
[b] These signals are overlapped.

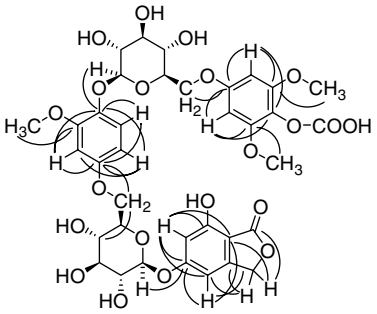


Figure 1. HMBC signal correlation of rhamnkoside A (**1**).

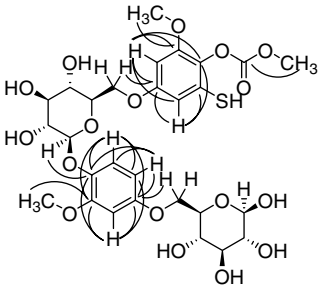


Figure 2. HMBC signal correlation of rhamnkoside B (**2**).

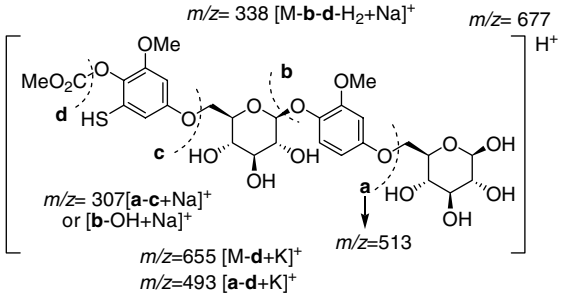


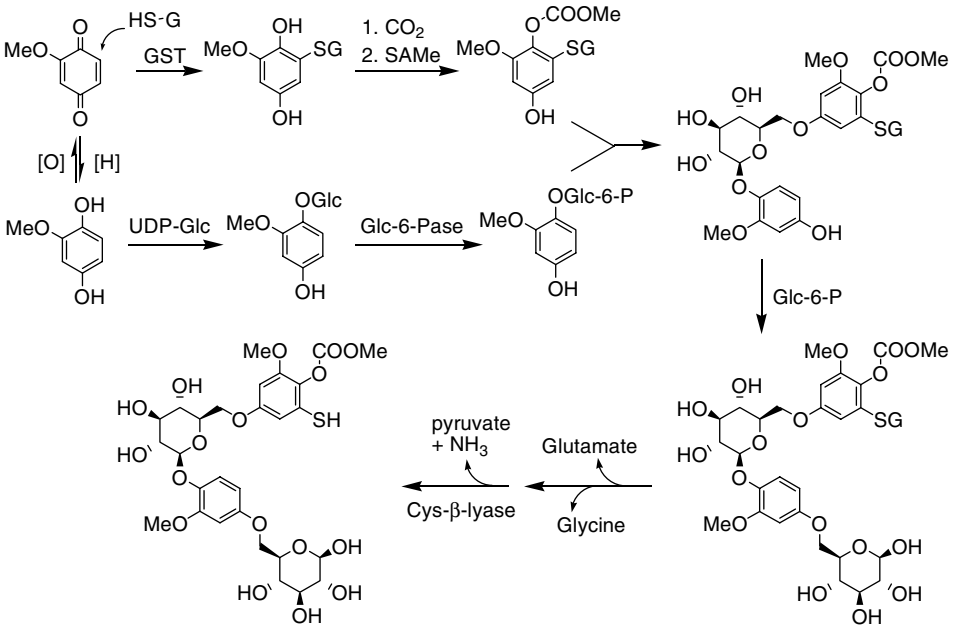
Figure 3. FABMS (pos. mode) fragmentation pattern of **2**.

evidences, the structure of **1** can be characterized as 5,7-dihydroxyphthalide 5-*O*-β-{6′-*O*-{3″-methoxy-4″-*O*-β-[6‴-*O*-(4⁗-*O*-carboxy-3⁗,5⁗-dimethoxy)phenyl]glucopyranosyl}phenyl]glucopyranoside (**1**), namely rhamnakoside A. The FABHRMS (pos. mode) of **1** found the quasi-molecular ion peak at *m*/*z*: 809.2137 (calcd for $C_{36}H_{40}O_{21} + H^+ = 809.2135$) confirmed the characterization. The carbonic acid monoester was never found from plant source. However, in the organic synthesis phenyl carbonic acid monoester had been used to prepare phenyl carbamate derivative under refluxing conditions in strong acid.[11] This interprets that phenyl carbonic acid monoester can be stable under vigorous condition and can be a support for the structure of **1**.

Rhamnakoside B (**2**) was isolated as colorless granules with an mp of 192 °C. The IR (KBr) absorption also showed carbonate carbonyl band at ν_{max} 1708 cm^{-1} together with other bands at ν_{max} 3372 (OH), 2915 (CH), and 1598 (aromatic C=C) cm^{-1}. The ^1H NMR (pyridine-d_5, 400 MHz) spectrum of **2** (Table 1) displayed two anomeric proton signals at δ 5.64 and 6.15 (each 1H, d, $J = 7.2$ Hz), and also two sets of lower field shift C-6 methylene signals at δ 5.31 (dd, $J = 11.6$, 2.0 Hz), 5.09 (t, $J = 10.8$ Hz) and 5.17 (dd, $J = 11.2$, 2.4 Hz), 4.54 (t, $J = 10.8$ Hz) interpreted two 6-O-substituted glucopyranosyl (Table 1).[10] The aromatic signals in the ^1H NMR of **2** appeared at δ 7.67 (1H, d, $J = 2.0$ Hz), 7.53 (1H, dd, $J = 8.4$, 2.0 Hz), and 7.43 (1H, d, $J = 8.4$ Hz) and δ 7.88, 7.52 (each 1H, d, $J = 1.6$ Hz) due to a 2-methoxyhydroquinonyl and a 6-substituted 2-methoxyhydroquinonyl moiety. The ^{13}C NMR (pyridine-d_5, 100 MHz, Table 1) spectrum of **2** supported the characterization of the above sub-structures and accordingly, the benzene systems were also connected by glucopyranose on head and tail. FABMS (pos. mode) of **2** showed quasi-molecular ion peak at *m*/*z*: 677 $[M+H]^+$ suggested the second benzene system as sulfur-containing 2-mercapto-6-methoxyhydroquinonyl, which was also confirmed by comparing the ^{13}C NMR data with 2-methoxyhydroquinone[9b] and thiophenol[12] as well as considering the possible metabolic pathway of glutathione adduct.[8a] The conjugation sequence of these sub-structures of **2** was established by the HMQC and HMBC spectra (Fig. 2) as 2-mercapto-6-methoxyhydroquinonyl (4→6) glucopyranosyl (1→1) 2-methoxyhydroquinonyl (4→6) glucopyranosyl, respectively. Furthermore, a methoxy signal at δ 3.48 (3H, s) showed a $^3J_{CH}$ cross peak with the signal at δ 155.0 in the HMBC spectrum of **2** (Fig. 2) suggested the di-esteric carbonate conjugation. Finally, the FABMS fragmentation pattern shown in Figure 3 supported that the methyl carbonate di-ester was conjugated at C-1 of the 2-mercapto-6-methoxyhydroquinonyl moiety in the manner of **1**, reasonably. Based on the above evidences, the structure of **2** was assigned as 6-*O*-{3′-methoxy-4′-*O*-β-[6″-*O*-(3‴-mercapto-5‴-methoxy-4‴-*O*-methylcarboxy)phenyl]glucopyranosyl}phenyl β-glucopyranose (**2**) and named rhamnakoside B. The FABHRMS (pos. mode) found quasi-molecular ion peak at *m*/*z*: 677.1750 (calcd for $C_{28}H_{36}O_{17}S + H^+$: 677.1746) also supported the characterization.

Carbon dioxide or carbonic acid has a well-known role of one carbon unit in biogenetic systems; however, phase II conjugative detoxicification in carbonate form is a novel example other than that of sulfate and/or glycosylation.[8b] No wonder these two compounds exist in the highly hydrophilic portion of the plant extract.[13] On the other hand, the biosynthesis of glycosides in higher plants is catalyzed by the enzyme glycosyl transferase that transfer sugar from the cofactor, uridine diphosphate (UDP)-glucose, to various aglycones and this transformation usually found at the anomeric carbon (C-1) of the sugar.[8b] These two compounds glucosylated by C-6 of the sugar could be a novel case of glycoside biogenesis at least to the author's knowledge.[14]

Quinones are known as pro-oxidants that can drive redox cycling to generate oxidation stress in the biosystems[8c] and glutathione plays a role to detoxicify these compounds by the enzyme glutathione *S*-transferase (GST).[8a] After the glutathione adducts were formed, peptidolytic enzymes hydrolyze the other amino acids and finally an enzyme called cysteine-*S*-β-lyase releases



Scheme 1. Possible biogenetic process of **2**; GSH: glutathione; UDP-Glc: uridine diphosphate glucose; GST: glutathione-*S*-transferase; SAMe: *S*-adenosyl methionine; G-6-P: glucose-6-phosphate; G-6-Pase: glucose-6-phosphatase; Cys-β-lyase: cysteine-*S*-β-lyase.

the free aromatic thiol.[8a] Obviously, the 2-mercapto-6-methoxyhydroquinonyl moiety in the structure of **2** must be a detoxified product of 2-methoxyquinone by the plant GST since there was no known precursor of aromatic thiol in biogenetic systems (Scheme 1). Rhamnakoside B (**2**) could also be a novel natural occurring case that contains an aromatic thiol especially as in plant sources.

Acknowledgments

The author is grateful for the financial supports by National Science Council, Taiwan ROC (NSC89-2320-B-127-010) and Tajen University. NMR spectra measured by Mr. Lin, P. R., Tajen University and FABHRMS measurement by Miss Lai, L. N., Department of Chemistry, National Cheng Kung University, Tainan, Taiwan are both gratefully acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.06.017.

References and notes

1. Chiu, N. Y.; Chang, K. H. In *The Illustrated Medicinal Plants of Taiwan*; Southern Materials Center: Taipei, 1998; Vol. 5, pp 135–136.
2. (a) Wei, B. L.; Lu, C. M.; Tsao, L. T.; Wang, J. P.; Lin, C. N. *Planta Med.* **2001**, *67*, 745; (b) Ko, W. C.; Shih, C. M.; Chen, M. C.; Lai, Y. H.; Chen, J. H.; Chen, C. M.; Lin, C. N. *Planta Med.* **2004**, *70*, 1123; (c) Jiang, J. S.; Shih, C. M.; Wang, S. H.; Chen, T. T.; Lin, C. N.; Ko, W. C. *J. Ethnopharmacol.* **2006**, *103*, 281.
3. (a) Ko, W. C.; Wang, H. L.; Lei, C. B.; Shih, C. H.; Chung, M. I.; Lin, C. N. *Planta Med.* **2002**, *68*, 30; (b) Ko, W. C.; Chen, M. C.; Wang, S. H.; Lai, Y. H.; Chen, J. H.; Lin, C. N. *Planta Med.* **2003**, *69*, 310.
4. Lin, C. N.; Lu, C. M.; Lin, H. C.; Ko, F. N.; Teng, C. M. *J. Nat. Prod.* **1995**, *58*, 1934.
5. Hsiao, G.; Ko, F. N.; Lin, C. N.; Teng, C. M. *Biochim. Biophys. Acta* **1996**, *1298*, 119.
6. Ng, L. T.; Lin, C. C.; Lu, C. M. *Chem. Pharm. Bull.* **2007**, *55*, 382.
7. Evans, W. C. In *Pharmacognosy*, 15th ed.; Harcourt Press: Edinburgh, 2002; Chapter 22, pp 229–244.
8. (a) Hodgson, E.; Smart, R. C. In *Introduction to Biochemical Toxicology*; LeBlanc, G. A., Dauterman, W. C., Eds.; Wiley-Interscience Press: New York, 2001; pp 126–131, Chapter 6; (b) Hodgson, E.; Smart, R. C. In *Introduction to Biochemical Toxicology*; LeBlanc, G. A., Dauterman, W. C., Eds.; Wiley-Interscience Press: New York, 2001; Chapter 6; p 117; (c) Hodgson, E.; Smart, R. C. In *Introduction to Biochemical Toxicology*; Reed, D. J., Ed.; Wiley-Interscience Press: New York, 2001; Chapter 10; pp 248–252.
9. (a) Otsuka, H.; Takeuchi, M.; Inoshiri, S.; Sato, T.; Yamasaki, K. *Phytochemistry* **1989**, *28*, 883; (b) Sajio, R.; Nonaka, G.-I.; Nishoka, I. *Phytochemistry* **1989**, *28*, 2443.
10. (a) Lu, C. M.; Yang, J. J.; Wang, P. Y.; Lin, C. C. *Planta Med.* **2000**, *66*, 374; (b) Breitmaier, E.; Voelter, W. In *Carbon-13 NMR Spectroscopy*; VCH Press: New York, 1989; Chapter 4, p 388.
11. Acklin', P.; Allgeier, H.; Auberson, Y. P.; Bischoff, S.; Ofner, S.; Sauer, D.; Schmutz, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 493.
12. Breitmaier, E.; Voelter, W. In *Carbon-13 NMR Spectroscopy*; VCH Press: New York, 1989; p 257.
13. Isolation of **1** and **2**. The isolation residue reported in Ref. 6 was washed by MeOH. The MeOH wash, which showed strong blue fluorescence under UV at 323 nm displayed in suspension and readily soluble in water. This residue was

condensed and dissolved in 30% MeOH/H_2O and chromatographed on an Rp-18 Si-gel (100 g, 60 μm, Merck, Germany) column (3 × 50 cm). Then elution with 30–85% of MeOH/H_2O and in 5% gradient per 500 ml. The elution fraction obtained by 40% of MeOH/H_2O (about first 250 ml) was further chromatographed by another Rp-18 Si-gel (30 g) column (1.5 × 55 cm) with 40% of MeOH/H_2O. The fraction was collected by 6 ml and fractions 12–29 obtained by **2** (21 mg) with an Rp-18 TLC R_f value 0.33, fractions 38–65 obtained by **1** (15 mg) with an Rp-18 TLC R_f value 0.19. General procedure and plant material extraction deposit in Supplemental data.

14. Data of rhamnakoside A (**1**) and B (**2**), please see text and NMR spectral data in Table 1.