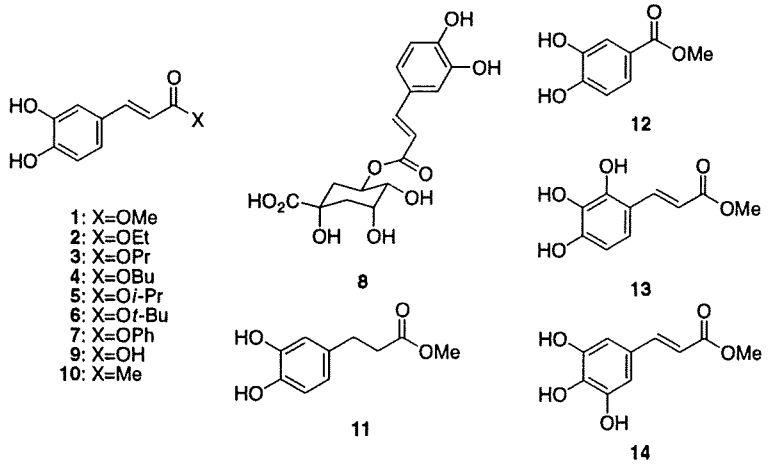

Fig. 1. Structures of Methyl Caffeate (**1**) and Related Compounds.

Table 2. Inhibitory Activity of **1**–**14** against Rat Intestinal Sucrase and Maltase

Compound	IC_{50} (mM)	
	Sucrase	Maltase
1	1.5	2.0
2	1.8	1.9
3	3.1	2.2
4	3.2	1.8
5	2.0	1.9
6	2.7	2.2
7	1.3	2.1
8	7.6	6.3
9	2.9	5.2
10	2.7	7.2
11	7.5	12.2
12	6.5	16.9
13	5.1	4.9
14	1.0	2.8

derivatives showed some variance in inhibitory activity, in spite of their low activity as compared to reported caffeoyl inhibitors having a multi caffeoyl or flavonol structure within the molecule.[3,7,24] Therefore caffeoyl ester itself showed some inhibitory activity, while the number of inhibitory active structures within the molecule might be influential.

In view of the results for **11** and **12**, the omission of a double bond in **1** led to a decrease in activity. Finally, an effect of the additional hydroxyl group in **1** was examined. Although the addition of a hydroxyl group to the C-5 position did not alter inhibitory activity much, the addition of a hydroxyl group to the C-2 position decreased its activity, as seen in the results for methyl 2,3,4-trihydroxycinnamate (**13**) and methyl 3,4,5-trihydroxycinnamate (**14**). Methyl 3,4,5-trihydroxycinnamate (**14**) is a C_2 symmetrical compound, and hence 3-OH and 5-OH are equivalent. This might be the reason **14** retained inhibitory activity. In contrast, an additional hydroxyl group of 2-OH might be sterically unfavorable or might have an detrimental electron-donating effect against the neighboring double bond, which was found to contribute to the inhibitory effect, resulting in a decrease in inhibitory activity.

In conclusion, methyl caffeate (**1**) was isolated as the active component in the α-glucosidase inhibitory activity of *S. torvum*. Methyl caffeate (**1**) and several derivatives (**2**–**14**) were tested for α-glucosidase inhibitory activity, and it was determined that **1** had a favorable structure for both sucrase and maltase inhibition, except for the slightly higher activity of **14** against sucrase. Its moderate α-glucosidase inhibitory action provides a promising application of *Solanum torvum* fruit as an antidiabetic agent.

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Flavonol Caffeoylglycosides as α-Glucosidase Inhibitors from *Spiraea cantoniensis* Flower

KAORI YOSHIDA,† ATSUYUKI HISHIDA,‡ OSAMU IIDA,‡ KEIZO HOSOKAWA,§ AND JUN KAWABATA†,*

Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan, Tsukuba Division, Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation, Tsukuba 305-0843, Japan, and Department of Nutritional Management, Faculty of Health Sciences, Hyogo University, Kakogawa 675-0195, Japan

In the screening experiments for rat intestinal α-glucosidase inhibitors in 218 plants cultivated in the Japanese temperate region, potent maltase-inhibiting activity was found in the extract of flowers of *Spiraea cantoniensis*. The enzyme assay guided fractionation of the extract led to the isolation of three flavonol caffeoylglycosides, quercetin 3-*O*-(6-*O*-caffeoyl)-β-galactoside (**1**), kaempferol 3-*O*-(6-*O*-caffeoyl)-β-galactoside (**2**), and kaempferol 3-*O*-(6-*O*-caffeoyl)-β-glucoside (**3**), as rat intestinal maltase inhibitors. This is the first report on the α-glucosidase-inhibitory activity of those flavonol caffeoylglycosides. Comparison in the activity of the isolates indicated the importance of caffeoyl substructures in the molecule for the α-glucosidase-inhibiting activity. The relatively high contents of the active isolates in the plant suggest that *S. cantoniensis* could be physiologically useful for treatment of diabetes.

KEYWORDS: ***Spiraea cantoniensis*; α-glucosidase inhibitor; flavonol acylglycoside; caffeoyl group**

INTRODUCTION

Diabetes mellitus is one of the most serious chronic diseases that is developing along with an increase in both obesity and aging in the general population (*1*). One of the therapeutic approaches for decreasing postprandial hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive organs (*2*). In recent years, many efforts have addressed the search for effective α-glucosidase inhibitors from natural sources in order to develop a physiological functional food or lead compounds for use against diabetes (*3*). In the course of our search for rat intestinal α-glucosidase-inhibiting principles from plants, we have isolated and identified several active compounds from a variety of plants grown not only in Japan (*4–6*) but also in Thailand (*7, 8*), China (*9, 10*), and Nepal (*11*). In this paper, we present results of a screen of temperate plants in Japan for α-glucosidase inhibition. In the screening experiments for rat intestinal maltase and/or sucrase inhibitors in 218 plants cultivated in Tsukuba, Japan, potent maltase-inhibiting activity was found in extracts of flowers of *Spiraea cantoniensis* (Rosaceae), an ornamental deciduous shrub. There have been only a few reports on the chemical constituents of this plant (*12–14*), but no medicinal usage is known, although some rosaceous plants are known to contain anthocyanins and soluble tannins showing antiglucosidase and antiamylase activities (*15*). Hence, the promising screening result prompted us to isolate and elucidate the structure of active compounds from this plant species.

MATERIALS AND METHODS

Materials. Two hundred and eighteen species of Japanese temperate plants were cultivated and collected in the experiment field in Tsukuba, Japan. All voucher specimens are deposited in Tsukuba Division, Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation, Tsukuba, Japan. All chemicals used were of reagent grade and were purchased from Wako Pure Chem. Co. (Osaka, Japan) unless otherwise stated. All solvents were distilled before use.

General Procedure. NMR spectra were recorded on a Bruker AMX500 instrument (^{1}H, 500 MHz; ^{13}C, 125 MHz). Chemical shifts were determined relative to residual signals of methanol-d_4 as a solvent (δ_H 3.3 ppm, δ_C 49.0 ppm). Field desorption (FD) and fast atom bombardment (FAB) mass spectra were determined by a JEOL SX102A instrument. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. Melting points were measured on a hot stage and are uncorrected.

Intestinal α-Glucosidase Inhibitory Activity Determination. The maltase- and sucrase-inhibitory activities designating an inhibition of maltose- and sucrose-hydrolyzing activities, respectively, in rat intestinal glucosidase complexes were measured as described previously (*10*). The crude enzyme solution prepared from rat intestinal acetone powder (Sigma-Aldrich Japan Co., Tokyo, Japan) was used as the small intestinal α-glucosidases, maltase and sucrase, showing specific activities of 0.70 and 0.34 U/mL, respectively. The reaction mixture consisted

* To whom correspondence should be addressed. E-mail: junk@chem.agr.hokudai.ac.jp.
† Hokkaido University.
‡ National Institute of Biomedical Innovation.
§ Hyogo University.

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Table 1. NMR Assignments of 1–3 (Methanol-d_4)

	δ_C, ppm			δ_H, ppm (mult, J in Hz)		
	1	2	3	1	2	3
2	158.4	159.2	159.4			
3	135.6	135.6	135.2			
4	179.1	179.6	179.4			
5	162.6	162.9	162.9			
6	99.7	100.1	100.1	6.26(d, 2.1)	6.14(d, 1.7)	6.14(d, 2.0)
7	165.2	166.2	166.0			
8	94.7	94.9	94.9	6.51(d, 2.1)	6.32(d, 1.7)	6.31(d, 2.0)
9	157.9	158.5	158.4			
10	105.3	105.5	105.6			
1′	122.7	122.6	122.7			
2′	117.4	132.3	132.2	7.87(d, 2.2)	8.05(d, 9.0)	7.98(d, 8.9)
3′	145.3	116.2	116.1		6.84(d, 9.0)	6.81(d, 8.9)
4′	149.3	161.6	161.5			
5′	115.7	116.2	116.1	6.92(d, 8.5)	6.84(d, 9.0)	6.81(d, 8.9)
6′	123.2	132.3	132.2	7.70(dd, 8.5, 2.2)	8.05(d, 9.0)	7.98(d, 8.9)
1″	105.3	105.2	104.1	5.22(d, 7.9)	5.07(d, 7.9)	5.20(d, 7.4)
2″	72.5	72.9	75.8	3.83(dd, 9.6, 7.9)	3.80(dd, 9.9, 7.9)	3.47(m)
3″	74.6	74.9	78.0	3.66(dd, 9.6, 3.3)	3.56(dd, 9.9, 3.3)	3.47(m)
4″	69.5	70.2	71.7	3.90(dd, 3.3, 1.2)	3.80(dd, 3.3, 1.5)	3.34(m)
5″	74.2	74.8	75.7	3.81(ddd, 7.1, 4.9, 1.2)	3.73(ddd, 8.0, 4.4, 1.5)	3.44(m)
6″	63.8	64.3	64.3	4.21(dd, 11.9, 7.1)	4.12(dd, 11.3, 8.0)	4.18(dd, 11.9, 6.7)
				4.26(dd, 11.9, 4.9)	4.32(dd, 11.3, 4.4)	4.28(dd, 11.9, 2.2)
1‴	127.5	127.7	127.7			
2‴	115.1	115.2	115.2	7.10(d, 2.0)	6.92(d, 1.6)	6.95(d, 1.7)
3‴	146.2	147.0	147.0			
4‴	148.7	149.5	149.5			
5‴	116.3	116.5	116.5	6.85(d, 8.1)	6.76(d, 8.1)	6.76(d, 8.4)
6‴	122.7	123.0	123.1	6.88(dd, 8.1, 2.0)	6.76(dd, 8.1, 1.6)	6.79(dd, 8.4, 1.7)
7‴	145.9	146.7	146.7	7.37(d, 16.0)	7.32(d, 16.0)	7.34(d, 15.8)
8‴	115.1	114.6	114.7	6.08(d, 16.0)	6.00(d, 16.0)	6.03(d, 15.8)
9‴	167.2	168.8	168.9			

of crude enzyme solution (0.05 mL of maltase and 0.2 mL of sucrase), substrate (maltose, 3.5 mM, 0.35 mL; sucrose, 56 mM, 0.2 mL) in 0.1 M potassium phosphate buffer (pH 6.3), and the test sample in 50% aqueous dimethyl sulfoxide (0.1 mL). After incubation for 15 min at 37 °C, the reaction was stopped by adding 0.75 mL of 2 M Tris-HCl buffer (pH 7.0). The reaction mixture was passed through a short column of basic alumina (ICN Alumina B, grade I, ICN Biomedical GmbH, Eschwege, Germany) to remove phenolic compounds, which might interfere with enzymatic glucose quantification in the following step. The amount of liberated glucose was measured by the glucose oxidase method, using a commercial test kit (Glucose B-test Wako, Wako Pure Chem. Co., Osaka, Japan).

Screening Experiment. The screening experiments for rat intestinal sucrase and maltase inhibition were carried out with extracts of 509 plant parts from 218 species. Each dried plant part was extracted with 50% aqueous methanol. The extracts were evaporated, redissolved in 50% aqueous dimethyl sulfoxide, and subjected as the test sample to the assay for rat intestinal α-glucosidase inhibitory activity at the final concentration of the extractable constituents obtained from 50 mg of plant material in 1 mL of solution.

Isolation of Quercetin 3-*O*-(6-*O*-Caffeoyl)-β-galactoside (1), Kaempferol 3-*O*-(6-*O*-Caffeoyl)-β-galactoside (2), and Kaempferol 3-*O*-(6-*O*-Caffeoyl)-β-glucoside (3) from *S. cantoniensis* Flower. Dried flowers (100 g) of *S. cantoniensis* were extracted with 50% aqueous methanol and the extracts were concentrated and partitioned between ethyl acetate and water. The inhibitory activity assay was carried out at the concentration of each fraction obtained from 0.1 g of plant material in 1 mL of reaction solution throughout the fractionation. The ethyl acetate fraction showed a strong inhibitory activity of 62% against maltase, whereas the sucrase-inhibitory activity was relatively low (36%). In contrast, the aqueous phase showed inconspicuous activities for both maltase (37%) and sucrase (26%). Hence, further fractionation was performed for isolating maltase inhibitors from the ethyl acetate fraction. This active fraction (6 g of dry weight) was charged onto a silica gel column and eluted with a chloroform–methanol gradient. The maltase-inhibitory activity was eluted in the chloroform–methanol (6:1) eluate (2.1 g of dry weight, 50% inhibition). The eluate was further purified by preparative HPLC (column, Inertsil PREP-ODS, 20 × 250 mm, GL-Science Co.; mobile phase, 27.5% MeCN in water; flow rate, 5.0 mL/min; detection, UV 254 nm). Eight principal peaks were detected in the region of t_R = 15–50 min. Among them, a peak eluted at t_R = 19.4 min, showing the highest activity of 38%, was rechromatographed under the same condition except for using 23% MeCN in water as the mobile phase to give **1** (33 mg, 0.03%, t_R = 39.2 min). A peak cluster that eluted at t_R = 22.5–25.4 min in the first HPLC, showing the second highest activity of 31%, was rechromatographed (column, Inertsil PREP-ODS, 20 × 250 mm × 2 (serial connection); mobile phase, 20% MeCN in water; flow rate, 7.0 mL/min (0–30 min) and 5.0 mL/min (30–120 min); detection, UV 254 nm) to give **2** (26 mg, 0.03%, t_R = 89.0 min) and **3** (29 mg, 0.03%, t_R = 101.8 min). **1**: yellow powder; mp 190–195 °C; $[\alpha]_D^{23}$ −11° (*c* 0.22, MeOH); FD-MS *m/z* 626 ($[M]^+$), 464 (M − 162), 302 (M − 162 × 2); FAB-HR-MS (negative) *m/z* 625.1185 ($[M - H]^-$, calcd for $C_{30}H_{25}O_{15}$, 625.1193). **2**: yellow powder; mp 210–212 °C; $[\alpha]_D^{23}$ −15° (*c* 0.22, MeOH); FD-MS *m/z* 611 ($[M + H]^+$), 448 (M − 162), 286 (M − 162 × 2); FD-HR-MS *m/z* 611.1423 (calcd for $C_{30}H_{27}O_{14}$, 611.1400). **3**: yellow powder; mp 205–208 °C; $[\alpha]_D^{23}$ −41° (*c* 0.24, MeOH); FD-MS *m/z* 611 ($[M + H]^+$), 448 (M − 162), 286 (M − 162 × 2); FD-HR-MS *m/z* 611.1397 (calcd for $C_{30}H_{27}O_{14}$, 611.1400). For ^1H and ^{13}C NMR data, see **Table 1**.

RESULTS AND DISCUSSION

In the screening experiment, 73 and 40 samples showed more than 50% enzyme–inhibitory activity for maltase and sucrase, respectively, out of 509 samples from 218 plant species. Among them, notable inhibitory activity (>90%) against rat intestinal maltase was observed in *Cicuta virosa* (leaf, 97%), *Akebia trifoliate* (berry, 97%), *Punica granatum* (seed, 95%; fruit skin, 100%), *Quercus myrsinaefolia* (seed, 90%), *Wisteria floribunda* (leaf, 95%), *S. cantoniensis* (flower, 91%), *Paeonia suffruticosa* (flower, 96%; leaf, 98%), *Paeonia lactiflora* (fruit, 97%; leaf, 94%), *Rheum unduratum* (root, 92%), and *Lythrum salicaria*

(leaf, 90%), and activity against sucrase was seen for *Akebia trifoliate* (berry, 96%), *Trichosanthes rostrata* (stem, 91%), *Chaenomeles sinensis* (leaf, 93%; stem, 99%), *Elaegnus umbellata* var. *rotundifolia* (fruit, 97%), and *Lythrum salicaria* (leaf, 93%). Among these promising species, we first chose extracts of *S. cantoniensis* flower for identifying active principles, since only a limited number of studies (*12–14*) have been carried out concerning chemical constituents of this species.

Dried flowers of *S. cantoniensis* were extracted with 50% aqueous methanol. After evaporation, the crude extracts were partitioned between ethyl acetate and water. The maltase-inhibiting activity was found principally in the ethyl acetate-soluble part. The active part was chromatographed on silica gel followed by HPLC purification to yield three major compounds, **1**–**3**.

Compound **1** showed a molecular ion at *m/z* 626 in FD-MS and the molecular formula was determined as $C_{30}H_{26}O_{15}$ from the high resolution FAB-MS analysis. The characteristic mass spectral fragments at *m/z* 464 (M − 162) and 302 (M − 162 × 2), resulting from successive loss of the 162 mass unit, suggested **1** to be a quercetin caffeoylglycoside. The ^{1}H NMR spectrum strongly supported this indication. The aromatic proton region (δ 6.0–8.0) contained 10 protons assignable to H-6, -8, -2′, -5′, and -6′ of quercetin and H-2‴, -5‴, -6‴, -7‴, and -8‴ of caffeic acid. These assignments were supported by the COSY cross peaks of two 1,2,4-trisubstituted benzenes and an isolated *trans*-olefin. The proton signals of a sugar unit appeared at δ 3.6–5.6 and could be fully correlated by COSY. The small coupling constants of 3.3 Hz between H-3″ (δ 3.66) and H-4″ (δ 3.90) and 1.2 Hz between H-4″ and H-5″ (δ 3.81) strongly supported the presence of galactose. In the HMBC spectrum, interunit cross peaks between the anomeric proton of the sugar (H-1″, δ 5.22) and C-3 (δ 135.6) of the aglycon and between the nonequivalent methylene protons (H-6″, δ 4.21 and 4.26) and the ester carbonyl (C-9‴, δ 167.2) were observed, which showed that the galactose was connected to the C-3 of quercetin and that caffeic acid was esterified with 6-OH of the galactose. Thus, **1** was concluded to be quercetin 3-*O*-(6-*O*-caffeoyl)-β-D-galactoside.

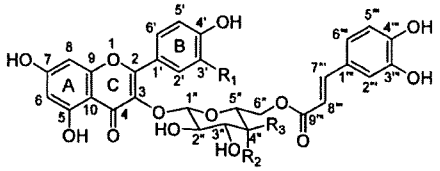
Compound **2** showed a pseudomolecular ion ($[M + H]^+$) at *m/z* 611 in FD-MS and the high-resolution analysis indicated the molecular formula of $C_{30}H_{26}O_{14}$. The mass spectral fragments at *m/z* 448 (M − 162) and 286 (M − 162 × 2) again indicated the presence of a caffeoylglycoside unit like **1**. The difference in molecular formula of **2** and **1** was one oxygen. Hence, **2** was suggested to be the kaempferol analog of **1**. The NMR spectrum of the aromatic region contained a pair of two-proton doublets (*J* = 9.0 Hz) at δ 6.84 and 8.05, being characteristic of a 4-hydroxyphenyl group in place of one of the 3,4-dihydroxyphenyls in **1**. The sugar unit was determined to be galactose in the similar manner as **1**, that is, *J* (H-3″/H-4″) = 3.3 Hz and *J* (H-4″/H-5″) = 1.5 Hz. The HMBC correlations confirmed the connectivity of each unit to be the same as that of **1**. The structure of **2** was determined to be kaempferol 3-*O*-(6-*O*-caffeoyl)-β-galactoside.

Compound **3** gave a similar spectral pattern to **2**, except for the sugar protons and carbons in the NMR spectra. Overlapping of H-2″, -3″, and -5″ at δ 3.44–3.47 and higher field resonance of H-4″ indicated the presence of glucose in **3** in place of galactose in **2** (*16, 17*). In addition, relatively higher chemical shifts of the sugar carbons also support the glucoside structure (*17, 18*). The structure of **3** was thus determined to be kaempferol 3-*O*-(6-*O*-caffeoyl)-β-glucoside.

Table 2. Inhibitory Activity of 1–3 and Related Compounds against Rat Intestinal Glucosidases

compd	IC_{50} (mM)	enzyme activity	ref
1	0.085	maltase	this work
2	0.35	maltase	this work
3	0.47	maltase	this work
4	(19%)[a]	maltase	(*29*)
5	(3%)[a]	maltase	(*29*)
6	(28%)[a]	maltase	(*29*)
7	(8%)[a]	maltase	(*29*)
8	0.029	sucrase	(*31*)
9	0.038	sucrase	(*31*)
10	1.89	maltase (immobilized)	(*3*)
11	1.91	maltase (immobilized)	(*3*)
12	18.9	maltase (immobilized)	(*3*)
13	0.024	maltase (immobilized)	(*3*)

[a] Percent inhibition at 0.5 mM.



1: R_1=R_2=OH, R_3=H
2: R_1=H, R_2=OH, R_3=H
3: R_1=R_2=H, R_3=OH

Figure 1. Structures of 1–3.

Quercetin 3-*O*-(6-*O*-caffeoyl)-β-galactoside (**1**) has been identified in several plants including *Hydrocotyle sibthorpioides* (*19*), *Scorzonera columnae* (*20*), *Polygonum viscosum* (*21*), *Blechnum novae-zelandiae* (*22*), *Monochaetum multiflorum* (*23*), and *Vaccinium corymbosum* (*24*), whereas kaempferol 3-*O*-(6-*O*-caffeoyl)-β-galactoside (**2**) was only isolated from *Conyza filaginoides* (*25*) and kaempferol 3-*O*-(6-*O*-caffeoyl)-β-glucoside from *Pteridium aquilinum* (*26*) and *Rubus ulmifolius* (*27*). However, this is the first report on the α-glucosidase-inhibitory activity of those compounds (**Table 2**). The similar extraction and fractionation of dried leaves of *S. cantoniensis* resulted in an isolation of **1** in 0.06% yield.

Among the isolates, the quercetin derivative **1** showed a higher activity (IC_{50} = 0.085 mM) than the kaempferol derivatives **2** (IC_{50} = 0.35 mM) and **3** (IC_{50} = 0.47 mM). The inhibition mode of **1** against rat intestinal maltase was determined to be mixed-inhibition type, as has been seen in other flavonoids (*29*) and the K_i value was calculated to be 110 μM. The moderate inhibitory activities of flavonols and their glycosides against mammalian intestinal glucosidases have been reported (*3, 30*). However, the maltase inhibitory activity (IC_{50} > 1 mM) of both quercetin and its glucoside was apparently lower than that of the caffeoylglycoside **1**. The substitution of the sugar moiety in flavonol glycosides by a phenolic acid, in particular, caffeic acid, could thus enhance their glucosidase inhibitory activity. In addition, the quercetin analogues having the caffeoyl substructure of C-2, -3, -4, and -1′–6′ in the B/C-rings of the flavonoid skeleton showed relatively higher glucosidase inhibitory activity than the corresponding kaempferol derivatives (*28, 29*). In the present case, the most potent compound, **1**, contains two caffeoyl substructures as the acyl substituent on the sugar and the B/C-rings of the aglycon, whereas **2** and **3** carry only one caffeoyl moiety. As shown in **Figures 1** and **2** and **Table 2**, the comparative study on the inhibitory activity of simple flavones and flavonols against rat intestinal glucosidase showed that luteolin (**4**) and quercetin (**6**) were more potent than apigenin (**5**) and kaempferol (**7**),

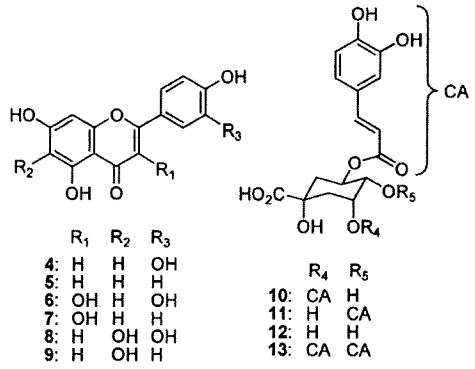


Figure 2. α-Glucosidase inhibitors having caffeoyl moieties.

respectively (*29*). This tendency was also seen in our structure–activity relationship study for baicalein (5,6,7-trihydroxyflavone) derivatives, in which 6-hydroxyluteolin (**8**) showed a higher activity than 6-hydroxyapigenin (**9**) (*31*). In any case, compounds possessing a caffeoyl substructure in the B/C ring of the flavone skeleton, **4**, **6**, and **8**, were more active than their *p*-coumaroyl counterparts, **5**, **7**, and **9**, respectively. In addition, in a series of caffeoylquinic acids, dicaffeoylquinic acids (**10**, **11**) showed a higher activity than monocaffeoyolquinic acid (chlorogenic acid, **12**), and a tricaffeoyl analogue (**13**) was much higher than those dicaffeoyl acids (*3*). These results strongly support the importance of a caffeoyl substructure in the molecule for exerting an effective glucosidase inhibitory activity. In contrast, the difference in sugar part, galactose and glucose, did not significantly affect the inhibitory activity.

In conclusion, the enzyme-assay guided fractionation of the extract from the dried flowers of *S. cantoniensis* led to the isolation of three flavonol caffeoylglycosides, **1**–**3**, as rat intestinal maltase inhibitors. The relatively high contents (0.03% each) of **1**–**3** in the plant compared to those in other plants (<0.002% *19*, *21*, *23*–*25*) suggest that *S. cantoniensis* could be physiologically useful for treatment of diabetes, although in vivo experiments are needed.

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Antileishmanial Compounds from *Cordia fragrantissima* Collected in Burma (Myanmar)

Kanami Mori,[†] Marii Kawano,[‡] Hiroyuki Fuchino,[§] Takashi Ooi,[⊥] Motoyoshi Satake,[∥] Yutaka Agatsuma,[∥] Takenori Kusumi,[⊥] and Setsuko Sekita*[,†]

Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University, Sanuki, Kagawa 769-2193, Japan, Faculty of Pharmaceutical Sciences, Josai International University, Togane, Chiba 283-8555, Japan, Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation, Tsukuba, Ibaragi 305-0843, Japan, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima 770-8505, Japan, and Institute of Environmental Science and Life, Ochanomizu University, Bunkyo, Tokyo 112-8610, Japan

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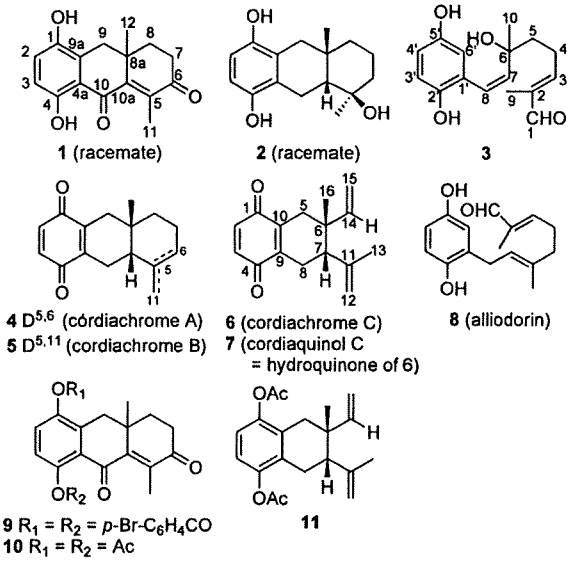
A methanol extract of the wood of *Cordia fragrantissima*, collected in Burma (Myanmar), was found to exhibit significant activity against *Leishmania major*. Bioassay-guided fractionation of this extract using several chromatographic techniques afforded three new compounds (**1–3**) and five known compounds (**4–8**). The structures of the new compounds were revealed on the basis of spectroscopic data interpretation and by X-ray crystallographic analysis. Interestingly, the new compounds, despite the presence of asymmetric carbons, were found to be racemates. The activities of the isolates from *C. fragrantissima* and several derivatives were evaluated against the promastigote forms of *Leishmania major*, *L. panamensis*, and *L. guyanensis*.

Leishmaniasis is a parasitic disease caused by protozoal species of the genus *Leishmania*, of which over 20 are known to be pathogenic to humans.[1] *Leishmania* spp. are all transmitted by small biting sandflies (*Phlebotomus* spp.). Twelve million people in 88 countries are afflicted by this disease.[2] *Leishmania major*, the causative agent of cutaneous leishmaniasis, is a digenetic parasite that exists as an extracellular promastigote within the insect vector, and as a nonmotile intracellular amastigote within the phagolysosome of macrophages and other cells of the reticuloendothelial system of the mammalian host.[3] Pentavalent antimonials are currently used for basic treatment of the disease, but these drugs are extremely toxic and generally expensive.[4] More economical and less toxic drugs seem to be necessary.

As part of a search for plant compounds that are active against *Leishmania* spp., it was found that a methanol extract of the wood of *Cordia fragrantissima* Kurz. (Boraginaceae), a plant from Burma (Myanmar), exhibited significant in vitro antileishmanial activity. This report deals with the isolation and structural determination of the active antileishmanial components of this plant and the activity of their derivatives of these naturally occurring compounds.



Results and Discussion

In a preliminary screening, a methanol extract of the wood of *C. fragrantissima* (local name "Sandawa"), used in Burma (Myanmar) for making furniture, was found to exhibit potent activity against *Leishmania major* [MLC (minimum lethal concentration): 25 μg/mL; MIC (minimum inhibitory concentration): 12.5 μg/mL].[5] Although secondary metabolites of other *Cordia* species such as *C. alliodora* and *C. multispicata* have been investigated previously,[6–12] the chemical composition of *C. fragrantissima* has not yet been reported.

Timber from *C. fragrantissima* was shaved, and the collected flakes were extracted with methanol. Bioassay-guided (in vitro leishmanicidal assay using *L. major*) fractionation of the methanol extract was performed by reversed-phase flash column chromatography, medium-pressure liquid chromatography (MPLC), and HPLC, yielding three new compounds, **1–3**, and five known compounds, cordiachromes A (**4**),[13,14] B (**5**),[13,14] and C (**6**),[11] cordiaquinol C (**7**),[8] and alliodorin (**8**).[7]

Compound **1** was obtained as a yellow, amorphous solid. Its molecular formula was established as $C_{16}H_{16}O_4$ by ESITOFMS (observed *m/z* 271.0952, calculated 271.0970, $[M - H]^-$). The 16 carbon signals present in the ^{13}C NMR spectrum were characterized by DEPT-90 and 135 spectra as two carbonyls [δ 195.8 (C-10), 199.6 (C-6)], two olefinic carbons [δ 138.8 (C-5), 150.1 (C-10a)], and four aromatic quaternary carbons [δ 117.3 (C-9a), 125.8 (C-4a), 144.8 (C-1), 157.6 (C-4)], as well as two aromatic methines [δ 116.1 (C-2), 125.2 (C-3)], three methylenes [δ 33.7 (C-7), 36.2 (C-8), 37.6 (C-9)], an sp^3-quaternary carbon [δ 37.8 (C-8a)], and two methyl carbons [δ 13.5 (C-11), 23.9 (C-12)]. The ^{13}C NMR chemical shifts of all hydrogenated carbons were assigned unambiguously using the HSQC spectrum. The ^1H NMR spectrum of **1** ($CDCl_3$) showed signals for two methyl groups at δ 1.25 (3H, s, H-12) and 2.12 (3H, s, H-11), two aromatic proton signals at δ 6.77 (1H, d, J = 9.0 Hz, H-3) and 7.01 (1H, d, J = 9.0 Hz, H-2), and four signals due to methylene protons at δ 2.08 (2H, t, J = 5.6 Hz, H-8), 2.61 (2H, t, J = 5.6 Hz, H-7), 2.82 (1H, d, J = 16.8 Hz, H-9b), and 3.06 (1H, d, J = 16.8 Hz, H-9a). Complete elucidation

* Corresponding author. Tel: +81-87-894-5111. Fax: +81-87-894-0181. E-mail: sekita@kph.bunri-u.ac.jp.
† Tokushima Bunri University.
‡ Josai International University.
§ National Institute of Biomedical Innovation.
⊥ The University of Tokushima Graduate School.
∥ Ochanomizu University.

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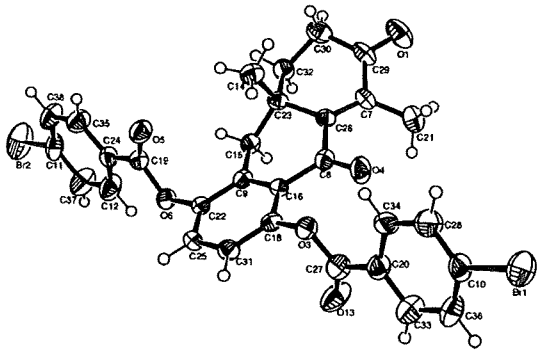

Figure 1. ORTEP drawing of **9**, the di-*p*-bromobenzoate derivative of **1**, as determined by X-ray analysis. Only one form of the racemate is shown.

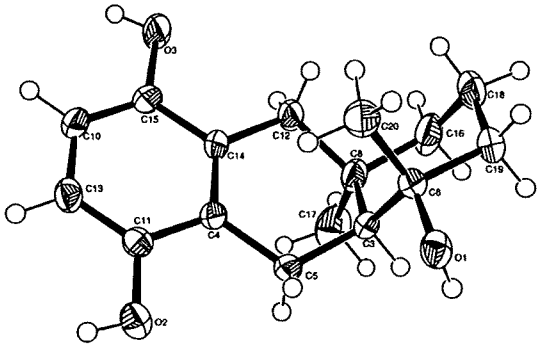

Figure 2. ORTEP drawing of **2** as determined by X-ray analysis. Only one form of the racemate is shown.

of the structure of **1**was achieved on the basis of HMBC correlations. $^{1}H/^{13}C$: Me-11/C-5, C-6, and C-10a; Me-12/C-8, C-9, and C-10a; H-7/C-8; H-8/C-7 and C-8a; H-9/C-1, C-4a, C-9a, and C-10a; H-2/C-1, C-4, and C-9a; H-3/C-4 and C-4a. In this way, a tricyclic structure including a hydroquinone moiety was established.

Compound **1** has an asymmetric carbon at position C-8a. In an attempt to elucidate its absolute configuration, **1** was converted to the di-*p*-bromobenzoate (**9**, obtained as yellow crystals) by addition of *p*-bromobenzoyl chloride in pyridine. An X-ray crystallographic analysis of **9** (Figure 1) confirmed the structure of **1**. Surprisingly, this work revealed that **9** exists as a racemate (space group $P\bar{1}$), which explained its optical sign: $[\alpha]_D$ −0.25 (*c* 0.13, $CHCl_3$). On acetylation under the usual conditions, compound **1** formed a diacetate derivative (**10**).

Compound **2**, $[\alpha]_D$ −0.84 (*c* 0.31, MeOH), was obtained as pale pink crystals. Its molecular formula, $C_{16}H_{22}O_3$, determined by ESITOFMS (observed *m/z* 285.1439, calculated 285.1467, $[M + Na]^+$), implied that this compound might be an analogue of **1**. This turned out to be the case; compound **2** was found to contain a *p*-hydroquinone moiety [^{13}C: δ 112.3 (d), 112.5 (d), 125.0 (s), 125.2 (s), 148.7 (s), 149.1 (s)] and two methyl groups [^{1}H: δ 0.92 (3H, s), 1.02 (3H, s)]. Although no carbonyls (^{13}C) were evident in **2**, a quaternary oxygenated carbon (^{13}C: δ 74.0) was present. The planar structure of **2** was elucidated by integrated analysis of the 1D and 2D NMR spectra. The following HMBC correlations were observed: Me-11 (δ 0.92)/C-5, C-6, and C-10a; Me-12 (δ 1.02)/C-8, C-9, C-8a, and C-10a; H-6 (δ 1.51)/C-5 and C-10a; H-7 (δ 1.69)/C-5 and C-8a; H-9 (δ 1.42)/C-12 and C-8a; H-8 (δ 2.27)/C-12, C-8a, and C-10a; H-10 (δ 3.18)/C-5, C-8a, and C-10a; H-10a (δ 1.65)/C-5, C-9, C-10, C-11, C-12, and C-8a; H-2 and H-3 (δ 6.47: overlapped)/C-1 and C-4, C-4a, and C-9a. The structure and relative configuration of **2** were eventually established by X-ray crystallography (Figure 2). Again the X-ray analysis indicated that compound **2** exists as a racemate (space group *Cc*), which is consistent with its small optical rotation value. The structures of compounds **1** and **2** are closely related to those of **4** and **5**, containing a benzocogeijerene skeleton (terpenoid benzoquinone), which have been isolated from other *Cordia* plants.[14] The present results represent the first examples of X-ray analysis of benzocogeijerene-type compounds. The monomethyl ether of **2** at the C-4 hydroxy group was obtained previously by acid treatment of a mushroom component.[15]

Compound **3**, $[\alpha]_D$ −1.80 (*c* 0.11, $CHCl_3$), $C_{16}H_{20}O_4$ (observed *m/z* 281.1151, calculated 281.1154, $[M + Na - H_2O]^+$) (ESITOFMS), was obtained as a pale yellow, amorphous solid. The ^{1}H NMR spectrum of **3**($CDCl_3$) showed signals similar to those of compounds **1** and **2**, with two aromatic proton signals at δ 6.53 (2H, overlapped), together with a new aromatic proton signal at δ 6.47 (1H, d, J = 2.0 Hz), suggesting the presence of a monosubstituted *p*-hydroquinone moiety. The presence of the *p*-hydroquinone moiety was evident from the carbon signals at δ 113.8 (d), 116.2 (d), 116.5 (d), 122.9 (s), 147.2 (s), and 150.9 (s), which confirmed that the *p*-hydroquinone group is monosubstituted.

Together with this moiety, the presence of the following groups was deduced from the NMR data (^{1}H; ^{13}C): an aldehyde [δ 9.30 (1H, s); δ 197.2 (d) (C-1)], an olefin [δ 6.61 (1H, tq, J = 7.8, 1.3 Hz); δ 140.3 (C-2) and 157.0 (C-3)] conjugated with the aldehyde, another olefin [δ 5.64 and 6.33 (AB pattern, J_{AB} = 10.0 Hz); δ 124.5 (C-8) and 131.0 (C-7)], a methylene [δ 2.51 (2H, q, J = 8.0 Hz); δ 25.2 (C-4)], another methylene [δ 1.48 (2H, t, J = 8.0 Hz); δ 40.3 (C-5)], two quaternary methyls [δ 1.36 (3H, s) and 1.64 (3H, d, J = 1.3 Hz); δ 9.0 (C-9) and 26.5 (C-10)], and a quaternary carbon bearing an oxygen (δ 78.9). COSY correlations between the proton signals at H-3 (δ 6.61), H-4 (2H, δ 2.51), and H-5 (2H, δ 1.48) and between H-7 (δ 5.64) and H-8 (δ 6.33) allowed partial elucidation of the structure. Complete elucidation of this structure was achieved by an HMBC experiment. $^{1}H/^{13}C$: H-1/C-2; H-3/C-9; H-4/C-3; H-5/C-4 and C-6; H-7/C-1′; H-8/C-6, C-2′, and C-6′; Me-9/C-2 and C-3; Me-10/C-6 and C-7; H-3′/C-5′; H-4′/C-5′. The structure of **3** is related to that of alliodorin (**8**). The small $[\alpha]_D$ value of **3** suggests that this compound may also exist as a racemate.

Of the known compounds isolated in the present investigation, **4**–**7** have asymmetric carbons. These compounds have been obtained previously from other *Cordia* species.[7,8,11,13,14] Since the $[\alpha]_D$ values of **4**–**7** (>99% pure by HPLC), which were obtained in this study, are all small (−0.26, −1.06, −1.06, and +0.02, respectively), they may be racemates, as in the case of compounds **1**–**3**. The $[\alpha]_D$ value reported for **6** is −1.11,[11] although the values of **4**, **5**, and **7** have not been reported.

The possible biosynthetic pathway[16] of the compounds obtained by this work is outlined in Figure S1, Supporting Information. Geranylhydroquinone (**a**) is oxidized to **b**, a precursor of **3** and **8**, the cyclization of which gives rise to a bicyclic diene (**c**). Cope rearrangement of **c** [**c**(**Cope**)] gives rise to a diene (**d**), which produces **6** and **7**. On the other hand, an ene-reaction of **c** [**c**(**Ene**)] would result in a tricyclic compound (**e**), a precursor of **1**, **2**, **4**, and **5**. The fact that an achiral precursor (**c**) is an intermediate in these reactions explains why compounds **1**, **2**, and **4**–**7** were obtained as racemates.

The activities of the new compounds (**1**–**3**) and the known compounds (**4**–**8**), together with some synthetic derivatives (**9**–**11**), were tested against the promastigote form of *Leishmania major*, *L. panamensis*, and *L. guyanensis*. All the tested samples exhibited leishmanicidal activity with IC_{50} values ranging from 1.4 to 81.4 μg/mL (Table 1). *Leishmania guyanensis* and *L. panamensis* seem to be more sensitive to these compounds than *L. major*, which is the easiest to handle (giving rapid and stable growth) and was used in the bioassay-guided isolation. In the *L. major* assay, **2**, **4**, **5**, **7**, **8**, and **11** showed good activity (1.4–7.0 μg/mL). The presence of a tricyclic framework or benzoquinone or hydroquinone functionality does not seem essential for leishmanicidal activity. No significant difference of the activities between natural (**1**–**8**) and synthetic

Table 1. In Vitro Leishmanicidal Activity and Cytotoxicity of Compounds **1–11**

	IC_{50} (μg/mL)			IC_{50} ± SD (μg/mL; μM)[d]	
compd	*L. major*	*L. guyanensis*	*L. panamensis*	COS-7	HuH-7
1	81.4	7.0	13.0	51.7 ± 0.8	71.8 ± 1.7
2	2.7	3.0	1.8	>100	41.7 ± 1.2
3	>25	NT[a]	NT	>100	>100
4	4.1	NT	NT	22.5 ± 0.3	61.1 ± 3.2
5	2.5	NT	NT	14.6 ± 0.3	71.5 ± 0.3
6	21.1	6.0	5.5	NT	NT
7	4.5	NT	NT	NT	NT
8	7.0	2.0	1.8	31.9 ± 7.2	44.8 ± 1.2
9	23.5	NT	NT	>100	>100
10	80.4	NT	NT	50.7 ± 0.7	65.5 ± 1.7
11	1.4	NT	NT	20.1 ± 0.3	60.9 ± 1.9
AmB[b]	<0.1	<0.1	<0.1		
MG132[c]				<10	<10

[a] NT = not tested. [b] AmB = amphotericin B. Positive control for antileishmanial assay. [c] Positive control for cytotoxicity assay. [d] Concentration in μg/mL for compounds **1–11** and AmB, and μM for MG132.

(**9–11**) compounds was found. The cytotoxicities of **1–5** and **8–11** were tested against COS-7 (African green monkey kidney cells, epithelial-like) and HuH-7 (human liver cancer cells, epithelial-like) cells (Table 1). None of these compounds was cytotoxic.

Experimental Section

General Experimental Procedures. Optical rotations were taken on a JASCO 1010 polarimeter. IR spectra were measured on a JASCO FT/IR-6300 spectrophotometer. UV spectra were taken on a JASCO International V-530 spectrophotometer. The 1D and 2D NMR spectra were obtained on Bruker AVANCE 400 MHz, 700 MHz, and Varian Unity INOVA 500 MHz spectrometers. ESITOFMS were measured on a JASCO International Q-TOF Micro mass spectrometer. For MPLC, reversed-phase material (Ultrapak, Yamazen Co., Ltd.) was used. ODS-flash column chromatography was carried out on Cosmosil C_{18} (Nakalai Tesque Co., Ltd.). For high-performance liquid chromatography (HPLC), columns of Shiseido Capcell pak C_{18} MG 5 μm, 20 × 250 mm, and C_{18} UG120 5 μm, 10 × 250 mm, Waters XTerra MS C_{18} 5 μm, 20 × 150 mm, Atlantis dC_{18} 5 μm, 20 × 100 mm, and Intact Unison UK-C_{18} 3 μm, 10 × 250 mm, and the HPLC system of JASCO Co., Ltd., were used. TLC was conducted on precoated silica gel 60 F_{254} (Merck) and/or RP-18 F_{254s} (Merck), and the spots were detected by heating after spraying with *p*-methoxybenzaldehyde–H_2SO_4 reagent.

Plant Material. The wood of *Cordia fragrantissima* was produced and kindly donated by the Ministry of Forestry of Myanmar in November 2004 and identified by Dr. Nyan Tun, taxonomist at the Institute of Forestry, Forest Department, Ministry of Forestry, Union Myanmar, where voucher specimens are deposited (accession number: 2.17-1b/cu. ft. 50).

Extraction and Isolation. The shaved timber of *C. fragrantissima* (850 g) was soaked in MeOH and extracted at 40 °C for 4 h three times. The MeOH extract was concentrated under reduced pressure to give a residue (70 g). The residue was treated with H_2O. The resultant aqueous suspension was partitioned with ethyl acetate. The EtOAc extract (64 g) was partitioned between hexane (18 g) and 90% MeOH (36 g). A part of the hexane extract (4.7 g) was subjected to silica gel column chromatography eluting with hexane–EtOAc (98:2 to 6:4) to give 10 fractions (Frs. 1–10). One third of fraction 4 (42 mg) was purified by recycle-HPLC [MeOH–distilled H_2O (95:5)] to afford cordiachrome A (**4**) (3 mg), $[\alpha]^{26}_D$ −0.26 (*c* 0.16, MeOH), cordiachrome B (**5**) (7 mg), $[\alpha]^{26}_D$ −1.06 (*c* 0.16, MeOH), and cordiachrome C (**6**) (5 mg), $[\alpha]^{26}_D$ −1.06 (*c* 0.19, MeOH) [lit. $[\alpha]^{23}_D$ −1.11 (*c* 0.27, $CHCl_3$)].[11] One third of the 90% MeOH extract (26 g) was subjected to ODS-flash column chromatography, eluting with MeOH–distilled H_2O (3:7 to 9:1), to give five fractions (Frs. 1-1–1-5). Fraction 1-2 (1.0 g) was applied to ODS-flash column chromatography with MeOH–distilled H_2O (1:1) to give five fractions (Frs. 2-1–2-5). One third of fraction 2-2 (100 mg) was purified by HPLC [MeOH–distilled H_2O (1:1)] to afford a new hydroquinone, cordiaquinol J (**2**, 3 mg). One third of the fraction 2-4 (55 mg) was purified by HPLC [MeOH–distilled H_2O (1:1)] to afford alliodorin (**8**) (3 mg). Fraction 2-5 (115 mg), mainly consisting of **8**, was also purified in a similar manner. Fraction 1-3 (2.1 g) was purified by MPLC with MeOH–distilled H_2O (7:3) to give three fractions (Frs. 3-1–3-3). Fraction 3-2 (467 mg) was purified by preparative HPLC with MeOH–distilled H_2O (1:1) to give eight fractions (Frs. 4-1–4-8). Fractions 4-4 and 4-6 constituted a new hydroquinone, cordiaquinol I (**1**, 60 mg), and cordiaquinol C (**7**) (648 mg), $[\alpha]^{27}_D$ +0.02 (*c* 0.19, MeOH), respectively, in pure states. One third of fraction 4-2 (11 mg) was purified by preparative HPLC with MeOH–distilled H_2O (6:4) to afford a new hydroquinone aldehyde, cordiaquinol K (**3**, 2 mg).

Cordiaquinol I (1): yellow, amorphous solid; $[\alpha]^{27}_D$ +0.82 (*c* 0.36, $CHCl_3$); IR ν_{max} (NaCl) 1683, 1670 cm^{-1}; UV (MeOH) λ_{max} (log ε) 294 (3.86) nm; ^1H NMR (400 MHz, $CDCl_3$) δ 12.13 (1H, s, OH-4, hydrogen-bonded with C=O-10), 7.01 (1H, d, *J* = 9.0 Hz, H-2), 6.77 (1H, d, *J* = 9.0 Hz, H-3), 3.06 (1H, d, *J* = 16.8 Hz, H-9a), 2.82 (1H, d, *J* = 16.8 Hz, H-9b), 2.61 (2H, t, *J* = 5.6 Hz, H-7), 2.12 (3H, s, Me-11), 2.08 (2H, t, *J* = 5.6 Hz, H-8), 1.25 (3H, s, Me-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.6 (C-6), 195.8 (C-10), 157.6 (C-4), 150.1 (C-10a), 144.8 (C-1), 138.8 (C-5), 125.8 (C-4a), 125.2 (C-3), 117.3 (C-9a), 116.1 (C-2), 37.8 (C-8a), 37.6 (C-9), 36.2 (C-8), 33.7 (C-7), 23.9 (C-12), 13.5 (C-11); HRESITOFMS (negative) *m/z* 271.0952 [M − H]$^-$ (calcd for $C_{16}H_{15}O_4$, 271.0970).

Cordiaquinol J (2): pale pink crystals; mp 205–208 °C; $[\alpha]^{26}_D$ −0.84 (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ε) 294 (4.02) nm; ^1H NMR (400 MHz, CD_3OD) δ 6.47 (2H, ABq, *J* = 9.2 Hz, H-2, H-3), 3.18 (1H, d, *J* = 19.1 Hz, H-10eq), 2.68 (1H, d, *J* = 17.6 Hz, H-9eq), 2.63 (1H, dd, *J* = 7.8, 19.1 Hz, H-10ax), 2.27 (1H, d, *J* = 19.1 Hz, H-9ax), 1.74 (1H, m, H-6), 1.69 (2H, m, H-7), 1.65 (1H, d, *J* = 7.8 Hz, H-10a), 1.62 (1H, m, H-8), 1.51 (1H, dt, *J* = 4.5 and 12.0 Hz, H-6), 1.42 (1H, dt, *J* = 5.2 and 12.6 Hz, H-8), 1.02 (3H, s, Me-12), 0.92 (3H, s, Me-11); ^{13}C NMR (100 MHz, CD_3OD) δ 149.1 (C-1), 148.7 (C-4), 125.2 (C-9a), 125.0 (C-4a), 112.5 (C-2), 112.3 (C-3), 74.0 (C-5), 50.1 (C-10a), 43.5 (C-6), 40.7 (C-8), 33.4 (C-8a), 32.6 (C-9), 31.6 (C-12), 22.8 (C-11), 21.7 (C-10), 21.2 (C-7); HRESITOFMS (positive) *m/z* 285.1439 [M + Na]$^+$ (calcd for $C_{16}H_{22}O_3Na$, 285.1467).

X-ray Crystallographic Analysis Data of 2. Crystal size, 0.30 × 0.30 × 0.10 mm; molecular formula, $C_{18}H_{28}O_4$; molecular formula moiety, $C_{16}H_{22}O_3$, C_2H_6O; crystal system, monoclinic; space group, *Cc*; unit cell dimensions (*a*, *b*, *c*), 11.474(3) Å, 13.935(3) Å, 10.813(2) Å; α = 90°, β = 104.93(2)°, γ = 90°, volume, 1670.5(7) Å^3; *Z* = 4; density, 1.226 mg m^{-3}; absorption coefficient, 0.085 mm^{-1}; *F*(000) = 672.0; diffractometer used, Rigaku RAXIS-RAPID; radiation (λ) Mo Kα (0.71073 Å); 2θ max 54.9°; reflections collected, 8225; independent reflections, 1911; observed reflections, 1612 [*R*(int) = 0.022]; final *R* indices, *R* = 0.0430 (obsd data), wR2 = 0.1080 (in-depth data); goodness of fit, 0.995; *T* = 173(1) K. The structure was solved by direct methods and refined by full matrix least-squares on F^2.[17]

Cordiaquinol K (3): dark yellow oil; $[\alpha]^{27}_D$ −1.80 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 264 (3.67) nm; ^1H NMR (400 MHz, $CDCl_3$) δ 9.30 (1H, s, H-1), 6.61 (1H, tq, *J* = 7.8, 1.3 Hz, H-3), 6.53 (2H, overlapped, H-3′and 4′), 6.47 (1H, d, *J* = 2.0 Hz, H-6′), 6.33 (1H, d, *J* = 10.0 Hz, H-8), 5.64 (1H, d, *J* = 10.0 Hz, H-7), 2.51 (2H, q, *J* = 8.0 Hz, H-4), 1.84 (2H, t, *J* = 8.0 Hz, H-5), 1.64 (3H, d, *J* = 1.3 Hz, Me-9), 1.36 (3H, s, Me-10); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.2 (C-1), 157.0 (C-3), 150.9 (C-5′), 147.2 (C-2′), 140.3 (C-2), 131.0 (C-7), 124.5 (C-8), 122.9 (C-1′), 116.5, 116.2 (C-3′, C-4′), 113.8 (C-6′), 78.9 (C-6), 40.3 (C-5), 26.5 (C-10), 25.2 (C-4), 9.0 (C-9); HRESITOFMS (positive) *m/z* 281.1151 [M + Na − H_2O]$^+$ (calcd for $C_{16}H_{18}O_3Na$, 285.1154).

1,4-*p*-Dibromobenzoylcordiaquinol I (9). To a solution of compound **1** (14 mg, 0.05 mmol) in pyridine (4.0 mL) was added *p*-bromobenzoyl chloride (58 mg, 0.26 mmol), and the reaction mixture was stirred at room temperature for 24 h. Workup as usual gave a residue, which was purified by silica gel column chromatography (hexane–EtOAc, 4:1) to give **9** (25 mg) as yellow crystals in hexane–CH_2Cl_2: mp 172–180 °C; $[\alpha]^{23}_D$ −0.25 (*c* 0.13, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.10 (4H, d, *J* = 8.4 Hz, benzoyl), 7.72, 7.69 (each 2H, d, *J* = 8.4 Hz, benzoyl), 7.46 (1H, d, *J* = 8.4 Hz, H-3), 7.21 (1H, d, *J* = 8.4 Hz H-2), 3.01 (1H, d, *J* = 16.8 Hz, H-9eq), 2.83 (1H, d, *J* = 16.8 Hz, H-9ax), 2.55 (2H, t, *J* = 5.6 Hz, H-7), 2.01 (2H, m, H-8), 1.94 (3H, s, Me-11), 1.28 (3H, s, Me-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.3 (C-6), 188.9 (C-10), 164.7 (benzoyl) 163.8 (benzoyl), 150.5 (C-10a), 148.1 (C-4), 146.4 (C-1), 137.5 (C-5), 134.7 (C-4a), 132.3 (benzoyl), 132.0 (benzoyl), 131.9 (benzoyl), 131.8

(benzoyl), 129.6 (benzoyl), 128.8 (benzoyl), 128.5 (benzoyl), 128.2 (C-3), 127.4 (benzoyl), 126.4 (C-9a), 123.1 (C-2), 37.6 (C-8a), 37.3 (C-9), 35.7 (C-8), 33.7 (C-7), 24.5 (C-12), 13.1 (C-11); HRESIMS (positive) *m/z* 660.9672 $[M + Na]^+$ (calcd for $C_{30}H_{22}O_6Na^{79}Br^{81}Br$, 660.9660).

X-ray Crystallographic Analysis Data of 9. Crystal size, 0.25 × 0.10 × 0.08 mm; molecular formula, $C_{30}H_{22}Br_2O_6$; crystal system, triclinic; space group, $P\bar{1}$; unit cell dimensions (*a*, *b*, *c*), 8.889(3) Å, 11.238(2) Å, 14.179(4) Å; α = 94.10(3)°, β = 100.838(5)°, γ = 109.55(3)°, volume, 1297.0(7) Å^3; *Z* = 2; density, 1.634 mg m^{-3}; absorption coefficient, 3.169 mm^{-1}; *F*(000) = 640.0; diffractometer used, Rigaku RAXIS-RAPID; radiation (λ) Mo Kα (0.71073 Å); 2θ max 55.0°; reflections collected, 12 761; independent reflections, 5919; observed reflections, 2778 [*R*(int) = 0.050]; final *R* indices, *R* = 0.0480 (obsd data), wR2 = 0.0730 (indept data); goodness of fit, 1.070; *T* = 173(1) K. The structure was solved by direct methods and refined by full matrix least-squares on F^2.[17]

Acetylcordiaquinol I (10). Compound **1** (8 mg, 0.03 mmol) was treated with pyridine (0.5 mL) and acetic anhydride (0.5 mL), and the mixture was allowed to stand overnight. The reagents were evaporated off, and the residue was purified by passage over an ODS cartridge (Waters Sep-Pak, C_{18}, MeOH−distilled H_2O), giving **10** (6 mg) as an oil; $[\alpha]^{23}_D$ −5.55 (*c* 0.18, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (1H, d, *J* = 8.7 Hz, H-2), 7.03 (1H, d, *J* = 8.7 Hz, H-3), 2.93 (1H, d, *J* = 16.5 Hz, H-9a), 2.78 (1H, d, *J* = 16.5 Hz, H-9b), 2.59 (2H, m, H-7), 2.41 (3H, s, OCO*CH*$_3$), 2.37 (3H, s, OCO*CH*$_3$), 2.05 (2H, m, H-8), 2.02 (3H, s, Me-11), 1.23 (3H, s, Me-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.3 (C-6), 189.1 (C-10), 168.7 (O*CO*CH$_3$), 168.1 (O*CO*CH$_3$), 150.6 (C-10a), 148.0 (C-4), 146.2 (C-1), 137.4 (C-5), 134.4 (C-4a), 128.1 (C-2), 126.0 (C-9a), 122.9 (C-3), 37.6 (C-9), 37.3 (C-8a), 35.7 (C-8), 33.7 (C-7), 24.3 (C-12), 21.1 (OCO*CH*$_3$), 20.8 (OCO*CH*$_3$), 13.1 (C-11); HRESIMS (positive) *m/z* 379.1133 $[M + Na]^+$ (calcd for $C_{20}H_{20}O_6Na$, 379.1158).

Acetylcordiaquinol C (11). Cordiaquinol C (**7**) (83 mg, 0.34 mmol) was treated with pyridine (2.0 mL) and acetic anhydride (2.0 mL), and the mixture was allowed to stand overnight. The reagents were evaporated off, and the residue was purified by passage over an ODS cartridge (Waters Sep-Pak, C_{18}, MeOH−distilled H_2O), giving **11** (58 mg); $[\alpha]^{23}_D$ −1.59 (*c* 0.73, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (2H, brs, H-2, H-3), 5.94 (1H, dd, *J* = 10.8 and 17.6 Hz, H-14), 4.98 (1H, d, *J* = 17.6 Hz, H-15a), 4.91 (1H, d, *J* = 10.8 Hz, H-15b), 4.89 (1H, s, H-12a), 4.78 (1H, s, H-12b), 2.66 (1H, d, *J* = 17.2, H-5β), 2.63 (2H, d, *J* = 6.8 Hz, H-8), 2.46 (1H, d, *J* = 17.2 Hz, H-5α), 2.45 (2H, m, H-7), 2.30 (3H, s, OCO*CH*$_3$), 2.29 (3H, s, OCO*CH*$_3$), 1.77 (3H, s, Me-13), 1.14 (3H, s, Me-16); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0 (O*CO*CH$_3$), 166.2 (O*CO*CH$_3$), 146.3 (C-4), 146.2 (C-1), 141.6 (C-14), 146.0 (C-11), 130.3 (C-9), 130.0 (C-10), 119.7 (C-15), 119.5 (C-12), 113.5, 113.2 (C-2, C-3), 49.8 (C-7), 37.7 (C-5), 37.7 (C-6), 27.2 (C-8), 26.0 (C-16), 23.1 (C-13), 20.7 (OCO*CH*$_3$), 20.7 (OCO*CH*$_3$); HRESITOFMS (positive) *m/z* 351.1563 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_4Na$, 351.1572).

Cultivation of *Leishmania* Promastigotes. Medium 199 was used for cultivation of promastigotes of *Leishmania major*, *L. guyanensis*, and *L. panamensis*. Promastigotes were cultured in medium [supplemented with heat-inactivated (56 °C for 30 min) fetal bovine serum (10%)] at 27 °C, in an atmosphere of 5% CO_2 in an incubator.

Leishmanicidal Assay. The leishmanicidal effects of the samples were assessed by an improved [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasodium bromide] (MTT) method as follows. Cultured promastigotes were seeded at 4 × 10^5 per 50 μL of medium per well in 96-well microplates, and then 50 μL samples of the test compounds at different concentrations, dissolved in a mixture of DMSO and medium, were added to each well. Each concentration was tested in triplicate. The microplate was incubated at 27 °C in 5% CO_2 for 48 h. Tetra Color One (10 μL) [a mixture of WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt) and 1-methoxy-PMS (1-methoxy-5-methylphenazinium methyl sulfate)] was added to each well, and the plates were incubated at 27 °C in 5% CO_2 for 6 h. Optical density values (test wavelength 450 nm, reference wavelength 630 nm) were measured using a Viento XS multispectrophotometer (Dainippon Pharmaceutical). Leishmanicidal activities were expressed as a minimum lethal concentration (MLC) and a minimum inhibitory concentration (MIC). The IC_{50} (50% inhibitory concentration) values for compounds were estimated from graphs. As positive control, amphotericin B was used.

Cytotoxicity Assay. Compounds were assayed for cytotoxicity against COS-7 and HuH-7 cells using the XTT method.[18-21] The IC_{50} is the concentration of agent that reduced cell growth by 50% under the experimental conditions. MG132 (carbobenzoxy-L-leucyl-L-leucyl-L-leucinal) was used as positive control.

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Supporting Information Available: Figure S1, showing the principal HMBC correlations observed in compounds **1–3**. Figure S2, showing a biogenetic pathway of compounds obtained in the present study. This material is available free of charge via the Internet at http://pubs.acs.org.

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A New Leishmanicidal Saponin from *Brunfelsia grandiflora*

Hiroyuki FUCHINO,*[,a] Setsuko SEKITA,[b] Kanami MORI,[b] Nobuo KAWAHARA,[c] Motoyoshi SATAKE,[d] and Fumiyuki KIUCHI[a]

[a] *Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation; 1–2 Hachimandai, Tsukuba, Ibaraki 305–0843, Japan:* [b] *Pharmaceutical Science at Kagawa Campus, Tokushima Bunri University; 1314–1 Shido, Sanuki, Kagawa 769–2193, Japan:* [c] *National Institute of Health Sciences; 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan: and* [d] *Institute of Environmental Science for Human Life, Ochanomizu University; 2–1–1 Otsuka, Bunkyo-ku, Tokyo 112–8610, Japan.* Received July 20, 2007; accepted October 16, 2007

A new furostan-type saponin (1) was isolated from the methanolic extract of *Brunfelsia grandiflora* leaves, together with four known compounds. The chemical structure of 1 was determined by spectroscopic analysis and chemical reaction to be 26-*O*-β-D-glucopyranosyl 22α-methoxyfurost-3β,26-diol 3-*O*-β-D-xylopyranosyl(1→3)-{β-D-glucopyranosyl(1→2)}-β-D-glucopyranosyl(1→4)-β-D-glucopyranoside. Compound 1 showed potent leishmanicidal activity *in vitro* against *Leishmania major*.

Key words *Leishmania*; *Brunfelsia grandiflora*; Solanaceae; chiricsanango; furostan

Brunfelsia grandiflora D. DON (Solanaceae), local name "chiricsanango", is widely cultivated in South America as an ornamental plant, and is also used in medicine. Its root is used against rheumatism and syphilis, and also has febrifuge activity. The leaf decoction is taken orally against arthritis and rheumatism in the Peruvian Amazon.[1] In the Amazon region, this plant is also used as an additive in the preparation of "ayahuasca". Although a few constituents of *B. grandiflora* have been reported,[2,3] the chemical details of this plant have not yet been investigated thoroughly.

Leishmaniasis is endemic in tropical regions, and currently affects 12 million people in 88 countries.[4] This disease is transmitted by small biting sandflies (*Phlebotomus* sp.). The first-line drugs for the treatment of leishmaniasis are pentavalent antimonials such as *N*-methylglucamine antimonate (Glucantime) and sodium stilbogluconate (Pentostam). However, these drugs are toxic and generally expensive.

In a screening of South American medicinal plants for leishmanicidal activity, we found that *B. grandiflora* showed potent activity. In this report, we describe the chemical constituents of *B. grandiflora* and their leishmanicidal activities.

Results and Discussion

A new furostan-type saponin, **1**, was isolated from the methanolic extract of *B. grandiflora* leaves, together with three known compounds: chlorogenic acid, scopoletin and 3-*O*-β-D-glucopyranosyl, 28-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl betulinate (cirensenoside P).

Compound **1** was determined to have the chemical formula $C_{57}H_{96}O_{28}$ by high-resolution FAB-MS. The IR spectrum revealed the presence of hydroxyl groups (3407 cm^{-1}). In the ^{13}C-NMR spectrum, 57 signals were observed, 26 of which were derived from oxygenated methines. Furthermore, five anomeric proton signals were observed in the ^1H-NMR spectrum at δ 4.83 (d, 7.8), 4.87 (d, 7.6), 5.17 (d, 7.8), 5.22 (d, 7.8), and 5.55 (d, 7.3). Thus **1** was thought to be a pentasaccharide. The ^{13}C- and ^1H-NMR spectra revealed the presence of a methoxyl group (δ_C=47.3 ppm, δ_H=3.23 ppm). Acid hydrolysis of **1** with 3% hydrochloric acid gave an aglycone (**1-a**) and a mixture of sugars, D-glucose and D-xylose. The ^{13}C-NMR data of **1-a** were identical to those of neotigogenin,[5] which has a spirostanol structure. Based on a comparison of the ^{13}C-NMR data of **1** and **1-a**, the genuine aglycone was deduced to be furostanol-type because of large differences in the chemical shifts from C-22 to 27, and this was confirmed by 2D-NMR (DQFCOSY, HMQC, and HMBC). Furthermore, enzymatic hydrolysis by β-glucosidase (from almond) gave **1-b**. Since **1-b** lacked 1 mol of glucose compared with **1** in the ^{13}C-NMR spectrum (Table 1), the latter molecule was surmised to possess a terminal glucose. A furostanol-type saponin bearing a sugar moiety at C-26 can release the sugar upon hydrolysis to form a spirostanol-type compound, and therefore a terminal glucose was determined to be located at C-26 by the correlation between C-26 (δ_C=74.9 ppm) and H′-1 (δ_H=4.82 ppm) in the HMBC spectrum. The anomeric configurations were deduced from the *J* values of the anomeric protons, and these values suggested the presence of four β-D-glucose units and one β-D-xylose unit. However, attempts to determine the glycosyl linkage of the sugar moiety at C-3 of **1** or **1-b** based on the 2D-NMR spectra did not succeed because of hard overlapping signals in key correlations. To determine the glycosyl linkage, the method reported by Jansson *et al.*[6] was applied. Compound **1** was converted to a permethylated compound (**1-c**) by Hakomori's method.[7] Compound **1-c** was subjected to acid hydrolysis and reduction with sodium borohydride followed by acetylation *in situ*, and the final reaction mixture was analyzed by GC-MS. In the GC-MS analysis, acetates of 2,3,4-trimethyl xylitol, 2,3,4,6-tetramethyl glucitol, 2,3,6-trimethyl glucitol, and 4,6-dimethyl glucitol were detected (see Experimental). Based on the results of GC-MS analysis, 1,4-disubstituted, 1,2,3-trisubstituted, and 1-monosubstituted glucoses and 1-monosubstituted xylose were deduced to be part of the sugar component of **1**. Atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) MS analysis of **1** and **1-b** using product ion scan experiments with various collision energies were examined. The results revealed the presence of a terminal xylose (Xyl-) (M-132), a terminal glucose (Glc-) (M-162), an inner glucose combined with a glucose, and a xylose [(Glc)Xyl-Glc-] (M-456) and an inner glucose combined with two glucoses and a xylose [Xyl-(Glc)Glc-Glc] (M-618), however, a terminal diglucosyl fragment (Glc-Glc-) (M-324) was not found in **1-b**. In addition, electron impact (EI)-MS analysis of acety-

* To whom correspondence should be addressed. e-mail: fuchino@nibio.go.jp

Table 1. ^{13}C- and ^{1}H-NMR Chemical Shifts for Sugar Moieties of **1** and **1-b** (Pyridine-d_5)

	1	**1-b**			**1**	**1-b**
	δ_C	δ_C	δ_H [a]		δ_C	δ_C
Glc				C-1	37.2	37.1
1	102.4	102.4	4.88 d (7.3)	2	29.9	29.8
2	73.2	73.2	4.41 t (7.8)	3	77.4	77.3
3	75.6	75.5	4.08—4.14	4	34.8	34.8
4	79.9	79.9	4.60—4.62	5	44.7	44.6
5	75.3	75.3	4.0—4.05	6	28.9	28.9
6	60.6	60.6	4.7 dd (16.1, 10.1)	7	32.1	32.4
			4.21—4.24	8	35.2	35.2
Glc′				9	54.4	54.3
1	105.1	105.2	5.2 d (7.8)	10	35.8	35.7
2	81.3	81.4	4.45 t (8.7)	11	21.2	21.2
3	86.7	86.6	4.18 t (8.7)	12	40.0	40.1
4	70.4	70.4	3.83 t (9.2)	13	41.0	40.7
5	77.7	77.7	3.87—3.90	14	56.3	56.4
6	62.9	63.0	4.01—4.05	15	32.4	32.1
			4.5—4.55	16	81.3	81.2
Glc″				17	64.3	62.8
1	104.8	104.8	5.58 d (7.4)	18	16.5	16.6
2	76.2	76.2	4.11 t (9.2)	19	12.3	12.3
3	78.6	78.6	4.03—4.10	20	40.5	42.4
4	71.0	70.9	4.21—4.24	21	16.3	14.9
5	78.7	78.7	3.90—3.93	22	112.6	109.7
6	62.4	62.4	4.6 dd (11.9, 2.7)	23	31.0	26.2
			4.36 dd (11.9, 5.9)	24	28.2	26.3
Xyl				25	34.4	27.5
1	104.9	104.9	5.24 d (7.8)	26	74.9	65
2	75.0	75.0	3.96 td (8.2, 3.7)	27	17.5	16.3
3	77.6	77.6	4.07—4.13	OCH_3	47.3	
4	70.7	70.7	4.05—4.13			
5	67.3	67.3	3.67 t (10.6)			
			4.21—4.24			
Glc‴						
1	105.0					
2	75.2					
3	78.6					
4	71.6					
5	78.5					
6	62.8					

All spectra recorded at 800 MHz. *a*) *J* values (in Hz) parentheses.

lated **1** showed no fragment 619, which was derived from a terminal diglucose heptaacetate [Glc(OAc)$_4$-Glc(OAc)$_3$-]. Based on this information, the glycosyl linkage position of **1-b** was determined by 1D-HOHAHA technique and 2D-NMR (800 MHz) (Fig. 1). The orientation of a methoxyl group at C-22 of **1** was determined to be α because the nuclear Overhauser effect (NOE) correlation between a methoxyl group and H-16 was observed. Thus the chemical structure of **1** was established as shown in Fig. 2. To our knowledge, this compound has not been reported previously.

The IC_{50} values of the leishmanicidal activities of **1** against *L. major*, *L. guyanensis*, and *L. panamensis* were 0.3, 5.5, and 8.0 μg/ml, respectively.

In conclusion, a new compound, **1**, was isolated together with three known compounds. **1** was found to show leishmanicidal activity against *L. major*, *L. guyanensis*, and *L. panamensis*, and was especially potent against *L. major*. Compound **1** possesses a methoxyl group at C-22, which was thought to be inserted during extraction with methanol.

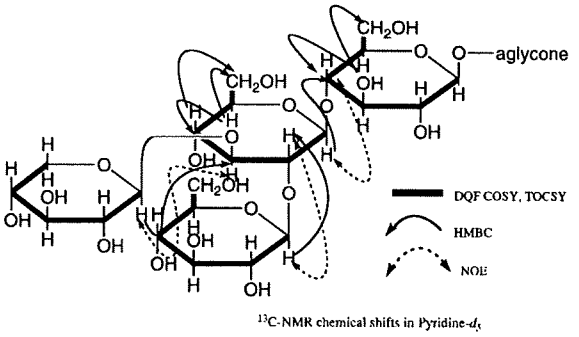

Fig. 1. DQF COSY, TOCSY, HMBC and NOESY Correlations for the Sugar Moiety of **1-b**

Experimental

Plant Material *Brunfelsia grandiflora* material was collected in the Peruvian Amazon region and identified by Dr. Elsa Rengifo (The Peruvian Research Institute of the Amazon, Iquitos, Peru). A voucher specimen (No. P07-01) is on file at the Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation (1–2 Hachimandai, Tsukuba, Ibaraki 305–0843, Japan).

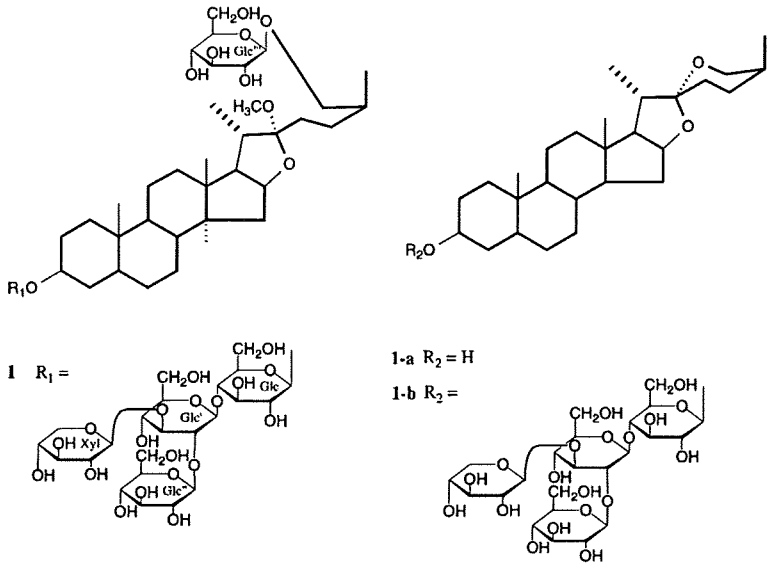

Fig. 2. Chemical Structures of **1**, **1-a** and **1-b**

General Experimental Procedures Melting points were determined by Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-370 automatic polarimeter. ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL Alpha 500 spectrometer (500 MHz) and JEOL ECA 800 (800 MHz). IR spectra were recorded on a JASCO FTIR-5300 spectrometer. GC-MS and LC-MS were performed with a SHIMADZU QP 5050A and an Applied Biosystems QSTAR XL with an APCI, and ESI ion source apparatus, respectively. FAB-MS was measured by JEOL HX110 spectrometer. HPLC was run on Shimadzu LC-10VP system with JASCO OR-2090 Plus chiral detector.

Extraction and Isolation The methanolic extract of *B. grandiflora* leaves was prepared in Peru by Dr. Victor Zorrilla (Institute of Tropical Medicine, Lima, Peru). The concentrated extract (367 g) was dissolved in methanol and the solution was passed through a column of activated charcoal (70 g). Methanol (10 l), 30% chloroform/methanol (10 l), and chloroform were then each used as an eluent to give 3 Fractions (FR. A—B). Each fraction was concentrated *in vacuo* to give a syrup. The fraction eluted with methanol (FR. A) was subjected to column chromatography on silica gel (developing solvent: gradient with chloroform–methanol system) to afford 20 Fractions (FR. 1—20). Fractions eluted with 30—40% methanol/chloroform (FR. 7—9) were combined and evaporated under reduced pressure, and the residue was crystallized from methanol to give ursolic acid (670 mg). Fractions eluted with 60—70% methanol/chloroform (FR. 12—16) were combined and concentrated then partitioned between *n*-buthanol and water. The *n*-buthanol layer was concentrated *in vacuo* and the residue was chromatographed on Sephadex LH-20 with 80% methanol/water as an eluent to give 83 fractions (FR. 1′—83′). FR. 67′—83′ were combined and concentrated to yield chlorogenic acid (565 mg). FR. 13′—33′ were combined and rechromatographed on silica gel with a mixture of chloroform–methanol–water (100 : 20 : 1) as an eluent to afford 294 fractions (FR. 1″—294″). FR. 148″—177″ were combined and the concentrated residue was subjected to HPLC (CapcelPak C-18, Shiseido, developing solvent: methanol/water (=3 : 1—4 : 1)) to afford cirensenoside P (12 mg). FR. 208″—242″ were combined and crystallized with methanol to yield **1** (1.08 g) as colorless fine needles. FR. B (eluted with 30% methanol/chloroform) was subjected to column chromatography on silica gel with a mixture of methanol and chloroform as an eluent then crystallized with chloroform–methanol to afford scopoletin (145 mg). **1**: colorless fine needles. mp 209—205 °C $[\alpha]_D^{23}$ −44.6° (c=1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.59 (3H, s, 19-CH_3), 0.76 (3H, s, 18-CH_3), 1.02 (3H, d, J=6.9 Hz, 27-CH_3), 1.14 (3H, d, J=6.9 Hz, 21-CH_3), 3.23 (3H, s, 22-OCH_3), 4.83 (1H, d, J=7.8 Hz, 26-Glc-1), 4.87 (1H, d, J=7.9 Hz, Glc-1), 5.17 (1H, d, J=7.3 Hz, Glc′-1), 5.22 (1H, d, J=7.8 Hz, Xyl-1), 5.55 (1H, d, J=7.4 Hz, Glc″-1). IR (KBr) cm^{-1}: 3407, 2927, 1654, 1451, 1378, 1073. HR-FAB-MS (positive mode) *m/z*: 1229.6195 $[M+H]^+$ (Calcd for $C_{57}H_{97}O_{28}$: 1229.6166). FAB-MS (positive mode) *m/z*: 1251 $[M+Na]^+$. APCI-TOF-MS (negative mode): *m/z* 1227 $[M-H]^-$, 1095 $[M-Xyl]^-$, 1065 $[M-Glc]^-$, 933 $[M-Glc-Xyl]^-$, 771 $[M-2Glc-Xyl]^-$, 609 $[M-3Glc-Xyl]^-$.

Acid Hydrolysis of 1 Compound **1** (32 mg) was dissolved in 3% hydrochloric acid (12 ml) and the mixture was refluxed for 4 h. After cooling, the mixture was poured into ice water, and the precipitate was filtered to give neotigogenin (10 mg).[5] The filtrate was evaporated *in vacuo*, then a half residue was analyzed by HPLC with chiral detector [column: high-performance carbohydrate column (Waters), solvent system: 80% acetonitrile/water, flow rate: 1.0 ml/min, Peak A: 5.0 min(+), Peak B: 4.5 min(+), D-glucose standard: 5.0 min(+), D-xylose standard: 4.5 min(+)]. A trimethylsilylating reagent (TMS-HT, Tokyo Kasei Kogyo Co., Ltd.) was added to another residue and the supernatant solution was analyzed by GC-MS [column: ZB-1701 capillary column (I.D. 0.25 mm×30 m) (Zebron), column temperature program: initial temperature 100 °C (0 to 5 min), rising from 100 to 245 °C (5 to 65 min)]. In GC-MS analysis, 4 peaks (17.5, 18.5, 20.7, 22.3 min) were detected. They were identified to be a trimethylsilylated glucose (TMSGlc) and a trimethylsilylated xylose (TMSXyl) by comparison of retention times and MS fragment patterns with standard samples (TMSGlc: 20.7, 22.3 min, TMSXyl: 17.5, 18.4 min).

Enzymatic Hydrolysis of 1 Compound **1** (30 mg) was suspended in citrate buffer solution (pH 4.25) (10 ml), β-glucosidase (from almond, Oriental Yeast Co., Ltd.) (30 mg) was added, and the mixture was stirred at 30 °C for 3 d. The mixture was poured into water, extracted with *n*-buthanol, and evaporated to afford **1-b** (10 mg). **1-b**: colorless amorphous powder. $[\alpha]_D$ −37.7° (c=0.19, MeOH). HR-FAB-MS (positive mode) *m/z*: 1035.5347 $[M+H]^+$ (Calcd for $C_{50}H_{83}O_{22}$ 1035.5376). FAB-MS (positive mode) *m/z*: 1058 $[M+Na]^+$. ESI-TOF-MS (negative mode) *m/z*: 1033 $[M-H]^-$, 901 $[M-Xyl]^-$, 871 $[M-Glc]^-$, 739 $[M-Glc-Xyl]^-$, 577 $[M-2Glc-Xyl]^-$, 415 $[M-3Glc-Xyl]^-$.

Permethylation, Acid Hydrolysis, Reduction and Acetylation of 1[8] A solution of sodium hydride (60% oil suspended) (100 mg) in dimethyl sulfoxide (dried with molecular sieves 4A) (7.5 ml) was heated at 65 °C with stirring for 60 min, and a solution of **1** (50 mg) in dimethyl sulfoxide (2.5 ml) was then added and the mixture was stirred for 1 h. Methyl iodide (0.75 ml) was added and the mixture was stirred at room temperature for 12 h. After the reaction, the mixture was poured into water and extracted twice with chloroform, and the organic layer was washed with water and evaporated *in vacuo*. The residue was subjected to preparative thin-layer chromatography (TLC) (development solvent system; chloroform/methanol (=20 : 1)) to give a permethylated compound (**1-c**). **1-c**: ^{1}H-NMR ($CDCl_3$) δ: 3.35, 3.37, 3.40, 3.44, 3.44, 3.48, 3.51, 3.52, 3.53, 3.56, 3.56, 3.58, 3.60, 3.61, 3.62, 3.62, 3.62 (each 3H, s), 4.19 (1H, d, J=7.3 Hz), 4.30 (1H, d, J=7.8 Hz), 4.70 (1H, d, J=7.3 Hz), 4.93 (1H, d, J=7.3 Hz), 4.99 (1H, d, J=7.8 Hz). **1-c** was dissolved in 0.5 N-sulfuric acid in 90% acetic acid (10 ml), and the solution was stirred at room temperature for 30 min then at 77 °C for 5 h. After being cooled, the reaction mixture was neutralized with silver carbonate and filtered. The filtrate was freeze-dried to give a residue. The residue was dissolved in water (5 ml) and sodium borohydride was added until halting foaming. The solution was stirred at room temperature for 2 h and then a few drops of acetic acid were added to stop the reaction. Methanol was added

and the solution was concentrated under reduced pressure to afford a residue. Acetic anhydride (2 ml) and pyridine (2 ml) were added to the residue and the mixture was heated at 70 °C for 2 h. After being cooled, water was added to the reaction mixture and the solution was stirred at room temperature for 30 min, extracted with chloroform, and evaporated *in vacuo* without heating to give a mixture of partially methylated alditol acetates. The mixture was analyzed by GC-MS. GC-MS analysis was performed with a DB-225 capillary column (I.D. 0.25 mm×30 m) (Agilent Technologies Co., Ltd.) at a column temperature of 170 °C. The relative retention times and mass fragmentation patterns of detected peaks were compared with the values in the literature.[6] The following peaks were detected and their mass fragmentation patterns were identical to those reported previously: 4,6-dimethylglucitol acetate (retention time; 3.29 min), 2,3,6-trimethylglucitol acetate (2.09 min), 2,3,4,6-tetramethylglucitol acetate (1.0 min), and 2,3,4-trimethylxylitol acetate (0.61 min).

Leishmanicidal Activity Assay Cultivation of *Leishmania* promastigotes and leishmanicidal activity assay were carried out as we previously described.[9] Amphotericin B was used as a positive control (IC_{50} 0.04 μg/ml). The moderate leishmanicidal activities for ursolic acid,[10] chlorogenic acid,[11] and scopoletin[12] have been reported in the literature. Activity for cirensenoside P was not carried out due to amount shortage.

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