Jacaranone-Derived Glucosidic Esters from *Jacaranda glabra* and Their Activity against *Plasmodium falciparum*

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In a survey of plants from Ecuador with antiprotozoal activity, *Jacaranda glabra* was found to show promising activity against the *Plasmodium falciparum* K1 strain. Subsequently, activity-guided isolation of the dichloromethane extract from the leaves of *J. glabra* afforded four new phenylethanoid glucosides containing jacaranone-type moieties (**1**−**4**), named jacaglabrosides A−D. Their chemical structures were identified using NMR spectroscopy and MS techniques. The compounds were found to be active in vitro against the *P. falciparum* K1 strain (IC_{50} **1**, 1.02; **2**, 0.56; **3**, 0.56; and **4**, 0.55 μg/mL) and generally possessed a low cytotoxicity toward L-6 cells, with the exception of compound **1** (IC_{50} **1**, 8.3; **2**, >90; **3**, 87; and **4**, 85 μg/mL).

The genus *Jacaranda* (Bignoniaceae) consists of mainly trees, which are native to Central and South America and the Caribbean region.[1] *Jacaranda* species are recognized around the world for the beauty of their flowers as well as for their use in the paper industry.[2,3] Recently, ethnobotanical and pharmacological uses of *Jacaranda* species have been reported, emphasizing skin ailments and protozoal-related diseases.[4]

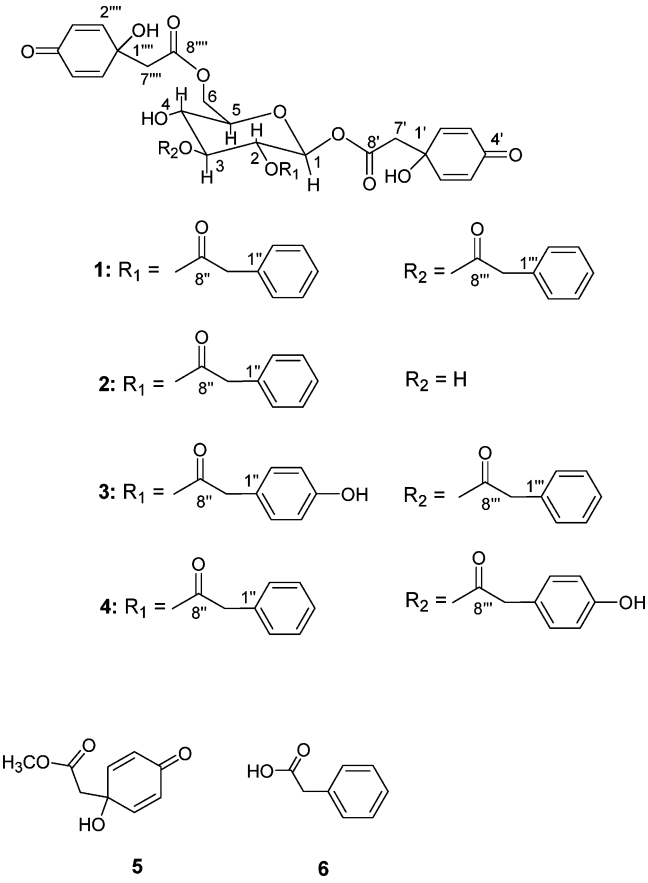
Jacaranda glabra (DC.) Bureau & Schumann is a small tree distributed in Colombia, Ecuador, Peru, Bolivia, and Brazil, growing at low altitudes (200−1100 m).[1] The leaves of *J. glabra* are used in traditional folk medicine to treat cutaneous leishmaniasis and skin illnesses.[5,6] The secondary metabolites of *J. glabra* are as yet unknown; however, the constituents that have been identified in the genus include acteoside derivatives, fatty acids, flavonoids, a neolignan, phenylethanoid glycosides, quinones, and triterpenes.[7−20] Among these chemical constituents, the benzoquinone derivative jacaranone has been described from several *Jacaranda* species.[7,11,12]

In an ongoing current project on the evaluation of antiprotozoal activity of plants traditionally used for medicine in Ecuador, the dichloromethane extract of the leaves of *J. glabra* showed promising activity against the *Plasmodium falciparum* K1 strain. Antiplasmodial activity has been previously reported from the methanolic extract of the leaves of *Jacaranda caucana*,[21] from which eight compounds have been identified.[20] The present article describes the bioassay-guided isolation, structure elucidation, and antiprotozoal evaluation of four new glucosidic esters with phenylacetic and benzoquinoid moieties (**1**−**4**) from *J. glabra*.

Results and Discussion

The dichloromethane extract of the leaves of *J. glabra* was subjected to several chromatographic separation steps, which led finally to the isolation of four new glucosidic ester derivatives (**1**−**4**), along with their degradation products **5** and **6**. Combined NMR and MS analysis allowed identification of these compounds.

Jacaglabroside A (**1**) was isolated as pale yellow oil. The positive ESIMS of **1** showed a molecular ion peak at *m/z* 717.01 corresponding to $[M + H]^+$, with the ion at *m/z* 734.2462 $[M + NH_4]^+$



5 **6**

and the monosodiated ion at *m/z* 739.2025 $[M + Na]^+$, suggesting a molecular formula of $C_{38}H_{36}O_{14}$ (calcd *m/z* 716.217). The ^1H and HSQC spectra indicated the presence of a highly substituted saccharide unit, and the triplet couplings of the proton resonances of H-2, H-3, and H-4, in combination with large three-bond homonuclear coupling constants, were consistent with the presence of glucopyranose in the β-form. The sign of optical rotation of the hydrolyzed sugar demonstrated that the glucose belongs to the D series. Two substituents were identified as phenylacetate moieties, while the other units were found to be jacaranone acid moieties. HMBC correlations between H-1 and C-8′, H-2 and C-8″, H-3 and C-8‴, and H-6 and C-8⁗ led to the structural assignment of

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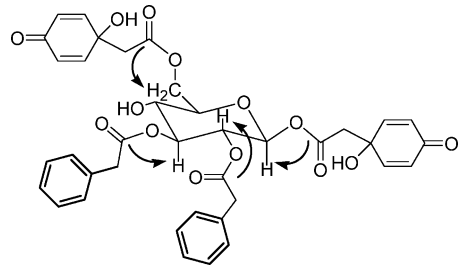

Figure 1. Relevant HMBC correlations of compound **1**.

jacaglabroside A (**1**) (Figure 1). The different shift values for the chemically equivalent positions in the quinoid moietiy and the benzylic moieties attached to C-1, C-2, and C-3 of the saccharide unit, respectively, indicated a hindered rotation of these substituents. The ^{1}H and ^{13}C NMR data for compound **1** data are presented in Table 1. The representative fragment ion at *m/z* 548.94 $[M - C_8H_7O_4 + H]^+$ pointed to the loss of one jacaranone acid moiety.

Compound **2** showed a molecular ion peak of *m/z* 599.1767 $[M + H]^+$ by positive-mode ESIMS, indicating a molecular formula of $C_{30}H_{30}O_{13}$ (calcd *m/z* 598.1679). Other representative ions were at *m/z* 616.2028 $[M + NH_4]^+$ and *m/z* 431.00 $[M - C_8H_7O_4 + H]^+$. The ^{1}H, ^{13}C, and HSQC NMR spectra shared the same characteristic data as for compound **1**, i.e., β-glucopyranose substituted by one phenylacetate and two jacaranone acid moieties. HMBC correlations between H-1 and C-8′, H-2 and C-8″, and H-6 and C-8‴ assisted in the structure determination of **2** (jacaglabroside B) as shown. (^{1}H and ^{13}C NMR chemical shifts are shown in Table 1).

ESIMS (positive mode) of **3** and **4** yielded molecular ion peaks at *m/z* 733.2148 and 733.2157 $[M + H]^+$, respectively, and very similar fragmentation patterns (e.g., *m/z* 750.2424 and 750.2392 $[M + NH_4]^+$, 564.97 and 565.02 $[M - C_8H_7O_4 + H]^+$, respectively), which confirmed the molecular formula, $C_{38}H_{36}O_{15}$ (calcd 732.2047). For both compounds the ^{1}H, ^{13}C, and HSQC NMR spectra of these isomers showed the same characteristic pattern described for compound **1**, which included two jacaranone acid and phenylacetate substituents (i.e., the observed three-bond HMBC correlations for positions H-1, H-2, H-3, and H-6). In contrast to compound **1**, one of the two phenylacetate moieties in **3** and **4** showed the presence of a hydroxy group in the *para*-position, as indicated by the carbon shifts at C-4″, C-3/5″, and C-1″ in compound **3** and at C-4‴, C-3/5‴, and C-1‴ in compound **4**, respectively. Thus the structures of the two compounds, jacaglabrosides C (**3**) and D (**4**), were assigned as shown. The ^{1}H and ^{13}C NMR chemical shifts are given in Table 1.

According to observations made in the present study, the four isolated quinoid glucosidic esters (**1**−**4**) were found to be unstable in MeOH to different degrees. This phenomenon became evident during the NMR analysis of compounds **2** and **4** in CD_3OD, the first solvent chosen. During the measurement of compound **2** using 2D NMR experiments, two compounds could be observed forming a mixture within a few hours. These degradation products corresponded to compound **2** after the loss of a jacaranone acid moiety at the anomeric carbon of the glucose, which was confirmed by MS data (*m/z* 448.98 $[M + H]^+$), resulting in a mixture of epimeric compounds. The free jacaranone acid was also identified in the NMR spectra. However, the loss of this moiety did not alter the antiplasmodial activity (see below), as demonstrated by the fact that the IC_{50} values of the artifacts were comparable to that for compound **2** (data not shown). Compound **4** was apparently even less stable, as shown by the recognition of several artifacts in both the NMR spectra and HPLC chromatograms, which made the elucidation difficult in CD_3OD. However, the main two artifacts of compound **4** showed a molecular mass of *m/z* 564.97 $[M + H]^+$, which suggested the loss of one jacaranone acid ($C_8H_7O_4$) and a molecular formula of $C_{30}H_{29}O_{11}$. LC-ESIMS and HPLC-DAD analysis of the isolated compounds from a fresh plant extract confirmed that free jacaranone (**5**), phenylacetic acid (**6**), and the artifacts did not occur in the original CH_2Cl_2 extract of *J. glabra*.

Similar chemical structures to those of compounds **1**−**4** have been identified from *Forsythia suspensa* (Oleaceae; forsythensides A and B)[20] and *Senecio scandens* (Compositae; mixtures of C-1 epimeric glucose derivatives).[21] Both investigators reported instability problems during the structure elucidation process, which could be attributed to the use of MeOH during isolation and for the NMR measurements. Therefore, it could be speculated that these reported compounds are actually degradation products of more highly substituted parent compounds, as described in the present work.

Data on the in vitro antiplasmodial activity and cytotoxicity against L-6 cells for compounds **1**−**6** are presented in Table 2. Compounds **2**−**4** showed similar IC_{50} values in both assays (moderate antiplasmodial activity and low cytotoxicity). In contrast, the IC_{50} value of **1** against L-6 cells was found to be approximately 1 order of magnitude lower than for **2**−**4**. Regarding jacaranone (**5**), the results were in agreement with known reports on unselective toxicity.[11,12,22,23] Phenylacetic acid (**6**) was not found to be active in either in vitro assay.

Recently, Martin et al. described phenylethanoid glycosides from *J. caucana*,[24] which were reported to have activity against *P. falciparum*.[25] These compounds share certain characteristics with those described in this paper, including the presence of β-glucose and of moieties structurally similar to jacaranone, namely, cyclohexanone and cyclohexanol moieties, which are attached to the central saccharide unit. It would be worthwhile, therefore, to determine the antiplasmodial potential of these compounds isolated from *J. caucana*.[24]

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH on a Perkin-Elmer polarimeter 341. UV−vis spectra were recorded on a UV-160A spectrophotometer (Shimadzu). IR spectra were taken as KBr pellets on a Perkin-Elmer 281 spectrophotometer. All 1D (^{1}H and ^{13}C) and 2D (COSY, HMBC, and HSQC) NMR spectra were recorded at 298 K on a Varian UNITY INOVA-600 MHz spectrometer using $(CD_3)_2CO$ as solvent with TMS as internal standard. EIMS were recorded on a Hewlett-Packard HP 7890 instrument fitted with a HP 7890 detector. ESIMS were measured in the positive mode on a Thermo Finnigan LQ Deca XP^{PLUS} mass spectrometer with autosampler using a SB-C18 Zorbax column (3.5 μm; 150 × 2.1 mm; Agilent Technologies), with a guard column, at a flow rate of 300 μL/min using an acetonitrile gradient in water. High-resolution ESIMS spectra were recorded on a MALDI Synapt HDMS system (Waters, Milford, MA) in positive ion time-of-flight mode using a LockSpray dual electrospray ion source. Leu-enkephalin was used for lock-mass correction.

Semipreparative and analytical HPLC separations were performed using an Agilent 1100 series instrument equipped with a diode-array detector. Compound mixtures were separated on a LiChrosorb RP-18 (7 μm, 250 × 10 mm, Merck, Darmstadt) HPLC preparative column. Analytical HPLC-DAD analysis was performed using a SB-C18 Zorbax column (3.5 μm; 150 × 2.1 mm; Agilent Technologies), equipped with a guard column, at a flow rate of 300 μL/min and a gradient elution program. Fractionation was performed on a fast centrifugal partition chromatograph (FCPC) with a rotor of 200 mL capacity, a 0.025−25 mL/min high-pressure pump, and an injection loop of 10 mL, coupled to a UV−vis detector with a flow cell and a fraction collector (FCPC-Kromaton-201, AlphaChrom OHG). For TLC analysis, precoated Si60 F254 plates (Merck) were used. Detection was performed under UV light at 254 and 366 nm, with visualization by spraying with vanillin-sulfuric acid reagent and heating.

Plant Material. Leaves of *Jacaranda glabra* were collected in August 2006 at the Estación Científica Jatun Sacha in the Province of Napo in the Ecuadorian Amazon. The plant was identified by Carlos Morales Cabrera, and a voucher (No. 227457) has been deposited at the Herbario Nacional del Ecuador in Quito.

***Plasmodium falciparum* in Vitro Assay.** Antiplasmodial activity was determined using the K1 strain of *P. falciparum* (resistant to

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**−**4** (600 and 150 MHz, in $(CD_3)_2CO$)

	1		**2**		**3**		**4**	
	δ_H mult. (*J*, Hz)	δ_C	δ_H mult. (*J*, Hz)	δ_C	δ_H mult. (*J*, Hz)	δ_C	δ_H mult. (*J*, Hz)	δ_C
β-glucose								
1	5.82 d (8.4)	92.6 d	5.67 d (8.4)	93.0 d	5.81 d (8.4)	92.7 d	5.81 d (8.4)	92.7 d
2	4.98 t (9.0)	71.5 d	4.90 t (9.0)	73.6 d	4.97 t (8.4)	71.3 d	4.97 t (8.4)	71.5 t
3	5.28 t (9.0)	75.8 d	3.74 m	75.3 d	5.27 t (9.0)	75.8 d	5.26 t (9.6)	75.7 d
4	3.76 t (9.6)	69.0 d	3.51 t (9.0)	71.1 d	3.75 s	69.0 d	3.74 t (9.6)	69.0 d
5	3.89 m	75.5 d	3.70 m	75.7 d	3.89 m	75.5 d	3.89 m	75.6 d
6	a 4.25 dd (5.4, 11.4)	63.8 t	a 4.19 dd (5.4, 10.8)	64.2 t	a 4.25 dd (5.4, 12.0)	63.8 t	a 4.24 dd (5.4, 12.0)	63.9 t
	b 4.42 d (11.4)		b 4.43 d (10.8)		b 4.42 d (12.0)		b 4.42 d (12.0)	
	jacaranone acid							
1′		67.6 s		67.7 s		67.5 s		67.6 s
2′[a]	7.03 d (10.2)	150.9 d	6.99 m	150.9 d	7.02 m	150.8 d	7.02 m	151.0 d
3/5′	6.09 m	128.3 d	6.08 d (10.8)	128.3 d	6.08 d (11.4)	128.1 d	6.08 m	128.3 d[a]
4′		185.5 s		185.5 s		185.5 s		185.5 s
6′[a]	7.01 d (10.2)	150.8 d	6.99 m	151.0 d	7.02 m	150.8 d	7.02 m	150.8 d
7′	a 2.60 d (15.0)	45.1 t	a 2.55 d (15.0)	45.2 t	a 2.64 d (15.0)	45.2 t	a 2.59 d (15.0)	45.1 t
	b 2.70 d (15.0)		b 2.67 d (15.0)		b 2.71 d (15.0)		b 2.70 d (15.0)	
8′		167.8 s		168.0 s		167.8 s		167.8 s
	phenylacetate				*p*-hydroxyphenylacetate			
1″		134.9 s		135.3 s		125.5 d		134.9 s
2/6″[b]	7.20 d (7.2)	130.3 d	7.30 m	130.2 d	7.02 m	131.2 d	7.20 d (7.2)	130.2 d
3/5″[c]	7.30 n.d.	129.2 d	7.30 m	129.2 d	6.77 d (8.4)	116.1 d	7.29 d (7.2)	129.3 d
4″[d]	7.25 n.d.	127.7 d	7.24 m	127.7 d		157.4 s	7.25 m	127.9 d
7″	a 3.41 d (15.6)	41.2 t	a 3.67 s	41.4 t	a 3.30 d (15.0)	40.4 t	a 3.41 d (15.0)	41.2
	b 3.49 d (15.0)		b 3.67 s		b 3.37 d (15.0)		b 3.47 d (15.0)	
8″		170.7 s		170.9 s		171.1 s		170.7 s
	phenylacetate						*p*-hydroxyphenylacetate	
1‴		135.1 s				135.2 s		125.8 s
2/6‴[b]	7.24 d (7.8)	130.3 d			7.24 m	130.3 d	7.07 d (11.4)	131.3 d
3/5‴[c]	7.30 n.d.	129.3 d			7.31 dd (6.6, 13.8)	129.2 d	6.78 d (8.4)	116.1 d
4‴[d]	7.25 n.d.	127.9 d			7.26 m	127.7 d		128.1 s
7‴	a 3.52 d (15.6)	41.3 t			a 3.51 d (16.2)	41.3 t	a 3.41 d (13.2)	40.5
	b 3.59 d (15.6)				b 3.58 d (16.2)		b 3.48 d (15.6)	
8‴		171.3 s				171.3 s		171.7 s
	jacaranone acid							
1⁗		67.8 s		67.9 s		67.9 s		67.8 s
2/6⁗	7.08 d (10.2)	151.3 d	7.08 d (10.6)	151.4 d	7.06 n.d.	151.3 d	7.08 d (13.2)	151.4 d
3/5⁗	6.09 m	128.1 d	6.10 d (10.6)	128.1 d	6.10 d (10.2)	128.4 d	6.10 d (9.6)	128.4 d[a]
4⁗		185.6 s		185.6 s		185.6 s		185.6 s
7⁗	2.81 s	45.7 t	2.79 s	45.8 t	2.81 s	45.7 t	2.81 s	45.8 t
8⁗		169.4 s		169.4 s		169.4 s		169.4 s

[a] Interchangeable signals. n.d. = not determined.

Table 2. Activities of Compounds **1**−**4** against *P. falciparum* and L-6 Cells

compound	IC_{50} (*P. falc.* K1)	IC_{50} (L-6 cells)
jacaglabroside A (**1**)	1.02 ± 0.29[a]	8.3 ± 4.8[a]
jacaglabroside B (**2**)	0.56	>90
jacaglabroside C (**3**)	0.56	87.0
jacaglabroside D (**4**)	0.55	84.8
jacaranone (**5**)	1.32	1.06
phenylacetic acid (**6**)	>5	>90
chloroquine[b]	0.06	
podophyllotoxin[c]		0.007

[a] Each sample was tested in two independent assays. Replicate values are within a factor of 2. Data are expressed in μg/mL. [b] $n = 8$. [c] Positive control.

chloroquine and pyrimethamine). A modification of the [^{3}H]-hypoxanthine incorporation assay was used.[26] Briefly, infected human red blood cells in RPMI 1640 medium with 5% Albumax were exposed to serial drug dilutions in microtiter plates. After 48 h of incubation at 37 °C in a reduced oxygen atmosphere, 0.5 μCi of ^{3}H-hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fiber filters and washed with distilled water. The radioactivity was counted using a Betaplate liquid scintillation counter (Wallac, Zurich, Switzerland). The results were recorded as counts per minute per well at each drug concentration and expressed as percentages of the untreated controls. From the sigmoidal inhibition curves, IC_{50} values were calculated. Each sample was tested in two independent assays. A third assay was performed if the calculated IC_{50} values differed by more than a factor of 2.

Cytotoxicity Assay on L-6 Cells. The rat skeletal myoblast cell line (L-6 cells) was used to assess cytotoxicity. The cells were grown in RPMI 1640 medium supplemented with 1% L-glutamine (200 nM) and 10% fetal bovine serum at 37 °C in 5% CO_2 in air. The assay was performed in 96-well microtiter plates, each well receiving 100 μL of culture medium with ca. 4×10^4 cells. After 24 h, the medium was removed from all wells, and serial drug dilutions were prepared covering a range from 90 to 0.123 μg/mL. After 72 h of incubation, the plates were inspected under an inverted microscope to ensure growth of the controls and sterile conditions. Then, 10 μL of Alamar blue (12.5 mg of resazurin dissolved in 100 mL of distilled water) was added to each well, and the plates were incubated for another 2 h, with the plates evaluated with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Corporation, Sunnyvale, CA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. IC_{50} values were determined using the microplate reader software Softmax Pro (Molecular Devices). Each sample was tested in two independent assays. A third assay was performed if the calculated IC_{50} values differed by more than a factor of 2.

Extraction and Isolation. The dried and powdered leaves (480 g) of *J. glabra* were extracted sequentially with CH_2Cl_2 and MeOH by percolation, to yield residues of 37 and 42 g, respectively. The CH_2Cl_2 extract, which showed activity against the *P. falciparum* K1 strain, was chosen for further purification. A portion (9 g) of the crude CH_2Cl_2 extract was fractionated on silica gel (VLC) using a gradient of *n*-hexane−EtOAc (each of 500 mL) from 100% *n*-hexane (F-1), with gradient steps of 5−10%, to 100% EtOAc (F-12), then with a final elution by 100% MeOH (F-13). The activity was found to be in fractions F-11 (840 mg) and F-12 (3.3 g), corresponding to 80% and 100% EtOAc, respectively.

Fraction F-11 was partitioned by FCPC (dual mode) using *i*-PrOH−*n*-hexane−EtOAc−MeOH (4:4:1:1:0.5) in the ascending mode (switching volume 360 mL, 1000 rpm, 4 mL/min flow rate). On the basis of UV detection at λ 254 nm and TLC analysis, 14 fractions were combined. Fractions F-11-H (465 mg) and F-11-I (110 mg) contained the same main compound. F-11-H was fractionated using the FCPC dual mode with *i*-PrOH−*n*-hexane−EtOAc−MeOH (4:4:1:4:0.5), in ascending mode (260 mL, 1000 rpm, 4 mL/min), to yield seven fractions, including F-11−H-5 (300 mg). A portion of F-11−H-5 (20.8 mg) was further purified by preparative HPLC (CH_3CN-H_2O, 45:55, 2.5 mL/min, t_R 18.2 min) to yield compound **1** (12.7 mg). Fraction F-11−I-5 yielded also 7 mg of jacaranone (**5**) (t_R 5.6 min). From fraction F-11-E (70 mg), phenylacetic acid (**6**) (18 mg) was isolated using preparative HPLC (CH_3CN-H_2O, 57:43; 2.5 mL/min, t_R 6.4 min).

A portion of VLC fraction F-12 (1 g) was separated by FCPC (dual mode) using *n*-hexane−EtOAc−MeOH−H_2O (3:7:4:6) in the descending mode (131 mL, 800 rpm., 1 mL/min), to yield six fractions. Fractions F-12-B (520 mg), F-12-D (220 mg), and F-12-E (861 mg) were further fractionated using preparative HPLC. F-12-B (36 mg) was fractionated using a CH_3CN-H_2O gradient (10:90 to 30:70 in 10 min, then isocratic for 20 min at 30:70, flow rate 2.5 mL/min, t_R 20.3 min) to yield compound **2** (12.7 mg). F-12-D (100 mg) was fractionated using isocratic conditions (CH_3CN-H_2O, 45:55, 2.5 mL/min, t_R 18.2 min) to yield **1** (15.2 mg). F-12 E (75 mg) was separated using a CH_3CN-H_2O gradient (25:75 to 35:65 in 10 min, then isocratic for 21 min at 35:65, flow rate 2.5 mL/min, t_R 29.5 min (**3**) and t_R 27.5 min (**4**), to yield compounds **3** (22.5 mg) and **4** (8.9 mg).

To determine the sugar configuration, 15 mg of compound **1** was dissolved and hydrolyzed in dioxane (1 mL) and 2 N H_2SO_4 (1 mL) and left stirring overnight. The aglycone was extracted with Et_2O from the sugar dissolved in H_2O. The H_2O was removed and the specific rotation was measured in MeOH.

Jacaglabroside A [1,6-bis(1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetyl)-2,3-bis(benzeneacetyl)-β-glucopyranoside] (1): slightly yellow oil; $[\alpha]^{20}_D$ −3.2 (*c* 0.19, CH_3OH); UV (CH_3CN) λ_{max} (log ε) 211 (4.7) nm; IR (KBr) ν_{max} 3425, 1745, 1671, 1625, 1152, 1076, 1014, 861, 714 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 717.01 $[M + H]^+$ (4), 733.94 $[M + NH_4]^+$ (100), 740.07 $[M + Na]^+$ (10), 548.94 $[M + H - C_8H_7O_4]^+$ (45), 430.94 $[M + H - C_8H_7O_3 - C_8H_7O_2]^+$; HRESIMS *m/z* 717.2198 $[M + H]^+$ (calcd for $C_{38}H_{37}O_{14}$, 717.2178), *m/z* 734.2462 $[M + NH_4]^+$ (calcd for $C_{38}H_{40}O_{14}N$, 734.2443), *m/z* 739.2025 $[M + Na]^+$ (calcd for $C_{38}H_{36}O_{14}Na$, 739.1997), *m/z* 549.1753 $[M - C_8H_8O_4 + H]^+$ (calcd for $C_{30}H_{29}O_{10}$, 549.1755).

Jacaglabroside B [1,6-bis(1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetyl)-2-benzeneacetyl-β-glucopyranoside] (2): slightly yellow oil; $[\alpha]^{20}_D$ +1.9 (*c* 0.16, CH_3OH); UV (CH_3CN) λ_{max} (log ε) 220 (4.4) nm; IR (KBr) ν_{max} 3422, 1738, 1671, 1626, 1398, 1261, 1152, 1079, 1012, 863, 715 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 598.93 $[M + H]^+$ (5), 615.92 $[M + NH_4]^+$ (100), 431.00 $[M + H - C_8H_7O_4]^+$ (77); HRESIMS *m/z* 599.1767 $[M + H]^+$ (calcd for $C_{30}H_{31}O_{13}$, 599.1759), *m/z* 616.2028 $[M + NH_4]^+$ (calcd for $C_{30}H_{34}O_{13}N$, 616.2025), *m/z* 621.1595 $[M + Na]^+$ (calcd for $C_{30}H_{30}O_{13}Na$, 621.1579), *m/z* 431.1336 $[M - C_8H_8O_4 + H]^+$ (calcd for $C_{22}H_{23}O_9$, 431.1337).

Jacaglabroside C [1,6-bis(1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetyl)-2-(*para*-hydroxybenzeneacetyl)-3-benzeneacetyl-β-glucopyranoside] (3): slightly yellow oil; $[\alpha]^{20}_D$ +2.2 (*c* 0.20, CH_3OH); UV (CH_3CN) λ_{max} (log ε) 220 (4.6) nm; IR (KBr) ν_{max} 3421, 1746, 1671, 1625, 1517, 1398, 1230, 1152, 1076, 1014, 862, 714 cm^{-1}; ^1H- and ^{13}C NMR data, see Table 1; ESIMS *m/z* 732.87 $[M + H]^+$ (5), 749.99 $[M + NH_4]^+$ (100), 564.97 $[M + H - C_8H_7O_4]^+$ (61); HRESIMS *m/z* 733.2148 $[M + H]^+$ (calcd for $C_{38}H_{37}O_{15}$, 733.2127), *m/z* 750.2424 $[M + NH_4]^+$ (calcd for $C_{38}H_{40}O_{15}N$, 750.2392), *m/z* 755.1987 $[M + Na]^+$ (calcd for $C_{38}H_{36}O_{15}Na$, 755.1946), *m/z* 565.1713 $[M - C_8H_8O_4 + H]^+$ (calcd for $C_{30}H_{29}O_{11}$, 565.1704).

Jacaglabroside D [1,6-bis(1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetyl)-2-benzeneacetyl-3-(*para*-hydroxybenzeneacetyl)-β-glucopyranoside] (4): slightly yellow oil; $[\alpha]^{20}_D$ +3.5 (*c* 0.16, CH_3OH); UV (CH_3CN) λ_{max} (log ε) 223 (4.6) nm; IR (KBr) ν_{max} 3423, 1750, 1671, 1625, 1517, 1232, 1152, 1076, 1018, 862, 714 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 733.01 $[M + 1]^+$ (5), 749.96 $[M + NH_4]^+$ (100), 565.02 $[M + 1 - C_8H_7O_4]^+$ (61); HRESIMS *m/z* 733.2157 $[M + H]^+$ (calcd for $C_{38}H_{37}O_{15}$, 733.2127), *m/z* 750.2423 $[M + NH_4]^+$ (calcd for $C_{38}H_{40}O_{15}N$, 750.2392), *m/z* 755.1994 $[M + Na]^+$ (calcd for $C_{38}H_{36}O_{15}Na$, 755.1946), *m/z* 565.1713 $[M - C_8H_8O_4 + H]^+$ (calcd for $C_{30}H_{29}O_{11}$, 565.1704).

2,5-Cyclohexadiene-1-acetic acid, 1-hydroxy-4-oxo-, methyl ester or jacaranone (5): yellow oil; ^1H and ^{13}C NMR in $CDCl_3$ compared with ref 12; GC EIMS $C_9H_{10}O_4$ *m/z* 182 $[M]^+$ (4), 109 in $[M - CH_2COOCH_3]^+$ (100).

Phenylacetic acid (6): yellow powder; ^1H NMR (400 CH_3OD, 400 MHz) δ 3.57 (3H, s), 7.28 (1H, m), 7.21 (1H, m), 7.26 (1H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.2 (C s), 41.6 (C t), 135.8 (C s), 128.7 (C d), 127.0 (C d), 129.5 (C d); GC EIMS $C_8H_8O_2$ *m/z* 136 $[M]^+$ (46), 91 $[M - CO_2 - H]^+$ (100).

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Supporting Information Available: NMR spectra of compounds **1**−**4**. This information is available free of charge via the Internet at http://pubs.acs.org.

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