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Two very unusual macrocyclic flavonoids from the water lily *Nymphaea lotus*

Awatif A. Elegami, Catharine Bates, Alexander I. Gray, Simon P. Mackay, Graham G. Skellern, Roger D. Waigh*

Department of Pharmaceutical Sciences, Strathclyde Institute for Biomedical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NR, UK

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Abstract

Three novel flavonols, myricetin-3′-*O*-(6″-*p*-coumaroyl)glucoside and two epimeric macrocyclic derivatives, as well as the known myricetin-3-*O*-rhamnoside and pentagalloyl glucose, have been isolated from the wild water lily *Nymphaea lotus* L. and identified using 2D NMR. This is the first report of such a macrocycle from any source.

Keywords: *Nymphaea lotus*; Nymphaeaceae; Water lily; Sudan; Flavonol; Glucoside; *p*-Coumaroyl; Macrocycle

1. Introduction

Nymphaea lotus L. is a water lily, widespread on the River Nile and tributaries in the centre and south of Sudan. The roots of the plant are used throughout this region as a cooked starchy vegetable, while the root and particularly the leaves have been used in traditional Sudanese medicine as a remedy for dysentery, to treat tumours (El Ghazali et al., 1994) and as an antibacterial (Elegami et al., 2001). As a part of a wider study of the Sudanese flora, in particular the use of indigenous plants for the treatment of bacterial infections, we have extracted the dried leaves of the plant and separated the constituents of the biologically active methanol fraction.

Some flavonoid glycosides (Fossen et al., 1998a, 1999; Fossen and Andersen, 1999), anthocyanins (Fossen et al., 1998b; Fossen and Andersen, 1997) and the hydrolysable tannin geraniin (Kurihara et al., 1993) have previously been isolated from the genus *Nymphaea* but there have been no reports concerning the chemistry of *N. lotus*. The present work describes the isolation and identification of two new macrocyclic flavonol glucoside esters and their open-chain precursor.

2. Discussion

Fractionation of the methanol extract of the leaves of *Nymphaea lotus*, using first VLC on silica, then Sephadex LH-20, gave a complex mixture of mainly phenolic metabolites which were separated on a reverse-phase HPLC column using methanol/water as the mobile phase. Two fractions were known compounds, pentagalloyl glucose and myricetin-3-*O*-rhamnoside. A third was an unidentified ellagic acid derivative. A fourth fraction eluting at 38 min showed the UV characteristics of a flavonoid, with a peak at 307nm and a shoulder at 359 nm. IR absorption at 1706 and 1653 cm^{-1} indicated two carbonyls. The mass spectrum suggested the molecular formula $C_{30}H_{26}O_{15}$. ^1H NMR data were consistent with a 5,7-dihydroxy flavonoid, showing two meta-coupled protons at 6.70 and 6.81 ppm, together with glucose protons, all showing *trans* diaxial coupling, in the range δ 4.3–5.8. The six protons of the glucose were much further downfield than in the unsubstituted sugar and the anomeric proton was also shifted downfield, consistent with esterification and acetal formation, respectively (Table 1). Application of 2D methods, particularly HMBC, established the structure as 6″-*p*-coumaroyl myricetin 3′-O-glucoside **1**, a new flavonol glucoside, belonging to a group which is fairly widely distributed. The 2′ and 6′ hydrogens of the flavonoid phenyl group were non-equivalent, confirming the position of glucosylation.

* Corresponding author.
E-mail address: eas98112@strath.ac.uk (R.D. Waigh).

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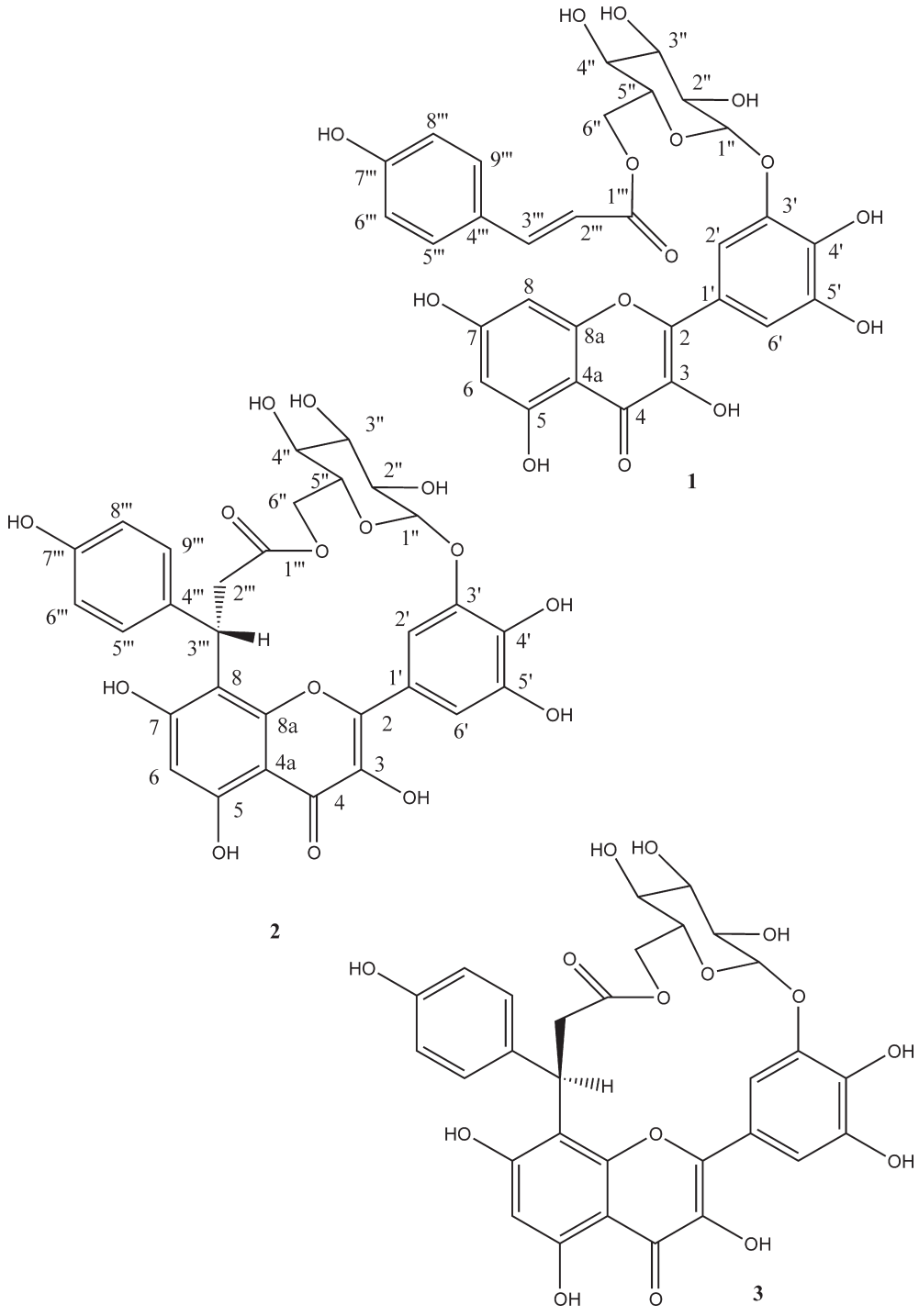

Fractions eluting at 43.9 and 36.4 min from HPLC were similar compounds (**2** and **3** respectively, for which we suggest the names nympholide A and nympholide B) and bore a familial relationship to **1**. In both cases one of the flavonoid A-ring protons had disappeared, the protons of the *p*-coumaroyl double bond had been replaced by a three-proton saturated system and the NMR signals of the 2′ and 6′ protons of the flavonoid phenyl ring had become further separated in frequency.

HMBC for **2** showed the correlations in Fig. 1 and suggested the unusual macrocyclic structure, since all parts of the molecule were connected. This structure, with the same mass as **1**, was confirmed by MS. Assuming that the glucose was stereochemically normal, i.e. 'D', molecular modelling indicated that the absolute stereochemistry at the one other chiral centre (3‴), arising from the *p*-coumaroyl residue, could be determined by nOe measurement. This was accomplished by use of

Table 1
Chemical shifts for compounds **1**, **2** and **3**; solvent pyridine-d_5

	1		**2**		**3**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2		147.9		147.9		147.6[a]
3		138.8		138.6		138.8
4		177.7		178.1		178.2
4a		104.9		104.9		104.9
5		162.9		160.9		160.9
6	6.70 (2)	99.8	6.69	100.1	6.79	100.1
7		166.1		164.0		164.3
8	6.81 (2)	94.9		110.7		110.7
8a		157.4		154.4		154.4
1′		123.5		124.4		124.5
2′	8.44 (2)	110.2	8.48 (2)	110.8	8.52 (2)	110.8
3′		148.5		148.6		149.4[a]
4′		140.5		141.3		141.2
5′		148.2		148.2		148.2[a]
6′	8.52 (2)	113.0	8.89 (2)	115.5	8.98 (2)	115.6
1″	5.72 (7.5)	104.8	5.57 (8)	107.1	5.43 (7.5)	108.2
2″	4.36[b]	75.4[c]	4.40 (8.8)	75.3	4.34[b]	75.5[d]
3″	4.36[b]	78.8	4.25 (8.8)	78.7	4.36[b]	78.7
4″	4.35[b]	72.0	4.03 (8.8)	72.3	3.96 (8.8)	72.6
5″	4.34[b]	76.5[c]	4.19 (8.8)	77.2	4.45 (8.8)	76.4[d]
6″a	4.88 (12.7)	65.1	4.48 (8.11)	65.3	4.56[b]	66.6
6″b	5.15 (12.2)		5.48 (11)		5.30 (10)	
1‴		168.0		172.8		173.2
2‴a	6.53 (16)	115.2	3.44 (15.3)	40.3	3.52 (14.3)	42.5
2‴b			4.67 (13.15)		4.57[b]	
3‴	7.82 (16)	145.9	6.05 (13.3)	38.1	6.05 (12.2)	38.9
4‴		126.4		135.5		135.6
5‴	7.34 (9)	131.0	8.10 (9)	130.4	8.24 (9)	130.6
6‴	7.09 (9)	117.2	7.11 (9)	116.7	7.17 (9)	116.9
7‴		161.9		158.0		158.2
8‴	7.09 (9)	117.2	7.11 (9)	116.7	7.17 (9)	116.9
9‴	7.34 (9)	131.0	8.10 (9)	130.4	8.24 (9)	130.6
5-OH	13.27		13.34		13.38	

[a] Interchangeable.
[b] Overlapped.
[c] Interchangeable.
[d] Interchangeable.

NOESY, which gave cross-peaks, some of which are illustrated in Fig. 2, establishing the stereochemistry in **2** at position 3‴ as *S*. With the *S* configuration at position 3‴, all the protons highlighted in Fig. 2 are nicely placed to produce the observed NOEs. The interaction between the 5″ and 5‴ protons is shown by molecular modelling to be very unlikely in the epimer **3**, unless a conformation is adopted which is both crowded and results in the removal of H-3‴ from proximity with H-2′.

Compound **3** was very similar to **2**, apart from some chemical shift differences, particularly at the 1″, 5″ and 6″ positions of the glucose moiety. HMBC suggested the same skeleton, which allowed only a difference in stereochemistry as the distinguishing feature. Once again, NOESY showed the expected cross-peaks, but, significantly, not between the 5″ and 5‴/9‴ protons.

In structures **2** and **3** the *p*-hydroxy phenyl groups of the cyclised *p*-coumaroyl units and the phenyl rings of the flavonoids exert shielding/deshielding effects on the sugar protons across the macrocycle (Table 1). The most affected protons are 1″, 4″, 5″, 6″a and 6″b, consistent with through-space, anisotropic effects, rather than electronic changes.

It seems certain that **1** is the precursor of **2** and **3**, probably through an acid-catalysed mechanism involving protonation of the carbonyl of the *p*-coumaroyl residue; a base catalysed process is also possible, via Michael addition of the phenolic flavonoid A-ring to the α,β-unsaturated ester. Although the isolation of both stereoisomers indicates rather loose conformational control in the cyclisation, the formation of a macrocycle requires that the precursor be coiled round in a sterically unfavourable manner, which seems very unlikely without enzymic catalysis: we are not aware of any previous reports of such a macrocycle.

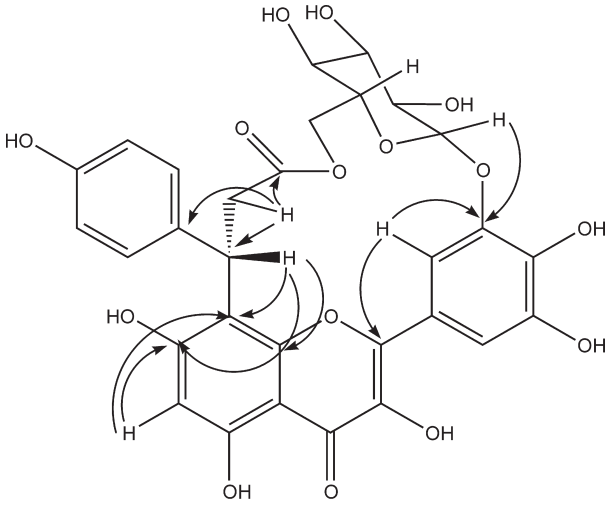

Fig. 1. Selected HMBC correlations for **2**.

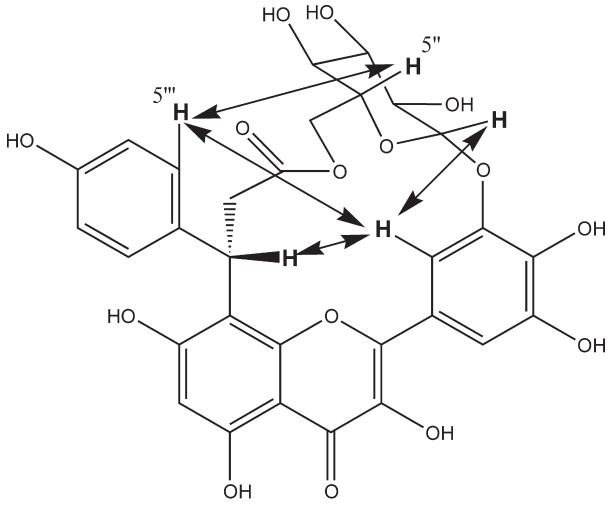

Fig. 2. Selected NOESY correlations for **2**.

None of the three new compounds showed significant activity against *Escherichia coli* or *Staphylococcus aureus*.

3. Experimental

3.1. General

UV spectra were recorded in absolute methanol using a Unicam UV 300 UV/visible spectrometer. IR data were recorded in KBr disks on a Mattson Genesis series FT-IR. NMR measurements were performed on a Brüker AMX-400 (400 MHz) instrument with C_5D_5N as solvent. Mass spectra were recorded on a Jeol JMS-AX 505 HA mass spectrometer, using FAB in either positive (nitrobenzyl alcohol matrix) or negative (glycerol matrix) ion mode.

3.2. Plant material

The plant material was collected from White Nile province (Sudan) in September 1998. The botanical identification was carried out by Dr. G.E.B. El Ghazali, Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum, Sudan. A voucher specimen, number G11/92, was deposited at the herbarium of the Institute.

3.3. Extraction and isolation

Dried ground leaves (750 g) of the plant were exhaustively extracted in a soxhlet with MeOH which was finally distilled in vacuo. The residue was successively extracted with petrol (bp 40–60°C), EtOA*c* and MeOH.

The MeOH extract was evaporated to obtain 26 g of residue which was subjected to VLC over silica gel (Merck type 60H) and eluted with *n*-hexane–EtOAc and EtOAc–MeOH with gradually increasing polarity. A fraction eluted with 3–9% MeOH in EtOAc yielded a mixture of flavonoids (5.6 g) which was further fractionated on a Sephadex LH-20 column using $CHCl_3$: MeOH, 8:2 as eluant to yield 3.1 g of mixed phenolics. This material was filtered through a membrane filter and separated by HPLC.

Preparative HPLC was carried out on a Gilson system with two reciprocating pumps (models 307 and 305), manometric module 806, dynamic mixer 811 C and UV/Vis Detector 118 with a Hewlett Packard HP 3395 integrator. Separation was effected with a Phenomenex semi-preparative C 18 reverse phase column (ECONO prep C18, 5 μ, 300×10 mm), fitted with a guard column (ECON 50×10mm). The elution profile consisted of a linear gradient from 20 to 80% MeOH in H_2O for 50 min, 80–100% MeOH in H_2O for 5 min, followed by further isocratic elution for 10 min at 100% MeOH and a linear gradient from 100 to 20% MeOH for 5 min. The flow-rate was 6 ml/min and aliquots of 0.5 ml of a 10 mg/ml solution in 20% MeOH were injected. The system was re-equilibrated for 5 min at 20% MeOH before each injection. Peaks were detected at 254 nm.

Material from 13 peaks was collected, of which six contained sufficient for possible identification. Two of these, myricetin-3-*O*-rhamnoside and pentagalloylglucose were known compounds and one was an unidentified ellagic acid derivative. The presently described compounds had retention times (mins) 36.43 (compound **3**), 38.10 (**1**) and 43.93 (**2**). Products from 30 runs were bulked, giving respectively, 11.0, 18.4 and 13.1 mg after evaporation.

3.4. 1,2,3,4,6-Pentagalloylglucose

Colourless amorphous powder with characteristic UV spectral maximum at $\lambda = 280$ nm in MeOH. The HR EIMS showed a molecular ion at m/z 940 consistent with the molecular formula $C_{41}H_{32}O_{26}$. Peaks at m/z: 787, 603, 391 and 270 indicated successive elimination of galloyl groups. The ^{1}H NMR spectrum revealed five aromatic proton singlets; each integrating for two protons, between δ 7.77 and 7.88. The spectrum also showed five clearly resolved resonances for the sugar and showed the α orientation of the 1-substituent. Comparison with literature data (Haddock et al., 1982) confirmed the structure.

3.5. Myricetin-3-O-rhamnoside (myricitrin)

Yellow needles, UV (MeOH) maxima at 201, 209, 257, and 352nm. HRMS gave the molecular ion as $C_{20}H_{21}O_{12}$. ^{1}H and ^{13}C NMR spectra were indicative of a flavonol rhamnoside; COSY, HMQC, HMBC and comparison with literature data established the structure, which has been reported previously from several sources (Zapesochnaya, 1982).

3.6. Myricetin-3′-O-(6″-p-coumaroyl)glucoside *1*

Brown amorphous solid, mp 130–136 °C (decomp.). UV (MeOH, λ_{max}, nm) 205, 256, 307, 359(sh). IR (NaCl disc, cm^{-1}) 3423, 2921, 1706, 1653, 1604, 1514, 1201, 1167, 1076, 830. MS 625.117; $C_{30}H_{25}O_{15}$ $[M-H]^-$ requires 625.119. For NMR data see Table 1. HMBC (set for J_{CH} 7 Hz): δ 6.70 (H-6) to 104.9 (4a), 162.9 (5), 166.1 (7), 94.9 (8); 6.81 (H-8) to 104.9 (4a), 99.8 (6), 166.1 (7), 157.4 (8a); 8.44 (H-2′) to 147.9 (2), 148.5 (3′), 140.5 (4′, 113.0 (6′); 4.88 (H-6″a) to 168.0 (1‴); 5.15 (H-6″b) to 168.0 (1‴); 6.53 (H-2‴a) to 126.4 (4‴); 7.82 (H-3‴) to 168.0 (1‴), 131.0 (5‴ and 9‴); 7.34 (H-5‴ and 9‴) to 161.9 (7‴), 131.0 (5‴ and 9‴); 7.09 (6‴ and 8‴) to 126.4

(4‴), 117.2 (6‴ and 8‴) δ 6.70 (H-6) had ^1J satellites with 99.8 (C-6).

3.7. Nympholide A **2**

Brown amorphous solid, mp 174–178 °C (decomp.). UV (MeOH, λ_{max}, nm) 209, 257, 311, 374. IR (NaCl disc, cm^{-1}) 3411, 2922, 1719, 1654, 1604, 1513, 1344, 1247, 1202, 1075, 1024, 832. MS 627.131; $C_{30}H_{27}O_{15}$ $[M+H]^+$ requires 627.135. For NMR data see Table 1. HMBC (set for J_{CH} 7 Hz): δ 6.69 (H-6) to 104.9 (4a), 160.9 (5), 164.0 (7), 110.7 (8); 8.48 (H-2′) to 148.6 (3′), 141.3 (4′), 115.5 (6′); 8.89 (H-6′) to 110.8 (2′), 141.3 (4′), 148.2 (5′); 5.57 (H-1″) to 148.6 (3′); 4.40 (H-2″) to 107.1 (1″), 78.7 (3″); 4.25 (H-3″) to 75.3 (2″); 4.03 (H-4″) to 77.2 (5″); 4.19 (H-5″) to 72.3 (4″); 3.44 (H-2‴a) to 172.8 (1‴), 38.1 (3‴), 135.5 (4‴); 4.67 (H-2‴b) to 172.8 (1‴), 38.1 (3‴), 135.5 (4‴); 6.05 (H-3‴) to 164.0 (7), 110.7 (8), 154.4 (8a), 40.3 (2‴), 135.5 (4‴), 130.4 (5‴ and 9‴); 8.10 (H-5‴ and 9‴) to 38.1 (3‴), 130.4 (5‴ and 9‴), 158.0 (7‴); 7.11 (H-6‴ and 8‴) to 135.5 (4‴), 116.7 (6‴ and 8‴); 13.34 (5-OH) to 104.9 (4a), 160.9 (5), 100.1 (6). There were ^1J satellites for H-6, H-2′, H-6′, H-1″, H-5‴/H-9‴ and H-6‴/8‴. NOESY (Helium degassed, 313 K, 1.1 sec mixing time) gave the following cross peaks: δ 8.48 (2′) to 5.57 (1″), 6.05 (3‴), 8.10 (5‴ and 9‴); 5.57 (1″) to 8.48 (2′), 4.40 (2″), 4.25 (3″), 4.19 (5″); 4.40 (2″) to 5.57 (1″); 4.25 (3″) to 5.57 (1″); 4.19 (5″) to 5.57 (1″), 5.48 (6″b), 8.10 (5‴ and 9‴); 4.48 (6″a) to 5.48 (6″b); 5.48 (6″b) to 4.19 (5″), 4.48 (6″a); 3.44 (2‴a) to 4.67 (2‴b), 6.05 (3‴); 4.67 (2‴b) to 3.44 (2‴a), 8.10 (5‴ and 9‴); 6.05 (3‴) to 8.48 (2′), 3.44 (2‴a), 8.10 (5‴ and 9‴), 8.10 (5‴ and 9‴) to 8.48 (2′), 4.19 (5″), 4.67 (2‴b), 6.05 (3‴), 7.11 (6‴ and 8‴).

3.8. Nympholide B **3**

Brown amorphous solid, mp 198 °C (decomp., softens at 168 °C). UV (MeOH, λ_{max}, nm) 204, 257, 298 (sh), 359 (sh). IR (NaCl disc, cm^{-1}) 3415, 2921, 1718, 1604, 1513, 1308, 1201, 1075, 972, 827. MS 625.124; $C_{30}H_{25}O_{15}$ $[M-H]^-$ requires 625.119. For NMR data see Table 1. HMBC (set for J_{CH} 7Hz): 6.79 (H-6) to 104.9 (4a), 160.9 (50, 164.3 (7), 110.7 (8); 8.52 (H-2′) to 141.2 (4′), 115.6 (6′); 8.98 (H-6′) to 110.8 (2′), 141.2 (4′); 4.34 (H-2″) to 78.7 (3″); 4.36 (H-3″) to 75.5 (2″); 3.52 (2‴a) to 173.2 (1‴), 38.9 (3‴), 135.6 (4‴); 6.05 (3‴) to 164.3 (6), 110.7 (8), 154.4 (8a), 42.5 (2‴), 135.6 (4‴), 130.6 (5‴ and 9‴); 8.24 (H-5‴ and 9‴) to 38.9 (3‴), 158.2 (7‴), 130.6 (5‴ and 9‴); 7.17 (H-6‴ and 8‴) to 135.6 (4‴), 116.9 (6‴ and 8‴). There were ^1J satellites for H-6, H-2′ and H-6′. NOESY (1.1 sec mixing time, 313 K) δ8.52 (2′) to 5.43 (1″), 6.05 (3‴), 8.24 (5‴ and 9‴); 5.43 (1″) to 8.52 (2′), 4.36 (3″), 4.45 (5″); 4.36 (3″) to 5.43 (1″); 4.45 (5″) to 5.43 (1″), 5.30 (6″b); 4.56 (6″a) to 5.30 (6″b); 5.30 (6″b) to 4.45 (5″), 4.56 (6″a); 3.52 (2‴a) to 6.05 (3‴); 6.05 (3‴) to 8.52 (2′), 3.52 (2‴a), 8.24 (5‴ and 9‴); 8.24 (5‴) to 8.52 (2′), 7.17 (6‴ and 8‴); 7.17 (6‴ and 8‴) to 8.24 (5‴ and 9‴).

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