JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Yucca gloriosa: A Source of Phenolic Derivatives with Strong Antioxidant Activity

CARLA BASSARELLO,[§] GIUSEPPE BIFULCO,[§] PAOLA MONTORO,[§] ALEXANDRE SKHIRTLADZE,[†] MARIAM BENIDZE,[†] ETHER KEMERTELIDZE,[†] COSIMO PIZZA,[§] AND SONIA PIACENTE*[,§]

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Melillo, 84084 Fisciano, Salerno, Italy, and Iovel Kutateladze, Institute of Pharmacochemistry, P. Sarajishvili st. 36, 0159 Tbilisi, Georgia

On the basis of the biological activities exhibited by the phenolic constituents of *Yucca schidigera*, the antioxidant activity of the methanol extract of *Yucca gloriosa* roots was evaluated in the TEAC assay. The strong activity exerted by this extract prompted investigation of its phenolic constituents, yielding three new phenolic derivatives, gloriosaols C, D, and E, along with gloriosaols A and B previously isolated from *Y. gloriosa* roots and yuccaols C−E isolated from *Y. schidigera*. ESIMS and NMR data of gloriosaols C−E closely resembled those reported for gloriosaols A and B, two diasteroisomers characterized by unusual spirostructures. Careful inspection of ROESY spectra revealed that gloriosaols C−E are diastereoisomers of gloriosaols A and B. A possible assignment of the relative configuration of gloriosaols C−E, derived according to an integrated NMR−quantum mechanical (QM) approach, which was already applied to the determination of the stereostructures of gloriosaols A and B, is also proposed. Gloriosaols A−E exhibited potent antioxidant activity measured by the TEAC assay, showing the potential use of *Y. gloriosa* as a source of antioxidant principles.

KEYWORDS: ***Yucca gloriosa*; gloriosaols A−E; GIAO NMR; DFT calculations; radical scavenging activity; TEAC**

INTRODUCTION

The *Yucca* genus (Agavaceae) comprises species characterized by the occurrence of steroidal saponins (*1*). Among these species the best known is *Y. schidigera*, commonly named yucca, native to the southwestern United States and Mexico. *Y. schidigera* is one of the two major commercial sources of saponins, the other being *Quillaia saponaria* (*2*). Two products obtained from the trunk of *Y. schidigera* are available on the market: yucca powder and yucca extract. These products possess a GRAS (generally recognized as safe) label given by the U.S. FDA, which allows their human dietary use (*3*). An important application of yucca extract is as a foaming agent in soft drinks, pharmaceuticals, cosmetics, and food industries. The foaming activity of yucca extract is due to the very high saponin content, comprising about 10% of dried material (*4*, *5*). The main application of yucca products is in animal nutrition, in particular as a food additive to reduce ammonia and fecal odors in animal excreta (*2*). The positive effects of dietary supplementation with yucca products on the growth rates, feed efficiency, and health of livestock seem to be due not only to the saponin constituents but also to other constituents. These observations prompted us to investigate the phenolic fraction of *Y. schidigera*, and this study led to the isolation of resveratrol, *trans*-3,3′,5,5′-tetrahydroxy-4′-methoxystilbene, and the spirobiflavonoid larixinol along with phenolic derivatives with very unusual spirostructures, named yuccaols A−E and yuccaone A (*3*, *6*−*8*).

The multifunctional activities of resveratrol together with the novelty of yuccaols A−E, structurally related to resveratrol, prompted us to carry out a program aimed to evaluate some of the activities exerted by resveratrol for yucca phenolics. A strong radical scavenging activity was observed for all yucca phenolics (*7*). Furthermore the evaluation of the inhibitory effects of yucca phenolics on thrombin-induced platelet aggregation revealed that these compounds showed even stronger antiplatelet activity than resveratrol (*9*). They also had an inhibitory effect on the thrombin-induced enzymic platelet lipid peroxidation and inhibited the generation of free radicals in blood platelets activated by thrombin or thrombin receptor activating peptide (TRAP). Comparative studies using in vitro tests showed that all of the phenolics from yucca bark exerted an antioxidant effect on different radical oxygen species (ROS) produced in resting blood platelets and blood platelets activated by thrombin or TRAP (*10*). Furthermore, yuccaol C was found to inhibit

* Author to whom correspondence should be addressed (telephone +39-089-969763; fax +39-089-969602; e-mail piacente@unisa.it).
[§] Università degli Studi di Salerno.
[†] Institute of Pharmacochemistry.

10.1021/jf071131n CCC: $37.00
Published on Web 07/11/2007

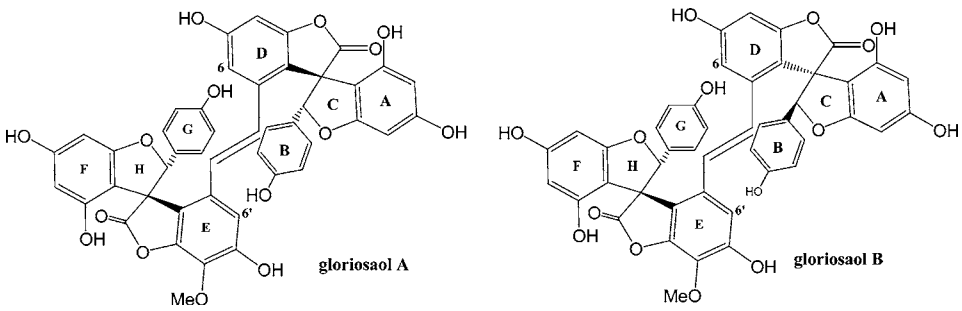

Figure 1. Gloriosaols A and B isolated from *Y. gloriosa* roots.

significantly and in a dose-related manner nitrogen oxide generation in activated macrophages and to reduce the expression of the inducible isoform of nitrogen oxide synthase (iNOS) (*11*). NO produced by iNOS is a key mediator in inflammatory processes, and its production is a crucial step in both the immunoresponsive cells. In a recent study, yuccaols A−C were found to inhibit in a dose-dependent manner vascular endothelial growth factor (VEGF)-induced proliferation, migration, and PAF biosynthesis in Kaposi's sarcoma (KS) cells. PAF is a potent mediator of inflammation, and it is known to promote angiogenesis and in vitro migration of endothelial and KS cells. These results provided the first evidence of the anticancer and antiinvasive properties of yuccaols (*12*). For these reasons we deemed it of interest to investigate another species of the same genus, *Y. gloriosa*. This species is largely cultivated in eastern Georgia, where industrial plantations occupy a total area of about 150 ha. In the past, the great interest in this plant was due to the very high content of steroidal sapogenins and their glycosides and the possibility of using these metabolites for the synthesis of 5α steroid hormones (*1*). Thus, investigations carried out until now on *Y. gloriosa* have been focused only on its saponin constituents.

A preliminary investigation of *Y. gloriosa* bark yielded two very unusual phenolic constituents named gloriosaols A and B (**Figure 1**) (*13*). The very high antioxidant activity exerted by the MeOH extract of *Y. gloriosa* in the Trolox equivalent antioxidant capacity (TEAC) assay encouraged us to further investigate the phenolic fraction, and this study led to the isolation of new phenolic constituents named gloriosaols C−E, along with yuccaols C−E previously isolated from *Y. schidigera* (*6*, *7*) and gloriosaols A and B (*13*). Gloriosaols are very unusual spirostructures made up of C_{15} units probably derived from a flavonoid skeleton and a C_{14} unit corresponding to *trans*-3,3′,5,5′-tetrahydroxy-4′-methoxystilbene linked via γ-lactone rings. They contain the same basic C_{15} and C_{14} structural units of yuccaols C−E but differ from yuccaols C−E in the occurrence of two C_{15} units instead of one. Gloriosaols A and B exhibited the two *p*-hydroxyphenyl rings of the C_{15} units at the opposite side of the stilbenic moiety, gloriosaol C showed the two *p*-hydroxyphenyl rings at the same side of the stilbenic moiety, and in gloriosaols D and E a *p*-hydroxyphenyl ring is oriented to the same side of the stilbenic moiety and the other one is located to the opposite side.

This study deals with the structure elucidation of gloriosaols C−E as well as the evaluation of the radical scavenging activity of the MeOH extract of *Y. gloriosa* bark and gloriosaols A−E in the TEAC assay. The structures of gloriosaols C−E were elucidated by extensive spectroscopic methods including 1D-(^{1}H and ^{13}C) and 2D NMR experiments (DQF-COSY, HSQC, HMBC, and ROESY) as well as HR-ESI-MS analysis. Corresponding to the general structure of gloriosaols A and B and being their diasteroisomers, a tentative configurational assignment for gloriosaols C−E has been proposed by employing the quantum mechanical approach performed on gloriosaols A and B (*13*−*17*).

MATERIALS AND METHODS

Instrumentation. Optical rotations were measured on a Jasco DIP 1000 polarimeter. UV spectra were recorded on a UV-2101PC Shimadzu UV−vis scanning spectrophotometer. IR measurements were obtained on a Bruker IFS-48 spectrometer. Exact masses were measured on a Q-Star Pulsar (Applied Biosystems, Foster City, CA) hybrid quadrupole orthogonal time-of-flight instrument. Electrospray ionization was used in TOF mode at 8.500 resolving power. Samples were dissolved in pure methanol, mixed with the internal calibrant, and introduced directly into the ion source by direct infusion. Calibration was performed on the peaks of cesium iodide and synthetic peptide (TOF positive ion calibration solution) (Bachem Distribution Services GmbH, Weil am Rhein, Germany) at *m*/*z* 132.9054 and 829.5398, respectively. Ions were detected in the mass range of 100−1000 atomic mass units. ESI-MS analyses were performed using a ThermoFinnigan (San Jose, CA) LCQ Deca ion trap mass spectrometer equipped with Xcalibur software. Samples were dissolved in methanol and infused into the ES ionization source using a syringe pump at a flow rate of 3 μL/min. The capillary voltage was at 40 V, the spray voltage was at 4.8 kV and the tube lens offset was at 35 V. The capillary temperature was 220 °C. Data were acquired in MS^1 and MS^n scanning mode operating in positive ion mode. The ^{1}H, gCOSY, ROESY, gHSQC, and gHMBC NMR experiments were run under standard conditions on a Bruker DRX-600 spectrometer at 300 K. The ROESY spectra were executed with a mixing time of 400 ms.

NMR samples were prepared by dissolving gloriosaol C (3.2 mg) and gloriosaols D−E (2.5 mg) in CD_3OD (99.96% D) (Sigma−Aldrich). The spectra were calibrated using the solvent signal as internal standard (^{1}H, δ 3.34 ppm; ^{13}C, δ 49.0 ppm). The NMR data were processed on a Silicon Graphic Indigo2 Workstation using UXNMR software.

Plant Material. The roots of *Y. gloriosa* were collected in December 2003 in the experimental field of the Institute of Pharmacochemistry of the Academy of Sciences, Tbilisi, Georgia. A voucher specimen (no. 259) was deposited at the Institute of Pharmacochemistry.

Extraction and Isolation Procedures. The powdered roots (100 g) of *Y. gloriosa* were extracted with 80% MeOH (1.5 L), yielding 14 g of extract, which was dissolved in water (300 mL) and partitioned with ethyl acetate (100 mL × 5), yielding 10 g of ethyl acetate extract. Part of this extract (2 g) was fractionated on Sephadex LH-20 (100 × 5 cm) using MeOH as mobile phase, and 150 fractions (8 mL) were obtained. Selected fractions were chromatographed by HPLC on an HP 1100 system (Agilent, Palo Alto, CA), on a 300 × 7.8 mm i.d. μ-Bondapack C18 semipreparative column (Waters) by gradient elution of solvent A (H_2O acidified with 0.05% CF_3COOH) and solvent B (CH_3CN acidified with 0.05% CF_3COOH) (Baker Mallinckrodt, Phillipsburg, NJ).

Elution was performed by means of a linear gradient of A/B from 20:80 to 70:30 over 30 min, then an isocratic portion of 70:30 for 10 min, a subsequent gradient to 60:40 in 30 min, and then an isocratic portion for 10 min, at a flow rate of 2 mL/min; the run time was 80 min. The column effluent was monitored at wavelengths of 310 nm, for phenols analysis, and 230 nm, for interfering compounds.

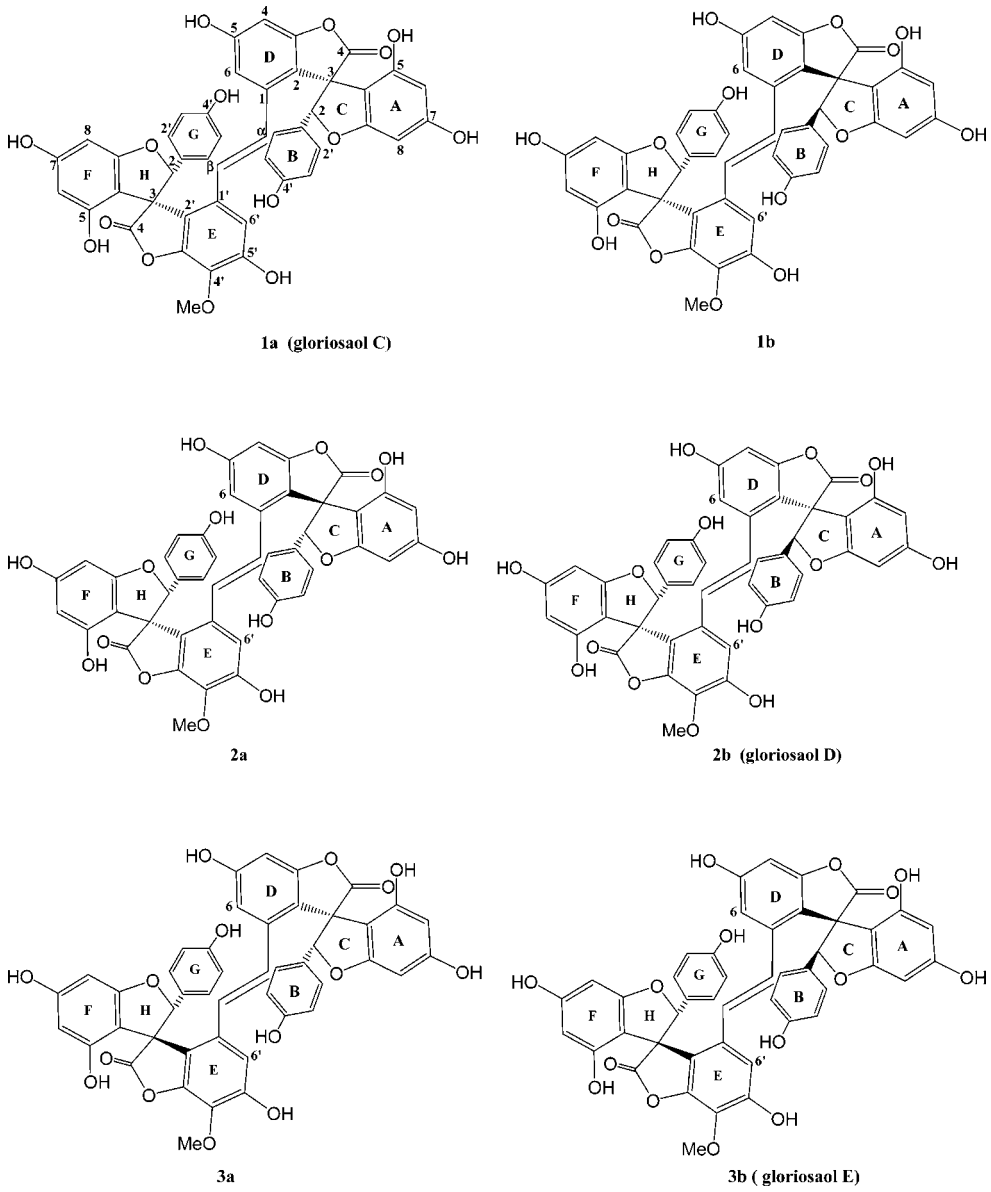

Figure 2. Calculated structures **1a** and **1b**, **2a** and **2b**, and **3a** and **3b** for gloriosaol C, gloriosaol D, and gloriosaol E, respectively.

Submitted to HPLC by using the gradient described above, fractions 74−80 (54 mg) yielded gloriosaols D and E (1.91 mg, t_R 63.51) as an inseparable mixture; fractions 134−138 (6.15 mg) yielded gloriosaol C (**Figure 2**) (3.15 mg, t_R 69.22); fractions 81−120 (115 mg) afforded gloriosaol B (2.5 mg, t_R 60.85) and gloriosaol A (3.72 mg, t_R 65.9); fractions 45−70 (130 mg) gave yuccaol C (3.92 mg, t_R 43.82), yuccaol D (2.85 mg, t_R 46.42), and yuccaol E (1.02 mg, t_R 30.01).

Physical data for gloriosaol C (**1a**): brown amorphous powder; $[\alpha]_D^{20}$ 20.4° (MeOH, *c* 0.1); UV (MeOH), λ_{max} (log ϵ) 320 (3.87), 230 (4.36); IR (KBr), ν_{max} 2918, 1791, 1623, 1509, 1455, 1397, 1202, 1132, 1074, 1020 cm^{-1}; ^1H and ^{13}C NMR data, see **Table 1**; ESI-MS, *m/z* 811 $[M + H]^+$, 833 $[M + Na]^+$; ESI-MS/MS, *m/z* 717 $[M + H - 94]^+$, 419 $[M + H - 392]^+$, 269 $[M + H - 542]^+$; HR-MS (ESI-Q-TOF) $[M + H]^+$, found 811.1688, $C_{45}H_{31}O_{15}$ requires 811.1662.

Physical data for the mixture of gloriosaols D (**2b**) and E (**3b**): brown amorphous powder; UV (MeOH), λ_{max} (log ϵ) 322 (3.85), 230 (4.30); IR (KBr), ν_{max} 2918, 1791, 1623, 1509, 1455, 1397, 1202, 1132, 1074, 1020 cm^{-1}; ^1H and ^{13}C NMR data, see **Table 1**; ESI-MS, *m/z* 811 $[M + H]^+$, 833 $[M + Na]^+$; ESI-MS/MS, *m/z* 717 $[M + H - 94]^+$, 419 $[M + H - 392]^+$, 269 $[M + H - 542]^+$; HR-MS (ESI-Q-TOF) $[M + H]^+$, found 811.1685, $C_{45}H_{31}O_{15}$ requires 811.1662.

Computational Details. To allow a full exploration of the conformational space, MM/MD calculations on each of the gloriosaols were performed using the MMFFs force field and the MonteCarlo Multiple Minimum (MCMM) method of the MacroModel package (*18*). All of the structures so obtained (ca. 1000) were minimized using the Polak-Ribier Conjugate Gradient algorithm (PRCG, maximum derivative of <0.05 kcal/mol). This led to the selection of the lowest energy minimum conformer for each gloriosaol. The initial geometries of the minimum energy conformers for gloriosaols C−E were optimized at the hybrid DFT MPW91PW91 level using the 6-31G(d) basis set (Gaussian 03 software package). GIAO ^1H calculations were performed using the mPW1PW91 functional and the 6-31G(d,p) basis set, using as input the geometry previously optimized at the mPW1PW91/6-31G(d) level (*19*).

Antioxidant Activity. The in vitro antioxidant activities of the compounds (gloriosaols A−E) and the methanol extract of *Y. gloriosa* roots were determined by the Trolox equivalent antioxidant capacity (TEAC) assay as previously reported (*7*). The antioxidant activities are expressed as TEAC values. The TEAC value is defined as the concentration of a standard Trolox solution with the same antioxidant capacity as a 1 mM concentration of the tested compound. In the case of the extract the TEAC value is defined as the concentration of a standard Trolox solution with the same antioxidant capacity as a 1 mg/mL of the tested extract.

Table 1. ^{13}C and ^{1}H NMR Data of Gloriosaols C–E in CD_3OD

	gloriosaol C					gloriosaol D					gloriosaol E				
	δ_C	δ_H (J, Hz)		δ_C	δ_H (J, Hz)		δ_C	δ_H (J, Hz)		δ_C	δ_H (J, Hz)		δ_C	δ_H (J, Hz)		δ_C	δ_H (J, Hz)
ring H			ring C			ring H			ring C			ring H			ring C		
2	94.7	5.99 s	2	94.6	5.98 s	2	93.5	6.03 s	2	91.9	5.58 s	2	91.9	5.58 s	2	93.3	6.01 s
3	60.9		3	60.9		3	61.1		3	60.4		3	60.4		3	61.1	
4	178.6		4	179.3		4	178.6		4	174.6		4	174.6		4	179.5	
ring F			ring A			ring F			ring A			ring F			ring A		
5	154.5		5	154.5		5	154.9		5	154.7		5	154.7		5	154.9	
6	97.3	5.85 d (1.8)	6	97.3	5.86 d (1.8)	6	95.5	5.83 d (2.0)	6	95.7	5.97 d (1.8)	6	95.6	5.96 d (1.8)	6	95.5	5.82 d (1.8)
7	160.9		7	160.9		7	161.5		7	161.5		7	161.5		7	161.5	
8	90.9	6.27 d (1.8)	8	90.9	6.26 d (1.8)	8	89.3	6.22 d (2.0)	8	89.4	6.09 d (1.8)	8	89.3	6.08 d (1.8)	8	89.3	6.22 d (1.8)
9	161.6		9	161.6		9	162.0		9	163.7		9	163.7		9	162.0	
10	106.8		10	106.8		10	105.8		10	103.5		10	103.5		10	105.8	
ring G			ring B			ring G			ring B			ring G			ring B		
1′	126.8		1′	126.8		1′	126.7		1′	127.6		1′	127.6		1′	126.7	
2′	127.3	7.03 d (8.4)	2′	127.3	7.02 d (8.4)	2′	125.9	7.12 d (8.5)	2′	126.8	6.82 d (8.5)	2′	126.8	6.81 d (8.5)	2′	125.9	7.10 d (8.5)
3′	115.6	6.64 d (8.4)	3′	115.6	6.65 d (8.4)	3′	114.3	6.62 d (8.5)	3′	114.3	6.70 d (8.5)	3′	114.3	6.72 d (8.5)	3′	114.3	6.61 d (8.5)
4′	157.6		4′	157.6		4′	157.5		4′	158.4		4′	158.4		4′	157.5	
5′	115.6	6.64 d (8.4)	5′	115.6	6.65 d (8.4)	5′	114.3	6.62 d (8.5)	5′	114.3	6.70 d (8.5)	5′	114.3	6.72 d (8.5)	5′	114.3	6.61 d (8.5)
6′	127.3	7.03 d (8.4)	6′	127.3	7.02 d (8.4)	6′	125.9	7.12 d (8.5)	6′	126.8	6.82 d (8.5)	6′	126.8	6.81 d (8.5)	6′	125.9	7.10 d (8.5)
ring E			ring D			ring E			ring D			ring E			ring D		
1′	130.3		1	136.6		1′	131.5		1	136.0		1′	131.5		1	135.5	
2′	117.9		2	116.4		2′	118.1		2	116.9		2′	118.0		2	116.0	
3′	144.1		3	153.4		3′	144.1		3	154.8		3′	144.9		3	153.8	
4′	132.1		4	97.7	6.38 d (1.8)	4′	132.4		4	96.8	6.40 d (2.0)	4′	132.1		4	96.8	6.35 d (2.0)
5′	150.2		5	150.2		5′	150.4		5	159.3		5′	150.8		5	158.3	
6′	108.2	6.61 s	6	107.4	6.52 d (1.8)	6′	106.7	6.27 s	6	106.9	6.90 d (2.0)	6′	107.7	6.98 s	6	105.8	6.18 d (2.0)
α	126.4	6.41 d (16.1)				α	127.5	6.74 d (16.0)				α	126.6	6.69 d (16.0)			
β	125.7	6.36 d (16.1)				β	122.7	6.67 d (16.0)				β	123.5	6.73 d (16.0)			
OMe	61.1	3.92 s				OMe	60.5	3.90 s				OMe	60.2	3.86 s			

Table 2. $\Delta\delta$ Values of Each Pair of Protons Symmetric with Respect to the Double Bond for Gloriosaols C–E and for the Calculated Structures (**1a**, **1b**, **2a**, **2b**, **3a**, and **3b**)[a]

	$\lvert\Delta\delta\rvert$ gloriosaol C	$\lvert\Delta\delta\rvert$ **1a**	$\lvert\Delta\delta\rvert$ **1b**	$\lvert(\lvert\Delta\delta\rvert$ gloriosaol C – $\lvert\Delta\delta\rvert$ **1a**$)\rvert$	$\lvert(\lvert\Delta\delta\rvert$ gloriosaol C – $\lvert\Delta\delta\rvert$ **1b**$)\rvert$
H-β–H-α	0.05	0.10	0.04	0.05	0.01
H6′ E–H6 D	0.09	0.07	0.78	0.02	0.69
H2 H–H2 C	0.01	0.01	0.45	0.00	0.44
H8 F–H8 A	0.01	0.01	0.44	0.00	0.43
H6 F–H6 A	0.01	0.03	0.18	0.02	0.17
H2′/6′ B–H2′/6′ G	0.01	0.03	0.04	0.02	0.03
H3′/5′ B–H3′/5′ G	0.01	0.01	0.06	0.00	0.05
$\sum \lvert(\lvert\Delta\delta\rvert$ exptl – $\lvert\Delta\delta\rvert$ calcd$)\rvert$				0.12	1.82
	$\lvert\Delta\delta\rvert$ gloriosaol D	$\lvert\Delta\delta\rvert$ **2a**	$\lvert\Delta\delta\rvert$ **2b**	$\lvert(\lvert\Delta\delta\rvert$ gloriosaol D – $\lvert\Delta\delta\rvert$ **2a**$)\rvert$	$\lvert(\lvert\Delta\delta\rvert$ gloriosaol D – $\lvert\Delta\delta\rvert$ **2b**$)\rvert$
H-β–H-α	0.07	0.10	0.01	0.03	0.06
H6′ E–H6 D	0.63	0.53	0.38	0.10	0.25
H2 H–H2 C	0.45	0.87	0.44	0.42	0.01
H8 F–H8 A	0.13	0.63	0.13	0.50	0.00
H6 F–H6 A	0.14	0.89	0.17	0.75	0.03
H2′/6′ B–H2′/6′ G	0.30	0.39	0.26	0.09	0.04
H3′/5′ B–H3′/5′ G	0.08	0.01	0.22	0.07	0.14
$\sum \lvert(\lvert\Delta\delta\rvert$ exptl – $\lvert\Delta\delta\rvert$ calcd$)\rvert$				1.96	0.53
	$\lvert\Delta\delta\rvert$ gloriosaol E	$\lvert\Delta\delta\rvert$ **3a**	$\lvert\Delta\delta\rvert$ **3b**	$\lvert(\lvert\Delta\delta\rvert$ gloriosaol E – $\lvert\Delta\delta\rvert$ **3a**$)\rvert$	$\lvert(\lvert\Delta\delta\rvert$ gloriosaol E – $\lvert\Delta\delta\rvert$ **3b**$)\rvert$
H-β–H-α	0.04	0.15	0.08	0.11	0.04
H6′ E–H6 D	0.80	1.32	0.56	0.52	0.24
H2 H–H2 C	0.43	0.91	0.46	0.48	0.03
H8 F–H8 A	0.14	0.63	0.15	0.49	0.01
H6 F–H6 A	0.14	0.92	0.09	0.78	0.05
H2′/6′ B–H2′/6′ G	0.29	0.38	0.28	0.09	0.01
H3′/5′ B–H3′/5′ G	0.11	0.01	0.16	0.10	0.05
$\sum \lvert(\lvert\Delta\delta\rvert$ exptl – $\lvert\Delta\delta\rvert$ calcd$)\rvert$				2.57	0.43

[a] Comparison for each pair of symmetric protons between experimental (gloriosaols C–E) and calculated (**1a**–**1b**, **2a**-**2b**, **3a**-**3b**) $\Delta\delta$ values.

Table 3. Antioxidant Activity of Methanolic Extract and Gloriosaols A–E in the TEAC Assay

	TEAC assay (mM) ± SD[a]
MeOH extract	5.78 ± 0.10
gloriosaol A	5.55 ± 0.07
gloriosaol B	3.00 ± 0.08
gloriosaol C	5.60 ± 0.01
gloriosaols D–E	4.91 ± 0.10
quercetin	2.60 ± 0.02

[a] $n = 3$.

RESULTS AND DISCUSSION

The MeOH extract of *Y. gloriosa* roots exhibited a strong antioxidant activity, much higher than that of quercetin, used as reference compound, measured in the TEAC assay (**Table 3**). This result prompted us to investigate its phenolic constituents, and the extract, submitted to Sephadex LH-20 and then to RP-HPLC, yielded the new gloriosaol C and the mixture of gloriosaols D and E in a ca. 1:1 ratio, along with yuccaols C−E and gloriosaols A and B (*13*).

Structural elucidation of gloriosaols A and B, previously isolated from *Y. gloriosa* roots, required a careful examination of the available NMR data in combination with quantum mechanical calculations because on the basis of only the extensive NMR analysis the same basic structure was established for the two compounds. The ^{1}H and ^{13}C NMR data for gloriosaol B were almost superimposable on those of gloriosaol A, and as no crucial cross-peak in the ROESY spectrum necessary to determine different structural features could be found, we first decided to clarify whether the compounds were two restricted rotational conformers of a single configurational isomer. Thus, semiempirical calculations of the potential energy surfaces on the configurational model structure suggested by biogenetic considerations, together with the ^{1}H NMR spectra recorded at various temperatures, allowed us to unambiguously exclude such a conformational hypothesis. Following these results, the relative configuration of gloriosaols was determined using a quantum mechanical (QM) strategy based on the calculation of ^{1}H chemical shift values performed on the fully optimized geometries of the diastereoisomers and on their comparison with the corresponding experimental chemical shift values (*14*−*17*). H-6$_{\text{ringD}}$ and H-6′$_{\text{ringE}}$ were identified as the only diagnostic for the configurational assignment of gloriosaols A and B, exhibiting more significant variations of their experimental chemical shift values. Indeed, a clear inversion in the comparisons between experimental (gloriosaols A and B) and calculated (model structures) chemical shift values was observed for such protons. The assignment so obtained was finally validated by careful analysis of the ROESY spectra, which proved to be in accordance with our computational method (*13*).

The molecular weights of gloriosaols C−E, obtained by ESI-MS spectrometry in the positive ion mode, corresponded to those of gloriosaols A and B, each with a pseudomolecular ion $[M + H]^+$ at *m*/*z* 811, corresponding to a molecular formula of $C_{45}H_{31}O_{15}$, and with a sodium adduct $[M + Na]^+$ at *m*/*z* 833 (molecular formula of $C_{45}H_{30}O_{15}Na$). Next, in the MS/MS spectra performed on the peak at *m*/*z* 811 of gloriosaols C−E an intense peak at *m*/*z* 269, interpreted as a protonated stilbene unit, and minor peaks at *m*/*z* 717 and 419 were detected. From these results, gloriosaols C−E were believed to have in common with gloriosaols A and B the basic spirostructure (*13*).

To elucidate their structural features, we then resorted to NMR spectroscopy. Analysis of ^{1}H and ^{13}C NMR data of gloriosaols C−E (**Table 1**), including 2D NMR experiments

(DQF-COSY, HSQC, HMBC), by comparison with those of gloriosaols A and B disclosed again the presence of two identical C_{15} fragments linked to a central stilbenic moiety. In fact, as described previously for gloriosaols A and B, the ^{1}H NMR spectrum of gloriosaols C−E (**Table 1**) also showed signals attributable to four doublets of ortho-coupled aromatic protons, to a trans-disubstituted double bond, to three pairs of meta-coupled protons, to three uncoupled protons, and to a methoxy group. Moreover, the long-range correlations observed in the HMBC spectra allowed us to ascertain the stilbenic core, corresponding to *trans*-3,3′,5,5′-tetrahydroxy-4′-methoxystilbene, already found in gloriosaols A and B. Similarly, the HMBC correlations clearly showed that the attachment of the two C_{15} units to the central stilbenic moiety was identical to that depicted in the general structure of gloriosaols (*13*).

Because we found for both diastereoisomeric gloriosaols A and B ROE effects between H-2 of ring H and H-α, H-*β*, and between H-2 of ring C and H-α, H-*β*, we envisaged the possibility that gloriosaols C−E were further stereoisomers likely differing in the orientation of H-2 and the *p*-hydroxyphenyl ring of the C_{15} unit with respect to the stilbenic moiety. To confirm this deduction, inspection of ROESY spectra was carried out on the three compounds, revealing some structural pieces of evidence, which were useful, as indicated below, in establishing their relative configurations at stereogenic centers C-2 and C-3 of rings H and C. Gloriosaol C showed no ROE cross-peaks between H-2 of ring H and H-α, H-*β* or between H-2 of ring C and H-α, H-*β*, a result suggesting that the two *p*-hydroxyphenyl rings G and B of the C_{15} units were both oriented to the same side of the stilbenic moiety; gloriosaol D exhibited no ROE cross-peaks between H-2 of ring H and H-α, H-*β*, whereas it showed ROE cross-peaks between H-2 of ring C and H-α, H-*β*, thereby allowing us to orient ring G at the same side and ring B at the opposite side of the stilbenic moiety; and, gloriosaol E exhibited ROE effects between H-2 of ring H and H-α, H-*β* but no ROE between H-2 of ring C and H-α, H-*β*, thereby indicating an orientation of ring G at the opposite side and ring B at the same side of the stilbenic moiety. Finally, the subtle structural differences in gloriosaols D and E suggested from the above ROESY data were in agreement with the chromatographic profile showing gloriosaols D and E eluted as a single chromatographic peak.

In the attempt to assign the relative configuration of gloriosaols C−E, a combination of NMR analysis and quantum mechanical method, already applied successfully to the relative assignment of gloriosaols A and B (*13*) was employed. First, for each gloriosaol we built the two possible relative diastereoisomers (**1a** and **1b**, **2a** and **2b**, and **3a** and **3b)** upon inversion of configurations at C-2 and C-3 of ring C. We attributed the label **1a**, **2a**, and **3a** to structures suggested by biogenetic considerations. After conformational search performed at semiempirical (PM3) level on both model structures of each gloriosaol, we optimized the geometries of the so obtained minimum energy conformers for the calculated structures (**1a**−**1b**, **2a**−**2b**, and **3a**−**3b**) employing the DFT MPW91PW91 level and using the 6-31G(d) basis set (Gaussian 03 software package) (*19*). Next, we computed for these model diastereoisomers the ^{1}H chemical shift values by the MPW91PW91/6-31G(d,p) method. Such computational strategy performed on gloriosaols A and B took advantage of the fact that they were diastereoisomeric compounds, and an inversion in the entity of $|\Delta\delta$ exptl − calcd| observed only for H-6_{ringD} and H-$6'_{ringE}$ was shown to be significant in the assignment of the relative configuration of gloriosaols A and B (*13*).

In the current case, the data analysis above outlined could not be performed on gloriosaols C−E, as these molecules are apparently not accompanied by their corresponding relative diastereoisomers. Nevertheless, an inspection of gloriosaols C−E suggested that these compounds, featuring two similar C_{15} units, may be pseudo symmetrical with respect to the double bond and that a comparison between the absolute chemical shift differences of each pair of symmetrical protons with respect to the double bond for each calculated diastereoisomer and the corresponding absolute chemical shift differences reported for each gloriosaol might be considered to be informative. In detail, for gloriosaol C, a careful analysis of the data reported in **Table 2** shows that the structure **1a**, displaying a pseudo C_2 symmetry, reproduced very well the experimental absolute chemical shift differences because both symmetrical protons of the gloriosaol C and **1a** exhibited very small deviations between themselves; in contrast, calculated chemical shift differences for **1b** would suggest a more asymmetrical situation for the natural compound. A similar situation is traced for gloriosaol D, for which the chemical shift calculated differences of all the symmetric protons for **2b** were of the same entity with respect to the corresponding experimental deviations observed in gloriosaol D, whereas more significant deviations were exhibited by the calculated symmetric protons of **2a**.

Finally, for gloriosaol E the calculated data analysis would suggest compound **3b** as the best candidate for the natural compound because the calculated absolute chemical shift differences for the pairs of protons of **3b** were comparable to the experimental, whereas the corresponding results obtained for model **3a** displayed larger differences with respect to the data collected for gloriosaol E. Moreover, a further indication of the relative configuration for gloriosaols C−E was given by comparison of the summation of the absolute values of the differences of the corresponding experimental and calculated chemical shift deviations (Σ |(|$\Delta\delta$| exptl − |$\Delta\delta$| calcd)|) for the calculated diastereoisomers, corroborating the qualitative observations outlined above. Thus, structures **1a**, **2b**, and **3b** have been proposed for gloriosaols C, D, and E, respectively.

The antioxidant activity of gloriosaols A−E was evaluated and compared to that of quercetin in the TEAC assay (**Table 3**). All of the tested samples exhibited strong radical scavenging activity, much higher than that of quercetin and of yuccaols C−E, previously evaluated (*7*). These results show the potential use of *Y. gloriosa* as a source of antioxidant principles. On the other hand, the similarity between the two species regarding the high steroidal saponins content and the occurrence of strong antioxidant phenolic principles suggests that the possible use of *Y. gloriosa* for the same applications as *Y. schidigera* should be explored.

LITERATURE CITED

(1) Skhirtladze, A.; Plaza, A.; Montoro, P.; Benidze, M.; Kemertelidze, E.; Pizza, C.; Piacente, S. Furostanol saponins from *Yucca gloriosa* L. rhizomes. *Biochem. Syst. Ecol.* **2006**, *34*, 809−814.

(2) Cheeke, P. R. Actual and potential applications of *Yucca schidigera* and *Quillaia saponaria* saponins in human and animal nutrition. *Proc. Am. Soc. Anim. Sci.* **1999**, 1−10.

(3) Piacente, S.; Pizza, C.; Oleszek, W. Saponins and phenolics of *Yucca schidigera* Roezl: chemistry and bioactivity. *Phytochem. Rev.* **2005**, *4*, 177−190.

(4) Miyakoshi, M.; Tamura, Y.; Masuda, H.; Mizutani, K.; Tanaka, O.; Ikeda, T.; Ohtani, K.; Kasai, R.; Yamasaki K. Antiyeast steroidal saponins from *Yucca schidigera* (Mohave yucca), a new anti-food-deteriorating agent. *J. Nat. Prod.* **2000**, *63*, 332−338.

(5) Oleszek, W.; Sitek, M.; Stochmal, A.; Piacente, S.; Pizza, C.; Cheeke, P. Steroidal saponins of *Yucca schidigera* Roezl. *J. Agric. Food Chem.* **2001**, *49*, 4392−4396.

(6) Oleszek, W; Sitek, M; Stochmal, A; Piacente, S; Pizza, C; Cheeke, P. Resveratrol and other phenolics from the bark of *Yucca schidigera* Roezl. *J. Agric. Food Chem*. **2001**, *49*, 747−752.

(7) Piacente, S; Montoro, P.; Oleszek, W.; Pizza, C. *Yucca schidigera* bark: phenolic constituents and antioxidant activity. *J. Nat. Prod.* **2004**, *67*, 882−885.

(8) Piacente, S; Bifulco, G.; Pizza, C.; Stochmal, A.; Oleszek, W. A novel phenolic spiro derivative, yuccaone A, from *Yucca schidigera* bark. *Tetrahedron Lett.* **2002**, *43*, 9133−9136.

(9) Olas, B.; Wachowicz, B.; Stochmal, A.; Oleszek, W. Anti-platelet effects of different phenolic compounds from *Yucca schidigera* Roezl. Bark. *Platelets* **2002**, *13*, 167−173.

(10) Olas, B.; Wachowicz, B.; Stochmal, A.; Oleszek, W. Inhibition of oxidative stress in blood platelets by different phenolics from *Yucca schidigera* Roezl. Bark. *Nutrition* **2003**, *19*, 633−640.

(11) Marzocco, S.; Piacente, S.; Pizza, C.; Oleszek, W.; Stochmal, A.; Pinto, A.; Sorrentino, R.; Autore, G. Inhibition of inducible nitric oxide synthase expression by yuccaol C from *Yucca schidigera* Roezl. *Life Sci.* **2004**, *75*, 1491−1501.

(12) Balestrieri, C.; Felice, F.; Piacente, S.; Pizza, C.; Montoro, P.; Oleszek, W.; Visciano, V.; Balestrieri, M. L. Relative effects of phenolic constituents from *Yucca schidigera* Roezl. bark on Kaposi's sarcoma cell proliferation, migration, and PAF synthesis. *Biochem. Pharmacol.* **2006**, *71*, 1479−1487.

(13) Bassarello, C.; Bifulco, G; Montoro, P.; Skhirtladze, A.; Kemertelidze, E.; Pizza, C.; Piacente, S. Gloriosaols A and B, two novel phenolics from *Yucca gloriosa*: structural characterization and configurational assignment by a combined NMR-quantum mechanical strategy. *Tetrahedron* **2007**, *63*, 148−154.

(14) Barone, G.; Duca, D.; Silvestri, A.; Gomez-Paloma, L.; Riccio R.; Bifulco, G. Determination of the relative stereochemistry of flexible organic compounds by *ab initio* methods: conformational analysis and Boltzmann-averaged GIAO ^{13}C NMR chemical shifts. *Chem. Eur. J.* **2002**, *8*, 3240−3245.

(15) Bifulco, G.; Bassarello, C.; Riccio, R.; Gomez-Paloma, L. Quantum mechanical calculations of NMR *J* coupling values in the determination of relative configuration in organic compounds. *Org. Lett.* **2004**, *6*, 1025−1028.

(16) Plaza, A.; Piacente, S.; Perrone, A.; Hamed, A.; Pizza, C.; Bifulco, G. Stemmosides C and D, two novel unusual pregnane glycosides from *Solenostemma argel*: structural elucidation and configurational study by a combined NMR-quantum mechanical strategy. *Tetrahedron* **2004**, *60*, 12201−12209.

(17) Bassarello, C.; Zampella, A.; Monti, M. C.; Gomez-Paloma, L.; D'Auria, M. V.; Riccio, R.; Bifulco, G. Quantum mechanical calculation of coupling constants in the configurational analysis of flexible systems: determination of the configuration of callipeltin A. *Eur. J. Org. Chem.* **2006**, 604−609.

(18) MacroModel, version 8.5, Schrödinger LLC, New York, 2003.

(19) Adamo, C.; Barone, V. Exchange functionals with improved long-range behavior and adiabatic connection methods without adjustable parameters: the mPW and mPW1PW models. *J. Chem. Phys.* **1998**, *108*, 664−675.

Received for review April 18, 2007. Revised manuscript received May 29, 2007. Accepted May 31, 2007.

JF071131N