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A novel phenolic spiro derivative, Yuccaone A, from *Yucca schidigera* bark

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Abstract—A new, very unusual, phenolic constituent based on a spiro benzopyran-4-cyclopentan-3-one system has been isolated from *Yucca schidigera* bark and its structure has been established by ESI-MS and NMR experiments. In particular the relative configuration of the molecule has been defined combining the NMR data with ab initio conformational studies; the minimum energy conformers for the eight possible diastereomers were compared to the NMR experimental data in order to assess the diastereomer fitting of all the *J* coupling values and the dipolar values.

Yucca schidigera is a plant that grows widely in Mexico, and is well known due to its very high (10% of DM) content of steroidal saponins. The extract of this plant is regarded as a *GRAS* (*generally recognized as safe*) product, approved by the FDA as a food additive and finds wide commercial application as a food, cosmetic and pharmaceutical additive.[1] *Yucca* extract and powder have been used for the reduction of ammonia and other odors from poultry excreta in poultry farms.[2]

Yucca schidigera is also known to contain resveratrol (*trans*-3,4′,5-trihydroxystilbene, THS),[3] a natural phytoalexin known for its antioxidant properties, occurring in the skin of grapes,[4,5] in mulberries, in peanuts,[6] and in some medicinal plants. It is reported that due to its antioxidant activity, at least in part, resveratrol is responsible for reduced risk of cardiovascular disease in man from long term moderate consumption of red wine.[7] Resveratrol is also reported to exert antimutagenic,[3] antiviral,[8] cancer chemopreventive,[9] apoptosis inductive,[10] dioxin toxicity preventing,[11] antiallergic[12] and phytoestrogenic[13] activities.

These multifunctional activities encourage research for compounds structurally related to resveratrol for possible pharmaceutical application. Thus, we investigated the phenolic constituents of yucca bark, finding three unusual spiro derivatives made up of a C_{15} and a C_{14} units closely related to a flavonoid skeleton and resveratrol, respectively.[14] We now wish to report the isolation and structure determination of yuccaone A, a spiro benzopyran-4-cyclopentan-3-one derivative.

Isolation. Yucca (*Y. schidigera*) bark was obtained from Desert King Int., Chula Vista, CA, USA. Powdered yucca bark (100 g) was extracted overnight at room temperature with MeOH (200 mL). The extract was condensed to 20 mL and loaded onto a Sephadex LH-20 (3×40 cm) column. The column was washed with MeOH and 10 mL samples were collected. Samples were monitored with TLC (cellulose, DC-Fertigplatten) developed with 15% AcOH and visualized under UV light (366 nm). Samples showing similar patterns were combined. Ten fractions were obtained. Fraction II (80 mg) was loaded onto a reversed phase C18 (1.2×30 cm) column. The column was washed with 22% MeCN in H_3PO_4 at a flow rate of 0.5 mL/min. 3 mL fractions were collected and monitored with HPLC (DAD detector, C18 Eurospher 4.6×250 column, 22% MeCN in H_3PO_4, flow rate 1 mL/min). Yuccaone A (12 mg), showing DAD absorption maxima at 210 and 276 nm was isolated.

Structure elucidation. Yuccaone A showed in the ESI MS spectrum (negative mode) a quasi-molecular ion peak at m/z 513. The molecular formula was unequivocally established to be $C_{29}H_{22}O_9$ by HREI MS (514.1256 found, 514.1264 calcd). The ^{13}C NMR spectrum showed 25 signals, four of which being for two carbons. On the basis of the DEPT spectrum, these signals were partitioned into one carbonyl carbon (δ 208.3), seven phenolic functions (δ 159.2, 158.7, 158.6,

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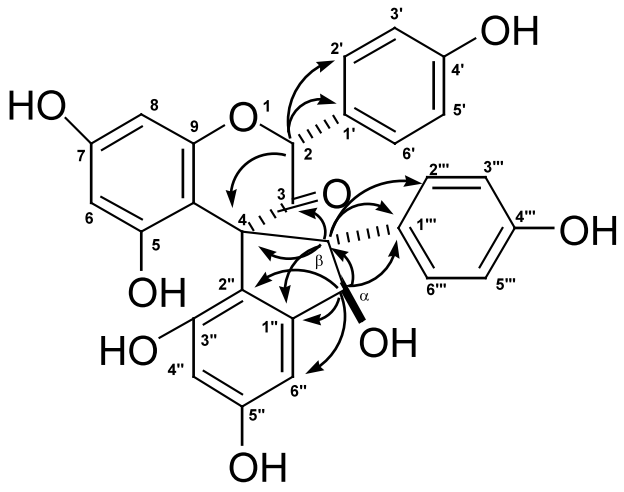

Figure 1. Diagnostic HMBC correlations for yuccaone A (**1**).

158.5, 158.0, 157.9, 154.6), five aromatic quaternary carbons (δ 149.3, 128.9, 127.2, 121.1, 106.7), one aliphatic quaternary carbon (δ 63.0), twelve methine aromatic carbons (δ 131.5×2, 130.7×2, 116.4×2, 115.6×2, 103.0, 102.7, 98.8, 96.4) and three methine aliphatic carbons (δ 84.4, 76.5, 64.0) The ^{1}H NMR spectrum of yuccaone A displayed four doublets at δ 6.95 (2H, d, J=8.0 Hz), 6.80 (2H, d, J=8.0 Hz), 6.78 (2H, d, J=8.0 Hz) and 6.67 (2H, d, J=8.0 Hz), typical of *ortho*-coupled aromatic protons. Also evident were four signals at δ 6.48 (1H, d, J=1.3 Hz), 6.10 (1H, d, J=1.3 Hz), 6.07 (1H, d, J=1.3 Hz), 5.89 (1H, d, J=1.3 Hz) ascribable to two pairs of *meta*-coupled protons and three signals at δ 5.49 (1H, d, J=9.2 Hz), 4.73 (1H, d, J=9.2 Hz) and 3.74 (1H, s). The DQF-COSY experiment allowed to establish the couplings between the signals at δ 6.67 and 6.78, 6.80 and 6.95, 6.10 and 6.48, 5.89 and 6.07, 4.73 and 5.49. From the HSQC correlations it was possible to deduce the occurrence of a monosubstituted phloroglucinol ring, a 4,5-disubstituted resorcinol ring and two *para*-hydroxy substituted phenyl rings. The complete elucidation of the structure of yuccaone A could be achieved by the HMBC experiment, which showed the correlations reported in Fig. 1. On the basis of the observed correlations it was possible to deduce that yuccaone A was made up of two portions: one of 15 carbons probably derived from a flavonoid skeleton and the other one of 14 carbons. The manner of the attachment of the units could be unambiguously derived from the long-range correlations between the proton signal at δ 3.74 and the carbon resonances at δ 63.0, 127.2, 130.7 and 208.3, the proton signal at δ 4.73 and the carbon resonances at δ 63.0, 76.5, 121.1, 128.9, 131.5, 149.3 and 208.3, the proton signal at δ 5.49 and the carbon resonances at δ 64.0, 121.1 and 149.9 which allowed us to deduce the spiro benzopyran-4-cyclopentan-3-one structure presented in Fig. 2.

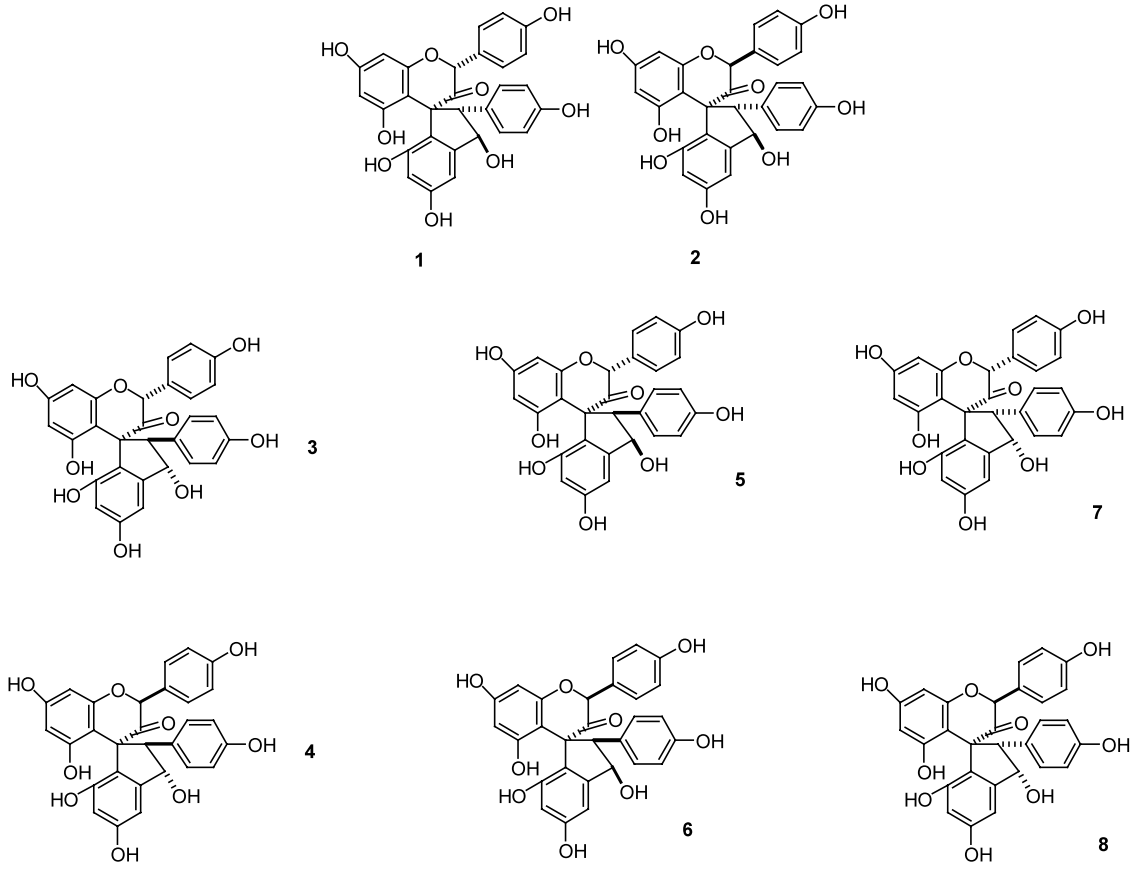

Figure 2. Yuccaone A (**1**) and all its possible relative diastereomers (**2**–**8**).

Table 1. Calculated parameters relative to the significant atoms H-α/H-β and H2/H2‴, for the set of diastereomers **1**–**8***

	Dihedral angle H-α-C–C-H-β	Calculated ^3J for H-α and H-β	Arrangement and distance for H-α and H-β	Distance H-2/H-2‴
Calculated:				
1	−164.3	**9.45**	***trans* 3.03**	**3.88**
2	−164.0	**9.45**	***trans* 3.01**	5.21
3	+102.4	0.95	***trans* 2.78**	4.45
4	+105.6	1.12	***trans* 2.78**	5.33
5	−30.8	**7.82**	*cis* 2.31	4.62
6	−31.3	**7.77**	*cis* 2.31	5.32
7	−39.3	4.53	*cis* 2.26	**3.78**
8	−38.3	4.69	*cis* 2.25	5.11

* The values compatible with the experimental data are shown in bold. *J* values are reported in Hz and interatomic distances in Ångstroms.

Determination of the relative configuration. Yuccaone A shows a very rigid central core, i.e. a spiro benzopyran-4-cyclopentan-3-one system, which allowed to speculate on the relative configuration of the molecule combining the available NMR data with ab initio conformational studies on the eight possible diastereomers, depicted in Fig. 2 as **1**–**8**. A preliminary molecular mechanics and dynamics study of all the eight diastereomers afforded eight minimum energy conformers that were further refined by a HF optimization, using the 6-31G* basis set. The minimum energy conformers for **1**–**8** were compared to the NMR experimental data in order to assess the stereomer fitting all the *J* coupling values and the dipolar values. Also, we have ideally grouped all the stereomers in four couples (**1**–**2**, **3**–**4**, **5**–**6**, **7**–**8**), because each of the two members of these couples shows a similar central core and differs just for the configuration of position 2. For the sake of simplicity, among the available experimental data, we considered three significant evidences: a ^3J H–H coupling of 9.2 Hz for the two protons H-α and H-β, the relative arrangement of the protons H-α and H-β, and a ROESY correlation between H-2 and H-2‴ (Table 1). In particular, the observed ^3J H–H coupling of 9.2 Hz relative to the protons H-α and H-β readily allowed to rule out diastereomers **3**–**4**, and **7**–**8**. Indeed, calculated ^3J values (the calculation of the vicinal coupling constants $^3J_{HH}$ from dihedral angles, taking into account the electronegativity of the substituents of the stereocenters, was done using the Altona equation)[15] of 0.95 and 1.12 Hz for stereomers **3** and **4**, and of 4.53 and 4.69 Hz for stereomers **7** and **8** (Table 1), were significantly different from the experimental. The dihedral angles of –30.8 and –31.3 for stereomers **5** and **6**, were indicative of a ^3J values of 7.82 and 7.77, respectively. These values differ from the experimental, but the entity of disagreement did not allow to safely rule out stereomers **5** and **6**. Nevertheless, diastereomers **5** and **6** showed a *cis* arrangement and a short distance (2.31 Å) between protons H-α and H-β, in contrast with the lack of a correlation in the ROESY spectra for these two resonances. Moreover, a ROESY correlation was observed between proton H-2 and H-2‴, which was not compatible with the geometries found for stereomers **5** and **6** (Table 1).

Stereomers **1** and **2** have a central skeleton in agreement with the data observed for H-α and H-β. Dihedral angles of −164.3 and −164.0 for **1** and **2** indicated calculated ^3J values for these protons very close to the experimental (9.45 versus 9.2) and a *trans* arrangement which was in agreement with the ROESY data. Nevertheless, among these two stereomers, only **1** shows a distance between the protons H2/H2‴ shorter than 4 Å, compatible with a cross-peak observed in the ROESY spectrum. All these data were in agreement with the relative configuration indicated in **1**. Thus, structure **1** has been assigned to yuccaone A.

The spiro-structures of yuccaone A (**1**) and of yuccaols A–C[14] are very unusual; they represent the only examples of naturally occurring phenolic spiro derivatives made up of C_{15} and C_{14} units. The only other case of a spiro derivative related to yucca phenolics is larixinol, a spirobiflavonoid isolated from *Larix gmelini*, made up of two C_{15} units both of flavonoidic origin.[16]

Yuccaone A (**1**), 0.012 g; ESI-MS *m*/*z* 513 $[M-H]^-$; HREI MS (514.1256 found, 514.1264 calcd). ^{1}H NMR (CD_3OD, 600 MHz) δ 3.74 (1H, s, H-2), 4.73 (1H, d. J=9.2 Hz, H-β), 5.49 (1H, d. J=9.2 Hz, H-α), 5.89 (1H, d, J=1.3 Hz, H-8), 6.07 (1H, d, J=1.3 Hz, H-6), 6.10 (1H, d, J=1.3 Hz, H-4″), 6.48 (1H, d, J=1.3 Hz, H-6″), 6.67 (2H, d, J=8.0 Hz, H-3′, H-5′), 6.78 (2H, d, J=8.0 Hz, H-2′, H-6′), 6.80 (2H, d, J=8.0 Hz, H-3‴, H-5‴) and 6.95 (2H, d, J=8.0 Hz, H-2‴, H-6‴); ^{13}C NMR (CD_3OD, 150 MHz) δ 208.3 (C-3), 159.2 (C-5″), 158.7 (C-5), 158.6 (C-4′), 158.5 (C-7), 158.0 (C-9), 157.9 (C-4‴), 154.6 (C-3″), 149.3 (C-1″), 131.5 (C-2‴, C-6‴), 130.7 (C-2′, C-6′), 128.9 (C-1‴), 127.2 (C-1′), 121.1 (C-2″), 116.4 (C-3‴, C-5‴), 115.6 (C-3′, C-5′), 106.7 (C-10), 103.0 (C-4″), 102.7 (C-6″), 98.8 (C-6), 96.4 (C-8), 84.4 (C-2), 76.5 (C-α), 64.0 (C-β), 63.0 (C-4).

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