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Spirocurcasone, a Diterpenoid with a Novel Carbon Skeleton from *Jatropha curcas*

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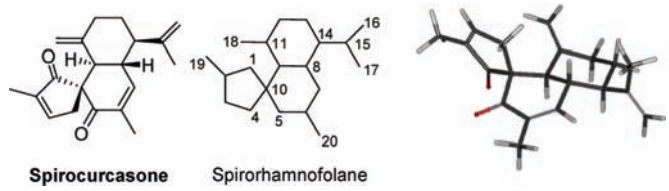
ABSTRACT



Spirocurcasone (14), a diterpenoid possessing the unprecedented "spirorhamnofolane" skeleton, was isolated from the root barks of *Jatropha curcas*, a plant extensively cultivated throughout the world, along with 11 known and two other new diterpenoids. The stereostructure of spirocurcasone was established using HRESIMS, NMR, and quantum mechanical calculation of the electronic circular dichroic (ECD) spectrum. Some of the isolated diterpenoids showed a potent activity against L5178Y, a mouse lymphoma cell line.

Jatropha curcas L. (Euphorbiaceae) is a drought-resistant shrub originating in Central and South America and now widely distributed in tropical and subtropical areas of Africa and Southeast Asia. *J. curcas* has long been used around the world as a source of lamp oil and soap and also as a hedging plant. In recent years interest in this plant has experienced an inconceivable explosion ("the Jatropha fever")[1] due to the possible use of its seeds as a sustainable source for biodiesel production, outperforming biodiesels from rapeseed, sunflower, and soya bean oil in terms of performance, efficiency, and emissions, also in standard diesel engines.

In many countries, extensive cultivation of *J. curcas* have been implanted in areas that do not compete with food cultivation, such as nonarable wastelands. For example, in 2007, China claimed to have 2 million hectares of *J. curcas* already under cultivation and announced plans to plant an additional 11 million hectares across its southern states by 2010.[1]

Extracts from *J. curcas* have also found a number of traditional medical uses and have been intensively investigated for their secondary metabolite content.[2−5] This plant continues to attract the interest of natural products chemists, and the most recent report about its chemical composition has disclosed the presence of jatrophalactam, a diterpenoid lactam possessing a novel tricyclic skeleton.[6]

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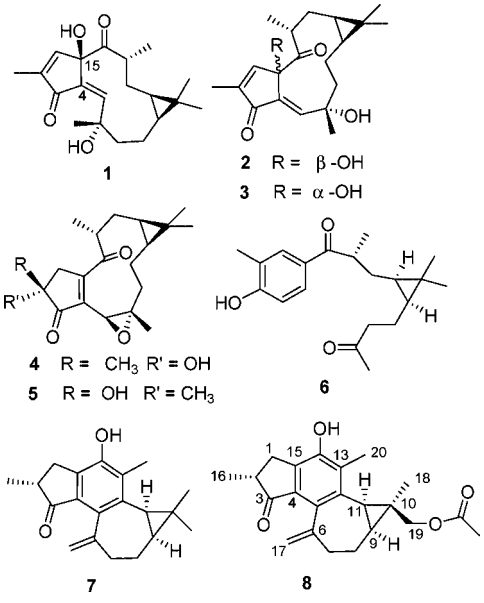
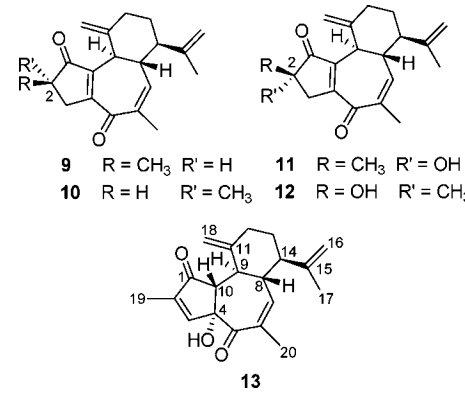
As a result of a bioassay-guided purification of crude extracts obtained from *J. curcas* root barks, we have now isolated 11 known (**1**−**7** and **9**−**12**) and three new (**8**, **13**, **14**) diterpenoids, including spirocurcasone (**14**), a novel diterpenoid possessing an unprecedented carbon framework that includes a spiro[4,5]decane junction. Herein we report the isolation and the structural elucidation of the new compounds together with data about the antiproliferative activity of all the isolated diterpenoids.

The dried and powdered root barks of *J. curcas* were extracted at room temperature sequentially with hexane and EtOAc. Both of the obtained crude extracts proved to be active in preliminary cytotoxicity tests and were subjected to column chromatography fractionation. Selected bioactive fractions were further purified over silica gel HPLC to yield five diterpenoids of the jatrogrossidentadione family (**1**−**5**),[7,8] multidione (**6**),[9] jatropholone B (**7**),[10] curcusones A−D (**9**−**12**),[11] and three new diterpenoids that we have named acetoxyjatropholone (**8**), curcusone E (**13**), and spirocurcasone (**14**).



Acetoxyjatropholone (**8**),[12] $C_{22}H_{26}O_4$ by HR-ESIMS, was isolated as a colorless amorphous solid. Its ^{1}H and ^{13}C NMR data were fully assigned[12] with the help of 2D NMR experiments and appeared strongly suggestive of a molecular architecture of the jatropholone type. Particularly, the network of the key HMBC cross-peaks (e.g., H_2-1 with C-4, C-14, and C-15; H-2 with the ketone carbonyl C-3, C-15, and C-4; the sp^2 methylene H_2-17 with C-5, C-6, and C-7; the cyclopropane methine H-11 with C-5, C-10, C-12, and the methyl-linking C-13) indicated a structure comprising a 5/6/7/3 ring system. Accordingly, the ^{1}H NMR spectrum of **8** closely resembled that of jatropholone B (**7**)[10] with the exception of the lack of one of the two high-field methyl singlets, and the appearance of an oxygenated diastereotopic methylene (H_2-19, δ 4.11 and 3.95) and an acetyl singlet (δ 2.12). The HMBC cross-peak of the above methylene protons with C-9, C-10, C-11, C-18, and the acetyl ester carbonyl indicated the attachment of an acetoxymethyl group at the cyclopropane unprotonated carbon C-10. The β-orientation of this group was deduced on the basis of the ROESY cross-peaks H_3-18/H-11, H_3-18/H-9, and H_2-19/H-8a.



Curcusone E (**13**),[13] $C_{20}H_{24}O_3$ by HR-ESIMS, is a new rhamnofolane diterpene of the curcusone type.[11] It differs from curcusones C (**11**) and D (**12**) in the positional isomerization of both the hydroxyl group and one of the double bonds. Both these changes occurred at the five-membered ring, and accordingly, the ^{1}H and ^{13}C NMR resonances of the six-membered ring nuclei (and those of the attached substituents), fully assigned through 2D NMR experiments (COSY, HSQC, HMBC) were suggestive of an unchanged arrangement compared to the other curcusones. On the other hand, in **13** Me-19 (δ_H 1.83) is an allylic methyl showing HMBC cross-peaks with the ketone carbonyl at C-1 (δ_C 204.1) and with two further sp^2 carbons, the unprotonated C-2 (δ_C 145.7) and the methine C-3 (δ_C 152.1). In turn, the methine proton H-3 showed HMBC cross-peaks with two ketone carbonyls, C-1 and C-5 (δ_C 206.3), with the oxygenated unprotonated carbon C-4 (δ_C 88.9) and with the methine at C-10 (δ_C 57.4), whose relevant proton (H-10, δ_H 3.32) was coupled with H-9 in the COSY spectrum. Once the planar structure of **13** was defined, its relative configuration could be established on the basis of 2D NMR ROESY cross-peaks. In particular, coupling of H-14 with H-9 and of Me-17 with H-8 indicated the relative arrangement around the six-membered ring, supported by the high proton−proton

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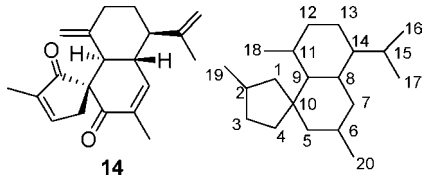
(12) **Acetoxyjatropholone** (**8**): colorless amorphous solid; $[\alpha]^{26}_D$ +18.1 (*c* 0.01 $CHCl_3$); UV (MeOH) λ_{max} (log ε) 275 (3.84), 220 (4.11) 204 (4.30) nm; ^{1}H and ^{13}C NMR, see Table S5 (Supporting Information); HR-ESIMS *m/z* 377.1734 ([M + Na]$^+$ calcd for $C_{22}H_{26}NaO_4$ 377.1729).

(13) **Curcusone E** (**13**): colorless amorphous solid; $[\alpha]^{26}_D$ −155.5 (*c* 0.03 $CHCl_3$); UV (MeOH) λ_{max} (log ε) 239 (3.55) 207 (4.05) nm; ^{1}H and ^{13}C NMR, see Table S6 (Supporting Information); HR-ESIMS *m/z* 335.1630 [M + Na]$^+$ (calcd for $C_{20}H_{24}NaO_3$ 335.1623).

coupling constants ($J = 8.5$ Hz) showed by H-8 with both H-9 and H-14, indicative of *pseudo* axial−axial relationships. Finally, the ROESY cross-peaks of OH-4 with H-9 and of H-10 with H-18a fully defined the relative configuration of the new curcusone E (**13**).



14

Figure 1. Spirocurcasone (**14**) and its new diterpene skeleton, named spirorhamnofolane.

Spirocurcasone (**14**, Figure 1)[14] was isolated as a colorless amorphous solid. HR-ESIMS established the molecular formula $C_{20}H_{24}O_2$, with nine degrees of unsaturation. The ^{1}H NMR spectrum of **14** (Table S4 in Supporting Information) showed the presence of six broad singlets in the region between δ 4.10 and 7.30, a series of multiplets between δ 1.50 and 3.20, and three allylic methyl singlets (δ 1.72, 1.74, and 1.84). The ^{13}C NMR spectrum of **14** (Table S4 in Supporting Information) showed 20 carbon signals that, with the help of the 2D NMR HSQC experiment, were sorted into two ketone carbonyls, eight alkene carbons (two methylenes, two methines, and four unprotonated carbons, indicative of four carbon−carbon double bonds), three methyls, three sp^3 methylenes, three sp^3 methines, and one sp^3 unprotonated carbon (δ 60.7). This preliminary analysis pointed to a tricyclic skeleton for spirocurcasone (**14**).

Analysis of the COSY spectrum indicates that the two structural moieties drawn with bold lines in Figure 2 are (1)

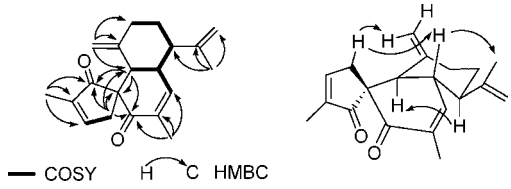


Figure 2. COSY and key HMBC (left) and ROESY (right) correlations of spirocurcasone.

a six-carbon fragment going from the sp^2 methine H-7 to H_2-12, with a methine branching, and (2) a two-carbon fragment including only the downfield shifted sp^2 methine H-3 (δ 7.27) and the diastereotopic methylene H_2-4 (δ 3.17 and 2.42). The HMBC cross-peaks (Figure 2) of H_2-18 with C-9, C-11, and C-12 defined the structure of the six-membered ring, which must link an isopropenyl group at C-14, as indicated by the cross-peaks of H_3-17 with C-14, C-15, and C-16. The allylic methyls H_3-19 and H_3-20 showed two parallel series of HMBC correlations, namely, with a ketone carbonyl (C-1 and C-5, respectively) and with two sp^2 carbons (C-2/C-3 and C-6/C-7, respectively). This allowed the extension of the two spin systems evidenced by the COSY spectrum, thus building two α-methyl-α,β-unsaturated ketones. Finally, the spiro-junction connecting a five- and a six-membered ring was unambiguously deduced by the HMBC correlations of H_2-4 and H-9 with the same carbon atoms, namely, the two ketone carbonyls (C-1 and C-5) and the unprotonated spiro-carbon at C-10 (Figure 2). Hence, the planar structure of **14** was determined as a diterpenoid with a novel carbon skeleton, for which we propose the trivial name of spirorhamnofolane, characterized by an unprecedented spiro-connection between a decalin system and a five-membered ring (see Figure 1).

The relative configuration of spirocurcasone has been assigned by the ROESY correlations H-14/H-9, H-4a/H-18b, H-8/Me-17, and H-8/H-4a (Figure 2). Owing to the unprecedented tricyclic framework of spirocurcasone (**14**), an unambiguous determination of its absolute configuration should be achieved.

We decided to face this problem by measurement of the electronic circular dichroism (ECD) spectrum and comparison with the ECD spectrum predicted from quantum mechanical time dependent density functional theory (TDDFT) calculations, a recent approach increasingly applied for the determination of absolute configurations of natural products.[15] The conformational analysis of one of the two possible enantiomers of spirocurcasone (the one shown in Figure 1 as **14**) was performed by using the simulated annealing procedure (INSIGHT II package). This afforded a set of conformers that were ranked on the basis of their conformational energy values and grouped into two families within 5 kcal/mol, which were further optimized with the software package Gaussian 03[16] by using DFT at the RB3LYP/6-31G(d) level (Figure 3). The excitation energies as well as

Figure 3. Optimized geometries of the conformational families (**14A**, left; **14B**, right) of **14** at the B3LYP/6-31G(d) level.

the oscillator and rotatory strengths of the electronic excitation were calculated for both conformational families using

(14) **Spirocurcasone** (**14**): $C_{20}H_{24}O_2$, colorless amorphous solid; $[\alpha]^{26}_D$ + 3.5 (*c* 0.02 $CHCl_3$); UV (MeOH) λ_{max} (log ε) 230 (3.32) nm; CD (MeOH) ε_{max} ($\Delta\varepsilon$) 208.0 (+ 2.9) 266.5 (- 5.6); ^{1}H and ^{13}C NMR see Table S4 (Supporting Information); ESI-MS *m*/*z* 297 $[M + H]^+$ 319 $[M + Na]^+$; HR-ESIMS *m*/*z* 319.1669 $[M + Na]^+$ (calcd for $C_{20}H_{24}NaO_2$ 319.1674).

(15) Stephens, P. J.; Pan, J. J.; Krohn, K. *J. Org. Chem.* **2007**, *72*, 7641.
(16) For details about Gaussian 03, see Supporting Information.

the TDDFT methodology, and their ECD spectra were then simulated by overlapping the Gaussian functions for each transition.[17]

In order to obtain the final ECD spectrum, the simulated spectra of the two conformational families were averaged on the basis of their Boltzmann distribution (**14A**, 65%; **14B**, 35%) and were UV corrected. Figure 4 shows the close

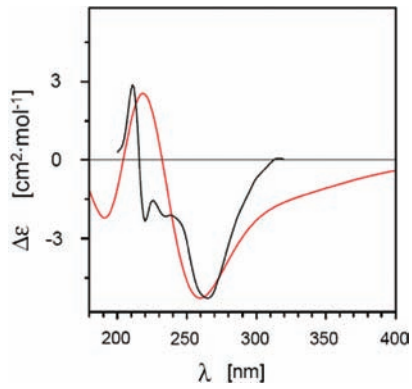


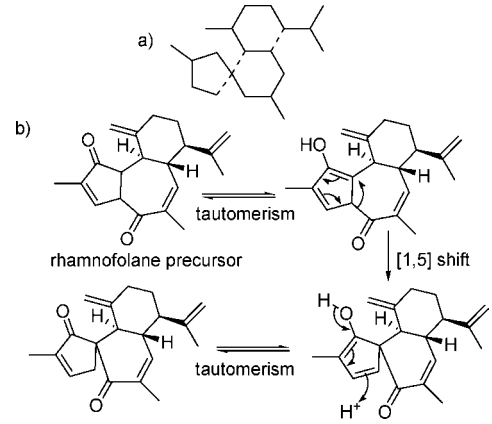
Figure 4. Experimental ECD spectrum (black) and conformationally averaged calculated ECD spectrum (red) of **14**.

similarity between the obtained theoretical curve and the experimental spectrum, which allowed a confident assignment of the absolute configuration of spirocurcasone as shown in **14**.

The novel spirorhamnofolane skeleton of **14** could biosynthetically derive from either a new folding of the linear diterpenoid precursor (Scheme 1, a) or a rearrangement of the rhamnofolane skeleton. A possible mechanism for the latter option, envisaging a [1,5]-sigmatropic alkyl shift, is proposed in Scheme 1b.

All of the 14 diterpenes obtained in this study have been tested for antiproliferative activity on the L5178Y (mouse lymphoma) cell line. A very potent activity (data expressed as IC_{50} in μg mL^{-1}) was exhibited by curcusones A (**9**, 0.21), B (**10**, 0.27), C (**11**, 0.08), and D (**12**, 0.16) and by compounds **1** (0.60), **3** (0.85), **4** (0.20), and **5** (0.24). On the other hand, multidione (**6**, 5.5), jatropholone (**7**, 7.5), acetoxyjatropholone (**8**, 2.5), and compound **2** (2.1) showed a moderate activity, whereas the remaining compounds **13** and **14** were practically inactive (>10). The mechanism(s) of this antiproliferative activity has not been investigated,

Scheme 1. Proposed Biogenetic Origins for the Spirorhamnofolane Skeleton of Spirocurcasone: (a) New Folding of the Diterpenoid Precursor; (b) Rearrangement of a Rhamnofolane Precursor



however, the presence of Michael acceptors, in some cases associated to cytotoxic activities,[18] should not fulfill the structural requirements of the pharmacophoric regions, since the inactive compounds **2**, **13**, and **14** all encompass more than one Michael acceptor sites in their structures.

In conclusion, analysis of root barks of *J. curcas*, a plant extensively cultivated throughout the world, afforded 11 known and 3 new diterpenoids, one of which showed an unprecedented carbon skeleton, named spirorhamnofolane. In addition, some of the isolated compounds showed a potent activity against the mouse lymphoma cell line, worthy of further investigation. In this regard, the millions of hectares cultivated with *J. curcas* are an incredible opportunity to gain access to potentially unlimited amounts of structurally intriguing and biologically interesting secondary metabolites.

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Supporting Information Available: Detailed isolation procedure, data about pharmacological tests and quantum mechanical ECD calculations, NMR spectra, and tables of NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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