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# A complex of 5-hydroxypyrrolidin-2-one and pyrimidine-2,4-dione isolated from *Jatropha curcas*

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**Abstract**

A complex of 5-hydroxypyrrolidin-2-one and pyrimidine-2,4-dione was isolated from the leaves of *Jatropha curcas* L. by extraction with ethyl acetate and subsequent fractionation of the extract by column chromatography on Sephadex LH20, silica gel and fractogel TSK HW 40. Final purification was carried out by HPLC on a preparative RP-18 column and the structure was proposed mainly by mass spectrometry, $^{1}$H- and $^{13}$C-NMR. © 1998 Elsevier Science Ltd. All rights reserved.

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## 1. Introduction

*Jatropha curcas* L. is cultivated as a medicinal plant in many tropical and subtropical countries. The leaves and also the other parts of the plant are used for the treatment of various diseases. Compounds that have been isolated from *J. curcas* leaves include the flavonoids apigenin and its glycosides vitexin and isovitexin, the sterols stigmasterol, β-D-sitosterol and its β-D-glucoside (Chhabra, Mahunnah, & Mshiu, 1990). Furthermore *J. curcas* leaves were reported to contain steroid sapogenins, alkaloids, the triterpenae alcohol, 1-triacontanol and a dimer of a triterpene alcohol (Neuwinger, 1994).

Although Kuhnt, Pröbstle, Rimpler, Bauer, and Heinrich, 1995 isolated (*R*)-5-hydroxypyrrolidin-2-one from the leaves of *Hyptis verticillata* Jacq. (Labiatae) (Kuhnt et al., 1995), there is no report in the literature concerning the isolation of this compound from plants belonging to the Euphorbiaceae.

## 2. Results and discussion

Fresh leaves were extracted with ethyl acetate and the extract subsequently fractionated on Sephadex LH 20, silica gel and fractogel TSK HW40. Bio-assay-guided isolation was done by testing the anti-inflammatory using a carrageenan-induced rat paw edema-test (Winter, Risley, & Nuss, 1962). Final purification of the substance was achieved by HPLC on a preparative RP-18 column. The pure compound was white, crystalline and soluble in water and methanol.

The use of 2-D NMR techniques allowed the assignment of all the $^{1}$H- and $^{13}$C-NMR resonances and a nonambiguous determination of the structure of 5-hydroxypyrrolidine-2-one (**1**) and pyrimidin-2,4-dione (uracil) (**2**). By means of an H,H-COSY (Aue, Bartholdi, & Ernst, 1976) spectrum the resonances were assigned to two spin systems (3-H2/4-H2/5-H, **1**; 45-H/6-H, **2**). On the basis of the known $^{1}$H chemical shifts and proton–proton couplings, the unambiguous assignment of the protonated carbon atoms was carried out using a HSQC (Bodenhausen & Ruben, 1980) spectrum, which reveals the one-bond C–H connectivities. The HMBC (Bax & Summers, 1986) spectrum enabled us to assign the nonprotonated carbon atoms.

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The NMR data of **1** are in good agreement with those of the synthetic racemate (Farina, Martin, Paredes, Ortega, & Tito, 1984) and the natural (*R*)-5-hydroxypyrroldin-2-one (Kuhnt et al., 1995). Additionally, the structures of **1** and **2** were corroborated by GC–MS analysis after trimethylsilylation.

From the foregoing, it was assumed that the two substances which occur in the ratio of 1:1 form a complex, which could not be separated by HPLC and TLC. Further investigations on the pharmacological action of this novel complex are in progress.

## 3. Experimental

### 3.1. General

NMR: NMR spectra were recorded with a Varian UNITYplus-400, 5 mm inverse probe head; solvent: $[D_4]$MeOH; *T* 303 K. The MeOH signal was used as an internal standard ($^1$H: $\delta = 3.3$, $^{13}$C, $\delta$ 49.0). The parameters were as follows: COSY: 45° mixing pulse. HMBC: phase-sensitive using TPPI, delay to achieve long-range couplings 71 ms.

GC–MS: Finigan MAT 312 system with Icis data system, EI mode, 70 eV, and Hewlett Packard 5890 Series II, using a 30 m × 0.32 mm DB-05 fused silica capillary column; helium was used as carrier gas; temp. program: 3 min isotherm. at 50, 50–100°C (4°C min$^{-1}$, 100–300°C (3°C min$^{-1}$).

### 3.2. Plant material

Fresh leaves of cultivated *J. curcas* were obtained from Proyecto Biomasa, Universidad Nacionál de Ingeniería, Managua, Nicaragua. A voucher specimen is deposited at the Institute of Pharmacognosy, University of Graz, (Herbar No. J95/01).

### 3.3. Isolation of the compound

Fresh leaves (200 g) were defatted with petrol in a Soxhlet apparatus, extracted with EtOAc at room temperature and the marc finally Soxhlet extracted with EtOAc. The extract was concentrated in vacuo and subjected to CC (Sephadex LH 20, isocratic elution with EtOAc–MeOH 3:2). Fractions of 20 ml were collected and monitored by TLC. Fraction 1–20 were combined, and the compounds separated by CC (silica gel, elution with EtOAc–MeCOEt 5:3, EtOAc–MeCOEt–MeOH 5:3:2, EtOAc–MeCOEt–MeOH–HCOOH 5:3:2:0.5). Fractions of 20 ml were collected and monitored by TLC. Fraction 26–28 were combined and the compound purified by CC (fractogel TSK HW(40), isocratic elution with 40% EtOH). Fractions of 10 ml were collected and monitored by TLC. Fraction 11 showed a pure compound on TLC. Final purification was carried out by HPLC on a preparative RP-18 column (isocratic elution with 10% $CH_3CN$) to give 3.0 mg pure white compound.

5-Hydroxypyrrolidin-2-one (**1**): $C_4H_7NO_2$; $^1$H-NMR: $\delta = 5.24$ (dd, $^3J_{5,4peq} = 1.7$ Hz, $^3J_{5,4pax} = 6.3$ Hz, H-5), 2.48 (m, $^2J_{3pax,3peq} = 16.7$ Hz, $^3J_{3pax,4pax} = 9.9$ Hz, $^3J_{3pax,4peq} = 7.9$ Hz, H-$3_{pax}$), 2.37 (m, $^2J_{4pax,4peq} = 13.5$ Hz, $^3J_{4pax,3pax} = 9.9$ Hz, $^3J_{4pax,3peq} = 9.7$ Hz, $^3J_{4pax,5} = 6.3$ Hz, H-$4_{pax}$), 2.19 (m, $^2J_{3peq,3ax} = 16.7$ Hz, $^3J_{3peq,4pax} = 9.7$ Hz, $^3J_{3peq,4peq} = 3.5$ Hz, H-$3_{peq}$), 1.90 (m, $^2J_{4peq,4pax} = 13.5$ Hz, $^3J_{4peq,3pax} = 7.9$ Hz, $^3J_{4peq,3pax} = 3.5$ Hz, $^3J_{4peq,5} = 1.7$ Hz); $^{13}$C-NMR: $\delta = 182.3$ (C-2), 80.9 (C-5), 31.3 (C-4), 29.5 (C-3). Pyrimidine-2,4-dione (uracil) (**2**): $C_4H_4N_2O_2$; $^1$H-NMR: $\delta = 7.38$ (d, $^3J_{6,5} = 7.6$ Hz, H-6), 5.59 (d, $^3J_{5,6} = 7.6$ Hz, H-5); $^{13}$C-NMR: $\delta = 167.8$ (C-4), 153.9 (C-2), 143.5 (C-6), 101.7 (C-5).

GC–MS analysis: 0.2 mg of the complex were transformed (3 h, room temperature) with 10 µl *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA). 2,4-bis[(trimethylsilyl)oxy]-pyrimidine: $C_{10}H_{20}N_2O_2Si_2$, *m*/*z* (rel. int.): 256 ($M^+$, 58), 241 (100), 207 (10), 147 (30). *N*-trimethylsilyl-5-trimethylsilyloxy-pyrrolidin-2-on: $C_{10}H_{23}NO_2Si_2$, *m*/*z* (rel. int.): 245 ($M^+$, 30), 230 (27), 156 (20), 147 (100).

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