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Deoxypreussomerins from *Jatropha curcas*: are they also plant metabolites? ☆

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Abstract

Three deoxypreussomerins, palmarumycins CP1, JC1 and JC2, have been isolated from a collection of the stems of *Jatropha curcas*. The second and third compounds are antibacterial constituents which were characterized from spectral evidence. The X-ray crystallographic structure of palmarumycin JC1 was also studied. Deoxypreussomerins have been obtained here from a plant source in appreciable quantities.

Keywords: *Jatropha curcas*; Deoxypreussomerins; Palmarumycins CP1; JC1 and JC2; X-ray analysis; Antibacterial activity

1. Introduction

Preussomerins and deoxypreussomerins belong to a relatively new and rare group of natural products (Weber et al., 1990; Weber and Gloer, 1991; Krohn et al., 1994a,b, 2001; Ragot et al., 1999; Soman et al., 1999; Wipf et al., 2001). The former are structurally consisted of two unsaturated decalin units connected via three oxygen bridges through two spiroketal carbons, one located at the upper decalin unit while the other at the lower. Deoxypreussomerins lack one of the bridged oxygen and one of the spiroketal carbons. Preussomerins and Deoxypreussomerins were generally reported as fungal metabolites (Weber et al., 1990; Weber and Gloer, 1991; Krohn et al., 1994a,b, 2001; Ragot et al., 1999; Soman et al., 1999; Wipf et al., 2001). Bipendensin was isolated (Connolly, 1991) in very small amounts from wood samples of the African tree *Afzelia bipendensis*. Preussomerin and deoxypreussomerins possess a wide range of biological properties including antibacterial, antifungal, herbicidal, antibiotic and antitumor activities (Wipf et al., 2001). Some of the compounds have been identified as novel inhibitors of *ras*-farnesyl-transferase (Singh et al., 1994).

Due to the interesting structural patterns and important biological properties a large number of investigations on preussomerins and deoxypreussomerins have been carried out in the last few years. Actually all the achievements in chemistry and biology of these molecules have been observed only in the last decade. It is interesting to mention that during our studies on the bioactive constituents of *Jatropha curcas* Linn (Euphorbiaceous), a plant grows wild in India, we have isolated three deoxypreussomerins along with the diterpenoids reported (Naengchomnong et al., 1986) earlier from the plant. One of the deoxypreussomerins was characterized as the known compound, palmarumycin CP1 (**1**) (Krohn et al., 1994a) while the other two constituents

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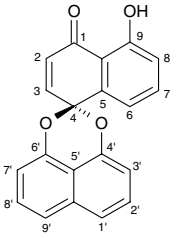
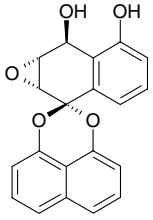
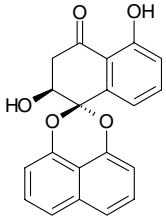
palmarumycins JC1 (**2**) and JC2 (**3**) are the new deoxypreussomerins. Here we report the isolation of the constituents of the plant and structure elucidation of its new constituents.



Palmarumycin CP1 (**1**)



Palmarumycin JC1 (**2**)



Palmarumycin JC2 (**3**)

2. Results and discussions

Palmarumycin JC1 (**2**) analyzed for $C_{20}H_{14}O_5$ from its elemental analysis and mass spectrum [M^+: *m*/*z* 334]. The IR spectrum displayed the absorption bands indicative of the presence of hydroxyl group and aromatic moiety. The ^1H and ^{13}C NMR spectra (Table 1) suggested the molecule to be a deoxypreussomerin related to palmarumycin CP1 (**1**). The three aromatic rings in both the molecules were similar but the substitution pattern in the non-aromatic ring was different. The ^1H NMR spectrum of **2** presented signals corresponding to three oxymethine protons at δ 3.56 (1H, *dd*, J=4.5, 3.0 Hz), 3.64 (1H, *d*, J=4.5 Hz) and 5.47 (1H, *d*, J=3.0 Hz). The ^1H-^1H COSY as well as HMBC experiments (Fig. 1) decided the presence of a hydroxyl group and an epoxide linkage at C-1 and C-2, C-3 respectively. The structure of palmarumycin JC1 (**2**) was also supported by its ^{13}C NMR spectrum which showed the signals for twenty carbons including three oxymethine carbons, sixteen sp^2-hybridized carbons and a spiroketal carbon.

The stereochemistry of palmarumycin JC1 (**2**) (HO-1, H-2 and H-3 — all β) was determined by comparison of its spectral data and optical properties with those of the related compounds (Barrett et al., 2002). The X-ray crystallographic analysis of **2** finally confirmed the proposed structure as well as the stereochemistry of the compound (the relevant data will be published in a related journal). The X-ray crystal structure (Fig. 2) demonstrated that the lower two aromatic rings are perpendicular to the upper one.

Palmarumycin JC2 (**3**), another new deoxypreussomerin has been identified by direct comparison of its spectra (IR, ^1H and ^{13}C NMR and MS) with those of **1**. Compound **3** analyzed for $C_{20}H_{14}O_5$ from its elemental analysis and mass spectrum ($M^{+\cdot}$; *m*/*z* 334). The molecular formula of the compound was similar to that of **2**. The IR spectrum of **3** was characteristic of a natural keto-hydroxy deoxypreussomerin (Krohn et al., 1994a,b; Ragot et al., 1999; Wipf et al., 2001). The ^1H and ^{13}C NMR spectra (Table 1) indicated that three aromatic rings present in compounds **1**–**3** were similar but substitution pattern at C-1 to C-3 was only different. The hydroxyl

Table 1
NMR spectral data of palmarumycins JC1 (**2**) and JC2 (**3**)

Position	Compound **2**		Compound **3**	
	δ_H (d_4-MeOH)	δ_C (d_4-MeOH)	δ_H ($CDCl_3$)	δ_C($CDCl_3$)
1	5.47 (*d*, J=3.0 Hz)	60.4		202.2
2	3.56 (*dd*, J=4.5, 3.0 Hz)	53.3	2.85 (*dd*, J=17.8, 3.2 Hz)	41.6
			3.15 (*dd*, J=17.8, 3.2 Hz)	
3	3.64 (*d*, J=4.5 Hz)	50.5	4.51 (*dd*, J=3.2, 2.8 Hz)	67.5
4		97.5		98.5
5		132.1		138.2
6	7.41 (*d*, J=8.0, 1.2 Hz)	120.6	7.26 (*dd*, J=8.0, 1.0 Hz)	121.8
7	7.24 (*t*, J=8.0 Hz)	127.6	7.45 (*t*, J=8.0 Hz)	137.5
8	6.92 (*dd*, J=8.0, 1.2 Hz)	120.6	7.00 (*dd*, J=8.0, 1.0 Hz)	120.2
9		155.6		162.8
10		121.6		121.5
1′	7.45 (*dd*, J=7.8, 1.2 Hz)	108.9	7.47 (*dd*, J=8.0, 1.0 Hz)	109.9
2′	7.36 (*t*, J=7.8 Hz)	127.4	7.40 (*t*, J=8.0 Hz)	128.1
3′	7.06 (*dd*, J=7.8, 1.2 Hz)	120.6	7.02 (*dd*, J=8.0, 1.0 Hz)	118.3
4′		147.5		147.0
5′		112.9		114.6
6′		147.5		147.8
7′	6.86 (*dd*, J=7.8, 1.2 Hz)	116.4	6.83 (*dd*, J=8.0, 1.0 Hz)	116.0
8′	7.26 (*t*, J=7.8 Hz)	127.3	7.37 (*t*, J=8.0 Hz)	128.1
9′	7.44 (*dd*, J=7.8, 1.2 Hz)	109.4	7.47 (*dd*, J=8.0,1.0 Hz)	109.2
10′		134.8		135.0
−OH			12.27 (*brs*)	

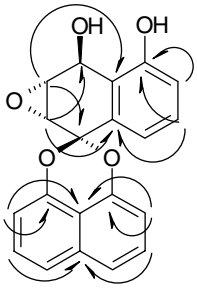


Fig. 1. Selected HMBC correlations of **2**.

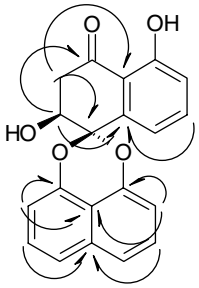


Fig. 3. Selected HMBC correlations of **3**.

group at C-1 in **2** has been oxidized to a keto group in **3**. This has also been supported by the presence of a chelated hydroxyl group (δ 12.27, 1H, *brs*) in the latter. The ^{1}H NMR spectrum also revealed that C-2 was not oxygenated in **3** but C-3 was oxygenated suggesting the placement of a hydroxyl group at C-3. The ^{1}H–^{1}H COSY spectrums clearly showed the correlation between these H_2-2 and H-3. The structure of **3** was also supported from the HMBC experiment on the molecule (Fig. 3). The β-configuration of the hydroxyl group at C-3 was concluded from observation of its almost opposite optical value to that of the compounds of similar system having $C_{3\alpha}$-OH (Bode et al., 2000).

Compounds **1**–**3** were found to exhibit significant antibacterial activity against the organism, *Staphylococcus aureus*. Their activity was comparable to that of the standard compound, penicillin-G. The inhibition zone (diameter in mm) was found to be 11, 10, 10 and 13 using the compounds, **1**–**3** and pencillin-G, respectively, and the bacteria, *Staph. aureus* (Srinivas et al., 2003). The concentration of each compound was taken as 30 μg/ml.

The interesting point to mention here is that palmarumycins CP1 (**1**), JC1 (**2**) and JC2 (**3**) were obtained from the plant *Jatropha curcas* in appreciable quantities (total amount: 172 mg from 3 kg of the stems). These data possibly excluded the occurrence of deoxypreussomerins in an endophytic fungus present in the plant but rather indicate the presence of these compounds in the plant as its constituents. Bipendensin (Connolly, 1991) (with the similar gross structure of **2**) was also previously reported in low yield from a plant source.

3. Experimental

3.1. General

Melting points were measured in a Buchi-510 apparatus and are uncorrected. The IR spectra were recorded on a Perkin–Elmer RX1 FT-IR spectrophotometer, the NMR spectra on a Varian Gemini-200 MHz spectrometer and the mass spectra on a Finnigan-Mat 1020 spectrometer. Optical rotations were determined on a JASCO DIP-360 polarimeter. Antibacterial activity

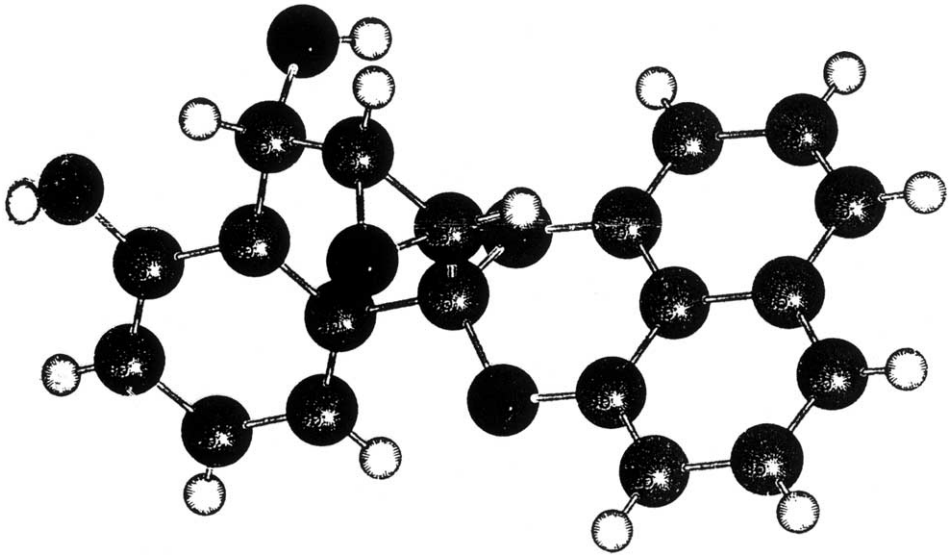


Fig. 2. X-ray crystal structure of compound **2**.

was studied following our reported method (Srinivas et al., 2003).

3.2. Plant material

The stems of *Jatropha curcas* were collected from Dhanasri, Andhra Pradesh in January, 2001 and identified by Professor C. Rajugopal, Department of Botany, Osmania University, Hyderabad. The voucher specimen of the sample (No. IICP-10801) has been preserved in the herbarium of IICT.

3.3. Extraction and isolation

The air dried and powdered stem (3 kg) was extracted thrice with CH_2Cl_2–MeOH (1:1), each extraction was continued for 72 h. The total extract was concentrated to a gummy brown mass (38 g). The material was subjected to column chromatography over silica gel using hexane-EtOAc as eluent. The fractions eluted with 5–10% EtOAc in hexane afforded the known diterpenoids, curcusones A (122 mg), B (52 mg), C (56 mg) and D (32 mg). Some steroids were eluted with 15–20% EtOAc in hexane. The subsequent fractions eluted with 25% EtOAc in hexane produced palmarumycins CP1 (**1**) (7 mg), JC1 (**2**) (42 mg) and JC2 (**3**) (123 mg). The physical and spectral properties of the known compounds were similar to those reported in the literature.

3.4. Palmarumycin JC1 (*2*)

White crystals, m.p. 208–210 °C; $[\alpha]_D^{25}+82.5$ ($c = 0.5$, MeOH); IR υ_{max} (KBr) cm^{-1}: 3050, 1605, 1455, 1409; ^1H and ^{13}C NMR Table 1; LSIMS: *m/z* 334 ($M^{+\cdot}$). Anal. Calcd. for $C_{20}H_{14}O_5$: C, 71.85; H, 4.22%. Found: C, 71.93; H, 4.20%.

3.5. Palmarumycin JC2 (*3*)

Semi solid, m.p. 192–194 °C (d), $[\alpha]_D^{25}+131.9$ ($c = 0.5$, $CHCl_3$); IR υ_{max} (KBr) cm^{-1}: 3539, 1655, 1609, 1515, 1454 ; ^1H and ^{13}C NMR : Table 1 ; EIMS : *m/z* (%). 334 ($M^{+\cdot}$, 35), 234 (19), 175 (14), 160 (24), 159 (55), 114 (97), 63 (100). Anal. Calcd. for $C_{20}H_{14}O_5$: C, 71.85; H, 4.22%; Found: C, 71.89; H, 4.26%.

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