New Lathyrane and Podocarpane Diterpenoids from *Jatropha curcas*[1)]

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Chemical investigation on *Jatropha curcas* resulted in the isolation of twenty constituents among which four diterpenoids were unknown and six compounds, tetradecyl-(*E*)-ferulate, 3-*O*-(*Z*)-coumaroyl oleanolic acid, heudelotinone, *epi*-isojatrogrossidione, 2α-hydroxy-*epi*-isojatrogrossidione, and 2-methyanthraquinone had not been reported earlier from this species. The structures of the new compounds were established by extensive studies of their 1D- and 2D-NMR spectra.

Key words *Jatropha curcas*; Euphorbiaceae; aerial part; diterpenoid; lathyrane; podocarpane

Jatropha curcas LINN, a small shrub, is found in different parts of India. The plant is known[2)] to possess anti-leukemia properties. Earlier researchers reported the isolation of several diterpenoids including phorbol esters,[3,4)] some coumarins,[5)] a triterpene[6)] and a flavone derivative.[7)] We recently examined the aerial parts of the plant and isolated twenty constituents. Four of the compounds **1**—**4** were unknown diterpenoids. Two of them, **1** and **2** were of the lathyrane type while the other two, **3** and **4** were of the podocarpane type. Six constituents presently identified were not reported earlier from the plant. Here we describe the isolation of the constituents and structure elucidation of the unknown compounds, **1**—**4**.

Compound **1** was obtained as a semi solid. Its molecular formula was deduced to be $C_{22}H_{30}O_5$ from its mass spectrum, elemental analysis and ^{13}C-NMR spectrum. The IR spectrum of the compound indicated the presence of hydroxyl and carbonyl group and unsaturation. The ^{1}H- and ^{13}C-NMR spectra (Table 1) of the compound suggested it to be a diterpene of lathyrane type.[8)] The ^{1}H-NMR spectrum showed the presence of a cyclopropane moiety, two olefinic protons, a hydroxyl, an acetoxy and five methyl groups. The ^{13}C-NMR spectrum recorded twenty two carbon signals including characteristic signals for two trisubstituted double bonds, one acetoxy and other two carbonyl groups, five methyls, three methylenes, three methines and three quarternary carbons. All of the signals for the protons and carbons in the ^{1}H- and ^{13}C-NMR spectra respectively, were assigned from 2D-NMR (^{1}H–^{1}H COSY, NOESY, HSQC and HMBC) and APT experiments. The comparison of these spectral values of **1** with those of the known constituent 15-*epi*-(4*E*)-jatrogrossidentadione (**5**)[8)] clearly revealed that the former is a mono acetyl derivative of

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **1** and **2**

Position	^{1}H-NMR (J in Hz)		^{13}C-NMR	
	1	**2**	**1**	**2**
1	7.85, dq (J=1.4, 1.2)	7.35, q (J=1.1)	149.5	149.5
2	—	—	147.3	142.0
3	—	—	193.8	204.0
4	—	2.79, d (J=9.6)	131.9	45.0
5	6.82, d (J=1.2)	2.90, d (J=9.6)	148.0	61.7
6	—	—	75.4	59.2
7	1.89, ddd (J=14.3, 5.0, 3.8)	2.29, ddd (J=14.3, 6.2, 1.2)	41.3	40.7
	1.78, ddd (J=14.3, 11.4, 3.8)	1.19, ddd (J=14.3, 12.9, 1.6)		
8	1.62, m	1.73, m	20.1	19.2
	0.78, m	0.98, m		
9	0.48, br dd (J=8.0, 10.0)	0.11, br t (J= 9.6)	27.6	27.7
10	—	—	17.1	17.2
11	0.61, m	0.52, ddd (J=9.0, 3.8, 11.8)	19.4	18.9
12	1.38, ddd (J=14.9, 5.0, 8.0)	1.75, ddd (J=12.2, 11.8, 3.8)	30.3	28.6
	1.34, ddd (J=14.9, 1.2, 3.2	1.08, ddd (J=12.2, 11.8, 5.0)		
13	2.83, m	2.50, ddq (J=11.8, 5.0, 7.0)	38.6	35.2
14	—	—	202.0	147.0
15	—	—	90.4	125.2
16	2.02, d (J=1.4)	1.90, d (J= 1.1)	11.0	10.6
17	1.35, s	1.41, s	29.7	24.3
18	0.97, s	1.00, s	28.4	28.9
19	0.62, s	0.87, s	15.0	15.1
20	1.13, d (J=7.0)	1.02, d (J= 7.0)	16.7	17.1
–OAc	2.19, s	2.24, s	21.6	20.6
			168.8	168.9

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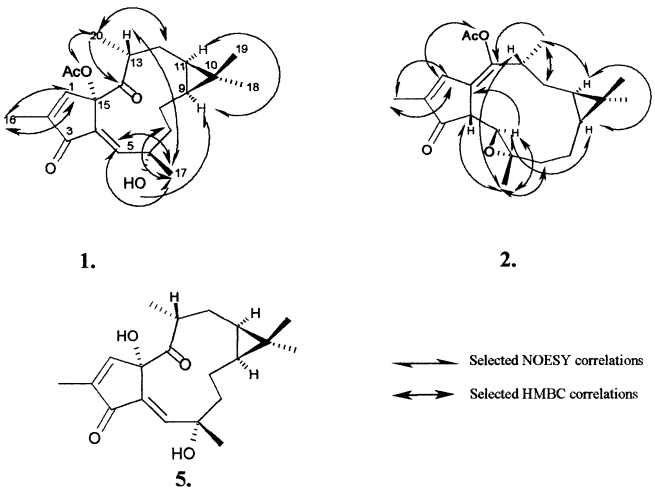
the latter which contains two hydroxyl groups at C-6 and C-15. The acetoxy group in the new compound **1** was reasonably placed at C-15 because in the ^{13}C-NMR spectrum this carbon (δ 90.4) showed a downfield shift compared to the position of the corresponding carbon (δ 84.2) of **5**. The H-1 also showed a rather downfield shift (δ 7.85 in **1** while δ 6.86 in **5**) due to the deshielding effect of –OAc-15. Moreover, in the NOESY experiment this –OAc group showed correlation with Me-20 while the –OH group with H-9. The HMBC spectrum also supported the correlation of the –OH group with C-5 and C-7 indicating the presence of this group at C-6. Additionally, the NOESY experiment revealed a similar relative stereochemistry of **1** as possessed by **5** (Fig. 1). The structure of compound **1** was thus established as 15-*O*-acetyl-15-*epi*-(4*E*)-jatrogrossidentadion.



1. **2.** **5.**

Fig. 1

Compound **2** was also obtained as a semi solid. Its molecular formula was deduced to be $C_{22}H_{30}O_4$ from its mass spectrum, elemental analysis and ^{13}C-NMR spectrum. The IR spectrum showed the presence of carbonyl group and unsaturation in the molecule. The ^{1}H- and ^{13}C-NMR spectral data (Table 1) indicated that both the compounds **1** and **2** have a similar general structure. Both the molecules contain cyclopentenone and cyclopropane moieties. However, compound **2** contained an epoxide ring at C-5, C-6 (instead of a double bond at C-4, C-5) generally observed in the lathyrane diterpenoid of *Jatropha* species.[8]

In the ^{1}H–^{1}H COSY experiments H-4 and H-5 were found to be related while in the HMBC experiment C-5 and Me-17. In the NOESY experiment H-4 showed a relation with Me-17 but not with H-5, which was related to H-9, indicating that H-4 and Me-17 had β-configuration while H-5 had α. The other difference between **1** and **2** was that the latter contained a tetrasubstituted double bond at C-14, C-15 with an acetoxy group at C-14 instead of a carbonyl group at C-14 in **1**. The position of this double bond in **2** was clearly determined from the HMBC spectrum, which revealed that C-14 was related to Me-20 while C-15 to H-5. The trans (*E*) configuration of the C-14, C-15 double bond was concluded by observation of the deshielding of H-1 (δ 7.35) as well as from the NOESY experiment which indicated that the acetyl group was weakly correlated to this proton. Considering all these spectral data the structure of the second new diterpenoid was deduced clearly as **2**.

Compound **3** was isolated as white crystals. Its molecular formula was suggested to be $C_{21}H_{28}O_4$ from mass spectrum, elemental analysis and ^{13}C-NMR spectrum. The IR spectrum indicated that the molecule contained carbonyl group and aromatic ring. The structure of the compound was derived from its ^{1}H- and ^{13}C-NMR spectra (Table 2) which sug-

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **3** and **4**

Position	^{1}H-NMR (*J* in Hz)		^{13}C-NMR	
	3	**4**	**3**	**4**
1	1.81, m 2.34, m	1.51, m 2.20, m	35.9	37.0
2	1.96, m 1.84, m	1.80, m 1.76, m	24.0	27.9
3	4.57, dd (*J*=11.1, 4.3)	3.30, dd (*J*=11.4, 4.7)	79.8	78.8
4	—	—	37.7	38.9
5	1.94, dd (*J*=11.8, 2.8)	1.28, dd (*J*=12.0, 2.5)	49.0	49.8
6	2.66, m 2.66, m	1.86, m 1.71, m	35.3	18.9
7	—	2.85, ddd (*J*=16.6, 6.5, 1.9) 2.75, ddd (*J*=16.6, 11.6, 7.5)	197.6	29.7
8	—	—	125.3	126.8
9	—	—	123.6	121.2
10	—	—	38.0	37.3
11	6.68, s	6.65, s	104.0	110.6
12	—	—	162.6	148.3
13	—	—	155.6	151.9
14	7.81, s	6.79, s	129.8	131.1
15	2.19, s	2.17, s	16.1	15.3
18	1.04, s	1.10, s	27.5	28.1
19	0.94, s	0.88, s	15.6	15.3
20	1.26, s	1.17, s	23.4	24.8
–OMe	3.88, s	—	55.6	—
–OAc	2.08, s	—	21.2 170.8	—

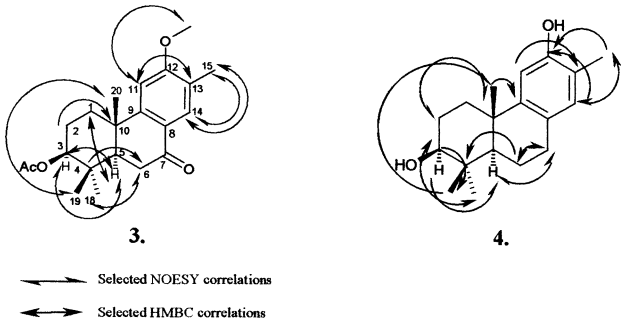


Fig. 2

gested[9] that the compound belongs to a diterpenoid with the dehydropodocarpane skeleton. The ^{1}H-NMR spectrum showed the presence of four methyl groups, one of which was in the aromatic C-ring. The spectrum also indicated that **3** contained two aromatic protons (each singlet), an acetoxy and a methyl groups. The acetoxy group was placed at C-3β as the proton at this position appeared as a doublet of a doublet. This was also supported from the NOESY experiment which showed a clear correlation between H-3 and H-5.

The methyl and methoxy groups were placed at C-12 and C-13 respectively in the ring C as decided from HMBC and NOESY experiments. In the HMBC spectrum the protons of the methyl group were related to C-12 and the latter was again related to H-14. The NOESY experiment also showed the correlations between H-11 and –OMe as well as H-14 and –Me. The ^{13}C-NMR spectrum of **3** pointed to the presence of another carbonyl group in the compound conjugated with the aromatic ring. This carbonyl group was suggested to be located at C-7 as a proton at C-6 appeared at the downfield region (δ 2.66). The structure of compound **3** was thus derived as 3β-acetoxy-12-methoxy-13-methyl-podocarpa-8,11,13-trien-7-one.

The C-3α isomer of **3** was reported[10] earlier as the synthetic compound. This isomeric compound showed a positive optical rotation. Its ^{1}H-NMR spectral values of all the protons except H-3 (δ 4.77, br s, H-3) and H-5 (δ 2.31, dd, 1H, J=10.3, 7.5 Hz) were similar to those of the corresponding protons of **3**.

Compound **4** was obtained as a semi solid. The compound was analyzed for $C_{18}H_{26}O_2$ from its mass spectrum, elemental analysis and ^{13}C-NMR spectrum. The IR spectrum showed the presence of hydroxyl and aromatic ring in the molecule. Comparison of ^{1}H- and ^{13}C-NMR spectral data of **4** (Table 2) with those of **3** suggested that the general structure of the two compounds is similar. However, compound **4** contained no acetoxy or methoxy group or any carbonyl group. The acetoxy group of **3** has been deacetylated and the methoxy group demethylated in **4**. Thus the latter contains two hydroxy groups at C-3 and C-12. The presence of two hydroxy groups in **4** was also supported by formation of its diacetate by acetylation of the compound. The β-configuration of the –OH group at C-3 was concluded from the splitting pattern of H-3 (doublet of a doublet) and from the NOESY experiment which suggested a clear correlation between H-3 and H-5. The position of the other –OH group at C-12 and the aromatic methyl group at C-13 (as in **3**) were supported from the observation that the methyl protons were related to C-12 and C-14 in the HMBC experiment while to the H-14 in the NOESY. The structure of compound **4** was thus determined to be 3β,12-dihydroxy-13-methyl-podocarpane-8,10,13-triene.

Along with compounds **1**—**4** sixteen other constituents: tetradecyl (*E*)-ferulate,[11] 3-*O*-(*Z*)-coumaroyl oleanolic acid,[12] heudelotinone,[13] *epi*-isojatrogrossidione,[8] 2α-hydroxy-*epi*-isojatrogrossidione,[8] 2-methoxyantraquinone,[14] scopaletin, tomentin,[5] curcasones A—D,[3] jatropholones A and B,[15] jatrophol[16] and 15-*epi*-(4*E*)-jatrogrossidentadion[8] were also isolated. The structures of the known compounds were settled by comparison of their physical and spectral properties with those reported in the literature. The occurrence of the first six compounds in the title species is reported here for the first time.

Experimental

General mp uncorr. The spectra were recorded with the following instruments; IR, Perkin Elmer Spectrum RX I FT-IR; 1D-NMR, Varian Gemini 200 MHz and 2D-NMR, Varian Unity INOVA 500 MHz; EI-MS, VG-micromass 7070 H and LSI-MS, Finnigan-MAT 1020 instrument. The optical rotations were measured with a JASCO DIP 360 digital polarimeter. Column chromatography was performed with silica gel (BDH, 100—200 mesh) and TLC with silica gel GF_{254}.

Plant Material The aerial parts of *J. curcas* were collected from Dhanasri, Andhra Pradesh, India in January 2001 and identified botanically. A voucher specimen (No. JC-AP-I) is preserved in our laboratory and another voucher specimen (IICP 150101) in the IICT herbarium.

Extraction and Isolation The shade dried plant material (3 kg) was powdered and extracted with hexane (5 l) for 72 h. The extract was filtered and concentrated with a rotavapour to produce a thick brown mass (72 g). The defatted plant material was subsequently extracted with $CHCl_3$–MeOH (1 : 1) for 120 h. The extract was filtered and concentrated to afford a brown gummy mass (44 g).

The residue of hexane extract was chromatographed over silica gel (100—200 mesh). The column was eluted with solvents of increasing polarity using hexane and EtOAc, separation of the components in the mixture being monitored by TLC. The elutes were collected in fractions of 100 ml each and similar fractions were combined. The later fractions eluted with hexane afforded tetradecyl (*E*)-ferulate (40 mg). The fractions eluted with hexane–EtOAc (20 : 1) showed the presence of four compounds (TLC) which were subsequently separated by repeated chromatography to yield curcasones A (120 mg), B (85 mg), C (35 mg), and D (40 mg). From the fractions eluted with hexane–EtOAc (10 : 1) a mixture of two other compounds was obtained. Purification of this mixture by rechromatography gave jatropholone A (100 mg) and B (70 mg). From the hexane extract of the plant no other compound could be isolated.

The residue of $CHCl_3$–MeOH extract was also chromatographed over silica gel following the method described above. The fractions eluted with hexane–EtOAc (10 : 1) showed the presence of two compounds, which were separated by rechromatography to afford more jatropholones A (30 mg) and B (25 mg). The subsequent fractions eluted with hexane–EtOAc (10 : 2) were found to be a mixture of four compounds which on rechromatographic separation produced 2-methoxyanthraquinone (15 mg), jatrophol (20 mg), scopaletin (25 mg) and heudelotinone (80 mg). A complex mixture consisting of six compounds was then eluted from the main column with hexane–EtOAc (5 : 1). The mixture was again subjected to column chromatography to achieve the separation and purification of the compounds to yield compound **4** (22 mg), compound **2** (20 mg), compound **1** (15 mg), 15-*epi*-(4*E*)-jatrogrossidentadien (26 mg), isojatrogrossidione (18 mg), and 2α-hydroxy-isojatrogrossidione (16 mg). The fractions eluted with hexane–EtOAc (10 : 3) were the mixture of two compounds, which on rechromatography afforded tomentin (12 mg) and compound **3** (22 mg). Finally the fractions eluted with hexane–EtOAc (1 : 1) yielded 3-*O*-(*Z*) coumaroyl oleanolic acid (22 mg).

Compound **1**: Semi solid, $[\alpha]_D^{25}$ −165.2° (c=0.5, $CHCl_3$); IR (KBr) ν_{max}: 3438, 1720, 1714, 1454 cm^{-1}. ^{1}H- and ^{13}C-NMR: Table 1; LSI-MS *m/z*: 375 [M+1]$^+$; EI-MS *m/z* (rel. int.): 314 (M$^+$−HOAc) (4), 271 (8), 161 (14), 43 (100). (Found: C, 70.52; H, 8.12. $C_{22}H_{30}O_5$ required: C, 70.59; H, 8.02%).

Compound **2**: Semi solid, $[\alpha]_D^{25}$ +68.2° (c=0.5, $CHCl_3$); IR (KBr) ν_{max}: 1760, 1707, 1506, 1455 cm^{-1}. ^{1}H- and ^{13}C-NMR: Table 1; LSI-MS *m/z*: 359 [M+1]$^+$; (Found: C, 73.82; H, 8.48. $C_{22}H_{30}O_4$ required: C, 73.74; H, 8.38%).

Compound **3**: White crystals, mp 158—159 °C, $[\alpha]_D^{25}$ −25.2° (c=0.5, $CHCl_3$); IR (KBr) ν_{max}: 1731, 1672, 1606, 1497, 1368 cm^{-1}. ^1H- and ^{13}C-NMR: Table 2; EI-MS m/z (rel. int.): 344 (M^+) (56), 269 (60), 201 (25), 119 (20), 43 (100). (Found: C, 73.32; H, 8.22. $C_{21}H_{28}O_4$ required: C, 73.26; H, 8.14%).

Compound **4**: Semi solid. $[\alpha]_D^{25}$ −12.6° (c=0.5, MeOH); IR (KBr) ν_{max}: 3439, 1649, 1461, 1374, 1287 cm^{-1}. ^1H- and ^{13}C-NMR: Table 2; EI-MS m/z (rel. int.): 274 (M^+) (72), 241 (100), 187 (32), 171 (50). (Found: C, 78.86; H, 9.64. $C_{18}H_{26}O_2$. required: C, 78.83; H, 9.49%).

Acetylation of Compound 4 Ac_2O (0.2 ml) and pyridine (2 drops) were added to compound **4** (5 mg) and kept overnight. After usual work-up the acetylation product of **4** was obtained as a viscous mass (5 mg). ^1H-NMR ($CDCl_3$): δ 6.82 (1H, s), 6.78 (1H, s), 4.52 (1H, dd, J=11.2, 4.2 Hz), 2.82 (2H, m), 2.24 (3H, s), 2.20 (1H, m), 2.02, 2.04 (3H each, s), 1.60—1.82 (5H, m), 1.4 (1H, m), 1.2 (3H, s), 0.98 (6H, s). LSI-MS m/z: 359 $[M+1]^+$.

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