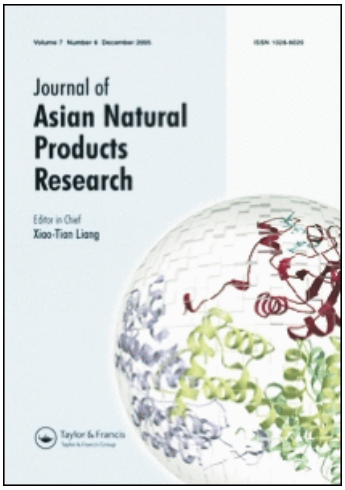

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Antihepatotoxic activity of debelalactone, a new oxirano-furanocoumarin from *Phyllanthus debilis*

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The whole plant of *Phyllanthus debilis* (Euphorbiacae) afforded a new oxirano-furanocoumarin, characterized as 5-hydroxy-7-methoxy-furanocoumarin-9(14)-cyclohex-12(13)-oxirano-11-one (**1**), named as debelalactone. Debelalactone exhibited a significant antihepatotoxic activity by reducing the elevated levels of serum enzymes such as serum glutamate oxaloacetate transaminase (SGOT) by 59.14%, serum glutamate pyruvate oxaloacetate transaminase (SGPT) by 61.84%, and alkaline phosphatase (ALP) by 85.93%; while the total protein (TP) levels were increased by 110.35%, when compared with standard drug silymarin that have decreased SGOT by 77.03%, SGPT by 69.67%, ALP by 93.18%, and increased TP levels by 100.48%, respectively, against CCl_4-induced toxicity in Wistar rats. These biochemical observations were also supplemented by histopathological examinations of the liver sections.

Keywords: *Phyllanthus debilis*; Euphorbiacae; debelalactone; oxirano-furanocoumarin; antihepatotoxic activity; histopathological studies

1. Introduction

Phyllanthus debilis (Euphorbiacae) is widely distributed in India, Sri Lanka, Burma, Indonesia, Pacific Islands, and the West Indies [1], and has been reported to possess immunomodulatory [2], analgesic, anti-inflammatory [3], and antihepatotoxic activities [4]. The petroleum ether extract exhibited significant anti-inflammatory activity in acute carrageenan-induced rat paw edema and the chronic granuloma pouch models. However, it was devoid of analgesic activity in the tail-flick model [3].

The literature survey has revealed that neither chemical investigation has been done nor any chemical compound has been isolated from the plant so far. We now report herein the isolation of a new oxirano-furanocoumarin characterized as 5-hydroxy-7-methoxy-furanocoumarin-9(14)-cyclohex-12(13)-oxirano-11-one and has been designated as debelalactone (**1**) (Figure 1), which exhibited a significant antihepatotoxic activity against CCl_4-induced toxicity in Wistar rats in comparison with standard silymarin.

2. Results and discussion

Compound **1** was obtained as yellow amorphous powder, which gave a single spot on TLC and exhibiting a molecular formula $C_{16}H_{10}O_7$ as established on the basis of FAB-MS at *m/z* 314.25 $[M]^+$, ^{13}C NMR, and DEPT spectra. The compound showed a green color with ferric chloride indicating it to be a phenolic compound. The UV, IR, and MS spectral data suggested that the compound

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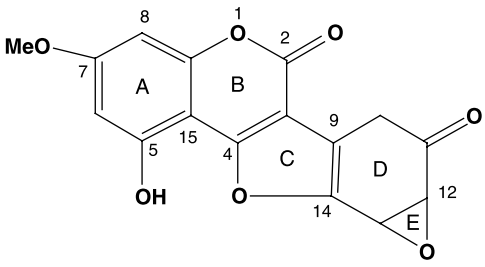

Figure 1. The structure of **1**.

was a coumestan derivative [5,6]. The UV spectrum showed characteristic absorption maxima at 230, 275, and 357 nm. The IR spectrum indicated the presence of hydroxyl group (3374 cm^{-1}), δ-lactone carbonyl (1722 cm^{-1}), C—O furan (1292 cm^{-1}), and C—O epoxide (1093 and 1040 cm^{-1}). The ^{13}C NMR and DEPT spectra [7] showed 16 carbon signals including eight quaternary, two carbonyls, four methine, one methylene, and one methoxy carbons (in total $C_{16}H_{10}$). The ^{1}H NMR spectrum exhibited two proton signals at δ 2.51 (d, $J = 7.5$ Hz) and 2.99 (dd, $J = 7.5, 7.8$ Hz) (δ 37.4) attributable to a methylene group at position-10. The position of the methylene group was further substantiated by long-range correlations in HMBC spectrum (Table 1; Figure 1), which exhibited long-range correlations with a carbonyl group (δ 172.0) assigned at position-11 and to a second carbonyl group ($\delta_4 J_{CH}$ 193.0) assignable at position-2 (Figure 2). The ^{1}H NMR spectrum also exhibited a doublet at δ 4.42 (d, $J = 7.5$ Hz, δ_C 41.4) due to a proton at position-12 and another doublet at δ 3.16 (d, $J = 7.5$ Hz, δ 49.1) due to one proton at position-13, which were correlated with each other in $^{1}H-^{1}H$ COSY spectrum indicating their vicinal position in ring D. The HMBC spectrum exhibited correlations of H-12 with C-11, C-13, and C-10, while H-13 showed

Table 1. 1D- and 2D-NMR spectral data of debelalactone (**1**).

Position	^{1}H NMR[a]	^{13}C NMR/ HMQC	$^{1}H-^{1}H$ COSY	DEPT[b]	HMBC[c] $2J_{CH}$	HMBC[c] $3J_{CH}$
1	–	–	–	–	–	–
2	–	193.0 s	–	C	–	–
3	–	139.0 s	–	C	–	–
4	–	148.0 s	–	C	–	–
5	–	159.0 s	–	C	–	–
6	7.50 d (2.0)	110.0 d	$^{3}J_{HH}$ – H-8	CH	C-5, C-7	C-8
7	–	160.0 s	–	C	–	–
8	7.30 d (2.0)	108.0 d	$^{3}J_{HH}$ – H-6	CH	C-7, C-16	C-6
9	–	140.0 s	–	C	–	–
10a	2.51 d (7.5)	37.4 t	H-10b	CH_2	C-9, C-11	C-14, C-2 ($_4J_{CH}$), C-3
10b	2.99 dd (7.5, 7.8)	–	H-10a	–	C-11, C-12	–
11	–	172.0 s	–	C	–	–
12	4.42 d (7.5)	41.4 d	H-13	CH	C-11, C-13	C-10
13	3.16 d (7.5)	49.1 d	H-12	CH	C-12, C-14	C-11
14	–	143.0 s	–	C	–	–
15	–	138.0 s	–	C	–	–
16	–	149.0 s	–	C	–	–
17	3.62 s	52.5 q	–	CH_3	C-7	–
OH	10.27 at 5	–	–	–	–	–

[a]Assignments were based on $^{1}H-^{1}H$ COSY and HMQC experiments; coupling constants in Hertz are given in parentheses; s, singlet; d, doublet; m, multiplet.
[b]DEPT chemical shifts are presented at $\theta = 3\pi/4$ when methylene groups reach negative maximum; s, C; d, CH; t, CH_2; q, CH_3.
[c]The HMBC correlations have been shown from protons to carbons.

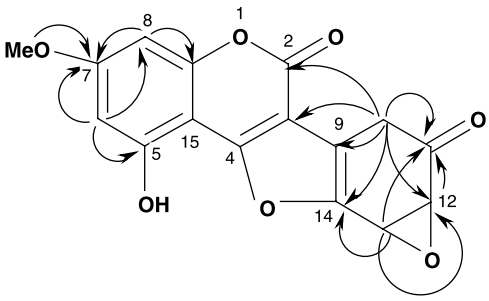

Figure 2. Significant HMBC correlations for **1**.

correlations with C-12, C-14 (δ 143.0), and C-11, which substantiated the structural assignment of ring D (Figure 2). The ^{1}H NMR spectrum also displayed two doublets of one proton each at δ 7.50 (d, $J = 2.0$ Hz, δ 110.0) and 7.30 (d, $J = 2.0$ Hz, δ 108.0), which were assigned at position-6 and -8, respectively. The position of these two protons was further confirmed by HMBC correlations of H-6 with C-5, C-7 and C-8, and H-8 with C-7, C-16 (δ 149.0) and C-6 indicating their *meta*-position in ring A. The ^{1}H NMR spectrum also displayed a three proton singlet at δ 3.62 (s, δ 52.5) due to a methoxyl group, which was placed at position-7 with the help of the long-range correlation of the methoxyl group with C-7 (δ 160.0). A broad singlet at δ 10.27 due to one proton of hydroxyl group, assignable at position-5, was also found in the ^{1}H NMR spectrum. The downfield shift of this proton was observed due to hydrogen bond with oxygen of ring C. Other peaks in ^{13}C NMR, DEPT, HMQC, and HMBC spectra were also supportive to the proposed structure of compound **1** (Figure 1).

Thus, on the basis of the above spectral data, the structure of the compound **1** has been elucidated as 5-hydroxy-7-methoxy-furanocoumarin-9(14)-cyclohex-12(13)-oxirano-11-one and has been designated as debelalactone (**1**).

As shown in Table 2, the activities of liver enzymes serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate oxaloacetate transaminase (SGPT), alkaline phosphatase (ALP) were markedly increased and total proteins (TP) were decreased in CCl_4-treated rats in comparison with normal values. Administration of silymarin (standard drug) and debelalactone (**1**) at the dose levels 10–50 mg/kg body weight, respectively, prevented CCl_4-induced elevation of SGPT, SGOT, ALP and also prevented decrease in total protein. Silymarin (10 mg/kg) had significantly decreased the level of SGOT, SGPT, and ALP and increased that in total protein by 70.80, 63.80, 46.67 U/ml, and 6.26 g/dl, respectively, whereas **1** (50 mg/kg) had a considerable decrease of 92.22, 71.88, 50.61 U/ml and an increase of 5.7 g/dl, respectively. The histopathological studies also showed significant recovery of hepatocytes of the liver in the standard drug and compound-treated animals (Table 3), which has again correlated with the biochemical parameters.

The results of the liver histopathological studies have been presented in Table 3, which showed hepatocytes swelling and necrosis in CCl_4-treated rats in comparison with normal control rats. Administration of **1** exhibited a significant protection of hepatocytes injury and showed complete normalization of the tissues as neither fatty accumulation nor necrosis was observed. The central vein appeared clearly indicating a potent antihepatotoxic activity. Debelalactone (**1**) was found to exhibit more potent antihepatotoxic activity comparable with standard drug silymarin. The liver section showed the structures of the portal triad and a normal liver parenchyma, and the central vein appeared clearly. There was no lymphocytic infiltration and fatty deposition indicating a potent antihepatotoxic activity, which has established a correlation that the active constituent debelalactone is present in the methanolic fraction responsible for antihepatotoxic activity of the ethanolic extract.

3. Experimental

3.1 General experimental procedures

Melting point was determined on Perfit melting point apparatus by open capillary

Table 2. Effect of **1** on liver enzymes in CCl_4-induced liver damage in rats.

Group ($n = 6$)	Treatment	Dose (p.o.)	SGOT (U/ml)	SGPT (U/ml)	ALP (U/ml)	TP (g/dl)
1	Normal/control	Nil	54.54 ± 1.26**	44.45 ± 1.36**	43.49 ± 1.73**	6.29 ± 0.40**
2	CCl_4-intoxicated control	1.5 mg/kg	140.66 ± 1.81	128.41 ± 2.48	69.99 ± 2.21	4.19 ± 0.38
3	Silymarin (standard drug)	10 mg/kg	70.80 ± 1.62** (77.03%)[a]	63.80 ± 2.09** (69.67%)[a]	46.67 ± 1.12** (93.18%)[a]	6.26 ± 0.46** (100.48%)[a]
4	Debelalactone (**1**) + CCl_4	50 mg/kg	92.22 ± 4.21** (59.14%)[a]	71.88 ± 2.06** (61.84%)[a]	50.61 ± 2.45* (85.93%)[a]	5.7 ± 0.18** (110.35%)[a]

SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvate transaminase; ALP, alkaline phosphatase; TP, total protein; p.o., per oral; $^{**}P < 0.01$; $^{*}P < 0.05$ vs. CCl_4; $P > 0.05$ ns. Values are mean ± SE of six animals. One way analysis and Dunnett's test. %, percent.
[a]Percentage with respect to normal.

method and is uncorrected. The UV spectrum was recorded on Beckman DU-64 spectrophotometer in methanol. The IR spectrum was recorded as KBr pellets on Hitachi-270 spectrophotometer. The ^{1}H (400 MHz) and ^{13}C and DEPT 90 and 135 NMR (400 MHz) and 2D-NMR (COSY, HMBC, and HMQC) were obtained on a Bruker DRX 400 spectrometer in DMSO-d_6 using TMS as internal standard reference; chemical shift in δ (ppm) and coupling constants (J values) are in Hertz. The MS spectrum was recorded on Finnegan MAT 300 mass spectrometer. Column chromatography was performed using silica gel (60–120 mesh; S.D. Fine, Mumbai, India) as an adsorbent. TLC was performed on silica gel G (S.D. Fine).

3.2 *Plant material*

The whole plant of *P. debilis* was collected from Karnataka, South India, and was authenticated by Dr M.P. Sharma, a taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard. A voucher specimen (No. PD-FP-21) has been deposited in the herbarium of Jamia Hamdard, New Delhi.

3.3 *Extraction and isolation*

The plant material (3 kg) was dried and crushed to coarse powder and extracted with ethanol using cold percolation till completely exhausted. The ethanolic extract was evaporated under reduced pressure and then successively fractionated into petroleum ether (60–80°C), chloroform, and methanol. The different fractions were dried under reduced pressure to get the crude dried fractions. The methanol fraction (160 g) was packed on the top of silica gel column packed in petroleum ether. The column was then eluted with petroleum ether, chloroform, and methanol successively in the order of increasing polarity. The eluent chloroform–methanol (90:10) afforded compound **1** (95 mg).

3.3.1 *Debelalactone (**1**)*

Yellow amorphous powder; mp 260–263°C; R_f 0.56 ($CHCl_3$–MeOH 8:2). IR ν_{max} cm^{-1}

Table 3. Histopathological changes in liver of Wistar rats.

Groups	Treatment	Microscopic observations
1	Normal control	Liver samples showed normal architecture without any degeneration, necrosis, or inflammation.
2	Toxic control	Liver samples showed prominent centrilobular necrosis with prominent and enlarged central vein. There is significant periportal inflammation reflecting liver damage.
3	Standard control	Liver samples showed a significant reduction in portal inflammation and sinusoidal dilatation. The central vein was clearly visible. Liver samples also showed good recovery with the absence of necrosis.
4	Debelalactone	Liver histology was almost normal with only very little sinusoidal dilatation seen in some hepatic lobules. Portal vein appeared clearly with the disappearance of necrosis thus indicating a potent antihepatotoxic activity.

(KBr): 3374 (OH), 3090 (OCH_3), 1722 (δ-lactone carbonyl), 1292 (C—O, furan), 1093, 1040 (C—O, epoxide), 1594, 1513, 1444, 1395, 1339, 1198, 985, 923, and 763; 1D- and 2D-NMR (DMSO) spectral data (see Table 1). FAB-MS *m/z* (rel. int.%): 314.25 (5) ($[M]^+$, $C_{16}H_{10}O_7$), 190 (20), 176 (75), 166 (30), 152 (85), 148 (80), 138 (30), 124 (10), and 108 (50). HR-MS (*m/z*): 314.0411 $[M]^+$ (calcd for $C_{16}H_{10}O_7$, 314.0426). Elemental analysis: found: C, 61.13%; H, 3.22%; calcd for $C_{16}H_{10}O_7$: C, 61.15%; H, 3.21%.

3.4 Experimental animals

Male Albino Wistar rats weighing 150–200 g were employed for assessing the antihepatotoxic activity. They were procured from the Central Animal House of Jamia Hamdard, New Delhi (Sanction Letter No. 173/CPCSEA), after approval under the project proposal number-326. They were fed with a standard pellet diet and water *ad libitum.*

3.5 Antihepatotoxic activity

The rats were divided into four groups, six rats in each group. Group I served as normal control, which received normal saline only. Group II as toxic control received CCl_4 diluted with liquid paraffin in a ratio of (1:1) [1.5 ml/kg b w, per oral (p.o.)] on the first day [8]. Group III was given a single dose of CCl_4 on the first day (1.5 ml/kg b w, p.o.) and then silymarin (Slybon-70, 10 mg/kg p.o.) once a day for 6 days. Group IV received a single dose of CCl_4 (1.5 ml/kg b w, p.o.) on the first day and then compound **1** at the dose of 50 mg/kg b w, p.o. for 6 days. On day 8, the blood samples were withdrawn by puncturing the orbital plexus first, and then the rats were killed by decapitation. The blood samples were allowed to clot for 30–40 min at room temperature.

3.6 Assessment of liver function

Biochemical parameters like SGOT and SGPT were determined by Reitman and Frankel [9], ALP and TP were determined by reported methods of Kind and King [10] and Wooton [11].

3.7 Statistical analysis

The data of biochemical estimations were reported as ± SE. For determining the statistical significance, one-way analysis of variance and Dunnett's test were employed. *P*-values of less than 0.05 were considered significant [12].

3.8 Histopathological studies of the liver

Rat livers were quickly removed after autopsy and fixed in 10% formalin. The sections

were cut and then stained by hematoxylin and eosin. These were observed under microscope [13].

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