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A lignan from the root of *Ecbolium linneanum* Kurz.

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Abstract

From the chloroform extract of the root of *Ecbolium linneanum* Kurz., a furofuran type of unsymmetrical lignan named as Ecbolin A was isolated. The structure was established by spectroscopic methods and confirmed by single crystal X-ray diffraction studies.

Keywords: *Ecbolium linneanum*; *Acanthaceae*; Unsymmetrical lignan; Furofuran type

1. Introduction

Ecbolium linneanum Kurz. (Fam. *Acanthaceae*), a shrub is reported to be useful in jaundice, menorrhoea, rheumatism (Chopra et al., 1966). The roots and leaves are used against tumours. 50% ethanol extract of the plant is used in cardiovascular affections (Nadkarni, 1954; Anon., 1967; Gamble, 1993). Four glycoflavones have been reported previously from this plant (Nair et al., 1975). In order to search for more bioactive natural products, the root of *E. linneanum* has been subjected to phytochemical examination in the present investigation.

2. Results and discussion

The chloroform extract of the root of *E. linneanum* was chromatographed over silica gel. Fractions 12–20 yielded a novel unsymmetrical furofuran type of lignan. The structure was established by the following spectroscopic and X-ray analyses. The electron impact mass spectrum of Ecbolin A (**1**) indicated the molecular weight of 444.05 corresponding to the molecular formula $C_{23}H_{24}O_9$. The UV spectrum of compound showed maxima at 232 and 280 nm which corresponds to the furofuran type of lignan viz. 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane (Matsushita et al., 1991). Infrared spectrum of the compound taken in $CHC1_3$ exhibited bands at 1650, 1615, 1489, 1510, 1450, 1150, 1102 cm^{-1} which is also in complete agreement with furofuran type of lignan reported earlier (Jaensch et al., 1989). The peak at 1102 cm^{-1} corresponds to O–C–O stretching; aromatic C–H stretching frequency appears at 1450 cm^{-1}. Infrared spectrum does not show peak at 3500 cm^{-1} indicating the absence of free hydroxyl group in the compound. ^1H NMR signal pattern in the aromatic region suggested the presence of a singlet at 6.87 δ and two *ortho* coupled protons at 6.82 δ and 6.78 δ respectively (Ayres and Loke, 1990; Abe and Yamauchi, 1989). It also showed three 3H singlets at 3.93 δ, 3.90 δ and 3.81 δ indicating the presence of three $-OCH_3$ groups. The two peaks at 5.91 δ and 5.94 δ correspond to $-O-CH_2-O-$ groups. ^1H–^1H COSY and 2D-ROESY correlations were also in support of the structure of Ecbolin A. In the long range COSY correlations, 6.82 (1H, *d*, $J=8.0$ Hz) is interacting with 4.75 (1H, *d*, $J=6.6$ Hz); 5.25 (1H, *d*, $J=6.4$ Hz) is interacting with 3.35 (1H, *m*); 4.75 (1H, *t*, $J=6.6$ Hz) is interacting with 3.12 (1H, *m*); 4.12 (1H, dd, $J=7.3$ and 8.4 Hz) is interacting with 3.85 (1H, *m*) and 3.35 (1H, *m*); 3.35 (1H, *m*) is interacting with 5.25 (1H, d, $J=6.4$ Hz); 4.12 (1H, dd, $J=7.3$ and 8.4 Hz) and 3.85 are interacting with 3.12 (1H, *m*) and 3.12 (1H, *m*) is interacting with 4.75 (1H, d, $J=6.6$ Hz), 4.31 (1H, *d*, $J=7.7$ Hz) and 3.35 (1H, *m*).

In the various 2D-ROESY correlations (NOE effects) (Table 1), 6.82 is correlating with 4.75 and 3.12; 6.87 is correlating with 4.75 and 3.12; 5.25 is correlating with 4.75; 5.25 is correlating with 3.35 (strong); 5.25 is cor-

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Table 1
^{1}H NMR data of Ecbolin A and its closely related compounds

Hydrogen atoms	Asarinin[a] (episesamin) (δ)	Eudesmin[a] (δ)	Ecbolin A (δ)
1,5	3.15 (*m*)	3.15 (*m*)	3.12 (*m*), 3.35 (*m*)
2,6	4.35 (*d*, *J* = 7.5 Hz)	4.75 (*d*, *J* = 4.0 Hz)	4.75 (*d*, *J* = 6.6 Hz)
	4.76 (*d*, *J* = 5.0 Hz)		5.25 (*d*, *J* = 6.4 Hz)
4,8	3.6–4.15 (m)	3.8–4.0 (*m*)	3.85 (*m*)
	3.6–4.15 (m)	4.2–4.4 (*m*)	4.12 (*dd*, *J* = 7.3, 8.4 Hz)
2″	–	–	6.87 (1H, *s*)
5″	–	–	6.78 (*d*, *J* = 8.0 Hz)
6″	–	–	6.82 (*d*, *J* = 8.0 Hz)
–OCH_3	–	3.86, 3.90 (3H, *s*)	3.81 (3H, *s*)
			3.90 (3H, s)
			3.93 (3H, s)
–OCH_2O–	5.87 (2H, *s*)	–	5.91 (2H, *s*)
			5.94 (2H, *s*)

[a] Pelter and Ward (1978).

relating with 4.12; 5.25 is correlating with 3.85 (strong); 5.25 is correlating with 3.12 (weak) and 5.25 is correlating with 3.85. These assignments are shown in Fig. 1.

^{13}C NMR data also confirmed the structure. The aryl carbon atoms of one ring were assigned with 134.8 δ, 106.4 δ, 146.0 δ, 147.8 δ, 108.0 δ and 119.2 δ. (C-1″–C-6″). The other aryl carbon atoms were assigned with 135.4 δ, 119.2 δ, 136.9 δ, 137.1 δ, 101.3 δ and 133.2 δ (C-1′–C-6′). The methylenedioxy carbon atoms were assigned with 100.9 δ, 101.3 δ. Three methoxyl carbon atoms were assigned with 60.1 δ, 60.2 δ, and 61.9 δ. From already reported unsymmetrical furofuran type of compounds (Cuenca and Catalan, 1991; Nishebe et al., 1977), it is evident that the compound, Ecbolin A is unsymmetrical due to the changes in the values of aryl carbon atoms of the two rings (Table 2). The ^{13}C NMR shift of 1′ and 1″-carbon atom of equatorial 3,4-methylenedioxyphenyl group comes at 134.9–135.6 ppm and that of an axial 3,4-methylenedioxyphenyl group comes at 132.0 ppm (Pelter and Ward, 1978). In the case of Ecbolin A, the 1′ and 1″ carbon atoms of 3,4-methylenedioxyphenyl group appears at 135.4 and 134.8 ppm respectively. Hence the diequatorial configuration of Ecbolin A is established. On the basis of mass, UV, IR, ^{1}H NMR, ^{1}H–^{1}H COSY, 2D-ROESY and ^{13}C NMR spectral data, the structure **1** was established for Ecbolin A and the name is 6-(3,4-methylenedioxyphenyl)-2-(2,5,6-trimethoxy-3,4-methylenedioxyphenyl)-3,7-dioxa-bicyclo[3.3.0] octane. Paucity of the material prevented determination of absolute streochemistiy.

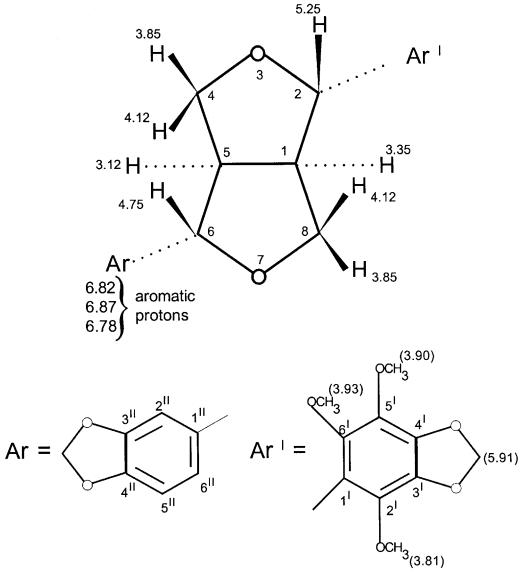


Fig. 1.

Table 2
^{13}C NMR spectra of Ecbolin A and its closely related compounds

Carbon atoms	Sesamin[a] (ppm)	Episesamin[a] (ppm)	Ecbolin A (ppm)
1	54.2	54.7	55.5
2	85.6	87.7	79.6
4	71.5	71.04	72.1
5	54.2	50.2	52.1
6	85.6	82.1	84.9
8	71.5	69.7	72.2
1″	134.9	135.6	134.8
2″	106.3	106.6	106.4
3″	146.8	146.8	146.0
4″	147.7	147.9	147.8
5″	107.9	108.2	108.0
6″	119.1	118.8	119.2
1′	134.9	132.6	135.4
2′	106.3	106.7	119.2
3′	146.8	147.4	136.9
4′	147.7	148.2	137.1
5′	107.9	108.2	101.3
6′	119.1	119.6	133.2
–OCH_2O–	100.89	101.14	100.9, 101.3
–OMe	–	–	60.1, 60.2, 61.9

[a] Pelter and Ward (1978).

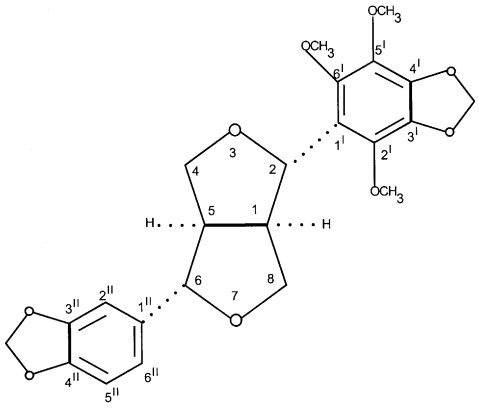
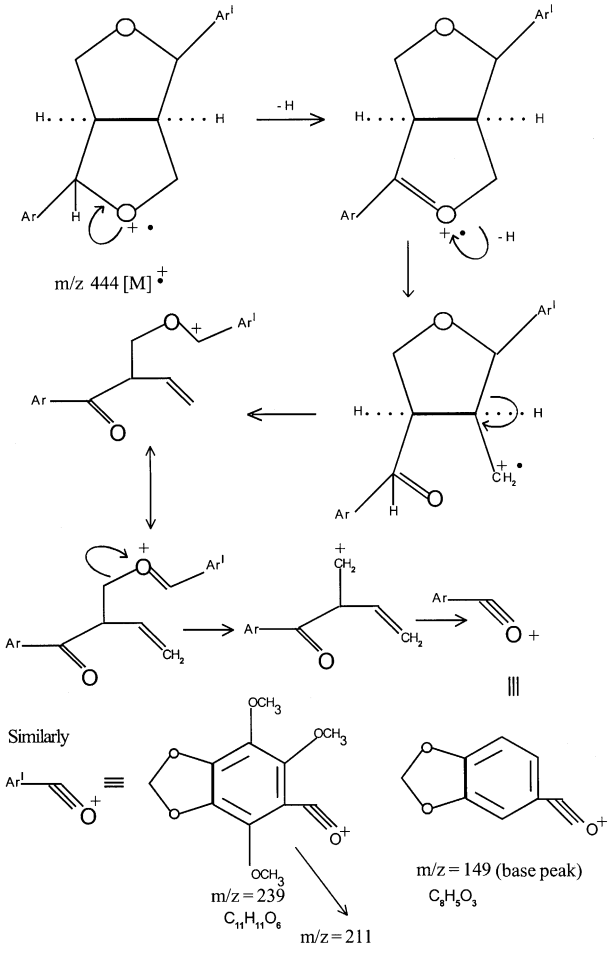


Fig. 2.



Scheme 1. Mass spectral fragmentation mode of Ecbolin A.

The structure for Ecbolin A (Fig. 2) was confirmed by the mass spectral fragmentation mode also and represented in Scheme 1.

The molecular ion peak $[M]^{+\bullet}$ occurred at m/z 444 and the base peak at m/z 149. The other significant peaks were appearing at m/z 239 and 211.

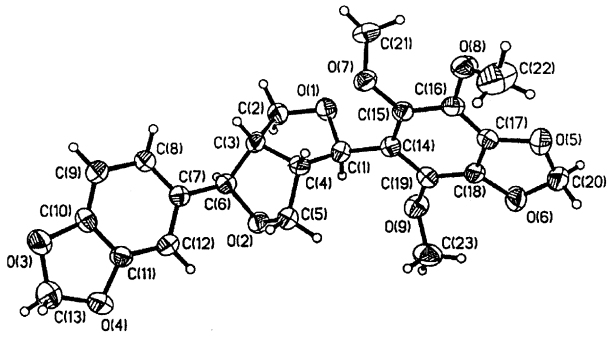


Fig. 3. Structure and solid-state conformation of Ecbolin A; small circles represent hydrogen atoms.

The complete structure of Ecbolin A was established by single crystal X-ray analysis (Fig. 3).

Ecbolin A was isolated from natural origin for the first time. Since lignans are known to have antitumour, antimitiotic and antiviral activities and also compounds containing methylenedioxy groups are reported to be useful in jaundice (Macrew and Towers, 1984), the biological activity of Ecbolin A was under investigation. However due to paucity of the material, the biological activity of Ecbolin A could not be proceeded further.

3. Experimental

Mps: uncorr. UV: EtOH, IR: $CHCl_3$, ^1H NMR and ^{13}C NMR: $CDCl_3$, TMS as int. std. except where noted, ms: a direct inlet system, X-ray: single crystal.

3.1. Plant material

The roots of *E. linneanum* Kurz. were collected from Courtallam Hills of Western Ghats of South India and identified by Dr. V. Chelladurai, Research officer (Botany), Survey of Medicinal Plants Unit, Siddha, CCRAS, Palayamkottai-627 002, Tamil Nadu, India. Voucher specimen (Voucher No. MSU 0021) has been deposited at Herbarium of the Department of Chemistry, Manonmoniam Sundaranar University, Tirunelveli-627 012, Tamil Nadu, India.

3.2. Extraction and separation

Air-dried roots (200 g) of *E. linneanum* were extracted (×5) with hot chloroform. The combined chloroform extract was concentrated under reduced pressure to give a green pasty mass. The green pasty mass was chromatographed over silica gel (60–120 mesh, BDH India Ltd.) and eluted with hexane and ethylacetate (8:2). Fractions 12–20 yielded a major compound which became homogeneous colourless needles

(mp 118–120 °C) upon recrystallisation with ethanol: acetone (8:2).

3.2.1. 6-(3,4-Methylenedioxyphenyl)-2-(2,5,6-trimethoxy-3,4-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0] octane

Ecbolin A (**1**) colourless needles mp 118–120°, HRMS: calcd. for $C_{23}H_{24}O_9$ m/z 444.05 $[M]^+_{\bullet}$ found 444.06 EIMS: m/z (rel. int.): 149 (100), 239 (18.15), 211 (9.46). UV λ_{max}^{EtOH} nm: 232 and 280, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1102, 1450, 3500 absent, ^1H NMR ($CDCl_3$, 500 MHz): see Table 1. ^{13}C NMR ($CDC1_3$, 500 MHz): see Table 2. *Crystal data*: Orthorhombic, space group $P2_12_12_1$; a = 7.401 (1), b = 9.468 (1) c = 29.665 (4) Å, V = 2085.3 $Å^3$, D = 1.416 mg/m^3 Crystal dimension 0.29×0.21×0.18 mm. The crystal structure was solved by direct methods using SHELX-97 package (anisotropic C,O; isotropic H) converged to R = 0.0399 over 1415 reflections with $[I > 2\sigma(I)]$.

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