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ALEURITIN, A COUMARINOLIGNOID, AND A COUMARIN FROM *ALEURITES FORDII**

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Key Word Index—*Aleurites fordii*; Euphorbiaceae; stem; alcuritin; angular coumarinolignoid; coumarin; 5,6,7-trimethoxycoumarin.

Abstract—A new coumarinolignoid and 5,6,7-trimethoxy coumarin have been isolated from stems of *Aleurites fordii* and their structures determined on the basis of chemical and spectroscopic evidence.

INTRODUCTION

Aleurites fordii, a member of the Euphorbiaceae is reported to be of medicinal importance [1]. Commercially, tung oil is isolated from the seeds of this plant, which on earlier investigations has yielded a sterol [2], amino acids [3] and two toxic and piscicidal diterpene esters [4].

RESULTS AND DISCUSSION

Materials used in this investigation were collected from the Campus of the Forest Research Institute, Dehradun (India) and identified there. Stem pieces were dried, and extracted successively with petrol (60–80°) and ethanol. The petrol extract on chromatography yielded **1** which was crystallized from methanol as colourless crystals, mp 75°. Its ^{1}H NMR spectrum exhibited signals representative of three methoxyl groups (δ3.87, 3.90 and 4.03, 3H each, *s*) and the three other protons, two of which appear as doublets at 6.22 and 7.90 (1H each *d*, $J = 9.5$ Hz) and the other at 6.61, as a singlet. The $[M]^+$ in the mass spectra of **1**, appeared at *m/z* 236 ($C_{12}H_{12}O_5$) leading to the formulation of **1** as 5,6,7-trimethoxy coumarin [5].

The concentrated ethanolic extract was chromatographed on a column of silica gel, elution of which with benzene–ethyl acetate (4:1) yielded a homogeneous white solid, mp 238–239° (aleuritin, **2a**). Aleuritin dissolves in aq. KOH from which it can be regenerated and fluoresces blue in UV light. Its ^{1}H NMR spectrum shows two pairs of doublets at 6.13 and 7.92 (1H each, *d*, $J = 9.5$ Hz) and its IR spectrum a prominent band at 1735 cm^{-1}. Aleuritin, is, therefore, a coumarin derivative. Additionally, the ^{1}H NMR spectrum indicates the presence of three methoxyl groups (3.93, 6H, *s*, and 3.98, 3H, *s*). Aleuritin on acetylation yields an acetate (**2c**) mp 190°. Apart from the presence of two coumarinic protons (6.23, 1H, *d*, and 7.92, 1H, *d*, $J = 9.66$ Hz) the ^{1}H NMR spectrum of **2c** reveals the presence of three methoxyl (3.84, 6H, *s* and 3.96, 3H, *s*) and two acetyl functions (2.07, 3H, *s* and 2.36, 3H, *s*) one of which can be presumed to be aliphatic in character. Furthermore, signals corresponding to three aryl protons (6.55, 1H, *s* and 6.62, 2H, *s*) and four deshielded non-aromatic protons (5.01, 1H, *d*, 7.66 Hz; 4.11, 1H, *dd*, 12.1 and 4.32 Hz; 4.42, 1H, *dd*, 12.1 and 3.3 Hz; 4.279, 1H, *p*) together with the appearance of a $[M]^+$ at *m/z* 416.1107 ($C_{21}H_{20}O_9$) in the mass spectra of **2a** leads to the conclusion that aleuritin is a coumarinolignoid. In support of this assumption, the four non aromatic protons appear as a four spin system as revealed by decoupling experiments (Table 1), consistent with the structural environment (**3**). Aleuritin, on the basis of its physical constants, however, appeared to be different from the isomeric aquillochin [6, 7]. Long distance coupling decipherable between the 1H aromatic signal at 6.55 and the coumarinic H-4 makes it possible to assign this singlet to H-8 [8], but the substitution pattern could be deduced only from NOE experiments (Table 2). Thus, irradiation of the H-8 at δ6.55 causes the enhancement of the methoxyl signal (3H) at 3.96 with the corollary being recorded in the reverse experiment. A methoxyl group is hence situated at C-7. Irradiation at H-4 does not cause enhancement of any methoxyl signal. The C_6–C_3 unit is, therefore, attached to the coumarin unit at C-5 and C-6. The extremely close resemblance of the UV spectrum of aleuritin with that of 5,6,7-trimethoxy coumarin further testifies to the fact that aleuritin is 5,6,7-trioxygenated. The other functional groups are, therefore, located on the C_6–C_3 moiety and the appearance of two aromatic protons and six methoxyl protons as singlets identifies this unit as syringyl alcohol. Aleuritin hence can be depicted as **2a**/**2b**. EI mass spectral data of aleuritin and its diacetate support such a formulation, especially in the appearance of fragment ions at *m/z* 167 ($C_9H_{11}O_3$; *a*), 208 ($C_{10}H_8O_5$; *b*) and 210 ($C_{11}H_{14}O_4$; *c*) in that of aleuritin [10]. Furthermore, ^{13}C NMR data obtained from the acetate is also in total agreement with the postulated structure, assignment of which were made by running spectra in the DEPT mode with ϕ as 90° and 135° and by resorting to $^1J_{CH}$-2D spectra correlations.

The $^3J_{CH}$-2D correlation spectrum does not make it possible to make a choice between **2a** or **2b** especially since the $^3J_{CH}$ coupling between the coumarinic carbons and the protons of the appendage are of the order of 1 Hz

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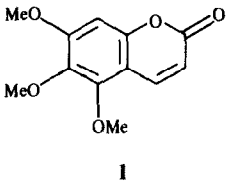

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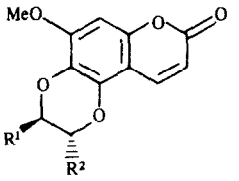
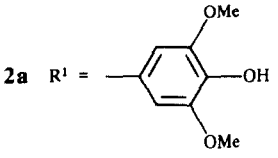
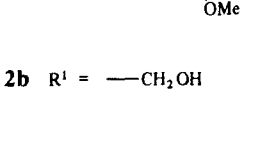




2a R^1 = —OH R^2 = —CH_2OH



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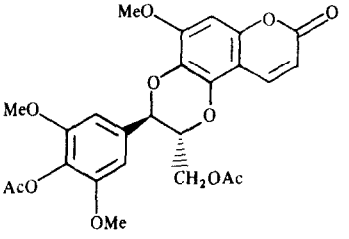
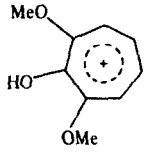
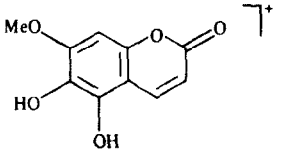


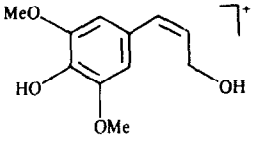
2c



a



b



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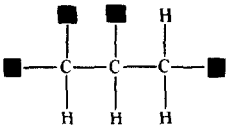

3

[7]. Low power heterodecoupling, however, helps in resolving this problem. Irradiation of 7′-H at 5.01 results in considerable sharpening of the C-6 signal at δ 128.90. Aleuritin, hence, can be represented as **2a**. The *trans* orientation of the phenyl and methylol groups is evident from the coupling constant of 7.66 Hz by which the 7′-H splits the 8′-H.

Table 1. Decoupling experiments on compound **2a**

Irradiated at	Effect at δ			
	5.01	4.279	4.42	4.11
5.01	—	$p \rightarrow q$	NE	NE
4.279	$d \rightarrow s$	—	$dd \rightarrow d$	$dd \rightarrow d$
4.42	NE	$p \rightarrow q$	—	—

NE = No effect.

Table 2. NOE (%)* and chemical shift observed for compound **2c**

Irradiated Signal (δ)	Observed signals			
	H-3	H-4	H-8	OMe
3.96	—	—	6.55 (26.66)	—
7.92	6.23 (18.75)	—	—	—
6.55	—	—	—	3.96 (10.0)

*Increase in signal heights are shown in parentheses.

Coumarinolignoids have been found to be cytotoxic [9, 10] and antihepatotoxic [11]. Nine coumarinolignoids have been reported so far, some of which have been isolated from more than one source, three having been isolated from Capparidaceae [11, 12, 13], three from Euphorbiaceae [14, 15, 16], two from Thymelaceae [6, 9], two from Hippocastanaceae [17] and one each from Burseraceae [18], Aceraceae [19], Sapindaceae [10], and Simaroubaceae [10]. One of these is of indeterminate structure [19] and another has been isolated from the closely related species, *A. moluccana* [16].

EXPERIMENTAL

5,6,7-Trimethoxy coumarin (**1**). Stem wood (5 kg) of *A. fordii* collected from the Campus of the Forest Research Institute, Dehradun, was cut into very small pieces and defatted × 4 by refluxing with petrol (60–80°). Petrol extracts were combined, concd and chromatographed on a column of silica gel. The column was eluted with C_6H_6 affording **1**, which was recrystallized from MeOH as colourless needles, mp 75°, UV λ_{max}^{MeOH} nm; 230 and 320. IR ν_{max}^{Nujol} cm^{-1}; 2900, 1730, 1609, 1450, 1370, 1250, 1190, 1120, 1020 and 820. ^1H NMR ($CDCl_3$) δ; 7.90 (1H, *d*, J = 9.5 Hz, H-4), 6.61 (1H, *s*, H-8), 6.22 (1H, *d*, J = 9.5 Hz, H-3), 3.87 (3H, *s*, OMe), 3.90 (3H, *s*, OMe), 4.03 (3H, *s*, OMe). ^{13}C NMR ($CDCl_3$) δ; 56.20 (OMe), 60.98 (OMe), 61.63 (OMe), 95.45 (C-8), 106.99 (C-10), 112.24 (C-3), 149.15 (C-5), 151.36 (C-7), 157.36 (C-9), 160.83 (C-2), 138.03 (C-6), 138.60 (C-4). MS *m/z*; 236 ($[M]^+$, $C_{12}H_{12}O_5$), 221, 193, 178, 150, 135, 95 and 67.

Aleuritin (**2a**). The plant material after defatting, was freed of residual solvent and then extracted by refluxing × 3 with EtOH. The extracts were combined and concd to give a gummy mass. The combined EtOH exts were chromatographed on a column of silica gel and eluted with C_6H_6 containing increasing quan-

tities of EtOAc. Frs collected were monitored by TLC [silica gel, MeOH–$CHCl_3$ (1:19) visualized with I_2 vapour]. Fr. nos 10–15 eluted with C_6H_6–EtOAc (4:1) were combined and evapd to yield a white solid which was crystallized from MeOH to give white crystals, mp 238–239° (**2a**) UV λ_{max}^{MeOH} nm; 240 and 320. IR ν_{max}^{KBr} cm^{-1}; 3470 (OH), 1735, 1620, 1460, 1229, 1120, 1040 and 820. ^1H NMR (60 MHz, DMSO-d_6) δ; 3.93 (6H, *s*, 2 × OMe), 3.98 (3H, *s*, OMe), 5.02 (1H, *d*, J = 7 Hz, H-7′), 6.13 (1H, *d*, J = 9.5 Hz, H-3), 6.66 (1H, *s*, H-8), 6.72 (2H, *s*, H-2′ and H-6′), 7.92 (1H, *d*, J = 9.5 Hz, H-4). MS *m/z*; 416.1107 ($[M]^+$, $C_{21}H_{20}O_9$), 398, 210, 208, 182, 167 and 154.

Diacetate **2c**. Compound **2a** (50 mg) was dissolved in a min. vol. of pyridine (1 ml) to which was added an equal vol. of freshly dist. Ac_2O. The soln was kept overnight and then heated at 100° for 30 min and worked-up in the usual manner. The powder obtained was crystallized from MeOH to give shining needles, mp 190°. ^1H NMR (200 MHz; $CDCl_3$) δ; 2.07 (3H, *s*, OAc), 2.36 (3H, *s*, OAc), 3.84 (6H, *s*, 2 × OMe), 3.96 (3H, *s*, OMe), 4.11 (1H, *dd*, J = 12.1 and 4.32 Hz, H-9′), 4.279 (1H, *p*, H-8′), 4.42 (1H, *dd*, J = 12.1 and 3.3 Hz, H-9′), 5.01 (1H, *d*, J = 7.66 Hz, H-7′), 6.23 (1H, *d*, J = 9.66 Hz, H-3), 6.55 (1H, *s*, H-8), 6.62 (2H, *s*, H-2′ and H-6′), 7.92 (1H, *d*, J = 9.66 Hz, H-4). ^{13}C NMR ($CDCl_3$) δ; 20.39 (COMe), 20.62 (COMe), 56.31 (2 × OMe), 56.60 (OMe), 62.59 (C-9′), 75.23 (C-8′), 77.40 (C-7′), 93.33 (C-8), 103.30 (C-10), 104.00 (C-2′ & C-6′), 112.32 (C-3), 128.90 (C-6), 129.10 (C-4′), 133.0 (C-1′), 137.64 (C-4), 139.50 (C-5), 149.60 (C-9), 152.30 (C-7), 152.74 (C-3′ and C-5′), 161.21 (CO), 168.36 (CO), and 170.22 (CO).

DEPT experiments. Two DEPT expts were performed using a polarization transfer pulse of 90 and 135°, respectively, obtaining in the former case only CH groups and in the later case positive signals for CH and CH_3 and negative ones for CH_2 groups. DEPT 90° C-CH δ; 75.24, 77.35, 93.25, 103.88, 112.32, 116.62 and 137.76. DEPT 135°C-J pair negative J. Impair positive. Positive signals δ; 20.46, 20.75, 56.31, 56.56, 75.24, 77.35, 93.25, 103.88, 112.32, 116.62 and 137.76. Negative signals δ; 62.64.

Non-decoupled spectrum of **2**. 56.31 (OMe, *q*, J = 145 Hz), 56.60 (OMe, *q*, J = 145 Hz), 62.59 (C-9′, *t*, J = 157.5 Hz), 75.23 (C-8′, *br d*, J = 155 Hz), 77.4 (C-7′, *br d*, J = 155 Hz), 93.33 (C-8, *d*, J = 165 Hz), 103.3 (C-10, *dd*, J = 8 and 5 Hz), 104.0 (C-2′ and C-6′, *dddd*, J = 157.5, 8 and 5 Hz), 112.31 (C-3, *d*, J = 172.5 Hz), 128.9 (C-6, *d*, J = 7.5 Hz), 129.10 (C-4′, *t*, J = 7.5 Hz), 133.0 (C-1′, *br s*), 137.64 (C-4, *d*, J = 167.5 Hz), 139.5 (C-5, *d*, J = 5 Hz), 149.64 (C-9, *t*, J = 6 Hz), 152.30 (C-7, *t*, J = 4 Hz), 152.74 (C-3′ and C-5′, *br s*), 161.21 (C-2, *dd*, J = 10 and 4 Hz). MS *m/z*; 500 ($[M]^+$, $C_{25}H_{24}O_{11}$), 458, 398, 252, 210, 181 and 167.

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