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LEPTODACTYLONE, A YELLOW COUMARIN FROM *LEPTODACTYLON* AND *LINANTHUS* SPECIES*

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Key Word Index—*Leptodactylon*; *Linanthus*; Polemoniaceae; leptodactylone; yellow pigment; 5,7-dimethoxy-8-hydroxycoumarin; NMR and solvent shifts; synthesis; chemotaxonomy.

Abstract—A new phenol in *Leptodactylon* and *Linanthus* leaf has been identified by spectral procedures as 5,7-dimethoxy-8-hydroxycoumarin. This structure has been confirmed by synthesis. Leptodactylone is unique among simple hydroxycoumarins in its yellow colour.

INTRODUCTION

During a chemosystematic survey of the Polemoniaceae for flavonoids and related phenolics, a novel yellow phenolic was found to occur in leaf of all three *Leptodactylon* species surveyed and in *Linanthus dichotomus* and *L. dianthiflorus* [1, 2]. Since spectral studies showed it to possess a carbonyl group, it was named leptodactylone. While its co-occurrence in these plants with daphnetin(7, 8-dihydroxycoumarin) suggested it might have a related coumarin structure, its yellow colour clearly distinguished it from any of the known naturally occurring simple coumarins.

Classification of species into *Leptodactylon* and *Linanthus* has provided taxonomists with difficulties because of the many morphological similarities between these two genera. New chemical evidence is thus of particular systematic value in this group of some 30 plants [2]. For this reason, identification of leptodactylone was of considerable interest. Its structural elucidation and synthesis is reported in the present paper.

* Parts 3 in the series 'Chemosystematics of the Polemoniaceae'; for Parts 1 and 2, see refs 1 and 2.

RESULTS

Mass spectral analysis established for leptodactylone the molecular formula $C_{11}H_{10}O_5$, the PMR spectrum (Table 1) and other properties indicating the presence of two methoxy groups, one phenolic hydroxy group, and one aromatic proton. The occurrence of doublets (J = 9 Hz) at δ 6.15 and 7.98 appropriate to a *cis*-alkene and IR absorption at 1700 cm^{-1} (KBr) then suggested that the compound might be a trisubstituted coumarin.

Table 1. PMR spectra at 220 MHz*

Compound	Solvent	Assignments							
		3-H	4-H	5-H	6-H		7-OMe	8-OMe	OH
7,8-Dimethoxycoumarin	$CDCl_3$	6.28†	7.64†	7.20†	6.90†		4.01	3.97	
	benzene-d_6	5.85	6.69	6.50	6.27		3.27‡	3.74‡	
8-Hydroxy-7-methoxycoumarin	$CDCl_3$	6.23†	7.62†	7.00	6.84§		3.96		4.7‖
(**12**)	benzene-d_6	¶	¶	¶	¶		3.01		
5,7,8-Trimethoxycoumarin	$CDCl_3$	6.17†	7.98†		6.35	3.97	3.92	3.91	
	benzene-d_6	5.85	7.49		5.74	3.15	3.29	3.73	
5,8-Dihydroxy-7-methoxycoumarin	acetone-d_6	6.05**	8.03**		6.51		3.87		8.88‖
(**14**)									7.52‖
Diacetate of (**14**)	$CDCl_3$	6.28	7.65		6.78	2.41††	3.90	2.41††	
8-Hydroxy-5,7-dimethoxycoumarin	$CDCl_3$	6.15§	7.98§		6.38	3.90	3.99		5.40‖
(leptodactylone)	benzene-d_6	¶	¶		¶	3.14	3.22		
(**8**)									
quinone (**13**)	$CDCl_3$	6.68**	7.91**		6.05		3.91		

* δ scale; † d, J = 10 Hz; ‡ assignments by analogy with the 4-methyl derivative [5]; § d, J = 9 Hz; ‖ removed by D_2O; ¶ saturated solution too dilute for reliable assignments of weak signals. ** d, J = 11 Hz; †† acetate Me.

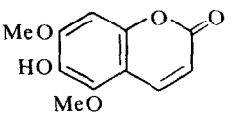
The UV spectrum and the long wave-length shift induced by alkali were similar to those of fraxinol [3] (**1**) and supported the conclusion that a phenolic coumarin was in hand.

Several studies [4, 5] of the PMR spectra of coumarins, especially those of González *et al.* [6], show that an oxygen substituent (OH or alkoxyl) at position 7 causes the resonance of the 3-proton to move to higher fields (*ca* 0.17 ppm); a 4-proton moves to a less extent (*ca* 0.10 ppm). Electron release by the substituent to the 3-position is probably partly responsible, as indicated in structure (**2**). A similar group at position 5 has a smaller effect upon the 3-proton, since electron release now involves a less favourable *ortho*-quinonoid distribution as in (**3**). It follows that oxygen substituents at positions 6 and 8 should have little or no effect, and shifts already recorded by other workers as well as those in Table 1 accord with this deduction.

The resonance of leptodactylone at δ 6.15 was therefore evidence that an oxygen atom must be located at position 7. Moreover, electron release as in (**2**) can occur only if the methoxy group is in the same plane as the benzene ring, a condition known not to be met when two flanking *ortho* substituents are present because steric hindrance prevents it [7]. Hence this resonance also pointed to a free 6- or 8-position, and consequently to the presence of a group at the 5-position.

It has been well established [6] that an oxygen substituent at the 5-position in a coumarin ring shifts the resonance of the adjacent 4-proton to much lower fields (*ca* 0.3 ppm) because of the peri effect also marked in chromenes [8]. Since leptodactylone showed such a shift we again had to locate one substituent at position 5 leaving the other to be placed at position 6 or at position 8.

If there is a substituent at position 6, three structures are possible. One corresponds to fraxinol [3] (**1**) but could be rejected because the mps differ widely. Another structure (**4**) had already been allocated [9] to a natural coumarin, the mp of which seemed not to have been recorded; however, leptodactylone did not respond to the Gibbs test so this structure was tentatively rejected.



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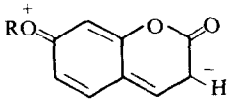

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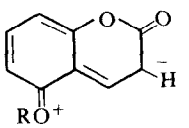

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The third structure (**5**) has been allocated [3] to a natural coumarin with an mp close to that of leptodactylone and a very similar mass spectral fragmentation pattern (Table 2), though the quantitative differences seemed too large for an identity.

With a substituent in position 8 another three structures are possible. Of these one (**6**) had been assigned [10] to a natural coumarin with a much higher mp than has leptodactylone and could therefore be dismissed leaving only the other two, (**7**) and (**8**). A final choice of structure was made by means of a solvent shift method, with $CDCl_3$ and benzene-d_6 as solvents.

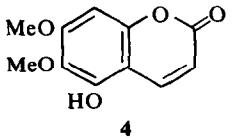
In benzene, association with solvent molecules results in a shift of methoxy proton resonances to higher fields provided that there is at least one free position *ortho* to the methoxy group [5, 11–13]. It appears that two *ortho* substituents provide enough steric hindrance to prevent the effect. Methoxy groups are well known to act as steric blocks in this sense, and there are some reports in which an hydroxy group [11] or coumarin ring oxygen [13] acts similarly. Our own work confirms these reports, and because in benzene-d_6 *both* methoxy resonances of leptodactylone were shifted upfield both have one free *ortho* position and only structure (**8**) satisfies this condition.

Because the solvent shift method is not unequivocal [11], however, we confirmed this conclusion by synthesizing coumarin (**8**) and showed it to be identical with leptodactylone. We first prepared 6,7-dihydroxycoumarin (**9**) by condensing pyrogallol with malic acid in the presence of H_2SO_4 as described by Günther *et al.* [14]; the results were poor. A recent modification [15] was no better; although less tar was formed, a dipyrone was a major component of the product and could not be separated readily. The use of acetylenic equivalents of malic acid also gave poor results [16]. One of the chief difficulties was that, in contrast to its 4-methyl derivative (**10**), the desired coumarin was rapidly darkened and

Table 2. Mass spectral collapse of leptodactylone compared with that of 7-hydroxy-5.6-dimethoxycoumarin [3] and of fraxinol [3]

Fragment ions *m/e*	Relative intensities		
	Lepto-dactylone	7-Hydroxy-5,6-dimethoxycoumarin	Fraxinol
222	100	100	100
207	60	99	24
194	5	7	54
179	20	53	56
151	20	38	32
136	5*	33	4
123	10	11	12
108	5	19	12
95	15	32	20
80	5	60	12

* A more prominent ion (rel int 20) occurs at *m/e* 135.



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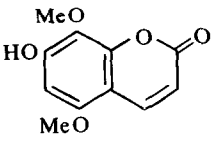

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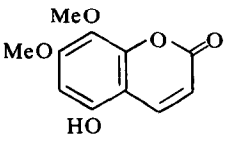

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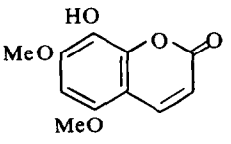

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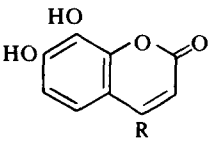
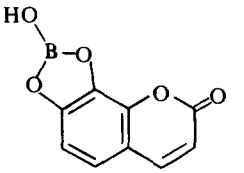


9 R = H
10 R = Me



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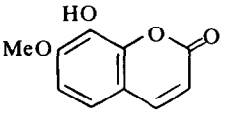

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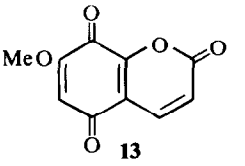

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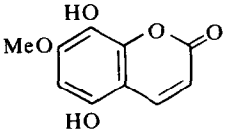

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destroyed by warm sulphuric acid. We therefore carried out some condensations in the presence of B_2O_3 in the hope of stabilising the product as the complex (**11**); the reaction was then much cleaner but the yields no better because the complex was not easy to separate from the excess of boric acid. Finally, we used the original method [14] after optimizing it with respect to temperature, concentration and time factors.

7,8-Dihydroxycoumarin was methylated and then selectively demethylated by warm H_2SO_4 giving 8-hydroxy-7-methoxycoumarin (**12**). Such demethylations are well known for methyl ethers having ether oxygen atoms at both *ortho* positions [17], but appear to be novel for ester (lactonic) oxygen. Oxidation by Frémy salt supplied the quinone (**13**) but since this deteriorated when kept for some hours it was immediately reduced with $NaBH_4$ to the quinol (**14**). Selective methylation of the quinol in the presence of a weak base was expected because, of the two hydroxy groups, only that at the 5-position could be rendered acidic by conjugation with the carbonyl group; it is also the less hindered. Methylation did yield 8-hydroxy-5,7-dimethoxycoumarin (**8**) identical with leptodactylone, though as much 5,7,8-trimethoxycoumarin was also formed.

A recent report [18] identifies a constituent of *Toddalia aculeata* as 5,7,8-trimethoxycoumarin, but the description of this compound does not match exactly that of our synthetic material. The natural substance has a lower mp and the PMR details are similar but not identical. The differences may be due to impurities in the natural sample, but some confirmation of its structure, or a direct comparison, would seem to be necessary.

We attach no special significance to the yellow colour of leptodactylone. Aromatic hydroxycarbonyl compounds commonly have UV absorption bands close to the visible region, and often with their tails running into it. A slight shift in the band is then enough to confer colour. Quinol derivatives show the effect best; 2,4-dihydroxybenzaldehyde is without colour whereas 2,5-dihydroxybenzaldehyde is yellow. The tendency of hydroxy groups to ionize also plays some part in the effect, which is usually diminished by methylation, e.g. 2,5-dimethoxybenzaldehyde is without colour. Similarly, the quinol (**14**) is more deeply yellow than leptodactylone or even the quinone (**13**), but its ether, 5,7,8-trimethoxycoumarin, is not coloured. In the same connection, it may be noted that leptodactylone occurs naturally mainly in bound form, presumably as an 8-glycoside, and this bound form is also not coloured.

EXPERIMENTAL

Leptodactylone was isolated from acid hydrolysed leaf tissue of *Leptodactylon californicum* by EtOAc extraction. The conc extract was separated on thick paper on *n*-BuOH–HOAc–H_2O (4:1:5) and the dark UV absorbing band, visible as a yellow band in daylight, of R_f 0.81 was cut out and eluted. Leptodactylone crystallized from EtOH as yellow needles, mp 149–152°. R_f 0.38 in H_2O, 0.70 in 15% HOAc. Measured mass 222.0530, $C_{11}H_{10}O_5$ requires 222.0528. λ_{max} (EtOH) 215, 269, 320, 350 nm; (EtOH–NaOEt) 221, 291, 330, 340, 412 nm. IR (KBr) 1335, 1490, 1500, 1600 (ene, aromatic) and 1705 (carbonyl) cm^{-1}. 2D PC of 95% EtOH extracts of *Leptodactylon* leaves showed that leptodactylone occurred mainly in colourless bound form. The presumed 8-glycoside was so closely associated on these chromatograms with co-occurring daphnetin glycosides [2] that no attempt was made to further characterize the bound form.

7,8-Dihydroxycoumarin (**9**). (i) Pyrogallol dihydrate (13 g) and malic acid (10.7 g) were stirred into H_2SO_4 (24.4 ml) and heated in an oil bath at 120–130° until gas evolution ceased (50–60 min). The mixture was allowed to cool and then added to H_2O (80 ml) and extracted with Et_2O (6 × 250 ml). The combined extracts were dried and the solvent removed *in vacuo* leaving a pink solid (7.8 g). Recrystallization from a small vol. of EtOH (charcoal) gave 7,8-dihydroxycoumarin as faintly tan prisms (3.8 g), mp 253–255° (lit. [14], 257–258°), imparting an intense green colour to ethanolic $FeCl_3$. (Found: C, 60.6; H, 3.6%; M^+, 178. Calc. for $C_9H_6O_4$: C, 60.7; H, 3.4%; M, 178). (ii) A stirred mixture of pyrogallol dihydrate (1.62 g), $ZnCl_2$ (0.67 g), B_2O_3 (0.69 g) and ethyl propiolate (1.47 g) was heated for 0.5 hr in an oil bath at 110°. The dark gum produced was dissolved in hot dil HCl (15 ml); when cooled, the soln deposited a mixture of a yellow powder and colourless plates. Recrystallization of the mixture from 5M aq. HCl gave the boric ester (**11**) as a faintly purplish power (0.6 g) mp 234°, that coloured EtOH–$FeCl_3$ green but resisted methylation by MeI and K_2CO_3 in Me_2CO (Found: M^+, 204. Calc. for $C_9H_5BO_5$: M, 204). The ester (3 gl was dissolved in the least vol. of hot H_2O, and mannitol (6 g) and a drop of HCl were added. After being kept at 100° for 10 min and then left *ca* 18 hr the soln deposited 7,8-dihydroxycoumarin as pinkish needles (0.55 g), mp 254–256°.

8-Hydroxy-7-methoxycoumarin (**12**). Methylation [14] of 7,8-dihydroxycoumarin (2.7 g) gave 7,8-dimethoxycoumarin (2.1 g), mp 118–119° (lit. [14], 117–119°) Trial experiments showed that demethylation affected the 8-OMe mainly but the 7-OMe sufficiently to give some dihydroxycoumarin if demethylation was prolonged 7,8-Dimethoxycoumarin (1.5 g) in H_2SO_4 (*d*, 1.84; 9 ml) containing H_2O (3 ml) was immersed in oil kept at 70° for 14 hr (i.e. until dihydroxycoumarin was perceptible by TLC) and then cooled and added to H_2O (150 ml). Extracted with CH_2Cl_2 (8 × 100 ml), washed with H_2O (2 × 100 ml), dried and recovered by evapn of the solvent, the product was a fawn solid (1 g) which, when purified from C_6H_6–MeOH (charcoal), supplied 8-hydroxy-7-methoxycoumarin as colourless prisms (0.56 g), mp 168–173°, contaminated by the dimethoxycoumarin but pure enough for preparative purposes. The pure coumarin derivative was obtained by chromatography from CH_2Cl_2–EtOAc on SiO_2 columns and then crystallized from C_6H_6–MeOH as prisms, mp 173–174° (lit. [19] 172–173°. (Found: M^+, 192. Calc. for $C_{10}H_8O_4$: M, 192).

5,6--Dihydroxy-7-methoxycoumarin (**14**). 8-Hydroxy-7-methoxycoumarin (384 mg; 2 mmol) in MeOH (25 ml) was oxidized at 22° by adding it to a mixture of Frémy salt (1.5 g; 4.4 mmol) in H_2O (70 ml) with aq. KH_2PO_4 (0.17 M; 10 ml) and aq. K_2HPO_4 (0.17 M; 10 ml). Light was excluded and the mixture stirred for 0.5 hr while crystals separated; further pptn occurred during 0.75 hr at 0°. The solid was collected at 0° and washed with a little H_2O giving the quinone (**13**) as dark red prisms (300 mg) pure enough for preparative purposes. Purification of this heat and light sensitive compound by crystallization or chromatography proved very troublesome, so an analytically pure specimen was obtained by conducting the oxidation with dust-free solns at half the above concns and omitting the chilling stage. This procedure gave *7-methoxy-2H-benzo [b] pyran-2,5,8-trione* (**13**) as deeply orange crystals, mp 204–205° (rapid heating) UV (EtOH 226 (3.98), 289 (4.10), and 300 (4.09) nm. IR (KBr): ν_{max} 1775, 1760, 1689, 1650, and 1595 cm^{-1}. (Found: C, 58.3; H, 3.2%; M^+, 206. $C_{10}H_6O_5$ requires C, 58.3; H, 2.9%; M, 206). The quinone (246 mg) in tetrahydrofuran (25 ml) was reduced by the addition of a freshly prepared soln (1M, 1.8 ml) of $NaBH_4$ in H_2O. The mixture blackened at once but soon became yellow again and was immediately adjusted to pH 4.5 with aq. HCl, diluted with H_2O (20 ml) and repeatedly extracted with Et_2O. Concn of the extract left the product as a yellow solid (210 mg), crystallising from CH_2Cl_2–tetrahydrofuran to give *5,8-dihydroxy-7-methoxycoumarin* as clusters of yellow needles, mp 207–209°. UV (EtOH) 215 (3.53), 225 (*sh*) (2.79), 270 (3.90), 320 (3.76), 360 (3.53), nm; IR: ν_{max} (KBr) 3000–3500 (bonded OH), 1690, 1625, 1610, 1570 cm^{-1}. (Found: M^+, 208.0363. $C_{10}H_8O_5$ requires M, 208.0372). The *diacetate* (Ac_2O–Py) separated from EtOAc as plates, mp 187–188.5°; UV (EtOH) 217 (4.01), 244 (3.73), 253 (3.73), and 315 (4.14) nm; IR ν_{max} (KBr) 1770 *sh*, 1755, 1725, 1615 and 1498 cm^{-1}. Found: C, 57.6; H, 4.1% M^+, 292. $C_{14}H_{12}O_7$ requires: C, 57.5; H, 4.1%; M, 292).

8-Hydroxy-5,7-dimethoxycoumarin (**8**). 5,8-Dihydroxy-7-methoxycoumarin (300 mg) was heated in refluxing Me_2CO (25 ml) with MeI (4 ml) and K_2CO_3 (2 g) for 0.75 hr and the product isolated as an oil which was chromatographed on Si gel (80 g) Elution with $CHCl_3$–EtOAc (4:1) gave fractions A and B and further elution with EtOAc alone gave fraction C. Fraction A crystallized from heptane–$CHCl_3$ giving *5,7,8-trimethoxycoumarin* which separated from heptane–$CHCl_3$ as needles (100 mg), mp 175.5–176°; UV: (EtOH) 254 *sh*, 260 (3.82), and 322 (3.89) nm; IR (KBr) 1718, 1615 *sh*, 1605 and 1505 cm^{-1}. (Found: C, 61.1; H, 5.1%; M^+, 236. $C_{12}H_{12}O_5$ requires: C, 61.0; H, 5.1%; M, 236). Fraction B formed an oily solid that was re-chromatographed as before and when crystallised from C_6H_6–MeOH provided *8-hydroxy-5,7-dimethoxycoumarin* as clusters of yellow needles (95 mg), mp 152–155° alone or in admixture with leptodactylone, and with the same spectroscopic properties as the natural product. (Found: C, 59.6; H, 4.6%; M^+, 222.0536. $C_{11}H_{10}O_5$ requires: C, 59.5; H, 4.5%; M, 222.0528). Fraction C contained unreacted 5,8-dihydroxy-7-methoxycoumarin (79 mg).

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