

MONOTERPENOID FURANOCOUMARIN LACTONES FROM *CLAUSENA ANISATA**

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Abstract—A reinvestigation of *Clausena anisata* has yielded imperatorin, xanthotoxol, lansamide-I and three new furanocoumarin lactone derivatives indicolactone, anisolactone and 2',3'-epoxyanisolactone. The structures of these compounds have been elucidated by a combination of spectroscopic and chemical methods.

INTRODUCTION

The roots and stems of *Clausena anisata* (Willd) Olive have been examined extensively in the past for the presence of furanocoumarins and carbazoles [1–5]. In the course of a systematic screening of indigenous plants for any biological activity, a 50% aqueous ethanolic extract of the aerial parts of *C. anisata* was found to show spasmolytic activity. On fractionation of the extract, the activity was found to become concentrated in the benzene and ethyl acetate fractions. Column chromatography of these fractions over silica gel furnished, apart from already reported imperatorin, xanthotoxol and lansamide-I [6], three new compounds which we have named indicolactone, anisolactone and 2',3'-epoxyanisolactone. The present paper deals with the essential data leading to the assignment of structures 1–3 to the new compounds.

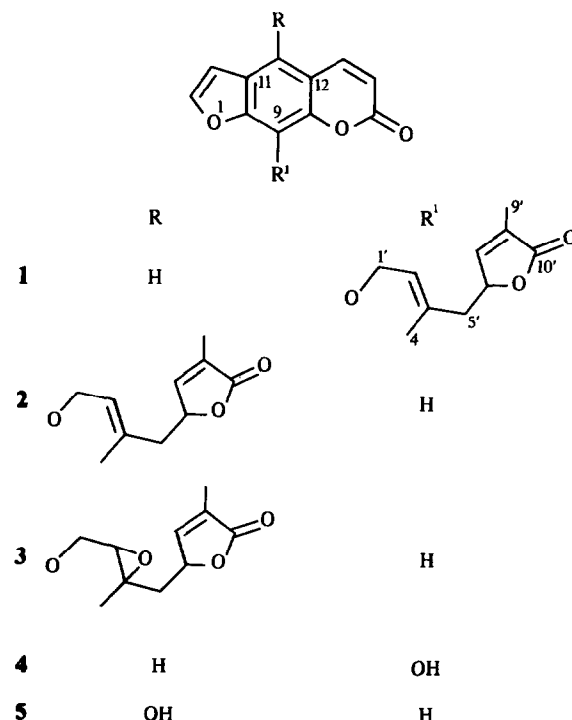
RESULTS AND DISCUSSION

Indicolactone (1) was obtained as a viscous mass which analysed for $C_{21}H_{18}O_6$ ($M^+ m/z$ 366), $[\alpha]_D^{25} + 26.3^\circ$ (c 2.66, $CHCl_3$). Its UV spectrum, λ_{max}^{MeOH} 212, 252, 268 and 304 nm ($\log \epsilon$ 3.71, 3.48, 3.29 and 3.20), was characteristic of linear furanocoumarins and remained unchanged on addition of alkali [7]. The presence of IR bands at 1750 and 1725 cm^{-1} indicated the presence of an α,β -unsaturated γ -lactone group and an α,β -unsaturated δ -lactone group respectively. When treated with 10 M HCl under reflux conditions, 1 afforded xanthotoxol (4) indicating that it was a C_{10} ether of 4.

The 90 MHz 1H NMR spectrum of 1 integrated for 18 protons. The protons at C-2 and C-3 in the xanthotoxol nucleus appeared as a pair of doublets at δ 7.50 and 6.80 ($J = 2.0$ Hz). Another characteristic pair of doublets at δ 7.64 and 6.16 ($J = 9.0$ Hz) was assigned to the C-5 and C-6 protons of the coumarin nucleus. As expected the C-4 proton was observed as a singlet at δ 7.22. A pair of doublets of two protons, which appeared at δ 4.88 ($J = 7.0$ Hz) was assigned to an $-OCH_2-$ group at 1', the methine protons appeared as a multiplet at δ 5.57 and the

5' methylene group was observed as a broad doublet at δ 2.24 ($J = 7.0$ Hz). A multiplet centred at δ 5.72 was assigned to the presence of a 6' proton of a 5-membered ring lactone. The singlet at δ 1.73 for three protons was assigned to the remaining methyl function at the α -position of the α,β -unsaturated- γ -lactone group. A three proton singlet at δ 1.68 was assigned to a methyl at the 4' position.

The proposed structure for 1 was fully supported by its ^{13}C NMR spectrum, which exhibited peaks for 21 carbon atoms (Table 1) (cf ^{13}C NMR of imperatorin) [8]. The MS fragmentation (EIMS) pattern of indicolactone also indicated that a double bond and α,β -unsaturated- γ -



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Table 1 ^{13}C NMR data of **1** (201 MHz, CDCl_3 , TMS as int standard)

C	δ	C	δ
2	144.37 (d)	13	146.76 (s)
3	106.87 (d)	1'	69.67 (t)
4	113.60 (d)	2'	123.39 (d)
5	145.20 (d)	3'	137.0 (s)
6	114.78 (d)	4'	10.58 (q)
7	159.30 (s)	5'	43.37 (t)
9	129.2 (s)	6'	79.58 (d)
10	148.37 (s)	7'	139.30 (d)
11	125.0 (s)	8'	130.8 (s)
12	116.60 (s)	9'	17.24 (q)
		10'	173.0 (s)

lactone were present in the C_{10} side chain [9]

Treatment of **1** with *m*-chloroperoxybenzoic acid gave an epoxide, in the ^1H NMR spectrum of which the 2'-proton was shifted from δ 5.7 to 4.94 (both multiplets), besides the other proton signals which were observed in the parent compound. When refluxed with 10% aqueous oxalic acid for two hours the epoxide furnished a diol, the molecular ion of which now appeared at m/z 400. The TLC, UV, $[\alpha]_D$, IR and ^1H NMR data were found to be identical in all respects to the previously described indicolactone diol isolated from *C. indica* [9]. On the basis of these observations indicolactone was assigned the structure **1**.

Anisolactone (**2**), mp 147–148°, analysed for $\text{C}_{21}\text{H}_{18}\text{O}_6$ (M^+ m/z 366), $[\alpha]_D^{25} + 19.8^\circ$ (c 2.7, CHCl_3). Its UV spectrum $\lambda_{\text{max}}^{\text{MeOH}}$ 224, 243, 250, 259, 267 and 307 nm (log ϵ 4.67, 4.44, 4.57, 4.30, 4.39 and 4.33 respectively) was characteristic of a linear furanocoumarin. The presence of IR bands at 1770 and 1730 cm^{-1} indicated the presence of α,β -unsaturated- γ - and δ -lactone groups respectively. When treated with 10 M HCl under reflux conditions, **2** afforded bergaptol (**5**), indicating that it was a C_{10} ether of bergaptol. The 90 MHz ^1H NMR spectrum of **2** integrated for 18 protons. The protons at C-2 and C-3 in the bergaptol nucleus appeared as a pair of doublets at δ 7.56 and 6.93 ($J = 2.0$ Hz) and the C-9 aromatic proton appeared as a singlet at δ 7.11. The signals for the rest of the protons in the C_{10} ether side chain and in the bergaptol nucleus were found to be identical to those of indicolactone (**1**). Its MS fragmentation pattern was identical to that of **1**. Based on these observations anisolactone was assigned structure **2**.

On reaction with *m*-chloroperoxybenzoic acid, **2** gave an epoxide, mp 171–172°, $\text{C}_{21}\text{H}_{18}\text{O}_7$ (M^+ m/z 382) which was found to be identical to 2',3'-epoxyanisolactone (**3**) in all respects (mp, mmp, UV, IR, ^1H NMR and MS).

EXPERIMENTAL

Mps uncorr, ^1H and ^{13}C NMR CDCl_3 , TMS as internal standard, MS direct inlet system.

Isolation of the constituents The ground dried aerial parts of *C. anisata* (60 kg) were extracted with 50% aq EtOH (3×101). The ethanolic extract was concd *in vacuo* and the residual mass diluted with H_2O and extracted with hexane (3×11), C_6H_6 (3×11), EtOAc (3×11) and *n*-BuOH (3×11) successively. The combined C_6H_6 extract was washed with H_2O , dried (Na_2SO_4) and the solvent removed. The residue so obtained (20 g) was chromatographed on a column of neutral alumina (10 kg) to afford xanthotoxol (**4**) (400 mg), mp 248–249°, imperatorin (60 mg), mp 98–99° and lansamide-I (60 mg), mp 123–125°. Similarly the EtOAc residue (14.0 g) on CC over silica gel (700 g) furnished indicolactone (**1**, 800 mg), anisolactone (**2**, 160 mg) and 2',3'-epoxyanisolactone (**3**, 60 mg).

Acid cleavage of indicolactone (1) A soln of **1** (50 mg) in 10 M HCl (15 ml) was refluxed on a water bath for 2 hr. After cooling, it was diluted with H_2O (20 ml) and extracted with Et_2O (3×25 ml). The ethereal layer was washed with H_2O and dried (Na_2SO_4), purified by prep TLC and crystallized from Me_2CO –hexane to afford xanthotoxol (**4**, 18 mg), mp 248–249°.

Indicolactone epoxide A soln of **1** (366 mg) in dry THF (15 ml) and *m*-chloroperoxybenzoic acid (75 mg) was stirred at room temp for 48 hr. The THF was removed under red pres and diluted with H_2O , and extracted with Et_2O (3×150 ml). The ethereal layer was washed with 5% aq NaHCO_3 soln, dried (Na_2SO_4) and concd under red pres. The residual mass was purified by prep TLC on silica gel plates to furnish the epoxide (250 mg), $[\alpha]_D^{25} - 25.6^\circ$ (c 0.82, CHCl_3), IR $\nu_{\text{max}}^{\text{neat}}$ cm^{-1} 1720, 1585, 1460, 1435, 1420, 1395, 1095, 1025 and 870. ^1H NMR (90 MHz, CDCl_3) δ 1.19 (3H, s, Me, H-4'), 1.35 (2H, d , $J = 4.5$ Hz, H-5'), 1.86 (3H, s, Me, H-9'), 4.52 (2H, d , $J = 6.5$ Hz, H-1'), 5.03 (1H, m , H-6'), 4.94 (1H, m , H-2'), 6.25 (1H, d , $J = 10.0$ Hz, H-6), 6.73 (1H, d , $J = 2.0$ Hz, H-3), 7.02 (1H, d , $J = 2.0$ Hz, H-2), 7.30 (1H, s, H-4) and 7.60 (1H, d , $J = 10.0$ Hz, H-5), MS m/z 382 [M] $^+$.

Indicolactone diol A soln of indicolactone epoxide (200 mg) in 10% aq oxalic acid (50 ml) was refluxed on a water-bath for 3 hr and usual work up afforded indicolactone diol (150 mg), $[\alpha]_D^{25} + 4.93^\circ$ (c 4.66, CHCl_3).

Anisolactone (2) ^1H NMR (90 MHz, CDCl_3) δ 1.80 (3H, s, Me, H-4'), 1.91 (3H, s, Me, H-9'), 2.41 (2H, d , $J = 6.0$ Hz, H-5'), 4.95 (2H, d , $J = 7.0$ Hz, H-1'), 5.03 (1H, m , H-6'), 5.67 (1H, m , H-2'), 6.25 (1H, d , $J = 10.0$ Hz, H-6), 6.93 (2H, m , H-7' and H-3), 7.11 (1H, s, H-9), 7.56 (1H, d , $J = 2.0$ Hz, H-2) and 8.08 (1H, d , $J = 10.0$ Hz, H-5), MS m/z (rel int) 366 [M] $^+$ (10), 348 (9), 333 (11), 202 (98), 174 (61), 165 (31), 164 (28), 147 (26), 145 (24), 136 (22), 121 (20), 118 (21), 97 (100%), 89 (24), 69 (40) and 41 (32).

Acid cleavage of anisolactone (2) A soln of **2** (50 mg) in 10 M HCl (15 ml) was refluxed for 2 hr. Usual work up gave bergaptol (**5**) which was purified by prep TLC on silica gel plates to give pure bergaptol (20 mg), mp 277–278°.

Epoxidation of anisolactone (2) A soln of **2** (60 mg) in dry THF (10 ml) and *m*-chloroperoxybenzoic acid (40 mg) was stirred in an ice-bath for 2 hr followed by 36 hr at room temp. The THF was removed and the residue was diluted with H_2O and extracted with Et_2O (5×15 ml). The ethereal layer was washed with 5% aq NaHCO_3 soln followed by H_2O , and then dried (Na_2SO_4). Removal of solvent afforded the crude product which was purified by CC on neutral alumina (30 g) to furnish the epoxide of **2** (20 mg), mp 171–172°. All the physical and chemical data of this compound were found identical with that of the natural 2',3'-epoxyanisolactone (**3**).

2',3'-Epoxyanisolactone (3) $[\alpha]_D^{25} - 16.9^\circ$ (c 0.7, CHCl_3), UV $\lambda_{\text{max}}^{\text{MeOH}}$ (log ϵ) 224 (4.55), 242 (4.42), 249 (4.46), 258 (4.39), 266 (4.35) and 305 (4.30) nm. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} 2920, 1750, 1715, 1610, 1595, 1570, 1375 and 1350. ^1H NMR (90 MHz, CDCl_3) δ 1.50 (3H, s, Me, H-4'), 1.91 (3H, s, Me, H-9'), 1.60–2.23 (2H, m (br), H-5'), 3.31 (1H, dd , $J = 6.0$ Hz and 4.0 Hz, H-2), 4.41 (1H, dd , $J = 10.0$ Hz and 6.0 Hz, H-1'), 4.70 (1H, d , $J = 4.0$ Hz and 10.0 Hz, H-1'), 5.06 (1H, m (br), H-6'), 6.26 (1H, d , $J = 10.0$ Hz, H-6), 6.93 (1H, d , $J = 2.0$ Hz, H-3), 7.03 (1H, m , H-7'), 7.11 (1H, s, H-9).

9), 7.56 (1H, *d*, *J* = 2.0 Hz, H-2) and 8.20 (1H, *d*, *J* = 10.0 Hz, H-5), MS *m/z* (rel int) 382 [*M*]⁺ (86), 202 (89), 181 (43), 174 (18), 173 (15), 145 (21), 141 (83), 137 (30), 97 (100%), 69 (51) and 41 (45)

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