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ISOCOUMARINS FROM STREPTOMYCES MOBARAENSIS

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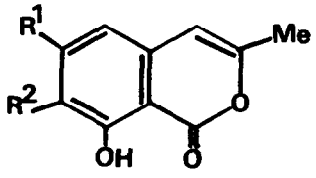
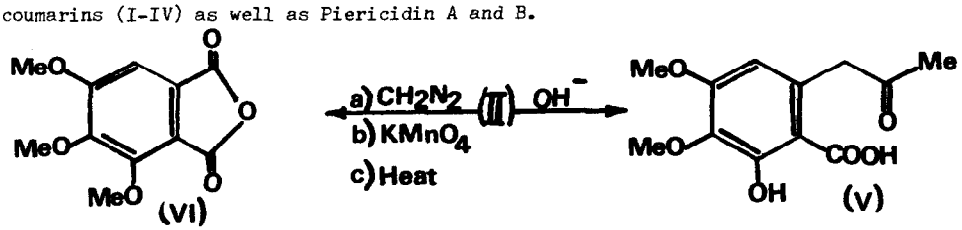
Isocoumarins are of less widespread natural occurrence than coumarins. Some have been obtained from micro-organisms (1-3) and 3,4-dihydroisocoumarins have been isolated from corn (4) and bitter carrots (5). We wish to report the isolation from Streptomyces mobaraensis of a series of isocoumarins (I-IV) which differ only in the substitution of the aromatic ring.



	R^1	R^2
I	OMe	H
II	OMe	OMe
III	OH	OMe
IV	OH	OH

S. mobaraensis, a micro-organism which produces the Piericidins (6), was grown aerobically for four days at 27° in a medium which contained glucose (2%), peptone (0.7%), dipotassium hydrogen phosphate (0.2%) and sodium chloride (0.2%). The cells were separated from the growth medium (20 l) by centrifugation, extracted with acetone (4 x 1 l), the extracts dried and evaporated to dryness in vacuo (Extract A). The growth medium after removal of the cells was brought to pH 5 with sulphuric acid and extracted with ethyl acetate (3 x 1 l). The ethyl acetate extracts were dried and evaporated to dryness in vacuo (Extract B).

Extracts A and B were then subjected to column chromatography on silicic acid. The chromatograms were eluted with benzene containing ethyl acetate (1-20%) to give the isocoumarins (I-IV) as well as Piericidin A and B.



TABLE

Compound and order of elution	Amount Present (mg) in 20 l medium	
	Extract A	Extract B
(I)	20	-
Piericidin B	20	-
(II)	200	40
Piericidin A	1000	40
(III)	-	200
(IV)	-	100

Evaporation of the eluants gave the enolic isocoumarins which were obtained as colourless crystalline solids after trituration with hexane. Compounds (III) and (IV) were not completely separated by the initial chromatographic procedure. Separation was finally achieved by tlc on silica using 2% methanol in chloroform ((III) R_F=0.60, (IV), R_F=0.34). The isocoumarins could be further purified by recrystallisation (ethanol) or by sublimation in vacuo.

Examination of the spectroscopic properties of (I)-(IV) suggested that they were probably oxygenated isocoumarins; e.g. their u.v. spectra were very similar to that of 6,8-dihydroxy-7-methoxy-3-methylisocoumarin(2). Their i.r. spectra contained bands in the region of 3400 and 1680 $cm.^{-1}$ indicating the presence of a hydroxyl group hydrogen-bonded to a carbonyl group. On methylation, the band at 3400 cm^{-1} disappeared while the band at 1680 cm^{-1} was displaced to 1720 $cm.^{-1}$ The n.m.r. spectra of (I-IV) were entirely consistent with their structures, in particular all spectra showed signals in the region of -1.0 (due to the hydrogen-bonded proton) and 7.7τ (due to the 3-methyl group).

The presence of the 3-methyl group which was suggested by biosynthetic considerations (1), was confirmed by the alkaline hydrolysis of (II). The resulting ketone (V) had an n.m.r. spectrum with singlets at 8.20 (3H) and 6.18τ (2H) due to the exocyclic methyl and the methylene groups.

The substitution pattern in the aromatic ring of the isocoumarins was demonstrated by methylation of (II) with diazomethane followed by oxidation of the product with permanganate to 3,4,5-trimethoxyphthalic acid which was then dehydrated by sublimation to 3,4,5-trimethoxyphthalic anhydride (VI) (7,8). The single aromatic proton appeared at much lower field in the n.m.r. spectrum of (VI) than in the spectrum of (II). Hence the proton is in position 5 and is deshielded in the anhydride (9).

The mass spectra of compounds (I-IV) were in agreement with the proposed structures. For example, the mass spectrum of (II) showed peaks at: 236 M(100%), 221 M-CH_3 (94), 193 M-CH_3-CO(51), 165 M-CH_3-2 CO(5), 150 M-2CH_3-2CO(16).

8-Hydroxy-6-methoxy-3-methylisocoumarin (I): m.p. 129°. λ_{max} (MeOH) 244 nm. (log ε 4.62), 277 (3.77), 326 (3.72). ν_{max} ($CHCl_3$) 1685 cm^{-1}. NMR 60 MHz ($CDCl_3$)τ -1.12 (1Hs), 3.51 (1Hd, J=2.6 Hz) 3.67 (1Hd, J=2.6 Hz), 6.15 (3Hs), 7.76 (3Hs). Mass spectrum M^+ 206.0567 $C_{11}H_{10}O_4$ requires 206.0579.

8-Hydroxy-6,7-dimethoxy-3-methylisocoumarin (II): m.p. 199°. λ_{max} (MeOH) 243 nm (log ε 4.61) 278 (3.84), 335 (3.69) ν_{max} (KCl) 3400, 1678 $cm.^{-1}$ NMR ($CDCl_3$)τ - 1.12 (1Hs), 3.64 (1Hs), 3.80 (1Hs) 6.02 (3Hs), 6.07 (3Hs) 7.72 (3Hs). Mass Spectrum M^+ 236.0687, $C_{12}H_{12}O_5$ requires 236.0685.

6,8-Dihydroxy-7-methoxy-3-methylisocoumarin(III) (3): m.p. 194°. λ_{max} (MeOH) 245 nm (log ε 4.68), 278 (3.86), 330 (3.76). ν_{max} (nujol) 3435, 1679 $cm.^{-1}$ NMR ($^2H_6$-DMSO)τ - 1.10 (1Hs), 3.53 (2Hs broad), 6.19 (3Hs), 7.77 (3Hs). Mass spectrum M^+ 222.0525, $C_{11}H_{10}O_5$ requires 222.0528.

6,7,8-Trihydroxy-3-methylisocoumarin (IV): m.p. 234°. λ_{max} (MeOH) 242 nm (log ε 4.69), 278 (3.43), 326 (3.76). ν_{max} (nujol) 3360, 3260, 1680 $cm.^{-1}$ NMR ($^2H_6$-DMSO)τ - 1.04 (1Hs) -0.70 (1Hs), 3.55 (1Hs), 3.69 (1Hs), 7.78 (3Hs). Mass spectrum M^+ 208.0370, $C_{10}H_8O_5$ requires 208.0371.

Methyl ester of (II): (II) (35 mg) was kept at -15° for 7 days in ether/$CHCl_3$ (1:1) containing a 10 fold excess of diazomethane. The methyl ester (35 mg, m.p. 119°) was isolated by tlc on silica using 10% ethyl acetate in benzene. λ_{max} (MeOH) 245 nm (log ε 4.66), 276 (3.83), 327 (3.60), 337 (3.57), ν_{max} ($CHCl_3$) 1725 cm^{-1}. NMR ($^2H_6$-DMSO)τ 3.48 (1Hs), 3.88 (1Hs) 6.00 (3Hs), 6.05 (3Hs), 6.09 (3Hs), 7.77 (3Hs). Mass spectrum M^+ 250.08466, $C_{13}H_{14}O_5$ requires 250.08412. Found C, 62.3, H, 5.48, O, 31.8%, $C_{13}H_{14}O_5$ requires C, 62.4, H, 5.64; O 32.0%.

Alkaline Hydrolysis of (II): (II) (30 mg) was heated under reflux for 1 hr. in 2N NaOH (15 ml). The cooled solution was acidified and continuously extracted with chloroform for 12 hr. The extracts were dried, evaporated to dryness, and the product (17mg, m.p. 180°) was recrystallised from methanol. ν_{max} (nujol) 1730 cm^{-1} NMR ($CDCl_3$) τ 3.61 (1Hs), 6.02 (3Hs), 6.07 (3Hs), 6.79 (2Hs), 8.20 (3Hs). Mass spectrum M^+ 254.0802, $C_{12}H_{14}O_6$ requires 254.0790.

3,4,5-Trimethoxyphthalic anhydride. The methyl ether of II (30 mg.) was oxidised with alkaline potassium permanganate (10) and the acidified solution, after removal of manganese dioxide with bisulphite, extracted with chloroform for 12 hr. The chloroform extract was dried, evaporated to dryness and the residue heated in vacuo, 3,4,5-trimethoxy phthalic anhydride sublimed as long colourless needles (16 mg. m.p. 144°). Lit. m.p. 144-5 (7), 147° (8). ν_{max} (nujol) 1838, 1770 $cm.^{-1}$ NMR (100 MHz, $^2H_6$-DMSO) τ 2.55 (1Hs), 5.93 (3Hs) 6.00 (3Hs), 6.15 (3 Hs). Mass spectrum M^+ 238.0474, $C_{11}H_{10}O_6$ requires 238.0477.

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