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Syntheses of Antifungal Isocoumarins. III.[1,2] Synthesis and Antifungal Activity of 3-Aryl-3,4-dihydro-4-substituted-isocoumarins

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Various *cis*- and *trans*-3-aryl-3,4-dihydro-8-(hydroxy or methoxy)isocoumarin-4-carboxylic acids (**3**—**16**) and their methyl esters (**21**—**29**) were prepared. In addition, *cis*- and *trans*-3,4-dihydro-8-(hydroxy or methoxy)-4-hydroxyacetyl-3-phenylisocoumarins (**33** or **32**, and **35** or **34**), *cis*- and *trans*-4-diazoacetyl-3,4-dihydro-8-methoxy-3-phenylisocoumarins (**30** and **31**), *trans*-4-acetoxyacetyl-3,4-dihydro-8-methoxy-3-phenylisocoumarin (**36**), and *trans*-4-acetyl-3,4-dihydro-8-(hydroxy or methoxy)-3-phenylisocoumarins (**38** or **37**) were prepared.

All the 3,4-dihydroisocoumarins thus prepared were examined *in vitro* for antifungal activity. The introduction of a carboxyl, carbomethoxy, acetyl, diazoacetyl or hydroxyacetyl group at the 4-position of the 3,4-dihydroisocoumarin nucleus resulted in the disappearance of the activity.

Keywords——antifungal activity; 3,4-dihydroisocoumarins; 3,4-dihydroisocoumarin-4-carboxylic acids; 3,4-dihydro-8-hydroxyisocoumarins; 3-hydroxyhomophthalic anhydride

It seemed desirable to synthesize a series of 3-arylisocoumarin derivatives having a carbonyl group at the 4-position for studies of their antifungal activity, since the powerful antifungal antibiotic oosponol[3] possesses a carbonyl group at position 4.

In Part I[4] of this series, we reported a convenient method for synthesizing *cis* and *trans* isomers of 3,4-dihydro-3-phenylisocoumarin-4-carboxylic acid in one step from homophthalic anhydride merely by shaking with powdered sodium carbonate and benzaldehyde at room temperature. Making use of this method, we synthesized from 3-hydroxyhomophthalic anhydride (**1**) eight 3,4-dihydroisocoumarin derivatives, that is, *cis*- and *trans*-3,4-dihydro-8-hydroxy-3-phenylisocoumarin-4-carboxylic acids (**3** and **11**), *cis*- and *trans*-3-(*p*-benzyloxyphenyl)-3,4-dihydro-8-hydroxyisocoumarin-4-carboxylic acids (**4** and **12**), *cis*- and *trans*-3,4-dihydro-8-hydroxy-3-(3-hydroxy-4-methoxyphenyl)isocoumarin-4-carboxylic acids (**5** and **13**), *cis*-3-(3-acetoxy-4-methoxyphenyl)-3,4-dihydro-8-hydroxyisocoumarin-4-carboxylic acid (**6**) and *cis*-3-(3-benzyloxy-4-methoxyphenyl)-3,4-dihydro-8-hydroxyisocoumarin-4-carboxylic acid (**7**). We also synthesized from 3-methoxyhomophthalic anhydride (**2**) six 3,4-dihydroisocoumarin derivatives, *i.e.*, *cis*- and *trans*-3,4-dihydro-8-methoxy-3-phenylisocoumarin-4-carboxylic acids (**8** and **14**), *cis*- and *trans*-3,4-dihydro-8-methoxy-3-(*p*-methoxyphenyl)isocoumarin-4-carboxylic acids (**9** and **15**) and *cis*- and *trans*-3,4-dihydro-3-(3,4-dimethoxyphenyl)-8-methoxyisocoumarin-4-carboxylic acids (**10** and **16**). The details of the synthetic compounds are shown in Tables I and II. The *cis* and *trans* configutraions of the products were determined from the *J*-value of the hydrogens located at the 3- and 4-positions of the 3,4-dihydroisocoumarin nucleus.

It was reported by Guyot *et al.*[5] that heating of 3-methoxyhomophthalic acid with acetic anhydride and pyridine at 40°C gave not 8-methoxyhomophthalic anhydride (**2**), but a mixture of 8-methoxy-3-methylisocoumarin-4-carboxylic acid and 4-acetyl-8-methoxy-1,3-isochromandione, and the present authors prepared the anhydride (**2**) by the reaction of 3-methoxyhomophthalic acid with a mixture of acetyl chloride and acetone at low temperature. Similarly, another starting material (**1**) was prepared.

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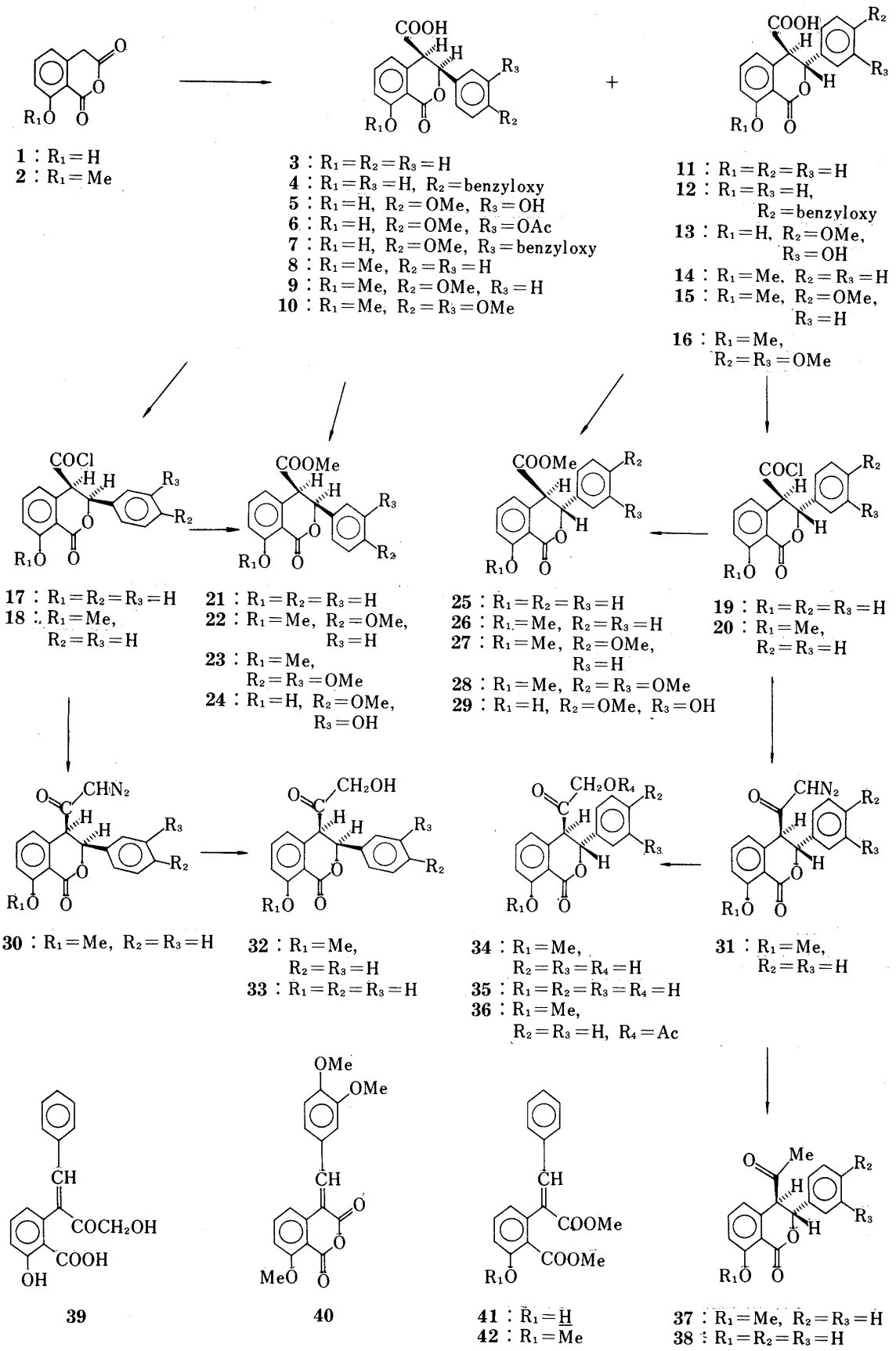

Chart 1

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TABLE I. 3,4-Dihydro-8-(hydroxy or methoxy)-3-arylisocoumarin-4-carboxylic Acids

Compd. No.	mp (°C)	Yield (%)	Recrystn. solvent[a]	Formula	Mass (*m/e*) M+	Analysis (%) Calcd (Found) C	H
3	220	20	Acetone[c]	$C_{16}H_{12}O_5$	284	69.60(67.33)	4.26(4.40)
4	213	60	EtOH[n]	$C_{23}H_{18}O_6 \cdot 1/2EtOH$	390	69.72(69.86)	5.12(5.50)
5	186	27	MeOH[m]	$C_{17}H_{14}O_6$	330	61.82(61.82)	4.27(4.21)
6	202	78	MeOH[pl]	$C_{19}H_{16}O_8 \cdot MeOH$	372	59.40(59.54)	4.00(4.59)
7	208	60	EtOH[m]	$C_{24}H_{20}O_7$	420	68.56(68.63)	4.80(4.99)
8	210	5	MeOH[pr]	$C_{17}H_{14}O_5$	298	68.45(67.90)	4.73(4.62)
9	182	19	MeOH[n]	$C_{18}H_{16}O_6$	328	65.85(66.16)	4.91(4.83)
10	190	24	MeOH[n]	$C_{19}H_{18}O_7$	358	63.68(63.27)	5.06(5.05)
11	140	72	Benzene[m]	$C_{16}H_{12}O_5$	284	67.60(67.45)	4.26(4.15)
12	177	28	MeOH[pr]	$C_{23}H_{18}O_6 \cdot MeOH$	390	68.24(68.43)	5.25(4.98)
13	129	54	MeOH[pl]	$C_{17}H_{14}O_6$	330.0730 (330.0739[b])		
14	210	82	MeOH[pr]	$C_{17}H_{14}O_5$	298	68.45(69.11)	4.73(4.72)
15	174	47	EtOH[pr]	$C_{18}H_{16}O_6$	328	65.85(65.45)	4.91(4.81)
16	189	44	$CHCl_3$[pr]	$C_{19}H_{18}O_7$	358	63.68(63.16)	5.06(5.02)

a) c, colorless cubes; m, colorless microcrystalline powder; n, colorless needles; pl, colorless plates; pr, colorless prisms.
b) Values in parenthesis are mass numbers required for the indicated molecular formulae.

TABLE II. Spectral Data for 3,4-Dihydro-8-(hydroxy or methoxy)-3-arylisocoumarin-4-carboxylic Acids

Compd. No.	IR ν_{max}^{KBr} cm^{-1} [a] (C=O)	PMR (in $CDCl_3$) δ (ppm)[b] Acetyl (3H, s)	MeO (3H, s)	C_4–H (1H, d)	Benzyl (2H, s)	C_3–H (1H, d)	Phenyl (m)	OH (1H, s)
3	1675, 1700[sh]			4.29 (J=3.3 Hz)		6.04 (J=3.3 Hz)	6.9—7.7(8H)	10.8
4	1678, 1700			4.22 (J=3.4 Hz)	5.11	5.96 (J=3.4 Hz)	6.8—7.6(7H)	10.9
5	1660, 1730		3.86	4.28 (J=3.4 Hz)		5.93 (J=3.4 Hz)	6.9—7.7(6H)	11.1
6	1660, 1730	2.26	3.86	4.34 (J=3.8 Hz)		6.01 (J=3.8 Hz)	6.9—7.7(6H)	11.0
7	1675, 1690[sh]		3.79	4.26 (J=3.5 Hz)	5.05	5.95 (J=3.5 Hz)	6.9—7.7(11H)	10.9
8	1685, 1735		3.94	4.17 (J=3.4 Hz)		5.76 (J=3.4 Hz)	6.9—7.8(8H)	
9	1710, 1730		3.81, 3.95	4.12 (J=3.4 Hz)		5.71 (J=3.4 Hz)	6.9—7.7(7H)	
10	1690, 1725		3.84, 3.85 3.95	4.12 (J=3.0 Hz)		5.70 (J=3.0 Hz)	6.9—7.7(6H)	
11	1670, 1720			4.61 (J=7.3 Hz)		6.01 (J=7.3 Hz)	6.7—7.7(8H)	10.8
12	1660, 1728			4.28 (J=7.6 Hz)	5.03	5.83 (J=7.6 Hz)	6.7—7.6(12H)	10.9
13	1685, 1700[sh]		3.83	4.54 (J=7.6 Hz)		5.92 (J=7.6 Hz)	6.8—7.7(6H)	11.1
14	1700, 1728		3.89	4.42 (J=6.3 Hz)		5.87 (J=6.3 Hz)	6.8—7.8(8H)	
15	1720		3.70, 3.90	4.39 (J=7.1 Hz)		5.77 (J=7.1 Hz)	6.8—7.7(7H)	
16	1700, 1735		3.79, 3.79 3.91	4.43 (J=7.1 Hz)		5.78 (J=7.1 Hz)	6.9—7.7(6H)	

a) sh, shoulder. *b*) s, singlet; d, doublet; m, multiplet.

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The methyl esters **21**, **25** and **26** were prepared from the corresponding acid chlorides (**17**, **19** and **20**) by treatment with methanol. The chlorides **17**, **19** and **20** were obtained by chlorination of **3**, **11** and **14**, respectively. In case of the acid (**16**), heating with thionyl chloride gave not its acid chloride but 4-(3,4-dimethoxybenzylidene)-8-methoxyisochroman-1,3-dione (**40**). The structure of **40** was elucidated by analysis of its proton magnetic resonance (PMR) spectrum, which did not show two characteristic doublet signals of hydrogens at the 3- and 4-positions of 3,4-dihydroisocoumarins but showed a one-proton singlet at 7.6 ppm assignable to an olefinic hydrogen, and also by analysis of its IR spectrum which showed two anhydride

Table III. 3,4-Dihydro-8-(hydroxy or methoxy)-3-arylisocoumarin-4-carboxylic Acid Methyl Esters

Compd. No.	mp (°C)	Yield (%)	Recrystn. solvent[a]	Formula	Mass (*m/e*) M^+	Analysis (%) Calcd (Found) C	H
21	176	89	MeOH[n]	$C_{17}H_{14}O_5$	298	68.45(68.78)	4.73(4.70)
22	168	84	MeOH[n]	$C_{19}H_{18}O_6$	342	66.66(66.17)	5.30(5.19)
23	158	77	MeOH[n]	$C_{20}H_{20}O_7$	372	64.51(65.20)	5.42(5.42)
24	161	82	Benzene[n]	$C_{18}H_{16}O_7$	344	62.79(63.05)	4.68(4.59)
25	132	80	MeOH[pr]	$C_{17}H_{14}O_5$	298	68.45(68.74)	4.73(4.65)
26	136	86	Acetone[m]	$C_{18}H_{16}O_5$	312	69.22(69.34)	5.16(5.03)
27	138	77	MeOH[n]	$C_{19}H_{18}O_6$	342	66.66(66.64)	5.30(5.25)
28	145	91	MeOH[n]	$C_{20}H_{20}O_7$	372	64.51(64.70)	5.42(5.41)
29	131 (lit.[6] 130.5—131.5)	87	MeOH[n]	$C_{18}H_{16}O_7$	344	—	—

a) n, colorless needles; m, colorless microcrystalline powder; pr, colorless prisms.

Table IV. Spectral Data for Methyl Esters of 3,4-Dihydro-8-(hydroxy or methoxy)-3-arylisocoumarin-4-carboxylic Acids

Compd. No.	IR ν_{max}^{KBr} cm^{-1} *a*) (C=O)	PMR[b,c] δ (ppm) COOMe (3H, s)	MeO (3H, s)	C_4–H (1H, d)	C_3–H (1H, d)	Phenyl (m)	OH (1H, s)
21	1675, 1725	3.39		4.40 (J=3.5 Hz)	6.08 (J=3.5 Hz)	6.9—7.7(8H)	
22	1725, 1740[sh]	3.50	3.82, 3.99	4.06 (J=3.2 Hz)	5.61 (J=3.2 Hz)	6.8—7.6(7H)	
23	1730, 1740[sh]	3.51	3.90, 3.91 4.00	4.03 (J=3.4 Hz)	5.61 (J=3.4 Hz)	6.8—7.7(6H)	
24	1670, 1720	3.50	3.91	4.07 (J=3.8 Hz)	5.69 (J=3.8 Hz)	6.7—7.5(6H)	5.66, 11.01
25	1650, 1720	3.69		4.31 (J=8.5 Hz)	5.89 (J=8.5 Hz)	6.6—7.6(8H)	11.01
26	1725, 1735	3.67	3.97	4.28 (J=8.1 Hz)	5.74 (J=8.1 Hz)	6.6—7.6(8H)	
27	1725, 1738[sh]	3.66	3.79, 3.97	4.26 (J=8.8 Hz)	5.65 (J=8.8 Hz)	6.6—7.6(7H)	
28	1735, 1745[sh]	3.67	3.87, 3.87 3.97	4.27 (J=8.5 Hz)	5.65 (J=8.5 Hz)	6.6—7.6(6H)	
29	1680, 1722	3.71	3.89	4.28 (J=8.2 Hz)	5.78 (J=8.2 Hz)	6.6—7.5(6H)	5.68, 11.03

a) sh, shoulder.
b) s, singlet; d, doublet; m, multiplet.
c) solvent: acetone-d_6 for **21**, $CDCl_3$ for **22**—**29**.

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absorptions at 1730 and 1770 cm^{-1}. Therefore the methyl ester, *i.e.*, methyl *trans*-3,4-dihydro-3-(3,4-dimethoxyphenyl)-8-methoxyisocoumarin-4-carboxylate (**28**), was produced directly from **16** by treatment with diazomethane. Similarly, methyl *cis*-3,4-dihydro-3-(3,4-dimethoxyphenyl)-8-methoxyisocoumarin-4-carboxylate (**23**), methyl *cis*- and *trans*-3,4-dihydro-8-methoxy-3-(*p*-methoxyphenyl)isocoumarin-4-carboxylates (**22** and **27**), and methyl *cis*- and *trans*-3,4-dihydro-8-hydroxy-3-(3-hydroxy-4-methoxyphenyl)isocoumarin-4-carboxylates (**24** and **29**) were prepared from the corresponding acids (**10**, **9** and **15**, **5** and **13**, respectively). Heating **11** with methyl iodide and potassium carbonate in acetone resulted in ring-opening of the lactone producing dimethyl 3-hydroxystilbene-2,α-dicarboxylate (**41**) and its methyl ether (**42**). Detailed descriptions of the methyl esters (**21**—**29**) are given in Tables III and IV.

The *cis* and *trans* acid chlorides (**18** and **20**) were changed into 4-diazoacetyl-3,4-dihydro-8-methoxy-3-phenylisocoumarins (**30** and **31**) with diazomethane. The diazoketones (**30** and **31**) showed IR absorptions at 2095 and 2090 cm^{-1} corresponding to a carbonyl group adjacent to a diazo group. Reaction of **30** and **31** with dil. sulfuric acid gave *cis*- and *trans*-3,4-dihydro-4-hydroxyacetyl-8-methoxy-3-phenylisocoumarins (**32** and **34**), which were converted to 3,4-dihydro-8-hydroxy-4-hydroxyacetyl-3-phenylisocoumarins (**33** and **35**) by demethylation with boron tribromide. Upon demethylation of compound **34**, by-product, 3-hydroxy-α-hydroxyacetyl-2-stilbenecarboxylic acid (**39**) was also obtained.

The diazoketone (**31**) was changed into *trans*-4-acetoxyacetyl-3,4-dihydro-8-methoxy-3-phenylisocoumarin (**36**) by heating with acetic acid, or into *trans*-4-acetyl-3,4-dihydro-8-methoxy-3-phenylisocoumarin (**37**) by heating with hydroiodic acid. The compound **39** was further transformed into *trans*-4-acetyl-3,4-dihydro-8-hydroxy-3-phenylisocoumarin (**38**) by demethylation with boron tribromide.

The isocoumarin derivatives thus prepared, and some other isocoumarins prepared in Part I[3] of this series, that is, *cis* (**43**) and *trans* (**44**) isomers of 3,4-dihydro-3-phenylisocoumarin-4-carboxylic acid, and methyl *trans*-3,4-dihydro-3-phenylisocoumarin-4-carboxylate (**45**), were examined *in vitro* for antifungal activity. The activity was determined by cylinder-agar plate assay according to the two-fold dilution method.[6] Compounds **4**, **5**, **6**, **8**, **9**, **13**, **14**, **21**, **24**, **25**, **26**, **29**, **30**, **31**, **32**, **33**, **34**, **35**, **43** and **44** did not show inhibitory activity against

Table V. *In Vitro* Antifungal Activities of Isocoumarins

Compd. No.	MIC[a], μg/ml		
	Alternaria maritima	*Fusarium splendens*	*Giberella zeae*
3	(100)	N[b]	(400)
7	(400)	(400)	(400)
10	N	(200)	(200)
11	N	(50)	N
12	N	200(100)	N
15	N	(200)	(200)
16	N	N	(200)
22	N	N	(100)
23	N	(200)	(200)
27	N	N	(200)
28	N	N	(200)
36	N	N	(100)
37	(200)	N	(12.5)
38	(100)	(200)	N
45	N	N	(50)

a) Minimum inhibitory concentration (MIC) is the lowest concentration of the compound that completely prevents visible growth of molds after 72 h of incubation at 27°C. The numbers in parenthesis are the lowest concentrations at which inhibition of growth was apparent.
b) N: No inhibition of growth was observed at concentrations below 400 μg/ml.

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the molds tested. Activities of other compounds prepared are shown in Table V. It was found that the introduction of a carboxyl, carbomethoxyl, acetyl, diazoacetyl, or hydroxyacetyl group at the 4-position of the 3,4-dihydroisocoumarin nucleus causes substantial inactivation.

Experimental

Melting points are not corrected. Infrared (IR) spectra were taken in KBr pellets in the case of solid samples, and by the film method in the case of liquid samples, with a Hitachi model 215 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained on a JEOL JMN-FT 100 FT NMR spectrometer at 100 MHz, using tetramethylsilane as an internal standard. Low resolution and high resolution mass spectra were obtained on a JEOL JMS-D 300 spectrometer.

3-Hydroxyhomophthalic Anhydride (1)——3-Hydroxyhomophthalic acid (5 g) was dissolved in a mixture of acetyl chloride (6 ml) and acetone (20 ml), and the whole was stirred for 30 min at room temperature. Concentration of the mixture *in vacuo* at a temperature below 50°C gave the product (**1**) in 96% yield. No purification was necessary before further reaction. An analytical sample was obtained by recrystallization from hexane–acetone; colorless microcrystalline powder mp 162°C. *Anal.* Calcd for $C_9H_6O_4$: C, 60.68; H, 3.40. Found: C, 60.61; H, 3.33. IR ν_{max}^{KBr} cm^{-1}: 1700, 1785 (C=O). PMR (in acetone-d_6) δ: 4.33 (2H, s, CH_2), 6.9—8.0 (3H, m, aromatic-H). MS *m/e*: 178 (M^+).

3,4-Dihydro-8-hydroxy-3-arylisocoumarin-4-carboxylic Acids (3—7, 11—13) and 3,4-Dihydro-8-methoxy-3-arylisocoumarin-4-carboxylic Acids (8—10, 14—16), General Procedure——A suspension of the appropriate homophthalic anhydride (**1** or **2**) (0.005 mol), appropriate arylaldehyde (0.0075 mol), and finely powdered anhydrous Na_2CO_3 (0.95 g, 0.009 mol) in benzene (20 ml) was shaken at room temperature for 15 h. Water (10 ml) was added to the suspension, then the whole was shaken, and the aqueous layer was separated and acidified with HCl. The separation of the precipitated solid, a mixture of *cis* and *trans* isomers, could generally be achieved by fractional crystallization (**3** and **11**, **4** and **12**, **8** and **14**, **9** and **15**, **10** and **16**), but in some cases (**5** and **13**) chromatography on silica gel with $CHCl_3$ elution was required. The details are given in Table I.

Methyl *cis*- and *trans*-3,4-Dihydro-8-hydroxy-3-phenylisocoumarin-4-carboxylates (21 and 25) and Methyl *trans*-3,4-Dihydro-8-methoxy-3-phenylisocoumarin-4-carboxylate (26), General Procedure——Compound **3**, **11** or **14** was boiled with $SOCl_2$ for 1.5 h. After removal of excess reagent, the residue was refluxed with MeOH for 2 h. Concentration of the mixture gave crude **21**, **25** or **26**, which was purified by recrystallization. Details are given in Tables III and IV.

4-(3,4-Dimethoxybenzylidene)-8-methoxyisochroman-1,3-dione (40)——Compound **16** (0.5 g) was boiled with $SOCl_2$ (5 ml) for 30 min. After removal of excess reagent, the residue was purified by recrystallization from benzene to give **40** as yellow needles, mp 174°C: yield, 0.2 g (42%). *Anal.* Calcd for $C_{19}H_{16}O_6$: C, 67.06; H, 4.74. Found: C, 66.80; H, 4.63. IR ν_{max}^{KBr} cm^{-1}: 1770 and 1730 (anhydride C=O). PMR (in $CDCl_3$) δ: 3.96 (6H, s, 2 × OMe), 4.01 (3H, s, OMe), 6.85—7.85 (6H, m, phenyl), 7.61 (1H, s, C_β–H). MS *m/e*: 340 (M^+).

Methyl *cis*- and *trans*-3,4-Dihydro-8-methoxy-3-(*p*-methoxyphenyl)isocoumarin-4-carboxylate (22 and 27), Methyl *cis*- and *trans*-3,4-Dihydro-3-(3,4-dimethoxyphenyl)-8-methoxyisocoumarin-4-carboxylate (23 and 28), and Methyl *cis* and *trans*-3,4-Dihydro-8-hydroxy-3-(3-hydroxy-4-methoxyphenyl)isocoumarin-4-carboxylate (24 and 29), General Procedure——Compound **9**, **15**, **10**, **16**, **5**, or **13** was dissolved in a small amount of MeOH and treated with excess ethereal diazomethane for 48 h at room temperature. The crude methyl ester obtained by concentration of the reaction mixture was purified by recrystallization. Details are given in Tables III and IV.

Dimethyl 3-Hydroxystilbene-2,α-dicarboxylate (41) and Dimethyl 3-Methoxystilbene-2,α-dicarboxylate (42)——Methyl iodide (2.5 ml) and powdered anhydrous K_2CO_3 (2.07 g) were added to a solution of **11** (1.42 g) in acetone (100 ml), and the whole was refluxed for 19 h. After removal of KI by filtration, the filtrate was concentrated. The solid residue was washed, dried, and chromatographed on silica-gel, with $CHCl_3$ as the eluent.

The first eluate gave **41** as colorless cubes of mp 110°C (from ether–pet·ether); yield, 0.93 g (65%). *Anal.* Calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.08; H, 5.10. IR ν_{max}^{KBr} cm^{-1}: 1665 (chelated ester C=O), 1705 (conjugated ester C=O). PMR (in $CDCl_3$) δ: 3.73 (3H, s, COOMe), 3.82 (3H, s, COOMe), 6.5—7.8 (8H, m, aromatic-H), 7.72 (1H, s, C_β–H), 11.23 (1H, s, OH) exchangeable with D_2O). MS *m/e*: 312 (M^+).

The second eluate gave **42** as a colorless oil; yield, 0.59 g (36%). IR $\nu_{max}^{cap.}$ cm^{-1}: 1710, 1725 (ester C=O). PMR (in $CDCl_3$) δ: 3.71 (3H, s, MeO or COOMe), 3.76 (3H, s, MeO or COOMe), 3.89 (3H, s, MeO or COOMe), 6.7—7.5 (8H, m, aromatic-H), 7.83 (1H, s, C_β–H). MS *m/e*: 326.1123 (required for $C_{19}H_{18}O_5$ 326.1153, M^+).

***cis*- and *trans*-4-Diazoacetyl-3,4-dihydro-8-methoxy-3-phenylisocoumarin (30 and 31)**——The acid chloride (**18** or **20**) (2 g) obtained from **8** or **14** was dissolved in dry acetone (10 ml), and reacted with an acetone solution of diazomethane (obtained from 20 g of nitrosomethylurea) overnight at room temperature. Concentration of the reaction mixture and crystallization of the residue from a small amount of acetone gave **30** or **31**.

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cis Isomer (**30**): yellow prisms, mp 139°C (dec.), yield, 1.1 g (54%). *Anal.* Calcd for $C_{18}H_{14}N_2O_4$: C, 67.07; H, 4.38; N, 8.69. Found: C, 66.52; H, 4.18; N, 8.63. IR ν_{max}^{KBr} cm^{-1}: 2095 ($COCHN_2$), 1730 (lactone C=O). PMR (in $CDCl_3$) δ: 4.00 (3H, s, OMe), 4.03 (1H, d, J=3.2 Hz, C_4–H), 4.97 (1H, s, CHN_2), 5.72 (1H, d, J=3.2 Hz, C_3–H), 6.8—7.7 (8H, phenyl). MS *m/e*: 294 (M^+-N_2).

trans Isomer (**31**): yellow prisms, mp 144°C (dec.), yield, 1.5 g (74%). *Anal.* Calcd for $C_{18}H_{14}N_2O_4$: C, 67.07; H, 4.38; N, 8.69. Found: C, 67.65; H, 4.35; N, 8.36. IR ν_{max}^{KBr} cm^{-1}: 2090 ($COCHN_2$), 1715 (lactone C=O). PMR (in acetone-d_6) δ: 3.89 (3H, s, OMe), 4.49 (1H, d, J=4.8 Hz, C_4–H), 5.88 (1H, s, CHN_2), 5.89 (1H, d, J=4.8 Hz, C_3–H), 6.8—7.7 (8H, m, phenyl). MS *m/e*: 294 (M^+-N_2).

***cis*- and *trans*-3,4-Dihydro-4-hydroxyacetyl-8-methoxy-3-phenylisocoumarin (32 and 34)**——Ten per cent H_2SO_4 (0.6 ml) was dropped into a suspension of diazoketone (**30** or **31**) (0.1 g) in dioxane (3.5 ml) under efficient stirring. The whole mixture was then heated at 40°C for 3.5 h and extracted with AcOEt (20 ml). The residue obtained by concentration of the solution *in vacuo* at room temperature was purified by chromatography on silica gel with $CHCl_3$ to give **32** or **34**.

cis Isomer (**32**): colorless oil, yield 0.075 g (77%). PMR (in $CDCl_3$) δ: 3.42 (1H, d, J=19.8 Hz, $-C\underline{H}_2-$OH), 3.98 (3H, s, OMe), 4.06 (1H, d, J=19.8 Hz, $-C\underline{H}_2-$OH), 4.22 (1H, d, J=3.2 Hz, C_4–H), 5.73 (1H, d, J=3.2 Hz, C_3–H), 6.7—7.7 (8H, m, phenyl). MS *m/e*: 312.0998 (required for $C_{18}H_{16}O_5$ 312.0998, M^+).

trans Isomer (**34**): colorless oil, yield 0.08 g (83%). PMR (in $CDCl_3$) δ: 3.91 (1H, d, J=19.0 Hz, $-C\underline{H}_2-$OH), 3.96 (3H, s, OMe), 4.29 (1H, d, J=19.0 Hz, $-C\underline{H}_2-$OH), 4.35 (1H, d, J=7.1 Hz, C_4–H), 5.80 (1H, d, J=7.1 Hz, C_3–H), 6.5—7.6 (8H, m, phenyl). MS *m/e*: 312.1006 (required for $C_{18}H_{16}O_5$ 312.0998, M^+).

***cis*-3,4-Dihydro-8-hydroxy-4-hydroxyacetyl-3-phenylisocoumarin (33)**——A solution of boron tribromide (1 ml) in CH_2Cl_2 (2 ml) was added dropwise into an ice-cooled solution of **32** (0.5 g) in CH_2Cl_2 (2 ml) under a nitrogen atmosphere. After being stirred for 5 min, the mixture was poured into ice-water (30 ml), and extracted with CH_2Cl_2. The residue obtained after removal of solvent was purified by chromatography on silica gel with benzene–acetone (50: 1), followed by recrystallization from MeOH, to give **33** as a colorless microcrystalline material, mp 166°C, yield 0.125 g (26%). *Anal.* Calcd for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.07; H, 4.60. PMR (in acetone-d_6) δ: 3.40 (1H, d, J=19.3 Hz, $-C\underline{H}_2-$OH), 4.10 (1H, d, J=19.3 Hz, $-C\underline{H}_2-$OH), 4.78 (1H, d, J=3.5 Hz, C_4–H), 6.11 (1H, d, J=3.5 Hz, C_3–H), 6.6—7.6 (8H, m, phenyl), 11.04 (1H, s, OH). MS *m/e*: 298 (M^+).

***trans*-3,4-Dihydro-8-hydroxy-4-hydroxyacetyl-3-phenylisocoumarin (35) and 3-Hydroxy-α-hydroxyacetyl-2-stilbenecarboxylic Acid (39)**——Compound **34** (0.5 g) was treated with boron tribromide in a manner similar to that described for the preparation of **33**. The reaction product was divided into benzene-soluble and insoluble fractions, and the soluble fraction was purified by passage through a column of silica gel with benzene–acetone (20: 1, v/v) as an eluent, followed by recrystallization from MeOH, to give pure **35** as colorless prisms, mp 128°C, yield 0.12 g (25%). *Anal.* Calcd for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.25; H, 4.63. IR ν_{max}^{KBr} cm^{-1}: 3520 (OH), 1725 (C=O), 1675 (chelated C=O). PMR (in $CDCl_3$) δ: 2.86 (1H, t, J=5.0 Hz, $-CH_2-O\underline{H}$), 3.93 (1H, dd, J=20.2 Hz, and 5.0 Hz, $-C\underline{H}_2-$OH), 4.27 (1H, dd, J=20.2 Hz, and 5.0 Hz, $-C\underline{H}_2-$OH), 4.36 (1H, d, J=7.0 Hz, C_4–H), 5.95 (1H, d, J=7.0 Hz, C_3–H), 6.5—7.6 (8H, m, phenyl), 11.01 (1H, s, OH). MS *m/e*: 298 (M^+).

The insoluble fraction was purified by recrystallization from benzene, to give pure **39** as a colorless microcrystalline material, mp 158°C (dec.) yield 0.23 g (49%). *Anal.* Calcd for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 67.83; H, 4.54. IR ν_{max}^{KBr} cm^{-1}: 3340 (OH), 3000—2400 (COOH), 1680 (C=O), 1660 (chelated C=O). PMR (in $CDCl_3$) δ: 4.31 (1H, d, J=22.0 Hz, $-C\underline{H}_2-$OH), 4.57 (1H, d, J=22.0 Hz, $-C\underline{H}_2-$OH), 6.19 (1H, broad, COOH), 6.6—7.5 (8H, m, phenyl), 7.57 (1H, s, C_β–H), 11.63 (1H, broad s, OH). MS *m/e*: 298 (M^+).

***trans*-4-Acetoxyacetyl-3,4-dihydro-8-methoxy-3-phenylisocoumarin (36)**——Diazoketone (**31**) (0.3 g) was added in portions to glacial acetic acid (0.6 ml) at 70°C, and the mixture was heated at 80°C till the evolution of nitrogen gas stopped. The reaction mixture was poured into ice-water (20 ml) and extracted with CH_2Cl_2 (40 ml). The extract was washed, dried and concentrated. After chromatography on silica gel with benzene–acetone (50: 1, v/v) and recrystallization from benzene–ether, pure **36** was obtained as colorless needles, mp 128°C, yield, 0.21 g (77%). *Anal.* Calcd for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12. Found: C, 67.30; H, 5.12. IR ν_{max}^{KBr} cm^{-1}: 1755 (C=O), 1735 (C=O), 1720 (C=O). PMR (in $CDCl_3$) δ: 2.13 (3H, s, acetyl), 3.97 (3H, s, OMe), 4.01 (1H, d, J=17.5 Hz, $-C\underline{H}_2-$OH), 4.39 (1H, d, J=8.3 Hz, C_4–H), 4.70 (1H, d, J=17.5 Hz, $-C\underline{H}_2-$OH), 5.68 (1H, d, J=8.3 Hz, C_3–H), 6.8—7.6 (8H, m, phenyl). MS *m/e*: 354 (M^+).

4-Acetyl-3,4-dihydro-8-methoxy-3-phenylisocoumarin (37)——A solution of diazoketone (**31**) (0.12 g) in $CHCl_3$ (2 ml) was added dropwise to an ice-cooled solution of 47% HI (0.15 ml) in $CHCl_3$ (0.32 ml) during a period of 1 h under efficient stirring. The mixture was stirred at room temperature for a further 30 min, then the $CHCl_3$ layer was separated, washed with 0.1 N $Na_2S_2O_3$ then water, dried and concentrated. Recrystallization of the product from MeOH–hexane gave **37** as a colorless microcrystalline powder, mp 119°C; yield, 0.1 g (91%). IR ν_{max}^{KBr} cm^{-1}: 1700, 1720 (C=O). PMR (in $CDCl_3$) δ: 2.11 (3H, s, COOMe), 3.95 (3H, s, MeO), 4.29 (1H, d, J=6.0 Hz, C_4–H), 5.80 (1H, d, J=6.0 Hz, C_3–H), 6.6—7.6 (8H, m, aromatic-H). MS *m/e*: 296.1047 (required for $C_{18}H_{16}O_4$ 296.1047, M^+).

4-Acetyl-3,4-dihydro-8-hydroxy-3-phenylisocoumarin (38)——This compound (**38**) was prepared from **37** (0.12 g) in a manner similar to that described for the preparation of **33**. After chromatography on silica gel with benzene and recrystallization from MeOH, **38** was obtained as colorless prisms, mp 130°C, yield

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0.095 g (83%). *Anal.* Calcd for $C_{16}H_{14}O_4$: C, 72.33; H, 5.00. Found: C, 71.80; H, 4.83. IR ν_{max}^{KBr} cm^{-1}: 1720 (acetyl C=O), 1680 (lactone C=O). PMR (in $CDCl_3$) δ: 2.12 (3H, s, COMe), 4.36 (1H, d, J=6.0 Hz, C_4–H), 6.00 (1H, d, J=6.0 Hz, C_3–H), 6.6—7.6 (8H, m, aromatic H). MS *m/e*: 282.0878 (required for $C_{16}H_{14}O_4$ 282.0890, M^+).

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