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Syntheses of Antifungal Isocoumarins. II.[1,2] Synthesis and Antifungal Activity of 3-Substituted Isocoumarins

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Various 3-arylisocoumarins (**5**, **6**, **8**, **10**, **11**, and **12**) were simply prepared in high yields by heating homophthalic acids (**1**—**4**) with aromatic acyl chlorides. 8-Hydroxy-3-phenylisocoumarin (**17**) and 8-acetoxy-3-phenylisocoumarin (**18**), and 8-hydroxy-3-(*p*-methoxyphenyl)isocoumarin (**19**) were obtained by alkaline hydrolysis of **10** and **11**, respectively, followed by treatment with acetic anhydride. 3,4-Dihydro-8-hydroxy-3-phenylisocoumarin (**24**) was prepared from **10** by alkaline hydrolysis followed by reduction with sodium borohydride then heating with acetic anhydride. 3-(*p*-Hydroxyphenyl)-isocoumarin (**7**), 8-hydroxy-3-(*p*-hydroxyphenyl)isocoumarin (**9**), 5-chloro-8-hydroxy-6-methoxy-3-phenylisocoumarin (**13**), and 3,4-dihydro-3-(*p*-hydroxyphenyl)isocoumarin (**23**) were prepared by demethylation of **6**, **8**, **12** and **22**, respectively. All the prepared isocoumarins and some other isocoumarin derivatives (**25**—**30**) were examined *in vitro* for antifungal activity. The structure-activity relationships are discussed.

Keywords——antifungal activity; 3-arylisocoumarins; 3,4-dihydroisocoumarins; 8-hydroxyisocoumarins; structure-activity relationship

Our previous finding that phyllodulcin possesses antifungal activity[3] prompted us to synthesize and examine the activity of various isocoumarins and 3,4-dihydroisocoumarins having an aryl group at position 3.

Various 3-arylisocoumarins were synthesized from homophthalic acids *via* two or three reaction steps by Tirodkar and Usgaonkar.[4] In this paper, however the authors report a convenient method for preparing 3-arylisocoumarins in only one step in high yields, simply by heating homophthalic acids with excess acyl chlorides. By this method, we synthesized 3-phenylisocoumarin (**5**) and 3-(*p*-methoxyphenyl)isocoumarin (**6**) from homophthalic acid (**1**), 8-methoxy-3-(*p*-methoxyphenyl)isocoumarin (**8**) from 3-methoxyhomophthalic acid (**2**), 8-benzoyloxy-3-phenylisocoumarin (**10**) and 8-(*p*-methoxybenzoyloxy)-3-(*p*-methoxyphenyl)-isocoumarin (**11**) from 3-hydroxyhomophthalic acid (**3**), and 5-chloro-6,8-dimethoxy-3-phenylisocoumarin (**12**) from 6-chloro-3,5-dimethoxyhomophthalic acid (**4**). The details of the syntheses and the properties of the products are given in Tables I and II. The starting material (**4**) was derived from 3,5-dimethoxybenzoic acid as reported in the previous paper.[5]

The 4′-methoxyl group of **6** was demethylated by treatment with boron tribromide in methylene chloride, with cooling, to yield 3-(*p*-hydroxyphenyl)isocoumarin (**7**). Similar reactions of **8** and **12** gave 8-hydroxy-3-(*p*-hydroxyphenyl)isocoumarin (**9**) and 5-chloro-8-hydroxy-6-methoxy-3-phenylisocoumarin (**13**), respectively.

When compound **6**, **10** or **11** was refluxed with 5% potassium hydroxide, the lactone ring opened to afford the keto-acid **14**, **15** or **16**, respectively. The keto-acids **15** and **16** were recyclized by heating with acetic anhydride to give 8-hydroxy-3-phenylisocoumarin (**17**) and 8-acetoxy-3-phenylisocoumarin (**18**), and 8-hydroxy-3-(*p*-methoxyphenyl)isocoumarin (**19**), respectively.

Following the procedures described in the literature,[8] 3,4-dihydro-3-(*p*-methoxyphenyl)-isocoumarin (**22**) or 3,4-dihydro-8-hydroxy-3-phenylisocoumarin (**24**) was synthesized from the keto-acid **14** or **15** *via* the compound **20** or **21**, by successive reduction with sodium borohydride and cyclization with acetic anhydride. The compound **22** was further derived to

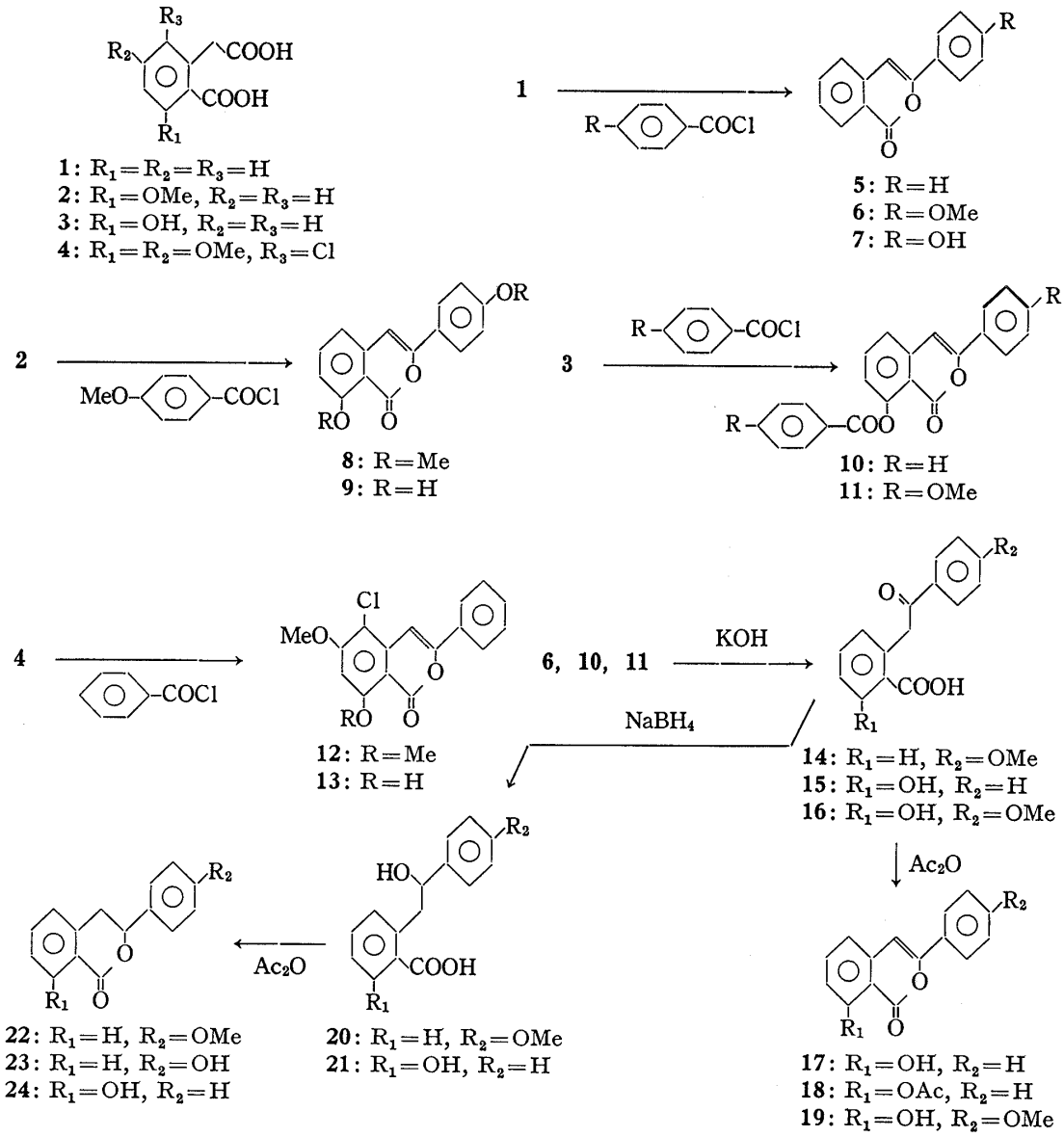

Chart 1

Table I. 3-Arylisocoumarins prepared from Homophthalic Acids and Acyl Chlorides

Compd. No.	mp (°C)	Yield (%)	Recrystn. solvent[a]	Chromato. solvent	Formula	Analysis (%) Calcd (Found) C	H
5	90 (lit.[1] 90)	83	EtOH[n]	Bz	$C_{14}H_{10}O_2$	—	—
6	121 (lit.[6] 116)	80	MeOH[n]	Bz	$C_{15}H_{12}O_3$	—	—
8	157 (lit.[7] 161—162)	78	MeOH[n]	Bz–Ac (3 : 1)	$C_{16}H_{14}O_4$	—	—
10	163	77	Bz[m]	$CHCl_3$–MeOH (3 : 1)	$C_{22}H_{14}O_4$	77.19 (77.44)	4.12 (4.49)
11	202	75	Bz[m]	Bz–Ac (100 : 1)	$C_{24}H_{18}O_6$	71.63 (71.17)	4.51 (4.35)
12	235	80	MeOH[n]	Bz–Ac (50 : 1)	$C_{17}H_{13}ClO_4$	64.47 (64.51)	4.14 (4.00)

a) n: colorless needles. m: colorless microcrystalline powder. Bz: benzene. Ac: acetone.

Table II. Spectral Data for 3-Arylisocoumarins prepared from Homophthalic Acids and Acyl Chlorides

Compd. No.	IR ν_{max}^{KBr} cm^{-1} (C=O)	NMR (in $CDCl_3$) (ppm) MeO (3H, s)	C_4–H (1H, s)	Phenyl-H (m)
5	1718		6.96	7.2—8.4(9H)
6	1735	3.87	6.83	6.9—8.4(8H)
8	1720	3.86 4.02	6.73	6.6—7.9(7H)
10	1720		6.95	7.1—8.3(13H)
11	1726	3.85 3.90	6.83	6.9—8.3(11H)
12	1722	4.03 4.04	6.53	7.2—8.0(6H)

3,4-dihydro-3-(*p*-hydroxyphenyl)isocoumarin (**23**) by treatment with boron tribromide in methylene chloride under cooling.

The isocoumarin derivatives thus prepared were examined *in vitro* for antifungal activity. The isocoumarins prepared by us previously,[9] *i.e.*, 8-hydroxy-3-methyl-6-methoxyisocoumarin (**25**), 5-chloro-8-hydroxy-6-methoxy-3-methylisocoumarin (**26**), 5-chloro-6,8-dimethoxy-3-methylisocoumarin (**27**), 5-chloro-3,4-dihydro-8-hydroxy-6-methoxy-3-methylisocoumarin (**28**), and 5-chloro-6,8-dimethoxy-3-methylisocoumarin (**29**) were examined. 3,4-Dihydro-3-phenylisocoumarin (**30**), prepared according to the literature,[8] was also examined. The antifungal activity was determined on agar plates by the two-fold dilution methed.[10] The results are listed in Table III. 3,4-Dihydro-derivatives (**23** and **30**) are slightly more active than the corresponding isocoumarins (**7** and **5**). In the case of isocoumarins having a hydroxyl group at the 8-position, the inclusion of a hydroxyl or a methoxyl group at the 4′-position increases the activity, as seen in **9** and **19** compared with **17**, but rather decreases the activity

Table III. *In Vitro* Antifungal Activity of Isocoumarins

Compd. No.	MIC,[a] μg/ml *Alternaria maritima*	*Cochliobolus miyabeanus*	*Fusarium splendens*	*Giberella zeae*
5	200(12.5)	(12.5)	(12.5)	(12.5)
6	(12.5)	N[b]	(100)	N[b]
7	N[b]	N[b]	(100)	N[b]
8	(12.5)	(12.5)	200(12.5)	(12.5)
9	(12.5)	(12.5)	(12.5)	(12.5)
12	N[b]	N[b]	N[b]	(200)
13	(100)	(100)	N[b]	N[b]
17	ND[b]	N[b]	(400)	N[b]
18	(12.5)	N[b]	(12.5)	(12.5)
19	(12.5)	N[b]	(12.5)	(12.5)
22	100	(50)	(12.5)	(50)
23	200	200	200	200
24	N[b]	N[b]	(200)	N[b]
25	(400)	ND[b]	200(50)	(50)
26	N[b]	ND[b]	200(12.5)	N[b]
27	50	ND[b]	200(50)	ND[b]
28	200(12.5)	ND[b]	200(12.5)	(12.5)
29	N[b]	ND[b]	N[b]	(50)
30	ND[b]	50(25)	100(12.5)	100(25)

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 more or less apparent.
b) N: No inhibition of growth was observed at concentrations below 400 μg/ml.
ND: Not determined.

in the case of isocoumarins or 3,4-dihydroisocoumarins having no hydroxyl group at the 8-position, as seen in **6** and **7** compared with **5**, and **23** and **22** compared with **30**, respectively.

Experimental

Melting points are uncorrected. Infrared (IR) spectra were taken in KBr pellets with a Hitachi model 215 grating spectrophotometer. Proton magnetic resonance (PMR) spectra were obtained on a JEOL LNM-FX 100 FT NMR spectrometer at 100 MHz, with tetramethylsilane as an internal standard. Mass spectra were obtained on a JEOL JMS-D 300 spectrometer.

3-Methoxyhomophthalic Acid (2)——3-Hydroxyhomophthalic acid (**1**) (25 g) was dissolved in 30% NaOH (88 ml), and dimethyl sulfate (47 ml) was added dropwise with stirring over a period of 30 min. Additional 20% NaOH (88 ml) was added, then the mixture was refluxed for 2 h, and concentrated *in vacuo*. The residue was washed with ether, dissolved in water and acidified to give **2** (27 g), mp 162°C. This product was sufficiently pure for further reaction. Crystallization from AcOEt gave pure **2** as colorless needles, mp 166°C (lit.[7] 164—166°C).

3-Arylisocoumarins (5 and 6), 3-Aryl-8-methoxyisocoumarins (8 and 12), and 8-Acyloxy-3-arylisocoumarins (10 and 11), General Procedure——A mixture of a homophthalic acid (**1**, **2**, **3**, or **4**) (0.001 mol) and an acyl chloride (0.004 mol) was heated at 190°C under stirring for 1.5 h. The mixture was cooled, CH_2Cl_2 (20 ml) and 5% NaOH (20 ml) were added, and the whole was shaken until all the solid had gone into solution. The CH_2Cl_2 layer was separated, washed, dried and stripped of solvent, and the residue was purified by chromatography on silica-gel, followed by recrystallization, to afford pure samples. Details are given in Table I.

3-(*p*-Hydroxyphenyl)isocoumarin (7), 8-Hydroxy-3-(*p*-hydroxyphenyl)isocoumarin (9), 5-Chloro-8-hydroxy-6-methoxy-3-phenylisocoumarin (13) and 3,4-Dihydro-3-(*p*-hydroxyphenyl)isocoumarin (23), General Procedure——A solution of boron tribromide (1 ml) in CH_2Cl_2 (4 ml) was added dropwise to an ice-cooled solution of **6**, **8**, **12** or **22**[8] (0.0005 mol) in CH_2Cl_2 (4.5 ml), under a nitrogen atmosphere. After stirring for 6 min, the whole mixture was poured into ice-water (100 ml) and extracted twice with CH_2Cl_2 (40 ml × 2) then with AcOEt (40 ml). The combined extracts were washed, dried, and concentrated. Chromatographic purification on silica-gel with benzene–acetone (30: 1 v/v), followed by recrystallization from $CHCl_3$ (**7**), or MeOH (**9**, **13** and **23**), gave colorless prisms (**7**), needles (**9** and **13**) or prisms (**23**).

Compound (**7**): mp 223°C (lit.[11] 211°C); yield, 18 mg. IR ν_{max}^{KBr} cm^{-1}: 1690 (C=O), 3275 (OH). PMR (in d_6-acetone) δ: 7.16 (1H, s, C_4–H), 6.9—8.3 (8H, m, phenyl). MS *m/e*: 238 (M^+).

Compound (**9**): mp 228°C; yield, 100 mg. *Anal.* Calcd for $C_{14}H_{10}O_4$: C, 70.86; H, 3.95. Found: C, 71.10; H, 3.75. IR ν_{max}^{KBr} cm^{-1}: 1662 (C=O). PMR (in d_6-acetone) δ: 6.87 (1H, s, C_4–H), 6.8—7.9 (7H, m, phenyl), 8.94 (1H, s, 4′-OH), 11.00 (1H, s, 8-OH). MS *m/e*: 254 (M^+).

Compound (**13**): mp 185°C; yield, 52 mg, *Anal.* Calcd for $C_{16}H_{11}ClO_4$: C, 63.48; H, 3.66. Found: C, 63.68; H, 3.52. IR ν_{max}^{KBr} cm^{-1}: 1680 (C=O). PMR (in $CDCl_3$) δ: 4.00 (3H, s, MeO), 6.56 (1H, s, C_4–H), 7.2—8.0 (6H, m, phenyl). MS *m/e*: 302 (M^+ for ^{35}Cl), 304 (M^+ for ^{37}Cl).

Compound (**23**): mp 156°C; yield, 103 mg. *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99; H, 5.03. Found: C, 75.32; H, 5.10. IR ν_{max}^{KBr} cm^{-1}: 1690 (C=O), 3300 (OH). PMR (in d_6-acetone) δ: 3.15 (1H, dd, J=4.0 and 16.5 Hz, C_4–H), 3.42 (1H, dd, J=11.1 and 16.5 Hz, C_4–H), 5.56 (1H, dd, J=4.0 and 11.1 Hz, C_3–H), 6.8—8.1 (8H, m, phenyl), 8.51 (1H, bs, OH). MS *m/e*: 240 (M^+).

8-Hydroxy-3-(*p*-methoxyphenyl)isocoumarin (19)——Compound **11** (140 mg) was suspended on a mixture of 5% KOH (10 ml) and EtOH (5 ml), and refluxed for 4 h. After cooling, the reaction mixture was acidified with HCl, and extracted with CH_2Cl_2 (50 ml) then AcOEt (50 ml). The combined extracts gave a crude solid (**16**) after usual work-up. Compound **16** was heated with acetic anhydride (2 ml) at 50°C for 30 min. After cooling, the reaction mixture was poured into ice-water (20 ml), and extracted with CH_2Cl_2 (50 ml). The crude **19** obtained after usual work-up was purified by chromatography on silica-gel with benzene elution, followed by recrystallization from MeOH to afford a pure sample as colorless needles of mp 151°C; 43 mg (overall yield, 46%). *Anal.* Calcd for $C_{16}H_{12}O_4$: C, 71.63; H, 4.51. Found: C, 71.44; H, 4.35. IR ν_{max}^{KBr} cm^{-1}: 1670 (C=O). PMR (in $CDCl_3$) δ: 3.87 (3H, s, MeO), 6.84 (1H, s, C_4–H), 6.8—7.9 (7H, m, phenyl), 11.00 (1H, s, OH). MS *m/e*: 268 (M^+).

8-Hydroxy-3-phenylisocoumarin (17) and 8-Acetoxy-3-phenylisocoumarin (18)——A mixture of **10** (0.85 g), 5% KOH (12 ml) and EtOH (4 ml) was heated under reflux for 4 h. Concentration and acidification with HCl gave a precipitate, which was washed and dried to give crude **15** (0.5 g). The crude **15** thus obtained was boiled with acetic anhydride (0.5 ml) for 2 h. The reaction mixture was poured into ice-water (10 ml), stirred for 2 h at room temperature, acidified with HCl, and extracted twice with CH_2Cl_2 (50 ml × 2). The extract was separated by chromatography on silica-gel, eluting with benzene.

The first eluate gave compound **17** as colorless needles, mp 142°C (from MeOH); yield, 100 mg (18%). *Anal.* Calcd for $C_{15}H_{10}O_3 \cdot 1/2H_2O$: C, 73.38; H, 4.23. Found: C, 72.86; H, 4.48. IR ν_{max}^{KBr} cm^{-1}: 1660 (chelated C=O). PMR (in $CDCl_3$) δ: 6.95 (1H, s, C_4–H), 6.9—8.0 (8H, m, phenyl), 10.99 (1H, s, OH). MS *m/e*: 238 (M^+).

Further elution gave pure **18** as colorless needles, mp 139°C (from MeOH); yield, 380 mg (58%). *Anal.* Calcd for $C_{17}H_{12}O_4$: C, 73.20; H, 4.49. Found: C, 72.85; H, 4.32. IR ν_{max}^{KBr} cm^{-1}: 1720 (C=O). PMR (in $CDCl_3$) δ: 2.45 (3H, s, $COCH_3$), 6.93 (1H, s, C_4–H), 6.9—8.0 (8H, m, phenyl). MS *m/e*: 280 (M^+).

3,4-Dihydro-8-hydroxy-3-phenylisocoumarin (24)——Compound **15** (2 g), prepared as described above, was dissolved in 1% NaOH (90 ml), and $NaBH_4$ (0.8 g) was added. The mixture was stirred for 1 h at room temperature. After being acidified with HCl, the whole mixture was extracted twice with AcOEt (50 ml × 2). Usual work-up gave crude **21** (1.9 g). This compound (**21**) was dissolved in acetic anhydride (2 ml) and heated under reflux for 2 hr. The mixture was cooled, water (20 ml) was added, and the whole was stirred continuously overnight. The crystals that deposited were collected by filtration, and the filtrate was extracted twice with CH_2Cl_2 (20 ml × 2). The extract was concentrated. The crystals obtained on the filter and from the filtrate were combined and purified by column chromatography on silica-gel with benzene, followed by recrystallization from MeOH. Colorless needles, mp 107°C; yield, 1.5 g. *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 75.29; H, 5.07. Found: C, 74.99; H, 5.03. IR ν_{max}^{KBr} cm^{-1}: 1650 (C=O). PMR (in $CDCl_3$) δ: 3.10 (1H, dd, J=4.4 and 16.4 Hz, C_4–H), 3.35 (1H, dd, J=11.1 and 16.4 Hz, C_4–H), 5.60 (1H, dd, J=4.4 and 11.1 Hz, C_3–H), 6.7—7.6 (8H, m, phenyl), 10.99 (1H, s, OH). MS *m/e*: 240 (M^+).

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