λ_{max} 223 nm (ϵ_{max} 27120), 274 (3890), 284 (39740), and 310 (3200); ir (KBr) 1630, 1450, 1383, 1525, and 1322 cm^{-1}.

(c) **3** (400 mg, 43%, GLC 70%, R_f 0.43), bp 93–95 (0.5 mm) and 78–80 (0.1 mm), which was identical with the material obtained from the chloroform reaction (^1H NMR, uv, and ir).

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Registry No.—**1**, 57173-97-4; **2**, 57173-98-5; **3**, 57173-99-6; **4**, 57174-00-2; **5**, 57174-01-3; **7**, 57174-02-4; **9**, 57174-03-5; 5-amino-*v*-triazole, 30132-90-2; acetylacetone, 123-54-6; *N*-bromosuccinimide, 128-08-5; iodine monochloride, 7790-99-0; *N*-chlorosuccinimide, 128-09-6.

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Fluorometric Reagents for Primary Amines. Syntheses of 2-Alkoxy- and 2-Acyloxy-3(2*H*)-furanones

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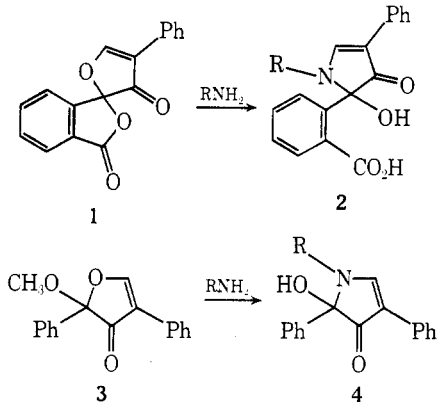
In recent years, fluorescamine, 4-phenylspiro[furan-2(3*H*),1′-phthalan]-3,3′-dione (**1**),[2] has become a widely used reagent for the fluorometric quantitation of primary amines. Fluorescamine reacts with primary amines (RNH_2) to form pyrrolinones of type **2** which upon excitation at 390 nm emit strong fluorescence at 475–490 nm (Scheme I). This reaction proceeds efficiently at room temperature in aqueous solutions[3] and allows the fluorometric estimation of submicromolar concentrations of amines, notably those of biological importance.[4] Many specific analytical applications of fluorescamine have been described in the recent literature, among them highly sensitive procedures for automated amino acid analyses[5,6] and for the assay of proteins.[7] Most recently, the use of fluorescamine has also been suggested for the colorimetric assay of amino acids.[8]

The structurally related compound, 2-methoxy-2,4-diphenyl-3(2*H*)-furanone (MDPF, **3**), which reacts similarly

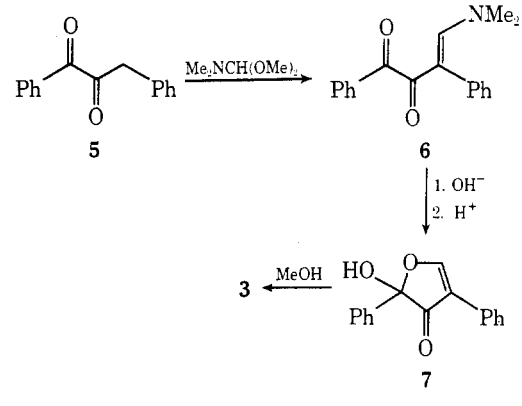
Scheme I



with primary amines to give fluorescent products of type **4**, was found particularly suitable for the fluorescent labeling of proteins and has been used in the preparation of fluorophoric immunoglobulin conjugates.[9] MDPF (**3**) has also been employed to derivatize α-amino acids for the purpose of determining their absolute configuration by chiroptical means.[10]

In this report we wish to describe in detail the syntheses of fluorescamine (**1**) and MDPF (**3**). As depicted in Schemes II and III, the carbon skeleton of these compounds was readily constructed by formylation of suitably substituted 1,2-propanediones.

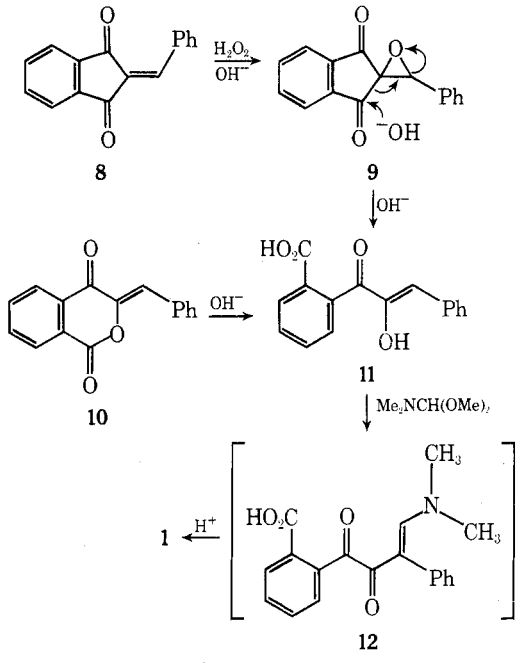
Scheme II



Thus, in the case of MDPF (**3**), 1,3-diphenyl-1,2-propanedione (**5**)[11] was converted by reaction with *N,N*-dimethylformamide dimethyl acetal to the dimethylaminomethylene derivative **6** (Scheme II). Alkaline hydrolysis of this enamine, followed by acidic work-up, gave the hydroxyfuranone **7**, which was smoothly converted to the desired methoxy derivative **3** by heating in methanol at reflux temperature. Proof for the structure of **3**, in particular the establishment of its cyclic nature, has already been outlined in a previous communication.[2]

The hydroxycinnamoylbenzoic acid **11**, which was required for the synthesis of **1**, was initially obtained by hydrolysis of 3-benzylidene-1,4-isochromandione (**10**)[2] (Scheme III). However, the preparation of **10** according to literature procedures[12] was found to be cumbersome and inefficient. We therefore chose to prepare **11** by a novel route. Thus, 2-benzylideneindan-1,3-dione (**8**)[13] was converted by base-catalyzed oxidation with hydrogen peroxide in methanol to the epoxide **9**. Hydrolysis of **9** with sodium hydroxide led to cleavage of both the indandione and the oxirane ring (cf. **9**, arrows) to afford the desired 1,2-propanedione derivative **11**. The formylation of **11** was carried

Scheme III



out with *N,N*-dimethylformamide dimethyl acetal,[14] much like the conversion of **5** to **6**. However, in this case, it was not possible to isolate the analogous enamine intermediate **12**. Instead, aqueous work-up of the reaction mixture at pH 4 resulted directly in the formation of the desired spirolactone fluorescamine (**1**).

Experimental Section

General. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer 621 or a Beckman IR-9 spectrometer. Ultraviolet spectra were recorded on a Cary Model 16 spectrophotometer. NMR spectra were measured on a Varian HA-100 instrument and are reported in parts per million downfield from internal tetramethylsilane.

1-Dimethylamino-2,4-diphenyl-1-butene-3,4-dione (6). A solution of 44.8 g of 1,3-diphenyl-1,2-propanedione (**5**)[11] in 90 ml of *N,N*-dimethylformamide dimethyl acetal was allowed to stand at room temperature for 2 hr. It was then poured into 1 l. of ice water and the aqueous mixture was extracted three times with ether. The combined extracts were washed with water, diluted with benzene, dried over Na_2SO_4, and evaporated under reduced pressure. The oily residue was crystallized from ether–petroleum ether to give 43.3 g (77.5%) of **6**: mp 108°C; uv max (CH_3OH) 250 nm (ϵ 12200) and 300 (13300); ir ($CHCl_3$) 1780, 1765, 1605, 1570 cm^{-1}.

Anal. Calcd for $C_{18}H_{17}NO_2$: C, 77.39; H, 6.13; N, 5.01. Found: C, 77.38; H, 6.10; N, 4.91.

2-Methoxy-2,4-diphenyl-3(2*H*)-furanone (3). To a solution of 43.3 g of 1-dimethylamino-2,4-diphenyl-1-butene-3,4-dione (**6**) in 500 ml of ethanol was added 500 ml of 2% aqueous potassium hydroxide. The mixture was stirred at room temperature for 2 hr. It was then diluted with 3 l. of H_2O and acidified with 10% HCl. Solid 2-hydroxy-2,4-diphenyl-3(2*H*)-furanone (**7**) precipitated. It was filtered off with suction and washed on the filter with water. [A dried sample had uv max (MeOH) 244 nm (ϵ 18400) and 292 (6250); NMR ($CDCl_3$) δ 8.59 ppm (s, =CHO–).] The filter cake was dissolved (without further purification) in 500 ml of methanol, and the methanolic solution was heated at reflux temperature for 20 hr and then concentrated on a steam bath to ca. 350 ml. The desired product crystallized upon refrigeration. After two recrystallizations from methanol, there was obtained 31.3 g (76%) of 2-methoxy-2,4-diphenyl-3(2*H*)-furanone (**3**): mp 93–95°C; uv max (MeOH) 241 nm (ϵ 18750) and 307 (3500); ir ($CHCl_3$) 1735, 1705, 1618, 1595 cm^{-1}; NMR ($CDCl_3$) δ 8.69 (s, =CHO–), 3.43 ppm (CH_3O).

Anal. Calcd for $C_{17}H_{14}O_3$: C, 76.67; H, 5.30. Found: C, 76.65; H, 5.48.

3-Phenylspiro[oxirane-2,2′-indan]-1′,3′-dione (9). Into a 2-l. three-necked flask, equipped with a stirrer and a dropping funnel, were placed 96.0 g of 2-benzylidene-1,3-indandione (**8**),[13] 1 l. of methanol, and 60 ml of 30% hydrogen peroxide. The mixture was cooled to 5°C and 10 ml of 1 *N* sodium hydroxide was added dropwise at such a rate as to keep the temperature below 15°C. After completed addition, stirring was continued at room temperature for 30 min. The mixture was then poured into 4.5 l. of water and the resulting crystalline precipitate was collected by filtration, washed repeatedly on the filter with water, and dried under high vacuum at room temperature, affording 101.0 g (98.5%) of the epoxide **9**, mp 154–156°C, pure enough for the next step. An analytical sample, recrystallized from ethyl acetate, had mp 158°C; ir ($CHCl_3$) 1765, 1750 (sh), 1735 (sh), 1725, 905 cm^{-1}; NMR ($CDCl_3$) δ 4.72 ppm (s, –CHO–).

Anal. Calcd for $C_{16}H_{10}O_3$: C, 76.79; H, 4.03. Found: C, 77.07; H, 4.02.

***o*-(α-Hydroxycinnamoyl)benzoic Acid (11).** To a stirred suspension of 20.0 g of 3-phenylspiro[oxirane-2,2α-indan]-1′,3′-dione (**9**) in 200 ml of 10% aqueous sodium hydroxide was added 50 ml of methanol. The reaction became slightly exothermic and the temperature was kept below 35°C by external cooling. After 3.5 hr, the reaction mixture was diluted with 2 l. of water, and the resulting alkaline solution was washed with 500 ml of ether, acidified with 10% HCl, and extracted with chloroform. The extract was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure. The crystalline residue was triturated with petroleum ether–ether (9:1) and filtered, giving 19.0 g (89%) of *o*-(α-hydroxycinnamoyl)benzoic acid (**11**): mp 106–115°C dec; uv max (Et_2O) 315 nm (ϵ 23300); ir (KBr) 1700, 1660, 1625, 1600 cm^{-1}; NMR (Me_2SO-d_6) δ 5.65 (1 s, –CH=), 7.21–8.17 (9 m, aromatic), 9.67 (1 s, –OH).

Anal. Calcd for $C_{16}H_{12}O_4$: C, 71.63; H, 4.51. Found: C, 71.73; H, 4.58.

4-Phenylspiro[furan-2(3*H*),1′-phthalan]-3,3′-dione (1). To a stirred solution of 16.08 g of *o*-(α-hydroxycinnamoyl)benzoic acid (**11**) in 60 ml of a 2 *N* dimethylamine solution in DMF was added 30 ml of *N,N*-dimethylformamide dimethyl acetal. The resulting mixture was kept at room temperature for 2 hr and was then poured into 500 ml of ice water. The aqueous solution was carefully acidified with 10% HCl to pH 4 and the resulting suspension was extracted with 3 × 600 ml of ether–benzene (1:1). After combination, the extracts were washed with 500 ml of 1% aqueous sodium bicarbonate and with water, dried over Na_2SO_4, and evaporated in vacuo. The solid residue was dissolved in 50 ml of warm methylene chloride and the resulting solution was diluted with 120 ml of ether. Upon refrigeration, 10.57 g (64%) of fluorescamine (**1**) precipitated; mp 154–155°C; uv max (Et_2O) 235 nm (ϵ 25900), 276 (3950), 284 (4100), and 306 (3800); ir ($CHCl_3$) 1810, 1745, 1722, 1625, 1600 cm^{-1}; NMR ($CDCl_3$) δ 8.71 (s, –OCH=).

Anal. Calcd for $C_{17}H_{10}O_4$: C, 73.38; H, 3.62. Found: C, 73.41; H, 3.57.

Registry No.—**1**, 38183-12-9; **3**, 50632-57-0; **5**, 23464-17-7; **6**, 36777-65-8; **7**, 54585-24-9; **8**, 5381-33-9; **9**, 43053-57-2; **11**, 43053-07-2; *N,N*-dimethylformamide dimethyl acetal, 4637-24-5.

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