## S 2. *Fluorescent Acylating Agents derived from 7-Hydroxycoumarin.*

By Wilson Baker and (the late) C. B. Collis.

Some carboxylic acids derived from 7-methoxycoumarin have been prepared with the object of using their chlorides as fluorescent reagents for hydroxy- and amino-compounds. Such fluorescent acyl derivatives can be used in the chromatographic separation of colourless compounds. *7-Methoxycoumarin-3-carboxylic chloride* is the most readily available of these reagents.

The chromatographic separation of colourless compounds can be facilitated in various ways, for example by conversion into coloured derivatives by means of benzeneazobenzoyl chloride or 2 : 4-dinitrophenylhydrazine. Another general possibility is to combine the colourless compound with a fluorescent reagent so that the derivative may be located on the column by its fluorescence in ultra-violet light. This procedure does not appear to have been investigated as yet, though it is mentioned by Strain (" Chromatographic Adsorption Analysis," New York, 1941, p. 76), and by Williams (" An Introduction to Chromatography," 1946, p. 50). What may be regarded as the reverse method, namely the adsorption of a colourless, non-fluorescent substance on a column of fluorescent material, has, however, been successfully carried out. The fluorescence of the column is quenched by many adsorbed materials, the location of which is shown as a dark band when the column is examined in ultra-violet light. Brockmann and Volpers (*Chem. Ber.*, 1947, **80**, 77) describe the use of, amongst other substances, morin on alumina, whilst Sease (*J. Amer. Chem. Soc.*, 1947, **69**, 2242) has used a fluorescent zinc sulphide on silica. A further application of fluorescence as a tracer technique is the combination of β-anthryl *iso*cyanate (Coons, Creech and Jones, *Proc. Soc. Exp. Biol. and Med.*, 1941, **47**, 200; Creech and Jones, *J. Amer. Chem. Soc.*, 1941, **63**, 1661, 1670) and of a crude fluorescein *iso*cyanate (Coons, Creech, Jones, and Berliner, *J. Immunology*, 1942, **45**, 159) with certain antibodies.

It is well known that 7-hydroxycoumarin (umbelliferone) and many of its derivatives are very strongly fluorescent (see Rangaswami and Seshadri, *Proc. Indian Acad. Sci.*, 1940, **12**, A, 375; Rangaswami, Seshadri, and Venkateswarlu, *ibid.*, 1941, **13**, A, 316; Balaiah, Seshadri, and Venkateswarlu, *ibid.*, 1942, **16**, A, 68), and the present experiments, mainly carried out in 1942—43, were designed to investigate the availability of carboxyl chlorides derived from this substance which could then be brought into reaction with hydroxy- and amino-compounds to give fluorescent acyl derivatives. In order to prevent self-condensation the 7-hydroxyl group was protected by methylation.

Attention was first directed towards 7-methoxycoumarin-3-carboxylic acid which was prepared by Rangaswami, Seshadri, and Venkateswarlu (*loc. cit.*) by condensing 2-hydroxy-4-methoxybenzaldehyde with malonic ester and hydrolysing the resulting ethyl 7-methoxycoumarin-3-carboxylate. Although the yields are not recorded, repetition of the experiments has shown that they are almost quantitative. This acid is also conveniently prepared from the same aldehyde by condensation with dilute, aqueous sodium cyanoacetate, and the resulting trans- (with respect to the Ar·C:C·$CO_2$H system) *2-hydroxy-4-methoxybenzylidenecyanoacetic acid* boiled with water for a few minutes, giving the ammonium salt of the

desired acid (for a discussion of this reaction and its stereochemical implications see Baker and Lapworth, *J.*, 1925, **127**, 561). The related *7-methoxycoumarin-3-carboxylic chloride* is readily prepared, and reacts with the corresponding alcohols giving the *methyl* and ethyl esters which exhibit strong blue fluorescence in solution or when absorbed on alumina. This reagent is the most readily available of its type.

*7-Hydroxy-4-methylcoumarin-3-carboxylic acid* has not previously been prepared, as the claim of Michael (*J. pr. Chem.*, 1888, **37**, 469) to have prepared it by hydrolysis of the ethyl ester formed by the interaction of resorcinol and ethyl malonate in presence of sodium has been proved incorrect by Dey (*J.*, 1915, **107**, 1610), who showed that Michael's acid was, in fact, 7-hydroxycoumarin-4-acetic acid. The first approach to 7-hydroxy-4-methylcoumarin-3-carboxylic acid was by the condensation of ethyl acetylmalonate with resorcinol in presence of alcoholic hydrogen chloride (cf. Appel, *J.*, 1935, 1031), but the yield was poor. No success attended experiments using concentrated sulphuric acid as condensing agent and either resorcinol or its monomethyl ether. Both the acid and its *methyl* ester show a strong blue fluorescence. A more successful synthesis of this type of acid was the Reformatski reaction between bromomalonic ester and a derivative of resacetophenone. Thus resacetophenone diacetate and bromomalonic ester yielded *ethyl 7-acetoxy-4-methylcoumarin-3-carboxylate*, and 2-acetoxy-4-methoxyacetophenone and bromomalonic ester yielded after hydrolysis the desired *7-methoxy-4-methylcoumarin-3-carboxylic acid*. It was then found that this acid did not react normally with thionyl chloride, but gave an unstable chloro-acid chloride, *7-methoxy-4-chloromethylcoumarin-3-carboxylic chloride*, which was not obtained in a completely pure state, but which reacted with ethanol in the cold to give *ethyl 7-methoxy-4-chloromethylcoumarin-3-carboxylate*. The position of the remaining chlorine atom in this ester is proved by the fact that it is eliminated readily on boiling with aqueous-alcoholic potassium hydroxide, and cannot therefore be present in the aromatic nucleus. In view of the failure to prepare the simple acid chloride, work with 7-methoxy-4-methylcoumarin-3-carboxylic acid was discontinued, but the acid and its *methyl* and *ethyl* esters, prepared by direct esterification, show an intense blue fluorescence in ultra-violet light in the solid state, in solution, and when adsorbed on alumina.

7-Methoxy-4-methylcoumarin-3-acetic acid was prepared by Dey and Sankaranarayanan (*J. Indian Chem. Soc.*, 1931, **8**, 823) by condensing resorcinol with acetylsuccinic ester, followed by hydrolysis and methylation. It is more conveniently prepared by the direct condensation of ethyl acetylsuccinate with resorcinol monomethyl ether and hydrolysis of the resulting ester. Attempts to prepare the chloride of this acid by a variety of methods were unsuccessful; these failures are undoubtedly due to the high degree of reactivity shown by the methyl group of 4-methylcoumarins, and the methylene group of coumarinacetic acids (see above, and Dey, *loc. cit.*). The *anilide*, prepared directly from the acid, is less strongly fluorescent than the ester.

*7-Methoxy-4-methylcoumarin-6-carboxylic acid* was prepared by hydrolysis of its methyl ester, the latter being obtained in 13% yield from ethyl acetoacetate and β-resorcylic acid (Shah, Sethna, Banerjee, and Chakravarti, *J. Indian Chem. Soc.*, 1937, **14**, 718).

A new coloured reagent for hydroxy- and amino-compounds is *cinnamylidenecyanoacetyl chloride*, $CHPh{:}CH{\cdot}CH{:}C(CN){\cdot}COCl$, a bright yellow compound readily available from the corresponding acid. It gives pale yellow derivatives. The *ethyl* and *phenyl* esters and the *anilide* and p-*nitroanilide* of azobenzene-4-sulphonic acid are described.

## Experimental.

trans-2-*Hydroxy-4-methoxybenzylidenecyanoacetic Acid.*—2-Hydroxy-4-methoxybenzaldehyde (1·5 g.; Robinson and Robinson, *J.*, 1932, 1441), water (10 c.c.), 2N-sodium hydroxide (10 c.c.), and an aqueous solution of sodium cyanoacetate made from chloroacetic acid (1·9 g.; 20 c.c.; Phelps and Tillotson, *Amer. J. Sci.*, 1908, **26**, 267) were kept at 35° for 1 hour, filtered, diluted, and acidified. The voluminous, bright yellow precipitate was collected, washed, and dried (1·9 g.). The *acid* has m. p. 196° (decomp.; rapid heating) (Found: C, 60·5; H, 4·3; N, 6·5. $C_{11}H_9O_4N$ requires C, 60·3; H, 4·1; N, 6·4%). Like other *o*-hydroxybenzylidenecyanoacetic acids (see Baker and Lapworth, *loc. cit.*) this acid cannot be recrystallised without undergoing chemical change.

*7-Methoxycoumarin-3-carboxylic Acid.*—The preceding acid (1·9 g.) was boiled with water (40 c.c.) for 5 minutes, filtered, acidified, and cooled. The precipitate (1·45 g.) separated from dilute alcohol in thin elongated, rectangular plates of a faint greenish-yellow colour, m. p. 195° (lit. 195°) (Found: C, 60·1; H, 3·7. Calc. for $C_{11}H_8O_5$: C, 60·0; H, 3·6%).

*7-Methoxycoumarin-3-carboxylic Chloride.*—The preceding compound (0·5 g.), dry chloroform (3 c.c.), and thionyl chloride (1 c.c.) were refluxed till reaction ceased, and the *acid chloride* precipitated by the addition of light petroleum (b. p. 60—80°). It separated from chloroform–light petroleum in pale yellow prismatic needles, m. p. 143° (Found: C, 55·7; H, 2·9; Cl, 14·6. $C_{11}H_7O_4Cl$ requires C, 55·4; H, 2·9; Cl, 14·9%).

*Methyl and Ethyl 7-Methoxycoumarin-3-carboxylates.*—These esters were prepared by boiling the acid chloride with the alcohol for 5 minutes. The *methyl* ester forms faintly yellow needles from methyl alcohol, m. p. 201—202° (Found: C, 61·5; H, 4·4. $C_{12}H_{10}O_5$ requires C, 61·5; H, 4·3%). The ethyl ester was obtained as thin, rhombic plates from ethyl alcohol, m. p. 134° (lit. 134°) (Found: C, 62·8; H, 4·9. Calc. for $C_{13}H_{12}O_5$: C, 62·9; H, 4·8%).

*7-Hydroxy-4-methylcoumarin-3-carboxylic Acid.*—A solution of ethyl acetylmalonate (14·6 g.; 1 mol.; Lund, *Ber.*, 1934, **67**, 935) and resorcinol (8 g.; 1 mol.) in absolute ethyl alcohol (100 c.c.) was saturated with anhydrous hydrogen chloride at 0°, left at room temperature for 40 hours, diluted with water at 0°, and extracted with ether. The ether-free product was hydrolysed on the water-bath for ½ hour with excess of 2N-sodium hydroxide, and acidification precipitated the crude acid (2·5 g.), which was crystallised twice from aqueous alcohol and obtained as colourless needles, m. p. 239—241° (decomp.; the m. p. varies with the rate of heating) (Found: C, 59·9; H, 3·8. $C_{11}H_8O_5$ requires C, 60·0; H, 3·6%). In solution or in the solid state this *7-hydroxy-4-methylcoumarin-3-carboxylic acid* exhibits an intense blue fluorescence in ultra-violet light.

*Methyl 7-Hydroxy-4-methylcoumarin-3-carboxylate.*—The preceding acid (0·8 g.) was refluxed with 3% methyl-alcoholic hydrogen chloride for 8 hours; after distillation to a small bulk, water was added, precipitating the solid *ester* (0·75 g.); this crystallised from methyl alcohol in long needles, m. p. 195° (Found: C, 61·5; H, 4·1. $C_{12}H_{10}O_5$ requires C, 61·5; H, 4·3%).

*Ethyl 7-Acetoxy-4-methylcoumarin-3-carboxylate* (with R. Banks).—Resacetophenone diacetate (3 g.), ethyl bromomalonate (3 g.), benzene (50 c.c.), and zinc turnings (5 g.) were heated on the water-bath for 6 hours with rigid exclusion of water. Owing to deposition of sticky material on the zinc, further small quantities of zinc were added at hourly intervals. After being shaken with dilute sulphuric acid, the benzene layer was dried and distilled finally in a vacuum till the distillate came over at 125°/0·08 mm. (this consisted largely of 4-*O*-acetylresacetophenone), and the residue triturated with alcohol leaving a solid, which was crystallised from dilute alcohol (charcoal) and then from alcohol (yield 0·5 g.). The *ethyl 7-acetoxy-4-methylcoumarin-3-carboxylate* forms needles, m.p. 130—131° (Found: C, 62·2; H, 5·1; Ac, 15·2. $C_{15}H_{14}O_6$ requires C, 62·1; H, 4·8; Ac, 14·8%).

*7-Methoxy-4-methylcoumarin-3-carboxylic Acid* (with R. Banks).—2-Acetoxy-4-methoxyacetophenone acetate (26 g.), benzene (180 c.c.), ethyl bromomalonate (30 g.; 1 mol.), and zinc turnings (12 g.) were warmed till a reaction set in, then cooled in ice till the vigour of the reaction had abated, and finally refluxed for 4 hours, a little more zinc being added after 2 hours. After addition of dilute sulphuric acid the benzene layer was steam-distilled to remove 2-hydroxy-4-methoxyacetophenone, and the residual oil hydrolysed by 8 hours' refluxing with aqueous-alcoholic hydrochloric acid; the alcohol was distilled off, and after the mixture had been made alkaline, boiled with charcoal and acidified, the colourless solid (10 g.) was collected. This *7-methoxy-4-methylcoumarin-3-carboxylic acid* was crystallised from 50% aqueous ethyl alcohol, and obtained as needles, m. p. 184—185° (Found: C, 61·7; H, 4·4. $C_{12}H_{10}O_5$ requires C, 61·5; H, 4·3%). It shows a strong blue fluorescence in the solid state and in solution. Esterification by the Fischer–Speier method readily gave the *methyl* ester as long prismatic needles, m. p. 129° (Found: C, 63·5; H, 5·0. $C_{13}H_{12}O_5$ requires C, 62·9; H, 4·8%), and the *ethyl* ester as flat, prismatic needles, m. p. 61—62°, from light petroleum (b. p. 40—60°) containing a little chloroform (Found: C, 64·2; H, 5·6. $C_{14}H_{14}O_5$ requires C, 64·1; H, 5·4%).

In an attempt to prepare the acid chloride by refluxing the acid with excess of thionyl chloride for ½ hour and adding dry ether, a compound separated in flat, yellow needles, m. p. 198—200° (decomp.) (Found: C, 49·4; H, 3·0; Cl, 25·4. $C_{12}H_8O_4Cl_2$ requires C, 50·2; H, 2·8; Cl, 24·7%). This *7-methoxy-4-chloromethylcoumarin-3-carboxylic chloride* underwent considerable decomposition when an attempt was made to crystallise it from light petroleum. When dissolved in cold ethyl alcohol and subsequently poured into water it yielded *ethyl 7-methoxy-4-chloromethylcoumarin-3-carboxylate*, which, after crystallisation from aqueous ethyl alcohol, had m. p. 117—119° (Found: Cl, 11·8; *M*, 289. $C_{14}H_{13}O_5Cl$ requires Cl, 11·9%; *M*, 296·5).

*7-Methoxy-4-methylcoumarin-3-acetic Acid.*—A mixture of ethyl acetylsuccinate (32 g.; 1 mol.; Ruhemann and Hemmy, *J.*, 1897, **71**, 330) and resorcinol monomethyl ether (18·5 g.; 1 mol.) was added dropwise with stirring at 0—5° to concentrated sulphuric acid (80 c.c.), and then kept at 0° overnight. After being poured on ice and water, the separated oily ester gradually solidified, and was hydrolysed by being boiled with 2N-sodium hydroxide for ½ hour. Acidification yielded the acetic acid derivative, which was crystallised twice from dilute ethyl alcohol (yield, 8·2 g.), and then from ethyl acetate, being obtained in long needles, m. p. and mixed m. p. with a specimen prepared by the method of Dey and Sankaranarayanan (*loc. cit.*) 199° (Found: C, 62·9; H, 4·8. Calc. for $C_{13}H_{12}O_5$: C, 62·9; H, 4·8%). It exhibits a strong blue fluorescence in ultra-violet light. The related *anilide*, prepared by refluxing the acid with aniline for 2 hours, formed long, colourless needles from alcohol, m. p. 222—223° (Found: N, 4·4. $C_{19}H_{17}O_4N$ requires N, 4·3%). Attempts to prepare the acid chloride by using phosphorus pentachloride, phosphorus trichloride, or thionyl chloride, or by the action of phosphorus oxychloride on the sodium salt of the acid, were unsuccessful.

*7-Methoxy-4-methylcoumarin-6-carboxylic Acid.*—Methyl 7-methoxy-4-methylcoumarin-6-carboxylate (0·5 g.; Shah, Sethna, Banerjee, and Chakravarti, *loc. cit.*) was hydrolysed by means of cold 2N-sodium hydroxide for 3 days. The free *acid* (0·4 g.) separated from water in thread-like crystals, m. p. 291° (Found: C, 61·6; H, 4·4. $C_{12}H_{10}O_5$ requires C, 61·5; H, 4·3%).

*Cinnamylidenecyanoacetyl Chloride.*—Cinnamylidenecyanoacetic acid (20 g.; Lapworth and McRae, *J.*, 1922, **121**, 1702), thionyl chloride (20 c.c.), and chloroform (20 c.c.) were refluxed for 1 hour, chloroform (30 c.c.) and light petroleum (50 c.c.; b. p. 60—80°) added, and the mixture cooled. The solid was collected, washed with chloroform–light petroleum (yield 12 g.), and crystallised slowly from carbon tetrachloride (110 c.c.). The *acid chloride* was obtained in orange, compact rhombic prisms (10·5 g.), m. p. 121—123° (Found: C, 66·7; H, 3·8; N, 6·6; Cl, 16·3. $C_{12}H_8ONCl$ requires C, 66·2; H, 3·7; N, 6·4; Cl, 16·3%). The compound is dimorphous, fairly rapid crystallisation giving small, bright-yellow, bipyramidal crystals which, on being kept in the mother-liquor, slowly pass into the compact, orange form of larger crystalline habit.

*Ethyl Azobenzene-4-sulphonate.*—Azobenzene-4-sulphonyl chloride (Pearl, *J. Org. Chem.*, 1945, **10**, 205; it is best crystallised from carbon disulphide, from which it separates in deep red, hexagonal plates) was warmed with ethyl alcohol and a few drops of aqueous sodium carbonate for 5 minutes; water was added, and the precipitated *ester* crystallised from ethyl alcohol (orange-yellow leaflets) and then from light petroleum (b. p. 60—80°); it formed orange, prismatic needles, m. p. 111° (Found: N, 9·5. $C_{14}H_{14}O_3N_2S$ requires N, 9·7%).

*Phenyl Azobenzene-4-sulphonate.*—Equimolecular quantities of the sulphonyl chloride and phenol were warmed with a little pyridine for 10 minutes, dilute hydrochloric acid added, and the precipitated *phenyl* ester crystallised from ethyl alcohol; long, flat, orange prisms, m. p. 131—132° (Found: N, 8·5. $C_{18}H_{14}O_3N_2S$ requires N, 8·3%).

*Anilide and* p-*Nitroanilide of Azobenzene-4-sulphonic Acid.*—These were prepared from aniline and *p*-nitroaniline as above. The *anilide* formed red needles from ethyl alcohol, m. p. 150—151° (Found: N, 12·5. $C_{18}H_{15}O_2N_3S$ requires N, 12·5%). The p-*nitroanilide* crystallised from ethyl alcohol in thick, orange-red plates, m. p. 229° (Found: N, 14·4. $C_{18}H_{14}O_4N_4S$ requires N, 14·7%).

THE DYSON PERRINS LABORATORY, OXFORD UNIVERSITY.
THE UNIVERSITY, BRISTOL.

[*Received, June* 14*th*, 1948.]