37. *Fluorescent Reagents. Acyl Chlorides and Acyl Hydrazides.*

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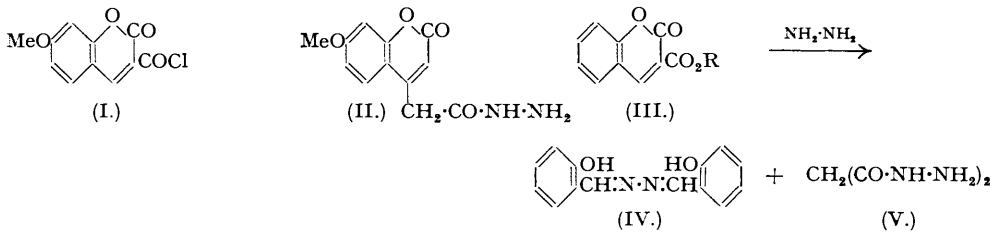
The paper describes some fluorescent acyl chlorides and hydrazides derived from coumarin-carboxylic acids, and a number of their derivatives formed by reaction with alcohols, amines, and carbonyl compounds. The fluorescence of these derivatives is designed to aid their separation by chromatographic methods. The two best reagents are 7-methoxycoumarin-3-carboxylic chloride (I), and *7-methoxycoumarin-4-acethydrazide* (II).

In a recent paper (*J.*, 1949, S 12) Baker, Collis, and Banks described some acid chlorides derived from 7-hydroxycoumarin (umbelliferone). These were prepared as fluorescent acylating agents for hydroxy- and amino-compounds, the separation and identification of which by chromatographic methods might thereby be facilitated. The most promising of these acyl chlorides was 7-methoxycoumarin-3-carboxylic chloride (I); the related esters and amides exhibit strong blue fluorescence in solution or when adsorbed on alumina, but the preparation of the reagent required the not very readily accessible 2-hydroxy-4-methoxybenzaldehyde. The present paper deals with other fluorescent reagents; no acid chloride superior to (I) has been found, but *7-methoxycoumarin-4-acethydrazide* (II) is an excellent reagent for carbonyl compounds, and is very easily prepared.

Derivatives of Salicylic Acid.—*o*-Anisoyl chloride gives weakly fluorescent esters and amides.

o-Hydroxybenz- and *o*-anis-hydrazide readily yield with a variety of aldehydes and ketones crystalline condensation products which show a rather weak blue fluorescence in ultra-violet



light. Acetaldehyde and propaldehyde gave resinous products only. *o*-Hydroxybenzoylazide (Bondi, *Z. physiol. Chem.*, 1907, **52**, 172) is unstable, but reacts with amino-acids to give their acyl derivatives (Bondi, *loc. cit.*), which are also fluorescent. Derivatives of nitrosalicylic acids are not fluorescent.

Derivatives of Coumarin-3-carboxylic Acid (III; R = H).—This acid, its chloride, esters, and amides are moderately fluorescent in ultra-violet light, but the fluorescence is much less intense than that shown by 7-methoxycoumarin-3-carboxylic acid and derivatives.

Unsuccessful attempts have been made to prepare coumarin-3-carboxyhydrazide. Methyl and ethyl coumarin-3-carboxylates (III; R = Me or Et) fail to react with aqueous-alcoholic hydrazine hydrate at room temperature, but at the boiling point they yield a mixture of *o*-hydroxybenzylideneazine (salazine) (IV) and malonhydrazide (V). This reaction formally involves the breakdown of the α-pyrone ring, under the influence of the strongly basic hydrazine, into the components from which it was formed, namely salicylaldehyde and a derivative of malonic acid. Although coumarin-3-carboxyl chloride readily yielded the amide by treatment with aqueous ammonia, it did not give the hydrazide on treatment with the much stronger base hydrazine hydrate; the only product isolated from the viscous reaction mass was *o*-hydroxybenzylideneazine.

Derivatives of 7-Methoxy-4-methylcoumarin-3-carboxylic Acid.—In attempting to prepare the chloride of this acid by the action of thionyl chloride, Baker, Collis, and Banks (*J.*, 1949, S 12) obtained only 7-methoxy-4-chloromethylcoumarin-3-carboxyl chloride, m. p. 198—200°. Repetition of this experiment has, however, now yielded the expected 7-methoxy-4-methylcoumarin-3-carboxyl chloride, which is rather unstable and melts at 94—95°; it was identified by conversion into the known, related methyl and ethyl esters. The original formation of the chloro-acid chloride was probably due to impurity in the thionyl chloride. Thus it has now been found that 7-methoxy-4-methylcoumarin-3-carboxylic acid is converted into the chloro-acid chloride by reaction with sulphuryl chloride in presence of a trace of benzoyl peroxide as catalyst (see Kharasch and Brown, *J. Amer. Chem. Soc.*, 1939, **61**, 2142).

Methyl 7-methoxy-4-methylcoumarin-3-carboxylate reacts with hydrazine hydrate to give *7-methoxy-4-methylcoumarin-3-carboxyhydrazide*, and this reagent gives strongly fluorescent derivatives with carbonyl compounds.

Derivatives of 7-Hydroxycoumarin-4-acetic Acid.—This acid was originally prepared by Burton (*Annalen*, 1891, **261**, 167) by condensing resorcinol with acetonedicarboxylic acid in presence of concentrated sulphuric acid, and later the procedure was simplified by Dey and Row (*J. Indian Chem. Soc.*, 1924, **1**, 112) who condensed resorcinol directly with citric acid in presence of sulphuric acid. A careful study of this reaction with particular attention to temperature control has raised the yield of 7-hydroxycoumarin-4-acetic acid to 80%. This acid and all derivatives are intensely fluorescent, even in daylight. *Methyl 7-hydroxycoumarin-4-acetate* proved very stable towards hydrazine hydrate, and the related hydrazide was not obtained.

Methylation of 7-hydroxycoumarin-4-acetic acid with methyl sulphate and alkali gave 7-methoxycoumarin-4-acetic acid. Resinous material only was obtained in attempts to prepare 7-methoxycoumarin-4-acetyl chloride, a result doubtless due to the highly reactive nature of the methylene group. The methyl ester was prepared by direct esterification, and yielded *7-methoxycoumarin-4-acethydrazide* (II) by reaction with hydrazine hydrate. Condensation of (II) with a variety of carbonyl compounds gave the corresponding highly fluorescent *acylhydrazones*.

Experiments with disc chromatograms of alumina have shown the possibility of separating mixtures of the fluorescent esters and hydrazones described.

Experimental.

Derivatives of o-*Hydroxybenzhydrazide.*—This hydrazide was prepared in 73% yield from methyl salicylate (Struve and Radenhausen, *J. pr. Chem.*, 1895, **52**, 239; see also Bondi, *Z. physiol. Chem.*, 1907, **52**, 170) by the use of 60% instead of pure hydrazine hydrate; it separated from ethyl alcohol in plates, m. p. 147°.

The following derivatives were prepared by addition of the carbonyl compound (1 mol.) to *o*-hydroxybenzhydrazide (0·3 g., 1 mol.) in warm alcohol (5 c.c.), collecting them after cooling, and recrystallising them from alcohol: benzylidene derivative, needles, m. p. 248° (Found: N, 11·8. Calc. for $C_{14}H_{12}O_2N_2$: N, 11·7%) (Struve and Radenhausen, *loc. cit.*, m. p. 232°); iso*propylidene* derivative, fine needles, m. p. 228° (Found: C, 62·7; H, 6·2; N, 14·8. $C_{10}H_{12}O_2N_2$ requires C, 62·5; H, 6·3; N, 14·6%); *piperonylidene* derivative, needles, m. p. 270° (Found: C, 63·3; H, 4·3; N, 10·1. $C_{15}H_{12}O_4N_2$ requires C, 63·4; H, 4·2; N, 9·9%); *acetophenone* o-*hydroxybenzoylhydrazone*, fine, fibrous needles, m. p. 212° (Found: C, 71·1; H, 5·7; N, 10·8. $C_{15}H_{14}O_2N_2$ requires C, 70·9; H, 5·5; N, 11·0%). When adsorbed from alcoholic solution on to alumina these derivatives all exhibited a blue fluorescence in ultra-violet light. Acetaldehyde and propaldehyde did not yield solid derivatives under the above conditions.

Hydrazide of 5-*Nitrosalicylic Acid.*—Nitration of methyl salicylate by the method of Barany and Pianka (*J.*, 1946, 965) gave not only methyl 5-nitrosalicylate, m. p. 99° from ether (40% yield), but also methyl 3-nitrosalicylate, m. p. 132° (25%), from methyl alcohol, separated by taking advantage of its very sparing solubility in ether. Methyl 5-nitrosalicylate (1 g.), in methyl alcohol (20 c.c.), was shaken and then refluxed for ¼ hour with 60% hydrazine hydrate (1 c.c.), cooled, and the solid collected and crystallised from methyl alcohol. The *hydrazide* formed yellow needles, m. p. 154° (decomp.) (Found: C, 42·4; H, 4·9; N, 18·4. $C_7H_7O_4N_3,CH_3{\cdot}OH$ requires C, 41·9; H, 4·8; N, 18·3%).

o-*Anishydrazide and Derivatives.*—Methyl *o*-anisate (30 g., 1 mol.) (Sachs and Harold, *Ber.*, 1907, **40**, 2718) and 60% hydrazine hydrate (26 c.c., 1·5 mols.) were refluxed for 2¼ hours and then cooled, and the solid was collected after some hours, washed with ether, and crystallised from benzene, giving o-*anishydrazide* (18 g., 60%) as fine needles, m. p. 85° (Found: C, 58·0; H, 5·9; N, 17·2. $C_8H_{10}O_2N_2$ requires C, 57·8; H, 6·0; N, 16·9%). The following derivatives were prepared by warming the reactants in alcoholic solution, completing the separation of solid by addition of water, and crystallising the products from dilute ethyl alcohol: *benzylidene* derivative, prismatic needles, m. p. 176° (Found: C, 71·0; H, 5·4; N, 11·1. $C_{15}H_{14}O_2N_2$ requires C, 70·9; H, 5·5; N, 11·0%); iso*propylidene* derivative, needles, m. p. 218° (Found: N, 13·8. $C_{11}H_{14}O_2N_2$ requires N, 13·6%). These compounds, when adsorbed on to alumina, showed a blue fluorescence in ultra-violet light. Crystalline derivatives were not obtained from *o*-anishydrazide and acetaldehyde or propaldehyde.

Derivatives of Coumarin-3-carboxylic Acid.—The acid chloride was prepared by refluxing coumarin-3-carboxylic acid (2 g.; Knoevenagel, *Ber.*, 1898, **31**, 2618) with thionyl chloride (15 c.c.) for 1 hour, cooling it, adding light petroleum (50 c.c.; b. p. 40—60°), collecting the solid, and crystallising it from benzene; it formed needles (1·7 g.), m. p. 147° (Boehm, Schumann, and Hansen, *Arch. Pharm.*, 1933, **271**, 490, record m. p. 147—148°). It was converted into the following derivatives which have been previously prepared in other ways: amide, needles from alcohol, m. p. 274° (decomp.) (Found: C, 63·4; H, 3·7; N, 7·2. Calc. for $C_{10}H_7O_3N$: C, 63·5; H, 3·7; N, 7·4%) (D.R.-P. 172,724 gives m. p. 268—269°; Bechert, *J. pr. Chem.*, 1917, **50**, 27, gives m. p. 236°); methyl ester, m. p. 116° (lit., m. p. 116—117°), which was also prepared by direct esterification of the acid (10 g.) in presence of hydrogen chloride (yield 11 g.); ethyl ester, m. p. 94° (lit., 94°); *n*-propyl ester, m. p. 75° (Found: C, 67·1; H, 5·2. Calc. for $C_{13}H_{12}O_4$: C, 67·2; H, 5·2%) (lit., m. p. 73°); *iso*propyl ester, m. p. 89° (lit., m. p. 89°); anilide, m. p. 248°, prepared in benzene solution (Found: C, 72·2; H, 4·2; N, 5·1. Calc. for $C_{16}H_{11}O_3N$: C, 72·5; H, 4·2; N, 5·3%) (lit., m. p. 250°); benzylamide, m. p. 154° (lit., 154°); piperidide, needles (from water), m. p. 168° (Found: C, 70·1; H, 5·7; N, 5·0. Calc. for $C_{15}H_{15}O_3N$: C, 70·0; H, 5·8; N, 5·45%) (von Werder, *Merck's Jahresbericht*, 1935—6, 98, gives m. p. 168—170°). The p-*toluidide* separates from ethyl acetate in fine, greenish-yellow needles, m. p. 230° (Found: C, 73·0; H, 4·7; N, 5·1. $C_{17}H_{13}O_3N$ requires C, 73·1; H, 4·7; N, 5·0%). The *morpholide* crystallises from water in fine needles, m. p. 92° (Found: C, 64·7; H, 5·0; N, 5·1. $C_{14}H_{13}O_4N$ requires C, 64·9; H, 5·4; N, 5·4%).

Reaction of Hydrazine with Methyl and Ethyl Coumarin-3-carboxylates.—No reaction occurred between alcoholic solutions of the esters and 60% aqueous hydrazine hydrate in the cold. The ester (2 g.) in alcohol (10 c.c.) was boiled under reflux for ½ hour with 60% hydrazine hydrate (2 c.c.), and after cooling, the mixture of yellow and colourless solids was collected, washed, and the former separated by solution in hot ethyl acetate. This yellow product, *o*-hydroxybenzylideneazine, separated from ethyl acetate in needles, m. p. and mixed m. p. with an authentic specimen (Borsche, *Ber.*, 1921, **54**, 668) 215° (Found: C, 69·8; H, 5·2; N, 11·4. Calc. for $C_{14}H_{12}O_2N_2$: C, 70·0; H, 5·0; N, 11·7%), and gave a dibenzoyl derivative, m. p. 189° (lit., 188—189°). The colourless compound after crystallisation from alcohol had m. p., and mixed m. p. with an authentic specimen of malonhydrazide (Curtius, Schöfer, and Schwan, *J. pr. Chem.*, 1895, **51**, 187), 152°; it further gave a dibenzylidene derivative, m. p. 226° (lit., 226°).

o-Hydroxybenzylideneazine was also obtained as the only readily isolable product by the action of 60% hydrazine hydrate upon coumarin-3-carboxylic chloride in various solvents.

Action of (a) *Thionyl and* (b) *Sulphuryl Chlorides on* 7-*Methoxy-*4-*methylcoumarin-*3-*carboxylic Acid.*—(*a*) The carboxylic acid (1 g.; Baker, Collis, and Banks, *loc. cit.*) was boiled under reflux for 1 hour with thionyl chloride (10 c.c.), excess of light petroleum (b. p. 40—60°) added, and the solid collected. A further precipitation from benzene by the addition of light petroleum gave the acid chloride as pale yellow needles, m. p. 94—95°, which rises on keeping the crystals even in a vacuum (after 24 hours the m. p. was 110—113°; after 48 hours, 180—182°). Owing to this instability the chloride was not analysed, but was identified by conversion into the methyl and ethyl esters, m. p.s and mixed m. p.s with authentic specimens, 128° and 57°, respectively.

(*b*) The acid (0·5 g.) was treated with sulphuryl chloride (5 c.c.) and a trace of benzoyl peroxide. After the vigorous reaction was over the mixture was boiled under reflux for 1 hour, and cooled, and the

solid collected after several hours, precipitated from warm toluene by the addition of light petroleum, and dried in a vacuum over sodium hydroxide. This 7-methoxy-4-chloromethylcoumarin-3-carboxyl chloride had m. p. 198°, and was shown to be identical with the material, m. p. 198—200°, previously prepared by Baker, Collis, and Banks; reaction with ethyl alcohol gave ethyl 7-methoxy-4-chloromethylcoumarin-3-carboxylate, m. p. 117° (lit., 117—119°).

7-Methoxy-4-methylcoumarin-3-carboxyhydrazide and Derivatives.—Methyl 7-methoxy-4-methylcoumarin-3-carboxylate (2·5 g.; Baker, Collis, and Banks, *loc. cit.*), dissolved in methyl alcohol (35 c.c.), was treated with 60% aqueous hydrazine hydrate (1·6 c.c., 2 mols.), and the solid which had separated after 3 hours was collected, washed with methyl alcohol and crystallised from methyl or ethyl alcohol. The *hydrazide* (yield 55—60%) formed pale yellow needles, m. p. 210° (Found: C, 58·4; H, 5·0; N, 11·0. $C_{12}H_{12}O_4N_2$ requires C, 58·1; H, 4·8; N, 11·3%). The following derivatives were prepared by warming the reactants in alcoholic solution: *benzylidene*, colourless needles (from alcohol), m. p. 250° (Found: C, 67·5; H, 4·8; N, 8·2. $C_{19}H_{16}O_4N_2$ requires C, 67·9; H, 4·8; N, 8·3%); iso*propylidene* (separated after heating for 1 hour and collected after addition of water), prismatic needles, m. p. 237°, from dilute alcohol (Found: N, 9·8. $C_{15}H_{16}O_4N_2$ requires N, 9·7%).

7-Hydroxycoumarin-4-acetic Acid.—Finely powdered citric acid (100 g.) and concentrated sulphuric acid (135 c.c.) were gently shaken for ½ hour, then slowly heated to 65—70° and maintained at this temperature for ¼ hour; the evolution of carbon monoxide had then abated. After the mixture had been cooled to room temperature (¼ hour) and then in a freezing mixture to about −5°, resorcinol (45 g., 1 mol.) was added (½ hour) with stirring at such a rate that the temperature remained below 5°, and then concentrated sulphuric (58 c.c.) was similarly added (½ hour), and the mixture placed in the refrigerator and next day poured into ice-water (2 l.). The solid was collected, washed, and dried, giving almost pure 7-hydroxycoumarin-4-acetic acid (83 g., 80%), m. p. 209°. Crystallisation from ethyl alcohol gave the pure acid as needles, m. p. 210°. The *methyl* ester, prepared by the Fischer–Speier method in 90% yield, crystallised from methyl alcohol in fine needles, m. p. 220° (Found: C, 61·1; H, 4·1. $C_{12}H_{10}O_5$ requires C, 61·5; H, 4·3%); attempts to convert it into the related hydrazide were unsuccessful.

7-Methoxycoumarin-4-acetic Acid.—This acid was prepared by Dey (*J.*, 1915, **107**, 1632) by hydrolysis of its methyl ester (itself prepared in unstated yield from 7-hydroxycoumarin-4-acetic acid, methyl sulphate, and concentrated alkali), or by condensing resorcinol monomethyl ether with acetonedicarboxylic acid. 7-Hydroxycoumarin-4-acetic acid (30 g.) was dissolved in a solution of sodium hydroxide (28 g., 5 mols.) in water (280 c.c.), and methyl sulphate (20 c.c., 1·5 mols.) added dropwise with stirring (½ hour). The mixture was heated on the water-bath for 1½ hours and then cooled, sodium hydrogen sulphite added to remove a red colour, and the whole acidified with dilute sulphuric acid (total volume *ca.* 1 l.). The 7-methoxycoumarin-4-acetic acid was collected after 2 hours, washed, and recrystallised from alcohol, giving thin prisms (18 g., 57%), m. p. 186° (lit., 187°). The methyl ester, prepared in 90% yield by the Fischer–Speier method, had m. p. 122° (lit., 122°). Unsuccessful attempts were made to prepare the acid chloride by the use of thionyl chloride under a variety of conditions, and by the action of phosphorus oxychloride on the sodium salt of the acid, but only coloured resinous products resulted.

7-Methoxycoumarin-4-acethydrazide.—To the preceding methyl ester (5 g.), dissolved in methyl alcohol (110 c.c.), was added 60% aqueous hydrazine hydrate (5 c.c.; 1·5 mols.), and the solid which separated was collected after 4 hours, washed with dilute methyl alcohol, and crystallised from methyl or ethyl alcohol, giving fibrous needles (2·8 g., 56%), m. p. 206° (Found: C, 58·1; H, 5·0; N, 11·5. $C_{12}H_{12}O_4N_2$ requires C, 58·1; H, 4·8; N, 11·3%). After melting, the substance solidified to a yellowish-brown mass which then had m. p. *ca.* 270—275°. The yield of hydrazide was diminished when the reaction mixture was heated.

The following derivatives were prepared by warming the acylhydrazide with the carbonyl compound in alcoholic solution in presence of a trace of acetic acid, and, if necessary, dilution with water, and crystallisation from alcohol except where otherwise stated: *benzylidene*, fine needles, m. p. 264° (Found: C, 68·1; H, 4·8; N, 8·1. $C_{19}H_{16}O_4N_2$ requires C, 67·9; H, 4·8; N, 8·3%); iso*propylidene*, fine needles (from dilute alcohol), m. p. 209° (Found: C, 62·3; H, 5·5; N, 9·6. $C_{15}H_{16}O_4N_2$ requires C, 62·5; H, 5·6; N, 9·7%); *ethylidene*, glistening plates, m. p. 194° (Found: C, 61·5; H, 5·2; N, 10·0. $C_{14}H_{14}O_4N_2$ requires C, 61·3; H, 5·1; N, 10·2%); *methylene*, fine prismatic needles, m. p. 156° (Found: C, 59·8; H, 4·8; N, 11·0. $C_{13}H_{12}O_4N_2$ requires C, 60·0; H, 4·6; N, 10·8%). *7-Methoxycoumarin-4-acethydrazones* were also prepared from: acetophenone, small prisms, m. p. 215° (Found: C, 68·3; H, 5·1; N, 7·9. $C_{20}H_{18}O_4N_2$ requires C, 68·6; H, 5·1; N, 8·0%); methyl ethyl ketone, fine needles (from dilute alcohol), m. p. 176° (Found: C, 63·4; H, 5·9; N, 9·4. $C_{16}H_{18}O_4N_2$ requires C, 63·6; H, 6·0; N, 9·3%); pyruvic acid (boiled under reflux for ½ hour), prisms (from dilute alcohol), m. p. 220° (Found: C, 56·3; H, 4·6; N, 8·5. $C_{15}H_{14}O_6N_2$ requires C, 56·6; H, 4·4; N, 8·8%); dihydro-orcinol (1 : 3-diketo-5-methyl-*cyclo*hexane) (boiled under reflux for ½ hour), faintly yellow needles (from dilute alcohol), m. p. 258° (decomp.) (Found: C, 64·0; H, 5·9; N, 8·2. $C_{19}H_{20}O_5N_2$ requires C, 64·0; H, 5·6; N, 7·9%).

Analyses are by Drs. Weiler and Strauss, Oxford, and by Mrs. W. M. Eno, Bristol.

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