A Convenient Synthesis of *N*-(7-Dimethylamino-4-methylcoumarin-3-yl)-maleimide Incorporating a Novel Variant of the Pechmann Reaction

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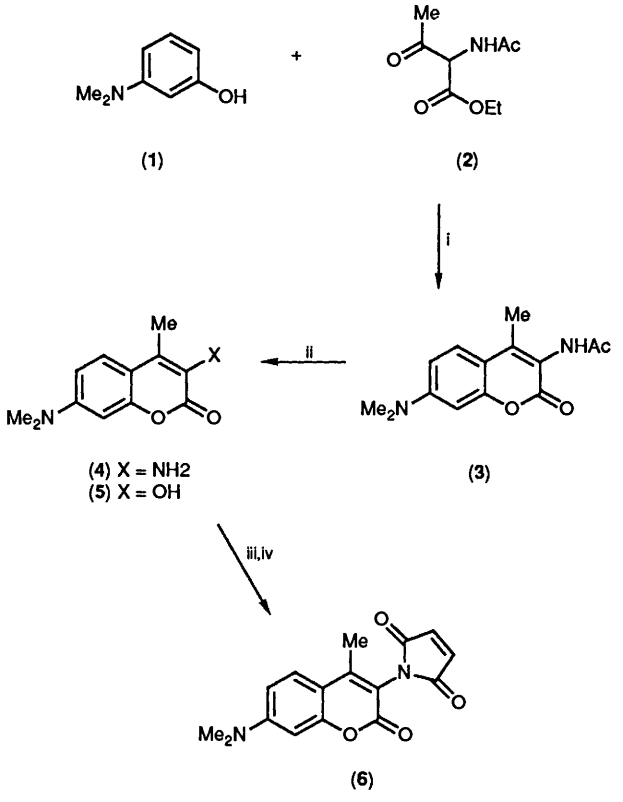
A new synthesis of the fluorescent, thiol-reactive compound *N*-(7-dimethylamino-4-methylcoumarin-3-yl)maleimide (**6**) is reported. Pechmann condensation of 3-dimethylaminophenol (**1**) and ethyl 2-acetamido-3-oxobutyrate (**2**) leads directly, in modest yield to the substituted 3-acetamidocoumarin (**3**) which is readily converted into the required compound (**6**) by standard means. In contrast to the previously reported synthesis of compound (**6**), no separation of structural isomers is required during the reaction sequence.

N-(7-Dimethylamino-4-methylcoumarin-3-yl)maleimide (DACM) (**6**), which reacts readily with thiols at neutral pH and thereby undergoes a marked increase in fluorescence,[1,2] has a range of uses in biological research. It has been applied *inter alia*



Reagents: i, $ZnCl_2$–EtOH; ii, conc. HCl; iii, maleic anhydride–$CHCl_3$; iv, Ac_2O–NaOAc

to the analysis of low molecular weight thiols and of thiol-containing peptides,[3] to histochemical staining,[4] and to structural studies.[5] However, large scale use of DACM is restricted by its high cost[6] which apparently arises from the need for chromatographic separation of isomers during its synthesis.[1] As part of a programme to develop and extend the technique of fluorescence photoactivation and dissipation for observing the movement of proteins within living cells (*cf.* ref. 7), we required several hundred milligrams of DACM or a closely similar compound and therefore examined alternative synthetic routes.

The problem in the existing synthesis of DACM is the introduction of a nitrogen substituent specifically onto C-3 of 7-dimethylamino-4-methylcoumarin and we therefore sought to incorporate such a group during construction of the coumarin ring so as to avoid the formation of isomeric mixtures. 3-Aminocoumarins or their *N*-acetyl derivatives (lacking the 4-Me group of DACM) have been prepared by condensation of salicylaldehydes with glycine and derivatives thereof in Perkin-like reactions[8] or with half esters of acetamidomalonic acid.[9] However, when applied to 4-dimethylaminosalicylaldehyde, which is deactivated towards electrophilic attack on the aldehyde group, none of these reaction conditions afforded better than trace amounts (< 1%) of the required coumarin.

The electron-donating properties of the dimethylamino group which disfavour the above reactions are however advantageous in the alternative coumarin synthesis *via* the Pechmann reaction, and Pechmann himself[10] reported a good yield from the condensation of 3-dimethylaminophenol and ethyl 3-oxobutyrate. However, the use of β-keto esters bearing an α-hetero substituent does not appear to have been described. When we treated 3-dimethylaminophenol with ethyl 2-acetamido-3-oxobutyrate (**2**)[11] in the presence of zinc chloride, the predicted acetamidocoumarin (**3**) was formed, albeit in modest yield. Since the pure compound was readily isolated from the product mixture by simple crystallisation, we have not attempted to optimise this reaction, although there are obvious opportunities available. Acidic hydrolysis of (**3**) readily gave the aminocoumarin (**4**) together with a small proportion (*ca.* 5%) of the hydroxycoumarin (**5**). Previous reports of similar hydrolyses of 3-acetamidocoumarins described either complete conversation into the hydroxy compounds[8b] or simply the production of the amino compounds.[8c,d] Brief hydrolysis clearly favours clean production of the amino product and the relatively long reaction time used herein, which was necessary to effect complete conversion of the starting material, evidently accounts for co-isolation of the amino- and hydroxy-coumarins. However, the presence of the latter compound did not interfere with construction of the maleimide ring and the final product (**6**) was readily prepared as previously described.[1]

This work provides ready access to the useful reagent DACM, which can now easily be prepared in gram quantities without recourse to chromatography. In the wider sense, further development of the novel Pechmann reaction described above may facilitate access to structures incorporating the 3-amino-coumarin moiety, some of which exhibit a range of biological activities.[12]

Experimental

Analyses were carried out by Butterworth Laboratories, Teddington, Middlesex. Melting points were determined on a Reichert hot-stage and are uncorrected. ^{1}H NMR spectra were determined in $CDCl_3$ on a JEOL FX90Q spectrometer with tetramethylsilane as internal standard.

3-Acetamido-7-dimethylamino-4-methylcoumarin (**3**).—A solution of redistilled 3-dimethylaminophenol (19.9 g, 144 mmol), ethyl 2-acetamido-3-oxobutyrate (28.6 g, 153 mmol; prepared according to ref. 11, method C) and anhydrous zinc chloride (9.9 g, 72 mmol) in absolute alcohol (90 ml) was heated under reflux for 7 h, then poured into water (320 ml) which contained concentrated HCl (3.6 ml). The mixture was extracted with CH_2Cl_2 and the extract washed with 0.2M NaOH and brine, dried, and evaporated. The residual oil was dissolved in EtOAc and again evaporated to remove traces of CH_2Cl_2; it was then dissolved in EtOAc (20 ml), diluted with Et_2O (100 ml) and allowed to stand overnight. The precipitate was filtered off, washed with Et_2O, and dried to give the *acetamidocoumarin* (**3**) as yellow flakes (4.7 g, 12.4%), m.p. 215–217 °C. A sample recrystallised from EtOAc–light petroleum gave fine yellow needles, m.p. 215–216 °C (Found: C, 64.4; H, 6.3; N, 10.8. $C_{14}H_{16}N_2O_3$ requires C, 64.6; H, 6.2; N, 10.8%); ν_{max}(Nujol) 3 280, 1 725, 1 655, and 1 620 cm^{-1}; λ_{max}(EtOH) 244 and 371 nm (ε 19 000 and 24 800 dm^3 mol^{-1} cm^{-1}); δ_H 7.44 (1 H, d, 5-H, $J_{5,6}$ 9.2 Hz), 7.41 (1 H, br s, NH, exchangeable with D_2O), 6.63 (1 H, dd, 6-H, $J_{6,8}$ 2.2 Hz), 6.47 (1 H, d, 8-H), 3.03 (6 H, s, NMe_2), 2.28 (3 H, s, MeCO), and 2.20 (3 H, s, 4-Me).

Acid Hydrolysis of 3-Acetamido-7-dimethylamino-4-methylcoumarin.—The crude acetamidocoumarin (**3**) (4.1 g, 15.8 mmol) was dissolved in concentrated HCl (50 ml) and heated under reflux for exactly 35 min after which it was cooled rapidly and neutralised with solid $NaHCO_3$. The mixture was extracted with CH_2Cl_2 and the extract was washed with brine, dried, and evaporated to leave a yellow solid (3.62 g) which was crystallised from EtOAc–light petroleum to afford 3-amino-7-dimethylamino-4-methyl-coumarin (**4**) as yellow blades (2.78 g), m.p. 148.5–150 °C (lit.,[1] m.p. 147–149 °C). The spectral properties (UV, IR, ^{1}H NMR) were identical with the published data [1] but ^{1}H NMR revealed the presence of *ca.* 5% of a contaminant, which separated from the major component by TLC [Kieselgel F_{254}, $CHCl_3$–EtOAc (4:1)] as a less polar, brightly fluorescent spot. This material was isolated by preparative TLC as above to give *7-dimethylamino-3-hydroxy-4-methylcoumarin* (**5**) as small buff prisms (MeOH), m.p. 229–231 °C (Found: C, 65.8; H, 6.15; N, 6.2. $C_{12}N_{13}NO_3$ requires C, 65.7; H, 6.0; N, 6.4%); ν_{max}(Nujol) 3 600, 1 690, 1610, 1 295, and 1 140 cm^{-1}; λ_{max}(EtOH) 246 and 357.5 nm (ε 16 100 and 17 500 dm^3 mol^{-1} cm^{-1}); λ_{max}(EtOH–NaOH) 255 and 365 nm (ε 17 500 and 18 900 dm^3 mol^{-1} cm^{-1}).

N-(7-Dimethylamino-4-methylcoumarin-3-yl)maleimide (**6**).—The aminocoumarin (**4**) from the above procedure, which contained the hydroxycoumarin contaminant (**5**), was converted as previously described [1] into the maleimide (**6**), m.p. 210–212 °C (from EtOAc–light petroleum) (lit.,[1] 218–219.5 °C). The m.p. was unchanged by further recrystallisation and the spectral properties (UV, ^{1}H NMR, fluorescence) were identical with those described.[1]

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References

1 M. Machida, N. Ushijima, T. Takahashi, and Y. Kanaoka, *Chem. Pharm. Bull.*, 1977, **25**, 1289.
2 M. Machida, M. I. Machida, T. Sekine, and Y. Kanaoka, *Chem. Pharm. Bull.*, 1977, **25**, 1678.
3 B. Kaagedal and M. Kaelberg, *J. Chromatogr.*, 1982, **229**, 409; E. C. Klasen, *Anal Biochem.*, 1982, **121**, 230.
4 S. K. Curtis and R. R. Cowden, *Histochemistry*, 1980, **68**, 23.
5 T. Ohyashiki, M. Takeuchi, M. Kodera, and T. Mohri, *Biochim. Biophys. Acta*, 1982, **688**, 16.
6 T. O. Sippel, *J. Histochem. Cytochem.*, 1981, **29**, 314.
7 T. J. Mitchison, *J. Cell Biol.*, 1989, **109**, 637; B. R. Ware, L. J. Brvenik, R. T. Cummings, R. H. Furukawa, and G. A. Krafft, in 'Applications of Fluorescence in the Biomedical Sciences,' eds. D. L. Taylor, A. S. Waggoner, F. Lanni, R. F. Murphy, and R. R. Birge, Liss, New York, 1986, p. 141.
8 (*a*) P. B. Mahajani and J. N. Ray, *J. Indian Chem. Soc.*, 1956, **33**, 455; (*b*) K. N. Trivedi and S. Sethna, *J. Org. Chem.*, 1960, **25**, 1817; (*c*) L. Reppel and W. Schmollack, *Arch. Pharm.*, 1963, **296**, 365; *f*) D. Chakravarty, S. P. Dutta, and A. K. Mitra, *Curr. Sci.*, 1965, **34**, 177.
9 H. Hellman and H. Piechota, *Liebigs Ann. Chem.*, 1960, **631**, 175.
10 H. von Pechmann and M. Schaal, *Ber. Deut. Chem. Ges.*, 1899, **32**, 3690.
11 N. F. Albertson, B. F. Tullar, J. A. King, B. B. Fishburn, and S. Archer, *J. Am. Chem. Soc.*, 1948, **70**, 1150.
12 D. T. Connor, U. S. P. 4 287 126; L. M. Ryzhenko, A. D. Sheldova, L. K. Kulikova, M. L. Khidekel, and O. N. Tsvetkova, *Khim.-Farm. Zh.*, 1981, **15**, 72; C. H. Yang, C. Chiang, K. C. Liu, S. H. Pang, and R. Wang, *Yao Hsueh T'ung Pao*, 1980, **15**, 48.

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