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1,8-DIAZAFLUORENONE AND RELATED COMPOUNDS. A NEW REAGENT FOR THE DETECTION OF α-AMINO ACIDS AND LATENT FINGERPRINTS.

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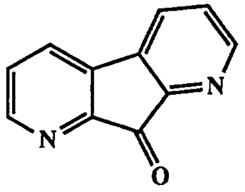
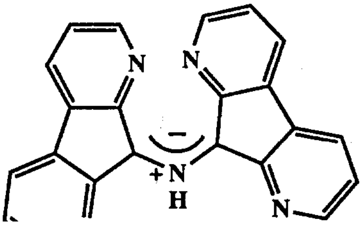
Summary 1,8-Diazafluorenone (DFO) reacts with α-amino acids and their esters via imine formation to give decarboxylated azomethine ylides and ester substituted azomethine ylides respectively. In the presence of N-methylmaleimide these azomethine ylides undergo stereospecific cycloaddition via endo-transition states. Analogues of DFO give similar cycloadducts. In the absence of dipolarophiles the α-amino acids and DFO give a red fluorescent dye thus providing a sensitive method for detecting latent fingerprints on paper.

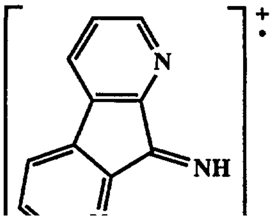
The detection and quantitation of α-amino acids plays a central role in peptide and protein chemistry and has led to the development of a number of reagents that react with α-amino acids to produce coloured and/or fluorescent species. Typical reagents include dansyl chloride,[1] fluorescamine,[2] 4-chloro-7-nitrobenzofuran (NBD-Cl),[3] o-phthalaldehyde,[4] and ninhydrin and its' benzo analogues.[5] Application of most of these reagents to the detection of latent fingerprints on paper is unsatisfactory due to interference by optical brighteners in the paper.[6] Currently ninhydrin is the most widely used reagent for the detection of latent fingerprints on paper. The reagent produces a coloured print due to formation of Ruhemann's purple via a process which involves formation of azomethine ylide intermediates.[7]

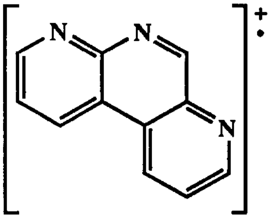
We now report a new reagent 1,8-diazafluorenone (1)[8] which reacts with α-amino acids to give a red coloured product which is highly fluorescent. The reagent is substantially more sensitive than ninhydrin and is particularly advantageous for the detection of latent fingerprints on paper.[9,10]

The red pigment has been isolated from reactions of several α-amino acids with (1) in boiling aqueous ethanol or methanol. Spectroscopic and analytical data accord with structure (2). The ^{1}H n.m.r. spectrum of the red pigment exhibits three signals at $\delta(CDCl_3)$ 7.40, 7.91 and 8.66, the mass spectrum shows the expected molecular ion at m/z 347, and structurally important fragment ions at m/z 319 (M-HCNH), 181, 168 and 154, e.g. m/z 181 corresponds to (3) or its' rearrangement product (4). The NH proton in (2) is assumed to be mobile with respect to the pyridine nitrogen atoms. The structure of (2) is closely related to that of protonated Ruhemann's purple (5),[7] and as expected (2) undergoes cycloaddition with N-methylmaleimide (NMM) in boiling wet toluene to afford (6a) (58%).









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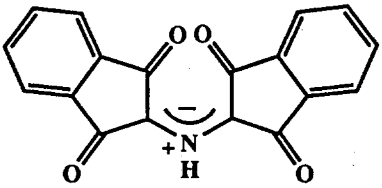

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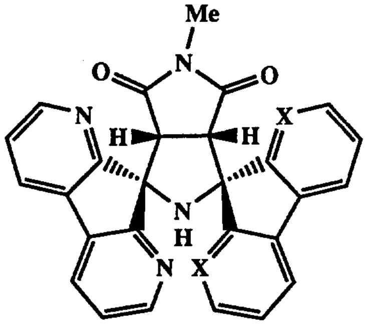
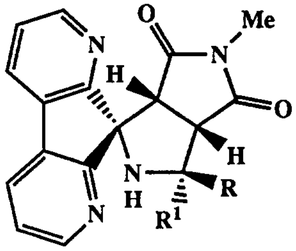
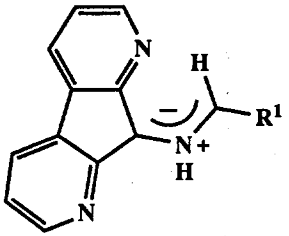


(6) a. X = N
b. X = CH



(7)



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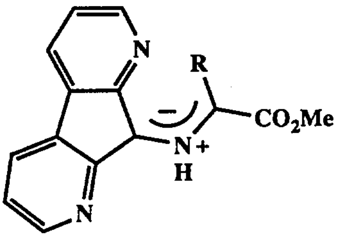
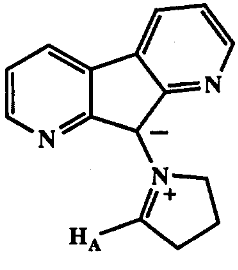
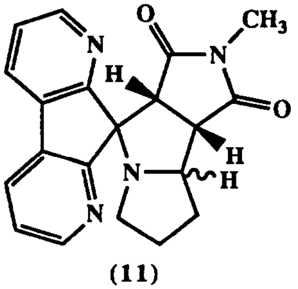


(9)



(10)



(11)

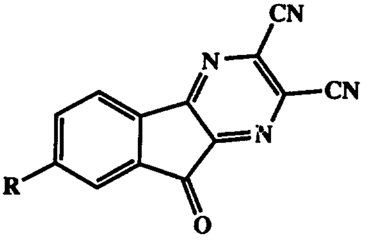
The mechanism of formation of (2) was probed by reacting DFO (1) with α-amino acids and their esters in boiling aqueous ethanol for 1-4 h. in the presence of NMM. Primary α-amino acids and their esters gave a single cycloadduct (7) in each case (Table).[11] Glycine differed from the other α-amino acids in that in aqueous ethanol it gave a cycloadduct (7, R=H, R^1=CO_2H) in which the carboxyl group was retained whilst the other α-amino acids underwent cycloaddition via the decarboxylated azomethine ylide (8) (Table). Formation of the decarboxylated azomethine ylide from glycine and (1) requires more forcing conditions (Table) i.e. 1,2-prototropy in the glycine imine is faster than decarboxylation.[7] The stereochemistry of the cycloadducts from the α-amino esters accords with the expected endo-cycloaddition to azomethine ylide (9).[12] Proline reacts with (1) in boiling methanol to give a blue product (10) [δ($CDCl_3$) 9.12, H_A] which undergoes cycloaddition with NMM to give (11)[11] as a 1.4:1 mixture of two stereoisomers. Analogous series of cycloadducts have been obtained from α-amino acids/esters, NMM, and the fluorenone derivatives (12) - (15).

Table Reaction of α-amino acids and their esters with DFO.

Amino acid/ester	Time(h)	Cycloadduct(7)	Yield(%)[a]
Glycine	1.0	R=H, R^1=CO_2H	62
Glycine	0.25[b]	R=R^1=H	70
Alanine	0.5	R=H, R^1=Me	77
Tryptophan	1.0	R=H, R^1=3-indolylmethyl	79
Methionine	2.0	R=H, R^1=$(CH_2)_2$ SMe	71
Glycine methyl ester	3.0	R=H, R^1=CO_2Me	52
Alanine methyl ester	2.0	R=Me, R^1=CO_2Me	61
Tryptophan methyl ester	2.0	R=3-indolylmethyl, R^1=CO_2Me	55
Methionine methyl ester	2.0	R=$(CH_2)_2$SMe, R^1=CO_2Me	53

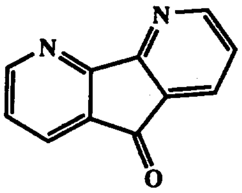
a. Isolated yield

b. Reaction carried out in boiling 20% aqueous DMF



(12) a. R = H

b. R = OMe



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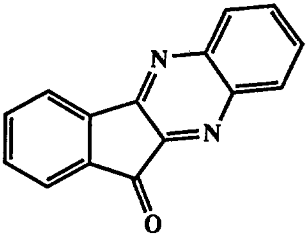

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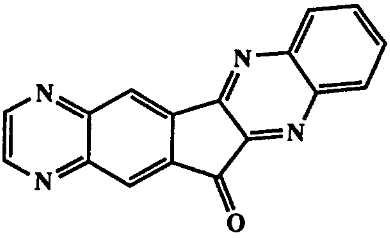

(15)

In the absence of a dipolarophile the reaction of α-amino acids and DFO (1) leading to (2) appears to parallel that of α-amino acids and ninhydrin.[7]

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9. The patented reagent is being widely used in forensic science laboratories throughout the world and is available from Lumichem Ltd., Fluorescent Chemicals, 10 Malone Road, Belfast BT9 5BN, Northern Ireland. The reagent produces 2-3 times more fingerprints than ninhydrin and would appear to have potential applications in automated amino acid analysis.
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13. Initial studies with DFO were carried out at Queens' University, Belfast.

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