Synthesis of Fluorigenic Reagent for the Detection of NH_2 Functional Groups

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Summary: New fluorigenic reagent, 1-phenylnaphthalene-2,3-dicarboxaldehyde (θNDA) has been synthesised. This has been found useful for fluorescent detection of both proteins and amino acids.

Introduction

Fluorigenic reagents with chemo selective anchor groups are valuable in detecting biologically important functional groups such as OH [1,2], NH_2 and SH [3,4] in proteins [5]. Sulphonyl chloride, e.g. dansyl chloride [6] as well as isothiocyanates, e.g. fluorescein isothiocyanate [7] are suitable for fluorescence marking of both primary and secondary amino groups. However, aromatic dialdehyde, e.g. ortho-phthalaldehyde OPA [8] has proved to be an excellent fluorigenic reagent, specific for primary amino group. For practical purposes, however, polyaromatic dialdehydes as fluorescent labelling molecules are also interesting. Synthesis of a new polyaromatic dialdehyde label for analytical fluorimetry has been described in this paper. This is specific for marking primary amino group in the presence of a thiol and is expected to exhibit fluorescence emission of its derivatives at longer wavelength than OPA-derivatives [9]. Also the dialdehyde label may be exploited to give modified reaction by replacing the thiol with sodium cyanide [10].

F. Weygand *et al.* [11] in 1950 and Y. Yagi [12] in 1951 reported synthesis of naphthalene-2,3-dicarboxaldehyde (NDA) using naphthalene-2,3-dicarboxylic acid as the starting material. Another investigation in 1956 W. Reid and H. Bodem [13] synthesized it by the bromination of 2,3-dimethylnaphthalene followed by hydrolysis of 2,3-di-(dibromomethyl)-naphthalene. However, all these methods suffer from various draw-backs such as being either time consuming or not easily followed in the laboratory.

In this paper for the synthesis of a new poly-aromatic dialdehyde (θNDA) we envisaged using the method which involved periodate cleavage of 1,2,3,4-tetrahydroxy-1,2,3,4-tetrahydro-9-phenylanthracene (tetrol) obtainable from 9-phenylanthracene by its oxidation with osmium tetroxide and hydrolysis of the osmate complex.

Results and Discussion

9-Phenylanthracene on oxidation with osmium tetroxide afforded an osmic ester complex (I). When this complex was hydrolysed with aq-alcoholic sodium sulphite, 1,2,3,4-tetrahydroxy-1,2,3,4-tetrahydro-9-phenylanthracene, tetrol (II) was formed.

The product had m.p. 214 °C with decomposition. Elemental analysis of the product agreed well with the composition of "tetraol" (II). When tetrol was treated with periodic acid an oxidised product (III) was obtained. The ^{1}H-NMR spectrum of this product showed a singlet at 9.95 in the aromatic resonance due to two protons from the two aldehyde groups providing evidence for the formation of a dialdehyde compound. The group of protons giving rise to signals at 7.25-8.15 are aromatic. The Infra red (IR) spectra of the compound (III) showed the carbonyl stretching band at 1700 cm^{-1} and the C-H band at 2880 cm^{-1}. Bands at 1620 cm^{-1} and 1460 cm^{-1} indicate the aromatic character. Mass spectral analysis showed the molecular ion peak at m/z 260, further supporting the structure with molecular formula $C_{18}H_{12}O_2$. The other fragment ions at m/z 231, 202 and 125 corresponding to M-CHO, M-2CHO and M-2CHO-C_6H_5 respectively confirmed the presence of two aldehyde groups and a phenyl group substituent. The identity of this compound was proved by its elemental analysis. The data thus support the assignment of the structure as (III).

Application of the fluorigenic reagent

1-phenylnaphthalene-2,3-dicarboxaldehyde has been used as a fluorigenic reagent for derivatising both proteins and amino acids in presence of a thiol (2-mercaptoethanol, ethanethiol or 2-methyl-2-propanethiol) in an alkaline medium of borate buffer, pH 10.0. The reaction product is an isoindole [14] which is fluorescent being soluble in water. The wavelength of excitation is 462 nm with a fluorescence emission of 552 nm. This fluorescence emission is at longer wavelength than the OPA-derivatives fluorescing at 450 nm, thus making it more suitable for use in the study of biological samples. The fluorescence of the θND-adduct (formed with the new fluorigenic reagent) has been found to be much enhanced by incorporating β-cyclodextrin into the reaction. This forms an inclusion complex [16] and provides a hydrophobic environment within its cavity to the adduct, thus increasing the fluorescence intensity.

The newly synthesized dialdehyde label has successfully been used for the fluorescent detection of amino acids following pre-column derivatisation and reversed phase high performance liquid chromatography.

This reagent is also suitable for derivatisation reactions with amino acids following flow injection analysis (FIA) [17]. This technique requires the use of a continuously flowing "Carrier stream" and then injecting a small volume of the sample directly into this flowing stream. The injected sample zone disperses in the reaction coil and is carried by the stream to an appropriate detector.

The reagent has been found equally useful for marking the proteins and may be employed for antigen-antibody reaction studies.

Experimental

Melting points were determined using a Gallenkamp Melting Point Apparatus MF-370 and are uncorrected. Infra red (I/R) spectra were obtained as solid dispersion in potassium bromide pellets or as suspension in Nujol between sodium chloride plates using Perkin-Elmer model 457 and 1310 spectrophotometers. Proton NMR spectra were obtained using 90 MHz Perkin Elmer R-32 Spectrometer in solutions of deutrochloroform and/or DMSO-d_6 with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on Kratos MS-80 mass spectrometer using a DS-55 data system. The elemental analysis were carried out by the Microanalytical Laboratory, Department of Chemistry, University of Manchester. Fluorescence measurements were made using an MPF-44B Perkin-Elmer Spectrofluorimeter.

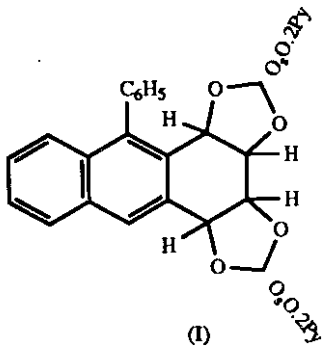
9-Phenylanthracene and Osmium tetroxide were from Aldrich Chemical Co. Ltd. whereas pyridine was from Fisons Ltd. Ethanethiol and 2-methyl-2-propanethiol were from Aldrich Chemical Co. Ltd. and the 2-mercaptoethanol from Sigma Chemical Co. Ltd. All other reagents were of analaR grade.

(A) Synthesis of 1-phenylnaphthalene-2,3-dicarboxaldehyde (θNDA)

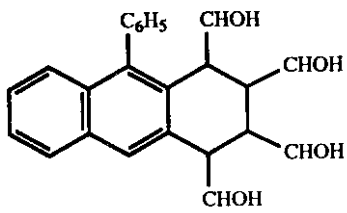
(i) 1,2,3,4-tetrahydroxy-1,2,3,4-tetrahydro-9-phenylanthracene-a 'tetrol'

Procedure

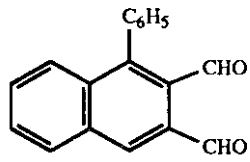
A solution of 9-phenylanthracene (0.635 g, 0.0025 mol), osmium tetroxide (1.27 g, 0.005 mol)



(I)



(II)



(III)

Fig.1: Synthesis of 1-phenylnaphthalene-2,3-dicarboxaldehyde (θNDA).

and pyridine (0.79 g, 0.01 mol) in benzene (30 ml) was kept at room temperature for 15-20 days with occasional shaking. The solid osmic ester complex (I) Fig. 1. separated out. This was filtered, washed with benzene and air dried (2.4 g).

The osmic ester complex (1.0 g) was hydrolysed with a solution of crystalline sodium sulphite (5 g) in water (30 ml) and ethanol (30 ml) by heating on a water bath for 1.5 hours. Sufficient ethanol was then added to precipitate out the excess sodium sulphite in solution. This was then filtered and the filtrate stored. The black residue on the filter paper was extracted several times with hot ethanol and the extracts combined with the original filtrate. The combined liquors allowed to stand overnight at room temperature to precipitate out further quantity of sodium sulphite from the solution. Using a 'Whatman' No. 40 this was filtered out and the filtrate was concentrated under reduced pressure. A solid separated after cooling, recrystallised from water and was obtained as a deep solid, tetrol (II) (90 mg), m.p. 214 °C with decomposition Found C, 74.21; H, 5.4% $C_{20}H_{18}O_4$ Required C, 74.5; H, 5.59%.

(ii) Periodate cleavage of 1,2,3,4-tetrahydroxyl, 2,3,4-tetrahydro-9-phenylanthracene (tetrol) to 1-phenylnaphthalene-2,3-dicarboxaldehyde (θNDA)

To a hot solution of the tetrol (II) Fig. 1 (70 mg) in water (20 ml) a solution of periodic acid (1.2 g) in water (5 ml) was added. A sticky solid was obtained on cooling. A little chloroform was added to this until it dissolved. The solution was allowed to stand in the open air when chloroform evaporated out leaving behind an orange coloured solid (III) Fig. 1 (43 mg), m.p. 105 °C.

IR (KBr) 2880, 1800, 1700, 1620, 1460, 1325, 750, 700 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 7.25-8.15; (m, 9H), 8.4 (s, 1H), 9.95 (S, 2H), M.S. (EI) 260 (M^+, 41.58%), 231 (M^+-CHO, 100%), 202 (M^+- 2CHO, 94.3%), 125 (M^+-2CHO-C_6H_5, 1.75%). 101 (C_8H_5, 45.1%), 88 (C_7H_4, 18.9%), 77 (C_6H_5, 6.08%), 76 (C_6H_4, 5.68%), 51 (C_4H_3, 4.96%), Found C, 83.3; H, 4.6%, $C_{18}H_{12}O_2$ Required C, 83.0; H, 4.6%.

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