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Nitro-Benzofurazanyl Ethers—A New Series of Fluorigenic Fingerprint Reagents

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ABSTRACT: Five 7-nitro-4-benzofurazanyl ethers have been prepared and examined as potential fluorigenic reagents for latent fingerprints on paper. All developed latent fingerprints with high sensitivity, similar to that of the parent compound 4-chloro-7-nitrobenzofurazan (NBD-chloride). Although development in solution is faster, vapor phase development is also possible, and it has certain advantages such as the avoidance of the use of solvents and the smaller background fluorescence and discoloration.

KEYWORDS: criminalistics, fingerprints, paper, reagents, luminescence

Luminescence derivatization procedures convert non- or weakly luminescent samples to highly luminescent products. The usual motivation for forming luminescent derivatives is to increase the sensitivity with which a sample can be detected. Analytical methods based on luminescence generally have detection limits from one to four orders of magnitude lower than corresponding methods based on absorption [*1*].

Research into fluorescence[2] techniques for latent fingerprints visualization started perhaps in 1976 with H. Ohki's report on the use of fluorescamine as a fluorigenic reagent [*2*]. A few other fingerprint fluorescors such as o-phthalaldehyde [*3*], naphthalene-2,3-dicarboxaldehyde [*4*], 4-chloro-7-nitrobenzofurazan (NBD-chloride) [*5*], and antimony trichloride [*6*] were also investigated in the late 1970s. Interest in fingerprint luminescence increased considerably after the introduction of lasers as the excitation source. Since the observation in 1977 by Dalrymple et al. that detectable luminescence from fingerprints could be obtained using an argon-ion laser as the primary light source [*7*], two other types of lasers and a number of alternative light sources for fingerprint detection have been introduced [*8–13*], providing further stimulus to the search for fluorigenic fingerprint reagents. Four procedures using fluorescence for latent fingerprint visualization are currently used by forensic science laboratories.

1. Detection based on inherent luminescence, with excitation being achieved by lasers or by alternative light sources [*7,10,13,14*].

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[2]While luminescence includes both fluorescence and phosphorescence, most of the applications involve fluorescence, and this is the dominant term throughout this text.

2. Enhancement of Super-Glue® developed fingerprints by treatment with fluorescing chemicals which adhere to the ridges [*15,16*]. (This is not a true fluorigenic process since the chemicals which are used, such as Rhodamine 6G and Coumarim 6, have a natural fluorescence, and they do not react chemically with the fingerprint material to produce a fluorescent product.)

3. Conversion of weak ninhydrin developed prints into luminescent metal complex by treatment with zinc chloride [*17,18*].

4. Direct treatment with the fluorigenic reagent 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole [known also as 4-chloro-7-nitrobenzofurazan or NBD-chloride (I)] [*5,12,19*].

It seems that of all the fluorigenic reagents, NBD-chloride (Table 1, Compound I) has obtained the most serious consideration. In their comprehensive reports, Carey et al. [5] and Warrener et al. [*12,19*] have clearly shown the advantages of this chemical which readily reacts with latent fingerprints under mild conditions to produce strong luminescent images. Both groups of researchers reported that in their experiments NBD-chloride gave results that were never worse than those obtained with ninhydrin.

Over the last few years, the authors of this article have been involved in a multifaceted research project whose aim is to study how systematic alterations of the molecular structure of fingerprint reagents affect their reactivity with latent fingerprints. This article reports on one type of modification—the change of the substituent at position 4 of NBD-chloride (Table 1, Compound I).

Five etheral derivatives of 7-nitrobenzofurazan, all bearing alkoxy or aryl-oxy groups at carbon 4 (Table 1, Compounds II to VI), were prepared and tested as potential fingerprint reagents.

Materials and Methods

Materials and Instrumentation

NBD-chloride (I), analytical grade, was purchased from Merck (Darmstadt, W. Germany).

The NBD-ethers (II to IV; VI) were prepared according to Johnson et al. [*20*]. Authentic samples for comparison were kindly provided by Dr. Lars Johnson of Bofors Nobel Kemi (Sweden). Methoxyethoxy-NBD [V], which to the best of our knowledge, is a new compound, was prepared similarly, from NBD-chloride (I) and 2-methoxyethanol in alkaline solution. It had the following properties: orange crystals, m.p. 80 to 82°C (from ethyl acetate/light petroleum); H^1 NMR ($CDC\ell_3$) 8.6 (d, $\mathcal{J}$ = 8.3 Hz, 1H), 6.8 (d, $\mathcal{J}$ = 8.4 Hz, 1H), 4.6 (t, $\mathcal{J}$ = 4.5 Hz, 2H), 3.9 (t, $\mathcal{J}$ = 4.5 Hz, 2H), 3.5 (s, 1H). Electron impact mass spectrum showed a molecular ion at m/z 239, corresponding to the above listed structure (V). The base peak at m/z 59 corresponds to the $CH_2CH_2OCH_3$ ion.

TABLE 1—*NBD-chloride (I) and some etheral derivatives of 7-nitrobenzofurazan, that were prepared and tested throughout this work.*

Compound	*R*	Chemical Name and Abbreviation
I	$-Cl$	4-chloro-7-nitrobenzofurazan (NBD-chloride)
II	$-OMe$	4-methoxy-7-nitrobenzofurazan (NBD-OMe)
III	$-OEt$	4-ethoxy-7-nitrobenzofurazan (NBD-OEt)
IV	$-OCH_2CH_2OH$	4-(2-hydroxyethoxy)-7-nitrobenzofurazan (NBD-OCH_2CH_2OH)
V	$-OCH_2CH_2OMe$	4-(2-methoxyethoxy)-7-nitrobenzofurazan (NBD-OCH_2CH_2OMe)
VI	$-OPh$	4-phenoxy-7-nitrobenzofurazan (NBD-OPh)

Trichlorotrifluoroethane (fluorisol) was purchased from I.C.I. (United Kingdom).

Fluorescence observations were made by VSC-1 (Video-Spectral Comparator, Foster & Freeman Ltd., United Kingdom) with a blue BG-2X12 excitation filter and FS-3 observation filter. The relative fluorescence emission curves for the derivatized fingerprints and for the paper were measured by a Docuspec TM/I microspectrophotometer with a blue Wratten 47B excitation filter and Wratten 12 observation filter. Results were photographed with Kodak Plus X film using a 75-W quartz-halogen lamp, with a Wratten 47B excitation filter and Wratten 12 observation filter. The film was developed by Kodak D-76 developer at 20°C for 6 min.

The negatives were enlarged on Agfa Brovira Speed paper (No. 4 or 5) and developed by Kodak D-163 developer diluted 1:2. No photo enhancement process was applied.

Fingerprint Development

Application by Dipping in Solution[3]—0.1% solutions in acetonitrile-fluorisol were obtained by dissolving 100 mg of the Compounds I to VI in 1 mL of acetonitrile and diluting to 100 mL by fluorisol. Fingerprint samples were dipped into the solution for 15 s, air-dried, and developed in an oven at 110 to 120°C for 10 to 15 min.

Vapor Phase Application—Crystals of Compounds I to VI (20 mg) were dispersed at the bottom of a 250-mL glass beaker. Fingerprint samples were suspended about 5 cm from the

FIG. 1—*Fluorescent impressions of latent fingerprints after development with NBD derivatives. Latents were taken from an "average" donor and developed 24 h after stamping. Development from white paper. NBD-chloride (I):* (left) *solution development and* (right) *vapor phase development (90 to 95°C, 10 min).*

[3]This is essentially the procedure used by Warrener et al. [*19*] with one modification—the solvent ratio here was acetonitrile-fluorisol 1:99 while Warrener used a 1:9 ratio.

crystals. The beaker was covered with a thin aluminum foil and heated in an oven to various temperatures from 60 to 95°C for periods ranging from 1 to 7.5 h.

Fingerprint Samples

Fingerprint samples of two donors, one good and one average (as determined by the quality of their ninhydrin developed prints), were collected on groundwood-free white paper and on checks of four major Israeli banks. All samples were kept at room temperature and developed after 24 h.

Results

All NBD-ethers (II to VI) developed latent fingerprints on paper with a high sensitivity, similar to that of the parent compound, NBD-chloride (I). This observation applies to both methods—dipping in solution and vapor phase development (Figs. 1 through 7). It seems that the selectivity of the NBD-ethers toward latent fingerprints on paper is somewhat better than that of NBD-chloride (I), namely background fluorescence and discoloration are somewhat smaller when developed by the etheral derivatives, but quantitative assessment of this property requires further experiments.

Vapor phase development gave similar results to development in solution. The optimal temperature range was 60 to 95°C, and heating periods between 2 and 7.5 h (lower temperatures requiring longer heating periods) were required for complete development. This process can, in fact, take place even at room temperature. Paper items bearing latent fingerprints that were kept in a closed vessel with some crystals of these compounds (I to VI) for a

FIG. 2—*Same as Fig. 1 except for NBD-OMe (II): solution development.*

FIG. 3—*Same as Fig. 1 except for NBD-OCH_2CH_2OMe (V): solution development.*

FIG. 4—*Same as Fig. 1 except for NBD-OPh (VI): vapor phase development (60 to 65°C; 7 1/2 h).*

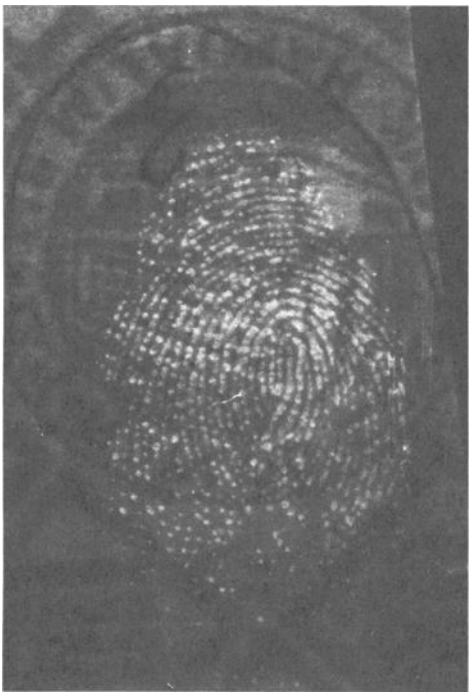

FIG. 5—*Same as Fig. 1 except development from checks. NBD-chloride (I): solution development.*

FIG. 6—*Same as Fig. 1 except development from checks. NBD-OMe (II): solution development.*

FIG. 7—*Same as Fig. 1 except development from checks. NBD-OCH_2CH_2OMe (V): solution development.*

few days showed nice fluorescent images of the fingerprint ridges under the same illumination conditions as above.

Discussion

It was not surprising to find that NBD-ethers develop latent fingerprints in a similar manner to NBD-chloride (I). Etheral derivatives of 7-nitrobenzofurazan were reported to react with amino acids in solution to form highly fluorescent compounds [*20,21*].

The chemical process that is responsible for the conversion of the nonfluorescent NBD-ethers (II to VI) to the fluorescent amino-derivatives is summarized in Fig. 8.

Microspectrofluorimetric measurements on the latent fingerprints that were developed by compounds (I to VI) suggest that fingerprint development on paper involves the same chemical process (Figs. 9 through 13).

Johnson et al. count four advantages to NBD-ethers over NBD-chloride (I) in their reaction with amino acids in solution [*20*]:

(1) higher reactivity,
(2) formation of neutral alcohols instead of hydrochloric acid as the other reaction product,
(3) higher concentration of reagents can be obtained in polar solutions, and
(4) smaller tendency to be involved in undesired side reactions.

The first and fourth advantages, reactivity and selectivity, could be very meaningful in fingerprint development. It appears, however, that in the reaction with fingerprint material on paper (as opposed to the reaction with amino acids in solution), these advantages are less

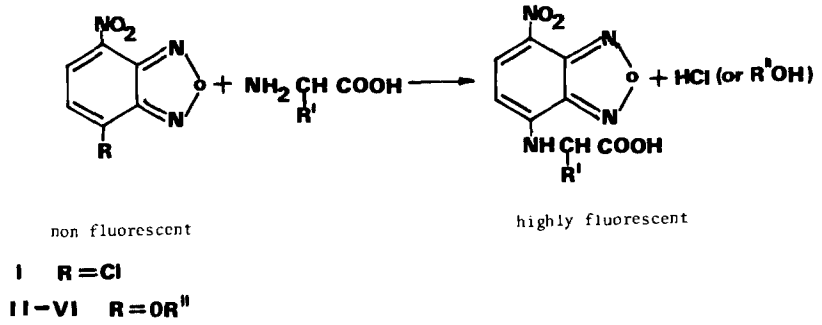

FIG. 8—*Reaction of NBD-chloride (I) and NBD-ethers (II–VI) with amino acids.*

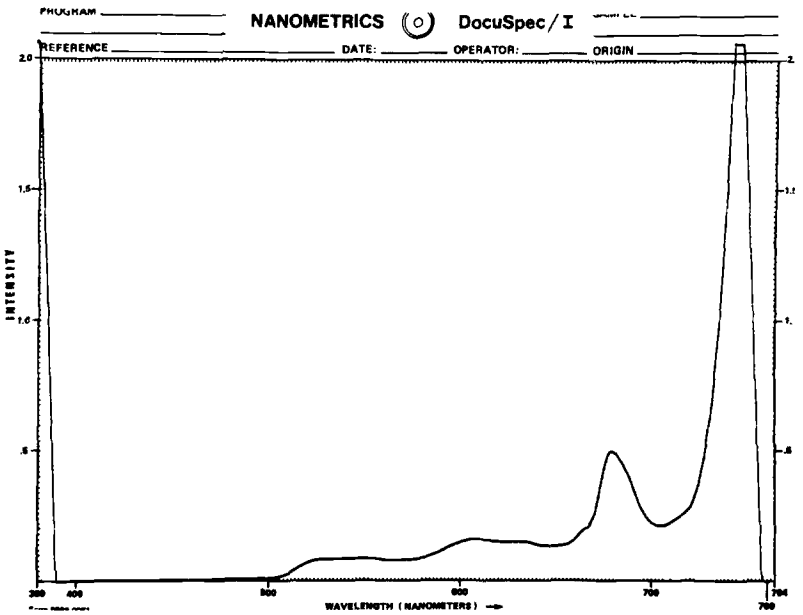

FIG. 9—*Microspectrofluorimetric curve of untreated white paper.*

pronounced. A possible reason for this might be the major difference in the reaction conditions in the two categories. While the reaction with amino acids [*20*] is carried out in a highly polar aqueous solution, the fingerprint development occurs with a substrate which is adsorbed on a solid surface, and the solvent system (where a solution is applied) is of low polarity. Nevertheless, the NBD-ethers seem to be at least as good as NBD-chloride (I) for the development of latent fingerprints on paper.[4]

[4]It must be emphasized that NBD-chloride(I) has been reported to be a potential mutagen [*22,23*] and should be handled with care.

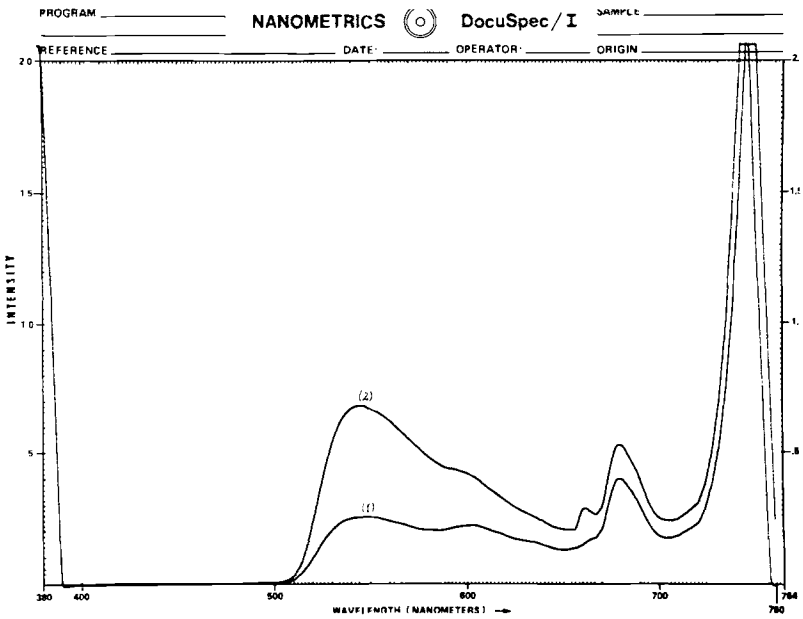

FIG. 10—*Microspectrofluorimetric curve of the fluorescent fingerprint image on paper with solution development by NBD-chloride (I):* (1) *paper background and* (2) *fingerprint ridge.*

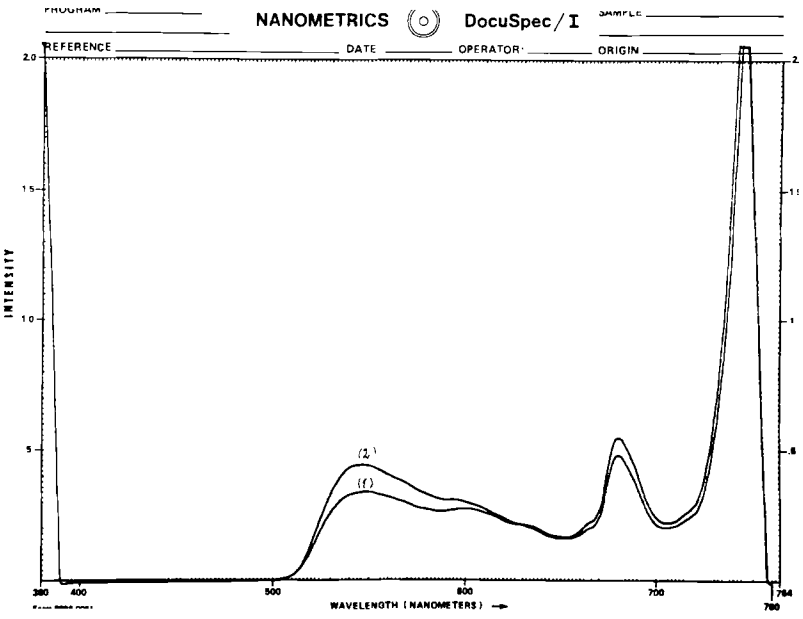

FIG. 11—*Microspectrofluorimetric curve of the fluorescent fingerprint image on paper by vapor phase development by NBD-chloride (I):* (1) *paper background and* (2) *fingerprint ridge.*

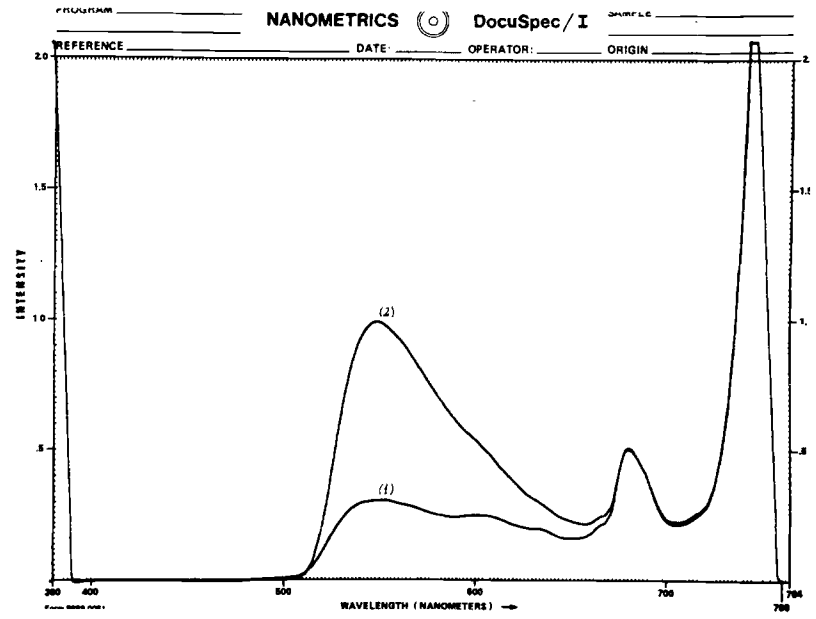

FIG. 12—*Microspectrofluorimetric curve of the fluorescent fingerprint image on paper with solution development by NBD-$OCH_2CH_2OCH_3$ (V):* (1) *paper background and* (2) *fingerprint ridge.*

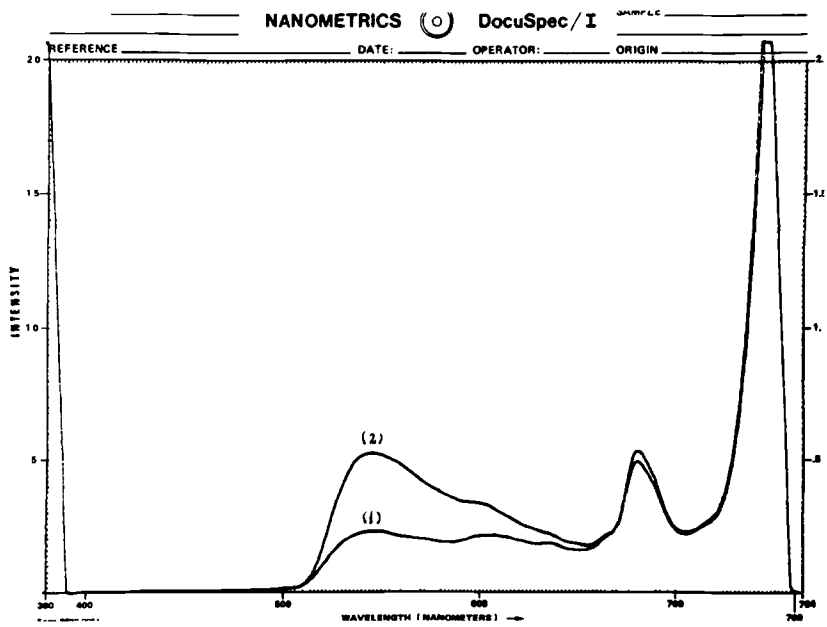

FIG. 13—*Microspectrofluorimetric curve of the fluorescent fingerprint image on paper with vapor phase development by NBD-$OCH_2CH_2OCH_3$ (V):* (1) *paper background and* (2) *fingerprint ridge.*

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