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Short Communication

Analysis of N-acyl aminonaphthalene sulphonic acid derivatives with potential anti-human immunodeficiency virus activity by thin-layer chromatography and flame ionization detection

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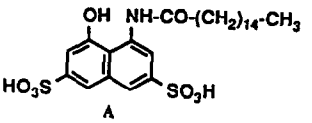
ABSTRACT

A method for checking the purity of N-acyl aminonaphthalene disulphonic acid derivatives was required for a systematic study of the anti-human immunodeficiency virus activity of these agents. We describe the use of thin-layer chromatography and flame ionization detection for the separation of these compounds, which are difficult to analyse by conventional methods. All the samples were prepared in methanol solutions (1 μl) containing 5 μg of aminonaphthalene derivative. These samples were applied to each type SIII Chromarod by a single injection and developed with pure methanol or a methanol–chloroform–ammonium hydroxide (35:55:10, v/v/v) solvent system.

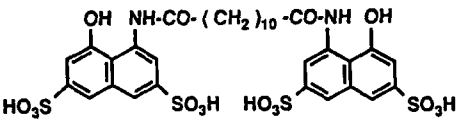
INTRODUCTION

In the quest for molecules with anti-human immunodeficiency virus (HIV) activity [1,2] new antiviral mechanisms have been discovered, and this has spurred the development of novel non-nucleoside agents. These agents inhibit both reverse transcriptase and absorption of virus [3]. Among such agents, the long-chain A, or bolaform B, naphthalene sulphonic acids have been shown to possess activity against both HIV-1 and HIV-2 [4,5].

In an attempt to alter the lipophilicity of these derivatives and optimize their activity, we carried out a study of the effect of the length of the



A



B

alkyl chain linked to the naphthalene disulphonic acid. We developed a new route [6] (Fig. 1) to the required derivatives **3a**, **b** and **i–k** in yields ranging from 25 to 60%. These yields are consistently higher than those obtained by conventional acylation [4]. The derivatives **3** are highly hygroscopic and are thus not readily purified or

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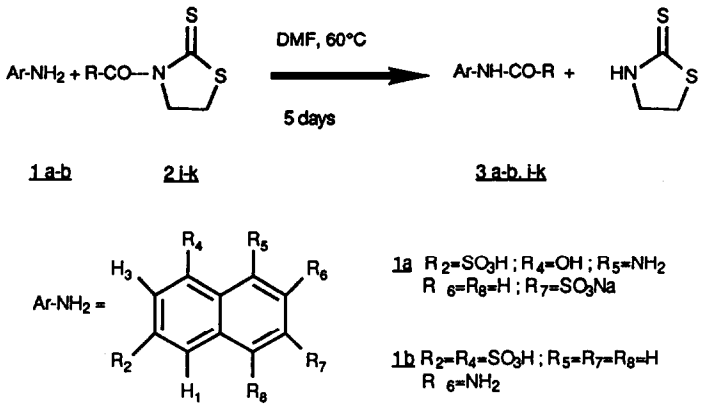

R	2	3
$C_{13}H_{27}$	2i	3a-b,i
$C_{15}H_{31}$	2j	3a-b,j
$C_{17}H_{35}$	2k	3a-b,k

Fig. 1. Acylation of aminonaphthalene disulphonic acids 1 by N-acyl thiazolidine-2-thiones.

crystallized [5]. Elemental analysis of compounds **3** produced inconsistent results, probably due to partial combustion of these high-molecular-mass substances. We therefore decided to employ thin-layer chromatography with flame ionization detection (TLC–FID) [7] to check the purity of the compounds prepared. This method has been used to analyse compounds of similar structure, such as sulphanilic acids [8,9] and various amphiphilic derivatives [10].

EXPERIMENTAL

TLC–FID was carried out using an Iatroscan TH-10 instrument (Iatron Labs., Tokyo, Japan), and the data were analysed by BOREAL software. The flame ionization detector was set up using the following parameters: hydrogen flow 160 ml/min, air flow 1 l/min, scan rate 4 mm/s, paper speed 28 mm/min. Samples (1 μl of a 0.5% solution of **1** or **3** in methanol) were placed on type S III Chromarods, which were activated immediately before use. The rods were developed with pure methanol (system 1) for compounds **3a**, **b** and **i–k**, or methanol–chloroform–ammonium hydroxide (35:55:10, v/v/v) (system 2) for compounds **1a** and **b**, in a chamber previously saturated with the solvent system.

RESULTS AND DISCUSSION

Typical chromatograms are shown in Fig. 2. The values of t_R obtained in the two solvent systems for both the starting compound **1b** and the derivatives synthesized, **3b** and **i–k**, are listed in Table I. Similar results were obtained with compounds **1a** and **3a** and **i–k**. It can be seen that the values of t_R were little affected by the nature of the alkyl chain linked to the aminonaphthalene sulphonic acid. On the other hand, the starting aminonaphthalene sulphonic acid **1** did not migrate in pure methanol ($t_R = 0.45$ min, see Table I). A mixture of methanol, chloroform and ammonium hydroxide was therefore employed to analyse the derivatives **1** ($t_R = 0.16$ min, see Table I). These compounds, like most

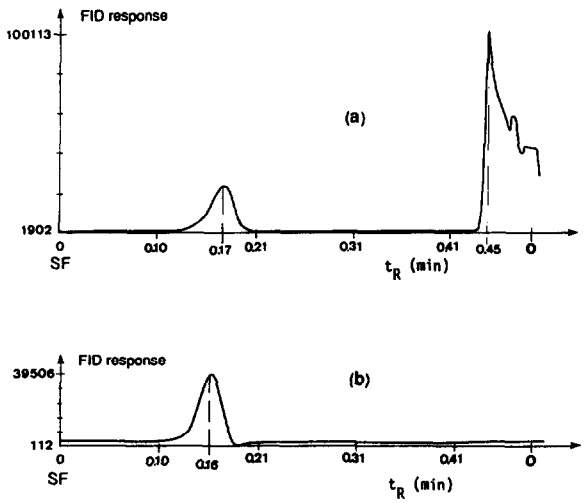

Fig. 2. TLC–FID analysis of mixture of compounds **3b** and **k** and **1b** in solvent system 1 (a) and pure compound **1b** in solvent system 2 (b). SF = Solvent front, $t_R = 0.00$; O = origin, $t_R = 0.50$ min.

TABLE I

TLC–FID ANALYSIS OF SEVERAL NAPHTHALENE SULPHONIC ACID DERIVATIVES

Compounds	t_R (min)	
	System 1	System 2
1b		
technical	0.17[a] + 0.45	0.16 + 0.27[a]
2 recrystallized	0.45	0.16
3b, i	0.18	–
3b, j	0.17	–
3b, k	0.17	0.12

[a] Oxidation product.

aminophenols, oxidize spontaneously in air [11]. They were thus recrystallized before use, and their purity was checked by TLC–FID, which readily detected oxidation products (Table I).

CONCLUSIONS

The Iatroscan system is both convenient to use and of high performance. It was successfully employed to analyse N-acyl aminonaphthalene disulphonic acid derivatives, which are not readily quantified by conventional methods.

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