CHROM. 4291

The detection of sulphonamido groups

Many sulphonamides of pharmacological interest also contain an aromatic amino group, which can be readily detected by diazotization and coupling, or by the formation of a Schiff base with an aldehydic reagent (such as *p*-dimethylaminobenzaldehyde in acid solution)[1,2]. Such reactions are used widely to estimate antibacterial sulphonamide drugs. The sulphonamide group itself is little used in analytical procedures, although a method involving chlorination and subsequent reaction with iodide has been described[3].

4-Ethylsulphonylnaphthalene-1-sulphonamide (ENS), which induces an acute proliferative response in the mouse bladder[4], lacks an aromatic amino group. For studies of its metabolism[5], a colour reaction based on the sulphonamido group itself had to be devised. It was observed that chromatograms of sulphonamides such as ENS showed yellow spots when they were sprayed with ethanolic, non-acidified *p*-dimethylaminobenzaldehyde (DAB) and then left in ammonia vapour. This paper describes the development of this finding into a satisfactory reagent for the detection of ENS and other compounds with unsubstituted sulphonamido groups on chromatograms.

Experimental

Reagent. 1.5 g *p*-dimethylaminobenzaldehyde or *p*-dimethylaminocinnamaldehyde (British Drug Houses Laboratory Reagent Grade) was dissolved in 75 ml ethanol and 25 ml ammonia, S.G. 0.88, was added. Ethanol and ammonia were British Drug Houses Analar.

Chromatography. Paper chromatograms were run on Whatman No. 1. Thin-layer chromatograms were 0.25 mm of Silica Gel N or Alumina G (Macherey, Nagel) on glass plates.

Results

Reagent. Spots of ENS sprayed with DAB developed a yellow colour with ammonia vapour very slowly. It was found that ammonia solution incorporated in the ethanolic spray coloured the spots yellow immediately. The concentrations of the reagents were not critical. A mixture of one part ammonia (S.G. 0.88) with three parts 2% DAB in ethanol was suitable; with more ethanol in the spray the spots faded more rapidly; with more aqueous component the colour did not appear so quickly. The reagent was considerably more sensitive if allowed to age for about 1 h before use; it was stable for at least a month. The spots began to fade slowly after about 5 min, particularly if spraying had been light. They could be regenerated by further spraying with the reagent.

If the same molar concentration of methylamine, diethylamine or triethylamine was used in place of ammonia, the reagent retained the ability to detect sulphonamido groups, though it was not as satisfactory. Replacement of ammonia by sodium hydroxide did not give an effective reagent, though addition of sodium hydroxide to the ammoniacal reagent did not prevent the development of a colour with sulphonamides.

p-Dimethylaminocinnamaldehyde (DAC) can replace DAB in the reagent. DAC gives red spots on an orange background. The yellow on white chromatograms with DAB reagent are less affected by uneven spraying, and detection of faint spots can be made with more confidence. Aldehydes, such as resorcylaldehyde, which are activated by hydroxy instead of dimethylamino groups do not appear to be suitable for detection of the sulphonamido group in the manner described, although in acid conditions they react well with the aromatic amino group[6].

Compounds detected. All compounds with unsubstituted sulphonamido groups tested gave a yellow colour when sprayed with the standard reagent on silica gel, alumina or paper.

TABLE I

DETECTION LIMITS OF SULPHONAMIDES CHROMATOGRAPHED ON SILICA GEL THIN LAYERS IN TOLUENE–ETHYL ACETATE (1:1)

Compound	*Detection limit (μg)*	*R_F value*
4-Ethylsulphonylbenzene-1-sulphonamide	0.5	0.25
4-Ethylsulphonylnaphthalene-1-sulphonamide	0.2	0.44
4-Methylsulphonylnaphthalene-1-sulphonamide	0.2	0.40
4-Ethylthionaphthalene-1-sulphonamide	0.2	0.59
Naphthalene-1-sulphonamide	0.4	0.57
p-Toluene-sulphonamide	1.0	0.55
N^4-Acetylsulphanilamide	1.0	0.06
4-Piperonyl-2,3,5,6-tetrafluorobenzene sulphonamide	0.3	0.77
4-(Cyclohexylamino)-2,3,5,6-tetrafluorobenzene sulphonamide	0.3	0.75
1-Oxo-3-(3'-sulphamyl-4'-chlorophenyl)-3-hydroxyisoindoline; chlorthalidone ("Hygroton", Geigy)	0.5	0.15
p-(Tetrahydro-2H-1,2-thiazin-2-yl)benzene sulphonamide dioxide; sulthiame	0.4	0.23

Table I shows the limits of detection for a number of chromatographed sulphonamides, with R_F values. The concentrations detected were less than 5 m*M* in each case. The compounds were applied in 1 μl spots to a 0.25-mm thick silica gel chromatogram run in toluene–ethyl acetate (1:1).

On alumina chromatograms, the reagent was slightly more sensitive. Less than 5 m*M* solutions could also be detected on paper chromatograms, run in butanol–1 *N* ammonia (1:1) for example, when the sulphonamides were applied in 5 μl spots. The spots were more stable on paper than on thin layers.

From Table I it can be seen that sulphonamido groups have been detected when: (a) attached to benzene or naphthalene rings, (b) the aromatic ring hydrogens are completely substituted, (c) the *para* position is substituted with either electron-attracting or electron-donating groups.

1-Ethylsulphonylnaphthalene and 1,4-diethylsulphonylnaphthalene contain the sulphonyl but not the sulphonamido group. They were not detected by the reagent.

Yellow colours were given by sulphathiazole and sulphapyridine, in which the sulphonamido group is substituted with a heterocyclic ring. The colours developed slowly as the chromatograms dried in the air and became neutral. These sulpha-drugs

also contain aromatic amino groups which can react with DAB in neutral or acid conditions. N-Acetyl- and N-methyl-ENS, and N-(2'-pyridyl)ethylthionaphthalene-1-sulphonamide were not detected by the reagent; each contains a substituted sulphonamido group, but lacks the aromatic amino group.

Discussion

CROWELL AND MCLEOD[7] have shown that the imine Me_2N–Ph–CH=NH is the dominant species in an ammoniacal methanolic solution of DAB, and that the hydrobenzamide can be formed from imine molecules with the elimination of ammonia. Reaction products such as these may be involved in the detection of sulphonamido groups by *p*-dimethylaminobenzaldehyde under the conditions used; it was found that the reagent was considerably more effective when allowed to stand before use. The finding that ammonia can be replaced by aliphatic amines without abolishing the activity of the reagent suggests that in some cases imine formation may not be involved. Diethylamine, for example, does not appear to react with DAB; dilution with *n*-hexane precipitates unchanged DAB from its solution in diethylamine.

The reaction appears sensitive enough for it to be useful in chromatographic studies of the metabolism of biologically active compounds with unsubstituted sulphonamido groups. A wide range of such substances seem likely to react in view of the successful detection of a variety of types of sulphonamide, which has been demonstrated.

Thanks are due to Dr. S. G. DE BAKER for a gift of 4-piperonyl- and 4-cyclohexylamino-2,3,5,6-tetrafluorobenzene sulphonamides, to Mr. TURNER for many of the other sulphonamides, and to Prof. D. B. CLAYSON for his interest.

This work was supported by the Yorkshire Council of the British Empire Cancer Campaign for Research.

Department of Experimental Pathology and Cancer Research, The School of Medicine, Leeds, LS2 9NL (Great Britain) L. R. A. BRADSHAW

1 A. C. BRATTON AND E. K. MARSHALL, *J. Biol. Chem.*, 128 (1939) 537.
2 J. BOOTH, E. BOYLAND AND D. MANSON, *Biochem. J.*, 60 (1955) 62.
3 G. BEISENHERZ, F. W. KOSS, L. KLATT AND B. BINDER, *Arch. Intern. Pharmacodyn.*, 161 (1966) 76.
4 D. B. CLAYSON, J. A. S. PRINGLE AND G. M. BONSER, *Biochem. Pharmacol.*, 16 (1967) 619.
5 L. R. A. BRADSHAW, *Biochem. J.*, 114 (1969) 338.
6 L. R. A. BRADSHAW, *Acta Unio Intern. Contra Cancrum*, 15 (1959) 137.
7 T. I. CROWELL AND R. R. MCLEOD, *J. Org. Chem.*, 32 (1967) 4030.

Received July 18th, 1969