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Note

Dicarboxidine [γ,γ′-(4,4′-diamino-3,3′-biphenylylenedioxy)dibutyric acid] dihydrochloride as a chromogen for the detection of barbiturates, meprobamate and some other sedatives on thin-layer chromatograms

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The chlorine-*o*-tolidine method[1,2] for the detection on thin-layer chromatograms of compounds that can be transformed into chloramines is frequently applied to various kinds of compounds containing the –CONH– group. Bösche[3] replaced *o*-tolidine with *o*-dianisidine and used the method for the detection of eighteen barbiturates and nine other sedatives and hypnotics (derivatives of urea or piperidinedione). We have recently shown[4] that the carcinogenic *o*-tolidine can be replaced with the safer alternative[5] γ,γ′-(4,4′-diamino-3,3′-biphenylylenedioxy)dibutyric acid dihydrochloride (dicarboxidine) for the detection of peptides. We have now compared dicarboxidine with *o*-dianisidine for the detection of a number of sedatives and hypnotics. The drugs used were allobarbital, hexobarbital, phenobarbital, pentobarbital, amobarbital, meprobamate, emylcamate, oxazepam, diazepam and methaqualone. Of these, allobarbital, hexobarbital, phenobarbital, pentobarbital, amobarbital and methaqualone had been tested by Bösche[3].

EXPERIMENTAL

All of the drugs were commercial products. They were dissolved in acetone in various concentrations and 1 μl of each solution was applied in a 2–3 mm wide spot to Silica gel 60 F_{254} pre-coated TLC plates (Merck, Darmstadt, G.F.R.). The amounts applied to the plates were 10, 1 and 0.2 μg; with meprobamate, samples of 0.1, 0.05, 0.02 and 0.01 μg were also applied. The plates were developed for 13 cm in chloroform–acetone (4:1)[3]. The *o*-dianisidine solution was prepared according to Bösche[3]. The dicarboxidine spray reagent was prepared by dissolving 500 mg of dicarboxidine in a mixture of 230 ml of water and 20 ml of acetic acid containing 1 g of potassium iodide. The chlorination step was performed in the usual manner[2,3].

RESULTS

The barbiturates could be detected when applied in 0.2–1.0-μg amounts and sprayed with either the *o*-dianisidine or the dicarboxidine spray reagents. According to Bösche[3], barbiturates could be detected when 10 μg of the substance were applied to the chromatogram. With dicarboxidine the colour of the spots was grey-brown to

violet. Phenobarbital could be detected under a UV lamp (254 nm) prior to chlorination and spraying when 10 μg were applied. Emylcamate and oxazepam had the same detection limit and colour as the barbiturates. Oxazepam could also be detected under a UV lamp (254 nm) prior to the chlorination and spraying when 0.2 μg was applied. Meprobamate could be detected when 0.02 μg was applied to the chromatogram. With 10 μg the grey-brown to violet spot had a red core.

Diazepam and methaqualone do not contain the –CONH– group but instead a –C=N– group, which reacts less easily to form chloramine, and therefore they are less sensitive to the tested detection method. They gave a red-violet colour with dicarboxidine, and with *o*-dianisidine diazepam gave a yellow colour and methaqualone a violet colour when 10 μg were applied to the chromatogram.

CONCLUSION

The sensitivity of the chlorine–dicarboxidine spray reagent is as high as that of the chlorine–*o*-dianisidine spray reagent for the required purpose.

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