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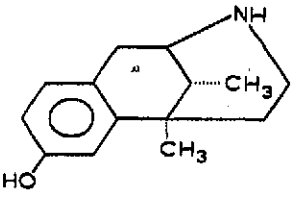
Separation and quantitative analysis of some benzomorphane derivatives by thin-layer chromatography

Synthetic benzomorphane derivatives, *e.g.* Pentazocine, are employed as effective analgesics in therapy. MULÉ[1] devised a method for the thin-layer chromatography (TLC) examination of this type of substance and reported R_F values of several benzomorphane derivatives in various solvent systems. A ninhydrin colour reaction in alkaline medium is used for both detection and quantitative assay[2,3].

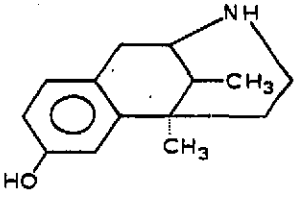
As *cis* and *trans* modifications frequently arise simultaneously during synthesis of these compounds, however, the pharmacological actions of both types of compound show great differences, and a method is needed for simultaneous detection and quantitative assay of the isomers in reaction mixtures as well as in isolated solid products.

In the present paper, TLC of the following benzomorphane derivatives is reported:

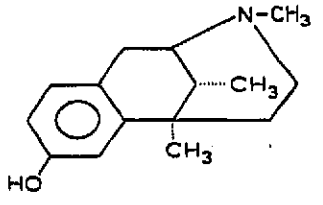
cis-2'-Hydroxy-2,9-dimethyl-6,7-benzomorphane (*cis*-DBM):



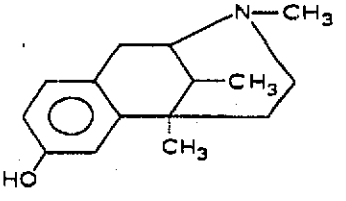
trans-2'-Hydroxy-2,9-dimethyl-6,7-benzomorphane (*trans*-DBM):



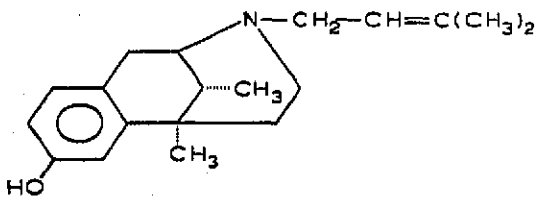
cis-2'-Hydroxy-2,5,9-trimethyl-6,7-benzomorphane (*cis*-TBM):



trans-2'-Hydroxy-2,5,9-trimethyl-6,7-benzomorphane (*trans*-TBM):



cis-2'-Hydroxy-2,9-dimethyl-5-(3-methyl-2-butenyl)-6,7-benzomorphane (Pentazocine):



TLC separation was carried out on a plate of Kieselgel $HF_{254+366}$. A solution of 10 mg/ml concentration was prepared from *cis*- and *trans*-DBM with an 8:2 mixture of chloroform and *n*-butanol; from *cis*- and *trans*-TBM with chloroform; and from Pentazocine with methanol. The dissolution of the substances was promoted by heating and stirring in a bath at 30–40°. Five μl of the solutions were applied onto the plates, which were dried by hot air. After examination of several solvent systems, the following proved to be the most useful:

Acetone–water–2*N* NH_4OH (16:4:1)	(I)
Methanol–water–2*N* NH_4OH (15:5:1)	(II)
n-Butanol–methanol–2*N* NH_4OH (5:15:4)	(III)

After a front distance of 15 cm had been reached, the plates were dried by hot air and examined in UV light at 254 nm. The spots could be detected by iodine vapour or by spraying with potassium permanganate–sulphuric acid reagent. R_F values in systems I, II and III were, respectively: *cis*-DBM, 0.12, 0.10, 0.18; *trans*-DBM, 0.24, 0.12, 0.36; *cis*-TBM, 0.39, 0.18, 0.52; *trans*-TBM, 0.74, 0.35, 0.70; and Pentazocine, 1.0, 0.50, 0.83.

Both TBM and DBM isomers were thus separated.

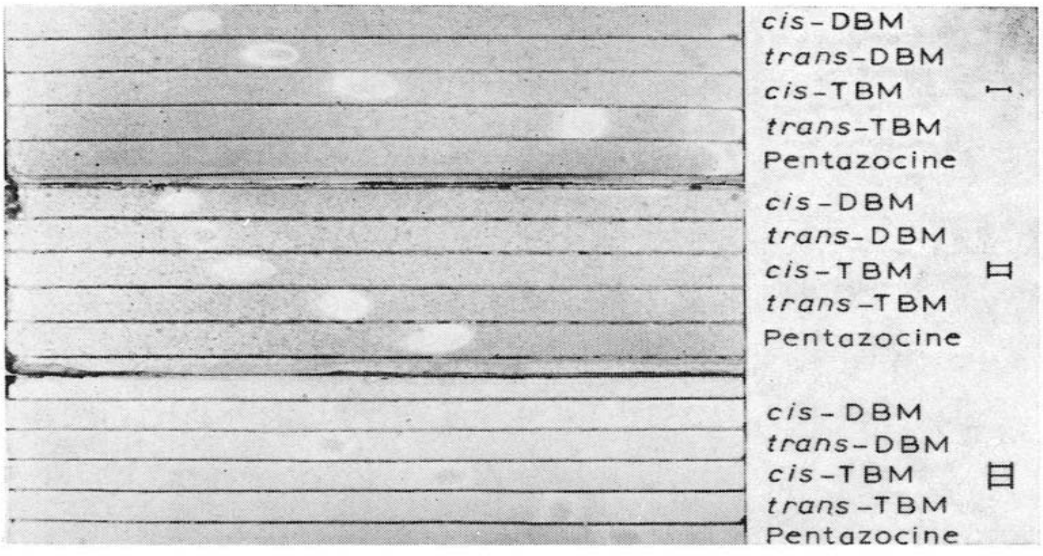


Fig. 1. Chromatograms of the benzomorphane derivatives examined in solvent systems I, II and III. Detection by potassium permanganate–sulphuric acid.

Chromatograms of the substances investigated in the three solvent systems are illustrated in Fig. 1. The spots were made visible by potassium–permanganate sulphuric acid reagent.

The quantitative analysis was rendered possible by the fact that these compounds showed a UV maximum at 285 nm in methanolic solution. In order to obtain the calibration curve, a dilution series was prepared from the substances in the concentration range 2–10 mg/ml. Each 20 μl of the solutions were applied onto the plates and developed in one of the three solvent systems. The spots were marked under UV light and sucked onto a glass filter by a suitable device. These glass filters were fixed on glass-stoppered test tubes which had a 5.0 ml graduation mark. The absorbents on the glass filters were eluted by 2 × 2 ml and subsequently by 1 × 1 ml of absolute methanol at room temperature, and the individual portions were sucked into the calibrated test tubes after waiting for several minutes. In order to prepare a blank test, a spot was marked on the developed plate which contained no substance and had a dimension identical with that of the substance. This spot was sucked in the same manner onto a glass filter and eluted as above in the case of spots containing a substance. The volumes of all eluates were made up to 5.0 ml and extinctions were read at 285 nm against the blank. The extinctions obtained were plotted as a function of the quantity applied on the plate. Linear calibration curves were obtained and a typical one is shown in Fig. 2.

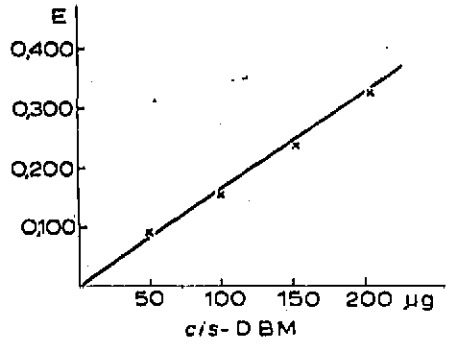


Fig. 2. Calibration curve of *cis*-DBM.

The unknown concentrations were determined by means of the calibration curves.

The TLC separation and quantitative analysis of *cis*- and *trans*-TBM were carried out before isolation from the reaction mixture in such a manner that 2 ml of the mixture were diluted with 2 ml of water, alkalinized by 1*N* sodium hydroxide and extracted with 5 × 4 ml of chloroform. The alkalinization was carried out after addition of the first 4-ml portion of chloroform to the sample, by portioning 1*N* sodium hydroxide under continuous shaking, while the pH was controlled by a universal pH indicator paper. The first portion of chloroform was removed after reaching a pH of 8–9 and the sample was further extracted by 4 × 4 ml of chloroform. The chloroform layers were combined, made up to 20.0 ml and the examination was carried out on this solution in the manner indicated above.

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