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Note

Thin-layer chromatography of pancuronium bromide and its hydrolysis products

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Pancuronium bromide (3α,17β-diacetoxy-2β,16β-dipiperidino-5α-androstane dimethobromide) is a potent curariform neuromuscular blocking agent of the competitive type. Although the discovery of this substance and its clinical usefulness were reported in 1967[1,2], the particular problems[3] associated with its assay delayed the appearance of papers on its determination.

We have studied the degradation of pancuronium bromide in order to elucidate the mechanism and the influence of hydrolysis on the preparation and the stability of parenteral solutions of the drug. Hydrolysis occurs at the two acetoxy-linkages, and based on the structure, a reaction scheme, of the type shown in Fig. 1 can be predicted.

Selective determination of the different compounds cannot be achieved by methods based on reactions involving the acetate groups (hydroxamic acid method)[4,5]
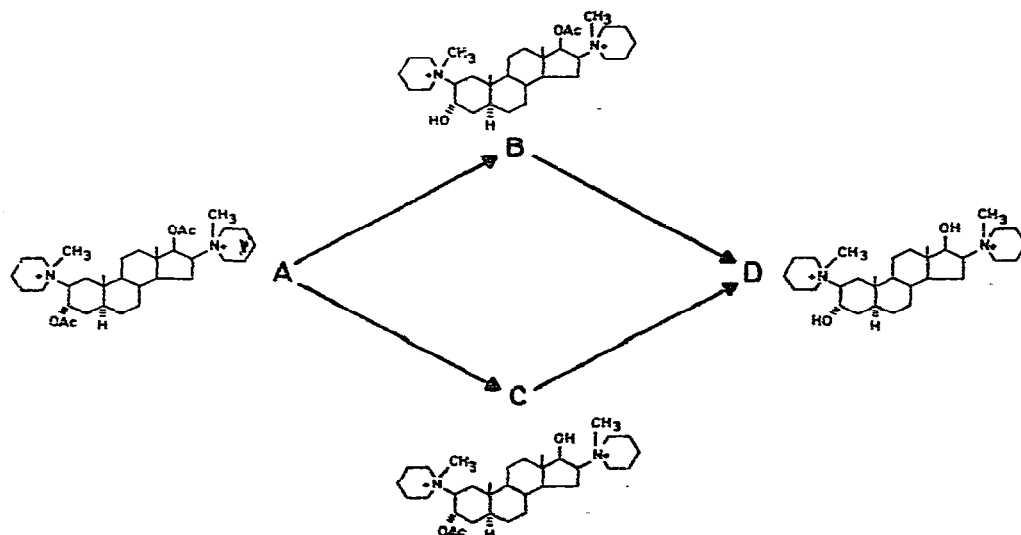

Fig. 1. Reaction scheme for hydrolysis of pancuronium bromide. A = 3α,17β-Diacetate; B = 3α-ol-17β-acetate; C = 3α-acetate-17β-ol; D = 3α,17β-diol.

or the quaternary ammonium groups (acid dye method)[3,5-7], so that preliminary separation is carried out by thin-layer chromatography (TLC).

The work reported here, which one of us has already mentioned briefly[8], is an attempt so to improve published separation methods[5-7] that the four compounds can be determined quantitatively, no matter in what proportions they are present in a mixture.

MATERIALS AND METHODS

Substances

Pancuronium bromide, the two monoacetates and the diol were supplied by Organon (Oss, The Netherlands); other chemicals, all of analytical-reagent grade, were purchased from E. Merck (Darmstadt, G.F.R.) and were used without further purification.

Chromatoplates

TLC was carried out on pre-coated plates (silica gel Woelm, 20 × 20 cm) or on laboratory-made plates. The latter plates (20 × 20 cm, or 20 × 30 cm) were coated (with use of Desaga apparatus) with a suspension of 18 g of silica gel G (Woelm), without a fluorescent indicator, in 36 ml of water to give a layer 0.25 mm thick. The developing tanks (21 × 21 × 9 cm) were purchased from Desaga.

Solvent

A mixture of 60 ml of *n*-butanol, 40 ml of pyridine, 12 ml of acetic acid and 48 ml of a 20% aqueous solution of ammonium chloride was prepared[9]; after complete phase separation, following storage overnight, the organic phase was used for development.

Procedures

Working conditions. The different working conditions investigated are summarised in Table I.

Chromatographic identification. Besides the sample, a mixture of authentic pancuronium bromide and the three degradation products was applied to the plate for reference. The spots were located by exposure to iodine vapour.

TABLE I

DEVELOPMENT CONDITIONS

Chromatogram No.	Type of plate*	Activation state**	Saturation state***	Run length, cm
1	+	+	+	15
2	—	+	+	15
3	—	—	+	15
4	—	—	—	15
5	—	—	—	26

* +, Pre-coated; —, laboratory-made.
** +, Heated 1 h at 110°; —, not activated.
*** +, Saturated; —, unsaturated.

Humidity control. Equilibration of the plates before development was carried out in an atmosphere having a relative humidity of 42%; this was achieved by placing the plates above a large shallow tank filled with 47% (w/w) sulphuric acid.

RESULTS AND DISCUSSION

Existing methods for separating the four compounds shown in Fig. 1 by TLC are part of metabolic studies[5–7,10]. Since the principal metabolic pathway is via the 17β-monoacetate, only very small amounts of the 3α-monoacetate are generally present in mixtures extracted from biological materials[5,10–13]; nevertheless, it seems that existing methods cannot separate the 17β- from the 3α-monoacetate sufficiently for individual determinations.

These workers used pre-coated plates of silica gel with the solvent system described above (see Materials and methods). Kersten *et al.*[6] allowed the chromatogram to run for 18–22 h, achieving this by lengthening the layer with a filter paper; only semi-quantitative results were obtained following visual comparison.

Tanaka *et al.*[5] developed the chromatogram in an open system for 16 h and evaluated the relative proportions of the different compounds by densitometry after spraying the layer with Dragendorff reagent; the two monoacetates were determined together, and the results were expressed as "monoacetyl derivatives".

Recently, Buzello[7] mentioned that he could not regularly separate the two monoacetates by using the method of Kersten *et al.* His attempts to improve the separation by using other adsorbents or layers twice as long were unsuccessful. In consequence, he also determined the two monoacetates together.

In spite of many attempts, we also failed to improve the separation by changing the solvent system or the adsorbent; we therefore paid further attention to the effects of different working conditions. The results, expressed by the relative R_F values of the degradation products, are shown in Table II.

The values obtained on chromatogram 2 in Table II show that better separations are obtained with laboratory-made plates, because of the more rapid flow of solvent through the layer. The spots, however, are less compact, but laboratory-made plates were used further because they were more responsive to changes in the working conditions.

TABLE II

EFFECT OF WORKING CONDITIONS ON SEPARATION OF PANCURONIUM BROMIDE AND ITS HYDROLYSIS PRODUCTS

Chromatogram No.	Relative R_F value* for hydrolysis product			R_F range for mixture	Distance between centres of spots for B and C, cm
	B^{**}	C^{**}	D^{**}		
1	1.15	1.19	1.27	0.19–0.24	0.1
2	1.15	1.25	1.40	0.20–0.28	0.2
3	1.15	1.23	1.42	0.21–0.30	0.2
4	1.18	1.27	1.54	0.23–0.35	0.2
5	1.16	1.29	1.49	0.27–0.41	0.9

* Ratio of the migration distance of the hydrolysis product to that of pancuronium bromide.
** B, 17β-Acetoxy-derivative; C, 3α-acetoxy-derivative; D, diol.

The results for chromatogram No. 3 show that activation of the plates has little or no effect on the separation; a decided improvement is achieved, however, by using unsaturated conditions. Chromatograms 1, 2 and 3 were developed in tanks lined with filter paper and equilibrated for 1 h with the solvent system. On the other hand, chromatogram 4 was developed in enhanced unsaturated conditions using a large unlined chamber consisting of two normal tanks superimposed. Development was started immediately after introduction of the solvent system in order to obtain reproducible results[14]. The improved resolution is mainly due to increased solvent transport, which results in a proportional increase of all R_F values[15]. The increase in R_F values on chromatogram 4, however, is more than proportional; thus, other, as yet unknown, effects also improve the resolution. Contrary to the observation of Buzello[7], we obtained further improvement on enlarged laboratory-made plates (20 × 30 cm), due to an effect similar to that in continuous TLC (chromatogram 5 in Table II). Since the relative R_F values are almost constant, better resolution is obtained because of the greater migration distance. The R_F values on chromatogram 5, of which a typical result is shown in Fig. 2, are: pancuronium bromide, 0.27; 17β-monoacetate, 0.31; 3α-monoacetate, 0.35; and 3,17-dihydroxy-derivative, 0.41.

A blank area of width 3–4 mm between the two monoacetates permits their separate determination by an elution method or by direct densitometry; we hope to report on this in a future paper.

Fig. 2. Chromatogram No. 5 (see Table II); for A, B, C and D, see Fig. 1.

CONCLUSION

The method described results in complete separation of pancuronium bromide from its hydrolysis products; this has been achieved by optimisation of the working conditions.

ACKNOWLEDGEMENTS

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