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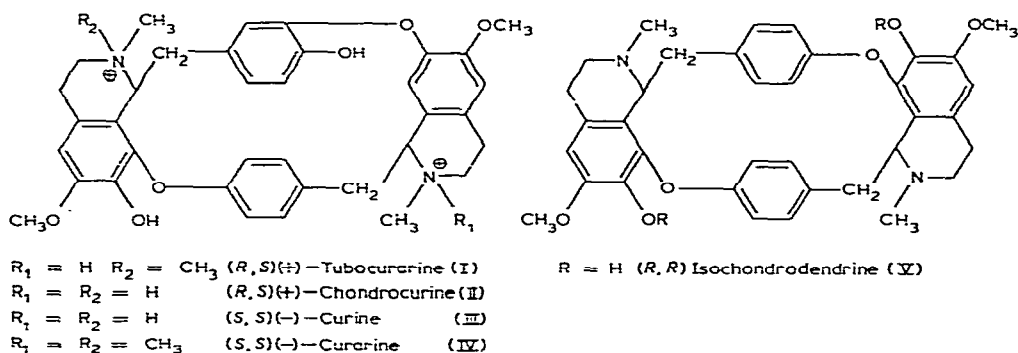
Improved thin-layer chromatographic system for evaluation of (+)-tubocurarine chloride and commercial curare

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The alkaloid (+)-tubocurarine chloride (I) is a potent neuromuscular blocking agent derived from the stems of *Chondrodendron tomentosum*¹⁻³, one of the menispermaceous plants traditionally used by South American Indians for preparing the arrow poison, curare. Curare resin, a concentrated aqueous extract of the plant pulp, is used as the raw material for the production of (+)-tubocurarine chloride to be used in surgery as a non-depolarising muscle relaxant.



Besides (+)-tubocurarine chloride, curare resin contains a large number of other bisbenzylisoquinoline alkaloids^{3,4}, the principal ones (II-V) being potential impurities in the refined drug.

A number of chromatographic methods have been applied to the analysis of curare resin including gradient-elution high-performance liquid chromatography (HPLC)⁵ and thin-layer chromatography (TLC)^{6,7}. TLC, potentially, offers a simple and rapid method for the semiquantitative evaluation of curare resin, however the published methods, in our hands, fail to resolve compounds I-V.

This paper describes a new TLC system giving compact spots and excellent resolution of the components of curare resin. The suitability of this system for analytical control of the purity of commercial (+)-tubocurarine chloride is demonstrated.

EXPERIMENTAL

Samples and reagents

Authentic samples of curare alkaloids were supplied by Diosynth (Apeldoorn, The Netherlands). (+)-Tubocurarine chloride USP reference standard was from U.S.P.C. (Rockville, MD, U.S.A.). All chemicals used were of analytical reagent grade.

TLC plates and chromatographic procedures

The TLC plates used were pre-coated silica gel 60 F-254, 20 × 20 cm, with a layer thickness of 0.25 mm (E. Merck, Darmstadt, G.F.R.).

Aliquots (5 μ l) of methanolic solutions containing 0.5–1.0% (w/v) of authentic alkaloids and curare were applied to the plates. The plates were then developed in an unlined chromatographic tank at ambient temperature. The developing solvent was the lower phase of a mixture of equal volumes of chloroform, methanol and 12.5% (w/v) aqueous trichloroacetic acid. The plates were developed over a distance of 15 cm. Following development the plates were dried at room temperature. Two spray reagents were found to be suitable. Equal volumes of an aqueous mixture of 0.1 *M* potassium ferricyanide solution and 0.1 *M* ferric chloride solution freshly prepared gave blue spots on a green background⁸. Orange spots on a yellow background were produced using Dragendorff's reagent for the detection of alkaloids⁹.

RESULTS AND DISCUSSION

The resolution of the fast running ditertiary alkaloids (II, III, V) improved using unsaturated conditions and therefore all TLC developments were performed in an unlined chamber.

The mobile phase gave rise to a secondary solvent front, but no significant spots were detected between the primary and secondary solvent fronts. The sensitivity of the colour reaction was such that 0.1 μ g of the alkaloids could be detected using both spray reagents. Curare and (+)-tubocurarine chloride showed a number of spots, some of which were identified from the R_F values of authentic alkaloids. By comparing the intensities of impurity spots with loadings of known concentrations of (+)-tubocurarine chloride, the levels of related substances in the USP reference standard and commercial samples from two sources were estimated. Table I gives impurity estimations together with assignments of known alkaloids. Some correlation between the chromatographic mobility (R_F) of the five alkaloids (I–V) and the degree of quaternisation of the two nitrogen centres was observed. The diquaternary curarine (IV) migrated more slowly than the ditertiary curine (III); (+)-tubocurarine chloride (I) with a quaternary and a tertiary nitrogen centre had an intermediate R_F value.

Some samples of curare contained a significant amount of an unidentified alkaloid at R_F 0.21, which suggests that the compound has a quaternary and a tertiary nitrogen centre. This compound has been extracted and purified, and an initial examination has shown it to be a laevorotatory alkaloid $[\alpha]_D^{20} - 199^\circ$ ($c = 1.0$, methanol) with a molecular formula of $C_{37}H_{40}Cl_2N_2O_6$.

TABLE I

TLC ESTIMATIONS OF RELATED SUBSTANCES IN SAMPLES OF (+)-TUBOCURARINE CHLORIDE

All estimations are expressed as a percentage in terms of standard loadings of (+)-tubocurarine chloride.

R_f	Assignment	USP Reference material	(+)-Tubocurarine chloride	
			Source A	Source B
0.52	Isochondrodendrine (V)	—	—	—
0.48	Curine (III)	—	—	—
0.44	Chondrocurine (II)	—	0.5	0.2
0.38		—	0.1	—
0.35		—	0.1	—
0.26	(+)-Tubocurarine chloride (I)			
0.21		0.1	0.5	0.1
0.17		—	0.5	0.1
0.14		0.3	0.8	0.3
0.11		0.2	0.5	0.2
0.08	Curarine (IV)	0.2	1.0	0.2
0.03		0.1	0.1	—
	Total impurity level	0.9	4.1	1.1

CONCLUSION

The TLC system described offers a simple method for the examination of curare resin and is applicable to the analytical control of foreign related alkaloids in commercial (+)-tubocurarine chloride.

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