Thin-layer chromatography of cinchona alkaloids

I. Separation and identification of vinyl-bases and their dihydro-derivatives

Various authors[1–11] have attempted to establish conditions for the separation of cinchona alkaloids by thin-layer chromatography. Their results, which were not always satisfactory, chiefly relate to the separation of the four principal bases of this group: quinine, quinidine, cinchonine and cinchonidine, or to their detection in a mixture with other alkaloids in compounded drugs or other preparations.

Until recently, the separation of mixtures of the diastereoisomers of these alkaloids was beset with overwhelming experimental difficulties. Nor was it possible to separate distinctly the vinyl-bases of cinchona alkaloids and their dihydro-derivatives.

In recent years, such methods as paper electrophoresis[12], gas chromatography[13] and thin-layer chromatography[14–16] have been applied with the aim of achieving such separations. The last mentioned method has been the most successful, since a mixture of the four cinchona bases[17] could be separated satisfactorily.

The aim of this paper is to describe in detail a method for the rapid separation of standard dihydro-derivatives from the various vinyl cinchona alkaloids.

Experimental

Materials. Standards of vinyl-bases and their dihydro-derivatives were obtained by preparative purification of commercial raw materials containing alkaloids of the cinchona bark. By means of repeated recrystallisation of the bases and their salts from several solvents, other vinyl alkaloids of the same group were removed from the mixture. In order to eliminate the dihydro-derivatives, the alkaloids were subjected to threefold purification according to THRON AND DIRSCHERL[18]. The method is based on the difference in solubility of the dihydro-derivative and of the addition compound of mercuric acetate with the vinyl alkaloids in aqueous ammonia.

Apparatus and reagents. The glass plates were coated with an adsorbent layer using a modification* of the Research Specialties Co.** applicator[19].

Adsorbent: Kieselgel G for thin-layer chromatography, Merck No. 7731.

Solvent: Chloroform p.a., methanol p.a. redistilled, diethylamine, twice purified.

Reagent: Dragendorff's reagent modified by MUNIER AND MACHEBOEUF[20].

Procedure. The adsorbent layer was prepared by Stahl's method. 35 g Kieselgel G, 60 ml of water and 10 ml 0.1 *N* sodium hydroxide were thoroughly mixed in a mortar. The slurry was poured on to the plates (100 × 200 mm) to a standard thickness of 0.3 mm, dried in air until opaque and then for 2.5 h at 50°, and finally activated for 30 min at 110°. The chromatoplates were developed by the ascending technique in a round chamber with ground lid. The chamber was lined with paper moistened with solvent. An 0.5 % butanol solution of the substances was applied to the starting line, which was 15 mm from the edge of the plates. A volume of either 0.45 μl (2.2 μg) was applied by means of a micropipette or 0.48 μl (2.4 μg) with a calibrated platinum wire loop, at intervals of about 1–1.5 cm. Immersion of the plate in the developing solvent was to a depth of 5 mm. The solvent system consisted of chloroform, methanol and

* Device with calibrated appliance for adjusting layer thickness.

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diethylamine (80:20:1). The time required to reach the front (10 cm) was about 25–26 min. After removing the solvent with a stream of hot air, the plates were observed in U.V. light and the positions of the fluorescent spots determined. The plates were then sprayed with Dragendorff's reagent, which yielded orange spots.

Discussion and results

From our experimental results and those of others, optimum conditions for the separation of cinchona alkaloids are obtained with kieselgel G adsorbent made alkaline with NaOH and when a solvent system containing diethylamine is used.

Preliminary experiments with solvent systems containing different amounts of diethylamine showed that the best separation was obtained using the solvent system chloroform–methanol–diethylamine (80:20:1). The development time was about 25 min.

The solvent systems chloroform–acetone–diethylamine (5:4:1)[15] and chloroform–methanol–diethylamine (80:20:0.2)[17] failed to give a satisfactory separation of the cinchona hydrobases and vinyl alkaloids under the conditions used here.

Satisfactory reproducibility of R_F values to within 0.03, for 8 alkaloids, was achieved (Table I). The $\Delta R_F \times 100$ values for the four mixtures differ, amounting to 7 for quinine and dihydroquinine, 9 for quinidine and dihydroquinidine, 10 for

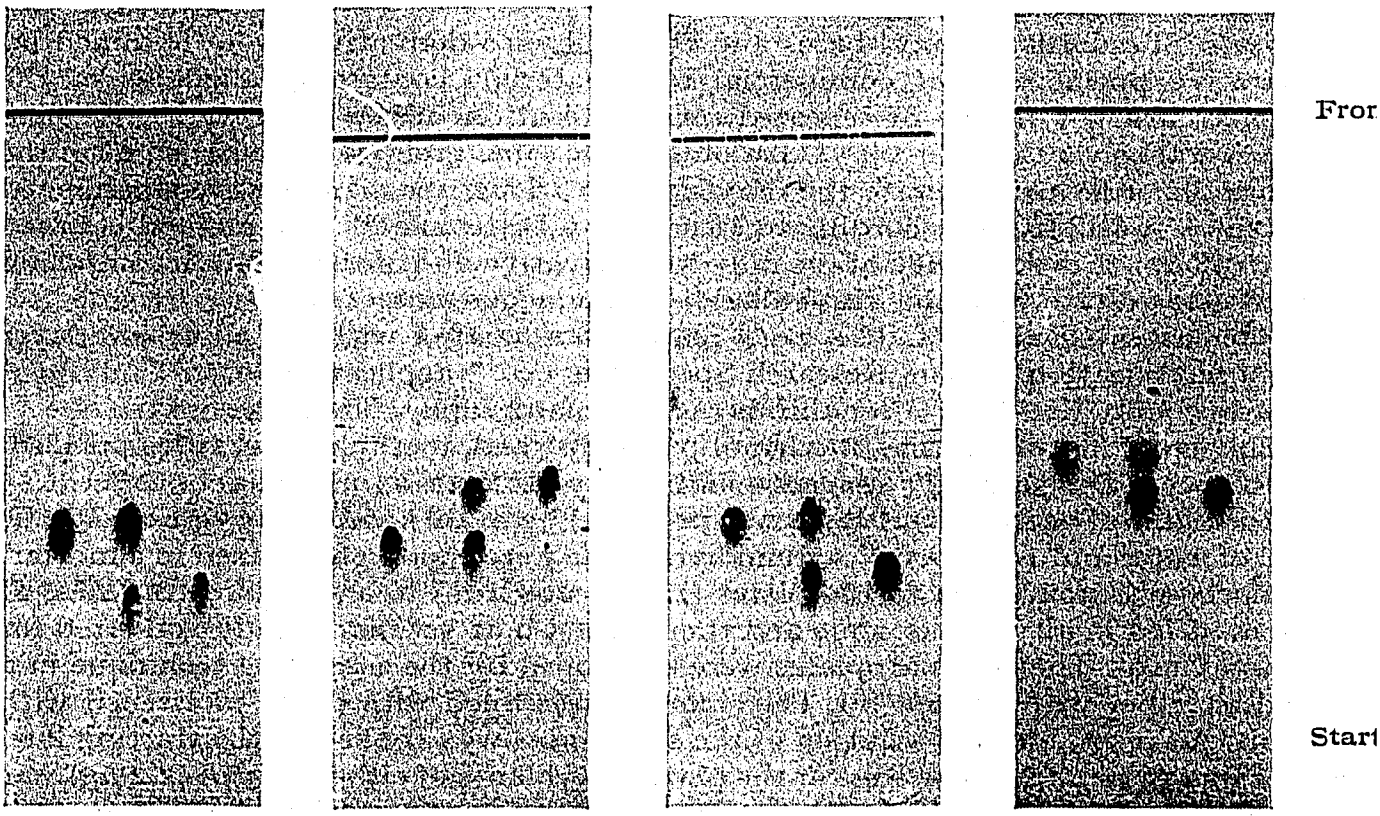

Fig. 1. Fig. 2. Fig. 3. Fig. 4.

Figs. 1–4. Solvent: chloroform–methanol–diethylamine (80:20:1). Kieselgel G Merck 7731. M_1 = mixture of quinine and dihydroquinine; M_2 = mixture of quinidine and dihydroquinidine; M_3 = mixture of cinchonidine and dihydrocinchonidine; M_4 = mixture of cinchonine and dihydrocinchonine. For explanation of the numbers, see Table I.

TABLE I

RANGE OF R_F VALUES FOR VARIOUS CINCHONA ALKALOIDS

Kieselgel G Merck 7731. Solvent: chloroform–methanol–diethylamine (80:20:1).

*No.**	*Alkaloids*	*R_F values*
1	Dihydroquinine	0.38–0.41
2	Quinine	0.45–0.47
3	Dihydroquinidine	0.36–0.39
4	Quinidine	0.46–0.48
5	Dihydrocinchonidine	0.29–0.32
6	Cinchonidine	0.39–0.41
7	Dihydrocinchonine	0.28–0.31
8	Cinchonine	0.38–0.41

* See also Figs. 1–4.

cinchonidine and dihydrocinchonidine, and 13 for cinchonine and dihydrocinchonine (Table II).

Figs. 1–4 show the central parts of developed chromatograms for which the R_F values are reproducible to within 0.01 (Table II), and where the values of $\Delta R_F \times 100$ for the mixtures are equal or almost equal to the average for the whole chromatogram (Table III).

Our experimental conditions were not greatly different from Stahl's standard conditions; this contributed considerably towards the reproducibility of our results. From the chromatograms shown, it is seen that at the concentrations applied sharp oval spots of sufficient intensity are obtained on development.

Alkaloids such as quinine and quinidine exhibit fluorescence with well-determined centre of intensity on illumination with a U.V. lamp; unlike paper chromatograms, these remain visible after spraying with the developing reagent. The coloured spots in the layer neutralized with acetic acid vapour were permanent over many months.

TABLE II

$\Delta R_F \times 100$ OF VINYL AND DIHYDROBASES OF CINCHONA ALKALOIDS SEPARATED BY THIN-LAYER CHROMATOGRAPHY

Numeration of alkaloids according to that of Table I and Figs. 1–4. Kieselgel G Merck 7731. Solvent system: chloroform–methanol–diethylamine (80:20:1).

No.	*R_F*			*$\Delta R_F \times 100$*	*Limits of R_F reproducibility*
	Vinyl bases	*Mixture*	*Dihydrobase*		
1		0.40	0.41		0.01
2	0.47	0.47		7	0.00
3	0.37	0.37			0.00
4		0.46	0.47	9	0.01
5		0.31	0.32		0.01
6	0.40	0.41		10	0.01
7		0.28	0.30		0.02
8	0.40	0.41		13	0.01

TABLE III

R_F AND $\Delta R_F \times 100$ OF CINCHONA ALKALOIDS SEPARATED BY THIN-LAYER CHROMATOGRAPHY

Numeration of alkaloids as in Table I and Figs. 1–4. Kieselgel G Merck 7731. Solvent system: chloroform–methanol–diethylamine (80:20:1). Solvent front: 10 cm. Time of run: 25 min.

No.		R_F values for the mixtures over entire chromatogram			$\Delta R_F \times 100$ for the mixtures		
1	M_1	0.38	0.40	0.40	7	7	7
2		0.45	0.47	0.47			
3	M_2	0.38	0.37	0.36	8	9	10
4		0.46	0.46	0.46			
5	M_3	0.29	0.31	0.30	10	10	10
6		0.39	0.41	0.40			
7	M_4	0.27	0.28	0.30	11	13	11
8		0.38	0.41	0.41			

Results of further work on the separation of four hydrobases and eight alkaloids will be published later.

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1 D. J. LUSSMAN, E. R. KIRCH AND G. L. WEBSTER, *J. Am. Pharm. Assoc.*, Sci. Ed., 40 (1951) 368; *J. Pharm. Pharmacol.*, 4 (1952) 129.
2 R. MUNIER, M. MACHEBOEUF AND N. CHERRIER, *Bull. Soc. Chim. Biol.*, 34 (1952) 204.
3 P. DE MOERLOOSE, *Pharm. Tijdschr. Belg.*, 29 (1952) 117; 30 (1953) 5; *Pharm. Weekbl.*, 89 (1954) 541.
4 P. CASTILLE, *Pharm. Weekbl.*, 89 (1954) 1.
5 J. REICHELT, *Pharmazie*, 11 (1956) 718.
6 J. REICHELT AND M. SARSUNOVA, *Pharmazie*, 13 (1958) 21.
7 T. BORKOWSKI AND A. DŁUZNIEWSKA, *Dissertationes Pharm.*, 15 (1963) 95.
8 Z. MARGASIŃSKI, A. SZYMAŃSKA AND L. WASILEWSKA, *Acta Polon. Pharm.*, 12 (1955) 65.
9 F. ADAMANIS, W. MIZGALSKI AND D. MALEJKA, *Acta Polon. Pharm.*, 16 (1959) 485.
10 F. ADAMANIS AND D. MALEJKA, *Bull. Soc. Amis Sci. Lettres Poznań, Ser. C*, 10 (1960) 77.
11 L. SYPER, *Dissertationes Pharm.*, 15 (1963) 411.
12 L. A. WILLIAMS, Y. M. BRUSOCK AND B. ŻAK, *Anal. Chem.*, 32 (1960) 1883.
13 H. A. LLOYD, H. M. FALES, P. F. HIGHET, W. J. A. VANDENHEUVEL AND W. C. WILDMAN, *J. Am. Chem. Soc.*, 82 (1960) 3791.
14 K. H. MÜLLER AND H. HONERLAGEN, *Arch. Pharm.*, 293 (1960) 202.
15 D. WALDI, K. SCHNATZKERZ AND F. MUNTER, *J. Chromatog.*, 6 (1961) 61.
16 J. BÄUMLER AND S. RIPPSTEIN, *Pharm. Acta Helv.*, 36 (1961) 382.
17 R. VAN SEVEREN, *J. Pharm. Belg.*, [N.S.] 17 (1962) 40.
18 H. THRON AND W. DIRSCHERL, *Ann.*, 515 (1935) 252.
19 H. MANGOLD, *J. Am. Oil Chemists' Soc.*, 38 (1961) 708.
20 R. MUNIER AND M. MACHEBOEUF, *Bull. Soc. Chim. Biol.*, 33 (1951) 846.

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