THIN-LAYER CHROMATOGRAPHY OF CINCHONA ALKALOIDS

II. QUALITATIVE EXAMINATION OF DIHYDRO-BASES OF THE CINCHONA ALKALOIDS IN COMMERCIAL PRODUCTS

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(Received May 11th, 1964)

Commercial preparations of quinine, quinidine, cinchonine and cinchonidine obtained on a technical scale from cinchona bark are not pure compounds but contain small amounts of other vinylic bases and their dihydro derivatives[1]. The constituents vary according to the species of tree and the technology of separation and preparation.

It was felt that the non-uniformity of these substances made it necessary to have a rapid and effective method of identification and qualitative assay of the impurities with regard to synthetic and analytical research work and pharmaceutical applications. Such a method could also serve for establishing new commercial standards for the production of these alkaloids and for the pharmaceutical industry.

Consequently, we attempted to apply earlier results[2] obtained by thin-layer chromatography to the qualitative assessment of alkaloid preparations from cinchona bark. In particular, this paper is aimed at presenting results on the content of dihydro-bases in various commercial preparations of the alkaloids under consideration.

EXPERIMENTAL

The following commercial preparations of quinine, quinidine, cinchonine and cinchonidine were examined:

(1) Quinine (Toscat Brand, Briddle Sawyer and Co. Ltd., London, Great Britain).

(2) Quinine hydrochloride (Toscat Brand, Briddle Sawyer and Co. Ltd., London, Great Britain).

(3) Quinine hydrochloride (N.V. Amsterdamsche Chininefabriek, Amsterdam, The Netherlands).

(4) Quinidine (E. Merck, Darmstadt, Germany).

(5) Quinidine (BDH Laboratory Reagent, The British Drug Houses Ltd., BDH Laboratory Chemical Group, Poole, Great Britain).

(6) Quinidine (Toscat Brand, Briddle Sawyer and Co. Ltd., London, Great Britain).

(7) Quinidine (N.V. Amsterdamsche Chininefabriek, Amsterdam, The Netherlands).

(8) Quinidine sulfate (N.V. Amsterdamsche Chininefabriek, Amsterdam, The Netherlands).

(9) Cinchonine (BDH Laboratory Reagent, The British Drug Houses Ltd., BDH Laboratory Chemical Group, Poole, Great Britain).

(10) Cinchonine (N.V. Amsterdamsche Chininefabriek, Amsterdam, The Netherlands).

(11) Cinchonine hydrochloride (Toscat Brand, Briddle Sawyer and Co., Ltd., London, Great Britain).

(12) Cinchonidine (BDH Laboratory Reagent, The British Drug Houses, Ltd., BDH Laboratory Chemical Group, Poole, Great Britain).

(13) Cinchonidine (W. Dembach and Co., Bad Ems, Germany).

(14) Cinchonidine (C. F. Boehringer and Soehne, GmbH, Mannheim, Germany).

Standards were obtained by preparative purification[2] of the purest commercial raw materials of cinchona bark alkaloids.

Preparation of the plates with adsorbent and development of the chromatograms were carried out according to the method already described[2]. Five per cent solutions in butyl alcohol of the standard substances and the commercial samples under investigation were obtained by dissolving 50 mg of the substance in 10 ml volumetric flasks. Single applications of the substances were made by means of a calibrated platinum wire loop, 2.4 μg of the alkaloid being applied to the 10 $\times$ 20 cm plates used. Two chromatograms were run simultaneously in the same chamber, providing for identical conditions for diastereoisomers. They were dried in a current of warm air and observed under a quartz lamp with filter to assess the fluorescent intensity of the spots corresponding to the hydro-bases. The centre of intensity was determined and the chromatograms were sprayed with Dragendorff's reagent according to MUNIER[3] after which observation was repeated in U.V. light. When the layer had dried, the relative sizes of the coloured spots and their colorations were determined.

Calibration of the wire was effected with respect to butyl alcohol, which was used as solvent for the standards and samples under investigation. From a volume of butanol whose weight had been determined to within 0.1 mg, sampling of the solvent with the wire was repeated 100 times, spreading the amount taken on paper or on a plate and weighing again. From the difference, the weight of a single loop probe was calculated as 0.388 mg, whence the volume of the platinum wire used was obtained in μl (0.48).

RESULTS AND DISCUSSION

In a series of experiments, the commercial preparations of cinchona bark alkaloids available were investigated for their dihydrobase content.

Almost all the samples were found to contain impurities whose R_F corresponded to the R_F values of the standard hydro-bases.

From the intensity of the fluorescence, size and coloration of the spots developed with the reagent, information could be obtained as to the quality of the preparation. Observation corroborated qualitatively data from the literature[1] relating to the hydro-base content in quinidine and cinchonine as determined by other methods[4]. Preparations of these two alkaloids (Figs. 2 and 3) revealed the highest hydrobase content, the latter attaining its highest value in quinidine.

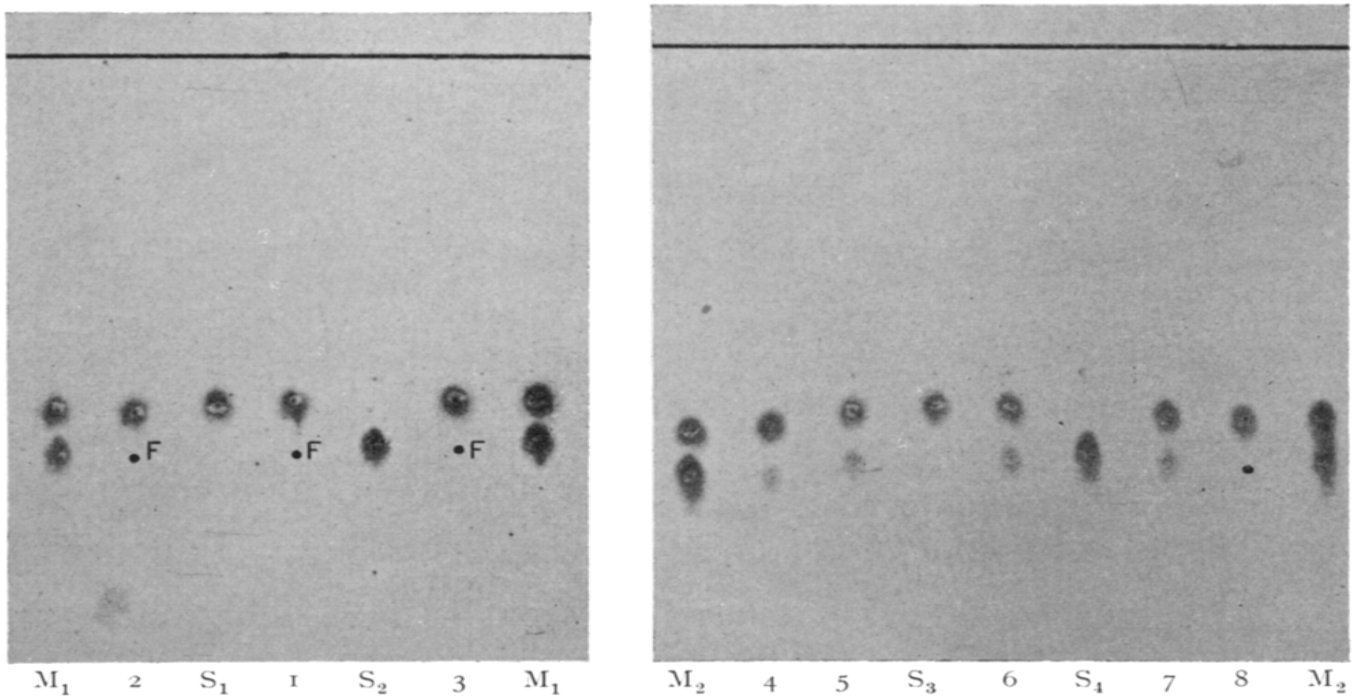

Fig. 1. Thin-layer chromatogram of commercial quinine 1, 2, 3. M_1 = mixture of quinine and dihydroquinine; S_1 = quinine; S_2 = dihydroquinine; F = centre of fluorescence of dihydroquinine in commercial quinine.

Fig. 2. Thin-layer chromatogram of commercial quinidine 4, 5, 6, 7, 8. M_2 = mixture of quinidine and dihydroquinidine; S_3 = quinidine; S_4 = dihydroquinidine.

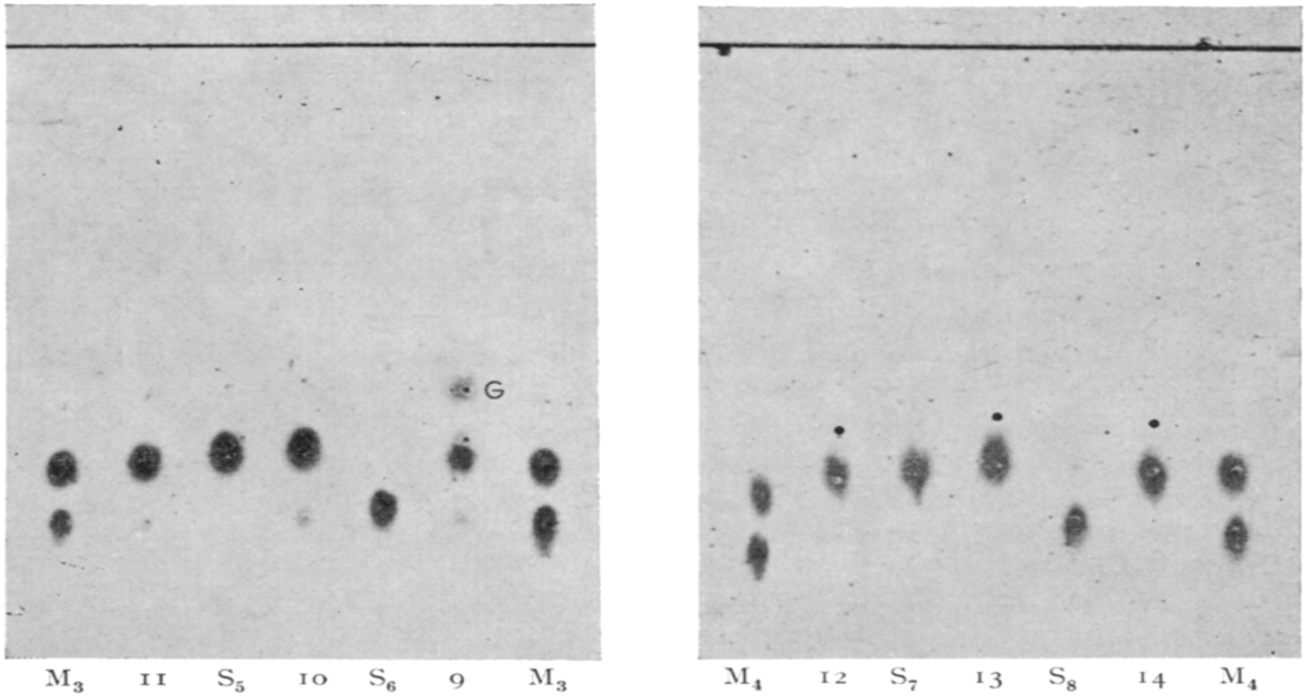

Fig. 3. Thin-layer chromatogram of commercial cinchonine 9, 10, 11. M_3 = mixture of cinchonine and dihydrocinchonine; S_5 = dihydrocinchonine; S_6 = dihydrocinchonine; G = additional component contaminating cinchonine hydrochloride.

Fig. 4. Thin-layer chromatogram of commercial cinchonidine 12, 13, 14. M_4 = mixture of cinchonidine and dihydrocinchonidine; S_7 = cinchonidine; S_8 = dihydrocinchonidine.

The technique of spotting with a calibrated platinum wire loop and a 25 min run produced qualitative results in a relatively short time.

Since butanol is a solvent in which all alkaloids of this group dissolve in the concentrations used, equal standards could be obtained. Moreover, butanol solutions of the alkaloids are well adapted to storage. The lower volatility of butanol as compared with methanol or chloroform provided for more accurate spotting of well-determined concentrations of the substances on the plates. Comparability of the results hinges on the accuracy achieved in preparing and spotting the solutions of the samples for investigation.

Preparations of salts of the alkaloids were found to be of higher purity than the free bases (Fig. 2, preparation 8).

Quinine

Dihydro-base impurities in commercial quinine preparations (Fig. 1) were detected primarily from their fluorescence and verified by the coloration (hardly visible at such concentrations) of the spot on spraying with Dragendorff's reagent. ΔR_F's for the mixture of standards and ΔR_F's for the preparations investigated amounted to 0.07–0.08. Qualitative chromatographic analysis of the quinine samples showed that dihydro-base fluorescence in the preparation of quinine hydrochloride F 3 was the weakest. More detailed observation showed that sample 3 had the lowest hydro-base content, whereas the free base preparation 1 had the highest (Table I).

TABLE I

ΔR_F AND R_F VALUES OF CINCHONA BARK ALKALOIDS IN COMMERCIAL PREPARATIONS, AND COMPARATIVE ASSESSMENT OF SAMPLES OF COMMERCIAL QUININE, QUINIDINE, CINCHONINE AND CINCHONIDINE

The numbers of the samples are those in the list in the text.

No.	*R_F of the dihydro-base*	*R_F of the vinyl-base*	*ΔR_F*	*Dihydro-base content in the commercial preparation*
1	0.42	0.50	0.08	1 > 2 > 3
2	0.41	0.49	0.08	max. min.
3	0.42	0.50	0.08	
4	0.36	0.44	0.08	6 > 7 > 5 > 4 > 8
5	0.38	0.46	0.08	max. min.
6	0.39	0.47	0.08	
7	0.38	0.46	0.08	
8	0.37	0.45	0.08	
9	0.29	0.38	0.09	10 > 9 > 11
10	0.30	0.40	0.10	max. min.
11	0.29	0.39	0.10	
12	—	0.36	0.43	
13	—	0.39	0.46	
14	—	0.37	0.46	

Kieselgel G Merck 7731. Solvent system: chloroform–methanol–diethylamine (80:20:1). Solvent front: 10 cm. The alkaloids were dissolved in butanol. 2.4 μg of alkaloid was applied to the plate. Time of run: 25 min. Spray reagent: Dragendorff by MUNIER[3].

Quinidine

In the case of quinidine (Fig. 2), the preparation of quinidine sulfate 8 showed the weakest hydro-base fluorescence with only weakly visible coloration of the spots, pointing to the lowest dihydroquinidine content. On the basis of the classification criteria assumed, the other samples were found to contain varying amounts of the latter, the quinidine preparation 6 containing the greatest amount of the dihydro-base while quinidine sulfate 8 had only traces (Table I).

In the case of the quinidine preparations, ΔR_F for separation of the base and its hydro derivative amounted to 0.08.

Cinchonine

Chromatographic analysis of cinchonine preparations (Fig. 3) revealed that cinchonine 10 contained the greatest amount of dihydro-base contamination, whereas the hydrochloride 11 had only traces (Table I).

It is noteworthy, however, that the above preparation of cinchonine hydrochloride contained yet another impurity giving an intense fluorescence and being intensely colored after spraying with the developing reagent. Its R_F value was found to be 0.49.

For the cinchonine preparations, ΔR_F for separation of the base and hydro-base ranged between 0.09 and 0.10 (Table I).

Cinchonidine

The cinchonidine preparations 12, 13 and 14 (Fig. 4) at the concentrations employed for spotting revealed no dihydro-derivatives. However, the preparations of this alkaloid gave a spot which fluoresced in U.V. light at an R_F value of 0.43–0.46 (Table I), pointing to the presence of an additional impurity. Similar fluorescence was found in some of the other samples dealt with. However, the problem requires further investigation.

The above experimental results show that qualitative chromatographic analysis on thin layers permits rapid detection of dihydroalkaloids and a comparative evaluation of their content in commercial preparations of cinchona bark.

The method as elaborated above has been applied by one of the authors for controlling the process of purification of commercial cinchona bark preparations by the THRON AND DIRSCHERL method[5].

ACKNOWLEDGEMENT

The authors wish to thank Prof. Dr. J. SUSZKO for making available the commercial preparations of the alkaloids.

SUMMARY

Fourteen commercial preparations of cinchona bark alkaloids were tested for their hydro-base content by thin-layer chromatography. From the intensity of fluorescence as well as the size and intensity of coloration of the spots, the content of dihydro-bases was assessed, thus establishing the quality of the commercial preparation in each case.

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