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INVESTIGATION OF *CINCHONA* LEAF ALKALOIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic (HPLC) system has been developed for the separation of thirteen *Cinchona* alkaloids. This system separates into two distinct groups the quinoline alkaloids, which are found predominantly in *Cinchona* bark, nd the indole alkaloids, which have been found to predominate in the leaves of one species. HPLC has been used to examine the alkaloid content of four plantation samples of *Cinchona* leaves obtained from different parts of the world. All four samples were found to contain different proportions of alkaloids and although the cinchophyllines were the major alkaloids found in two samples of *Cinchona ledgeriana*, quinoline alkaloids were also present. However, quinoline alkaloids were the major alkaloids of *Cinchona succirubra* from Thailand and *C. succirubra* × *C. ledgeriana* from Guatemala. In addition, quinamine and 3-epiquinamine were present in the four leaf samples investigated.

INTRODUCTION

Alkaloids from the bark of *Cinchona* species have been subjected to extensive studies since the discovery of the clinically useful compounds quinine and quinidine[1]. *Cinchona* leaf alkaloids, however, received relatively little attention until Potier *et al.*[2] discovered indole alkaloids in the leaves of *Cinchona ledgeriana*. More recently, Zeches *et al.*[3,4] established the structures of the cinchophyllines, a series of indole alkaloids present in the leaves of *C. ledgeriana* from a plantation at Rwanda. Zeches *et al.* did not detect any of the quinoline alkaloids in their sample of *C. ledgeriana*. Quinine and quinidine have, however, been found in leaf samples of *C. ledgeriana* from Kenya[5] and a study of *C. succirubra* from Thailand has revealed quinine, quinidine, cinchonine and cinchonidine as the major alkaloids[6].

It has been proposed that both the quinoline alkaloids and the indoles of the quinamine, cinchonamine and cinchophylline series (Fig. 1) are derived from the same biosynthetic precursors, tryptamine and secologanin[1,3]. In view of the production of these two distinct groups of alkaloids from the same biosynthetic precursors, it would be of interest to determine the relative proportions of these alkaloids in different species of *Cinchona*.

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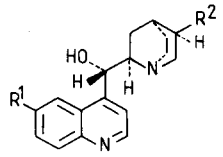
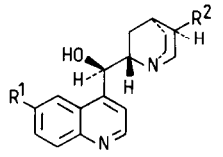
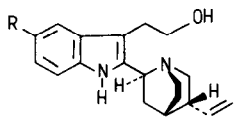


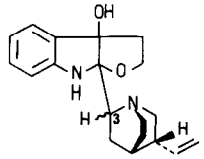
	R^1	R^2
CINCHONINE	H	$CH=CH_2$
QUINIDINE	OCH_3	$CH=CH_2$
DIHYDROQUINIDINE	OCH_3	CH_2CH_3



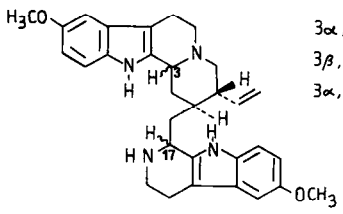
	R^1	R^2
CINCHONIDINE	H	$CH=CH_2$
QUININE	OCH_3	$CH=CH_2$
DIHYDROQUININE	OCH_3	CH_2CH_3



	R
CINCHONAMINE	H
10 METHOXY CINCHONAMINE	OCH_3



	C-3H
QUINAMINE	α
3-EPIQUINAMINE	β



	C-3H	3-17H
$3\alpha,17\alpha$ CINCHOPHYLLINE	α	α
$3\beta, 17\beta$ CINCHOPHYLLINE	β	β
$3\alpha, 17\beta$ CINCHOPHYLLINE	α	β

Fig. 1. Structures of quinoline and indole alkaloids of *Cinchona* species.

High-performance liquid chromatography (HPLC) has proved to be an ideal method for the separation of the *Cinchona* quinoline alkaloids. In general, HPLC studies have been concerned with the separation of either quinine, quinidine and their metabolites from plasma[7,8] or alternatively with the separation of quinine, quinidine, cinchonine and cinchonidine[9–12] which are the major alkaloids of *Cinchona* barks. These HPLC separations have utilised both normal and reversed-phase systems.

In this communication the separation of indole and quinoline types of *Cinchona* alkaloids, using an isocratic HPLC system, is reported. The technique has been used to compare the alkaloid composition of four *Cinchona* leaf samples obtained from plantations in different geographical regions.

MATERIALS AND METHODS

Apparatus

An Altex liquid chromatograph (Model 330) with a valve loop injection (Rheodyne 7125, 20 μl) was used. Detection was by UV absorbance at 280 nm.

Column packing

A stainless-steel tube (25 cm × 4.5 mm I.D.) was packed with 5-μm Li-Chrosorb Si 60 (Jones Chromatography Ltd.) using the slurry technique with a Jones HPLC packing instrument.

Operation procedure

The instrument was operated at laboratory temperature. The solvent system used consisted of chloroform–methanol–conc. ammonium hydroxide (500:7:1) at a flow-rate of 1.5 ml/min.

Extraction of leaf material

Leaf material (10 g) was powdered and macerated in methanol (50 ml × 2). The methanol extracts were combined, evaporated to dryness and then extracted (25 ml × 3) with 2% sulphuric acid. The aqueous acidic portions were combined, made alkaline with ammonium hydroxide solution and then extracted (× 3) into an equal volume of chloroform. The chloroform portions were combined, washed with water, dried over anhydrous sodium sulphate and evaporated to dryness. The alkaloid extracts were weighed and then re-dissolved in chloroform to give a final concentration of 5 mg/ml for analysis by HPLC.

Reference alkaloids

Quinine, quinidine, cinchonine and cinchonidine were obtained from BDH; dihydroquinine and dihydroquinidine were kindly supplied by Mr. Duncan McGregor of Lake and Cruickshank Ltd.; 3α,17α-cinchophylline, 3α,17β-cinchophylline and 3β.17β-cinchophylline were gifts from Professor L. Le Men Olivier, Faculté de Pharmacie, Reims, France.

Quinamine, 3-epiquinamine and 10-methoxycinchonamine were isolated from leaves of *Cinchona* species in our laboratories and authenticated by mass spectrometry, proton magnetic resonance, ultraviolet (UV) and circular dichroism spectroscopy[13].

RESULTS AND DISCUSSION

Interest in the production of the commercially important alkaloids, quinine and quinidine, by cell cultures of *Cinchona* species[5,12] has led us to investigate the alkaloid content of a number of *Cinchona* leaf samples. Four samples of leaves were obtained from plantations in different geographical locations.

Preliminary thin-layer chromatographic examination of the leaf alkaloid extracts revealed complex mixtures. Whilst the components could be tentatively identified by colour reactions with a range of spray reagents and fluorescence under UV light, quantitative estimation proved difficult. Gas–liquid chromatography (GLC) has been largely unsuccessful for the analysis of quinoline alkaloids and the most successful separation of the four major alkaloids has involved the use of capillary columns[14].

HPLC has proved to be one of the most useful techniques for the separation of quinoline alkaloids, consequently this technique was adopted to analyse the leaf extracts. Although the indole alkaloids of *Cinchona* have not previously been ana-

TABLE I

DISTRIBUTION PATTERNS AND t_R VALUES OF ALKALOIDS IN *CINCHONA* LEAF SAMPLES

For chemical structures, see Fig. 1. Leaf extracts: 1 = *Cinchona ledgeriana* (Kenya); 2 = *C. ledgeriana* (Zaire); 3 = *C. succirubra* (Thailand); 4 = *C. succirubra* × *C. ledgeriana* (Guatemala). − Not detected; + alkaloid detected.

Alkaloid	t_R *(min)*	*Leaf extract*			
		1	*2*	*3*	*4*
Quinamine	2.0	+	+	+	+
3-Epiquinamine	3.0	+	+	+	+
3α,17β-Cinchophylline	4.0	+	+	−	−
Cinchonamine	6.0	−	−	−	−
10-Methoxycinchonamine	7.0	−	−	+	−
3α,17α-Cinchophylline	8.5	+	+	−	−
3β,17β-Cinchophylline	12.0	+	+	−	−
Quinidine	19.0	+	−	+	+
Quinine	25.0	+	−	+	+
Cinchonine/cinchonidine	31.0	+	+	+	+
Dihydroquinidine	37.0	+	−	+	+
Dihydroquinine	45.0	+	−	+	+

lysed by this procedure, good separation was achieved. In total, thirteen alkaloids were resolved into two distinct groups using an isocratic HPLC method on microparticulate silica. The indole alkaloids, such as the quinamines, cinchonamines and cinchophyllines, eluted first from the column while the quinoline alkaloids had relatively longer retention times (Table I). Good resolution was achieved for all the reference alkaloids except cinchonine and cinchonidine which overlapped in this system.

HPLC analysis showed the four leaf samples to have markedly different alkaloid patterns (Table I, Fig. 2). All four alkaloid extracts were found to contain quinoline alkaloids, contrasting with the results of Zeches *et al.*[3] who did not detect any of the quinoline alkaloids in their sample of *C. ledgeriana* leaves from Rwanda. The relative levels of the quinoline alkaloids vary significantly between the four leaf samples examined in the present work (Fig. 2). In particular, the *C. succirubra* × *C. ledgeriana* from Guatemala contains high levels of quinine whereas the *C. ledgeriana* leaves from Zaire and *C. succirubra* from Thailand produce relatively little quinine. Despite the presence of quinine and quinidine in these *Cinchona* leaves the levels are very low when compared to those of the barks and so leaves cannot be considered as a commercial source of these alkaloids. However, knowledge of the leaf alkaloids may provide valuable information to assist the selection of suitable plant material for initiating cell and organ cultures.

All four leaf samples produced the indole alkaloids quinamine and 3-epiquinamine. Cinchonamine was not detected in any of the leaf extracts and 10-methoxycinchonamine was only identified in the *C. succirubra* leaves from Thailand. 10-Methoxycinchonamine has recently been reported as a novel, minor alkaloid in the leaves of *C. ledgeriana*[15] but this is the first report of the alkaloid occurring in the leaves of *C. succirubra*.

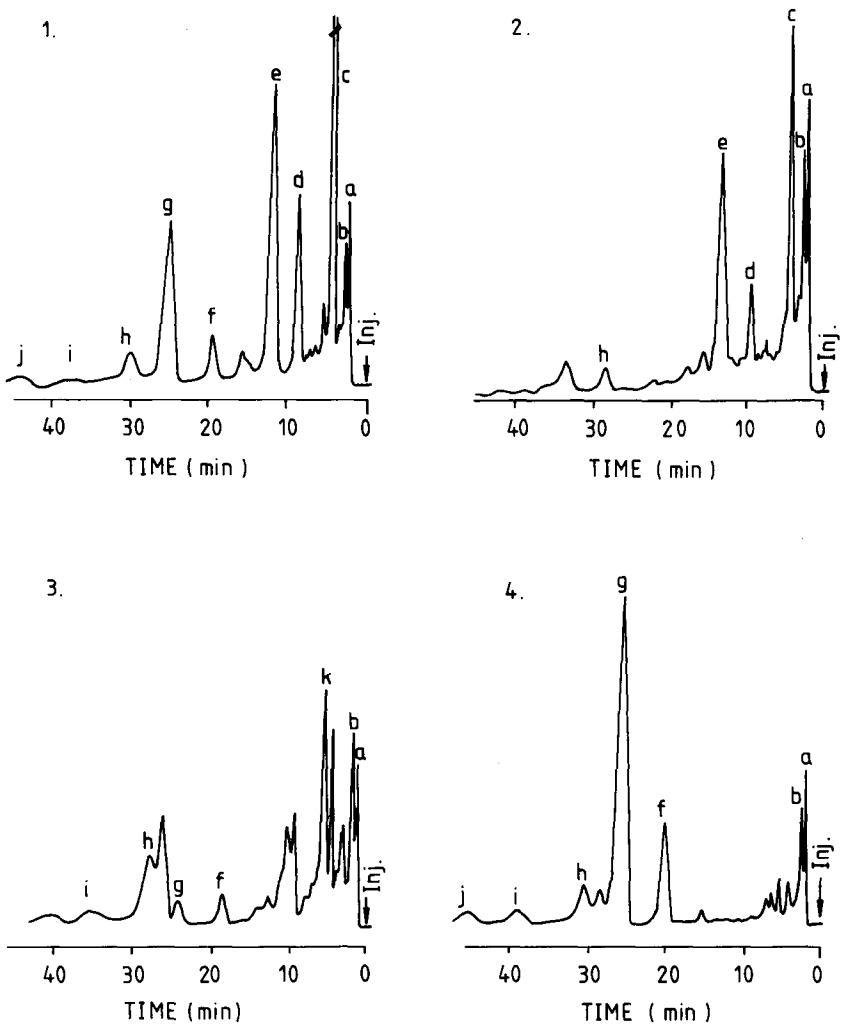


Fig. 2. HPLC separations of *Cinchona* leaf extracts: 1 = *C. ledgeriana* (Zapote variety, Kenya); 2 = *C. ledgeriana* (Zaire); 3 = *C. succirubra* (Thailand); 4 = *C. succirubra* × *C. ledgeriana* (Guatemala). Peaks: a = quinamine; b = 3-epiquinamine; c = 3α,17β-cinchophylline; d = 3α,17α-cinchophylline; e = 3β,17β-cinchophylline; f = quinidine; g = quinine; h = cinchonine/cinchonidine; i = dihydroquinidine; j = dihydroquinine; k = 10-methoxycinchonamine.

Three cinchophylline isomers were identified as relatively major components of the two *C. ledgeriana* leaf extracts. These cinchophylline alkaloids were not detected in either the *C. succirubra* or the *C. succirubra* × *C. ledgeriana* leaves. These findings correlate with the results of Zeches *et al.*[3] who found the cinchophylline isomers in their *C. ledgeriana* leaf sample and with Mulder-Krieger *et al.*[15] who detected two of the isomers, 3α,17α- and 3α,17β-cinchophylline, in a sample of *C. ledgeriana* of undefined geographical origin.

It is not known whether the difference in the alkaloid patterns of the four leaf samples are due to genetic or edaphic factors. Until now, the cinchophylline-type alkaloids have only been found in samples of *C. ledgeriana* leaves. Apart from *C. succirubra* none of the other 30 or more species of *Cinchona* have been evaluated for these rare alkaloids. It would be of interest to investigate whether the cinchophyllines are widely distributed throughout the genus or if they are unique to one species. The latter situation is found with the closely related roxburghine-type alkaloids, similarly derived from one secologanin and two tryptamine units, which are found in only one of the 34 species of the neighbouring genus, *Uncaria*[16].

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