Journal of Chromatography, 234 (1982) 521–527
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,292

Note

Thin-layer and short-column chromatography of partially reduced Cinchona alkaloids

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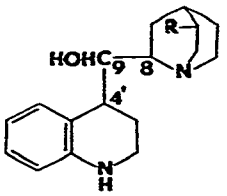
(Received August 13th, 1981)

Partial reduction of Cinchona alkaloids in the 1′,2′,3′,4′ positions of the molecules gives rise to a new chiral centre at C-4′, and consequently from each parent alkaloid two epimeric 1′,2′,3′,4′-tetrahydro derivatives, designated α and β, are

TABLE I

STRUCTURE OF PARTIALLY REDUCED CINCHONA ALKALOIDS



In the 9-deoxy compounds the chiral centre at C (9) is replaced by a methylene group. This structural modification is designated in abbreviation by the prefix 9D. The absolute configuration around the 4′ chiral centre was unambiguously established for α-1′,2′,3′,4′,10,11-hexahydrocinchonine[11]. Other assignments were derived from data for αHHC, comprehensive optical information and chemical interconversions being available. In a pair of epimeric free bases, the prefix α is given to the one having the more positive specific rotation.

Compound	*Abbreviation*	*Absolute configuration*			*R*
		C (8)	*C (9)*	*C (4′)*	
					10 11
Derivatives of cinchonine	C	R	S	–	$CH=CH_2$
and 10,11-dihydrocinchonine	HC	R	S	–	$CH_2–CH_3$
α-1′,2′,3′,4′-Tetrahydrocinchonine	αTHC	R	S	R	$CH=CH_2$
β-1′,2′,3′,4′-Tetrahydrocinchonine	βTHC	R	S	S	$CH=CH_2$
α-1′,2′,3′,4′,10,11-Hexahydrocinchonine	αHHC	R	S	R	$CH_2–CH_3$
β-1′,2′,3′,4′,10,11-Hexahydrocinchonine	βHHC	R	S	S	$CH_2–CH_3$
Derivatives of cinchonidine	Cd	S	R	–	$CH=CH_2$
and 10,11-dihydrocinchonidine	HCd	S	R	–	$CH_2–CH_3$
α-1′,2′,3′,4′-Tetrahydrocinchonidine	αTHCd	S	R	R	$CH=CH_2$
β-1′,2′,3′,4′-Tetrahydrocinchonidine	βTHCd	S	R	S	$CH=CH_2$
α-1′,2′,3′,4′10,11-Hexahydrocinchonidine	αHHCd	S	R	R	$CH_2–CH_3$
β-1′,2′,3′,4′,10,11-Hexahydrocinchonidine	βHHCd	S	R	S	$CH_2–CH_3$

0021-9673/82/0000–0000/$02.75

formed (Table I). These partially reduced alkaloids are of interest for their antiinflammatory activity[1,2] and their synthetic utility for a number of transformations which are not otherwise possible directly[3–7].

Previous methods of separation of the C-4′ epimers employed conversion into crystalline derivatives, fractional crystallization of the latter and, in a few cases, subsequent regeneration of a free base[2,8–10]. These methods are rather tedious and frequently failed to provide both epimers. This was due mainly to the complexity of the mixtures after reduction, the desired compounds having usually accompanied by a pair of 9-deoxy analogues.

In the present paper we report a simple thin-layer chromatographic (TLC) method for complete separation and identification of a series of partially reduced Cinchona alkaloids derived from cinchonine and cinchonidine, together with TLC and short-column preparative procedures for their isolation.

The possibilities of chromatographic separation were studied with mixtures obtained by chemical reduction with sodium and ethanol. This method of reduction gives the most complex mixtures.

EXPERIMENTAL

Compounds and reagents

Parent alkaloids were reduced with sodium and ethanol as described earlier[12]. The resulting crude product was reduced again using 18 g sodium and 250 ml ethanol per 5 g of starting alkaloid. All solvents were purified by standard methods and redistilled.

Solvent systems

Solvent systems suitable for the TLC separation of other Cinchona alkaloids[13], together with several new ones, were tested on the crude mixtures of reduction products. The following systems gave the best separations:

S1 chloroform–methanol–25% ammonia (85:14:1)
S2 dichloromethane–diethyl ether–diethylamine (20:15:5)
S3 acetone–25% ammonia (58:2)
S4 ethyl acetate–isopropanol–25% ammonia (45:35:5)
S5 carbon tetrachloride–*n*-butanol–methanol–10% ammonia (12:9:9:1)
S6 dichloromethane–isopropanol–triethylamine (90:10:15)
S7 chloroform–methanol–triethylamine (85:5:10)
S8 ethyl acetate–isopropanol–triethylamine (5:2:1)
S9 chloroform–methanol–triethylamine (85:14:1)
S10 ethyl acetate–isopropanol–triethylamine (17:2:1)

Analytical TLC

The TLC plates were 20 × 20 cm or 20 × 5 cm silica gel 60 F_{254} pre-coated glass plates, with a layer thickness of 0.25 mm (E. Merck, Darmstadt, G.F.R.). They were not activated before use. The chromatograms were developed at room temperature in a chamber lined with filter-paper and pre-saturated with the solvents for at least 30 min. The alkaloids were spotted 3.0 cm above the bottom of the plates and the plates were developed over a distance of 10 cm. The hR_F values given in Table II were determined using pure compounds isolated by preparative TLC (see below) and calculated from at least four chromatograms.

Preparative TLC

The TLC plates were 20 × 20 cm silica gel 60 F_{254} Merck glass plates with a layer thickness of 2 mm, not activated before use. Samples (0.15 g) of the mixture of alkaloids were dissolved in ethanol and applied as a thin band 3 cm from the bottom of the plate, and developed in a carefully saturated chamber with solvent S7. The solvent was allowed to rise 15 cm from the starting line. The plates were dried at ambient temperature. The bands of resolved compounds were located in UV light, and then scraped off with a spatula and eluted with ethanol. The composition of the resulting eluates was checked by TLC in systems S4, S7 and S8. The separation was not complete. Mixed fractions were rechromatographed in solvent S4 in the case of the reduction products of cinchonine and dihydrocinchonine, and in solvents S6, S7 and S8 for derivatives of cinchonidine and dihydrocinchonidine (Table III). The isolated components of the mixture after reduction of dihydrocinchonine were identified by comparison with reference compounds isolated earlier by chemical procedures[14,15]. Other assignments were derived from mass spectra and optical rotation data.

Short-column chromatography

The columns were prepared as follows. A slurry of silica gel (Macherey, Nagel & Co. HF_{254}, *ca.* 70 g) in solvent S9 (300 ml) was poured into a glass column (3.5 cm I.D.) and allowed to settle. Solvent S9 was then pumped through the system at flow-rate 1.5 ml/min until the absorbent became uniform, the flow was stopped and the column was kept overnight before use. The crude mixture of alkaloids (0.6–0.9 g) was dissolved in solvent S9 (5 ml), applied on the column and eluted with the same solvent. The volumes of the eluted fractions collected and the flow-rates varied for individual groups of reduction products (Table IV).

The completeness of separation was monitored by TLC in solvents S7, S8 and S9. To achieve complete separation, some fractions were rechromatographed —the most mobile fraction from the mixture of cinchonidine derivatives in solvent S10, and the less mobile fraction from the mixture of dihydrocinchonidine derivatives in solvent S7 (Tables III and IV). Chromatographically homogeneous fractions were pooled, evaporated to dryness and coevaporated with isopropanol until the smell of triethylamine was no longer present. The individual components were identified as described under *Preparative TLC*.

RESULTS AND DISCUSSION

Table II summarizes the results with eight solvent systems found to give the best separations of partially reduced derivatives of cinchonine and cinchonidine. Two TLC systems, S6 and S7, are suitable, with all the groups of investigated modified alkaloids, for the analytical separation of four partially hydrogenated products within the particular groups. Two other solvents, S1 and S4, also give good separations with the exception of HCd derivatives.

There are distinct differences in the separations between cinchonine and cinchonidine derivatives. The latter show much higher differences in chromatographic mobility between oxy and deoxy compounds than do the former, *e.g.*, in solvents S1, S6 and S7. Nevertheless, system S7 gives satisfactory results also for cinchonine

TABLE II

hR_F VALUES OF PARENT AND PARTIALLY REDUCED CINCHONA ALKALOIDS

The values were calculated from at least four chromatograms run under the following conditions: silica gel 60 F_{254} pre-coated glass plates, 20 × 20 cm (Merck); temperature 21 ± 3°C; normal chromatography chamber, saturated for 30 min before use; distance travelled 10 cm.

Alkaloid	*Solvent*							
	S1	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*
Cinchonine (C)	40	37	41	52	76	53	37	41
αTHC	30	47	44	47	56	51	37	41
βTHC	23	47	36	33	35	46	31	31
α9DTHC	50	58	53	60	73	71	60	50
β9DTHC	34	47	37	39	46	55	49	32
10,11-Dihydrocinchonine (HC)	27	28	34	42	58	38	23	31
αHHC	19	43	34	36	41	40	23	33
βHHC	14	43	24	22	22	32	20	23
α9DHHC	38	57	44	49	57	66	57	45
β9DHHC	24	43	29	30	35	46	36	25
Cinchonidine (Cd)	36	27	38	48	73	42	30	34
αTHCd	29	50	44	45	51	56	41	43
βTHCd	23	42	41	41	50	46	29	38
α9DTHCd	41	54	45	51	62	64	58	44
β9THCd	45	60	52	55	66	70	62	51
10,11-Dihydrocinchonidine (HCd)	25	25	33	44	65	35	22	30
αHHCd	18	46	35	34	35	43	25	32
βHHCd	17	38	33	31	42	37	21	32
α9DHHCd	30	55	33	44	48	62	51	38
β9DHHCd	36	61	44	46	57	69	60	46

derivatives. In contrast, the separation of the epimers within each pair is much easier to achieve in the series of cinchonine derivatives (solvents S4, S5, S6 and S7 for pairs of deoxy compounds, S4 and S5 for pairs of oxy derivatives) than in the cinchonidine series.

The expected order of mobility is that deoxy alkaloids precede compounds with 9-hydroxy substituents. This is found in the cinchonidine series, but only partly for cinchonine derivatives. In some chromatographic systems (S3, S4, S5, S8), αTHC and αHHC are more mobile than their β-deoxy congeners. Because of this effect the differences in hR_F values between epimers become favourable, *e.g.*, Δ21 for α- and β9DTHC in solvent S4 and for α- and βTHC in solvent S5.

Of the epimeric reduced alkaloids, the α compounds, *i.e.*, those whose partial rotatory contribution at C-4′ is positive, usually have higher R_F values than their counterparts in all solvent systems tested. In two deoxy pairs derived from cinchonidine this order is reversed, β9DTHCd and β9DHHCd being more mobile than the respective α compounds. Solvent S5 is exceptional, in that βHHCd is more mobile than αHHCd.

All the newly developed solvent systems S6, S7 and S8 contain triethylamine.

TABLE III

ROUTES OF CHROMATOGRAPHIC SEPARATIONS OF MIXTURES OF CINCHONA ALKALOIDS PARTIALLY REDUCED BY SODIUM AND ETHANOL

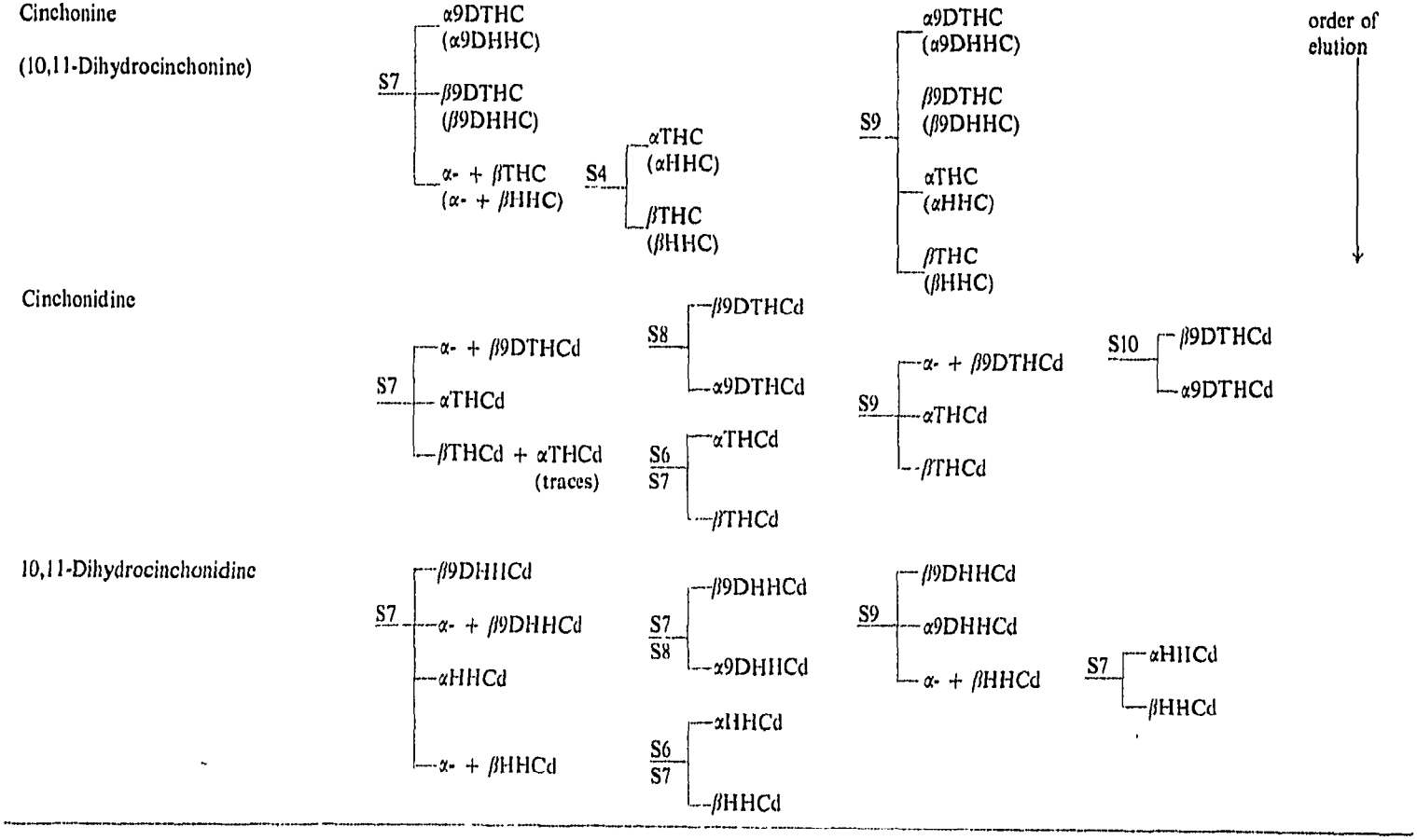


TABLE IV

SHORT-COLUMN CHROMATOGRAPHY OF MIXTURES OF CINCHONA ALKALOIDS PARTIALLY REDUCED BY SODIUM AND ETHANOL

Solvent: S9

Parent alkaloid	*Height of column (cm/0.1 g)*	*Flow-rate*	*Volume of fractions collected (ml)*	*Components isolated* and % of starting mixture*				*Total recovery (%)*
Cinchonine (C)	3.0	1.1	5	α9DTHC	β9DTHC	αTHC	βTHC	70
				19	10	11	30	70
Dihydrocinchonine (HC)	2.2	1.2	15	α9DHHC	β9DHHC	αHHC	βHHC	
				9	16	16	26	67
Cinchonidine (Cd)	2.2	1.4	10	β9DTHCd**	α9DTHCd**	αTHCd	βTHCd	
				11	4	26	13	54
Dihydrocinchonidine (HCd)	3.0	1.1	10	β9DHHCd	α9DHHCd	αHHCd***	βHHCd***	
				17	18	22	6	63

* In order of elution.

** After rechromatography in solvent S10.

*** After rechromatography in solvent S7.

the presence of which is necessary to obtain successful separations of partly reduced alkaloids. Triethylamine was not used as a component of the systems giving the best results in the TLC of Cinchona alkaloids. Solvents S6 and S7 may find broader application as new systems for the separation of the vinyl and dihydro alkaloids. The differences between the R_F values in these systems are equal to or higher than those in the literature[13].

For preparative purposes, thin-layer and short-column approaches were investigated (Table III) TLC may be recommended for small samples or for fast isolation of some compounds e.g. α9DTTC or α9DHHC. Generally, however, the short-column procedure is superior because of the smaller number of chromatographic steps needed to obtain chromatographically homogeneous compounds. This approach was therefore studied in more detail.

The results are given in Table IV. Solvent S9 on a short silica gel column is able to separate in one operation four partly reduced alkaloids derived from cinchonine or 10,11-dihydrocinchonine. In the case of the cinchonidine series, α- and β9DTHCd as well as α- and βHHCd are not resolved and have to be rechromatographed in solvents S10 and S7 respectively.

CONCLUSION

The application of the described chromatographic methods enables the rapid, simple and complete separation of partially reduced Cinchona alkaloids. In addition to known 10,11-dihydrocinchonine derivatives, tetra- and hexahydro bases derived from cinchonine, cinchonidine and 10,11-dihydrocinchonidine were isolated for the first time. Their chemical and spectroscopic characterization will be described elsewhere.

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