PAPER CHROMATOGRAPHIC ANALYSIS OF *STRYCHNOS* ALKALOIDS*

G. B. MARINI-BETTÒLO
Istituto Superiore di Sanità, Rome (Italy)

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INTRODUCTION

The importance of chromatography for the study of natural substances is well known, and in many cases chromatographic methods have been of the greatest importance in the approach to several problems. For example the study of calabash curares and of *Strychnos* alkaloids used by South American Indians for the preparation of these curares, was only made possible in recent years by the use of both analytical and preparative chromatographic methods. The most complex mixtures of alkaloids so far known were separated and identified by these methods.

The raw alkaloid extract has in many cases been found to contain 20–30 different substances, some of which are rather unstable, and each of them being present in only small amounts.

In a previous report a complete account of methods both on an analytical and a preparative scale was given[1].

We shall now review the application of paper chromatographic methods to the study of *Strychnos* alkaloids.

Recent chemical research has demonstrated that no substantial differentiation can be made between *Strychnos* alkaloids of the strychnine group that are found in African and Asiatic species of this genus and the quaternary alkaloids from South American species. In recent studies by KING, CARNEIRO, WIELAND, KARRER, SCHMID and MARINI-BETTÒLO and coworkers it has been demonstrated that the quaternary alkaloids from South American species are identical with the quaternary alkaloids of curares (for literature see ref.[2]).

Actually the structure of strychnine (I) can now be related biogenetically to the quaternary alkaloids of curares (III) through the Wieland-Gumlich aldehyde, which was found as caracurine VII in a *Strychnos toxifera* from Venezuela. The Wieland-Gumlich aldehyde may under certain conditions be converted into dihydrotoxiferine, as demonstrated by KARRER AND SCHMID[3], while on the other hand it is known that the same aldehyde can be transformed into strychnine as shown by ROBINSON[4].

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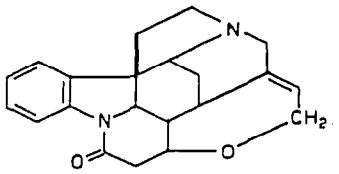
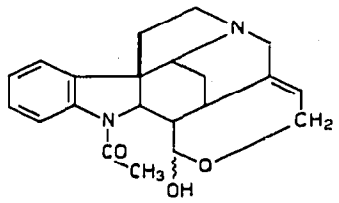
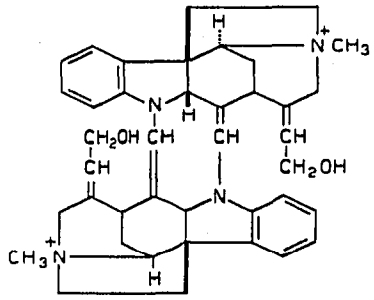
Diaboline, an alkaloid characteristic of South American *Strychnos*[5] was recently found[6] in a Malayan *Strychnos*, *Strychnos* KL 1929. BATTERSBY AND HODSON[7] have shown that diaboline (II) is the N-acetyl derivative of the Wieland-Gumlich aldehyde.



(I) STRYCHNINE



DIABOLINE (II)



TOXIFERINE (III)

This alkaloid common to both South American and Asiatic *Strychnos*, may represent the missing link between the strychnine and curare alkaloids[8]. For this reason it must be assumed that henceforth all *Strychnos* alkaloids should be considered and studied as a single group. The only differentiation that can be made is based on their chemical behaviour, *i.e.* tertiary and quaternary alkaloids, and anhydronium bases should be considered separately. Separation of these three groups can be achieved by chemical methods based on the extraction of their solutions at different pH's, with organic solvents, and by precipitation from the previously extracted solution with Reinecke's salt or picric acid.

PAPER CHROMATOGRAPHY AND ELECTROPHORESIS OF TERTIARY BASES

The *Strychnos* tertiary alkaloids so far known belong to the strychnine group and to some less studied bases from African species.

The separation of strychnine alkaloids has been studied by BÜCHI AND SCHUMACHER[9], who have established conditions for separating brucine and strychnine.

JAMINET[10] has proposed isobutanol, isopropanol with acetic acid or NH_4OH for the separation of alkaloids from *S. holstii*. With these solvents he was able to separate strychnine, brucine, genostrychnine, dihydrobrucine, α- and β-colubrine, vomicine and holstiine.

Recently we came to consider this problem again in order to find a way to follow the transformation of the Wieland-Gumlich aldehyde by paper chromatography, and to analyse rapidly the alkaloid mixture of Asiatic *Strychnos* species and of arrow

poisons used in Malaya. For this purpose MARINI-BETTÒLO AND CAGGIANO[11] used four different solvents, namely butanol–water, butanol–acetic acid–water and KARRER's solvents C and D. The results are reported in Table I.

From the R_F values it can be deduced that it is rather difficult to separate strychnine from its homologues, such as α- and β-colubrine, whereas it is easier to separate brucine, and even more so vomicine, when the pH of the solvent is alkaline, diaboline

TABLE I

Alkaloid	R_F			
	BuOH–AcOH–H_2O	*BuOH–H_2O*	*Solv. C*	*Solv. D*
Desacetyldiaboline	0.67	0.61	0.26	0.58
Diaboline	0.61	0.36	0.24	0.34
Strychnine	0.67	0.49	0.42	0.42
α-Colubrine	0.67	0.44	0.20	0.49
β-Colubrine	0.69	0.42	0.33	0.46
Brucine	0.57	0.44	0.26	0.53
Dihydrobrucine	0.61	0.41	0.28	0.29
Vomicine	0.66	0.70	0.82	0.83
N-Oxystrychnine	0.70	0.60	0.26	0.33
Retuline	0.69	0.53	0.24	0.37
Holstiine	0.66	0.47	0.33	0.40
Alkaloid C from *S. icaja*	0.56	0.57	0.61	0.65

can be more easily differentiated from strychnine, especially in solvent C and D.

On the other hand we have not succeeded in establishing a relationship between the R_F values of the different compounds.

Also in the case of structurally related compounds such as strychnine, α- and β-colubrine, and brucine, it is not possible to find any additive properties. We have, however, found paper electrophoresis advantageous for a rapid identification of these alkaloids.

By performing experiments at different pH values it was possible to obtain migration values for each alkaloid, from which several compounds can be rapidly recognized, *viz.* strychnine, diaboline, vomicine, etc.; on the other hand it is rather difficult in this case also to differentiate strychnine from α- and β-colubrine and brucine (Table II).

PAPER CHROMATOGRAPHY OF QUATERNARY BASES

The separation by paper chromatography of *Strychnos* quaternary alkaloids, *i.e.* of a curare alkaloid, was studied by SCHMID AND KARRER[12], WIELAND AND MERZ[13] and MARINI-BETTÒLO and coworkers[14].

The presence of a quaternary group in the molecule greatly affects the physicochemical behaviour of these substances and renders rather difficult the separation of several alkaloids that are also structurally related.

TABLE II

Alkaloid	*Migration in mm (in 3 h)*		
	pH 2.5	*pH 6.8*	*pH 10.4*
Diaboline	58	64	38
Strychnine	58	59	24
α-Colubrine	51	52	25
β-Colubrine	53	52	21
Brucine	51	51	20
Dihydrobrucine	50	51	22
Vomicine	52	17	9
N-Oxystrychnine	46	13	19
Pseudostrychnine	47	19	16
Retuline	60	68	50
Holstiine	57	62	25
Alkaloid C from *S. icaja*	36	16	15

A great improvement in the paper chromatographic separation of these alkaloids was introduced by KARRER, SCHMID and coworkers, by the use of mixed solvent systems for two-dimensional separations. One-dimensional techniques for this group of substances are of little value, because of the great number of compounds present. Moreover, reproducibility of R_X data is rather difficult to obtain, owing to the fact that the chromatogram must flow for many hours. Usually galactose or curarine are used as reference substances, but also in this case there are many limitations, such as, for instance, the amount of substance used for the chromatogram, which greatly affects the R_X value.

An observation of first importance was that the quaternary alkaloids can be divided into two classes, the "fast running" and the "slow running". The most impressive fact in this respect, brought to light by the pharmacological tests of Prof. WASER, was that many of the "slow running" alkaloids are much more active in causing curarization than the "fast running" ones[15]. This was explained years later when the structure of some of these alkaloids was elucidated.

Slow running alkaloids are generally biquaternary ammonium derivatives, whereas the fast running alkaloids are monoquaternary ammonium salts.

This is an excellent example of the influence of the structure on the chromatographic behaviour of a substance, although it is rather difficult to establish a strict correlation between structure and R_M value because of the high complexity of the molecules, and because there is no common fundamental group for all the alkaloids of these plants.

Melinonines are related to the yohimbane systems, whereas alkaloids such as hemitoxiferine are related to strychnine, and fluorocurine to indole compounds.

We may take some synthetic quaternary salts as a model for natural quaternary alkaloids, and consider the R_F values, for instance of some quaternary salts studied by MARINI-BETTÒLO[16] and MARINI-BETTÒLO AND MIRANDA[17]. It can be observed in this case that the R_F values increase with the molecular weight of the compounds, whereas the diammonium salts have smaller R_F values (Table III).

TABLE III

$A—O\overset{+}{N}(CH_3)_3I^-$		$I^-(CH_3)_3\overset{+}{N}—O—B—O—\overset{+}{N}(CH_3)_3I^-$		$A—\overset{+}{N}(CH_3)_3I^-$	
A	R_F	*B*	R_F	*A*	R_F
CH_3—	0.61			CH_3—	0.43
C_2H_5—	0.64			C_2H_5—	0.61
C_3H_7—	0.72			C_3H_7—	0.67
C_4H_9—	0.76				
C_5H_{11}—	0.81	—C_5H_{10}—	0.64		
C_6H_{13}—	0.85	—C_6H_{12}—	0.52		
$C_{10}H_{21}$—	0.92	—$C_{10}H_{20}$—	0.88		

PAPER CHROMATOGRAPHY ON AN ANALYTICAL SCALE OF QUATERNARY ALKALOIDS FROM *Strychnos* AND CURARE

For the separation of curare and *Strychnos* quaternary alkaloids by paper chromatography a number of solvents were used, as reported in a previous review[1]. The most important were KARRER's solvents C (methyl ethyl ketone–water) and D (pyridine–ethyl acetate), and butanol–water as proposed by BOEKELHEIDE.

MARINI-BETTÒLO AND LEDERER[18] also employed aqueous solvents as previously suggested by BOSCOTT, for *Rauwolfia* alkaloids. In this case it is possible to obtain a clear inversion of some spot sequences that may present the resolution of different alkaloid mixtures.

Paper electrophoresis has also proved valuable in analytical work; this was also reviewed in a former paper[1,19].

Although a large number of experiments were performed in order to separate these alkaloids, it has so far been impossible to identify by paper chromatography all the constituents of a natural mixture, with the exception of a few alkaloids, for the following reasons.

(a) Many quaternary alkaloids have been described up to the present, but there are many others, exhibiting similar chemical behaviour, that have not yet been identified.

(b) The long developing time for the solvent (from 14 to 18 h) affects the R_C values of the different compounds under certain conditions, so that these values are only reproducible with difficulty (influence of the displacement of the reference substance[20], and of the quantity of substances employed[11]).

(c) The R_C values of various alkaloids are affected by the presence of other alkaloids; *i.e.* in a mixture of three or more alkaloids the R_C of one of these may be different from the value obtained when it is chromatographed alone. These interactions are much more evident than in the case of other mixtures of natural substances, such as for example amino acids.

(d) It is difficult to obtain pure samples of the minor alkaloids for purposes of control or for comparing R_C values under different conditions.

(e) It is generally very difficult to obtain an alkaloid in a pure state. Repeated paper chromatography on the successively purified samples may lead to the identi-

fication of minor compounds. This is due to the fact that by successive concentration of alkaloids present in traces, the minimum concentration for detection by colour reactions may be reached.

Another possibility is that during the various operations unstable alkaloids may give rise to transformation products. For the identification it is advisable in this case to carry out first a separation by one-dimensional paper chromatography or paper electrophoresis. The single fractions are then eluted and submitted to two-dimensional chromatography.

In this case, the identification of several constituents is based for the above-mentioned reasons, not only on the R_C value, but also on chromatic reactions and the spectroscopic behaviour of the single spot.

Fluorescent spots are first detected by observation under U.V. light, while observation with U.V. light with special Dow Corning 9863 filters, permits the recognition of the substances as black spots*.

For this reason in these particular cases the colour reaction and the fluorescence of these compounds are of great importance in connection with the R_C value. Elution of the spot and spectrophotometry may be used to confirm the fundamental structure of the compound.

The methods discussed here were largely used in the identification and characterisation of chromatograms of many alkaloid mixtures of South American *Strychnos*.

PREPARATIVE PAPER CHROMATOGRAPHY AND ELECTROPHORESIS OF QUATERNARY ALKALOIDS FROM *Strychnos* AND CURARE

For preparative purposes paper chromatography is widely used for the separation of *Strychnos* alkaloids. The paper band technique was employed successfully in the case of the alkaloids from *S. solimoesana*[21], *S. guianensis*[22] and *S. subcordata*[23].

Whatman paper sheets No. 1 or 3 MM are used, the substance being placed along a line on the paper, and successively developed with an adequate solvent. Bands are obtained, which have the advantage over other separation methods in that the formation of tails and mixing of two or more compounds is avoided.

The bands are detected as described above, and the zones containing the alkaloids are cut out, eluted and subjected to further chromatography until pure alkaloids are obtained.

In this case it is advisable to avoid using pyridine as solvent, because it is difficult to eliminate and also causes difficulties in the spectrophotometric control of the product.

Paper band electrophoresis has also been used with success in conjunction with paper band chromatography, which sometimes yields a better purification of single fractions.

* This property is common to substances absorbing around 250 mμ.

The band method was largely used by KARRER AND SCHMID[24] to follow transformations of these alkaloids effected on a small scale.

This method was also applied with success to the final purification of the alkaloids of *S. parvifolia*[25], used alternately with chromatography on ion-exchange resins, the purification of several alkaloids being achieved.

SUMMARY

A critical review of the paper chromatography of *Strychnos* alkaloids has led to the following conclusions:

1. Paper chromatography is one of the most important methods of approach in elucidating the chemistry of *Strychnos* alkaloids.

2. Paper chromatography and electrophoresis, in the case of *Strychnos* tertiary alkaloids, are of great value for the rapid analytical identification of the various compounds. In the case of quaternary alkaloids these methods can give general information, but no absolute conclusions, about a great number of alkaloids so far known of this group, and facilitate the identification of some of the more common bases.

3. No absolute relation between R_M value and structure can so far be derived from the R_C values of *Strychnos* alkaloids. This is due to the extremely complicated structures of these alkaloids, which belong to several chemical groups, and to the difficulty of obtaining standard R_C values for many quaternary alkaloids.

4. Paper electrophoresis of *Strychnos* tertiary alkaloids is of great importance for the identification of the different strychnine derivatives.

5. Paper chromatography and electrophoresis must be considered as the best methods hitherto used for the preparative purification of small quantities of *Strychnos* quaternary alkaloids.

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