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GAS–LIQUID CHROMATOGRAPHY OF TERTIARY *STRYCHNOS* ALKALOIDS

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SUMMARY

The gas–liquid chromatography of tertiary *Strychnos* alkaloids is described. Examination of the alkaloids from *S. nux-vomica* L. has shown that the method can be readily used for the detection and estimation of strychnine in brucine and *vice versa*; a new minor base, icajine, has been detected and isolated.

Alkaloids from *S. icaja* Baill. have also been examined. JAMINET's alkaloid A from this plant has been shown to be a mixture of vomicine and icajine.

Relationships between the relative retention times and chemical structures are discussed.

INTRODUCTION

In 1960 VANDENHEUVEL and co-workers[1] showed that gas–liquid chromatography (GLC) could be applied to the separation of many different types of alkaloids. They used a 6 ft. × 4 mm I.D. column packed with 2–3 % SE-30 (a methylsilicone polymer) on 80–100 mesh Chromosorb W and worked at temperatures mostly between 204° and 222°. Under these conditions strychnine had a retention time of 25.9 min and brucine of 80.0 min.

Later studies have been concerned with the use of GLC in toxicological work. PARKER *et al.*[2] and BROCHMANN-HANSSEN AND FONTAN[3] investigated the application to the separation of alkaloids of other stationary phases, in order of increasing polarity: XE-60 (a cyanosilicone polymer), EGSS-Y (a polyester methylsilicone polymer), and HI-EFF-8B (a cyclohexane dimethanol succinate polyester). It was found that while a polar stationary phase tended to be more selective, the retention times were significantly increased with increasing polarity of the stationary phase. Table I records the retention times of strychnine for these various phases.

Another study[4] with dichlorodimethylsilane-coated Gas-Chrom Q coated with 1 % neopentyl glycol succinate with or without 1 % polyvinylpyrrolidone (PVP) showed that alkaloids containing alcoholic or phenolic groups had their retention times greatly prolonged by the inclusion of PVP and bases with an aromatic ring

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TABLE I

THE RETENTION TIMES OF STRYCHNINE ON COLUMNS WITH STATIONARY PHASES OF INCREASING POLARITY

Borosilicate glass column 3 ft. × 0.07 in., filled with Gas-Chrom P treated with HMDS + 1% stationary phase[3].

Phase	*Temperature (°C)*	*Retention time (min)*
SE-30	225	11.6
XE-60	220	52.9
EGSS-Y	230	75.0
HI-EFF-8B	240	90.2

system were also affected in the same way but to a lesser degree. Thus, without PVP strychnine had a retention time of 59.4 min and with PVP of 97.9 min. Again the application was primarily to toxicological analysis and a wide range of products was examined.

TERTIARY *Strychnos* ALKALOIDS

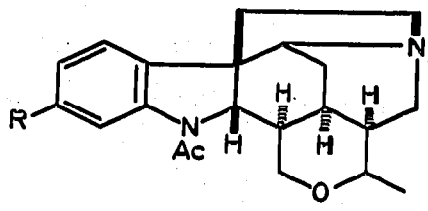
The best known tertiary *Strychnos* alkaloids are strychnine and brucine[5], which occur abundantly in the pharmaceutically important seeds of *S. nux-vomica* L. and *S. ignatii* Berg. Altogether nine tertiary alkaloids, whose formulae are shown in Fig. 1, have been obtained from *S. nux-vomica*, but quantitatively strychnine and brucine predominate by far. Much effort has been directed towards the analysis of these two bases. Many methods have been elaborated, including gravimetric and titrimetric analyses[6], paper chromatography[6,7,23] thin-layer chromatography (TLC)[8], and even nuclear magnetic resonance spectrometry[9]. Analysis of *S. nux-vomica* seeds by paper chromatography or TLC enables strychnine and brucine to be separated, but to do the same for the subsidiary alkaloids may require the use of several different solvent systems.

During studies on *Strychnos* alkaloids, the possibility of applying GLC to the analysis of their mixtures has been investigated. One of the aims was to develop a rapid method for the detection and estimation of small amounts of strychnine in brucine and *vice versa*. Brucine is currently added to denatured alcohol as a tracer and the purity for the brucine must be rigidly controlled. Another aim was to examine the minor alkaloids in the mother liquors from the crystallization of brucine sulphate.

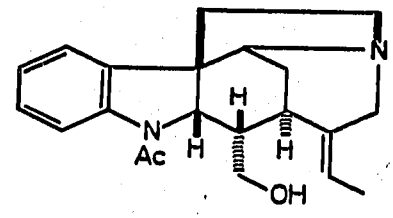
Experimental conditions and retention times

The work discussed in the introduction shows that if brucine and less polar bases like vomicine and novacine are chromatographed on any very polar stationary phase, their retention times will be very long. Such stationary phases would not be suitable for a rapid analysis. The slightly polar SE-52 (a methylphenylsilicone polymer) was therefore tried and proved satisfactory for the purpose in view.

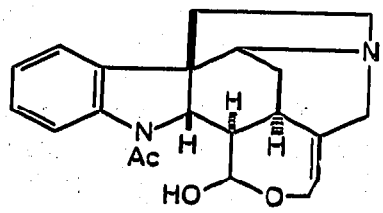
The apparatus used in the experiments was an Aerograph 204-1B, provided with a hydrogen-flame ionization detector (hydrogen flow rate 25 ml/min). The carrier gas was nitrogen at a flow rate of 35 ml/min. The recorder was a Honeywell. A 2 ft. × 1/8 in. Inox (stainless steel) column filled with 100–120 mesh Aeropak 30 coated with



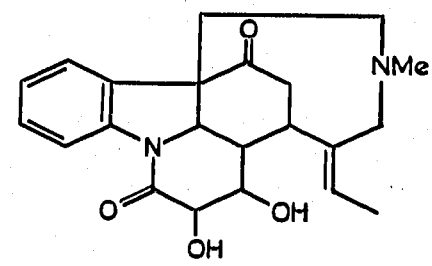
R = H Spermostrychnine
R = OMe Strychnospermine



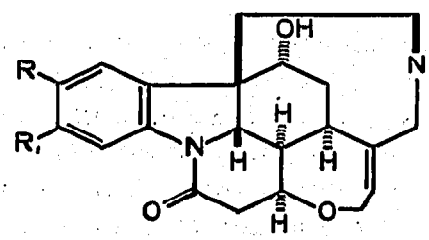
Retuline



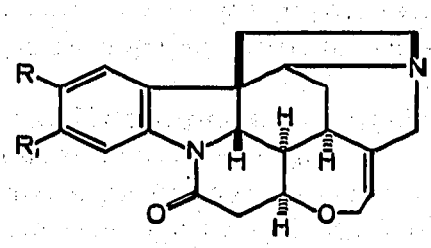
Diaboline



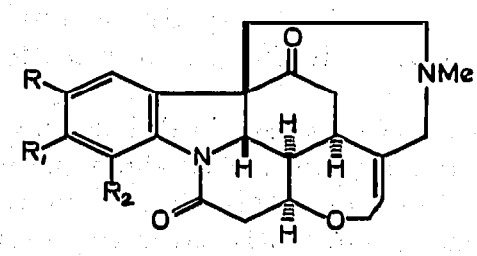
Holstiine ?



R = R, = H Pseudostrychnine
R = R, = OMe Pseudobrucine



R = R, = H Strychnine
R = H, R, = OMe α-Colubrine
R = OMe, R, = H β-Colubrine
R = R, = OMe Brucine



R = R, = R₂ = H Icajine
R = R, = H, R₂ = OH Vomicine
R = R, = OMe, R₂ = H Novacine

Fig. 1. Tertiary *Strychnos* alkaloids.

5 % SE-52 was used. The alkaloids being examined were injected as 1 % solutions in ethanol, 0.4 μl of solution generally being used. In some of the quantitative work an Aerograph 471 Digital Integrator was available.

Table II shows the retention times relative to strychnine (R_{Stry}) and to brucine (R_{Bru}) at column temperatures of 230°, 250°, and 280°. The order of elution is the reverse of that on TLC, *i.e.* the more polar alkaloids such as diaboline and retuline have short retention times, while the less polar bases like vomicine and novacine

TABLE II

THE RELATIVE RETENTION TIMES OF TERTIARY *Strychnos* ALKALOIDS

Inox (stainless steel) column 2 ft. × 1/8 in. filled with 100–120 mesh Aeropak 30 coated with 5% SE-52.

Column temperature	*230°*	*250°*		*280°*	
Injector temperature	*260°*	*280°*		*310°*	
	R_{Stry}	R_{Stry}	R_{bru}	R_{Stry}	R_{bru}
Spermostrychnine (324)[a]	0.45	0.44	0.17		
Retuline (338)	0.50	0.49	0.19		
Diaboline (352)	0.76	0.69	0.26	0.69	0.31
Strychnospermine (354)	0.82	0.75	0.29		
Strychnine[b] (334)	1.00	1.00	0.38	1.00	0.45
Holstiine (382)	1.03	0.94	0.36		
α-Colubrine (364)		1.71	0.66	1.59	0.71
β-Colubrine (364)		1.82	0.70	1.66	0.74
Icajine (364)		1.96	0.78	1.88	0.84
Brucine[c] (394)		2.61	1.00	2.24	1.00
Vomicine (380)		3.62	1.41	3.10	1.39
Novacine (424)		5.48	2.18	4.24	2.12

[a] Molecular weight.
[b] Actual retention times: 230°, 11.0, 250°, 5.85, 280°, 2.07 min.
[c] Actual retention times: 250°, 15.2 and 280°, 4.55 min.

have much longer retention times. Since the column is relatively non-selective, the increase in molecular weight will also play a part in increasing the retention times.

A column temperature of 250° gives a satisfactory scale of retention times, as the last base to be eluted, novacine, emerges after about 31 min.

On the other hand, the first six alkaloids, which all have very short retention times, are not all readily separated. Thus, the pairs spermostrychnine[5] and retuline[10–12], diaboline and strychnospermine[5], and strychnine and holstiine[13–15] are incompletely separated from each other. Lowering the column temperature to 210° gives some improvement but β-colubrine is then no longer eluted. The use of a 3-ft. column does not lead to any improvement in the separation either. Not all these alkaloids occur in the same plant at the same time and with the help of TLC it is in fact possible to unravel such a mixture. Further work is clearly necessary to deal with these bases and it may well be that use of a somewhat more polar stationary phase will give the desired separations.

Alkaloids of Strychnos nux-vomica L.

All the *S. nux-vomica* alkaloids—strychnine, α- and β-colubrine, brucine, icajine (N-methyl-*sec.*-pseudostrychnine), vomicine, novacine (N-methyl-*sec.*-pseudobrucine)[5,16]—are well separated with the exception of the two colubrines. The two most important bases, strychnine and brucine, are completely separated, with short retention times and with well-formed peaks which are symmetrical and show no tailing (*cf.* Fig. 2). At a column temperature of 250° strychnine comes out after only 5.85 min and brucine after 15.20 min; at 280° strychnine emerges after 2.07 min

and brucine after 4.55 min. Taking the retention time of strychnine as 1.00, brucine has a relative retention time of 2.61 at 250° and of 2.24 at 280°, which underlines the effectiveness of the separation. The detector responses to strychnine and brucine are in the ratio 100:84 (10 ng strychnine can be readily detected), so that quantitative determinations are readily made.

Clearly, GLC can be of great use in the analysis and purity control of strychnine and brucine.

Isolation of icajine from S. nux-vomica alkaloids

One result of the present work has been the detection in and the isolation from *S. nux-vomica* alkaloids of icajine. The starting material was a partially purified fraction from the mother liquors of brucine sulphate crystallization. The chromatogram of this material is shown in Fig. 2. Peak 2 corresponds to strychnine but together with the little peak 1 in front probably means that some pseudostrychnine or a derivative is present as well (see below). Peaks 3 and 4 belong to the colubrines, peak 5 corresponds in position with icajine, while peaks 6, 7, and 8 represent brucine, vomicine, and novacine, respectively. Small, additional peaks in front of 1, between 2 and 3, and between 7 and 8 may belong to trace amounts of further alkaloids which have not yet been isolated.

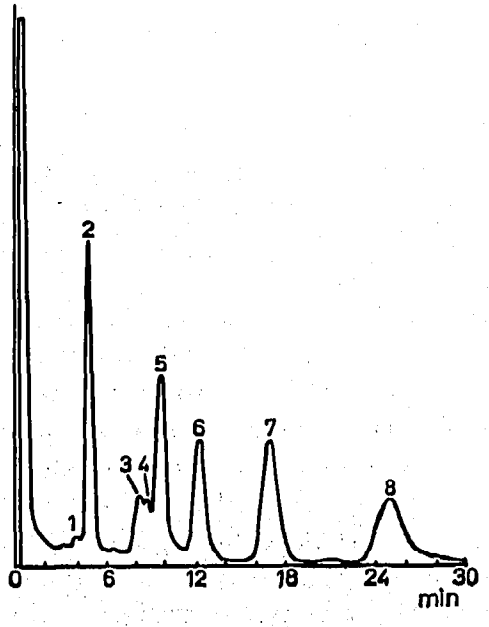


Fig. 2. Chromatogram of a partially purified fraction of the free bases from the mother liquors of brucine sulphate crystallization. 1 = pseudostrychnine subsidiary peak (?); 2 = strychnine; along with peak 1 may indicate that pseudostrychnine is present as well; 3 = α-colubrine; 4 = β-colubrine; 5 = icajine (N-methyl-*sec.*-pseudostrychnine); 6 = brucine; 7 = vomicine; 8 = novacine (N-methyl-*sec.*-pseudobrucine).

With the exception of icajine, all the bases indicated have already been isolated from *S. nux-vomica*. By means of a counter-current distribution between 70% aqueous ethanol and carbon tetrachloride, fractions rich in icajine and vomicine were obtained. Several recrystallizations from methanol eliminated much of the more soluble vomicine, and the icajine was finally obtained pure by preparative TLC.

Comparison with an authentic specimen of N-methyl-*sec.*-pseudostrychnine (m.p. and mixed m.p., optical rotation, TLC and GLC, and infrared spectrum) confirmed the identity[17,18].

The alkaloid has recently been isolated independently by DELLE MONACHE *et al.*[16].

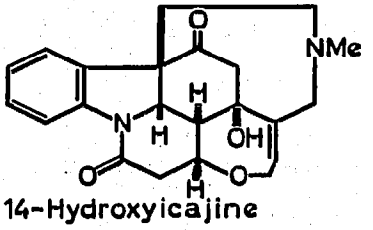
N-Oxides and alkaloids of the pseudo-series

Pseudostrychnine (16-hydroxystrychnine) and pseudobrucine (16-hydroxybrucine) are both known to occur in *S. nux-vomica*[16]. The behaviour on GLC of N-oxystrychnine and pseudostrychnine and its O-ethyl and O-methyl ethers has therefore also been examined.

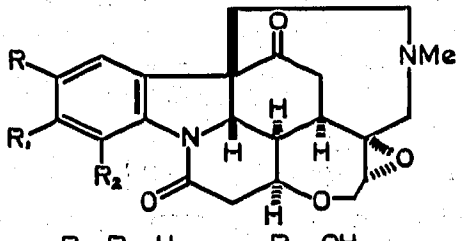
Here again, there have been difficulties, for all of these under the conditions used probably undergo decomposition and in each case the retention time of the major peak is the same as that of strychnine itself. However, in addition to this major peak, N-oxystrychnine shows two small peaks, one on each side of the main one. With pseudostrychnine and its O-ethers, a narrow peak in front of the main one but poorly separated from it is observed.

N-Oxybrucine and pseudobrucine behave similarly.

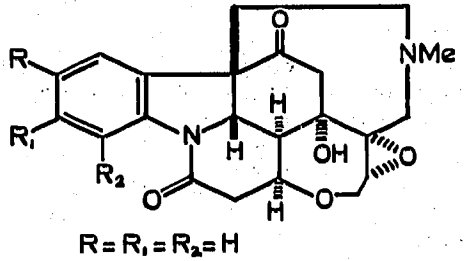
Probably the use of glass injectors and glass columns, rather than stainless steel ones, would reduce the pyrolysis. Perhaps also the use of a more polar column operating at a lower temperature would be advantageous. The point has not been investigated further. However, the complementary application of TLC solves the difficulty, as the N-oxides and derivatives of the pseudo-series are easily separated from the parent bases.



14-Hydroxyicajine
(14-Hydroxy-N-methyl-pseudo-series)



R = R₁ = H , R₂ = OH
R = OMe , R₁ = R₂ = H
R = R₁ = OMe , R₂ = H
(21,22-Epoxy-N-methyl-pseudo-series)



R = R₁ = R₂ = H
R = R₁ = H , R₂ = OH
R = R₁ = OMe , R₂ = H
(14-Hydroxy-21,22-epoxy-N-methyl-pseudo-series)

Fig. 3. Alkaloids from *Strychnos icaja* Baill.

Alkaloids of Strychnos icaja Baill.

This *Strychnos* species, from West and Central Africa, has been, and still is, much used in the preparation of ordeal and arrow poisons[19]. Numerous early investigations of its alkaloids were almost all interpreted in terms of strychnine and brucine. However, in 1951 JAMINET[20] showed by means of paper chromatography that neither of these two bases was present. Later, he[21] reported the isolation of three alkaloids, A, B′, and C. A gave two spots on paper chromatography, but on recrystallization appeared not to be altered. Study of it was difficult with the small amount (15 mg) available. The hypothesis that it might be pseudostrychnine, which could conceivably behave as if it were a mixture of carbinolamine and keto-amine forms, was disproved by comparison with authentic material. Professor A. DENOËL, Liège, provided a small sample of JAMINET's product and examination by GLC and TLC suggested that it was a mixture of roughly equal amounts of vomicine and icajine. Separation of the two components by preparative TLC and comparison with authentic vomicine and icajine verified the identities.

Since JAMINET's original work on his alkaloid A, the above two bases have been isolated from the plant and identified[18].

Further alkaloids have been obtained from *S. icaja*[22] and their structures are shown in Fig. 3. So far, representatives of five groups are known and they are in part more complex than those found in *S. nux-vomica*:

(i) pseudo-series (pseudostrychnine, isolated as its O-methyl ether);
(ii) N-methyl-pseudo-series (icajine and vomicine);
(iii) 14-hydroxy-N-methyl-pseudo-series;
(iv) 21,22-epoxy-N-methyl-pseudo-series; and
(v) 14-hydroxy-21,22-epoxy-N-methyl-pseudo-series.

The retention times of these and some related bases relative to that of brucine and at a column temperature of 280° are shown in Table III.

TABLE III

THE RELATIVE RETENTION TIMES (R_{bru}^{280}) OF *Strychnos icaja* ALKALOIDS

Inox (stainless steel) column 2 ft. × 1/8 in. filled with 100–120 mesh Aeropak 30 coated with 5% SE-52.

Member	*Series*				
	"Normal"	*N-Me-ψ*	*14-OH-N-Me-ψ*	*21,22-Ep-N-Me-ψ*	*14-OH-21,22-Ep-N-Me-ψ*
Strychnine	0.45 (334)[a]	0.84 (364)	1.00 (380)	—	1.06 (396)
Vomicine	—	1.44 (380)	—	1.57 (396)	1.82 (410)
α-Colubrine	0.71 (364)	—	—	—	—
β-Colubrine	0.74 (364)	—	—	1.49 (410)	—
Brucine	1.00 (394)	2.12 (424)	—	2.28 (440)	2.66 (456)

[a] Molecular weight.

Relative retention times and chemical structure

Examination of Tables II and III reveals some interesting connections between the relative retention times and the chemical structure.

Table II shows the general tendency of higher molecular weight alkaloids to have longer retention times. The first six bases with the exception of strychnine all have an acetyl and/or free aliphatic hydroxyl groups present and are evidently rather polar.

Substitution by methoxyl groups in the aromatic ring as in the series spermostrychnine–strychnospermine, strychnine–colubrines–brucine, etc., leads to notable increases in the relative retention times. On the other hand, on going from the "normal" series to the N-methyl-pseudo-series there is a rather greater increase in relative retention time: R^{280}_{bru} 0.45 for strychnine (mol.wt. 334) increases to 0.71 and 0.74 for the colubrines (mol.wt. 364) but to 0.84 for icajine (mol.wt. also 364).

Vomicine (mol.wt. 380) with its hydroxyl group at C-4 in the aromatic ring forms an exception. It has R^{280}_{bru} 1.44, while the isomeric 14-hydroxyicajine has the much smaller value 1.00. 21,22-α-Epoxyvomicine (mol.wt. 396) has R^{280}_{bru} 1.57, but 21,22-epoxy-N-methyl-*sec.*-pseudo-β-colubrine (mol.wt. 410) has the smaller value 1.49. Here, the differences are not due simply to molecular weight effects. It is known that the phenolic hydroxyl group in vomicine is strongly hydrogen-bonded to the amide carbonyl function[5] and this has the effect of reducing the polarity of the base, as compared with the non-hydroxylated base icajine; this can also be observed in TLC where in methylene dichloride–methanol systems vomicine has a greater R_F value than icajine. Even though at the high column temperature of 280° the intramolecular hydrogen bond is presumably still present, the consequent reduction in polarity is not sufficient to reduce the R^{280}_{bru} value below that of the corresponding higher molecular weight colubrine derivative.

Changes in the alicyclic part of the molecule in the N-methyl-pseudo-series also lead to increasing R^{280}_{bru} values. While epoxidation of the 21,22-double bond brings about a small increase:

Vomicine	1.44	21,22-α-Epoxyvomicine	1.57
Novacine	2.12	21,22-α-Epoxynovacine	2.28,

the introduction of a 14-hydroxyl group, whether the 21,22-double bond is epoxidized or not, causes a somewhat greater increase:

Icajine	0.84	14-Hydroxyicajine	1.00
21,22-α-Epoxyvomicine	1.57	14-Hydroxy-21,22-α-epoxyvomicine	1.82
21,22-α-Epoxynovacine	2.28	14-Hydroxy-21,22-α-epoxynovacine	2.66.

In contrast, epoxidation of the 21,22-double bond, when the 14-hydroxyl group is already present, has only a slight effect:

14-Hydroxyicajine	1.00	14-Hydroxy-21,22-α-epoxyicajine	1.06.

Not all the members of the various series are known, so that it is not easy to make meaningful comparisons. However, the indications available so far suggest that as further data are acquired, it may be possible to obtain hints about the chemical structure of alkaloids belonging to these series from their relative retention times.

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