morphine. Contact with strong alkali is minimal; it occurs only during the separation of morphine from the non-phenolic alkaloids. The alkali column is covered with chloroform and thus protected from contact with air during this operation.

The morphine used as spectrophotometric standard was analyzed by the proposed procedure. The material is slightly off-white, and contained a small amount (about 0.5%) of codeine; it was used in the absence of a pure standard. Recoveries of 98.3–99.5% were obtained (Table 3).

A synthetic paregoric was prepared, containing all of the components other than opium, together with added meconic acid and the opium alkaloids codeine, thebaine, narceine, narcotine, papaverine, and morphine in amounts normally present in opium. Recoveries of morphine were comparable to those for the morphine alone.

Table 3. Recoveries of morphine standard alone and in synthetic paregoric

Sample	mg Present	mg Found	Per Cent Recovered
Morphine, standard	4.110	4.04	98.3
		4.09	99.5
		4.07	99.0
Paregoric, synthetic	4.248	4.17	98.1
		4.21	99.1
		4.16	97.9
Paregoric, synthetic	6.372	6.30	98.8
		6.24	97.9
		6.25	98.1

Table 4. Per cent morphine found in opium samples

Sample	Proposed Method	USP XVII	I. Ph.,[a] 2nd Ed.
Powder 1	11.94	10.19	11.89
	12.02		
	11.98		
Powder 5	7.67	5.97	7.30
	7.63		
	7.61		
Powder 2A	15.15	14.46	14.52
	15.08		
	15.04		
Powder 2B	15.16		
	15.12		
Iranian 1	10.94	9.67	
	10.98		
	11.00		
Iranian 2	11.47	9.53	
	11.47		
	11.36		
Turkish	6.24	6.32	
	6.26		
	6.18		

[a] See reference (18).

Table 5. Results of analysis of paregoric

Sample	Proposed Method: mg Found	% Label	USP % Label
A	6.17	154.3	152.0
	6.17	154.3	
	6.23	155.8	
A[a]	3.06	153.0	
	3.07	153.5	
	3.09	154.5	
B	4.05	101.1	102.6
	4.00	100.0	
	4.00	100.0	
C	4.16	104.0	
	4.22	105.5	
	4.18	104.5	
D	3.94	98.5	
	4.00	100.0	
	3.97	99.3	
E	4.31	107.7	
	4.34	108.5	
	4.32	108.0	
F	4.31	107.8	
	4.35	108.8	
	4.37	109.2	

[a] Sample A diluted with equal volume of synthetic paregoric without morphine.

Three samples of powdered opium, three of opium,[1] and five samples of USP Paregoric were analyzed (Tables 4 and 5). In addition to these, a paregoric sample of unknown age, but at least 30 years old, which had lost much of its alcoholic content by evaporation, was analyzed (Sample A in Table 5).

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[1] We are grateful to W. P. Butler and F. J. Feeny, Alcohol and Tobacco Tax Laboratory, Internal Revenue Service, for the USP and the International Pharmacopeia assays of these samples.

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Separation and Identification of Minor Alkaloids of *Strychnos nux vomica*

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The various alkaloids of *Strychnos nux vomica* L. are separated and identified by the use of silica gel or alumina columns and/or thin layer plates. The groups correspond to these three: strychnine, pseudostrychnine, and icajine. Alkaloidal composition of the column chromatographic eluates and R_f values for the various TLC solvent systems are given.

During a systematic investigation of the alkaloids of *Strychnos,* a biogenetic relationship was postulated between the tertiary and quaternary alkaloids; these compounds have diaboline (Fig. 1) as the common precursor (1). This was confirmed by finding diaboline in various *Strychnos* species (2–9).

Recently we reported the presence in

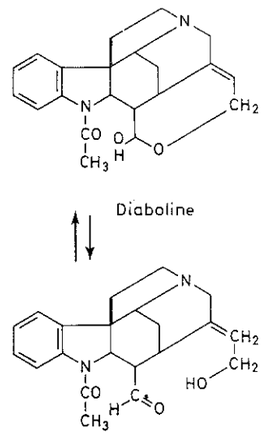


Fig. 1—**Diaboline structure**

Strychnos nux vomica L. (10) of two alkaloids, icajine (N-methyl-*sec*-pseudostrychnine) and pseudobrucine, previously obtained from *S. icaja* Baill (11) and *S. gaultheriana* Pierre (12), respectively, in addition to the other alkaloids thus far described; i.e., strychnine (13), brucine (13), vomicine (14), α- and β-colubrine (15), pseudostrychnine (15), and novacine [N-methyl-*sec*-pseudobrucine] (16).

The fact that two alkaloids were detected only now in the seeds and bark of *S. nux vomica*, investigated for about 150 years by chemists, indicates the difficulties in separating and identifying these substances.

In this paper, we report methods for separation and identification, both on the analytical and the preparative scale, of the alkaloids of *Strychnos* and, particularly, the alkaloids of *S. nux vomica*.

The separation of *S. nux vomica* alkaloids was studied by Büchi and Schumacher (17), who established the conditions for the separation of brucine and strychnine.

Jaminet (18) proposed a paper chromatographic method using isobutanol or isopropanol with acetic acid or ammonium hydroxide for the separation of alkaloids from *S. holstii*.

Caggiano and Marini-Bettòlo (19) used four different solvents for paper chromatography: butanol:water, butanol:acetic acid: water, and Karrer's solvents C and D. Strychnine was not sufficiently separated (Table 1) from the colubrines, whereas the former can be easily separated from diaboline in alkaline medium. Separation by paper electrophoresis was proposed for a rapid identification of these alkaloids. Migration values at different pH's are reported in Table 2; these values show that strychnine is not well separated from α- and β-colubrine nor from brucine.

The use of silica gel and alumina columns for the fractionation of *Strychnos* alkaloids and the use of thin layer chromatography have greatly improved the possibility of separating and detecting *Strychnos* tertiary alkaloids (8, 9).

We now can separate and identify *Strychnos* tertiary alkaloids by means of TLC, an improvement on the previous methods; R_f values are reported in Table 3. The alkaloids can be detected on the plates with the Dragendorff reagent when solvents *c* and *d* are used. Pseudobrucine and novacine are not detected when solvents *a* and *b* are used, but they are visible with a 2% solution of cesium sulfate in sulfuric acid. Macheboeuf's reagent, 3% solution of cesium sulfate in phosphoric acid, makes all the alkaloids visible; novacine and pseudobrucine react immediately, but the others show a coloration only after 30 minutes.

The total alkaloid mixture extracted from various samples of *S. nux vomica*[1] was fractionated on a silica gel column, with chloroform:methanol (96:4) as eluant; *see* Table 4. If the elution is continued, brucine, α- and β-colubrine, and strychnine are eluted in that order. The retention rate of these alkaloids is inversely proportional to their pK_a values (20), also given in Table 4. Alkaloids with low pK_a values like pseudostrychnine and vomicine are eluted in the first fractions (Table 4), whereas strychnine and brucine, which are strongly retained, have higher pK_a values.

This separation can be utilized for fractionating the tertiary *Strychnos* alkaloids into three main groups; i.e., strychnine, brucine, and the colubrines; pseudobrucine and pseudostrychnine; and vomicine, icajine, and novacine. These alkaloids can be related structurally to three main groups (Fig. 2): strychnine (I), pseudostrychnine (II), and icajine (III). Thus, this method is very convenient for a rapid separation of the strychnine group from the other alkaloids.

[1] *S. nux vomica* seeds, *S. nux vomica* root bark, and mother liquors from industrial preparation of strychnine sulfate from *S. nux vomica* seeds.

Table 1. R_f values for *Strychnos* alkaloids, using four different solvent systems

Alkaloid	R_f BuOH:AcOH:H_2O	R_f BuOH:H_2O	R_f Solv. C	R_f 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

Table 2. Electrophoretic migration values of *Strychnos* alkaloids in various pH systems

Alkaloid	Migration in mm (in 3 hr) 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

Table 3. Thin layer chromatographic separation (R_f values) of *Strychnos nux vomica* alkaloids in various solvent systems

Alkaloid	Solvent[a] (a)	(b)	(c)	(d)
Pseudostrychnine	0.37	0.64	0.64	0.69
Pseudobrucine	0.31	0.54	0.57	0.50
Vomicine	0.40	0.76	0.46	0.76
Icajine	0.50	0.78	0.41	0.67
Novacine	0.44	0.66	0.31	0.45
Strychnine	0.38	0.58	0.11	0.12
Brucine	0.23	0.39	0.06	0.03
Diaboline	0.42	0.48	0.02	0.05
Desacetyldiaboline	0.28	0.38	0.0	0.0

[a] (a) = Cyclohexane:chloroform:diethylamine (5:4:1); (b) = benzene:ethyl acetate:diethylamine (7:2:1); (c) = chloroform:methanol (96:4); (d) = pyridine:ethyl acetate:water (11.5:75:16.5, upper phase).

The first separation on silica gel permits the separation of *S. nux vomica* alkaloids into three main groups which correspond to the three fundamental structures (I, II, III; Fig. 2) of tertiary *Strychnos* alkaloids. The method used thus far for the work on the alkaloids of various Strychnos species (7–9) can also form the basis for the analysis and determination of *Strychnos* alkaloids in drugs.

Several pharmacopoeia mention *Nux vomica* and strychnine; the methods for the determination of the alkaloids could be considerably improved by applying a chromatographic separation under the conditions reported above. Moreover, the same methods could be extremely useful to establish the impurities present in strychnine.

Experimental

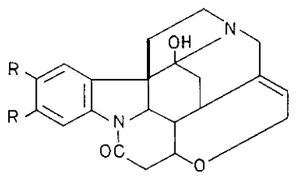
Materials and Methods

Strychnos nux vomica L. seeds from Indonesia; *Strychnos nux vomica* L. root bark from Madras area, supplied by General Drug Institute, Lucknow, India; and a concentrate of mother liquors from the crystallization of strychnine sulfate, were used for the separation.

Silica gel GF-254 (Merck, Darmstadt) pre-

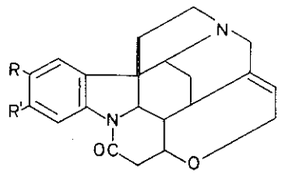
Table 4. Retention sequence and R_f values of *Strychnos nux vomica* alkaloids

Alkaloid	PK_a	R_f (TLC) in $CHCl_3$: CH_3OH (96:4)	Retention Sequence
Pseudostrychnine	5.60	0.64	
Pseudobrucine		0.54	1 pseudostrychnine + pseudobrucine
Vomicine	5.88	0.46	2 vomicine
Icajine	6.07	0.41	3 icajine + novacine
Novacine	6.88	0.31	
Brucine	7.37	0.06	4 brucine + strychnine
Strychnine	7.45	0.11	
α-Colubrine		0.08	
β-Colubrine		0.08	



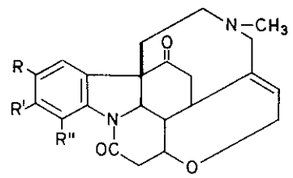
Pseudostrychnine R = H
Pseudobrucine R = O-CH_3

(II)



Strychnine R = R' = H
Brucine R = R' = OCH_3
α-Colubrine R = H, R' = OCH_3
β-Colubrine R = OCH_3, R' = H

(I)



Vomicine R = R' = H, R" = OH
Icajine R = R' = R" = H
Novacine R = R' = OCH_3, R" = H

(III)

***Fig. 2*—Types of *Strychnos* alkaloids.**

pared according to Stahl was used for TLC. The plates (250 mμ layer) were activated for 30 min at 105°C before use.

Silica gel for preparative column chromatography was prepared from SiO_2 (Carlo Erba R. P.) which passed through a 575 M/cm sieve but was retained on a 1560 M/cm sieve. Before use, it was heated at 105°C for 24 hr.

Alumina for preparative column chromatography was prepared from standardized aluminum oxide (Merck, Darmstadt). The material was heated for 24 hr at 105°C and alumina of activity III was prepared according to Brockmann (adding 6% water and agitating for 12 hr).

Chloroform, methanol, benzene, and ethyl acetate were Carlo Erba R. P. products and were used without any further purification.

Extraction

S. nux vomica L.—20 powdered seeds of *S. nux vomica* L. were extracted three times with 2*N* acetic acid for 24 hr. The extracts were pooled, concentrated to a small volume, made alkaline with concentrated ammonia, and extracted with chloroform. The chloroform concentrate was placed on a silica column and eluted with chloroform:methanol (97:3) to eliminate most of the strychnine, brucine, and the colubrines which are retained, under these conditions, on the column. The column eluate submitted to TLC, using silica gel and ethyl acetate:pyridine:water (75:14.5:16.5) as solvent, yielded five spots. Three of these were identified as vomicine, pseudostrychnine, and novacine. In this solvent, strychnine, brucine, and colubrines have R_f values of <0.1.

S. nux vomica.—50 g root bark was treated and extracted as above. The chloroform extract was fractionated on silica gel; the eluate was submitted to TLC with the same solvent as above. Two spots were observed and identified as pseudostrychnine and pseudobrucine. Vomicine, icajine, and novacine were either not present or present only in trace amounts.

Mother liquors of the strychnine crystallization.—20 g concentrate from mother liquors of industrial crystallization of strychnine sulfate, obtained from the extraction of *S. nux vomica*, was dissolved in water, and the solution was filtered; the filtrate was made alkaline with concentrated ammonia and extracted with chloroform. The solution was dried on anhydrous sodium sulfate and was evaporated by vacuum, yielding 16 g residue.

TLC on silica gel plates of this residue with the solvent mentioned above yielded five spots, like the extracts, and in the same proportion—except novacine—as *S. nux vomica* seeds.

Separation

Vomicine.—1.5 g basic extract dissolved in chloroform was adsorbed on 6.5 g silica gel and dried at room temperature. This material was placed on a 4 × 60 cm column containing 360 g silica gel in chloroform:methanol (98:2). The column was eluted with the same solvent in 10 ml fractions; after fraction 200, chloroform:methanol (95:5) was used as eluant. The eluate composition was controlled by silica gel TLC; *see* Table 5.

Further elution showed the presence of brucine, the colubrines, and strychnine (slow eluters in ethyl acetate:pyridine:water).

The yield of the alkaloids (fast eluters in ethyl acetate:pyridine:water) is 37–38%, consisting of 17–18% pseudostrychnine, 25–26% pseudobrucine, 38–39% vomicine, 7–8% icajine, and 11–12% novacine.

Table 5. Composition of fractions of *Stychnos nux vomica* extract

Fraction	Alkaloid(s) Present	Amt, mg
	A—Vomicine Fraction	
66–105	Pseudostrychnine, pseudobrucine	205
106–125	Vomicine, pseudobrucine (traces)	105
126–126	Vomicine	105
168–200	Icajine, novacine, vomicine	45
201–380	Icajine, novacine	100
	Total	560
	B—Pseudostrychnine and Pseudobrucine	
95–160	Pseudostrychnine	145
161–200	Pseudostrychnine, pseudobrucine	165
201–300	Pseudobrucine	160
	C—Icajine and Novacine	
31–37	Vomicine	15
40–57	Icajine	100
84–103	Novacine	150

Vomicine isolated in fractions 127–167 was purified from ethyl acetate, m.p. 282–283° [literature: 282° (14); 281° (16); 281–282° (11)]; $[\alpha]_D$ = +99.2° in $CHCl_3$ [literature: +80.4° in ethanol (14); +84° in ethanol (16); +100° in $CHCl_3$ (11)]; λ_{max}: 283 mμ (log Σ = 4.14); 259 mμ (log Σ = 3.91) shoulder; 294 mμ (log Σ = 3.56) shoulder.

Analysis. Calculated (%) for $C_{22}H_{24}O_4N_2$: C, 69.45; H, 6.36; N, 7.36. Found (%): C, 69.39; H, 6.20; N, 7.40.

Pseudostrychnine and pseudobrucine.—0.6 g pseudostrychnine and pseudobrucine mixture obtained from the above separation on silica gel dissolved in chloroform was adsorbed on 10 g aluminum oxide and dried first at room temperature and then in a vacuum desiccator. This mixture was placed on a 3 × 50 cm column containing 250 g aluminum oxide (activity III, according to Brockmann) in benzene:ethyl acetate (75:25). The column was eluted with the same solvent, and 10 ml fractions were collected; the composition was checked by TLC. The fractions obtained are shown in Table 5B. The separation can be improved by using a mixture of benzene:ethyl acetate (80:20) as eluants.

Pseudostrychnine.—When crystallized from ethyl acetate, it melts at 262–264° (dec); [literature: 253–257° isomer I (21); 235-237°

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isomer II (21); 236° (12); 266–268° (15); 262–268° (22)]; $[\alpha]_D = -72°$ in $CHCl_3$ [literature: −63° in $CHCl_3$ (21); −79° in $CHCl_3$ (12); −43.8° in ethanol (15); −44.2° in ethanol (22)]; λ_{max}: 255 mμ (log Σ = 4.13); 279 mμ (log Σ = 3.70) shoulder; 280 mμ (log Σ = 3.58) shoulder.

Analysis. Calculated (%) for $C_{21}H_{22}O_3N_2$: C, 71.98; H, 6.33; N, 8.00. Found (%): C, 71.65; H, 6.33; N, 7.96.

IR and NMR data are in accordance with those of a synthetic sample (4).

Pseudobrucine.—Crystallized from ethyl acetate, it melts at 255–260° (dec); [literature: 256° (12); 255–258° (21); 258–268° (23); $[\alpha]_D = -55°$ in $CHCl_3$ [literature: −70° in $CHCl_3$; −64° in $CHCl_3$; −65.5° in $CHCl_3$]; λ_{max}: 265 (log Σ = 4.21); 299 (log Σ = 6.03) shoulder.

A synthetic specimen of pseudobrucine (23) melts at 252–260°; undepressed in mixture with pseudobrucine; $[\alpha]_D = -52°$ in $CHCl_3$.

Analysis. Calculated (%) for $C_{23}H_{26}O_5N_2$: C, 67.30; H, 6.59; N, 6.83. Found (%): C, 67.34; H, 6.33; N, 6.63.

UV, IR, and NMR spectra of natural and synthetic samples are identical.

Icajine and Novacine.—0.3 g icajine-novacine mixture obtained from the above separation on silica gel column, dissolved in chloroform, was adsorbed on 5 g aluminum oxide and dried first at room temperature and then under vacuum.

This material was placed on a column (2 × 50 cm) containing 110 g aluminum oxide (activity III, according to Brockmann) in benzene:ethyl acetate (85:15). Using the same solvent, 70 fractions of ca 10 ml were separated; elution was continued with benzene:ethyl acetate (70:30). The composition of the fractions was controlled by TLC. The fractions shown in Table 5C were separated.

Icajine. — N-Methyl-*sec*-pseudostrychnine; crystallized from ethyl acetate, melts at 275–276° [literature: 271–272° (11); 273° (24)]; m.p.: undepressed in mixture with a synthetic sample of icajine (24); $[\alpha]_D = -9°$ in $CHCl_3$ [literature: −10° in $CHCl_3$ (11)]; λ_{max}: 254 mμ (log Σ = 4.39), 291 (log Σ = 3.64) shoulder.

Analysis. Calculated (%) for $C_{22}H_{24}O_3N_2$: C, 72.36; H, 6.66; N, 7.14. Found (%): C, 72.50; H, 6.64; N, 7.69.

UV, IR, and NMR spectra of natural and synthetic samples are identical.

Novacine. — N-Methyl-*sec*-pseudobrucine; crystals from ethyl acetate melt at 232–233° [literature: 228–230° (16); 231–232° (25)]; m. p.: undepressed in mixture with a synthetic sample of novacine; $[\alpha]_D = -17°$ in $CHCl_3$ [literature: −17.7° in $CHCl_3$ (16)]; λ_{max}: 264 mμ (log Σ = 4.16); 301 mμ (log Σ = 3.27).

Analysis. Calculated for $C_{24}H_{28}O_5N_2$: C, 67.90; H, 6.60; N, 6.65. Found (%): C, 67.95; H, 6.69; N, 6.55.

UV, IR, and NMR spectra of natural and synthetic samples are identical.

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Selection of Partition Chromatographic Systems from Distribution Diagrams: Determination of Dextromethorphan in Cough Sirups

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Ionization and extraction constants for dextromethorphan and 14 other alkoids are given. By constructing the logarithmic distribution diagrams for mixtures of these compounds, Celite partition chromatographic systems can easily be selected for their separation; both free base and ion-pair extraction provide useful systems. A general procedure for the isolation of dextromethorphan from any of those alkoids with which it is currently formulated is presented; the results of analyses of standard samples and of commercial sirups are given. Some other separations do not appear to be feasible. However, the distribution diagrams clearly show the reasons for this fact.

The analysis of the components of cough and cold remedies is a difficult problem. Most of these formulations contain an antihistamine, a sympathomimetic, and an antitussive; the chief problem is to separate these alkoids[1] from each other. Some form of chromatography is generally employed; the gas chromatographic analysis of such mixtures has been reported (2, 3) and Celite partition chromatography is the basis of official methods for several combinations (4).

In the official methods, an alkoid is selectively extracted from aqueous acid solution (in contrast to the more general and hence nonselective extraction from alkaline solution). The extracted species in these cases is frequently the associated salt (ion-pair) of the alkoid with a given acid; this has been the subject of considerable attention in the recent literature (1, 5, 6). Alternatively, the free base may be extracted, even when the amine nitrogen exists overwhelmingly in the protonated form in the aqueous phase.

The quantitative mathematical description of both salt and base extraction is straightforward in theory but tends to become algebraically complex. In an attempt to overcome this difficulty, we have recently shown (7) how, by determining a small number of ionization and extraction constants, linear logarithmic distribution diagrams can be constructed. These diagrams give an essentially complete picture of the overall distribution behavior of a given alkoid and provide a convenient method for selecting partition chromatographic systems to separate pharmaceutical mixtures.

We have applied this approach to the determination of dextromethorphan in cough sirups. This synthetic, non-narcotic antitus-

[1] Because alkaloids and many pharmaceutically important synthetic organic bases present identical analytical problems, and since the word "alkaloid" is reserved for the natural product, it has been proposed (1) that the word "alkoid" be used to encompass the entire group.