Processing of Nux Vomica. II. Changes in Alkaloid Composition of the Seeds of *Strychnos nux-vomica* on Traditional Drug-Processing

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In the course of our study on the drug-processing of the seeds of *Strychnos nux-vomica* L. (Loganiaceae), the alkaloid composition of the heat-treated seeds of *S. nux-vomica* was compared to that of the untreated seeds. On heat treatment, the contents of the major alkaloids such as strychnine and brucine declined significantly with increases in the amounts of isostrychnine, isobrucine, strychnine *N*-oxide and brucine *N*-oxide. The cleavage of an ether linkage and the occurrence of *N*-oxidation were demonstrated by heat treatment of authentic strychnine and brucine.

Keywords brucine; drug-processing; isobrucine; isostrychnine; Loganiaceae; strychnine; *Strychnos nux-vomica*; strychnos alkaloids

Nux vomica, the dried seeds of *Strychnos nux-vomica* L. (Loganiaceae), has been used for promoting blood circulation, alleviating blood stasis and relieving pain in traditional Chinese medicine. Nux vomica contains extremely poisonous alkaloids, strychnine (**1**) and brucine (**2**), which have a strong convulsant action in animals and humans. Therefore, when nux vomica is used as an internal medicine in China the seeds are generally processed to reduce their toxicity.[1)]

For the purpose of evaluating this traditional processing of nux vomica, we investigated the changes in akaloid composition between the processed and unprocessed seeds. In a previous paper,[2)] we reported the isolation and identification of four new alkaloids, isobrucine, isobrucine *N*-oxide, isostrychnine *N*-oxide (isostrychnine-I *N*-oxide) and 2-hydroxy-3-methoxystrychnine, which may be transformed from the major alkaloids by thermal reaction through the processing.

In the present paper, we describe the quantitative analysis of strychnos alkaloids in the processed and unprocessed seeds when the processing was carried out according to the two methods described in the Chinese Pharmacopoeia.[1)] In addition, we report the thermal transformation of strychnine (**1**) and brucine (**2**) into ring-opened and *N*-oxidized compounds.

Materials and Methods

Apparatus A dual-wavelength chromatoscanner (CS-910, Shimadzu, Kyoto, Japan) was used for quantitative analysis of alkaloids in the processed and unprocessed seeds of *S. nux-vomica*.

Materials The seeds of *S. nux-vomica* were purchased from Koshiro Seiyaku Co. (Osaka, Japan).

Chemicals Strychnine and brucine were purchased from Sigma Chem. Co. (St. Louis, U.S.A.) and Wako Pure Chemical Co. Osaka, Japan). Brucine *N*-oxide, strychnine *N*-oxide and pseudostrychnine were purchased from Carl Roth K. G. (Karlsruhe, FRG). Isobrucine, isostrychnine, β-colubrine, pseudostrychnine, novacine, icajine and vomicine were isolated from the seeds of *S. nux-vomica* as reported in a previous paper.[2)] All reagents used were of analytical grade.

Chromatography Merck Kieselgel 60 F_{254} plates (layer thickness, 0.25 mm; E. Merck, Darmstadt, FRG) were used for thin layer chromatography (TLC). The plates were developed with three solvent systems: A, *n*-hexane–EtOAc–MeOH–Et_2NH (8 : 6 : 0.3 : 1.5); B, EtOAc–MeOH (20 : 0.7); C, MeOH–H_2O (8 : 2).

Processing of the Seeds of *S. nux-vomica* (Processing A) According to the procedure described in the Chinese Pharmacopoeia,[1)] sand (3 kg) in an iron pan was heated up to 220, 240 and 260 °C (the temperature at the bottom of the sand). The seeds of *S. nux-vomica* were then put into the sand, and parched for 3 min. The seeds foamed, became dark yellow in color and were well swollen.

Processing of the Seeds of *S. nux-vomica* (Processing B) Sesame oil (500 ml) in an iron pan was heated at 220, 240 and 260 °C. The seeds of *S. nux-vomica* were then put into the hot oil and kept there for 3 min. The seeds became swollen and the color changed to brown (at 220 °C) or dark yellow (at 260 °C). The oil was wiped off the surface of the seeds.

Extraction of Strychnos Alkaloids Aqueous ammonia (10%; 3.0 ml) was added to carefully weighed powder (2.0 g) of processed or unprocessed seeds and the mixture was kept for 1.5 h at room temperature. Chloroform (80 ml) was then added, and the suspension was well dispersed with an ultrasonicator for 20 min and kept for 2 d at room temperature. The $CHCl_3$ phase was separated and the residue was re-extracted a further three times with $CHCl_3$ in a similar manner. The combined organic phases were evaporated *in vacuo* to give a residue. The residue was dissolved in $CHCl_3$ to give exactly 10 ml of solution.

Analytical Procedure of Strychnos Alkaloids A sample solution (30 μl) was applied to a TLC plate and the plate was developed twice with the following solvent systems: (1) *n*-hexane–EtOAc–MeOH–Et_2NH (8 : 6 : 0.3 : 1.5; solvent system A) for analysis of strychnine, brucine, β-colubrine, novacine and icajine; (2) EtOAc–MeOH (20 : 0.7; solvent system B) for analysis of pseudostrychnine and vomicine; (3) MeOH–H_2O (8 : 2; solvent system C) for analysis of brucine *N*-oxide, strychnine *N*-oxide, isostrychnine and isobrucine. Identification of spots on the plate was performed on the basis of their *Rf* values, and the intensity was measured with a chromatoscanner at 260 nm relative to a reference wavelength of 465 nm. The peak height was measured manually and the quantity was calculated by using a standard curve prepared as described below.

Standard Curves An authentic sample was dissolved in $CHCl_3$ and diluted with the same solvent to give five different concentrations. An aliquot of the dilution was applied to a TLC plate and the plate was developed with the solvent system described above. The intensity of spots was measured by densitometry. A standard curve was plotted as peak height *versus* alkaloid concentration. Linear regression analysis was performed to determine the slope, intercept and correlation coefficient.

Recovery Experiment An exactly weighed authentic sample was added to the extract of the seeds of *S. nux-vomica* dissolved in $CHCl_3$. An aliquot of the solution was applied to a TLC plate, which was developed with the solvent systems described above. The net amount of added compound was calculated from the standard curve and the percentage recovery is shown in Table I.

Heat Treatment of Strychnine, Brucine and a Mixture of Both Compounds Glass test tubes (50 mm × 6 mm i.d.) containing crystalline powder of strychnine, brucine or a mixture of both compounds were heated for 1.5, 3.0, 4.5, 6.0 and 7.5 min on a fusible alloy (Wood's metal) bath at 235 °C. Tubes were then immediately cooled, and $CHCl_3$ (800 μl) was added to each tube. The mixture was dispersed by using an ultrasonicator. An aliquot (30 μl) of the solution was applied to a TLC plate. The plate was developed with solvent system C and analyzed by TLC-densitometry.

Results

Quantitative Analysis of Strychnos Alkaloids Among various approaches to analyze strychnos alkaloids present in the processed and unprocessed seeds of *S. nux-vomica*, a TLC-densitometric method was found to be the most convenient, enabling us to separate all the strychnos alkaloids (Chart 1) on a TLC plate of silica gel with three different solvent systems and to analyze them quantitatively. Calibration curves (standard curves) showed good linearity between peak-height and concentration. The regression equations are listed in Table I. The accuracy of each equation was examined by means of recovery experiments (see Table II).

A Comparison of Alkaloid Contents between the Processed and Unprocessed Seeds of *S. nux-vomica* Table III shows the alkaloid contents (mean) and their coefficients of variation (C.V.; in parentheses) in the processed and unprocessed seeds of *S. nux-vomica*. Two different processings

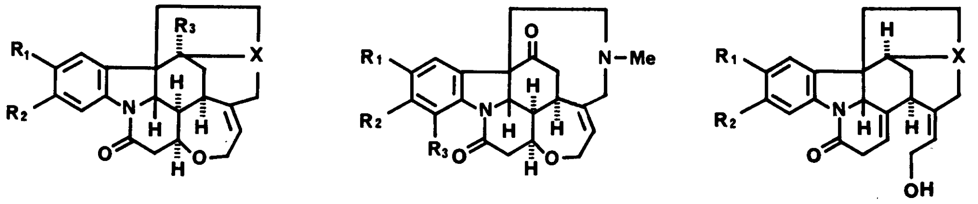
Table I. Regression Equations and Their Correlation Factors for Quantitative Analysis of Strychnos Alkaloids

Compound	Range of concentration (μg/spot)	Equation of regression line	Correlation coefficient
Strychnine (**1**)	2.5—12.5	$Y=0.324X-0.362$	$r=0.999$
Brucine (**2**)	2.3—11.5	$Y=0.199X+0.017$	$r=0.9997$
β-Colubrine (**3**)	3.6—18.2	$Y=0.149X+0.033$	$r=0.999$
Pseudostrychnine (**4**)	1.5— 7.5	$Y=0.699X-0.205$	$r=0.995$
Strychnine *N*-oxide (**5**)	1.6— 8.0	$Y=0.528X-0.014$	$r=0.995$
Brucine *N*-oxide (**6**)	2.0—10.0	$Y=0.556X+0.037$	$r=0.999$
Novacine (**7**)	1.5— 7.5	$Y=0.838X-0.282$	$r=0.991$
Icajine (**8**)	2.0—10.0	$Y=0.236X-0.095$	$r=0.996$
Vomicine (**9**)	1.8— 9.0	$Y=0.593X-0.128$	$r=0.999$
Isostrychnine (**10**)	2.2—11.0	$Y=0.587X-0.080$	$r=0.992$
Isobrucine (**11**)	2.0—10.0	$Y=0.734X-0.260$	$r=0.994$

X and Y represent the peak height (mm) and the added amount of alkaloid (μg), respectively.

Table II. Accuracy of the Analysis of Strychnos Alkaloids by TLC-Densitometry

Compound	Added amount (μg)	Found (μg)	Recovery (%)
Strychnine (**1**)	9.02	8.18	97.1
Brucine (**2**)	11.60	11.46	98.8
β-Colubrine (**3**)	18.16	17.54	96.6
Pseudostrychinine (**4**)	6.01	5.04	84.0
Strychnine *N*-oxide (**5**)	6.42	6.63	103.2
Brucine *N*-oxide (**6**)	8.16	8.03	98.4
Novacine (**7**)	6.19	5.97	96.5
Icajine (**8**)	10.2	8.87	86.9
Vomicine (**9**)	5.98	5.70	95.3
Isostrychnine (**10**)	11.2	9.80	87.5
Isobrucine (**11**)	8.24	7.04	85.4



1: $R_1=R_2=R_3=H$, $X=N$
2: $R_1=R_2=OMe$, $R_3=H$, $X=N$
3: $R_1=OMe$, $R_2=R_3=H$, $X=N$
4: $R_1=R_2=H$, $R_3=OH$, $X=N$
5: $R_1=R_2=R_3=H$, $X=N\rightarrow O$
6: $R_1=R_2=OMe$, $R_3=H$, $X=N\rightarrow O$

7: $R_1=R_2=OMe$, $R_3=H$
8: $R_1=R_2=R_3=H$
9: $R_1=R_2=H$, $R_3=OH$

10: $R_1=R_2=H$, $X=N$
11: $R_1=R_2=OMe$, $X=N$
12: $R_1=R_2=H$, $X=N\rightarrow O$
13: $R_1=R_2=OMe$, $X=N\rightarrow O$

Chart 1. Structures of Alkaloids Isolated from the Processed Seeds of *S. nux-vomica*

Table III. Alkaloid Compositions in the Processed and Unprocessed Seeds of *S. nux-vomica*

Compound	Average content (%) and C.V. (%)						
	Unprocessed	Processing A (in sand)			Processing B (in oil)		
		220 °C	240 °C	260 °C	220 °C	240 °C	260 °C
Strychnine (**1**)	1.670[a] (4.8)[b]	1.550 (3.5)	1.402 (4.6)	0.585 (0.8)	1.337 (3.3)	0.918 (3.5)	0.334 (2.8)
Brucine (**2**)	1.317 (6.4)	1.138 (5.2)	0.898 (5.3)	0.463 (6.5)	0.894 (5.9)	0.730 (5.3)	0.278 (1.9)
β-Colubrine (**3**)	0.401 (2.9)	0.355 (3.0)	0.209 (5.1)	0.115 (6.5)	0.357 (3.4)	0.166 (3.5)	0.098 (3.7)
Pseudostrychnine (**4**)	0.024 (4.2)	0.021 (4.2)	0.017 (6.9)	0.016 (6.1)	0.015 (6.5)	0.013 (3.9)	0.007 (6.5)
Strychnine *N*-oxide (**5**)	0.063 (4.7)	0.089 (5.7)	0.102 (5.1)	0.138 (4.7)	0.080 (5.6)	0.110 (4.9)	0.065 (3.3)
Brucine *N*-oxide (**6**)	0.028 (5.1)	0.049 (5.5)	0.050 (6.1)	0.115 (2.0)	0.060 (5.6)	0.084 (5.2)	0.053 (3.4)
Novacine (**7**)	0.030 (5.0)	0.025 (2.8)	0.027 (4.3)	0.057 (3.0)	0.018 (5.4)	0.025 (6.2)	0.019 (5.8)
Icajine (**8**)	0.028 (3.5)	0.026 (5.4)	0.024 (6.8)	0.022 (3.7)	0.024 (6.3)	0.023 (6.7)	0.013 (4.8)
Vomicine (**9**)	0.054 (4.9)	0.047 (3.7)	0.034 (3.5)	0.020 (6.6)	0.032 (2.2)	0.031 (3.8)	0.017 (6.2)
Isostrychinine (**10**)	0.008 (4.5)	0.022 (4.2)	0.027 (6.3)	0.057 (3.2)	0.064 (4.1)	0.086 (4.9)	0.049 (4.2)
Isobrucine (**11**)	0.0004 (25)	0.005 (4.8)	0.013 (6.4)	0.025 (8.0)	0.030 (6.2)	0.047 (4.4)	0.020 (5.3)

a) The content of each alkaloid in the processed and unprocessed seeds is indicated as a percentage (wt%). Each value is the mean of three determinations. *b*) Coefficient of variation (C.V.), given in parentheses, is expressed by the following equation:

$$\text{C.V. (\%)}=\frac{\text{standard deviation (S.D.)}}{\text{mean value}}\times 100$$

Processings A and B were carried out by using a sand bath and an oil bath, respectively, as described in Materials and Methods.

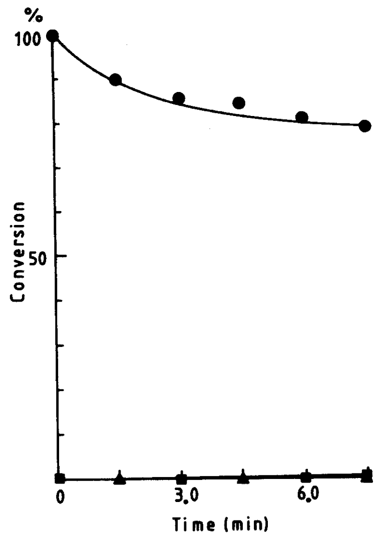


Fig. 1. Time Course of the Thermal Transformation of Strychnine (1) into Isostrychinine (10) and Strychinine N-Oxide (5)

The amounts of ring-opened and N-oxidized compounds are represented as conversion percentages from added strychnine. (●), strychnine; (▲), isostrychnine; (■), strychnine N-oxide.

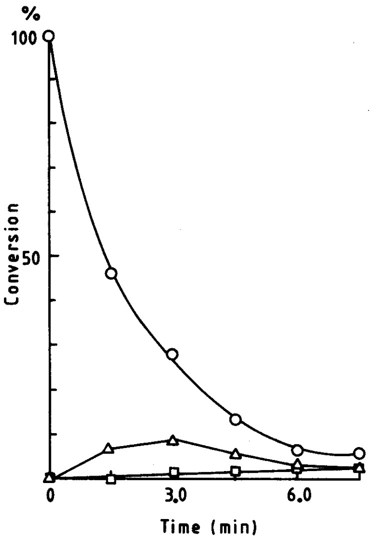


Fig. 2. Time Course of the Thermal Transformation of Brucine (2) into Isobrucine (11) and Brucine N-Oxide (6)

The amounts of ring-opened and N-oxidized compounds are represented as conversion percentages from added brucine. (○), brucine; (△), isobrucine; (□), brucine N-oxide.

(processings A and B) were carried out according to the Chinese Pharmacopoeia.[1] The contents of the major alkaloids, strychnine (1) and brucine (2), in the unprocessed seeds, were similar to those reported previously.[3-5] Processings A and B, carried out using a sand bath and an oil bath, respectively, both caused the contents of strychnine (1), brucine (2) and β-colubrine (3) to decrease significantly, the decreases being greater with increase of temperature from 220 to 260 °C. In the processing A, the contents of major alkaloids were *ca.* 85—90%, 50—80% and 30—35% of the original amounts in the unprocessed seeds at 220, 240 and 260 °C, respectively, while in the processing B, they were *ca.* 70—90%, 40—55% and 20—25% at the respective temperatures. The temperature-dependent decreases in amounts of these alkaloids were greater in the latter processing than in the former processing. The contents of minor alkaloids, pseudostrychnine (4), novacine (7), icajine (8) and vomicine (9) were also decreased by both processings. On the other hand, strychnine N-oxide (5), brucine N-oxide (6), isostrychnine (10) and isobrucine (11) appreciably increased in amounts. The maximum amounts of these compounds were obtained at 260 °C in processing A, but at 240 °C in processing B. The identification of the above products (5, 6, 10 and 11) was performed by direct comparison with authentic samples isolated in the previous experiment.[2]

Thermal Transformation of Strychnine (1) and Brucine (2) For the purpose of investigating the thermal stability of strychnine (1) and brucine (2), and the time course of the transformation of both compounds into ring-opened and N-oxidized compounds, both samples were incubated at intervals at 235 °C. Powdered strychinine (1) itself was fairly stable at 235 °C and only 20% of it was decomposed in 7.5 min (Fig. 1). On the other hand, brucine (2) was decomposed rapidly under these conditions (half life time ($T_{1/2}$) = 1.4 min) and small amounts of isobrucine (11) and brucine N-oxide (6) were formed (Fig. 2). However, when a 1 : 1 mixture of both compounds was heated under the same conditions, decomposition of strychinine (1) was significantly accelerated ($T_{1/2}$ = 5.2 min), while that of brucine (2) was suppressed ($T_{1/2}$ = 5.3 min) (Fig. 3). In addition, the amounts of ring-opened and N-oxidized compounds derived from both compounds increased to a considerable extent. The maximum amounts of isostrychinine (10), isobrucine (11) and brucine N-oxide (6) were obtained at 3.0—4.5 min.

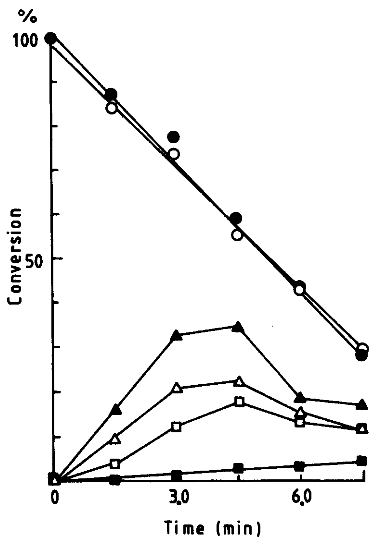


Fig. 3. Time Course of the Thermal Transformation of Strychnine (1) and Brucine (2) in the Presence of Both Compounds

The amounts of products are represented as conversion percentages from the respective added alkaloids. (●), strychnine; (○), brucine; (▲), isostrychnine; (△), isobrucine; (■), strychnine N-oxide; (□), brucine N-oxide.

Discussion

Drug-processing occupies an important part in the traditional Chinese medicinal system. Crude drugs such as Ginseng Radix, Rhei Rhizoma, Glycyrrhizae Radix, Aconiti Tuber, Rehmanniae Radix, nux vomica *etc.* are processed in different ways as the needs of the case demand. Nux vomica is processed by parching in a sand bath or frying in an oil bath, both at 235 °C. The purposes of processing nux vomica are said to be as follows: (1) to reduce its toxicity; (2) to eliminate irritant hair on the surfaces of seeds, so that the drug and its pieces can be swallowed safely; (3) to make it easier to crush the seeds into pieces, because the dried seeds are quite hard.

Sha *et al.*[3] and Zeng *et al.*[4] reported that the contents of major alkaloids and the toxicity decreased during processing of the seeds of *S. nux-vomica*. In our present experiments, we not only confirmed their observation, but also found that styrchnine (**1**) and brucine (**2**) were transformed in part into isostrychnine (**10**), isobrucine (**11**), strychnine *N*-oxide (**5**) and brucine *N*-oxide (**6**). The former two (**10** and **11**) were formed by cleavage of the ether linkage located at the beta position to the N-1 lactam carbonyl in the original alkaloids and the latter two (**5** and **6**) might be formed by air-oxidation of both alkaloids.

In a comparison of processing A (sand bath method) and processing B (oil bath method), the decomposition of the major alkaloids was found to be faster in the latter than in the former at corresponding temperatures (220, 240 and 260 °C), their analytical values being quite reproducible, with a C.V. of less than 6.5%. The maximum amounts of transformed products (**5**, **6**, **10** and **11**) were observed at 260 and 240 °C in processings A and B. The difference in temperature may be due to the difference in heat-conductive medium (sand and oil). In the present paper, we can not conclusively discuss the merits and demerits of processings A and B, but the processed seeds were easily separated from the sand by using a sieve in processing A. In processing B, it was troublesome to wipe away the surface oil. So, processing A is more convenient.

In the model experiment performed by heating authentic samples at various temperatures, strychnine (**1**) was fairly stable at 235 °C, while brucine (**2**) was unstable. On the other hand, the decomposition of strychnine (**1**) and the transformation of strychnine (**1**) and brucine (**2**) into the respective ring-opened and *N*-oxidized compounds were significantly accelerated in the mixture of both compounds. As the melting points of strychnine (**1**) and brucine (**2**) are 281—283 °C and 180—182 °C, respectively,[2] the accelerated decomposition of strychinine (**1**) in the mixture may be due to the change in physical state (solid to liquid), which may be caused by depression of the melting point in the presence of two different compounds. Strychnine (**1**) in aqueous solution was reported to be readily converted to isostrychnine (**10**) at 170—180 °C.[6,7] These findings reveal that the range of temperature adopted for processing of the seeds of *S. nux-vomica*[1] is quite adequate for the purpose of reducing the toxicity of the drug. Under the conditions employed, the significant amounts of ring-opened and *N*-oxidized compounds were produced.

In a previous paper, we reported the isolation of isostrychnine *N*-oxide (**12**) and isobrucine *N*-oxide (**13**) from a large amount of processed seeds of *S. nux-vomica*. However, we could not determine the quantity of these compounds in this experiment, due to their extremely low contents. These compounds (**12** and **13**) may be formed from strychnine (**1**) and brucine (**2**), respectively, by thermal cleavage and air-oxidation reactions, or from the corresponding *N*-oxides (**5** and **6**) present in the unprocessed seeds, by thermal cleavage reaction.

In contrast with a vast number of studies on strychnine (**1**), few studies have been conducted on ring-opened and *N*-oxidized compounds. It would be worthwhile to investigate the biological activities of these compounds produced by drug-processing, in order to understand the significance of the processing of the seeds of *S. nux-vomica*.

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