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Note

High-performance liquid chromatographic determination of the alkaloids in betel nut

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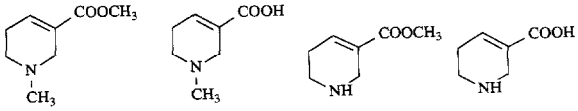
The nuts of the areca palm, *Areca catechu* L., are commonly chewed by the peoples indigenous to South-Eastern Asia and the Pacific. The nuts have been shown to contain at least six related pyridine alkaloids[1], of which four have been conclusively identified. Arecoline is the major constituent, being present at 0.1–0.5%. The other alkaloids are present in much lower levels[1,2]. The structures of the four major alkaloids are shown in Fig. 1.



I II III IV

Fig. 1. Structures of the *Areca* alkaloids: (I) arecoline, (II) arecaidine, (III) guvacoline and (IV) guvacine.

Betel nuts have been used for a variety of purposes including appetite suppression[3], increasing stamina and general well-being[2,3], even as a treatment for tapeworm[2,4]. Probably because it is present in the largest quantities most of the effects of chewing betel nuts have been attributed to arecoline, although this has yet to be proved. Arecoline has a stimulating parasympathetic action[5], cardiovascular[6] and ocular[7] effects and as a veterinary preparation it is used as an anthelminthic. More recently it has been suggested that some of the psychic effects observed upon chewing betel nuts may in fact be due to arecaidine and guvacine, which are both potent γ-aminobutyric acid (GABA) uptake inhibitors[8].

It would be of interest to correlate the levels of the individual alkaloids in betel nuts with the observed pharmacological response. Although a gas–liquid chromatographic (GLC) method of quantitation has been described for arecoline in capsule preparations[9] the determination of arecoline levels in nuts has generally involved steam distillation and titration[10]. Paper and thin-layer chromatography have both been used to separate the other related alkaloids[2] however there is no satisfactory method for quantitative analysis. This paper describes the development of a method suitable for the simultaneous determination of the four major alkaloids in betel nuts.

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EXPERIMENTAL

Materials

Arecoline–HBr and arecaidine–HCl were purchased from Sigma (St. Louis, MO, U.S.A.). Guvacoline and guvacine were prepared by N-demethylation of arecoline and arecaidine, respectively. Satisfactory spectral data were obtained for both compounds. All solvents were of analytical reagent or HPLC grade. Water was purified by a Milli-RO4 water purification system or a Milli-Q reagent grade water system (Millipore, Bedford, MA, U.S.A.).

Procedures

Fresh betel nut (2–3 g) was placed in a 50-ml test-tube containing 0.001 *M* orthophosphoric acid (30 ml). Following grinding with an homogenizer (Polytron, Kinematica, Lucerne, Switzerland) the mixture was stirred for 4 h (room temperature) and then centrifuged (2000 *g*) for 10 min. A 1-ml volume of supernatant was mixed with 1 ml 85% orthophosphoric acid and passed through a 0.22-μm filter (Millipore Type GS). This solution was diluted (1:160) into a buffer [0.16% ammonium hydroxide–methanol (60:40)] at pH 3–4. An injection volume of 100 μl was used.

Chromatographic conditions

A Waters Model 510 pump was used in conjunction with a Model 481 variable-wavelength UV detector (Waters Assoc., Milford, MA, U.S.A.). The pump was fitted with a Rheodyne 7125 loop injection system equipped with a 100 μl loop. Peak areas were determined using a Shimadzu C-R4A integrator. Peak heights were measured with the integrator or an Omniscribe recorder. For the stationary phase a cation-exchange column (Whatman Partisil SCX particle size 10 μm, 25 cm $\times$ 4.6 mm I.D.) was used at ambient temperature. The mobile phase consisted of 85% orthophosphoric acid–methanol–water (3:400:590), and was adjusted to pH 3.8 with 14% ammonium hydroxide. The flow-rate was 1.8 ml/min and the eluent was monitored at 215 nm.

RESULTS AND DISCUSSION

As there had been no previous reports on the high-performance liquid chromatography (HPLC) of *Areca* alkaloids a number of columns were examined under a variety of conditions. An ion pairing procedure using 1-heptanesulphonic acid and a reversed-phase column showed promising results, however the simplest and best separation was achieved using a strong cation-exchange (SCX) column. The retention times, shown in Table I, are all highly reproducible and demonstrate the ready separation of the four alkaloids.

Calibration graphs were prepared for all four alkaloids. Responses were linear over the range 5–5000 ng/ml (correlation coefficients > 0.995). The limits of sensitivity (signal-to-noise ratio of 4) are listed in Table I.

In betel nuts arecoline has been estimated by steam distillation of the nuts followed by titration of the distillate[10]. This procedure involved steam distillation of a solution containing arecoline at pH 9.0–9.1. When this methodology is followed by HPLC both arecoline and guvacoline showed a considerable degree of hydrolysis to

TABLE I
RETENTION TIMES AND SENSITIVITY LIMITS OF ALKALOID STANDARDS

Alkaloid	*Retention time (min)*	*Detection limit (ng/ml)*
Guvacine	3.3	5.2
Arecaidine	4.3	5.2
Guvacoline	5.0	7.9
Arecoline	7.7	12.2

their corresponding acid[11]. These acids are not steam volatile and as a consequence the results obtained using steam distillation methods may be misleading.

To overcome this problem we have developed a mild extraction procedure using dilute (0.001 *M*) orthophosphoric acid. Under these conditions both arecoline and guvacoline are quite stable. Although stirring for 4 h ensures complete extraction, the time may be reduced to 2 h without significant decrease in efficiency. As shown in Fig. 2, this extract does not produce a clean chromatogram, however interfering impurities

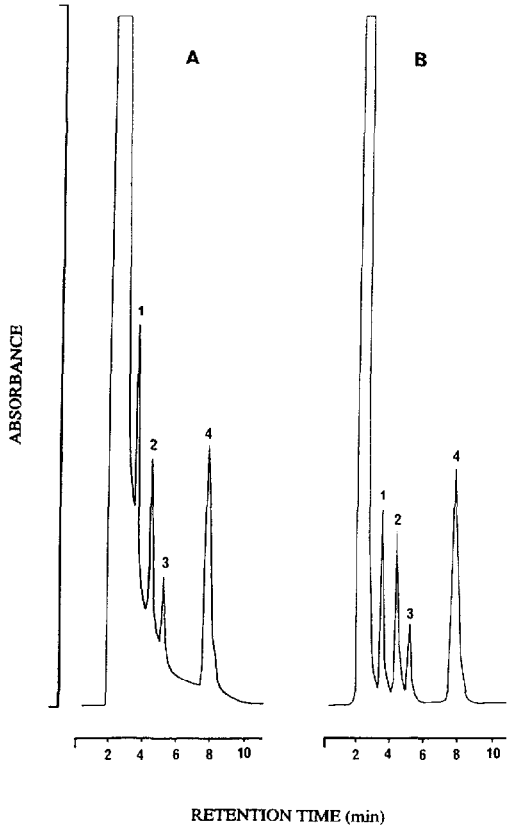

Fig. 2. Chromatogram of the *Areca* alkaloids, (1) guvacine, (2) arecaidine, (3) guvacoline and (4) arecoline, before (A) and after (B) treatment with concentrated orthophosphoric acid. The injection volume was 100 μl. Compounds were detected by UV spectroscopy (λ = 215 nm; 0.05 a.u.f.s.).

may be removed by precipitation with concentrated orthophosphoric acid and subsequent filtration.

Analysis of fresh betel nuts (obtained from Darwin, Australia) showed alkaloid contents of: arecoline (0.30–0.63%), arecaidine (0.31–0.66%), guvacoline (0.03–0.06%) and guvacine (0.19–0.72%). The arecoline levels are marginally higher than those obtained using the steam distillation method for Indian[12] and New Guinea[3] nuts. Individual levels for the other alkaloids have not previously been reported although the total alkaloid content is certainly higher than observed in prior studies[3]. This may be due to seasonal and/or geographical variation. Both possibilities are currently under investigation.

CONCLUSIONS

A simple and rapid HPLC method has been developed for the estimation of *Areca* alkaloids. Levels for the four major alkaloids have been determined in fresh nuts and shown to be higher than previously thought.

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