AN INVESTIGATION OF PANAMANIAN IPECAC: BOTANICAL SOURCE AND ALKALOID ANALYSIS

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ABSTRACT.—Contrary to current opinion, *Cephaelis ipecacuanha*, the botanical source of Brazilian Ipecac, was also found to be the source of the Panamanian form of the drug. Hplc analysis showed that the alkaloid content of Panamanian Ipecac samples was typical of that previously reported for Central American and Columbian Ipecac; that is the percentage of cephaeline usually exceeds that of emetine (8 of 11 samples). Since the opposite is generally true for Brazilian Ipecac, it appears that *C. ipecacuanha* produces different emetine to cephaeline ratios in the two regions where the plant is often collected for drug use.

The crude drug Ipecac or Ipecacuanha is usually defined as the dried roots and rhizomes of either *Cephaelis acuminata* Karsten or *Cephaelis ipecacuanha* (Brotero) A. Richard (1–3). The medicinal value of this drug rests mainly on its content of cephaeline and emetine (4–6). Although Ipecac contains several other isoquinoline alkaloids, these compounds are largely responsible for the emetic, antiamebic, expectorant, and diaphoretic effects for which the drug is noted. The persisting importance of Ipecac is attested to by the fact that it remains official in numerous pharmacopeias throughout the world.

Although Panamanian Ipecac is an important form of the drug, very little has been published which deals with its characteristics. This study was undertaken to clarify the botanical source and alkaloid content of Panamanian Ipecac.

Traditionally, Ipecac from Panama has been grouped with commercial forms of the drug from Central America and Colombia, such as Cartagena, Nicaragua, and Savanilla Ipecac. All of these forms have been said to be derived from *C. acuminata* (1–3). The other botanical source of Ipecac, *C. ipecacuanha* (or *Psychotria ipecacuanha* Stokes), yields Rio or Brazilian Ipecac. A number of minor morphological differences have been noted for the crude drugs obtained from these two plant sources (1), but the only major difference appears to be in alkaloid composition. The Colombian and Central American drug usually contains more alkaloid than the Brazilian or Rio form, and the ratio of emetine to cephaeline differs in the two types (4, 7–9). In most cases Brazilian Ipecac contains more emetine than cephaeline, while the reverse is generally true for the Colombian and Central American drug.

Contrary to current opinion regarding the botanical source of Panamanian Ipecac (1–3, 10), this investigation has established the source to be *C. ipecacuanha* and not *C. acuminata*. Panamanian herbarium material was studied as well as eleven additional collections obtained from the Darien and Panama provinces of Panama. These latter collections were later analyzed for alkaloid content. All of this material was quite uniform taxonomically and agrees with other herbarium material from South America which has been going under the name of *C. ipeca-*

cuanha. A full description of this species, as well as an illustration prepared from the Panamanian herbarium collections, was recently published elsewhere (11).

As mentioned above, Ipecac derived from other regions of Central America and Colombia have also been thought to be derived from *C. acuminata*. Because these forms of the drug have long been recognized as being similar to Panamanian Ipecac in crude drug morphology and alkaloid composition, the botanical source of Cartagena, Nicaragua, and other related forms of Ipecac is now in question. This situation is further complicated by the fact that the name *Cephaelis acuminata* appears to be a *nomen nudum* in that a description of this species has not been validly published from a taxonomic point of view (11, 12).

This investigation was also concerned with the alkaloid content of Panamanian Ipecac, in particular the emetine to cephaeline ratio. A number of analytical procedures have been published for Ipecac. The USP XX (10) procedure utilizes a multiple column partition system to separate the alkaloids, followed by an assay of emetine and cephaeline by ultraviolet absorption. Although the accuracy and reproducibility of this procedure is well documented (13), it is a very time-consuming assay. Numerous other methods have been reported for the separation and subsequent quantitation of emetine and cephaeline (7, 8, 14–18). However, a procedure based on high pressure liquid chromatography (hplc) appeared to provide the best opportunity for both a rapid and accurate method of analysis. High pressure liquid chromatography of emetine and cephaeline has been previously reported, but these methods were not suitable because of the excessively long retention time of cephaeline in one system (19) and the need for derivatization in the other (20).

Thus, a new hplc assay was developed for the alkaloids of Ipecac. This system employed 10 μm microparticulate silica columns which were eluted with a dichloromethane-methanol-ammonium hydroxide solvent. The solvent flow rate was programmed from 1 to 4 ml per minute and eluted alkaloids were detected with a uv detector at 280 nm (19). With the exception of psychotrine, this system provided baseline separation of the principal alkaloids of Ipecac in less than 14 minutes. The retention time of psychotrine was in excess of 20 minutes and, because of peak broadening, this system could not be used to quantify this minor alkaloid of Ipecac. A typical chromatogram of an Ipecac extract sample containing internal standard is shown in figure 1. The retention times and k' values (21) of the various Ipecac alkaloids are given in the experimental section.

Strychnine was used as an internal standard for the hplc assay and quantitation of emetine and cephaeline was achieved through the preparation of standard curves relating chromatogram peak area to alkaloid concentration. The minor alkaloids of Ipecac (e.g., O-methylpsychotrine) were not quantified. Analytical data for the Panamanian Ipecac samples assayed are shown in table 1. The variation in the alkaloid content between samples could be due to a number of factors such as differences in the age of the samples and in the season and geographical area of collection. However, in spite of the variation, these data show that the alkaloid content of the Panamanian samples is typical of that previously reported for Central American and Colombian Ipecac (4, 7–9); that is, the percentage of cephaeline usually exceeds that of emetine (8 of 11 samples).

Thus, it appears that *C. ipecacuanha*, the source of both Panamanian and Brazilian Ipecac, produces different emetine to cephaeline ratios in the two regions where the plant is often collected for drug use. This difference could be due to environmental and/or genetic factors and further study will be needed to clarify its bases.

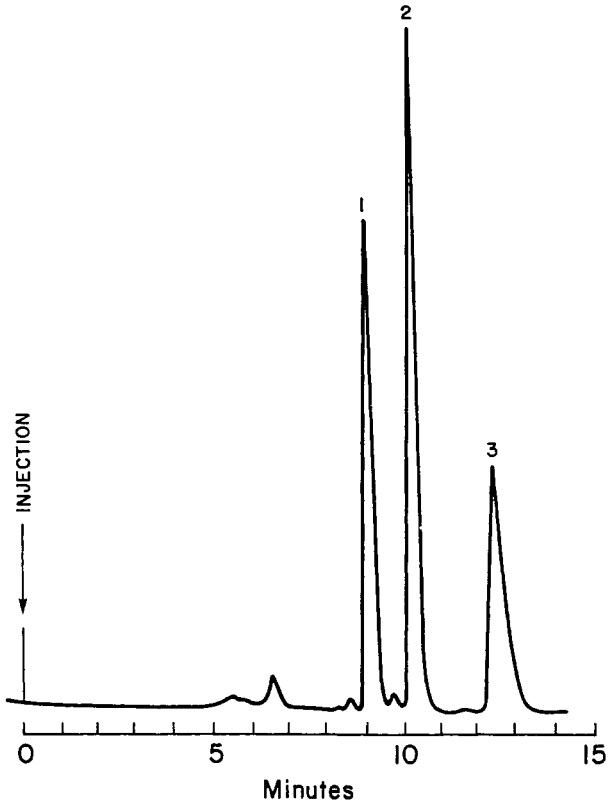

FIG. 1. Chromatogram of Ipecac extract residue. Column: 10 μm silica, 3.9 mm (ID) X 60 cm. Eluate: dichloromethane-methanol-NH_4OH (250:10:1), programmed 1–4 ml/min. UV detector at 280 nm, 0.2 aufs. Peaks: 1=emetine; 2=strychnine (internal standard); 3=cephaeline.

TABLE 1. Emetine and cephaeline content of Panamanian Ipecac samples.

Sample	Province[a]	Collection date	Emetine (%)[b]	Cephaeline (%)[b]	E/C[c]
1	D	03-02-76	0.76±0.03	1.06±0.04	0.72
2	D	21-03-76	0.42±0.02	0.76±0.04	0.55
3	D	12-06-76	0.55±0.04	0.77±0.04	0.72
4	D	14-09-76	0.78±0.01	1.31±0.05	0.60
5	D	18-11-76	0.82±0.03	1.06±0.04	0.77
6	P	26-07-78	1.12±0.03	1.37±0.14	0.82
7	P	10-08-78	1.29±0.02	1.21±0.02	1.07
8	P	24-08-78	1.49±0.09	1.35±0.05	1.10
9	P	26-10-78	1.60±0.04	1.33±0.03	1.20
10	P	12-01-79	1.27±0.08	1.82±0.02	0.70
11	P	21-09-79	1.15±0.04	1.33±0.03	0.86

[a]Samples were collected in the Darien (D) and Panama (P) provinces of Panama.
[b]Two extracts of each sample were prepared and each was assayed twice using hplc. The mean of the four assays (±1 standard derivation) is given in the table.
[c]Ratio of the amount of emetine to the amount of cephaeline.

EXPERIMENTAL

PLANT MATERIAL.—The samples of Panamanian Ipecac were collected in Punta Alegre and Cucunati (Province of Darien) and in Chorrera (Province of Panama). The plants were identified by Professor Mireya Correa, Curator of the Herbarium of the University of Panama, and the identification was further confirmed by one of us (John Dwyer). Samples of these plants were deposited in the Herbarium of the University of Panama. The roots were dried, stored in air-tight containers, and protected from light until used.

HPLC ASSAY.—The hplc system consisted of a Waters Associates 6000A solvent pump, 660 flow programmer, U6K sample injector, 440 uv detector (280 nm) and a Fischer Recordal Series 5000 recorder. The system employed two 3.9 mm (ID) X 30 cm μporasil® (10 μm silica, Waters Associates) columns connected in series. These columns were preceded by a 3.2 mm (ID) X 5 cm precolumn containing HC Pellosyl® (30–50 μm pellicular silica, Whatman).

The solvent system consisted of dicloromethane-methanol-ammonium hydroxide (250:10:1). The solvent flow was programmed (660 flow program 8) from 1 ml/min to 4 ml/min over a ten-minute period.

The retention time and k' values for the various Ipecac alkaloids and strychnine are as follows: emetamine (8.0 min, 0.45), O-methylpsychotrine (8.5, 0.54), emetine (9.0, 0.64), strychnine (10.0, 0.82), isoemetine (10.8, 0.96), and cephaeline (11.6, 1.11). Psychotrine was not eluted within 20 minutes in this system. Chromatographic analysis of an extract prepared from alkaloid-free Ipecac (*vide infra*) did not show peaks which overlapped with any of the alkaloids listed above.

Standard response curves were prepared with solutions (dichloromethane-methanol, 25:1) containing 2 mg/ml strychnine and known amounts of emetine or cephaeline free base. An injection volume of 7 μl and a uv detector sensitivity of 0.2 absorbance units full scale were used to establish chromatograms for each standard solution. The areas of peaks in the chromatograms were calculated by multiplying peak heights by peak widths at half height. From these data, curves were prepared which related the concentration of emetine or cephaeline (mg/ml) in the injected solution to the peak area ratios of the alkaloid and internal standard. This ratio was calculated for each standard solution by dividing the emetine or cephaeline peak area by that of strychnine and multiplied by 100. Each standard solution was assayed twice, and the standard curves were then prepared. The emetine standard curve gave a correlation coefficient of 0.998, a slope of 54.29, and an intercept of 0.92. These values for cephaeline were 0.999, 43.57, and 0.86.

DETERMINATION OF EMETINE AND CEPHAELINE IN PANAMANIAN IPECAC SAMPLES.—Ipecac samples were ground in a Waring blender and sieved to give a 30 mesh powder. The ground samples were then extracted by means of the following modification of the USP XX procedure for Ipecac alkaloid extraction (10). A one gram aliquot of each sample was placed in a 25 ml glass stoppered flask with 10 ml of ethyl ether. This material was mixed thoroughly, and 1 ml of ammonium hydroxide TS (23%) was added to the flask. After its weight was determined, the extraction mixture was shaken for two hours at room temperature. It was then allowed to stand overnight. The extraction mixture was shaken again for a few minutes and, if necessary, the weight of the mixture was adjusted to the initial value by the addition of ether. Two milliliters of the ether extract were then placed in a 20 ml screw-cap vial and reduced to dryness in a stream of nitrogen. The resulting residue was dried under vacuum and redissolved in 2 ml of dichloromethane-methanol (25:1) containing 4 mg of strychnine base (internal standard). This solution was then assayed for emetine and cephaeline using the hplc system described above. Each sample of Ipecac was extracted twice, and each extract was assayed in duplicate. For the four assay results, the average and standard deviation ($\pm 1\ \sigma$) were determined for each sample. The results of these assays are shown in table 1. Figure 1 shows a typical chromatogram produced by an alkaloid extract sample containing internal standard.

The efficiency of alkaloid extraction from Ipecac powder could not be determined with strychnine as an internal standard due to the low ether solubility of the strychnine base. Thus, alkaloid-free Ipecac powder to which known quantities of emetine and cephaeline were added was used to determine this efficiency. The alkaloid-free Ipecac was prepared by exhaustively extracting the basified crude drug with ether in a Soxhlet extractor. Alkaloids were then removed from the ether extract by extraction with 5% sulfuric acid. This extraction was repeated until the acid extract was no longer positive to Dragendorff's reagent. The acid extracts were discarded and the remaining ether extract was washed to remove excess acid. The alkaloid-free ether extract was then combined with the Ipecac marc, and the solvent was removed by vacuum evaporation. The resulting powder was dried under vacuum for 12 hours before use. Chromatographie analysis of this powder showed that it did not contain detectable quantities of alkaloid.

To determine the alkaloid extraction efficiency, 1 g samples of the alkaloid-free powder were mixed with 500 μl of a 60% ethanol solution containing 34.6 mg of cephaeline and 15.0 mg of emetine. The powder samples were then dried under vacuum and assayed for emetine and cephaeline as described above. The mean extraction efficiency for emetine was found to be $99.17 \pm 4.76\%$ and 96.61 ± 4.49 for cephaeline.

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