Determination of Pyrrolizidine Alkaloids in Commercial Comfrey Products (*Symphytum* sp.)

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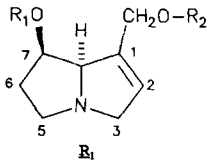
Received December 22, 1992, from the *Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC 20204.* Accepted for publication October 7, 1993[⊗].

Abstract □ The presence of hepatotoxic pyrrolizidine alkaloids in comfrey (*Symphytum* sp.) and the widespread use of decoctions of this plant as a beverage (herbal tea) are of increasing concern. A method for the extraction and solid-phase concentration and capillary gas chromatographic determination of these alkaloids and their *N*-oxides in botanical materials has been developed and was applied to eleven comfrey-containing products purchased from retail health-food outlets in the Washington, DC, area during May–June 1989. Nine of the 11 products were found to contain measurable quantities of one or more of the alkaloids, in ranges from 0.1 to 400.0 ppm. Products containing comfrey leaf in combination with one or more other ingredients were found to contain the lowest alkaloid levels. Highest levels were found in bulk comfrey root, followed by bulk comfrey leaf. The species of the bulk material was verified by thin-layer chromatography and other means.

The medicinal use of herbs has increased greatly during the past 30 years. This surge in the popularity of alternative medicine arises from disillusionment with the conventional medical establishment and from the belief that traditional and natural ingredients are inherently safer and more healthful than are synthetic ingredients.[1-4] Unfortunately, historical use of medicinal herbs and plants does not guarantee safety. Many of the plants previously used medicinally are mentioned in monographs on poisonous plants, and their continued use was based primarily on the lack of safer, more effective alternatives.[5] Botanical products that make no health claims are classified as foods by current U.S. law, and they are therefore not required to undergo the rigorous testing for safety and efficacy mandated for drugs.

One of the more popular of these botanical remedies is comfrey (*Symphytum officinale* L., Boraginaceae). This plant is a tall perennial with large hairy leaves and small purple flowers. As a poultice or ointment, comfrey root has been used to promote healing of broken bones, ulcers, and bruises.[6-8] When used internally, comfrey teas or tablets are claimed to have a soothing and healing effect on the digestive tract.[8] For this reason comfrey is often combined with other botanicals, such as pepsin and fenugreek, that have a reputed effect on gastrointestinal activity. In the past, comfrey was one of the most popular herbal teas in the world. Fortunately, as its dangers have become known, its popularity has declined, but it is still available commercially in several forms.

Concerns over the adverse health effects of comfrey products stem from their content of pyrrolizidine alkaloids (PAs) and pyrrolizidine alkaloid *N*-oxides (Figure 1). A hydroxymethylene group is normally attached to C-1 and a hydroxy group at C-7. One or both hydroxyl groups are commonly esterified, and a double bond between C-1 and C-2 is a common feature that is apparently necessary for toxicity.[9,10] According to SAR studies, the unsaturated PAs are toxic because they are very rapidly converted to the corresponding pyrroles by the mixed-function oxidases of the liver.[11-14] These pyrroles are potent alkylating agents that react very rapidly with cell constituents, this reaction resulting in cellular destruction or abnormal growth patterns.[15]



Name	R_1	R_2	R_3
retronecine	H	H	H
lycopsamine	H	III	H
intermedine	H	IV	H
7-acetyllycopsamine	CH_3CO	III	H
7-acetylintermedine	CH_3CO	IV	H
symphytine	I	III	H
symlandine	II	III	H
echimidine	II	III or IV	OH
uplandicine	CH_3CO	III or IV	OH

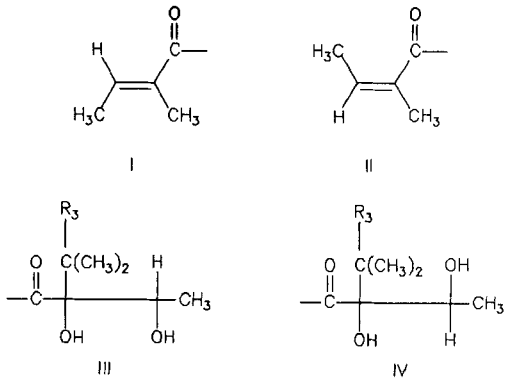


Figure 1—Alkaloids of *S. officinale* L. and *S. x uplandicum* Nyman.[5,34]

Accumulation of this cellular damage results in a syndrome known as hepatic veno-occlusive disease (HVOD).[11-13] A review of comfrey toxicity has been provided by Weisner.[16] More recent and more comprehensive reviews have been provided by the World Health Organization[17] and by Awang.[18]

Livestock poisonings caused by PA-containing plants belonging to the same family as comfrey have provided many reports on the acute and chronic toxicity of this group of compounds.[17] Human poisonings by PA-containing plants have also been reported. Fox et al.[19] reported the death of a 2-month-old child who had been given an infusion of a PA-containing plant called *Senecio longilobus*. Stillman et al.[20] and Huxtable[21] reported the development of cirrhosis in a 6-month-old also given an infusion of *S. longilobus*. More germane to the current discussion are reports by Ridker et al.,[22] Weston et al.,[23] and Bach et al.[24] of human HVOD caused by ingestion of herbal infusions of *S. officinale*. Huxtable et al.[25] analyzed commercially available comfrey–pepsin capsules (root and leaf) and found PA levelsthat could be expected to produce HVOD after a few months of regular consumption.

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Table 1—Comfrey Products[a] Analyzed for Pyrrolizidine Alkaloids

Product	Form	Brand	Labeled Ingredients	Directions for Use
Comfrey-oatstraw	Capsules	1	Comfrey leaves, oatstraw, horsetail (shavegrass), *Lobelia*	Take two capsules three times daily with a large glass of water.
Comfrey-pepsin	Capsules	1	Comfrey leaves, pepsin (372 mg + 48 mg)	Take two capsules before every meal with a large glass of water.
Herbal comfrey	Capsules	1	Comfrey root, fenugreek seeds, hyssop, yerba santa, wild cherry bark	Take two capsules three times daily with a large glass of water.
Comfrey root	Capsules	1	*S. officinale*	Take two capsules three times daily with a large glass of water.
Imported comfrey, leaf tea	Bulk tea	2	(No ingredients listed)	Prepare as ordinary tea, using 1–1 1/2 teaspoonsful per cup. Add boiling water and let tea steep for at least 3 min.
Comfrey leaf, natural leaf tea	Tea bags	3	*S. officinale*	Pour fresh boiling water over a teabag in a cup and steep for 3–5 min.
Comfrey root, natural herb tea	Bags	3	*S. officinale*	Use 1–1 1/2 teaspoonsful of tea per cup. Steep 3–5 minin fresh boiling water.
Comfrey-pepsin	Capsules	4	Comfrey leaves and 100 mg pepsin	Take two capsules three times daily with water at mealtime or prepared as a tea. Recommended for occasional use only.
Comfrey leaf/fenugreek seed	Tablets	4	Fenugreek seed and comfrey leaves	Take two or three tablets every 3 h with water.
Comfrey root[b]	Bulk powder		*S. officinale*	None provided.
Comfrey leaf[c]	Dried leaf		*S. officinale*	None provided.

[a] Purchased from retail health food outlets in Washington, DC, in May–June 1989. [b] Gas chromatogram of extract is shown in Figure 2. Authentic material. [c] Gas chromatogram of extract is shown in Figure 3. Authentic material.

Unfortunately, the mechanism of action of the PAs renders them potent hepatocarcinogens, and even if acute intoxication does not occur, the likelihood of increased incidence of liver cancer must be considered. *S. officinale* fed to rats by Hirono et al.[26] produced 81 adenomas and three hemangiosarcomas of the liver in 175 animals autopsied at the end of the study. A large rat-feeding study, also conducted by Hirono et al.,[27] used several concentrations of *S. officinale* leaf and root in the diet. No liver adenomas or hemangiosarcomas were found in 130 control animals, but the incidence of these tumors in both leaf- and root-fed animals was extremely high. In a study on symphytine administered intraperitoneally to rats, one liver adenoma and three liver hemangiosarcomas were produced in a total of 20 male animals, whereas no liver tumors were found in 20 control males.[28] A detailed summary of these and other toxicological data may be found in the volume on pyrrolizidine alkaloids published by the World Health Organization.[17]

Analytical methods for the determination of PAs include thin-layer chromatography (TLC),[29–32] high-pressure liquid chromatography (HPLC),[14,31,33–37] and gas chromatography (GC).[29,38–41] Of these, GC offers the combination of high resolution, high sensitivity, and the capability to be interfaced with a variety of selective techniques such as mass spectrometry (MS), Fourier transform infrared spectroscopy (FTIR), and nitrogen/phosphorus detection (NPD). These selective techniques eliminate interferences commonly encountered in plant extracts. The purpose of the current study was to develop a gas chromatographic method for the separation and quantitation of PAs in herbal products and to examine the PA content of several commercially available comfrey-containing products.

Experimental Section

Materials—Intermedine, lycopsamine, acetylintermedine, acetyllycopsamine, and ridelline were obtained from R. J. Molyneux, U.S. Department of Agriculture (USDA) (Albany, CA). Bulk ground comfrey (*S. officinale*) root and leaf material were purchased from a local dealer in bulk botanicals. The identity of the ground bulk material was confirmed by the use of several criteria. The chemotaxonomic work of Huizing et al.[42] firmly established the lack of echimidine in *S. officinale* L. and ruled out the possibility that the material was *Symphytum asperum* or *Symphytum x uplandicum*. In addition, the pattern of alkaloids found in the bulk leaf and root was consistent with that reported by Huizing et al.[42] and by Awang et al.[43] The allantoin content, alkaloid pattern, and microscopic appearance of this material were considered sufficient for confirmation of the identity of the root powder. The alkaloid pattern and microscopic appearance of the leaf powder (especially of the characteristic epidermal trichomes)[44] were sufficient to establish the identity of the leaf material. Comfrey-containing products were purchased from a Washington, DC, retail health foods outlet during May–June 1989. Table 1 lists these products and the form in which they were sold, the labeled ingredients, and the directions for use. Brand names of the herbal products are not identified here. Chloroform and methanol were purchased from Baxter Health Care Corporation (McGaw Park, IL). Zinc dust, sulfuric acid, and ammonium hydroxide were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ). Diatomaceous earth (Celite) and Whatman No. 1 filter paper were purchased from Fisher Scientific Co. (Pittsburgh, PA). ChemElut CE-1020 columns were purchased from Varian Sample Preparation Products, (Harbor City, CA).

Extraction—For botanical materials, 10 g of botanical and 5 g of Celite were shaken for 1 h with 10 mL of ammonium hydroxide and 250 mL of chloroform–methanol (85:15, v/v). The mixture was filtered and the filtrate was evaporated at 45 °C on a rotary evaporator. The *N*-oxides in the residue were reduced to the free bases by dissolving the residue in 50 mL of 2 N sulfuric acid and adding 2.5 g of zinc dust to the solution. The resulting slurry was stirred for 2 h and then filtered. After 15 mL of filtrate was collected, 5 mL of ammonium hydroxide was added to the filtrate with mixing. The resulting basic solution was poured into a ChemElut CE-1020 column and allowed to equilibrate for 5 min. The PAs were eluted from the column with 100 mL of chloroform–ammonium hydroxide (99:1, v/v), and the eluate was evaporated to dryness at 45 °C on a rotary evaporator. The residue was resuspended in exactly 1 mL of methanol for GC and GC/MS determination.

Because comfrey is often consumed as an herbal tea rather than ingested whole, hot water infusions of the bulk root and leaf were prepared and then analyzed. In this method, 2 g (about 1 teaspoon) of root or leaf was added to 250 mL of hot water, and the mixture was allowed to steep for 5 min. The resulting infusion was carefully decanted, cooled to room

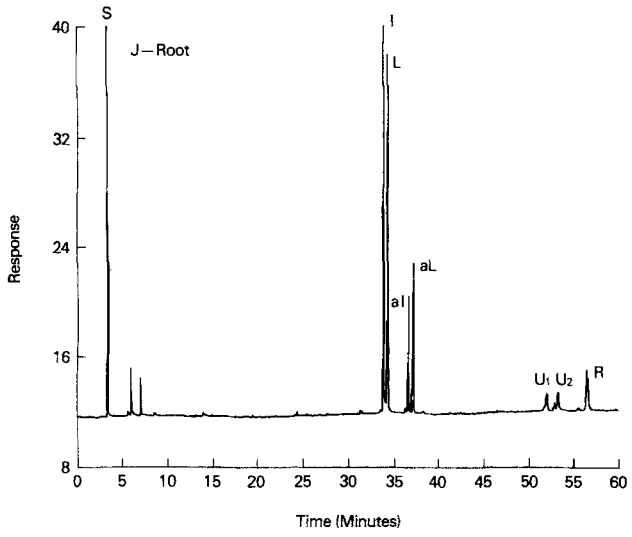

Figure 2—Gas chromatogram of comfrey root (*S. officinale* L.) extract. Key: S = solvent, I = intermediate, L = lycopsamine, aI = acetylintermedine, aL = acetyllycopasmine, U_1 = unknown 1, U_2 = unknown, 2, R = ridelline (internal standard).

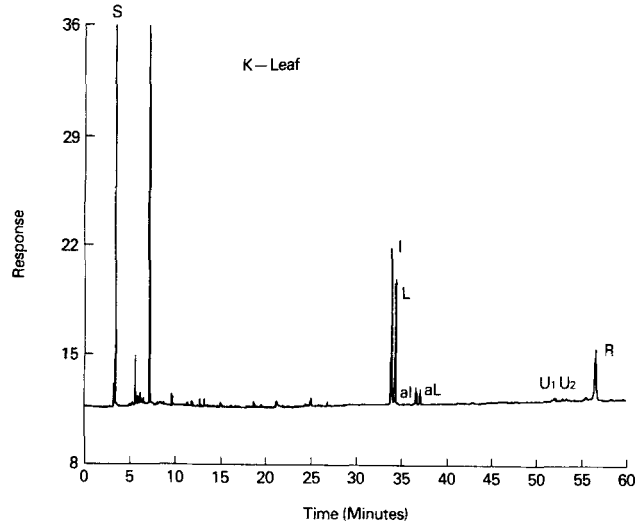

Figure 3—Gas chromatogram of comfrey leaf (*S. officinale* L.) extract. Key: S = solvent, I = intermediate, L = lycopsamine, aI = acetylintermedine, aL = acetyllycopsamine, U_1 = unknown 1, U_2 = unknown 2, R = ridelline (internal standard).

temperature, and extracted three times with 250 mL of chloroform–ammonium hydroxide (99:1, v/v). This solution was rotary evaporated to dryness at 45 °C and the residue was resuspended in 1 mL of methanol for GC and GC/MS determination. Ridelline was used as the internal standard.

Instruments—A Hewlett-Packard (HP) Model 5890 gas chromatograph was equipped with a nitrogen/phosphorus detector, an HP Model 7673A autosampler, and an RSL-200 bonded phase fused silica capillary column (50 m × 0.32 mm i.d.) (Alltech Associates, Deerfield, IL). The injector and detector temperatures were 190 and 280 °C, respectively. The GC oven temperature was held at 120 °C for 1 min and programmed to increase 4 °C/min to a final temperature of 230 °C, which was held for 31 min. The helium carrier gas flow rate was 28 cm/s, with an injector split ratio of 1:100.

GC/MS determinations were performed on a Finnigan MAT TSQ-46 quadrupole mass spectrometer interfaced to an INCOS 2300 data system equipped with Revision C TSQ software. Electron ionization (EI) data were acquired by using the following conditions: 70 eV electron energy, 0.35 mA emission current, 140 °C source temperature, 10^{-8} A/V preamplification, and −5 kV conversion dinode. Full-scan data were acquired by scanning the first quadrupole from *m/z* 40 to 540 in 1.0 s. Components in comfrey product extracts were separated as described for GC, except that the injection port was configured for splitless injection at 190 °C and the helium carrier gas flow rate was increased to 40 cm/s. Splitless injections were made at 40 °C. After 1 min the oven temperature was ramped to 120 °C and then programmed to increase to 240 °C at 4 °C/min, as described for the GC determination. The column outlet was coupled directly to the ion source of the mass spectrometer through a heated transfer line (280 °C).

Results

Representative gas chromatograms of extracts of authentic comfrey root and leaf are shown in Figures 2 and 3, respectively. Intermedine, lycopsamine, acetyllycopsamine, and acetylintermedine have been identified in these extracts by comparison of their GC retention times (t_R) and full-scan EI mass spectra with the same data obtained for authentic standards. Molecular ions (M^+) and base peak ions (B^+) were observed for these compounds (Table 2). Both M^+ and B^+ were the same as reported for those compounds by Stelljes et al.[41] in their identifications of a number of PAs. The mass spectra of two small late-eluting components showed M^+ and B^+ with *m/z* values of 381 and 220, respectively. These compounds could be symphytine or symlandine, consistent with data reported by Culvenor et al.[38] and Pedersen.[45] Lack of standards prevented us from determining which, if any, of the compounds was symphytine and prevented identification of all of the PAs known to occur in *Symphytum* sp. For these reasons, total PA contents were not calculated, and quantitative data are listed only for individual alkaloids. None of the peaks in any of the chromatograms corresponded to the M^+ or B^+ reported by Stelljes et al.[41] for echimidine.

Table 2—GC/MS Data for Pyrrolidine Alkaloids Found in Comfrey Products[a]

Compound	t_R (min)	M^+ (*m/z*)	B^+ (*m/z*)
Intermediate	33.98	299	138
Lycopsamine	34.40	299	138
7-Acetylintermedine	36.71	341	180
7-Acetyllycopsamine	37.21	341	180
Unknown 1	52.92	381	220
Unknown 2	53.37	381	220

[a] t_R = retention time; M^+ = molecular ion; B^+ = base peak ion.

Peak areas in chromatograms of authentic PAs and corresponding peak areas in chromatograms of comfrey product extracts were measured. A standard curve for each available authentic PA was constructed and linear regression was used to convert peak area to mass for calculation of PA levels in comfrey products. The lowest level of an individual PA found in any product was 0.1 ppm. As shown in Table 3, the PA content of commercially available comfrey products varies considerably. As expected, the highest PA levels were found in the authentic powdered root. Levels of intermedine and lycopsamine were approximately 4 times lower in authentic leaf than in root, whereas levels of acetyllycopsamine and acetylintermedine (the 7-acetyl derivatives) were roughly 8 and 11 times lower, respectively. All but two of the commercial products contained measurable amounts of alkaloid. PAs were also present at measurable levels in the decoctions prepared from the authentic leaf and root (Table 3).

Discussion

As noted above, there is extreme variability in the PA content of commercially available comfrey products. Low levels or a

Table 3—Pyrrolizidine Alkaloid Content of Comfrey Products (ppm) Determined by GC and GC/MS Analyses

Product	Intermedine	Lycopsamine	Acetylintermedine	Acetyllycopsamine
Comfrey–oatstraw	0.2	*a*	*a*	*a*
Comfrey–pepsin	0.3	*a*	*a*	*a*
Herbal comfrey	*a*	*a*	*a*	*a*
Comfrey root	*a*	*a*	*a*	*a*
Imported comfrey	0.1	0.1	*a*	*a*
Comfrey leaf	0.2	0.1	*a*	*a*
Comfrey root	0.2	0.2	1.3	*a*
Comfrey–pepsin	3.3	5.3	6.0	*a*
Comfrey leaf/ fenugreek seed	0.2	*a*	0.7	*a*
Comfrey root[b]	400.0	309.7	280.3	234.0
Comfrey leaf[b]	95.0	70.3	35.3	19.3
Root decoction	14.0	8.0	42.5	30.0
Leaf decoction	0.3	0.4	1.0	2.5

[a] Below detectable limits. [b] Authentic material also listed in Table 1. Used to prepare decoction.

total lack of alkaloids in the test products may reflect natural biological variation, age of plant material,[18] dilution of the *Symphytum* with non-PA-containing plant material, or mislabeling. Chemotaxonomically, these compounds should be present in roots and leaves of *Symphytum* sp.[42,43] Most of the comfrey products examined contained quantifiable levels of hepatotoxic and carcinogenic PAs. Echimidine was not detected in any of the commercially available products examined in the current study. This is an important point since Awang et al.[43] have demonstrated that the comfrey of commerce is not always *S. officinale*. The absence of echimidine indicates that the products examined were not prickly comfrey (*S. asperum*) or Russian comfrey (*S. x uplandicum*). Initially, an early Canadian ban on the sale of Russian comfrey was based on its content of echimidine, one of the most carcinogenic PAs. Ridker et al.[22] report that a patient who had consumed an estimated total of 85 mg of PAs over 4 months developed veno-occlusive disease. Huxtable et al.[25] reported total alkaloid levels in two brands of comfrey–pepsin preparations and found that a person consuming these products according to package directions would receive the toxic dose in 3 months for a comfrey leaf–pepsin product and 9 days for a comfrey root–pepsin product. A person consuming two 250-mg capsules of the bulk comfrey root used in this study three times a day would receive approximately 1.8 mg/day of alkaloid (excluding symphytine). Consumption of a similar amount of bulk leaf in capsule form would provide approximately 0.3 mg/day. Consumption of the two comfrey–pepsin formulations as per Huxtable et al.[25] would result in a daily dose of 0.9 μg/day and 44 μg/day. Although *S. officinale* lacks echimidine, the other alkaloids that it contains possess the structural characteristics necessary for hepatotoxicity and carcinogenicity, and as mentioned previously, both *S. officinale* and symphytine have been shown to be rodent carcinogens.[26–28] The Delaney clause of the U.S. Federal Food, Drug, and Cosmetic Act establishes a zero tolerance for proven carcinogens added to foods.

Proponents of comfrey as a medicinal tea have maintained that, although the root may not be safe for internal use because of its high alkaloid levels, the leaf is harmless because its PA levels are low and the PAs are not particularly water soluble. The present study confirms previous reports[24,42,43,45–47] that comfrey leaf definitively contains PAs and that the most common method of preparing the leaf for consumption (aqueous decoction, or tea) produces an aqueous solution that also contains alkaloids at measurable levels (probably in the form of the more water-soluble *N*-oxides).

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