Isolation of Minor Lupin Alkaloids. 1. A Simple Procedure for the Isolation of Angustifoline from *Lupinus angustifolius* (Cv. Fest) Seeds, with Application to Other Lupin Alkaloids

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The lupins, especially *Lupinus angustifolius*, are an important source of high protein food, which also benefit the soil by nitrogen fixation during growth. Angustifoline, a minor alkaloid of *L. angustifolius*, has been isolated by derivitization as the trichloroacetate followed by base hydrolysis. Other derivatives, such as the acetate, could not be satisfactorily hydrolyzed to the base alkaloid without extensive decomposition. A second method of isolation was developed using solvent extraction from pH-controlled solutions, from pH 7 to 13, yielding relatively pure crystalline angustifoline. This pH-controlled procedure has been successfully applied to the isolation of epilupinine and multiflorine from the mixed alkaloids of *Lupinus atlanticus*.

Keywords: *Lupin alkaloids; Lupinus angustifolius; angustifoline; 13-hydroxylupanine; trichloroacetylangustifoline; isolation; derivitization*

The production of lupin seed accounts for ~1% of the total worldwide grain legume production. However, in Australia ~1 million tonnes of lupin seed are grown commercially, with between 60 and 70% of the annual crop being exported. The main use of lupin seed is as stockfeed, although lupin seed is slowly acquiring increasing acceptance as a source of high-protein food for human consumption, particularly in Asian countries.

Lupins, especially *Lupinus angustifolius*, play an important role in Western Australian agricultural management by contributing to soil nitrogen levels. As an added bonus, the grower produces lupin seed as a commercial commodity which is high in protein and dietary fiber. The alkaloid content of lupin seed appears to be the only antinutritional component of significance, as the levels of hemagglutinins, phytate, α-galactoside, tannins, and trypsin inhibitors are less than those found in other legumes and are comparable with those in cereals (Petterson et al., 1986). As the alkaloids are the main antinutritional component in *L. angustifolius* seed, a concerted breeding program in Western Australia has reduced the alkaloid levels from over 1% to as low as 0.001%.

Toxicity studies on the alkaloids of *L. angustifolius* have found them to be neither carcinogenic nor teratogenic and their LD50 levels are well above the levels that would be consumed in a normal diet by rats (Culvener and Petterson, 1986; Petterson et al., 1986, 1987). The pharmacological effects of the lupin alkaloids have been documented (Kinghorn and Balandrin, 1984), and relatively low toxicity has been observed. However, elevated concentrations (>0.02%) impart a bitter taste to the palate, especially to pigs (Godfrey et al., 1985). Consequently, considerable effort at the Chemistry Centre (WA) has gone into the development of suitable analytical methods for determining the alkaloid levels in lupin seeds of the yearly commercial harvest and breeding programs (Harris, 1988; Harris and Wilson, 1988; Greirson et al., 1991) to ensure the production of the so-called "sweet", low-alkaloid cultivars of *L. angustifolius*. Capillary gas chromatography is the standard laboratory method of analysis (Priddis, 1983; Harris and Spadek, 1986; Harris and Wilson, 1988) for lupin alkaloids. An enzyme-linked immunosorbent assay has also been developed to provide a rapid screening analytical technique (Allen et al., 1990; Greirson et al., 1991).

To maintain quantitative analytical results on the levels of main alkaloids present in "sweet" *L. angustifolius* seed, pure alkaloid standards are required. The main alkaloids of commercial varieties of *L. angustifolius* grown in Western Australia as shown in Chart 1 are (+)-lupanine (**1**, 44%), (+)-13-hydroxylupanine (**2**, 38%), angustifoline (**3**, 16%), and α-isolupanine (**4**, 1%); none of which are commercially available in their pure form for use by analytical laboratories.

A procedure was therefore required to isolate and purify these alkaloids for use as analytical standards. The objectives for a useful procedure were that it be inexpensive, easily applicable to large scale workup, and of relatively low technology.

This paper presents two simple procedures using derivitization and adjustments in pH to isolate the minor alkaloid angustifoline (**3**). An added benefit of the procedures is that (+)-lupanine (**1**), as the perchlorate salt, and (+)-13-hydroxylupanine (**2**) are also obtained in relatively pure forms during the workup.

The pH-controlled extraction procedure was later used to isolate epilupinine (**5**) and multiflorine (**6**) at more than 99% purity from the "bitter" seed of another potentially commercial lupin crop, *Lupinus atlanticus*. It is considered by the authors that the procedures presented in this paper have wider application to the general isolation of other alkaloids.

MATERIALS AND METHODS

Materials and Reagents. Technical grade dichloromethane (DCM) was redistilled to give the equivalent of chromatographic grade DCM; the fraction collected was in the boiling

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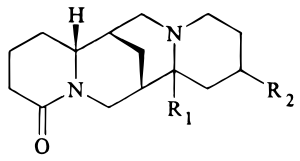
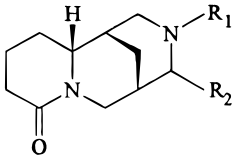
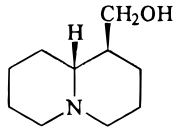
Chart 1



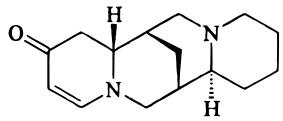
		R_1	R_2
(1)	(+)-Lupanine	····H	—H
(2)	(+)-13-Hydroxylupanine	····H	····OH
(4)	(+)- alpha-Isolupanine	—H	—H



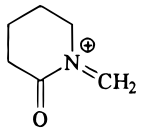
		R_1	R_2
(3)	Angustifoline	—H	—$CH_2CH{=}CH_2$
(7)	Acetylangustifoline	—$COCH_3$	—$CH_2CH{=}CH_2$
(8)	Trichloroacetylangustifoline	—$COCCl_3$	—$CH_2CH{=}CH_2$
(9)	15-Hydroxyangustifoline	—H	—$CH_2CHOHCH_3$
(10)	16-Hydroxyangustifoline	—H	—$CH_2CH_2CH_2OH$
(11)	Tosylangustifoline	—SO_2Ph	—$CH_2CH{=}CH_2$
(12)	Isoangustifoline	—H	····$CH_2CH{=}CH_2$
(13)	Trichloroisoangustifoline	—$COCCl_3$	····$CH_2CH{=}CH_2$



(5) Epilupinine



(6) Multiflorine



(14)

point range 38−41 °C. All other solvents were chromatography grade and chemicals were of analytical grade. Silica gel thin-layer chromatography (TLC) [Merck Art. 5553: TLC aluminium sheets silica gel 60 (without fluorescent indicator precoated) cut to 5 × 10 cm; layer thickness 0.2 mm] was used to monitor the composition of crude alkaloid mixtures and reaction products. The developing solvent used was chloroform−MeOH−aqueous ammonia (95:4:1) with visualization by spraying with Dragendorf Reagent.

Lupanine perchlorate prepared during the isolation procedure should be regarded as a hazardous material. Organic perchlorates may be unstable and can form an explosive mixture with carbonaceous materials; they may also be a fire hazard. Organic perchlorates are also irritable to the skin and mucous membranes and care should be taken to avoid contact (Sax, 1984).

The "bitter" *L. angustifolius* (Cv Fest) seed was obtained from a commercial source (The Grain Pool of Western Australia). The "bitter" *L. atlanticus* seed was obtained from the Western Australian Department of Agriculture. The lupin seed was coarsely milled using a hammer mill and was not prepared any further.

Gas Chromatography Procedures. Gas chromatography was performed on a Hewlett-Packard (HP) 5890 gas chromatograph (GC) equipped with flame ionization detection and HP 7673 autosampler or a HP 5890 series II GC with a 5971 series

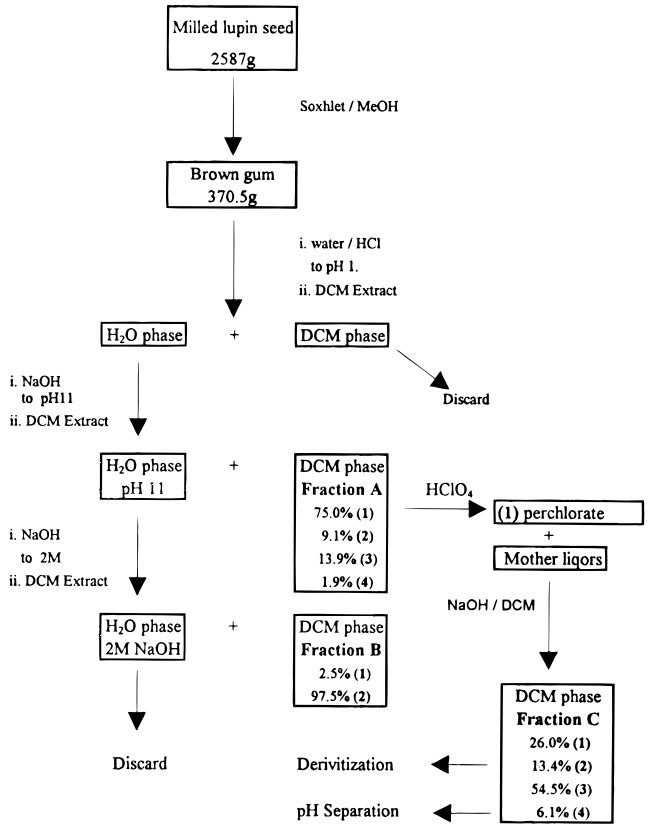


Figure 1. Pathway for primary fractionation of lupin alkaloids.

mass-selective detector (MSD) and HP 7673 autosampler. The carrier gas was helium at 25 kPa head pressure on a HP1 column (methyl silicone, 12 m × 0.2 mm × 0.3 μm film thickness). The GC conditions were those of Priddis (1983) and Harris and Wilson (1988).

Infrared Spectra and Melting Points. Infrared spectra were determined as KBr disks using a Shimadzu IR-408 spectrometer. Melting points were determined in capillary tubes using an Electrothermal IA900 digital melting point apparatus.

Extraction of Alkaloids. This was carried out using the method of Petterson et al. (1987) in the initial stages and is shown in Figure 1. Accordingly, milled *L. angustifolius* (cv. Fest) seed (2587 g) was subject to Soxhlet extraction using methanol solvent for 24 h. The extract was concentrated by evaporation under reduced pressure at 50 °C to yield a dark brown gum (370.5 g), containing some water and proteinaceous material. Water (~2 L) was added and the aqueous mixture adjusted to pH 1 using hydrochloric acid. The aqueous solution was extracted with DCM (500, 500, and 200 mL). The mixture readily emulsified, but this was broken by addition of sodium chloride or small volumes of ethanol. The residual aqueous acidic phase was basified to ~2 M concentration by addition of 75% sodium hydroxide and the resulting solution extracted with DCM (500, 500, and 200 mL) and reduced *in vacuo* at 50 °C to yield fraction A (23.0 g). The composition of this crude alkaloid mixture was determined by GC to be 75.0% **1**, 13.9% **3**, 9.1% **2**, and 1.9% **4**.

It was later determined that the pH of the aqueous solution after basification was actually pH 11, causing significant retention of **2** in the aqueous phase. **2** could be extracted from the remaining aqueous phase by further basification to pH 13 and solvent extraction using DCM. An oil, fraction B (1.71 g), was obtained after drying and concentration by evaporation under reduced pressure. Fraction B had the composition 97.5% **2** and 2.5% **1**, as determined by GC. **2** could be further purified by recrystallization from dry acetone.

The alkaloid mixture from fraction A (23.0 g) was dissolved in methanol (20 mL) and a methanolic solution of perchloric acid (methanol 13.2 mL, 70% aqueous perchloric acid 13.2 mL) was added. Seed crystals of lupanine perchlorate were added and the solution refrigerated over 2 days to yield crystals of

lupanine perchlorate. These were removed by filtration and washed with cold methanol to yield lupanine perchlorate as an almost white crystalline solid (17.9 g). After removal of a further yield of crystals (2.6 g), water (100 mL) was added to the mother liquors together with sodium hydroxide (12 g) and the solution extracted with DCM (4 × 30 mL). The organic phase was dried and concentrated under reduced pressure at 50 °C to yield a yellow-orange oil (fraction C, 5.81 g) with the following composition: 54.5% **3**, 26.0% **1**, 13.4% **2**, and 6.1% **4**, as determined by GC.

TLC analysis demonstrated the following R_f values: **2**, 0.22; **3**, 0.46; **1**, 0.72; **4**, 0.79. This system proved generally useful in monitoring alkaloid extractions and synthetic procedures involving the individual alkaloids.

Acetylangustifoline (7). Typically, a sample of mixed alkaloid bases from fraction C (1.038 g), containing ~54.5% **3**, was dissolved in excess acetic anhydride (2.0 mL) and glacial acetic acid (2 mL) and left at room temperature overnight. TLC examination of the reaction mixture indicated the absence of **3**. Water (10 mL) was added, the solution adjusted to pH 1 by addition of 0.45 M hydrochloric acid, and the mixture subjected to DCM solvent extraction (4 × 10 mL). Each DCM extraction was washed with two volumes of dilute hydrochloric acid (10 mL) and then with saturated sodium bicarbonate solution (10 mL). The yellow organic fraction (DCM) was dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to yield an oil (0.785 g) which deposited colorless feather-shaped crystals. The crystals were dissolved in a few drops of acetone, to which diethyl ether was added to induce crystallization. After cooling, almost colorless feather-shaped crystals of **7** (0.405 g, mp 145.4−147.5 °C) were obtained. A second yield of crystals (0.033 g) was obtained. The total yield was ~65.6%.

Workup of the bases remaining after acetylation was carried out by basifying the aqueous solution remaining after DCM extraction of **7** and extracting with DCM to yield an oil (0.480 g) which was demonstrated by GC and TLC to be free of **3**.

Acid Treatment of Acetylangustifoline (7). A solution of **7** (0.099 g) in 20% (w/v) sulfuric acid (10 mL) was heated at reflux temperature for 8 h, basified by careful addition of sodium hydroxide solution, and then extracted using DCM (3 × 10 mL) to yield an oil (0.104 g) after drying and concentration under vacuum. GC examination of this oil showed a relative composition of 10.0% **3**, 21.6% **7**, and 66.3% of a previously unknown compound (**9**/**10**).

The oil could be further separated into a neutral crystalline fraction (0.029 g) identifiable by GC as **7**, and a colorless basic oil (0.048 g). GC examination of the basic oil demonstrated a relative composition of 14.0% **3**, 81% (**9**/**10**), and 2.9% **7**.

Trichloroacetylangustifoline (8). A sample of fraction C mixed alkaloid bases (0.841 g) was heated under reflux with trichloroacetic anhydride (40% excess, 1.00 g) in petroleum ether solvent (bp 60−80 °C) for 30 min. The white precipitate, which formed immediately, dissolved on heating. The reaction was monitored using TLC to show the disappearance of **3**.

The reaction mixture was evaporated to dryness at 70 °C under vacuum, water (10 mL) was added, and the solution was adjusted to pH 1 by addition of dilute hydrochloric acid. The resulting mixture was extracted using three 10 mL volumes of DCM, the combined DCM extracts washed with saturated sodium bicarbonate solution, dried with anhydrous sodium sulfate, and concentrated under vacuum to yield an almost colorless oil (0.609 g). Crystallization of the oil from diethyl ether gave a first yield of buff-colored crystals (0.374 g) which were recrystallized from DCM−diethyl ether to yield buff-colored crystals of **8** (0.306 g, total yield 34.9%), mp 167.6−168.6 °C.

Conversion of Trichloroacetylangustifoline (8) to Angustifoline (3). Typically, **8** (2.395 g) was heated under reflux in a mixture of methanol (10 mL) and 10% aqueous sodium hydroxide (10 mL) for a period of 15 min, or until TLC examination showed loss of **8** and complete appearance of Dragendorf-positive **3**. The reaction mixture was cooled and acidified to pH 1 using dilute hydrochloric acid. Extraction with DCM (3 × 10 mL) removed unrequired acidic and or neutral materials, and the remaining aqueous solution was basified to pH 9 using dilute sodium hydroxide solution and extracted using DCM, which upon drying and evaporation yielded **3** as a colorless oil which crystallised upon addition of petroleum ether (bp 60−80 °C) to give **3** as a pale yellow solid (1.020 g, 69.0%). A further yield of less pure **3** (0.308 g) was obtained, containing ~84.4% **3** by GC analysis.

Angustifoline Hydrochloride (3·HCl). A saturated methanolic solution of hydrogen chloride was added dropwise to a sample of recrystallized **3** (1.029 g) in acetone (10 mL) to a final pH of 7, using indicator paper to monitor addition. Spontaneous crystallization of **3**·HCl occurred to yield white crystals (0.948 g, 80.0%, mp 127.2−131.8 °C) after vacuum filtration. GC analysis after base treatment and DCM extraction determined the composition of **3**·HCl to be 95.9% **3** and 4.1% isoangustifoline (**13**).

Isolation of Angustifoline (3) Using a pH Gradient with Solvent Extraction. A sample of mixed alkaloid bases from fraction C (2.876 g) was dissolved in methanol (40 mL) and titrated to pH 7 (indicator paper) using methanolic 15% perchloric acid (~4.5 mL). Overnight refrigeration yielded crystals of lupanine perchlorate (0.394 g, equivalent to 0.28 g of **1**) and a further yield (0.082 g, equivalent to 0.058 g of **1**) was obtained on standing of the mother liquors.

The mother liquors, containing ~2.54 g of mixed alkaloids, were concentrated by vacuum evaporation and adjusted to pH 7 by addition of dilute sodium hydroxide solution. This aqueous solution was extracted using DCM (3 × 10 mL), adjusting back to pH 7, after each extraction. The DCM extracts were combined, dried, and evaporated in vacuo in tared flasks. The remaining aqueous solution was adjusted to pH 7.5 and the extraction procedure repeated. This procedure was repeated through 0.5 pH increments to pH 11.5, when the remaining aqueous phase was adjusted to contain ~2 M sodium hydroxide (pH >13) by the addition of solid sodium hydroxide.

RESULTS AND DISCUSSION

The method of Petterson et al. (1987) was used to prepare a crude mixture of the alkaloids from milled *L. angustifolius* (cv. Fest) seeds using Soxhlet extraction with 100% methanol solvent.

Workup using DCM extraction from an aqueous solution of the alkaloid mixture at pH ~11 yielded fraction A, which was deficient in **2**, it having remained in the aqueous phase. **2** could be recovered from the aqueous phase by basification to pH ~13 followed by DCM extraction.

1 was partially removed from fraction A by preparation and separation of the perchlorate salt. The residual alkaloids contained in the resulting fraction C were enriched in **3** (54.5%) and provided a suitable starting point for our studies on the isolation of **3**.

That **3** is a secondary amine occurring in mixture with tertiary amine alkaloids suggested the use of derivitization to aid isolation of a neutral product from an otherwise basic mixture.

Previous experiments (Spadek, 1991) had successfully produced pure tosylangustifoline (**11**), which could not be cleaved under accepted hydrolysis conditions to give **3**. Accordingly, **7** was prepared by acetylating a sample of fraction C crude alkaloid mixture, to yield neutral **7**. TLC analysis demonstrated the absence of angustifoline in the postacetylation reaction mixture.

7 is a known compound, first prepared (Bohlmann and Winterfeldt, 1960) as a derivative of **3**, which had been purified by crystallization after vacuum distillation of a crude *Lupinus polyphillus* alkaloid mixture.

The identity of **7** was confirmed by infrared (IR), gas chromatography/mass-selective detection (GC/MSD), and melting point measurement. The MS displayed most of the fragmentation characteristics of **3**, in that the molecular ion (m/z = ~276) was generally absent, but a fragment at m/z = 235 (M^+ = 41) was present,

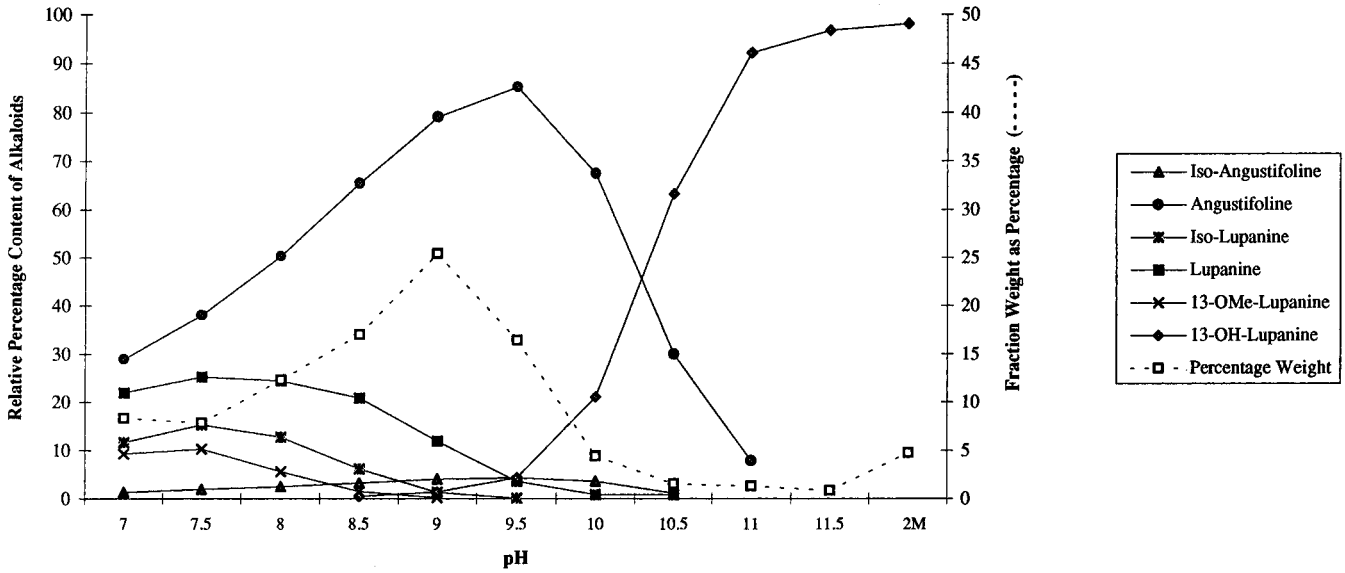


Figure 2. pH gradient solvent extraction from mixed angustifoline-rich alkaloids.

generated by loss of propene ($m/z = 41$). Loss of acetyl ($m/z = 42$) occurred to yield a major fragment at $m/z = 193$, which is very characteristic of angustifoline fragmentation in which the molecular ion ($M^+ = 234$) is generally absent and the base peak is at $m/z = 193$ ($M^+ - 41$, 100%), caused by facile loss of the propene fragment ($m/z = 41$). A major fragment at $m/z = 112$, characteristic of angustifoline, tetrahydrorhombifoline, synthetic *N*-methylangustifoline, and certain other closely related alkaloids has been assigned to the ion (**14**) (Kinghorn and Balandrin, 1984). The IR spectrum showed strong carbonyl absorption as a single band at 1620 cm^{-1}; the presence of a shoulder at 1640 cm^{-1} was indicative of the acetylamide carbonyl group. The spectrum showed the absence of NH stretching in the region 3000−3500 cm^{-1}.

A variety of attempts was made to deacetylate **7**, principally relying on acid hydrolysis at reflux temperature. In summary, hydrolytic conditions, such as hot, refluxing hydrochloric acid (1.5 M, 9 h heating), 37% concentrated hydrochloric acid under reflux, and sulfuric acid (10, 20, or 70%) under reflux, all failed to yield angustifoline base in anything but low concentrations, together with evidence of extensive degradation. Acetylangustifoline was also very resistant to refluxing 10% (w/v) sodium hydroxide solution, yielding only low concentrations of angustifoline and much unchanged starting material.

The use of 37% hydrochloric acid and 20% sulfuric acid in particular gave a characteristically low yield of angustifoline, some unreacted acetylangustifoline, and a new compound which was partially characterized as a "hydroxyangustifoline". Typically, heating acetylangustifoline with 20% sulfuric acid for 4 h produced an organic mixture comprising 10% **3**, 22% unreacted **7**, and 66% hydroxyangustifoline (**9**/**10**). Solvent extraction for bases gave a product containing ~81% hydroxyangustifoline and 14% angustifoline. Further characterization of the hydroxyangustifoline was not carried out, but consideration of the mass spectrum and the theoretical course of reaction has suggested structure **10**. The mass spectrum shows a small molecular ion at $m/z = 252$, with a fragment at $m/z = 219$ possibly representing an originally low concentration of the alternative structure **9**, which has lost $m/z = 33$ in an initial fragmentation from its molecular ion ($M^+ = 252$). A major fragment at $m/z = 193$ is characteristic of angustifoline, suggesting its origin by loss of a fragment $m/z = 59$, "propan-2-ol" (C_3H_7O).

Following the observation of the general failure of **7**, to undergo ready clean cleavage to **3**, recourse was made to the synthesis and hydrolysis of **8**. Haloacetamides are reported (Greene, 1991) to be more easily cleaved by acid (0.1 M hydrochloric acid) and base (0.01 M potassium hydroxide or 15% ammonia/methanol) than the corresponding unsubstituted amides, due to the inductive effect of the halo atoms ensuring a hyperactivity of the amide toward hydrolytic cleavage.

It was routinely found that **8** invariably contained ~4% (trichloroacetyl)isoangustifoline (**12**) as a contaminant, displaying different GC retention times but an identical mass spectrum. Repeated crystallization of **8** failed to separate the isomers. The presence of isoangustifoline has been inferred (Harris et al., 1986) in earlier examinations of crude alkaloid mixtures extracted from *L. angustifolius*. The authors do not consider isoangustifoline to be an artifact of the extraction process but rather a natural compound. Just as **1** occurs naturally together with **4**, so it may be expected that **13** can arise via the angustifoline biosynthetic pathway. It has also been observed (Harris and Lee, 1995) that **1** is not easily converted to **4** via **2**, which would further suggest that **12** is not an artifact of the extraction process.

GC/MS examination of **8** showed the absence of a molecular ion at $m/z = \sim 379$, but displayed fragments at $m/z = 338$ ($M^+ - 41$) and 339. As in the mass spectra of **3** and **7**, it appeared that this fragmentation had occurred by loss of m/z 41, representing a propene-derived unit ($C_3H_5^+$). The fragment at m/z 193, seen as the base peak in the mass spectra of **3** and (**7**) was of low percentage in the spectrum of **8**. The base peak for **8** was at m/z 112, a fragment characteristic of angustifoline and certain closely related alkaloids (Kinghorn and Balandrin, 1984). An important feature of the GC of **8** was the presence of other compounds in minor concentration. Later experiments demonstrated that these were degradation products of **8** arising by loss of chlorine atoms, probably catalyzed by base residues on the injection port and column of the GC instrument.

8 recrystallized to constant melting point gave these extra peaks if chromatographed on aged columns, while injection into a clean column with a cleaned injection port generally gave a characteristic single peak and

Table 1. pH Fractionation of *L. atlanticus* Crude Alkaloid Mixture

pH	rel fraction yield (g)	rel % content	
		epilupinine (**5**)	multiflorine (**6**)
7	0.32	0.0	5.0
8	0.14	1.2	70.0
9	0.66	1.3	92.7
10	1.20	18.0	80.0
11	0.40	83.0	15.0
12	0.23	97.0	1.3
2 M NaOH	0.25	98.0	0.1

appropriate mass spectrum. The IR spectrum clearly demonstrated the presence of the trichloroacetyl carbonyl functionality by strong absorption at 1665 cm^{-1}, complementing the expected strong C_2 amide carbonyl absorption occurring at 1630 cm^{-1}. The spectrum showed the absence of characteristic NH stretching in the region 3000−3500 cm^{-1}.

8 was easily hydrolyzed by boiling in methanolic 10% (w/v) sodium hydroxide solution and monitoring the appearance of Dragendorf-positive angustifoline after TLC separation. The reaction was very clean and gave a single basic product identified as **3**. Crystalline **3** prepared in this manner was found to be a very hygroscopic low melting point solid. Conversion to angustifoline hydrochloride gave stable, nonhygroscopic crystals with a product composition of 95.9% **3**·HCl and 4.1% **12**·HCl, as determined by GC/MS. This product has been accepted as a suitable standard for GC analysis purposes.

As noted previously, an objective of this study was to investigate methods of isolation of angustifoline which were inexpensive, applicable to large-scale workup, and of relatively low technology. To this end, a pH gradient/solvent extraction procedure was used to attempt purification of **3** from the **3**-rich alkaloid base mixture fraction C.

The general adopted procedure was to commence with an aqueous solution of the mixed alkaloids at pH 7.0 and extract three times with DCM, readjusting the pH as necessary between extractions. Sequences of DCM extractions were carried out at intervals as the pH was raised in 0.5 unit steps. The relative alkaloid content of each weighed fraction was determined by GC. The results of a series of such pH controlled extractions for one sample of the **3**-rich alkaloid mixture are shown in Figure 2. Repetition of this procedure yielded similar results each time with respect to the alkaloid composition of each fraction. The fractions obtained at pH's 9.0 and 9.5, containing 79.1 and 85.2% **3**, respectively, which could be isolated by crystallization to give hygroscopic crystals of **3** of 97.4% purity, as shown by GC examination.

The pH gradient/DCM solvent extraction, so described, provided a rapid, simple method of fractionating the lupin alkaloid mixture. The method of trichloroacetyl derivatization can be applied as a scavenging system for angustifoline from those remaining fractions containing only low concentrations of the alkaloid.

The pH gradient procedure was later applied to a similar problem when attempting to isolate **5** and **6** from the crude mixed alkaloid gum obtained from *L. atlanticus*. In this application using pH steps of 7, 8, 9, and 10, with DCM solvent extraction, multiflorine and epilupinine could be obtained separately and in acceptable yield. These results are summarized in Table 1. The successful isolation and purification of multiflorine and epilupinine using the pH gradient/solvent extraction technique has confirmed the general usefulness and application of this procedure in the studies on lupin alkaloids.

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