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ANALYSIS OF PLANT ALKALOID MIXTURES BY AMMONIA CHEMICAL IONIZATION MASS SPECTROMETRY

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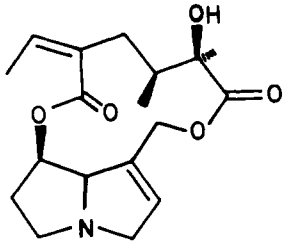
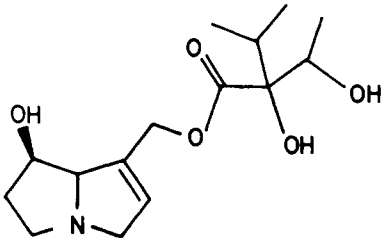
ABSTRACT.—Chemical ionization mass spectrometry with NH_3 as a reagent gas provides an excellent method for qualitative analysis of plant alkaloid mixtures. Plant alcoholic extracts are purified by a differential pH extraction to yield a crude alkaloid mixture which can then be analyzed by NH_3/ci direct insertion probe mass spectrometry. Good quasimolecular ion (M+1) peaks are observable for individual alkaloid components of the mixture. The technique is shown to apply to analysis of plant extracts containing pyrrolizidine, quinolizidine, diterpenoid, bishordeninyl terpene, and indole alkaloids, but should be adaptable to any alkaloid mixture.

Chemical ionization (ci) mass spectrometry with ammonia as a reagent gas is almost as old as ci itself. With the exception of sporadic individual reports of its use, however, the technique has not been adopted generally by alkaloid chemists. We have evaluated NH_3/ci ms as a general technique for the analysis of a number of different alkaloid mixtures from plants and report here some of our results.



senecionine



supinine

RESULTS AND DISCUSSION

PYRROLIZIDINE ALKALOIDS.—The rapid detection and analysis of these toxic alkaloids remains an important goal because they are known as components of herbal teas and medicines, of range plants poisonous to livestock, and, occasionally, of honey and milk. Results of ei, CH_4/ci, isobutane/ci, and NH_3/ci ms measurements on the pure alkaloids senecionine and supinine are given in figures 1 and 2. Numerous other examples are available (1), but figures 1 and 2 are typical of extreme cases. Senecionine represents an example of molecular ion (*m/z* 335) being detected under regular ei conditions, but in which marked improvement is obtainable with any of the reagent gases in the ci mode. The *m/z* 364 and 376 peaks in the CH_4/ci spectrum (figure 1) represent adduct formation, as does the *m/z* 378 peak in the isobutane/ci spectrum. Note that adduct formation is absent in the NH_3/ci spectrum. Supinine (figure 2) represents a case in which *no* molecular ion is visible in the ei spectrum. Although M+1 quasimolecular ions are observed with methane and isobutane, the superiority of ammonia as a reagent gas is evident in figure 2. Again, adduct formation is observed with methane and isobutane, but not with ammonia. The lack of fragmentation will be valuable in the analysis of alkaloid mixtures. Such an analysis is exemplified by the next study.

Reports of deaths among horses that had consumed hay from a pasture near Eagle, Colorado, in the summer of 1982 led to the hypothesis (2) that the poisoning could be due to two plant components of the hay: *Cynoglossum officinale* and *Senecio multilobatus*. *C officinale* is known elsewhere as a toxic range plant, from which two major alkaloids,

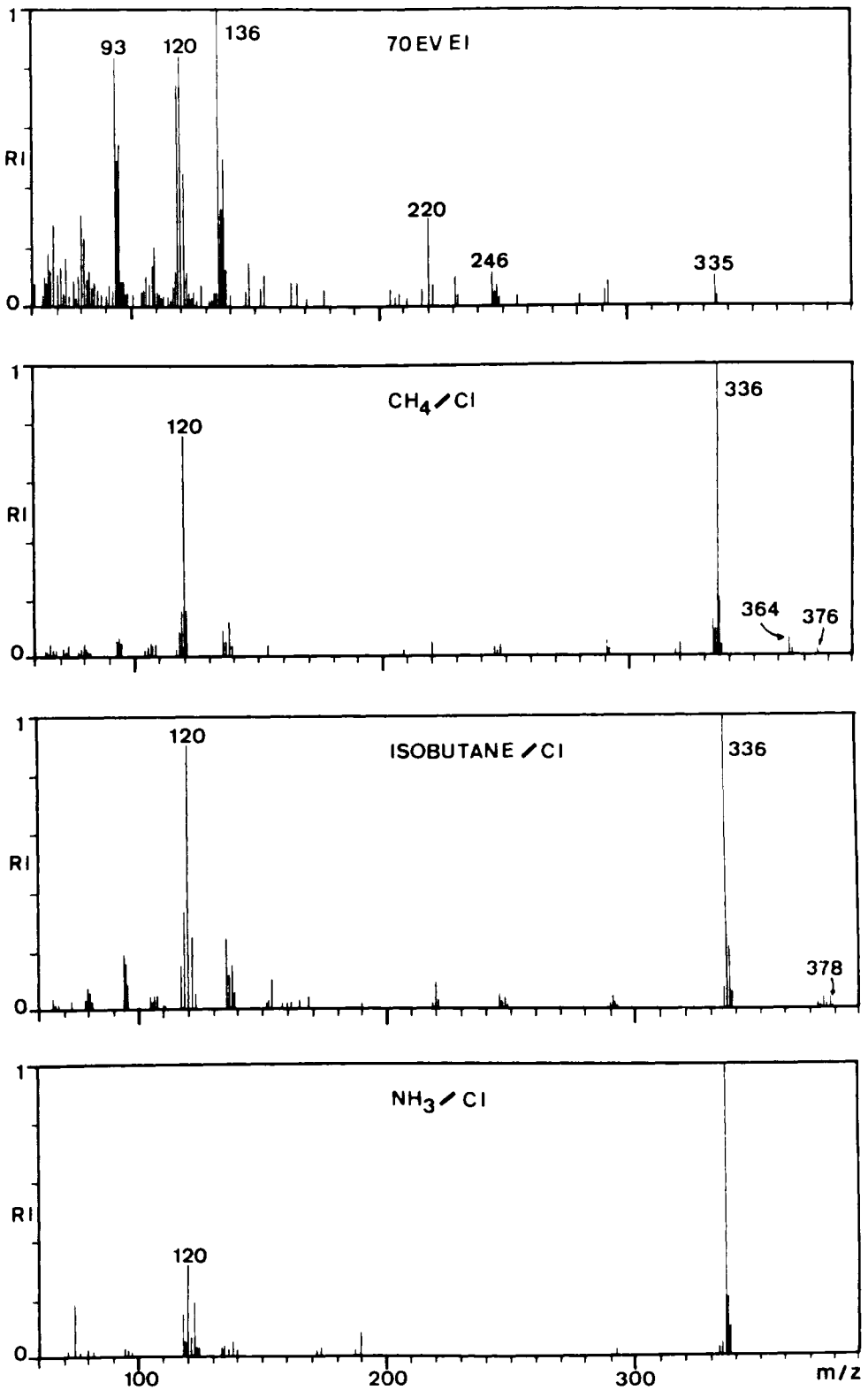


FIGURE 1. Electron impact and methane, isobutane, and ammonia chemical ionization mass spectra of senecionine.

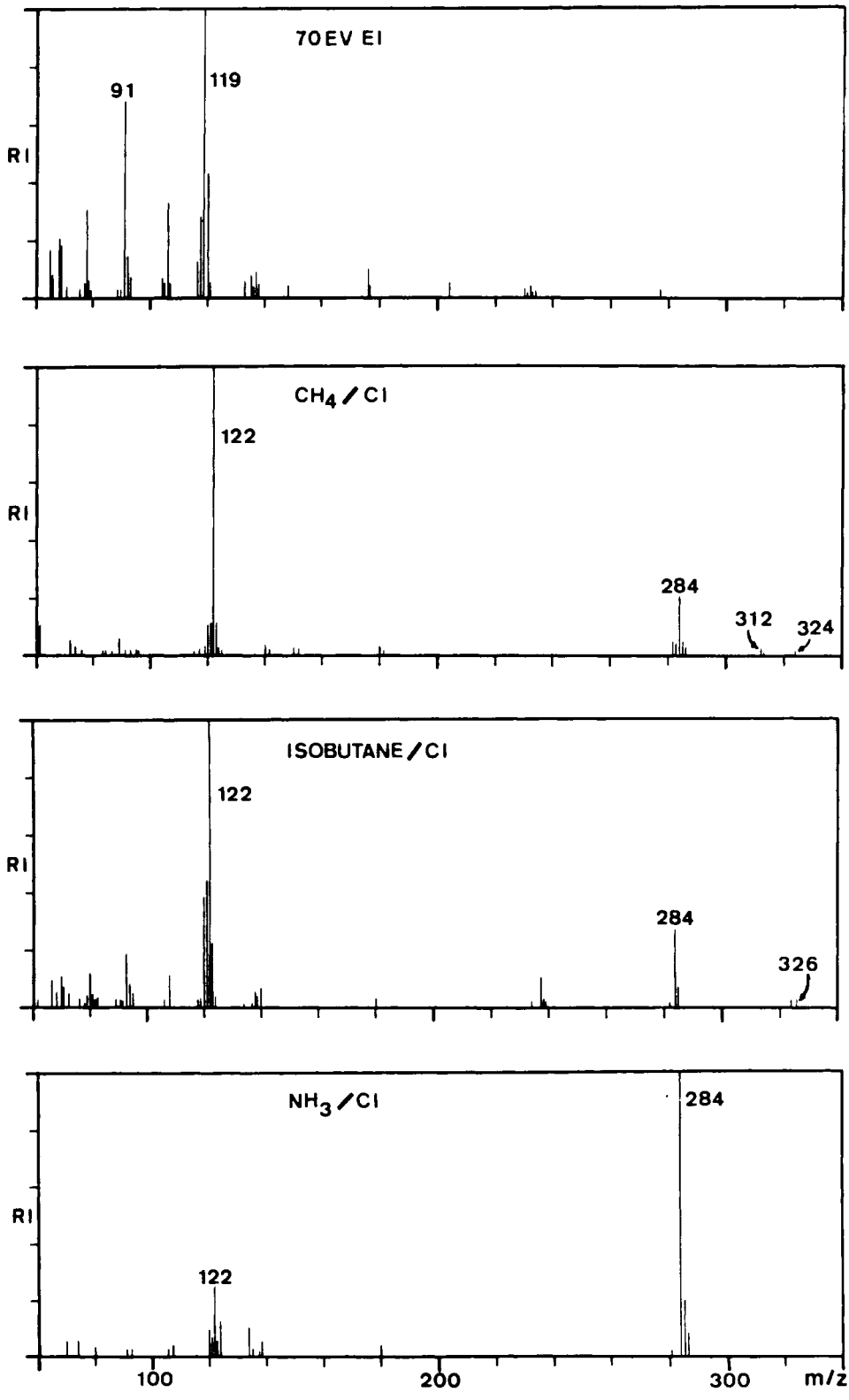


FIGURE 2. Electron impact and methane, isobutane, and ammonia chemical ionization mass spectra of supinine.

echinatine (MW 299) and heliosupine (MW 397), previously have been isolated. The known minor alkaloids include 7-angelylheliotridine (MW 237). Figure 3 shows the ei and NH_3/ci mass spectral results on the crude alkaloid mixture isolated from *C. officinale*. Because of differences in volatility, ionization cross sections, and fragmentations for the alkaloids, the observed intensities of the M+1 peaks do not necessarily reflect amounts of the given alkaloids. Quasimolecular ions are seen for each of the above three alkaloids as well as peaks indicating the presence of alkaloids of MW 285 and 321. None of the other known trace alkaloids from *C. officinale* have these molecular weights. A pyrrolizidine alkaloid of MW 285 (cynaustraline) is, however, known from *Cynoglossum australe*. Classical separation and identification techniques (column and thin layer

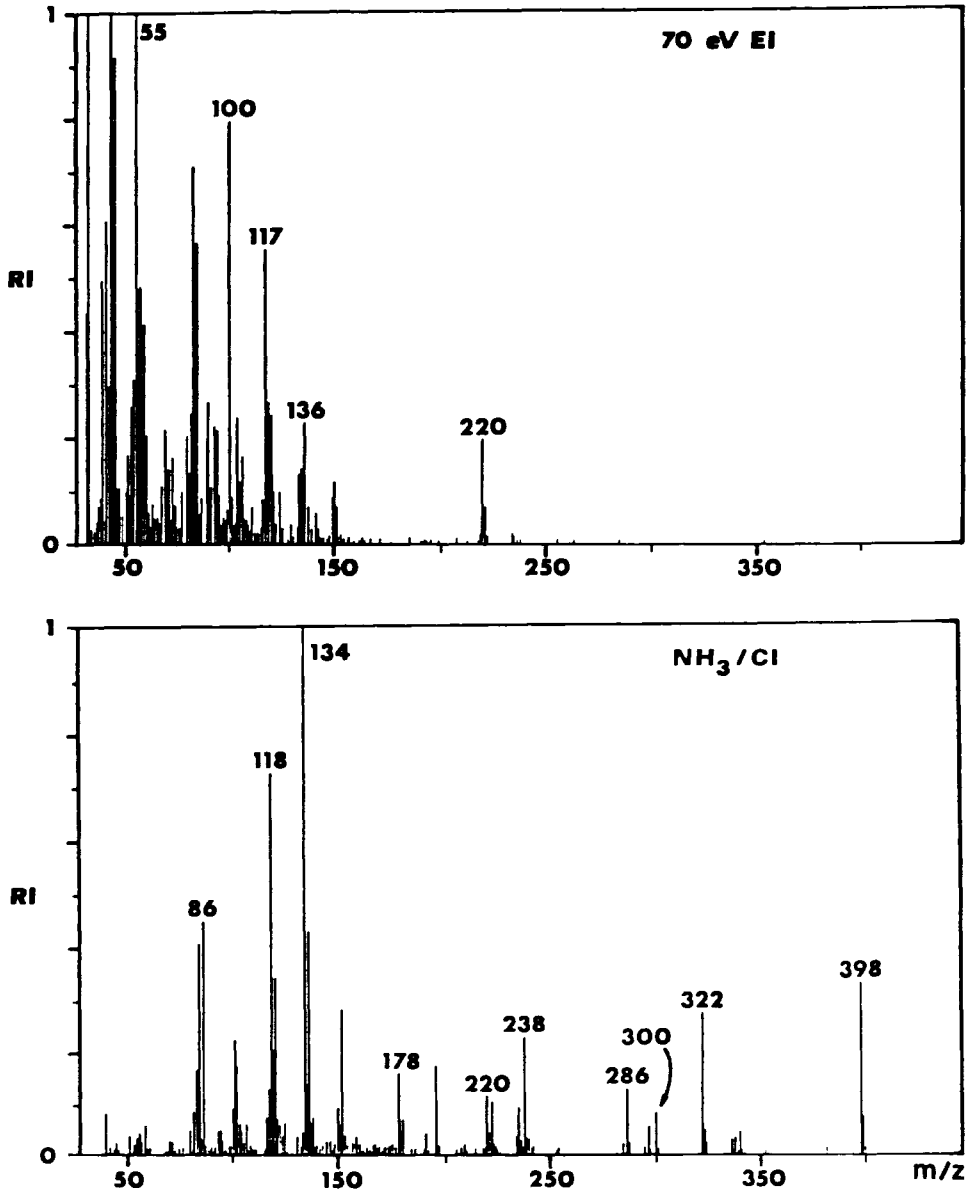


FIGURE 3. Electron impact and ammonia chemical ionization mass spectra of the crude alkaloid mixture from *Cynoglosom officinale*.

chromatography; nmr analysis) allowed absolute identification of heliosupine (MW 397) as a major alkaloid (3).

A previous alkaloid analysis of *S. multilobatus* could not be found in the literature. NH_3/ci mass spectral analysis of the crude alkaloid mixture gave the results of figure 4. (Again, direct ei analysis gave no useful information.) The *m/z* peak at 336 indicated the

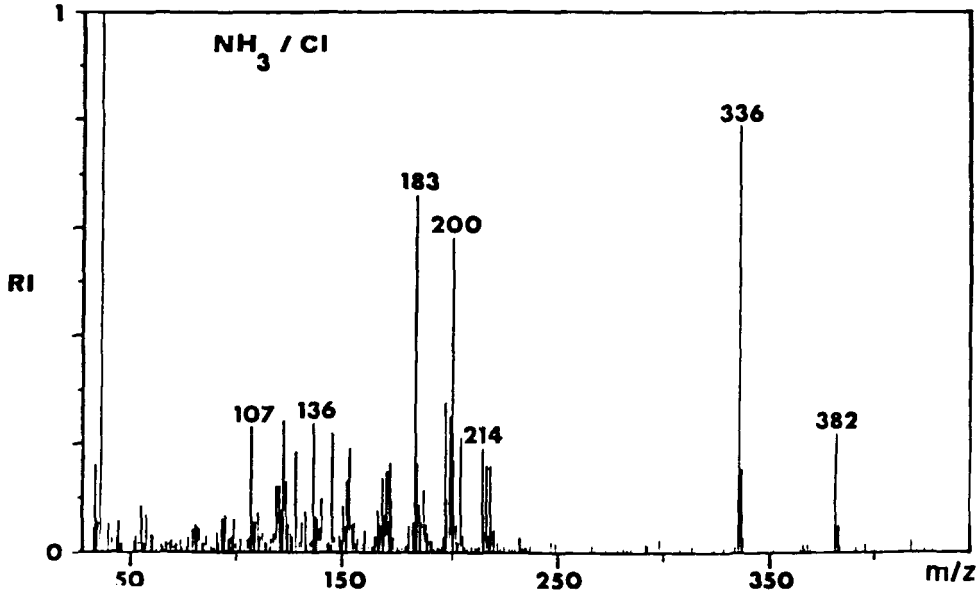


FIGURE 4. Ammonia chemical ionization mass spectrum of the crude alkaloid mixture from *Senecio multilobatus*.

presence of senecionine (MW 335), and this was confirmed to be the major alkaloid by classical techniques (3). Analysis by tlc showed mainly one minor component, which is probably that represented by the *m/z* 382 quasimolecular ion seen in figure 4.

QUINOLIZIDINE AND RELATED ALKALOIDS.—We had earlier shown (4) that *Castilleja rhexifolia* contained senecionine as well as other alkaloids. The closely related species *Castilleja miniata* was found to also contain alkaloids, but classical isolation and structure techniques showed them to be quinolizidines, rather than pyrrolizidines (5). We, therefore, applied NH_3/ci mass spectral screening to the crude alkaloid mixture with the result seen in figure 5 (ei screening gave no useful information). Alkaloids of MW 207, 222, 248, and 264 have, indeed, been obtained from the crude mixture.

Quinolizidine alkaloid samples are often analyzed by gc (6), and hence we extended our technique to NH_3/ci gc/ms. For this experiment, NH_3 gas was bled into the mass spectrometer source after the gc effluent connection. Figure 6 gives the total ion current gc/ms trace on crude alkaloid mixture. Each peak gave an NH_3/ci mass spectrum characteristic of the indicated alkaloid. Details on complete structure elucidation of each component are in the accompanying paper (5).

DITERPENE ALKALOIDS.—The toxic range plant *Delphinium geyeri* is being studied in our group (7). Figure 7 shows the NH_3/ci ms of the crude alkaloid fraction from the flowers of *D. geyeri*. The presence of two major and three minor alkaloids was evident *via* tlc. By classical isolation techniques we have found (7) that the *m/z* 428 peak is accounted for by a new C-20 diterpene alkaloid ($C_{25}H_{33}NO_5$), whose structure will be discussed when work on this and the other components is finished. It is clear from the

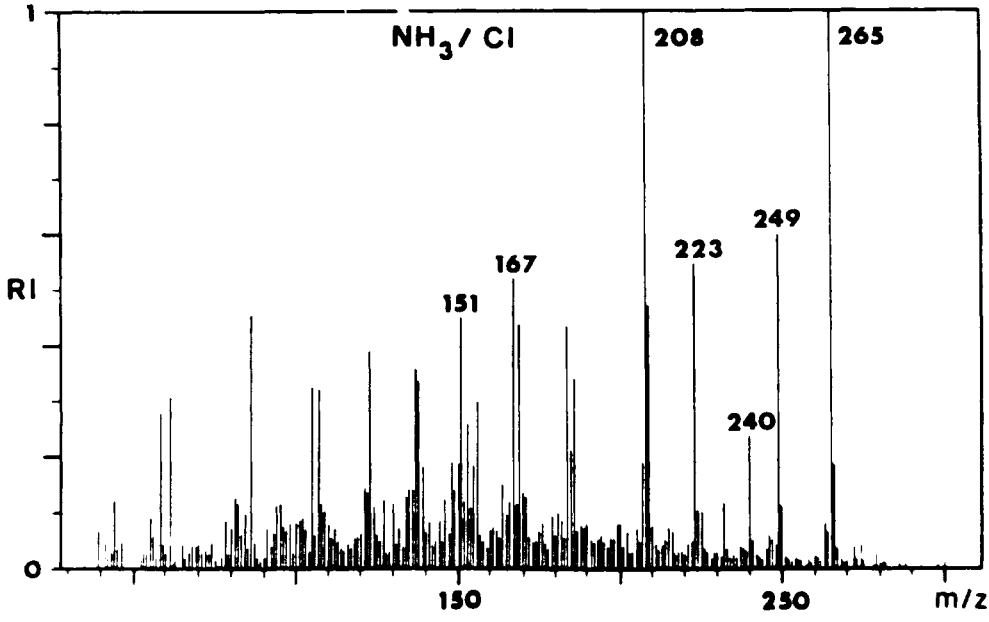


FIGURE 5. Ammonia chemical ionization mass spectrum of the crude alkaloid mixture from *Castilleja miniata*.

present data, however, that the NH_3/ci ms screening technique can be extended to these complex, high molecular weight alkaloids.

OTHER OBSERVATIONS.—The above examples show rather specific detection of alkaloids in the acid-base partition purified crude alkaloid mixture. If considerable quantities of nonalkaloidal material is present, false positives may be obtained. Thus, a similar mixture from flowers of *Penstemon whippleanus* contained cinnamamide and a va-

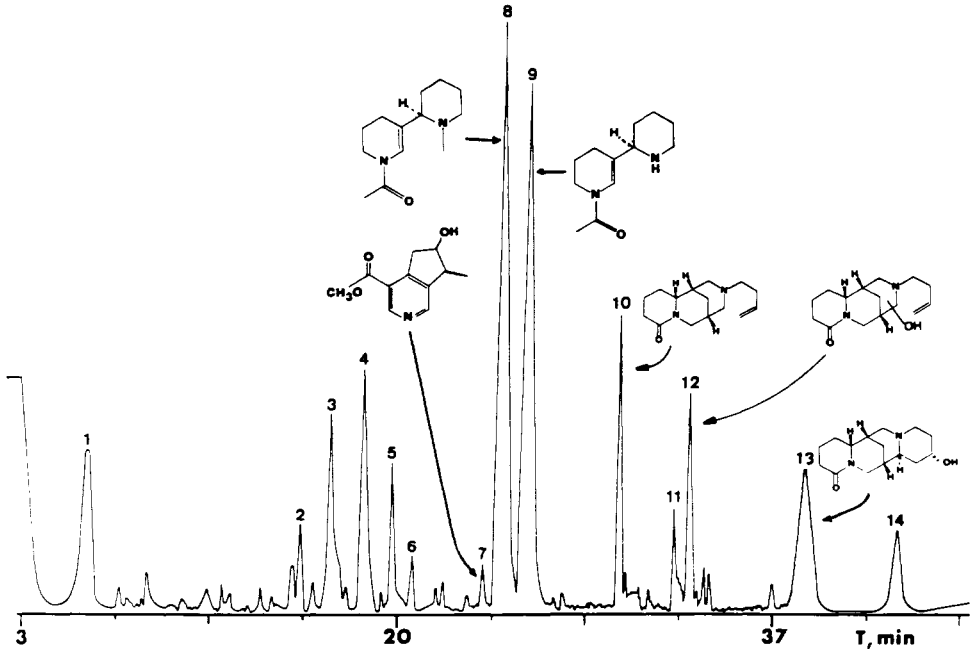


FIGURE 6. TIC gc/ms-NH_3/ci trace of the crude alkaloid mixture from *Castilleja miniata*. (Peak 7: cantleyine, 8: *N'*-methylammodendrine, 9: ammodendrine, 10: rhombifoline, 12: hydroxyrhombifoline, 13: hydroxylupanine).

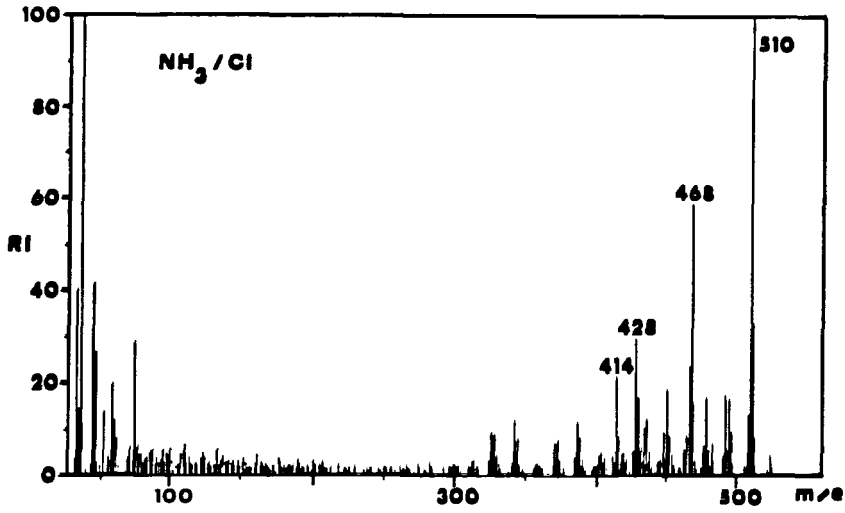


FIGURE 7. Ammonia chemical ionization mass spectrum of the crude alkaloid mixture from flowers of *Delphinium geyeri*.

riety of cinnamates as well as alkaloids, and the nonalkaloidal components also gave relatively high M+1 pseudomolecular ions (1).

As mentioned above, the simple direct probe technique does not give a true picture of the relative amounts of each alkaloid. For best results, spectra should be monitored over time. In one case this has proved useful, inasmuch as we have been able to detect (8) small amounts of *N,N*-dimethyltryptamine (MW 188) in a mixture of mainly bishordeninyl terpene alkaloids (MW 490, 508). Early scans were dominated by an *m/z* 189 peak, while the *m/z* 491 and 509 peaks became dominant in the late scans.

Although our major use of this technique has been with pyrrolizidine and quinolizidine alkaloids, the last case discussed shows that it is applicable to an indole and the bishordeninyl terpene alkaloids. One pyridine monoterpene is seen in figure 6 and several others have been detected by this method in *P. whippleanus* (5) and *C. rhexifolia* (9). From this wide sampling of structures, it seems likely that it will be applicable to any alkaloid class. It should be particularly valuable in providing more information on the types of alkaloids present when screening herbarium material (10).

EXPERIMENTAL

MASS SPECTROMETRIC METHODS.—Routine ei and ci spectra were taken with a Vacuum Generators model MM16 spectrometer equipped with a Systems Industries interface and disk drive with a digital PDP 8-A Computer. All routine ei and ci spectra were obtained by use of a direct insertion probe, with the sample contained in a glass capillary. The capillary tubes were cut to 7 mm, so that 1 mm would protude from the probe tip. Samples were applied as a chloroform and/or methanol solution to the open end tip of the capillary and were carefully evaporated.

Unless otherwise stated, all ci spectra were recorded under protonating conditions with neat NH_3. We used an ion source temperature of 180-200°, and a reagent gas pressure sufficient to obtain a ratio of parent to fragment ions of at least 5:1 (for NH_3/ci of a standard alkaloid). The pressure required to achieve this varied according to the cleanliness of the ion source, but a source housing pressure of 3 or 4 x 10^{-6} torr was generally used for NH_3/ci. Methane/ci and isobutane/ci tuning was far more temperamental and often required a pressure on the order of 2 x 10^{-5} torr. Xylene was introduced into the liquid reservoir for CH_4/ci tuning, with the ratio of $(M+1)^+/M^+$ being optimized. Perfluorokerosene (PFK) was used as a calibration standard.

Gas chromatograph ms experiments used a Perkin-Elmer Model Sigma 2 Chromatograph coupled to a modified v.g. capillary interface. AJ. and W. Bonded Phase (DB-5) Fused Silica Capillary Column (30 m) was used. Further details are available in a thesis (1).

SAMPLE PREPARATION.—Dried and ground plant material was extracted with methanol or ethanol in a Soxhlet and then concentrated to dryness *in vacuo*. The residue was triturated well with a mixture of $CHCl_3$ and 1 M H_2SO_4, the layers were separated, and the acidic aqueous layer was washed with additional $CHCl_3$. The aqueous solution was made basic to pH 9 with NH_4OH or NaOH and extracted three times with $CHCl_3$. The $CHCl_3$ layers were combined, dried over Na_2SO_4, and evaporated to dryness *in vacuo*. The resulting crude alkaloid mixture was used for analysis.

ACKNOWLEDGMENTS

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