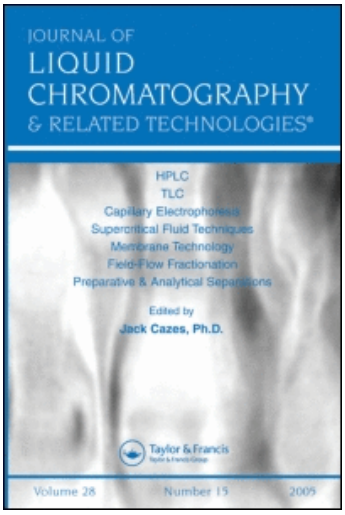

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Recent Chromatographic Methods to Isolate Pyrrolizidime Alkaloids

H. J. Segall[a]
[a] Department of Veterinary Pharmacology and Toxicology, University of California, Davis, California

To cite this Article Segall, H. J.(1984) 'Recent Chromatographic Methods to Isolate Pyrrolizidime Alkaloids', Journal of Liquid Chromatography & Related Technologies, 7: 10, 377 — 392
To link to this Article: DOI: 10.1080/01483918408073943
URL: http://dx.doi.org/10.1080/01483918408073943

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(S-2), 377-392 (1984)

RECENT CHROMATOGRAPHIC METHODS TO ISOLATE PYRROLIZIDINE ALKALOIDS

H. J. Segall
Department of Veterinary Pharmacology and Toxicology
University of California
Davis, California 95616

ABSTRACT

The application of recent chromatographic techniques to isolate various pyrrolizidine alkaloids are evaluated. Alkaloids which have been successfully separated include monoesters, diesters, and macrocyclic pyrrolizidine alkaloids exhibiting the retronecine base as well as the otonecine base. Both analytical and preparative high performance liquid chromatography methods are discussed.

INTRODUCTION

Pyrrolizidine alkaloid (PAs) are indigenous to a variety of plant species which are widely distributed throughout the world (Figs. 1 and 2). Plants containing these alkaloids include such diverse botanical families as the Compositae, Leguminosae, and Boraginaceae (1). The genera which exhibit the greatest toxicity to humans and livestock are Senecio, Crotalaria, Heliotropium, and Amsinckia (1,2). Pyrrolizidine alkaloids have been identified in a variety of human food sources [teas, medicinal herbs, milk, honey, cereals, and grains (3-11)] and have been identified as the source of injury (liver, lung) and death to livestock for many years (11). This contamination may be highly significant since PAs are responsible for numerous syndromes and are proven mutagens and carcinogens (12-14).

The difficulty in studying PAs has been the lack of available compounds to the investigator as only monocrotaline is sold commercially. Various isolation and extraction processes are

 0148-3919/84/07S2-0377$3.50/0

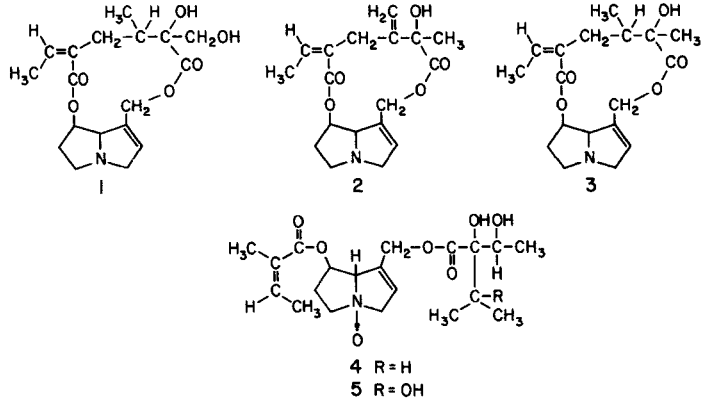

Figure 1. Examples of macrocyclic pyrrolizidine alkaloids exhibiting a retronecine base are: 1. retrorsine, 2. seneciphylline, 3. senecionine. Examples of diester pyrrolizidine alkaloids are: 4. symphytine-N-oxide, 5. echimidine-N-oxide.

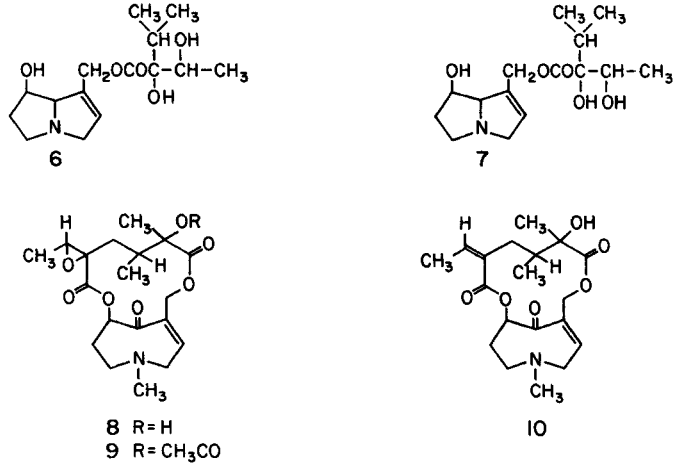

Figure 2. Examples of monoester pyrrolizidine alkaloids are: 6.intermedine and 7. lycopsamine. Examples of macrocyclic pyrrolizidine alkaloids exhibiting an otonecine base are: 8. petasitenine, 9. neopetasitenine, 10. senkirkine.

required prior to separating a mixture of PAs and have been summarized by numerous authors (15-17). To facilitate the initial extractions of PAs, some investigators have used large-scale apparati coupled with ion exchange columns while others have used smaller soxhlets (18,19). This paper will discuss recent chromatographic procedures for isolating PAs including analytical as well as preparative high performance liquid chromatography (HPLC).

DISCUSSION

My own laboratory expressed an interest in the rapid isolation of PAs in the mid 70s to aid our veterinary school with suspected PA poisonings. Two plants which contribute to livestock toxicities in California are Senecio vulgaris (common groundsel) and Amsinckia intermedia (fiddleneck).

A publication by Qualls and Segall was the first HPLC method to successfully separate PAs and used a single 10 μ Bondapak CN column (Waters Associates) and a THF/0.01 M ammonium carbonate (pH 7.8) solvent system (19). This procedure described the isolation of the macrocyclic PAs (retrorsine, seneciphylline, senecionine) from S. vulgaris (Fig. 3) and was also applied to two other Senecio species, S. longilobus (threadleaf groundsel) and S. jacobaea, commonly known as tansy ragwort (20,21). Senecio longilobus contains the same macrocyclic PAs as S. vulgaris plus an additional PA riddelline, which is structurally similar to retrorsine. Using this method the PAs derived from S. jacobaea were also isolated (20), Fig. 4. The procedure was further modified by the addition of another 10 μ CN Bondapak column in tandem as described in a later publication (22). The disadvantage of this chromatographic method was the relative high pH used (pH 7.8). Horvath et al. had stated that "the reproducibility and efficiency of these columns could be maintained as long as the mobile phase was kept at pH <7" (23). Reproducibility was not a problem, but maintaining the 10 μ CN Bondapak column efficiency for lengthy periods while operating at pH 7.8 was difficult (19-22).

Due to the above mentioned difficulties, a 10 μ C18 Bondapak column (3.9 mm x 30 cm) was utilized in conjunction with a 55:45

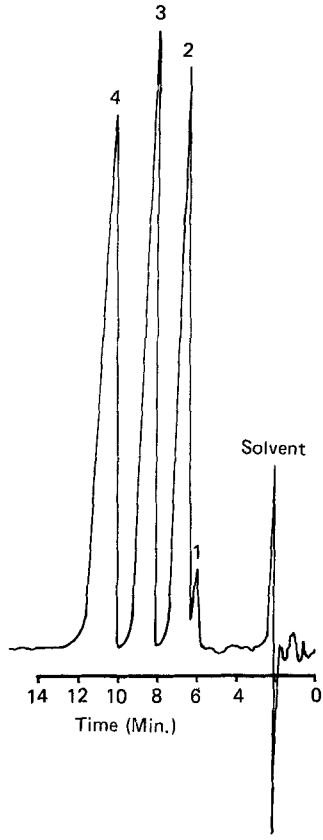

Figure 3. Isolation of pyrrolizidine alkaloids from Senecio vulgaris. Column 10 μ C18 Bondapak CN (Waters Associates), 3.9 mm x 30 cm. Solvent THF-0.01 M $(NH_4)_2CO_3$ (pH 7.8), linear gradient 13% → 26% THF over 30 min, flow rate 1.8 ml/min. Detector, SF770 (Kratos) at 235 nm. Peak 1 = Unknown, 2 = retrorsine, 3 = seneciphylline, 4 = senecionine.

methanol 0.01 M PO_4 (pH 6.3) solvent system (24). Excellent sensitivity was obtained and the lower pH was an important factor in extending column efficiency (Fig. 5). Another advantage of this technique was the low solvent costs, especially when this technique was later applied to semipreparative as well as preparative separations. Contrary to other reports (25), proper care of the

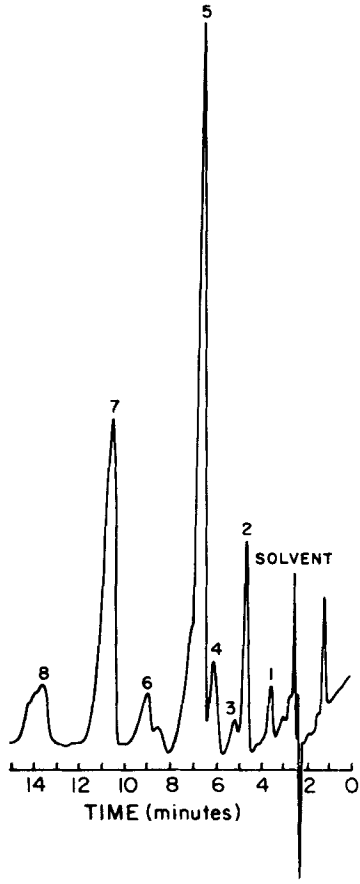

Figure 4. Isolation of pyrrolizidine alkaloids from Senecio jacobaea. Chromatography conditions as in Fig. 1. Peak 1 = jacoline, 2 = jacozine, 3 & 4 = jacobine and ? 5 = m/z 351 and m/z 385, 6 = jaconine, 7 = seneciphylline, 8 = senecionine.

reversed phase columns (flushing with water and programming to 100% MeOH at the end of each day) plus the advantage of using the low-cost solvents (water-methanol), make this a practical method.

Tittel et al. developed a quantative HPLC method to isolate the diester PAs (symphytine-N-oxide and echimidine-N-oxide) from Symphyti Radix (26). They used a 10 MN-Nucleosil® C18 column with solvents methanol/water (45:55). The methanol/water

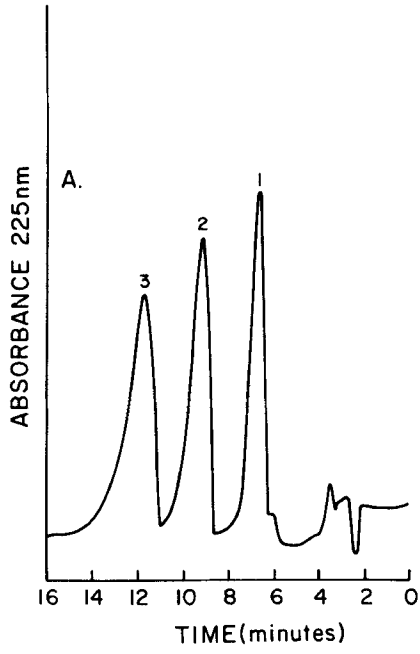

Figure 5. Separation of pyrrolizidine alkaloids from Senecio vulgaris. Column, 10 μ C18 Bondapak (Waters Associates), 3.9 mm x 30 cm. Solvent, MeOH/0.01 M KH_2PO_4 (pH 6.3), isocratic 55:45, flow rate 1.2 ml/min. Detector SF770 (Kratos) at 225 nm. Peak 1 = retrorsine, 2 = seneciphylline, 3 = senecionine.

solvent system was monitored at 220 nm which allowed a detectability of 40 ng of echimidine-N-oxide and 70 ng symphytine-N-oxide. In addition, the reduced PAs symphytine and echimidine were separated using a MN-Nucleosil®-NH_2 column with dichloromethane/propanol. The dichloromethane/propanol solvent system was monitored at 238 nm, which did not afford the sensitivity obtained when monitoring at 220 nm (26).

Huizing and Malingre (27) have successfully purified both monoester and diester PAs from the Boraginaceae using a polystyrene-divinylbenzene resin (XAD). The authors used a gradient elution of acidified methanol-water mixture to separate echimidine and symphytine, which are major alkaloids in the Symphytum species.

In addition, echimidine plus an unknown alkaloid fraction was isolated from Radix consolidae

Other investigators (25) have used a PRP-1 reversed phase resin column (HPLC) with a 10-30% acetonitrile and 0.1 M NH_4OH gradient in attempting to isolate the PAs from S. vulgaris and S. jacobaea (Fig. 6). The major disadvantage of this technique was the high pH (due to 0.1 M NH_4OH) as PAs are sensitive to alkaline pH.

Niwa and colleagues have recently developed a HPLC reversed phase system to separate the macrocyclic PAs of the otonecine type from the plant Petasites japonicus (29). P. japonicus is used as a folk medicine and food stuff in Japan and the PAs appear to be stable for at least one year at room temperature, according to Niwa et al. (28). Fortunately, it appears that the general population boil the young flower stalks of P. japonicus to remove the harsh taste (also known as processing) which removes slightly more than half of the PAs prior to use.

The authors were successful in separating the purified PAs neopetasitenine, senkirkine, and petasitenine as well as otosenine (otosenine is the stereoisomer of petasitenine with respect to the epoxy group). In addition, an alkaloidal mix from the ethanolic extracts of P. japonicus containing neopetasitenine, senkirkine, and petasitenine was successfully separated (Fig. 7). Previous HPLC methods which had been performed with PAs exhibiting the retronecine base (examples are senecionine and jacobine) were not applicable to the PAs exhibiting on otonecine base. The conditions used to separate the otonecine PAs are as follows: column, Cosmosil 5 pH (5μ, 15 cm x 4.6 mm), solvent system-isocratic methanol-0.02 M ammonium carbonate (45:55, pH 8.2) with a flow rate of 1.0 ml/min (Fig. 7). The relative high pH of 8.2 may lead to a premature destruction of the column according to the Cosmosil column bulletin, which advises maintaining a pH between 2 and 8 (29). A personal communication from Dr. Yamada stated that the pH of 8.2 did not appear to markedly decrease the efficiency of the Cosmosil 5 pH 5μ column.

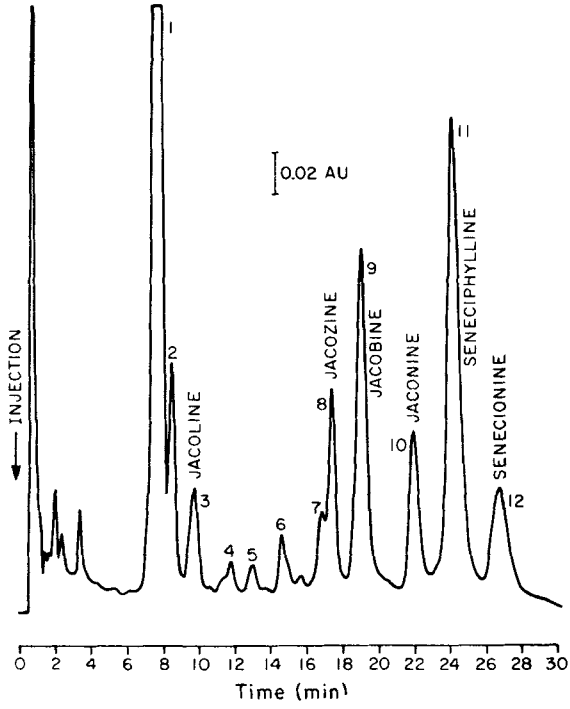

Figure 6. Separation of Senecio jacobaea extract. Column, PRP-1 (Hamilton), 4.1 mm x 15 cm. Solvent CH_3CN/0.01 M NH_4OH, linear gradient 10% to 30% CH_3CN over 20 min, flow rate 1 ml/min. Detector SF770 (Kratos) at 220 nm. Peaks labelled are jacoline, jacozine, jacobine, jaconine, seneciphylline, and senecionine.

Frahn et al. developed two preparative procedures to separate the diastereoisomers intermedine and lycopsamine which are found in the genera Amsinckia and Echium of the Boraginaceae and the genus Parsonsia of the Apocynaceae (30). Their procedures are based on the difference in the degree to which the vicinal glycol groups of different configurations (erythro and threo) complex with borate. The first method used a column of glass powder premoistened with a borax solution and eluted with chloroform (Fig. 8). The second method used a Bio-Rad AG 50 W-X2 resin (cation exchange) impregnated with 0.1 M borax and eluted with a 0.1 M borate solution

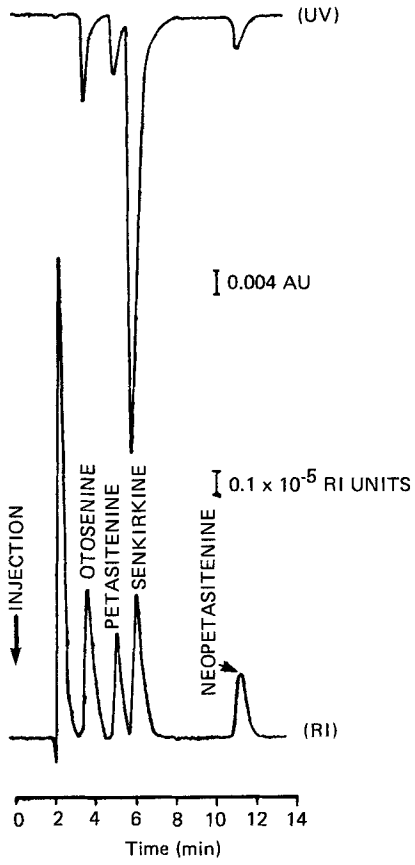

Figure 7. Separation of a mixture of otonecine pyrrolizidine alkaloids. Column, Cosmosil 5 Ph (Nakarai Chemicals), 4.6 mm x 15 cm. Solvent, MeOH/0.01 M $(NH_4)_2CO_3$ (pH 8.2), isocratic 45:55, flow rate 1 ml/min. Detectors, Uvidec-100-II UV spectrometer at 215 nm and Model SE^{-5}II RI differential refractometer at sensitivity of 4 x 10^{-5} RI units. Peaks labelled are otosenine, petasitenine, senkirkine, and neopetasitenine.

(Fig. 9). Both methods may be used to isolate gram quantities of PAs, but aliquots must be taken from each tube, spotted on filter paper, and sprayed with manganese sulphate-potassium permanganate-sulphuric acid reagent to detect the alkaloid(s).

Mohanraj et al. (1982) proposed to separate diastereomeric PAs using alkalized silica gel (Silica gel C or G) with solvents

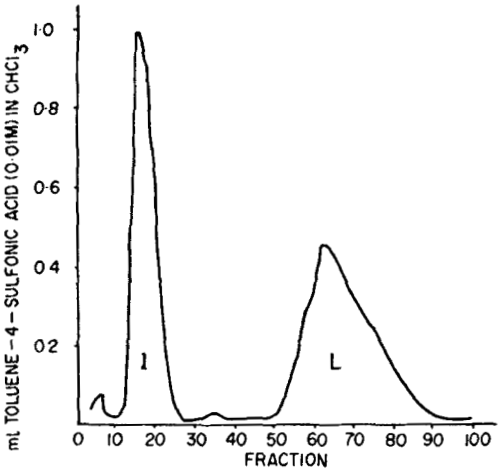

Figure 8. Elution pattern of intermedine (I) and lycopsamine (L) from borate partition column. Solvent, $CHCL_3$.

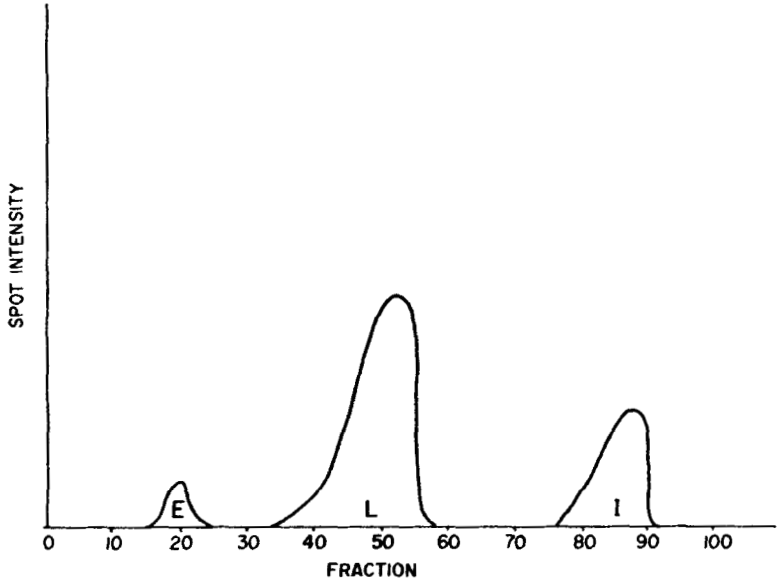

Figure 9. Elution pattern of echimidine (E), lycopsamine (L), and intermedine (I) from Bio-Rad AG 50W-X2 ion-exchange column. Solvent 0.1 M di-sodium tetraborate.

chloroform-methanol-25% ammonia (31). The authors used the PAs from Heliotropium curassavicum to separate curassavine, heliocurassavinine, coromandaline, heliocoromandaline, heliovicine, heliocurassavicine, and heliocurassavine. In addition to the required extraction of the PAs from H. curassavicum, PAs were fractionated on a column of neutral alumina prior to column chromatography on alkalized silica gel. Thin-layer chromatography was performed on silica gel C with the previously stated solvent system. Spots were visualized with iodine and/or Dragendorff's reagent.

Few investigators have successfully used HPLC to isolate large quantities of PAs. Our laboratory has used a reversed phase system which was based on our previously published analytical system (24). This method used a Waters Associates prep 500 system with a 0.005 M KH_2PO_4 pH 6.3/methanol solvent system (40:60) at 150 ml/min to separate retrorsine, seneciphylline, and senecionine from S. vulgaris (32).

To ensure that all three PAs were pure, recycling was used (Fig. 10). The advantage of this preparation were the low solvent costs (methanol/water), speed of separation, and the fact that no derivatives had to be made. The disadvantages were that not all investigators have access to a preparative system and the reverse phase columns required for this instrument are expensive. An adaptation of this method is to use the packing material found in the C18 prep 500 columns (Bondapak C18 50 → 125 μ particular packing material, Waters Associates) and bulkpack two 22.5 mm x 50 cm stainless steel columns, Whatman, Clifton, N.J. This method used a 0.01 M PO_4 pH 6.3/methanol (50:50) solvent system with a flow rate of 9 ml/min to isolate two PA metabolites from an in vitro hepatic microsomal mix (33). ^{14}C-labelled senecionine, ^{14}C-seneciphylline, and ^{14}C-retrorsine were isolated in part from S. vulgaris using a 7.8 mm x 300 cm column system, the Bondapak C18 50 → 125 μ particular packing material, and solvent system as previously described. (7).

Huizing et al. described the preparative ion-pair high performance liquid chromatography method to isolate the macrocyclic

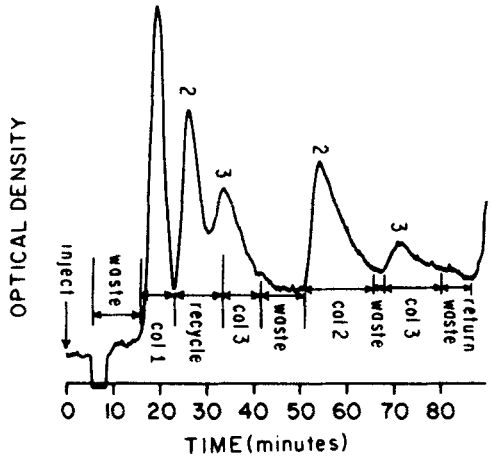

Figure 10. Preparative analysis of pyrrolizidine alkaloids derived from Senecio vulgaris. Columns, two prep-500/C18 reverse phase. Solvent, MeOH/0.005 M KH_2PO_4 (pH 6.3) isocratic (60:40), flow rate 150 ml/min. Detector RI (Waters Associates). Peak 1 = retrorsine, 2 = seneciphylline, 3 = senecionine.

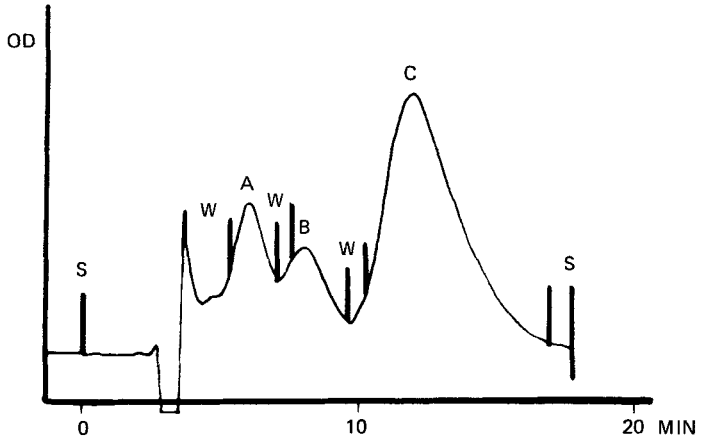

Figure 11. Preparative analysis of pyrrolizidine alkaloids from comfrey as ion pairs. Column, silica gel 60, 230-400 mesh, 2.5 cm x 53 cm stainless steel (Waters Associates). Solvent, .075 M lithium chloride in $CHCL_3$/MeOH isocratic (85:15), flow rate 50 ml/min. Detector RI (Waters Associates). Peak S = injection, W = waste, A = lycopsamine-intermedine, b = acetyllycopsamine + acetylintermedine, C = symphytine and/or isomer.

PAs from ground comfrey roots (34). A 25 mm x 53 cm stainless steel column (Waters Associates) was packed with dry silica gel 60 (230-400 mesh, Merck) connected to a Waters preparative LC/system 500A with solvent consisting of 0.075 M lithium chloride in chloroform/methanol (85:15) at a flow rate of 50 ml/min (Fig. 11). To reduce their solvent costs, the authors redistilled their solvents and added additional chloroform, or methanol to ensure a 85:15 solvent ratio.

An investigator who does not have access to HPLC can take comfort that many of the PAs were initially separated using silica gel columns and TLC techniques. Although more time consuming than "newer" chromatography techniques, they should not be overlooked.

ACKNOWLEDGMENT

The author gratefully acknowledges the excellent technical assistance of Crawford Brown and Thomas Krick. This work was supported by grants from the University of California (Faculty Research Grants, Livestock Disease Research Grants), the National Science Foundation (PFR-7806924), and the National Institute of Health (ES-03343).

Special thanks is extended to the publishers for permission to use the following figures and figure legends:

Figs. 3, 6, 7, 8, 9, & 11; J. Chromatogr., Elsevier Science Publishers.

Fig. 4.; Toxicol. Lett., Elsevier Science Publishers

Figs. 5 & 10.; J. Liquid Chromatogr., Marcel Dekker, Inc.

REFERENCES

1. Bull, L. B., Culvenor, C. C. J., and Dick, A. T., The pyrrolizidine alkaloids, North-Holland, Amsterdam, 1968.

2. McLean, E. K., The toxic actions of pyrrolizidine (Senecio) alkaloids, Pharm. Rev. 22, 429-483, 1970.

3. Hill, K. R., Rhodes, K., Stafford, J. L., and Aub, R., Liver diseases in Jamaican children (serous hepatosis), West Indian Med. J., 1, 49-63, 1951.

4. Huxtable, R. J., Herbal teas and toxins: Novel aspects of pyrrolizidine poisoning in the United States, Perspect. Biol. Med., 24, 1-4, 1980.

5. Culvenor, C. C. J., Clarke, J. A., Edgar, J. A., Frahn, J. L., Jago, M. V., Peterson, J. E. and Smith L. W., Structure and toxicity of the alkaloids of Russian comfrey (Symphytum and Uplandicum Nyman), a medicinal herb and item of human diet, Experientia, 15, 377-379, 1980.

6. Mattocks, A. R., Toxic pyrrolizidine alkaloids in comfrey, Lancet, 1136-1137, 1980.

7. Eastman, D. F., Dimenna, G. P., and Segall, H. J., Covalent binding of senecionine and seneciphylline to hepatic macromolecules and their distribution, excretion and transfer into milk of lactating mice, Drug Metab. Dispos., 10, 236-240, 1982.

8. Fowler, M. E., Pyrrolizidine alkaloid poisoning in calves, J. Am. Vet. Med. Assoc., 152, 1131-1137, 1968.

9. Deinzer, M. L., Thomson, P. A., Bergett, D. M., and Isaacson, D. L., Pyrrolizidine alkaloids: Their occurrence in honey from tansy ragwort (Senecio jacobaea L.). Science, 195, 497-499, 1977.

10. Mohabbat, O., Srivastava, R. M., Younos, M. S., Sediq, G. G., Merzad, A. A. and Aram, G. N., An outbreak of hepatic veno-occlusive disease in northwestern Afghanistan, Lancet, 2, 269-271, 1976.

11. Tandon, B. M., Tandon, R. K., Tandon, H. D., Narndranathan, M. and Joshi, Y. K., An epidemic of veno-occlusive disease of liver in central India, Lancet, 2, 271-272, 1976.

12. Harris, P. M. and Chen, K. K., Development of hepatic tumors in rats following ingestion of Senecio longilobus, Cancer Res., 30, 2881-2886, 1970.

13. Schoental, R., Toxicity and carcinogenic action of pyrrolizidine alkaloids, Cancer Res., 28, 2237-2246, 1968.

14. Yamanaka, H., Nagao, M., Sugimura, T., Furuya, T., Shirai, A., and Matsushima, T., Mutagenicity of pyrrolizidine alkaloids in the Salmonella/Mammalian microsome test, Mutation Res., 68, 211-216, 1979.

15. Koekemoer, M. J. and Warren, F. L., The Senecio alkaloids. Part VIII. The occurrence and preparation of the N-oxides. An improved method of extraction of the Senecio alkaloids, J. Chem. Soc. (Lond), 66-68, 1951.

16. Bradbury, R. B. and Culvenor, C. C. J., The alkaloids of Senecio jacobaea L. I. Isolation of the alkaloids and identification of jacodine as seneciphylline, Aust. J. Chem., 7, 378-383, 1954.

17. Mattocks, A. R., Extraction of heat-labile alkaloids from plants, Nature, 191, 1281-1282, 1961.

18. Deagen, J. T. and Deinzer, M. L., Improvements in the extraction of pyrrolizidine alkaloids, J. Natural Prod. (Lloydia), 40, 395-397, 1977.

19. Qualls, C. W. and Segall, H. J., Rapid isolation and identification of pyrrolizidine alkaloids (Senecio vulgaris) utilizing high pressure liquid chromatography, J. Chromatogr., 150, 202-206, 1978.

20. Segall, H. J., and Molyneux, R. J., Identification of pyrrolizidine alkaloids (Senecio longilobus), Res. Commun. Chem. Pathol. Pharm., 19, 545-548, 1978.

21. Segall, H. J., Pyrrolizidine alkaloids derived from Senecio jacobaea, Toxicol. Lett., 1, 279-284, 1978.

22. Segall, H. J. and Krick, T. P., Pyrrolizidine alkaloids organohalogen derivative isolated from Senecio jacobaea (tansy ragwort), Toxicol. Lett., 4, 193-198, 1979.

23. Horvath, C., Melander, W., and Molnár, I., Solvophobic interactions in liquid chromatography with nonpolar stationary phases. J. Chromatogr., 125, 129-156, 1976.

24. Segall, H. J., Reverse phase isolation of pyrrolizidine alkaloids, J. Liquid Chromatogr., 2, 424-436, 1979.

25. Ramsdell, H. S. and Buhler, D. R., High-performance liquid chromatographic analysis of pyrrolizidine (Senecio) alkaloids using a reversed-phase styrene-divinylbenzene resin column, J. Chromatogr. 210, 154-158, 1981.

26. Tittel, G., Hinz, H. and Wagner H., Quantitative bestimmung der pyrrolizidinalkaloide in Symphyti Radix durch HPLC, Planta Medica., 37, 1-8, 1979.

27. Huizing, H. J. and Malingre, T. M., Purification and separation of pyrrolizidine alkaloids from Boraginaceae on a polystyrene-divinylbenzene resin, J. Chromatogr., 176, 274-279, 1979.

28. Niwa, H., Ishiwata, H., and Yamada, K., Separation and determination of macrocyclic pyrrolizidine alkaloids of the otonecine type present in the edible plant Petasites

japonicus by reversed-phase high-performance liquid chromatography, J. Chromatogr., 257, 146-150, 1983.

29. Nakarai Chemical Bulletin, Specially Prepared Reagents, Cosmosil packed columns, Nakarai Chemicals, LTD, Nijyo Karasuma Nakagyo-Ku Kyoto, 1983.

30. Frahn, J. L., Culvenor, C. C. J., and Mills, J. A., Preparative separation of the pyrrolizidine alkaloids, intermedine and lycopsamine, as their borate complexes, J. Chromatogr, 195, 379-383, 1979.

31. Mohanraj, S., Herz, W. and Subramanian, P.S., Separation of diastereomeric pyrrolizidine alkaloids by chromatography on alkalized silica gel, J. Chromatogr., 238, 530-534, 1982.

32. Segall, H. J., Preparative isolation of pyrrolizidine alkaloids derived from Senecio vulgaris, J. Liquid Chromatogr., 2, 1319-1323, 1979.

33. Segall, H. J., Dallas, J. L. and Haddon, W. F., Two dihydropyrrolizine alkaloid metabolites isolated from mouse hepatic microsomes in vitro, Drug Metab. Dispos., 12, 68-71, 1984.

34. Huizing, H. J., DeBoer, F., and Malingre, Th. M., Preparative ion-pair high-performance liquid chromatography and gas chromatography of pyrrolizidine alkaloids from comfrey, J. Chromatogr., 214, 257-262, 1981.