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Note

Rapid high-performance liquid chromatography isolation of monoesters, diesters and macrocyclic diester pyrrolizidine alkaloids from *Senecio jacobaea* and *Amsinckia intermedia*

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Pyrrolizidine alkaloids (PAs) are plant-derived toxins which may contaminate human and animal food sources[1]. They are capable of causing severe liver necrosis and cirrhosis in animals and man[2,3]. Some *Senecio* and *Amsinckia* species (*S. vulgaris*, common groundsel; *A. intermedia*, fiddleneck) are commonly found in northern California in early spring. *Senecio jacobaea* (tansy ragwort) has invaded large areas of the Pacific Northwest and has become a dominant summer plant.

In a series of articles dealing with the isolation and purification of PAs utilizing high-performance liquid chromatography (HPLC)[4–9], we concluded that the most efficient method for the isolation of macrocyclic diesters (retrorsine, senecionine) is a methanol–0.01 *M* KH_2PO_4 buffer (pH 6.3) reversed-phase system[7]. However, purifying and isolating diesters, monoesters and epoxide type PAs with a reversed-phase system was not effectively resolved. The PAs from *A. intermedia* and some from *S. jacobaea* also exhibit a gum or tar-like constituency following extraction, which makes them difficult to purify.

Amsinckia intermedia has been reported to contain 4 PAs. These are echiumine [molecular ion (M^+) = 381] which is a diester and intermedine (M^+ = 299), lycopsamine (M^+ = 299) and sincamidine (M^+ = 313) which are monoesters[10]. *Senecio jacobaea* has been previously reported to contain 6 or more PAs; these are senecionine (M^+ = 333), senecionine (M^+ = 335), jacoline (M^+ = 369), jaconine (M^+ = 387) plus the epoxide containing PAs jacobine (M^+ = 351), and jacozine (M^+ = 349) (refs. 11, 12). In a recent article additional PAs were isolated from *S. jacobaea*[9].

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In a prior experiment PAs from *S. jacobaea* were isolated utilizing two 10-μm Bondapak C_{18} CN columns in series with a tetrahydrofuran–0.01 *M* ammonium carbonate buffer system[9]. This paper will introduce a reversed-phase HPLC procedure using a single 10-μm reversed-phase column to isolate PAs from *S. jacobaea and A. intermedia.*

MATERIALS AND METHODS

Amsinckia intermedia (fiddleneck) was collected near Putah Creek, adjacent to the University of California Davis campus. *Senecio jacobaea* (tansy ragwort) was a gift from Dr. Dickinson, Washington State University, Pullman, Wash. to C.W. Qualls at the University of California at Davis.

A 120-g amount of dried ground *A. intermedia* was subjected to the usual isolation and extraction procedure[7]. Approximately 74.8 mg of a dark brown tar (non-recrystallized) was isolated and dissolved in 2.5 ml methanol. Each sample injected was 100 μl. A similar amount of *S. jacobaea* was prepared in the customary fashion eventually yielding 75 mg. The sample concentration of *S. jacobaea* was 28.5 mg/ml of methanol and 50 μl were injected per run.

A Waters Assoc. 6000 A pump plus 660 programmer, 1 semiprep 10-μm C_{18} reversed-phase column (30 cm × 7.8 mm), 1 UK-6 injector, a Schoeffel 770 UV detector (218 nm) plus Varian recorder A-25 were used.

The solvent system was isocratic methanol–0.01 *M* KH_2PO_4 (17.5:82.5) (pH 4.79). The HPLC flow program used was gradient No. 7 (concave) which increased the flow-rate from 1 to 7 ml/min over a 60-min period. Ten complete runs were made and all numbered peaks (Figs. 1 and 2) were collected. For each sample the methanol was removed under reduced pressure, the pH adjusted to 9 with NH_4OH and extracted with chloroform. The chloroform was removed under reduced pressure.

Mass spectra were obtained for each numbered peak (Figs. 1, 2) using an LKB-9000 gaschromatograph–mass spectrometer interfaced to a PDP-8e computer with spectrometer conditions of ion source 290°, separator 290° and an electron voltage of 20 eV. The samples were run using both the direct probe inlet and the gas chromatographic (GC) inlet.

For GC–mass spectrometric (MS) analysis, all samples were run underivatized isothermally at 230° on a 5 ft. × 2 mm i.d. glass column packed with 3% SP 2100 DB on 100–120 mesh Supelcoport. Additionally, trimethylsilyl (TMS) derivatives of *A. intermedia* were made by adding either 50 μl Sylon BTZ (Supelco, Belleforte, Pa., U.S.A.) or 50 μl bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Regis, Morton Grove, Ill., U.S.A.) to a fraction of the sample, then heating at 60° for 15 min prior to injection. The derivatized samples were run on a 4 ft. × 2 mm I.D. glass column packed with 3% OV-1 on 100–120 mesh Supelcoport programmed at a temperature of 180 to 280° at 10°/min.

RESULTS

Senecio jacobaea

The order of HPLC resolution of the PAs derived from *S. jacobaea* (Fig. 1) generally followed the previous results[5,9]. Each HPLC sample peak was examined

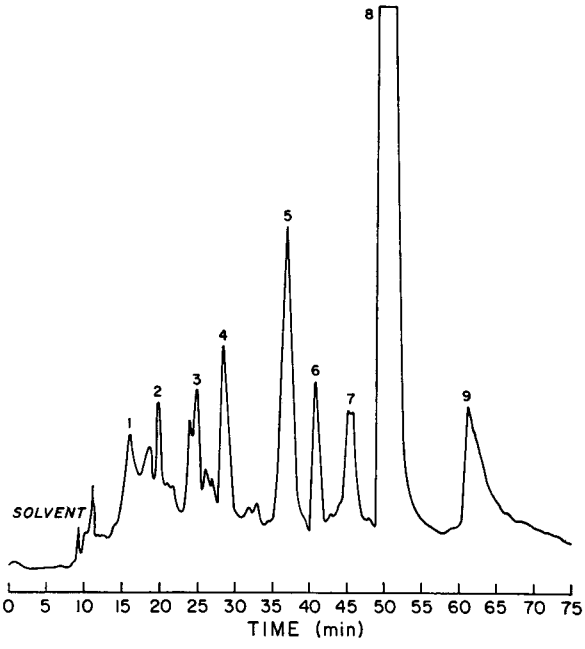

Fig. 1. Gradient analysis. Column, 30 cm × 7.8 mm semiprep 10-μm C_{18} reversed-phase (Waters Assoc.). Sample, 1.43 mg of *S. jacobaea* extract in 50 μl of methanol. Solvent, methanol–0.01 *M* KH_2PO_4 (pH 4.79) (17.5:82.5) Flow-rate, 1 to 7 ml/min over a 60-min period (see text). Column temperature, 25°. Detector, Model SF 770 Spectroflow monitor (Schoeffel) operated at 218 mm. Peaks: 1 = 369, 335, 333 (M^+); 2 = unknown; 3 = 351; 4 = 387, 351; 5 = 387, 351; 6 = 387; 7 = 333; 8 = 335; 9 = 335.

by GC–MS (see Materials and methods). The molecular ions (M^+) for each of the HPLC peaks are: peak No. 1 = 369, 335, 333; 2 = unknown; 3 = 351; 4 = 387, 351; 5 = 387, 351; 6 = 387; 7 = 333; 8 = 335 and 9 = 335. No M^+ = 349 or 385 were detected in this sample.

Amsinckia intermedia

The PAs of *A. intermedia* eluted off the HPLC in the following fashion (Fig. 2): peak No. 1 = unknown; 2 = possible 313; 3 = unknown; 4 = 299 and possible 381; 5 = 299; 6 = 299, 313 and 7 = 299. The mass spectra of the underivatized 299 and 313 peaks agreed well with published spectra[1,3]. However, since these monoesters did not chromatograph well underivatized, TMS derivatives were made. The BSTFA only derivatized two of the hydroxyl groups whereas the BTZ fully derivatized all three (Fig. 3). This spectrum fits well with the structure giving a molecular ion at (M^+ = 515), losses of 15 (CH_3), 43 ($CH(CH_3)_2$), 116 (C(OTMS) (CH_3) and peaks at 210, 117 and 94 which correspond to characteristic ions of the molecule (Fig. 3). The base peak at *m/e* 210 corresponds to the base peak 138 of the underivatized sample with a TMS group on the ring hydroxyl.

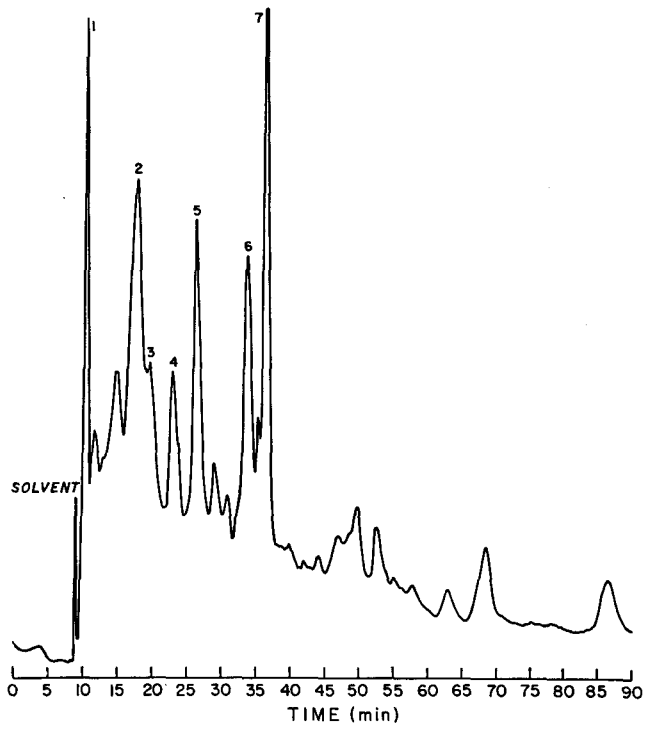

Fig. 2. Gradient analysis. Sample, 2.99 mg of *A. intermedia* extract in 100 μl of methanol. Conditions as in Fig. 1. Peaks: 1 = unknown; 2 = possible 313; 3 = unknown; 4 = 299; possible 381; 5 = 299, 313; 7 = 299.

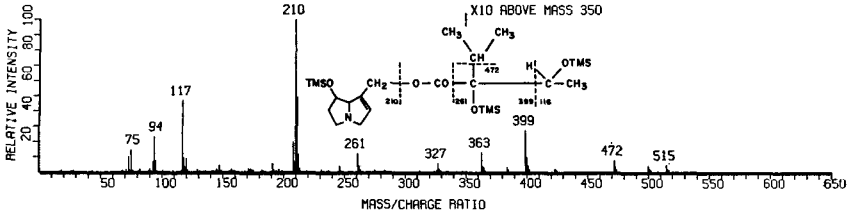

Fig. 3. Mass spectrometry of intermedine (M^+ = 299) TMS derivative (see text, M^+ = 515). The fragmentation pattern is 15 (CH_3), 43 ($CH(CH_3)_2$), 116 ($C(OTMS)(CH_3)$) while peaks 210, 117 and 94 correspond to characteristic ions of the molecule.

DISCUSSION

This paper describes our first successful attempt in purifying PAs which contain esters and diesters (*A. intermedia*). These techniques are also capable of isolating epoxide type PAs (jacobine, jacozine). Prior systems which we have utilized have not provided the resolution this system has. As an example, the previous method we utilized to isolate epoxide containing PAs from *S. jacobaea* required two CN columns, which created a high backpressure on the HPLC system. The utilization of a single 10-μm C_{18} reversed-phase column rather than two 10-μm C_{18} CN columns is indicative of the increased efficiency of this particular method.

Another advantage is that only 1 pump plus a flow programmer are necessary, instead of the customary two pumps plus programmer. Also, the method we have

described will probably be adaptable for a semipreparative or preparative scale up, which has been difficult to do with many of the PAs of *S. jacobaea* and *A. intermedia*. For preparative scale ups, a reversed-phase system of methanol–buffer is quite economical.

Our results indicate that no $M^+ = 349$ (jacozine) or $M^+ = 385$ peaks were isolated from the *S. jacobaea* samples. These samples were treated identically to prior samples, but originated from Washington (Dickinson) rather than Oregon. It has been our experience that plants collected in different areas of the Western United States will exhibit slight differences in PA content. Both samples (*S. jacobaea* and *A. intermedia*) had also been in the laboratory for quite some time. The effects of long-term storage and drying also lead to the decomposition of pyrrolizidine alkaloids.

The major problems associated with the PAs of *S. jacobaea* or *A. intermedia* are that they are difficult to crystallize and are usually isolated as a gum or tar. It would be advantageous to attempt to crystallize *A. intermedia* by the method of Culvenor and Smith[10] which results in the isolation of the acid salt.

By lowering the pH of 0.01 *M* KH_2PO_4 buffer from 6.3 to 4.79 plus decreasing the amount of methanol, we effectively increased the retention time of the macrocyclic diesters, seneciphylline and senecionine. In prior reversed phase experiments [methanol–0.01 *M* KH_2PO_4 (pH 6.3) (60:40)] using a semi-preparative column, seneciphylline and senecionine eluted respectively at 11.4 and 14.0 min using a flow-rate of 3.5 ml/min. In these experiments seneciphylline and senecionine eluted at 45.1 and 50.2 min, even though the flow-rate was increased to 7 ml/min over a 60-min period.

Reversed-phase HPLC appears to be a valuable technique in the isolation and identification of PAs. Through slight modifications of pH, solvent and flow-rate, various types of PAs may be isolated from different plants.

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REFERENCES

1 L. B. Bull, C. C. J. Culvenor and A. T. Dick, *The Pyrrolizidine Alkaloids*, North-Holland, Amsterdam, 1968.
2 A. R. Mattocks, in J. B. Harborne (Editor), *Phytochemical Ecology*, Academic Press, New York, 1972, Ch. 11, p. 179.
3 O. H. Muth, *J. Amer. Vet. Assoc.*, 153 (1968) 310.
4 C. W. Qualls, Jr., and H. J. Segall, *J. Chromatogr.*, 150 (1978) 202.
5 H. J. Segall, *Toxicol. Lett.*, 1 (1978) 279.
6 H. J. Segall and R. J. Molyneux, *Res. Commun. Chem. Pharm. Tox.*, 19 (1978) 545.
7 H. J. Segall, *J. Liquid. Chromatogr.*, 2 (1979) 429.
8 H. J. Segall, *J. Liquid. Chromatogr.*, 2 (1979) 1319.
9 H. J. Segall and T. P. Krick, *Toxicol. Lett.*, 4 (1979) 193.
10 C. C. J. Culvenor and D. W. Smith, *Aust. J. Chem.*, 19 (1966) 1955.
11 R. Adams and M. Gianturco, *J. Amer. Chem. Soc.*, 78 (1956) 398.
12 R. B. Bradbury and C. C. J. Culvenor, *Aust. J. Chem.*, 7 (1954) 378.
13 S. R. Heller and G. W. A. Milne, *EPA/NIH Mass Spectral Data Base*, U.S. Government Printing Office, Washington, D.C., 1978, pp. 2224 and 2370.