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HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF STEROIDAL ALKALOIDS

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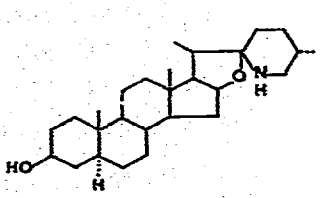
SUMMARY

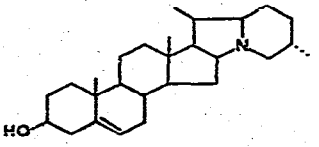
High-pressure liquid chromatography was used to separate the following steroidal alkaloids: tomatidine, solanidine, solasodine, rubijervine, veratramine and jervine. The method was used to prepare crystalline solanidine from a crude mixture of aglycones obtained from *Solanum chacoense*, and to separate radioactive solanidine from extracts of potato plants fed with [4-^{14}C]cholesterol.

INTRODUCTION

In addition to its high separating efficiency and adaptability to various chromatographic systems, high-pressure liquid chromatography (HPLC) has the potential for preparative uses. This technique has been applied to various classes of steroids[1], but so far no method for the steroidal alkaloids has been published. In our work on the biosynthesis and metabolism of this group of plant products[2] we have felt the need for a chromatographic method capable of separating adequate amounts of plant extracts for the determination of the structure or specific radioactivity of steroids and for the isolation of radioactive products to be re-administered to plants.



I Tomatidine



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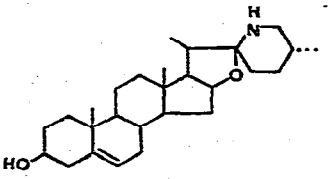
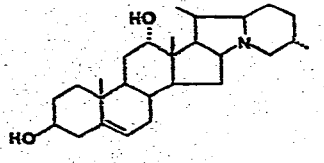
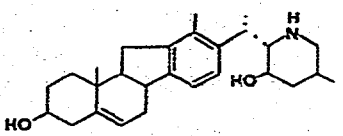


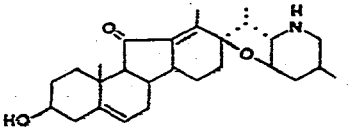
III Solasodine



IV Rubijervine



V Veratramine



VI Jervine

The structures of the steroidal alkaloids used in this work are shown in Formulae I–VI. Such compounds are best separated by adsorption chromatography, and excellent results have indeed been obtained by thin-layer chromatography (TLC)[3]. However, large differences in polarity between individual members of the class of steroidal alkaloids require the use of different mobile phases for TLC. In HPLC, however, gradient elution can advantageously be employed.

EXPERIMENTAL

The apparatus was assembled from commercially available components. The gradient elution device was a Beckman Model 141 high-capacity gradient pump (Beckman, Irvine, Calif., U.S.A.)*, modified by replacing the plastic ball joints by glass ball joints and the vinyl tubing by 1/8 in. O.D. polyethylene tubing, suitably attached to the glass and metal connections of the instrument. The gradient pump was geared to deliver a total gradient volume of 4 l. A concave gradient was prepared by cutting a phosphor bronze cam as shown in Fig. 1.

Two solvent reservoirs were connected to the gradient pump, one containing a 2:1 mixture of acetone and *n*-hexane, and the other one containing 97% aqueous acetone. Both solvents were of "distilled in glass" quality (Burdick and Jackson, Muskegon, Mich., U.S.A.). The output of the gradient pump was mixed with a magnetic stirrer in a solvent reservoir containing a nearly constant volume of 250 ml of the mixture. The pumping system, a Waters Associates' Model C-900 pump (Waters Assoc., Milford, Mass., U.S.A.) capable of operating at pressures up to 3000 p.s.i., drew solvent from this reservoir and delivered it through the sampling valve to the chromatographic column.

For sample injection, a Disc Model 706 sample valve (Disc, Costa Mesa, Calif., U.S.A.) was used. Depending on the sample volume, sample loops of various sizes were attached to the six-port rotary type valve. For samples up to 0.5 ml a piece of PTFE tubing, 1/8 in. O.D., with a capacity slightly more than 0.5 ml, served as the sample loop. For samples up to 70 ml a piece of polyethylene tubing, 4 mm I.D. (1/4 in. O.D.) and up to 18 ft. long was attached to the valve by Imperial Polyflo tube fittings (American Brass and Copper, Cleveland, Ohio, U.S.A.).

The column was assembled from 2-ft. sections of 3/8 in. O.D. stainless-steel tubes, capped with 10-μm end fittings (Waters Assoc.). Each section was packed with Porasil A, 37–75 μm (Waters Assoc.) by pouring small portions into the tube and tapping its end against the floor. The sections were then connected by 0.009 in. I.D. tubes with lock nuts and ferrules, after wrapping all threaded portions with PTFE tape.

The column effluent was collected with an LKB Radirac Model 3400 B fraction collector (LKB, Bromma, Sweden), operated on a time schedule, in 18-mm × 150-mm test tubes.

In the absence of a suitable detector, an aliquot of each fraction was analyzed by TLC[3]. Precoated silica gel G plates (250-μm layer) (Uniplates; Analtech, Newark, Del., U.S.A.) were scribed to give 1-cm-wide bands and cut across to provide two 8.5-cm path lengths. The developing solvents used were, for the less polar alkaloids, *n*-

* Reference of a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

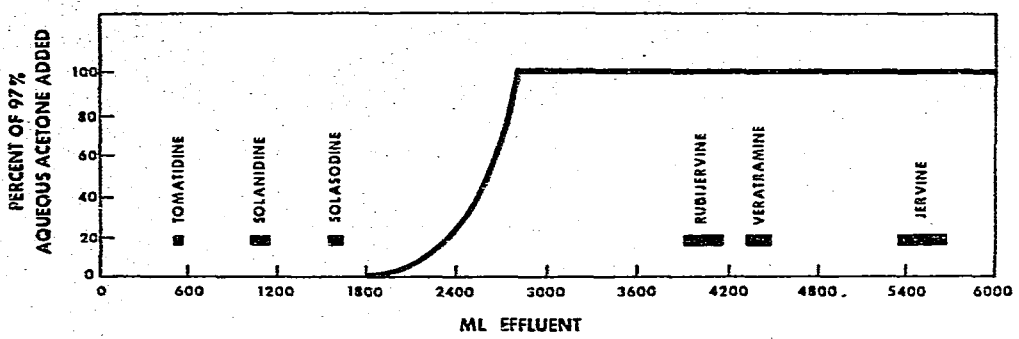


Fig. 1. Elution pattern of steroidal alkaloids. An 8 ft. × 3/8 in. O.D. stainless-steel column packed with Porasil A was used. Gradient elution, with acetone–*n*-hexane (2:1) and 97% aqueous acetone, was applied.

hexane–ethyl acetate (1:1) and, for the more polar ones, acetone. For detection, the plates were sprayed with 50% aqueous sulfuric acid and then heated[4].

After a variety of solvent systems had been tried, the method was standardized as follows: An 8-ft. packed column was washed with 500 ml acetone followed by 500 ml *n*-hexane and then 200 ml acetone–*n*-hexane (2:1). The sample, in acetone–*n*-hexane (2:1) solution, was then injected and isocratic elution with this solvent mixture was started. The pumps were adjusted to give a constant flow-rate of 2.5 ml/min at an initial pressure of 500 ± 100 p.s.i. and a final pressure of 1100 ± 100 p.s.i. Fractions were collected at the rate of 8 min per tube. After 800 ml of effluent had been collected, a concave gradient of increasing amounts of 97% acetone in acetone–*n*-hexane (2:1) was started, ending at 2000 ml of effluent with 97% acetone. Finally, isocratic elution with 97% acetone was used until 6000 ml of effluent had been collected.

RESULTS AND DISCUSSION

Fig. 1 shows the location of the steroidal alkaloids in the liquid chromatogram. Tomatidine and solasodine, which are isomeric at C-22, are well separated. As expected, rubijervine (12α-hydroxysolanidine) is much more polar than solanidine, requiring the addition of 97% acetone to elute it. Also, jervine is more polar than veratramine.

Table I is a summary of the elution volumes of several steroidal alkaloids observed with columns 4, 8, and 16 ft. in length under various elution programs. In all cases, isocratic elution with acetone–*n*-hexane (2:1) was followed by an elution gradient of increasing amounts of 97% acetone up to a certain volume and then by isocratic elution with 97% acetone.

When 100 μg of each of the alkaloids is chromatographed, the reproducibility of elution volumes under similar experimental conditions is satisfactory for all but identification purposes. As expected, with increasing column length the elution volumes increase and the resolution increases in spite of some zone broadening.

We have applied HPLC on an 8-ft. column with a concave elution gradient in our biosynthetic experiments[5] for the isolation of radioactive solanidine from 49- and 127-mg samples of non-saponifiable material from potato plants fed with [4-^{14}C]-

TABLE I

ELUTION VOLUMES OF STEROIDAL ALKALOIDS UNDER VARIOUS ELUTION PROGRAMS

Column length (ft.)	*Isocratic up to (ml)*	*Gradient*		*Elution volume (ml)*				
		Kind	*Up to (ml)*	*Tomatidine*	*Solanidine*	*Solasodine*	*Rubijervine*	*Jervine*
4	800	linear	3200	220–240	380– 460	620– 720	1760–2060	2240–2800
4	1000	linear	2600	200–220	380– 440	580– 700	1720–1920	3520–3840
4	800	concave	2000	200–240	400– 440	640– 720	2080–2320	3520–3760
8	800	concave	2000	540–620	1040–1160	1760–1840	2860–3060	3900–4020
8	800	concave	2000	520–580	1060–1160	1600–1700	2640–2800	3440–3660
16	3500	—	—	900–980	1820–1980	2960–3260		

cholesterol. In related work, we have had occasion to use the 16-ft. column for the isolation of solanidine from 2.09 g of a crude mixture of aglycones obtained from *Solanum chacoense* foliage. The crude solanidine obtained by isocratic elution with 800 ml of acetone–*n*-hexane (2:1), weighing 961 mg, was re-chromatographed under the same conditions and yielded 688 mg of crystalline material.

In order to investigate the preparative potential of HPLC for steroidal alkaloids further, we have determined the elution characteristics of various amounts of pure tomatidine up to 1 g on the 16-ft. column, eluted isocratically with acetone–*n*-hexane (2:1). In every case a sharp peak was obtained and for 1 g of tomatidine 90% of the sample was recovered in 300 ml of effluent. Although the 3/8-in. columns are undoubtedly somewhat overloaded, the zones are sufficiently compact for preparative purposes.

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