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Note

High-performance liquid chromatography of *Solanum* and *Veratrum* alkaloids

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Since our first publication on high-performance liquid chromatography (HPLC) of steroidal alkaloids in 1976[1], only two papers have appeared on this subject. Bushway *et al.*[2] have described the separation of potato glycoalkaloids, and Crabbe and Fryer[3] have applied HPLC to the quantitative analysis of solasodine glycosides. While we have used adsorption chromatography on a silica column for the aglycones, reversed-phase partition chromatography was applied in both cases where the more polar glycoalkaloids were analyzed.

We have continued to rely on adsorption, but the substitution of Zorbax-Sil for the coarser Porasil A has greatly improved resolution and shortened the analysis time. Instead of increasing the polarity of the eluent, we now use an increase in flow-rate for accelerating the elution of more polar steroids. The use of a UV detector is an obvious improvement over the testing of fractions by thin-layer chromatography, practiced earlier.

EXPERIMENTAL*

The HPLC apparatus was assembled from commercially available components. A solvent reservoir was connected to the inlet of a single-piston reciprocating pump (Model 110; Altex, Berkeley, CA, U.S.A.). From the pump outlet stainless-steel tubing led to a sample injection valve (Model 7125; Rheodyne, Berkeley, CA, U.S.A.) with a loop volume of 100 μl and from there to the column inlet.

The column consisted of two stainless-steel tubes, 250 × 4.6 mm I.D., prepacked with Zorbax-Sil (particle size 6 μm, DuPont, Wilmington, DE, U.S.A.), connected in series. The column outlet was connected to the inlet of a variable-wavelength detector (Model 155, Altex) with a flow cell having a 10-mm pathlength and a 20-μl volume, which was set at 213 nm, 0.2 full scale. The effluent from the detector was returned to the reservoir.

The signal from the detector was fed into a single-channel recorder (Model 335; Linear, Irvine, CA., U.S.A.), which was set at 10 mV. Only *n*-hexane was HPLC

* Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

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grade ("Distilled-in-Glass" quality; Burdick & Jackson Labs., Muskegon, MI, U.S.A.); the other solvents were of reagent grade.

We have tested 14 *Solanum* and *Veratrum* alkaloids in two groups. The less polar compounds, mainly *Solanum* alkaloids, were separated by use of *n*-hexane–methanol–acetone (18:1:1) as the eluent (Fig. 1). After the elution of the first four steroids, the flow-rate was increased from 0.3 ml/min to 1.5 ml/min. The more polar *Veratrum* alkaloids were separated by use of *n*-hexane–ethanol–acetone (18:1:1) as the eluent (Fig. 2) at a flow-rate of 1.0 ml/min. After the elution of the first two steroids, the flow-rate was increased to 1.6 ml/min.

Rubijervine was included in both groups and served as a reference for the determination of the relative retention times. These were found to vary by no more than $\pm$ 5%. As expected, rubijervine, having 2 hydroxyl groups, was more polar than any of the monohydroxysteroids in the first group (Fig. 1). Tomatillidine, which

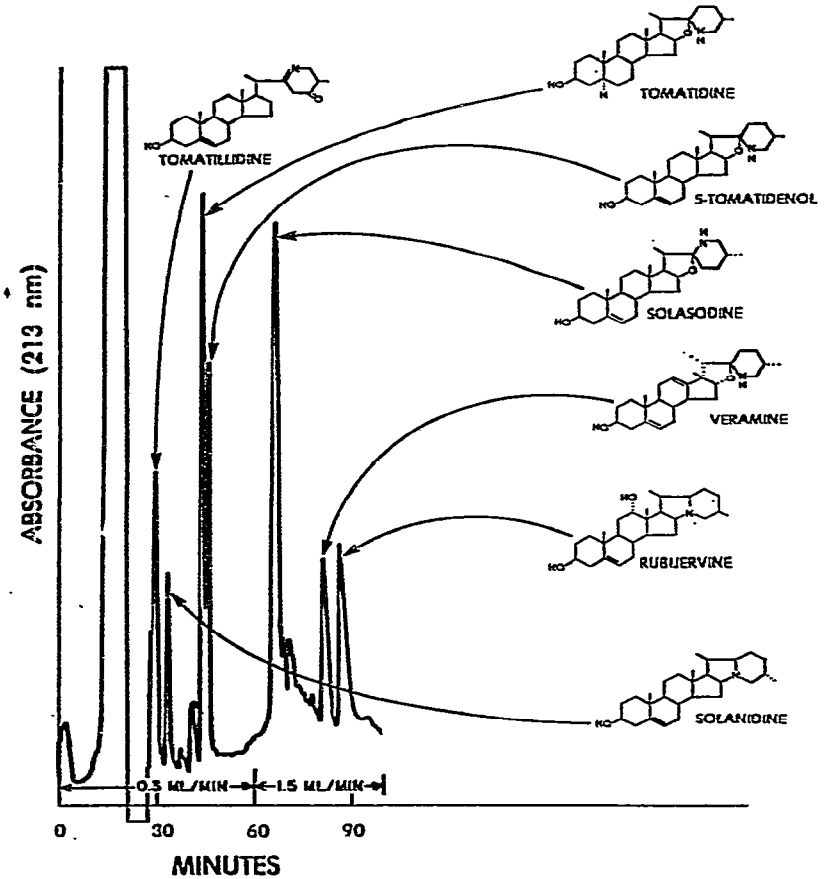

Fig. 1. Separation of the less polar steroidal alkaloids by HPLC. A mixture of 6.3 μg tomatillidine, 13 μg solanidine, 210 μg tomatidine, 13 μg 5-tomatidenol, 84 μg solasodine, 70 μg veramine, and 40 μg rubijervine in 20 μl dichloromethane-2-propanol (1:1) was applied to a Zorbax-Sil column, 500 × 4.6 mm I.D. Eluent, *n*-hexane–methanol–acetone (18:1:1); flow-rate, 0.3 ml/min; pressure, 800 p.s.i.; for 60 min. Afterwards, flow-rate, 1.5 ml/min; pressure 5000 p.s.i. Detector at 213 nm, range 0.2; recorder speed 6 cm/h, span 10 mV.

would be expected to show an intermediate polarity, being a monohydroxymonoketone, turned out to be the least polar compound, apparently because it has a tertiary nitrogen. This is followed by solanidine with its condensed ring system. Tomatidine was slightly less polar than its Δ^5-analog, 5-tomatidenol. However, solanidine was not separated from its 5α-analog, demissidine (not shown). The separation of the two 22-epimers, 5-tomatidenol and solasodine, is remarkable. In contrast to earlier results[1], the order of elution of solanidine and tomatidine was reversed.

Among the more polar alkaloids (Fig. 2), the condensed-ring isomers isorubijervine and rubijervine were eluted ahead of the C-*nor*-D-*homo*-steroids, as expected. However, isorubijervine with its primary hydroxyl at C-18 would have been expected to be more polar than rubijervine with its secondary hydroxyl at C-12. Certainly,

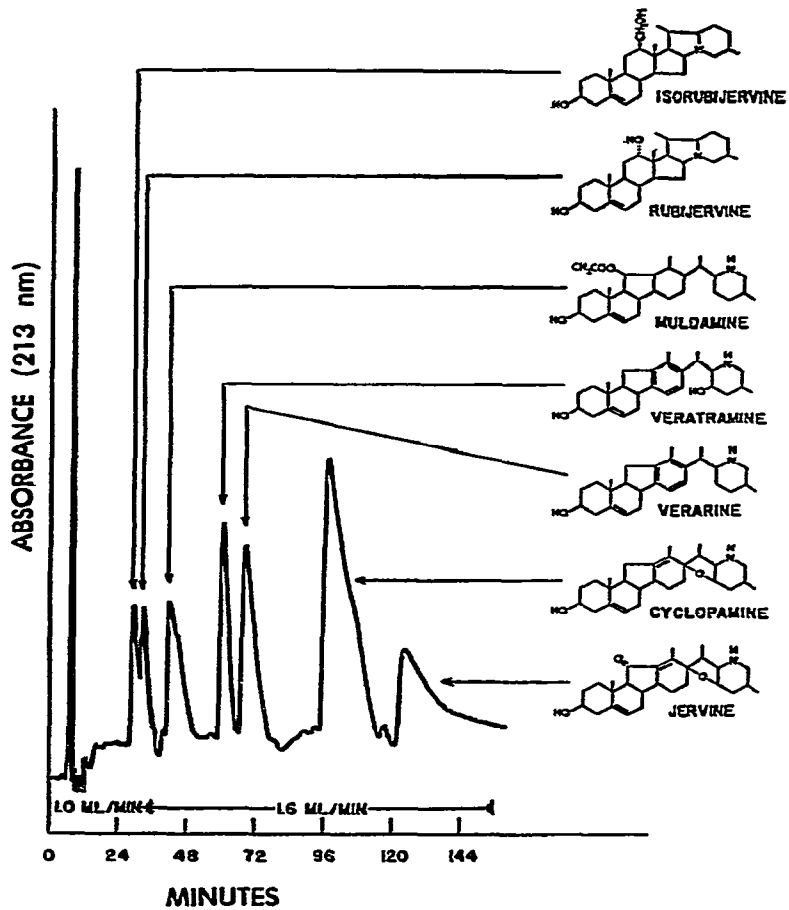

Fig. 2. Separation of the more polar steroidal alkaloids by HPLC. A mixture of 16 μg rubijervine, 56 μg isorubijervine, 210 μg muldamine, 55 μg veratramine, 30 μg verarine, 183 μg cyclopamine, and 540 μg jervine in 31 μl 2-propanol was applied to the same column as in Fig. 1. Eluent, *n*-hexane–ethanol–acetone (18:1:1); flow-rate, 1.0 ml/min; pressure, 2800 p.s.i.; for 36 min. Afterwards, flow-rate, 1.6 ml/min; pressure, 5000 p.s.i. For all other conditions, see Fig. 1.

steric phenomena are at play here, as is evident from the totally unexpected elution order of veratramine (2 hydroxyls) and verarine (1 hydroxyl). Otherwise, the sequence is as expected. The method obviously lends itself to the purification of alkaloids. Most of our reference compounds were found to contain impurities, which were difficult to remove by the methods available heretofore.

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3 P. G. Crabbe and C. Fryer, *J. Chromatogr.*, 187 (1980) 87.