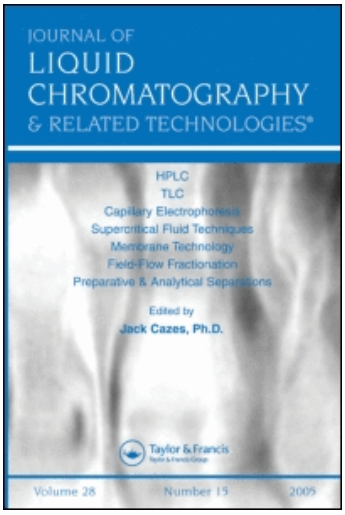

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Analysis of Dopamine-Derived Tetrahydroisoquinoline and Tetrahydro-Protoberberine Alkaloids by Cation-Exchange Liquid Chromatography

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ANALYSIS OF DOPAMINE-DERIVED TETRAHYDROISOQUINOLINE AND TETRAHYDROPROTOBERBERINE ALKALOIDS BY CATION-EXCHANGE LIQUID CHROMATOGRAPHY

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ABSTRACT

Liquid chromatographic characteristics of twelve selected dopamine-derived tetrahydroisoquinoline and tetrahydroprotoberberine alkaloids on commercially available 10 μ cation-exchange columns (Partisil 10 SCX, Vydac TP 401 SCX and Nucleosil SA) were examined. Effects of mobile phase ionic strength, pH, addition of organic modifiers, and column temperature on retention and efficiency were determined. Capacity factors (k') were linear with respect to 1/ionic strength for Nucleosil and Vydac, but not for Partisil columns. Retention of compounds varied as follows: Nucleosil > Vydac > Partisil. Mobile phase pH had little effect on retention or selectivity. However, chromatographic efficiency was adversely affected by mobile phase pH greater than 5.5. Organic modifiers decreased elution time in the following order: 1-butanol > dioxane > 2-propanol ≈ acetonitrile > ethanol. Elevated column temperature decreased capacity factors and increased efficiency. Conditions for the separation and detection of low levels (less than 1 pmole) of these alkaloids were developed.

INTRODUCTION

The 1-benzyltetrahydroisoquinolines (1,2) and tetrahydroprotoberberines (3,4) are among the best known of the many classes of isoquinoline alkaloids (5) occurring in the plant kingdom. Additionally, endogenous formation of tetrahydroisoquinoline alkaloids in mammals has been suggested as a factor in development of dependence on alcohol (6,7) and other sedative hypnotic drugs (8).

The benzyltetrahydroisoquinoline and tetrahydroprotoberberine alkaloids affect various biochemical systems and elicit diverse pharmacological effects (9-11). Most interestingly, infusion of minute amounts of tetrahydropapaveroline (THP, IIIa, Figure 1) into the lateral ventricle of the brain of Sprague-Dawley rats results in a marked and long lasting increase in ethanol preference (12,13).

Formation of THP has been reported *in vitro* (7,14) and *in vivo* in experimental animals (15) and in man (16). Additionally, certain tetrahydroprotoberberines have been identified in the urine of rats after administration of THP and in urine from human patients receiving L-dopa therapy for Parkinson's disease (17).

The difficulties inherent in analysis of very low levels of these polyphenolic alkaloids have seriously hampered their detection and quantitative assessment in mammalian systems. Until recently, application of HPLC to analysis of these compounds has been limited to salsolinol and tetrahydropapaveroline (18) and

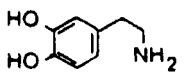
previously reported analyses have predominantly utilized gas chromatography (19) or combined gas chromatography/mass spectrometry (15,16,20). These latter techniques suffer from the necessity of derivatization prior to gas chromatographic separation. Additionally, derivatizing reagents such as trimethylsilyl donors are not compatible with the aqueous milieu of biological samples and preliminary extractions and evaporations to dryness are necessary. Reversed phase and cation-exchange liquid chromatography appeared to hold promise for the analysis of these alkaloids with minimal sample manipulation. Accordingly, the HPLC characteristics of selected dopamine-derived tetrahydroisoquinoline and tetrahydroprotoberberine alkaloids (Figure 1) on commercially available cation-exchange packings were determined.

EXPERIMENTAL

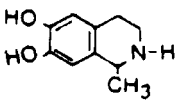
Dopamine (3,4-dihydroxyphenethylamine, I, Figure 1) was purchased from Calbiochem, LaJolla, CA, and salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, II, Figure 1) from Aldrich Chemical Co., Milwaukee, WI. The remaining alkaloids were synthesized in this laboratory (21).

Chromatographic Equipment:

Analyses were carried out using several high pressure liquid chromatographs: an ALC-202 equipped with a M-6000 pump (Waters Associates, Milford, MA) and valve-loop injector (Glenco Scientific, Houston, TX); and three modular chromatographs utilizing piston pumps rated at 1000 psi (Laboratory Data



I. DOPAMINE



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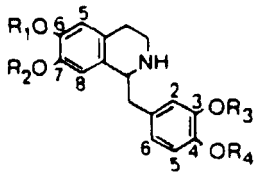
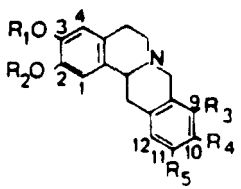


		R_1	R_2	R_3	R_4
III. a.	TETRAHYDROPAPAVEROLINE (THP)	H	H	H	H
b.	6-OMe-THP	CH_3	H	H	H
c.	7-OMe-THP	H	CH_3	H	H
d.	3'-OMe-THP	H	H	CH_3	H
e.	4'-OMe-THP	H	H	H	CH_3



		R_1	R_2	R_3	R_4	R_5
IV. a.	2,3,9,10-TETRAHYDROXYBERBINE (2,3,9,10-THB)	H	H	OH	OH	H
b.	2,3,10,11-THB	H	H	H	OH	OH
c.	2-OMe-THB	H	CH_3	H	OH	OH
d.	3-OME-THB	CH_3	H	H	OH	OH
e.	10-OMe-THB	H	H	H	CH_3O	OH
f.	11-OMe-THB	H	H	H	OH	CH_3O

FIGURE 1. Dopamine and related 1-methyltetrahydroisoquinoline, 1-benzyltetrahydroisoquinoline and tetrahydroprotoberberine alkaloids.

Control, Riviera Beach, FL), 3000 psi (Glenco Scientific, Inc., Houston, TX) or 5000 psi (Altex Inc., Berkeley, CA). Solutes were monitored by uv absorption at 280 nm (Waters Associates or Altex)

or by electrochemical detection with a carbon paste electrode maintained at a potential of + 0.8 V vs. Ag/AgCl (Bioanalytical Systems, West Lafayette, IN). Samples were introduced on the modular chromatographs by either valve-loop injection (Glenco Scientific, Inc.) or "off column" septum injection (Altex).

All solutions were filtered through 0.45 μ filters (Millipore Corp., Bedford, MA or Waters Associates) before use. Mobile phases were prepared by neutralizing phosphoric acid with ammonium hydroxide to specific pH values or by dissolving solid monobasic ammonium phosphate.

Three different prepacked 10 μ strong cation-exchangers were employed: three Partisil 10 SCX columns, 4.6 mm i.d. x 25 cm (Whatmann, Inc., Clifton, NJ); four Vydac TP 401 SCX columns, 3.2 mm i.d. x 25 cm (Altex or Separations Group, Hesperia, CA); and two Nucleosil SA columns, 3.2 mm i.d. x 25 cm (Altex).

RESULTS AND DISCUSSION

Effect of Counter-Ion Concentration:

The ion-exchange capacities of the three ion exchangers employed are: Vydac, 0.01 meq/g; Partisil, 0.1 meq/g; and Nucleosil, 1.0 meq/g (22). However, the retention powers of the packing materials did not follow this ranking but was in the following order: Partisil < Vydac < Nucleosil. Chromatography of THP as a test solute, for example, with 0.01 M ammonium phosphate, pH 4.5 on Partisil, or with 0.5 M ammonium phosphate, pH 4.5 on

Vydac, or with 1.0 M ammonium phosphate, pH 4.5 on Nucleosil columns gave approximately equivalent retention volumes.

Plots of capacity factors, k', (23) and reciprocal of mobile phase ionic strength should be linear with intercept (k_o') of zero if chromatographic retention of the solute is based solely on ion-exchange mechanisms (24). Values of k_o', therefore, provide a measure of the degree of non-ion-exchange interactions between solutes and stationary phase. Plots of k' values of selected tetrahydroisoquinoline and tetrahydroprotoberberine alkaloids against the reciprocal of mobile phase ionic strength obtained with Vydac and Nucleosil columns were linear whereas plots obtained with Partisil columns were non-linear. Intercept values (k_o') for salsolinol (II), THP (IIIa), 2,3,10,11-THB (IVb), 2,3,9,10-THB (IVa), Figure 1, were 0.4, 3.3, 9.2 and 12.1 on Vydac columns; 1.4, 6.0, 12.9 and 15.4 on Nucleosil columns; and approximately 0.3, 0.1, 0.8 and 0.5 on Partisil columns, respectively. A significant portion of the mechanism of retention of these alkaloids on Vydac and Nucleosil columns thus appears to be based on non-ion-exchange factors.

Effect of Eluent pH:

Manipulation of mobile phase pH, particularly in the region equivalent to the pKa of the solute molecules, should lead to changes in retention and selectivity. However, only a limited portion of the pH range is accessible since basic mobile phases lead to deterioration of silica-based packings and, in this instance, also to decomposition of polyphenolic solutes. Capacity

factor values for selected compounds at pH values in the region 2 - 6 are given in Table 1. As can be seen, the effect of pH over the range studied on retention was minimal. Indeed, the most important effect was related to column efficiency rather than solute retention. Mobile phases of pH above 5.5 gave peaks with increased tailing and corresponding decreased efficiency on all columns.

Effect of Organic Modifiers:

Addition of selected organic solvents to ammonium phosphate mobile phases decreased retention time for all solutes on all columns. The relative effectiveness of the organic modifiers in decreasing solute retention time, as illustrated in Figure 2, was found to be ethanol < 2-propanol ≈ acetonitrile < dioxane < 1-butanol.

TABLE 1

Effect of pH on Capacity Factors (k')

Column	Mobile Phase	pH	k' SAL	THP	2,3,9,10-THB	2,3,10,11-THB
Nucleosil	0.5 M $NH_4H_2PO_4$	2.7	2.4	9.3	27.9	21.1
		4.5	2.4	8.6	26.0	20.4
		6.1	2.4	7.9	23.3	20.1
Vydac	0.5 M $NH_4H_2PO_4$	3.0	1.0	5.0	15.9	13.0
		4.0	1.0	4.9	15.9	13.0
		5.0	1.0	4.7	15.9	13.1
		6.0	1.0	4.5	15.9	13.2
Partisil	0.1 M $NH_4H_2PO_4$	2.1	0.9	0.6	1.0	1.4
		2.9	1.0	0.6	1.1	1.5
		4.5	1.2	0.8	1.4	1.9
		6.2	2.1	1.7	2.1	2.9

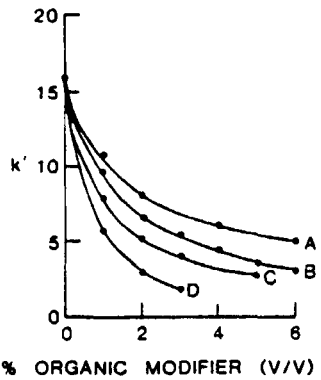


FIGURE 2. Effect of organic modifiers on capacity factors. 2,3, 9,10-THB test solute, Vydac TP 401 SCX, 0.5 M $NH_4H_2PO_4$, pH 4.5, 0.5 ml/min. Modifiers: (A) ethanol, (B) 2-propanol, acetonitrile, (C) dioxane, (D) 1-butanol.

The decrease in retention time obtained on incorporation of organic modifiers in the mobile phase was greater for the strongly retained more lipophilic compounds such as the mono-O-methyl analogs of THP and 2,3,10,11-THB than for the early eluting, more hydrophilic parent compounds. For this reason, addition of organic modifiers best serves to decrease analysis time of samples with lipophilic constituents. Significant changes in selectivity were not observed. The three different cation-exchangers gave qualitatively comparable results. Preliminary experiments with n-alkane reversed phase packings gave similar results both in respect to effect on k' values and the relative eluting strength of the various organic modifiers.

Column Efficiencies:

Height equivalent to a theoretical plate (HETP) values were determined routinely (25). At ambient temperature, Vydac and Nu-

cleosil columns gave HETP values in the ranges of 0.11 - 0.18 and 0.15 - 0.20 mm, respectively, for solutes with k' between 0 and approximately 25. HETP values were essentially constant with regard to k' for the two materials, although Vydac gave slightly higher efficiencies (ca. 10%) for compounds with large k' values. In contrast, Partisil exhibited much greater efficiency at low values of k' (HETP = 0.1 mm at k' = 0) than at higher k' (HETP = 0.55 mm at k' = 10).

For this series of solutes and under the conditions employed, these data suggest that partition isotherms are more nearly linear for Nucleosil and Vydac than for Partisil. The increase in HETP with k' exhibited by Partisil presumably arises from unfavorable thermodynamic interactions between the solute molecules and stationary phase and not from kinetic factors (26).

No attempt was made to exploit "infinite column diameter effects" (27). Columns and injection systems with more optimal geometries should lead to increased efficiencies. Smaller diameter packing materials, presently not available commercially, should also substantially improve the chromatographic efficiencies.

Effect of Column Temperature:

Increased column temperatures substantially decreased k' values, particularly for strongly retained compounds. For example, 3-OMe-THB and 10-OMe-THB gave respective k' values of 20.2 and 19.2 at 23° on Nucleosil with 0.5 M $NH_4H_2PO_4$, pH 4.5 + 5% 1-butanol. The k' values for these compounds at 62° were 6.2 and 6.0,

respectively, with an accompanying reduction in column HETP values (approximately 30-40%). Comparable decreases in retention volume and improvement in column efficiencies were observed for Vydac and Partisil.

Column Longevity:

The life expectancy of expensive pre-packed ion-exchange columns is of general concern. In addition to the obvious economic burden that frequent replacement of columns entails, there is also the problem of batch-to-batch variation. The life expectancy of Partisil columns, in particular, has been questioned (24,28). The columns have been reported to last only a few months before loss of ion-exchange capacity becomes critical (24). Our experience agrees with this observation, however, the pre-column recommended by the manufacturer for maximum column survival was not employed in either case. Only one of the four Vydac columns used in these studies failed after approximately two years of daily operation. This particular column had been employed frequently in preparative mode with much larger sample volumes (100 - 200 µl) and mobile flow rates (3 - 5 ml/min) than those used for analytical separations (10 - 25 µl, 0.5 ml/min). Nucleosil columns appeared to equal Vydac columns in longevity.

Representative Separations:

Separation of a mixture of 13 test solutes by Nucleosil, Vydac and Partisil cation-exchangers is illustrated in Figures 3, 4 and 5, respectively. As shown in Figures 3 and 4, the selectivity

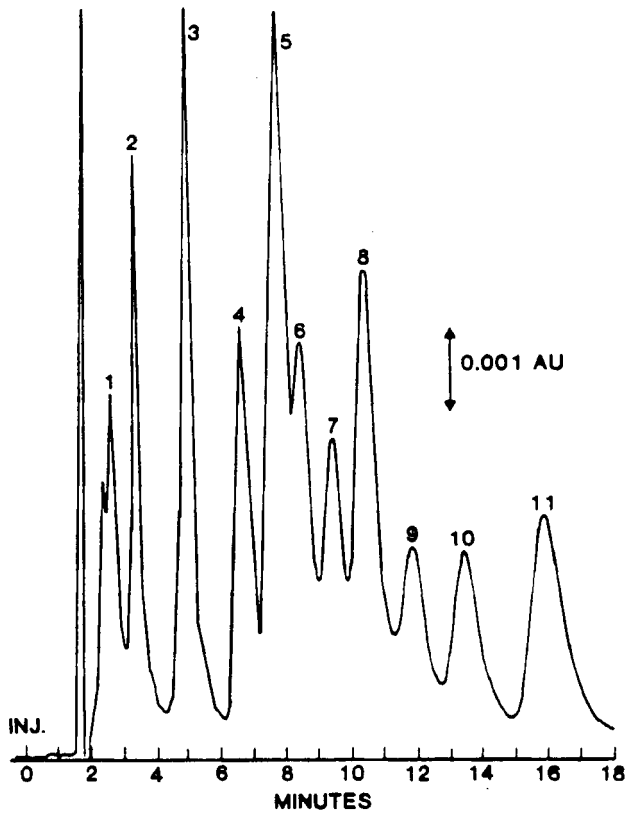


FIGURE 3. Separation of dopamine-derived alkaloids on Nucleosil SA. Peak identities: (1) Dopamine, (2) SAL, (3) THP, (4) 2,3,10, 11-THB, (5) 2,3,9,10-THB, 3'-OMe-THP, (6) 7-OMe-THP, (7) 4'-OMe-THP, (8) 6-OMe-THP, (9) 2-OMe-THB, (10) 11-OMe-THB, (11) 3-OMe-THB, 10-OMe-THB. Conditions: 0.5 M $NH_4H_2PO_4$ + 5% 1-butanol, 1.0 ml/min, 2000 psi, chart 1 cm/min.

of Vydac and Nucleosil columns for these compounds is strikingly similar and the general order of elutions is essentially identical. In contrast, the representative chromatogram derived with Partisil (Figure 5) indicates substantial differences in selectivity compared to the other materials, particularly in the elu-

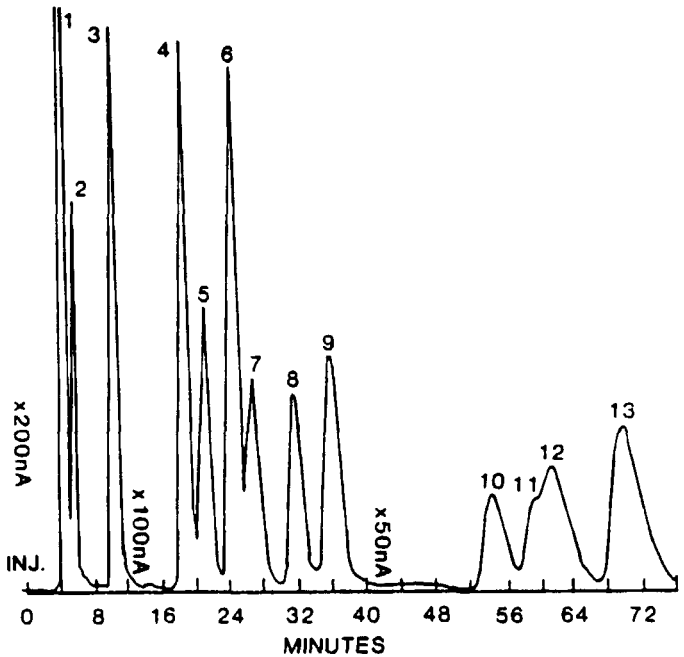


FIGURE 4. Separation of dopamine-derived alkaloids on Vydac TP 401 SCX. Peak identities: (1) Dopamine, (2) SAL, (3) THP, (4) 2,3,10,11-THB, (5) 3'-OMe-THP, (6) 2,3,9,10-THB, (7) 7-OMe-THP, (8) 4'-OMe-THP, (9) 6-OMe-THP, (10) 2-OMe-THB, (11) 11-OMe-THB, (12) 10-OMe-THB, (13) 3-OMe-THB. Conditions: 0.2 M $NH_4H_2PO_4$, 0.5 ml/min, 500 psi, column temperature 51.5 C, chart 15 cm/hr.

tion order of salsolinol and THP and of 2,3,9,10- and 2,3,10,11-THB. These differences in selectivity may be of value for certain specific applications.

None of the packing materials afforded a complete resolution of this complex mixture of compounds which contained materials widely divergent in nature as well as isomeric sets with very subtle structural differences. However, the resolutions depicted in Figures 3 and 4 indicate that cation-exchange HPLC is well suited for analysis of dopamine-derived alkaloids.

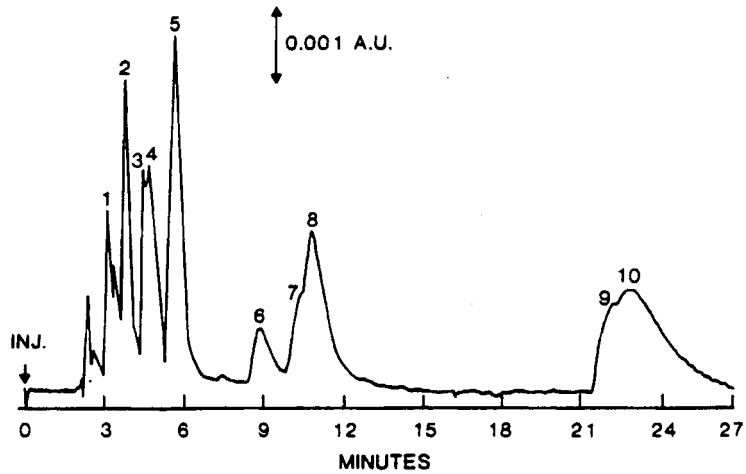


FIGURE 5. Separation of dopamine-derived alkaloids on Partisil 10 SCX. Peak identities: (1) dopamine, (2) THP, (3) SAL, (4) 2,3,9, 10-THB, (5) 2,3,10,11-THB, 3'-OMe-THP, 7-OMe-THP, (6) 4'-OMe-THP, (7) 2-OMe-THB, (8) 6-OMe-THP, 11-OMe-THB, (9) 3-OMe-THB and (10) 10-OMe-THB. Conditions: 0.1 M $NH_4H_2PO_4$, 1.5 ml/min, 1000 psi, chart 20 in/hr.

Method Sensitivity:

The absolute sensitivity of a chromatographic system depends on a number of variables, which are dependent not only on detector response but also on the efficiency of the entire system. Figure 6 is representative of the sensitivity attainable with the electrochemical detector on a routine basis. Under the conditions employed, readily detectable amounts for THP, 2,3,10,11-THB, 7-OMe-THP and 2-OMe-THB were approximately 35, 85, 120 and 250 picograms, respectively. Absolute minimal detectable amounts may be a factor of 10 or more lower, perhaps to the sub-picogram level. The uv detector, even when employed at 280 nm near the absorption maxima characteristic of these compounds, is approximately 1000 times less sensitive than the electrochemical detector.

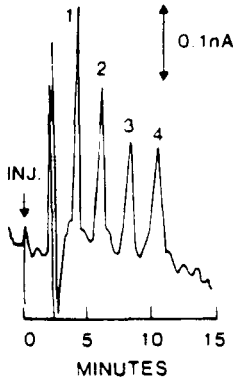


FIGURE 6. Separation and detection of low levels of dopamine-derived alkaloids. Peak identities (amount injected): (1) THP (5×10^{-13} mole), (2) 2,3,10,11-THB (10^{-12} mole), (3) 7-OMe-THP (10^{-12} mole), (4) 2-OMe-THB (2×10^{-12} mole). Conditions: Vydac TP 401 SCX, 0.5 M $NH_4H_2PO_4$ + 5% dioxane, 0.5 ml/min, 750 psi, ambient temperature, chart 10 in/hr.

Mechanism of Retention:

The cation-exchange packing materials employed in this study are proprietary, and few details of their physical nature and manufacture are available. From the data presented, however, it appears that the retention power of these materials is due to hydrophobic interactions as well as to ion-exchange mechnaisms. In fact, these materials can be considered as reversed phase packings with ion-exchange capability, rather than solely as ion-exchangers. This viewpoint is useful in selecting conditions for a particular analysis. Thus, mobile phase ionic strength is varied to affect retention of hydrophilic compounds (low k') while addition of organic modifiers is employed to decrease retention of hydrophobic solutes (large k'). Figure 6 shows an example of the application of this procedure. The preliminary selection of conditions can be followed by adjustments in other parameters (e.g. column tempera-

ture, flow rate and further changes in mobile phase ionic strength and organic modifiers) to optimize resolution.

Conclusion:

Cation-exchange chromatography, especially when coupled with the highly sensitive electrochemical detector, is a very powerful technique for analysis of basic, highly-polar dopamine-derived alkaloids (29). The technique is particularly useful for mixtures of these closely related compounds. The term cation-exchange chromatography, however, may be considered a partial misnomer because hydrophobic interactions appear to contribute significantly to the retention properties of the packing materials.

ACKNOWLEDGMENTS

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29. For an example of the analysis of alkaloids in biological samples see Ref. 11.