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Note

Gas chromatographic method for separation of nine polyhydroxy alkaloids

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Polyhydroxy derivatives of pyrrolidine, piperidine and indolizidine alkaloids have recently been isolated from plants[1] and microorganisms[2–4]. A number of these compounds have been shown to be potent glycosidase inhibitors owing to their structural resemblance to monosaccharides[5,6].

We describe the separation of the trimethylsilyl derivatives of nine naturally occurring polyhydroxy alkaloids by gas–liquid chromatography (GLC).

EXPERIMENTAL

Chemicals

(2*R*,3*S*)-2-Hydroxymethyl-3-hydroxypyrrolidine (CYB-3) and (1*S*,6*S*,7*R*,-8*R*,8a*R*)-1,6,7,8-tetrahydroxyoctahydroindolizine (castanospermine) were isolated from *Castanospermum australe*[7,8]; (2*S*,3*R*,4*R*,5*S*)-2-carboxy-3,4,5-trihydroxypiperidine (BR-1) from *Baphia racemosa*[9]; 1,5-dideoxy-1,5-imino-D-glucitol (deoxynojirimycin; DNJ) from *Morus nigra*[10]; 1,2,5-trideoxy-1,5-imino-D-arabino-hexitol (fagomine; FAG) was produced by acid hydrolysis of its 4-O-glucoside from *Xanthocercis zambesiaca*[11]; (2*R*,3*R*,4*R*,5*R*)-2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) from *Lonchocarpus costaricensis*[10]; and (1*S*,2*R*,8*R*,8a*R*)-1,2,8-trihydroxyoctahydroindolizine (swainsonine) from *Swainsona canescens*[12]. Synthetic 1,5-dideoxy-1,5-imino-D-mannitol (DMJ) and 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB-1), known to occur naturally, were for this study synthesized and kindly donated by Drs. G. Legler and G. Kinast (DMJ)[13] and G. W. J. Fleet (D-AB-1)[6].

Derivatization procedures

Trimethylsilyl (TMS) derivatives of the polyhydroxy alkaloids were formed by the addition of 200 ml of Sigma Sil A (trimethylchlorosilane–hexamethyldisilazane–pyridine, 1:3:9) to 1 mg of each compound[14]. BR-1 was heated at 60°C for 1 h. All other compounds, including glucose, were heated to 50°C for 15 min. TMS derivatives were rapidly formed. All derivatives remain stable under ambient conditions over a 24-h period.

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Chromatography

GLC was carried out on a Pye-Unicam 104 gas chromatograph. Glass columns (5 ft. × 4 mm I.D.) were prepacked with Chromosorb W HP coated with stationary phases of 3% OV-1 or 3% OV-17 (Phase Separations). Flame ionization detector

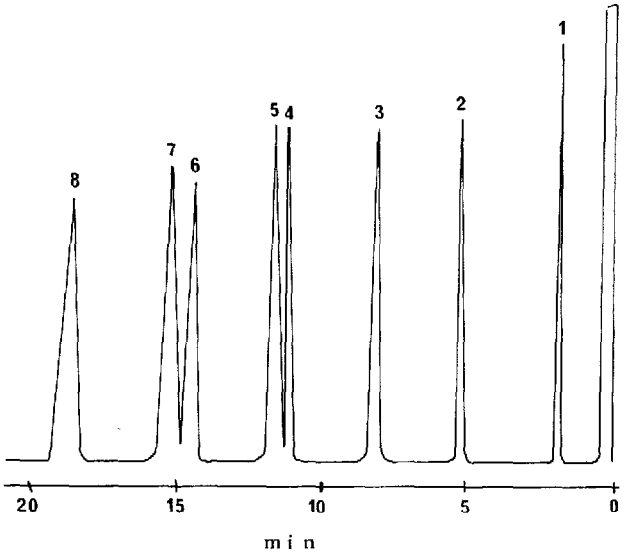

Fig. 1. Gas chromatogram of TMS derivatives of (1) CYB-3, (2) D-AB-1, (3) FAG, (4) DMDP, (5) DMJ, (6) glucose, (7) DNJ, (8) castanospermine. Column (5 ft. × 4 mm I.D.) coated with OV-1; carrier gas, nitrogen; column temperature programmed from 135°C (3 min) to 175°C (6 min) at 4°C/min.

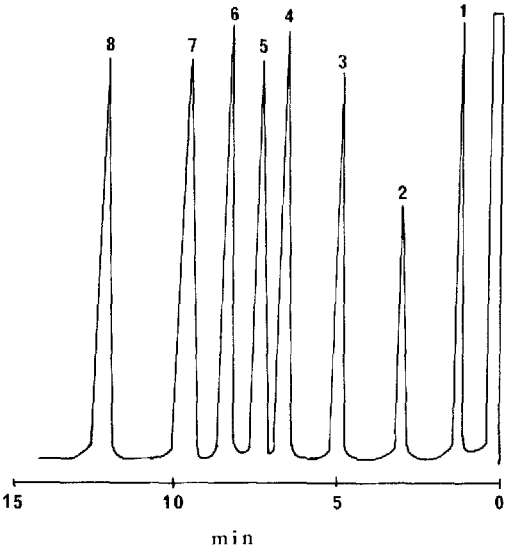

Fig. 2. Gas chromatogram of TMS derivatives of (1) CYB-3, (2) D-AB-1, (3) FAG, (4) DMDP, (5) swainsonine, (6) glucose, (7) DNJ, (8) castanospermine. Column (5 ft. × 4 mm I.D.) coated with OV-17; carrier gas nitrogen; column temperature programmed from 125°C (2 min) to 200°C (15 min) at 5°C/min.

TABLE I

RETENTION TIMES OF POLYHYDROXY ALKALOIDS ON OV-1 AND OV-17 COATED COLUMNS

OV-1 column (5 ft. × 4 mm I.D.) programmed from 135°C (3 min) to 175°C (6 min) at 4°C/min. OV-17 column (5 ft. × 4 mm I.D.) programmed from 125°C (2 min) to 200°C (15 min) at 5°C/min.

Compound	*Retention time (min)*	
	OV-1	*OV-17*
CYB-3	2.0	1.3
D-AB-1	5.2	3.2
FAG	7.9	5.3
DMDP	11.0	7.0
DMJ	11.2	7.0
Swainsonine	11.0	7.8
DNJ	14.5	10.1
BR-1	17.9	11.0
Castanospermine	17.9	12.9

oven temperature was 240°C and nitrogen carrier flow-rate 40 ml/min. The samples (0.1–1.0 μl) were injected directly onto the columns.

The following temperature programmes were used: OV-1 column 135°C for 2 min, rising at 4°C/min to 175°C and held for 6 min; OV-17 column, 125°C for 2 min, rising at 5°C/min to 200°C and held for 15 min.

RESULTS AND DISCUSSION

Fig. 1 illustrates the separation of the derivatives on OV-1 and Fig. 2 of that on OV-17. Table I lists the retention times of the compounds on each column. BR-1 and castanospermine have identical retention times on OV-1 as do swainsonine and DMDP. These compounds can, however, be separated on OV-17. DMDP and DMJ cannot be separated on OV-17.

The results obtained indicate that polyhydroxy alkaloids form stable TMS derivatives which can be successfully identified by judicious use of OV-1 and OV-17 silicone phases. The methods proposed can also be applied to the quantification of polyhydroxy alkaloids.

REFERENCES

1 L. E. Fellows, S. V. Evans, R. J. Nash and E. A. Bell, *ACS Symp. Ser.*, 296 (1986) 72.
2 M. J. Schneider, F. S. Ungemach, H. P. Broquist and T. M. Harris, *Tetrahedron*, 39 (1983) 29.
3 S. Murao and S. Miyata, *Agric. Biol. Chem.*, 44 (1980) 219.
4 D. D. Schmidt, W. Frommer, L. Müller and E. Truscheit, *Naturwissenschaften*, 66 (1979) 584.
5 U. Fuhrmann, E. Bause and H. Ploegh, *Biochim. Biophys. Acta*, 825 (1985) 95.
6 G. W. J. Fleet, S. J. Nicholas, P. W. Smith, S. V. Evans, L. E. Fellows and R. J. Nash, *Tetrahedron Lett.*, 26 (1985) 3127.
7 R. J. Nash, E. A. Bell, G. W. J. Fleet, R. H. Jones and J. M. Williams, *J. Chem. Soc., Chem. Commun.*, (1985) 738.
8 L. D. Hohenschutz, E. A. Bell, P. J. Jewess, D. P. Leworthy, R. J. Pryce, E. Arnold and J. Clardy, *Phytochemistry*, 20 (1981) 811.

9 K. S. Manning, D. G. Lynn, J. Shabanowitz, L. E. Fellows, M. Singh and B. D. Schrire, *J. Chem. Soc., Chem. Commun.*, (1985) 127.
10 S. V. Evans, L. E. Fellows, T. K. M. Shing and G. W. J. Fleet, *Phytochemistry*, 24 (1985) 1953.
11 S. V. Evans, A. R. Hayman, L. E. Fellows, T. K. M. Shing, A. E. Derome and G. W. J. Fleet, *Tetrahedron Lett.*, 26 (1985) 1465.
12 S. M. Colegate, P. R. Dorling and C. R. Huxtable, *Aust. J. Chem.*, 32 (1979) 2257.
13 U. Fuhrmann, E. Bause, G. Legler and H. Ploegh, *Nature (London)*, 307 (1984) 755.
14 C. C. Sweeley, R. Bentley, M. Makita and W. W. Wells, *J. Am. Chem. Soc.*, 85 (1963) 2497.