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Note

Separation of hydroxy and polyhydroxy derivatives of 2-carboxy and 2-hydroxymethyl piperidine and pyrrolidine by high-performance liquid chromatography

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Several hydroxy and polyhydroxy derivatives of 2-carboxy and 2-hydroxymethyl piperidine and pyrrolidine have been found in plants and microorganisms[1–4]. Certain of the 2-hydroxymethyl derivatives bear a structural resemblance to pyranose and furanose sugars with the ring oxygen replaced by nitrogen and have been shown to be glycosidase inhibitors[5,6]. As such they are of interest as research tools in many areas of biology and medicine, including AIDS (HIV) infectivity *in vitro*[7,8]. Some have anti-insect activity[6] and it is likely that they contribute to the chemical defence of the plants in which they occur.

The ecological role of 2-carboxy piperidine derivatives, also widespread in higher plants, in less fully understood. However, these compounds also exhibit anti-insect effects[9,10] and may be important in the chemical ecology of the plants in which they are found.

Many of these compounds frequently occur as mixtures (refs. 11 and 12, and observations in this laboratory). There is currently a need for a simple analytical method to facilitate the search for these potentially valuable compounds.

We describe here a simple, rapid separation of derivatives of pyrrolidine and piperidine by high-performance liquid chromatography (HPLC), as their 9-fluorenylmethyl chloroformate derivatives.

EXPERIMENTAL

Chemicals

2*S*-Carboxy-3*R*,4*R*,5*S*-trihydroxypiperidine (BR1), 1,5-dideoxy-1,5-imino-D-glucitol (1-deoxnojirimycin; DNJ), 1,5-dideoxy-1,5-imino-D-mannitol (1-deoxymannojirimycin; DMJ), 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP), 1,2,5-trideoxy-1,5-imino-D-arabino-hexitol (fagomine), 2*R*-hydroxymethyl-3*S*-hydroxypyrrolidine (CYB-3) and 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1) were obtained as previously described[13], as were 2*S*-carboxy-4**S**,5*S*-dihydroxypiperidine, 2*S*-carboxy-4*R*,5*S*-dihydroxypiperidine, 2*S*-carboxy-4*R*,5*R*-dihydroxypiperidine, 2*S*-

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carboxy-4*S*,5*R*-dihydroxypiperidine, 2*S*-carboxy-5*R*-hydroxypiperidine, 2*S*-carboxy-4*R*-hydroxypiperidine, 2*S*-carboxy-4*S*-hydroxypiperidine, and 2*S*-carboxy-4*R*-acetylaminopiperidine[11,14–16].

2*S*-Carboxy-5*S*-hydroxypiperidine and L-pipecolic acid were obtained from Sigma. All other chemicals were of analytical reagent grade, all solvents were HPLC-grade.

Derivatization procedure

This method was adapted from one reported for amino acids[17]. To 100 μl of aqueous solutions of the compounds (0.1 mg/ml) was added 100 μl of 200 m*M* aqueous sodium bicarbonate. After vigorous mixing, 200 μl of 5 m*M* 9-flourenylmethyl chloroformate (Sigma) in acetone was added and the mixture incubated for 30 min at 30°C. The mixture was extracted with ethyl acetate–hexane (20:80, v/v) and the upper phase discarded after phase separation.

Aliquots (10 μl) of the lower phase were injected directly into the HPLC system.

High-performance liquid chromatography

HPLC was carried out using a Varian Model 5020 liquid chromatograph (Varian; Walnut Creek, CA, U.S.A.) connected to a Spherisorb C_{18} column (5 μm ODS, 25 cm × 5 mm I.D.) (HPLC Technology; Macclesfield, U.K.) protected with a Vydac reversed-phase pre-column (5 cm × 5 mm I.D.). Detection was with a Varian UV-50 detector (absorption wavelength = 264 nm) connected to a Pharmacia (Uppsala, Sweden) dual-channel chart recorder. Separation was achieved using a linear gradient of 100% acetonitrile (buffer A) and 75 m*M* trisodium citrate, pH 4.2 (buffer B), rising from 25% buffer A at $t = 0$ min to 62.5% buffer A at $t = 30$ min, with a constant flow-rate of 1.8 ml/min at a column temperature of 32°C.

RESULTS AND DISCUSSION

The retention times obtained are listed in Tables I and II. This method has been used to particular advantage in the analysis of mixtures of piperidine and pyrrolidine glycosidase inhibiting alkaloids which are resolved only with difficulty[1]. It complements a previously published gas chromatographic separation[13], in particular

TABLE I

RETENTION TIMES OF 2-HYDROXYMETHYL DERIVATIVES OF PYRROLIDINE AND PIPERIDINE

Compound	*Retention time (min)*
1,5-Dideoxy-1,5-imino-D-mannitol	7.9
1,5-Dideoxy-1,5-imino-D-glucitol	8.5
2*R*,5*R*-Dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine	9.4
1,2,5-Trideoxy-1,5-imino-D-arabino-hexitol/ 1,4-dideoxy-1,4-imino-D-arabinitol	10.5
2*R*-Hydroxymethyl-3*S*-hydroxypyrrolidine	13.4

TABLE II

RETENTION TIMES OF 2-CARBOXY DERIVATIVES OF PIPERIDINE

Compound	*Retention time (min)*
2*S*-Carboxy-4*R*,5*S*-dihydroxypiperidine	6.0
2*S*-Carboxy-4*R*,5*R*-dihydroxypiperidine	6.1
2*S*-Carboxy-3*R*,4*R*,5*S*-trihydroxypiperidine	6.9
2*S*-Carboxy-4*S*,5*S*-dihydroxypiperidine	8.4
2*S*-Carboxy-4*S*,5*R*-dihydroxypiperidine	9.3
2*S*-Carboxy-5*S*-hydroxypiperidine	9.9
2*S*-Carboxy-5*R*-hydroxypiperidine	10.3
2*S*-Carboxy-4*R*-acetylaminopiperidine	11.0
2*S*-Carboxy-4*S*-hydroxypiperidine	11.5
2*S*-Carboxy-4*R*-hydroxypiperidine	13.7
L-Pipecolic acid	20.2

giving a more satisfactory separation of DMDP and DMJ which have been frequently found to occur together[18].

In addition, the method provides an improved analytical separation of the pipecolic acids which hitherto required a more complicated combination of chromatographic and electrophoretic techniques for identification[11,19]. All but two of the dihydroxypipecolic acids are resolved.

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REFERENCES

1 L. E. Fellows and G. W. J. Fleet, in G. H. Wagner and R. Cooper (Editors), *Natural Products Isolation*, Elsevier, Amsterdam, 1988, in press.
2 L. E. Fellows, *Pestic. Sci.*, 17 (1986) 602.
3 J. T. Romeo, in P. R. Cheeke (Editor), *Toxicants of Plant Origin, Vol. III, Proteins and Amino Acids*, CRC Press, Boca Raton, FL, in press.
4 S. Hunt, in G. C. Barrett (Editor), *Chemistry and Biochemistry of Amino Acids*, Chapman and Hall, London, 1985, Ch. 4, p. 55.
5 A. D. Elbein, *CRC Crit. Rev. Biochem.*, 16 (1984) 21.
6 L. E. Fellows, C. H. Doherty, J. M. Horn, G. C. Kite, R. J. Nash, J. T. Romeo, M. S. J. Simmonds and A. M. Scofield, in L. F. James, A. D. Elbein, R. J. Molyneux and C. D. Warren (Editors), *Swainsonine and Related Glycosidase Inhibitors Symposium, Logan, UT, August 10–14, 1987*, in preparation.
7 A. S. Tyms, E. M. Berrie, T. A. Ryder, R. J. Nash, M. P. Hegarty, D. L. Taylor, M. A. Mobberley, J. M. Davis, E. A. Bell, D. J. Jeffries, D. Taylor-Robinson and L. E. Fellows, *Lancet*, Oct. (1987) 1025.
8 R. A. Gruters, J. J. Neefjes, M. Tersmette, R. E. Y. de Goede, A. Tulp, H. G. Huisman, F. Niedema and H. L. Ploegh, *Nature (London)*, 330 (1987) 74.
9 J. T. Romeo, *Biochem. Syst. Ecol.*, 12 (1984) 293.
10 M. S. J. Simmonds and J. T. Romeo, personal communication.
11 J. T. Romeo, L. A. Swain and A. B. Bleecker, *Phytochemistry*, 22 (1983) 1615.

12 J. T. Romeo, *Ann. Mo. Bot. Gard.*, 73 (1986) 764.
13 R. J. Nash, W. S. Goldstein, S. V. Evans and L. E. Fellows, *J. Chromatogr.*, 366 (1986) 431.
14 A. B. Bleecker and J. T. Romeo, *Phytochemistry*, 20 (1981) 1845.
15 A. B. Bleecker and J. T. Romeo, *Phytochemistry*, 22 (1983) 1025.
16 M. Marlier, G. Dardenne and J. Casimir, *Phytochemistry*, 18 (1979) 479.
17 S. Einarsson, B. Josefsson and S. Lagerkvist, *J. Chromatogr.*, 282 (1983) 609.
18 S. V. Evans, L. E. Fellows, T. K. M. Shing and G. W. J. Fleet, *Phytochemistry*, 24 (1985) 1953.
19 A. B. Bleecker and J. T. Romeo, *Anal. Biochem.*, 121 (1982) 295.