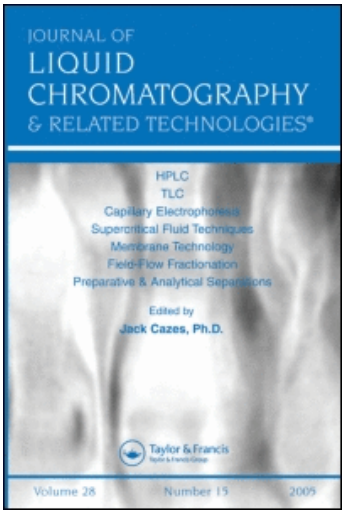

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Resolution of Synthetic ( )- and (-)-Epibatidine by Chiral High Performance Liquid Chromatography and Identification of the Natural Enantiomer

A. P. Watt[a]; H. M. Verrier[a]; D. O'Connor[a]
[a] Department of Medicinal Chemistry, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Harlow, Essex

To cite this Article Watt, A. P. , Verrier, H. M. and O'Connor, D.(1994) 'Resolution of Synthetic ( )- and (-)-Epibatidine by Chiral High Performance Liquid Chromatography and Identification of the Natural Enantiomer', Journal of Liquid Chromatography & Related Technologies, 17: 6, 1257 — 1264
To link to this Article: DOI: 10.1080/10826079408013760
URL: http://dx.doi.org/10.1080/10826079408013760

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(6), 1257-1264 (1994)

RESOLUTION OF SYNTHETIC (+)- AND (-)-EPIBATIDINE BY CHIRAL HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND IDENTIFICATION OF THE NATURAL ENANTIOMER

ALAN P. WATT, HUGH M. VERRIER, AND DESMOND O'CONNOR
Department of Medicinal Chemistry
Merck Sharp and Dohme Research Laboratories
Neuroscience Research Centre
Terlings Park, Eastwick Road
Harlow, Essex, CM20 2QR

ABSTRACT

Methods are described for the separation of the enantiomers (+)- and (-)-epibatidine and (+)- and (-)-N-acetylepibatidine by high performance liquid chromatography using Chiral-AGP and Chiralcel OD chiral stationary phases respectively. Comparison of synthetic material with authentic epibatidine.HCl using retention times and UV spectra allows the unambiguous assignment of the natural product as the (+)- isomer.

INTRODUCTION

Epibatidine, *exo*-2-(6-chloro-3-pyridyl)-7-azabicyclo[2.2.1]heptane (Figure 1), is an alkaloid isolated from the skin of the Ecuadoran poison frog, *Epipedobates*

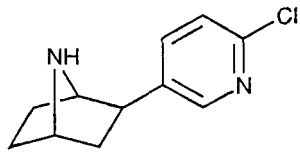

Figure 1. Structure of epibatidine [*exo*-2-(6-chloro-3-pyridyl)-7-azabicyclo[2.2.1]heptane].

tricolor which has been reported by Daly *et al* (1) to possess potent non-opioid analgesic properties. Since the first reported isolation and structural determination, interest has been generated in synthesising sufficient material to confirm the reported biological properties (2,3). In our laboratories the synthesis of (+)- and (-)-epibatidine was carried out since the enantiomer corresponding to the natural product was unknown (3). In this study the development of a chiral high performance liquid chromatographic separation of (+)- and (-)-epibatidine is described. Using this methodology, authentic epibatidine extracted from natural sources could be compared on the basis of retention time and UV spectra. Hence, by identifying which peak corresponds to the (+)- isomer and which to the (-)-isomer, it is possible to assign the enantiomer corresponding to the natural product.

As a further confirmation, separation of (+)- and (-)-N-acetylepibatidine was undertaken. By comparison with authentic material in an analogous fashion and also by spiking of the racemate with authentic N-acetylated material, the enantiomer corresponding to natural epibatidine was unambiguously assigned.

EXPERIMENTAL

Materials

(+)- and (-)-Epibatidine and (+)- and (-)-N-acetylepibatidine were synthesised as previously described (3) with identity and purity confirmed by NMR, MS, HPLC and elemental analysis. Absolute ethanol was obtained from Hayman Limited (Witham, UK). Acetonitrile and hexane were of HPLC grade and

all other reagents were of Analytical Grade, obtained from Fisons (Loughborough, UK). Water was of Millipore MilliQ grade and all solvents were filtered using a glass Millipore system with a 0.45μm filter.

Instrumentation

An HP1090M series high performance liquid chromatograph was used (Hewlett Packard, Avondale, USA). The system comprises an autoinjector, consisting of a Rheodyne 7010 injection valve fitted with a 250 μl loop, an autosampler and a DR-5 solvent delivery system. Detection was by UV at 215nm using a built-in linear photodiode array detector and data was processed using a 79994A PASCAL workstation.

Resolution of (+)- and (-)-epibatidine

Epibatidine was separated on a Chiral-AGP column (Chromtech AB, Sweden) (100 mm x 4.0 mm i.d., 5μm) using a mobile phase of 3% acetonitrile in 10mM dipotassium hydrogen phosphate with 5mM pentane sulphonic acid (PSA), adjusted to pH 7.4 with *ortho*-phosphoric acid. The flow rate was 1ml min^{-1} and all analyses were performed at ambient temperature. Samples were dissolved at approximately 1.0mg ml^{-1} in ethanol and 2μl injection were made corresponding to ca. 1μg of each enantiomer on the column.

Resolution of (+)- and (-)-N-acetylepibatidine

N-acetylepibatidine was separated on a Chiralcel OD column (Diacel Chemical Industries Ltd., Japan) (250 mm x 4.6 mm, 10μm) using a mobile phase of 5% ethanol in hexane (pre-mixed). The flow rate was 2ml min^{-1} and all analyses were performed at ambient temperature. Samples were dissolved at approximately 1.5mg ml^{-1} in ethanol and 5μl injection were made corresponding to ca. 3.5μg of each enantiomer on the column.

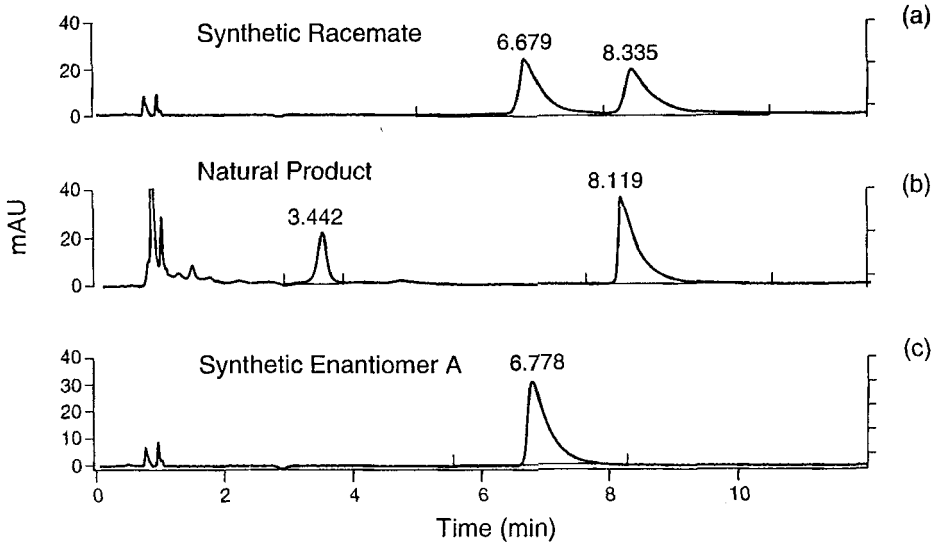

Figure 2. Chromatographic chiral separation using Chiral AGP of underivatised epibatidine corresponding to (a) Synthetic racemic material (b) the natural product and (c) the opposite enantiomer to the natural product.

RESULTS AND DISCUSSION

Initial attempts to resolve epibatidine focused on the application of cyclodextrin and derivatised cyclodextrin bonded CSPs to include the azabicycle within the cyclodextrin cavity, but the basicity of the secondary nitrogen of epibatidine appeared to preclude inclusion hence no resolution was observed. Reports that N-BOC-amino acids, where the basicity of the nitrogen has been removed, could be resolved on hydroxypropyl substituted β-cyclodextrin CSPs (5) led to attempts to resolve the N-BOC-derivative of epibatidine in an analogous fashion, but only a partial separation was ever attained. However, it was found that the N-BOC-derivative could easily be separated on a Chiralcel OD stationary phase (3) leading to the discovery that the N-acetyl derivative behaves in an analogous fashion.

Resolution of epibatidine was attempted using a Chiral-AGP column as development work had demonstrated that the presence of lipophilic substituents and the availability of hydrogen bonding groups were important for chiral recognition (6). Figure 2a shows that baseline resolution of racemic epibatidine

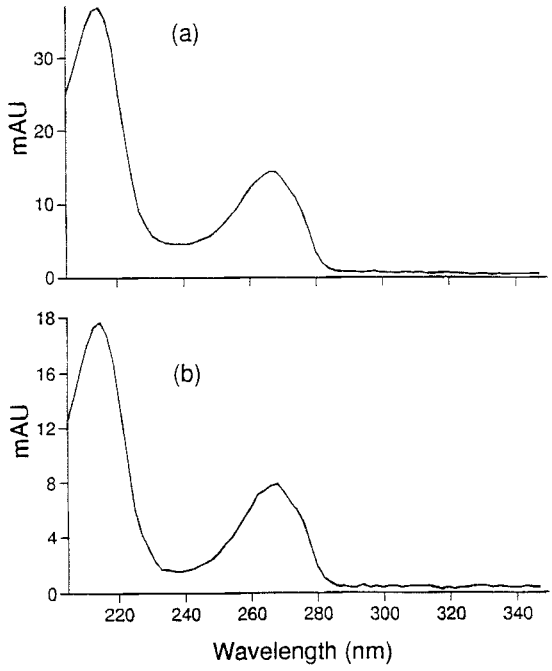

Figure 3. UV spectra obtained from on-line diode array detector of (a) enantiomer B from racemic mixture and (b) natural product.

could be achieved (t_1 = 6.68 mins, t_2= 8.34 mins; α = 1.28, R_S = 2.32). The use of the lipophilic ion-pair reagent pentane sulphonic acid in this system is worth noting. It was found that resolution could not be achieved using simple aqueous buffer systems as insufficient retention could be obtained but by addition of PSA, a significant increase in both retention and separation were gained. This allowed addition of an organic modifier which resulted in more efficient peaks thereby improving resolution. By injection of the racemate then injection of both the (+)- and (-)-enantiomers individually, the peaks are assigned as the first eluting enantiomer (-)-epibatidine.HCl and the second as (+)-epibatidine.HCl (4).

Figure 2b shows the injection of ca. 500ng of authentic epibatidine onto an identical HPLC system. The sample was known not to be pure as is evident from the chromatogram. Correlation of retention times from the racemate (t_2= 8.34 mins, epibatidine = 8.12 mins) strongly suggests that the natural product is the

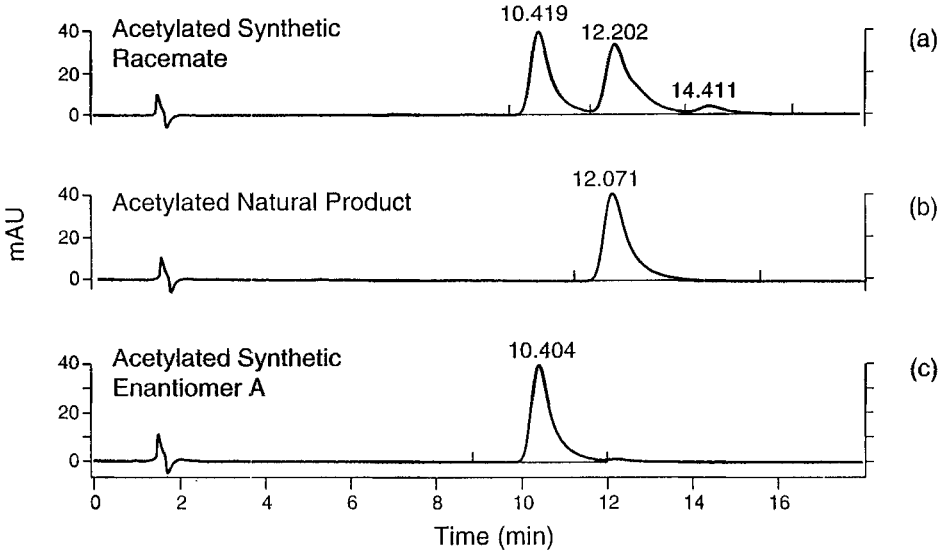

Figure 4. Chromatographic chiral separation using Chiralcel OD of N-acetyl epibatidine corresponding to (a) Synthetic racemic material (b) the natural product and (c) the opposite enantiomer to the natural product.

second eluting enantiomer which possesses the (+)- rotation as its HCl salt. Figure 2c shows that injection of the pure first eluting enantiomer indeed gives a peak at 6.78 minutes confirming that the natural product is the second eluting. Data obtained using the photodiode array detector showed that the UV spectra of the second eluting peak of the racemate (Figure 3a) exactly matched the UV spectrum of epibatidine (Figure 3b) under identical chromatographic conditions.

The resolution of N-acetylepibatidine is shown in figure 4a. The loss of the basic nitrogen through acetylation precludes separation on the Chiral-AGP, but methodology had already been devised for the resolution of N-BOC-epibatidine using a Chiralcel OD stationary phase (3). This was applied and optimised for the N-acetyl derivative (t_1 = 10.42 mins, t_2= 12.20 mins; α = 1.20, R_S = 1.59). Assignment of the peak elution order proved to be as for epibatidine i.e. the (-)-isomer elutes before the (+)-isomer. In an analogous experiment to that described above, natural N-acetylepibatidine is shown to correspond to the second eluting

peak (t_2 = 12.20 mins, N-acetylepibatidine = 12.07 mins; Figure 4b) demonstrating that this enantiomer corresponds to the natural product. Further confirmation is provided by injection of the pure (-)- enantiomer which is shown to elute at 10.40 minutes (Figure 4c) and by spiking approximately equal amounts of racemic N-acetylepibatidine with authentic material and chromatographing to give an increase in the peak area of the second eluting peak over the first.

CONCLUSIONS

Comparison of authentic epibatidine.HCl with synthetic (+)- and (-)- isomers shows the natural product to be the (+)- isomer. Efforts are continuing to elucidate the absolute configuration of this molecule by X-ray crystallography.

ACKNOWLEDGEMENTS

The authors would like to thank Dr S. Fletcher for his advice and comments on this manuscript, Dr. M. Chambers and Ms. S. Hobbs for the provision of (+)- and (-)-epibatidine and (+)- and (-)-N-acetylepibatidine and Dr. J. Daly for the kind provision of authentic material.

REFERENCES

1. T.F. Spande, H.M. Garraffo, M.W. Edwards, H.J.C. Yeh, L. Pannell and J.W. Daly, *J. Amer. Chem Soc.*, 1992, **114**, 3475-78.

2. C.A. Broka, *Tetrahedron Lett.*, 1993, **34**, 3251-3254.

3. S.R. Fletcher, R. Baker, M.S. Chambers, S.C. Hobbs and P.J. Mitchell, *J. Chem. Soc., Chem. Comm.*, 1993, 1216-1218.

4. (+)-Epibatidine.HCl, $[\alpha]_D^{24}$ +34.7° (c=0.36, MeOH); t_r = 8.34 mins on Chiral- AGP system; t_r = 12.20 mins as N-acetyl derivative on Chiralcel OD system.

   (-)-Epibatidine.HCl, $[\alpha]_D^{24}$ -33.7° (c=0.16, MeOH); t_r = 6.68 mins on Chiral- AGP system; t_r = 10.42 mins as N-acetyl derivative on Chiralcel OD system.

5. Stalcup, A.M., Chang, S., Armstrong, D.W., Pitha, J. *J. Chromatogr.*, **513**, 181-194 (1990).

6. Hermansson, J., *Trends in Analytical Chemistry*, Vol. 8, No. 7, (1989).

Received: September 31, 1993
Accepted: October 19, 1993