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# Direct optical resolution of vesamicol and a series of benzovesamicol analogues by high-performance liquid chromatography

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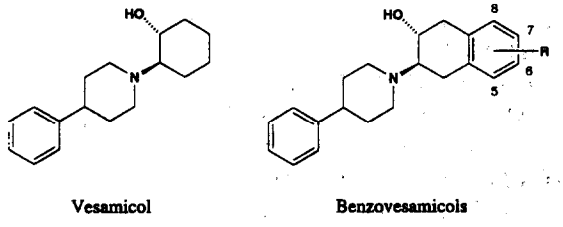
**Abstract**

The direct optical resolution of the vesicular acetylcholine uptake inhibitors vesamicol and benzovesamicol and nine benzovesamicol analogues were performed by HPLC on a commercially available cellulose tris(3,5-dimethylphenyl carbamate) chiral stationary phase. Separation of each enantiomeric pair was optimized with respect to solvent strength and flow-rate, using mobile phase mixtures of hexane–2-propanol–diethylamine. The method has been successfully applied to the analysis of the optical purity of benzovesamicol intermediates and products, including (−)-5-[$^{123}$I]iodobenzovesamicol which is currently undergoing clinical evaluation as a tracer for mapping central cholinergic neurons, and the purification of both antipodes of (±)-7-[$^{125}$I]iodobenzovesamicol.

## 1. Introduction

The availability of radiolabeled tracers to visualize cholinergic function *in vivo* could add significantly to our knowledge of parasympathetic neuronal dysfunction and the relationship between cholinergic neuronal degeneration and the onset of Alzheimer's disease. It could also potentially provide a means to monitor the efficacy of future neuron-sparing drugs in the treatment or prevention of this disease.

Vesamicol (AH 5183), (−)-*trans*-2-(4-phenylpiperidino)cyclohexanol (Fig. 1), is a potent, non-competitive inhibitor of acetylcholine uptake into the vesicles of cholinergic neurons [1–4]. Structural variants of vesamicol, especially those based on benzovesamicol [*trans*-2-hydroxy-3-(4-phenylpiperidino)tetralin], exhibit even higher affinity and selectivity for the vesicular binding site [4]. We have previously reported that 5-iodobenzovesamicol (5-IBVM), labeled with iodine-125, is a highly specific *in vivo*, presynaptic marker for cholinergic neurons in the brain [5]. It exhibits an avid and prolonged uptake into cholinergic-rich regions in mouse brain that is highly stereospecific for the (−)-




Vesamicol Benzovesamicols

Fig. 1. Structures of vesamicol and benzovesamicol analogues. R = H, I, $NH_2$, NH-Boc, $NHCH_3$, OH, $OCH_3$.

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isomer. Labeled with iodine-123, (−)-5-IBVM has been successfully used to obtain *in vivo* tomographic maps of the cholinergic nerve pattern in human brain [6,7]. Two additional tracers, (−)-5-[$^{11}$C]methylaminobenzovesamicol ([$^{11}$C]MABV) and (−)-5-[$^{18}$F]fluoroethoxybenzovesamicol ([$^{18}$F]FEOBV), are undergoing preclinical trials in our laboratories for use in positron emission tomography (PET). The [$^{11}$C]MABV tracer is synthesized from either (−)-5-aminobenzovesamicol [8] or (−)-5-N-Boc-aminobenzovesamicol [9]; [$^{18}$F]FEOBV is synthesized from (−)-5-hydroxybenzovesamicol [10].

The presence of the radiolabeled (+)-enantiomers would complicate the tomographic images and pharmacokinetic results obtained with these tracers. This could obscure differences between normal and diseased tissues. Therefore, it is essential that methods be developed both to resolve the synthetic precursors for the tracers of interest and to ensure that the final radiolabeled product is enantiomerically pure.

We previously described the use of the Chiracel OD column for the resolution of (±)-5-IBVM [5]. In support of our efforts to develop additional medical tracers for the non-invasive mapping of cholinergic nerves, this method has been extended to the resolution of additional benzovesamicol analogues. This paper describes the direct resolution, by normal-phase chiral HPLC, of the stereoisomers of (±)-benzovesamicol (BVM), (±)-5-aminobenzovesamicol (5-ABVM), (±)-5-N-methylaminobenzovesamicol (MABVM), (±)-5-N-Boc-aminobenzovesamicol (5-N-Boc-ABVM), (±)-5-iodobenzovesamicol (5-IBVM), (±)-6-iodobenzovesamicol (6-IBVM), (±)-7-iodobenzovesamicol (7-IBVM), (±)-8-iodobenzovesamicol (8-IBVM), (±)-5-hydroxybenzovesamicol (5-HOBVM) and (±)-5-methoxybenzovesamicol (5-MOBVM), as well as (±)-vesamicol (VM) itself. It also describes the application of the methods to the analysis of (−)-5-[$^{123}$I]-IBVM and the purification of the (−) and (+) antipodes of 7-[$^{125}$I]IBVM, whose (+) antipode has been found to be a highly selective marker for sigma binding sites in the brain [11].

## 2. Experimental

### 2.1. Reagents and chemicals

(±)-VM, (±)-BVM and (±)-5-ABVM were synthesized by the method of Rogers *et al.* [4]. The following compounds were synthesized by methods which we have described elsewhere: (±)-5-IBVM [5,12], (±)-6-IBVM [12] (±)-7-IBVM [12], (±)-8-IBVM [12], (±)-5-N-MABVM [8], (±)-5-N-Boc-ABVM [8], (±)-5-HOBVM [9] and (±)-5-MOBVM [13]. The enantiomers of 5-IBVM, 7-IBVM, 5-HOBVM and 5-ABVM were resolved by either preparative TLC or flash chromatography of their diastereomeric (*S*)-(−)-α-trifluoromethylphenylacetyl (MTPA) derivatives, followed by cleavage of the MTPA group [5,9,10,14]. (−)-BVM was synthesized from (−)-5-IBVM by reductive cleavage of the carbon–iodine bond with 10% palladium on activated carbon and hydrogen at atmospheric pressure. (−)-VM was obtained from Research Biochemicals (Natick, MA, USA). The racemic, positional isomers of IBVM were radiolabeled with iodine-125 by the solid-phase exchange method, which we have previously described [12,15,16].

Chromatographic solvents of HPLC grade were obtained from either EM Science (Gibbstown, NJ, USA) or Mallinckrodt Specialty Chemicals (Paris, KY, USA). Diethylamine of analytical-reagent grade was obtained from Fisher Scientific (Fair Lawn, NJ, USA) and was used without further purification. 1,3,5-Tri-*tert.*-butylbenzene was purchased from Aldrich (Milwaukee, WI, USA).

### 2.2. Apparatus and HPLC conditions

A Perkin-Elmer Model 241 automatic polarimeter was used to determine the identity of the optical isomers in ethanol solvent. The HPLC system consisted of a Waters Model 510 HPLC pump with U6K injection valve, and Model 486 UV–Vis detector (Millipore, Milford, MA, USA) Data were recorded on either a Spectra-Physics Model SP4400 ChromJet dual-channel integrator (San Jose, CA, USA) or

Table 1
Chromatographic conditions for analytical resolution of vesamicol and benzovesamicol analogues

| Compound | *n*-Hexane–2-propanol (v/v) | Flow-rate (ml/min) |
|---|---|---|
| VM | 99:1 | 1.0 |
| BVM | 95:5 | 1.0 |
| 5-IBVM | 95:5 | 1.0 |
| 6-IBVM | 95:5 | 1.0 |
| 7-IBVM | 95:5 | 1.0 |
| 8-IBVM | 95:5 | 1.0 |
| 5-HOBVM | 85:15 | 1.5 |
| 5-MOBVM | 99:1 | 1.5 |
| 5-ABVM | 50:50 | 1.0 |
| 5-N-Boc-ABVM | 95:5 | 1.0 |
| 5-MABVM | 90:10 | 1.0 |

Millenium 2010 Chromatography Manager (Millipore). Where required, a radioactivity flow detector, consisting of a Bicron (Newbury, OH, USA) Frisk-Tech rate-meter/ monitor, fitted with a G1LE probe and a 3-$\mu$l PTFE flow-cell, was attached to the outlet of the UV–Vis detector to monitor for radioactivity eluting from the column. The column was a Chiracel OD (Daicel; 10 $\mu$m particle size, 250 × 4.6 mm I.D.) from J.T. Baker (Phillipsburg, NJ, USA). The mobile phase consisted of a mixture of *n*-hexane, 2-propanol and diethylamine as described in Table 1. Flow-rates are also listed in Table 1. Column effluent was monitored at 254 nm. The column void volume ($t_0$) was determined for each mobile phase mixture and flow-rate combination by injecting 10 $\mu$g of 1,3,5-tri-*tert*.-butylbenzene in 10 $\mu$l of *n*-hexane–2-propanol (98:2, v/v). Resolutions ($R_s$), separation factors ($\alpha$) and capacity factors ($k'$) were calculated as previously described [17].

## 3. Results and discussion

With the current large selection of commercially available chiral stationary phases (CSPs), choosing the proper CSP can be difficult. No one CSP is capable of resolving all classes of compounds. Fortunately, vesamicol and the benzovesamicols possess a hydroxyl group at the chiral center, much like the $\beta$-adrenergic blockers atenolol, propanolol, pindolol and metoprolol. Very efficient direct resolution of these $\beta$-blockers has been obtained on a tris(3,5-dimethylphenylcarbamate) cellulose coated silica column (Chiracel OD) [18–20]. Therefore, the Chiracel OD column was selected for this work.

As recommended by the column manufacturer, the mobile phase was limited to mixtures of *n*-hexane and 2-propanol. The mobile phase composition was optimized for each racemic pair by varying the concentration of 2-propanol until the desired capacity factors were obtained (Table 1); a small amount of diethylamine (0.1%, v/v) was added to reduce potential solute tailing, especially for 5-ABVM and its derivatives. The flow-rates were then varied to optimize column efficiency but no attempt was made to optimize the temperature, despite the observation that the efficiency of this type of chiral column can be increased by operating at an elevated temperature (40°C) [21].

The Chiracel OD column resolved all eleven racemic compounds that were tested; the results are summarized in Table 2. Resolution of the lead compounds in this series, VM and BVM, is illustrated in Fig. 2. The enantiomers of both compounds were well resolved with a resolution of 1.5 or larger. In Fig. 3, the resolution of the enantiomers of the four positional isomers of IBVM is shown. For the purposes of comparison, the experimental conditions (Table 1) were optimized for the resolution of the antipodes of (±)-8-IBVM and were then applied to the other three isomers. These compounds exhibited the most efficient resolution on this column, and the enantiomers of 5-IBVM and 7-IBVM were the most readily resolved of all the compounds tested. The resolution of the enantiomers of 8-IBVM was larger than 1.0 while that of the other three isomers was larger than 1.5. The electron-withdrawing effect of the large halogen group may be responsible for the greater resolution exhibited by this series of compounds. This hypothesis will be tested on future BVM derivatives bearing a variety of large, electron-withdrawing groups.

The enantiomers of compounds that bear an

Table 2
Capacity factors ($k'_1$ and $k'_2$), separation factors ($\alpha$), resolution factors ($R_s$) and retention times ($t_{R1}$ and $t_{R2}$) in the chiral resolution of vesamicol and benzovesamicol analogues

| Compound | $k'_1$ | $k'_2$ | $\alpha$ | $R_s$ | $t_{R1}$ (min)[a] | $t_{R2}$ (min)[a] |
|---|---|---|---|---|---|---|
| VM | 2.13 | 2.40 | 1.13 | 1.49 | 9.41 (−) | 10.23 (+) |
| BVM | 2.30 | 2.67 | 1.16 | 1.64 | 9.93 (−) | 11.03 (+) |
| 5-IBVM | 1.79 | 2.82 | 1.58 | 2.56 | 8.22 (−) | 11.29 (+) |
| 6-IBVM | 3.33 | 4.45 | 1.34 | 1.70 | 12.72 | 16.02 |
| 7-IBVM | 4.59 | 7.12 | 1.55 | 2.46 | 16.44 (−) | 23.86 (+) |
| 8-IBVM | 3.28 | 4.01 | 1.22 | 1.12 | 12.58 | 14.72 |
| 5-HOBVM | 2.55 | 3.11 | 1.22 | 0.78 | 6.94 (−) | 8.04 (+) |
| 5-MOBVM | 2.19 | 2.59 | 1.18 | 0.97 | 9.27 (−) | 10.44 (+) |
| 5-ABVM | 1.83 | 2.24 | 1.22 | 1.38 | 8.25 (+) | 9.44 (−) |
| 5-N-Boc-ABVM | 4.39 | 5.64 | 1.28 | 1.43 | 15.69 (−) | 19.33 (+) |
| 5-MABVM | 3.47 | 3.98 | 1.15 | 1.02 | 13.00 (−) | 14.50 (+) |

Column: 250 × 4.6 mm I.D. Chiracel OD; eluent mixture and flow-rate as described in Table 1.
[a] When known, the identity of the drug enantiomer is given.

electron-donating group, the amino and hydroxyl group in particular, were also resolved by the Chiracel OD column. Fig. 4 illustrates the separation the enantiomers of (±)-5-ABVM, as well as the enantiomers of its N-methyl (5-MABVM) and N-Boc (5-N-Boc-ABVM) derivatives. The enantiomers of all three compounds exhibited resolutions larger than 1.0. The separation of the enantiomers of the 5-hydroxyl derivative of BVM (5-HOBVM) and its methoxy derivative (5-MOBVM) are shown in Fig. 5. The 5-MOBVM enantiomers were separated with a resolution of almost 1.0. While being the least resolved of all of the eleven vesamicols tested, the separation of the enantiomers of 5-HOBVM, with a resolution of 0.78, was sufficient for monitoring the purification of the (−)-enantiomer as its MTPA derivative as described above. Since (−)-5-HOBVM is only an intermediate in the synthesis of (−)-5-MOBVM, additional proof of enantiomeric purity is provided by analysis of its O-methylated product (−)-5-MOBVM. It is apparent that the enantiomers of compounds that possess either electron-withdrawing or electron-donating groups can be resolved on this column.

The elution order of the enantiomers was

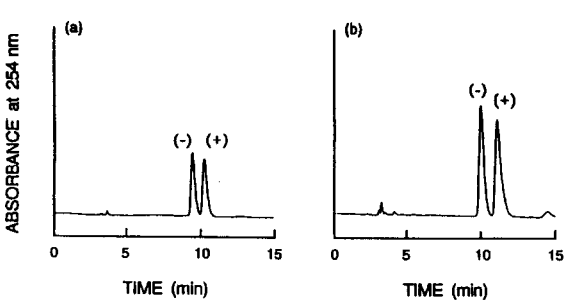


Fig. 2. Resolution of (a) vesamicol and (b) benzovesamicol enantiomers. Chromatographic conditions as described in Table 1.

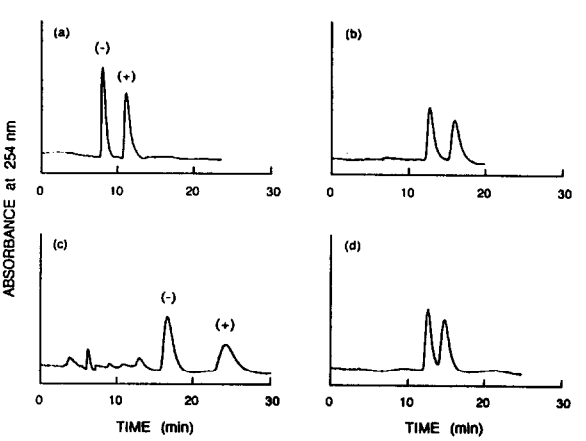


Fig. 3. Resolution of the enantiomers of positional isomers of IBVM: (a) 5-IBVM, (b) 6-IBVM, (c) 7-IBVM and (d) 8-IBVM. Chromatographic conditions as described in Table 1.

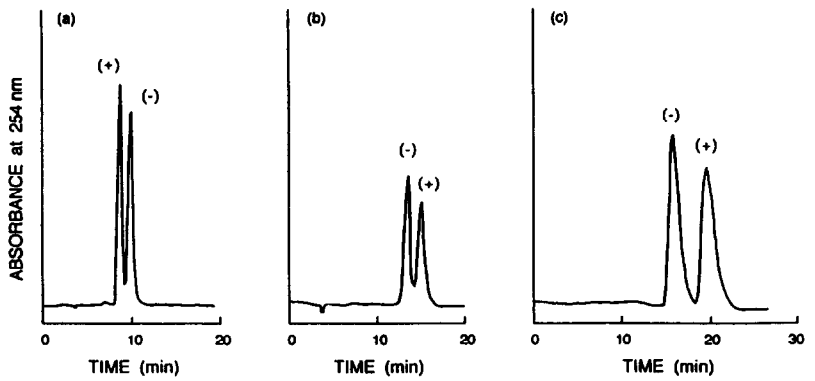


Fig. 4. Resolution of the enantiomers of (a) 5-ABVM, (b) 5-MABVM and (c) 5-N-Boc-ABVM. Chromatographic conditions as described in Table 1.

determined for nine of the eleven racemic pairs. In all cases but one, the (−)-enantiomer, which may be assigned the *R,R* configuration, eluted first. The single exception was (±)-5-ABVM, whose (+)-(*S,S*)-enantiomer eluted first. However, after derivatization of the aniline group with either a methyl group (5-MABVM) or Boc group (5-N-Boc-ABVM), the elution order reverts to the (−)-enantiomer eluting first. The mechanism responsible for this phenomenon has yet to be determined, but appears not to be due to the presence of an electron-donating group since the elution order for the antipodes of 5-HOBVM were not inverted.

This method was applied both to the direct analysis of enantiomeric purity and to the chiral purification of radiolabeled derivatives of benzovesamicol. Fig. 6 illustrates the application of this method to the analysis of optical purity of iodine-123 labeled (−)-5-IBVM, which had been synthesized via iododestannylation of the respective tributyltin precursor [22]. The 5-tributyltin-BVM precursor was itself synthesized from (−)-5-IBVM that was >98% optically pure. The radiolabeled product was identified as the (−)-enantiomer and determined to also be >98% optically pure, thus preserving the optical integrity and purity of the synthetic precursors. Fig. 7 illustrates the application of this method to

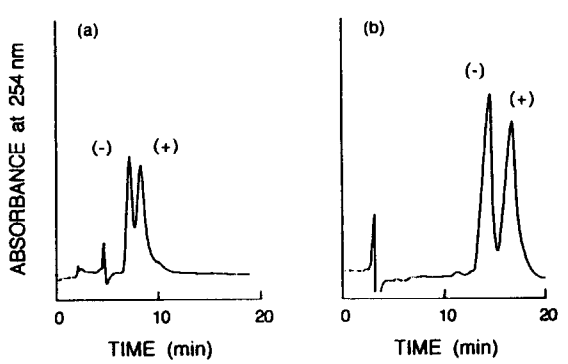


Fig. 5. Resolution of the enantiomers of (a) 5-HOBVM and (b) 5-MOBVM. Chromatographic conditions as described in Table 1.

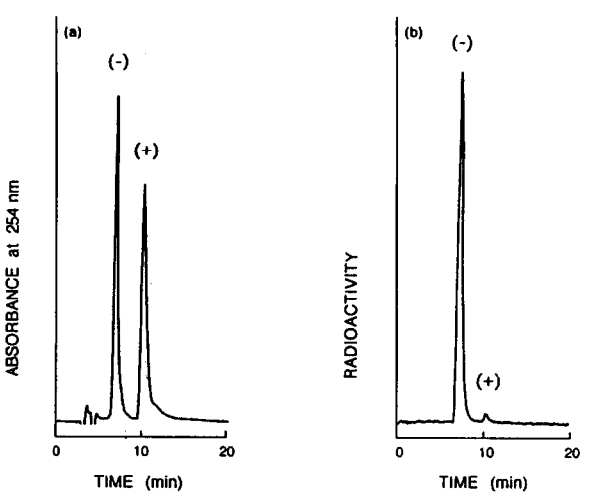


Fig. 6. Test of the optical purity of (−)-5-[$^{123}$I]iodobenzovesamicol spiked with carrier (±)-5-IBVM: (a) UV trace of racemic 5-IBVM carrier and (b) radioactive trace of the iodine-123 labeled product which preserves the optical purity of the precursor tin compound (optical purity >98%). Chromatographic conditions as described in Table 1.

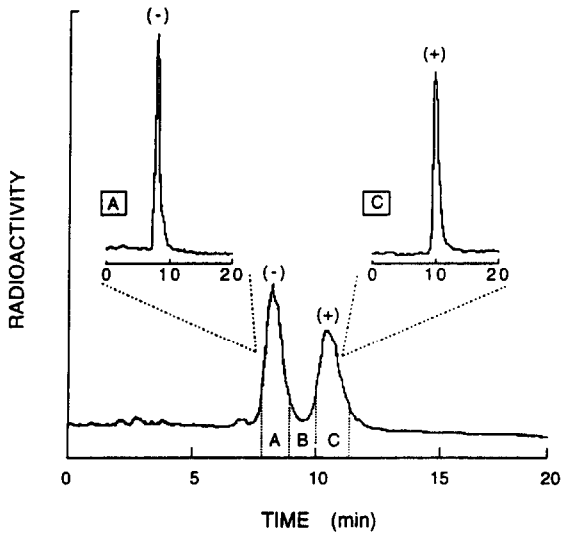


Fig. 7. Chiral purification of the ( − ) and ( + ) enantiomers of racemic 7-[$^{125}$I]iodobenzovesamicol. Insets: Test of enantiomeric purity of the purified antipodes. Mobile phase: *n*-hexane–2-propanol (90:10, v/v); flow-rate, 1.5 ml/min.

the purification of the ( − ) and ( + ) antipodes of iodine-125 labeled ( ± )-7-IBVM (specific activity > 140 Ci/mmol) and the analysis of the optical purity of the isolated enantiomers, which is shown in the two insets. To improve recovery of the later eluting peak, the chromatographic conditions, described under the figure, were modified from those described in Table 1. Three fraction cuts, heart fractions of each enantiomer with an intermediate fraction containing a mixture of both, were made on the basis of the response levels of the radioactivity detector and are indicated in the figure. Reinjection of an aliquot of each of the isolated peaks onto the same column showed that both enantiomers were > 99% optically pure (see insets).

In summary, the chiral HPLC technique described makes possible the rapid, direct optical resolution of all eleven vesamicols tested. This approach is especially effective in resolving ( − )-5-IBVM and ( + )-7-IBVM, which in radioiodinated form are selective *in vivo* markers for the vesamicol and sigma binding sites, respectively. The methodology is not only applicable to the routine determination of the chiral purity of these two radiotracers, but because of the associated low mass levels, it can also be used to purify multi-mCi amounts of chirally pure tracer from racemic mixtures for research purposes.

## 4. Acknowledgements

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