Acknowledgment. We thank Dr. M. Shiro and his staff for the single-crystal X-ray analyses and N. Ishizuka for his helpful advice on the computer graphics. We also thank H. Shintaku and Y. Takahara for assistance with the binding assays.

Registry No. **1a**, 104679-66-5; **1b**, 104679-67-6; **1c**, 104679-75-6; **1d**, 119945-92-5; **1e**, 119945-93-6; **1f**, 119945-94-7; **1g**, 119945-95-8; **2a**, 104679-79-0; **2b**, 104635-74-7; **2c**, 119945-96-9; **2d**, 119945-97-0; **3b**, 104635-65-6; **3c**, 104635-66-7; **3d**, 119945-98-1; **3e**, 119945-99-2; **3f**, 104635-69-0; **3g**, 119946-00-8; **4**, 13720-94-0; **5d**, 119946-01-9; **5e**, 119946-02-0; **5f**, 119946-03-1; **5g**, 119946-04-2; **6d**, 119946-09-7; **6e**, 119946-10-0; **6f**, 119946-11-1; **6g**, 119946-12-2; **7d**, 119946-18-8; **7e**, 119946-19-9; **7f**, 119946-20-2; **7g**, 119946-21-3; **8c**, 119946-05-3; **8d**, 119946-06-4; **9d**, 119970-50-2; **9e**, 119946-07-5; **9g**, 119946-08-6; **10c**, 119946-13-3; **10d**, 119946-14-4; **11d**, 119946-15-5; **11e**, 119946-16-6; **11g**, 119946-17-7; **12c**, 119946-22-4; **12d**, 119946-23-5; **13d**, 119946-24-6; **13e**, 119946-25-7; **13g**, 119946-26-8.

Synthesis, in Vitro Acetylcholine-Storage-Blocking Activities, and Biological Properties of Derivatives and Analogues of *trans*-2-(4-Phenylpiperidino)cyclohexanol (Vesamicol)

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Eighty-four analogues and derivatives of the acetylcholine-storage-blocking drug *trans*-2-(4-phenylpiperidino)-cyclohexanol (vesamicol) were synthesized, and their potencies were evaluated with the acetylcholine active-transport assay utilizing purified synaptic vesicles from *Torpedo* electric organ. The parent drug exhibits enantioselectivity, with (-)-vesamicol being 25-fold more potent than (+)-vesamicol. The atomic structure and absolute configuration of (+)-vesamicol were determined by X-ray crystallography. The absolute configuration of (-)-vesamicol is 1*R*,2*R*. Structure–activity evidence indicates that (-)-vesamicol does not act as an acetylcholine analogue. Alterations to all three rings can have large effects on potency. Unexpectedly, analogues locking the alcohol and ammonium groups trans-diequatorial or trans-diaxial both exhibit good potency. A potent benzovesamicol family has been discovered that is suitable for facile elaboration of the sort useful in affinity labeling and affinity chromatography applications. A good correlation was found between potencies as assessed by the acetylcholine transport assay and LD_{50} values in mouse.

The biochemical and physiological mechanisms of acetylcholine (ACh) storage by nerve terminal synaptic vesicles are being studied by many groups.[1] In addition to a proton-pumping ATPase and ACh transporter, a receptor for the compound *trans*-2-(4-phenylpiperidino)cyclohexanol is present.[2] When the receptor is occupied by drug, noncompetitive inhibition of ACh storage occurs.[3] The drug (formerly called AH5183 but now called vesamicol[1,4]) has been of particular value to the study of ACh metabolism in intact nerve terminal preparations (reviewed in ref 1). While it is entirely satisfactory for biochemical studies utilizing highly purified *Torpedo* electric organ synaptic vesicles, vesamicol exhibits some nonspecificity in intact preparations. For example, before neuromuscular block sets in as a result of the ACh storage block, the amplitude of muscle contraction in response to indirect stimulation actually increases.[5] This has been shown in α-bungarotoxin-blocked preparations to be nonneural in origin,[6] and thus to be unrelated to the primary site of action.

Because vesamicol is proving to be an important tool in both biochemical and physiological studies of the cholinergic nerve terminal, we sought to understand its mode of action and to develop its potential further through a structure–activity study. No structure–activity work has been published previously on this drug. We sought to determine whether the pharmacological potency of the drug exhibits enantioselectivity and whether the drug is mimicking ACh to inhibit uptake. To exploit vesamicol for receptor identification and purification in the future, we need to know which parts of the drug are critical to its potency and where we can attach steric bulk without compromising the potency. Also, we would like to increase the drug potency and specificity in order to facilitate physiological studies on and anatomical mapping[7] of cholinergic nerve terminals in mammalian preparations. Thus, we report here the synthesis of a number of new vesamicol derivatives and analogues. Their potencies as inhibitors of ACh active transport were assessed in the purified *Torpedo* electric organ synaptic vesicle assay and, for some of the compounds, in vivo toxicities also were determined. The X-ray crystallographic structure of (+)-vesamicol also was determined.

Chemistry

Most of the new analogues were synthesized by addition of secondary amines (usually substituted piperidines or piperazines) to epoxides under S_N2 conditions followed in some cases by further derivatization. Thus, except where noted, the amino alcohol substituents in the products are in the trans relationship. A number of required specialized epoxides[8] were generous gifts from Dr. Bruce F. Rickborn (Department of Chemistry, University of California, Santa Barbara, CA 93106) and were used to make compounds

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52–55, 58, and 65–68. No attempt was made to optimize yields except of intermediates in some multistep pathways.

Compounds 1–36, 82, and 83 were synthesized with cyclohexene oxide. These compounds were produced from precursors having variations in the phenyl and/or piperidinyl rings of the parent, and they hold the cyclohexanol ring constant. Compounds 40, 43, 49, 51–58, 63, 65, 66, and 69 were synthesized by condensing 4-phenylpiperidine with the appropriate epoxide (or sulfide), and thus they explore the consequences of structural variations in the cyclohexanol ring of the parent while holding the rest of the structure constant. Compounds 67, 68, and 70–75 were prepared by the condensation of various amines with epoxides derived from derivatives of tetralin or decalin. Compound 50 was obtained by reduction of the enamine, *cis*-vesamicol by reduction of the amino ketone (48), and compounds 59 and 60 by reduction of the keto amide. Compounds 37–39, 41, 42, 44–47, 61, 62, and 76–81 were prepared by derivatization of the appropriate precursors.

Biochemistry

ACh active transport assays at a fixed time of uptake were carried out with highly purified *Torpedo californica* electric organ synaptic vesicles and [^{3}H]ACh as reported.[3] Hyperbolic titration curves were fit to inhibition data by nonlinear regression analysis to determine apparent inhibition constants, IC_{50} values, which are listed in Table I. Lower IC_{50} values indicate a more potent drug. More than 160 drug titrations were performed with potent or pivotal drugs being titrated several times. Some of the analogues were studied as iodide salts, and it was considered possible that the iodide ion might contribute to the observed inhibition. However, potassium iodide had no effect on ACh active transport up to 10 mM. All drugs that have been tested multiple times with the same preparation of vesicles show good reproducibility of potency. However, with different preparations of vesicles, repetitive testing shows some variability in potency, and the potency is even dependent upon the vesicle concentration used in the assay.[9] This indicates that inherent variability in the properties of the vesicle preparations affects the results, and these effects currently are under study. To minimize the biochemical variability, the vesicle concentration used to assay the drugs in this study generally was about 0.2 mg of protein/mL. On the basis of our experience, differences in IC_{50} values by a factor greater than 2 are significant. Because of this biochemical variability, the IC_{50} values are given here to only one significant figure without confidence limits even though the actual titrations provided data of higher precision.

Pharmacology

Test compound was injected intraperitoneally (0.10 mL/10 g body weight) into five male Swiss Webster mice (15–20 g) for each of at least four dose levels. Solvent composition was normal saline or a mixture of normal saline and phospate-buffered saline. For some drugs EtOH was used in solubilization to a final concentration of no greater than 2.5%. The animals were observed continuously for at least 1 h postinjection and then intermittently for the remainder of the day of injection and the two following days. A control group was injected with solvent only and similarly observed. Data were analyzed with Pharm/PCS-Version 4.[10]

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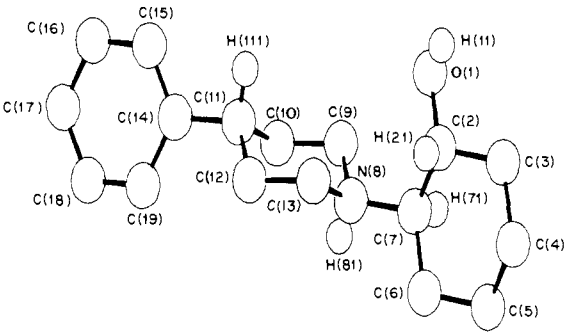

Figure 1. Atomic structure of (−)-vesamicol which was derived by inversion of the experimentally determined coordinates of the hydrogen (*S,S*)-di-*p*-toluoyl-D-tartrate salt of (+)-vesamicol. For clarity, only the cation structure is shown and only five of the hydrogen atoms.

Results and Discussion

Enantioselectivity of the Vesamicol Receptor. Since a specific receptor for vesamicol is thought to exist, the drug action should exhibit enantioselectivity. The enantiomers of vesamicol were resolved by fractional crystallization of diasteriomeric salts which proceeded readily in high yield. The hydrogen di-*p*-toluoyltartrate salts were converted to the hydrochlorides to obtain optical rotation measurements and to determine IC_{50} values uncomplicated by the ditoluoyltartrate anion. The (−)-enantiomer of vesamicol was obtained from the hydrogen (−)-di-*p*-toluoyl-L-tartrate. The specific rotations were −22.8° and +22.2° for the (−)- and (+)-enantiomer hydrochlorides, respectively.

Both enantiomers were assayed for the ability to inhibit active transport of [^{3}H]ACh. They exhibited typical hyperbolic titration curves. As listed in Table I the (−)-enantiomer had an IC_{50} value of 20 nM with a typical vesicle preparation. The (+)-enantiomer exhibited an IC_{50} value of 500 nM, or 25-fold less potent than the (−)-enantiomer, and it has been shown to displace (−)-[^{3}H]vesamicol from vesicle membranes.[11] The 25-fold difference in potency observed here for the vesamicol enantiomers should be viewed as a minimum difference since a small amount of the more active enantiomer as a contaminant in the less active enantiomer could greatly affect the accuracy of the apparent IC_{50} value. Thus, inhibition is quite sensitive to the drug configuration, which suggests that vesamicol interacts with a stereoselective receptor. Nonspecific interaction of vesamicol with the membrane or simple neutralization of the transmembrane pH gradient is not likely to provide a significant contribution to the ACh active transport inhibition activity.

The unsaturated analogue of vesamicol, compound 16, also was resolved into its enantiomeric components in a manner entirely analogous to that used for vesamicol. The (−)-enantiomer was shown to be more active. This material was smoothly reduced to (−)-vesamicol by catalytic hydrogenation over 10% Pd/C. This provided a route to highly tritiated (−)-vesamicol.

X-ray Crystallographic Structure and Absolute Configuration of Vesamicol Enantiomers. The absolute configuration of (+)-vesamicol was determined by X-ray diffraction measurements carried out on the (+)-di-*p*-toluoyl-D-tartrate salt. It was determined to have the 1*S*,2*S* absolute configuration. The atomic structure of the pharmacologically potent 1*R*,2*R* (−)-enantiomer, which was derived from the crystal structure of the (+)-enantiomer,

(11) Kaufman, R.; Parsons, S. M. Unpublished observation.

is shown in Figure 1. These results confirm our earlier assignment of absolute configuration which was based on consideration of optical activities of model *trans*-2-aminocyclohexanols.[12-15] The most pertinent conformational parameters for (-)-vesamicol are given in Table II. Both the piperidinium and the cyclohexanol rings exist in the chair conformation with diequatorial substituents. From the dihedral angles which describe the spatial relationship of the phenyl ring to the piperidinyl ring (+18.7°) and the cyclohexanol ring to the piperidinyl ring (-169.5°) it is apparent that the phenyl and cyclohexyl rings are nearly coplanar with each other and perpendicular to the plane of the piperidinyl ring defined by the methylene carbons, C(9)-C(10)-C(12)-C(13). Another feature of interest is the absence of a hydrogen bond between the ammonium proton and the alcohol. In the crystal, the ammonium proton is hydrogen bonded to the carboxylate of the anion, while the alcohol proton is hydrogen bonded to the other carboxyl group of the same anion.

Is Vesamicol an Analogue of Acetylcholine? The chemical structure of vesamicol includes a core resembling desmethylcholine. One obvious possibility for the drug action is that it is an analogue of ACh which binds to the ACh recognition site in the synaptic vesicle membrane, thus inhibiting transporter function. Such a role would be analogous to the action of reserpine on catecholamine storage. If this were its mechanism of action, analogues of vesamicol which more closely resemble ACh are expected to have a greater binding affinity for the transporter active site. We have tested this possibility by synthesizing and characterizing 11 analogues of vesamicol which more closely resemble ACh. In Table I these analogues are numbers **37-47**.

Briefly, it is apparent that changes which make the drug more closely resemble ACh lower the potency. Methylation (**37**) of vesamicol causes a 25-fold decrease in potency, acetylation (**38**) a 50-fold decrease, and both methylation and acetylation (**39**) a 125-fold decrease. Removal of two or four of the methylene groups from the cyclohexanol ring (compounds **40** and **43**) leads to decreases of 7- or 250-fold, respectively. It is pertinent to note here that compound **43** is indeed a vesamicol analogue since in work to be reported elsewhere we have shown that it displaces [^{3}H]-vesamicol from its specific binding site in vesicles. Similarly to the vesamicol series, methylation or acetylation of compound **40** lowers potency by 7- and 3-fold, respectively (**41** and **42**). Finally, the analogue which mimics the ACh structure best (**43**) also has been studied as methylated and acetylated derivatives (compounds **44-47**). Here we see that the modifications are not deleterious, but neither do they increase potency significantly. This behavior should be contrasted to that of choline where acetylation promotes a 100-fold increase in transporter binding affinity.[16]

In addition to the structure-activity data presented above, there are kinetic data which bear on the question of the vesamicol binding locus. Initial velocity, steady-state inhibition data indicate that vesamicol is a noncompetitive inhibitor of transport with respect to ACh.[3] Noncompetitive inhibition is not consistent with binding of the drug to the transporter ACh recognition site on the outside of the synaptic vesicle since this would give competitive inhibition. It is consistent with allosteric binding or binding to the transporter active site on the inside of the vesicle membrane. Since vesamicol is a tertiary amine, the small proportion which is uncharged at neutral pH (about 0.1%) could mediate nonspecific permeation through the membrane to reach an internal receptor. However, the absence of a significant effect from methylation of compound **43**, which should make product **44** less membrane permeant, suggests that vesicle membrane permeation is not required for drug action. This observation, in conjunction with the kinetic data, leads to the conclusion that drug binding is not to the ACh transporter ACh recognition site on either side of the membrane and therefore that vesamicol is not likely an analogue of ACh. This deduction is supported by results obtained with analogue **78** discussed below.

Additional Structure-Activity Relationships. A. Alterations to the Phenyl Ring. The most fundamental and profound alteration of the phenyl ring is to remove it completely. In doing so (**15**) drug potency is lowered by 3 orders of magnitude. However, compound **1** shows that a saturated ring serves equally well, and even another piperidinocyclohexanol (**11**) is moderately tolerated, especially considering that there is a second positive charge on the additional piperidinyl nitrogen. It is also possible that the charge on the second piperidinyl ring promotes a favorable interaction with a distinct binding site not occupied by vesamicol. The conclusion is that a variety of hydrophobic rings might be placed in the 4-position of the piperidinyl ring with a resulting potency similar to that of vesamicol. In addition, if compounds **3** and **4** are compared with vesamicol and compounds **12** and **13** with compound **11**, then it appears from the corresponding IC_{50} values that there are at least two favorable binding interactions possible for a hydrophobic ring with the putative receptor. That is, while one or two methylene groups as spacers between the piperidinyl and phenyl (or other ring) is quite unfavorable, a third methylene group restores potency.

From an examination of the various analogues which contain single or double substitutions in the phenyl ring of vesamicol, it is apparent that all modifications to date decrease drug potency. This diminuition ranges from a factor of 2.5 (for *p*-chloro or *p*-nitro, **2** and **5**) to 500 (for *p*-acetamido, **10**). One might also attempt to draw conclusions by comparing the various substituted phenylpiperazine analogues with compound **17** as the reference structure, but this could be misleading. For example, by comparing compound **27** with **17**, one might conclude that placing a methyl group in the ortho position of the phenyl ring of vesamicol increases potency by a factor of 3. However, it first should be noticed that two changes in the piperidinyl ring which serve to alter the dihedral angle with the phenyl ring lower the drug potency, namely, the styryl analogue (**16**) by a factor of 5 and the piperazinyl analogue itself (**17**) by a factor of 7. Thus, it is highly likely that the addition of the methyl group to compound **17** to give **27** merely forces the phenyl ring to rotate back into a more favorable conformation for receptor binding and regain some of the lost potency caused by orbital overlap with the nitrogen.[17] It is encouraging to our future endeavors,

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Table I. Structures and Biochemical Activities of Vesamicol Analogues and Other Related Compounds[a]

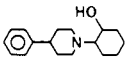
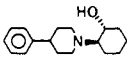
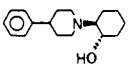
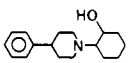
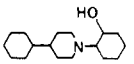
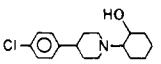
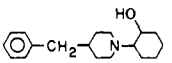
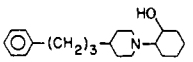
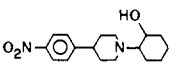
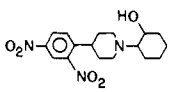
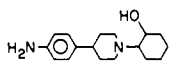
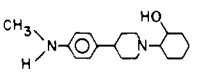
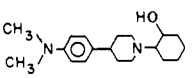
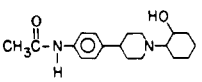
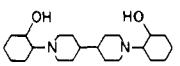
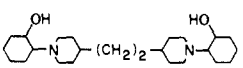
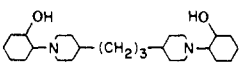
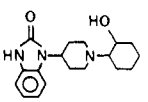
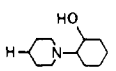
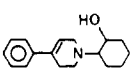
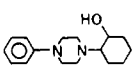
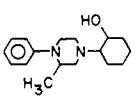
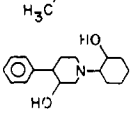
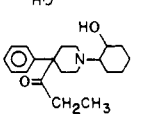
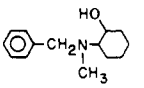
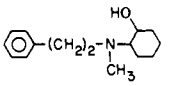
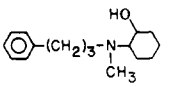
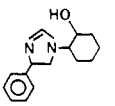
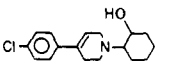
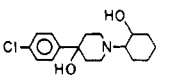
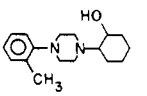
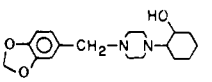
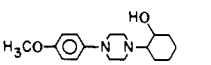
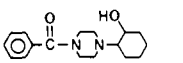
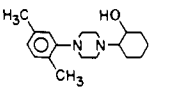
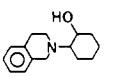
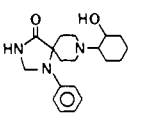
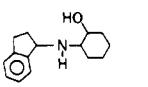
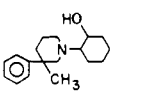
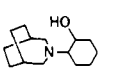
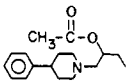
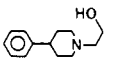
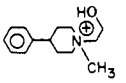
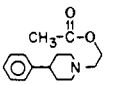
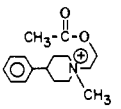
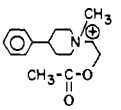
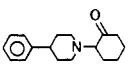
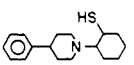
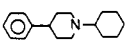
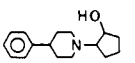
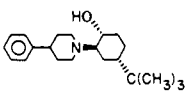
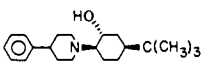
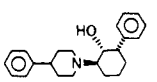
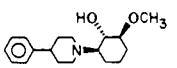
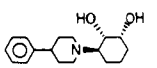
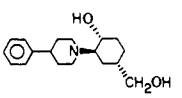
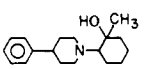
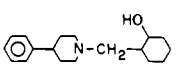
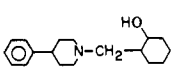
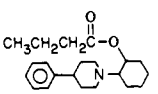
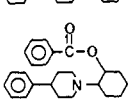
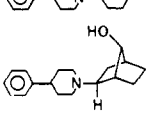
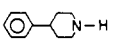
compound designation	structure	IC_{50}, nM
(±)-trans		40
R,R[b]		20
S,S[b]		500
(±)-cis		3000
1		40
2		100
3		300
4		70
5		100
6		300
7		500
8		400
9		300
10		20000
11		200
12		≥1000
13		200
14		1000
15		≥40000
16		200
17		300
18		300
19 (trans)		200
20		50
21		10000
22		20000
23		2000
24		40000
25		200
26		500
27		100
28		300
29		300
30		10000
31		100
32		5000
33		1000
34		3000
35		4000
36		20000
37 (*E,Z*)		≥1000
38		2000
39[c] (*Z*)		5000
40		300
41 (*E,Z*)		2000

Table I (Continued)

compound designation	structure	IC_{50}, nM
42		800
43		10000
44 (*E*,*Z*)		8000
45		9000
46 (*Z*)		6000
47 (*E*)		4000
48		40000
49		4000
50		1000
51		2000
52[d]		20
53[d]		90
54[d]		600
55[d,e]		2000
56[d]		100
57[d]		800
58		6000
59 (cis)		2000
60 (trans)		1000
61		3000
62		3000
63		10000
64		90000

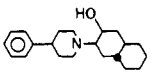
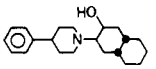
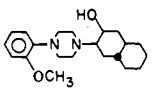
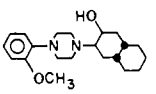
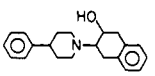
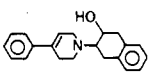
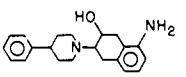
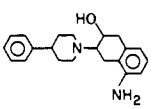
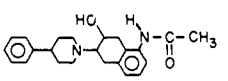
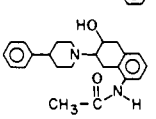
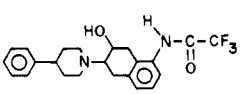
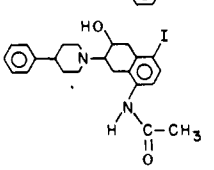
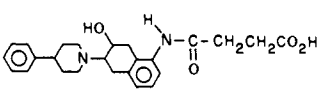
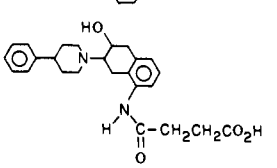
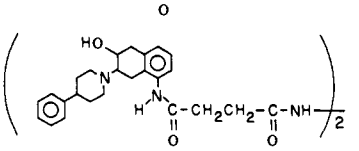
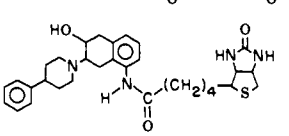
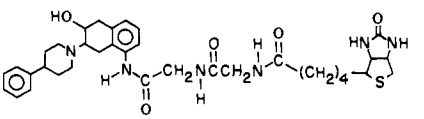
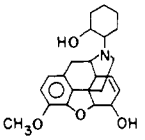
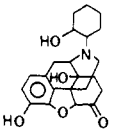
compound designation	structure	IC_{50}, nM
65		10
66		60
67		200
68		300
69		50
70		100
71		300
72		100
73		≥2000
74		50
75		2000
76		300
77[f]		500
78[f]		50
79		1000
80		100
81		200

Table I (Continued)

compound designation	structure	IC_{50}, nM	compound designation	structure	IC_{50}, nM
82		80000	83		>100000

[a] Unless otherwise indicated, all compounds were *trans*-2-amino alcohols and were tested as racemic mixtures. [b] Due to a printing error, these compounds were shown with interchanged IC_{50} values in Parsons et al. *Ann. N.Y. Acad. Sci.* **1987**, *483*, 220. [c] See footnote 26. [d] Only a single enantiomer is shown for stereochemical clarity. The compounds were tested as racemates. [e] In earlier publications (Parsons et al. in footnote *b* and ref 21) this compound was shown with the methoxy group in the 3-position. Subsequent high-field NMR experiments have shown that it is in the 6-position. [f] In an earlier publication (Parsons et al. *Synaptic Transmitters and Receptors*; Tucek, S., Ed.; Academia: Publishing House of the Czechoslovak Academy of Sciences, Praha, 1987) the assignment of the position of the anilino amino group was based on a steric analysis of the epoxide ring opening. Subsequent NMR experiments comparing compounds **71** and **72** to **69** (see footnote 28) revealed that assignment to be wrong.

Table II. Selected Conformational Parameters for (−)-Vesamicol[a]

atoms (1–2–3–4)	dihedral angle,[b] deg
H(111)–C(11)–C(14)–C(15)	+18.7
H(81)–N(8)–C(7)–C(2)	−169.5
N(8)–C(7)–C(2)–O(1)	−57.4

[a] These angles were obtained after coordinate inversion of the (+)-vesamicol structure actually solved. [b] Dihedral angles are positive for anticlockwise rotation from atom 1 when viewed from atom 3 to atom 2.

however, that some modifications involving the phenyl ring have a virtually neutral influence on drug potency (compare **28** and **29** to **17**, and **31** to **27**).

B. Alterations to the Piperidinyl Ring. As pointed out above, two changes in the piperidinyl ring which would alter the dihedral angle with the phenyl ring, namely, replacement of the benzylic carbon with a nitrogen (**17**) or changing the hybridization at that site to sp^2 (**16**), lower the drug potency by factors of 7 and 5, respectively. Certainly, replacing the piperidinyl ring with acyclic methylene spacers as in compounds **21–23** proved to be disastrous to the potency. From compound **35** it appears that moving the phenyl ring to the 3-position of the piperidinyl ring lowers potency by about 100-fold since the methyl group in that position probably is tolerated (compare compounds **17** and **18**). Lastly, if compound **26** is compared to **2**, then it appears that the addition of a hydroxyl group at the 4-position is unfavorable by a factor of about 5, while the addition of a propionyl group at the same position (**20**) has virtually no effect on potency. The rationalization of this observation is not obvious, but could involve lower solvation requirements for the ketone relative to the alcohol or additional hydrophobic interactions of the ketone with the receptor.

C. Alterations to the Cyclohexanol Ring. In the discussion above, the point was made that the enantiomeric relationship of the alcohol to the amine group is very important to drug potency. What else can we deduce about the alcohol group? In order to answer this question it is informative to compare each vesamicol enantiomer to deoxyvesamicol (compound **50**), wherein the hydroxyl group is absent. Such a comparison demonstrates that in the pharmacologically more potent *R,R* enantiomer the hydroxyl group provides a factor of 50 greater potency, while in the less active enantiomer only a factor of 2. On the other hand, location of the hydroxyl in a cis relationship to the amine [(±)-*cis*-vesamicol] lowers potency by a factor of 3 compared to deoxyvesamicol and by a factor of 75 compared to vesamicol. Thus, a *cis*-hydroxyl group exerts a slight negative effect on potency in the racemate. Some uncertainty in the detailed analysis exists since, possibly, one enantiomer is of equal potency to deoxyvesamicol and the other enantiomer is very much less potent.

The function of the hydroxyl group in ACh transport inhibition cannot be served adequately by a sulfhydryl (**49**), nor by a variety of esters (compounds **38**, **61**, and **62**) even when they greatly increase the hydrophobicity of the drug. A further comparison of deoxyvesamicol with phenylpiperidine (**64**), one of the worst drugs encountered, shows that the cyclohexyl ring itself asserts a nearly 100-fold increase in drug potency. Replacement of the cyclohexanol ring with cyclohexanone (**48**) dramatically lowers potency nearly to that of phenylpiperidine, confirming that the cyclohexyl ring makes critical van der Waals contacts with the receptor. Moreover, replacement of the cyclohexanol ring with cyclopentanol (**51**) decreases potency by 50-fold. Rather than suggesting the importance of a precise cyclohexyl ring conformation (the acyclic analogue **40**, devoid of two methylene groups, has only a 8-fold drop in potency, and analogue **69**, containing unsaturation distal to the amino alcohol group, is equipotent), this last result could mean that the van der Waals contour of the receptor discriminates against a 5-membered ring and certain other fixed conformations.

In solution, greater than 99% of the vesamicol molecules will exist in the cyclohexyl chair conformaton possessing diequatorial alcohol and ammonium substituents,[18] which would suggest that it is this conformation which likely is bound by the receptor. A number of analogues were synthesized in order to explore this possibility. Compounds **52** and **53** contain *cis*-4- or *trans*-5-*tert*-butyl substituents, respectively, which will overwhelmingly prefer an equatorial position on the cyclohexyl ring, thus placing the alcohol and ammonium substituents in the diaxial conformation. Unexpectedly, both analogues are effective drugs, suggesting that the diaxial conformation of vesamicol can act on the receptor. This conclusion is supported by compound **65**. This *trans*-decalin analogue (that is, a trans-ring junction) strongly favors the diaxial conforma-

(18) Hirsch, J. A. *Top. Stereochem.* **1967**, *1*, 199. Values herein were used to calculate a free energy difference of 3.3 kcal/mol favoring the diequatorial conformation. This simple calculation assumes no electrostatic interaction between the two substituents. However, on the basis of molecular mechanics calculations (see ref 19), we conclude that any such interaction would only further favor the diequatorial conformation. In solution (D_2O/DCl) at 25 °C, the 300-MHz ^1H NMR spectrum of vesamicol reveals that the methine proton α to the alcohol [H(21) of Figure 1] is in an axial conformation with coupling constants of 4.5 Hz (t) and 10.2 Hz (d). Thus the alcohol and ammonium substituents in vesamicol are diequatorial in solution as well as the solid state.

tion for the alcohol and ammonium substituents,[19] and it is our most potent drug. Nevertheless, other comparisons show that access to the diaxial conformation is not a necessary factor in drug potency. Compound **66** contains the *cis*-decalin ring junction, which will strongly promote the diequatorial conformation of the alcohol and ammonium substituents,[19] and yet it is nearly as potent as vesamicol and within a factor of 6 in potency of the *trans*-decalin analogue. Compounds **67** and **68**, another pair of *cis*- and *trans*-decalin derivatives, show an even lower potency differential. Thus, it appears that either diequatorial or diaxial conformations can act on the receptor with similar IC_{50} values. These perplexing observations may suggest that the correlation of structure and activity is not a function simply of binding.

Not all *trans*-2-(4-phenylpiperidino)cyclohexanols investigated were potent drugs. Compounds **54**, **55**, **57**, and **58** contain substituents in the 1-, *cis*- and *trans*-6-, or *cis*-4-positions in the cyclohexanol ring which reduce potency substantially. This possibly is due to adverse steric interactions with the binding site. On the other hand, the moderate potency of 6-hydroxyvesamicol (**56**) reinforces the conclusion reached above that appropriately positioned hydroxyl groups are tolerated. Also, since the 6-hydroxyl group of **56** is cis to the 1-hydroxyl, it should be possible to destroy the vesamicol receptor binding potency of this analogue by using agents such as periodate or borate.

As discussed above, substitution of hydrophobic groups at positions 4 or 5 is well tolerated, and it was realized that a benzo substituent here might present the best opportunity for useful structural diversity in the vesamicol family of drugs. Thus, compound **69** was synthesized and found to be nearly equipotent with vesamicol. Because of the opportunities for structural variations, additional compounds derived from benzovesamicol were synthesized (**70–81**). A most significant advance in this subgroup has been achieved through use of anilino derivative **72**. Note that the substituted benzovesamicol series based on **72** is uniformly more potent than the series based on the isomeric **71**, suggesting that the edge of the benzo ring syn with the hydroxyl group points toward the receptor surface. This conclusion is supported by the observation above that bulky substituents in the 6-position both cis and trans to the hydroxyl group (**54** and **55**, respectively) lower drug potency markedly. Starting with **72** we synthesized the zwitterionic (at the pH of our assay conditions) analogue, compound **78**. It is very unlikely that the ammonium/carboxylate form of **78** which exists at pH 7.8 could permeate nonspecifically through the vesicle membrane. Since this analogue has a potency nearly equal to that of vesamicol and displaces bound [^{3}H]vesamicol,[20] it can be concluded that the vesamicol receptor resides on the outside of the vesicle membrane.

Table III. Compounds Tested in Vivo[a]

compound	LD_{50},[b] μmol/kg	IC_{50}, nM
(±)-vesamicol	16 (10–26)	40
(−)-vesamicol	12 (7.5–18)	20
(+)-vesamicol	>190	500
3	>180	300
25	>340	200
37	>180	>1000
40	300[c]	300
51	>200	2000
52	5.7 (3.1–10)	20
55	240[c]	2000
58	>180	6000
65	2.0 (1.3–3.0)	10
67	6.2 (5.0–7.8)	200
68	12 (8.4–17)	300
69	1.2 (0.81–2.8)	50
74	1.7 (0.52–5.5)	50

[a] Tested in mouse ip. [b] 95% confidence limits are given in parentheses. [c] Limited data set did not allow statistical analysis.

The next three analogues to be considered take further advantage of the ability to attach a variety of substituents to benzovesamicol through the anilino nitrogen. Specifically, we have been successful in synthesizing two biotinylated derivatives (compounds **80** and **81**) as well as a symmetric dimer (**79**). As can be seen from Table I, the simple biotinylated derivative is only slightly less potent than vesamicol. However, we have shown that binding of analogue **80** or **81** to the vesamicol receptor (**80** does displace [^{3}H]vesamicol[21]) and avidin is mutually exclusive. This is readily explained as being due to steric hindrance between the vesamicol receptor and avidin, which binds biotin in a 9 Å deep pocket.[22] Hence, even for analogue **81**, the vesamicol end of the molecule would only just reach the surface of avidin. This steric interaction should allow study of the dissociation of the biotinylated vesamicol analogue from receptor by utilizing avidin occlusion. The substantially lower potency of the dimer (**79**) relative to the comparison compounds **74** and **78** is difficult to explain since either the meso product or a racemic mixture of enantiomers should have potencies approximately the same as or slightly lower than the parent monomer compounds. Perhaps a strong hydrophobic interaction between the ends of the dimer in solution promotes a drug conformation which interferes with productive binding to the receptor.

Is Vesamicol an Opiate Analogue? Earlier studies[16] showed that levorphanol and several other opiates inhibit ACh active transport into synaptic vesicles with IC_{50} values in the low μM range. Since opiates contain the 4-phenylpiperidine moiety, it seemed possible that a vesamicol analogue based on the opiate family would exhibit good potency. Thus, analogues based on codeine (**82**) and oxymorphone (**83**) were synthesized. The potencies determined for these two analogues (our least potent) make

(19) Molecular mechanics calculations carried out with the Allinger and co-workers MM2 program (QCPE No. 395) show that for *trans*-decalin the diaxial conformation is 2.6 kcal/mol more stable than the twist boat conformation. *cis*-Decalin also was studied. In order to include electrostatic effects for *cis*-decalin we used the MOLMEC program which is based on the empirical potential developed by Oie et al.: Oie, T.; Maggiora, G. M.; Christofferson, R. E. *Int. J. Quantum Chem.* **1981**, *S8*, 1. (The SRI Molecular Theory Lab version of this program, containing modifications by D. J. DuChamp and P. W. Payne, was kindly provided to us by G. H. Loew and G. Frenking.) Without electrostatics, the diequatorial conformation is more stable than diaxial by 3.1 kcal/mol, while with electrostatics, the energy difference increases to 4.6 kcal/mol. The charges employed were determined from an MNDO calculation at the MOLMEC geometry. Since the torsional angles are greatly affected by electrostatic interactions, it was necessary to iterate several times in order to obtain consistent geometry and charges. In addition, a careful search was done to ensure that no low-energy portions of the potential energy surfaces were overlooked. The increased stability found for the diequatorial conformation relative to the diaxial due to electrostatic interactions is the basis for the statement made in ref 18 concerning the analogous conformations of vesamicol.

(20) Kornreich, W. D.; Parsons, S. M. *Biochemistry* **1988**, *27*, 5262.

(21) Rogers, G. A.; Nilsson, L.; Bahr, B. A.; Kornreich, W. D.; Parsons, S. M. *Cellular and Molecular Basis of Cholinergic Function*; Dowdall, M. J., Hawthorne, J. N., Eds.; Ellis Horwood Ltd.: Chichester, 1987; p 333.

(22) Green, N. M.; Konieczny, L.; Toms, E. J.; Valentine, R. C. *Biochem. J.* **1971**, *125*, 781.

it unlikely that the vesamicol receptor bears any resemblance to an opiate receptor.

Comparison of IC_{50} Values with LD_{50} Values. Vesamicol and some of its analogues have been tested for acute toxicity in mice. Modifications in structure produce a broad range of lethality with greater than a 100-fold difference between the least and most potent drugs. The behavior induced by these compounds, however, remains consistent. Administration of a lethal dose produces immediate onset of tremors followed by convulsions of the hind limbs and death within 5 min. At marginally lethal doses a more detailed course of action is seen. A short period of sedation preceeds onset of tremors, cyanosis is evident along with tremors, Straub tail occurs during convulsions, and convulsions are followed by flaccid paralysis, respiratory paralysis, and death. Listed in Table III are LD_{50} values for a number of drugs, some of which were both too low in potency and solubility to determine anything but a lower LD_{50} limit. For those compounds potent enough to yield LD_{50} values, a reasonable correlation with the IC_{50} values was found with a coefficient of 0.75. This strongly suggests that the lethal drug target in mouse is very similar to the vesamicol receptor of *Torpedo* cholinergic synaptic vesicles.

Summary. A few compounds in Table I of low potency and generally large structural deviation were not discussed, but in no case did we observe exceptions to the trends which were noted above. While much progress has been made in developing an understanding of structure–activity relationships in the vesamicol family of drugs, more data are desirable. There are compounds readily visualized which could test why the diequatorial and diaxial amino alcohol conformations both are effective inhibitors of ACh active transport. Also, as the factors that influence the biochemical assay variability are understood, greater accuracy will be achieved. Application of computational techniques such as QSAR then may allow a more rational approach to the design of even more potent and useful drugs. Nevertheless, despite the stated limitations, the current work provides many new candidate compounds which can be screened for increased specificity and potency of ACh storage block in intact cholinergic preparations. In addition, discovery of the potent benzovesamicol derivatives opens new biochemical opportunities. By extending the length of the spacer arm in the biotinylated drugs it should be possible to construct a reasonably potent drug that can simultaneously bind to avidin and the receptor. This might facilitate histochemical high-resolution localization of the vesamicol receptor in cholinergic nerve terminal sections. Furthermore, given the variety of substituents that can be incorporated with either compound **72** or **78**, construction of a successful covalent affinity label for the vesamicol receptor should be possible, as well as an affinity matrix useful for its isolation and purification.

Experimental Section

X-ray Crystallography. The hydrogen (*S,S*)-di-*p*-toluoyl-D-tartrate salt of (+)-vesamicol cation, $C_{17}H_{26}NO^+C_{20}H_{17}O_8^-\cdot CH_3CN$, FW = 685.8, was crystallized from hot acetonitrile. A clear, colorless plate of dimensions 1.05 mm × 0.60 mm × 0.10 mm was used for the structural determination. A triclinic cell with a = 7.628 (1) Å, b = 9.055 (1) Å, c = 14.464 (2) Å, α = 95.550 (4)°, β = 81.637 (5)°, γ = 72.837 (4)°, V = 934.0 (6) Å^3, Z = 1, and D_{calcd} = 1.22 g/mL was determined. Diffraction data were obtained at room temperature on a Huber four-circle diffractometer controlled with the Crystal Logic automation system utilizing a graphite monochromator and Mo Kα radiation (0.71069 Å). A total of 6550 reflections (entire sphere) were measured in the $\theta/2\theta$ scan mode to a maximum 2θ of 50° at a scan speed of 6°/min. Three standard reflections were measured after every 97 reflections and showed no evidence of crystal decomposition. The data were corrected for Lorentz and polarization effects but not for absorption (μ = 0.81 cm^{-1}).

Data reduction was performed with the program CARESS of the UCLA Computing Package.[23] The structure was solved by direct methods (SHELXS)[24] and refined with the program CRYSTALS.[25] In the triclinic system, the possible space groups are $P1$ and $P\bar{1}$. Both the volume of the unit cell and the presence of only one optical isomer are consistent with the noncentrosymmetric space group, $P1$. The absolute configuration of the anion, in the trial structure obtained from SHELXS, was found to be *S,S*; the refinement was continued with that starting model. The remaining non-hydrogen atoms, including an acetonitrile solvate, were located by successive cycles of least-squares refinement and difference Fourier syntheses. Positions were calculated (C–H = 1.0 Å) for hydrogen atoms bonded to carbon and nitrogen. Hydrogen atoms bonded to oxygen atoms were located in difference maps. In the final refinement, the positions and anisotropic thermal parameters of all non-hydrogen atoms and the positions of hydrogens H(11), H(21), H(71), H(81), H(111), H(301), H(321), and H(371) were allowed to vary. All other hydrogens were constrained to ride on the carbon atom to which they are bonded (C–H = 1.0 Å). The thermal parameters of all hydrogen atoms were fixed to 0.05 Å^2. In order to fix the origin in this space group, the sum of the shifts in each of the x, y, and z directions was constrained to be zero. The final agreement factors for the 5680 reflections with $I > 3.0\sigma(I)$ were R = 0.057 and R_w = 0.052. A final difference map showed no significanct features.

Anomalous dispersion effects are too small in this case to determine the absolute configuration of the molecule. However, by use of a counterion of known absolute configuration, the absolute configuration of (+)-vesamicol was determined to be *S,S*. The atomic structure (and numbering scheme) for the pharmacologically potent (−)-vesamicol cation of *R,R* absolute configuration was derived from the structure of (+)-vesamicol by coordinate inversion and is shown in Figure 1.

Chemistry. General Comments. Melting points were determined in open capillaries on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on either a Varian EM360A, a Nicolet NT-300, or a General Electric GN-500 with Me_4Si as internal standard. UV spectra were recorded on a Perkin-Elmer Lambda 3 spectrophotometer and IR spectra on a Perkin-Elmer 1330 infrared spectrophotometer. Mass spectra were run on a VG Analytical 70-250 HF mass spectrometer in either the FAB, EI, or DCI modes. TLC analyses were performed on Kodak 13181 silica gel plates and visualized with UV light and I_2 vapor. Column chromatography was performed on Merck silica gel 60. Elemental analyses (C, H, and N) were performed (Galbraith Laboratories, Inc., Knoxville, TN) where appropriate (*cis*-vesamicol and compounds **4**, **5**, 11, **20**, **37**, **38**, **48**, **50**, **53**, **65**, **66**, **72**, and **78**). Results were within ±0.4% of the calculated values. All other products (except compounds **19**, **41**, **44**, **68**, **70**, **76**, and **82**) were analyzed by high-resolution mass spectroscopy in the CI mode, except compounds **79** and **81**, for which FAB was employed.

(±)-*trans*-2-(4-Phenylpiperidino)cyclohexanol (Vesamicol). 4-Phenylpiperidine (15.0 g, 93.0 mmol) and cyclohexene oxide (20 mL, 198 mmol) were dissolved in 50 mL of EtOH and refluxed for 22 h. Upon cooling of the solution, white crystals formed, which were collected by filtration and washed with cold EtOH and petroleum ether. Yield of product (MS, ^{1}H NMR) was 17.2 g (mp 122–123 °C). A second crop gave 4.0 g for a total yield of 88%.

(−)-*trans*-2-(4-Phenylpiperidino)cyclohexanol. Racemic vesamicol (1.3 g, 5.0 mmol) in 30 mL of acetone was added dropwise with stirring to (−)-di-*p*-toluoyl-L-tartaric acid monohydrate (2.1 g, 5.4 mmol) in 30 mL of acetone and set aside to

(23) Strouse, C. E., Department of Chemistry, UCLA, Los Angeles, CA 90024.
(24) Sheldrick, G. M.; Krueger, C.; Goddard, R. *Crystallographic Computing 3*; Oxford University Press: Oxford, 1985; pp 175–189.
(25) Watkin, D. J.; Carruthers, J. R.; Betteridge, P. W. *CRYSTALS Users Guide*; Chemical Crystallography Laboratory: Oxford University Press: Oxford, 1986.

crystallize at 23 °C. It should be noted that the sign of rotation for L-tartaric acid changes upon diesterification with *p*-toluic acid. Crystals were collected by filtration and washed with cold acetone (1.8 g yield, mp 173.5–174.5 °C). The filtrate was evaporated to an oil under reduced pressure and partitioned between 1 M NaOH and benzene. The benzene layer containing vesamicol enriched in the pharmacologically inactive (+)-isomer was dried over Na_2SO_4, evaporated to an oil, taken up in 25 mL of acetone, and added dropwise with stirring to (+)-di-*p*-toluoyl-D-tartaric acid monohydrate (1.1 g) in 25 mL of acetone and set aside to crystallize at 23 °C. Crystals were collected as above (1.3 g yield, mp 175.5–176.5 °C) and reserved for pooling. The same procedure was carried out with another portion of racemic vesamicol, starting with (+)-di-*p*-toluoyl-D-tartaric acid. The hydrogen (−)-di-*p*-toluoyl-L-tartrate salts were pooled and recrystallized two times from CH_3CN [2.4 g yield, mp 176–176.5 °C, $[\alpha]_D = -55°$ (c = 1, MeOH)]. The resolved amine was converted to the free base as above and precipitated from Et_2O with anhydrous HCl and the amine hydrochloride recrystallized from hot EtOH/Et_2O [94% recovery, mp 238.5–240 °C, $[\alpha]^{23}_D = -22.8°$ (c = 1.4, EtOH)] for an overall yield of 70% of the (−)-enantiomer of vesamicol. The (+)-enantiomer obtained from the pooled hydrogen (+)-di-*p*-toluoyl-D-tartrate salts behaved similarly [hydrochloride $[\alpha]^{23}_D = +22.2°$ (c = 1.3, EtOH)].

[^{3}H]-(−)-*trans*-2-(4-Phenylpiperidino)cyclohexanol. The pharmacologically active enantiomer of the precursor was obtained by fractional crystallization of the hydrogen (−)-di-*p*-toluoyl-L-tartrate of compound **16** (68% yield, mp 199–200 °C) and conversion to the hydrochloride salt [mp 228–229 °C, $[\alpha]^{23}_D = -47.0°$ (c = 1.7, EtOH)]. This enantiomer was reduced (commercially) to (−)-vesamicol with $^3H_2$ over 10% Pd/C in EtOH. The specific activity was determined to be 32.6 Ci/mmol by mass spectroscopic analysis.

(±)-*cis*-2-(4-Phenylpiperidino)cyclohexanol (*cis*-Vesamicol). 2-(4-Phenylpiperidino)cyclohexanone (compound **48**) (1.0 g, 3.9 mmol) was dissolved in 10 mL of absolute EtOH to which was added 50 mg of PtO_2. Hydrogenation was carried out at 3 atm and room temperature for 3 h. The PtO_2 was removed by filtration and 350 mg (35%) of product (^{1}H NMR, FAB MS) was obtained as the hydrochloride [crystallized from MeOH/CCl_4 (mp 277–280 °C)]. TLC showed no trans isomer.

(±)-*trans*-2-(4-Cyclohexylpiperidino)cyclohexanol (1). Vesamicol hydrochloride was reduced in EtOH with 100 atm H_2 over Pt_2O at 60 °C for 3 h. The product hydrochloride (MS) was obtained in 79% yield from EtOH/Et_2O with mp 285–287 °C.

(±)-*trans*-2-[4-(4-Chlorophenyl)piperidino]cyclohexanol (2). Compound **25** (below) was reduced as the free base in EtOH with 1 atm H_2 over Pd/C (10%) at 25 °C. Product (MS, ^{1}H NMR, UV) was obtained in 79% yield from EtOH/H_2O with mp 101–107 °C.

(±)-*trans*-2-(4-Benzylpiperidino)cyclohexanol (3). 4-Benzylpiperidine (free base) was combined with cyclohexene oxide in a modification of the procedure for vesamicol. Product (MS) was obtained as the free base (MeOH/H_2O) in 58% yield with mp 75.5–77 °C.

(±)-*trans*-2-[4-(3-Phenylpropyl)piperidino]cyclohexanol (4). Synthesis was as for compound **3** with 4-(3-phenylpropyl)-piperidine. Product (MS) was obtained as the free base (EtOH) in 97% yield with mp 202–205 °C.

(±)-*trans*-2-[4-(4-Nitrophenyl)piperidino]cyclohexanol (5). 4-Phenylpiperidine (0.82 g, 5.0 mmol) was added in portions to 5 mL of concentrated H_2SO_4. The solution was cooled in an ice bath and 0.8 mL of a fuming HNO_3/concentrated H_2SO_4 mixture (equivalent to 5 mmol of HNO_3 and 10 mmol of H_2SO_4) was added dropwise over a period of 10 min. The solution was stirred for 2.5 h, allowed to come to ambient temperature slowly, and then poured over 30 g of ice. The solution was made basic by the addition of 8 g of NaOH (dissolved in H_2O) after which a brown oil formed. The mixture was extracted twice with $CHCl_3$ and the organic layer then washed with water and dried over Na_2SO_4. When the solvent was removed under reduced pressure, a residual yellow-brown oil separated and crystallized to yield 0.633 g (61% yield) of product which consisted of only the para isomer (by ^{1}H NMR).

4-(4-Nitrophenyl)piperidine (4.50 g, 21.8 mmol) was dissolved in 40 mL of absolute EtOH together with cyclohexane oxide (4.28 g, 43.6 mmol) and refluxed for 12.5 h. Upon being cooled, the solution deposited 5.17 g (78% yield) of light yellow crystalline product (MS, ^{1}H NNR) which was collected by filtration, washed twice with absolute EtOH, and dried at 110 °C; mp 151–155 °C.

(±)-*trans*-2-[4-(2,4-Dinitrophenyl)piperidino]cyclohexanol (6). 4-Phenylpiperidine was converted to an amide with acetic anhydride. Product (^{1}H NMR) was obtained in 95% yield after purification by column chromatography (silica gel) with mp 75 °C. The amide (2.0 g, 9.8 mmol) was suspended in cold concentrated H_2SO_4 to which was added 1 equiv of 90% HNO_3 (0.7 g). The mixture was allowed to come to room temperature (homogeneous at this time) and was stirred for 12 h. The solution was then heated to 100 °C for 1 h and poured into ice. The dinitro derivative (^{1}H NMR) was isolated as a yellow/orange oil by extraction of the aqueous mixture with $CHCl_3$ and Et_2O. Crystallization from CCl_4 afforded 1.3 g (45% yield) of reasonably pure material with mp 122–132 °C.

N-Acetyl-4-(2,4-dinitrophenyl)piperidine (1.3 g, 4.4 mmol) was taken up in concentrated HCl and refluxed overnight. Solvent was removed under reduced pressure and the resulting oil partitioned between aqueous NaOH and $CHCl_3$. The $CHCl_3$ solution was dried over Na_2SO_4 and removal of solvent gave product (^{1}H NMR) in quantitative yield with mp 109–119 °C. Condensation of the free piperidinyl derivative with cyclohexene oxide was carried out as for vesamicol and gave compound **6** in 69% yield with mp 167–171 °C.

(±)-*trans*-2-[4-(4-Aminophenyl)piperidino]cyclohexanol (7). *trans*-2-[4-(4-Nitrophenyl)piperidino]cyclohexanol (1.00 g, 3.29 mmol) was added to 50 mL of EtOH together with 85 mg of Pd/C (5%). The mixture was stirred under an H_2 atmosphere at low pressure for 15 h and then filtered in order to remove catalyst. Removal of most of the solvent under reduced pressure afforded 0.58 g of crystals (washed with petroleum ether) while a second crop raised the yield to 0.76 g (84% yield); mp 141–142 °C. Recrystallization from EtOH/H_2O raised the melting point to 145.5–146 °C.

(±)-*trans*-2-[4-[4-(Methylamino)phenyl]piperidino]-cyclohexanol (8) and (±)-*trans*-2-[4-[4-(Dimethylamino)-phenyl]piperidino]cyclohexanol (9). *trans*-2-[4-(4-Aminophenyl)piperidino]cyclohexanol (100 mg, 0.364 mmol) was dissolved in 4 mL of CH_3CN to which was added 0.37 mmol of CH_2O (37% diluted 10 times with CH_3CN) dropwise. The solution was stirred for 10 min, after which $NaBH_3CN$ (37 mg, 0.58 mmol) was added, and stirring was continued for an additional 10 min. Glacial acetic acid was added until a white precipitate formed. The mixture was stirred for 3 h and then acetic acid sufficient to dissolve the precipitate was added. The solution was made basic with 2 M NaOH and extracted with CH_2Cl_2/Et_2O (1:1). The organic layer was washed with water and then extracted two times with 5 mL of 1 M HCl. The aqueous solution was made basic with 2 M NaOH and the product extracted four times with 5 mL of CH_2Cl_2. The organic solution was dried over Na_2SO_4 and concentrated under reduced pressure to yield 70 mg of crystals. Both ^{1}H NMR and MS show the product to be a mixture of mono- and dimethylated derivatives. The mixture was recrystallized from EtOH/H_2O, but the recovered material (45 mg) was still a mixture. Separation of the mixture was performed by silica gel HPLC with hexane/Et_2O/Et_2NH (70:30:0.75) as the mobile phase. The dimethylated derivative (^{1}H NMR) (22 mg, 20% yield) eluted first, followed by the monomethylated compound (^{1}H NMR) (11 mg, 10% yield). The two products had mp 125–128 °C and 143–146 °C, respectively.

(±)-*trans*-2-[4-(4-Acetamidophenyl)piperidino]cyclohexanol (10). *trans*-2-[4-(4-Aminophenyl)piperidino]cyclohexanol (71 mg, 0.26 mmol) was dissolved in 2 mL of AcOH to which was added 4-nitrophenyl acetate (50 mg, 7% excess). Heating at 70 °C for 4 h caused complete release of nitrophenol. AcOH was removed via rotary evaporator and replaced with $CHCl_3$. This solution was extracted with aqueous Na_2CO_3 in order to remove nitrophenol. The organic phase was dried and concentrated to an oil. A ^{1}H NMR spectrum indicated both O- and N-acetylation had occurred and therefore the product was heated to reflux in MeOH for 30 min which effected transesterification. MeOH was removed and the resulting solid residue was crystallized from CH_3CN. Yield of product (^{1}H NMR, IR) was 61% with mp 244–245 °C.

(±)-*trans,trans*-*N,N'*-Bis(2-hydroxycyclohexyl)-4,4'-bipiperidine (11). 4,4'-Bipiperidine dihydrochloride was combined with cyclohexene oxide and 2,6-lutidine in a modification of the procedure for vesamicol. Product (MS) was obtained as the free base (benzene/petroleum ether) in 55% yield with mp 235–239 °C.

(±)-*trans,trans*-*N,N'*-Bis(2-hydroxycyclohexyl)-4,4'-ethylenedipiperidine (12). Synthesis was as for compound 3 with 4,4'-ethylenedipiperidine (free base). Product (MS) was obtained as the free base (EtOH) in 64% yield with mp 180–182 °C.

(±)-*trans,trans*-*N,N'*-Bis(2-hydroxycyclohexyl)-4,4'-trimethylenedipiperidine (13). Synthesis was as for compound 3 with 4,4'-trimethylenedipiperidine. Product (MS) was obtained as the free base (CH_3OH/H_2O) in 54% yield with mp 103.5–105 °C.

(±)-*trans*-2-[4-(2-Keto-1-benzimidazolinyl)piperidinyl]-cyclohexanol (14). Synthesis was as for compound 3 with 4-(2-keto-1-benzimidazolinyl)piperidine. Product (MS) was obtained as the free base (benzene) in 41% yield with mp 232–235 °C.

(±)-*trans*-2-Piperidinocyclohexanol (15). Synthesis was as for compound 3 with piperidine. Product (MS) was obtained as the hydrochloride ($EtOH/Et_2O$) in 59% yield with mp 271–272.5 °C.

(±)-*trans*-2-(4-Phenyl-1,2,3,6-tetrahydropyridino)cyclohexanol (16). Synthesis was as for vesamicol with 4-phenyl-1,2,3,6-tetrahydropyridine. Product (MS, ^1H NMR, UV) was obtained as the free base (95% EtOH) in 85% yield with mp 118–119.5 °C.

(±)-*trans*-2-(4-Phenyl-1-piperazinyl)cyclohexanol (17). Synthesis was as for compound 3 with 1-phenylpiperazine (free base). Product (MS, ^1H NMR) was obtained as the free base (Et_2O/petroleum ether) in 80% yield with mp 132.5–133 °C.

(±)-*trans*-2-(3-Methyl-4-phenyl-1-piperazinyl)cyclohexanol (18). Synthesis was as for vesamicol with 3-methyl-4-phenylpiperazine. Product (MS) was isolated as the hydrochloride (MeOH) in 80% yield with mp 193–196 °C.

(±)-*trans*-2-(3-Hydroxy-4-phenylpiperidino)cyclohexanol (19). Compound 16 (257 mg, 1.0 mmol) was dissolved in 4 mL of tetrahydrofuran containing 4 mmol of BH_3. The solution was stirred at 23 °C for 30 min during which time a white precipitate formed. Subsequently the solution was refluxed for 10 min, cooled (23 °C), quenched with excess NaOH, and treated with 0.25 mL of 30% H_2O_2 (0 °C) for 5 min. Finally the reaction mixture was poured into 100 mL of 0.2 M phosphate buffer (pH 6.8) and stirred at 23 °C overnight. Organic components were extracted from the aqueous solution with $CHCl_3$, concentrated, and separated by silica gel chromatography. The desired product (MS, ^1H NMR) was isolated as an oil in ca. 30% yield.

(±)-*trans*-2-(4-Phenyl-4-propionylpiperidino)cyclohexanol (20). Synthesis was as for compound 3 with 4-phenyl-4-propionylpiperidine. Product (MS) was isolated as the free base ($MeOH/H_2O$) in 66% yield with mp 85.5–87 °C.

(±)-*trans*-2-(*N*-Benzyl-*N*-methylamino)cyclohexanol (21). Synthesis was as for vesamicol with *N*-benzylmethylamine. Product (^1H NMR) was isolated as a colorless oil (after purification on silica gel) in 69% yield.

(±)-*trans*-2-[*N*-(2-Phenethyl)-*N*-methylamino]cyclohexanol (22). Synthesis was as for vesamicol with *N*-methylphenethylamine. Product (^1H NMR) was isolated as the hydrochloride ($EtOH/Et_2O$) in 74% yield with mp 177–179 °C.

(±)-*trans*-2-[*N*-(3-Phenylpropyl)-*N*-methylamino]cyclohexanol (23). 1-Bromo-3-phenylpropane (10.0 g, 50.3 mmol) was added to a solution of 50 mL of *i*-PrOH containing 50 mL of 40% aqueous $MeNH_2$. The mixture was stirred for 16 h and then concentrated under reduced pressure. Residual water was removed with the aid of EtOH (repeated azeotropic distillation) and the product 1-(methylamino)-3-phenylpropane (^1H NMR) isolated as the hydrobromide ($EtOH/Et_2O$) in 69% yield.

The secondary amine (free base) from above was combined with cyclohexene oxide and treated as for vesamicol. Product (^1H NMR, IR) was isolated as a colorless oil (after silica gel chromatography) in 70% yield.

(±)-*trans*-2-(4-Phenyl-1-imidazolyl)cyclohexanol (24). Synthesis was as for compound 3 with 4-phenylimidazole (free base). Product (MS) was isolated as the free base ($CHCl_3$/petroleum ether) in 40% yield with mp 181–183 °C.

(±)-*trans*-2-[4-(*p*-Chlorophenyl)-1,2,3,6-tetrahydropyridino]cyclohexanol (25). Synthesis was as for compound 11 with 4-(*p*-chlorophenyl)-1,2,3,6-tetrahydropyridine hydrochloride. Reaction workup was by partitioning between benzene and 1 N NaOH. Product (MS, ^1H NMR, UV) was isolated as the free base ($MeOH/H_2O$) in 74% yield with mp 132–133.5 °C.

(±)-*trans*-2-[4-(*p*-Chlorophenyl)-4-hydroxypiperidino]-cyclohexanol (26). Synthesis was as for compound 3 with 4-(*p*-chlorophenyl)-4-hydroxypiperidine. Product (MS) was isolated as the hydrochloride ($EtOH/Et_2O$) in 55% yield with mp 239.5–240.5 °C.

(±)-*trans*-2-[4-(*o*-Tolyl)-1-piperazinyl]cyclohexanol (27). 1-(*o*-Tolyl)piperazine dihydrochloride was treated as for compound 11. Product (MS) was isolated as the free base ($MeOH/H_2O$) in 70% yield with mp 111–112.5 °C.

(±)-*trans*-2-(4-Piperonyl-1-piperazinyl)cyclohexanol (28). Synthesis was as for compound 3 with 1-piperonylpiperazine. Product (MS) was isolated as the free base (petroleum ether) in 83% yield with mp 121–125 °C.

(±)-*trans*-2-[4-(4-Methoxyphenyl)-1-piperazinyl]cyclohexanol (29). Synthesis was as for compound 11 with 1-(4-methoxyphenyl)piperazine. Product (MS) was isolated as the free base (petroleum ether) in 66% yield with mp 138–139 °C.

(±)-*trans*-2-(4-Benzoyl-1-piperazinyl)cyclohexanol (30). Piperazine was monobenzoylated in 32% yield by the method of Schotten and Baumann and condensed with cyclohexene oxide as in the preparation of vesamicol. The product was isolated as the hydrochloride ($EtOH/Et_2O$) in 50% yield with mp 256.5–257.5 °C.

(±)-*trans*-2-[4-(2,5-Dimethylphenyl)-1-piperazinyl]-cyclohexanol (31). Synthesis was as for vesamicol with 4-(2,5-dimethylphenyl)piperazine. Product (MS) was recrystallized from $EtOH/H_2O$ in 80% yield with mp 125–126 °C.

(±)-*trans*-2-(1,2,3,4-Tetrahydro-2-isoquinolyl)cyclohexanol (32). Synthesis was as for compound 3 with 1,2,3,4-tetrahydroisoquinoline (free base). Product (MS) was isolated as the hydrochloride ($EtOH/Et_2O$) in 60% yield with mp 209–212 °C.

(±)-8-(*trans*-2-Hydroxycyclohexyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (33). Synthesis was as for compound 3 with 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. Product (MS) was isolated as the free base (EtOH/benzene) in 37% yield with mp 250–254 °C.

(±)-*trans*-2-(1-Indanylamino)cyclohexanol (34). Synthesis was as for compound 3 with 1-aminoindan. Product (MS) was isolated as the hydrochloride ($EtOH/Et_2O$) in 48% yield with mp 175–177.5 °C.

(±)-*trans*-2-(3-Methyl-3-phenylpiperidino)cyclohexanol (35). Synthesis was as for compound 3 with 3-methyl-3-phenylpiperidine. Product (MS) was isolated as the hydrochloride ($EtOH/Et_2O$) in 49% yield with mp 248–250 °C.

(±)-*trans*-2-(3-Azabicyclo[3.2.2]non-3-yl)cyclohexanol (36). Synthesis was as for compound 3 with 3-azabicyclo[3.2.2]nonane. Product (MS) was isolated as the free base ($MeOH/H_2O$) in 72% yield with mp 118–119.5 °C.

(±)-*N*-(*trans*-2-Hydroxycyclohexyl)-*N*-methyl-4-phenylpiperidinium Iodide (37). Vesamicol (1.0 g, 3.9 mmol) and excess CH_3I (4 mL) were dissolved in 4 mL of EtOH and allowed to stand at 23 °C for 24 h. Et_2O (10 mL) was added and the precipitated product (^1H NMR) was collected by filtration, washed with Et_2O, and recrystallized from $EtOH/Et_2O$ in 40% yield with mp 171–174 °C.

(±)-*O*-Acetyl-*trans*-2-(4-phenylpiperidino)cyclohexanol (38). Vesamicol (0.20 g, 0.77 mmol) was acetylated with a mixture of triethylamine (0.1 mL), 4-(dimethylamino)pyridine (10 mg), and 1 mL of acetic anhydride with which it is incubated at 23 °C for 1 day. The product was partitioned between 100 mL each of cold saturated aqueous Na_2CO_3 and benzene. The benzene layer was washed twice with water, dried over Na_2SO_4, and evaporated to an oil under reduced pressure. The oil was dissolved in 10 mL of Et_2O which was bubbled with excess anhydrous HCl. The precipitated hydrochloride (MS) was collected by filtration, washed with Et_2O, and recrystallized from $CHCl_3/Et_2O$ in 98% yield with mp 227–229 °C.

(±)-*O*-Acetyl-*N*-(*trans*-2-hydroxycyclohexyl)-*N*-methyl-4-phenylpiperidinium Trifluoromethanesulfonate

(39). Vesamicol (1.0 g, 3.86 mmol) was dissolved in 15 mL of CH_2Cl_2 and cooled in ice. Acetyl chloride (0.40 g, 30% excess) was added dropwise during 2 min. After the solution was allowed to reach ambient temperature, solvent and excess reactant were removed under reduced pressure. The ester was partitioned between CH_2Cl_2 and aqueous K_2CO_3. The organic phase was dried over Na_2SO_4 and K_2CO_3. After removal of solvent under reduced pressure, the ester (300-MHz ^1H NMR) was obtained in nearly quantitative yield and further purified by silica gel chromatography.

Acetate ester (0.3 g, 1 mmol) was dissolved in 2 mL of Et_2O to which was added 1 mL of methyl trifluoromethanesulfonate. Within minutes an orange oil deposited, which slowly solidified. The supernatant was decanted and the residual solid was washed with Et_2O, dried, and weighed (0.35 g). Chromatographic purification on silica gel gave two isomers, only one of which (the less polar, *Z* isomer[26]) was obtained in a pure form. The *Z* isomer (300-MHz ^1H NMR, FAB MS) was crystallized from dimethyl sulfoxide/$CHCl_3$/Et_2O to yield 0.14 g of a white solid with mp 162–162.5 °C. A mixture (3:1) of the *E* and *Z* isomers (300-MHz ^1H NMR) also was isolated.

(±)-*N*-(2-Hydroxybutyl)-4-phenylpiperidine (40). 4-Phenylpiperidine was combined with 1-butene oxide in a modification of the procedure for vesamicol. Product (MS) was isolated as the hydrochloride (EtOH/Et_2O) in 36% yield with mp 182–183 °C.

(±)-*N*-(2-Hydroxybutyl)-*N*-methyl-4-phenylpiperidinium Iodide (41). Synthesis was as for compound **37** with compound **40**. Product could not be obtained as a crystalline solid and was therefore converted to the chloride form (35% yield) which was also an oil (^1H NMR).

(±)-*N*-(2-Acetoxybutyl)-4-phenylpiperidine (42). Synthesis was as for compound **38** with compound **40**. Product (DCI MS) was isolated as the hydrochloride ($CHCl_3$/Et_2O) in 69% yield with mp 209–210 °C.

***N*-(2-Hydroxyethyl)-4-phenylpiperidine (43).** Ethylene oxide (1.1 g, 25 mmol), 4-phenylpiperidine (8.05 g, 50 mmol), and 10 mL of heptane were placed in a glass-lined steel cylinder at 90 °C for 7 h. The solvent was removed under reduced pressure and the product purified by silica gel chromatography. The desired product (3.5 g, 68% yield) was eluted as an oil with hexane/$CHCl_3$/*i*-PrOH (7:7:6) and exhibited only one spot on silica gel TLC. The amine was converted into the hydrochloride salt (MS) and crystallized from EtOH/Et_2O (mp 138–143 °C).

***N*-(2-Hydroxyethyl)-*N*-methyl-4-phenylpiperidinium Iodide (44).** Synthesis was as for compound **37** with compound **43**. Product (FAB MS) was isolated as the iodide (EtOH/Et_2O) in 65% yield with mp 159–162 °C.

***N*-(2-Acetoxyethyl)-4-phenylpiperidine (45).** Synthesis was as for compound **38** with compound **43**. Product was isolated as the hydrochloride (EtOH/Et_2O) in 25% yield with mp 138–142 °C.

(*Z*)-*N*-(2-Acetoxyethyl)-*N*-methyl-4-phenylpiperidinium Bromide (46) and (*E*)-*N*-(2-Acetoxyethyl)-*N*-methyl-4-phenylpiperidinium Bromide (47). 4-Phenylpiperidine (10 mmol) was N-methylated with CH_2O in formic acid (Eschweiler–Clarke reaction). The tertiary amine (^1H NMR) was isolated as the free base and purified by silica gel chromatography which gave a colorless oil in 74% yield. Alkylation of *N*-methyl-4-phenylpiperidine (1.3 g, 7.4 mmol) was carried out in an excess (4 mL) of bromoethyl acetate which gave crystals after 1 day. Product was isolated by filtration under a stream of nitrogen and washed with Et_2O. The yield of product composed of both isomers (an equal mixture of *E* and *Z* by ^1H NMR) was 28%. The isomers were separated by silica gel chromatography ($CHCl_3$/EtOH) with the *Z* isomer (300- and 500-MHz ^1H NMR, FAB MS) eluting before the *E* isomer (300- and 500-MHz ^1H NMR, FAB MS).[26]

(±)-2-(4-Phenylpiperidino)cyclohexanone (48). Cyclohexanone (50 g, 0.51 mol) was added to a solution of 11.5 g (93.5 mmol; 10% excess) $KClO_3$ in water. One mL of concentrated HCl was added followed by dropwise addition of 40 g (0.5 equiv) of Br_2. The reaction proceeded smoothly at 40 °C. The organic layer was separated from the aqueous solution, dried, and distilled under vacuum (ca. 3 Torr). Unreacted cyclohexanone distilled at 28 °C while product 2-bromocyclohexanone (^1H NMR) was collected at 58–65 °C in 40% yield.

2-Bromocyclohexanone (6.0 g, 33.7 mmol) and 11.6 g (72 mmol) of 4-phenylpiperidine were dissolved in 50 mL of $CHCl_3$ and stirred for 7 days. The 4-phenylpiperidine hydrobromide was removed by filtration and the resulting oil distilled to yield 6.5 g (75%) of 2-(4-phenylpiperidino)cyclohexanone (^1H NMR, IR, FAB MS).

(±)-*trans*-2-(4-Phenylpiperidino)cyclohexanethiol (49). 4-Phenylpiperidine was combined with cyclohexene sulfide under N_2 in a modification of the procedure for vesamicol. Product (MS, ^1H NMR) was isolated as the hydroperchlorate (EtOH/H_2O/dithiothreitol) in 38% yield with mp 199–203 °C. The IC_{50} value was determined in the presence of 1 mM dithiothreitol.

***N*-Cyclohexyl-4-phenylpiperidine (50).** *N*-Cyclohexyl-4-phenylpiperidine was prepared by the method of De Benneville and McCartney[27] in 30% yield by reduction of the intermediate enamine. Product (^1H NMR, MS) was isolated as the hydrochloride which had mp 288–290 °C (recrystallized from EtOH/Et_2O).

(±)-*trans*-2-(4-Phenylpiperidino)cyclopentanol (51). Synthesis was as for compound **3** with cyclopentene oxide. The crude reaction mixture was chromatographed on silica gel and product (MS, ^1H NMR) obtained as the free base (EtOH/H_2O) in 50% yield with mp 103–105 °C.

(±)-*cis*-4-*tert*-Butyl-*trans*-2-(4-phenylpiperidino)cyclohexanol (52). Synthesis was as for compound **3** with *cis*-4-*tert*-butylcyclohexene oxide. Product (MS, ^1H NMR) was isolated as the hydrochloride (EtOH/Et_2O) in 46% yield with mp 233–235 °C.

(±)-*trans*,*trans*-5-*tert*-Butyl-2-(4-phenylpiperidino)-cyclohexanol (53). Synthesis was as for compound **3** with *trans*-4-*tert*-butylcyclohexene oxide. Product (MS) was isolated as the hydrochloride (EtOH) in 55% yield with mp 297.5–298.5 °C.

(±)-*cis*-6-Phenyl-*trans*-2-(4-phenylpiperidino)cyclohexanol (54). Synthesis was as for compound **3** with a mixture (1:3) of *cis*- and *trans*-3-phenylcyclohexene oxide. The crude reaction mixture was chromatographed on silica gel and product from the cis isomer (MS, ^1H NMR) isolated as the hydrochloride (EtOH/Et_2O) in 55% yield with mp 190–192 °C.

(±)-*trans*,*trans*-6-Methoxy-2-(4-phenylpiperidino)cyclohexanol (55). Synthesis was as for compound **3** with *trans*-3-methoxycyclohexene oxide. The crude reaction mixture was chromatographed on silica gel and product (300-MHz ^1H NMR) isolated as the hydrochloride (EtOH/Et_2O) in 66% yield with mp 240–241 °C.

(±)-*cis*-6-Hydroxy-*trans*-2-(4-phenylpiperidino)cyclohexanol (56). 2-Cyclohexenol was epoxidized in CH_2Cl_2/Et_2O with 3-chloroperoxybenzoic acid. The epoxide was combined with 4-phenylpiperidine as for vesamicol. The product (300-MHz ^1H NMR), isolated in 24% yield after chromatography on silica gel,

(26) The structures of the isomers shown in Table I were assigned by use of ^1H NMR. It was observed that there are large, reciprocal chemical shift differences between the *N*-methyl (0.29 ppm) and the exocyclic methylene (0.38 ppm) resonances of compounds **46** and **47**. For the less polar isomer (**46**) the resonances appear at 3.43 ppm (methyl) and 4.51 ppm (methylene), while for the more polar isomer (**47**) at 3.72 ppm (methyl) and 4.13 ppm (methylene). The most obvious explanation for this observation is deshielding by steric compression (Cheney, B. V. *J. Amer. Chem. Soc.* **1968**, *90*, 5386 and references therein). Hence, substituents cis to the phenyl ring (which is equatorial in both isomers) will be in an axial position and experience steric interactions with the axial protons. The resultant steric compression will cause deshielding of the axial substituents on the nitrogen atom as well as the axial ring protons. Therefore, the isomer with the lower resonance position for the methyl group (**47**) was judged to have the methyl substituent cis to the phenyl ring and hence to be the *E* isomer. The assignment of the isomeric structure for compound **39** was made by comparisons to compounds **46** and **47**.

(27) De Benneville, P. L.; Macartney, J. H. *J. Am. Chem. Soc.* **1950**, *72*, 3073.

was derived from the *cis*-epoxide and had mp 120.5–121.5 °C.

(±)-*cis*-4-(Hydroxymethyl)-*trans*-2-(4-phenylpiperidino)cyclohexanol (57). Synthesis was as for compound **56**, starting with (±)-3-cyclohexene-1-methanol. The product (300-MHz ^{1}H NMR), isolated in 33% yield after chromatography on silica gel, was derived from the *cis*-epoxide and had mp 139.5-141 °C.

(±)-*trans*-1-Methyl-2-(4-phenylpiperidino)cyclohexanol (58). Synthesis was as for compound **3** with 1-methylcyclohexene oxide. The crude reaction mixture was chromatographed on silica gel and product (MS) isolated as the hydrochloride (EtOH/Et_2O) in 23% yield with mp 258–258.5 °C.

(±)-*cis*-2-[(4-Phenylpiperidino)methyl]cyclohexanol (59) and (±)-*trans*-2-[(4-Phenylpiperidino)methyl]cyclohexananol (60). Ethyl 2-cyclohexanone carboxylate (1.0 g, 6 mmol) plus 4-phenylpiperidine (1.2 g, 7.5 mmol) were heated to reflux in 1 mL of methoxyethanol for 16 h. The crude reaction mixture was dissolved in CCl_4, washed with 1 M HCl, dried over Na_2SO_4, and chromatographed on silica gel. Product amide (^{1}H NMR) eluted with $CCl_4/CHCl_3/Et_2O$ (8:1:1) and crystallized from CCl_4 in 67% yield with mp 98–101 °C.

The keto amide (1.15 g, 4.03 mmol) from above was dissolved in 12 mL of dry Et_2O/THF and the flask flushed with N_2. Lithium aluminum hydride (0.200 g, 5.26 mmol) was added in portions so as to maintain gentle reflux, after which the solution was refluxed for 16 h. An additional 30 mg of lithium aluminum hydride was added and reflux continued for 3 h and then quenched with wet Et_2O followed by H_2O and concentrated NaOH. The product was extracted from the reaction mixture with Et_2O and diluted with CCl_4. The Et_2O was removed under reduced pressure and the addition of petroleum ether (30–60 °C) initiated crystallization. Product was collected by filtration and washed with petroleum ether. Yield of light yellow solid was 0.49 g (42% yield) with mp 115–122 °C. Silica gel TLC indicated two impurities. Recrystallization from CCl_4/petroleum ether raised the melting point to 127–128 °C. Both IR (by comparison with known cis- and trans-2-substituted cyclohexanols) and ^{1}H NMR (300 MHz) showed this to be the cis isomer (**59**).

The mother liquor and petroleum ether washes were combined and the solvents removed under reduced pressure to give 0.61 g (52% yield) of light yellow oil which TLC showed to be one main component. Both IR and ^{1}H NMR suggest that this is the trans isomer. The oil was chromatographed on silica gel and the product (**60**) converted to the hydrochloride in Et_2O and crystallized from *i*-PrOH/petroleum ether (60–90 °C); mp 186–186.5 °C.

(±)-*O*-Butyryl-*trans*-2-(4-phenylpiperidino)cyclohexanol (61). Preparation was as for compound **38** with butyric anhydride. Product (MS) was isolated as the hydrochloride ($CHCl_3/Et_2O$) in 50% yield with mp 181–185 °C.

(±)-*O*-Benzoyl-*trans*-2-(4-phenylpiperidino)cyclohexanol (62). Synthesis was as for compound **38** with benzoic anhydride. Product (MS) was isolated as the hydrochloride ($CHCl_3/Et_2O$) in 79% yield with mp 218–224 °C.

(±)-*cis*-(4-Phenylpiperidino)-7-norborneol (63). *exo*-2,3-Epoxynorbornane (1.8 g, 16 mmol) was combined with 4-phenylpiperidine (4.0 g, 25 mmol) plus 3 drops of CF_3COOH in 2 mL of absolute EtOH. The mixture was heated to 155 °C in a stainless steel reaction vessel for 48 h. Product was partitioned into $CHCl_3$ from aqueous base, purified via silica gel chromatography, and finally crystallized from $CHCl_3$/petroleum ether in 18% yield (^{1}H NMR, MS) with mp 143.5–145 °C.

4-Phenylpiperidine (64). 4-Phenylpiperidine is commercially available.

(±)-*trans*-2-Hydroxy-3-(4-phenylpiperidino)-*trans*-decalin (65). Synthesis was as for compound **3** with a 1:1 mixture of *cis*- and *trans*-bicyclo[4.4.0]decene oxide. Product from the trans isomer (MS, ^{1}H NMR) was isolated as the hydrochloride (EtOH/Et_2O) in 55% yield with mp 217–220 °C. The small amount of cis isomer which was formed was not isolated.

(±)-*trans*-2-Hydroxy-3-(4-phenylpiperidino)-*cis*-decalin (66). Synthesis was as for compound **65** except that 1-propanol was used as solvent for the condensation. The product mixture was fractionated by silica gel chromatography, and those fractions which contained only the cis isomer (TLC) were pooled. The pure cis product (FAB MS, ^{1}H NMR) was isolated as the hydrochloride (13% yield) and recrystallized (EtOH/Et_2O); mp 275–278 °C.

(±)-*trans*-2-Hydroxy-3-[4-(2-methoxyphenyl)-1-piperazinyl]-*trans*-decalin (67) and (±)-*trans*-2-Hydroxy-3-[4-(2-methoxyphenyl)-1-piperazinyl]-*cis*-decalin (68). Synthesis was as for compound **65**, using 1-(2-methoxyphenyl)-piperazine. The crude reaction mixture was chromatographed on silica gel and products (MS) separated and isolated (hydrochlorides from EtOH/Et_2O) in 59% and 4% yields with mp 238–240 and 200–200.5 °C for the trans (**67**) and cis (**68**) compounds, respectively.

(±)-*trans*-2-Hydroxy-3-(4-phenylpiperidino)tetralin (69). 1,4-Dihydronaphthalene was prepared via Birch reduction (see compounds **71** and **72** below for procedure) of naphthalene in Et_2O/*t*-BuOH using Na/NH_3. Yield of product (^{1}H NMR) was 75% with 25% starting material recovered. The subsequent epoxidation of the alkene was performed with 3-chloroperoxybenzoic acid in $CCl_4/CHCl_3$ without purification to remove naphthalene. The epoxide product contained no other detectable (^{1}H NMR) impurities.

4-Phenylpiperidine was combined with an excess of the 1,4-dihydronaphthalene oxide in a modification of the procedure for vesamicol. The reaction product was chromatographed on silica gel and precipitated from Et_2O with HCl gas. The hydrochloride was recrystallized first from EtOH/Et_2O and then from EtOH/CCl_4 to give the desired product (MS, ^{1}H NMR, UV) in 37% yield with mp 229–229.5 °C.

(±)-*trans*-2-Hydroxy-3-(4-phenyl-1,2,3,6-tetrahydropyridino)tetralin (70). 4-Phenyl-1,2,3,6-tetrahydropyridine (free base) was combined with 1,4-dihydronaphthalene oxide in a modification of the procedure for vesamicol. The crude reaction mixture was chromatographed on silica gel and product (MS, ^{1}H NMR) isolated as the free base in 32% yield with mp 159.5–164 °C.

(±)-*trans*-5-Amino-3-hydroxy-2-(4-phenylpiperidino)tetralin (71) and (±)-*trans*-5-Amino-2-hydroxy-3-(4-phenylpiperidino)tetralin (72). A 1-L, three-necked, round-bottom flask was equipped with a large cold-finger condenser cooled with dry ice. To the flask were added 1-aminonaphthalene (79 g, 0.55 mol), Et_2O (300 mL), *t*-BuOH (50 mL), and NH_3 (200–300 mL). Sodium (30 g, 1.3 mol) was added in portions over a 4-h period, followed by an additional 50 mL of *t*-BuOH. After 1 h absolute EtOH (100 mL) was added slowly. This mixture was allowed to stir overnight and was then quenched by careful addition of NH_4Cl (50 g) and H_2O (400 mL). The two liquid layers were separated, and the aqueous layer was extracted two times with ether. The ether extracts were combined with the organic layer, and this solution was extracted twice with water, once with a saturated NaCl solution, dried over Na_2SO_4, and finally filtered through $MgSO_4$.

Ether and most of the *t*-BuOH were removed at 65 °C on a rotary evaporator. The ^{1}H NMR spectrum of this material showed *t*-BuOH as the only impurity. However, colorless 1-amino-5,8-dihydronaphthalene with mp 37–39 °C may be obtained in 97% yield after vacuum distillation.

1-Amino-5,8-dihydronaphthalene (60.1 g, 0.414 mol) was dissolved in 200 mL of benzene and cooled to 0 °C. Trifluoroacetic anhydride (89 g, 0.42 mol) was added slowly due to the exothermicity of the reaction. The ammonium salt began to precipitate almost immediately, but the reaction solution was homogeneous upon complete addition of the anhydride. The solution was maintained at 0 °C for 1 h after which benzene and trifluoroacetate acid were removed under reduced pressure. More benzene was added and again evaporated in order to aid the removal of trifluoroacetic acid.

The amide from above was dissolved in 300 mL of Et_2O to which was added 3-chloroperoxybenzoic acid (85.0 g, 80–85% pure). The solution was maintained near 10 °C throughout the addition and then allowed to warm to 23 °C and stirred for 5 h. The product epoxide containing some 3-chlorobenzoic acid was collected by filtration and washed with ether. The solid was resuspended in 400 mL of Et_2O, thoroughly washed, and recollected by filtration to yield 89.0 g of epoxide with mp 174–178.5 °C. After extraction of 3-chlorobenzoic acid from the ethereal mother liquor, an additional 2.1 g of product can be isolated for an overall yield of 85%. Purification of the epoxide by crystallizing from $CHCl_3$ and washing with Et_2O raises the melting point to 179.5–181 °C.

N-(Trifluoroacetyl)-1-amino-5,8-dihydronaphthalene oxide (1.9 g, 7.4 mmol) was dissolved in 25 mL of absolute EtOH to which was added 4-phenylpiperidine (3.0 g, 19 mmol). The solution was maintained at 45 °C for 17 h and then refluxed for 3 h. After 7 h at 23 °C a crystalline solid was collected by filtration and washed with EtOH and CCl_4. The yield of this polar isomer (**71**)[28] which results from deacylation during the reaction was 0.78 g (33% yield). The mother liquor was evaporated to an oil which was taken up in CCl_4. This process was repeated in order to remove most of the EtOH. When the oil was again dissolved in CCl_4, crystallization commenced and yielded 0.7 g of 4-phenylpiperidinium trifluoroacetate. The remaining mother liquor was chromatographed on silica gel where *N*-(trifluoroacetyl)-4-phenylpiperidine eluted with CCl_4 and a second less polar product isomer with $CCl_4/CHCl_3$. The yield of this isomer (**72**) was 0.84 g (35% yield). The polar isomer could be crystallized from $CHCl_3$/EtOH and had mp 217–218 °C while the less polar isomer had mp 174–175 °C. Their R_f values on silica gel TLC in $CHCl_3$ were 0.30 and 0.42, respectively.

(±)-*trans*-5-Acetamido-3-hydroxy-2-(4-phenylpiperidino)tetralin (73) and (±)-*trans*-5-Acetamido-2-hydroxy-3-(4-phenylpiperidino)tetralin (74). Synthesis was as for compounds **71** and **72** except acetic anhydride was employed in the acylation step and the acetyl amide is resistant to attack by 4-phenylpiperidine under the conditions of the condensation reaction. As above, two products were obtained which had different mobilities on silica gel (by which they were separated). The less polar isomer (**74**) (MS, ^{1}H NMR) was obtained in 32% yield and crystallized (free base) from EtOH/H_2O with mp 201–201.5 °C. The more polar isomer (**73**) (MS, ^{1}H NMR) was obtained in 23% yield and crystallized (free base) from EtOH/H_2O with mp 191–192 °C.

(±)-*trans*-3-Hydroxy-2-(4-phenylpiperidino)-5-(trifluoroacetamido)tetralin (75). Isolated only as a side product (9% yield) in the synthesis of compounds **71** and **72**, this compound is apparently related to the more polar isomers (**71** and **73**) regarding the position of the anilino group (by ^{1}H NMR). Crystallized from CH_2Cl_2/CCl_4, the material has mp 175 °C.

(±)-*trans*-5-Acetamido-2-hydroxy-8-iodo-3-(4-phenylpiperidino)tetralin (76). Compound **74** (36.4 mg) was incubated with 3-chloroperoxybenzoic acid in 0.5 mL of acetic acid at 23 °C. After 10 min ICl (50 mg) was added and the reaction allowed to proceed an additional 25 min and then quenched with aqueous bisulfite. Product was extracted into CH_2Cl_2, which was then washed with NaOH and dried over Na_2SO_4. Solvent was removed under reduced pressure and the residue dissolved in 1 mL of acetic acid to which was added triphenylphosphine (26 mg). This solution was allowed to stand overnight and then poured into H_2O. The aqueous solution was washed with benzene to remove triphenylphosphine oxide and then made basic with NaOH. Product was extracted into CH_2Cl_2. This organic layer was dried, concentrated under reduced pressure, and applied to a semipreparative silica gel HPLC column. Elution was carried out with hexane/CH_2Cl_2/*i*-PrOH (5:4:1), and the appropriate fractions (determined by TLC) were pooled and concentrated to yield the iodinated product (EI and DCI MS, ^{1}H NMR) with mp 130 °C. The yield was not quantitated.

(±)-*trans*-3-Hydroxy-2-(4-phenylpiperidino)-5-succinamidotetralin (77) and (±)-*trans*-2-Hydroxy-3-(4-phenylpiperidino)-5-succinamidotetralin (78). Succinic anhydride (78.7 mg, 0.787 mmol) was dissolved in 3 mL of acetic acid to which was added 231 mg (0.718 mmol) of compound **72** (the less polar isomer). After 1 h CCl_4 was added and then removed under reduced pressure. This was repeated several times in order to aid in the removal of acetic acid as an azeotrope. The resulting oil was crystallized from EtOH/H_2O and dried in vacuo to give 302 mg (100% yield) of the desired amide (**78**) (mp 151 °C). The more polar isomer (compound **71**) was similarly succinylated in quantitative yield and gave a product (**77**) with mp 191–193 °C.

(±)-*trans*,*trans*-*N*,*N*′-Bis[4-oxo-4-[[5,6,7,8-tetrahydro-6-hydroxy-7-(4-phenylpiperidino)-1-naphthalenyl]amino]butanoyl]hydrazine (79). A solution of compound **78** (100 mg, 0.237 mmol) in a mixture of 10 mL of $CHCl_3$/2 mL of EtOH was cooled to 0 °C and treated with $SOCl_2$ (66 mg, 0.55 mmol). After stirring overnight at room temperature, the solution was refluxed for 15 min. Removal of the solvent left an oil whose ^{1}H NMR spectrum was consistent with the expected ethyl ester. The oil was redissolved in 10 mL of EtOH to which was added 6 drops of 85% aqueous hydrazine. This solution was stirred at 23 °C for 6 h followed by 30 min at reflux. An additional 3 drops of hydrazine was added during the reflux period. The solvent was removed under reduced pressure and the crude product partitioned between H_2O and CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered through K_2CO_3, and concentrated under reduced pressure. Product hydrazide (MS, ^{1}H NMR) crystallized from CH_2Cl_2/CCl_4 in 39% yield and had mp 148–149 °C. A second crop of crystals of lower purity could be obtained.

A solution of compound **78** (50 mg, 0.118 mmol) plus the hydrazide (52 mg, 0.118 mmol) in 5 mL of $CHCl_3$ was cooled to 0 °C and treated with dicyclohexylcarbodiimide (29 mg, 0.14 mmol). After 10 min the solution was allowed to reach room temperature and stirred overnight. The urea byproduct was removed by filtration and the concentrated solution applied to a column of silica gel. Product (FAB MS) was eluted from the column with $CHCl_3$/MeOH and crystallized from MeOH in 35% yield with mp 225–226 °C.

(±)-N^5-*d*-Biotinyl-*trans*-5-amino-2-hydroxy-3-(4-phenylpiperidino)tetralin (80). Compound **72** (the less polar isomer) (55.4 mg, 0.166 mmol) was dissolved in 0.4 mL of acetic acid to which was added 66.7 mg (0.183 mmol) of biotin *p*-nitrophenyl ester. Heating was commenced at 60 °C for 3 h followed by 70 °C for 3 h. Analysis for release of nitrophenol at this time indicated that only one-third of the ester had reacted. Heating was continued at 70 °C for 14 h and then 80 °C for 24 h. Acetic acid was removed azeotropically by repeated evaporation with CCl_4. The resulting oil was dissolved in $CHCl_3$ and 75 mg of light green solid precipitated upon addition of Et_2O. The solid was redissolved in $CHCl_3$ and the solution washed with aqueous Na_2CO_3

(28) The structures of the isomers **71** and **72**, shown in Table I, were assigned by ^{1}H NMR. It had been noted in ^{1}H NMR spectra at 60 MHz that the two compounds could be differentiated on the basis of the shape of largely unresolved resonances between 2.2 and 3.2 ppm. At higher field strengths (300 and 500 MHz) all the protons of interest (namely those in the cyclohexanol ring) could be assigned. The most striking difference in the spectra of compound **71** compared to **69** (the analogue lacking the anilino amine) and **72** compared to **69** was an upfield shift of one proton in each by approximately 0.4 ppm. Employing two-dimensional *J*-correlation (2-D COSY) analysis of compound **72** with selective decoupling experiments on compound **71**, it was determined that the proton giving the upfield resonance (relative to **69**) is not the same proton in **71** and **72**. In compound **71**, the resonance could be assigned to the benzylic, axial proton adjacent to the hydroxyl group and in compound **72**, to the benzylic, axial proton adjacent to the piperidinyl ring. Since the only structural difference involved is the position of the anilino group, the observed differences in proton resonances must arise as a consequence of the position of that amino group. Simple resonance theory predicts that sites ortho to the anilino nitrogen will experience greater electron density compared to the meta position. The increased electron density on an aromatic carbon bonded to the cyclohexanol ring should influence the local magnetic environment of the protons on the neighboring benzylic carbon. If models of these structures are examined, the bonding orbitals of the benzylic, axial protons are predicted to be nearly periplanar to the π orbitals of the aniline ring and hence the axial proton should be more strongly influenced by any change in electron density on the neighboring aromatic carbon than would the corresponding equatorial proton. Since in each structure in question, one benzylic position is ortho to the anilino group while the other is in a meta relationship, only one proton in each structure is expected to experience shielding predicted by the resonance effect. Therefore we can unequivocally assign structures **71** and **72** on the basis of the observed upfield shift of a single, different proton in each relative to the corresponding proton in compound **69**. This phenomenon also is evident in the spectra of 2,3-dimethylaniline where the methyl protons differ by 0.22 ppm and 1-amino-5,6,7,8-tetrahydronaphthalene where the benzylic protons differ by 0.35 ppm (Pouchert, C. J.; Campbell, J. R. *The Aldrich Library of NMR Spectra, Vol. V*, 1974).

and dried with Na_2SO_4. Chromatography on silica gel and elution with $CHCl_3$/MeOH gave 28 mg (30% yield) of white product (^1H NMR, MS, UV).

(±)-N^5-Biotinylglycylglycyl-*trans*-5-amino-2-hydroxy-3-(4-phenylpiperidino)tetralin (81). Glycylglycine (158 mg, 1.2 mmol) was dissolved in 2.5 mL of H_2O and diluted with 2.5 mL of pyridine. Aqueous KOH was added until a pH meter reading of 8.1. Biotin *p*-nitrophenyl ester (373 mg, 1.02 mmol) was added at once and the indicated pH adjusted to 8.7. The mixture was vortexed for 45 min with three additions of KOH used to maintain a pH reading of 8.7. At this time the solution was homogeneous. After an additional 30 min, the solution was diluted with water, washed with CCl_4 (5×), and Et_2O (1×), acidified to pH 4.6 with concentrated HCl, and again washed with Et_2O (3×). Finally the solution was warmed to remove residual Et_2O and acidified to pH 1.75 with concentrated HCl. Within minutes white crystals began to form. After 90 min product was collected by filtration and washed with cold water. The mother liquor was chilled to 0 °C and a second crop of crystals was collected. The combined yield of biotinylglycylglycine was 298 mg (83%) (mp 235–235.5 °C). The ^1H NMR spectrum was significantly different (more complex) than an authentic sample of biotin.

Biotinylglycylglycine (100 mg, 0.279 mmol) was dissolved in 2.0 mL of dry pyridine, to which was added 1 equiv *p*-nitrophenol (39 mg) and 4 equiv of *p*-nitrophenyl trifluoroacetate (262 mg). The resulting solution was heated for 9 h at 50 °C and then diluted with Et_2O in order to precipitate the product. The precipitate was collected by filtration, washed with Et_2O, resuspended in Et_2O and again isolated by filtration, and finally dried in vacuo. Yield of white product was 97 mg. This ester is quite reactive (readily undergoes transesterification) and was used immediately in the subsequent reaction.

Compound **72** (92 mg, 0.286 mmol) was dissolved in 0.1 mL of acetic acid (50 °C) to which was added 97 mg of the *p*-nitrophenyl biotinylglycylglycinate. The solution was heated to 60 °C for 12 h, cooled, and diluted with aqueous HCl. Most of the precipitate dissolved and the remainder was removed by filtration (5 mg). The solution was neutralized with concentrated NH_4OH and cooled, and the solid was collected by filtration. The solid was dissolved in hot $CHCl_3$, which was then dried over Na_2SO_4, filtered through K_2CO_3, and applied to a column of silica gel. Unreacted compound **72** eluted with 4% EtOH/$CHCl_3$ and the desired product with 10% MeOH/$CHCl_3$. The solid product was dissolved in aqueous HCl, precipitated with NH_4OH, collected by filtration, and dried in vacuo. Yield of product (FAB MS) was 9 mg (7%).

(±)-*N*-(*trans*-2-Hydroxycyclohexyl)norcodeine (82). Synthesis was as for vesamicol, using norcodeine and a 5-fold excess of cyclohexene oxide for 24 h at 60 °C. The product mixture was chromatographed on silica gel and eluted with $CHCl_3$/EtOH. The product was dissolved in EtOH, converted to the hydrochloride with HCl gas, and then crystallized from EtOH/Et_2O at −20 °C. A mass spectrum (FAB) of the product exhibited only one mass at 384 (P + 1).

(±)-*N*-(*trans*-2-Hydroxycyclohexyl)noroxymorphone (83). Noroxymorphone (50 mg, 0.17 mmol) was heated to reflux for 24 h in 3 mL of *n*-PrOH containing 1 mL (a large excess) of cyclohexene oxide. The solution was taken to dryness and the residue chromatographed on silica gel. The free base was dissolved in $CHCl_3$/Et_2O and precipitated with anhydrous HBr. The precipitate was collected by filtration, washed with Et_2O, and crystallized from EtOH/Et_2O at −20 °C. A mass spectrum (FAB) of the product exhibited only two significant masses at 386 (P + 1) and 367 (P − H_2O).

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Supplementary Material Available: Atomic positions, thermal parameters, bond distances, and bond angles for the (*S*,*S*)-vesamicol cation, hydrogen (*S*,*S*)-di-*p*-toluoyltartrate anion (Tables IV–VII), and copies of high-field NMR spectra of vesamicol and vesamicol hydrochloride, as well as of compounds **39**, **46**, **47**, **54–57**, **69**, **71**, and **72** (33 pages). Ordering information is given on any current masthead page.