Proof of the assigned structures took the form of infrared (IR), ^1H and ^{13}C NMR, and mass spectrometric analyses. Upon enolization, the long-wavelength carbonyl stretching of progesterone (1672 cm^{-1}) underwent a hypsochromic shift to 1732 cm^{-1}, consistent with the formation of **1a**. The 17-acetyl moiety is unaffected by this manipulation. In the ^1H NMR spectra, the proton attached to the central carbon of the α,β-unsaturated ketone is shifted downfield by δ 0.23 and a second proton (δ 5.44) attached to an unsaturated carbon appeared. The ^{13}C NMR reveals alterations consistent with the assigned conversion including the appearance of an ester carbonyl absorbance at δ 163.4, shielding of the 3-pregnane carbon (δ 197.3 → 146.7), and appearance of an additional unsaturated (pregnane C-6) carbon (δ 117.3). Similar changes were manifested upon enolization in medroxyprogesterone acetate and hydrocortisone 21-acetate. Compound **2a** was also examined by electron impact (EI) mass spectrometry. Proposed fragmentation patterns are given in Scheme II. On the basis of the spectral interpretation of the parent steroid,[22] a mass shift of m/z 105 was observed for structurally significant ions, consistent with the structure proposed for the 3-enol nicotinate. Spectroscopic analyses of 1b[18] and **1a**[19] were consistent with their assigned structures.

The conversion of 1b to the parent compound was examined in various biological matrices including whole human blood, whole rat blood, rat liver homogenate, and rat brain homogenate.[23] As shown in Table I, hydrolysis of 1b to 1 occurred at varying rates in various media. Conversion of 1b was faster in rat blood than human blood, consistent with the high enzymatic activity of the rodent systems. Compound **1c** was relatively stable in human blood but converted to the quaternary salt with a $t_{1/2}$ of 16 min in rat liver homogenate and about 1.7 h in brain homogenate.

(21) ^1H and ^{13}C NMR and MS assignments for **3a** were given as follows: ^1H NMR (300 MHz, DMSO-d_6) δ 9.16 (d, 1 H, pyridine C-2), 8.84 (m, 1 H, pyridine C-6), 8.34 (d, 1 H, pyridine C-4), 7.57 (m, 1 H, pyridine C-5), 5.93 (s, 1 H, alkenic (4) proton), 5.50 (s, 1 H, alkenic (6) proton), 4.95 (q, 2 H, 21-CH_2, J = 8 Hz), 2.10 (s, 3 H, CH_3CO), 1.18 (s, 3 H, 19-CH_3), 0.52 (s, 3 H, 18-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) 205.45 (pregnane C-20), 169.69 (acetate carbonyl)*, 163.17 (nicotinate carbonyl)*, 153.04 (pyridine C-2), 150.33 (pyridine C-6), 146.84 (pregnane C-3), 137.09 (pregnane C-4), 136.98 (pyridine C-4), 136.82 (pregnane C-5), 125.18 (pyridine C-3), 123.77 (pyridine C-5), 116.38 (pregnane C-6), 67.79 (pregnane C-11), 48.99 (pregnane C-21), 46.35 (pregnane C-9); MS m/z (EI, 70 eV) 491 ($M^+ - H_2O$), 390 ($M^+ - H_2O - COCH_2OCOCH_3$), 372 (390 − H_2O), 332 (M^+ − D ring − H_2O), 267 (M^+ − C and D ring). An asterisk indicates exchangeable assignments.

(22) Zaretskii, Z. *Mass Spectrometry of Steroids*; Wiley: New York, 1976.

(23) Sprague–Dawley rats, Charles Rivers Lboratories, were the source of brain, liver, and blood samples. Freshly obtained brain or liver was homogenized with pH 7.4 phosphate-buffered saline to give a final concentration of 20% w/v. These matrices, as well as rat and human blood, were maintained at 37 °C. The test compound (5×10^{-3} M) was then introduced in a small volume (25 μL) of dimethyl sulfoxide to 2.5 mL of the matrix. After various time intervals, samples (100 μL) of homogenate were withdrawn, treated with cold acetonitrile (400 μL), and centrifuged at 13000g for 5 min. The supernatant was then analyzed by HPLC. The analytical method utilized a Toya Soda ODS 120T C-18 reversed-phase column operating at ambient temperature. The mobile phase consisted of 75:25 acetonitrile–0.05 M KH_2PO_4 and the flow rate was 1.0 mL/min. Under this condition, progesterone (1) eluted at 9.5 min and 1**b** eluted at 5.7 min. Compounds were quantitated with ultraviolet detection at 266 nm. In the case of 1c, a mobile phase containing 90:10 acetonitrile–water was required and the compound had a retention time of 13.1 min. The compound was detected at 360 nm.

These studies indicate that enol esters of α,β-unsaturated ketones are viable bridges for chemical delivery systems. These compounds can be prepared from the ketone and nicotinoyl chloride hydrochloride in the presence of an acid catalyst. The dihydropyridines were shown to be oxidized in biological media to yield the quaternary salts. Likewise, the salts were quickly hydrolyzed to yield the parent compound.

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† This is contribution no. 36 in the series Improved Delivery through Biological Membranes.
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A Radiotracer for Mapping Cholinergic Neurons of the Brain

Sir:

A γ-emitting radiotracer that localizes selectively in vivo in brain cholinergic neurons could provide an externally detectable map of regional neuronal degeneration in Alzheimer's disease. Such a radiopharmaceutical might also be used to monitor the neuronal-sparing efficacy of future drugs developed for treating this devastating disease. Vesamicol is a potent inhibitor of the vesicular sequestration of acetylcholine (Ach).[1-3] Structural studies and kinetic data suggest that vesamicol binds to the outside of the storage vesicle membrane at a locus distinct from the active site of the Ach transporter. The inhibition of Ach storage by vesamicol is stereoselective and noncompetitive, suggesting that a specific receptor exists which can allosterically modulate the Ach transporter.[2] Parsons and co-workers recently reported an elegant study of the Ach blocking activity of 84 analogues of vesamicol.[1] A number of derivatives in the benzovesamicol series were equipotent with vesamicol. Additionally, it was found that the edge of the benzo ring anti to the hydroxyl group in benzovesamicol was a territory of high bulk tolerance; seemingly this region of the compound points away from the receptor surface. In this communication we describe the synthesis and preliminary in vivo screening of racemic 5-[^{125}I]iodobenzovesamicol (IBVM) and its enantiomers as possible mapping agents for central cholinergic neurons.

(1) Rogers, G. A.; Parsons, S. M.; Anderson, D. C.; Nilsson, L. M.; Bahr, B. A.; Kornreich, W. D.; Kaufman, R.; Jacobs, R. S.; Kirtman, B. *J. Med. Chem.* **1989**, *32*, 1217.
(2) Marshall, I. G.; Parsons, S. M. *TINS* **1987**, *10*, 174.
(3) Marien, M. R.; Parsons, S. M.; Altar, C. A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 876.

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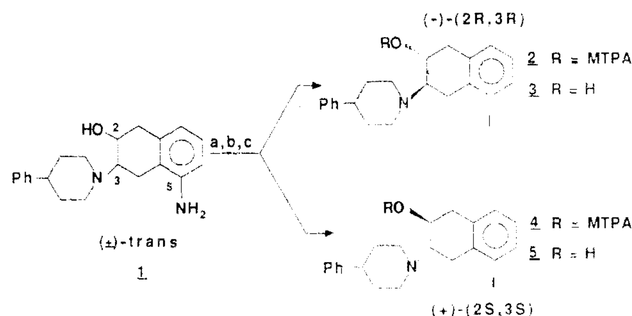
Table I. Radiotracer Concentration in Mouse Tissue 4 h after Intravenous Injection[a]

	concentration, % dose/g (±SC)			% of dose	concentration ratios			
tracer	cerebral cortex	striatum	cerebellum	injected in whole brain	[striatum]/ [cerebellum]	[cortex]/ [cerebellum]	[cortex]/ [blood]	[l atrium]/ [l ventricle]
[^{125}I]-(−)IBVM	2.56 ± 0.21	6.26 ± 0.46	0.23 ± 0.04	1.03 ± 0.07	27.2	11.1	6.2	5.5
[^{125}I]-(±)-IBVM	1.17 ± 0.20	3.29 ± 0.52	0.20 ± 0.04	0.56 ± 0.07	16.5	5.9	2.3	4.7
[^{125}I]-(+)IBVM	0.12 ± 0.01	0.26 ± 0.04	0.06 ± 0.01	0.06 ± 0.01	4.3	2.0	0.3	0.9
[^{3}H]-(−)vesamicol	0.42 ± 0.03	0.40 ± 0.11	0.10 ± 0.02	0.24 ± 0.02	4.0	4.2	0.8	0.1

[a] Female CD-1 mice (n = 4 or 5/tracer) weighing 18–27 g were injected under ether anesthesia via the tail vein with 7.2–10.8 μCi of ^{125}I-labeled tracer or 5.0 μCi of [^{3}H]-(−)vesamicol. Animals were sacrificed 4 h later and tissues were excised, weighed, and counted in an autogamma counter or a liquid-scintillation counter.[14] Tissue concentrations are normalized to a 25 g mouse. Specific activities of the ^{125}I-labeled tracers ranged from 105 to 170 Ci/mmol[15] as determined by comparison of a known amount of radioactivity with a UV standard curve of unlabeled compound. [^{3}H]-(−)-Vesamicol (piperidinyl-*3,4-^{3}H*) was obtained from New England Nuclear; specific activity 57 Ci/mmol.

Scheme I[a]



[a] Reagents: (a) $NaNO_2$/HCl, I_2, KI, 68%; (b) MTPA, 4-(dimethylamino)pyridine (cat.), Et_3N; TLC separation of diastereomers, 90%; (c) 2 N NaOH, MeOH, 89%.

Racemic IBVM[4] was synthesized from (±)-5-aminobenzovesamicol (1)[1] by diazotization and reaction with potassium iodide (Scheme I). Reaction of (±)-IBVM with (−)-(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride[5] gave a mixture of the diastereomeric esters **2** and **4**; separation of the esters was achieved by preparative TLC on silica gel after several developments with hexane/EtOAc (19/1).[6] Base hydrolysis of the MTPA esters provided pure enantiomers **3** and **5**.[7] ^{125}I-labeling of the enantiomers was accomplished by solid-phase exchange at 150 °C with Na[^{125}I] in the presence of ammonium sulfate as promoter.[8] Radiochemical purities of [^{125}I]-**3** and [^{125}I]-**5** following silica Sep-Pak purification were 97% and 92%, respectively, as determined by radio-HPLC.[9] The optical purity of the radiolabeled enantiomers was confirmed before and after exchange labeling by chiral radio-HPLC; no racemization occurred during radiolabeling.[9] The chiral tracers, dissolved in either absolute ethanol or in ethanol/0.005 M sodium acetate (1/9) buffer (pH 4.5) at 4 °C in the dark, showed <1% deiodination for up to 4 weeks at concentrations of approximately 1 mCi/mL.

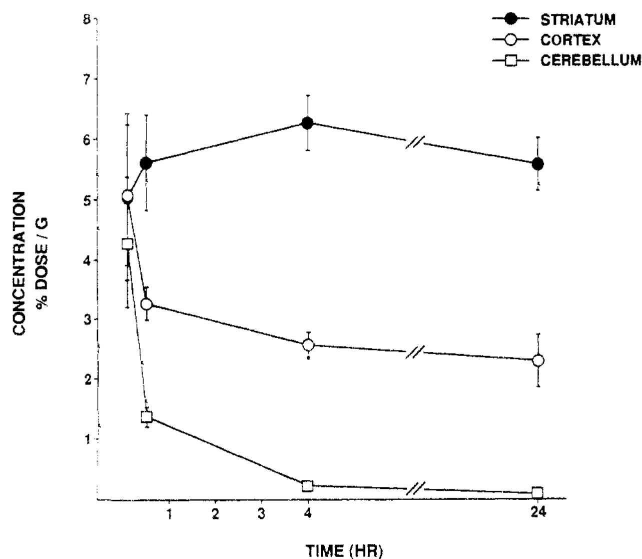


Figure 1. Temporal pattern of distribution of [^{125}I]-(−)-IBVM in brain of female CD-1 mice (n = 4 or 5 per time point). Tissue concentrations are normalized to a 25-g mouse.

Table I compares the accumulation of radioactivity in three regions of mouse brain[10] following tail vein administration of racemic [^{125}I]IBVM and its optical isomers **3** and **5**. The marked accumulation of radioactivity in the striatum and cerebral cortex following intravenous injection of the (−)-enantiomer [^{125}I]-**3** is consistent with the high density of cholinergic innervation in these two brain regions. Choline acetyltransferase (ChAT) activity, the best marker for cholinergic nerves, is present in striatum,

(4) Satisfactory IR, NMR (^{1}H and ^{13}C), mass spectra, and elemental analysis were obtained for (±)-IBVM: white solid; mp 122 °C (recrystallized from ether/hexane); IR (KBr) 3610–3275 (br) 3059, 3025, 2929, 2907, 1645, 1602 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.68–1.97 (m, 4 H), 2.44–3.03 (m, 9 H), 3.25 (d, d, J = 16.1, 5.7 Hz, 1 H), 3.84 (d, d, d, J = 16.1, 10.2, 5.6 Hz, 1 H), 4.3 (br s, OH), 6.84 (t, J = 7.7 Hz, 1 H), 7.10 (d, J = 7.6 Hz, 1 H), 7.19–7.35 (m, 5 H), 7.70 (d, J = 7.8 Hz, 1 H) ppm; ^{13}C NMR ($CDCl_3$, 90.56 MHz) δ 32.88, 33.86, 34.35, 38.36, 42.82, 44.94, 53.61, 65.27, 67.17, 102.92, 126.25, 126.81, 127.78, 128.47, 129.56, 135.87, 137.10, 137.44, 146.06 ppm; MS (EI, 70 eV) m/z (relative intensity) 433 (15.12), 306 (1.73), 231 (1.10), 216 (1.13), 202 (6.32), 186 (1.53), 174 (100.00), 160 (22.81), 146 (6.25), 128 (14.46), 115 (26.41), 91 (21.44); High-resolution MS (EI, 70 eV) calcd for $C_{21}H_{24}INO$ 433.0903, found 433.0910. Anal. Calcd for $C_{21}H_{24}INO$: C, 58.21; H, 5.58; N, 3.23. Found: C, 58.27; H, 5.65; N, 3.29.

(5) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.

(6) (a) For compound **2**: R_f = 0.35 (silica TLC, EtOAc/hexane 1/9); $[\alpha]_D^{23}$ = −56.7° (c = 1.5, EtOH). (b) For compound 4: R_f = 0.41 (silica TLC, EtOAc/hexane 1/9); $[\alpha]_D^{23}$ = +36.7° (c = 1.5, EtOH).

(7) (a) The specific rotation of compound 3 was $[\alpha]_D^{23}$ = −45.3° (c = 1.5, EtOH). (b) The specific rotation of compound 5 was $[\alpha]_D^{23}$ = +44.0° (c = 1.5, EtOH). (c) Optical purities of 3 (>98%) and 5 (>98%) were determined by chiral HPLC using a Chiracel OD column (4.6 × 250 mm) eluted with n-hexane-/2-propanol (9/1) at a flow rate of 1 mL/min with UV detection at 254 nm. Retention times of 3 and 5 were 6.8 and 10.0 min, respectively.

(8) Mangner, T. J.; Wu, J.-L.; Wieland, D. M. *J. Org. Chem.* **1982**, *47*, 1484.

(9) Same chiral HPLC system as described in ref 7 except column effluent was monitored with a Flo-One Model DR/IC radioactivity detector fitted with a 340-μL solid scintillator cell (Radiomatic Instruments, Tampa, FL).

(10) A very similar distribution of [^{125}I]-(±) IBVM has also been observed in rat brain (data not shown).

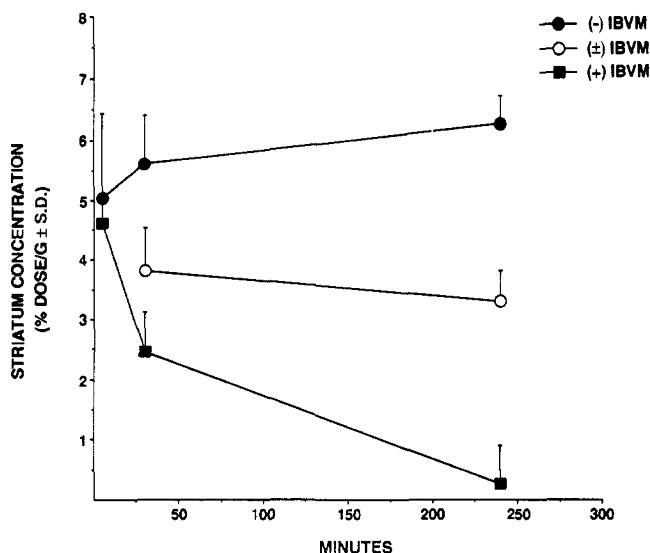


Figure 2. Comparison of the striatum concentrations of ^{125}I-labeled enantiomers of IBVM in female CD-1 mice (n = 4 or 5 animals per time point). Tissue concentrations are normalized to a 25-g mouse.

cerebral cortex, and cerebellum of the adult rat in approximate ratios of 26/9/1, respectively.[11] The low striatal and cortical accumulation of the (+)-enantiomer [^{125}I]-**5** is consistent with the stereospecificity of vesamicol blockade of acetylcholine storage observed in vitro.[1] It is notable that accumulation of radioactivity in the striatum and cortex 4 h after intravenous injection of [^{3}H]-(-)-vesamicol is markedly less than that observed after injection of [^{125}I]-(-)-IBVM. Also, the neuronal selectivity of [^{3}H]-(-)-vesamicol, estimated either by the striatum/cerebellum concentration ratio in the brain or the left atrium/left ventricle concentration ratio in the heart, is substantially lower than that obtained for [^{125}I]-(-)-IBVM. Whether these differences are due to lower in vivo instability of the tritium label or to differences in binding characteristics or metabolic fate of vesamicol itself remains to be determined. Using mouse thyroid radioactivity concentration as an estimate of in vivo deiodination, roughly 2.5% deiodination of [^{125}I]-(-)-IBVM occurs 2 h after intravenous injection (data not shown). Chromatographic analysis of the mouse brain 4 h after injection of [^{125}I]-(-)-IBVM revealed that >95% of the radioactivity is present as unchanged tracer.[12]

Figure 1 shows that from 4 to 24 h after injection of [^{125}I]-**3** the radioactivity in the cerebral cortex and striatum remains at a high and fairly constant level. With the cerebellar concentration during this time period as an approximation of nonneuronal binding, it can be seen from Figure 1 that the specific neuronal binding of [^{125}I]-**3** in cortex and striatum from 4 to 24 h is >95%. Figure 2 compares the time–activity curves of the two labeled enantiomers in mouse striatum. The two tracers show nearly identical initial striatal uptake, but the (+)-isomer rapidly washes out of the striatum and within 4 h is nearly absent from this tissue. If one uses the 4 h cortical or striatal activity of the (+)-enantiomer as an estimate of nonneuronal binding of the (-)-enantiomer in these two brain areas, the same value calculated above (i.e. >95%) is obtained for the 4–24 h neuronal specificity of [^{125}I]-**3**. Further support for the high neuronal specificity of [^{125}I]-**3** can be obtained by comparison of the regional distribution pattern of the enantiomers in heart tissue. The concentrations of Ach and ChAT in mammalian heart indicate a density of cholinergic innervation in the ventricles that is about $^1/_5$ of that in the atria.[13] At 4 h the left atrium/left ventricle concentration ratio of (-)-isomer **3** is 5.5 compared to a ratio of 0.9 for (+)-isomer **5** (Table I).

Pretreatment of mice with unlabeled (-)-vesamicol hydrochloride (605 μg/kg ip) 10 min prior to tracer injection lowers the 4-h striatal and cortical accumulation of [^{125}I]-**3** by 65%. However, this percent decrease is most likely an underestimation of the extent of neuronal binding of [^{125}I]-**3** since larger doses of (-)-vesamicol could not be injected due to toxicity limitations. Also, as indicated in Table I, the retention of [^{3}H]-(-)-vesamicol in brain at 4 h is comparatively low, suggesting that putative blocking doses of vesamicol would likely clear more rapidly from the brain than tracer levels of IBVM. The autoradiographic distribution of racemic [^{125}I]-**3** in rat brain 90 min following intravenous injection parallels the in vitro regional brain distribution of [^{3}H]-(-)-vesamicol.[16] Autoradiography studies in brain-lesioned rats will hopefully confirm the high neuronal specificity of [^{125}I]-**3** suggested by the regional tissue distribution results presented here.

These preliminary studies lend strong support to the possible use of (-)-IBVM labeled with ^{123}I (γ-ray energy = 159 kev; $T_{1/2}$ = 13.3 h) as an imaging agent for assessing the extent of neuronal damage in Alzheimer's disease. Autoradiographic analysis and metabolism studies of [^{125}I]-(-)-IBVM in rat brain and imaging studies in monkeys with [^{123}I]-(-)-IBVM will be described in a future publication.

Acknowledgment. We gratefully acknowledge the National Institutes of Health for support of this research

(11) Stavinoha, W. B.; Weintraub, S. T.; Modak, A. T. *J. Neurochem.* **1974**, *23*, 885.

(12) Two CD-1 mice under light ether anesthesia were each injected intravenously with 400 μCi of [^{125}I]-(-)-IBVM in 0.10 mL of ethanol/acetate buffer (1/9) and sacrificed 4 h later. The brains were removed, frozen at -80 °C, homogenized in a 9-fold volume of acetonitrile for 4 min, and then centrifuged at 10000g for 5 min. Extraction efficiency was 96%. The acetonitrile was concentrated in vacuo, spotted on Whatman silica gel coated glass TLC plates (Whatman K6F, 250 μm), and eluted with $CHCl_3$/ethanol/ammonium hydroxide (97/3/0.1). Radiopeak analysis, performed on a Berthold Model LB2832 TLC linear analyzer, showed that >97% of the radioactivity coeluted with authentic IBVM (R_f = 0.65). Radio-HPLC analysis of the acetonitrile concentrates, using a Waters μBondapak CN column (3.9 × 300 mm) eluted with 0.1 M ammonium acetate/acetonitrile (1/1) at a flow rate of 1 mL/min with UV detection at 254 nm, showed >95% of the radioactivity coeluted with authentic IBVM (t_R = 11.3 min).

(13) Loffelholz, K.; Pappano, A. J. *Pharmacol. Rev.* **1985**, *37*, 1.
(14) Wieland, D. M.; Rosenspire, K. C.; Hutchins, G. D.; Van Dort, M.; Rothley, J. M.; Mislankar, S. G.; Lee, H. T.; Massin, C. C.; Gildersleeve, D. L.; Sherman, P. S.; Schwaiger, M. *J. Med. Chem.*, **1990**, *33*, 956.

(15) Since the specific activity of [^{125}I]-(-)-IBVM was only 105–170 Ci/mmol, a 10 μCi injected dose would contain close to 100 pmol of unlabeled (-)-IBVM. This quantity of (-)-IBVM likely represents a subpharmacological dose level and should not affect the biodistribution pattern of the radiolabeled compound. Support for this, as suggested by one of the referees, was obtained by comparing the regional brain radioactivity concentrations obtained 2 h after intravenous injection of 0.9 μCi to one group of CD-1 mice (n = 5) and 8.1–8.9 μCi (nearly a 10-fold higher mass dose) to a second group (n = 4). The brain radioactivity concentrations in striatum and cerebral cortex of both groups were within one standard deviation. Although the specific activity of [^{125}I]-(-)-IBVM was sufficiently high for preliminary in vivo screening in animals, a no-carrier-added synthesis of [^{123}I]-(-)-IBVM will be employed for clinical studies which should allow specific activities approaching the theoretical maximum of 2.4 × 10^5 Ci/mmol.

(16) Frey, K. A.; Wieland, D. M., unpublished findings.

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Methylsulfamic Acid Esters. A New Chemical Class of Oral Antiarthritic Agents

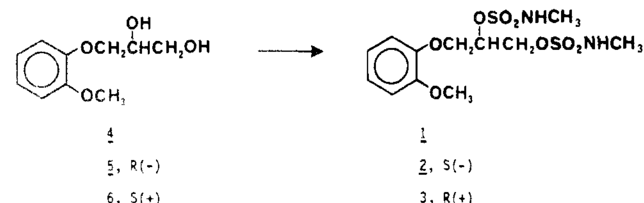
Sir:

Arthritis is a term used to describe a variety of disorders which manifest themselves in painful, inflamed joints.[1] Over the past 40 years therapies have been developed which treat various aspects of the disease states. These therapies include adrenal corticosteroids and the more potent synthetic derivatives; agents which interfere with arachidonic acid metabolism, the nonsteroidal antiinflammatory drugs (NSAIDs); the so-called disease-modifying antiarthritic drugs (DMARDs), the mechanism of action of which remains unclear; and the immunoregulatory agents, which affect the immune response. In this communication, we report on methylsulfamic acid 3-(2-methoxyphenoxy)-2-[[(methylamino)sulfonyl]oxy]propyl ester (**1**) and its optical isomers [**2**, *S*-(–); **3**, *R*-(+)], examples of the sulfamate ester pharmacophore, which have antiarthritic activity in the rat adjuvant-induced arthritis (AA) model[2] and which have a pharmacological profile that suggests that they exert their effects by a mechanism distinct from any agents previously reported.

The synthesis of **1** [mp 53.0–54.5 °C (isopropyl ether/1-propanol)] from commercially available **4** was readily accomplished in 82% yield as depicted in Scheme I. The optical isomers of **4**[3] were used to prepare **2** [mp 50.0–51.5 °C, $[\alpha]^{22}_D = -5.3°$ (c = 1, CH_3OH)] and **3** [mp 50.5–52.0 °C, $[\alpha]^{22}_D = +5.3°$ (c = 1, CH_3OH)]. Compound **4**, **5**, or **6** was dissolved in methylene chloride and treated over a 0.5-h period with simultaneous, dropwise additions of solutions of methylsulfamoyl chloride and diisopropylethylamine in methylene chloride. The reaction mixture was stirred at ambient temperature for an additional 2 h and then purified by column chromatography on silica gel to provide **1**, **2**, or **3**, respectively, as white solids.

Compounds **1**–**4** were tested in the adjuvant-induced arthritic (AA) rat model of chronic inflammation[2] by using a therapeutic dosing regimen[4] (dosing from day 18 through day 50 after adjuvant injection; Table I). Indomethacin at 3.16 mg/kg orally was used as a positive control. At

Scheme I[a]



[a] Reaction conditions: CH_2Cl_2, CH_3NHSO_2Cl, $C_2H_5N[CH(CH_3)_2]_2$, 25 °C, 2.5 h.

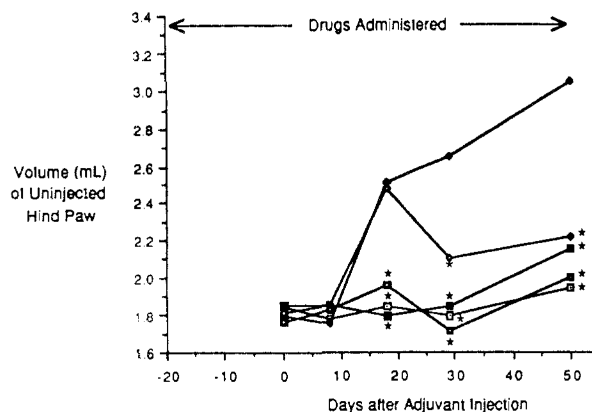


Figure 1. Effect of 1 (◇, 10 mg/kg), indomethacin (□, 3.16 mg/kg), and cyclosporin (■, 10 mg/kg) on the development of adjuvant arthrits in rats (◆, positive control; ⊡; negative control). The drugs were administered once daily to groups of rats beginning 18 days prior to the injection of the adjuvant. The volume of the uninjected hind limb was determined on days 0, 8, 18, 29, and 50 post adjuvant injection. An asterisk indicates that $p < 0.05$, compared to positive-control group, by Dunnett's t test.

doses as low as 3.16 mg/kg, **1** showed significant activity against both edema (volume change of uninjected paw) and bone destruction (X-ray of uninjected joint). Interestingly, the *S*-(–)-isomer (**2**) was active at 10 mg/kg (the results for the 3.16 mg/kg dose of **2** were close to being statistically significant, but a larger number of animals will need to be tested to give statistical results comparable to those of the 3.16 mg/kg dose of **1**) while the *R*-(+)-isomer (**3**) was inactive at 31.6 mg/kg, the highest dose tested. Since **4** had no activity in the adjuvant arthritic rat assay at 100 mg/kg, the antiarthritic activity seen in **1** is not due to **4**, a potential metabolite.

When given daily at 10 mg/kg po, beginning 18 days prior to adjuvant injection, **1** did not inhibit generation of the arthritic state (unlike an immunomodulator or a steroid, but it did suppress the arthritic lesions once they were present (as in the therapeutic dosing regimen) (Figure 1). Histological analysis[5] revealed that **1** at 10 mg/kg po significantly suppressed the following parameters in adjuvant arthritic rats: osteolysis, chrondrolysis, synovitis, connective-tissue proliferation, and cellular inflammatory reaction. In addition, **1** at 10 mg/kg po had no significant effect on serum diagnostic values[6] (electrolytes, enzymes, etc.) of rats dosed from day 18 through day 50 after adjuvant injection or on weight changes in treated rats. These results (lack of effect on the development of arthritis

(1) A current text which gives a good overview of the subject: *Inflammation: Basic Principles and Clinical Correlates*; Gallin, J. I., Goldstein, I. M., Synderman, R., Eds.; Raven: New York, 1988.
(2) Walz, D. T.; DiMartino, M. J.; Misher, A. *J. Pharmacol. Exp. Ther.* **1971**, *178*, 223–231.
(3) Nelson, W. L.; Wennerstrom, J. E.; Sankar, S. R. *J. Org. Chem.* **1977**, *42*, 1006-1012.
(4) Sancilio, L. F.; Reese, D. L.; Cheung, S.; Alphin, R. S. *Agents Actions* **1977**, *7*, 133-144.
(5) Histological analyses on stained sections (from formalin-fixed tissues) were made by Dr. Geoffrey T. Mann, LL.B, M.D., Lauderdale-by-the-Sea, FL. The cellular inflammatory reaction, osteolysis/chrondialysis, connective-tissue proliferation, and synovitis were scored on a 0–3 scale (0 = normal to 3 = marked) in a blinded manner.
(6) Roche Biomedical Laboratories, Richmond, Virginia.

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