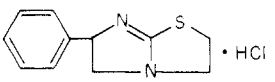
Selenium–Sulfur Analogues. 1. Synthesis and Biochemical Evaluation of Selenotetramisole

Robert N. Hanson,*[1a,b] Roger W. Giese,[1a] Michael A. Davis,[1a,b] and Sheila M. Costello[1a]

Department of Medicinal Chemistry and Pharmacology, College of Pharmacy and Allied Health Professions, Northeastern University, Boston, Massachusetts 02115, and Division of Nuclear Medicine, Department of Radiology, Harvard Medical School, Boston, Massachusetts 02115. *Received November 10, 1977*

(±)-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]selenazole (1-selenotetramisole) was prepared from 2-aminoselenazoline in a three-step synthetic sequence. Resolution with *d*-10-camphorsulfonic acid yielded the optical isomers which were compared with (+)- and (−)-tetramisole as inhibitors of alkaline phosphatase isoenzymes. At 8.7×10^{-5} M the (−) isomer of both tetramisole and 1-selenotetramisole produced significant inhibition of bovine liver and placental isoenzymes but not of calf intestinal or human placental isoenzymes. The (+) isomers demonstrated no inhibition at these concentrations. The similarity in inhibitory activity of the (−) isomers indicates the virtual interchangeability of selenium for sulfur in the thiazolidine ring of the parent drug and the likelihood that ^{75}Se-radiolabeled selenotetramisole can provide an in vivo tracer to tetramisole biodistribution.

The degree to which the biologic activity drugs can be altered by replacing sulfur with selenium has received considerable attention.[2] Our research interests are focused on the preparation of selenium compounds in which the biologic activity is not markedly different from that of the parent sulfur drug. Because of the similarity in the chemical and physical properties of selenium and sulfur and the excellent scintigraphic detectability of the γ-ray emitting ^{75}Se radionuclide, the use of ^{75}Se–selenium analogues facilitates biodistribution and pharmacokinetic studies. In contrast, the labeling of drugs with other γ-emitting radionuclides such as ^{125}I, while maintaining good detection capability, tends to significantly change the biologic activity. Labeling with β emitters, such as ^{14}C or ^{3}H, retains biologic activity but sacrifices the ease with which tissue concentrations can be measured.



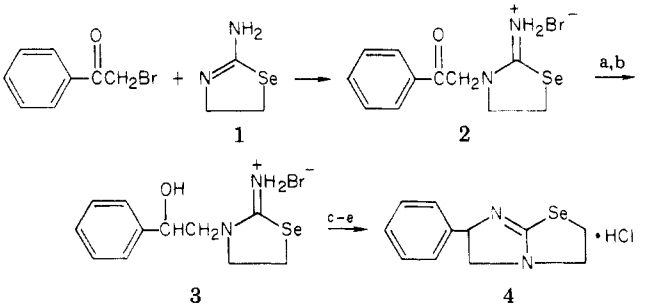
I

Tetramisole (I) is one of the sulfur-containing drugs that we have chosen to investigate initially. This agent differentially inhibits isoenzymes of alkaline phosphatase,[3] shows anthelminthic activity,[4] acts as an immunopotentiator,[5] and offers some control of rheumatoid arthritis.[6] In this paper we report the synthesis and resolution of (±)-1-selenotetramisole and a comparison of its effect relative to that of the corresponding tetramisole enantiomers on the enzymatic activity of alkaline phosphatase isoenzymes.

Chemistry. The (±)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]selenazole was synthesized following the procedure described by Raeymaekers et al.[7] for the preparation of tetramisole. The introduction of the selenium atom was accomplished by the substitution of 2-aminoselenazoline, prepared by the procedure described by Chu and Mautner,[8] for 2-aminothiazoline. The synthetic scheme involved the alkylation of 2-aminoselenazoline with α-bromoacetophenone, reduction of the resulting ketone with sodium borohydride, and cyclization of the carbinol with concentrated sulfuric acid (Scheme I). The resolution of the (±)-selenazole 4 was achieved with *d*-10-camphorsulfonic acid by the procedure reported for tetramisole.[9] Conversion to the free base followed by treatment with hydrogen chloride–2-propanol gave the desired (+)- and (−)-selenotetramisole hydrochlorides.

Biological Results and Discussion. The assay concentration of (+)- and (−)-tetramisole and (+)- and (−)-selenotetramisole was maintained at 8.7×10^{-5} M throughout these studies. Preliminary work showed this concentration to produce significant inhibition of the

Scheme I[a]



[a] a = $NaBH_4$; b = HBr; c = H_2SO_4; d = NH_4OH; e = HCl.

Table I. Inhibition of Alkaline Phosphatase Isoenzymes by (−)-Tetramisole and (−)-Selenotetramisole

Sample	Inhibition, %[a] (−)-Tetramisole	Inhibition, %[a] (−)-Selenotetramisole
Human serum		
64% L, 10% B, 26% I[b]	65	70
60% L, 40% B	71	78
86% L, 14% B	56	63
92% L, 8% B	76	80
Bovine liver ALP[c]	67	76
Bovine placenta ALP	75	78
Calf intestine ALP	10	10
Human placenta ALP[d]	0	0

[a] The assay concentration of each inhibitor was 8.7×10^{-5} M. [b] L, B, and I represent the liver, bone, and intestinal isoenzymes of alkaline phosphatase, respectively. [c] ALP is alkaline phosphatase. [d] Dade Enzatrol (Dade Division, American Hospital Supply Corp., Miami, Fla.); the ALP activity is derived from human placenta.[3]

enzymatic activity by the (−) enantiomers in the susceptible samples. No change in percent inhibition was seen for incubation times (samples plus inhibitor) of 0.5, 15, and 35 min; 5 min was arbitrarily selected. The results of the experiments are presented in Table I except for (+)-tetramisole and (+)-selenotetramisole, which were not inhibitory at 8.7×10^{-5} M. Others have reported no inhibition by (+)-tetramisole at concentrations up to 2×10^{-3} M.[10] The strong inhibition by (−)-tetramisole at this concentration for all isoenzymes of alkaline phosphatase investigated except those of calf intestine and human placenta is in agreement with the results of Van Belle,[3] who has reported that (−)-tetramisole is a potent inhibitor of the nonintestinal isoenzymes in a variety of animals. In man, however, both the intestinal and placental isoenzymes are resistant to this inhibitor.

0022-2623/78/1821-0496$01.00/0

(−)-Selenotetramisole has a slightly greater ability than (−)-tetramisole to inhibit the enzymatic activity of the susceptible isoenzymes. This difference was confirmed by repeating the assays not only on the original day but on two subsequent days, each involving the preparation of fresh solutions of the inhibitors.

The configuration of the asymmetric carbon is the most significant factor in determining the inhibitory activity of the tetramisole-like compounds, as (*S*)-(−)/(*R*)-(+) potency ratios of 100–1000/1 are observed.[11] In addition, as demonstrated by Bhargava et al., the nature of the aromatic moiety at the 6 position is important, as indicated by the variation of the IC_{50} values over a 50-fold range.[12] Compared to these effects, the change from the thiazolidine to the selenazolidine ring system represents a minor perturbation. The reason for this small observed difference remains to be defined. Most important for our purposes is the essential equivalence in the biologic activity of (−)-tetramisole and its selenium-containing analogue in this enzyme system. This supports our further biologic evaluation of the latter, including in vivo studies. The preparation of radiolabeled selenotetramisole for biodistribution and pharmacokinetic studies also is underway.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Analyses were performed by Schwartzkopf Microanalytical Laboratory, Woodside, N.Y., and were within ±0.4% of calculated values unless otherwise noted. Spectral data were obtained using a Perkin-Elmer infrared spectrophotometer Model 700 and a Varian T-60 NMR spectrometer. Optical rotations were determined using a Perkin-Elmer polarimeter Model 141. Tetramethylsilane was used as an internal standard for NMR spectra. NMR and IR spectra were consistent with the proposed structures. The kinetic assays for enzymatic activity were carried out on a Gilford 240 spectrophotometer equipped with a Gilford 6050 recorder. The (−)-tetramisole hydrochloride was provided by American Cyanamid Co. (stated purity, 99.7%) and was assayed at 99+% purity [$[\alpha]^{25}_D$ −120.4° (lit. −122.9°)].

Inhibition of Alkaline Phosphatase. Human sera were obtained from SmithKline Laboratory, Waltham, Mass., where the determination of percentage composition of alkaline phosphatase isoenzymes by cellulose acetate electrophoresis was performed according to the commercial procedure of Helena Laboratories, Beaumont, Texas. Samples of pure isoenzymes (calf intestine Type VII, beef liver Type IX, and bovine placenta Type XV) were obtained from Sigma Chemical Co., St. Louis, Mo.

These samples were each diluted with 0.85 M 2-amino-2-methyl-1-propanol, pH 10.3 (MAP), prior to the inhibition assays in order to obtain comparable total levels of alkaline phosphatase activity. The substrate solution was prepared by dissolving 400 mg of *p*-nitrophenyl phosphate in 10.0 mL of 1.5 mM magnesium chloride in water. The assays were conducted at room temperature by simultaneously monitoring the activities in four cuvettes: (A) 1.1 mL of MAP plus 0.05 mL of alkaline phosphatase (ALP) as a blank control; (B) 1.0 mL of MAP plus 0.05 mL of ALP plus 0.1 mL of substrate solution for total activity; (C) 1.0 mL of MAP containing tetramisole plus 0.05 mL of ALP plus 0.1 mL of substrate solution for tetramisole inhibition; and (D) 1.0 mL of MAP containing selenotetramisole plus 0.05 mL of ALP plus 0.1 mL of substrate for selenotetramisole inhibition.

The order of addition was as cited; mixing followed by a 5-min incubation period was carried out after sample addition, prior to the addition of substrate. The rate of increased absorbance was recorded at 404 nm over the initial 6–8 min and was found to be completely linear in all cases. The degree of inhibition was calculated by dividing the average rate in each cuvette containing the inhibitor by the average total activity obtained in cuvette B for each set of repeated assays. The results of repeated assay agreed to within ±2%.

Chemical Syntheses. 2-Imino-3-phenacylselenazolidine Hydrobromide (2). To a solution of 5.16 g (34.2 mmol) of 2-aminoselenazoline in 100 mL of acetonitrile was added portionwise 7.1 g (35 mmol) of α-bromoacetophenone. The reaction mixture was heated at reflux for 1 h and then cooled. The product was collected by filtration, washed with acetonitrile, and air-dried to yield 10.31 g (29.5 mmol, 86%) of a white solid, mp 194.5–195.5 °C. Anal. ($C_{11}H_{13}N_2OSeBr$) C, H, N, Se.

(±)-2-Imino-3-(2-phenyl-2-hydroxyethyl)selenazolidine Hydrobromide (3). To a stirred suspension of 5.26 g (15.0 mmol) of **2** in methanol at 5 °C was added portionwise 0.63 g of sodium borohydride. After 1 h the reaction mixture was concentrated under reduced pressure to a white solid. The solid was dissolved in 100 mL of hot, 1 M HBr and the solution was filtered. The hydrobromide salt crystallized upon cooling to yield 4.66 g (13.2 mmol, 88%) of a colorless solid, mp 187.0–189.0 °C. Anal. ($C_{11}H_{15}N_2OSeBr$) C, H, N, Se.

(±)-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]selenazole Hydrochloride (4). To 2.5 mL of H_2SO_4, cooled to 0–5 °C, was added 1.77 g (5.0 mmol) of **3**. The mixture was warmed to ambient temperature and stirred for 1 h. The solution was poured over ice and made basic with ammonium hydroxide, and the resulting suspension was extracted with chloroform. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The resulting solid was dissolved in acetonitrile and HCl–2-propanol was added. The colorless hydrochloride salt that precipitated was collected by filtration, washed with acetonitrile, and air-dried to yield 1.09 g (3.8 mmol, 76%) with mp 270–274 °C. Anal. ($C_{11}H_{13}N_2SeCl$) C, H, N, Se.

(+)-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]selenazole (+)-10-Camphorsulfonate (5). A warm stirred solution of the free base of **4** (2.49 g, 9.9 mmol) and (+)-10-camphorsulfonic acid (2.31 g, 9.95 mmol) in 20 mL of chloroform was allowed to cool and stand at −20 °C overnight. The crystals which formed were collected by filtration, washed with 5 mL of cold chloroform, and air-dried for 24 h to yield 2.25 g (94%) of the salt, mp 158–161 °C. Recrystallization from chloroform gave an analytical sample: mp 159–162 °C; $[\alpha]^{25}_D$ +80.1° (*c* 2.2, H_2O). Anal. ($C_{21}H_{26}N_2O_4SSe$) C, H; N: calcd, 5.79; found, 6.35.

(−)-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]selenazole (+)-10-Camphorsulfonate (6). The mother liquor from the above preparation was concentrated to a syrup, treated with 15 mL of hot acetone, and heated at reflux for 10 min. The precipitate that formed initially was removed by filtration and washed with 5 mL of hot acetone to yield 0.46 g (10%) of crude (±) salts, mp 200–210 °C. The acetone filtrate was heated to reflux and then cooled at −28 °C for 16 h. The precipitate that formed was collected by filtration, washed with cold acetone, and air-dried to yield 1.60 g (67%) of the salt, mp 145–149 °C. Recrystallization from acetone gave an analytical sample: mp 154–156 °C; $[\alpha]^{25}_D$ −49.0° (*c* 1.5, H_2O). Anal. ($C_{21}H_{26}N_2O_4SSe$) C, H, N.

(+)-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]selenazole Hydrochloride (4). To a solution of 0.483 g (1.0 mmol) of the (+)-10-camphorsulfonate salt **5** in 10 mL of H_2O was added 2 mL of 1 N Na_2CO_3. The turbid mixture was extracted with chloroform; the organic phase was dried over $MgSO_4$ and evaporated to dryness. The colorless oil was dissolved in acetone and the hydrochloride salt formed by the addition of HCl–2-propanol. The product was collected by filtration, washed with cold acetone, and air-dried overnight to yield 0.243 g (0.83 mmol, 83%) of (+)-**4**: mp 237.0–238.5 °C; $[\alpha]^{25}_D$ +112.8° (*c* 0.9, H_2O). Anal. ($C_{11}H_{13}N_2SeCl$) C, H, N.

(−)-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]selenazole Hydrochloride (4). The (−)-**4** was prepared from the (+)-10-camphorsulfonate salt **6** by the same method: mp 236.5–238.5 °C; $[\alpha]^{25}_D$ −110.0° (*c* 0.5, H_2O). Anal. ($C_{11}H_{13}N_2SeCl$) C, H, N.

Acknowledgment. The authors wish to thank Dr. Stephen Dungan and Mr. Patrick Burns of SmithKline Laboratory in Waltham, Mass., for providing us with the serum samples and results of electrophoretic analysis. This work has been supported in part by NIH Grants CA 19898 and GM 18674, ERDA Contract E(11-1)4115, given to Harvard Medical School, and HEW Grant RR07143 given to Northeastern University.

References and Notes

(1) (a) Northeastern University; (b) Harvard Medical School.
(2) (a) D. L. Klayman, "Organic Selenium Compounds: Their

Chemistry and Biology", D. L. Klayman and W. H. H. Gunther, Ed., Wiley, New York, N.Y., 1973, p 727; (b) T. C. Stadtman, *Science*, **183**, 915 (1974).
(3) H. Van Belle, M. E. DeBroe, and R. J. Wieme, *Clin. Chem.*, **23**, 454 (1977).
(4) D. Thienpont, O. F. J. Vanparijs, A. H. M. Raeymaekers, J. Vandenberk, P. J. A. Demoen, F. T. N. Allewijn, R. P. H. Marsboom, C. J. E. Niemeggers, K. H. L. Schellekens, and P. A. J. Janssen, *Nature (London)*, **209**, 1084 (1966).
(5) F. G. vonStaber, *Fortschr. Med.*, **94**, 791 (1976).
(6) M. Rosenthal et al., *Scand. J. Rheumatol.*, **5**, 216 (1976).
(7) A. H. M. Raeymaekers, F. T. N. Allewijn, J. Vandenberk, P. J. A. Demoen, T. T. T. Van Offenwert, and P. A. J. Janssen, *J. Med. Chem.*, **9**, 545 (1966).
(8) S. H. Chu and H. G. Mautner, *J. Org. Chem.*, **27**, 2899 (1962).
(9) M. W. Bullock, J. J. Hand, and E. Waletzky, *J. Med. Chem.*, **11**, 169 (1968).
(10) H. Van Belle, *Clin. Chem.*, **22**, 972 (1976).
(11) H. Van Belle, *Biochim. Biophys. Acta*, **289**, 158 (1972).
(12) K. K. Bhargava, M. H. Lee, Y.-M. Huang, L. S. Cunningham, K. C. Agrawal, and A. C. Sartorelli, *J. Med. Chem.*, **20**, 563 (1977).

Book Reviews

Liquid Chromatography of Polymers and Related Materials. Edited by Jack Cazes. Marcel Dekker, New York, N.Y. 1977. viii + 180 pp. 16 × 23 cm. $19.75.

In general, this is a good reference work for researchers working on applications of liquid chromatography to polymers and related materials. The topics and quality of the various chapters in this book are both of mixed interest and quality since this volume is reporting on the proceedings of an international symposium in this subject area. This reviewer found particularly useful the chapters on "The Gel Permeation Chromatography of Oligomers" by Ambler and Mate and the chapter on "Chromatographic Analysis of Epoxy Resins" by Crabtree and Hewitt. The latter chapter, in addition to discussing standard gel permeation chromatography (GPC), also contains an excellent presentation of the applications of high-pressure liquid chromatography (HPLC), particularly reverse-phase techniques. Some of the chapters, such as that on "Intrinsic Viscosity by Gel Permeation Chromatography: Method and Application", by M. Y. Hellman, are highly theoretical and may be of less interest to the reader primarily interested in the utilization of GPC or HPLC to the analysis of polymers. In any event, this volume should provide useful reading to most workers interested in the characterization of polymers and should certainly be in every polymer reference library.

Research Triangle Institute **Monroe E. Wall**

Drug Metabolism—From Microbe to Man. Edited by D. V. Parke and R. L. Smith. Taylor and Francis, Ltd., London, and Crane, Russak & Co., New York, N.Y. 1977. xii + 460 pp. 18 × 25.5 cm. $57.50.

In April of 1976, a group of prominent research scientists participated in a symposium which was organized in honor of Professor R. T. Williams. The proceedings of the symposium are presented in this volume which consists of 22 chapters covering a wide range of topics, followed by some brief observations by Professor Williams on future developments in the field of drug metabolism.

Book reviewers frequently feel compelled to note that the title of a given book is rather misleading. Such is not the case with this book. The topics include, among others, metabolism in fish, birds, plants, microbes, and man, as well as mechanisms of drug metabolism. The considerable emphasis given to the troublesome area of species variation is a welcome feature. The chapters are 10–25 pages in length and tend to serve as reviews of the state of various subareas of xenobiotic metabolism rather than as presentations of recent laboratory data or new hypotheses, although several authors do include data which had not been published at the time of the symposium. Most chapters are accompanied by 2–4 pages of pertinent references.

Several of the subject areas (e.g., conjugations, nitrogen oxidation, carbon oxidation, pharmacokinetics, and pharmacogenetics) are among those which have been reviewed in greater depth by many of the same authors in other recent books, symposia proceedings, and review journals. Therefore, their presence here may not be sufficient reason for individuals to purchase the book for their personal libraries. However, the chapters on xenobiotic metabolism in plants, fish, invertebrates, and various mammalian species contain useful information which is not frequently reviewed elsewhere, rendering this volume a particularly useful reference source. It therefore deserves a place in the libraries of institutions which house active research programs in drug metabolism.

As befits a tribute to such a notable individual, the book has been carefully produced. It contains both a subject index and an author index and is marred by few typographical errors. Thanks are due the editors and publisher for providing us with the symposium proceedings within a reasonable length of time without resorting to the use of photoreproductions of the authors' typescripts. Presumably, discussions of the presentations took place at the symposium, but these are not included in the proceedings. In conclusion, it can be stated that the editors and symposium organizers have achieved their declared goal of presenting a "worthy and fitting tribute" to R. T. Williams.

University of Minnesota **Patrick E. Hanna**

Advances in Chromatography. Volume 13. Edited by J. C. Giddings, E. Grushka, R. A. Keller, and J. Cazes. Marcel Dekker, New York, N.Y. 1975. xiv + 324 pp. 15 × 23.5 cm. $28.50.

Volume 13 of this distinguished series probably will leave the reader wondering if the title of the series might be more appropriately named, "Advances in Physicochemical Chromatography". Of the six chapters contained in this volume, four pertain to the use of chromatography to generate physicochemical data, to the application of physical chemistry principles to effect molecular separations by the use of supercritical fluids, or to interpret GPC elution curves.

The initial chapter coauthored by Gouw and Jentoft represents the fifth review of the use of supercritical fluids in chromatographic separations in a 3-year period. Unfortunately, such literary attention overemphasizes the relative importance of the field which is wanning due to the rapid development of high-pressure liquid chromatography. However, the principles and experimental observations set forth in this chapter may be applicable to the application of supercritical fluids in the solvent extraction of thermally labile substances and in the production of high-purity organic chemicals, such as pharmaceuticals, free from residual solvents.

The largest chapter in this volume by Altgelt and Gouw concerns itself with the chromatography of petroleum fractions. The extensive theoretical treatment of liquid–solid chromatography is probably not warranted here and violates the authors statement on p 73 of the text. The authors also attempt to give a small primer on ion exchange, in terms of experimental techniques, which probably is not germane to the title subject.