ATP compound 1 contained an equimolar amount of $MgSO_4$. Apparent enzyme–inhibitor dissociation constants (K_i values; for competitive inhibitors) and I_{50} values (for noncompetitive inhibitors) were obtained from replots of inhibitor concentrations vs. slopes of the Lineweaver–Burk plots. The K_i or I_{50} values were reproducible to within ±12%. The amount of inhibition remained unchanged when the levels of pyruvate kinase and lactate dehydrogenase were increased twofold, showing that the inhibitory effect was exerted solely on the adenylate kinase.

Acknowledgment. This work was supported by Public Health Service Research Grant CA-11196 from the National Cancer Institute and by grants to The Institute for Cancer Research (USPHS Grants CA-06927 and RR-05539 and an appropriation from the Commonwealth of Pennsylvania).

References and Notes

(1) For Paper 2 of this series, see: A. Hampton, F. Kappler, and R. R. Chawla, *J. Med. Chem.*, **22**, preceding paper in this issue.
(2) W. E. Criss, G. Litwack, H. P. Morris, and S. Weinhouse, *Cancer Res.*, **30**, 370 (1970).
(3) V. Sapico, G. Litwack, and W. E. Criss, *Biochim. Biophys. Acta*, **258**, 436 (1972).
(4) A. Hampton, F. Kappler, M. Maeda, and A. D. Patel, *J. Med. Chem.*, **21**, 1137 (1978).
(5) A. Hampton, L. A. Slotin, and R. R. Chawla, *J. Med. Chem.*, **19**, 1279 (1976).
(6) M. Ikehara and S. Uesugi, *Chem. Pharm. Bull.*, 17(2), 348 (1969).
(7) A. Hampton and K. Nealy, unpublished results.

Notes

Syntheses and Activities of Sulfur and Selenium Isosteric Substitution Analogues of Retinol

Steven C. Welch* and John M. Gruber

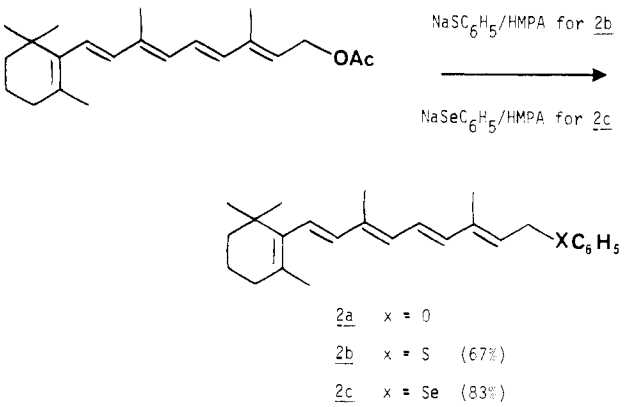
Department of Chemistry, University of Houston, Houston, Texas 77004. *Received July 11, 1979*

The syntheses of sulfur and selenium isosteric substitution analogues of retinol, namely, retinyl phenyl thioether (**2b**), retinyl phenyl selenoether (**2c**), and retinyl thioacetate (**2e**) are described. These retinoid derivatives were examined for activity in terms of "chemoprevention" of cancer by measuring the reverse keratinization of epithelial cells in vitro. Retinoid analogues **2b**, **2c**, and **2e** were found to be active in 20, 80, and 33.3% of the cultures, respectively, as compared to 72.7% activity for *trans*-retinol.

The active form of vitamin A (retinol) appears to differ depending on the target tissues.[1] Retinol, which is required for healthy reproductive functions,[2] is reversibly oxidized to retinal, which is utilized in visual proteins as photoreceptor molecules.[3] Retinal is then further oxidized, irreversibly, to retinoic acid which exhibits hormonal-like properties in the control of the normal growth, development, and differentiation of epithelial tissues.[4-6] These epithelial tissues make up the membranes that cover, enclose, and protect the major organs of the body. Well over half of cancer begins in these epithelial tissues. Natural retinoids, as well as synthetic retinoid analogues, have been shown to prevent or delay the onset of certain forms of epithelial cancer, such as, bladder, breast, lung, and skin cancer in animals, which previously were given doses of chemical carcinogens.[7-12] Natural retinoids have limited usefulness for "chemoprevention"[13] of cancer, because of excessive toxicity and inadequate tissue distribution. Therefore, it would be advantageous to explore the possibility of utilizing new synthetic retinoid derivatives with better therapeutic indexes and pharmokinetics in order to prevent or delay the onset of epithelial malignancies. With this background information in mind, sulfur and selenium analogues, retinyl phenyl thioether (**2b**), retinyl phenyl selenoether (**2c**), and retinyl thioacetate (**2e**) depicted in Schemes I and II, were synthesized and examined by tracheal organ culture in order to determine what effect isosteric substitution would have on activity.

Both retinyl phenyl thioether (**2b**) and retinyl phenyl selenoether (**2c**) are conveniently prepared (Scheme I) by substitution nucleophilic bimolecular reactions of sodium thiophenoxide or sodium phenylselenide, respectively,

Scheme I



upon retinyl acetate (**1**) in hexamethylphosphoric triamide. Both sodium thiophenoxide and sodium phenylselenide are good nucleophiles, and hexamethylphosphoric triamide is an excellent solvent for S_N2 reactions.[14]

The synthesis of *trans*-retinyl thioacetate (**2e**) employs the Wittig reaction[15] (Scheme II). Treatment of the Wittig salt **3**[16] with 1 equiv of *n*-butyllithium in anhydrous tetrahydrofuran, followed by the addition of freshly prepared 2-methyl-4-(thioacetyl)-2-(*E*)-butenal (**5**), affords both 11-*cis*-retinyl thioacetate (**2d**) and 11-*trans*-retinyl thioacetate (**2e**). Aldehyde **5** was prepared from 2-methyl-4-chloro-2-(*E*)-butenal[17] by displacement of the chlorine substituent with potassium thioacetate in ethanol.

These sulfur and selenium isosteric substitution analogues of retinol were examined for activity by an in vitro hamster tracheal organ culture assay.[18] This tracheal organ

0022-2623/79/1822-1532$01.00/0 © 1979 American Chemical Society

Scheme II

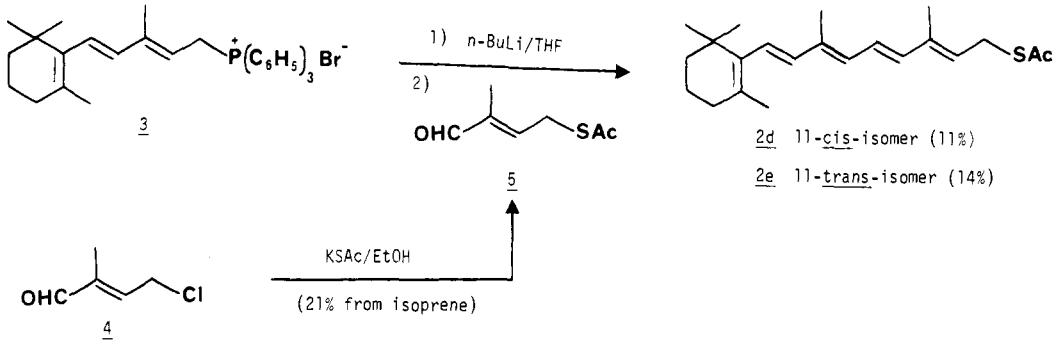


Table I. Tracheal Organ Culture Assay of Retinoids 2b,c,e

retinoid	no.	concn, M	active/total cultures, %[a]
trans-retinol[b]		10^{-8}	16/22 (72.7)
trans-retinyl acetate[b]	1	10^{-8}	31/31 (100)
trans-retinyl phenyl ether[b]	2a	10^{-8}	1/11 (9.1)
trans-retinyl phenyl thioether	2b	10^{-8}	1/5 (20.0)
trans-retinyl phenyl selenoether	2c	10^{-8}	4/5 (80.0)
trans-retinyl thioacetate	2e	10^{-8}	2/6 (33.3)

[a] See ref 18. [b] See ref 20.

culture assay measures the instrinsic ability of retinoids to control epithelial cell differentiation by determining reverse keratinization. Tracheas are removed from hamsters that are in very early stages of vitamin A deficiency and placed in organ cultures.[18] For 3 days the tracheas are grown in a medium containing no retinoid. After that time, the tracheas are treated with each synthetic retinoid analogue in dimethyl sulfoxide[19] with appropriate control cultures. The tracheal organ cultures were scored by microscopic examination for the presence of keratin and keratohyaline granules. Approximately 90% of the control cultures that receive no retinoids have keratin and keratohyaline granules present. The retinoid analogues are scored as active if neither keratin nor keratohyaline granules are seen or if keratohyaline granules alone are present.[18]

As can be seen from the data listed in Table I,[18-20] *trans*-retinyl phenyl ether (**2a**)[20] is active in only 9.1% of the cultures and *trans*-retinyl phenyl thioether (**2b**) is active in 20% of the cultures, whereas *trans*-retinyl phenyl selenoether (**2c**) is active in 80% of the cultures at 10^{-8} M. *trans*-Retinyl thioacetate (**2e**) was only active in 33.3% of the cultures. These trachael organ culture assays listed in Table I are extremely sensitive. They measure the intrinsic abilities of these synthetic retinoids to control epithelial cell differentiation. It is interesting to note that as the isosteric substitution proceeds down group 6A of the periodic table from oxygen (**2a**), to sulfur (**2b**), to selenium (**2c**) the activity increases dramatically. In fact, *trans*-retinyl phenyl selenoether (**2c**) is slightly more active than *trans*-retinyl acetate (**1**). Selenoether **2c** is expected to be a very lipiophilic retinoid which would have a tendency to localize in organs with high concentrations of adipose tissue; however, only further studies on the toxicology and pharmokinetics of this analogue will either prove or disprove its usefulness as a cancer "chemopreventative" agent.

Experimental Section

Materials and Techniques. Melting points were determined on a Büchi melting point apparatus and are uncorrected. Analyses were performed by Spang Micro-analytical laboratory, Eagle Harbor, Mich. Silica gel 60, F-254 (E. Merck no. 5765), and silica gel 60 (E. Merck no. 7734, 70–230 mesh), available from Brinkmann Instruments, were used for thin-layer and column chromatography, respectively. Ultraviolet (UV) spectra were recorded on a Cary-14 spectrometer in 95% ethanol. Infrared (IR) spectra were recorded on a Perkin-Elmer 237B spectrometer in spectroquality solvents as 10% solutions using 0.10-mm sodium chloride cells or as thin films between sodium chloride crystals. Nuclear magnetic resonance (NMR) spectra were measured on Varian Associates Models T-60 and XL-100 spectrometers. High-resolution mass spectra (HRMS) were recorded on Dupont Flash CEC 21-110B spectrometer at 70 eV and low-resolution mass spectra (LRMS) were recorded on a Finnigan 3000 spectrometer at 25 eV. Vinyl-β-ionol, which is utilized in the synthesis of the Wittig salt **3**,[16] was obtained from BASF Aktiengesellschaft, 6700 Ludwigshafen am Rhein, Germany. Retinyl acetate was obtained from Hoffmann-La Roche, Nutley, N.J. For all reactions performed under an atmosphere of dry nitrogen, the equipment was dried in an oven at 120 °C for several hours and then allowed to cool in an atmosphere of dry nitrogen. All liquid transfers were made with nitrogen-filled syringes. The term "petroleum ether" refers to Baker "analyzed reagent", bp 30–60 °C. The terms "dry ether" and "dry tetrahydrofuran" (THF) refer to purification of commercial anhydrous diethyl ether and tetrahydrofuran by distillation from lithium aluminum hydride under nitrogen. "Dry hexamethylphosphoric triamide" (HMPA) was obtained by vacuum distillation of commercial material from calcium hydride (~40 mesh) on to activated 4Å molecular sieves.

Retinyl Phenyl Thioether (2b). To retinyl acetate (300 mg, 0.915 mmol) and sodium thiophenoxide (300 mg, 2.27 mmol) at 0 °C was added HMPA (2.1 mL). After 1 h at 0 °C, the reaction mixture was allowed to stir at room temperature for 14 h and then diluted with ether (150 mL). The ethereal solution was washed with water (50 mL), saturated sodium bicarbonate solution (2 × 15 mL), and water (30 mL), then dried ($MgSO_4$), and concentrated in vacuo to afford a viscous yellow-orange oil which crystallized upon standing. Recrystallization from hexane/ether gave 230 mg (67%) of ether **2b**: mp 91–94 °C; UV (95% C_2H_5OH) λ_{max} 332 nm (ε 23 000); NMR ($CDCl_3$) δ 1.03 (s, 6 H, C-1 methyls), 1.4–1.8 (m, 4 H), 1.73 (br s, 6 H, C-5 and C-13 methyls), 1.98 (br s, 5 H, C-9 $-CH_3$ and C-4 $-CH_2-$), 3.68 (d, 2 H, J = 8 Hz, C-15 $-CH_2S-$), 5.63 (t, 1 H, J = 8 Hz), 5.9–6.8 (m, 5 H), 7.1–7.6 (m, 6 H, C_6H_5-). Anal. Calcd for $C_{26}H_{34}S$: C, 82.48; H, 9.05. Found: C, 82.38; H, 9.05.

Retinyl Phenyl Selenoether (2c). To retinyl acetate (300 mg, 0.915 mmol) and sodium phenylselenide (300 mg, 1.68 mmol; prepared from diphenyldiselenide and sodium metal in anhydrous THF) at 0 °C was added HMPA (2.1 mL). After 1 h at 0 °C, the reaction mixture was allowed to stir at room temperature for 14 h and then diluted with ether (150 mL). The ethereal solution was washed with water (50 mL), saturated sodium bicarbonate solution (2 × 15 mL), and water (30 mL), then dried ($MgSO_4$), and concentrated in vacuo to afford, after crystallization from hexane/ether, 320 mg (83%) of ether **2c**: mp 68–72 °C; UV (95% C_2H_5OH) λ_{max} 338 nm (ε 30 000); NMR ($CDCl_3$) δ 1.03 (s, 6 H, C-1 methyls), 1.4–1.8 (m, 4 H), 1.64 (s, 3 H, C-13 $-CH_3$), 1.73 (s, 3 H, C-5 $-CH_3$), 1.96 (s, 3 H, C-9 $-CH_3$), 3.70 (d, 2 H, J = 8 Hz, C-15 $-CH_2Se-$), 5.73 (t, 1 H, J = 8 Hz), 6.0–6.8 (m, 5 H), 7.2–7.8 (m, 5 H, C_6H_5-). Anal. Calcd for $C_{26}H_{34}Se$: C, 73.39; H, 8.05. Found: C, 73.16; H, 7.78.

2-Methyl-4-(thioacetyl)-2-(*E*)-butenal (5). To potassium thioacetate [20 mmol; prepared by neutralizing 1.52 g (20 mmol) of thioacetic acid with 1.32 g of 85% potassium hydroxide in 95% ethanol] in 95% ethanol (30 mL) was added 2-methyl-4-chloro-2-(*E*)-butenal [4; 2.1 g, bp 30–42 °C (0.5 mmHg), ~70% pure by NMR][17] dissolved in 95% ethanol (10 mL). After 1 h at room temperature, most of the ethanol was removed in vacuo and the residue was partitioned between ether (200 mL) and saturated sodium bicarbonate solution (100 mL). After separation, the ethereal solution was dried ($MgSO_4$) and concentrated in vacuo, followed by bulb to bulb distillation to afford 0.651 g (21% overall yield from isoprene) of aldehyde **5**: bp 140–150 °C (2.0 mmHg); IR (film) 1690 (br), 1643 cm^{-1}; NMR (CCl_4) δ 1.78 (br s, 3 H, CH_3–), 2.33 (s, 3 H, CH_3CO), 3.72 (d, 2 H, J = 8 Hz, $-CH_2-$), 6.30 (br t, 1 H, J = 8 Hz, –CH=C), 9.35 (s, 1 H –CHO). Anal. Calcd for $C_7H_{10}O_2S$: C, 53.14; H, 6.37. Found: C, 53.16; H, 6.30.

Retinyl Thioacetate (2d,e). To a solution of the C_{15} Wittig salt **3**[16] (300 mg, 0.55 mmol) in anhydrous tetrahydrofuran (5.0 mL) cooled to −78 °C (dry ice–acetone bath) was added *n*-butyllithium (0.34 mL, 0.55 mmol, 1.6 M in hexane). After stirring the solution for 15 min, 2-methyl-4-(thioacetyl)-2-(*E*)-butenal (**5**; 61 mg, 0.385 mmol) dissolved in THF (5.0 mL) was added. The reaction mixture was then allowed to stir at −78 °C for 1 h and then 0 °C for 6 h, followed by the addition of ether (150 mL). The ethereal solution was washed with saturated sodium bicarbonate solution (2 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo. Column chromatography on silica gel using 3% ether/petroleum ether as an eluant gave 14 mg (11%) of cis isomer **2d** and 18 mg (14%) of trans isomer **2e** as yellow oils. The cis isomer **2d** exhibited the following spectral data: UV (95% C_2H_5OH) λ_{max} 327 nm (ϵ 30 000); IR ($CHCl_3$) 1680 cm^{-1}; NMR (100 MHz, $CDCl_3$) δ 1.03 (s, 6 H, C-1 methyls), 1.4–1.8 (m, 4 H), 1.71 (s, 3 H, C-5 $-CH_3$), 1.91 (s, 8 H C-9 and C-13 methyls and C-4 CH_2), 2.33 (s, 3 H, CH_3CO), 3.66 (d, 2 H, J = 8 Hz, $-CH_2S$), 5.55 (t, 1 H, C-14, J = 8 Hz), 5.83 (1 H, C-12), 6.36 (1 H, C-11), 6.51 (1 H, C-10), 6.13 (1 H, C-8), 6.19 (1 H, C-7). MS Calcd for $C_{22}H_{32}OS$: 344.2174. Found: 344.2168; 1.7 ppm error by HRMS.

The trans isomer **2e** exhibited the following spectral properties: UV (95% C_2H_5OH) λ_{max} 330 nm (ϵ 30 000); IR ($CHCl_3$) 1680 cm^{-1}; NMR (100 MHz, $CDCl_3$) δ 1.02 (s, 6 H, C-1 methyls), 1.4–1.8 (m, 4 H), 1.70 (s, 3 H, C-5 $-CH_3$), 1.87 (s, 3 H, C-13 $-CH_3$), 1.94 (s, 3 H, C-9 $-CH_3$), 2.32 (s, 3 H, CH_3CO), 3.68 (d, 2 H, J = 8.2 Hz, $-CH_2S$), 5.52 (t, 1 H, C-14, J = 8 Hz), 6.30 (1 H, C-12), 6.63 (1 H, C-11), 6.12 (1 H, C-10), 6.12 (1 H, C-8), 6.21 (1 H, C-7). This vinyl pattern resembles *trans*-retinyl acetate. MS Calcd for $C_{22}H_{32}OS$: 344.2174. Found: 344.2170; 1.2 ppm error by HRMS.

Acknowledgment. We graciously thank the National Cancer Institute, Division of Cancer Cause and Prevention, for the funds (CP 75935) to support this research program. We also thank Dr. Michael B. Sporn for the tracheal organ culture assay of these synthetic retinoids. We express our sincere gratitude to Hoffmann-La Roche, Nutley, N.J., and BASF, Germany, for generous gifts of retinoid starting materials. We also thank Professor E. Wenkert and Mr. Perry Marriott, Rice University, for the mass spectra.

References and Notes

(1) Marks, J. V. *Vitam. Horm. (N.Y.)* **1974**, *32*, 131–254.
(2) Thompson, J. N.; Mc C. Howell, J.; Pitt, A. J. *Proc. R. Soc. London, Ser. B* **1964**, *159*, 510–535.
(3) Wald, G. *Science* **1968**, *162*, 230–239.
(4) Morton, R. A. *Vitam. Horm.* **1974**, *32*, 155–166.
(5) Pitt, G. A. J. in "Carotenoids", Isler, O., Ed.; Burkhauser Verlag: Basel, 1971; pp 717–742, and references cited therein.
(6) (a) Olson, J.A.; Lakshmanan, M. R. in "The Fat-Soluble Vitamins", DeLuca, H. F.; Suttie, J. W., Ed.; University of Wisconsin Press: Madison, 1970; pp 213–226. (b) Wolf, G.; De Luca, L. *ibid.* pp 257–265. (c) Thompson, J. N. *ibid.* pp 267–281.
(7) Sporn, M. B.; Clamon, G. H.; Dunlop, N. M.; Newton, D. L.; Smith, J. M.; Saffiotti, U. *Nature (London)* **1975**, *253*, 47–50.
(8) Sporn, M. B.; Dunlop, N. M.; Newton, D. L.; Henderson, W. R. *Nature (London)* **1976**, *263* 110–113, and references therein.
(9) Wynder, E. L. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1976**, *35*, 1309–1315.
(10) Sporn, M. B.; Dunlop, N. M.; Newton, D. L.; Smith, J. M. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1976**, *35*, 1332–1338.
(11) Sporn, M. B.; Squire, R. A.; Brown, C. C.; Smith, J. M.; Wenk, M. L.; Springer, S.; *Science* **1977**, *195*, 487–489.
(12) Pawson, B. A.; Cheung, H.-C.; Han, R.-J. L.; Trown, P. W.; Buck, M.; Hansen, R.; Bollag, W.; Ineichen, U.; Pliel, H.; Rüegg, R.; Dunlop, N. M.; Newton, D. L.; Sporn, M. B. *J. Med. Chem.* **1977**, *20*, 918–925.
(13) The term "chemoprevention" simply means the utilization of chemical substances as prophylactic agents in the prevention of disease; see ref 10.
(14) Kelly, T. R.; Dali, H. M.; Tsang, W.-G. *Tetrahedron Lett.* **1977**, 3859–3860.
(15) Maercker, A. *Org. React.* **1964**, *14*, 270–490.
(16) Schwieter, U.; von Planta, C.; Rüegg, R.; Isler, O. *Helv. Chim. Acta* **1962**, *45*, 541–548. The Wittig salt **3** is prepared from vinyl-β-ionol utilizing triphenylphosphonium bromide in methanol.
(17) Eletti-Bianchi, G.; Centini, F.; Re, L. *J. Org. Chem.* **1976**, *41*, 1648–1650.
(18) These retinoid analogues were submitted to Dr. Michael B. Sporn, Chief, Lung Cancer Branch, Division of Cancer Cause and Prevention, National Cancer Institute. See: Newton, D. L.; Henderson, W. R.; Sporn, M. B.; "Structure-Activity Relationships of Retinoids" (Tracheal Organ Culture Assay of Activity of Retinoids), Laboratory of Chemoprevention, Division of Cancer Cause and Prevention, National Cancer Institute: Bethesda, Md., Oct 11, 1978.
(19) The final concentration of Me_2SO in the culture medium is never greater than 0.1%.
(20) These compounds were submitted to Dr. Michael B. Sporn, National Cancer Institute, by Hoffmann-La Roche.