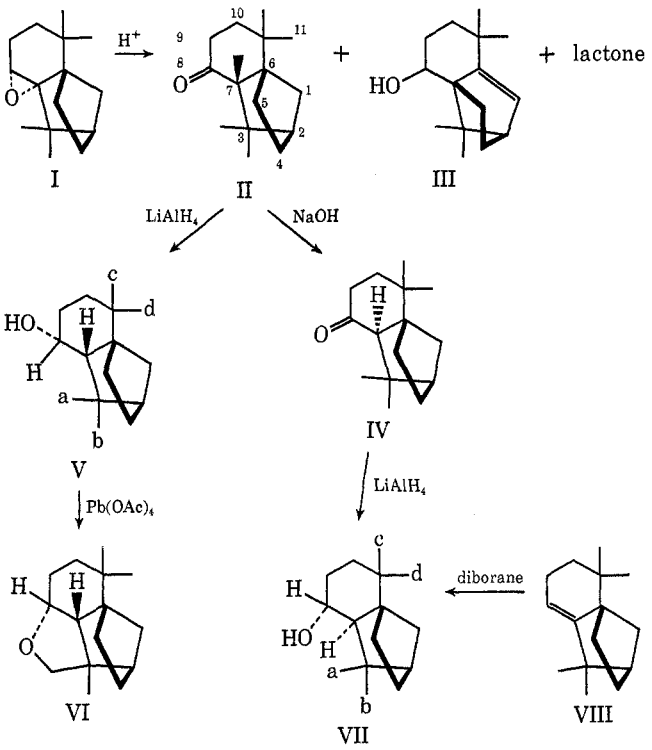
SCHEME I



alcohol VII is further supported by the fact that hydroboration[5] of the isolongifolene VIII gave a compound which was identical in all respects with alcohol VII, mp 124–126° (lit.[5] 122°).

The formation of VII by hydroboration could be attributed to the fact that diborane would preferably attack from the least hindered α side. The stereochemistry of the hydroxyl group at C-8 of alcohols V and VII is further supported by applying the nmr europium complexing technique described by Hinckley[6] and Williams.[7]

We conclude from these results that the more stable epimeric ketone has stereochemistry at C-7, as depicted in structure IV, and the less stable epimer has stereochemistry as shown in structure II. Thus the stereochemistry of the above two ketones and isolongifolene epoxide has been rightly assigned by Dev.[1]

Experimental Section[8]

Isolongifolene Alcohol V.—In a three-necked flask fitted with a condenser, stirrer, and addition funnel were placed 400 ml of dry tetrahydrofuran and 8.2 g (0.215 mol) of lithium aluminum hydride. A solution containing 91 g (0.41 mol) of the isolongifolene ketone II in 100 ml of dry tetrahydrofuran was added dropwise during 45 min to the above mixture. After the addition was over, the mixture was refluxed for 9 hr. The reaction mixture was cooled and 8 ml of water was added slowly followed by 8 ml of 15% sodium hydroxide followed by 24 ml of water. The crude mixture was filtered and the solvent was removed under vacuum. The crude oil was distilled to give a colorless oil: bp 114° (2 mm) (80% yield); infrared (Nujol) λ_{max} 2.94 (OH); nmr ($CDCl_3$) 0.93 $\left(6\text{ H, s, C}<^{CH_3}_{CH_3}\right)$, 1.02 (3 H, s, CCH_3), 1.12 (3 H, s, CCH_3), 1.2–1.73 (12 H, m, CH, CH_2), 4.15 (1 H, broad m, CHOH).

Anal. Calcd for $C_{15}H_{26}O$: C, 81.02; H, 10.98. Found: C, 81.58; H, 11.71.

Isolongifolene Cyclic Ether VI.—In a three-necked flask fitted with stirrer, thermometer, and reflux condenser were placed 11.0 g (0.025 mol) of lead tetraacetate, 5 g of calcium carbonate, and 185 ml of dry benzene. The mixture was refluxed for 1 hr and then 5.5 g (0.025 mol) of isolongifolene alcohol III was added and the mixture was refluxed for 22 hr. It was then cooled to 25°, 10 ml of ethylene glycol was added, and the mixture was heated to 80° for 1 hr. The mixture was then cooled, the solids were filtered off, and the filtrate was washed twice with 25 ml os 1% sodium hydroxide. The combined aqueous layers were extracted twice with ether and the organic layers were combined and dried over magnesium sulfate. The solvent was removed under vacuum. The crude product was distilled to give colorless liquid: bp 100° (0.2 mm) (60% yield); infrared (film) shows no hydroxyl band at 2.93 μ; nmr ($CDCl_3$) 0.95 (3 H, s, CCH_3), 0.99, 1.00 $\left(6\text{ H, s, C}<^{CH_3}_{CH_3}\right)$, 1.08–1.8 (11 H, m, CH_2, CH), 4.15–3.88 (3 H, m, $HCOCH_2$).

Anal. Calcd for $C_{15}H_{24}O$: C, 81.76; H, 10.98. Found: C, 81.48; H, 10.95.

Isolongifolene Alcohol VII.—The lithium aluminum hydride reduction of ketone IV, under the similar conditions as described for alcohol V, gave alcohol VII, mp 124–126°, as a major product in 60% yield. It was found to be identical in all respects with the one obtained by the hydroboration of isolongifolene (Table I).

TABLE I

NMR SPECTRAL DATA OF ALCOHOLS V AND VII COMPLEXES WITH Eu(DPM)$_3$[a]

	Alcohol V		Alcohol VII	
	Δ[Eu-(DPM)$_3$]	R, Å	Δ[Eu-(DPM)$_3$]	R, Å
(a) H_3C–C	2.06	2.5	2.27	3.0
(b) H_3C–C–	0.77	4.0	1.60	3.4
(c) H_3C C–	0.77	4.7	1.04	5.1
(d) CH_3 C–	0.60	4.80	0.64	5.4
HC–O–	4.86	1.35	6.06	1.35

[a] Log-log plot of Δ(Eu) *vs.* R (Å) gives best fit for the configurations assigned to the alcohols V and VII.

Registry No.—II, 29641-13-0; IV, 29461-14-1; V, 30469-89-7; VI, 30545-64-3; VII, 30469-90-0.

Acknowledgment.—The author wishes to express his thanks to Dr. W. I. Taylor for his continued interest and encouragement, to Professor G. Stork for his helpful discussions, and to M. Jacobs for the nmr data. The technical assistance of Mr. R. Santangelo is appreciated.

(5) P. Bani Bai, S. Y. Kamat, B. B. Ghatge, K. K. Chakravarti, and S. C. Battacharyya, *Tetrahedron*, **21**, 629 (1965).

(6) C. C. Hinckley, *J. Amer. Chem. Soc.*, **91**, 5160 (1969).

(7) J. K. M. Sanders and D. H. Williams, *Chem. Commun.*, **7**, 422 (1970).

(8) All the nmr spectra were run on Varian HA-100 spectrometer. TMS was used as an internal standard. The C and H analyses were run by Schwarzkopf Microanalytical Laboratory, N. Y.

Selenomethionine, a Potential Catalytic Antioxidant in Biological Systems[1,2]

RODERICH WALTER* AND J. ROY

Department of Physiology, The Mount Sinai Medical and Graduate Schools of the City University of New York, New York, New York 10029

Received January 20, 1971

This report describes the first isolation and characterization of products resulting from the oxidation of sel-

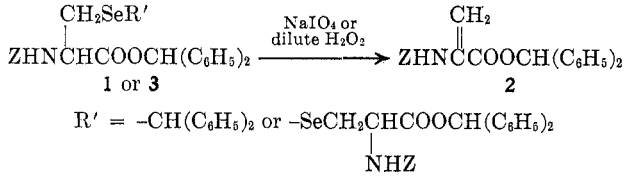
(1) This work was supported by U. S. Public Health Service Grants AM-13567 and AM-10080.

(2) The following abbreviations have been adopted: Z = $C_6H_5CH_2$-OCO; AcOH = acetic acid; MeOH = methanol; EtOAc = ethyl acetate.

enium-containing α-amino acids which possess propionic acid and butyric acid skeletons. Specifically, we investigated the oxidation of selenocysteine, selenocystine, and selenomethionine derivatives by sodium metaperiodate[3] and dilute hydrogen peroxide. Such a study seemed appropriate in view of the conclusion by Caldwell and Tappel[4] that selenocystine is oxidized by hydroperoxides to yield either alanine and metallic selenium or selenocystine diselenoxide, selenocysteine seleninic acid, selenocysteic acid, and alanine and selenate as final products; it was suggested that the conversion of selenocystine to selenocystine diselenoxide is reversible and that selenocystine may express its catalytic antioxidative reactivity in biological systems.

When *N*-carbobenzoxy-*Se*-diphenylmethyl-L-selenocysteine diphenylmethyl ester (**1**) was treated with a slight molar excess of sodium metaperiodate at ice-bath temperature, only starting material was recovered but not the expected selenoxide of **1**. Repetition of the experiment at room temperature likewise did not result in the selenoxide of **1** but rather in the diphenylmethyl ester of *N*-carbobenzoxydehydroalanine (**2**) (Scheme I). Similar results were obtained when **1** was oxidized

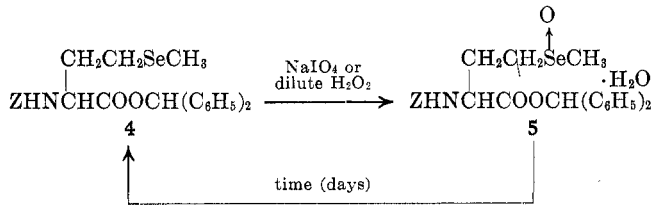
SCHEME I



with an excess of hydrogen peroxide, although the oxidation reaction proceeds even at lower temperature than when sodium metaperiodate was the oxidizing agent. In order to determine whether the diphenylmethyl group alkylating the selenium moiety has any significant influence on dehydroalanine formation, bis(diphenylmethyl) bis(*N*-carbobenzoxy)-L-selenocystinate (**3**) was oxidized with hydrogen peroxide at room temperature. Again the dehydroalanine derivative **2** was the only product. At this point it was concluded that the selenoxide of the β-selenopropionic acid derivative had been formed but that the electron-withdrawing power of the Se → O moiety is so strong as to evoke an instantaneous β elimination.

To test this contention we next subjected a selenomethionine derivative to a similar oxidation procedure, since a Se → O moiety attached to the γ carbon of an α-amino acid should have a greatly reduced tendency for β elimination. Reaction of *N*-carbobenzoxy-DL-selenomethionine diphenylmethyl ester (**4**) with either sodium metaperiodate or hydrogen peroxide gave the corresponding selenoxide (**5**)[5] in practically quantitative yield (Scheme II). Significantly, compound **5** in the dry state or in solution slowly lost the oxygen

SCHEME II



which is attached to the selenium moiety, restoring compound **4**. In acetone this deoxidation proceeds particularly smoothly in terms of purity of product. The mechanism of transformations of **5** to **4** is certainly of interest and warrants further study.

In summary, the intrinsic chemical lability of selenocystine and selenocysteine militates against their role as catalytic antioxidants, while selenomethionine has the potential for such a function in biological systems.

Experimental Section[7]

Oxidation of *N*-Carbobenzoxy-*Se*-diphenylmethyl-L-selenocysteine Diphenylmethyl Ester (**1**). **A.**—*N*-Carbobenzoxy-*Se*-diphenylmethyl-L-selenocysteine diphenylmethyl ester[8] (200 mg) was dissolved in acetone (10 ml). To the stirred solution, cooled in an ice bath, 30% H_2O_2 (0.5 ml) was added. After 45 min of continued stirring at 4° the reaction mixture was diluted with ice-cold water (50 ml) and extracted with EtOAc. The organic layer was separated, washed with water (three 25-ml portions), dried, and concentrated *in vacuo*. Examination of the resultant oil on tlc with C_6H_6–EtOAc (9:1, v/v) as solvent system revealed two compounds when developed with tolidine/KI following chlorination of the amides.[9] The oil was chromatographed on a silica gel column; the two compounds were eluted with *n*-hexane–C_6H_6 (1:3, v/v) (compound **a**) and C_6H_6 (compound **b**), respectively. Compound **a** was crystallized from a mixture of EtOAc–*n*-hexane, yield 75 mg (61.5%), mp 78.5–79.5°. Ir and nmr revealed the compound **a** as the diphenylmethyl ester of *N*-carbobenzoxydehydroalanine (**2**): ir 3435 (NH), 1745 (ester), and 1700 cm^{-1} (urethane); nmr τ 2.67 (15, aromatic), 3.05 (1, ester CH), 3.67 (1) and 4.0 (1) (methylene), 4.85 (2) (benzyl CH_2).

Anal. Calcd for $C_{24}H_{21}NO_4$: C, 74.4; H, 5.46; N, 3.62. Found: C, 74.7; H, 5.48; N, 3.61.

Compound **b**, crystallized from MeOH (80 mg, 40%), was identified as starting material by melting point, mixture melting point, and ir. If the reaction mixture is kept at room temperature for 15 min in the presence of hydrogen peroxide prior to work-up, the starting material is completely converted to **2**.

B.—To another sample of **1** (210 mg) dissolved in ice-cold acetone (10 ml), sodium metaperiodate [78 mg (1.1 *M*) in 3 ml of water] was added. The reaction mixture was stirred in an ice bath for 15 min and then at room temperature for 6 hr. Isolation of products yielded **1** and **2** in approximately equal amounts.

Oxidation of Bis(diphenylmethyl) Bis(*N*-carbobenzoxy)-L-selenocystinate (**3**).—To a stirred solution of ice-cold acetone

(3) M. Cinquini, S. Colonna, and R. Giovini, *Chem. Ind. (London)*, 1737 (1969).

(4) K. A. Caldwell and A. L. Tappel, *Biochemistry*, **3**, 1643 (1964); A. L. Tappel, *Fed. Proc.*, **24**, 73 (1965).

(5) The ir of **5** (see Experimental Section) exhibits the characteristic Se → O resonance and in the nmr the methyl group is shifted downfield by 0.52 ppm, as would be expected from the electron-withdrawing character of the Se → O moiety. Elementary analysis of the product would correspond to the structure being a selenone or the monohydrate of a selenoxide. A broad absorption in the ir in the region characteristic for hydroxyl groups is indicative of the selenoxide monohydrate structure of the oxidized product. Aliphatic sulfoxides similarly absorb 1 mol of water.[6]

(6) D. Barnard, J. M. Fabian, and H. P. Koch, *J. Chem. Soc.*, 2442 (1949).

(7) All melting points were determined with a Thomas-Hoover capillary melting point apparatus and are corrected. The infrared spectra were recorded on a Perkin-Elmer 457 infrared spectrophotometer in pressed disks of KBr at a concentration of 0.3% for solids and in films between NaCl windows for liquids. Nmr spectra were recorded on a Varian T-60 nmr spectrometer in $CDCl_3$. Amino acids were chromatographed on a Beckman 120C amino acid analyzer using a Beckman Custom Research Resin PA-28 packed in a 56 × 0.9 cm column. The buffer flow rate was set at 68.0 ml/hr, the ninhydrin flow rate at 34 ml/hr; temperature was maintained at 55°. The elementary analyses were carried out by Galbraith Laboratories Knoxville, Tenn. The plates were coated with silica gel.

(8) J. Roy, W. Gordon, I. L. Schwartz, and R. Walter, *J. Org. Chem.*, **35**, 510 (1970).

(9) H. Zahn and E. Rexroth, *Z. Anal. Chem.*, **148**, 181 (1955).

(5 ml) containing 50 mg of **3**, 30% H_2O_2 (0.2 ml) was added. Stirring was continued in ice bath for 30 min and then at room temperature for 1 hr. Isolation of product by the usual procedure gave **2** in almost quantitative yield.

***N*-Carbobenzoxy-DL-selenomethionine.**—DL-Selenomethionine (0.5 g) was carbobenzoxylated as described for its sulfur analog.[10] The product crystallized from EtOAc–*n*-hexane: yield 0.5 g (60%); mp 114–116°; nmr τ 0.67 (carboxyl H), 2.66 (aromatic), 4.35–4.75 (amide), 4.88 (benzyl CH_2), 5.25–5.78 (C α H), 7.2–7.98 (C β H_2 and C γ H_2), 8.04 (methyl).

Anal. Calcd for $C_{18}H_{17}NO_4Se$: C, 47.3; H, 5.19; N, 4.24. Found: C, 47.4; H, 5.30; N, 4.25.

***N*-Carbobenzoxy-DL-selenomethionine Diphenylmethyl Ester (4).**—*N*-Carbobenzoxy-DL-selenomethionine (0.66 g) was dissolved in EtOAc (50 ml); to the ice-cold, stirred solution diphenyldiazomethane[11] (0.35 g, 0.9 *M*) was added. Stirring was continued while the reaction mixture was allowed to warm to room temperature. After several hours the solution was washed with 5% $NaHCO_3$ (three 25-ml portions) and water (three 25-ml portions). Following drying over Na_2SO_4, removal of solvent under vacuum gave an oil (0.90 g) which was purified by chromatography on a silica gel column. The product was eluted with C_6H_6–EtOAc (98:2, v/v): yield 0.8 g (91%); nmr τ 2.7 (aromatic), 3.12 (ester CH), 4.4–4.75 (amide), 4.94 (benzyl CH_2), 5.2–5.66 (C α H), 7.35–8.0 (C β H_2 and C γ H_2), 8.17 (methyl).

Anal. Calcd for $C_{26}H_{27}NO_4Se$: C, 62.9; H, 5.48; N, 2.82. Found: C, 62.9; H, 5.60; N, 2.72.

Oxidation of *N*-Carbobenzoxy-DL-selenomethionine Diphenylmethyl Ester. A.—To an ice-cold, stirred solution of acetone (10 ml) containing **4** (265 mg), sodium metaperiodate (143 mg, 1.25 *M*) dissolved in water (5 ml) was added. After stirring at ice-bath temperature for 30 min and at room temperature for 2 hr the aqueous phase was extracted with EtOAc. The organic layer was washed with water saturated with NaCl (three 25-ml portions), dried (Na_2SO_4), and concentrated under vacuum. *N*-Carbobenzoxy-DL-selenomethionine selenoxide diphenylmethyl ester monohydrate (**5**) was obtained as an oil (270 mg, 95% yield), which was examined for purity after chromatography in the solvent systems $CHCl_3$–MeOH (6:1, v/v) and C_6H_6–EtOAc (25:1, v/v) by both uv and Zahn reagent: ir 3500–3100 (broad OH and NH), 810 cm^{-1} (selenoxide);[6,12,13] nmr τ 2.68 (aromatic), 3.1 (ester CH), 4.95 (benzyl CH_2), 5.35–5.75 (C α H), 7.10–8.0 (C β H_2 and C γ H_2), 7.65 (methyl).

Anal. Calcd for $C_{26}H_{29}NO_6Se$: C, 58.9; H, 5.51; N, 2.64. Found: C, 59.1; H, 5.12; N, 2.61.

B.—Another aliquot of **4** was allowed to react with 0.2 ml of 30% hydrogen peroxide. After stirring the mixture in the ice bath for 30 min, the oxide **5** was isolated as product in nearly quantitative yield.

Conversion of the Oxide of *N*-Carbobenzoxy-DL-selenomethionine Diphenylmethyl Ester to *N*-Carbobenzoxy-DL-selenomethionine Diphenylmethyl Ester.—The selenoxide **5** (270 mg) was dissolved in acetone (10 ml) and the solution was stored with exclusion of light at room temperature. Aliquots of the solution were examined by tlc from time to time in solvent systems $CHCl_3$–MeOH (6:1, v/v) and C_6H_6–EtOAc (25:1, v/v). After 7 days the selenoxide had totally disappeared and the reaction mixture contained essentially one compound. The acetone was removed under vacuum and the residue was purified by chromatography on a silica gel column. Elution with C_6H_6–EtOAc (99:1, v/v) yielded 175 mg (69%) of *N*-carbobenzoxy-DL-selenomethionine diphenylmethyl ester **4**, which was identified by superimposable ir, nmr, and also by elementary analysis.

Anal. Calcd for $C_{26}H_{27}NO_4Se$: C, 62.9; H, 5.48; N, 2.82. Found: C, 63.0; H, 5.55; N, 2.74.

A portion of the above compound (5 mg), dissolved in anhydrous AcOH (0.3 ml), was decarbobenzoxylated and deesterified by treatment with 0.3 ml of 4 *N* HBr in AcOH. After 20 min the reaction mixture was evaporated to dryness. The resulting residue was dissolved in 10 ml of citrate buffer (pH 2.2). A 0.25-ml aliquot was analyzed for ninhydrin-active material by amino acid analysis as described.[14] Two ninhydrin-active components were detected, one corresponding to selenomethionine[14] while the other (emerging at 297 ml of buffer after the start of the chromatogram) was identified as selenohomocystine by comparison with authentic DL-selenohomocystine. The fact that selenohomocystine has been identified as one of the ninhydrin-active components leads to the conclusion that selenomethionine (or one of its intermediates) is partially demethylated during the acid treatment and subsequently oxidized to the diselenide.

Registry No.—**2**, 29751-58-4; **4**, 29875-98-7; **5**, 29751-59-5; DL-selenomethionine, 2578-28-1; *N*-carbobenzoxy-DL-selenomethionine, 29751-61-9.

Acknowledgment.—The authors are thankful to Mr. D. H. Schlesinger for performing the amino acid analyses. We are also grateful to Dr. D. F. Petersen, Los Alamos Scientific Laboratory, for kindly supplying us with a sample of DL-selenohomocystine and to Dr. K. D. Gibbons, Rockefeller University, for 220-MHz nmr spectra and discussion.

(10) K. Hofmann, A. Jöhl, A. E. Furlenmeier, and H. Kappeler, *J. Amer. Chem. Soc.*, **79**, 1636 (1957).
(11) J. B. Miller, *J. Org. Chem.*, **24**, 560 (1959).
(12) K. A. Jensen and V. Krishnan, *Acta Chem. Scand.*, **21**, 1988 (1967).
(13) R. Steudel, *Z. Naturforsch., B*, **25**, 645 (1970).
(14) R. Walter, D. H. Schlesinger, and I. L. Schwartz, *Anal. Biochem.*, **27**, 231 (1969).

Microbiological Transformation of 2,2,4-Trimethyl-7-*tert*-octyl-6-hydroxychroman

J. R. SCHAEFFER* AND C. T. GOODHUE

Research Laboratories, Eastman Kodak Company, Rochester, New York 14650

Received December 28, 1970

Recent studies on the microbiological transformation of 6-hydroxychromans yielded a mixed culture of soil microorganisms that was able to convert 2,2,4-trimethyl-7-*tert*-octyl-6-hydroxychroman to an *o*-quinone and an *o*-nitrophenol.

We have found that biotransformation of **1** has produced **2** in 3.5% yield and **3** in 5.1% yield (Scheme I). Also a trace amount of product with a molecular weight of 606 was isolated by glc. Attempts to isolate a larger quantity of the compound for structure determination were unsuccessful. A molecular weight of 606 suggests that the compound is a dimer of **1**. Support for this conclusion is found in the reported oxidative dimerization of the 6-hydroxychroman ring of α-tocopherol.[1]

A single aromatic proton was observed in the nmr spectrum of **2** which suggested that either the 5- or 8-position proton had been replaced. Elemental analytical and mass spectral data were in agreement with the molecular formula $C_{20}H_{31}NO_4$. Confirmation of the presence of a nitro group was obtained by nitrosation of **1** followed by a nitric acid oxidation of the nitroso group.[2] That the nitro group is in the 5 position was shown by reductive cyclization of the acetate of **2** to benzoxazole **4**. The physical properties of the nitrochromanol obtained by chemical synthesis are identical with those of the fermentation product.

The nmr spectrum of **3** has a single vinylic proton and no hydroxyl group absorption, which suggests disruption of the aromatic character of the benzene ring in **1**. A molecular formula, $C_{20}H_{30}O_3$, is consistent with the

(1) W. A. Skinner and P. Alaupovic, *J. Org. Chem.*, **28**, 2854 (1963).
(2) C. F. Koelsch, *J. Amer. Chem. Soc.*, **66**, 2019 (1944).