(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.

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

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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

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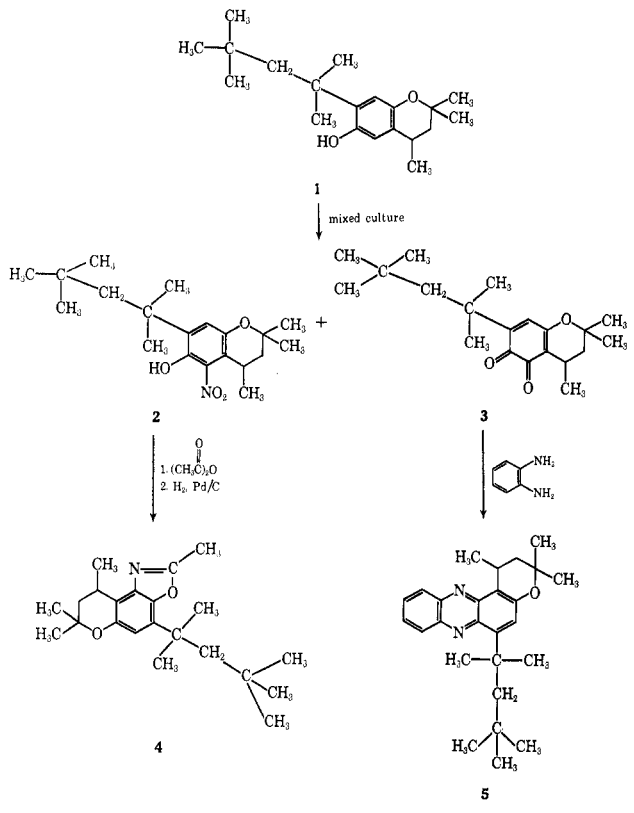
SCHEME I



elemental analytical and mass spectral data. Oxidation of the 6-hydroxychroman ring of α-tocopherol to a quinone has been reported.[3] Nitric acid oxidation of **1** yielded a quinone with physical properties identical with fermentation product **3**. Confirmation of an *o*-quinone structure was obtained by synthesis of phenazine **5** from **3**.

It was not possible to isolate pure cultures that transformed **1**, because no solid medium containing chromanol that would selectively support growth of the active organisms could be found. Plating of the mixed culture onto nutrient agar (1% glucose, 1% yeast extract) separated about 20 different colonies, but none of these organisms was able to transform the chromanol. Consequently, we were forced to maintain the active mixed culture by serial transfer in a liquid medium.

Even though the transformation reactions are rather slow, control experiments indicated that no significant reactions occur in the absence of the mixed culture. It seems probable that the first reaction involves the hydroxylation of chromanol **1**, since this type of reaction is a common method of aromatic hydrocarbon oxidation in microorganisms.[4]

Experimental Section[5]

Isolation of Culture.—An active mixed culture was obtained by percolating soil with a medium containing 0.5% of chromanol **1**, 1% $(NH_4)_2SO_4$, 1% K_2HPO_4, and mineral salt solution (10 ml/l).[6] The percolator was run for 2 weeks at room temperature. During this time the percolation medium became cloudy and orange-yellow insoluble material appeared. A 5-ml portion of this culture was transferred to an erlenmeyer flask containing 25 ml of the percolation medium plus 0.1% yeast extract. Just prior to inoculation chromanol **1** was added to the sterile medium as a fine powder. This first transfer was incubated at 30° with shaking on a rotary shaker at 400 rpm for 28 days. During this time the culture medium became deep orange and the insoluble material turned red. This active culture was maintained by serial transfer at monthly intervals in the same medium.

Microbiological Production of 2 and 3.—Cultures were grown in 2.8-l. wide-mouthed indented Fernbach flasks that contained 1 l. of percolation medium with 0.5% chromanol **1**, 0.1% yeast extract, and 0.05% Tween 80. Each flask was inoculated with 100 ml of an active culture grown in the same medium until it became deep orange. The Fernbach flask cultures were incubated at 30° on a rotary shaker at 200 rpm for approximately 30 days. Transformation products were obtained by extraction as described below.

Control Fermentations.—These experiments were performed in Fernbach flasks containing 1 l. of percolation medium (without chromanol **1**) for the basic mineral medium. Additions were made as follows: flask A, 500 mg of chromanol **1**; flask B, 500 mg of chromanol **1**; flask C, 500 mg of chromanol **1** and 200 mg of *o*-quinone **3**; flask D, 200 mg of *o*-quinone **3**. Only flask A was inoculated with 150 ml of an active culture. All flasks were incubated together on a shaker in the usual manner for 11 days. The contents of each flask then was analyzed by thin layer chromatography of chloroform extracts.[7] Flask A contained compounds **1**, **2**, and **3** and a trace of an unknown. Flask B contained only **1** and traces of the unknown (R_f value 0.73 in system described in footnote 7). Flask C contained **1** and **3** and traces of the unknown. Flask D contained only **3**.

Isolation of Products from 2,2,4-Trimethyl-7-*tert*-octyl-6-hydroxychroman (1) Fermentation.—The fermentation broth (2.4 l.) was extracted with chloroform. The chloroform-extracted broth was then continuously extracted with ether for 48 hr. The chloroform extract was dried and concentrated to an oil and then it was chromatographed on 300 g of silica. Elution was carried out with hexane–chloroform (95:5, then 85:15). Rechromatography of the three main eluate fractions, each on 60 g of silica, eluting with hexane–chloroform (95:5), furnished 0.34 g of product. Chromatography of the ether extract in the manner described above yielded an additional 0.14 g of product. The total yield of pure **2** was 0.48 g (3.5%): mp 81–82°; mass spectrum *m/e* 349; $\tau_{tms}^{CDCl_3}$ 5.2 (s, 1, OH), 3.0 (s, 1, aromatic).

Anal. Calcd for $C_{29}H_{31}NO_4$: C, 68.7; H, 9.0; N, 4.0; mol wt, 349.4. Found: C, 68.9; H, 8.8; N, 3.9; mol wt, 341.

Crude **3** obtained along with pure **2** in the manner described above was further purified by chromatography on silica. Elution was carried out with increasing concentrations of chloroform in hexane.[8] The yield of **3** was 0.63 g (5.1%): mp 92–94°; mass spectrum *m/e* 318; $\tau_{tms}^{CDCl_3}$ 3.5 (s, 1, quinone H).

Anal. Calcd for $C_{20}H_{30}O_3$: C, 75.4; H, 9.5; mol wt, 318.4. Found: C, 75.1; H, 9.3; mol wt, 313.

Preparation of 2,2,4-Trimethyl-5-nitro-7-*tert*-octyl-6-hydroxychroman (2).—2,2,4-Trimethyl-7-*tert*-octyl-6-hydroxychroman

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(5) Melting points are uncorrected. Molecular weights were obtained in acetone by the ebullometric method. All evaporations were carried out under reduced pressure. The drying agent was sodium sulfate. Yeast extract was obtained from Difco Laboratories, silica gel G from Brinkmann Instruments, and all other chemicals were obtained from Eastman Organic Chemicals. The soil percolator was obtained from Belco Glass Co. The nmr spectra were determined with a Varian Model A-60 spectrometer, tetramethylsilane being used as an internal standard. The mass spectra were determined on a 60° sector-type, single-focusing instrument equipped with an all-glass heated inlet system operated at 235°, a modified design of the system described by Caldecourt [V. J. Caldecourt, *Anal. Chem.*, **27**, 1670 (1955)].

(6) The mineral salt solution was prepared by dissolving 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.17 g of $MnSO_4 \cdot 7H_2O$, 0.028 g of $FeSO_4 \cdot 7H_2O$, 0.006 g of NaCl, 0.001 g of $CaCl_2 \cdot 2H_2O$, and 0.006 g of $ZnSO_4 \cdot 7H_2O$ in 1 l. of 0.1 *N* HCl.

(7) Thin layer chromatography of compounds **1**, **2**, and **3** was carried out on Eastman Chromagram silica gel 6061. The developing solvent was hexane–chloroform (95:5); rhodamine G was the indicator. The following R_f values were obtained: **1**, R_f 0.30; **2**, R_f 0.92; **3**, R_f 0.11.

(8) A purple band was observed on the column during purification of **3**. Silica was extruded from the column. The band was cut out and eluted with chloroform. Examination of a peak at 322° on an F & M Model 810 gas chromatograph (Se-30, He flow 30 ml/min program 100–360° at 15°/min) by mass spectrometry indicated a mass of 606 amu.

(1) (10.0 g, 0.033 mol) was dissolved in 250 ml of methanol. The solution was cooled to 5° and 70 ml of glacial acetic acid was added. Sodium nitrite (20 g, 0.29 mol) dissolved in 200 ml of water was added to the stirred solution in several portions over a period of 5 min, the temperature of the reaction mixture being maintained below 10° during the addition. The reaction mixture was stirred for 15 min and then poured onto ice. The product was extracted into ligroin. The extract was evaporated to a red oil and then dissolved in 350 ml of methanol. To the stirred solution, 20 ml of concentrated nitric acid was added in five equal portions. After the reaction mixture had been stirred for 1 hr, it was poured onto water and extracted with ether. The ether extract was dried and evaporated. The crude product was chromatographed on 170 g of silica. Elution was carried out with hexane–chloroform (95:5, then 90:10). The red solid product weighed 4.5 g (39.4%), mp 82–84°, mmp (with fermentation sample) 82–84°.

Anal. Calcd for $C_{20}H_{31}NO_4$: C, 68.7; H, 9.0; N, 4.0; mol wt, 349.4. Found: C, 68.4; H, 9.1; N, 4.0; mol wt, 341.

Preparation of 2,2,4-Trimethyl-7-*tert*-octylchroman-4,6-dione (3).—To a stirred solution containing 10 g (0.033 mol) of **1** in 50 ml of methanol was added 30 ml of nitric acid in six equal portions. The reaction mixture was stirred for 1 hr, poured onto 500 ml of water, and extracted with ether. The ether extract was dried and evaporated to red oil. The oil was chromatographed on 170 g of silica. Elution was carried out with increasing concentrations of chloroform in hexane. The red solid product weighed 8.7 g (83%), mp 92–94°, mmp (with fermentation product) 92–94°.

Anal. Calcd for $C_{20}H_{30}O_3$: C, 75.4; H, 9.5; mol wt, 318.4. Found: C, 75.7; H, 9.5; mol wt, 312.

8,9-Dihydro-2,7,7,9-tetramethyl-4-*tert*-octyl-7*H*-pyrano[3,2-*e*]-benzoxazole (4).—Compound **2** (0.46 g, 0.0013 mol) was dissolved in 9 ml of a 1:1 mixture of acetic anhydride and pyridine. The reaction mixture was stirred 2.5 hr at 55° and then poured onto ice water. The diluted reaction mixture was extracted with ether and the extract dried and evaporated to an oil. The oil was dissolved in 100 ml of ethanol and 0.2 g of 15% palladium on charcoal was added. The mixture was hydrogenated at 500 psi for 4–5 hr (room temperature). After removal of the catalyst and evaporation of the solvent, **4** was isolated on a Varian Aerograph Autoprep gas chromatograph using a $^3/_8$ in. by 20 ft aluminum column of 10% Se-30, 250° column temperature, and 150-ml/min He flow. The product has a 9-min retention time. Compound **4**, an off-white solid, weighed 0.078 g (22%): mp 84–87°; $\tau_{tms}^{CDCl_3}$ 7.4 (s, 3, $CH_3C(O)=N$), 3.4 (s, 1, aromatic).

Anal. Calcd for $C_{22}H_{33}NO_2$: C, 77.1; H, 9.7; N, 4.1; mol wt, 343.5. Found: C, 76.9; H, 9.4; N, 4.4; mol wt, 329.

2,3-Dihydro-1,3,3-trimethyl-6-*tert*-octyl-1*H*-pyrano[3,2-*a*]-phenazine (5).—In a mixture of 75 ml of glacial acetic acid and 300 ml of toluene were dissolved 3.0 g (0.0093 mol) of **3** and 2.0 g (0.0186 mol) of *o*-phenylenediamine.[9] During a 26-hr reflux, 2.2 ml of water was collected. The solvent mixture was evaporated to 50 ml. The concentrated solution was dissolved in ether and the resulting solution washed with water. The combined water wash was neutralized with sodium bicarbonate and extracted with ether. The ether extracts were combined, washed with a saturated sodium bicarbonate solution and then with water, dried, and evaporated to an oil. The oil was chromatographed on 75 g of silica. Elution was carried out with increasing concentrations of chloroform in hexane. The yellow solid product weighed 0.37 g (10.2%): mp 48–50°; $\tau_{tms}^{CDCl_3}$ 2.8 (s, 4, C=CHC=C), 2.9 (symmetrical multiplet around 2.1, 4, aromatic).

Anal. Calcd for $C_{26}H_{34}N_2O$: C, 80.0; H, 8.8; N, 7.2; mol wt, 390.6. Found: C, 80.0; H, 8.5; N, 7.2; mol wt, 375.

Registry No.—**1**, 18403-59-3; **2**, 30469-74-0; **3**, 30469-75-1; **4**, 30469-76-2; **5**, 30469-77-3.

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The Conversion of Hydroxamic Acids to *N,O*-Diacylhydroxylamines

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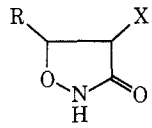
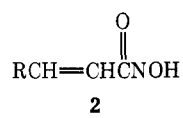
As an approach to the synthesis of substituted 3-isoxazolidones (**1**), it was predicted that the treatment of α,β-unsaturated hydroxamic acids (**2**) with electrophilic reagents would cause a cyclization to the desired compounds. This reaction would be analogous to the halolactonization reactions of β,γ-unsaturated acids.[2-4]



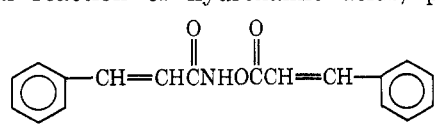
1



2

3, R = C_6H_5

Cinnamohydroxamic acid **3** was the initial compound with which this reaction was attempted. A suspension of **3** in sodium bicarbonate was treated with potassium triiodide; however, the product isolated was not the expected 3-isoxazolidone but rather *N,O*-dicinnamoylhydroxylamine (**4**). In order to determine if this is a general reaction of hydroxamic acids, potassium



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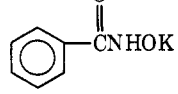
benzohydroxamate (**5**) was treated under the same conditions and was found to be converted to *N,O*-dibenzoylhydroxylamine (**6**). When this same reaction



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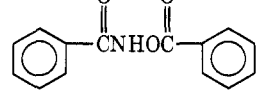

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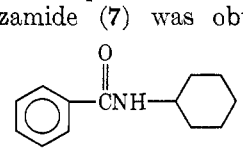
was repeated in the presence of cyclohexylamine, *N*-cyclohexylbenzamide (**7**) was obtained. Hydroxamic acids are readily converted to the corresponding carboxylic acids and nitrogen or nitrous oxide by such reagents as bromine water and aqueous periodic acids.[5,6] On the basis of these observations and of the products obtained, a plausible mechanistic interpretation of this reaction is as follows.



7

(1) Taken in part from the theses presented by N. A. Dahle, 1965, and V. D. Warner, Sept 1970, to the Graduate School of the University of Kansas in partial fulfillment of the requirements for the Doctor of Philosophy Degree.

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