Synthesis and Some Toxicity and Oxidation Studies of a Fatty Acid Hydroquinone and Analogs of Tocol and δ-Tocopherol[1]

ROBERT A. STEIN

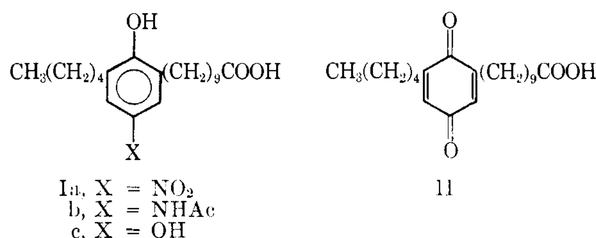
Department of Biological Chemistry, School of Medicine, University of California at Los Angeles, Los Angeles, California 90024

Received August 9, 1966

Sodium nitromalonaldehyde was condensed with 12-oxooctadecanoic acid to form 2-(ω-carboxynonyl)-6-pentyl-4-nitrophenol. Reduction to the amine and subsequent oxidation with ferric ion gave 2-(ω-carboxynonyl)-6-pentyl-*p*-quinone. Reduction with zinc in acetic acid converted the quinone into 2-(ω-carboxynonyl)-6-pentyl-*p*-hydroquinone. Similarly, sodium nitromalonaldehyde was condensed with phytylacetone and 1-phytyl-2-butanone to form 2-phytyl-4-nitrophenol and 2-phytyl-6-methyl-4-nitrophenol, which were cyclized to the 6-nitro-2-methyl-2-(4,8,12-trimethyltridecyl)chromane and 6-nitro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-chromane, respectively. These nitro analogs of tocol and δ-tocopherol were hydrogenated to the corresponding amines. An oxidation product of 6-amino-2-methyl-2-(4,8,12-trimethyltridecyl)chromane is described. The effectiveness of 2-(ω-carboxynonyl)-6-pentyl-*p*-hydroquinone, as an antioxidant for methyl linolenate was greater than that of α-tocopherol. The fatty acid hydroquinone was fed to mice in amounts up to 0.1% of the diet for 15 weeks without toxic effect. Mice on diets containing 0.3% and more of the hydroquinone decreased their food intake and excreted a metabolite that changed to a red color on standing in air. The corresponding fatty acid quinone in the diet at the 3% level was fatal to mice in 2 weeks.

Hydroquinone derivatives are important antioxidants although many of them are too toxic to be used in biological systems.[2] A hydroquinone derivative with lipid properties, and which could be metabolized to nontoxic compounds would be a potentially useful antioxidant for edible fats. A fatty acid hydroquinone, the synthesis of which is described here, has these potentialities. The antioxidant capacity of the hydroquinone is superior to that of α-tocopherol and, in the course of a 15-week feeding experiment with mice, appeared to have a low toxicity.

In the fatty acid hydroquinone synthesis, the benzene ring forming reaction was a sodium hydroxide catalyzed condensation of sodium nitromalonaldehyde[3] with 12-oxooctadecanoic acid to give *p*-nitrophenol Ia.

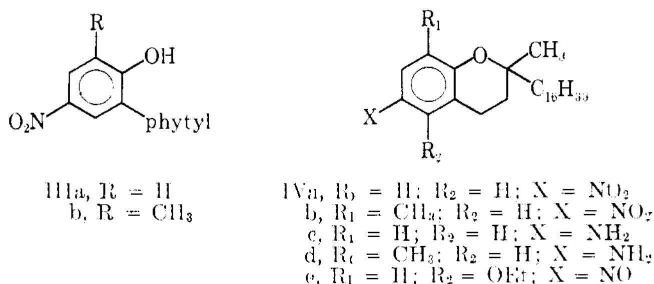


This was reduced with stannous chloride in acetic acid and subsequently acetylated to form the N-acetyl derivative (Ib). *p*-Quinone II was formed by ferric sulfate oxidation of Ib and was reduced to hydroquinone (Ic) by zinc in acetic acid. The quinone and hydroquinone were used in toxicity tests with mice and are described below.

The usual approach to the synthesis of the tocopherols is phytylation of a hydroquinone.[4] The scheme of the fatty acid hydroquinone synthesis described here was used as a novel method of synthesis in the tocopherol series. To this end, phytylacetone and 1-phytyl-2-butanone were condensed with sodium nitromalonaldehyde to give nitrophenol IIIa and IIIb, respectively.

During the prolonged alkaline conditions used for the benzene ring forming reaction, it seemed possible that the double bond in the phytyl side chain could be isomerized into conjugation with the ring. This possibility was ruled out as being of major importance by ozonizing IIIb and phytol and identifying the same ketone from each by vapor phase chromatography.

Cyclization of *p*-nitrophenols IIIa and IIIb to 6-nitrochromanes (IVa and IVb), which are the nitro analogs of tocol and δ-tocopherol, respectively, was accomplished by refluxing in acidic alcohol solutions.



Aminochromanes IVc and IVd, the amine analogs of tocol and δ-tocopherol, respectively, were prepared by hydrogenation of IVa and IVb. These reductions are in contrast to the unreactive nature of the nitro group in a similarly constituted nitrochromane in which methyl groups in the positions *ortho* to the nitro group apparently are steric inhibitors to the reduction.[5]

Oxidations of aminochromanes IVc and IVd with silver and ferric ion were uniformly unsuccessful for preparation of either the quinone of tocol or δ-tocopherylquinone. The tarry products were usually red or purple and may have contained compounds similar in structure to α-tocored[6] and α-tocopurple[7] obtained by ferric chloride oxidation of α-tocopherol. An exception was a brief oxidation of IVc with silver acetate

(1) Supported in part by contract DA-49-007-MD-579 with the U. S. Army Office of the Surgeon General and in part by Public Health Service Research Grant H-4120 from the National Heart Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

(2) J. G. Bierl, *Progr. Chem. Fats Lipids*, **7**, 247 (1964).

(3) For a review of the chemistry of sodium nitromalonaldehyde, see P. E. Fanta and R. A. Stein, *Chem. Rev.*, **60**, 261 (1960).

(4) O. Isler, P. Schudel, H. Mayer, V. Würsch, and R. Rüegg in *Vitamins Hormones*, **20**, 389 (1962).

(5) L. I. Smith, H. H. Hoehn, and H. E. Ungnade, *J. Org. Chem.*, **4**, 351 (1939).

(6) V. L. Frampton, W. A. Skinner, Jr., and P. S. Bailey, *J. Am. Chem. Soc.*, **76**, 282 (1954).

(7) V. L. Frampton, W. A. Skinner, Jr., P. Cambour, and P. S. Bailey, *J. Am. Chem. Soc.*, **82**, 4632 (1960).

in ethanol. The yellow oxidation product had ultraviolet absorption maxima consistent with those of 2,4-dimethoxynitrosobenzene[8] but not with *p*-nitrosoanisole. Structure IVe for the yellow oxidation product accounts for the known properties. The ethoxy group, arising from an attack by solvent during the oxidation, is pictured at position 5 instead of 7 of the chromane ring, because in formation of both α-tocored and α-tocopurple oxidation occurs at the 5 position.

The antioxidant effectiveness of the fatty acid hydroquinone was measured by determining the induction time of autoxidation of methyl linolenate at 100°. In these experiments, Ic was about seven times more effective on a weight basis than α-tocopherol.[9]

No toxicity of the fatty acid hydroquinone was found for Swiss white mice over a period of 15 weeks when the animals consumed diets containing up to 0.1% (w/w) Ic in the diet. At levels of 0.3, 1, and 3% in the diet for 16 days, the animals decreased their food intake and gained less weight than the controls. The results are similar to those found for hydroquinone fed to rats.[10]

After 5 days on the diet the mice consuming the 0.3, 1, and 3% diets began excreting material that turned red on the paper under the cages after it had been exposed to air for 18 hr. The intensity of the red color increased with the amount of hydroquinone in the diet.

Fatty acid quinone II was definitely toxic at the 3% level in the diet. Within 14 days, 3 of 4 rats had died, with death being preceded by a period of trembling and hunching of the back. At the 0.3% level, weight gain of the animals was not as rapid as the controls, and at the 1% level there was a weight loss.

Experimental Section[11]

2-(ω-Carboxynonyl)-6-pentyl-4-nitrophenol (Ia).—12-Oxooctadecanoic acid[12] (11.95 g, 0.04 mole) was dissolved in 50 ml 95% ethanol and mixed with a solution of sodium nitromalonaldehyde monohydrate[13] (6.28 g, 0.04 mole) and 4 g of NaOH in 50 ml of water. The resulting red solution was allowed to react for 1 week at 60°. This reaction time is probably longer than necessary, because, in the case of the tocopherol analogs described below, the yield of nitrophenol did not change between 3 and 6 days. The reaction mixture was poured into 300 ml of water containing 20 ml of concentrated HCl, and the precipitated product was collected in a Büchner funnel, washed with water, and dried in air (13.01 g). Crystallization from 25 ml of ethanol at −15° gave 1.43 g of keto acid starting material. Removal of the mother liquor solvent *in vacuo* and crystallization of the residue from a 1:1 solution of benzene–petroleum ether (30–60°) gave 9.25 g (0.024 mole, 61% yield) of the *p*-nitrophenol derivative, mp 84–85°. A sample crystallized several times from the benzene–petroleum ether solvent melted at 87.6–88.4°, λ_{max}^{EtOH} 326 mμ (log ε 3.97).

Anal. Calcd for $C_{21}H_{33}NO_5$: C, 66.5; H, 8.76; N, 3.69. Found: C, 66.4; H, 8.67; N, 3.42.

N-Acetyl-2-(ω-carboxynonyl)-6-pentyl-*p*-aminophenol (Ib).—To 5.0 g (0.0132 mole) of Ia in 75 ml of acetic acid was added 20 g of $SnCl_2 \cdot 2H_2O$ in 20 ml of concentrated HCl. The solution was heated on the steam bath for 1.5 hr and then diluted with 75 ml water. After cooling to room temperature, 5 ml of acetic anhydride was added, and the material was allowed to crystallize in the refrigerator. The colorless crystals were collected in a Büchner funnel and washed with two 50-ml portions of distilled water. After drying *in vacuo* ($CaCl_2$), the product weighed 5.4 g. It contained Cl^- and could be stored for several months in a screw-capped bottle without much apparent autoxidation, although the surface of the lumps of material developed a purple color which did not extend into the interior of the pieces.

2-(ω-Carboxynonyl)-6-pentyl-*p*-quinone (II).—To 2.5 g (0.0064 mole) of Ib in 100 ml of acetic acid was added 10 g of $Fe_2(SO_4)_3 \cdot H_2O$ in 25 ml of water, and the mixture was allowed to stand for 24 hr at room temperature. During this time, the color became dark red and then finally orange-red. After 24 hr, all of the ferric salt had dissolved, and crystals of the quinone had appeared. The product was dissolved by heating and recrystallized in the refrigerator. The collected quinone, washed with water and dried in air, weighed 1.5 g (0.0043 mole, 67% yield). Recrystallization from dilute methanol gave II, mp 68–69°, λ_{max}^{EtOH} 259 mμ (log ε 4.15).

Anal. Calcd for $C_{21}H_{32}O_4$: C, 72.5; H, 9.25. Found: C, 72.2; H, 9.26.

2-(ω-Carboxynonyl)-6-pentyl-*p*-hydroquinone (Ic).—Zinc dust (0.2 g) added to 0.75 g (0.0023 mole) of II and 1 g of fused sodium acetate in 25 ml of acetic acid caused the disappearance of the yellow color of the quinone within a few minutes. Unreacted zinc was removed by filtration, and the solution was diluted with water and cooled in a refrigerator. The hydroquinone derivative was obtained as nearly colorless crystals. After collecting, washing with water, and drying *in vacuo* the yield was 0.75 g (0.0023 mole, 100%). An analytical sample was crystallized from aqueous methanol, mp 93.4–94.4°, λ_{max}^{EtOH} 290 mμ (log ε 3.52).

Anal. Calcd for $C_{21}H_{34}O_4$: C, 72.1; H, 9.78. Found: C, 71.9; H, 9.45.

1-Phytyl-2-butanone.—In a manner similar to that described for phytylacetone,[14] phytyl bromide[15] from 25 g (0.084 mole) of phytol was treated with the sodium salt of ethyl propioacetate (10 g, 0.070 mole). After hydrolysis and decarboxylation of the condensation product, 1-phytyl-2-butanone distilled in a molecular still at 100 μ in an oil bath at 190°. The yield was 16.3 g (0.047 mole, 67% from ethyl propioacetate).

Anal. Calcd for $C_{24}H_{46}O$: C, 82.2; H, 13.2. Found: C, 82.0; H, 13.0.

4-Nitro-2-phytylphenol (IIIa).—Sodium nitromalonaldehyde monohydrate (7.85 g, 0.05 mole) was dissolved in warm dilute ethanol (150 ml of 95% ethanol and 25 ml of water). To this was added 16.8 (0.05 mole) of phytylacetone[14] in 25 ml of 95% ethanol followed by 3 g (0.075 mole) of NaOH pellets. After complete solution was attained by heating and stirring, the solution was kept at 60° for 1 week. Crude IIIa was isolated by pouring the reaction mixture into 200 ml of water containing 10 ml of concentrated HCl and extracting with 30–60° petroleum ether. The extract was washed and dried ($MgSO_4$), and, after evaporation of the solvent, weighed 17.23 g. The absorbance at 322 mμ showed that the crude material contained 49% of the expected product. In another preparation using 0.032-mole quantities, the nitrophenol concentration in the reaction mixture was 64% after 3 days and had not changed in 6 days.

The crude product was purified in 5-g batches by silicic acid chromatography on a 7.5 × 16 cm column, the nitrophenol being eluted by 8% (v/v) ether in pentane. A sample was further purified by silicic acid rechromatography on a smaller scale to give a pale yellow oil: λ_{max}^{EtOH} 321 mμ (log ε 3.88), $\lambda_{max}^{0.02N\ [illegible]-EtOH}$ 322 mμ (log ε 3.97), $\lambda_{max}^{0.01N\ NaOH-EtOH}$ 418 mμ (log ε 4.26).

Anal. Calcd for $C_{26}H_{43}NO_3$: C, 74.8; H, 10.4. Found: C, 74.9; H, 10.1.

Ozonization[16] followed by vapor phase chromatography of a pure sample showed only one large oxidation fragment identified as 6,10,14-trimethyl-2-pentadecanone by comparison with the product from a similar oxidation of phytol.

(8) A. Burawoy, M. Cais, J. T. Chamberlain, F. Liversedge, and A. R. Thompson, *J. Chem. Soc.*, 3721 (1955).

(9) With another method for measuring antioxidant effectiveness (H. L. Olcott and E. Einset, *J. Am. Oil Chemists' Soc.*, **35**, 161 (1958)), it was found that the fatty acid hydroquinone was less effective than α-tocopherol (private communication from Dr. Olcott).

(10) A. J. Carlson and N. R. Brewer, *Proc. Soc. Exptl. Biol. Med.*, **84**, 684 (1953).

(11) Elemental analyses were performed at Elek Microanalytical Laboratories, Torrance, Calif. Melting points were observed in a Thomas–Hoover capillary melting point apparatus and are corrected. Ultraviolet absorption measurements were made with a Beckman DU spectrophotometer. Silicic acid adsorption chromatographic columns were prepared by pouring a slurry of silicic acid in acetone into a glass chromatographic tube and were activated by washing in sequence with two column volumns each of acetone, ether, and pentane.

(12) R. Perrotte, *Compt. Rend.*, **199**, 358 (1934).

(13) P. E. Fanta, "Organic Syntheses," Coll. Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1963, p 844.

(14) P. Karrer and K. S. Yap, *Helv. Chim. Acta*, **24**, 639 (1941).

(15) L. I. Smith and H. E. Ungnade, *J. Org. Chem.*, **4**, 298 (1939).

(16) R. A. Stein and N. Nicolaides, *J. Lipid Res.*, **3**, 476 (1962).

6-Methyl-4-nitro-2-phytylphenol (IIIb). In a similar manner to that described for IIIa, 5.0 g (0.014 mole) of 1-phytyl-2-butanone in 10 ml of 95% ethanol was added to 2.25 g (0.014 mole) of sodium nitromalonaldehyde in 50 ml of 95% ethanol and 10 ml of water. NaOH pellets (0.7 g) were added and the mixture was allowed to stand for 7 days at 60°. After the usual isolation procedure, 6.27 g of dark amber oil was obtained which was found by ultraviolet spectroscopy to contain 52.8% nitrophenol. A small sample was purified by silicic acid chromatography, the nitrophenol being eluted by 8% (v/v) ether in pentane: λ_{max}^{EtOH} 321 mμ (log ε 3.85), $\lambda_{max}^{0.01N\,NaOH-EtOH}$ 432 mμ (log ε 4.24).

Anal. Calcd for $C_{27}H_{45}NO_3$: C, 75.1; H, 10.5. Found: C, 75.1; H, 10.4.

2-Methyl-6-nitro-2-(4,8,12-trimethyltridecyl)chromane (IVa).—Crude IIIa (12.17 g) (64% nitrophenol) was refluxed 24 hr in 200 ml of methanol containing 10 ml of concentrated HCl. The product was isolated by diluting the reaction mixture with water and extracting with petroleum ether (30–60°). The extract was washed, dried ($MgSO_4$), and freed of solvent by evaporation. The amount of ring closure that had occurred was calculated by measuring the decrease in absorption in ethanolic NaOH at 418 mμ, which is a maximum for the nitrophenol but at which the nitrochromane is transparent. The ring closure was 50% complete.

The mixture of IIIa and IV was refluxed for 56 hr in 200 ml of methanol containing 5 ml of H_2SO_4. After isolation, the oil contained 20% unreacted nitrophenol. A further reaction for 24 hr in 200 ml of 1-propanol containing 5 ml of concentrated H_2SO_4 completed the cyclization. The crude chromane (10.35 g) was divided into two portions for chromatography and purified on a 7.5 × 16 cm silicic acid column, the chromane being eluted by 1 l. of 2% (v/v) ether in pentane but not by 400 ml of pentane. The 2% fractions totaled 7.86 g of 96% pure IVa. An analytical sample was rechromatographed on a small silicic acid column to give a pale yellow oil: λ_{max}^{EtOH} 321 mμ (log ε 4.06), $\lambda_{max}^{0.02N\,HCl-EtOH}$ 321 mμ (log ε 4.05). There is no absorption at 418 mμ in alkaline ethanol.

Anal. Calcd for $C_{26}H_{43}NO_3$: C, 74.8; H, 10.4. Found: C, 75.0; H, 10.5.

2,8-Dimethyl-6-nitro-2-(4,8,12-trimethyltridecyl)chromane (IVb).—Cyclization of crude (1.00 g) IIIb was brought about by refluxing in 25 ml of 1-propanol containing 1 ml of concentrated H_2SO_4 for 56 hr. After isolation as described for IVa, the chromane (0.96 g) was divided into four nearly equal amounts and chromatographed on small silicic acid columns. For each column, after an initial elution with 75 ml of pentane, the fractions eluted by 75 ml each of 2 and 6% (v/v) ether in pentane were collected and combined (0.33 g). The combined fractions were further purified by dividing the sample in half and chromatographing each half on a small column of silicic acid. The combined fractions eluted by 75 ml of 2% (v/v) ether in pentane, but not by 75 ml of pentane, weighed 0.22 g, λ_{max}^{EtOH} 321 mμ (log ε 4.00), $\lambda_{max}^{0.02N\,HCl-EtOH}$ 325 mμ (log ε 4.02). There is no absorption at 432 mμ in alkaline ethanol.

Anal. Calcd for $C_{27}H_{45}NO_3$: C, 75.1; H, 10.5 Found: C, 75.3; H, 10.2.

6-Amino-2-methyl-2-(4,8,12-trimethyltridecyl)chromane (IVc).—Pure IVa hydrogenated in ethanol over prereduced Pd (10% on C) absorbed 3 molar equiv of H_2. The solution was filtered while N_2 was blown over the funnel. Evaporation of the solvent left a nearly colorless oil. The hydrochloride of IVc was formed by bubbling dry HCl into an ether solution containing the amine. Evaporation left tan crystals, mp 153–155°. An appropriate solvent for crystallization was not found; λ_{max}^{EtOH} 235 mμ (log ε 4.20), 304 mμ (log ε 3.48). Hydrogen of IVa over reduced PtO_2 in ethanol brought the absorption of 6 molar equiv of H_2. The product, which did not absorb in the ultraviolet, was probably the hexahydro derivative of IVc.

6-Amino-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)chromane (IVd). Pure IVb was hydrogenated over prereduced Pd (10% on C). The isolated amine formed a hydrochloride which did not crystallize; λ_{max}^{EtOH} 241 mμ (log ε 3.75), 305 mμ (log ε 3.25).

Oxidation of IVc with Silver Acetate.—Compound IVa (0.108 g) was hydrogenated over prereduced Pd (10% on C) in 10 ml of ethanol. The catalyst was removed by filtration, and 0.20 g of freshly prepared silver acetate was added. The mixture was swirled for 10 min, and the insoluble material was removed by filtration and washed with ether. The combined filtrates were evaporated *in vacuo* at room temperature, and the dark red residual oil was dissolved in 25 ml of 4% (v/v) ether in pentane. The solution was passed through a small silicic acid column; the column was washed with 50 ml of 4% (v/v) ether in pentane. The bright yellow eluate was evaporated *in vacuo* leaving an oil weighing 54 mg. A purple band remained at the top of the silicic acid column.

Ultraviolet absorption measurements were made in ethanol, but a small amount of a red oil that accompanied the yellow product was not soluble; λ_{max}^{EtOH} 254 mμ (log ε 3.57), 371 mμ (log ε 3.83), 377 mμ (log ε 3.82).

The yellow oxidation product (10 mg) was hydrogenated in ethanol over Pd (10% on C). As soon as the yellow color disappeared (very rapidly), the hydrogenation was stopped. After filtering, the solution was diluted to 100 ml with ethanol and showed absorption maxima at 233 and 303 mμ, which are similar to IVc.

A small sample of yellow oil in benzene was reduced by shaking with aqueous sodium hydrosulfite to give an oil which formed a hydrochloride soluble in ether, insoluble in pentane, and melting at 150–152°.

Antioxidant Capacity of Ic.—Ic (0.6 mg) dissolved in 0.4 ml of a mixture consisting of 1 ml of methyl linolenate and 3 ml of ethyl laurate was used as a substrate for O_2 uptake experiments. Assuming a density for the fatty ester mixture of 0.87, Id had a nominal concentration of 0.17 wt %. Similarly, an α-tocopherol solution (2.0 mg/0.4 ml) of 0.57 wt % was prepared. Duplicate aliquots (0.1 ml) of Id and α-tocopherol solutions were placed in flasks of a Warburg apparatus. The remaining solutions were diluted with 0.2 ml of ester substrate and duplicate aliquots (0.1 ml) of these were put in Warburg flasks.

Oxygen uptake at constant pressure was measured at 100°. The induction period for the autoxidation was measured by extrapolating the constant reaction velocity portion of the autoxidation curve to zero O_2 uptake. Ic at 0.17 and 0.086 wt % gave induction times of 141 ± 32 min and 79 ± 9 min, respectively. α-Tocopherol in 0.57 and 0.29 wt % gave induction times of 62 ± 4 min and 37 min (one value), respectively.

Toxicity of Ic and II. A diet consisting of sucrose (60%), casein (20%), corn oil (5%), lard (10%), salt mix W (4%), and vitamins (1%) was fed to groups of Swiss white mice. Each group consisted of two males and two females. The compounds to be tested were dissolved in warm corn oil (20 mg/1 ml), and an appropriate amount of this oil was used to replace corn oil in the basic diet. Ic was present in the diet groups in 0.0125, 0.025, 0.05, 0.1, 0.3, 1.0, and 3.0 wt %. II was fed in diets containing 0.3, 1.0, and 3.0 wt %. The animals were fed on Monday, Wednesday, and Friday, and were weighed on Monday and Friday.

Acknowledgments.—The author is indebted to Mrs. Dorothy Thompson and Mrs. Mary Louise Fantel for aiding in the autoxidation and feeding experiments.