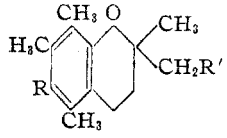
[JOINT CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA AND THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

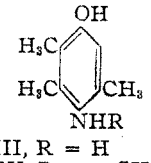
The Chemistry of Vitamin E. XXXVIII.[1,2] α-Tocopheramine,[3] a New Vitamin E Factor

BY LEE IRVIN SMITH, W. B. RENFROW, JR., AND J. W. OPIE

α-Tocopherol, the most active vitamin E factor, possesses structure I, and it has been prepared by condensation of trimethylhydroquinone with phytyl halides, phytol, or phytadiene.[4]



I, R = OH; R^1 = 3,7,11-trimethyldodecyl-1
II, R = NH_2; R^1 = 3,7,11-trimethyldodecyl-1

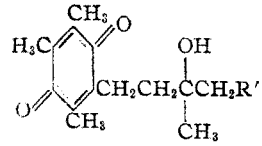


III, R = H
IV, R = —CHO

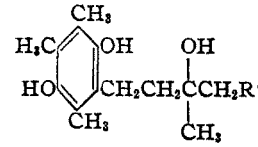
Since α-tocopherol is an oil, its conversion into a solid derivative of high biological activity would offer many advantages. Solid derivatives of the vitamin are few in number, and the easiest of these to prepare—the allophanate[5] and the 3,5-dinitrophenylurethan,[6] are obviously not suitable for biological purposes.[7]

It occurred to us that 2,3,5-trimethyl-4-aminophenol (III), an intermediate in the preparation of trimethylhydroquinone, might condense with various derivatives of phytol in a manner analogous to that exhibited by trimethylhydroquinone. If so, the product II would be the amino-analog of α-tocopherol, and this substance might possess a high biological activity and might be a solid, or at least might form a solid salt. These expectations have been realized, and it has been found that the condensation of phytol with the aminophenol III—or better with its formyl derivative IV—takes place in formic acid, leading to a fair yield of the amino compound II.

The structure of the amino compound (II) was shown by its conversion into α-tocopherol. Oxidation of the amine with ferric chloride produced the known yellow tocopherylquinone (V), which was reduced to the hydroquinone (VI) and cyclized to α-tocopherol I, according to the procedure of Tishler and Wendler.[8]



V



VI

α-Tocopheramine hydrochloride is insoluble in water, although an opalescence results when 0.01 g. of it is shaken with 5 cc. of water. If a drop or two of hydrochloric acid is added to the mixture, the hydrochloride dissolves, although the solution remains somewhat turbid. The salt is quite soluble in alcohol and in ether. In the bio-assay, carried out by Dr. H. M. Evans of the University of California,[9] the hydrochloride showed a vitamin E activity quite comparable to that of α-tocopherol. The substance was dissolved in water acidified with hydrochloric acid to *p*H 3, and fed at a level of 5 mg. to each of 9 rats. There resulted 5 good litters with an average of 10.4 living young (average weight, 5.2 g.); 2 poor litters of 2 dead young each; 1 litter cast but eaten; and 1 resorption. Thus, on a molecular basis, the substance is approximately as active biologically as is α-tocopherol itself.

The biological activity of α-tocopheramine raises some interesting questions as to the mechanism whereby α-tocopherol exerts its biological effect. If the phenolic hydroxyl group in α-tocopherol is absent,[10] or is masked as an ether or as the allophanate, complete inactivity results. But many carboxylic esters of α-tocopherol are as active as the parent substance itself.[11] These facts

(1) Paper XXXVII, THIS JOURNAL, **64**, 646 (1942).

(2) Presented at the 103rd meeting of the American Chemical Society, Memphis, Tenn., April, 1942.

(3) The Authors acknowledge with thanks the suggestion of Dr. C. R. Addinall, that this substance be named "α-tocopheramine."

(4) (a) Karrer, Fritzsche, Ringier and Salomon, *Helv. Chim. Acta*, **21**, 520 (1938); (b) Smith, Ungnade and Prichard, *Science*, **88**, 37 (1938); (c) Bergel, Jacob, Todd and Work, *Nature*, **142**, 36 (1938); *J. Chem. Soc.*, 1382 (1938); (d) Smith and Ungnade, *J. Org. Chem.*, **4**, 298 (1939); (e) Smith, Ungnade, Hoehn and Wawzonek, *ibid.*, **4**, 311 (1939); (f) Smith, Ungnade, Stevens and Christman, THIS JOURNAL, **61**, 2615 (1939).

(5) Evans, Emerson and Emerson, *J. Biol. Chem.*, **113**, 319 (1936).

(6) Smith and Sprung, THIS JOURNAL, **64**, 433 (1942).

(7) The allophanate is inactive biologically.

(8) Tishler and Wendler, THIS JOURNAL, **63**, 1532 (1941).

(9) We wish to thank Dr. Evans for his aid in this investigation.

(10) v. Werder, Moll and Jung, *Z. physiol. Chem.*, **257**, 129 (1939).

(11) Demole, Isler, Ringier, Salomon and Karrer, *Helv. Chim. Acta*, **22**, 65 (1939).

indicate that the hydroxyl group is of fundamental importance in bringing about the biological activity, for if it is missing, or is masked in such a way that the body cannot remove the masking group, the activity is lost. There have been speculations as to the possible connection between the biological activity of α-tocopherol and the oxidation–reduction potential of the related quinone (V)[12]; these speculations were current at the time when the quinone (V) was believed to possess vitamin E activity. But recently it has been conclusively shown[13] that α-tocopherylquinone (V), when carefully purified, is devoid of any biological activity whatsoever; so speculations as to the mechanism of the biological action of the vitamin which involved this quinone, have had to be abandoned. Yet α-tocopheramine hydrochloride is a very active vitamin E factor, as active as α-tocopherol, and the only apparent chemical link between these two substances is this very quinone V, which is obtained from both substances by gentle oxidation. In spite of the biological inactivity of the quinone (V), the great activity of α-tocopheramine makes it impossible to discard the quinone as a factor of importance in producing vitamin E activity, but much more work will have to be done before the complete picture emerges.

In order to explore further these effects, we have under way the preparation of other analogs of the tocopherols.

Experimental[14]

2,3,5-Trimethyl-4-aminophenol (III) (54 g.) was prepared from 2,3,5-trimethylphenol (68 g.) according to the procedure previously described.[15] After crystallization from toluene, the substance melted at 149–152°.

2,3,5-Trimethyl-4-formylaminophenol (IV).—The aminophenol hydrochloride (81 g.) and sodium formate (34 g.) were refluxed for two and three-quarters hours in formic acid (88%, 277 cc.). Additional formic acid (25 cc.) was added during the period of refluxing in order to compensate for losses by evaporation. The reaction mixture was poured into cold water, and the solid was removed and washed four times with water. This material weighed 65 g. (84%) and melted at 212–213°. The analytical sample, prepared by crystallizing a small amount of the crude material twice from ethanol, melted at 213–214°.

Anal. Calcd. for $C_{10}H_{13}O_2N$: C, 67.02; H, 7.31. Found: C, 66.75; H, 7.44.

α-Tocopheramine Hydrochloride (II).—The formylaminophenol (IV) (14.4 g.) was placed in a three-necked flask equipped with a mechanical stirrer and an inlet for nitrogen. Anhydrous formic acid (120 cc.) was distilled from anhydrous copper sulfate directly into the flask, and then phytol (24 g.) was added. The mixture was surrounded by a bath at 135° and was stirred for twenty-two hours while a slow stream of dry nitrogen was passed through the apparatus. The reaction mixture was poured over ice and the dark oil was taken up in ether. The ether solution was washed successively once with water, three times with sodium hydroxide (10%, 60 cc. each time), then twice with saturated sodium chloride (60 cc. each time). After drying over magnesium sulfate, the ether solution was filtered and evaporated. The residue was dissolved in petroleum ether (200 cc., b. p. 90–100°) and poured through an 8 inch tube packed with alumina (Brockmann) and the chromatograph was developed with 400 cc. (b. p. 90–100°) of petroleum ether. The dark bands at the top and bottom were removed and the rest of the column was eluted with 400 cc. of ether–methanol mixture (9:1). The light brown eluate was filtered and evaporated, and the semi-solid residue was refluxed for four hours with absolute ethanol (180 cc.) and hydrochloric acid (50 cc.). This solution, when placed in a refrigerator for forty-eight hours, deposited a crystalline solid. The solid was removed, washed twice with cold absolute alcohol (20 cc. each time) and recrystallized from a mixture of absolute alcohol (30 cc.) and hydrochloric acid (20 cc.). The product, at this point, was colored and melted at 78–106°. It was dissolved in dry ethanol (50 cc.) containing hydrochloric acid (2 cc.), and the solution was boiled with Norit (1 g.) for a minute. The hot mixture was filtered and the Norit was washed three times with hot alcohol (20 cc. each time). After a second treatment with Norit, the combined filtrates and washings were colorless. Hydrochloric acid (30 cc.) was added and the mixture was set aside in a refrigerator. The solid was removed and washed several times with petroleum ether, after which it was dried for three hours at 100° under high vacuum. It weighed 2.13 g., and melted at 155–157°.

Anal. Calcd. for $C_{29}H_{52}ONCl$: C, 74.69; H, 11.25. Found: C, 75.24; H, 11.38.

This product was recrystallized from alcoholic hydrochloric acid (7:3) and dried at room temperature for fifty-six hours under high vacuum in the presence of sodium hydroxide and calcium chloride. The material then weighed 1.51 g. and melted at 156–158°.

Anal. Calcd. for $C_{29}H_{52}ONCl$: C, 74.69; H, 11.25; N, 3.00. Found: C, 74.84; H, 11.45; N, 3.10.

The free amine formed a stable oil which boiled at 285–288° under 1–2 mm., and by action of anhydrous oxalic acid (1.58 g.) in alcohol (25 cc.) upon a solution of the distilled amine (8 g.) in alcohol (225 cc.), there resulted a crystalline oxalate which melted at 153–154°.

Frequently, the preparation of the amine hydrochloride led to a product which had the composition of a hydrochloride hemihydrate. No quantitative experiments were made upon this material to show that it actually was a definite hydrate, nor was it possible to predict which form of the hydrochloride would result from a given procedure. The two forms had approximately the same melting point and appearance, and one form may be merely an impure form of the other. Yet, it is curious that the analyses in-

(12) Smith, *Chem. Reviews*, **27**, 323 (1940).

(13) Karrer and Geiger, *Helv. Chim. Acta*, **23**, 455 (1940).

(14) Microanalyses by E. E. Renfrew and D. Hayman.

(15) (a) Smith, Opie, Wawzonek and Prichard, *J. Org. Chem.*, **4**, 318 (1939); (b) Smith and Opie, *ibid.*, **6**, 427 (1941).

variably checked well either for the anhydrous hydrochloride or for the hemihydrate.

Anal. Calcd. for $C_{29}H_{52}ONCl \cdot {}^1/_2H_2O$: C, 73.28; H, 11.24. Found: C, 72.73, 73.63, 73.13; H, 11.43, 11.06, 10.83.

Conversion of α-Tocopheramine to α-Tocopherol.—A suspension of the amine hydrochloride (0.95 g.) in water (25 cc.), methanol (10 cc.), hydrochloric acid (1 cc.) and ferric chloride hexahydrate (1.62 g.) was heated to 70° and stirred for thirty minutes. The mixture was cooled and extracted with ether. The ether solution was dried (Drierite) and evaporated, and the residue of viscous oil was stirred for two and one-half hours with methanol (20 cc.) to which had been added a solution of sodium hydrosulfite (1 g.) in water (2 cc.). The mixture was poured into a separatory funnel containing water (70 cc.) and petroleum ether (10 cc.) and was shaken thoroughly. The petroleum ether layer was removed and chilled in an ice-bath. The voluminous white precipitate of tocopherylhydroquinone (VI) was separated by centrifuging and washed twice with a little cold petroleum ether. The solid was refluxed for four hours with dioxane (10 cc.), hydrochloric acid (1.7 cc.) and stannous chloride dihydrate (2.3 g.). This mixture was poured into water and extracted with petroleum ether. The extract was washed with water, dried (Drierite) and the solvent was evaporated. There remained 0.37 g. (43%) of faintly straw-colored α-tocopherol.

Summary

1. α-Tocopheramine, the amino analog of α-tocopherol, has been prepared by condensation of 2,3,5-trimethyl-4-formylaminophenol with phytol.

2. The hydrochloride of this amine has been converted into α-tocopherol by the procedure of Tishler and Wendler.

3. The amine hydrochloride exhibits vitamin E activity in approximately the same degree as does α-tocopherol.

4. Although tocopherylquinone possesses no vitamin E activity, it cannot be eliminated as a possible factor in producing biological activity, in view of its close relationship to both α-tocopherol and α-tocopheramine.

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The Chemistry of Vitamin E. XXXIX. Calcium α-Tocopheryl Succinate[1]

BY LEE IRVIN SMITH, W. B. RENFROW, JR., AND J. W. OPIE

The rather large number of simple esters of α-tocopherol which have been prepared[2] include many which have a high biological activity, but all of these are liquids. It appeared likely that the metallic salts of the acid esters of α-tocopherol and dibasic acids would be stable, solid substances with a high degree of biological activity. This paper reports the results of experiments along these lines, and includes a description of the preparation and properties of calcium α-tocopheryl succinate.

2,2,5,7,8-Pentamethyl-6-hydroxychroman was used in the model experiments, and it was found that this chroman failed to give an ester with ethyl chlorocarbonate, alone or in the presence of alcoholic potassium hydroxide or sodium methoxide in methanol. However, the bromomagnesium derivative of the chroman reacted readily with ethyl chlorocarbonate to give the mixed carbonate ester, a solid melting at 50–52°. This ester was extremely sensitive to alkali, hydrolyzing completely in a minute or two when in contact with dilute alkali.

Using the bromomagnesium derivative of the chroman, the acid succinate and the chloroacetate were prepared by action of succinic anhydride and chloroacetyl chloride, respectively. These esters, like the carbonate, were hydrolyzed with extreme ease. The succinate dissolved in 2% sodium hydroxide to give a clear solution, but within thirty seconds this solution became cloudy and the chroman began to precipitate. It had been planned to use the chloroacetate as an intermediate in the preparation of the glycine ester of the chroman, but action of ammonia upon a solution of the chloroacetate in methanol gave only the chroman, and no glycine ester.

Succinic, maleic and phthalic anhydrides reacted with the bromomagnesium derivative of α-tocopherol to give the corresponding acid esters. The acid succinate formed a pasty solid which was converted into potassium, calcium and barium

(1) Presented at the 103rd meeting of The American Chemical Society, Memphis, April, 1942; Paper XXXVIII, THIS JOURNAL, **64**, 1082 (1942).

(2) Demole, Isler, Ringier, Salomon and Karrer, *Helv. Chim. Acta*, **22**, 65 (1939).