123. *Studies on Vitamin E. Part VI. Synthesis of Lower Homologues of α-Tocopherol.*

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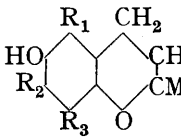
In continuation of the work described in Part V (J., 1938, 1382) two racemic tocopherols (I; $R_1 = R_3 = Me$; $R_2 = H$) and (II; $R_1 = H$; $R_2 = R_3 = Me$) have been synthesised. Satisfactory yields of these are obtained by condensing p- and o-*xyloquinol monobenzoates* with phytol or phytyl bromide in presence of zinc chloride. The products are characterised as crystalline p-*nitrophenylurethanes* and show biological activity comparable with that of β- and γ-tocopherol.

It is now generally accepted that α-tocopherol, the most active anti-sterility factor (vitamin E) known to occur in nature, is to be regarded as 6-hydroxy-2 : 5 : 7 : 8-tetramethyl-2-(4′ : 8′ : 12′-trimethyltridecyl)chroman (I; $R_1 = R_2 = R_3 = Me$). Its formulation as a chroman rather than a coumaran derivative is justified on the basis of certain degradative evidence (Fernholz, *J. Amer. Chem. Soc.*, 1938, **60**, 700; John, Dietzel, Günther, and Emte, *Naturwiss.*, 1938, **26**, 366) and by the methods used for its synthesis (Karrer, Salomon, and Fritzsche, *Helv. Chim. Acta*, 1938, **21**, 520; Bergel, Copping, Jacob, Todd, and Work, J., 1938, 1382; Smith, Ungnade, and Pritchard, *Science*, 1938, **88**, 37).



(I.)

The naturally occurring anti-sterility factors β- and γ-tocopherol are to be regarded as isomeric lower homologues of α-tocopherol bearing only two methyl groups on the aromatic nucleus (cf. Part V, *loc. cit.*), the side chain in both being the same as in α-tocopherol (Emerson, *J. Amer. Chem. Soc.*, 1938, **60**, 1741). In Part V it was pointed out that three isomers fulfilling these conditions were possible, derived from *o*-, *m*-, and *p*-xyloquinol, and one of these was synthesised by condensation of phytol with *m*-xyloquinol. The tocopherol had a vitamin E activity roughly comparable with those of β- and γ-tocopherol. Consequent on this observation we undertook the synthesis of the remaining isomers. Direct condensation of *o*- or *p*-xyloquinol with phytol or phytyl bromide was not a practicable method; the products were complex mixtures and separation of the pure tocopherols was extremely difficult. Karrer and Fritzsche (*Helv. Chim. Acta*, 1938, **21**, 1234) recorded rather similar results; by direct condensation of the xyloquinols with phytol or phytyl bromide, followed by chromatographic analysis, they obtained vitamin E-active oils, which, although they gave analytical figures approximating to the calculated values for $C_{28}H_{48}O_2$, were apparently impure, since the Swiss workers failed to obtain pure derivatives from them.

Our initial approach to the synthesis of the desired substances was by condensation of the *quinol monobenzyl ethers* with phytyl bromide, since the benzyl group, which would prevent condensation with two phytyl residues, could be removed subsequently by catalytic hydrogenation. Using this method, we obtained oils showing vitamin E activity, but the products were difficult to purify and the yields rather low. A further inconvenience lay in the fact that the benzyl ethers were difficult to prepare in good yield. A variety of methods were tried for their preparation, but none was really satisfactory. An interesting feature of the direct benzylation of *p*-xyloquinol was the formation of a violet-red *substance*, m. p. 111—112°, as a by-product. This substance was not further investigated, but analysis and absorption spectrum suggest that it may be a derivative of a diquinone analogous to the diduroquinone of v. Pechmann (*Ber.*, 1889, **22**, 2115).

Condensation of the *monobenzoates* with phytol or phytyl bromide, followed by the removal of benzoyl groups by hydrolysis, proved entirely satisfactory, chromatographic analysis of the products yielding the desired tocopherols as yellowish oils showing full vitamin E activity in rats at a dose of 10 mg. From both of them, crystalline p-*nitrophenylurethanes* were readily prepared. All these synthetic tocopherols are, of course, racemic about carbon atom 2 in the heterocyclic ring and have not yet been resolved. The product from *p*-xyloquinol (I; $R_1 = R_3 = Me$; $R_2 = H$) shows vitamin E activity

in a dose of 5 mg., which corresponds to the minimum active dose of natural β-tocopherol (cf. Part II, *Biochem. J.*, 1937, **31**, 2257); the minimum active dose of the others is at present being determined.

John, Günther, and Schmeil (*Ber.*, 1938, **71**, 2637) have described experiments carried out in the course of their efforts to synthesise α-tocopherol and simpler 6-hydroxychroman derivatives. We record here briefly some results of experiments, carried out early in 1938, which more or less confirm the observations of the German workers. In an endeavour to synthesise 6-hydroxy-2 : 2 : 5 : 7 : 8-pentamethylchroman we condensed ψ-cumoquinol with ββ-dimethylacrylyl chloride in the presence of aluminium chloride; the only crystalline product was a small amount of a substance, m. p. 232°, spectroscopically unlike a chromanone. Similarly we failed to isolate any chromanone in a similar condensation of phytenyl chloride with ψ-cumoquinol. Among other unsuccessful attempts to synthesise α-tocopherol was an endeavour to condense 3-bromophytan 1-acetate with ψ-cumoquinol in the presence of sodium ethoxide, followed by removal of the acetyl residue and ring closure; no pure products could be isolated.

Experimental.

p-*Xyloquinol Benzyl Ethers.*—*p*-Xyloquinol (30 g.) was dissolved in a solution of sodium (4·9 g.; 1 atom) in absolute alcohol (150 c.c.), benzyl chloride (27·4 g.) added, and the mixture refluxed for 4 hours in a nitrogen atmosphere. After dilution with ether and filtration from sodium chloride the solution was evaporated, and the residue dissolved in the minimum quantity of hot methyl alcohol. On cooling, p-*xyloquinol dibenzyl ether* separated in colourless needles (12 g.); recrystallised from methyl alcohol, it had m. p. 130° (Found: C, 82·9; H, 6·6. $C_{22}H_{22}O_2$ requires C, 83·0; H, 6·9%).

The combined methyl-alcoholic mother-liquors were evaporated, and the residue extracted with light petroleum (b. p. 40—60°); the extract was allowed to flow through a column of activated aluminium oxide (Merck), the column being then washed with more light petroleum. From the filtrate and washings a further amount of dibenzyl ether (2 g.) was obtained. The residue of the light petroleum extraction was now dissolved in benzene, filtered from *p*-xyloquinol (5 g.), and poured through the same column as was used above. The chromatogram formed was washed with benzene until nothing more came through. Unchanged *p*-xyloquinol remained adsorbed and could be eluted with ether. The benzene filtrate and washings, which were yellow, were concentrated to small bulk and diluted with light petroleum. On standing, two compounds separated simultaneously, one forming warty aggregates of colourless prisms (A) and the other rosettes of violet-red needles (B). These were separated mechanically. (A) on recrystallisation from ligroin gave p-*xyloquinol monobenzyl ether* (3·5 g.), colourless prisms, m. p. 92—93° (Found: C, 78·6; H, 6·9. $C_{15}H_{16}O_2$ requires C, 78·9; H, 7·0%). (B) crystallised from nearly colourless solutions in ligroin in violet-red needles (2 g.), m. p. 111—112° (Found: C, 76·0; H, 6·6. $C_{23}H_{24}O_4$ requires C, 75·8; H, 6·6%). This *substance*, which was quite stable, dissolved in aqueous alkali to give solutions from which it was reprecipitated with acids, and on oxidation with chromic acid gave *p*-xyloquinone together with other products. Alcoholic solutions showed two well-marked absorption maxima at 2520 A.($E^{1\%}_{1\,cm.}$ = 616) and 2920 A.($E^{1\%}_{1\,cm.}$ = 113) with a band of negligible persistence at about 4300 A. Variation in the conditions of the above experiment or the use of benzyl bromide had little effect on the yield of monobenzyl ether. Other methods tried included benzylation of *p*-xyloquinol monobenzoate, use of the potassium derivative of *p*-xyloquinol, and partial debenzylation of the dibenzyl ether with aluminium chloride.

o-*Xyloquinol Benzyl Ethers.*—Benzylation of *o*-xyloquinol (10·5 g.) in the manner described above yielded in similar fashion o-*xyloquinol dibenzyl ether* (3·5 g.), crystallising from methyl alcohol in colourless needles, m. p. 109° (Found: C, 82·7; H, 6·9%), and o-*xyloquinol monobenzyl ether* (3·5 g.), crystallising from benzene–light petroleum in colourless needles, m. p. 116° (Found: C, 78·5; H, 6·9%). No red compound was formed.

Condensation of o-Xyloquinol Monobenzyl Ether with Phytyl Bromide.—A mixture of phytyl bromide (3 g.), *o*-xyloquinol monobenzyl ether (2 g.), anhydrous zinc chloride (2 g.), and hexane (50 c.c.) was gently refluxed for 2½ hours in a nitrogen atmosphere, by which time evolution of hydrogen bromide had ceased. The product, dissolved in light petroleum (b. p. 40—60°), was adsorbed on a column of activated aluminium oxide (Merck), the column being washed with the same solvent. From the washings a small quantity of a yellow solid was obtained as well as an

oil. The solid separated from ligroin in yellow needles, m. p. 111° (Found: C, 82·8; H. 6·1%). In alcoholic solution it showed a single absorption band at 2630 A.($E^{1\%}_{1\,cm.}$ = 532) with a second band of negligible persistence at about 4450 A.

The material adsorded on the alumina was eluted with benzene–acetone–methyl alcohol (8 : 1 : 1), hydrogenated with a palladised charcoal catalyst to remove benzyl groups, and again submitted to chromatographic adsorption. A brownish oil was finally obtained showing vitamin E activity in rats at a dose of 30 mg. On pyrolysis it gave a poor yield of ψ-cumoquinol, m. p. 165—167°, and allophanation did not give a homogeneous product.

Condensation of p-*Xyloquinol Monobenzyl Ether with Phytyl Bromide.*—Condensation as described above gave oils showing some vitamin E activity in doses of 10 mg., but allophanation did not give a homogeneous product.

p-*Xyloquinol Benzoates.*—To a solution of *p*-xyloquinol (2 g.) in dry pyridine (10 c.c.), benzoyl chloride (3 g.; 1·5 mols.) was added, and the mixture left at room temperature for 20 hours. The crude benzoate mixture obtained on working up was dissolved in hot methyl alcohol. On cooling, p-*xyloquinol dibenzoate* (1 g.) separated; it crystallised from methyl alcohol in colourless needles, m. p. 159° (Found: C, 76·5; H, 5·4. $C_{22}H_{18}O_4$ requires C, 76·3; H, 5·2%). After separation of the dibenzoate the mother-liquor was evaporated; the residue, recrystallised from light petroleum, furnished p-*xyloquinol monobenzoate* (1·9 g.) in colourless leaflets, m. p. 162—163° (Found: C, 74·7; H, 5·6. $C_{15}H_{14}O_3$ requires C, 74·4; H, 5·8%).

o-*Xyloquinol Benzoates.*—Benzoylated as above described, *o*-xyloquinol yielded a *dibenzoate*, m. p. 182° after recrystallisation from acetone–methyl alcohol (Found: C, 76·2; H, 5·3%), and a *monobenzoate*, m. p. 174—175° after recrystallisation from acetone–light petroleum (Found: C, 74·4; H, 5·9%).

p-*Xyloquinol Acetates.*—Acetylation of *p*-xyloquinol in pyridine solution with acetic anhydride (1·5 mols.) gave the diacetate, m. p. 135° (Found: C, 64·9; H, 6·4. Calc. for $C_{12}H_{14}O_4$: C, 64·9; H, 6·3%), and the *monoacetate*, m. p. 117° (Found: C, 66·4; H, 6·5. $C_{10}H_{12}O_3$ requires C, 66·7; H, 6·7%); the yield of the latter was, however, poor.

Condensation of p-*Xyloquinol Monobenzoate with Phytol.*—A mixture of the monobenzoate (2·3 g.), phytol (3 g.), anhydrous zinc chloride (1·5 g.), and decalin (25 c.c.) was heated at 170° in an oil-bath for 2½ hours, cooled, and diluted with light petroleum (b. p. 40—60°). After several hours the solution was filtered. From the filter residue, after removal of zinc chloride, unchanged monobenzoate (1·4 g.) was recovered. The filtrate was evaporated, the decalin removed in a vacuum, and the oily residue hydrolysed by refluxing for 2 hours in a hydrogen atmosphere with methyl-alcoholic potassium hydroxide (25 c.c. of 5%). The solution was cooled and extracted with ether, and the extract dried over sodium sulphate and evaporated. The residual oil was dissolved in light petroleum (b. p. 40—60°) and allowed to flow through a column of activated aluminium oxide (Merck), the chromatogram being developed with benzene until nothing further came through. Evaporation of the filtrate gave a quantity of yellowish-brown oil having no reducing properties; it probably consisted of aliphatic material. The narrow brownish layer at the top of the column was discarded and the remainder, which was approximately uniform in colour save for a yellowish ring near the base, was cut into three parts of equal length. These were separately eluted with acetone. The lowest layer yielded an oil which had no reducing properties and was therefore not further examined. The top and the middle layer yielded respectively 450 mg. and 500 mg. of crude tocopherol, which reduced neutral silver nitrate solution on warming and gave a yellow colour with concentrated sulphuric–glacial acetic acids. On pyrolysis at about 350° they yielded ψ-cumoquinol, which was identified by m. p. and mixed m. p.

When phytyl bromide was substituted for phytol in the above experiment, and the condensation carried out by refluxing a mixture of the reactants with light petroleum (b. p. 80—100°) for 5 hours, similar results were obtained. The crude tocopherol obtained in these experiments showed full vitamin E activity in rats at a dose of 10 mg.; it was not analysed, but converted directly into the p-*nitrophenylurethane*. The oil (450 mg.) was heated at 90—100° during 1 hour with *p*-nitrophenyl *iso*cyanate (0·5 g.) in a nitrogen atmosphere. The mixture was cooled, diluted with acetone containing a drop of water to destroy any *iso*cyanate, and left for an hour or two. The mixture was now evaporated, and the urethane extracted from the residue with light petroleum (b. p. 40—60°). The extract gave on evaporation a crystalline product (550 mg.) still contaminated with *p*-nitroaniline. This could be removed either by crystallisation from methyl alcohol or by absorption from light petroleum solution on a short column of aluminium oxide; when the column was washed with benzene, the urethane was eluted much more rapidly than the amine. Recrystallised from methyl alcohol, the *p*-nitrophenylurethane of tocopherol

(I; $R_1 = R_3 = Me$; $R_2 = H$) [6-hydroxy-2 : 5 : 8-trimethyl-2-(4′ : 8′ : 12′-trimethyltridecyl)-chroman] was obtained in globular aggregates of colourless crystals (450 mg.), m. p. 111—112° (Found: C, 72·5; H, 9·0; N, 4·9. $C_{35}H_{52}O_5N_2$ requires C, 72·4; H, 9·0; N, 4·8%). In alcoholic solution the absorption spectrum showed a maximum at 3160 A.(ε mol., *ca.* 16000) and a minimum at 2500 A.

Hydrolysis of the ester (100 mg.) with 5% methyl-alcoholic potassium hydroxide yielded the pure tocopherol as a slightly yellowish oil (58 mg.) corresponding closely in chemical behaviour to natural β-tocopherol. In alcoholic solution its absorption spectrum showed a maximum at 2960 A.(ε mol., *ca.* 3600) and a minimum at 2580 A. It showed full vitamin E activity when tested in rats in doses of 5 mg.

Condensation of o-*Xyloquinol Monobenzoate with Phytyl Bromide.*—The ester (3·2 g.), phytyl bromide (4·8 g.), and anhydrous zinc chloride (2 g.) were refluxed in dry benzene (30 c.c.) for 3 hours in a hydrogen atmosphere, by which time evolution of hydrogen bromide had ceased. The mixture was diluted with ether, and the solution decanted, washed, dried, and evaporated. The residue was completely soluble in light petroleum, *i.e.*, contained no unchanged *o*-xyloquinol monobenzoate. The product was subjected to the same process of hydrolysis and chromatographic analysis as in the previous experiment. The crude tocopherol (1·2 g.) was a yellowish oil showing full vitamin E activity in rats at a dose of 10 mg. It reduced neutral silver nitrate solution on warming, gave a yellow colour with concentrated sulphuric–glacial acetic acids, and on pyrolysis at about 350° yielded ψ-cumoquinol, identified by m. p. and mixed m. p. Heated with *p*-nitrophenyl *iso*cyanate either alone or in benzene solution, the product gave the p-*nitrophenylurethane* of the tocopherol (I; $R_1 = H$; $R_2 = R_3 = Me$) [6-hydroxy-2 : 7 : 8-trimethyl-2-(4′ : 8′ : 12′-trimethyltridecyl)chroman]. Recrystallised from methyl alcohol, the ester had m. p. 100° (Found: C, 72·6; H, 9·1; N, 4·8%). In alcoholic solution the absorption spectrum showed a maximum at 3130 A.(ε mol., *ca.* 13000) and a minimum at 2530 A. Hydrolysis with 5% methyl-alcoholic potassium hydroxide yielded the pure tocopherol as a slightly yellowish oil corresponding closely in chemical behaviour to natural β-tocopherol. Alcoholic solutions showed an absorption maximum at 2970 A. (ε mol., *ca.* 3400) and a minimum at 2640 A. Tests are in progress to determine the minimum active dose of this product.

p-*Nitrophenylurethane of Tocopherol from* m-*Xyloquinol and Phytol.*—The crude tocopherol (I; $R_1 = R_2 = Me$; $R_3 = H$) [6-hydroxy-2 : 5 : 7-trimethyl-2-(4′ : 8′ : 12′-trimethyltridecyl)-chroman], prepared by condensing *m*-xyloquinol with phytol (Part V, J., 1938, 1382) and biologically active in a dose of 10 mg., was heated with *p*-nitrophenyl *iso*cyanate, and the product worked up as above described. The p-*nitrophenylurethane* had m. p. 89° after recrystallisation from methyl alcohol (Found: C, 72·4; H, 8·9; N, 5·0%). In alcoholic solution the absorption spectrum showed a maximum at 3160 A. (ε mol., *ca.* 18000) and a minimum at 2520 A.

The biological tests were carried out at the Lister Institute by Miss A. M. Copping (M.R.C. grantee), to whom we are indebted. We have also to thank Messrs. Hoffmann La Roche and Company for generous gifts of material.

(*Note added, February 27th,* 1939.) Since the above was written a paper by Karrer and Fritzsche (*Helv. Chim. Acta*, 1939, **22**, 260) has appeared in which the authors describe derivatives of products obtained by direct condensation of *m*- and *p*-xyloquinol with phytol or phytyl bromide. The data given for one of these (*p*-nitrophenylurethane of I; $R_1 = R_2 = Me$, $R_3 = H$) are in good agreement with our own, but their *p*-nitrophenylurethane of (I; $R_1 = R_3 = Me$, $R_2 = H$) is apparently impure, since it melts some 20° lower than the corresponding material prepared by us *via* the benzoate. In view of this fact the argument advanced by these workers for the disposition of methyl groups in β-tocopherol is hardly conclusive.

In biological tests now in progress on our product (I; $R_1 = R_2 = Me$, $R_3 = H$) from *m*-xyloquinol, positive results have been obtained at a dosage as low as 3 mg., *i.e.*, the substance seems to have an activity approaching that of α-tocopherol. We are therefore unable to agree with the statement of Karrer and Fritzsche (*loc. cit.*, p. 261) that the synthetic isomers of β-tocopherol are three to four times less active than α-tocopherol. Their statements on constitutional specificity must be accepted with considerable reserve, since their biological tests have not been carried out with material regenerated from pure derivatives.

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