67. *Studies on Vitamin E. Part VII. Further Investigations on Homologues of α-Tocopherol.*

By (Miss) A. Jacob, F. K. Sutcliffe, and A. R. Todd.

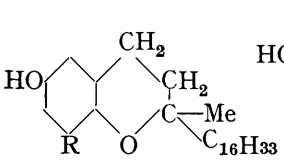
The synthesis of lower homologues of α-tocopherol by condensation of quinol and toluquinol monobenzoates with phytol, followed by removal of the benzoyl groups, is described; the tocopherols obtained showed no vitamin E activity in doses of 50 mg. In the case of quinol monobenzoate the material obtained on condensation with phytol, followed by hydrolysis, appeared to consist largely of the tocopherylquinol (II), which could be cyclised by acid treatment, yielding the tocopherol (I; R = H). Condensation of 1 : 4-dihydroxy-2-methylnaphthalene with phytol under conditions normally used for tocopherol syntheses gave an oil which apparently consisted largely of quinones related to vitamin K; this oil appeared to contain little or no vitamin E active material.

Earlier observations (Part VI; J., 1939, 542) on the high degree of activity shown by *m*-xylotocopherol have been confirmed by further biological tests; this substance closely approaches α-tocopherol in physiological potency.

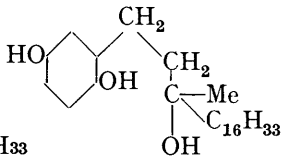
In previous publications (Part V, J., 1938, 1382; Part VI, J., 1939, 542) dealing with homologues of α-tocopherol syntheses of tocopherols containing but two methyl substituents in the aromatic nucleus were described. These substances may be regarded as condensation products of the three isomeric xyloquinols with phytol. In extension of this work tocopherols derived from toluquinol and from quinol itself were synthesised and the results are now recorded.

6-*Hydroxy*-2 : 8-*dimethyl*-2-(4′ : 8′ : 12′-*trimethyltridecyl*)*chroman* (I; R = Me) was synthesised by condensation of 2-*hydroxy*-5-*benzoyloxytoluene* with phytol, followed by removal of the benzoyl group and purification by chromatographic analysis. The product resembled the natural tocopherols in chemical properties, but neither the allophanate nor the *p*-nitrophenylurethane could be obtained in a satisfactory crystalline condition. The substance showed no biological activity in rats when tested in 50 mg. doses. Karrer and

Fritzsche (*Helv. Chim. Acta*, 1939, **22**, 260) have described a product obtained by direct condensation of toluquinol with phytyl bromide, but their product may well have been a mixture of three isomers (methyl group at 5, 7 or 8). The preparation of 6-*hydroxy*-2-*methyl*-2-(4′ : 8′ : 12′-*trimethyltridecyl*)*chroman* (I; R = H) from quinol monobenzoate and phytol proved more troublesome. The usual procedure of heating in decalin with anhydrous zinc chloride being used, a product was obtained which, after hydrolysis and repeated adsorption on aluminium oxide, furnished a low yield of product which evidently consisted mainly of the required substance. An attempt was made to effect condensation of the benzoate with phytol by heating with anhydrous formic acid, but the materials were recovered unchanged. This method has been successfully used by Karrer and his collaborators for the condensation of free quinols with phytol : its failure here is doubtless due to the lower reactivity of the monobenzoate as compared with the quinol. Repetition of the condensation with zinc chloride and decalin under milder conditions showed that, after hydrolysis, the main product was an oil, $C_{26}H_{46}O_3$, having the reducing properties of a quinol. The most probable explanation seemed to be that this product had the monocyclic structure (II); an active-hydrogen estimation gave low results, suggesting that, as isolated, the product is a mixture of (II) with the corresponding quinone, which may arise during the hydrolysis of the condensation product. In accordance with this view of its nature, the oil, on refluxing with zinc dust and hydrobromic acid in acetic acid solution, afforded a pale yellowish oil which had the properties expected of the required chroman derivative. It was characterised as its *acetate*, obtained as a stable colourless liquid. Biological tests were carried out on rats with a slightly impure preparation which had not been acetylated; doses of 50 mg. were ineffective.



(I.)



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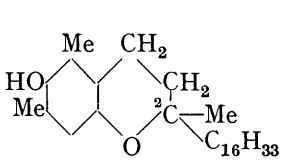
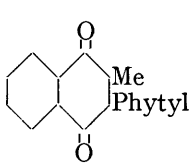


(III.)



(IV.)

In Part VI (J., 1939, 542) it was reported that interim results of biological tests on the condensation product of *m*-xyloquinol and phytol (III) indicated that it was fully active in rats at a dosage of 3 mg.; this result was confirmed. In view of the high degree of activity of this material it was desirable to have the tests repeated in another laboratory, since differences in biological activity recorded by different investigators are frequently due to differences in technique when no standard preparation is in general use. Dr. V. Demole of Messrs. Hoffmann-La Roche and Co., Basle, to whom we are deeply grateful, kindly tested our material (purified *via* the *p*-nitrophenylurethane) in his laboratory. He reported, in accordance with our results, that 3 mg. and 5 mg. doses of the substance gave 100% response and 2 mg. doses 70% response in rats. Karrer, Koenig, Ringier, and Salomon (*Helv. Chim. Acta*, 1939, **22**, 1139) report that their preparation of this tocopherol, purified *via* the allophanate, showed 100% activity at a dose of 10 mg. (confirming their earlier value) and only 66% activity at a dose of 5 mg. It is difficult to explain these discrepancies on the basis of variations in the tests, since our results were obtained in two different laboratories, one of which also carried out biological tests for the Swiss workers. We must therefore adhere to the view that our substance (III) has a biological activity almost as great as that of natural α-tocopherol. The difference is the more remarkable as our values for the activity of the tocopherols from *o*- and *p*-xyloquinols (5 mg. and 10 mg.) agree substantially with those of the Swiss workers (6 mg. and 10 mg.). The only apparent difference in the experiments of the two groups of workers is that Karrer and his collaborators purified their tocopherol before testing *via* the allophanate while we used the *p*-nitrophenylurethane. Since the condensation of *m*-xyloquinol with natural (racemic) phytol, which contains two asymmetric centres, introduces a third asymmetric centre (at position 2), it is clear that the synthetic material cannot be a simple racemate or racemic

mixture but must be a mixture of stereoisomers. This raises the possibility that the different degrees of physiological activity recorded might have their origin in stereochemical differences in the synthetic materials tested. The fact that natural α-tocopherol and the synthetic product have approximately the same biological activity does not necessarily preclude the possibility of stereochemical configuration being of importance in the vitamin E group, particularly when it is realised that the biological test method has a considerable margin of error. This point seems worthy of further investigation, especially as the stereochemistry of the tocopherols must be worked out before rigid identification of the natural vitamins E is possible.

Karrer, Koenig, Ringier, and Salomon (*loc. cit.*), referring to the synthesis of tocopherols from *o*- and *p*-xyloquinols, state that, contrary to our view, the use of monobenzoates as starting materials is unnecessary. We wish to take this opportunity of pointing out that we have never claimed the use of quinol esters to be necessary, although we still consider it preferable to the use of free quinols save where the location of substituents (as in *m*-xyloquinol) precludes the possibility of side reactions.

As regards the structural specificity of vitamin E activity it seems clear that removal of two or three methyl groups from the aromatic nucleus of α-tocopherol gives compounds of negligible activity. Removal of one methyl group from α-tocopherol reduces the activity somewhat, but in the case of the 5 : 7-dimethyl compound the diminution in activity is very small. It seemed, therefore, of interest to attempt the synthesis of a tocopherol analogue from 1 : 4-dihydroxy-2-methylnaphthalene and phytol; this substance, like α-tocopherol, would have all positions in the hydroxylated aromatic nucleus substituted. Condensation of the two substances was carried out in decalin solution in presence of zinc chloride. The product proved to be a complex mixture consisting to a large extent of a yellowish-brown oil with the reactions of a quinone and a spectrum reminiscent of that exhibited by the antihæmorrhagic factor, vitamin K. Attempts to isolate homogeneous materials from it by adsorption on aluminium oxide, followed by reductive acetylation and distillation in a high vacuum, were unsuccessful. At the period when these experiments were being carried out (July—August, 1939) it became known through the work of Doisy *et al.* (*J. Amer. Chem. Soc.*, 1939, **61**, 1928, 2558), Fieser *et al.* (*ibid.*, pp. 1925, 2559), and Almquist and Klose (*ibid.*, p. 2558) that vitamin K_1 is 2-methyl-3-phytyl-1 : 4-naphthaquinone (IV) and can be synthesised from 1 : 4-dihydroxy-2-methylnaphthalene and phytol by condensation under mild conditions in presence of anhydrous oxalic acid (Fieser, *loc. cit.*, p. 2559). It was thus clear that our products contained, as we suspected, mainly quinones related to vitamin K_1 together probably with some of the desired tocopherol. As samples of our partly purified products showed no vitamin E activity in doses of 250 mg., and in view of the synthetic work on vitamin K_1 in other laboratories, the condensation was not further investigated. A brief account of our results is given in the experimental section of this paper.

It is an interesting fact that both vitamin E and K are condensation products of phytol with quinols or quinones, although their functions in the animal organism are apparently quite different. There is at present little information available as to the mode of action of these vitamins, but it seems reasonable to suppose that they form components of oxidation–reduction systems. It might therefore be possible for vitamins E and K to show some degree of interchangeability. The reported activity of α-tocopherylquinone as vitamin E (Emerson, Emerson, and Evans, *J. Biol. Chem.*, 1939, **131**, 409) and as vitamin K (Kuhn, Wallenfels, Weygand, Moll, and Hepding, *Naturwiss.*, 1939, **57**, 518) would, if confirmed, be of considerable importance in this connection.

Experimental.

Benzoylation of Toluquinol.—Benzoyl chloride (4·8 c.c.) was gradually added with shaking to an ice-cooled solution of toluquinol (5 g.) in dry pyridine (50 c.c.). The mixture was maintained at 0° during 2 hours, left overnight, and poured on ice and dilute sulphuric acid. The aqueous suspension was extracted with ether, and the extract washed successively with acid, sodium carbonate solution, and water, dried, and evaporated. The residual oil was dissolved in the minimum quantity of methyl alcohol: on cooling, *toluquinol dibenzoate* separated in

colourless needles. Recrystallised from methyl alcohol, it had m. p. 122° (Found : C, 75·8; H, 5·0. $C_{21}H_{16}O_4$ requires C, 75·9; H, 4·8%). Yield, 1·6 g.

The methyl-alcoholic mother-liquors from the above yielded on dilution with water toluquinol monobenzoate. After several recrystallisations from light petroleum (b. p. 80—100°) it had m. p. 113—114° (Found : C, 73·7; H, 5·3. $C_{14}H_{12}O_3$ requires C, 73·6; H, 5·3%). Yield, 4·5 g. The orientation of this substance as *2-hydroxy-5-benzoyloxytoluene* is deduced by analogy with the mono-alkylation of toluquinol (cf. Nietzki, *Annalen*, 1882, **215**, 165; Bamberger, *ibid.*, 1912, **390**, 175).

6-Hydroxy-2 : 8-dimethyl-2-(4′ : 8′ : 12′-trimethyltridecyl)chroman (I; R = Me).—2-Hydroxy-5-benzoyloxytoluene (2·3 g.) and phytol (3·5 g.) were heated under reflux in dry decalin (30 c.c.) in presence of anhydrous zinc chloride (1·5 g.) during 4 hours in a nitrogen atmosphere. The mixture was cooled, and the solution decanted and diluted with light petroleum (b. p. 40—60°). After filtering from a trace of unchanged benzoate, the solution was evaporated, the decalin removed under reduced pressure, and the residual oil hydrolysed by refluxing during 45 minutes in a nitrogen atmosphere with methyl-alcoholic potassium hydroxide (25 c.c. of 5%). The mixture was poured into N/2-hydrochloric acid (50 c.c.) and extracted with peroxide-free ether. After being washed thoroughly with sodium carbonate solution and with water, the ethereal extract was evaporated, and the brown oil redissolved in light petroleum (b. p. 40—60°). After filtering from toluquinol (0·2 g.), the solution was allowed to flow down a column of activated aluminium oxide (Merck) (30 g.), the column being then washed with benzene until the runnings were colourless. The combined runnings on evaporation yielded a brownish oil (3·2 g.) devoid of reducing properties; this was discarded. After removal of a narrow brown layer at the top the column was cut into three approximately equal portions, which were separately eluted with acetone–ether (1 : 1). The lowest layer gave a trace of material with very feeble reducing properties. The top and the middle portion gave brownish oils (650 mg. and 500 mg.), both of which reduced neutral silver nitrate solution on warming and had similar absorption spectra. These were combined and purified by a second adsorption on aluminium oxide. The product was a yellowish oil (Found : C, 79·9; H, 11·2. $C_{27}H_{46}O_2$ requires C, 80·7; H, 11·4%). It reduced methyl-alcoholic silver nitrate solution on warming and gave a yellow colour with a mixture of concentrated sulphuric acid and glacial acetic acid. Its absorption spectrum in alcohol showed a maximum at 3010 A. (ε 3560) and a minimum at 2610 A.

6-Hydroxy-2-methyl-2-(4′ : 8′ : 12′-trimethyltridecyl)chroman (I; R = H).—(*a*) Condensation of quinol monobenzoate (2·1 g.) with phytol (3·5 g.) was carried out under the same conditions as above. After hydrolysis of the product a brown oil (3·9 g.) was obtained which was submitted to adsorption on activated aluminium oxide (Merck). As before, the upper two-thirds of the column contained an oil (750 mg.) which had reducing properties. Repeated purification by adsorption gave ultimately a yellowish oil which seemed to consist mainly of the desired product (Found : C, 80·6; H, 12·1. $C_{26}H_{44}O_2$ requires C, 80·4; H, 11·3%). It reduced methyl-alcoholic silver nitrate solution on warming, and with a mixture of concentrated sulphuric and glacial acetic acid gave a yellow colour rapidly becoming green. Its alcoholic solution had an absorption maximum at 3005 A. (ε 2440) and a minimum at 2400 A. The *p*-nitrophenylurethane and the allophanate prepared from this product could not be crystallised.

(*b*) In an attempt to improve the preparation, condensation of quinol monobenzoate with phytol was attempted by refluxing the mixture with formic acid (99—100%) during 5 hours, a current of nitrogen being passed continuously through the reactants to ensure proper mixing; all the monobenzoate was recovered unchanged.

(*c*) Quinol monobenzoate (6 g.), phytol (8 g.), and anhydrous zinc chloride (4 g.) were heated together in dry decalin (100 c.c.) in a nitrogen atmosphere during 4 hours at 140—160° (bath temp.). After cooling and decanting, unchanged monobenzoate (3 g.) was precipitated by addition of light petroleum (b. p. 40—60°), and the filtrate washed with water, dried over sodium sulphate, and evaporated, the decalin being removed in a vacuum. The dark brown oil obtained was hydrolysed by refluxing in a nitrogen atmosphere with methyl-alcoholic potassium hydroxide (4%), and the product extracted with peroxide-free ether, transferred to light petroleum (b. p. 40—60°), and submitted to chromatographic analysis.

1st Chromatogram. The light-petroleum solution was allowed to flow through a column of aluminium oxide (200 g.; Birmingham Electric Co., Ltd.), and the column washed first with benzene–light petroleum (1 : 1) and then with benzene until nothing further came through. The runnings gave in all 2·8 g. of a brownish non-reducing oil, which was discarded. After removal of a small brownish ring at the top the pale tan-coloured column was cut into two equal parts, which were separately eluted with acetone. The upper portion gave an oil (A)

(2·1 g.) which reduced methyl-alcoholic silver nitrate readily in the cold and gave with concentrated sulphuric and glacial acetic acid a yellowish-green colour becoming olive-green. The lower part gave an oil (B) (2·1 g.) with feebler but distinct reducing properties towards cold silver nitrate; with concentrated sulphuric–glacial acetic acid it gave a yellowish-green colour.

2nd Chromatogram. The oil (A), dissolved in light petroleum, was resubmitted to adsorption on aluminium oxide (Merck), the column being washed with benzene–light petroleum (1 : 1). The chromatogram was washed with benzene until nothing more came through. From the runnings and washings a little oil was obtained (250 mg.). The column, apart from a narrow brownish ring at the top, which was discarded, was tan-coloured and was cut arbitrarily into four parts (numbered 1—4 downwards), each part being eluted separately with acetone to yield a yellowish oil.

Eluate 1 (0·27 g.) reduced neutral silver nitrate readily in the cold and gave an olive-green colour with concentrated sulphuric–glacial acetic acid (Found: C, 76·9; H, 11·3. $C_{26}H_{46}O_3$ requires C, 76·9; H, 11·4%). Its absorption spectrum in alcohol had a maximum at 2990 A. ($E^{1\%}_{1\,cm.}$ = 82·8) and a minimum at *ca.* 2600 A. These properties correspond to those of a quinol derivative, but active-hydrogen estimation gave values corresponding to two hydroxyl groups. *Eluate* 2 (0·38 g.) was similar in properties (Found: C, 76·9; H, 11·4%), as was *eluate* 3 (0·37 g.). *Eluate* 4 (0·30 g.) reduced neutral silver nitrate in the cold rather more slowly than the others (Found: C, 76·3; H, 10·5%).

3rd Chromatogram. The oil (B), dissolved in light petroleum, was adsorbed on aluminium oxide (Merck) in the same way as oil (A). The quantity of non-reducing oil washed through in this case was considerable (0·9 g.), but the material remaining in the column (1·1 g.) was similar to that obtained from the column in the 2nd chromatogram (above) (Found: C, 77·0; H, 11·3%).

The eluted materials from chromatograms 2 and 3 were combined (2·4 g.) and dissolved in glacial acetic acid (20 c.c.). After addition of a solution of hydrogen bromide in acetic acid (2 c.c. of 50%) and zinc dust (0·7 g.) the mixture was refluxed for 15 minutes, the brownish solution becoming nearly colourless. The mixture was cooled, poured on ice, and extracted with peroxide-free ether. After evaporation of the ethereal extract the yellowish oil (2·1 g.) was dissolved in light petroleum and adsorbed on aluminium oxide (Merck), the column being washed with benzene–light petroleum (1 : 1). The washings were discarded together with the small coloured layer at the top of the column. The remainder of the column, on elution with acetone, gave an oil which, like the natural tocopherols, did not reduce neutral silver nitrate in the cold, although reduction took place slowly on warming. Analysis indicated that it was not quite pure (Found: C, 79·3; H, 11·5. $C_{26}H_{44}O_2$ requires C, 80·4; H, 11·4%). The oil was oxidised fairly rapidly in air, becoming brownish. It was purified by acetylation with acetic anhydride in presence of fused sodium acetate and a trace of zinc dust. The acetate, a stable colourless oil, distilled at 190—195° (bath temp.)/10^{-2} mm. (Found: C, 77·9; H, 10·7. $C_{28}H_{46}O_3$ requires C, 78·1; H, 10·8%). The absorption spectrum of the acetate in alcoholic solution had a maximum at 2860 A. (ε 2590) and a minimum at 2580 A.

Condensation of 1 : 4-*Dihydroxy*-2-*methylnaphthalene with Phytol.*—1 : 4-Dihydroxy-2-methylnaphthalene (6 g.), phytol (10 g.), and anhydrous zinc chloride (9 g.) were heated in boiling decalin (150 c.c.) during 4 hours. After working up in the usual manner, the clear brownish oil (14 g.) was dissolved in light petroleum (b. p. 40—60°) and submitted to chromatographic analysis on aluminium oxide (Merck) (400 g.). After being developed with light petroleum, the column showed two narrow brown and yellow bands at the top, followed by larger blue-grey, pale yellow, grey, vivid yellow, and purple bands. The column was washed with benzene–light petroleum (1 : 1); a reddish-yellow oil (6·5 g.) passed through. Increasing the benzene concentration to 80% gave a further small amount (0·5 g.) of similar material. Subsequent washing with pure benzene eluted negligible quantities. This oil gave a red colour with a mixture of concentrated sulphuric and glacial acetic acid and showed no reducing properties towards neutral silver nitrate. It was reduced readily by zinc and dilute sulphuric acid to a pale oil with strong reducing properties towards cold neutral silver nitrate and its alcoholic solution showed absorption maxima at 2480 A. ($E^{1\%}_{1\,cm.}$ = 271) and 2940 A. The material remaining in the column (*ca.* 2·5 g.) possessed marked reducing properties, which were increased by treatment with zinc and sulphuric acid. In alcoholic solution it showed absorption maxima at 2420 A., 2750 A., and 3210 A.

Efforts to obtain homogeneous products from these materials by chromatography or molecular distillation gave unsatisfactory results. Reductive acetylation, followed by molecular distillation, gave products with principal absorption maxima at 2750 A. ($E^{1\%}_{1\,cm.}$ = 427) and

2650 A. ($E^{1\%}_{1\,cm.}$ = 259). These acetylated products deposited small amounts of waxy solids on standing in contact with methyl alcohol for long periods.

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