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New Tocopherol Dimers

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Four new tocopherol dimers have been discovered during fractionation of fresh corn oil. Structural studies allow these dimers to be assigned as 5-(γ-tocopheryloxy)-γ-tocopherol (*1a*), 5-(δ-tocopheryloxy)-γ-tocopherol (*1b*), 5-(γ-tocotrienyl)-γ-tocopherol (*2a*), and 5-(γ-tocopheryl)-γ-tocopherol (*2b*). Dimers *1b* and *2a* are "mixed" dimers involving two different tocopherols or tocotrienols. These mixed dimers are a new type of tocopherol dimer. Dimer *2a* appears to be the first observation of tocotrienol in corn oil. The tocopherol dimers from corn oil may form intact biological systems during normal metabolism, or they may be artefacts of the isolation procedure, or conceivably they are both naturally occurring and artefactual. It has not yet been possible to differentiate between these possibilities because of the ease and non-specificity of their formation.

Four tocopherol and tocotrienol [1] dimers have been discovered during fractionation of fresh corn oil.[2] These dimers are of two structural types represented by the generic formulas *1* and *2*. Structural studies are consistent with the formulation of these products as 5-(γ-tocopheryloxy)-γ-tocopherol (*1a*), 5-(δ-tocopheryloxy)-γ-tocopherol (*1b*),5-(γ-tocotrienyl)-γ-tocopherol (*2a*) and 5-(γ-tocopheryl)-γ-tocopherol (*2b*). Three of these compounds (*1b*, *2a*, and *2b*) were previously unknown. Mixed tocopherol or tocotrienol dimers (*i.e.*, *1b*) are a new type of dimer not previously known either synthetically or by isolation. Also, dimer *2a* appears to be the first indication of the presence of a tocotrienol in corn.

Four tocopherols, α(*3a*), β(*3b*), γ(*3c*), and δ(*3d*), and their corresponding unsaturated analogs, the tocotrienols (*4a*—*4d*), are presently recognized as constituents of living systems.

McHale and Green [3] have isolated a nonpolar tocopherol-like substance from cotton seed oil deodorizer scum to which they assigned structure *1a*, 5-(γ-tocopheryloxy)-γ-tocopherol, on the basis of ultraviolet and infrared spectra and chemical evidence. Other tocopherol-like substances were noted,[3] but

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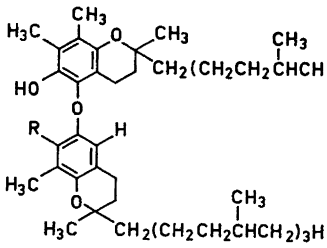
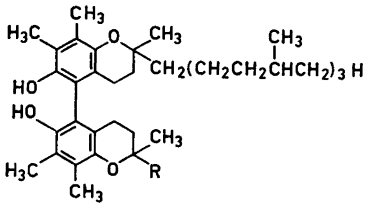


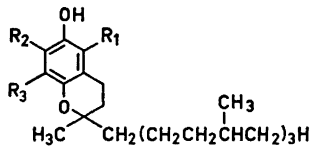
1a, R = $-CH_3$ *(77)*

1b, R = $-H$ *(87)*



2a, R = $-CH_2(CH_2CH_2\underset{\displaystyle CH_3}{\underset{|}{C}}HCH_2)_3H$

2b, R = $-CH_2(CH_2\underset{\displaystyle CH_3}{\underset{|}{C}}{=}CHCH_2)_3H$



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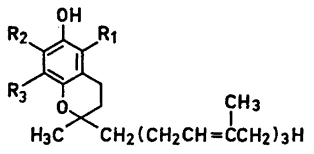
a, R_1, R_2 and $R_3 = -CH_3$ (α)

b, R_1 and $R_3 = -CH_3$; $R_2 = H$ (β)

c, R_2 and $R_3 = -CH_3$; $R_1 = H$ (γ)

d, $R_3 = -CH_3$; R_1 and $R_2 = H$ (δ)



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were not characterized. A product apparently similar to that isolated from cotton seed oil deodorizer scum, but not fully characterized, has been isolated from tung oil.[4] A metabolite of α-tocopherol, found in rat liver,[5] was at one time considered to be a dimer, but is now known to be a trimer.[6,7] No other reports of tocopherol dimers from natural sources were found, but several other tocopherol oxidation products including *p*-quinones [13b,14] and *o*-quinones [15] have been obtained by fractionation of biological material and characterized.

The reactivity of the tocopherols as general antioxidants is well recognized.[9] whether the tocopherols possess other, coenzymatic roles in metabolism has been subject to continuing research.[10] The biological effects of various tocopherol oxidation products of both natural and synthetic origin have been studied. Several of these products (chiefly of quinoid structure) have exhibited weak vitamin E (tocopherol) [11] activity and interesting physiological effects including apparent antivitamin K activity.[12] It has recently been suggested that the tocopherylquinones derived from β- and γ-tocopherol (which are closely related structurally to plastoquinone) could function in electron

transfer in chloroplasts.[13] However, no intrinsic metabolic role for these or other products derived from the tocopherols has been established.

The origin of the dimers reported here may be dual. They may be artefacts of the isolation procedure even though the steps were very mild; it was found that 5-(γ-tocopheryloxy)-γ-tocopherol (*1a*) was formed in small amounts when γ-tocopherol was chromatographed in a manner similar to that used in the fractionation of corn oil. The dimers (*1a* and *2a*) were also formed by air oxidation when γ-tocopherol was stored for three months in a closed container in the refrigerator. It was considered possible that dimerization might occur during Soxhlet extraction of the corn particularly since corn oil contains high concentrations (about 0.2 mg/g) of ubiquinone-9, which could act as an oxidant. However, when hexane solutions of γ-tocopherol were heated for 4 h either in the presence or absence of hexahydroubiquinone-4 dimers were not formed in detectable quantities. The dimers described here are formed readily under a variety of oxidative conditions, and it seems likely that they may also be formed in intact biological systems in the course of the functioning of normal metabolism.

As part of an investigation of biologically active constituents of corn oil, a careful fractionation of freshly prepared corn oil was undertaken. Early fractions obtained by silica gel chromatography yielded three phenolic tocopherol-like products (*1a*, *1b*, and *2*) as evidenced by positive reactions with diazotized sulfanilic acid, Gibb's[16] and Emmerie-Engel[17] reagents. "Product *2*" was later found to be a mixture of two compounds (*2a* and *2b*). These products possess very similar spectral and chromatographic properties (Table 1). The infrared spectrum of each product exhibits an OH-stretching band at ~3500 cm^{-1}. The ultraviolet spectra of the three products are essentially identical and very similar to the spectra of the tocopherols. Products *1a* and *1b* exhibit λ_{max}^{hexane} at 297 mμ with a second band appearing as a shoulder at 292 mμ, and "product *2*" had λ_{max}^{hexane} at 300 and 292 (sh) mμ. α-Tocopherol (*3a*) exhibits corresponding maxima at 297 and 292 mμ.

Products *1a*, *1b*, and "*2*" were readily differentiated from the tocopherols by thin layer chromatography; relative R_F values (silica gel G developed in ether/hexane 1:9) are *1a*, 0.80; *1b*, 0.60; "*2*", 0.67; α-tocopherol (*4a*), 0.23; and β-tocopherol (*4b*), 0.19.

Mass spectra of products *1a*, *1b*, and "*2*" show that these products are tocopherol dimers. The mass spectrum of product *1a* shows a molecular ion at *m/e* 830 (2β- or γ-tocopherols − 2H) and an intense peak at *m/e* 416 (β- or γ-tocopherol monomer). Peaks due to monomers have been observed in the spectra of all the tocopherol dimers so far investigated;[18] the nature of the process giving rise to these peaks is not fully understood, but it appears to be in the spectral determination on the basis of the apparent chromatographic purity of the samples. Other intense peaks characteristic of β- or γ-tocopherol (*3b*, *3c*) appear at *m/e* 191 and 151.[18] The mass spectrum of product *1b* exhibits a parent ion at *m/e* 816 (β- or γ-tocopherol + δ-tocopherol − 2H) and intense peaks at *m/e* 416 (β- or γ-tocopherol) and 402 (δ-tocopherol). Similarly, the mass spectrum of "product *2*" reveals the presence of two compounds giving rise to parent ions at *m/e* 830 (2β- or γ-tocopherols − 2H) and 824 (β- or γ-

Table 1. Chromatographic and spectral data for isolated and synthetic tocopherol dimers.

Compound	TLC [a] R_F	UV λ_{max}^{hexane} (mμ)	NMR [b] (τ values)						Mass spectra	
			Aromatic	OH	Aromatic methyl				M^+ (dimer) *m/e*	M^+ (monomer) *m/e*
Product *1a*	0.80	297 292(sh)[c,d]	4.11	5.34	7.74	7.86	7.92	7.96	830	416
Synthetic $\gamma\gamma$ (*1a*) [e]	0.80	297 293(sh)	4.11	5.34[f]	7.74	7.86	7.92	7.96		
Product *1b*	0.60	297 292(sh)							816	416 402
Synthetic $\delta\gamma$ (*1b*) [e]	0.60	297 292(sh)	3.62 3.79	5.34	7.80	7.96				
Product "*2*" [g]	0.67	300 292(sh)	—	5.80	7.87	7.92			830 824 [h]	416 410 [h]
Synthetic $\gamma\gamma$ (*2a*) [e]	0.67	300 292(sh)	—	5.80[f]	7.87	7.92				

[a]) Thin layer chromatography (silica gel G plates, 0.3 mm, developed in hexane/ether, 9:1).
[b] All signals listed in this table appeared as singlets.
[c] (sh) = shoulder.
[d] Product *1a* gave λ_{max}^{EtOH} 292 mμ. Reported:[3] 293 mμ.
[e] See Ref. 19.
[f] Signal disappeared upon shaking with D_2O.
[g] Weak NMR signals due to the presence of the minor component *2b* were observed at τ 5.0 (vinyl) and 8.44 (vinylic methyl).
[h] Peaks arising from dimer *2b*.

tocopherol + β- or γ-tocotrienol — 2H). Corresponding intense peaks due to the monomers appear at *m/e* 416 and 410.

The NMR spectra of the dimeric products *1a*, *1b*, and "*2*" are similar to the spectra of the tocopherols, but exhibit significant differences. In the spectrum of product *1a*, four distinct aromatic methyl groups appear as singlets at τ 7.74, 7.86, 7.92, and 7.96. In addition, one-proton singlets at τ 4.11 and 5.34 indicate the presence of a single aromatic proton and one phenolic hydroxyl. These data are consistent with the formulation of product *1a* as a dimer in which two γ-tocopheryl residues are joined by an ether linkage involving the phenolic oxygen of one of the γ-tocopherol moieties.

That product *1a* is 5-(γ-tocopheryloxy)-γ-tocopherol, a dimer of γ-tocopherol (*3c*), and not a dimer of β-tocopherol (*3b*) or a mixed dimer of β- and γ-tocopherols was established by a study of the oxidative dimerization of the various tocopherols. Oxidative dimerization of γ-tocopherol (*3c*) yielded a compound [19] which is identical to product *1a* by co-chromatography and by comparison of their UV, IR, and NMR spectra (Table 1).

Product *1b* was assigned structure 5-(δ-tocopheryloxy)-γ-tocopherol on the basis of its UV-spectrum which is identical to that of product *1a*, and its mass spectrum. Compound *1b* was synthesized,[19] and shown to be identical with isolated product *1b* by co-chromatography and comparison of IR and UV spectra (Table 1).

The NMR spectrum of "product *2*" (Table 1) is significantly different from that of dimer *1a*. No signals due to aromatic protons are observed, and a singlet at τ 5.80 (2H) was shown by deuterium exchange to be due to hydroxyl protons. The area of the aromatic methyl groups shows two singlets at τ 7.86 and 7.91. Additional signals (weak) due to the tocopherol-tocotrienol minor product (see discussion of mass spectral data above) are observed at τ 5.0 (vinyl) and τ 8.44 (side chain vinylic methyl groups). These data are consistent with the formulation of the major component of "product *2*" as 5-(γ-tocopheryl)-γ-tocopherol (*2a*), a dimer of γ-tocopherol isomeric with dimer *1a*. Structure *2a* accounts for the absence of aromatic protons, and its symmetry explains the equivalence of the hydroxyl groups and the observance of only two aromatic methyl signals in the NMR spectrum. Dimer *2a* was attained synthetically by dimerization of γ-tocopherol (*3c*).[19] This synthetic compound exhibited chromatographic and spectral data (Table 1) which establish its identity with the major component of "product *2*".

Structure *2b*, 5-(γ-tocotrienyl)-γ-tocopherol, is assigned to the minor component of "product *2*" on the basis of its mass spectrum and its chromatographic identity with *2a*. In the chromatographic systems used (see Experimental and Table 1), the degree of unsaturation in the isoprenoid side chain makes little or no difference in chromatographic properties.[20]

EXPERIMENTAL

General comments. The ultraviolet absorption spectra were measured with a Cary Model 14 M spectrophotometer. The infrared absorption spectra were determined with a Beckman-IR5A spectrophotometer. Nuclear magnetic resonance spectra were measured using CCl_4 solutions with a Varian Associates HA 100 spectrometer. Chemical shifts are

expressed as τ units relative to tetramethyl silane as an internal standard. A deuterium exchange experiment was carried out by shaking a CCl_4 solution of the compound with D_2O and after separation of the layers, recording a new spectrum using the organic phase. Mass spectra were determined using a CEC Model 103-C mass spectrometer modified as earlier described.[21] Thin layer chromatography was performed using silica gel G plates of 0.3-mm (analytical) and 1-mm (preparative) thickness. The plates were activated by heating at 130° for 1.5 h and were stored in a dry cabinet until used. Freshly distilled hexane was used throughout.

Preparation of fresh corn oil. Fresh corn (12 kg) was ground to a flour, and then extracted in 1-kg batches with hexane in a Soxhlet apparatus for 24 h per kg. After evaporation of the solvent, 378 g of a turbid orange colored corn oil was obtained.

Fractionation of corn oil. The fresh corn oil was dissolved in 800 ml of hexane and placed on a column of 500 g of Al_2O_3 (Alumina Merck 71707). The column was then washed with an additional 1.5 liter of hexane to remove neutral lipids. Acidic substances, including phenols, remained on the column.[2] The column was then eluted with 3 liters of ether/methanol (3:1) to remove phenolic substances. Evaporation of solvent from this eluate yielded 30 g of a dark red oil which was dissolved in 100 ml of hexane. The hexane solution was applied to a column (5.5 × 100 cm) packed with 800 g of silica gel. The column was eluted with ether/hexane mixtures of increasing polarity and the eluate was collected in 1-liter fractions. (The fractionation scheme has been previously presented).[2] Each fraction was scanned for phenols by spraying a thin layer chromatogram with phenol color reagents (diazotized sulphanilic acid, Gibb's reagent,[16] and Emmerie-Engel reagent [17]).

Isolation and identification of tocopherol dimers. In fractions eluted from the column with 3 % ether in hexane,[2] three phenolic products *1a*, *1b*, and *2* were detected. In the same fractions, α- and γ-tocopherol were also detected and identified by IR and UV spectra and co-chromatography with authentic samples. After purification of the three phenolic products by preparative thin layer chromatography using ether:hexane, benzene, and chloroform as developing solvents, products *1a* (38 mg), *1b* (3 mg), and *2* (39 mg) were obtained as pale yellow oils. Structure elucidation of these products was accomplished by analysis of their IR, UV, NMR, and mass spectra and by comparison with authentic synthetic samples.[19] These data are summarized in Table 1.

Formation of dimer 1a *under conditions similar to the isolation procedure.* Twenty mg of γ-tocopherol was applied to a column of 300 g Al_2O_3 (Alumina Merck 71707). The column was eluted with 1000 ml of hexane and then with 500 ml of ether:methanol (3:1). The latter eluate was evaporated and the residue, dissolved in hexane, was placed on a column of 200 g silica gel which was eluted with 1000 ml of 3 % ether in hexane. The eluate was evaporated and the residue was subjected to preparative thin layer chromatography on silica gel G developed in ether:hexane (1:9). When the edge of the plate was sprayed with Gibb's reagent it showed two phenolic compounds, one corresponding to γ-tocopherol (*3a*) and another corresponding to 5-(γ-tocopheryloxy)-γ-toco-pherol(*1a*). The area corresponding to *1a* was removed from the plate and eluted with ether. After evaporation of the ether, the residual material weighed about 1 mg.

γ-Tocopherol was stored in the refrigerator in a closed container for three months. A sample was then chromatographed on silica gel G in ether:hexane (1:9). When the plate was sprayed with Gibb's reagent, it showed three phenolic spots, corresponding to γ-tocopherol, dimer *1a* and *2a*, respectively.

A hexane solution of γ-tocopherol was heated in a sealed container on a steam bath for 4 h. When this sample was chromatographed as described above, no dimer was detected. The same experiment was repeated with one equivalent of hexahydroubiquinone-4 present. No dimer was detected when the reaction mixture was chromatographed as before.

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