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Isolation of γ-Tocotrienol Dimers from *Hevea* Latex*

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ABSTRACT: Two novel reducing compounds were isolated from *Hevea* latex lipid and shown to be dimers of γ-tocotrienol. They were obtained synthetically by oxidation of γ-tocotrienol with *p*-benzoquinone. Structural characterization indicated that the compounds are 5-(γ-tocotrienyloxy)-γ-tocotrienol and 5-(γ-tocotrienyl)-γ-tocotrienol.

Since the isolation of a dimer of α-tocopherol from mammalian tissues by Csallany and Draper (1963) the occurrence of other dimers of the tocopherols has been actively investigated. McHale and Green (1963) isolated a dimer of γ-tocopherol from cottonseed oil deodorizer scum and proposed that it was identical with a compound detected earlier by Shone (1963) in tung oil. They concluded that the compound was 5-(γ-tocopheryloxy)-γ-tocopherol (Figure 1, Ia). Komoda and Harada (1969) have reported a similar compound in soybean oil. In a study of the chemical oxidation products of various tocopherols, Nilsson *et al.* (1968) reported the synthesis of nine dimers and three trimers. Two dimers obtained by oxidation of γ-tocopherol with *p*-benzoquinone were identified as 5-(γ-tocopheryloxy)-γ-tocopherol and 5-(γ-tocopheryl)-γ-tocopherol (Figure 1, IIa). These two compounds also were found in corn oil.

Latex of the rubber tree *Hevea brasiliensis* contains an extraordinary concentration of tocopherols (about 8% of total lipids), mainly in the form of the unsaturated isomers (tocotrienols). During the isolation of these compounds for reference purposes, two novel reducing substances of low polarity were detected on thin-layer chromatograms. These compounds were isolated and found to be dimers of γ-tocotrienol. Evidence is presented that they are 5-(γ-tocotrienyloxy)-γ-tocotrienol (Figure 1, Ib) and 5-(γ-tocotrienyl)-γ-tocotrienol (Figure 1, IIb).

Experimental Section

Isolation of Compounds A and B. Samples (50 g) of *Hevea* latex were extracted with 750 ml of CH_3OH–$CHCl_3$ (1:2) according to the Folch *et al.* (1951) procedure. After being homogenized with a solvent in a Waring laboratory blendor for 3 min the samples were filtered and 0.2 volume of H_2O was added to the filtrate. The two solvent phases were allowed to separate overnight, whereupon the lower phase was evaporated to dryness under vacuum at 50° and the residue was taken up in diethyl ether. The ethereal solution was washed three times with water, dried over anhydrous sodium sulfate, and evaporated under N_2.

Approximately 700 mg of lipid was chromatographed on a 50 g Bio-Rad neutral alumina column deactivated with 6% water. The column was developed with 250-ml volumes of petroleum ether (twice-distilled Skellysolve F, bp 40–60°) containing the following proportions of peroxide-free diethyl ether: 0, 2, 4, 6, 8, 10, 12, 20, 40, and 100%. The residue from each fraction was chromatographed on thin layers of silica gel G using $CHCl_3$ as solvent. The plates were prepared with a 0.002% aqueous solution of 2′,7′-dichlorofluorescein. In addition to bands corresponding to standard tocopherols, two unidentified compounds were detected at R_F positions 0.9 and 0.8 by spraying with Emmerie–Engel reagent. These brownish yellow oils (A and B, respectively) were eluted with diethyl ether and purified by thin-layer chromatography on silica gel G until they gave a single spot in several systems. Isopropyl

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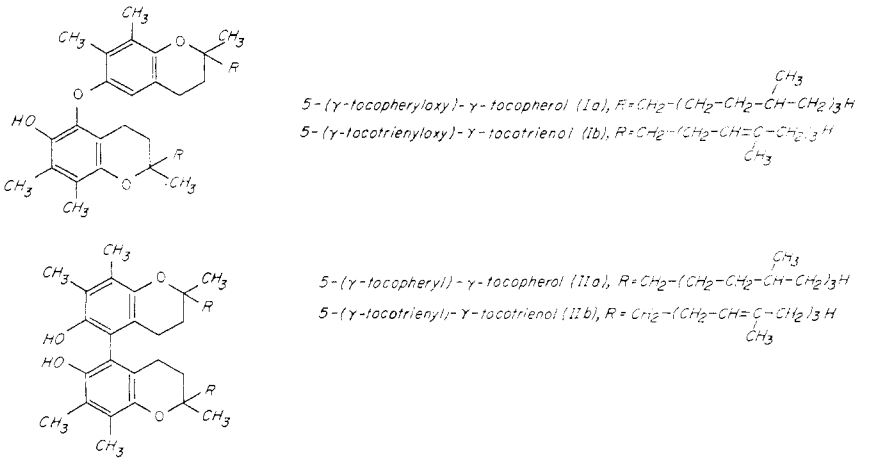

FIGURE 1: Structural formulas of dimers γ-tocopherol and γ-tocotrienol.

ether (10%) in hexane, 6% isopropyl ether in hexane, and 1% methanol in cyclohexane were used as solvents.

Synthesis of Compounds A and B. Reports of the occurrence of tocopherol dimers in natural oils (see above) suggested that compounds A and B might be dimers of γ-tocotrienol, the predominant isomer of vitamin E in latex. γ-Tocotrienol (60 mg) isolated from latex lipid and 60 mg of *p*-benzoquinone were dissolved in 10 ml of benzene and refluxed for 10 min. After evaporation of the solvent under N_2, the residue was dissolved in petroleum ether and filtered to remove excess *p*-benzoquinone. Upon chromatography of the brownish oily residue in the thin-layer systems described above, two reducing compounds were separated having R_F values which corresponded to those of compounds A and B from latex. After purification by repeated thin-layer chromatography, the yields of the synthetic compounds were 22% and 10%, respectively.

Characterization of Compounds A and B. The ultraviolet absorption spectra of the isolated and synthetic compounds were recorded in a Beckman DB-G spectrophotometer. Infrared spectra were recorded in a Beckman IR-3 spectrophotometer using samples smeared on NaCl disks. $LiAlH_4$ reduction of 0.5-mg samples was carried out according to the procedure of Duggan (1959). The product was chromatographed on silica gel G against γ-tocotrienol using 10% isopropyl ether in hexane as solvent. The reducing activity of the compounds toward ferric ion was measured spectrophotometrically according to the modified Emmerie–Engel reaction of Tsen (1961) using a 3-min reaction time.

Trimethylsilyl derivatives were prepared by reacting 5 mg of each compound with 1 ml of pure *N,O*-bis(trimethylsilyl)acetamide overnight at room temperature. The excess *N,O*-bis-(trimethylsilyl)acetamide was removed under vacuum. Mass spectra of the compounds and their trimethylsilyl derivatives were obtained in a Hitachi Perkin–Elmer RMU-6E spectrometer at an electron energy of 70 eV and an accelerating voltage of 3 kV.

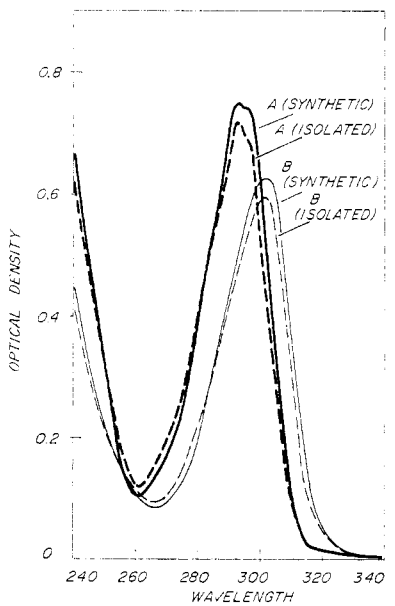

FIGURE 2: Ultraviolet absorption spectra of isolated and synthetic compounds A and B.

Results

The yield of lipid from latex was 0.8%. Thin-layer chromatography of the column eluates showed that tocopherol esters were present in the 2% diethyl ether fraction, compound A mainly in the 6 and 8% fractions, and compound B in the 10 and 12% fractions. γ-Tocotrienol was eluted with 12 and 20% ether. The recovery of sample from the column was 79% by weight. After purification by thin-layer chromatography 1.48 mg of compound A and 1.07 mg of compound B were obtained per gram of lipid.

The ultraviolet absorption spectra of the isolated and synthetic compounds are shown in Figure 2. The spectrum of compound A from latex is similar to that of one of the *p*-benzoquinone oxidation products of γ-tocotrienol (λ_{max} 292 mμ, λ_{min} 261 mμ) and the spectrum of latex compound B is similar to the other (λ_{max} 301 mμ, λ_{min} 266 mμ). The infrared spectra of the isolated compounds are essentially identical with those of their synthetic counterparts (Figure 3). Absorp-

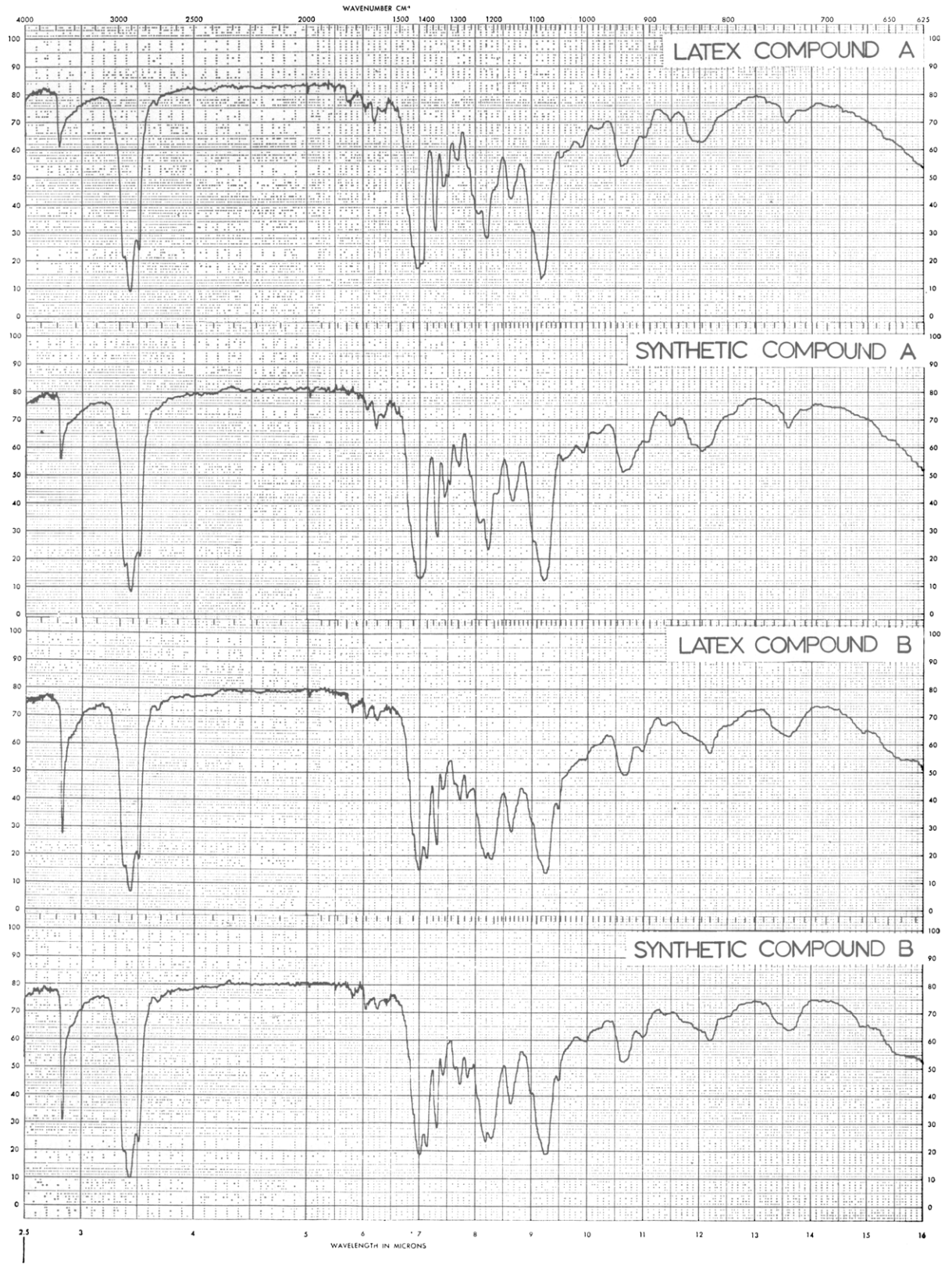

FIGURE 3: Infrared absorption spectra of isolated and synthetic compounds A and B.

tion bands are present at 3540 cm^{-1} (hydroxyl), 1650 cm^{-1} (alkene), 1240 cm^{-1} (aromatic ether), 1200 and 1220 cm^{-1} (aromatic alcohol), and 1085 cm^{-1} (cyclic ether).

The R_F values of isolates A and B were identical with those of the analogous synthetic compounds. These values were, respectively, 0.9 and 0.8 with $CHCl_3$ as solvent, 0.7 and 0.5 with 10% isopropyl ether in hexane, 0.6 and 0.5 with 1% methanol in cyclohexane. On paraffin-coated Kieselguhr G plates de-

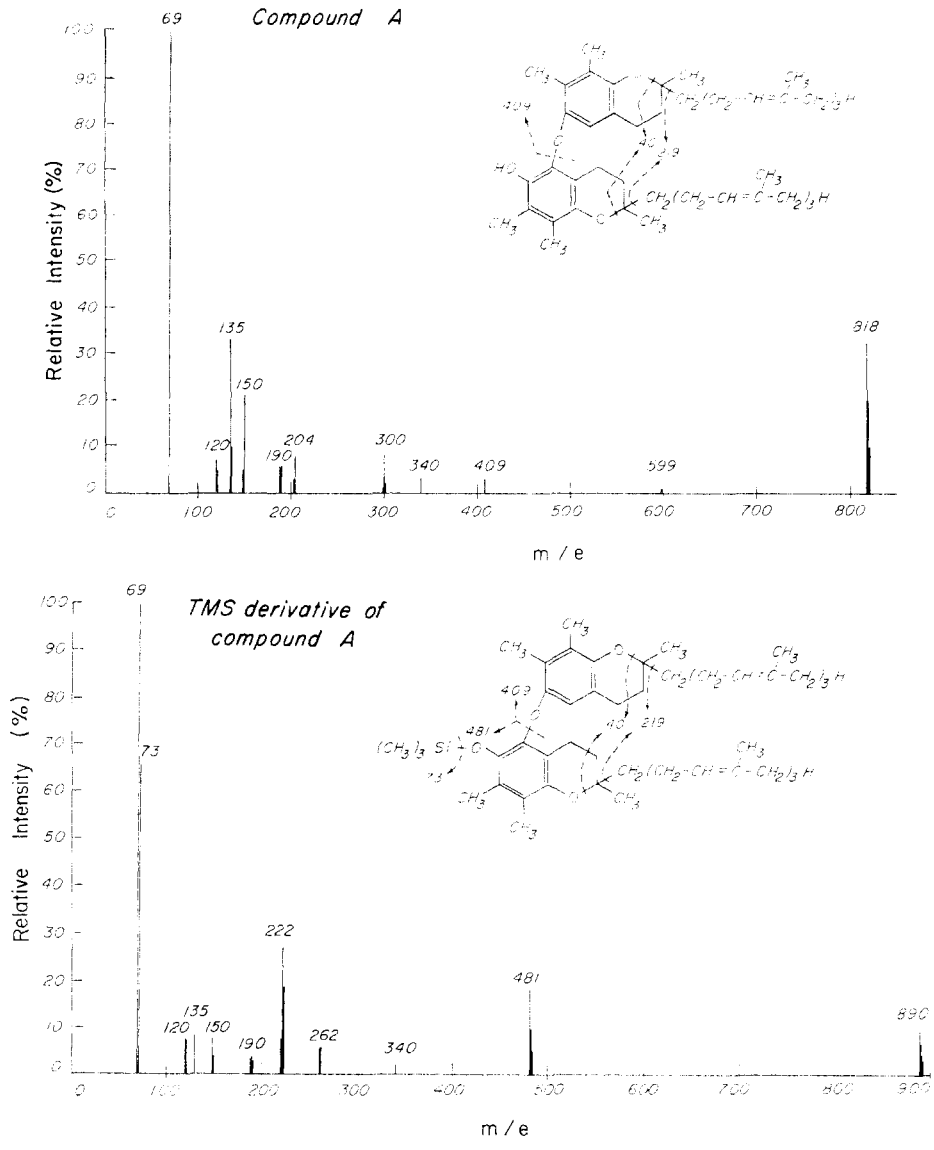

FIGURE 4: Mass spectra of compound A and its trimethylsilyl derivative. Numerous peaks of low intensity are omitted.

veloped with 75% ethanol the R_F values were 0.0 and 0.1, respectively.

The reducing activity of compound A, calculated on an equal weight basis, was only half that of γ-tocotrienol (47% for the isolate from latex, 49% for the synthetic material). In contrast, the isolated and synthetic forms of compound B were found to have 80% and 84%, respectively, of the reducing power of γ-tocotrienol. $LiAlH_4$ failed to reduce either compound A or B to γ-tocotrienol.

The mass spectrum of synthetic compound A (Figure 4) contains a peak at *m/e* 818 corresponding to the molecular ion of compound Ib in Figure 1 ($C_{56}H_{82}O_4$). The fragmentation pattern also contains peaks at *m/e* 599 attributable to loss of part of the side chain and at *m/e* 409 arising from cleavage of the aromatic ether linkage. The peak at *m/e* 340 indicates the loss of both side chains, cleavage of the chroman structure with rearrangement of hydrogen and loss of a $CH_3C{\equiv}CH$ fragment. Peaks at *m/e* 204 and 190 are due to loss of the side chain from a γ-tocotrienol ion; the ion at *m/e* 150 is due to cleavage of the chroman structure and loss of another $CH_3C{\equiv}CH$ fragment. The intense peak at *m/e* 69 is attributable to an isoprenoid unit from the side chain (Nair and Luna, 1968).

The mass spectrum of the trimethylsilyl derivative of compound A (Figure 4) contains a molecular ion at *m/e* 890 which corresponds to the molecular weight of compound A plus a trimethylsilyl group minus one hydrogen. The fragmentation pattern contains peaks at *m/e* 481 (γ-tocotrienol plus a trimethylsilyl group) formed by rupture of the aromatic ether linkage, and at *m/e* 262 attributable to further degradation of the side chain. The ion with mass 222 arises from rupture of the chroman structure of the silylated monomer. The strong peak at *m/e* 73 is due to the trimethylsilyl group.

The mass spectrum of compound B (Figure 5) indicates that its molecular weight is the same as that of compound A.

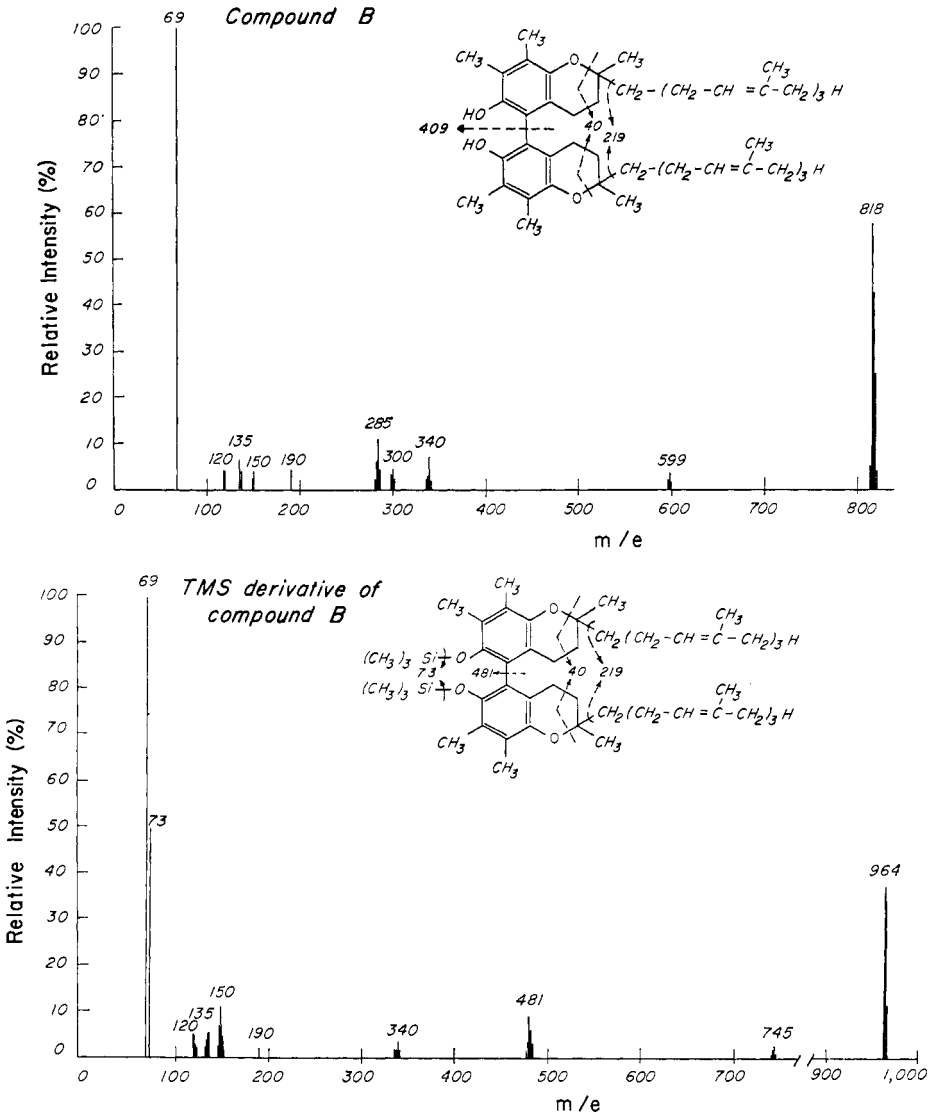

FIGURE 5: Mass spectra of compound B and its trimethylsilyl derivative. Numerous peaks of low intensity are omitted.

The molecular ion (*m/e* 818) is compatible with either structure Ib or IIb of Figure 1 ($C_{56}H_{82}O_4$). However, the spectrum of the trimethylsilyl derivative of compound B reveals the presence of two trimethylsilyl groups (*m/e* 964) as opposed to one in the silyl derivative of compound A.

Discussion

The results show that the compounds isolated from latex are dimers of γ-tocotrienol which are analogous to the dimers of γ-tocopherol isolated previously from other oils (Shone, 1963; McHale and Green, 1963; Komoda and Harada, 1969; Nilsson *et al.*, 1968). The molecular weights of both compounds are equivalent to twice that of γ-tocotrienol less two hydrogens. The presence of one free hydroxyl group in compound A in contrast to two in compound B is demonstrated by the fact that compound A has only about half the reducing activity (w/w) of compound B and γ-tocotrienol. It also has weaker absorption at 3540 and 1200 cm^{-1}, and forms a monosilyl as opposed to a disilyl derivative. The presence of an aromatic ether group in compound A is indicated by increased absorption at 1240 cm^{-1}. Compound B is also slightly more polar than compound A. The combined data indicate that in compound A the γ-tocotrienol monomers are joined by an ether bond between the 5 and 6 positions of the two chroman rings, and in compound B by a carbon–carbon bond between the two 5 positions (Figure 1). This proposal is in harmony with the mechanism of formation of dimeric and trimeric products of the tocopherols postulated by Nilsson *et al.* (1968). Elemental analysis yielded the following values: 82.26% C, 10.06% H, and (by difference) 7.68% O for compound A; 82.24% C, 9.99% H, and (by difference) 7.77% O for compound B. The theoretical values for both compounds are: 82.10% C, 10.09% H, and 7.81% O.

It is uncertain whether compounds A and B are natural constituents of latex lipid or are formed by oxidation of γ-toco-

trienol during storage. The same uncertainty applies to the dimers of γ-tocopherol isolated from other oils. The sources from which these dimers have been isolated are rich in the corresponding monomers. Oxidation of α-tocopherol in the presence of methyl linoleate has been found to generate a dimer and a trimer (A. S. Csallany, M. Chiu, and H. H. Draper, 1969). The occurrence of dimers of other tocopherols in stored oils may be anticipated. The present dimers were shown not to be artifacts of chromatography by recovery tests on γ-tocotrienol internal standards. Addition of 25 mg of monomer to 700 mg of latex lipid before chromatography did not lead to an increase in the yield of either compounds A or B.

The presence of hydroxy dimers of the tocopherols in natural oils is a complication in chemical assays for the various isomers of vitamin E by the usual reduction reactions. Unless these compounds are removed beforehand they will contribute to an overestimation of vitamin E potency.

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