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Synthesis and Mass Spectrometry of New Compounds Related to the Tocopherols and Plastoquinones[1]

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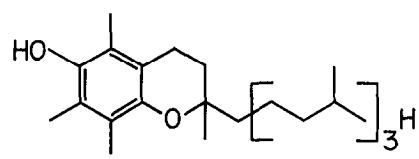
Seven new isomers of α-tocopherol acetate have been synthesized from di- and trimethylated phenols. The compounds synthesized are: 2,5,6,7-tetramethyl-8-acetoxy-2-(4′,8′,12′-trimethyltridecyl) chroman ("ortho tocopherol acetate"), **7**, and the six possible structural isomers of dimethylphytyl-1-methoxy-4-acetoxybenzene, **12**, **13**, **14**, **15**, **18**, and **19**. Upon mass spectral determination, the seven synthetic compounds and natural α-tocopherol acetate produced the same fragment ions, the only differences in the spectra being in the relative intensities of some of the fragments.

INTRODUCTION

α-Tocopherol has been known as a vitamin E factor since 1922 (*1*) although its chemical structure was not elucidated until 1938 (*2*). Since then, there has been considerable interest in the role of α-tocopherol and synthetic tocopherol analogs in mammalian metabolism (*3*). Structurally related materials which occur in a variety of plants are the β-, γ-, and δ-tocopherols, and as well the tocotrienols with unsaturated side chains. All of these materials can be considered as members of the larger class of isoprenoid-substituted quinones (and derivatives thereof), which includes other biologically active materials such as vitamin K, ubiquinone, and plastoquinone (*4*).



α-TOCOPHEROL

Recently, we isolated a new isomer of α-tocopherol from *Euglena gracilis* and characterized it as an acetyl derivative of a new structural type (*5*, *6*). This material contains a benzene ring substituted with the following groups (see preceding paper): -OH, $-OCH_3$, two $-CH_3$, phytyl. To aid in the identification of this new compound we have synthesized the acetates of several unusual isomers of α-tocopherol; these

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materials may also have potential interest as vitamin E analogs. These syntheses, which involve the introduction of an oxygen substituent and a phytyl group into the appropriate positions of various methylated phenols, are described in this paper. The compounds synthesized were "ortho tocopherol" (2,5,6,7-tetramethyl-8-hydroxy-2-[4′, 8′,12′-trimethyltridecyl]chroman), **6**, and its acetyl derivative, **7**, and the six isomers of dimethylphytyl-1-methoxy-4-acetoxybenzene, **12–15**, **18**, and **19**.

MATERIALS AND METHODS

Materials for chromatography and instrumentation for spectroscopic measurements were those described in the preceding paper, except that a Bruker 90 MHz spectrometer was used for some nuclear magnetic resonance spectra. The following conditions were used for combined gas chromatography–mass spectrometry:

Instrument: LKB 9000
Columns: 6 ft × ¼-in. glass, 3% OV-1 or 3% OV-17 coated on Gas-Chrom Q (60–80 mesh)
Ionization potential: 70 eV
Multiplier: 1.9
Trap current: 60 μA

High-resolution mass spectra were obtained through the courtesy of Dr. C. Hignite, Massachusetts Institute of Technology, using a CEC-110B mass spectrometer.

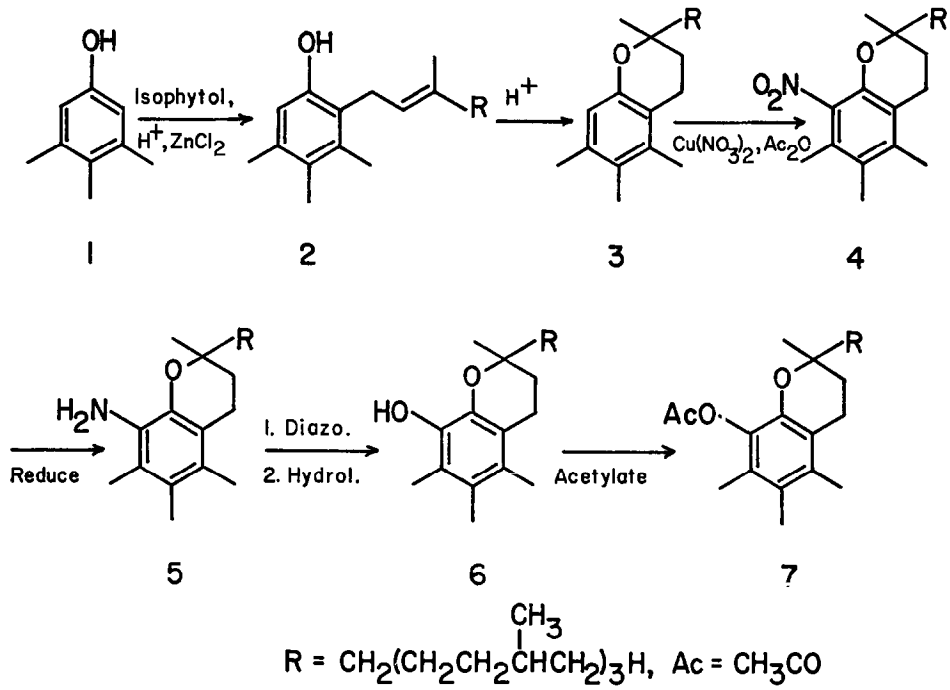
The methylated phenols used as starting materials for the syntheses were obtained from the Aldrich Chemical Company, Milwaukee, Wisconsin. The melting points (uncorrected) were determined on a Koffler Micro Hot-Stage Melting Point apparatus.

"Ortho Tocopherol Acetate" (2,5,6,7-Tetramethyl-8-Acetoxy-2-[4′,8′,12′-Trimethyltridecyl] Chroman)-Scheme 1

2-Phytyl-3,4,5-Trimethylphenol, **2**

3,4,5-Trimethylphenol, **1**, (mp, 106–107°C, 13.6 g), dissolved in 500 ml dry ether in a dry flask, was treated with isophytol (28 g) and zinc chloride (2 g); the latter, which had been fused and desiccated immediately before use, was added to the reaction mixture with 0.2 ml glacial acetic acid. It was important to maintain anhydrous conditions throughout the reaction. The mixture was stirred at room temperature overnight (18–20 hr), after which the ether was evaporated and the contents of the flask were heated at 50°C for 30 min. The pink oil was dissolved in a mixture of petroleum ether (40–60°C) and 75% aqueous methanol; the petroleum ether layer was washed several times, first with 75% methanol, then saturated sodium chloride solution, and finally with water. On evaporation of the solvents a brown oil was obtained which contained a mixture of 2-phytyl-3,4,5-trimethylphenol, **2**, and the corresponding cyclized product, **3**, 2,5,6,7-tetramethyl-2-(4′,8′,12′-trimethyltridecyl) chroman. Yield of mixture, 41.5 g. Separation of **2** and **3** could be achieved by elution with petroleum ether from a Brockmann Grade III alumina column or by thin-layer chromatography in chloroform on Rhodamine 6G-impregnated silica gel G plates. Pure **2** was a colorless oil, which on gas chromatography (6-ft, 3% OV-1 column, 245°C) showed a single peak, retention time 6.9 min.

ir[3] spectrum (CCl_4): 2.77, 2.87 (broad), 3.39, 3.43, 3.48, 6.81, 7.23 μ.
Mass spectrum: M^+ 414 m/e (9.7), 412 (18.7), 189 (13.3), 187 (100), 149 (95.4).[4]



SCHEME 1

2,5,6,7-Tetramethyl-2-(4',8',12'-Trimethyltridecyl) Chroman, **3**

The mixture of **2** and **3** (40 g) from the previous reaction, dissolved in 325 ml glacial acetic acid and 4 ml sulfuric acid, was refluxed for 1.5 hr. The solution was then cooled and extracted several times with ether. The combined ethereal extracts were washed with water, 5% sodium bicarbonate solution, and finally with water. The ether solution was dried over anhydrous sodium sulfate, filtered, and evaporated to give a light-brown oil. The chroman, **3**, was purified by chromatography of the product on a column of 350 g of Brockmann Grade III alumina; elution was with petroleum ether (40–60°). Yield of colorless oil, 35 g (88%). The product gave a single peak (retention time, 4.2 min) on gas chromatography using a 6-ft, 1½% OV-1 column at 245°C.

ir spectrum (CCl_4): 3.39, 3.43, 3.48, 6.83, 7.25, 8.02 μ.

Mass spectrum: M^+ 414 m/e (11.8), 412 (90.5), 189 (10.8), 187 (4.2), 149 (100), 147 (2.9).

[3] Abbreviations used: TMSi, trimethylsilyl; uv, ultraviolet; ir, infrared; nmr, nuclear magnetic resonance; $[\alpha]_{330}$, specific optical rotation at 330 nm.

[4] In the mass spectra, M^+ refers to the molecular ion; m/e is the mass to charge ratio. The figures in parentheses refer to the relative intensities of the fragment ions. High-resolution mass spectral data are quoted only for the molecular ion; however, the m/e values for the other fragment ions were also obtained and were within the acceptable limits of error (± 3 mmass units).

2,5,6,7-Tetramethyl-8-Nitro-2-(4',8',12'-Trimethyltridecyl) Chroman, **4**

For nitration, **3**, (10 g) was dissolved in 100 ml redistilled acetic anhydride and maintained in an ice bath with constant stirring. Gradually, powdered cupric nitrate (5 g) was added over a period of 20 min. When the addition was complete, the mixture was allowed to warm up to room temperature until brown fumes were visible. Immediately, the mixture was poured over crushed ice and extracted with ether. The bright-yellow ethereal solution was washed with water, dried, filtered, and evaporated to a brown oil. The nitro chroman, **4**, was obtained as a yellow oil by elution with 2% diethyl ether in petroleum ether from a 100 g silicic acid column. Yield, 4.1 g (38%). Gas chromatographic analysis of the product (6-ft column, 1½% OV-1, 245°C) showed a single peak, retention time, 10.8 min.

ir spectrum (CCl_4): 3.38, 3.42, 3.48, 6.08, 6.50, 6.79, 8.05 μ.

Mass spectrum: M^+ 459 m/e (27.7), 442 (6.0), 429 (4.1), 424 (7.6), 234 (23.9), 194 (100.0), 177 (26.9), 164 (24.6), 163 (23.7).

2,5,6,7-Tetramethyl-8-Amino-2-(4',8',12'-Trimethyltridecyl) Chroman, **5**

The nitro chroman, **4** (4 g), was dissolved in 160 ml ethanol, and with constant stirring, a solution of 32 ml of 20% aqueous sodium hydroxide was added; the pale yellow solution was heated to reflux on a water bath. The flask was removed from the heat and zinc dust (2 g) was carefully added, after which the mixture was returned to the water bath and refluxed for 1.5 hr. The hot reaction mixture was filtered and the aqueous/ethanolic layer was extracted with ether and finally the ethereal solution was washed with water. Evaporation of the ether yielded a dark-yellow oil. The amino chroman was purified on a 35 g silicic acid column by elution with 5% diethyl ether in petroleum ether. Yield, 2.4 g (66%). Gas chromatography (6 ft, 1½% OV-1, 245°C) of the *N*-trimethylsilyl (TMSi) derivative (formed by co-injection of the amine with bis-[trimethylsilyl] acetamide) or the *N*-acetyl derivative (formed by reaction of the amine with pyridine: acetic anhydride, 1:1 at room temperature for 1 hr) each gave a single peak.

Mass spectrum of *N*-acetylated derivative: M^+ 471 m/e (23.3), 429 (6.3), 206 (100.0), 188 (37.8), 164 (72.2), 163 (39.0).

Mass spectrum of *N*-trimethylsilyl derivative: M^+ 501 (14.1), 429 (15.4), 236 (32.2), 221 (32.0), 220 (27.8), 164 (19.9), 163 (40.8), 147 (26.9), 73 (100.0).

2,5,6,7-Tetramethyl-8-hydroxy-2-(4',8',12'-Trimethyltridecyl) Chroman, **6**

One gram of amino chroman, **5**, dissolved in 200 ml methanol, was cooled to 0–5°C, and concentrated sulfuric acid (30 ml) was added with constant stirring. Sodium nitrite (10 g) in 20 ml water was added over a 20-min period and the mixture was stirred for 15 min at 0–5°C. After the addition of an equal volume of water, the mixture was stirred for 1 hr at room temperature then left at room temperature overnight; the yellow color of the diazonium salt disappeared as hydrolysis to the phenol occurred. The phenol was extracted from the aqueous solution with ether, and the ethereal extract was washed with water, dried over anhydrous sodium sulfate, filtered, and evaporated to give a colorless oil. Gas chromatography of this oil (co-injected with bis-[trimethylsilyl] acetamide) on a 6-ft, 1½% OV-1 column at 250°C, revealed two major peaks. The compound with retention time 4.2 min was identified as the deaminated product, **3**. The other component had a retention time of 6.8 min and was identified as the TMSi ether of 2,5,6,7-tetramethyl-8-hydroxy-2-(4',8',12'-trimethyltridecyl) chroman, "ortho tocopherol," **6**. For final purification, the "ortho tocopherol" was eluted with 1% ether in

petroleum ether (150 ml) from a 15-g Brockmann Grade III alumina column. Yield, 260 mg (26%).

Mass spectrum of the TMSi ether: M^+ 502 m/e (32.6), 277 (2.4), 237 (32.8), 221 (100.0), 73 (23.6).

2,5,6,7-Tetramethyl-8-Acetoxy-2-(4',8',12'-Trimethyltridecyl) Chroman, **7**

"Ortho tocopherol," **6**, (260 mg) was treated with 10 ml dry pyridine and 10 ml dry acetic anhydride at room temperature for 30 min. The mixture was extracted with ether and the ether extracts were washed first with water, then with 6 *N* HCl, and finally with water. The acetylated product, **7**, was chromatographed on a 10-g silicic acid column from which it was eluted by 100 ml benzene. Yield, 250 mg (96%). Small amounts of **7** were purified for spectral studies by thin-layer chromatography in chloroform: benzene, 1:1. A single peak (retention time 11.2 min) resulted on gas chromatography of **7** on a 6-ft, 1½% OV-1 column at 245°C.

ir spectrum (CCl_4): 3,38, 3.42, 3.48, 5.68, 6.83, 8.23 μ.

Mass spectrum: M^+ 472 m/e, 430, 207, 205, 165, 164, 43.

High-resolution mass spectrum: M^+, m/e 472.389 (calcd for $C_{31}H_{52}O_3$, 472.3916).

uv spectrum (EtOH): λ_{max} 285 nm, shoulder at 277 nm.

nmr spectrum: τ 9.22, 9.12 (m), 8.82 (s), 8.29 (2H, t), 8.05 (3H, s), 7.95 (3H, s), 7.88 (3H, s), 7.45 (2H, t).[5]

$[\alpha]_{330}$ (*n*-heptane), zero.

1-Methoxy-2,3-Dimethyl-4-Acetoxy-5-Phytylbenzene, **12** *and 1-Methoxy-2,3-Dimethyl-4-Acetoxy-6-Phytylbenzene*, **13**—*Scheme 2*

2,3-Dimethyl-1,4-Benzoquinone, **8**

2,3-Dimethyl-1,4-benzoquinone, **8**, was prepared from 28 g 2,3-dimethylphenol (mp, 71–73°C) via 2,3-dimethylaminophenol using the diazo-coupling reaction of Smith and Austin (*7*). The yield of product was 6.0 g (24%); mp, 56–57°C, with sublimation (lit. 56.5–57.5°C).

ir spectrum (CCl_4): quinone C=O, 6.05 μ; no absorption in the region 2.7–3.1 μ.

uv spectrum (EtOH): λ_{max}, 247 nm, shoulder at 255 nm. On addition of $NaBH_4$ the maximum at 247 nm disappeared with the formation of a new maximum at 290 nm.

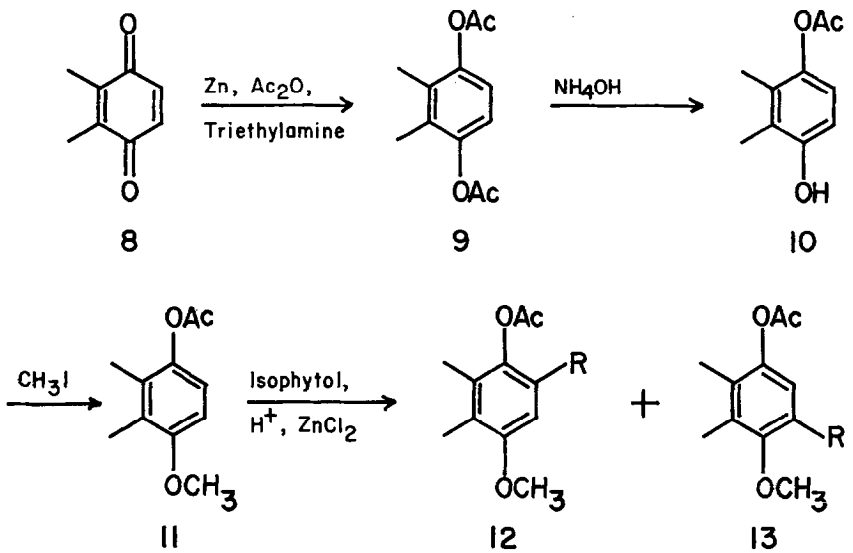
Mass spectrum: M^+ 136 m/e (100.0), 138 (4.8), 108 (35.1), 107 (31.0), 82 (49.2), 54 (54.0).

2,3-Dimethyl-1,4-Diacetoxybenzene, **9**

The quinone, **8**, (6.0 g) was dissolved in 30 ml of redistilled acetic anhydride and 20 ml of dry redistilled triethylamine, then treated gradually with 5.0 g zinc dust and more triethylamine (10 ml). The mixture was heated on a steam bath for 15 min as the color changed from yellow to colorless. The diacetate was extracted with diethyl ether and the ethereal extracts were washed with water, 6 *N* HCl, and finally with water. The diacetate, **9**, was a white, crystalline product which on gas chromatography (6 ft, 3% OV-1, 120°C) gave a single peak, with a retention time of 10 min. Yield, 7.9 g (81%); mp, 107–108°C.

Mass spectrum: M^+ 222 m/e (3.4), 180 (9.1), 138 (100.0), 123 (6.0), 91 (3.6), 81 (3.7), 79 (3.2), 77 (3.1), 43 (55.4).

[5] The chemical shifts in the nuclear magnetic resonance spectra are expressed in τ values relative to tetramethylsilane. The multiplicities of the bands are expressed as follows: s, singlet; d, doublet; t, triplet; m, multiplet.



R = PHYTYL, Ac = CH_3CO

SCHEME 2

2,3-Dimethyl-4-Acetoxyphenol, **10**

A mixture of 28% ammonia (6 ml) and a solution of 2,3-dimethyl-1,4-diacetoxybenzene in 100 ml methanol was allowed to stand at room temperature in an atmosphere of nitrogen. The course of the reaction was followed by gas chromatography of aliquots taken after various time intervals of hydrolysis; the reaction was found to be essentially complete after 2 hr. After the solution was acidified with dilute HCl, the monoacetate, **10**, was extracted with chloroform, the chloroform solution was washed with water, and then evaporated to yield a light-brown oil. Purification by elution from a 60-g silicic acid column by chloroform gave white crystals of **10**. Yield, 2.9 g (72%); mp, 124°C. Gas chromatography on a 6-ft, 3% OV-1 column gave a single peak (retention time, 6.8 min).

ir spectrum (CCl_4): 2.77, 2.90 (broad), 5.76 μ.

Mass spectrum: M^+ 180 m/e (7.9), 138 (100.0), 123 (18.2), 109 (3.1), 95 (4.7), 91 (4.4), 77 (4.0), 43 (43.7).

1-Methoxy-2,3-Dimethyl-4-Acetoxybenzene, **11**

2,3-Dimethyl-4-acetoxyphenol, **10**, (1.0 g) dissolved in 25 ml freshly distilled acetone, was refluxed with anhydrous potassium carbonate (1.4 g) and methyl iodide (2.5 ml) for 6 hr. The cooled solution was decanted and acidified (litmus) with 10% sulfuric acid. Extraction of the aqueous solution with diethyl ether, followed by drying (anhydrous sodium sulfate) and evaporation, yielded a light-brown oil. The compound, **11**, was eluted from a 50-g silicic acid column with benzene:chloroform, 1:1, and **11** was thus separated from the starting material and other impurities. The product, a colorless oil, produced a single peak (retention time 4.9 min) on gas chromatography using a 6-ft, 3% OV-1 column at 120°C. Yield, 435 mg (41%).

ir spectrum (CCl_4): 5.70 (C=O), 8.33 (ether); no absorption in the region 2.7–3.1 μ.

Mass spectrum: M^+ 194 m/e (21.1), 152 (100.0), 137 (77.9), 121 (4.6), 107 (5.0), 91 (12.7), 77 (14.0), 43 (39.6).

1-Methoxy-2,3-Dimethyl-4-Acetoxy-5-Phytylbenzene, **12** *and 1-Methoxy-2,3-Dimethyl-4-Acetoxy-6-Phytylbenzene*, **13**

1-Methoxy-2,3-dimethyl-4-acetoxybenzene, **11** (300 mg) dissolved in 20 ml dry ether, was treated with 500 mg isophytol, 100 mg zinc chloride (which had been fused and desiccated immediately before use) and 2 drops of glacial acetic acid. Anhydrous conditions were maintained throughout the reaction. After the mixture had been stirred at room temperature for 18–20 hr, the ether was evaporated, and the residue was heated at 50°C for 30 min. The resulting oil was dissolved in a mixture of light petroleum and 75% aqueous methanol; the petroleum ether layer was washed several times first with 75% methanol, then saturated sodium chloride, and finally with water. On evaporation of the solvents, a brown oil was obtained. This oil, containing components **12** and **13**, was applied to a 30-g silicic acid column, and both **12** and **13** were eluted by a total of 350 ml benzene. Fractions (5 ml) were collected from the column which separated components **12** and **13**; the minor product of the condensation of **11** with isophytol was **13**, which was eluted from the column by benzene before the major product, **12**. Further purification of **12** and **13** by thin-layer chromatography in chloroform yielded colorless oils. Each purified component gave a single gas chromatographic peak (6 ft, 3% OV-1, 250° or 6 ft, 3% OV-17, 260°); product **13** had a retention time of 0.89 relative to that of **12** on the OV-1 column.

Product **12**: Yield, 178 mg (22%).

ir spectrum (CCl_4): 3.38, 3.42, 3.48, 5.68, 6.80, 7.35, 8.31 μ.

uv spectrum (EtOH): λ_{max}, 277 nm, 269 nm.

Mass spectrum: M^+ 472 m/e, 430, 207, 205, 165, 151, 135, 57, 43.

High-resolution mass spectrum: M^+, m/e 472.390 (Calcd for $C_{31}H_{52}O_3$, 472.3916).

nmr spectrum: τ 9.20, 9.10 (12–15H, m), 8.80 (20H, s), 8.35 (3H, s), 8.07 (3H, s), 7.85 (6H, s), 6.78 (2H, d), 6.42 (3H, s), 4.82 (1H, m), 3.55 (1H, s).

$[\alpha]_{330}$ (*n*-heptane), + 6.7 deg cm^2 dg^{-1}.

Product **13**: Yield, 60 mg (7.5%).

ir spectrum (CCl_4): 3.38, 3.42, 3.48, 5.68, 6.80, 7.35, 8.31 μ.

uv spectrum (EtOH): λ_{max}, 277 nm, shoulder 270 nm.

Mass spectrum: M^+ 472 m/e, 430, 207, 205, 165, 151, 135, 57, 55, 43.

High-resolution mass spectrum: M^+, m/e 472.390 (Calcd for $C_{31}H_{52}O_3$, 472.3916).

nmr spectrum: τ 9.20, 9.10 (m), 8.82 (m), 8.31 (3H, s), 8.06 (3H, s), 8.02 (3H, s), 7.84 (3H, s), 6.73 (2H, d), 6.42 (3H, s), 3.54 (1H, s). There was some evidence for the vinyl H in the region of 5.0 τ, but an exact value could not be assigned.

1-Acetoxy-2,5-Dimethyl-4-Methoxy-6-Phytylbenzene, **14**[6]

Using the same reaction sequence and the same methods as described for the syntheses of **12** and **13**, the following compounds were prepared from 2,5-dimethylphenol.

2,5-Dimethyl-1,4-benzoquinone. Yield, 25%; mp, 123–125°C, with sublimation (lit. 125°C), crystallized from ethanol.

ir spectrum (CCl_4): quinone C=O, 6.05 μ. No absorption in the region 2.7–3.1 μ.

uv spectrum (EtOH): λ_{max} 252 nm, shoulder at 260 nm. The addition of $NaBH_4$ made the maximum at 252 nm disappear with the formation of a new maximum at 285 nm.

Mass spectrum: M^+ 136 m/e (6.0), 138 (2.0), 122 (100.0), 121 (43.5), 107 (89.5), 91 (17.7), 77 (30.2), 65 (8.9).

[6] For structures, see summary Scheme 5.

2,5-Dimethyl-1,4-diacetoxybenzene. Yield, 75%; mp, 134°C (lit. 134–135°C).

Mass spectrum: M^+ 222 m/e (4.9), 180 (12.3), 138 (100.0), 123 (6.4), 91 (2.1), 81 (1.7), 79 (2.1), 77 (1.9), 43 (40.1).

2,5-Dimethyl-4-acetoxyphenol. Yield, 85%; mp, 116°C (lit. 117°C).

ir spectrum (CCl_4): 2.77, 2.90 (broad), 5.76 μ.

Mass spectrum: M^+ 180 m/e (13.7), 138 (100.0), 123 (21.4), 109 (3.7), 95 (4.5), 91 (3.3), 77 (3.1), 43 (2.2).

1-Methoxy-2,5-dimethyl-4-acetoxybenzene. Yield, 41%.

ir spectrum (CCl_4): 3.31, 5.69, 8.35 μ. No absorption in the region 2.7–3.1 μ.

Mass spectrum: M^+ 194 m/e (14.8), 152 (100.0), 137 (81.1), 123 (2.1), 121 (2.1), 108 (3.4), 91 (5.0), 77 (4.9), 43 (10.0).

1-Acetoxy-2,5-Dimethyl-4-Methoxy-6-Phytylbenzene, **14**

As described above for the synthesis of **12**, isophytol (1.5 g) was condensed with 1-methoxy-2,5-dimethyl-4-acetoxybenzene (940 mg) to yield **14**. After purification on a silicic acid column, the product, a colorless oil, gave a single gas chromatographic peak (6-ft column, 3%, OV-1, 250°C) with a retention time of 9.5 min. Yield, 250 mg (11%).

ir spectrum (CCl_4): 3.38, 3.42, 3.48, 5.67, 6.78, 7.30, 8.28 μ.

uv spectrum (EtOH): λ_{max} 269 nm, shoulders at 277 and 274 nm.

Mass spectrum: M^+ 472 m/e, 430, 207, 205, 165, 135, 43.

High-resolution mass spectrum: M^+, m/e 472.392 (Calcd for $C_{31}H_{52}O_3$, 472.3916).

nmr spectrum: τ 9.21, 9.14 (m), 8.84 (m), 8.29 (3H, s), 8.11 (3H, s), 7.98 (3H, s), 7.82 (3H, s), 6.70 (2H, d), 6.39 (3H, s), 5.00 (1H, m), 3.43 (1H, s).

1-Acetoxy-2,6-Dimethyl-3-Phytyl-4-Methoxybenzene, **15**[6]

Using the same reaction sequence and the same methods as described for the synthesis of **12**, the following compounds were prepared from 2,6-dimethylphenol.

2,6-Dimethyl-1,4-benzoquinone. Yield, 31.4%; mp, 71–72°C (lit. 72–73°C).

ir spectrum (CCl_4): quinone C=O, 6.05 μ. No absorption in the region 2.7–3.1 μ.

uv spectrum (EtOH): λ_{max} 253 nm. On addition of $NaBH_4$ the maximum at 253 nm disappeared with the formation of a new maximum at 280 nm.

2,6-Dimethyl-1,4-diacetoxybenzene. Yield, 82%; mp, 93–94°C.

Mass spectrum: M^+ 222 m/e (3.9), 180 (12.2), 138 (100.0), 123 (2.5), 91 (1.3), 81 (1.0), 79 (1.2), 77 (1.2), 43 (18.1).

3,5-Dimethyl-4-acetoxyphenol. Yield, 53.3%; mp, 110–111°C.

ir spectrum (CCl_4): 2.77, 2.80 (broad), 5.70 μ.

Mass spectrum: M^+ 180 m/e (5.8), 138 (100.0), 123 (7.8), 109 (1.9), 95 (2.0), 91 (1.6), 77 (1.4), 43 (9.7).

1-Acetoxy-2,6-dimethyl-4-methoxybenzene. Yield, 57%.

Mass spectrum: M^+ 194 m/e (10.5), 152 (100.0), 137 (48.8), 123 (4.0), 108 (3.4), 91 (6.6), 77 (5.9), 43 (15.3).

1-Acetoxy-2,6-Dimethyl-3-Phytyl-4-Methoxybenzene, **15**

Isophytol (500 mg) was condensed with 1-acetoxy-2,6-dimethyl-4-methoxybenzene (275 mg) using the reaction conditions described for the synthesis of **12**. Gas chromatographic assay of the purified product on a 6-ft, 3% OV-1 column at 250°C, resulted in one peak with a retention time of 11.6 min. Yield, 250 mg (40.5%).

ir spectrum (CCl_4): 3.38, 3.42, 3.48, 5.66, 6.80, 7.30, 8.32 μ.

uv spectrum (EtOH): λ_{max} 282 nm, shoulders at 285 and 277 nm.

Mass spectrum: M^+ 472 m/e, 430, 207, 205, 165, 152, 151, 135, 57, 43.

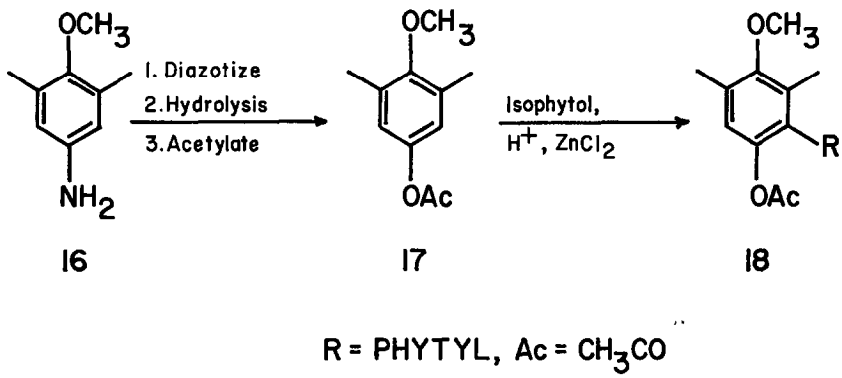
High-resolution mass spectrum: M^+, m/e 472.392 (Calcd for $C_{31}H_{52}O_3$, 472.3916).

nmr spectrum: τ 9.19, 9.14 (m), 8.83 (m), 8.31 (3H, s), 8.04 (3H, s), 7.96 (3H, s), 7.79 (3H, s), 6.74 (2H, d), 6.24 (3H, s), 4.95 (d ?), 3.55 (1H, s).

1-Methoxy-2,6-Dimethyl-4-Acetoxybenzene, **18**—*Scheme 3*

1-Methoxy-2,6-Dimethyl-4-Acetoxybenzene, **17**

4-Amino-2,6-dimethylanisole, **16**, required for this synthesis was itself prepared by the sequence, 2,6-dimethylphenol ——→ 2,6-dimethylanisole ——→ 4-amino-2,6-dimethylanisole, using standard methods; it was obtained as a white crystalline



SCHEME 3

material, mp 136°C (lit. 136–137°C). A solution of 4-amino-2,6-dimethylanisole (1 g) in 200 ml methanol was cooled to 0–5°C and after the dropwise addition of 30 ml concentrated sulfuric acid, a solution of 10 g sodium nitrite in 20 ml water was added over a 20-min period. The solution was allowed to stir for 30 min in the ice bath. After the addition of 200 ml water, the mixture was stirred at room temperature for 1 hr, then it was heated slowly to 65°C until the evolution of nitrogen had ceased. The product was extracted from the aqueous: methanolic solution with ether and the combined ethereal extracts were washed with water and evaporated to give a pale-yellow oil containing the free phenol. The yellow oil was dissolved in 50 ml pyridine: acetic anhydride and the solution was left at room temperature for 1 hr. The product was extracted with ether; the ethereal solution was washed first with water, then 6 *N* HCl, and finally with water. It was dried over anhydrous sodium sulfate, filtered, and evaporated to yield a pale-yellow oil (400 mg), which was purified by elution from a 20-g silicic acid column with chloroform. The product, **17**, a colorless oil, gave a single peak on gas chromatography (6-ft column, 3% OV-1, 120°C). Yield, 300 mg (24%).

Mass spectrum: M^+ 194 m/e (22.9), 152 (72.6), 137 (100.0), 121 (3.8), 109 (5.7), 107 (3.3), 91 (6.9), 77 (8.1), 43 (25.7).

1-Methoxy-2,6-Dimethyl-3-Phytyl-4-Acetoxybenzene, **18**

Isophytol (250 mg) was condensed with **17** (150 mg) using the conditions outlined above for the synthesis of **12** from **11**. Gas chromatographic assay of the product, purified by column chromatography, showed one peak (6-ft column, 3% OV-1, 250°C) with a retention time of 10.6 min. Yield, 59 mg (16%).

ir spectrum (CCl_4): 3.38, 3.42, 3.48, 5.70, 6.81, 7.33, 8.29 μ.
uv spectrum (EtOH): λ_{max} 277 nm, shoulders at 273 and 268 nm.
Mass spectrum: M^+ 472 m/e, 430, 207, 205, 165, 152, 151, 135, 43.
High-resolution mass spectrum: M^+ m/e, 472.390 (Calcd for $C_{31}H_{52}O_3$, 472.3916).
nmr spectrum: τ 9.20, 9.13 (m), 8.81 (m), 8.34 (s), 7.86 (s), 7.84 (s), 7.80 (s), 6.90 (d), 6.38 (s), 3.43 (s). There was evidence for the vinyl H in the region of 4.8–5.0 τ.

RESULTS AND DISCUSSION

2,5,6,7-Tetramethyl-8-Acetoxy-2-(4',8',12'-Trimethyltridecyl) Chroman, "Ortho Tocopherol Acetate"

In the first step of the synthesis of ortho tocopherol acetate, **7** (see Scheme 1) monophytylation rather than disubstitution of the symmetrical molecule, 3,4,5-trimethylphenol, was achieved using a 1 : 1 molar ratio of isophytol and the phenol in the presence of the Friedel–Crafts catalyst, zinc chloride. Gas chromatographic analysis of the product on a 6-ft, 3% OV-1 column at 235°C showed two components which on combined gas chromatography–mass spectrometry had the same molecular weight (414) and very similar fragmentation patterns, corresponding to structures **2** and **3**. Compound **2** formed a TMSi ether when the mixture was co-injected into the gas chromatograph with bis-(trimethylsilyl) acetamide, whereas the cyclized compound, **3**, remained unchanged. The identity of **2** was confirmed by the mass spectrum of its TMSi ether which showed a molecular weight of 486, with fragment ions at m/e 471, 261 (base peak), 246, 221, 208, 207, 205, and 73.

Although **2** and **3** could be separated from each other it was convenient for the purpose of this synthesis to subject the mixture of **2** and **3** to the cyclization reaction. This reaction is similar to the method used by Fieser et al. (*8*) and Tishler et al. (*9*) for the cyclization of phytylnaphthohydroquinone to "naphthotocopherol". After the cyclization reaction, gas chromatography and ir spectroscopy, demonstrated that any uncyclized compound **2** had been converted to **3**.

Nitration in the remaining position of the benzene ring was achieved by reaction with cupric nitrate in acetic anhydride (*10*). The chroman ring of the nitrated compound, **4**, was shown to be intact since there was no absorption in the –OH stretching region of the ir spectrum. A reduction of the nitrochroman with zinc powder and alcoholic sodium hydroxide yielded the aminochroman, **5**, the structure of which was confirmed by mass spectrometry of the *N*-acetyl and *N*-trimethylsilyl derivatives.

The low yield of phenol **6** (26%) obtained by diazotization followed by hydrolysis of the diazonium salt, was a result of the formation of some chroman **3**. This was an overall deamination of the aminochroman, which is a common side-reaction in the formation of phenols from diazonium salts. Acetylation of the phenol yielded the final product, ortho tocopherol acetate, **7**, which was pure according to gas and thin-layer chromatography. The mass spectrum of **7** (shown in Fig. 1) confirmed that there was only one free hydroxyl group in the ortho tocopherol, i.e., the chroman ring was intact. A proposed mass spectral fragmentation scheme for **7** is shown in Scheme 4.

It should be noted that the specific optical rotation $[\alpha]_{330}$ of **7** was zero, whereas for a natural sample of α-tocopherol acetate, $[\alpha]_{330}$ was +10.0 deg cm^2 dg^{-1}. α-Tocopherol acetate contains three chiral centers (C-2, C-4', C-8') and the absolute configuration of naturally occurring α-tocopherol is known to be 2*R*, 4'*R*, 8'*R* (*11*). The stereochemistry at C-4' and C-8' is the same as in naturally occurring phytol and this contributes

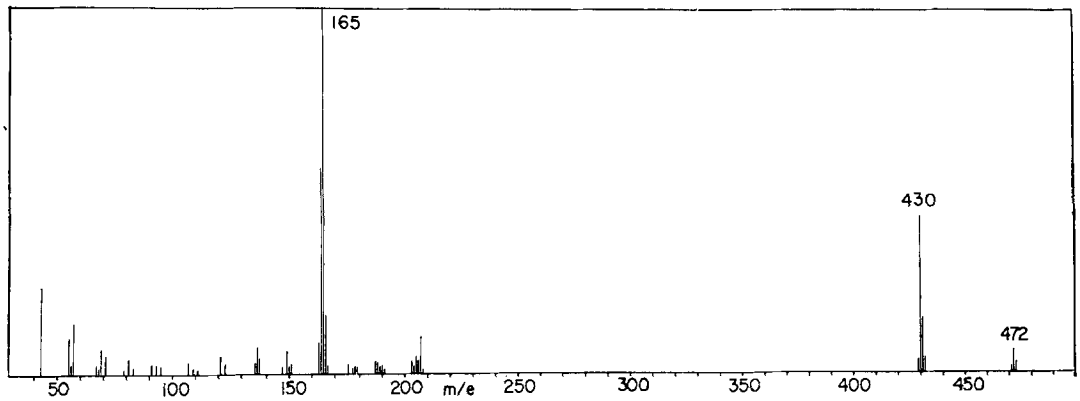
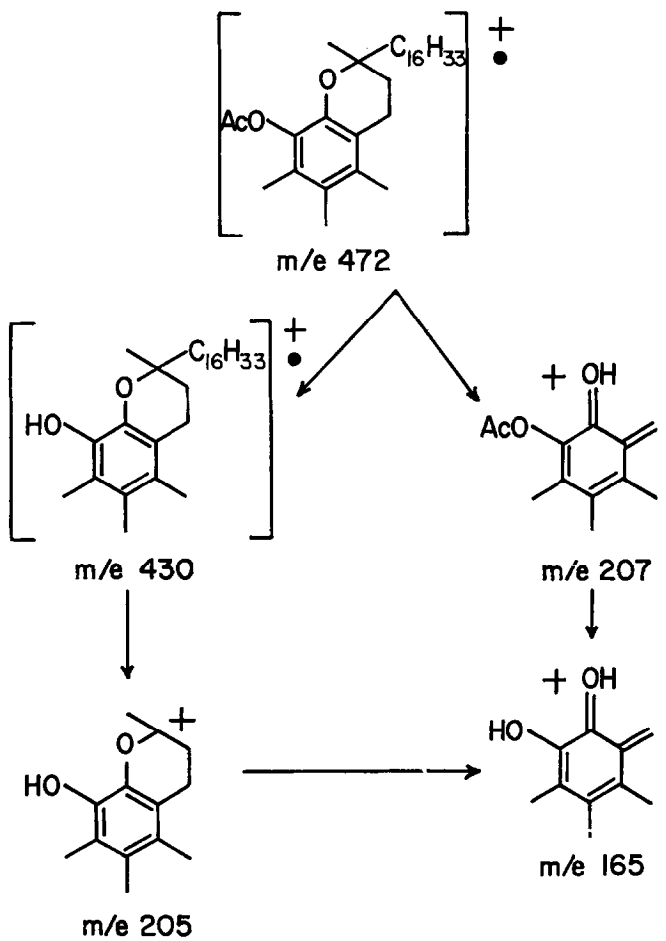


FIG. 1. Mass spectrum (70 eV) of ortho tocopherol acetate, 7.

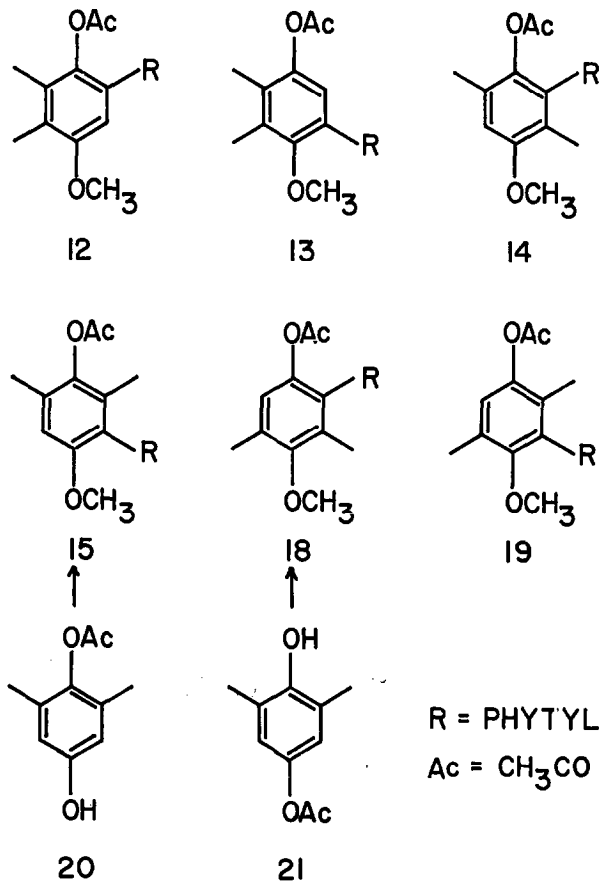


SCHEME 4

very little to the optical rotation of α-tocopherol acetate; its optical activity is almost entirely due to the chirality at C-2 (in the chroman ring). Since the optical rotation of synthetic ortho tocopherol acetate was zero, this implies that the cyclization of 2-phytyl-3,4,5-trimethylphenol was not stereospecific, so that an approximately equimolar mixture of two diastereoisomers of **7**, which were racemic at C-2, was produced. (See structure **7** in Scheme 1 for numbering system.)

The Isomeric Dimethylphytyl-1-Methoxy-4-Acetoxybenzenes

The six possible structural isomers of dimethylphytyl-1-methoxy-4-acetoxybenzene are compared in Scheme 5 (**12–15**, **18**, **19**). The objectives in each synthesis were: (a) to introduce an oxygen atom *para* to the hydroxyl of the dimethylphenol; (b) to acetylate one hydroxyl; (c) to methylate the other hydroxyl; and (d) to introduce the phytyl group in the appropriate position.



SCHEME 5

The sequence of reactions for the synthesis of **12** and **13** outlined in Scheme 1 was also used for the synthesis of **14**, **15**, and **19** with the appropriate dimethylphenol. Compound **18**, however, was synthesized by a different route as discussed below.

In the synthesis of **12**, **13**, **14**, **15**, and **19**, the second oxygen atom was introduced by a diazo coupling reaction *para* to the original hydroxyl, followed by reduction of the *p*-aminophenol and finally oxidation of the amine and steam distillation of the product to yield the crystalline dimethyl-1,4-benzoquinone. The formation of the dihydroquinone diacetate by reductive acetylation of the quinone was almost quantitative in each case.

A controlled, mild hydrolysis of the diacetate, under conditions similar to those described by Baker et al. (*12*), removed one of the acetyl groups and left the other intact. The course of the hydrolysis was monitored by gas chromatography of aliquots taken from the reaction mixture at various time intervals. Usually, 2 hr was sufficient

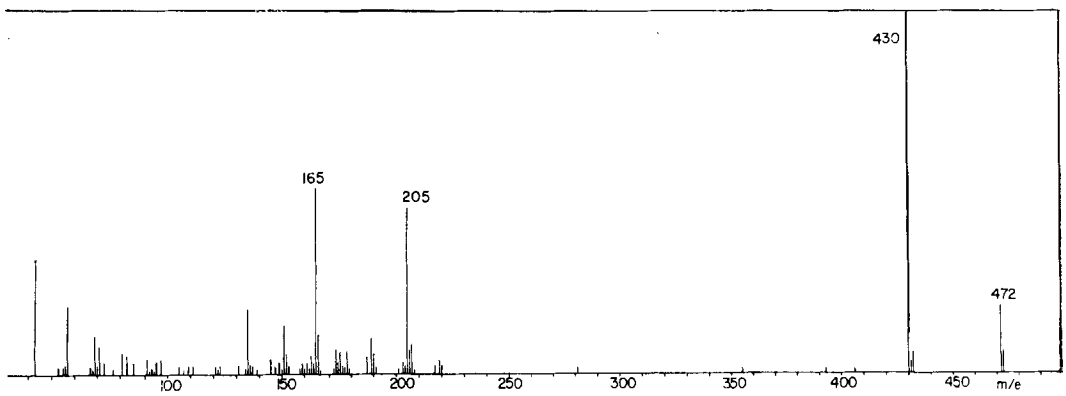


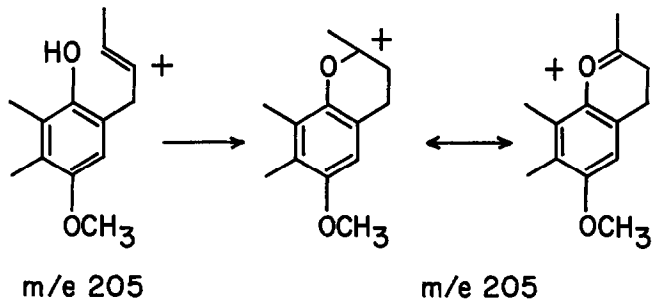
FIG. 2. Mass spectrum (70 eV) of 1-methoxy-2,3-dimethyl-4-acetoxy-5-phytylbenzene, **12**.

time to form the monoacetyl derivative without significant production of the dihydroquinone. These gas chromatographic conditions allowed a rapid assay of the dihydroquinone, the diacetate and the monoacetate in a single injection. Combined gas chromatography–mass spectrometry was used to identify these three components.

The free hydroxyl group was methylated with methyl iodide and anhydrous potassium carbonate in acetone. The reaction mixture was not sufficiently basic to hydrolyze the phenolic acetate, and allowed the formation of the methyl ether in adequate yield (40–50%). The desired product was purified by silicic acid chromatography.

In the phytylation reaction, the final step, it was important to use a 1:1 molar ratio of isophytol:methoxyphenol acetate since there were two vacant positions available for substitution of the aromatic moiety. In the synthesis of **12**, the intermediate 1-methoxy-2,3-dimethyl-4-acetoxy-benzene, **11**, was an unsymmetrical molecule so that monophytylation at position 5 or 6 would be expected to result in two different products, **12** and **13**. In fact, gas chromatography of the crude phytylated product revealed two major peaks, the areas of which were in the ratio 75:25. Both components had the same molecular weight (472) and similar mass spectral fragmentation patterns, the only differences being in the relative intensities of certain fragment ions. The mass spectrum obtained for product **12** is presented in Fig. 2, and a mass spectral fragmentation scheme was proposed in the previous paper (*13*). The fragment ion at m/e 205 in the spectrum of the major component was more intense (44.7% of the intensity of the base peak) than m/e 205 for the minor product (22.0% of the intensity of the base peak). This fact can be explained in terms of structural differences as follows. A loss of ketene from the parent molecular ion at m/e 472 yields a fragment ion at m/e 430. A further

loss of 225 mass units from m/e 430 gives the fragment at m/e 205, which can be stabilized by the formation of a cyclic chroman-like structure, as indicated in Scheme 6. The compound with structure **12** produces an ion at m/e 205 which can be stabilized in this manner, since the loss of ketene would yield a fragment with the –OH group adjacent to the phytyl moiety. On the other hand, structure **13** has the methoxyl group



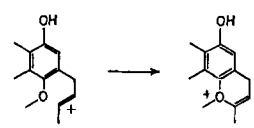
SCHEME 6

adjacent to the phytyl group; after the loss of ketene from the parent molecular ion, the free –OH group would not be in a position suitable for the formation of this stable cyclic structure. Similarly, the fragment ion at m/e 430, derived from compound **12** would be stabilized by the formation of a chroman-like structure, whereas this would not be possible for compound **13**. In the mass spectrum of the major product of the synthesis, the base peak was at m/e 430, yet in the spectrum of the minor product, the intensity of this fragment was only 46% of the base peak. Therefore, since we would expect the abundance of m/e 430 and 205 to be greater for compound **12** than compound **13**, we assign structure **12** to the major component of the synthesis and structure **13** to the minor component.[7]

Compounds **12** and **13** were separated from each other by column chromatography and were further purified by thin-layer chromatography in 100% chloroform and benzene:petroleum ether, 2:3. Additional spectral evidence (uv, ir, and nmr spectra) agreed with the proposed structures.

In a like manner, monophytylation of 1-methoxy-2,5-dimethyl-4-acetoxybenzene would theoretically result in two different products, **14** and **19**. Gas chromatography of the reaction mixture before final purification revealed one major peak and a minor one (5% of the area of the major one) with a retention time of 0.91 relative to the major one. Although the minor component could not be isolated completely free from the major component, it was possible to obtain the mass spectrum of each by combined gas

[7] A referee has suggested stabilization of the m/e 205 ion from **13** by the process shown in Scheme 7. A consideration of the overall fragmentation pattern, however, supports our proposal that it is the ion



SCHEME 7

from **12** which is stabilized (Scheme 6). Thus, loss of ketene from m/e 207 to form the m/e 165 ion is much more pronounced in the compound with a low intensity for m/e 205, and conversely, m/e 165 is less pronounced in the compound with the higher intensity for m/e 205. This observation agrees with the stabilization of **12** by formation of the chroman-like structure.

chromatography–mass spectrometry. Each compound had a molecular weight of 472 and the mass spectral fragmentations were similar. In the mass spectrum of the major product, the ions at m/e 205 and 430 were more abundant than in the spectrum of the minor product. Therefore, on the basis of the reasoning used for compounds **12** and **13**, structure **14** was assigned to the major component and structure **19** to the minor component (see Table 1). The uv, ir, and nmr spectra of compound **14** were in agreement with the proposed structure.

In the synthesis of compounds **15** and **18**, two possible structures, **20** and **21**, had to be considered for the 4-acetoxydimethylphenol derived from 2,6-dimethylphenol. During the hydrolysis of 2,6-dimethyl-1,4-diacetoxybenzene, only one isomer was obtained. We assigned structure **20** to this product since the acetyl group situated between two hydrogen atoms would be more susceptible to hydrolysis than the sterically hindered acetyl group between the two methyl groups. An *O*-methylation of **20** followed by phytylation, yielded the compound **15**. The nmr, uv, ir, and mass spectra of the synthesized compound were in agreement with the proposed structure.

The alternative 4-acetoxydimethylanisole (the methyl ether of **21**) for the synthesis of **18** was made from 2,6-dimethylphenol by the independent, unambiguous route outlined in Scheme 3. First, 2,6-dimethylphenol was *O*-methylated to yield 2,6-dimethylanisole which was nitrated in the 4-position according to the method of Rowe et al. (*14*). Reduction gave 2,6-dimethyl-4-aminoanisole, **16**, after which diazotization, hydrolysis, and acetylation yielded **17**. The final step in the synthesis of **18** was the condensation of isophytol with the methyl ether, **17**. In addition to the unambiguous route of synthesis, the uv, ir, nmr, and mass spectra supported the structure **18** proposed for the final product. The nonidentity of the two compounds, synthesized as structures **15** and **18**, was confirmed by their nmr spectra as well as by their separation on gas chromatography.

Table 1 is a summary of the relative intensities of the major fragment ions from the mass spectra of the six structural isomers. Since the same fragment ions occur in the mass spectra derived from each of the six isomers, we assume that the pathways for the mass spectral fragmentations of the six isomers are similar to that shown for **12** in the previous paper (13). Although ortho tocopherol acetate and α-tocopherol acetate

TABLE 1

RELATIVE INTENSITIES OF SOME FRAGMENT IONS FROM THE MASS SPECTRUM OF THE SIX SYNTHETIC DIMETHYLPHYTYL-1-METHOXY-4-ACETOXY-BENZENES, α-TOCOPHEROL ACETATE AND ORTHO TOCOPHEROL ACETATE

Compound	% Relative intensity of ion at m/e: [a]					
	472	430	207	205	165	43
α-Tocopherol acetate	14.2	100.0	28.4	8.2	87.8	1.7
7	4.4	32.6	9.6	3.6	100.0	28.2
12	18.4	100.0		44.7	50.0	30.6
13	9.8	46.3	8.9	22.2	100.0	17.4
14	21.0	100.0	14.8	26.8	92.4	33.6
15	14.2	100.0	17.4	33.1	37.6	25.5
18	15.6	100.0	33.1	46.1	49.9	29.2
19	5.2	19.4	42.9	1.3	100.0	10.6

[a] The intensities are relative to the fragment ion of highest intensity.

contain a chroman ring, their mass spectra are very similar to the mass spectra of the six structural isomers, with some differences in the relative intensities of some fragment ions. A mass spectrum cannot reliably distinguish between a compound containing a chroman ring, such as **7**, and an isomer containing an uncyclized phytyl side-chain, such as the six synthetic compounds **12**, **13**, **14**, **15**, **18**, and **19**.

In summary, of the six possible structural isomers for dimethylphytyl-1-methoxy-4-acetoxybenzene, five (**12**, **13**, **14**, **15**, and **18**) were obtained and purified in sufficient amounts so that their spectral properties could be compared. Compound **19**, obtained only in small amounts as a by-product of the synthesis of **14**, could not be separated from **14** by column chromatography or thin-layer chromatography; however, **14** and **19** separated on gas chromatography so that their mass spectra could be compared. It is noteworthy that a combination of the spectral parameters which are commonly determined (uv, ir, nmr, and mass spectra) is not sufficient for determination of the orientation of the groups in a compound which contains a multisubstituted benzene ring, such as the six structural isomers described in this paper.

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