Dye-Sensitized Photooxidation of α-Tocopherol

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Abstract: The photooxidation of α-tocopherol with visible light requires the presence of a dye sensitizer (proflavin). α-Tocopherol photooxidized smoothly in methanol to isomers of 4a,5-epoxy-8a-methoxy-α-tocopherone (34%), 8a-methoxy-α-tocopherone (14%), α-tocoquinone 2,3-oxide (6%), and α-tocoquinone (<1%). Two geometric isomers of 4a,5-epoxy-8a-methoxy-α-tocopherone were isolated and characterized and a possible mechanism involving singlet oxygen is proposed.

The oxidation of biological antioxidants such as α-tocopherol has been studied extensively to gain information on the mechanism by which tissues and lipids are protected from oxidative degradation.[2] Autoxidation of tocopherols in vegetable oils gives oxidation products[3] that have been obtained chemically from free radical initiated reactions with the use of inorganic oxidizing agents and peroxides.[4]

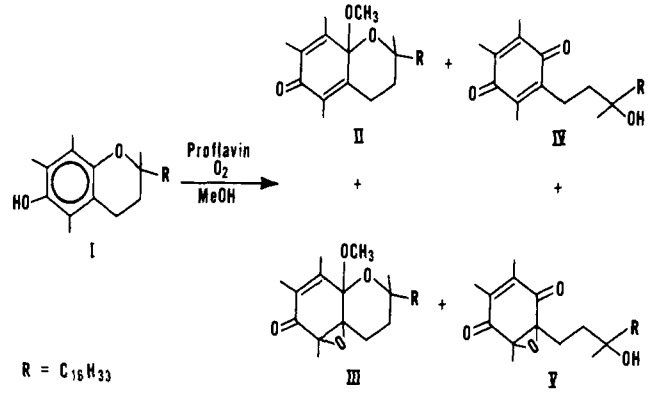
Singlet oxygen generated by dye sensitization or by sodium hypochlorite–hydrogen peroxide has been implicated as a possible *in vivo* oxidant.[5] Some enzymatic oxidations occur by a similar mechanism and may involve singlet oxygen.[5,6]

The reaction of α-tocopherol with singlet oxygen was investigated, and the oxidation products from this reaction were characterized.

Results and Discussion

In preliminary experiments α-tocopherol proved to be insensitive to incandescent light during photolysis in a water-cooled Pyrex reaction vessel at 25° for 16 hr. When the methanolic solution of α-tocopherol was saturated with oxygen or nitrogen gas during photolysis, α-tocopherol remained unchanged. Dye (proflavin), oxygen, and light were required for the photooxidation of α-tocopherol. After photolysis for 3 hr under oxygen in the presence of proflavin, α-tocopherol was converted into two fractions (Scheme I). The major fraction is composed of less polar compounds than α-tocopherol and, after purification, was determined to be a mixture of substituted 8a-methoxy-α-tocopherones.[7]

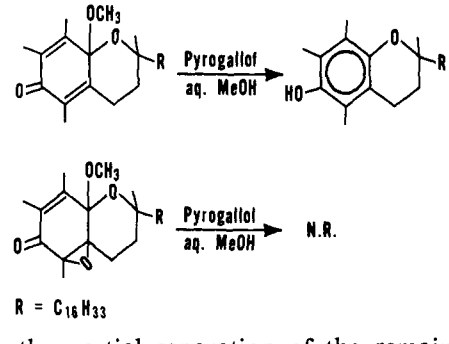
Scheme I



The minor fraction is composed of more polar compounds than α-tocopherol and is a mixture of α-tocoquinone (IV) and a new compound, α-tocoquinone oxide (V).

By thin-layer cochromatography with an authentic sample, 8a-methoxy-α-tocopherone (II) was characterized as one of five compounds present in the major fraction. Reduction of the photomixture with methanolic pyrogallol selectively reduced II to α-tocopherol. This reaction is characteristic of 8a-alkoxy-α-tocopherones (Scheme II).[8] Selective reduction of II made

Scheme II



possible the partial separation of the remaining four compounds into two pure geometric isomers (IIIa and IIIb) and one pair of geometric isomers (IIIc and IIId) of 4a,5-epoxy-8a-methoxy-α-tocopherone (see Figure 1).

The fragmentation pattern of IIIa obtained by low-resolution mass spectrometry was identical with that of IIIb and the mixture of IIIc–d and showed that an

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(2) (a) For a recent review see: J. Green and D. McHale in "Biochemistry of Quinones," R. A. Morton, Ed., Academic Press, London, 1965, pp 261; (b) J. Lars G. Nilsson, H. Sievertsson, and H. Selander, *Acta Chem. Scand.*, **23**, 859 (1969); (c) W. A. Skinner and R. M. Parkhurst, *Lipids*, **5**, 184 (1969).

(3) A. S. Csallany, M. Chiu, and H. H. Draper, *ibid.*, **5**, 63 (1969).

(4) (a) W. A. Skinner and R. M. Parkhurst, *J. Org. Chem.*, **31**, 1248 (1966); W. A. Skinner, *Biochem. Biophys. Res. Commun.*, **15**, 469 (1964); (b) J. L. G. Nilsson, H. Sievertsson, and H. Selander, *Tetrahedron Lett.*, 5023 (1968); (c) G. E. Inglett and H. A. Mattill, *J. Amer. Chem. Soc.*, 77, 6552 (1955); (d) C. T. Goodhue and H. A. Risley, *Biochem. Biophys. Res. Commun.*, **17**, 549 (1964); (e) C. T. Goodhue and H. A. Risley, *Biochemistry*, **4**, 854 (1965).

(5) (a) C. S. Foote, *Accounts Chem. Res.*, **1**, 104 (1968), and references therein; (b) C. S. Foote and R. W. Denny, *J. Amer. Chem. Soc.*, **90**, 6233 (1968); (c) C. S. Foote, Y. C. Chang, and R. W. Denny, *ibid.*, **92**, 5216, 5218 (1970).

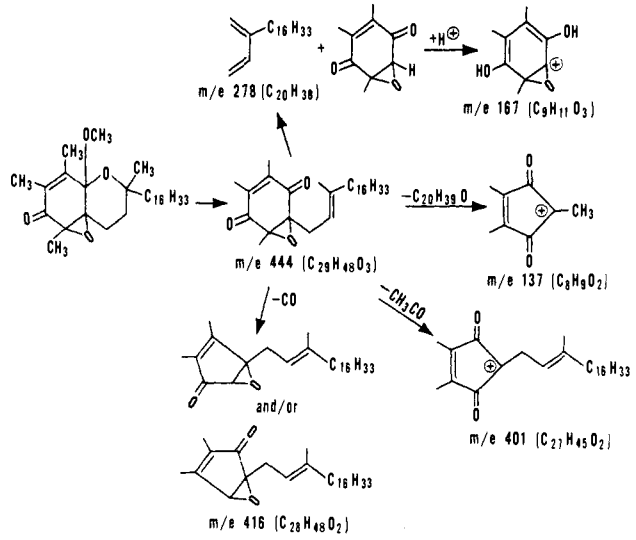
(6) (a) B. Samuelson, *ibid.*, **87**, 3011 (1965), and references therein; (b) J. E. Baldwin, H. H. Basson, and H. Krauss, Jr., *Chem. Commun.*, 984 (1968), and references cited therein; (c) I. Saito, S. Kato, and T. Matsuura, *Tetrahedron Lett.*, 239 (1970).

(7) The nomenclature follows that of C. T. Goodhue and H. A. Risley[4e] and W. Dürckheimer and L. A. Cohen,[8] for substituted chromanones.

(8) W. Dürckheimer and L. A. Cohen, *J. Amer. Chem. Soc.*, **86**, 4388 (1964).

additional oxygen atom was present in the molecule as an epoxide group (Scheme III). High-resolution

Scheme III



mass spectral data confirmed the elemental composition of the major fragments of IIIa resulting from the fragmentation pattern proposed in Scheme III. The ir, uv, and nmr spectra of IIIa, IIIb, and IIIc–d were in agreement with the structure proposed.

By interpretation of the nmr spectra of IIIa, IIIb, and IIIc–d we were able to establish the geometric relationship among the four isomers (Figure 1). Molecular models show that cis ring fusion (IIIa, IIIb) leads to compounds whose structures have the methoxy group in a similar magnetic environment (δ_{MeO} 3.20, 3.20). When the methoxy group is cis to the 2-methyl group (IIIa), deshielding of the 2-methyl group (δ_{CH_3} 1.37) is observed and may be due to 1,3-diaxial interaction of this group with the methoxy group.[9] When the methoxy group is trans to the 2-methyl group, the 2-methyl group is localized in a shielding environment above the plane of the enone system.[10] Only one of the four possible isomers has a shielded 2-methyl group (IIIb, δ_{CH_3} 1.06). The ratio of IIIa to IIIb is 7:3 (Table I).

When the ring fusion is trans (IIIc, IIId), the methoxy group lies above the enone plane and an upfield shift

Table I. Yield of Photooxidation Products

Compound	Yield, %
cis-4a,5-Epoxy-*cis*-8a-methoxy-α-tocopherone (IIIa)[a]	7
trans-4a,5-Epoxy-*trans*-8a-methoxy-α-tocopherone (IIIb)	3
trans-4a,5-Epoxy-*cis*-8a-methoxy-α-tocopherone (IIIc)	10
cis-4a,5-Epoxy-*trans*-8a-methoxy-α-tocopherone (IIId)	14
8a-Methoxy-α-tocopherone (II)	14[b]
α-Tocoquinone 2,3-oxide (V)	6
α-Tocoquinone (IV)	1[c]
α-Tocopherol (I), recovered	29

[a] The structure of the four geometric isomers is related to the 2β-methyl group. If the 2-methyl group were α, the four enantiomers would result. [b] The yield of II is based on the yield of α-tocopherol from the reduction of the purified photomixture of 8a-alkoxy-α-tocopherones. [c] The small amount of IV may be due to oxidation during isolation.

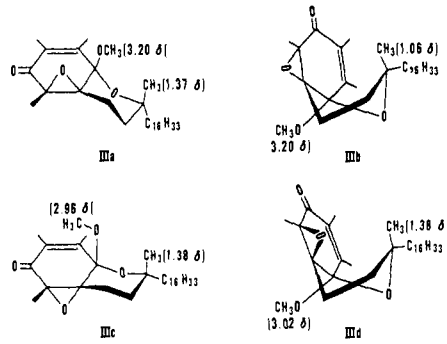


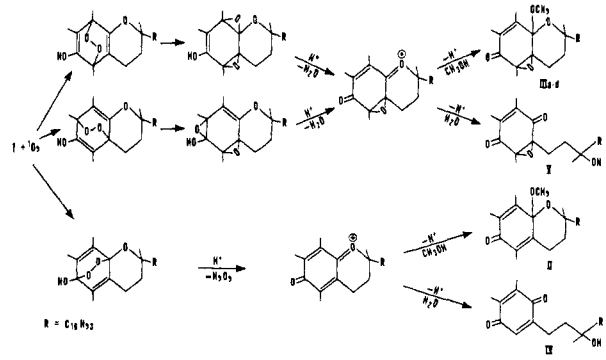
Figure 1. Geometric isomers of 4a,5-epoxy-8a-methoxy-α-tocopherone (IIIa–d). Structures of the geometric isomers shown are related to the 2β-methyl group. If the 2-methyl group were α, the four enantiomers would result.

is observed in the methoxy resonance (δ_{MeO} 2.96, 3.02).[10] In both isomers trans ring fusion leads to deshielding of the 2-methyl group owing to interaction with the oxygen atoms of the methoxy group in IIIc (δ_{CH_3} 1.38) and the epoxy group in IIId (δ_{CH_3} 1.37).[11] By integration of the methoxy group resonance, the ratio of IIIc to IIId in the mixture is approximately 4:3. The isomers in which methoxy is trans to the epoxy group predominate over the cis in approximately the same ratio (IIIc:IIIb = 10:3 ≈ IIId:IIIa = 14:7).

The minor fraction is a mixture of α-tocoquinone (IV) and α-tocoquinone 2,3-oxide (V), which could be separated by reverse-phase thin-layer chromatography (tlc) on hexadecane-impregnated silica gel. The ratio of α-tocoquinone to α-tocoquinone oxide was 1:23. The stereochemistry of V was not investigated at this time. Acid hydrolysis of IIIa, IIIb, and IIIc–d gave α-tocoquinone 2,3-oxide. The mass spectrum of α-tocoquinone oxide is similar to that reported for phylloquinone oxide,[12] and uv, ir, and nmr spectra are consistent with this structure. The yields of photooxidation products characterized are listed in Table I.

The distribution of products suggests that singlet oxygen attacks α-tocopherol to give an intermediate oxonium ion which is in turn attacked by solvent to give the observed products. A plausible mechanism is illustrated in Scheme IV. The 1,4-cycloaddition of oxygen, followed by endoperoxide ring opening, may lead to diepoxide intermediates similar to those proposed in other phenolic oxidations.[6c] Protonation of the di-

Scheme IV



(9) K. Tori and E. Kondo, *Tetrahedron Lett.*, 645 (1963), and references therein.

(10) N. S. Bhacca and D. H. Williams, "Application of NMR Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, Calif., 1964, p 88; W. L. Meyer and R. W. Huffman, *Tetrahedron Lett.*, 691 (1962).

(11) K. Tori, K. Aono, K. Kitahonoki, Y. Takano, H. Tanida, and T. Tsuji, *ibid.*, 2921 (1966).

(12) J. T. Matschiner, R. G. Bell, J. M. Amelotti, and T. E. Knauer, *Biochim. Biophys. Acta*, 201, 309 (1970).

epoxide intermediate followed by loss of water leads to an oxonium ion intermediate. Solvolysis leads to the observed products. Product analysis shows that addition of methanol to form the trans fused ring system is favored.

Our investigation shows that α-tocopherol is susceptible to photooxidation in the presence of a dye sensitizer and that this oxidation in methanol leads to several compounds including α-tocoquinone 2,3-oxide and the four possible geometric isomers of 4a,5-epoxy-8a-methoxy-α-tocopherone. From these results it seems possible that α-tocopherol may play a role in the protection of biological lipids from the effects of photodynamic action.

Experimental Section[13]

Materials. *dl*-α-Tocopherol came from City Chemical Co., New York, N. Y., and was homogeneous by tlc. *dl*-α-Tocoquinone was purchased from Eastman Kodak. Proflavin dye was obtained as the free base from Pfaltz and Bauer, Inc. Baker Reagent Grade methanol was used as received.

Photooxidation Procedure. *dl*-α-Tocopherol (320 mg) was dissolved in 160 ml of methanol containing proflavin (2 mg). Dry oxygen was bubbled through the reaction solution in a Pyrex reaction vessel fitted with a cooling jacket circulating water and the temperature maintained at 25°. The reaction vessel was photolyzed externally for 3 hr with two G.E. 300-W tungsten lamps in a Rayonet photochemical reactor. When the reaction time was extended to 16 hr without dye, oxygen, or light, no reaction was observed. The photolyzed reaction mixture was concentrated under reduced pressure below 40° to a small volume (~20 ml). Ethyl ether (100 ml) was added and the proflavin dye was extracted with 5% aqueous $NaHCO_3$ (three 50-ml portions). The organic layer was concentrated under reduced pressure, dried over anhydrous Na_2SO_4 after the addition of petroleum ether (bp 40–60°), and concentrated to approximately 5 ml. The reaction mixture was placed on an alumina column (basic, activity III, 30-ml dry volume, 15 mm i.d.). After the column was washed with 50 ml of petroleum ether, the major fraction (172 mg) was eluted with 100 ml of 5% ethyl ether in petroleum ether. Unreacted α-tocopherol (93 mg) was eluted with 25% ethyl ether (200 ml) and the minor fraction (18 mg) was eluted with ethyl ether (100 ml).

Separation and Characterization of Photoproducts. 8a-Methoxy-α-tocopherone (II). After a portion of the major fraction was chromatographed on an alumina tlc plate (250 μ × 20 cm × 20 cm, aluminum oxide, basic, type T, activated at 110° for 1 hr) in 5% ethyl ether–petroleum ether solvent, with the aid of a short-wave uv lamp, four compounds were observed with R_f 0.53, 0.64, 0.71, and 0.76. The compound with R_f 0.64 was characterized as 8a-methoxy-α-tocopherone by cochromatography with an authentic sample prepared by the method of Goodhue and Risley.[4e] Characteristic of 8a-alkoxy-α-tocopherones, II was reduced to α-tocopherol in methanolic pyrogallol.[8] The yield of α-tocopherol obtained from the selective reduction of II in the photomixture is used to estimate the amount of II present in the photomixture (see below).

Reduction of Photomixture. The major fraction (260 mg) was dissolved in 2 ml of petroleum ether (bp 40–60°) and the solution was added to 100 ml of 5% methanolic pyrogallol and 10 ml of water before being set aside for 2.5 hr. The solution was diluted with 200 ml of distilled water and the desired product was extracted with petroleum ether (three 100-ml portions. The petroleum ether extract was washed with water (four 50-ml portions), dried over Na_2SO_4, and concentrated to a small volume. The mixture was placed on an alumina column (basic, activity IV, i.d. 15 mm, 20-ml dry volume) and the mixture of photoproducts (156 mg) and α-tocopherol (52 mg) was eluted with 1 l. of petroleum ether. Owing to acid hydrolysis during the pyrogallol reduction, a small amount of quinones (27 mg) was formed that could be eluted from the column with 100 ml of ethyl ether. By repeated chromatography as outlined above the geometric isomers of 4a,5-epoxy-8a-methoxy-α-tocopherol could be separated into two pure isomers, *cis*-4a,5-epoxy-*cis*-8a-methoxy-α-tocopherone (IIIa, R_f 0.76, 30 mg) and *trans*-4a,5-epoxy-*trans*-8a-methoxy-α-tocopherone (IIIb, R_f 0.71, 15 mg), and one isomeric pair (IIIc, IIId, R_f 0.53, 106 mg), *trans*-4a,5-epoxy-*cis*-8a-methoxy-α-tocopherone (IIIc) and *cis*-4a,5-epoxy-*trans*-8a-methoxy-α-tocopherone (IIId).

***cis*-4a,5-Epoxy-*cis*-8a-methoxy-α-tocopherone (IIIa).** The ir spectrum (CCl_4) of the colorless oil showed bands at 2950, 2780, 1680, 1660 (sh), 1465, 1385, 1080 (sh), 1065, and 975 cm^{-1}; uv (C_6H_{12}) λ_{max} 248 nm (ϵ_{max} 7.1 × 10^3); nmr (CCl_4) δ 0.75–0.95 (m, 12 H, 4 CH_3), 1.00–1.66 (m, 25 H, 11 CH_2, 3 CH), 1.37 (s, 3 H, deshielded 2-CH_3), 1.44 (s, 3 H, CH_3), 1.76–1.80 (m, 6 H, vinyl, CH_3), 3.2 (s, 3 H, methoxy); mass spectrum (70 eV) *m/e* 444 ($C_{29}H_{48}O_3$, 4%), 416 ($C_{28}H_{48}O_2$, 16%), 401 ($C_{27}H_{45}O_2$, 12%), 306 (3), 278 ($C_{20}H_{38}$, 4%), 263 (1), 233 (2), 219 (5), 205 ($C_{13}H_{17}O_2$, 13%), 167 ($C_9H_{11}O_3$, 100%), 149 (7), 139 ($C_8H_{11}O_2$, 12%), 138 ($C_8H_{10}O_2$, 15%), 137 ($C_8H_9O_2$, 22%), 123 (16), 109 (19), 81 (C_6H_9, 40%), 57 (51), 43 (62).

Anal. Calcd for $C_{30}H_{52}O_4$: C, 75.58; H, 10.99. Found: C, 75.87; H, 11.21.

***trans*-4a,5-Epoxy-*trans*-8a-methoxy-α-tocopherone (IIIb).** The ir spectrum (CCl_4) of the colorless oil showed bands at 2950, 2770, 1680, 1660 (sh), 1465, 1385, 1080, 1065 (sh), and 970 cm^{-1}; uv (C_6H_{12}) λ_{max} 243 (ϵ_{max} 7.4 × 10^3); nmr (CCl_4) δ 0.80–0.95 (m, 12 H, 4 CH_3), 1.00–1.66 (m, 25 H, 11 CH_2, 3 CH), 1.06 (s, 3 H, shielded 2-CH_3), 1.43 (s, 3 H, CH_3), 1.77 (s, 3 H, vinyl, CH_3), 1.82 (s, 3 H, vinyl, CH_3), 3.20 (s, 3 H, methoxy); mass spectrum (70 eV) *m/e* 444 (8), 416 (20), 401 (18), 278 (11), 263 (12), 219 (8), 205 (16), 167 (100), 149 (40), 139 (16), 138 (22), 137 (27), 123 (23), 109 (25), 81 (44), 57 (74), 43 (23).

Anal. Calcd for $C_{30}H_{52}O_4$: C, 75.58; H, 10.99. Found: C, 75.23; H, 11.15.

***trans*-4a,5-Epoxy-*cis*-8a-methoxy-α-tocopherone (IIIc) and *cis*-4a,5-Epoxy-*trans*-8a-methoxy-α-tocopherone (IIId).** The ir spectrum (CCl_4) of the colorless oil showed bands at 2950, 2770, 1685, 1650 (sh), 1465, 1385, 1085, 1060, and 1020 cm^{-1}; uv (C_6H_{12}) λ_{max} 248 nm (ϵ_{max} 7.1 × 10^3); nmr (CCl_4) δ 0.80–0.95 (m, 12 H, 4 CH_3), 1.00–1.66 (m, 25 H, 11 CH_2, 3 CH), 1.38 (s, 6 H, 2 CH_3), 1.76 (s, 3 H, vinyl, CH_3), 1.84 (s, 3 H, vinyl, CH_3), 2.96 (s, 9/7 H, *cis*-methoxy), 3.02 (s, 12/7, *trans*-methoxy); mass spectrum (70 eV) *m/e* 444 (7), 416 (19), 401 (17), 278 (7), 263 (8), 219 (7), 205 (16), 167 (100), 149 (28), 139 (14), 138 (23), 137 (26), 123 (18), 109 (21), 81 (44), 57 (65), 43 (72).

Anal. Calcd for $C_{30}H_{52}O_4$: C, 75.58; H, 10.99. Found: C, 75.50; H, 11.29.

α-Tocoquinone 2,3-Oxide (V). When IIIa, IIIb, or IIIc-d was acid hydrolyzed according to the procedure of Boyer,[14] the product was V. The stereochemistry of V was not investigated. The ir spectrum (CCl_4) of the light yellow oil showed bands at 3500, 2950, 2780, 1680, 1635 (w), 1465, and 1380 cm^{-1}; uv (C_6H_{12}) λ_{max} 271 (ϵ_{max} 4.05 × 10^3); nmr (CCl_4) δ 0.80–0.90 (m, 12 H, 4 CH_3), 1.00–1.66, (m, 28 H, 11 CH_2, 3 CH, 1 CH_3), 1.55 (s, 3 H, CH_3; 1 H, —OH), 1.92 (s, 6 H, 2 vinyl, CH_3); mass spectrum (70 eV) *m/e* 462 (2), 444 (1), 419 (49), 402 (25), 237 (4), 139 (9), 138 (21), 137 (19), 99 (24), 83 (17), 81 (14), 69 (26), 57 (34), 43 (100).

Anal. Calcd for $C_{29}H_{50}O_4$: C, 75.28; H, 10.89. Found: C, 75.61; H, 10.93.

The minor fraction from alumina chromatography of the photoproduct mixture contained two compounds, which could only be separated by reverse-phase chromatography. The oily mixture (500 μg) was dissolved in methanol, the solution spotted on a hexadecane-impregnated Eastman (6060) silica gel plate (20 cm × 10 cm × 0.1 mm) prepared according to Hammond and White,[15] and the plate developed in 80% aqueous acetone. Two spots were observed corresponding to α-tocoquinone and α-tocoquinone oxide. The bands were cut out and the compounds eluted with methanol. The absorbance at 270 nm was measured against a blank prepared by elution of an equal area of the silica gel plate with methanol. The ratio of α-tocoquinone (ϵ_{max} 16.2 × 10^3)[16] to tocoquinone oxide (ϵ_{max} 4.05 × 10^3) was 1:23.

Acknowledgments. We are grateful for the services of D. Weisleder, nmr spectra, W. K. Rohwedder, mass spectra, and C. E. McGrew, elemental analysis.

(13) Ir spectra were recorded on a Perkin-Elmer Model 700 spectrophotometer equipped with sodium chloride cells, uv spectra, on a Beckman Model DB spectrophotometer and a Cary 14 spectrophotometer, nmr spectra, on a Varian Model HA-100 spectrometer, and mass spectra, on a Nuclide Model 12-90 DF spectrometer. The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

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