C. Signor for the biochemical data, and Dr. B. Watkins, Dr. D. Chen, and D. Miller and their staffs for the in vivo biological data.

**Registry No.** 4 ($R^1$, $R^2$ = H; $R^3$ = *i*-Pr; $R^4$ = OH), 32806-63-6; 4 ($R^2$, $R^3$ = H; $R^1$ = *i*-Pr; $R^4$ = OEt), 82924-31-0; *meso*-4 ($R^1$, $R^3$ = $CH_3$; $R^2$ = H; $R^4$ = OEt), 3891-70-1; *dl*-4 ($R^1$, $R^3$ = $CH_3$; $R^2$, $R^4$ = H), 3891-69-8; (2*R*,4*R*)-4 ($R^1$, $R^3$ = $CH_3$; $R^2$ = H; $R^4$ = OH) (+)-α-methylbenzylamine salt, 86309-43-5; (2*R*,4*R*)-4 ($R^1$, $R^3$ = $CH_3$; $R^2$ = H; $R^4$ = OH), 24018-75-5; (2*R*,4*R*)-4 ($R^1$, $R^3$ = $CH_3$; $R^2$ = H; $R^4$ = OEt), 82924-08-1; **5a**·HCl, 82923-76-0; **5b**·HCl, 79854-42-5; **6a**, 82923-77-1; **6b**, 82923-90-8; **6c**, 82923-79-3; **6d**, 82924-01-4; **6e**, 82924-13-8; **6f**, 82924-06-9; **6g** (isomer 1), 86309-39-9; **6g** (isomer 2), 86309-42-4; **6h**, 82923-92-0; **6i**, 82924-33-2; **6j**, 82923-88-4; **6k**, 82923-87-3; **6l**, 82923-94-2; **6l** (base), 82923-93-1; **6m**, 82923-95-3; **6o**, 82950-75-2; **6p**, 82924-03-6; **6q**, 82950-74-1; **6r**, 82923-85-1; **6s**, 82924-29-6; **6t**, 82924-28-5; **6u**, 82950-76-3; **6v**, 82924-14-9; **6** ($R^1$ = *i*-Pr; $R^2$, $R^3$ = H; $R^4$, $R^5$ = Et) (isomer 1), 86309-40-2; **6** ($R^1$ = *i*-Pr; $R^2$, $R^3$ = H; $R^4$, $R^5$ = Et) (isomer 2), 86309-41-3; **7**, 82923-86-2; **11**, 82924-16-1; **15**, 72297-80-4; **16**, 86309-44-6; **17**, 82924-18-3; **18**, 82924-20-7; **19**, 82950-73-0; **21a**, 86390-74-1; **21a** (base), 86362-11-0; **21b**, 86390-75-2; **21b** (base), 86362-12-1; **21c**, 86390-76-3; **21c** (base), 86362-13-2; (2*R*,4*R*)-4-(ethoxycarbonyl)-2,4-dimethylbutyryl chloride, 82924-02-5; 4-phenylbutyryl chloride, 18496-54-3; ethyl (2*R*,4*R*)-5-hydroxy-4-methyl-2-(2-phenylethyl)pentanoate, 82924-19-4; (2*R*,4*R*)-4-(ethoxycarbonyl)-2-methyl-4-(2-phenylethyl)butyryl chloride, 82924-21-8; 3,3-dimethylglutaric anhydride, 4160-82-1; 2-isopropylglutaric anhydride, 57280-77-0; L-prolinol, 23356-96-9; angiotensin converting enzyme, 9015-82-1.

# Aromatic Retinoic Acid Analogues. 2. Synthesis and Pharmacological Activity

Marcia I. Dawson,*,† Rebecca Chan,† Peter D. Hobbs,† Wan-ru Chao,† and Leonard J. Schiff‡

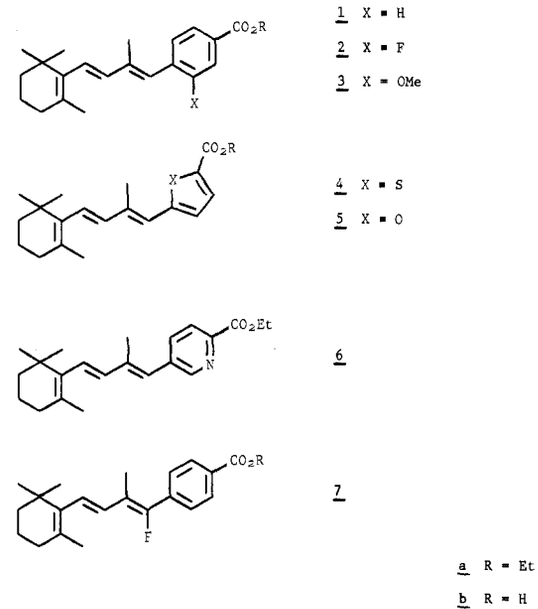
*Bio-Organic Chemistry Laboratory, SRI International, Menlo Park, California 94025, and Life Sciences Research, IIT Research Institute, Chicago, Illinois 60616.* *Received December 13, 1982*

Aromatic analogues of (*E*)-1-(4-carboxyphenyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (**1b**) and its ethyl ester (**1a**) were synthesized as potential chemopreventive agents for the treatment of epithelial cancer and such skin diseases as psoriasis and cystic acne. The phenyl ring of **1** was replaced by 2-fluorophenyl, 2-methoxyphenyl, thienyl, furanyl, and pyridyl groups. The 1-fluorobutadiene analogue of **1** was also synthesized. With exception for the furanyl analogue, these compounds demonstrated good activity in reversing keratinization in hamster tracheal organ culture and in inhibiting the induction of ornithine decarboxylase in mouse epidermis by a tumor promoter.

Retinoids have pharmaceutical importance because of their potential value as chemopreventive agents in the treatment of epithelial cancer, psoriasis, and cystic acne.[1] We recently reported the synthesis of aryl triene **1b** and its ethyl ester **1a**.[2] These compounds displayed significant activity in two bioassays: (1) the reversal of keratinization in hamster tracheal organ culture (TOC assay) and (2) the inhibition of the induction of ornithine decarboxylase in mouse dorsal epidermis by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (ODC assay). Positive activity in these assays has been shown to correlate with the ability of a retinoid to reverse the process of neoplastic transformation in vivo.[3,4] We now report the synthesis and biological testing results for aromatic ring modified analogues of **1**.

**Synthesis.** The proton at the 2-position of the phenyl ring was replaced by either a fluoro or a methoxy group to determine the effect of an electron-withdrawing or -donating group, respectively, on biological activity (compounds **2** and **3**). The phenyl ring was replaced by a thienyl, furanyl, or pyridyl ring to determine the effects of ring polarity and aromaticity on activity (compounds **4–6**). In addition, the proton at the $10_R$-position[5] of **1** (position 1 of the butadiene chain) was replaced by fluorine (compound **7**). This substitution was made because Pawson and co-workers reported that $10_R$-fluoro analogues of the 4-methoxy-2,3,6-trimethylphenyl retinoids have enhanced activity.[6] The structures of analogues **2** to **7** are shown in Chart I.

Chart I




Analogues **2** to **6** were readily prepared by the reaction sequences outlined in Scheme I. Because $9_R$ double-bond isomers in this type of series are unusually difficult to separate, the synthetic scheme was designed so that this bond was introduced stereospecifically by employing the

† SRI International.
‡ IIT Research Institute.

(1) (a) Sporn, M. B.; Dunlop, N. M.; Newton, D. L.; Smith, J. M. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1976**, *35*, 1332. (b) Lotan, R. *Biochim. Biophys. Acta* **1980**, *605*, 33. (c) Bollag, W. *Cancer Chemother. Rep.* **1971**, *55*, 53. (d) Bollag, W. *Cancer Chemother. Pharmacol.* **1979**, *3*, 207.
(2) Dawson, M. I.; Hobbs, P. D.; Chan, R. L.; Chao, W.-R.; Fung, V. A. *J. Med. Chem.* **1980**, *24*, 583.

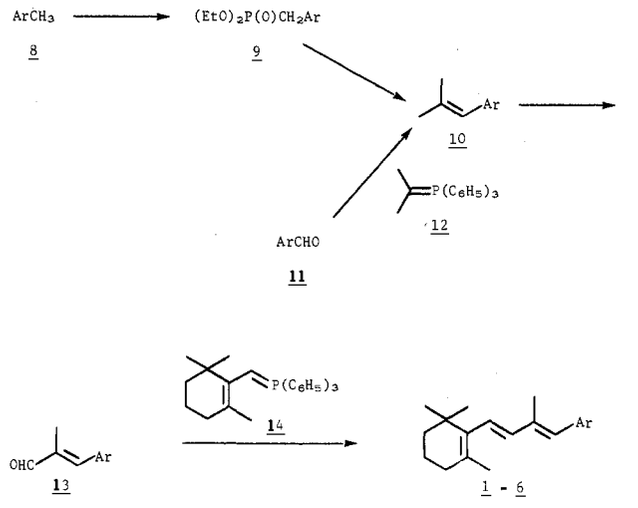
0022-2623/83/1826-1282$01.50/0 

Table I. $^1$H NMR Spectral Shifts of Retinoids 1 to 7[a]

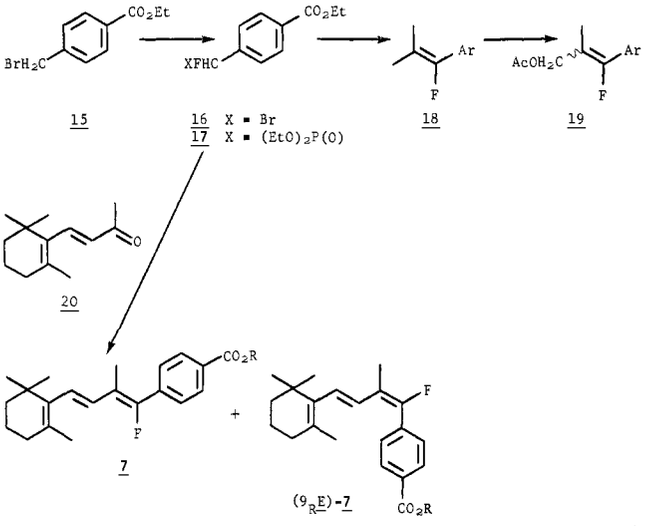
| compd | vinylic signals | | | methyl signals | | |
|---|---|---|---|---|---|---|
| | $7_R$ | $8_R$ | $10_R$ | $16_R$, $17_R$ | $18_R$ | $19_R$ |
| 1a | 6.29 (d, 16) | 6.20 (d, 16) | 6.47 (s) | 1.05 (s) | 1.74 (s) | 2.07 (d, 1) |
| ($9_R$*Z*)-1a | 6.43 (m) | 6.43 (m) | 6.43 (s) | 1.03 (s) | 1.70 (s) | 2.08 (d, 1.5) |
| 1b | 6.31 (d, 16) | 6.22 (d, 16) | 6.49 (s) | 1.06 (s) | 1.75 (s) | 2.09 (s) |
| ($9_R$*Z*)-**1b** | 6.39 (d, 16) | 6.47 (d, 16) | 6.45 (s) | 1.02 (s) | 1.72 (s) | 2.10 (s) |
| 2a | 6.32 (d, 16) | 6.24 (d, 16) | 6.46 (s) | 1.05 (s) | 1.75 (s) | 2.02 (s) |
| 2b | 6.34 (d, 16) | 6.26 (d, 16) | 6.47 (s) | 1.06 (s) | 1.75 (s) | 2.04 (s) |
| 3a | 6.13 (d, 16) | 6.40 (d, 16) | 6.57 (s) | 1.05 (s) | 1.75 (s) | 2.03 (s) |
| 3b | 6.15 (d, 16) | 6.42 (d, 16) | 6.58 (s) | 1.05 (s) | 1.76 (s) | 2.04 (s) |
| 4a | 6.34 (d, 16) | 6.18 (d, 16) | 6.57 (s) | 1.04 (s) | 1.73 (s) | 2.19 (s) |
| ($9_R$*Z*)-**4a** | 6.44 (d, 16) | 6.86 (d, 16) | 6.21 (s) | 1.06 (s) | 1.79 (s) | 2.08 (s) |
| 4b | 6.39 (d, 16) | 6.24 (d, 16) | 6.77 (s) | 1.03 (s) | 1.70 (s) | 2.13 (s) |
| 5a | 6.43 (d, 16) | 6.17 (d, 16) | 6.25 (s) | 1.05 (s) | 1.72 (s) | 2.22 (s) |
| 5b | 6.38 (d, 16) | 6.17 (d, 16) | 6.29 (s) | 1.04 (s) | 1.72 (s) | 2.22 (s) |
| 6 | 6.36 (d, 16) | 6.23 (d, 16) | 6.43 (s) | 1.05 (s) | 1.75 (s) | 2.09 (d, 1) |
| **7a** | 6.27 (d, 16) | 6.76 (dd, 16, 2) | | 1.06 (s) | 1.77 (d, 0.5) | 1.98 (d, 2.8) |
| ($9_R$*E*)-**7a** | 6.35 (d, 16) | 6.15 (dd, 16, 2) | | 1.00 (s) | 1.68 (s) | 2.04 (d, 3.5) |
| **7b** | 6.26 (d, 16) | 6.71 (dd, 16, 2) | | 1.06 (s) | 1.75 (s) | 2.02 (d, 2.8) |

[a] Shifts are reported relative to $Me_4Si$ ($\delta$ 0) in $CDCl_3$, with the exception of 7b ($CDCl_3/Me_2SO$-$d_6$). 300-MHz $^1$H NMR spectra were taken, except for ($9_R$*Z*)-1a and 5a when 100- and 60-MHz spectra, respectively, were run.

Scheme I




Scheme II




selective oxidation[7] of the less-hindered methyl group of 1,1-dimethyl olefinic intermediates (**10**). Accessibility of starting materials determined the route used for the preparation of **10**. In the case of the fluoro- and methoxy-substituted aryl trienes, the correspondingly substituted toluenes (**8**) were employed. Bromination of the benzylic methyl group of **8** with NBS and treatment with $(EtO)_3P$ afforded the desired diethyl benzylphosphonates (**9**). Horner–Emmons reaction of the anion of **9** with acetone gave **10**. This reaction was readily effected with LDA in THF in the case of the fluoro-substituted benzylphosphonate; however, NaH in DMF was required for the methoxy-substituted benzylphosphonate. For the heteroaromatic compounds, a Wittig reaction between the aromatic aldehydes **11** and isopropylidenetriphenylphosphorane (**12**) produced the 1,1-dimethyl olefins **10**. $SeO_2$ oxidation of the less-hindered methyl group of **10** readily afforded the (2*E*)-propenals (**13**) contaminated with 5–9% of their 2*Z* isomers, which were removed by chromatography. It is interesting to note that the heteroaromatic rings were stable to these oxidation conditions.

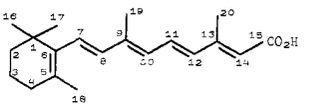
Wittig reaction of **13** with $\beta$-cyclogeranylidenetriphenylphosphorane (**14**) afforded the aryl triene esters (**2a** to **6a**) with the desired *all-E* bond geometry. Base hydrolysis (KOH, $H_2O$/MeOH) gave the target acids (**2b** to **5b**). The thienyl analogues were also synthesized by a nonstereospecific route involving reaction of 5-carbethoxythienyl-2-carboxaldehyde with $\beta$-ionylidenetriphenylphosphorane.

The double-bond geometry of these esters and acids was determined from their $^1$H and $^{13}$C NMR spectra, which were compared with reported spectra.[8,9] The $7_R$*E* dou-

(3) (a) Sporn, M. B.; Newton, D. L.; Smith, J. M.; Acton, N.; Jacobson, A. E.; Brossi, A. In "Carcinogens: Identification and Mechanism of Action", Annual Symposium on Fundamental Cancer Research, 31st, M. D. Anderson Hospital and Tumor Research Institute, Houston, 1978; Griffin, S. C.; Shaw, C. R., Eds.; Raven Press: New York, 1979; pp 445–453. (b) Newton, D. L.; Henderson, W. R.; Sporn, M. B. *Cancer Res.* **1980**, *40*, 3413.

(4) (a) Verma, A. K.; Shapas, B. G.; Rice, H. M.; Boutwell, R. K. *Cancer Res.* **1979**, *39*, 419. (b) Verma, A. K.; Rice, H. M.; Shapas, B. G.; Boutwell, R. K. *Ibid.* **1978**, *38*, 793. (c) Verma, A. K.; Boutwell, R. K. *Ibid.* **1977**, *37*, 2196.

(5) For structural comparisons standard retinoid numbering has been used:




Similar proton and carbon atoms in the aromatic analogues have been denoted by the subscript R.

(6) Pawson, B. A.; Chan, K.-K.; DeNoble, J.; Han, R. L.; Piermattie, V.; Specian, A. C.; Srisethnil, S.; Trown, P. W.; Bohoslawec, O.; Machlin, L. J.; Gabriel, E. *J. Med. Chem.* **1979**, *22*, 1059.

ble-bond configuration for this series of compounds was established from the 16-Hz coupling constants[7] for the $7_R$ and $8_R$ protons (Table I). The sharper of the two doublets was assigned to the $8_R$ proton. The $9_RE$ double-bond configuration was verified by the position of the signal for the $8_R$ proton. In $9_RZ$ isomers, this signal is shifted downfield.[2,8] In addition, the $^{13}C$ NMR chemical shifts for the $8_R$ and $19_R$ carbons were in agreement with those observed for 1 rather than for $(9_RZ)$-1.[2]

A stereoselective $SeO_2$ oxidation could not be achieved in the preparation of the $10_R$-fluoro retinoid **7** (Scheme II). No oxidation of the 1,1-dimethyl-2-fluoro olefin **18** occurred with $SeO_2$ in refluxing 95% ethanol or dioxane. Evidently, the fluoro group deactivated the allylic methyl group to oxidation. However, oxidation was accomplished in refluxing HOAc to give a 1:1 $E/Z$ mixture of allylic acetates **19** by $^1H$ NMR. Because this oxidation was nonstereospecific, a more direct nonstereoselective route was followed. The anion of fluorobenzylphosphonate **17**, which was prepared by displacement of 4-carbethoxybenzyl bromide (**15**) with KF, followed by bromination with NBS and treatment with $(EtO)_3P$, was allowed to undergo a Horner–Emmons reaction with $\beta$-ionone to give a 1:1 mixture of $9_RZ/9_RE$ double-bond isomers. The ratio of these isomers was not changed significantly on treatment with $I_2$ in ether/toluene for 22 h. However, these esters were separated more readily by preparative LC than were the corresponding $10_R$-H aryl trienes. The double-bond geometry of the esters was assigned from their $^1H$ NMR spectra. The 16-Hz coupling constant for the $7_R$ and $8_R$ protons was indicative of a $7_RE$ double bond.[7] The $J_{^1H-^{19}F}$ allylic-coupling constant of the $19_R$-methyl protons and the $10_R$-fluorine of **7a** (2.8 Hz) was smaller than that of its $9_RE$ isomer (3.5 Hz). These coupling assignments are consistent with those reported by Pawson and co-workers.[10] The downfield shift of the $8_R$ proton of **7a** ($\delta$ 6.76) relative to that for $(9_RE)$-**7a** ($\delta$ 6.15) confirmed the bond assignment. Such a shift has been observed in 10-fluororetinal isomers.[11]

**Pharmacological Activity.** Compounds were screened in both the TOC and ODC assays by using procedures developed by the groups of Sporn,[3b,12] and Verma and Boutwell,[13] respectively. The TOC assay measures the ability of retinoids to reverse the process of keratinization in tracheal organ cultures. The tracheas were obtained from month-old Syrian golden hamsters that were in the early stages of vitamin A deficiency. Retinoids were considered "active" if, after treatment, keratohyaline granules were absent. Experiments were usually conducted in duplicate with six or seven tracheas per concentration.

Studies on the mechanism of carcinogenesis suggest that the induction of ornithine decarboxylase is one of the essential events in carcinogenesis.[4] The ODC assay measures the ability of topically applied retinoids to inhibit the induction of the enzyme by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate applied to the dorsal skin of 7–9 week old CD-1 female mice. Experiments were performed in triplicate with three groups of three mice for each dose.

The results of these assays are presented in Table II. With the exception of the furanyl analogues, the aromatic ring modifications of **1a** did not greatly affect activity in the TOC assay. The aryl region could be modified considerably without eliminating activity. For example, both the fluoro- and methoxy-substituted aryl trienes had similar activity profiles in the TOC assay and were more active than **1a**. Activity was also enhanced with the thienyl analogues **4a** and **4b** and the aryl fluorotrienes **7a** and **7b**. The pyridyl analogue **6** had reduced activity. The furanyl analogues were inactive in both assays. These analogues may be hydrolytically unstable under the assay conditions. With the exception of **4a**, **7a**, and **7b**, the analogues showed reduced activity in the ODC assay. The reduced activity may be the result of differences between the two bioassay systems. The $9_RZ$ isomer of **4a** also displayed moderate activity in both assays. The amount of activity was unusual because most 9*Z* isomers of retinoids having a tetraene chain are inactive.[14] The aryl trienes of this type may undergo bond isomerization more readily in these assays than do their polyolefinic counterparts. In contrast, the $9_RE$ isomer of **7a** was essentially inactive in both bioassays.

In summary, these results indicate that modification can be made in the region of the aryl ring of **1a** without eliminating activity in two retinoid bioassays.

## Experimental Section

Melting points are uncorrected. IR spectra were recorded with a Perkin-Elmer 710B infrared spectrophotometer. NMR spectra were obtained with a Varian A-60A, EM360A, or XL-100-F spectrometer or with a 300-MHz Nicolet spectrometer, with $(CH_3)_4Si$ as an internal standard ($\delta$ 0) and solvent as specified. High-resolution mass spectral analyses were conducted on a CEC-21-110B high-resolution mass spectrometer equipped with facilities for combination GC-MS. LC analyses were done on a Waters Associates ALC 210 equipped with either a $\mu$Bondapak $C_{18}$ column or a Radialpak A or B cartridge. Detection was by a Schoeffel Instrument Model 770 variable-wavelength UV monitor. Analyses were performed at ambient temperature at a flow rate of 2 mL/min. Preparative work was done on a Waters Associates Prep LC/System 500 instrument, with Prep Pak 500/silica cartridges at a flow rate of 0.2 L/min. Detection was by UV absorption or refractive index. UV spectra were taken on a Perkin-Elmer 552 spectrophotometer. Unless otherwise mentioned, compounds were determined to be homogeneous by TLC, analytical LC, and GC–MS analyses before submission for high-resolution mass spectral analysis.

Where required, reactions and purifications were conducted with deoxygenated solvents and under inert gas (argon) and either subdued light or photographic red light. Retinoid intermediates were stored at −40 °C. Solvents were dried or distilled before use. TLC analyses were performed on Analtech silica gel analytical plates. Merck silica gel 60 was used for chromatography. Spectral signal designations were based on the retinoid numbering system.[5] $^1H$ NMR[8] and $^{13}C$ NMR[9] signals were assigned by comparison with those reported for other retinoids. The stereochemical assignments were supported by the larger $\epsilon$ values for the *all-E* isomers.[15]

(7) Miller, C. H.; Katzenellenbogen, J. A.; Bowlus, S. B. *Tetrahedron Lett.* **1973**, 285.
(8) (a) Vetter, W.; Englert, G.; Rigassi, N.; Schwieter, U. In "Carotenoids"; Isler, O.; Gutmann, H.; Solms, U., Eds.; Birkhaeuser-Verlag: Basel, 1971; pp 216–225. (b) Mousseron-Canet, M.; Mani, J.-C. *Bull. Soc. Chim. Fr.* **1966**, 3285. (c) Mousseron-Canet, M.; Mani, J.-C. *Ibid.* **1966**, 3291. (d) Patel, D. J. *Nature (London)* **1969**, *211*, 825.
(9) Englert, G. *Helv. Chim. Acta* **1975**, *58*, 2367.
(10) Lovey, A. J.; Pawson, B. A. *J. Med. Chem.* **1982**, *25*, 71.
(11) Asato, A. E.; Matsumoto, H.; Denny, M.; Liu, R. S. H. *J. Am. Chem. Soc.* **1978**, *100*, 5957.
(12) (a) Clamon, G. H.; Sporn, M. B.; Smith, J. M.; Saffiotti, U. *Nature (London)* **1974**, *250*, 64. (b) Sporn, M. B.; Clamon, G. H.; Dunlop, N. M.; Newton, D. L.; Smith, J. M.; Saffiotti, U. *Ibid.* **1975**, *253*, 47. (c) Sporn, M. B.; Dunlop, N. M.; Newton, D. L., Henderson, W. R. *Ibid.* **1976**, *263*, 110.
(13) (a) Raineri, R.; Simsiman, R. C.; Boutwell, R. K. *Cancer Res.* **1973**, *33*, 134. (b) Verma, A. K.; Lowe, N.; Boutwell, R. K. *Ibid.* **1979**, *39*, 1035.
(14) Newton, D. L.; Henderson, W. R.; Sporn, M. B. "Structure Activity Relationships of Retinoids"; Laboratory of Chemoprevention, Division of Cancer Cause and Prevention, National Cancer Institute: Bethesda, MD, 1978.

Table II. Activity of Retinoids in the TOC and ODC Assays

| retinoid | TOC concn, M | TOC active/total cultures, % | TOC rel act.[a] | ODC dose, nmol | ODC % inhibn of control |
|---|---|---|---|---|---|
| 1a | $10^{-8}$ | 6/6 (100) | 1 | 17.0 | 80 |
| | $10^{-9}$ | 13/13 (100) | | | |
| | $10^{-10}$ | 4/13 (31) | | | |
| | $10^{-11}$ | 0/7 (0) | | | |
| 1b | | | 2[b] | 170.0 | 87[c] |
| | | | | 17.0 | 77 |
| | | | | 1.7 | 62 |
| ($9_R$ *Z*)-1b | | | <0.1[b] | 170.0 | 64[c] |
| | | | | 17.0 | 43 |
| | | | | 1.7 | 33 |
| 2a | $10^{-8}$ | 6/6 (100) | 5 | 17.0 | 46 |
| | $10^{-9}$ | 11/13 (85) | | 1.7 | 8 |
| | $10^{-10}$ | 9/14 (64) | | | |
| | $10^{-11}$ | 1/6 (17) | | | |
| 2b | $10^{-8}$ | 6/6 (100) | 5 | 17.0 | 78 |
| | $10^{-9}$ | 12/12 (100) | | 1.7 | 37 |
| | $10^{-10}$ | 3/11 (27) | | | |
| | $10^{-11}$ | 0/6 (0) | | | |
| 3a | $10^{-8}$ | 7/7 (100) | 5 | 17.0 | 39 |
| | $10^{-9}$ | 10/12 (83) | | 1.7 | 21 |
| | $10^{-10}$ | 8/13 (62) | | | |
| | $10^{-11}$ | 2/6 (33) | | | |
| 3b | $10^{-9}$ | 5/6 (83) | 3 | 17.0 | 37 |
| | $10^{-10}$ | 3/6 (50) | | 1.7 | 9 |
| | $10^{-11}$ | 1/6 (17) | | | |
| 4a | $10^{-8}$ | 13/13 (100) | 3 | 17.0 | 77 |
| | $10^{-9}$ | 10/14 (71) | | 1.7 | 67 |
| | $10^{-10}$ | 6/14 (43) | | | |
| ($9_R$ *Z*)-4a | $10^{-8}$ | 11/13 (85) | 0.3 | 17.0 | 76 |
| | $10^{-9}$ | 6/14 (43) | | 1.7 | 35 |
| | $10^{-10}$ | 5/14 (36) | | | |
| 4b | $10^{-8}$ | 15/15 (100) | 2 | 17.0 | 67 |
| | $10^{-9}$ | 14/15 (93) | | 1.7 | 42 |
| | $10^{-10}$ | 7/15 (47) | | | |
| 5a | $10^{-8}$ | 4/14 (29) | <<0.1 | 17.0 | 9 |
| | $10^{-9}$ | 3/12 (25) | | 1.7 | 9 |
| | $10^{-10}$ | 1/6 (17) | | | |
| 5b | $10^{-8}$ | 5/11 (45) | <<0.1 | 17.0 | 5 |
| | $10^{-9}$ | 5/13 (38) | | 1.7 | 7 |
| | $10^{-10}$ | 4/13 (31) | | | |
| 6 | $10^{-8}$ | 7/7 (100) | 0.4 | 17.0 | 68 |
| | $10^{-9}$ | 8/14 (57) | | 1.7 | 29 |
| | $10^{-10}$ | 4/14 (29) | | | |
| 7a | $10^{-8}$ | 11/12 (92) | 2 | 17.0 | 82 |
| | $10^{-9}$ | 10/12 (83) | | 1.7 | 56 |
| | $10^{-10}$ | 6/13 (46) | | | |
| ($9_R$ *E*)-7a | $10^{-8}$ | 2/12 (17) | <<0.1 | 17.0 | 16 |
| | $10^{-9}$ | 1/13 (8) | | 1.7 | 5 |
| | $10^{-10}$ | 0/12 | | | |
| 7b | $10^{-8}$ | 13/13 (100) | 3 | 17.0 | 80 |
| | $10^{-9}$ | 12/13 (92) | | 1.7 | 70 |
| | $10^{-10}$ | 5/13 (38) | | | |

[a] Relative activity is defined as the ratio of $ED_{50}$ (analogue)/$ED_{50}$(retinoic acid) divided by the ratio of $ED_{50}$ (1a)/$ED_{50}$ (retinoic acid), where $ED_{50}$ is the molarity of retinoid required to effect reversal of keratinization in 50% of the cultures. The $ED_{50}$ values for the retinoic acid controls were $6 \times 10^{-12}$ M (1a, 2a, 2b, and 3a), $8 \times 10^{-12}$ M (**6**), $3 \times 10^{-11}$ M [1b and ($9_R$ *Z*)-1b], $1 \times 10^{-11}$ M (3b), and $2 \times 10^{-11}$ M (other analogues). [b] Reference 3b. [c] Reference 2.

**Preparation of (*E*)-1-(4-Carboxy-2-fluorophenyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (2b) and Its Ethyl Ester (2a).** **Ethyl 4-(Bromomethyl)-3-fluorobenzoate.** A solution of 10.2 g (66.2 mmol) of 3-fluoro-4-methylbenzoic acid in 50 mL of EtOH and 25 mL of toluene containing 0.2 mL of $H_2SO_4$ was heated gradually to 120 °C (oil bath temperature). The solvent was removed by distillation, through a 10-cm Vigreux column, over a period of 2.25 h. The residue was cooled and treated with a further 50 mL of EtOH and 25 mL of toluene, and the distillation was repeated. The residual solution, containing some white solid, was poured into 75 mL of aqueous $NaHCO_3$ and extracted with hexane (75 mL, then 50 mL). The extract was washed with water (2 × 75 mL), dried ($Na_2SO_4$), and concentrated. The pale-yellow liquid was distilled, bp 49–58 °C (0.01–0.05 mm), to give 11.1 g (92% yield) of ethyl 3-fluoro-4-methylbenzoate as a colorless liquid: IR ($CHCl_3$) 1720 (C=O), 1580, 1420, 1370, 1290, 1190, 1130, 1090, 1020, 940, 895 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.40 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 2.32 (d, $J$ = 2 Hz, 3, $ArCH_3$), 4.37 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 7.22 (dd, $J$ = 8 and 8 Hz, 1, 6′-H), 7.68 (m, 2, 3′- and 5′-H); MS calcd for $C_{10}H_{11}FO_2$, 182.0743; found, 182.0729.

To a solution of 10.95 g (60.1 mmol) of ethyl 3-fluoro-4-methylbenzoate in 60 mL of $CCl_4$ was added, over a period of 30 min, a mixture of 12.8 g (72 mmol) of recrystallized (water) NBS and 125 mg (0.52 mmol) of dibenzoyl peroxide (5-mL $CCl_4$ rinse). The suspension was heated at reflux with stirring for 14 h, cooled, and filtered. The precipitate of succinimide was washed with 200 mL of hexane. The combined filtrates were filtered again, and

(15) (a) Sebrell, W. H.; Harris, R. S. "The Vitamins"; Academic Press: New York, 1967; Vol. 1. (b) VonPlanta, C.; Schwieter, U.; Chopard-dit-Jean, L.; Rüegg, R.; Kofler, M.; Isler, O. *Helv. Chim. Acta* **1962**, *45*, 548.

the filtrate was concentrated to give a light-orange liquid. Distillation through a 10-cm Vigreux column yielded, successively, 0.50 g (4.5% recovery) of unreacted ethyl ester, bp 55–60 °C (0.01 mm), 8.13 g (52% yield) of the 4-(bromomethyl)benzoate as a colorless liquid, bp 94–104 °C (0.01 mm), and 5.75 g of a mixture of the product and ethyl 4-(dibromomethyl)benzoate, bp 104–115 °C (0.01 mm). The latter fraction was redistilled to give 3.41 g (22% yield) of additional ethyl 4-(bromomethyl)-3-fluorobenzoate, bp 101–108 °C (0.05 mm). The total yield was 11.54 g (74%) of white crystals, mp 42.5–44 °C (pentane): IR ($CHCl_3$) 1720 (C=O), 1585, 1425, 1375, 1290, 1105, 1090, 1020, 950, 900 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.40 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 4.40 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 4.52 (s, 2, $CH_2Br$), 7.60 (m, 3, 3′-, 5′-, and 6′-H); MS calcd for $C_{10}H_{10}{}^{79}BrFO_2$, 259.9849; found, 259.9860.

**Diethyl (4-Carbethoxy-2-fluorobenzyl)phosphonate [9(2)].** To 9.0 g (54.2 mmol) of degassed (argon) $(EtO)_3P$ heated under a stream of argon in a 150 °C oil bath was added, over a 25-min period, 9.4 g (36.0 mmol) of ethyl 4-(bromomethyl)-3-fluorobenzoate. A pale yellow solution resulted. The heating bath temperature was raised to 200–205 °C over a period of 25 min and was maintained there for 55 min. The bright yellow liquid was then cooled and chromatographed on a silica gel column (4 × 40 cm) with 1.5-L portions of 25 and 75% EtOAc/hexane and EtOAc to give 9.8 g of the crude phosphonate **9(2)** as a yellow viscous oil. Evaporative distillation at 130–140 °C (0.05–0.1 mm) produced 9.34 g (82% yield) of phosphonate **9(2)** as a colorless, viscous oil: IR ($CHCl_3$) 1710 (C=O), 1580, 1420, 1390, 1365, 1280 (1240 sh), 1090, 1020 (1040 sh), 960, 895, 850 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.28 [t, $J$ = 7 Hz, 6, $P(OCH_2CH_3)_2$], 1.40 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 3.30 (d, $J$ = 22 Hz, 2, $CH_2PO$), 4.03 [q, $J$ = 7 Hz, 4, $P(OCH_2CH_3)_2$], 4.40 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 7.65 (m, 3, 3′-, 5′-, and 6′-H); MS calcd for $C_{14}H_{20}FO_5P$, 318.1033; found, 318.1058.

**1-(4-Carbethoxy-2-fluorophenyl)-2-methylpropene [10(2)].** To 2 mL (14 mmol) of $(i\text{-}Pr)_2NH$ in 5 mL of $Et_2O$, which was kept at ice-bath temperature under argon, was added 8.0 mL of a 1.39 M solution of $n$-BuLi (11.1 mmol) in hexane. This solution of LDA was stirred for 45 min, cooled in a −20 °C bath, and treated with a solution of 3.61 g (11.3 mmol) of phosphonate **9(2)** in 6 mL of THF. The reaction mixture was stirred for 30 min to give a red gum and solution. Next, a solution of 0.85 mL (11.6 mmol) of acetone in 3 mL of THF was added, and the cooling bath was removed. Then the reaction mixture was shaken to give a red-brown solution, stirred at room temperature for 65 min, quenched with 20 mL of water containing 1 mL (17.5 mmol) of HOAc, diluted with 50 mL of brine, and extracted with $Et_2O$ (2 × 25 mL). The extract was washed with water (2 × 20 mL), dried ($MgSO_4$), and concentrated. Chromatography on a 3 × 35 cm silica gel column with 500 mL of 3%, 300 mL of 10%, and 800 mL of 75% EtOAc/hexane gave 1.405 g (56% yield) of olefin **10(2)** as a colorless liquid: IR ($CHCl_3$) 1720 (C=O), 1665 (C=C), 1625, 1575, 1505, 1450, 1420, 1375, 1290, 1195, 1115, 1090, 1020, 945, 895, 860 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.39 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 1.83 and 1.97 [2 s, 6, $C{=}C(CH_3)_2$], 4.40 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 6.29 (s, 1, C=CH), 7.33 (dd, $J$ = 8 and 8 Hz, 1, 6′-H), 7.77 (m, 2, 3′- and 5′-H); UV (EtOH) $\lambda_{max}$ 270 nm ($\epsilon$ 1.61 × $10^4$); MS calcd for $C_{13}H_{15}FO_2$, 222.1056; found, 222.1062. In addition, 0.73 g (20%) of unreacted phosphonate **9(2)** was recovered.

**(*E*)-3-(4-Carbethoxy-2-fluorophenyl)-2-methylpropenal [13(2)].** A suspension of 1.28 g (11.54 mmol) of $SeO_2$ in 65 mL of wet dioxane containing 1.72 g (7.7 mmol) of olefin **10(2)** was degassed three times under argon, heated at reflux for 3.25 h, and cooled. The precipitate of Se was removed by filtration and washed with 20 mL of dioxane. The filtrate and wash were concentrated. The residue was chromatographed on a 3 × 30 cm silica gel column with 800-mL volumes of 8 and 10% EtOAc/hexane. Fractions containing mixtures of the 2*E*- and 2*Z*-aldehydes were rechromatographed on a 2 × 25 cm silica gel column with 8% EtOAc/hexane to yield (a) 16 mg (1% yield) of the 2*Z*-aldehyde [IR ($CHCl_3$) 1710 (C=O), 1680 (C=O), 1610, 1570, 1410, 1370, 1345, 1280 (1240 sh), 1180, 1110 (1120 sh), 1015, 890, 855 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.45 (t, $J$ = 7 Hz, 3 $CH_2CH_3$), 2.02 (d, $J$ = 1.5 Hz, 3, $C{=}CCH_3$), 4.40 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 7.57 (m, 3, 3′-, 5′-, and 6′-H), 9.85 (d, $J$ = 2 Hz, 1, CHO)], (b) 44 mg (2% yield) of a mixture of the 2*E*- and 2*Z*-aldehydes, and (c) 1.16 g (63% yield) of the 2*E*-aldehyde **13(2)** as white crystals, mp 46.5–47 °C (EtOAc/hexane): IR ($CHCl_3$) 1680 (C=O) (1710 sh, C=O), 1630, 1610, 1570, 1400, 1370, 1285 (1240 sh), 1180, 1105, 1010, 890, 860 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.43 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 2.05 (s, 3, $C{=}CCH_3$), 4.43 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 7.48 (m, 1, 6′-H), 7.70 (dd, $J$ = 7 and 1 Hz, 1, 3′-H), 7.90 (s, 1, C=CH), 7.97 (dd, $J$ = 7 and 2 Hz, 1, 5′-H), 9.72 (s, 1, CHO); UV (MeCN) $\lambda_{max}$ 278 nm ($\epsilon$ 1.94 × $10^4$); MS calcd for $C_{13}H_{13}FO_3$, 236.0849; found, 236.0835.

**(*E*)-1-(4-Carbethoxy-2-fluorophenyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (2a).** To a suspension of 3.6 g (7.5 mmol) of β-cyclogeranyltriphenylphosphonium bromide in 15 mL of THF at −25 °C was added 5.0 mL (6.95 mmol) of a 1.39 M solution of $n$-BuLi in hexane. The reaction was allowed to warm to 0 °C over a 45-min period. Then, 1.11 g (4.7 mmol) of aldehyde **13(2)** in 6 mL of THF was added. The mixture was allowed to warm to room temperature over 1.5 h before the dark-orange suspension was quenched with 10 mL of 10% aqueous HOAc and diluted with 50 mL of water. The product was extracted into 30 mL of 10% EtOAc/hexane, washed with water (2 × 30 mL), dried ($Na_2SO_4$), and concentrated. The crude product was eluted through a 3 × 30 cm silica gel column with 3% EtOAc/hexane to give 1.68 g of pale-yellow liquid, which was purified twice by preparative LC (2% $Et_2O$/hexane) using the recycle technique to give 1.13 g (68% yield) of ester **2a** as a pale-yellow gum: LC (Radialpak B, 1% $Et_2O$/hexane, 2.0 mL/min, 280 nm) $t_R$ 4.1 min (100%); LC (Radialpak A, 5% $H_2O$/MeCN, 2.0 mL/min, 280 nm) $t_R$ 11.9 (sh, 1.2%), 12.5 (97.6%), 13.3 min (sh, 1.2%); IR ($CHCl_3$) 1705 (C=O), 1605, 1555, 1435, 1410, 1360, 1285, 1180, 1110, 1080, 1010, 960, 935, 885, 855 $cm^{-1}$; 300-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.05 (s, 6, $16_R$- and $17_R$-$CH_3$), 1.40 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 1.48 and 1.63 (2 m, 4, $2_R$- and $3_R$-$CH_2$), 1.75 (s, 3, $18_R$-$CH_3$), 2.02 (s, 3, $19_R$-$CH_3$), 2.03 (m, 2, $4_R$-$CH_2$), 4.38 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 6.24 (d, $J$ = 16 Hz, 1, $8_R$-HC=C*H*), 6.32 (d, $J$ = 16 Hz, 1, $7_R$-*H*C=CH), 6.46 (s, 1, $10_R$-C=CH), 7.40 (dd, $J$ = 8 and 8 Hz, 6′-H), 7.71 (dd, $J$ = 10.5 and 1.5 Hz, 1, 3′-H), 7.80 (dd, $J$ = 8 and 1.5 Hz, 1, 5′-H); $^{13}C$ NMR ($CDCl_3$), 14.2 and 14.3 ($19_R$, $CH_2CH_3$), 19.2 ($3_R$), 21.7 ($18_R$), 28.9 ($16_R$, $17_R$), 33.0 ($4_R$), 34.2 ($1_R$), 39.5 ($2_R$), 61.2 ($CH_2CH_3$), 116.3 (d, $J$ = 25 Hz, 3′), 121.1 (5′), 124.7 (6′), 128.8 ($10_R$), 129.7 ($5_R$), 130.1 (4′), 130.5 ($7_R$, 1′), 137.4 ($6_R$, $8_R$), 140.0 ($9_R$), 159.8 (d, $J$ = 248 Hz, 2′), 165.5 (C=O) ppm; UV (EtOH) $\lambda_{max}$ 318 nm ($\epsilon$ 2.31 × $10^4$); MS calcd for $C_{23}H_{29}FO_2$, 356.2151; found, 356.2163.

**(*E*)-1-(4-Carboxy-2-fluorophenyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (2b).** To a degassed (argon) solution of 0.2 g (3.0 mmol) of 85% KOH in 1.5 mL of EtOH and 0.5 mL of water was added a solution of 0.293 g (0.82 mmol) of ester **2a** in 1 mL of EtOH. The mixture was heated to 80 °C (bath temperature) over a 15-min period. After heating at 80 °C for 15 min more, the pale yellow solution was cooled to room temperature. The reaction mixture was quenched with 4 mL of 25% aqueous HOAc, diluted with 10 mL of water, and extracted with 10 mL of $Et_2O$. The $Et_2O$ solution was washed with water (2 × 5 mL), dried ($Na_2SO_4$), and concentrated. The yellow solid was recrystallized from 2 mL of MeOH under argon to give 0.211 g (78% yield) of yellow crystals of carboxylic acid **2b**: mp 141–142 °C; LC (Radialpak A, 40% $H_2O$/MeCN, 2.0 mL/min, 280 nm) $t_R$ 2.1 min (100%); IR ($CHCl_3$) 3300–2300 (OH), 1690 (C=O), 1610, 1560, 1420, 1280, 1210, 1115, 1080, 965, 935, 895 $cm^{-1}$; 300-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.06 (s, 6, $16_R$- and $17_R$-$CH_3$), 1.49 and 1.64 (2 m, 4, $2_R$- and $3_R$-$CH_2$), 1.75 (s, 3, $18_R$-$CH_3$), 2.04 (s, 3, $19_R$-$CH_3$), 2.04 (m, 2, $4_R$-$CH_2$), 6.26 (d, $J$ = 16 Hz, 1, $8_R$-HC=C*H*), 6.34 (d, $J$ = 16 Hz, 1, $7_R$-*H*C=CH), 6.47 (s, 1, $10_R$-C=CH), 7.44 (dd, $J$ = 8 and 8 Hz, 1, 6′-H), 7.78 (dd, $J$ = 10.5 and 1 Hz, 1, 3′-H), 7.88 (dd, $J$ = 8 and 1 Hz, 1, 5′-H); $^{13}C$ NMR ($CDCl_3$) 14.3 ($19_R$), 19.2 ($3_R$), 21.7 ($18_R$), 28.9 ($16_R$, $17_R$), 33.0 ($4_R$), 34.3 ($1_R$), 39.5 ($2_R$), 117.0 (d, $J$ = 24 Hz, 3′), 121.0 (5′), 125.4 (6′), 128.8 (d, $J$ = 8 Hz, 4′), 129.1 ($10_R$), 129.9 ($5_R$), 130.7 ($7_R$), 131.9 (d, $J$ = 14 Hz, 1′), 137.3 and 137.4 ($6_R$, $8_R$), 140.5 ($9_R$), 159.9 (d, $J$ = 248 Hz, 2′), 171.3 (C=O) ppm; UV (EtOH) $\lambda_{max}$ 313 nm ($\epsilon$ 2.30 × $10^4$); MS calcd for $C_{21}H_{25}FO_2$, 328.1838; found, 328.1818.

**Preparation of (*E*)-1-(4-Carboxy-2-methoxyphenyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (3b) and Its Ethyl Ester (3a). Methyl 3-Methoxy-4-methylbenzoate.** A mixture of 30 g (0.20 mol) of 3-hydroxy-4-methylbenzoic acid, 68.1 g (0.49 mol) of $K_2CO_3$, and 123 mL (1.98

mol) of MeI in 250 mL of DMF was heated at reflux for 19 h. After cooling to room temperature, the reaction mixture was diluted with 500 mL of water and extracted with $Et_2O$ (2 × 300 mL). The organic phase was washed with water and brine, dried ($MgSO_4$), and concentrated to 37 g of orange oil: TLC (5% $Et_2O$/hexane) $R_f$ 0.13 (acid), 0.63 (ester), 0.72. The oil was chromatographed on 450 g of silica gel with 5% $Et_2O$/hexane (100-mL fractions) to give 19.5 g of ester and 6.6 g of ester contaminated with the higher $R_f$ material. The impure fraction was chromatographed on 250 g of silica gel to afford an additional 5.6 g of ester. The total yield of white crystalline solid, mp 50–51 °C, was 25.1 g (71%). A sample from an earlier experiment was characterized: IR (film) 1700, 1400, 1290, 1270, 1100 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 2.25 (s, 3, $ArCH_3$), 3.85 and 3.88 (2 s, 6, $CO_2CH_3$, $ArOCH_3$), 7.2–7.9 (m, 3, ArH); MS calcd for $C_{10}H_{12}O_3$, 180.0786; found, 180.0779.

**Ethyl 4-(Bromomethyl)-3-methoxybenzoate.** A 19.0 g (0.11 mol) portion of methyl 3-methoxy-4-methylbenzoate was dissolved in 100 mL of EtOH, and 2 mL of concentrated $H_2SO_4$ was added. This solution was heated at reflux for 4 days, at which time GC analysis (0.125 in. × 6 ft 3% OV-1 column, 100 to 250 °C, 16 °C/min) of an aliquot, which had been diluted with $Et_2O$ and washed with water, indicated two peaks at 3.25 and 3.75 min, corresponding to the methyl and ethyl esters, respectively. Another 2-mL portion of acid was added and heating was continued for 4 more days, when GC analysis indicated the disappearance of starting material. The cooled reaction mixture was diluted with $Et_2O$ and washed with water and brine, dried ($Na_2SO_4$), and concentrated to afford 19.7 g (96% crude yield) of the ethyl ester as a white solid: IR ($CHCl_3$) 1710, 1470, 1410, 1290, 1270, 1110, 1040 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.38 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 2.32 (s, 3, $ArCH_3$), 3.87 (s, 3, $OCH_3$), 4.40 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 7.1–7.8 (m, 3, ArH).

A mixture of 17.9 g (0.09 mol) of crude ethyl 3-methoxy-4-methylbenzoate, 19.7 g (0.11 mol) of NBS (recrystallized from water), and 4 grains of benzoyl peroxide in 250 mL of $CCl_4$ was heated at reflux for 1 h, at which time TLC (7% $Et_2O$/hexane) indicated two spots with $R_f$ values of 0.41 (product) and 0.52 (starting material). More benzoyl peroxide (4 grains) was added to the reaction mixture, and heating was continued for 2 h more, when TLC indicated only a trace of starting material. The reaction mixture was cooled to room temperature and filtered ($CCl_4$ rinse). Concentration of the filtrate afforded 27.9 g of an oil, which was chromatographed on 800 g of silica gel with 10% $Et_2O$/hexane (500-mL fractions). Fractions 9 to 15 contained 24.6 g (98% yield) of a white solid: mp 57–63 °C; IR ($CHCl_3$) 1710, 1410, 1290, 1100 $cm^{-1}$; 60-MHz $^1H$ NMR $\delta$ 1.37 (t, $J$ = 6 Hz, 3, $CH_2CH_3$), 3.97 (s, 3, $OCH_3$), 4.40 (q, $J$ = 6 Hz, 2, $CH_2CH_3$), 4.85 (s, 2, $CH_2Br$), 7.3–8.1 (m, 3, ArH); MS calcd for (M − 1) $C_{11}H_{12}O_3{}^{79}Br$, 270.9970; found, 270.9962.

**Diethyl (4-Carbethoxy-2-methoxybenzyl)phosphonate [9(3)].** A mixture of 48.2 g (0.176 mol) of the benzyl bromide, prepared as described above, and 46.0 mL (0.27 mol) of $(EtO)_3P$ was heated under a stream of argon in an oil bath at 50–55 °C for 20 min. The bath temperature was then raised to 200 °C for 2 h while the EtBr was removed by distillation. The bath temperature was next raised to 250 °C to remove unreacted $(EtO)_3P$ at 14 mm. The dark brown residue was chromatographed on 450 g of silica gel with 15.5 L of 50% EtOAc/hexane, 7 L of 75% EtOAc/hexane, and 100% EtOAc. Fractions 9 to 38 (500 mL) contained, after concentration, 36.0 g of crude product, which was rechromatographed on 1400 g of silica gel with 21 L of 75% EtOAc/hexane, followed by EtOAc. Fractions 15 to 44 contained, after concentration, 32.3 g of a viscous yellow oil: TLC (50% EtOAc/hexane) $R_f$ 0.25. Evaporative distillation at 144–150 °C (0.20–0.25 mm) afforded 30.6 g (52% yield) of benzylphosphonate **9(3)** as a colorless, viscous oil: IR (film) 1720, 1415, 1280, 1230, 1100, 1040, 960 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.24 [t, $J$ = 7 Hz, 6, $P(OCH_2CH_3)_2$], 1.35 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 3.26 (d, $J$ = 22 Hz, 2, $PCH_2Ar$), 3.90 (s, 3, $OCH_3$), 4.12 [q, $J$ = 7 Hz, 4, $P(OCH_2CH_3)_2$], 4.40 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 7.33 (d, $J$ = 8 Hz, 1, 6′-H), 7.58 (br s, 1, 3′-H), 7.62 (d, $J$ = 8 Hz, 1, 5′-H); MS calcd for $C_{15}H_{23}O_6P$, 330.1234; found, 330.1212.

**1-(4-Carbethoxy-2-methoxyphenyl)-2-methylpropene [10(3)].** A 3.42 g (83.8 mmol) portion of 59% NaH–mineral oil dispersion was washed with pentane (2 × 10 mL). To the NaH remaining was added, with stirring, 85 mL of DMF, followed by 27.8 g (84.2 mmol) of benzyl phosphonate **9(3)** in 10 mL of DMF (5-mL DMF rinse). The reaction mixture turned deep yellow. After 3.5 h of stirring, when hydrogen evolution ceased, 32.0 mL (436 mmol) of acetone was added, with cooling in a cold-water bath to maintain the internal temperature at 20–30 °C. The orange reaction was then stirred at room temperature for 13 h before dilution with 600 mL of $Et_2O$ and 400 mL of $H_2O$ containing 4 mL of HOAc. The aqueous layer was extracted with 400 mL of $Et_2O$. The combined $Et_2O$ extracts were washed with water (3 × 100 mL) and brine (2 × 100 mL), dried ($MgSO_4$), and concentrated to 19.8 g of a yellow oil. This material was chromatographed on 500 g of silica gel with 10% $Et_2O$/hexane to afford in fractions 15 to 23 (125-mL volume) 14.4 g (73% yield) of a colorless oil (TLC $R_f$ 0.27), which solidified to off-white prisms: mp 42 °C; IR (film) 1720, 1470, 1420, 1300, 1280, 1260, 1110, 1040 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.40 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 1.82 (d, $J$ = 0.5 Hz, 3, C=$CCH_3$), 1.98 (d, $J$ = 0.5 Hz, 3, C=$CCH_3$), 3.90 (s, 3, $OCH_3$), 4.42 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 6.38 (br s, 1, C=CH), 7.26 (d, $J$ = 8 Hz, 1, 5′-H), 7.60 (d, $J$ = 2 Hz, 1, 2′-H), 7.70 (dd, $J$ = 8 and 2 Hz, 1, 6′-H); MS calcd for $C_{14}H_{18}O_3$, 234.1256; found, 234.1259. Further elution of the column afforded a viscous yellow oil ($R_f$ 0.19).

**(*E*)-3-(4-Carbethoxy-2-methoxyphenyl)-2-methylpropenal [13(3)].** A mixture of 1.0 g (4.3 mmol) of 1,1-dimethyl olefin **10(3)**, 1.24 g (11.2 mmol) of $SeO_2$, and 0.2 mL of water in 15 mL of dioxane was heated in a 110 °C oil bath for 1.5 h. After cooling to room temperature, the reaction mixture was filtered through Celite ($Et_2O$ rinse) to remove excess $SeO_2$. Concentration afforded an orange semisolid residue, which was dissolved in a small amount of toluene and chromatographed on 25 g of silica gel with 30% $Et_2O$/hexane to afford a small amount of a yellow oil ($R_f$ 0.64, *Z* isomer), followed by 0.60 g of a yellow solid ($R_f$ 0.56, *E* isomer). The solid was rechromatographed on 20 g of silica gel (toluene) to remove colored byproducts ($R_f$ 0.0, 0.38) and to isolate the aldehyde ($R_f$ 0.18), which was submitted to evaporative distillation at 110–120 °C (0.005 mm) to afford 0.45 g (42% yield) of very pale yellow solid. The $^1H$ NMR spectrum indicated that none of the *Z* isomer was present. A sample was purified by crystallization from EtOAc/hexane: white prisms; mp 59–61 °C; IR ($CHCl_3$) 2840, 1705, 1675, 1410, 1290, 1110, 1010 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.42 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 2.0 (d, $J$ = 0.5 Hz, 3, C=$CCH_3$), 3.95 (s, 3, $OCH_3$), 4.42 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 7.3–7.85 (m, 4, C=CH, 3′-, 5′-, and 6′-H), 9.67 (s, 1, CHO); MS calcd for $C_{14}H_{16}O_4$, 248.1049; found, 248.1058.

**(*E*)-1-(4-Carbethoxy-2-methoxyphenyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (3a).** To a suspension of 10.59 g (22.1 mmol) of $\beta$-cyclogeranyltriphenylphosphonium bromide in 225 mL of THF, which was stirred in a dry ice/$CCl_4$ bath, 12.7 mL (19.6 mmol) of 1.54 M *n*-BuLi in hexane was added over a period of 15 min. This deep-red reaction mixture was next stirred in an ice bath for 10 min before a solution of 4.98 g (20.1 mmol) of aldehyde **13(3)** in 25 mL of THF (5-mL THF rinse) was added. The reaction mixture was degassed three times under argon and stirred at room temperature for 19 h, at which time the color had faded to light orange. The reaction mixture was diluted with 500 mL of hexane, 250 mL of $Et_2O$, and 500 mL of $H_2O$. The aqueous phase was extracted with 500 mL of hexane. The organic extracts were washed with two 250-mL portions of water and brine, dried ($MgSO_4$), and concentrated to a yellow semisolid, which on extraction with 10% $Et_2O$/hexane and concentration gave 8.3 g of a yellow oil, which was chromatographed on 400 g of silica gel with 10% $Et_2O$/hexane (200-mL fractions). Fractions 8 to 11 afforded 6.8 g of a pale-yellow oil. Further elution with 30% $Et_2O$/hexane afforded 1.3 g of recovered aldehyde. The crude diene mixture was purified by preparative LC by using the recycle technique (2% ether/hexane) to afford 2.9 g (39% yield) of pale-yellow, viscous oil, which solidified to off-white prisms, mp 74–76 °C, on standing: LC (Radialpak B, 2.5% $Et_2O$/hexane, 2 mL/min, 260 nm) $t_R$ 10.0 (0.5%), 11.1 (99.2%), 12.1 min (0.3%); LC (Radialpak A, 20% water/MeCN, 2 mL/min, 260 nm) $t_R$ 3.0 (2.7%), 34.0 min (97.3%); IR ($CHCl_3$) 1705 (C=O), 1290, 1270, 1110 $cm^{-1}$; 300-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.05 (s, 6, $16_R$- and $17_R$-$CH_3$), 1.40 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 1.48 and 1.63 (2 m, 4, $2_R$- and $3_R$-$CH_2$), 1.75 (s, 3, $18_R$-$CH_3$), 2.03 (s, 3, $19_R$-$CH_3$), 2.03 (m, 2, $4_R$-$CH_2$), 3.90 (s, 3, $OCH_3$), 4.39 (q, $J$ = 7 Hz, 2, $CH_2CH_3$),

6.13 (d, $J$ = 16 Hz, 1, $7_R$-*HC*=CH), 6.40 (d, $J$ = 16 Hz, 1, $8_R$-HC=C*H*), 6.57 (s, 1, $10_R$-C=CH), 7.33 (d, $J$ = 9 Hz, 1, 6′-H), 7.53 (d, $J$ = 1.3 Hz, 1, 3′-H), 7.65 (dd, $J$ = 1.7 and 9 Hz, 1, 5′-H); $^{13}$C NMR ($CDCl_3$) 14.2 and 14.4 ($19_R$, $CH_2CH_3$), 19.3 ($3_R$), 21.8 ($18_R$), 29.0 ($16_R$, $17_R$), 33.1 ($4_R$), 34.3 ($1_R$), 39.7 ($2_R$), 55.6 ($OCH_3$), 60.9 ($CH_2CH_3$), 111.0 (3′), 121.5 (1′), 124.4 (5′), 127.6 (6′), 129.7 ($7_R$), 130.1 ($5_R$), 131.9 (4′), 137.7 ($6_R$), 138.0 ($8_R$, $9_R$, $10_R$), 157.1 (2′), 166.5 (C=O) ppm; UV (EtOH) $\lambda_{max}$ 230 nm (shoulder, $\epsilon$ 1.34 × $10^4$), 330 (2.76 × $10^4$); MS calcd for $C_{24}H_{32}O_3$, 368.2351; found, 368.2377.

**(*E*)-1-(4-Carboxy-2-methoxyphenyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (3b).** A solution of 1.2 g (18.2 mmol) of 85% KOH in 3 mL of $H_2O$ and 5 mL of EtOH was degassed four times under argon and added to a suspension of 2.0 g (5.42 mmol) of ester **3a** in 5 mL of EtOH. The mixture was degassed four times and heated at 80 °C for 30 min. The cooled solution was acidified with 12 mL of 50% HOAc and diluted with 30 mL of $H_2O$ and 35 mL of $Et_2O$. The aqueous layer was extracted again with 35 mL of $Et_2O$. The combined $Et_2O$ layers were washed with brine (2 × 50 mL) and dried ($Na_2SO_4$). Concentration gave 1.84 g (100% yield) of pale yellow solid. Recrystallization from cold EtOAc and hexane yielded 764 mg (41% yield) of pale yellow powder, mp 156–157 °C. The mother liquor was concentrated and recrystallized from cold EtOAc, affording 450 mg (24% yield) of fine yellow crystals, mp 157–158 °C. The overall yield was 65%: LC (μBondapak $C_{18}$, 50% $H_2O$/MeCN, 2 mL/min, 260 nm) $t_R$ 7.6 min (99.7%, both crops); IR ($CHCl_3$) 3520, 2930 (broad), 2600 (broad), 1685, 1600, 1575, 1500, 1460, 1420, 1360, 1300–1200 (broad), 1180, 1120, 1040, 975, 920, 880 $cm^{-1}$; 300-MHz $^1$H NMR ($CDCl_3$) δ 1.05 (s, 6, $16_R$- and $17_R$-$CH_3$), 1.46–1.65 (m, 4, $2_R$- and $3_R$-$CH_2$), 1.76 (s, 3, $18_R$-$CH_3$), 2.04 (s, 3, $19_R$-$CH_3$), 2.04 (m, 2, $4_R$-$CH_2$), 3.92 (s, 3, $OCH_3$), 6.15 (d, $J$ = 16 Hz, 1, $7_R$-*HC*=CH), 6.42 (d, $J$ = 16 Hz, 1, $8_R$-HC=C*H*), 6.58 (s, 1, $10_R$-C=CH), 7.38 (d, $J$ = 8 Hz, 1, 6′-H), 7.59 (d, $J$ = 1 Hz, 1, 3′-H), 7.74 (dd, $J$ = 1 and 8 Hz, 1, 5′-H), 12.15 (very broad s, 0.5, $CO_2H$); $^{13}$C NMR ($CDCl_3$) 14.3 ($19_R$), 19.3 ($3_R$), 21.8 ($18_R$), 29.0 ($16_R$, $17_R$), 33.1 ($4_R$), 34.3 ($1_R$), 39.7 ($2_R$), 55.6 ($OCH_3$), 111.4 (3′), 122.8 (1′), 124.2 (5′), 127.9 (6′), 128.3 (4′), 129.4 ($7_R$), 130.2 ($5_R$), 133.1 ($10_R$), 137.6 ($6_R$), 138.0 and 138.4 ($8_R$, $9_R$), 151.7 (2′), 172.4 (C=O) ppm; UV (MeCN) $\lambda_{max}$ 328 nm ($\epsilon$ 2.5 × $10^4$); MS calcd for $C_{22}H_{28}O_3$, 340.2038; found, 340.2036.

**Preparation of (*E*)-1-(5-Carboxythien-2-yl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (4b) and Its Ethyl Ester (4a). (1*E*,3*E*)- and (1*Z*,3*E*)-1-(5-Carbethoxythien-2-yl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadienes [4a and ($9_R$*Z*)-4a].** To a suspension of 3.5 g (6.74 mmol) of (*E*)-1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1-buten-3-yl]triphenylphosphonium bromide (β-ionyltriphenylphosphonium bromide) in 30 mL of THF cooled to −78 °C was added, over a 5-min period, 4.46 mL (6.73 mmol) of a 1.51 M solution of *n*-BuLi in hexane. The mixture was gradually warmed to room temperature and stirred for another 15 min. A solution of 1.24 g (6.74 mmol) of 2-carbethoxy-5-thiophenecarboxaldehyde [11(4)][16] in 6 mL of THF (4-mL THF rinse) was added. The mixture was stirred at room temperature for 16 h and at 50 °C for 1 h. Water (50 mL) was added to the mixture, which was then extracted with 50% $Et_2O$/hexane (4 × 50 mL). The organic layer was washed with brine (2 × 100 mL), dried ($MgSO_4$), and concentrated to give a yellow oil, which was passed over a precolumn of 150 g of silica gel (5% EtOAc/hexane) to give 1.70 g (73% yield) of a bright yellow oil. Analytical LC (Radialpak B, 1% $Et_2O$/hexane, 2 mL/min, 260 nm) indicated two isomers: $t_R$ 9.60 (68%), 10.04 min (32%). These isomers were separated by multiple passes on preparative LC (0.25% $Et_2O$/hexane) to give 0.6 g (26% yield) of the 1*Z*,3*E* isomer and 0.3 g (13% yield) of the 1*E*,3*E* isomer **4a** as bright yellow oils.

**1*Z*,3*E* isomer**: LC (Radialpak B, 0.5% $Et_2O$/hexane, 2 mL/min, 260 nm) $t_R$ 16.85 (98%), 17.88 min (2%); LC (Radialpak A, 5% $H_2O$/MeOH, 2 mL/min, 260 nm) $t_R$ 5.75 (2%), 6.7 min (98%); IR (film) 2950, 1720, 1450, 1270, 1100, 750 $cm^{-1}$; 300-MHz $^1$H NMR ($CDCl_3$) δ 1.06 (s, 6, $16_R$- and $17_R$-$CH_3$), 1.37 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 1.45–1.55 and 1.6–1.7 (2 m, 4, $2_R$- and $3_R$-$CH_2$), 1.79 (s, 3, $18_R$-$CH_3$), 2.05–2.15 (m, 2, $4_R$-$CH_2$), 2.08 (s, 3, $19_R$-$CH_3$), 4.34 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 6.21 (s, 1, $10_R$-C=CH), 6.44 (d, $J$ = 16 Hz, 1, $7_R$-*HC*=CH), 6.86 (d, $J$ = 16 Hz, 1, $8_R$-HC=C*H*), 6.88 (d, $J$ = 4 Hz, 1, 3′-C=CH), 7.65 (d, $J$ = 4 Hz, 1, 4′-C=CH); $^{13}$C NMR ($CDCl_3$) 14.3 ($CH_2CH_3$), 19.1 ($3_R$), 21.4 and 21.9 ($18_R$, $19_R$), 28.9 ($16_R$, $17_R$), 33.1 ($4_R$), 34.2 ($1_R$), 39.6 ($2_R$), 60.9 ($CH_2CH_3$), 120.1 (3′), 127.4 ($10_R$), 130.1, 130.4, 132.1, 133.0, 137.1 ($9_R$),* 137.5 ($6_R$),* 147.4 (5′), 162.2 (C=O) ppm; UV (EtOH) $\lambda_{max}$ 239 nm ($\epsilon$ 1.4 × $10^4$), 272 (1.1 × $10^4$), 351 (2.0 × $10^4$); MS calcd for $C_{21}H_{28}O_2S$, 344.1810; found, 344.1831.

**4a**: LC (Radialpak B, 0.5% $Et_2O$/hexane, 2 mL/min, 260 nm) $t_R$ 17.9 min (100%); LC (Radialpak A, 5% $H_2O$/MeOH, 2 mL/min, 260 nm) $t_R$ 5.6 (99%), 6.6 min (1%); IR (film) 2950, 1720, 1450, 1280, 1230, 1100, 960, 750, $cm^{-1}$; 300-MHz $^1$H NMR ($CDCl_3$) δ 1.04 (s, 6, $16_R$- and $17_R$-$CH_3$), 1.38 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 1.45–1.55 and 1.6–1.7 (2 m, 4, $2_R$- and $3_R$-$CH_2$), 1.73 (s, 3, $18_R$-$CH_3$), 2.0–2.1 (m, 2, $4_R$-$CH_2$), 2.19 (s, 3, $19_R$-$CH_3$), 4.35 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 6.18 (d, $J$ = 16 Hz, 1, $8_R$-HC=C*H*), 6.34 (d, $J$ = 16 Hz, 1, $7_R$-*HC*=CH), 6.57 (s, 1, $10_R$-C=CH), 6.96 (d, $J$ = 4 Hz, 1, 3′-C=CH), 7.69 (d, $J$ = 4 Hz, 1, 4′-C=CH); $^{13}$C NMR ($CDCl_3$) 14.4 ($CH_2CH_3$, $19_R$), 19.2 ($3_R$), 21.7 ($18_R$), 28.9 ($16_R$, $17_R$), 33.0 ($4_R$), 34.2 ($1_R$), 39.6 ($2_R$), 60.9 ($CH_2CH_3$), 122.5 (3′), 127.7 ($7_R$), 129.0 ($5_R$, $10_R$), 133.1, 137.3, 137.5, 137.8, 148.1 (5′), 162.3 (C=O) ppm; UV (EtOH) $\lambda_{max}$ 235 nm ($\epsilon$ 9.2 × $10^3$), 353 (3.1 × $10^4$); MS calcd for $C_{21}H_{28}O_2S$, 344.1810; found, 344.1820.

**1-(5-Carbethoxythien-2-yl)-2-methyl-1-propene [10(4)].** A suspension of 17.28 g (40 mmol) of isopropyltriphenylphosphonium iodide in 50 mL of dry THF was cooled to −40 °C while 26 mL (40 mmol) of a 1.54 M solution of *n*-BuLi in hexane was added over a 15-min period. The dark brown mixture was warmed to room temperature over 30 min, and then a solution of 7.4 g (40 mmol) of 11(4) in 15 mL of THF was added dropwise to the reaction mixture. The deep violet mixture was stirred at room temperature overnight and at 55 °C for 1 h. It was poured into 100 mL of ice–water. The product was extracted with $Et_2O$ (3 × 60 mL). The ethereal extracts were washed with brine (2 × 150 mL), dried ($MgSO_4$), and evaporated to give 14.5 g of a dark brown oil, which was passed through 200 g of silica gel (10% $Et_2O$/hexane) to give 5.66 g (67% yield) of 1-(5-carbethoxythien-2-yl)-2-methyl-1-propene [10(4)] as a pale yellow oil: IR (film) 2950, 1710 (C=O), 1650, 1520, 1450, 1370, 1320, 1270, 1250, 1240, 1170, 1100, 850, 750 $cm^{-1}$; 60-MHz $^1$H NMR ($CDCl_3$) δ 1.40 (t, 3, $J$ = 7 Hz, $CH_2CH_3$), 2.02 and 2.07 [2 s, 6, $(CH_3)_2C$=C], 4.43 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 6.47 (broad s, 1, C=CH), 6.92 (d, $J$ = 4 Hz, 1, 3′-C=CH), 7.75 (d, $J$ = 4 Hz, 1, 4′-C=CH).

**(*E*)-3-(5-Carbethoxythien-2-yl)-2-methyl-2-propenal [13(4)].** A mixture of 3.8 g (18.1 mmol) of 1-(5-carbethoxythien-2-yl)-2-methyl-1-propene and 5.0 g (45 mmol) of $SeO_2$ in 60 mL of dioxane and 0.8 mL (44 mmol) of $H_2O$ was degassed three times under argon and heated under reflux for 45 min. Cooling, filtering over Celite, and concentrating gave 6.8 g of a light-brown solid, which was diluted with 50 mL of $Et_2O$ and filtered. The filtrate was concentrated and passed over a column of 150 g of silica gel (15% EtOAc/hexane) to give 2.35 g (58% yield) of the aldehyde as a pale yellow solid, a portion of which was recrystallized from EtOAc/hexane to give white needles: mp 98 °C; IR (mull) 1720, 1680, 1620, 1380, 1290, 1250, 1180, 1160, 1100, 1020, 890, 850, 815, 750 $cm^{-1}$; 60-MHz $^1$H NMR ($CDCl_3$) δ 1.42 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 2.17 (s, 3, C=$CCH_3$), 4.47 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 7.40 (d, $J$ = 4 Hz, 1, 3′-C=CH), 7.43 (s, 1, C=CH), 7.83 (d, $J$ = 4 Hz, 1, 4′-C=CH), 9.63 (s, 1, CHO); MS calcd for $C_{11}H_{12}O_3S$, 224.0507; found, 224.0519.

**(*E*)-1-(5-Carbethoxythien-2-yl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (4a).** A suspension of 4.95 g (10.3 mmol) of β-cyclogeranyltriphenylphosphonium bromide in 40 mL of THF was degassed twice under argon and cooled to −78 °C before 6.65 mL (10.2 mmol) of 1.54 M *n*-BuLi in hexane was added over a 10-min period. The mixture was warmed to 0 °C over a 45-min period. Next, a solution of 2.3 g (10.3 mmol) of aldehyde 13(4) in 10 mL of THF (3-mL THF rinse) was added to the reaction mixture over a 10-min period. The mixture was stirred at room temperature for 16 h and at 55–60 °C for 1 h, then cooled, diluted with 100 mL of ice–water, and extracted with $Et_2O$ (3 × 30 mL). The $Et_2O$ layer was washed with brine (2 × 100 mL), dried ($MgSO_4$), and evaporated to give 7 g of crude product, which was dissolved in hexane and filtered. The filtrate was concentrated to give 3.8 g of a yellow oil, which was passed through

(16) Thames, S. F.; McCleskey, J. E. *J. Heterocycl. Chem.* **1966**, *3*, 104.

100 g of silica gel (5% EtOAc/hexane) to give 3.06 g of a yellow oil. Further purification by preparative LC (1% $Et_2O$/hexane) gave 2.0 g (57% yield) of triene **4a** as a yellow oil, which crystallized on standing: mp 68–70 °C; LC (Radialpak B, 1% $Et_2O$/hexane, 2 mL/min, 260 nm) $t_R$ 12.2 (2.5%), 12.8 min (97.5%); LC (Radialpak A, 5% $H_2O$/MeOH, 2 mL/min, 260 nm) $t_R$ 4.71 (0.6%), 6.05 (1.1%), 7.26 min (98.3%); the IR, $^1H$ NMR, $^{13}C$ NMR, and UV spectra were identical with the previously prepared sample.

**(*E*)-1-(5-Carboxythien-2-yl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (4b).** A solution of 1.0 g (15.2 mmol) of 85% KOH in 3 mL of $H_2O$ and 5 mL of EtOH was added to a suspension of 1.8 g (5.23 mmol) of ester **4a** in 5 mL of EtOH. The mixture was degassed under argon twice and heated at 80 °C for 30 min. The clear yellow solution was cooled and acidified with 12 mL of 50% aqueous HOAc. The precipitated acid was extracted with 100 mL of $Et_2O$, and the $Et_2O$ layer was washed with 50 mL of $H_2O$ and 50 mL of brine, dried ($Na_2SO_4$), and evaporated to give 1.7 g of a bright yellow solid, which was recrystallized from 75 mL of MeOH to give two crops (0.75 g and 0.65 g, 85%) of bright yellow crystals: mp 215–216 °C; LC (Radialpak A, 20% $H_2O$/MeOH, 2 mL/min, 260 nm) $t_R$ 8.78 min (100%); IR (mull) 1680 (C=O), 1520, 1440, 1380, 1310, 1100, 1030, 970, 880, 810, 750 $cm^{-1}$; 300-MHz $^1H$ NMR ($Me_2SO$-$d_6$) $\delta$ 1.03 (s, 6, $16_R$- and $17_R$-$CH_3$), 1.4–1.5 and 1.55–1.65 (2 m, 4, $2_R$- and $3_R$-$CH_2$), 1.70 (s, 3, $18_R$-$CH_3$), 2.0–2.1 (m, 2, $4_R$-$CH_2$), 2.13 (s, 3, $19_R$-$CH_3$), 6.24 (d, $J$ = 16 Hz, 1, $8_R$-HC=C*H*), 6.39 (d, $J$ = 16 Hz, 1, $7_R$-*H*C=CH), 6.77 (s, 1, $10_R$-C=CH), 7.15 (d, $J$ = 4 Hz, 1, 3′-C=CH), 7.67 (d, $J$ = 4 Hz, 1, 4′-C=CH); $^{13}C$ NMR ($Me_2SO$-$d_6$) 14.3 ($19_R$), 19.0 ($3_R$), 21.7 ($18_R$), 29.0 ($16_R$, $17_R$), 32.8 ($1_R$, $4_R$), 39.4 ($2_R$), 122.9 (3′), 128.6 ($7_R$, $10_R$), 129.5 ($5_R$), 133.2, 137.1, 137.2, 147.2 (5′), 163.1 ppm (C=O); UV (EtOH) $\lambda_{max}$ 233 nm ($\epsilon$ 9.4 × $10^3$), 347 (3.2 × $10^4$); MS calcd for $C_{19}H_{24}O_2S$, 316.1497; found, 316.1509.

**Preparation of (*E*)-1-(5-Carboxy-2-furanyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (5b) and Its Ethyl Ester (5a). 2-Carbethoxy-5-furaldehyde [11(5)].** To a solution of 17.0 g (0.1 mol) of 2-furfural diethyl acetal[17] in 300 mL of anhydrous $Et_2O$ at −15 °C was added 67 mL (0.1 mol) of a 1.5 M solution of *n*-BuLi in hexane over a period of 30 min, while the temperature of the reaction mixture was maintained between −10 and −15 °C. The reaction mixture was gradually raised to room temperature over a period of 1 h, and then gaseous $CO_2$ was bubbled through for 4 h. Dilute HCl (200 mL) was next added, and the mixture was heated under reflux for 1 h. The $Et_2O$ layer was separated, and the aqueous phase was washed with 100 mL of EtOAc. The combined organic phase was washed with brine (2 × 200 mL), dried ($Na_2SO_4$), and concentrated to give 8.0 g of a dark-colored solid. This solid was dissolved in 100 mL of anhydrous $Me_2SO$ and then stirred at room temperature with 15 g (0.11 mol) of $K_2CO_3$ and 8 mL (0.1 mol) of EtI for 60 h. The reaction mixture was diluted with 500 mL of brine and extracted with EtOAc (3 × 100 mL). The extract was dried ($Na_2SO_4$) and concentrated to give 10.0 g of a dark oil, which was eluted through 180 g of silica gel (50% EtOAc/hexane) to give 6.2 g of a brown oil, which was again purified on 150 g of silica gel (25% $Et_2O$/hexane) to give 5.8 g of a yellow solid. The solid was recrystallized from $Et_2O$/hexane to give 5.0 g of **3** as white needles (30% overall yield): mp 39–40 °C; IR (mull) 1740, 1700, 1590, 1520, 1420, 1320, 1270, 1230, 1160, 1030, 980, 880, 850, 780 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.35 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 2.73 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 7.27 (s, 2, C=CHHC=C), 9.80 (s, 1, CHO); MS calcd for $C_8H_8O_4$, 168.0423; found, 168.0432.

**1-(5-Carbethoxy-2-furanyl)-2-methyl-1-propene [10(5)].** To a suspension of 24.2 g (0.056 mol) of isopropyltriphenylphosphonium iodide in 300 mL of THF at −20 °C was added 37.3 mL of a 1.5 M (0.056 mol) solution of *n*-BuLi in hexane over a 20-min period. The dark brown solution was warmed to 0 °C and maintained at this temperature for 30 min, when almost all of the solid phosphonium salt had disappeared. A solution of 9.42 g (0.056 mol) of aldehyde 11(5) in 20 mL of THF was added over a period of 10 min. The resulting clear yellow solution was stirred at room temperature for 20 h and at 50–60 °C for 1 h. The mixture was diluted with 500 mL of brine and 300 mL of $Et_2O$. The organic layer was washed twice with 300 mL of brine, dried ($Na_2SO_4$), and evaporated to afford 11.0 g of the crude product, which was purified on 150 g of silica gel (12.5% $Et_2O$/hexane) to give 9.0 g (83% yield) of the pure product as a very pale yellow oil: IR (film) 2990, 2950, 1720, 1670, 1590, 1510, 1460, 1380, 1350, 1320, 1270, 1230, 1190, 1160, 1140, 1030, 980, 850, 800, 760 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.25 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 1.85 and 1.95 [2 s, 6, C=C($CH_3$)$_2$], 4.25 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 6.05 (s, 1, C=CH), 6.17 (d, $J$ = 4 Hz, 1, 3′-H), 7.07 (d, $J$ = 4 Hz, 1, 4′-H); MS calcd for $C_{11}H_{14}O_3$, 194.0943; found, 194.0946.

**(*E*)-3-(5-Carbethoxy-2-furanyl)-2-methyl-2-propenal [13(5)].** A mixture of 8.2 g (0.042 mol) of olefin **10(5)**, 10 g (0.09 mol) of $SeO_2$, and 120 mL of wet dioxane was degassed three times under argon and heated at 90 °C for 1.5 h. The reaction mixture was cooled and filtered through Celite, and the filtrate was evaporated to remove most of the dioxane. The residue was filtered again and washed with 50 mL of dioxane. The filtrate and washings were concentrated to a small volume and chromatographed on 170 g of silica gel (25% $Et_2O$/hexane) to give 3.9 g (44% yield) of a white solid. Recrystallization from EtOAc/hexane gave long white needles: mp 83 °C; IR (mull) 1740, 1690, 1650, 1320, 1280, 1240, 1200, 1040, 980, 780 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.43 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 2.18 (s, 3, C=C$CH_3$), 4.48 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 6.88 and 7.35 (2 d, $J$ = 4 Hz, 2, 3′- and 4′-H), 7.15 (s, 1, C=CH), 9.60 (s, 1, CHO); MS calcd for $C_{11}H_{12}O_4$, 208.0736; found, 208.0728.

**(*E*)-1-(5-Carbethoxy-2-furanyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (5a).** A suspension of 8.87 g (0.019 mol) of β-cyclogeranyltriphenylphosphonium bromide in 200 mL of THF was cooled to −20 °C. A solution of 12.3 mL (0.019 mol) of 1.5 M *n*-BuLi in hexane was added over a period of 10 min. When the addition was complete, the mixture was stirred at 0 °C for another 10 min. To the resulting deep brown solution was added a solution of 3.85 g (0.019 mol) of **(13)5** in 10 mL of THF (5-mL THF rinse). The mixture was stirred overnight at room temperature and for 1 h at 50–60 °C. The clear yellow reaction mixture was concentrated to about 20 mL. $Et_2O$ (200 mL) was added. The precipitated triphenylphosphine oxide was removed by filtration through Celite. The filtrate upon evaporation gave 6.5 g of a yellow oil, which was purified on 100 g of silica gel (10% $Et_2O$/hexane) to give 5.3 g (87% yield) of the product as a bright yellow oil. Analytical LC (1% $Et_2O$/hexane, Radialpak B, 2 mL/min, 260 nm) indicated one major and three minor peaks: $t_R$ 13.0 (0.7%), 13.8 (4.3%), 15.6 (93.2%), and 19.0 min (1.8%). Further purification on preparative LC (0.8% $Et_2O$/hexane and 2% EtOAc/hexane) gave 2.9 g of the pure product (**5a**) as a light yellow oil: LC (Radialpak B, 1% $Et_2O$/hexane, 2 mL/min, 260 nm) $t_R$ 15.6 min (100%); reverse-phase LC (Radialpak A, 20% $H_2O$/MeCN, 2 mL/min, 260 nm) $t_R$ 10.7 min (100%); IR (film) 2950, 1720, 1580, 1500, 1380, 1310, 1230, 1190, 1140, 1030, 980, 880, 810, 770 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.05 (s, 6, $16_R$- and $17_R$-$CH_3$), 1.37 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 1.4–1.8 (m, 4, $2_R$- and $3_R$-$CH_2$), 1.72 (s, 3, $18_R$-$CH_3$), 1.9–2.15 (m, 2, $4_R$-$CH_2$), 2.22 (s, 3, $19_R$-$CH_3$), 4.37 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 6.17 (d, $J$ = 16 Hz, 1, $8_R$-HC=C*H*), 6.25 (s, 1, $10_R$-C=CH), 6.42 (d, $J$ = 4 Hz, 1, 3′-H), 6.43 (d, $J$ = 16 Hz, 1, $7_R$-*H*C=CH), 7.22 (d, $J$ = 4 Hz, 1, 4′-H); $^{13}C$ NMR ($CDCl_3$) 14.1, 14.3 ($19_R$, $CH_2CH_3$), 19.2 ($3_R$), 21.6 ($18_R$), 28.9 ($16_R$, $17_R$), 33.0 ($4_R$), 34.2 ($1_R$), 39.5 ($2_R$), 60.6 ($CH_2CH_3$), 110.8, 116.8, 119.4 ($10_R$, 3′, 4′), 129.2, 129.9 ($5_R$, $7_R$), 137.0 ($8_R$), 137.4 ($6_R$), 138.9 ($9_R$), 142.8, 157.1 (2′, 5′), 158.7 (C=O) ppm; UV (EtOH) $\lambda_{max}$ 339 nm ($\epsilon$ 3.17 × $10^4$); MS calcd for $C_{21}H_{28}O_3$, 328.2038; found, 328.2041.

**(*E*)-1-(5-Carboxy-2-furanyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (5b).** A solution of 1.2 g (18.2 mmol) of 85% KOH in 3 mL of $H_2O$ and 5 mL of EtOH was degassed three times and added to a suspension of 1.8 g (5.49 mmol) of ester **5a** in 5 mL of EtOH. The mixture was degassed three times under argon and heated at 80 °C for 30 min. The cooled solution was then acidified with 12 mL of 50% HOAc and extracted with $Et_2O$. The ethereal layer was washed with brine (2 × 50 mL), dried ($Na_2SO_4$), and concentrated to give 1.6 g of a yellow gel (97% yield), which gradually solidified upon trituration with hexane. Recrystallization from EtOAc and hexane yielded 1.1 g (67% yield) of bright yellow crystals: mp 109 °C; reverse-phase LC (Radialpak A, 20% $H_2O$/MeCN, 2 mL/min, 260 nm) $t_R$ 3.59 min (100%); IR (mull) 1690, 1590, 1510, 1320, 1280, 1240, 1190, 1170, 1050, 980, 770, 740 $cm^{-1}$; 300-MHz $^1H$ NMR

(17) Thames, S. F.; Odom, H. C. *J. Heterocycl. Chem.* **1966**, *3*, 490.

($CDCl_3$) $\delta$ 1.04 (s, 6, $16_R$- and $17_R$-$CH_3$), 1.48 and 1.63 (2 m, 4, $2_R$- and $3_R$-$CH_2$), 1.72 (s, 3, $18_R$-$CH_3$), 2.03 (m, 2, $4_R$-$CH_2$), 2.22 (s, 3, $19_R$-$CH_3$), 6.17 (d, $J$ = 16 Hz, 1, $8_R$-HC=C*H*), 6.29 (s, 1, $10_R$-C=CH), 6.38 (d, $J$ = 16 Hz, 1, $7_R$-*H*C=CH), 6.45 (d, $J$ = 4 Hz, 1, 3′-H), 7.34 (d, $J$ = 4 Hz, 1, 4′-H), 11.58 (s, 1, $CO_2H$); $^{13}C$ NMR ($CDCl_3$) 14.2 ($19_R$), 19.2 ($3_R$), 21.7 ($18_R$), 28.9 ($16_R$, $17_R$), 33.0 ($4_R$), 34.2 ($1_R$), 39.5 ($2_R$), 111.2, 116.6, 121.9 ($10_R$, 3′, 4′), 129.8, 130.1 ($5_R$, $7_R$), 136.9 ($8_R$), 137.4 ($6_R$), 139.9 ($9_R$), 141.8, 158.4 (2′, 5′), 163.8 (C=O) ppm; UV (EtOH) $\lambda_{max}$ 335 nm ($\epsilon$ 3.66 × $10^4$); MS calcd for $C_{19}H_{24}O_3$, 300.1725; found, 300.1737.

**Preparation of (*E*)-1-(2-Carbethoxy-5-pyridyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (6). Diethyl Pyridine-2,5-dicarboxylate.** To a suspension of 100 g (0.60 mol) of 2,5-pyridinedicarboxylic acid in 300 mL of absolute EtOH was added 100 mL of concentrated $H_2SO_4$ over a period of 1.5 h. During this time, the solid gradually all went into solution. The brown reaction mixture was refluxed for 16 h. Then 200 mL of benzene was added, and the benzene/EtOH/$H_2O$ azeotrope was removed at the rate of 300 mL/30 min, with more 1:1 benzene/EtOH mixture added at 30-min intervals. After 5 h, the reaction mixture was poured onto 3 L of ice–water. Solid $NaHCO_3$ was then added until the mixture was neutralized. The product was extracted into EtOAc. The EtOAc layer was washed with brine, dried ($Na_2SO_4$), and concentrated to 99.8 g of a yellow solid, which was purified on 400 g of silica gel (35% $Et_2O$/hexane and 50% $Et_2O$/hexane) to give 95 g of solid. Recrystallization from $Et_2O$/hexane gave 90.0 g (67% yield) of diethyl ester as pale yellow crystals: mp 46–47 °C (lit.[18] 46.5–48 °C); IR (mull) 1730, 1710, 1600, 1375, 1280, 1250, 1100, 1030, 750 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.47 and 1.50 (2 t, $J$ = 7.5 Hz, 6, $CH_2CH_3$), 4.55 and 4.62 (2 q, $J$ = 7.5 Hz, 4, $CH_2CH_3$), 8.17 (d, $J$ = 8 Hz, 1, 3′-H), 8.47 (dd, $J$ = 2.5 and 8 Hz, 1, 4′-H), 9.33 (d, $J$ = 2.5 Hz, 1, 6′-H).

**2-Formyl-5-(hydroxymethyl)pyridine.** A patented procedure[19] for the preparation of 2,5-pyridinedimethanol was modified. To a solution of 33.5 g (0.15 mol) of diethyl pyridine-2,5-dicarboxylate in 300 mL of absolute EtOH was added 11.4 g (0.3 mol) of $NaBH_4$ in several portions over a 30-min period. Then a solution of 33.3 g (0.3 mol) of anhydrous $CaCl_2$ in 200 mL of EtOH was added dropwise over a 1-h period. The mixture was stirred at room temperature for 16 h, and then it was neutralized with 12 to 13 mL of concentrated $H_2SO_4$. The $CaSO_4$, which precipitated, was removed by centrifugation (two EtOH washings). The combined supernatant was concentrated, dissolved in about 10 mL of $H_2O$, and applied to a 4.5 × 18 cm column of Dowex 50W-X8 cation exchange resin ($H^+$ form). The column was eluted with $H_2O$ until the eluate was no longer acidic, and then it was eluted with dilute $NH_4OH$, which removed 2,5-pyridinedimethanol from the column. Evaporation afforded 10.6 g (51% yield) of this diol as a light brown hygroscopic solid: 60-MHz $^1H$ NMR ($Me_2SO$-$d_6$) $\delta$ 4.55 and 4.59 (2 s, 4, $CH_2OH$), 5.23 (broad s, 2, $CH_2OH$), 7.42 (d, $J$ = 8 Hz, 1, 3′-H), 7.72 (dd, $J$ = 2 and 8 Hz, 1, 4′-H), 8.42 (d, $J$ = 2 Hz, 1, 6′-H).

The crude diol (10.5 g, 0.076 mol) was dissolved in 80 mL of dioxane and 2 mL of $H_2O$, and 4.0 g (0.036 mol) of $SeO_2$ was added. The mixture was degassed three times under argon and then heated at 100 °C for 3 h. The cooled reaction mixture was filtered through Celite (30-mL dioxane wash). The filtrate and washings were concentrated to about a 10-mL volume and chromatographed on 150 g of silica gel (50% EtOAc/hexane and 75% EtOAc/hexane) to give 6.2 g (59% yield) of the product aldehyde as a pale yellow solid. A portion was recrystallized from EtOAc/hexane to give white plates: mp 75 °C; IR (mull) 3250, 1700, 1600, 1580, 1460, 1230, 1190, 1060, 1030, 830, 770 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 4.23 (broad s, 1, OH), 4.90 (s, 2, $CH_2$), 7.95 and 7.97, (2 s, 2, 3′- and 4′-H), 8.77 (s, 1, 6′-H), 10.07 (s, 1, CHO); MS calcd for $C_7H_7NO_2$, 137.0478; found, 137.0471.

**2-Carbethoxy-5-(hydroxymethyl)pyridine.** 2-Formyl-5-(hydroxymethyl)pyridine (5.7 g, 0.042 mol) was dissolved in 40 mL of $H_2O$ and treated with 10 mL of aqueous 30% $H_2O_2$ at room temperature for 16 h. The mixture was concentrated to about 20 mL and cooled, and the precipitated acid was collected by filtration. The acid was obtained in 97% yield (6.1 g). A portion was recrystallized from $H_2O$ to obtain an analytical sample. These white crystals sublimed at about 220 °C (lit.[20] mp 216 °C dec); IR (mull) 3200, 1670, 1600, 1380, 1070, 870, 850, 800 $cm^{-1}$; 60-MHz $^1H$ NMR ($Me_2SO$-$d_6$) $\delta$ 4.73 (s, 2, $CH_2OH$), 7.9–8.2 (m, 2, 3′- and 4′-H), 8.67 (m, 1, 6′-H), MS calcd for $C_7H_7NO_3$, 153.0426; found, 153.0429.

To a solution of 10 g (0.065 mol) of the acid in 80 mL of EtOH and 10 mL of $Et_3N$ was added a solution of 3.65 g (0.033 mol) of $CaCl_2$ in 25 mL of EtOH. The mixture was evaporated to dryness under vacuum and then refluxed for 4 h with a mixture of 80 mL of EtOH and 25 mL of $H_2SO_4$. The combined filtrate and washings were concentrated to about 40 mL and diluted with 100 mL of ice–water. The mixture was neutralized with solid $NaHCO_3$ and extracted with $CHCl_3$ (6 × 70 mL). The organic extract was dried ($Na_2SO_4$) and concentrated to give 11.0 g of the ester as a light yellow solid (93% yield): mp 64 °C (lit.[21] mp 67–68 °C); IR (mull) 3300, 1725, 1595, 1590, 1480, 1390, 1310, 1290, 1150, 1070, 1030, 860, 780, 705 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.42 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 4.43 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 4.82 (s, 2, $CH_2OH$), 4.95 (broad s, 1, OH), 7.82 (dd, $J$ = 2 and 8 Hz, 1, 4′-H), 8.03 (d, $J$ = 8 Hz, 1, 3′-H), 8.67 (d, $J$ = 2 Hz, 1, 6′-H); MS calcd for $C_9H_{11}NO_3$, 181.0739; found, 181.0739.

**2-Carbethoxy-5-formylpyridine [11(6)].** To a stirred solution of 30 mL (0.37 mol) of pyridine in 150 mL of $CH_2Cl_2$ at 0 °C was added 18.0 g (0.18 mol) of $CrO_3$ over a period of 30 min. The orange mixture was stirred at 0 °C for another 20 min before a solution of 11.0 (0.061 mol) g of 2-carbethoxy-5-(hydroxymethyl)pyridine in 20 mL of $CH_2Cl_2$ was added. The mixture was stirred at room temperature for 4 h and filtered through Forisil. The filtrate was concentrated and purified on 150 g of silica gel (1 L of 50% EtOAc/hexane, followed by 75% EtOAc/hexane) to give 6.4 g (59% yield) of the product as white crystals: mp 59 °C; IR (melt) 1720, 1600, 1570, 1370, 1310, 1250, 1120, 1020, 835, 795, 700 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.48 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 4.52 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 8.33 (broad s, 2, 3′- and 4′-H), 9.20 (broad s, 1, 6′-H), 10.27 (s, 1, CHO); MS calcd for $C_9H_9NO_3$, 179.0582; found, 179.0572.

**1-(2-Carbethoxy-5-pyridyl)-2-methyl-1-propene [10(6)].** A suspension of 15.21 g (35.0 mmol) of isopropyltriphenylphosphonium iodide in 200 mL of THF was cooled to −20 °C, while 23.4 mL (35.1 mmol) of a 1.5 M solution of *n*-BuLi in hexane was added over a 10-min period. The mixture was gradually warmed to 0 °C over a period of 30 min before 6.3 g (35.2 mmol) of 2-carbethoxy-5-formylpyridine in 15 mL of THF was added. The reaction mixture was stirred at room temperature for 16 h and at 55–60 °C for 1 h. The cooled reaction mixture was filtered through Celite. The filtrate was concentrated and filtered again and chromatographed on 150 g of silica gel (50% EtOAc/hexane, 75% EtOAc/hexane) to give 4.7 g of the product as a yellow oil (65% yield): IR (film) 3000, 2950, 1730, 1670, 1600, 1580, 1470, 1410, 1390, 1330, 1280, 1230, 1190, 1150, 1120, 1040, 890, 800, 730 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.45 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 1.92 and 1.99 [2 s, 6, C=C($CH_3$)$_2$], 4.50 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 6.30 (s, 1, C=CH), 7.67 (dd, $J$ = 2 and 8 Hz, 1, 4′-H), 8.13 (d, $J$ = 8 Hz, 1, 3′-H), 8.67 (d, $J$ = 2 Hz, 1, 6′-H); MS calcd for $C_{12}H_{15}NO_2$, 205.1103; found, 205.1118.

**(*E*)-3-(2-Carbethoxy-5-pyridyl)-2-methyl-2-propenal [13(6)].** A mixture of 5.0 g (45.0 mmol) of $SeO_2$ and 4.25 g (20.7 mmol) of olefin **10(6)** in 50 mL of dioxane and 2 mL of $H_2O$ was degassed three times under argon and then heated at 100 °C for 2 h. The mixture was filtered through Celite and washed with dioxane. The filtrate was concentrated and chromatographed on 150 g of silica gel (50% EtOAc/hexane and 75% EtOAc/hexane) to give 2.95 g of product as a yellow solid. Analytical LC (Radialpak B, 25% EtOAc/hexane) indicated about 5% of the 2*Z* isomer was present. Recrystallization from EtOAc/hexane gave 2.4 g (53% yield) of the pure 2*E* isomer as white crystals, mp 72–73 °C; IR (mull) 1740, 1700, 1330, 1300, 1260, 1200, 1170, 1140, 1120,

(18) Isagawa, K.; Kawai, M.; Fushizaki, Y. *Nippon Kagaku Zasshi* **1967**, *88*, 553.
(19) Matsumoto, I.; Yoshizawa, J. Japan Kokai 73 29 783, 1973; *Chem. Abstr.* **1973**, *79*, 31912*j*.
(20) Matsumoto, I.; Yozhizawa, J. Japan Kokai 73 13 370, 1973; *Chem. Abstr.* **1973**, *78*, 136100*a*.
(21) Matsumoto, I.; Yoshizawa, J. Japan Kokai 73 54 072, 1973; *Chem. Abstr.* **1973**, *79*, 105071*h*.

1020, 920, 880, 830, 810, 720 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.50 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 2.10 (s, 3, C=$CCH_3$), 4.50 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 7.35 (s, 1, C=CH), 8.02 (dd, $J$ = 2 and 8 Hz, 1, 4'-H), 8.27 (d, $J$ = 8 Hz, 1, 3'-H), 8.93 (d, $J$ = 2 Hz, 1, 6'-H), 9.70 (s, 1, CHO); MS calcd for $C_{12}H_{13}NO_3$, 219.0895; found, 219.0907.

**(*E*)-1-(2-Carbethoxy-5-pyridyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (6).** To a suspension of 5.25 g (11.0 mmol) of $\beta$-cyclogeranyltriphenylphosphonium bromide in 150 mL of THF at −20 °C was added 7.2 mL (11.0 mmol) of a 1.54 M solution of *n*-BuLi in hexane. The dark brown mixture was stirred at 0 °C for 30 min. Then 2.40 g (10.95 mmol) of aldehyde **13(6)** in 10 mL of THF was added. The mixture was stirred overnight at room temperature and at 50–60 °C for 1 h. The resulting yellow solution was concentrated to about 20 mL, diluted with 50 mL of EtOAc, and filtered through Celite. The filtrate was washed with 100 mL of brine, dried ($Na_2SO_4$), and concentrated to afford about 6.0 g of a yellow oil. The oil was purified on 120 g of silica gel (25% EtOAc/hexane) to give 2.8 g (75% yield) of a bright yellow oil, which was further purified by preparative LC (10% $Et_2O$/hexane containing 0.3% *i*-PrOH) to give 2.2 g of the product as a light yellow oil: LC (Radialpak B, 10% EtOAc and 0.5% *i*-PrOH in hexane, 2 mL/min, 260 nm) $t_R$ 4.0 (97.5%), 4.6 min (2.5%); LC (Radialpak A, 10% $H_2O$/MeCN, 2 mL/min, 260 nm) $t_R$ 6.42 (97%), 7.47 min (3%); IR (film) 2950, 1720, 1620, 1600, 1570, 1470, 1380, 1320, 1260, 1200, 1150, 1130, 1040, 980, 950, 890, 800, 720 $cm^{-1}$; 300-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.05 (s, 6, $16_R$- and $17_R$-$CH_3$), 1.48 and 1.65 (2 m, 4, $2_R$- and $3_R$-$CH_2$), 1.51 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 1.72 (s, 3, $18_R$-$CH_3$), 2.04 (m, 2, $4_R$-$CH_2$), 2.09 (d, $J$ = 1 Hz, 3, $19_R$-$CH_3$), 4.49 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 6.23 (d, $J$ = 16 Hz, 1, $8_R$-HC=C*H*), 6.36 (d, $J$ = 16 Hz, 1, $7_R$-*H*C=CH), 6.43 (s, 1, $10_R$-C=CH), 7.74 (dd, $J$ = 2 and 8 Hz, 1, 4'-H), 8.10 (d, $J$ = 8 Hz, 1, 3'-H), 8.70 (d, $J$ = 2 Hz, 1, 6'-H); $^{13}C$ NMR ($CDCl_3$) 13.8, 14.2 ($19_R$, $CH_2CH_3$), 19.0 ($3_R$), 21.5 ($18_R$), 28.7 ($16_R$, $17_R$), 32.8 ($4_R$), 34.0 ($1_R$), 39.3 ($2_R$), 61.5 ($CH_2CH_3$), 124.3, 124.7 ($10_R$, 3', 5'), 129.2, 129.7 ($5_R$, $7_R$), 136.1, 137.0, 137.2 ($6_R$, $8_R$, 4'), 140.2 ($9_R$), 145.0 (6'), 150.0 (2'), 164.9 (C=O) ppm; UV (EtOH) $\lambda_{max}$ 334 nm ($\epsilon$ 2.22 × $10^4$), 266 (1.2 × $10^4$); MS calcd for $C_{22}H_{29}NO_2$, 339.2198; found, 339.2206.

**Preparation of (1*Z*,3*E*)-1-(4-Carboxyphenyl)-1-fluoro-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (7b) and Its Ethyl Ester (7a). 4-Carbethoxybenzyl Fluoride.** To a solution of 7.9 g (30 mmol) of 18-crown-6 in 150 mL of MeCN was added 35.4 g (0.60 mol) of anhydrous KF (oven dried at 120 °C). The suspension was stirred at room temperature for 1 h and treated with 76.3 g (0.31 mol) of 4-carbethoxybenzyl bromide (**15**; 10-mL MeCN rinse). The reaction was heated at reflux for 92 h, cooled, and filtered. The potassium salts were washed with MeCN, and the combined filtrates were evaporated. The residue was extracted with $Et_2O$ (2 × 200 mL) from 300 mL of water. The extract was washed with water (2 × 100 mL), dried ($MgSO_4$), and concentrated to a pale yellow oil. Evaporative distillation [bath temperature 93–110 °C (1.7 mm)] yielded the benzyl fluoride (50.0 g, 87%) as a colorless liquid: IR ($CHCl_3$) 1710 (C=O), 1610, 1580, 1460, 1410, 1365, 1275 (1230 sh), 1170, 1105, 1010, 850 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.38 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 4.36 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 5.40 (d, $J$ = 48 Hz, 2, $CH_2F$), 7.39 (d, $J$ = 8 Hz, 2, ArH), 8.05 (d, $J$ = 8 Hz, 2, ArH ortho to $CO_2CH_2CH_3$); MS calcd for $C_{10}H_{11}FO_2$, 182.0743; found, 182.0748.

**Ethyl 4-(Bromofluoromethyl)benzoate (16).** To a solution of 50.0 g (0.275 mol) of 4-carbethoxybenzyl fluoride in 300 mL of $CCl_4$ at reflux was added 51.0 g (0.286 mol) of NBS (recrystallized from water) containing 0.5 g (2 mmol) of dibenzoyl peroxide in about 1-g portions over a 1.25-h period with stirring. $CCl_4$ (50 mL total volume) was added periodically to wash in the reagent. The red suspension was heated at reflux for a further 5.5 h, cooled, and allowed to stand overnight. The succinimide was removed by filtration and rinsed with 200 mL of $CCl_4$. The combined filtrates were evaporated, and the residue was extracted with 1 L of hexane, allowed to stand for 1 h, and filtered. Concentration afforded 75.1 g of a red liquid, which was evaporatively distilled [bath temperature 85 to 95 °C (0.07–0.08 mm)]. The 68.7 g of pale yellow distillate was a mixture of the product and starting material by $^1H$ NMR. This liquid was redistilled at 0.01 mm to give (1) a mixture of starting material and product (bp 61–87 °C) and (2) 50.3 g (70% yield) of ethyl 4-(bromofluoromethyl)benzoate (bp 87–95 °C) as a colorless liquid: IR ($CHCl_3$) 1715 (C=O), 1615, 1580, 1415, 1365, 1275, 1120, 1105, 1045, 1015, 860 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.40 (*t*, $J$ = 7 Hz, 3, $CH_2CH_3$), 4.38 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 7.45 (d, $J$ = 8 Hz, 2, ArH), 8.10 (d, $J$ = 50 Hz, 1, C*H*F), 7.55 (d, $J$ = 8 Hz, 2, ArH ortho to $CO_2CH_2CH_3$); MS calcd for $C_{10}H_{10}{}^{79}BrFO_2$, 259.9849; found, 259.9872.

**Diethyl (4-Carbethoxy-α-fluorobenzyl)phosphonate (17).** To 9.0 g (54 mmol) of degassed (four times, argon) $(EtO)_3P$ at 150–155 °C (bath temperature) was added dropwise, under a stream of argon, 9.4 g (36 mmol) of **16** over a 20-min period. The bath temperature was raised to 200 °C and maintained there for 2.25 h. The solution, which became yellow at 200 °C, was allowed to cool overnight. $(EtO)_3P$ was removed by distillation by heating to 130 °C (bath temperature) at 35 mm. The crude phosphonate was chromatographed on a 5 × 50 cm silica gel column eluted successively with 2-L portions of 50% and 75% EtOAc/hexane and EtOAc to give 1.39 g of a byproduct, followed by 7.9 g of the phosphonate. The product was evaporatively distilled at 125–135 °C (0.01 mm) to yield 7.72 g (68% yield) of colorless, viscous liquid: IR ($CHCl_3$) 1710 (C=O), 1615, 1580, 1415, 1390, 1365, 1275 (1220 sh), 1105, 1020 (1050 sh), 975, 860 $cm^{-1}$; 60-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.29 [dd, $J$ = 7 and 3 Hz, 6, $P(OCH_2CH_3)_2$], 1.40 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 4.05 [dd, $J$ = 7 and 3 Hz, 4, $P(OCH_2CH_3)_2$], 4.39 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 5.77 (dd, $J$ = 45 and 9 Hz, 1, CHFP=O), 7.55 (dd, $J$ = 8 and 1.5 Hz, 2, ArH ortho to CHFP=O), 8.10 (d, $J$ = 8 Hz, 2, ArH ortho to $CO_2CH_2CH_3$); MS calcd for $C_{14}H_{20}FO_5P$, 318.1033; found, 318.1045.

**(1*Z*,3*E*)-1-(4-Carbethoxyphenyl)-1-fluoro-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (7a).** A solution of LDA was prepared by the addition of 10 mL of 1.5 M *n*-BuLi in hexane (15 mmol) to 2.0 g (20 mmol) of diisopropylamine in 5 mL of THF at 0 °C, followed by stirring at this temperature for 20 min. Then a solution of 5.1 g (16 mmol) of diethyl (4-carbethoxy-α-fluorobenzyl)phosphonate in 5 mL of THF was added over a 5-min period; after 15 min, a brown suspension resulted. To the reagent was added at −78 °C a solution of 4.8 g (25 mmol) of $\beta$-ionone (**20**) in 5 mL of THF, and the reaction mixture was degassed (two times, argon). After 30 min, the temperature of the reaction mixture was allowed to rise slowly from −78 °C to room temperature overnight. The resultant orange solution was poured into 250 mL of 1:1 water/saturated brine containing 2 mL of HOAc and then extracted with 9:1 hexane/$Et_2O$ (2 × 100 mL). The extract was washed with 100 mL of 1:1 water/saturated brine and then with 100 mL of water, dried ($Na_2SO_4$), and concentrated to yield a viscous yellow oil. The crude product was chromatographed on a 5 × 45 cm silica gel column with 4% $Et_2O$/hexane to give 4.62 g (81% yield) of a mixture of esters as a yellow oil. The isomers were separated by LC in 1% $Et_2O$/hexane by using the recycle technique to yield 2.14 g (37% yield) of the 1*E* isomer and 2.13 g (37% yield) of the 1*Z* isomer **7a**. A 3.03-g sample of **7a** obtained from two combined reactions was finally purified by a second chromatography with 1% $Et_2O$/hexane to yield 2.69 g (33% overall yield) of pure ester: LC (Radialpak B, 1% $Et_2O$/hexane, 2.0 mL/min, 280 nm) $t_R$ 9.1 min (100%); LC (Radialpak A, 5% $H_2O$/MeOH, 2.0 mL/min, 280 nm) $t_R$ 2.5 (0.7%), 3.6 (0.4%), 4.2 (2.1%), 7.3 min (96.8%); IR ($CHCl_3$) 1710 (C=O), 1610, 1370, 1280, 1110, 1085, 1005, 970, 860 $cm^{-1}$; 300-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.06 (s, 6, $16_R$- and $17_R$-$CH_3$), 1.40 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 1.4–1.75 (m, 4, $2_R$- and $3_R$-$CH_2$), 1.77 (d, $J$ = 0.5 Hz, 3, $18_R$-$CH_3$), 1.98 (d, $J$ = 2.8 Hz, 3, $19_R$-$CH_3$), 1.9–2.15 (m, 2, $4_R$-$CH_2$), 4.38 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 6.27 (d, $J$ = 16 Hz, 1, $7_R$-*H*C=CH), 6.76 (dd, $J$ = 16 and 2 Hz, 1, $8_R$-HC=C*H*), 7.55 (d, $J$ = 8.5 Hz, 2, ArH), 8.07 (d, $J$ = 8.5 Hz, 2, ArH ortho to $CO_2CH_2CH_3$); $^{13}C$ NMR ($CDCl_3$) 13.1 (d, $J$ = 3 Hz, $19_R$), 14.3 ($CH_2CH_3$), 19.3 ($3_R$), 21.7 ($18_R$) 28.9 ($16_R$, $17_R$), 33.0 ($4_R$), 34.2 ($1_R$), 39.6 ($2_R$), 61.0 ($CH_2CH_3$), 115.5 (d, $J$ = 12 Hz, $8_R$), 127.3, 127.7, 128.1, 128.3, 128.9, 129.2, 129.8, 137.1 (d, $J$ = 28 Hz, $9_R$), 137.7 (1'), 152.6 (d, $J$ = 247 Hz, $10_R$), 166.0 (C=O) ppm; UV (EtOH) $\lambda_{max}$ 244 nm ($\epsilon$ 1.22 × $10^4$), 322 (2.21 × $10^4$); MS calcd for $C_{23}H_{29}FO_2$, 356.2151; found, 356.2181.

A sample of the 1*E* isomer was similarly purified in 30% overall yield: LC (Radialpak B, 1% $Et_2O$/hexane, 2.0 mL/min, 280 nm) $t_R$ 6.9 min (100%); LC (Radialpak A, 5% $H_2O$/MeOH, 2.0 mL/min, 280 nm) $t_R$ 2.2 (0.3%), 3.7 (0.7%), 4.2 (2.1%), 8.2 min (96.9%); IR ($CHCl_3$) 1710 (C=O), 1610, 1370, 1275, 1105, 1075,

1010, 860 $cm^{-1}$; 300-MHz $^1H$ NMR ($CDCl_3$) $\delta$ 1.00 (s, 6, $16_R$- and $17_R$-$CH_3$), 1.39 (t, $J$ = 7 Hz, 3, $CH_2CH_3$), 1.35–1.7 (m, 4, $2_R$ and $3_R$-$CH_2$), 1.68 (s, 3, $18_R$-$CH_3$), 1.9–2.1 (m, 2, $4_R$-$CH_2$), 2.04 (d, $J$ = 3.5 Hz, 3, $19_R$-$CH_3$), 4.38 (q, $J$ = 7 Hz, 2, $CH_2CH_3$), 6.15 (dd, $J$ = 16 and 2 Hz, 1, $8_R$-HC=CH), 6.35 (d, $J$ = 16 Hz, 1, $7_R$-HC=CH), 7.52 (d, $J$ = 8 Hz, 2, ArH), 8.08 (d, $J$ = 8 Hz, 2, ArH ortho to $CO_2CH_2CH_3$); $^{13}C$ NMR ($CDCl_3$) 11.1 (d, $J$ = 8 Hz, $19_R$), 14.3 ($CH_2CH_3$), 19.2 ($3_R$), 21.7 ($18_R$), 28.9 ($16_R$, $17_R$), 32.9 ($4_R$), 34.2 ($1_R$), 39.5 ($2_R$), 61.0 ($CH_2CH_3$), 116.8 (d, $J$ = 20 Hz, $8_R$), 128.4, 128.6, 129.1, 129.3, 129.5, 130.3, 136.5 (d, $J$ = 25 Hz, $9_R$), 137.8 (1'), 154.6 (d, $J$ = 241 Hz, $10_R$), 165.9 (C=O) ppm; UV (EtOH) $\lambda_{max}$ 231 nm ($\epsilon$ 1.59 × $10^4$), 252 (1.46 × $10^4$), 323 (1.28 × $10^4$); MS calcd for $C_{23}H_{29}FO_2$, 356.2151; found, 356.2181.

**(1*Z*,3*E*)-1-(4-Carboxyphenyl)-1-fluoro-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (7b).** To a degassed (four times, argon) solution of 0.6 g (9.1 mmol) of 85% KOH in 1.5 mL of water and 4 mL of EtOH was added a solution of 1.26 g (3.5 mmol) of (1*Z*,3*E*)-1-(4-carbethoxyphenyl)-1-fluoro-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (**7a**) in 3 mL of EtOH. This suspension was again degassed (two times, argon). The reaction mixture was heated to 80 °C (bath temperature) over a 15-min period, and the temperature was maintained there for 30 min to produce a dark red solution and a precipitate. The cooled reaction mixture was quenched with 3 mL of HOAc in 5 mL of water and allowed to stand for 65 h. The yellow suspension was diluted with 30 mL of water and extracted with 50 mL of $Et_2O$. The extract was washed with water (2 × 25 mL) and dried ($Na_2SO_4$), and the solvent was evaporated. The resultant yellow powder was not very soluble in $Et_2O$, $CHCl_3$, or acetone and was therefore crystallized from 70 mL of MeOH under argon, washed with MeOH (2 × 3 mL), and dried to yield 896 mg (77% yield) of yellow crystals, mp 193–194 °C. The crystallization liquor was concentrated to 10 mL of cooled to yield a second crop of **7b** (141 mg, mp 193–194 °C). The total yield of acid **7b** was 1.037 g (90%): LC (Radialpak A, 30% $H_2O$/MeOH, 2.0 mL/min, 280 nm) $t_R$ 3.2 min (100%); IR (mull) 3100–2200 (carboxyl OH), 1675 (C=O), 1595, 1450, 1420, 1370, 1315, 1285, 1180, 1075, 960, 865, 770 $cm^{-1}$; 300-MHz $^1H$ NMR ($CDCl_3$/$Me_2SO$-$d_6$) $\delta$ 1.06 (s, 6, $16_R$- and $17_R$-$CH_3$), 1.4–1.75 (m, 4, $2_R$- and $3_R$-$CH_2$), 1.75 (s, 3, $18_R$-$CH_3$), 2.02 (d, $J$ = 2.8 Hz, 3, $19_R$-$CH_3$), 1.9–2.15 (m, 2, $4_R$-$CH_2$), 6.26 (d, $J$ = 16 Hz, 1, $7_R$-HC=CH), 6.71 (dd, $J$ = 16 and 2 Hz, 1, $8_R$-HC=CH), 7.55 (d, $J$ = 8.5 Hz, 2, ArH), 8.05 (d, $J$ = 8.5 Hz, 2, ArH ortho to $CO_2H$), 7.5–9.0 (very broad s, 1, OH, exchanged $D_2O$); $^{13}C$ NMR ($CDCl_3$/$Me_2SO$-$d_6$) 10.8 (d, $J$ = 4 Hz, $19_R$), 16.8 ($3_R$), 19.4 ($18_R$), 26.6 ($16_R$, $17_R$), 30.6 ($4_R$), 31.8 ($1_R$), 37.2 ($2_R$), 113.0 (d, $J$ = 13 Hz, $8_R$), 124.8, 125.2, 125.6, 125.8, 126.5, 126.7, 127.0, 127.3, 134.3 (d, $J$ = 29 Hz, $9_R$), 135.3 (1'), 155.1 (part of d, $10_R$), 165.1 (C=O) ppm; UV (EtOH) $\lambda_{max}$ 241.5 nm ($\epsilon$ 1.05 × $10^4$), 317 (2.02 × $10^4$); MS calcd for $C_{21}H_{25}FO_2$, 328.1828; found, 328.1809.

**Tracheal Organ Culture Assay.** This assay procedure was established to measure the ability of retinoids to control cell differentiation and reverse keratinization in retinoid-deficient tracheal epithelium.[12] Syrian golden hamsters were maintained on a vitamin A deficient diet from birth until 30–32 days old, at which time tracheas were removed and cultured in serum-free medium (CMRL medium 1066 with crystalline bovine insulin, 1.0 μg/mL; hydrocortisone hemisuccinate, 0.1 μg/mL; glutamine, 2 mM; penicillin, 100 units/mL; and streptomycin, 100 μg/mL). Culture dishes containing one explant each were placed in a controlled atmosphere culture chamber (Bellco, Vineland, NJ) and gassed with a mixture of 50% $O_2$, 45% $N_2$, and 5% $CO_2$. The chamber was placed on a rocker platform (Bellco) in a 36 °C incubator, and rocked at 10 cycles/min, permitting exposure of the trachea surface to the gas phase (50% of the rocking cycle).

All tracheas were cultured for 3 days in medium without retinoid. At the end of this time, some tracheas were fixed for histological evaluation, while the remaining tracheas were cultured for an additional 7 days in medium containing either cyclic AMP dissolved in dimethyl sulfoxide ($Me_2SO$) or an equivalent amount of $Me_2SO$. (*all-E*)-Retinoic acid was used as the reference substance. The culture medium and gas atmosphere were replaced three times during the final 7 days in culture. All tracheas were harvested on the 10th day of culture, fixed, and processed for histology. Sections were assessed with respect to the presence or absence of keratin and keratohyalin granules. The compounds were scored as *inactive* if both keratin and keratohyaline granules were seen, and scored as *active* if neither keratin nor keratohyaline granules were seen or if keratohyaline granules alone were absent.

**Ornithine Decarboxylase Assay.** The ODC assay is rapid, reliable, and inexpensive. Female Charles River CD-1 mice were obtained from Charles River Breeding Laboratories, Wilmington, MA, and were used when they were 7 to 9 weeks old. The dorsal hair of the mice was shaved 1 to 2 days before testing, and only mice showing no hair regrowth were used. A single dose of 12-*O*-tetradecanoylphorbol-13-acetate (TPA; 10.5 μg, 15 nmol) in 0.2 mL of acetone was applied topically to the back of each mouse. The synthetic retinoid, at one of three dose levels (1.7, 17, and 170 nmol), dissolved in 0.2 mL of acetone was applied 1 h before the TPA treatment to the test groups; the control group was treated with acetone alone. The mice were killed by cervical dislocation 5 h after TPA treatment. Determinations were done in triplicate.

The epidermis was obtained essentially as described by Raineri et al.[13a] To obtain sufficient material, we pooled the dorsal skins from three mice in each treatment group. The depilatory agent Nudit (Helena Rubinstein, NY) was applied to the shaved area of the skin; after 5 min, it was washed off thoroughly with cold tap water. Then the skin was excised and plunged immediately into ice-cold water; it was then placed in a 55 °C water bath for 30 s and reimmersed in ice-cold water for at least another 30 s. The skin was placed epidermis side up on a cold plate, and the epidermis was scraped off with a razor blade. The pooled epidermal sheets were homogenized (Polytron PT-10 homogenizer) at 0 to 4 °C for 15–20 s in 50 mM sodium phosphate buffer (pH 7.2) containing 0.1 mM pyridoxal phosphate and 0.1 mM EDTA, at a volume of 1 mL/skin.

The supernatant fraction remaining after centrifugation of the homogenate at 10000*g* for 30 min at 0 °C was used for the enzyme assay. Enzyme activity was determined by using the microassay for ODC as described by Verma et al.[13b] to measure the release of $^{14}CO_2$ from DL-[1-$^{14}$C]ornithine (58 mCi/mmol) after incubation with the 10000*g* supernatant. The incubations were carried out by decanting, with a Pasteur pipet, 100 μL of the supernatant containing 100 to 120 μg of protein into two or three 15-mL Corex tubes in a shaking water bath at 37 °C. The assay mixture in the tubes consisted of 50 μL of 100 mM sodium phosphate buffer (pH 7.2), 10 μL of 4 mM pyridoxal phosphate, 40 μL of 25 mM dithiothreitol, and 1 μL of 0.1 M EDTA. The center wells in the tubes were filled with 200 μL of a 2:1 solution (v/v) of ethanolamine–2-methoxyethanol. The reaction was started by adding 50 μL of substrate (0.5 μCi of DL-[1-$^{14}$C]ornithine in 2 mM cold ornithine) at 1-min intervals by injection to each of the stoppered tubes. Incubations were routinely carried out at 37 °C for 30 to 60 min. The reaction was stopped by addition of 0.5 mL of 2 M citric acid, and incubation was then continued for 1 h without heating to ensure complete absorption of $^{14}CO_2$.

We measured radioactivity by using a toluene-based scintillant (4 g of PPO and 50 mg of POPOP/L of toluene) in a Beckman LS-250 liquid scintillation counter. Enzyme activity was determined in triplicate and expressed as nanomoles of $CO_2$ released in 30 min per milligram of protein. Enzyme activity was linear for the protein concentration used. The protein concentrations of the epidermal extracts were determined by the Lowry procedure, with bovine serum albumin as the standard.

**Acknowledgment.** This work was supported in part by Public Health Service Grant CA30512 and Contracts N01-CP-05600 and N01-CP-05610, which were awarded by the Division of Cancer Cause and Prevention, National Cancer Institute, DHHS. This support is gratefully acknowledged. We thank Dr. Craig Barnes for running the 100- and 300-MHz NMR spectra and Dr. David Thomas for conducting mass spectral analyses.

**Registry No.** 1a, 77837-56-0; **1b**, 75664-66-3; **2a**, 86238-75-7; **2b**, 86238-76-8; **3a**, 86238-77-9; **3b**, 86238-78-0; **4a**, 86238-79-1; **4b**, 86238-80-4; **5a**, 86238-81-5; **5b**, 86238-82-6; **6**, 86238-83-7; **7a**, 86238-84-8; **7b**, 86238-85-9; **9(2)**, 86238-86-0; **9(3)**, 86238-87-1; **10(2)**, 86238-88-2; **10(3)**, 86238-89-3; **10(4)**, 86238-90-6; **10(5)**, 86238-91-7; **10(6)**, 86238-92-8; **11(4)**, 67808-65-5; **11(5)**, 22551-91-3; **11(6)**, 53574-57-5; **13(2)**, 86238-93-9; **13(3)**, 86238-94-0; **13(4)**, 86238-95-1; **13(5)**, 86238-96-2; **13(6)**, 86238-97-3; **15**, 26496-94-6;

16, 86238-98-4; 17, 86238-99-5; 3-fluoro-4-methylbenzoic acid, 350-28-7; ethyl 3-fluoro-4-methylbenzoate, 86239-00-1; ethyl 4-(dibromomethyl)benzoate, 26496-95-7; ethyl 4-(bromomethyl)-3-fluorobenzoate, 86239-01-2; methyl 3-methoxy-4-methylbenzoate, 3556-83-0; 3-hydroxy-4-methylbenzoic acid, 586-30-1; ethyl 4-(bromomethyl)-3-methoxybenzoate, 86239-02-3; ethyl 3-methoxy-4-methylbenzoate, 86239-03-4; β-ionyltriphenylphosphonium bromide, 66556-69-2; diethyl pyridine-2,5-dicarboxylate, 5552-44-3; 2-formyl-5-(hydroxymethyl)pyridine, 40749-33-5; 2-carbethoxy-5-(hydroxymethyl)pyridine, 50501-35-4; 4-carbethoxybenzyl fluoride, 86239-04-5; acetone, 67-64-1; 2,5-pyridinedicarboxylic acid, 100-26-5; β-ionone, 29-77-6.

# New Myocardial Imaging Agents: Stabilization of Radioiodine as a Terminal Vinyl Iodide Moiety on Tellurium Fatty Acids

F. F. Knapp, Jr.,*† M. M. Goodman,† A. P. Callahan,† L. A. Ferren,† G. W. Kabalka,‡ and K. A. R. Sastry‡

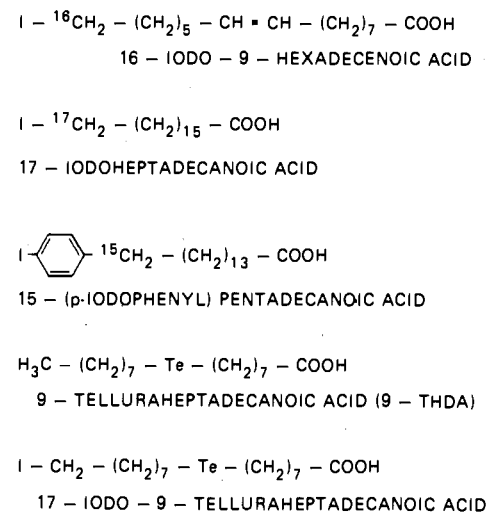
*Nuclear Medicine Group, Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, and Chemistry Department, University of Tennessee, Knoxville, Tennessee 37916.* *Received July 26, 1982*

To determine the myocardial uptake and retention properties of radioiodinated tellurium fatty acids, we prepared two new tellurium fatty acids in which iodine-125 has been chemically stabilized by attachment as a *trans*-vinyl iodide (I–CH=CH–R–Te–R′–COOH) and evaluated them in rats. Fabrication of 18-iodo-13-tellura-17-octadecenoic acid was accomplished by coupling 1,5-diiodo-1-pentene with sodium 12-(methoxycarbonyl)-*n*-dodecan-1-yl telluride. The [5-$^{125}$I]-1,5-diiodo-1-pentene was prepared by an organoborane technique involving $^{125}I^+$ treatment of 5-iodo-1-penten-1-ylboronic acid [$I(CH_2)_3CH{=}CHB(OH)_2$]. The absolute heart uptake of this agent was moderate (1.47–2.52% dose/g after 60 min), but the heart/blood ratios were low (~2.6:1). Only marginal in vivo deiodination occurred, since the thyroid uptake was low (15–18% dose/g after 60 min). The effect of tellurium in position 13 was unexpected. To determine if the low heart specificity and low heart/blood ratios were dependent upon the position of the tellurium, we prepared an analogue with the same chain length, 18-[$^{125}$I]iodo-7-tellura-17-octadecenoic acid, in the same manner by reaction of [11-$^{125}$I]-1,11-diiodo-1-undecene with sodium 6-(methoxycarbonyl)-*n*-hexan-1-yl telluride. This agent showed pronounced heart uptake (2.47–3.94% dose/g after 60 min) and prolonged retention (1.76–3.14% dose/g after 4 h) in rats. Furthermore, the heart/blood ratios remained high for several hours (13:1 after 60 min; 9:1 after 4 h). Iodine-123 labeled 18-iodo-7-tellura-17-octadecenoic acid is an attractive new compound for evaluation as a myocardial imaging agent.

Radioiodinated long-chain fatty acids are important agents for the clinical evaluation of regional myocardial perfusion and fatty acid metabolism.[1] 17-[$^{123}$I]Iodoheptadecanoic acid[2-5] and 16-[$^{123}$I]iodo-9-hexadecenoic acid[6,7] have been widely used as myocardial imaging agents (Chart I). Clinical studies with 16-[$^{123}$I]iodohexadecanoic acid have also been reported.[8,9] The problem of deiodination of these agents results in relatively rapid loss of radioactivity from the myocardium with accumulation of radioiodide in the thyroid and blood. Radioactivity in the blood pool interferes with the measurement of myocardial fatty acid uptake, so a correction method is required to account for blood levels of free radioiodide.[3-5] In order to overcome the problem of radioiodide loss, iodine has been chemically stabilized by attachment to the para position of the phenyl ring of 15-phenylpentadecanoic acid.[10-12] Tissue distribution studies in mice with 15-(*p*-[$^{123}$I]iodophenyl)pentadecanoic acid have shown that this agent is relatively stable to facile in vivo deiodination and shows moderate myocardial washout.[12,13] This agent has also been used in humans.[14]

A different strategy that has been studied involves the introduction of the tellurium heteroatom in the fatty acid to inhibit β-oxidation and "trap" the fatty acid in the myocardium.[15] Tellurium-123m labeled 9-telluraheptadecanoic acid (9-THDA) shows rapid and pronounced myocardial uptake in rats[15-17] and dogs.[18,19] The unique properties of 9-THDA and similar tellurium fatty acids are the prolonged myocardial retention and high heart/blood ratios. In order to take advantage of the more attractive radionuclidic properties of the iodine-123 radioisotope (13.3 h half-life) in comparison to tellurium-123m (119 days half-life), we have explored the development of radioiodinated fatty acids containing stable tellurium.[1,16] Evaluation in rats indicated that the myocardial uptake

Chart I. Structures of the Iodinated Long-Chain Fatty Acids




† Oak Ridge National Laboratory.
‡ University of Tennessee.

(1) Knapp, Jr., F. F.; Goodman, M. M.; Elmaleh, D. R.; Okada, R. D.; Strauss, H. W. In Proceedings of the International Symposium on the Developing Role of Short-Lived Radioisotopes in Clinical Nuclear Medical Practice, U.S. Department of Energy, Food & Drug Administration, in press.
(2) Machulla, H. J.; Stocklin, G.; Kupfernagel, W.; Freundlieb, Ch.; Hock, A.; Vyska, K.; Feinendegen, L. E. *J. Nucl. Med.* 1978, *19*, 298.
(3) Freundlieb, W.; Hock, A.; Vyska, K.; Feinendegen, L. E.; Machulla, H.-J.; Stocklin, G. *J. Nucl. Med.* 1980, *21*, 1043.
(4) Feinendegen, L. E.; Vyska, K.; Freundlieb, W.; Hock, A.; Machulla, H.-J.; Kloster, G.; Stocklin, G. *Eur. J. Nucl. Med.* 1981, *6*, 191.

0022-2623/83/1826-1293$01.50/0 