Inhibition of Papilloma Formation by Analogues of 7,8-Dihydroretinoic Acid

Y. Fulmer Shealy,* James M. Riordan, Jerry L. Frye, Linda Simpson-Herren, Brahma P. Sani, and Donald L. Hill†

Southern Research Institute, P.O. Box 55305, Birmingham, Alabama 35255-5305

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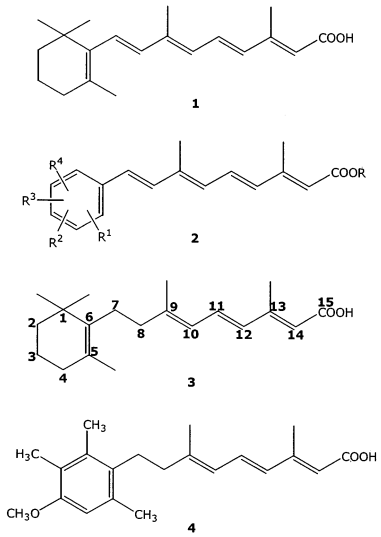
The design of analogues of 7,8-dihydroretinoic acid (7,8-dihydro-RA) was based on reported biological activities of this retinoid and its dihydro-TMMP[1] analogue and on structural hypotheses. 7-Oxa-7,8-dihydroretinoids (**5, 6**) were prepared by O-alkylation of phenoxides by methyl 8-bromo-3,7-dimethyl-2,4,6-octatrienoate. In some cases, C-alkylation also occurred. 7-Aza-8-oxo-7,8-dihydroretinoids (**12**, **13**) were synthesized from benzeneamines and the acyl cyano or bromo derivative of the monomethyl ester of 3,7-dimethyl-2,4,6-octatriene-1,8-dioic acid. These monomethyl ester precursors were synthesized from the known analogous aldehyde via an *O*-trimethylsilyl cyanohydrin. 7-(2,3,5-Trimethylphenoxy)-3,5-dimethyl-2,4,6-octatrienoic acid (**6b**) was the most active of the 7-oxa-7,8-dihydro-RAs in inhibiting DMBA-initiated and TPA-promoted mouse-skin papillomas. The ED_{50} was about 4-fold that of etretinate. Two additional 7-oxa-7,8-dihydro-RAs exhibited modest activity in the papilloma assay. Some of the 7-oxa-7,8-dihydro-RAs bind to CRABP and RARα.

Introduction and Rationale

In the type of retinoid analogues described in this report, a heteroatom occupies position 7 of the retinoid side chain, a methylene or carbonyl group occupies position 8, and an aromatic group replaces the cyclohexenyl group. The original rationale was based on both biological assay results and structural considerations. It is well-known that retinoic acid (RA, **1**) (Chart 1)and certain of its aromatic analogues (**2**) are highly active in inhibiting DMBA[1]-initiated and TPA[1]-promoted mouse-skin papillomas[2−4] and in reversing keratinization of cultured hamster tracheas.[5] It was reported, also, by Pawson et al.[6] that 7,8-dihydroretinoic acid (**3**) and the TMMP[1] analogue (**4**) of 7,8-dihydro-RA were moderately active in both the mouse-skin papilloma assay and the hamster-trachea organ-culture assay. Therefore, RA-activity was partially retained when the 7,8-double bond was saturated.[7]

When the 7,8-π bond of the normal (nonatetraene) side chain of a retinoid is replaced by a σ-7,8 bond, the resulting nonatriene side chain can rotate about the 7,8-bond. The side chain is more flexible and, consequently, has the capability of assuming various conformations; certain of these conformations might be more selective in fitting to binding sites of carrier proteins or retinoid receptors. On the other hand, the 7,8-region of the normal retinoid side chain is an electron-rich center by virtue of the π-electrons of the system of conjugated double bonds. When the 7,8-bond is saturated, as it is in 7,8-dihydro-RA, an electron-rich center is removed. The decreased electron density at the 7,8-bond could partly offset whatever advantage may result from greater flexibility of the side chain. If a heteroatom (O,N) is placed at position 7 of a 7,8-saturated side chain, an electron-rich center is reintroduced because of the nonbonding electrons of the oxygen or nitrogen atom. Therefore, placing a heteroatom at position 7 will combine the increased side-chain flexibility of the 7,8-saturated side chain with the high electron density of the normal retinoid side chain. Obviously, in these structures, there will be differences in the flexibility potential, i.e., differences in restriction to rotation about the 7,8-bond, depending on the individual structure. Restriction to rotation should be greater in 7-aza-8-oxo-7,8-dihydroretinoids (**12**, **13**, Scheme 1) than in the 7-oxa-7,8-dihydroretinoids (**5**, **6**, Scheme 2).

Chart 1



Chemistry

The 7-oxa-7,8-dihydroretinoids were prepared from the appropriate phenoxide (**10**) and methyl 8-bromo-3,7-dimethyl-2,4,6-octrienoate (**9**). Precursor **9** was obtained by reducing the aldehyde ester (**7**) (prepared by a known

* To whom correspondence should be addressed at Southern Research Institute, Drug Discovery Division, Birmingham, AL 35255-5305. Tel: 205-581-2000. Fax: 205-581-2870. E-mail: muglach@sri.org.
† Present address: University of Alabama at Birmingham, Birmingham, AL 35294.

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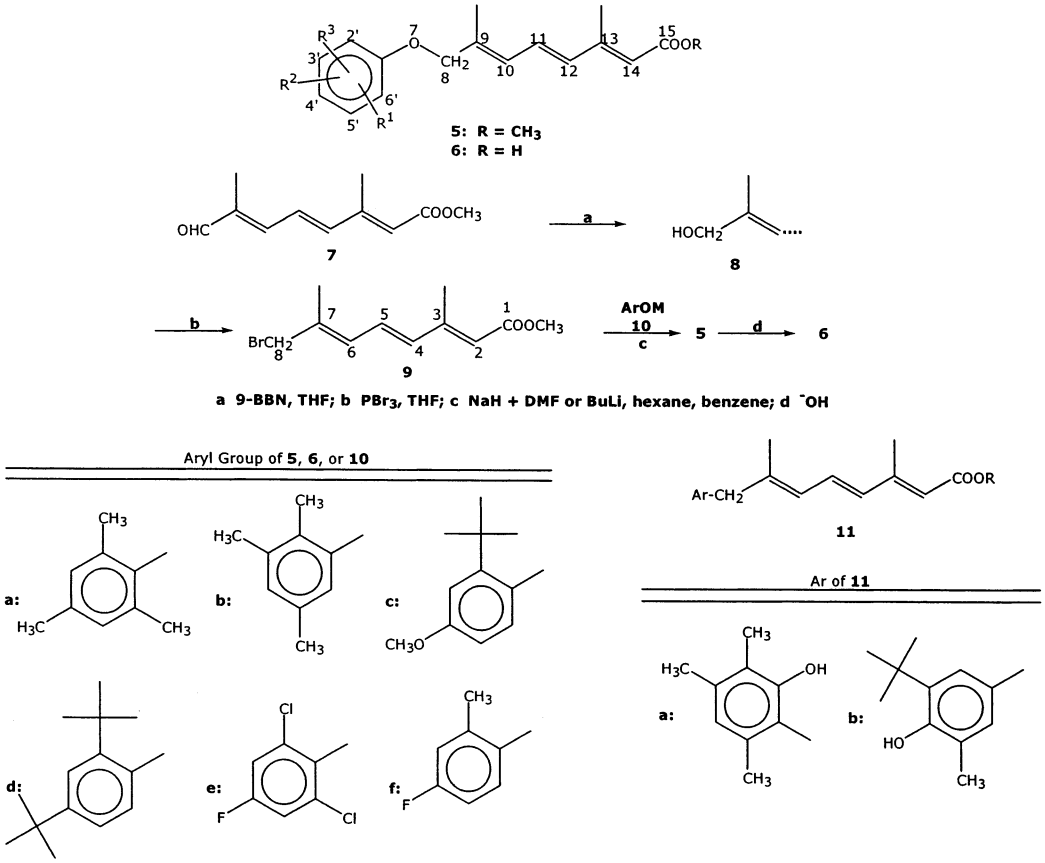
Scheme 1

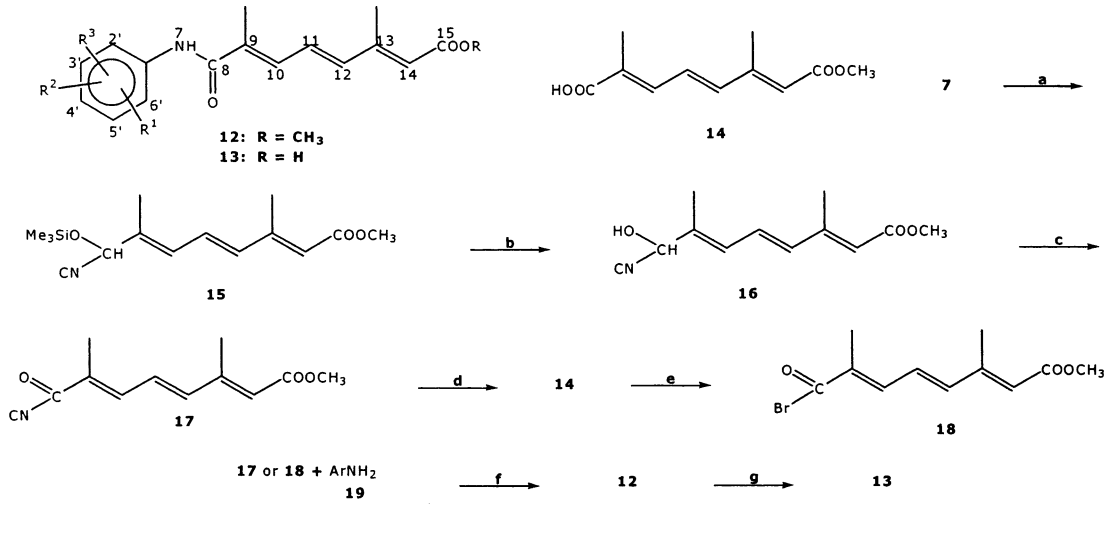


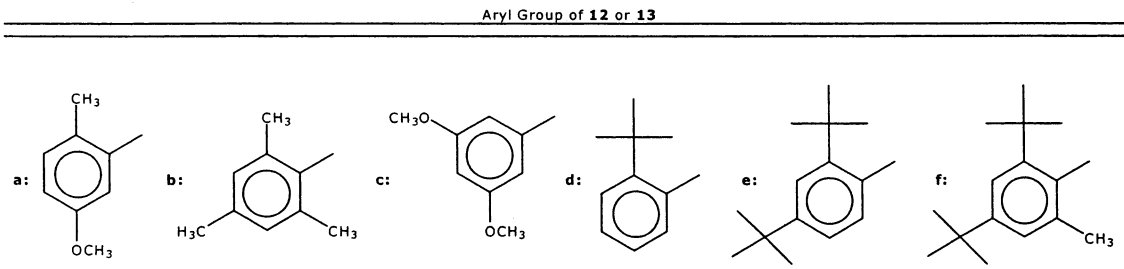
six-step synthesis route[8−10] from methyl 3-formyl-2-butenoate) to the corresponding alcohol (**8**) and converting the latter intermediate to **9** with phosphorus tribromide (Scheme 1). If an ortho or a para position of the phenol is open, C-alkylation or Claisen rearrangement might occur. Usually, alkylation of the phenoxides with the unsaturated bromomethyl compound (**9**) resulted principally in O-alkylation. If there was hindrance by an ortho substituent and if the other ortho or the para position was open, some C-alkylation occurred. The following examples illustrate O- and C-alkylation. Methyl 8-(2,3,5-trimethylphenoxy)-3,7-dimethyl-2,4,6-octatrienoate (**5b**), the O-alkylation product, was the major product obtained from sodium 2,3,5-trimethylphenoxide. HPLC analysis of a total reaction residue indicated that the ratio of the O-alkylation product (**5b**) to the C-alkylation product (**11a**, R = CH_3) was 9:1; small amounts of similar compounds, assumed to be cis-isomers, were also observed. (Small amounts of cis isomers might have originated at certain stages during the synthesis of side-chain synthons (**7**−**9**) from methyl 3-formyl-2-butenoate.) Alkylation of 2,4-bis(2-*tert*-butyl)phenoxide proceeded slowly; the only material isolated was the O-alkylation product (**5d**). Only C-alkylation (at the para position) was observed when 2-*tert*-butyl-6-methylphenoxide was alkylated (**11b**, R = CH_3).

Infrared spectra were consistent with structures **11** and **5**; the infrared spectra of the C-alkylation products included a strong OH-stretching band, whereas the O-alkylation products showed only CH-stretching bands in that region. *O*- and C-alkylation products were distinguished unequivocally by the proton NMR spectra ($CDCl_3$). The chemical shift of *O*-CH_2 of the O-alkylation products (**5**) appeared at δ 4−4.5, whereas the shift of CH_2 of the C-alkylation products (**11**) was at δ 3.4−3.5 and the OH shift was approximately δ 4.8. Also, **11a** had only one aromatic hydrogen at 6.63 ppm, and **11b** had two aromatic hydrogens, H-2′ and H-6′, at 6.91 and 6.78 ppm, respectively. In **11b**, the coupling between H-2′ and H-6′ of $^4J_{2',6'} = 2.1$ Hz confirmed that H-2′ and H-6′ are meta to each other and that the alkylation occurred at C-1′. For **11a**, the site of alkylation was confirmed by NOE's. The irradiation of the signals of 4′-CH_3 and 6′-CH_3 gave an enhancement of H-5′ of 9.6% and 10.2%, respectively. Similarly, irradiation of the signal H-8 showed an enhancement of the signals of 6′-CH_3 and 2′-OH of 2.7% and 7%, respectively, again showing that alkylation occurred at C-1′. The NMR, therefore, confirmed that the principal rearrangement is a [1,3]-sigmatropic shift rather than a [3,3] shift (Claisen rearrangement).[11,12]

The synthesis of 7-aza-8-oxo-7,8-dihydroretinoids (**12**, Scheme 2) requires a reactive derivative of the monoester (**14**) of 2,6-dimethyl-2,4,6-octatriene-1,8-dioic acid; reaction of such a derivative (acyl halide or *N*-acylimidazole) with an aromatic amine would furnish the desired retinoid analogues. Treatment of aldehyde-ester **7** with sodium cyanide and manganese dioxide or Ag_2O_2[13,14] did not afford significant amounts of **14**. However, an alternative route was postulated and brought to fruition. The route consists of treatment of **7** with trimethylsilyl cyanide to produce a trimethylsilyl cyanohydrin[15−17] (**15**), weakly acidic hydrolysis of **15** to cyanohydrin **16**, and oxidation of **16** (an allylic-type alcohol) with MnO_2 to acyl cyanide **17**. The latter

Scheme 2





compound proved to be sufficiently reactive to acylate most of the aromatic amines (**19**), but acylation of some of the representatives of **19** proceeded slowly. Therefore, some of the 7-aza-8-oxo-7,8-dihydroretinoates (**12**) were prepared by employing the acyl bromide (**18**), which was obtained by hydrolyzing **17** and converting the resulting **14** to **18**.

Biological Evaluation

Replacement of the trimethylcyclohexenyl group of the vitamin-A family of compounds with a substituted-benzene group relates the retinoids of this report to the aromatic retinoids,[3,4,18,19] many of which have high activity in the mouse-skin papilloma assay and in other retinoid assays. Methyl and methoxy groups on the benzene ring of the 7-hetero-7,8-dihydroretinoids were chosen to resemble the pattern of substituents on some of the aromatic retinoids (e.g., the TMMP analogues) and the cyclohexenyl group of RA; also, the *tert*-butyl group was selected because of its important influence on the activity of the "retinobenzoic acids" of Shudo and co-workers.[20,21]

The 7-oxa-7,8-dihydroretinoids (**6**) and three of the 7-aza-8-oxo-7,8-dihydroretinoids (**13**) were evaluated for inhibition of papilloma development in mouse skin. The retinoid assay developed and employed by Bollag and co-workers[2−4] was performed by applying DMBA in croton oil to a shaved area of the dorsal skin of mice and then administering a retinoid orally. Subsequently, Verma, Boutwell, and co-workers[22,23] modified the assay by using TPA (the most active tumor-promotor in croton oil) after initiating tumor development with DMBA and by applying the retinoid topically to the same shaved-skin area. The Verma−Boutwell modification was employed in dose−response papilloma assays of **6** and **13**.

Table 1. Mouse-Skin Papilloma Assay[a] of 8-(2,3,5-Trimethylphenoxy)-3,7-dimethyl-2,4,6-octatrienoic Acid (**6b**). Day 87[b]

treatment	no. of mice without tumors	no. of tumors/mouse: range	no. of tumors/mouse: mean no. ± SD	% of control
acetone	10/10			
acetone, DMBA, TPA	0/20	8−29	13.9 ± 6.2	
6b, nmol				
135.7	5/10	0−5	1.4 ± 2.0	10
45.9	4/10	0−15	5.0 ± 5.6	36
15.3	2/9	0−22	9.2 ± 6.8	66
5.1	2/10	0−29	12.4 ± 10.3	89
RA[c] 15.3	7/10	0−6	1.1 ± 2.0	8

[a] CD-1 Female mice. Tumor development was initiated with DMBA (0.2 μmol in 0.2 mL of acetone) and promoted 2x/week with TPA (10 nmol in acetone). Retinoids were applied in 0.2 mL of acetone. [b] Day 87 after the beginning of promotion, which began 14 days after initiation. [c] Data from the same assay in which **6b** was evaluated. In nine such assays that included representatives of **6** or **13**, the average of the mean nos. when 15.3 nmol of RA was applied was 2.4 tumors/mouse (18% of the mean nos. of control groups).

The most active dihydro-RA analogue was 8-(2,3,5-trimethylphenoxy)-3,5-dimethyl-2,4,6-octatrienoic acid (**6b**) (Table 1). This analogue effectively inhibited tumor development at the higher doses. The dose−response pattern was consistent, and significant numbers of treated mice were without tumors. At the highest dose (135.7 nmol) of **6b**, its activity was similar to that of RA at 15.3 nmol, and its ED_{50} (Table 2) was about 4-fold

Table 2. Biological Assays of 7-Hetero-7,8-dihydroretinoic Acids[a]

compound	mouse-skin papilloma assay[b], ED_{50}, nmol[c]	CRABP assay avg. % inhibition[d] (no. of assays)	RARα assay[e]
6a	60	40 (3)	30
6b	27	35 (3)	30
6c	>100	35 (2)	25
6d	>45.9[f]	10 (3)	5
6e	>100	50 (2)	ND
6f	~45.9[f]	15 (2)	0
etretinate	7.2[g]	NA	NA
RA	5.2, 3.5	100	100
13a	ND	0 (2)	ND
13b	>100	0 (3)	ND
13c	>45.9[f]	0 (3)	ND
13e	>100[h]	5−10 (3)	0
13f	ND	0 (2)	15
12e	>100	NA	NA

[a] ND = not determined. NA = not applicable. [b] Same as footnote *a* of Table 1. [c] Except as indicated, the ED_{50} was calculated from the results of dose−response assays that included 3 doses of 137.7, 45.9, and 15.3 nmol or four doses as shown for **6b** in Table 1. Duration of the assays was 83−89 days. [d] Percent inhibition of binding of tritiated retinoic acid by a 100-fold molar excess of unlabeled assayed retinoids. [e] Inhibition of binding of tritiated retinoic acid to RARα by a 200-fold molar excess of unlabeled retinoids. [f] One dose of 45.9 nmol. [g] Five doses. [h] Two doses, 137.7 and 45.9 nmol.

of that of etretinate, the well-known and highly active analogue of ethyl retinoate.[3,4,19] The 2,4,6-trimethylphenoxy analogue (**6a**) was modestly active (ED_{50}, 60 nmol), and the 2-methyl-4-fluorophenoxy analogue (**6f**) appeared to have modest activity at the only dose (45.9 nmol) at which it was tested (Table 2). None of the three 7-aza-8-oxo-dihydro-RA analogues (**13b**−**d**) inhibited papilloma development at the tested doses.

In assays for binding of the dihydro-RA analogues to cellular retinoic acid-binding protein (CRABP I), 7-oxa-7,8-dihydro-RA analogues **6a**,**b**,**c**,**e** exhibited moderate binding affinity and **6d** and **6f** were weak binders (Table 2). The representatives of **13** were without significant binding affinity for CRABP I. Retinoids **6a**,**b**,**c** also showed moderate binding to nuclear retinoic acid receptor RARα (Table 2).

Experimental Section

General Methods. All operations involved in the preparation, isolation, purification, and transfer of retinoids were performed in an atmosphere, or under a current, of nitrogen or argon. All such operations were also performed in dim light or photographic darkroom light and, insofar as possible, with containers wrapped with aluminum foil or with black cloths. All retinoids were stored in an atmosphere of argon or nitrogen in hermetically sealed containers at −20 °C or −80 °C. Commercial solutions of butyllithium were used. Chromatographic purifications were performed on columns of silica gel 60 or deactivated, neutral alumina. Deactivated alumina consisted of anhydrous neutral alumina and water mixed in proportions of 9:1.

Melting temperatures were determined in capillary tubes heated in a Mel-Temp apparatus. Ultraviolet spectra were recorded with a Perkin-Elmer Model Lambda 9 spectrophotometer. Mass spectral (MS) data were taken from fast-atom-bombardment spectra determined with a Varian MAT Model 311A double-focusing spectrometer; M = molecular ion. Some of the other peaks are identified as probable fragments, e.g., M minus a fragment. Infrared spectra were obtained from specimens in pressed potassium bromide disks and were recorded with a Nicolet Model 10MXE Fourier Transform IR spectrometer; vs = very strong, br = broad. Proton nuclear magnetic resonance spectra (^{1}H NMR) were determined at 300.635 MHZ and carbon-13 NMR spectra were determined at 75.602 MHz with a Nicolet Model NT 300 NB NMR spectrometer; tetramethylsilane was the internal reference. Assignments of chemical shifts are designated by the position numbers shown on structures **5** and **12**. For ^{1}H NMR data, the multiplicity, the number of hydrogens, and the positions are given parenthetically with each chemical shift. The positions of the hydrogens are shown as *H*X, and multiplicity is designated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, b = broad. For compounds represented by **5**, **6**, **12**, and **13**, X = positions 7−15 or 2′−6′. The position numbers of intermediates and **11** are the same as those of the names of those compounds. Thin-layer chromatography (TLC) was performed on plates of fluorescing silica gel, and developed plates were examined with UV lamps (254 nm). Typically, chloroform or dichloromethane was the developing solvent; chloroform−methanol was also used. High-pressure liquid chromatography (HPLC) was performed with components by Waters Associates systems and a Hewlett-Packard Model 3380-S integrator or with a Hewlett-Packard Model 1084B system. HPLC was performed on columns packed with octadecylsilylated silica (Spherisorb ODS), 5 μm particle size. Unless indicated otherwise, the eluting solvent was 85:15 acetonitrile−1% aqueous ammonium acetate, isocratic, 1 mL/min flow rate; and elution was monitored by UV absorption at the designated wavelengths.

7-Oxo-7,8-dihydroretinoids (5,6). The preparation of the 7-oxa-7,8-dihydroretinoates (**5**) is exemplified by the procedure for **5a**. Compounds **5b**−**f** were obtained by similar procedures; listed yields are based on the phenol. Similarly, the preparation of the dihydro-RA analogues (**6**) is exemplified by the procedure for **6a**. Reaction times and purification procedures for **5** and **6** were monitored by TLC. When the formula listed for elemental analysis includes water, the presence of water was confirmed by the ^{1}H NMR spectrum.

Methyl 3,7-Dimethyl-8-(2,4,6-trimethylphenoxy)-2,4,6-octatrienoate (5a). Heptane-washed sodium hydride (60 mg of 60% NaH in mineral oil = 36 mg, 1.5 mmol) was added slowly to a solution, in an ice bath, of 2,4,6-trimethylphenol (177 mg, 1.3 mmol) in DMF (3 mL), and the reaction mixture, under a stream of nitrogen, was stirred during 1 h. A solution of the bromomethyltrienyl ester (**9**, 360 mg, 1.4 mmol) in dry benzene (3 mL) was added to the phenoxide mixture, and the resulting mixture was stirred at 0−5 °C during 1.5 h. The reaction mixture was poured into a water−ice mixture, and the resulting mixture was extracted with ether (2×). The ether extract was washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure to a pale red oil that partially crystallized (wt. 400 mg; TLC, one spot plus several faint impurity spots). The crude product in chloroform was subjected to column chromatography on silica gel 60, and the best eluted fractions, determined by TLC, were combined and chromatographed in the same way. The product-containing fractions were concentrated under reduced pressure to a nearly colorless syrup that crystallized: yield, 170 mg (41.7%); mp 71−72 °C; HPLC (340 or 280 nm), 98%; MS *m*/*z* 315 (M + H), 283 (M − OCH_3), 179 (M − trimethylphenoxy), 135 (trimethylphenoxy), 119 (trimethylphenyl); IR (KBr disk, strong bands, cm^{-1}) 3070−2825 (CH, multiple bands), 1714 (C=O), 1607, 1485, 1436, 1344, 1236, 1226, 1211, 1148, 997, 952, OH absent in 3550−3350 region (cf. **11a**); UV (MeOH) λ_{max} 305 (ϵ 42600); ^{1}H NMR ($CDCl_3$) δ 6.91 (dd, 1H, *H*11, $J_{10,11}$ = 11.1 Hz, $J_{11,12}$ = 15.2 Hz), 6.82 (bs, 2H, *H*3′, *H*5′), 6.37 (d, 1H, *H*10, $J_{10,11}$ = 11.1 Hz), 6.31 (d, 1H, *H*12, $J_{11,12}$ = 15.2 Hz), 5.79 (bs, 1H, *H*14), 4.22 (bs, 2H, *H*8), 3.72 (s, 3H, $COOCH_3$), 2.36 (d, 3H, $13CH_3$, $J_{13-CH3,14}$ = 1.1 Hz), 2.24(s, 9H, $2'CH_3$, $4'CH_3$, $6'CH_3$), 1.99 (s, 3H, $9CH_3$). Anal. ($C_{20}H_{26}O_3$) C, H.

Methyl 3,7-dimethyl-8-(2,3,5-trimethylphenoxy)-2,4,6-octatrienoate (5b) was prepared from 2,3,5-trimethylphenol and **9**. The reaction mixture was stirred overnight and then poured into a water−ice mixture; the resulting mixture was extracted with ether. The solid residue obtained by concentrat-

ing the ether extract to dryness was recrystallized from a small amount of ether: yield, 27%; HPLC (340 or 254 nm), 99−100%.

HPLC (254 nm) of the total residue from the ether extract of another reaction mixture indicated that it consisted of 64.2% of **5b**, 3.4% of a similar compound assumed to be the 13-cis isomer of **5b**, 10.8% of the starting phenol, 6.9% of **11a**, 7.7% of a similar compound assumed to be a cis isomer of **11a**, and 7% of an unidentified compound assumed to be a contaminant of the starting phenol. This specimen was triturated with pentane and then recrystallized from acetonitrile: mp 116−117 °C; HPLC, 100% (340 or 254 nm); MS *m/z* 315 (M + 1), 283 (M − OCH_3), 179 (M − trimethylphenoxy), 135 (trimethylphenoxy), 119 (trimethylphenyl); IR (KBr disk, strong bands, cm^{-1}) 3050−2825 (CH, multiple bands), 1704 (C═O), 1603, 1590, 1433, 1359, 1320, 1240, 1163, 1148, 1121, 952, OH absent in 3550−3350 region (cf. **11a**); UV (MeOH) λ_{max} 305 (ϵ 41, 600); ^{1}H NMR ($CDCl_3$) δ 6.90 (dd, 1H, *H*11, $J_{10,11}$ = 11.1 Hz, $J_{11,12}$ = 15.2 Hz), 6.62 (s, 1H, *H*4′), 6.50 (s,1H, *H*6′), 6.31 (d, 1H, *H*10, $J_{10,11}$ = 11.1 Hz), 6.28 (d, 1H, *H*12, $J_{11,12}$ = 15.2 Hz), 5.79 (s, 1H, *H*14), 4.46 (bs, 2H, *H*8), 3.71 (s, 3H, COOCH_3), 2.35 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 1.1 Hz), 2.27(s, 3H, 5′CH_3), 2.24 (s, 3H, 2′CH_3), 2.14 (s, 3H, 3′CH_3), 1.95 (s, 3H, 9CH_3). Anal. ($C_{20}H_{26}O_3$·0.1H_2O) C, H.

Methyl 8-[2-(1,1-dimethylethyl)-4-methoxyphenoxy]-3,7-dimethyl-2,4,6-octatrienoate (5c) was prepared from 4-methoxy-2-*tert*-butylphenol and **9**, as described for **5a**, and was recrystallized from hexane: mp 73−74 °C; HPLC, 99.5% (254, 270, or 340 nm); MS *m/z* 358 (M), 327 (M − OCH_3), 179 (M − 4-methoxy-2-*tert*-butylphenoxy); UV (MeOH) λ_{max} 304 nm (ϵ 42100), 226 nm (ϵ 10000); ^{1}H NMR ($CDCl_3$) δ 6.90 (dd, 1H, *H*11, $J_{10,11}$ = 11.2 Hz, $J_{11,12}$ = 15.2 Hz and d, 1H, *H*3′, $J_{3',5'}$ = 3.0 Hz), 6.77 (d, 1H, *H*6′, $J_{5',6'}$ = 8.9 Hz), 6.66 (dd, 1H, *H*5′, $J_{5',6'}$ = 8.9 Hz, $J_{3',5'}$ = 3.0 Hz), 6.32 (d, 1H, *H*10, $J_{10,11}$ = 11.2 Hz), 6.27 (d, 1H, *H*12, $J_{11,12}$ = 15.2 Hz), 5.79 (s, 1H, *H*14), 4.48 (bs, 2H, *H*8), 3.77 (s, 3H, 4′OCH_3), 3.71 (s, 3H, COOCH_3), 2.35 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 1.2 Hz), 1.97 (s, 3H, 9CH_3), 1.39 (s, 9H, 2′C(CH_3)$_3$). Anal. ($C_{22}H_{30}O_4$) C, H.

Methyl 8-[2,4-bis(1,1-dimethylethyl)phenoxy]-3,7-dimethyl-2,4,6-octatrienoate (5d) was prepared from 2,4-bis-(*tert*-butyl)phenol and **9** and was recrystallized from acetonitrile−water: 22% yield; mp 108 °C; HPLC (340 or 254 nm), 100%; MS *m/z* 384 (M), 383 (M − H), 353 (M − OCH_3), 327 (M − *tert*-butyl), 179 (M − bis(*tert*-butyl)phenoxy); UV (MeOH) λ_{max} 305 nm (ϵ 42200), 223 nm (ϵ 11500); ^{1}H NMR ($CDCl_3$) δ 7.34 (d, 1H, *H*3′, $J_{3',5'}$ = 2.5 Hz), 7.16 (dd, 1H, *H*5′, $J_{3',5'}$ = 2.5 Hz, $J_{5',6'}$ = 8.5 Hz), 6.91(dd, 1H, *H*11, $J_{10,11}$ = 11.0 Hz, $J_{11,12}$ = 15.2 Hz), 6.77 (d, 1H, *H*6′, $J_{5',6'}$ = 8.5 Hz), 6.32 (bd, 1H, *H*10, $J_{10,11}$ = 11.0 Hz), 6.28 (d, 1H, *H*12, $J_{11,12}$ = 15.2), 5.79 (bs, 1H, *H*14), 4.51 (bs, 2H, *H*8), 3.71 (s, 3H, COOCH_3), 2.36 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 1.2 Hz), 1.98 (s, 3H, 9CH_3, $J_{9-CH3,10}$ = 1.0 Hz), 1.41 (bs, 9H, 2′C(CH_3)$_3$), 1.30 (s, 9H, 4′C(CH_3)$_3$). Anal. ($C_{25}H_{36}O_3$) C, H.

Methyl 8-(2,6-dichloro-4-fluorophenoxy)-3,7-dimethyl-2,4,6-octatrienoate (5e) was prepared from 2,6-dichloro-4-fluorophenol and **9**. The white solid obtained by concentrating the ether extract to a small volume was collected, washed with a small amount of ether, and dried: 62% yield; mp 125−127 °C; HPLC (254 or 340 nm), 99−99.5%; MS *m/z* 359 (M + H), 179 (M − dichlorofluorophenoxy); UV (EtOH) λ_{max} 304 nm (ϵ 42800); ^{1}H NMR ($CDCl_3$) δ 7.08 (d, 2H, *H*3′, *H*5′, $J_{3',4'-F} = J_{5',4'-F}$ = 7.8 Hz), 6.89 (dd, 1H, *H*11, $J_{10,11}$ = 11.0 Hz, $J_{11,12}$ = 15.2 Hz), 6.33 (d, 1H, *H*10, $J_{10,11}$ = 11.0 Hz), 6.32 (d, 1H, *H*12, $J_{11,12}$ = 15.2 Hz), 5.80 (s, 1H, *H*14), 4.44 (s, 2H, *H*8), 3.72 (s, 3H, COOCH_3), 2.35 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 1.1 Hz), 2.05 (d, 3H, 9CH_3, $J_{9-CH3,\ 10}$ = 1.0 Hz).

Methyl 8-(4-fluoro-2-methylphenoxy)-3,7-dimethyl-2,4,6-octatrienoate (5f) was prepared from 0.6 g of 4-fluoro-2-methylphenol and 1 g of crude **9** as described for **5b**. The yellow solid obtained by concentrating the ether extract was triturated with acetonitrile−water: yield, 0.5 g (34%); mp 76−77 °C; HPLC, 99.5% (254 nm), 98.7% (340 nm); UV (EtOH) λ_{max} 305 nm (ϵ 41, 100); ^{1}H NMR ($CDCl_3$) δ 6.93−6.83 (m, 2H, *H*11, *H*5′), 6.78 (dd, 1H, *H*3′, $J_{3',5'}$ = 3.1 Hz, $J_{3',4'-F}$ = 8.3 Hz), 6.70 (dd, 1H, *H*6′, $J_{4'-F,6'}$ = 4.7 Hz, $J_{5',6'}$ = 8.9 Hz), 6.28 (bd, 1H, *H*10, $J_{10,11}$ = 11.4 Hz and d, 1H, *H*12, $J_{11,12}$ = 15.0 Hz), 5.79 (bs, 1H, *H*14), 4.46 (s, 2H, *H*8), 3.71 (s, 3H, COOCH_3), 2.34 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 1.1 Hz), 2.25 (s, 3H, 2′CH_3), 1.94 (bs, 3H, 9CH_3). Anal. ($C_{18}H_{21}FO_3$) C, H.

Flash chromatography (silica gel 60-chloroform) of the residue from the trituration filtrate, and then trituration of the **5f** fraction with pentane afforded additional pure **5f** (170 mg; total yield, 46.9%).

3,7-Dimethyl-8-(2,4,6-trimethylphenoxy)-2,4,6-octatrienoic Acid (6a). A solution of **5a** (260 mg) in methanol (10 mL) and 1 N NaOH (1.5 mL) was boiled under reflux during 5 h and then concentrated under reduced pressure to remove methanol. The residue was dissolved in water (20 mL), the water solution was extracted with ether, and the aqueous layer was acidified to pH 3.5 with dilute HCl. A precipitate was collected by filtration, washed with water, and dried in vacuo (wt. 170 mg) and then recrystallized from acetonitrile: wt., 130 mg (52.4%); mp 168−169 °C; HPLC, 100% (254 or 340 nm); MS *m/z* 301 (M + H), 165 (M − trimethylphenoxy), 119 (trimethylphenyl); UV (MeOH) λ_{max} 303 nm (ϵ 39700); ^{1}H NMR ($CDCl_3$) δ 12.62 (bs, 1H, COO*H*), 6.96 (dd, 1H, *H*11, $J_{10,11}$ = 11.0 Hz, $J_{11,12}$ = 15.1 Hz), 6.83 (s, 2H, *H*3′, *H*5′), 6.39 (d, 1H, *H*10, $J_{10,11}$ = 11.0 Hz), 6.34 (d, 1H, *H*12, $J_{11,12}$ = 15.1 Hz), 5.82 (s, 1H, *H*14), 4.22 (s, 2H, *H*8), 2.37 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 0.9 Hz), 2.24 (s, 9H, 2′CH_3, 4′CH_3, 6′CH_3), 2.00 (bs, 3H, 9CH_3). Anal. ($C_{19}H_{24}O_3$) C, H.

3,7-Dimethyl-8-(2,3,5-trimethylphenoxy)-2,4,6-octatrienoic acid (6b) was obtained as described for **6a** except that the hydrolysis was allowed to proceed overnight. The precipitated acid (yield, 84%) was recrystallized from ethyl acetate: yield, 42%; mp 190−191 °C; HPLC, 100% (254 or 340 nm); MS *m/z* 301 (M + H), 300 (M), 165 (M − trimethylphenoxy); UV (MeOH) λ_{max} 302 nm (ϵ 38600); UV (EtOH)[24] λ_{max} 295 nm (ϵ 39100); ^{1}H NMR (Me_2SO-d_6) δ 12.04 (bs, 1H, COO*H*), 6.92 (dd, 1H, *H*11, $J_{10,11}$ = 11.0 Hz, $J_{11,12}$ = 15.2 Hz), 6.60 (bs, 1H, *H*4′), 6.58 (bs, 1H, *H*6′), 6.39 (d, 1H, *H*12, $J_{11,12}$ = 15.2 Hz), 6.30 (d, 1H, *H*10, $J_{10,11}$ = 11.0 Hz), 5.80 (s, 1H, *H*14), 4.50 (s, 2H, *H*8), 2.26 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 0.8 Hz), 2.20 (s, 3H, 5′CH_3), 2.17 (s, 3H, 2′CH_3), 2.06 (s, 3H, 3′CH_3), 1.91 (s, 3H, 9CH_3). Anal. ($C_{19}H_{24}O_3$) C, H.

8-[2-(1,1-Dimethylethyl)-4-methoxyphenoxy]-3,7-dimethyl-2,4,6-octatrienoic acid (6c) was obtained by hydrolysis of **5c**, and the acidified aqueous layer (cf. **6a**) was extracted with ether. The ether extract was washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure to a solid that was recrystallized from acetonitrile: HPLC, 98% (270 nm); UV (EtOH),[24] λ_{max} 293 nm (ϵ 41600), 228 nm (ϵ 10200); ^{1}H NMR ($CDCl_3$) δ 14.67 (bs, 1H, COO*H*), 6.95 (dd, 1H, *H*11, $J_{11,12}$ = 15.0 Hz, $J_{10,11}$ = 11.1 Hz), 6.90 (d, 1H, *H*3′, $J_{3',5'}$ = 3.0 Hz), 6.77 (d, 1H, *H*6′, $J_{5',6'}$ = 8.9 Hz), 6.66 (dd, 1H, *H*5′, $J_{5',6'}$ = 8.9 Hz, $J_{3',5'}$ = 3.0 Hz), 6.35 (d, 1H, *H*10, $J_{10,11}$ = 11.1 Hz), 6.30 (d, 1H, *H*12, $J_{11,12}$ = 15.0 Hz), 5.82 (bs, 1H, *H*14), 4.89 (s, 1H, *H*8), 3.77 (s, 3H, 4′OCH_3), 2.36 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 1.2 Hz), 1.98 (bs, 3H, 9CH_3), 1.39 (s, 9H, 2′C(CH_3)$_3$). Anal. ($C_{21}H_{28}O_4$·0.25 H_2O) C, H.

8-[2,4-Bis(1,1-dimethylethyl)phenoxy]-3,7-dimethyl-2,4,6-octatrienoic acid (6d) was obtained from **5d** as described for **6a** and **6c** and was recrystallized from acetonitrile−water: yield, 40%; mp 181−182 °C; HPLC, 100% (340 or 254 nm); MS *m/z* 371 (M + H), 370 (M), 353 (M − OH), 165 (M-bis(*tert*-butyl)phenoxy), 147; UV (MeOH) λ_{max} 301 (ϵ 38700), 224 nm (10900); ^{1}H NMR ($CDCl_3$) δ 12.95 (bs, 1H, COO*H*), 7.34 (d, 1H, *H*3′, $J_{3',5'}$ = 2.5 Hz), 7.16 (dd, 1H, *H*5′, $J_{3',5'}$ = 2.5 Hz, $J_{5',6'}$ = 8.5 Hz), 6.95 (dd, 1H, *H*11, $J_{10,11}$ = 11.0 Hz, $J_{11,12}$ = 15.2 Hz), 6.77 (d, 1H, *H*6′, $J_{5',6'}$ = 8.5 Hz), 6.35 (bd, 1H, *H*10, $J_{10,11}$ = 11.0 Hz), 6.29 (d, 1H, *H*12, $J_{11,12}$ = 15.2 Hz), 5.82 (bs, 1H, *H*14), 4.51 (bs, 2H, *H*8), 2.36 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 1.0 Hz), 1.99 (bs, 3H, 9CH_3), 1.41 (s, 9H, 2′C(CH_3)$_3$), 1.30 (s, 9H, 4′C(CH_3)$_3$). Anal. ($C_{24}H_{34}O_3$·0.2 H_2O) C; H: calcd. 9.16; found, 9.65.

8-(2,6-Dichloro-4-fluorophenoxy)-3,7-dimethyl-2,4,6-octatrienoic Acid (6e). A mixture of **5e**, methanol, and 1 N NaOH was boiled under reflux during 6.5 h, and methanol was evaporated under reduced pressure. Water was added to

the residue, and the mixture was filtered to remove a small amount of unchanged **5e** (12% recovery). The filtrate was acidified and **6e** was obtained and recrystallized as described for **6c**: HPLC, 98.5% (270 nm); UV (EtOH)[24] λ_{max} 294 nm (ϵ 41600); ^{1}H NMR ($CDCl_3$) δ 9.82 (bs, 1H, COO*H*), 7.08 (d, 2H, *H*3′, *H*5′, $J_{3',4'-F} = J_{5',4'-F} = 7.8$ Hz), 6.93 (dd, 1H, *H*11, $J_{10,11} = 10.9$ Hz, $J_{11,12} = 15.3$ Hz), 6.35 (d, 1H, *H*10, $J_{10,11} = 10.9$ Hz and d, 1H, *H*12, $J_{11,12} = 15.3$ Hz), 5.83 (s, 1H, *H*14), 4.45 (s, 2H, *H*8), 2.37 (d, 1H, 13CH_3, $J_{13-CH3,14} = 1.0$ Hz), 2.06 (d, 3H, 9CH_3, $J_{9-CH3,10} = 0.7$ Hz). Anal. ($C_{16}H_{15}Cl_2FO_3$).

8-(4-Fluoro-2-methylphenoxy)-3,7-dimethyl-2,4,6-octatrienoic acid (6f) was obtained from **5f** by the procedure described for **6c** and was recrystallized from ether: yield, 62%; mp 185−186 °C; HPLC, 98.3% (254 nm); UV (EtOH)[24] λ_{max} 293 nm (ϵ 38900); ^{1}H NMR ($CDCl_3$ + two drops of Me_2SO-d_6) δ 6.90−6.81 (m, 2H, *H*11, *H*5′), 6.78 (dd, 1H, *H*3′, $J_{3',5'} = 2.9$ Hz, $J_{3',4'-F} = 8.0$ Hz), 6.73 (dd, 1H, *H*6′, $J_{5',6'} = 8.8$ Hz, $J_{4'-F,5'} = 4.7$ Hz), 6.29 (d, 1H, *H*10, $J_{10,11} = 10.8$ Hz and d, 1H, *H*12, $J_{11,12} = 15.2$ Hz), 5.73 (bs, 1H, *H*14), 4.48 (s, 2H, *H*8), 3.02 (bs, COO*H* + H_2O from solvent) 2.32 (d, 3H, 13CH_3, $J_{13-CH3,14} = 1.1$ Hz), 2.25 (s, 3H, 2′CH_3), 1.95 (s, 3H, 9CH_3). Anal. ($C_{17}H_{19}FO_3$) C, H.

Methyl 7-formyl-3-methyl-2,4,6-octatrienoate (7) was prepared from methyl 3-formyl-2-butenoate by a six-step route (which was outlined by Pommer[8] and by Mayer and Isler[9]) and was recrystallized from cyclohexane: mp 89−90 °C; HPLC, 99.5−100% (340 or 254 nm); IR (KBr disk, strong bands, cm^{-1}) 1711 (vs), 1668 (vs), 1620 (vs), 1442, 1359, 1241, 1195, 1155 (vs), 1016, 963; UV (MeOH) λ_{max} 315 nm (ϵ 51900), 327 (sh, ϵ 46500), 228 nm (ϵ 3500).

Methyl 8-hydroxy-3,7-dimethyl-2,4,6-octatrienoate (8) was obtained by reduction of **7** with 9-borabicyclo[3.3.1]nonane in THF and purification of the crude product by chromatography on silica gel 60 with gradient elution by ethyl acetate−hexane: two mp were observed, 56−58 °C and 110−111 °C; IR (KBr disk, lower-melting form, strong and medium bands, cm^{-1}) 3350−3250 (OH), 1710, 1605, 1434, 1350, 1242, 1191, 1156 (vs), 1011, 964, higher-melting form similar; UV (MeOH) λ_{max} 306 nm (ϵ 37000). ^{1}H NMR ($CDCl_3$) δ 6.86 (dd, 1H, *H*5, $J_{5,6} = 11.2$ Hz, $J_{4,5} = 15.1$ Hz), 6.27 (d, 1H, *H*4, $J_{4,5} = 15.1$ Hz), 6.21 (bd, 1H, *H*6, $J_{5,6} = 11.2$ Hz), 5.78 (bs, 1H, *H*2), 4.14 (s, 2H, *H*8), 3.71 (s, 3H, COOCH_3), 2.34 (d, 3H, 3CH_3, $J_{3-CH3,2} = 1.1$ Hz), 1.87 (s, 3H, 7CH_3).

Methyl 8-Bromo-3,7-dimethyl-2,4,6-octatrienoate (9). Phosphorus tribromide (2 g, 0.7 mL) was added dropwise to a cold (5−10 °C) stirring solution of **8** (2 g, 10 mmol) in dry THF (100 mL) under a slow stream of N_2. The reaction mixture was stirred during 2.5 h and then poured into a water−ice mixture. The aqueous mixture was quickly extracted with ether (2×), and the ether solution was dried ($MgSO_4$) and concentrated to a yellow oil, wt., 2.6 g. This total product was stored under N_2 at −20 °C and was employed, without further purification, usually during the same or the following day for the preparation of representatives of **5**.

Methyl 8-(2-Hydroxy-3,4,6-trimethylphenyl)-3,7-dimethyl-2,4,6-octatrienoate (11a). A solution of *n*-butyllithium in hexane (1.6 M, 1.8 mL, 2.8 mmol) was added dropwise to a stirred solution of 354 mg (2.6 mmol) of 2,3,5-trimethylphenol in 20 mL of anhydrous benzene. The viscous mixture was stirred during 0.5 h under nitrogen and at 5 °C. A solution of 0.8 g (3 mmol) of **9** in 3 mL of benzene was added dropwise, and the reaction mixture was stirred during 20 h and then poured into cold water. The aqueous mixture was extracted with ether (2×), and the ether solution was washed with brine, dried, and concentrated to a solid: wt., 0.8 g; HPLC (340 nm), 87%. The crude product was triturated with pentane, and the resulting solid (0.4 g) was recrystallized from acetonitrile: mp 121−122 °C; HPLC, 100% (340 or 254 nm); UV (MeOH) λ_{max} 316 nm (ϵ 39, 300); IR (KBr disk, strong bands, cm^{-1}) 3510 (OH), 3070−2825 (CH, multiple bands), 1700 (C=O), 1609, 1570, 1442, 1434, 1356, 1244, 1203, 1162, 967; MS *m*/*z* 315 (M + H), 314 (M), 149 (2-hydroxy-3,4,6-trimethylphenyl-CH_2); ^{1}H NMR ($CDCl_3$) δ 6.85 (dd, 1H, *H*5, $J_{5,6} = 10.9$ Hz, $J_{4,5} = 15.1$ Hz), 6.63 (s, 1H, *H*5′), 6.13 (d, 1H, *H*4, $J_{4,5} = 15.1$ Hz), 5.86 (bd, 1H, *H*6, $J_{5,6} = 10.9$ Hz), 5.71 (bs, 1H, *H*2), 4.79 (s, 1H, 2′O*H*), 3.69 (s, 3H, OCH_3), 3.45 (bs, 2H, *H*8), 2.31 (d, 3H, 3CH_3, $J_{3-CH3,2} = 1.2$ Hz), 2.24 (s, 3H, 4′CH_3), 2.20 (s, 3H, 6′CH_3), 2.12 (s, 3H, 3′CH_3), 1.89 (d, 3H, 7CH_3, $J_{7-CH3,6} = 0.6$ Hz); ^{13}C NMR ($CDCl_3$) δ 167.50 (C=O), 152.91 and 152.48 (*C*3 and *C*2′), 141.55 (*C*7), 135.63 (*C*4′), 134.58 (*C*6′), 143.39 (*C*4), 130.44 (*C*5), 124.71 and 123.97 (*C*6 and *C*5′), 120.01 and 119.89 (*C*1′ and *C*3′), 118.01 (*C*2), 50.93 (- COOCH_3), 36.87 (C8), 19.93, 19.58 and 17.53 (3′CH_3, 4′CH_3, 6′CH_3), 17.14 (3CH_3), 13.77 (7CH_3). Anal. ($C_{20}H_{26}O_3$) C, H.

Methyl 8-[4-Hydroxy-3-(1,1-dimethylethyl)-5-methylphenyl]-3,7-dimethyl-2,4,6-octatrienoate (11b). A reaction mixture prepared (exactly as described for **5a**) from 2-*tert*-butyl-6-methylphenol (570 mg), DMF (5 mL), NaH (60%, 124 mg), **9** (800 mg), and benzene (5 mL) was allowed to warm to room temperature, stirred during 3 h, warmed at 35−40 °C during 2.5 h, and then poured into cold water. The aqueous mixture was extracted with ether (2×), washed with brine, dried ($MgSO_4$), and concentrated to a syrup that partially crystallized. The crude product was triturated with acetonitrile−water (3:1), and the resulting solid (227 mg) was recrystallized from acetonitrile−water and then from acetonitrile: mp 144−145 °C; HPLC, 98.6% (340 nm); MS *m*/*z* 343 (M + H), 342 (M), 179 (M − 2-*tert*-butyl-4-hydroxy-6-methylphenyl-CH_2), 147 (2-*t*.-butyl-4-hydroxy-6-methylphenyl); UV (EtOH) λ_{max} 315 nm (ϵ 41, 100); IR (KBr disk, strong bands, cm^{-1}) 3444 (OH), 2960, 2952, 1692 (vs), 1601, 1436, 1362, 1359, 1250, 1222, 1198, 1192, 1176, 1168, 1147, 972; ^{1}H NMR ($CDCl_3$) δ 6.91 (d, 1H, *H*2′, $J_{2',6'} = 2.1$ Hz), 6.86 (dd, 1H, *H*5, $J_{4,5} = 15.3$ Hz, $J_{5,6} = 11.0$ Hz), 6.78 (dq, 1H, *H*6′, $J_{2',6'} = 2.1$ Hz, $J_{6',5'-CH3} = 0.6$ Hz), 6.21 (d, 1H, *H*4, $J_{4,5} = 15.3$ Hz), 6.01 (bd, 1H, *H*6, $J_{5,6} = 11.0$ Hz), 5.76 (bs, 1H, *H*2), 4.64 (s, 1H, 4′O*H*), 3.70 (s, 3H, OCH_3), 3.30 (bs, 2H, *H*8), 2.33 (d, 3H, 3CH_3, $J_{3-CH3,2} = 1.1$ Hz), 2.22 (bs, 3H, 5′CH_3), 1.79 (d, 3H, 7CH_3, $J_{7-CH3,6} = 1.0$ Hz), 1.40 (s, 9H, 3′C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) δ 168.28 (C=O), 155.50 (*C*4′), 150.67 (*C*3), 144.31 (*C*7), 135.53 (*C*3′), 133.41 (*C*4), 131.76 (*C*5), 130.10 (*C*1′), 128.77 (*C*2′), 125.49 (*C*6), 125.67 (*C*6′), 122.97 (*C*5′), 117.02 (*C*2), 50.99 (COOCH_3), 46.10 (*C*8), 34.46 (*C*(CH_3)$_3$), 29.78 (C(CH_3)$_3$), 17.08, (3CH_3), 16.00 (3′CH_3), 14.07 (7CH_3). Anal. ($C_{22}H_{30}O_3$) C, H.

Methyl 7-[(5-Methoxy-2-methylphenyl)aminocarbonyl]-3-methyl-2,4,6-octatrienoate (12a). A solution of **17** (125 mg), 5-methoxy-2-methylbenzenamine (100 mg), anhydrous THF (5 mL), and triethylamine (0.2 mL) was stirred magnetically under N_2 in a stoppered flask at room temperature during 24 h. The reaction mixture was then concentrated in vacuo to a yellow solid that was triturated with ethyl acetate. The residual solid was collected and dried, wt. 126 mg. The filtrate residue in dichloromethane was subjected to flash chromatography on silica gel 60. The product-containing fractions yielded 30 mg. The two portions were combined and triturated with ether: yield of the dried solid, 143 mg (76%); mp 167−168 °C; ^{1}H NMR, **12a** containing a small amount of the 2Z (13-cis) isomer. The remainder of this specimen was recrystallized from acetonitrile: wt. 107 mg; HPLC (340 nm), 99.9%; MS *m*/*z* 330 (M + H), 298 (M − OCH_3); UV (EtOH) λ_{max} 314 nm (ϵ 44, 900); IR (KBr disk, strong bands, cm^{-1}) 1703 (ester CO), 1623 (amide I), 1603, 1584, 1523 (amide II), 1484, 1454, 1434, 1353, 1288, 1240, 1162, 1155, 1135, 1045, 966; ^{1}H NMR ($CDCl_3$) δ 7.72 (d, 1H, *H*6′, $J_{4',6'} = 2.7$ Hz), 7.34 (bs, 1H, 7N*H*), 7.12 (bd, 1H, *H*10, $J_{10,11} = 11.0$ Hz), 7.08 (bd, 1H, *H*3′, $J_{3',4'} = 8.5$ Hz), 6.89 (dd, 1H, *H*11, $J_{11,12} = 15.0$ Hz, $J_{10,11} = 11.0$ Hz), 6.65 (dd, 1H, *H*4′, $J_{4',6'} = 2.7$ Hz, $J_{3'4} = 8.5$ Hz), 6.56 (d, 1H, *H*12, $J_{11,12} = 15.0$ Hz), 5.90 (bs, 1H, *H*14), 3.81 (s, 3H, 5′OCH_3), 3.74 (s, 3H, − COOCH_3), 2.37 (d, 3H, 13CH_3, $J_{13-CH3,14} = 1.1$ Hz), 2.32 (s, 3H, 2′CH_3), 2.18 (d, 3H, 9CH_3, $J_{9-CH3,10} = 1.0$ Hz). Anal. ($C_{19}H_{23}NO_4$) C, H, N.

Methyl 3-Methyl-7-[(2,4,6-trimethylphenyl)aminocarbonyl]-2,4,6-octatrienoate (12b). The procedure was like that for **12a** except that the reaction mixture, under nitrogen and with a reflux condenser, was heated at 70 °C during 6 h and at 85 °C during 3 h and then was maintained in the range of 70−85 °C overnight. The mixture was concentrated in vacuo to a semisolid that was triturated twice with small portions

of ether. The undissolved solid (TLC, 1 spot, 39% yield) was recrystallized from acetonitrile: HPLC (1:1 MeCN−1% aqueous NH_4OAc, 340 nm), 99%; MS *m/z* 327 (M + H), 296 (M − OCH_3); UV (EtOH) λ_{max} 314 nm (ϵ 44600); ^{1}H NMR ($CDCl_3$) δ 7.12 (bd, 1H, *H*10, $J_{10,11}$ = 11.1 Hz), 6.95 (bs, 1H, 7N*H*), 6.91 (s, 2H, *H*3′ and *H*5′), 6.90 (dd, 1H, *H*11, $J_{10,11}$ = 11.1 Hz, $J_{11,12}$ = 15.3 Hz), 6.54 (d, 1H, *H*12, $J_{11,12}$ = 15.3 Hz), 5.89 (bs, 1H, *H*14), 3.73 (s, 3H, OCH_3), 2.37 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 1.1 Hz), 2.28 (s, 3H, 4′CH_3), 2.20 (s, 6H, 2′CH_3 and 6′CH_3), 2.18 (bs, 3H, 9CH_3). Anal. ($C_{20}H_{25}NO_3$) C, H, N.

Methyl 7-[(3,5-dimethoxyphenyl)aminocarbonyl]-3-methyl-2,4,6-octatrienoate (12c) was prepared according to the procedure outlined for **12a**. The reaction mixture was concentrated in vacuo to a solid that was recrystallized from ethyl acetate−hexane: yield, 61%; TLC, 1 spot; mp 122−123 °C; MS *m/z* 346 (M + H); UV (EtOH) λ_{max} 315 nm (ϵ 40100); IR (KBr disk, strong bands, cm^{-1}) 3295 (OCH_3, broad), 1719, 1645, 1604, 1583, 1552, 1455, 1420, 1292, 1259, 1237, 1204, 1160, 1152, 1066, 970, 834; ^{1}H NMR ($CDCl_3$) δ 7.39 (bs, 1H, 7N*H*), 7.04 (dq, 1H, *H*10, $J_{10,11}$ = 11.1 Hz, $J_{10,9-CH3}$ = 1.1 Hz), 6.87 (dd, 1H, *H*11, $J_{10,11}$ = 11.1 Hz, $J_{11,12}$ = 15.0 Hz), 6.83 (d, 2H, *H*2′, *H*6′, $J_{2',4'} = J_{4',6'}$ = 2.2 Hz), 6.54 (d, 1H, *H*12, $J_{11,12}$ = 15.0 Hz), 6.26 (t, 1H, *H*4′, $J_{2',4'} = J_{4',6'}$ = 2.2 Hz), 5.90 (bs, 1H, *H*14), 3.80 (s, 6H, 3′OCH_3, 5′OCH_3), 3.74 (s, 3H, COOCH_3), 2.37 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 1.2 Hz), 2.14 (d, 3H, 9CH_3, $J_{9-CH3,10}$ = 1.1 Hz). Anal. ($C_{19}H_{23}NO_5$) C, H, N.

Methyl 7-[[2-(1,1-Dimethylethyl)phenyl]aminocarbonyl]-3-methyl-2,4,6-octrienoate (12d). A reaction mixture prepared, as described for **12a**, from 2-(1,1-dimethylethyl)-benzenamine and **17** was heated under reflux overnight. The solid obtained from the reaction mixture residue was triturated with ether and then recrystallized from acetonitrile: HPLC (7:3 MeCN−1% aqueous NH_4OAc, 340 nm), 99.8%; MS *m/z* 342 (M + H), 326 (M − CH_3), 310 (M − OCH_3), 193 (M − *tert*-butylphenylamino); UV (EtOH) λ_{max} 314 nm (ϵ 43000); IR (KBr disk, strong bands, cm^{-1}) 3309, 1712, 1649, 1612, 1599, 1500, 1483, 1442, 1390, 1288, 1239, 1160, 970; ^{1}H NMR ($CDCl_3$) δ 7.76 (dd, 1H, *H*6′, $J_{4',6'}$ = 1.7 Hz, $J_{5',6'}$ = 7.9 Hz), 7.55 (bs, 1H, N*H*), 7.41 (dd, 1H, *H*3′, $J_{3',5'}$ = 1.7 Hz, $J_{3',4'}$ = 7.9 Hz), 7.25 (dt, 1H, *H*5′, $J_{4',5'}$ = 7.8 Hz, $J_{5',6'}$ = 7.9 Hz, $J_{3',5'}$ = 1.7 Hz), 7.17 (dt, 1H, *H*4′, $J_{4',6'}$ = 1.7 Hz, $J_{4',5'}$ = 7.8 Hz, $J_{3',4'}$ = 7.9 Hz), 7.16 (bd, 1H, *H*10, $J_{10,11}$ = 11.1 Hz), 6.91 (dd, 1H, *H*11, $J_{10,11}$ = 11.1 Hz, $J_{11,12}$ = 15.0 Hz), 6.56 (d, 1H, *H*12, $J_{11,12}$ = 15.0 Hz), 5.90 (bs, 1H, *H*14), 3.74 (s, 3H, OCH_3), 2.37 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 1.1 Hz), 2.19 (d, 3H, 9CH_3, $J_{9-CH3,10}$ = 0.9 Hz), 1.43 (s, 9H, 2′$C(CH_3)_3$). Anal. ($C_{21}H_{27}NO_3 \cdot {}^3/_4CH_3CN$) C, H, N.

Methyl 7-[[2,4-Bis(1,1-dimethylethyl)phenyl]aminocarbonyl]-3-methyl-2,4,6-octatrienoate (12e). 2,4-Bis(1,1-dimethylethyl)benzenamine was prepared as described by Keidel et al.[25] from the corresponding nitrobenzene.[26] Compound **12e** was obtained from the benzenamine and **17** as described for **12a** except that the reaction mixture was boiled under reflux during 6.5 h. The solid obtained by concentrating the reaction mixture in vacuo was triturated with small amounts of acetonitrile, and the residue (57% yield) was recrystallized from the same solvent: HPLC (7:3 MeCN−1% aq NH_4OAc, 340 nm), 99.5%; UV (EtOH) λ_{max} 314 nm (ϵ 47000); IR (KBr disk, strong bands cm^{-1}) 3255 (broad), 2961, 1721, 1648, 1601, 1507, 1361, 1290, 1244, 1237, 1162, 963; ^{1}H NMR ($CDCl_3$) δ 7.53 (d, 1H, *H*6′, $J_{5',6'}$ = 8.2 Hz), 7.46 (bs, 1H, N*H*), 7.43 (d, 1H, *H*3′, $J_{3',5'}$ = 2.2 Hz), 7.27 (dd, 1H, *H*5′, $J_{3',5'}$ = 2.2 Hz, $J_{5',6'}$ = 8.2 Hz), 7.15 (bd, 1H, *H*10, $J_{10,11}$ = 11.4 Hz), 6.90 (dd, 1H, *H*11, $J_{10,11}$ = 11.4 Hz, $J_{11,12}$ = 15.0 Hz), 6.55 (d, 1H, *H*12, $J_{11,12}$ = 15.0 Hz), 5.90 (bs, 1H, *H*14), 3.73 (s, 3H, COOCH_3), 2.37 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 1.1 Hz), 2.18 (s, 3H, 9CH_3), 1.43 (s, 9H, 2′$C(CH_3)_3$), 1.32 (s, 9H, 4′$C(CH_3)_3$). Anal. ($C_{25}H_{35}NO_3$) C, N; H: calcd. 8.87; found 9.28.

Methyl 7-[[2,4-Bis(1,1-dimethylethyl)-6-methylphenyl]-aminocarbonyl]-3-methyl-2,4,6-octatrienoate (12f). 2,4-Bis(1,1-dimethylethyl)-6-methylbenzenamine was prepared as described by Keidel et al.[25] from the corresponding nitrobenzene.[26] Ester-acid **14** (40 mg, 0.19 mmol) was suspended in benzene (5 mL), phosphorus tribromide (40 mg, 0.15 mmol) was added, and the mixture was warmed to effect solution and was stirred during 1.5 h. A solution of the benzenamine (40 mg, 0.18 mmol) in DMF (3 mL) was added to the acid bromide (**18**) solution, and the resulting mixture was stirred at room temperature during 2 h and at 50 °C during 2 h. The reaction mixture was poured into water, the aqueous mixture was extracted with ether, and the ether layer was washed with brine, dried ($MgSO_4$), and concentrated to an oil that crystallized (yield 38 mg). The solid was triturated with acetonitrile and then with ether: yield, 28 mg (36%); mp 185−186 °C; HPLC (7:3 MeCN − 1% aqueous NH_4OAc, 340 nm), 100%; MS *m/z* 412 (M + H), 396 (M − CH_3), 380 (M − OCH_3), 356 (M − $Me_2C{=}CH_2$); UV (EtOH) λ_{max}, 314 nm (ϵ 46700); IR (KBr disk, strong bands, cm^{-1}) 3314, 2956, 1717, 1701, 1649, 1608, 1600, 1497, 1435, 1361, 1280 sh, 1275, 1238, 1225, 1165, 1151, 974; ^{1}H NMR ($CDCl_3$) δ 7.31 (d, 1H, *H*3′, $J_{3',5'}$ = 2.1 Hz), 7.16 (d, 1H, *H*5′, $J_{3',5'}$ = 2.1 Hz), 7.14 (bd, 1H, *H*10, $J_{10,11}$ = 11.3 Hz), 7.10 (bs, 1H, N*H*), 6.91 (dd, 1H, *H*11, $J_{10,11}$ = 11.3 Hz, $J_{11,12}$ = 15.2 Hz), 6.56 (d, 1H, *H*12, $J_{11,12}$ = 15.2 Hz), 5.89 (bs, 1H, *H*14), 3.73 (s, 3H, OCH_3), 2.38 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 0.9 Hz), 2.20 (bs, 6H, 6′CH_3, 9CH_3), 1.38 (s, 9H, 2′$C(CH_3)_3$), 1.31 (s, 9H, 4′$C(CH_3)_3$). Anal. ($C_{26}H_{37}NO_3 \cdot 1/3\ H_2O$) C, H, N.

7-[(5-Methoxy-2-methylphenyl)aminocarbonyl]-3-methyl-2,4,6-octatrienoic Acid (13a). A solution of **12a** (80 mg), methanol (5 mL), and 1 N sodium hydroxide (3 mL) was boiled under reflux during 4.5 h and then concentrated in vacuo to a solid. The residue was dissolved in water, the solution was extracted with ether, and the aqueous layer was acidified to pH 3 and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and concentrated to a pale yellow solid [yield, 72 mg (93.5%); HPLC (340 nm), 100%] that was recrystallized from acetonitrile: mp 191−192 °C; UV (EtOH)[24] λ_{max} 312 nm (ϵ 43100); IR (KBr disk, strong bands, cm^{-1}) 3360, 3350, 2920, 1697, 1650, 1618, 1601, 1587, 1530, 1491, 1457, 1442, 1418, 1297, 1252, 1239, 1187, 1163, 1036, 966; ^{1}H NMR (Me_2SO-d_6) δ 12.3 (bs, 1H, COO*H*), 9.39 (s, 1H, N*H*), 7.12 (d, 1H, *H*3′, $J_{3',4'}$ = 8.4 Hz), 7.05 (dq, 1H, *H*10, $J_{10,11}$ = 11.3 Hz, $J_{10,9-CH3}$ = 1.1 Hz), 6.97 (dd, 1H, *H*11, $J_{10,11}$ = 11.3 Hz, $J_{11,12}$ = 14.3 Hz), 6.93 (d, 1H, *H*6′, $J_{4',6'}$ = 2.7 Hz), 6.72 (dd, 1H, *H*4′, $J_{4',6'}$ = 2.7 Hz, $J_{3',4'}$ = 8.4 Hz), 6.68 (d, 1H, *H*12, $J_{11,12}$ = 14.3 Hz), 5.92 (bs, 1H, *H*14), 3.71 (s, 3H, 5′OCH_3), 2.29 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 0.7 Hz), 2.11 (s, 3H, 2′CH_3), 2.09 (s, 3H, 9CH_3). Anal. ($C_{18}H_{21}NO_4$) C, H, N.

3-Methyl-7-[(2,4,6-trimethylphenyl)aminocarbonyl]-2,4,6-octratrienoic Acid (13b). A mixture of **12b** (125 mg), methanol (10 mL), and 1 N sodium hydroxide (5 mL) was heated at 60 °C during 2 h, boiled under reflux during 8 h, and then concentrated to remove methanol. Additional water was added to the aqueous residue, and the solution was filtered and acidified to pH 3. A precipitate was collected, dried, and recrystallized from acetonitrile: yield, 110 mg (91.6%); mp 248−250 °C dec; HPLC (1:1 MeCN − 1% aq. NH_4OAc, 340 nm), 100%; MS *m/z* 314 (M + H), 179 (M − trimethylphenyl − NH); UV (EtOH)[24] λ_{max} 310 nm (ϵ 45500); IR (KBr disk, strong bands) 3266, 2915, 1701, 1639, 1623, 1599, 1585, 1505, 1280, 1258, 1238, 1191, 962; ^{1}H NMR (Me_2SO-d_6) δ 12.18 (bs, 1H, COO*H*), 9.17 (s, 1H, N*H*), 7.04 (d, 1H, *H*10, $J_{10,11}$ = 11.3 Hz), 6.97 (dd, 1H, *H*11, $J_{10,11}$ = 11.3 Hz, $J_{11,12}$ = 14.7 Hz), 6.89 (s, 2H, *H*3′ and *H*5′), 6.66 (d, 1H, *H*12, $J_{11,12}$ = 14.7 Hz), 5.91 (s, 1H, *H*14), 2.30 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 0.9 Hz), 2.23 (s, 3H, 4′CH_3), 2.08 (s, 9H, 2′CH_3, 6′-CH_3, 9CH_3). Anal. ($C_{19}H_{23}NO_3$) C, H, N.

7-[(3,5-Dimethoxyphenyl)aminocarbonyl]-3-methyl-2,4,6-octatrienoic acid (13c) was obtained from **12c** by a procedure similar to that described for **13b**: yield, 66%; HPLC (1:1 MeCN−1% aq NH_4OAc, 340 nm), 99.8%; UV (EtOH)[24] λ_{max} 315 nm (ϵ 40200); MS *m/z* 332 (M + H); ^{1}H NMR (Me_2SO-d_6) δ 12.22 (bs, 1H, COO*H*), 9.71 (bs, 1H, N*H*), 7.00 (d, 2H, *H*2′, *H*6′, $J_{2',3'} = J_{3',6'}$ = 2.2 Hz), 6.96 (m, 2H, *H*10, *H*11), 6.68 (m, 1H, *H*12), 6.21 (t, 1H, *H*4′, $J_{2',3'} = J_{4',6'}$ = 2.2 Hz), 5.91 (s, 1H, *H*14), 3.71 (s, 6H, 3′OCH_3, 5′OCH_3), 2.30 (d, 3H, 13CH_3, $J_{13-CH3,14}$ = 1.1 Hz), 2.07 (s, 3H, 9CH_3). Anal. ($C_{18}H_{21}NO_5$) C, H, N.

7-[[2,4-Bis(1,1-dimethylethyl)phenyl]aminocarbonyl]-3-methyl-2,4,6-octatrienoic acid (13e) was obtained from

12e by a procedure similar to that described for **13a**. The crude product was recrystallized from acetonitrile, a solution of the solid in dichloromethane was filtered through a short column silica gel 60, and the eluate was concentrated to a crystalline residue: yield, 58%; mp 213-215 °C dec; HPLC (7:3 CH_3CN − 1% aq NH_4OAc, 340 nm), 99%; UV (EtOH)[24] λ_{max} 311 nm (ϵ 43200); IR (KBr, strong bands, cm^{-1}) 3280, 2959, 2927, 2868, 1691, 1667, 1640, 1621, 1602, 1588, 1512, 1480, 1442, 1350, 1290, 1265, 1252, 1243, 1192, 1167, 961; ^1H NMR ($CDCl_3$) δ 7.53 (d, 1H, *H*6′, $J_{5',6'}$ = 8.3 Hz), 7.47 (s, 1H, N*H*), 7.43 (d, 1H, *H*3′, $J_{3',5'}$ = 2.2 Hz), 7.27 (dd, 1H, *H*5′, $J_{5',6'}$ = 8.3 Hz, $J_{3',5'}$ = 2.2 Hz), 7.16 (bd, 1H, *H*10, $J_{10,11}$ = 11.3 Hz), 6.95 (dd, 1H, *H*11, $J_{10,11}$ = 11.3 Hz, $J_{11,12}$ = 15.1 Hz), 6.58 (d, 1H, *H*12, $J_{11,12}$ = 15.1 Hz), 5.93 (s, 1H, *H*14), 2.39 (d, 3H, 13C*H*$_3$, $J_{13-CH3,14}$ = 0.7 Hz), 2.19 (s, 3H, 9C*H*$_3$), 1.43 (s, 9H, 2′C(C*H*$_3$)$_3$), 1.32 (s, 9H, 4′C(C*H*$_3$)$_3$). Anal. ($C_{24}H_{33}NO_3$) C, H, N.

7-[[2,4-Bis(dimethylethyl)-6-methylphenyl]aminocarbonyl]-3-methyl-2,4,6-octatrienoic acid (13f) was obtained by a procedure similar to that described for **13a**: yield, 80%; HPLC (7:3 CH_3CN−1% aq NH_4OAc, 340 or 254 nm), 100%; MS *m*/*z* 398 (M + H), 342 (M − $Me_2C{=}CH_2$); UV (EtOH)[24] λ_{max} 309 nm (ϵ 44500); IR (KBr disk, strong bands, cm^{-1}) 3315, 2966, 2931, 2868, 1689, 1650, 1600, 1502, 1444, 1362, 1287, 1247, 1185, 968; ^1H NMR ($CDCl_3$) δ 7.31 (d, 1H, *H*3′, $J_{3',5'}$ = 2.3 Hz), 7.16 (d, 1H, *H*5′, $J_{3',5'}$ = 2.3 Hz), 7.15 (d, 1H, *H*10, $J_{10,11}$ = 11.4 Hz), 7.12 (s, 1H, N*H*), 6.95 (dd, 1H, *H*11, $J_{10,11}$ = 11.4 Hz, $J_{11,12}$ = 15.1 Hz), 6.59 (d, 1H, *H*12, $J_{11,12}$ = 15.1 Hz), 5.92 (bs, 1H, *H*14), 2.39 (d, 3H, 13C*H*$_3$, $J_{13-CH3,14}$ = 1.0 Hz), 2.20 (s, 6H, 9C*H*$_3$, 6′C*H*$_3$), 1.39 (s, 9H, 2′C(C*H*$_3$)$_3$), 1.31 (s, 9H, 4′C(C*H*$_3$)$_3$). Anal. ($C_{25}H_{35}NO_3$·0.1 CH_3CN) H, N; C: calcd, 75.35; found, 74.94.

7-Methoxycarbonyl-2,6-dimethyl-2,4,6-heptatrienoic acid (14) was obtained by hydrolyzing the acyl cyanide (**17**) in a mixture of ether and aqueous sodium bicarbonate that was shaken in a separatory funnel. The aqueous layer was separated and acidified to pH 3. A precipitate was collected by filtration and dried: mp 160−161 °C; MS *m*/*z* 211 (M + H), 193 (M − OH), 179 (M − OCH_3); UV (EtOH) λ_{max} 313 nm (ϵ 41400), 231 (ϵ 4300); IR (KBr disk, strong bands, cm^{-1}) 3150−2750 broad, 2953, 1718, 1684, 1618, 1436, 1425, 1360, 1295, 1246, 1189, 1166, 972; ^1H NMR ($CDCl_3$) δ 7.38 (bd, 1H, *H*3, $J_{3,4}$ = 11.3 Hz), 6.87 (dd, 1H, *H*4, J $_{3,4}$ = 11.3 Hz, $J_{4,5}$ = 15.1 Hz), 6.59 (d, 1H, *H*5, $J_{4,5}$ = 15.1 Hz), 5.92 (bs, 1H, *H*7), 3.74 (s, 3H, 7COOC*H*$_3$), 2.36 (d, 3H, 6C*H*$_3$, $J_{6-CH3,7}$ = 1.2 Hz), 2.04 (d, 3H, 2C*H*$_3$, $J_{2-CH3,3}$ = 1.3 Hz). Anal. ($C_{11}H_{10}O_4$·1/6 H_2O) C, H.

Methyl 8-Cyano-8-(trimethylsilyloxy)-3,7-dimethyl-2,4,6-octatrienoate (15). Trimethylsilyl cyanide (3.65 mL, 27 mmol) was added to a solution of aldehyde **7** (5.3 g., 27 mmol) in dry acetonitrile (50 mL) containing a few milligrams of zinc iodide.[15] The reaction solution was stirred under an atmosphere of nitrogen at room temperature during 2.5 h, treated with charcoal, filtered through Celite, and concentrated to a syrup that was dried in vacuo: yield, 7.6 g (95%); MS *m*/*z* 294 (M + H), 267 (M − CN), 262 (M − OCH_3), 235 (M − $COOCH_3$ + H), 75 (Me_3Si); ^1H NMR ($CDCl_3$) δ 6.78 (dd, 1H, *H*5, $J_{4,5}$ = 15.1 Hz, $J_{5,6}$ = 11.0 Hz), 6.37 (d, 1H, *H*4, $J_{4,5}$ = 15.1 Hz), 6.32 (bd, 1H, *H*6, $J_{5,6}$ = 11.0 Hz), 5.83 (s, 1H, *H*2), 4.86 (s, 1H, *H*8), 3.73 (s, 1H, COOC*H*$_3$), 2.33 (d, 3H, 3C*H*$_3$, $J_{3-CH3,2}$ = 1.2 Hz), 1.95 (d, 3H, 7C*H*$_3$, $J_{7-CH3,6}$ = 1.3 Hz), 0.23 (s, 9H, −Si(C*H*$_3$)$_3$). Very weak peaks in the ^1H NMR spectrum indicated that a small amount of the 2,3-cis isomer was present. This material was used without further purification for the preparation of **16**.

Methyl 8-Cyano-8-hydroxy-3,7-dimethyl-2,4,6-octatrienoate (16). Compound **15** (6 g 20.4 mmol) was suspended in 3 N HCl (80 mL), and the mixture was stirred under a nitrogen atmosphere during 2 h at 50−60 °C. A pale yellow solid was collected by filtration, washed with water, and dried in vacuo: yield, 4.2 g (93%); MS *m*/*z* 222 (M + H), 204 (M − OH), 195 (M − CN), 190 (M − OCH_3), 172 (M − $COOCH_3$). A portion of the product was triturated with small portions of pentane (3×), and the residual white solid was recrystallized from ethyl acetate−hexane: UV (EtOH) λ_{max} 305 nm (ϵ 50, 900), 227 nm (ϵ 3700); IR (KBr disk, strong bands, cm^{-1}) 3355 (broad, OH), 1689 (ester C═O), 1598 (ester $COCH_3$), 1251, 1168, 1145, 1092, 975, weak 2240 (CN); ^1H NMR ($CDCl_3$) δ 6.77 (dd, 1H, *H*5, $J_{4,5}$ = 15.1 Hz, $J_{5,6}$ = 11.0 Hz), 6.43 (bd, 1H, *H*6, $J_{5,6}$ = 11.0 Hz), 6.39 (d, 1H, *H*4, $J_{4,5}$ = 15.1 Hz),), 5.84 (s, 1H, *H*2), 4.95 (d, 1H, *H*8, $J_{8,8-OH}$ = 6.3 Hz), 3.73 (s, 3H, COOC*H*$_3$), 2.59 (d, 1H, 8O*H*, $J_{8,8-OH}$ = 6.3 Hz), 2.33 (d, 3H, 3C*H*$_3$, $J_{3-CH3,2}$ = 1.1 Hz), 2.00 (d, 3H, 7C*H*$_3$, $J_{7-CH3,6}$ = 1.0 Hz). Anal. ($C_{12}H_{15}NO_3$) C, H, N.

Methyl 7-(Cyanocarbonyl)-3-methyl-2,4,6-octatrienoate (17). Activated MnO_2 (7.5 g) was added to a solution of **16** (2.3 g, 10.4 mmol) in tetrahydrofuran (100 mL), and the mixture was stirred under a nitrogen atmosphere during 2.5 h at room temperature. The MnO_2 was removed by filtration and washed with THF. The filtrate, including washings, was concentrated under reduced pressure to a syrup that crystallized: wt. 2.2 g; TLC, major spot (**17**) plus a weak, slower component. This material in CH_2Cl_2 was subjected to flash chromatography on silica gel 60, and product-containing fractions were combined and concentrated under reduced pressure to a pale yellow solid: wt. 750 mg; mp, 91−92 °C; TLC, 1 spot; HPLC (340 nm), 99%; UV (EtOH) λ_{max} 311 nm (ϵ 48300), 221 nm (ϵ 4200); IR (KBr disk strong bands, cm^{-1}), 1655 (ester CO), 1613, 1596 (ester C − OCH_3), 1218, 1172, 1160, medium 2220 (CN); MS *m*/*z* 220 (M + H), 193 (M − CN), 188 (M − OCH_3), 161 (M − CN − MeOH); ^1H NMR ($CDCl_3$) δ 7.61 (dq, 1H, *H*6, $J_{6,8}$ = 1.1 Hz, $J_{5,6}$ = 10.4 Hz), 6.98 (dd, 1H, *H*5, $J_{4,5}$ = 15.1 Hz, $J_{5,6}$ = 10.4 Hz), 6.88 (d, 1H, *H*4, $J_{4,5}$ = 15.1 Hz), 6.06 (s, 1H, *H*2), 3.77 (s, 3H, COOC*H*$_3$), 2.38 (d, 3H, 3C*H*$_3$, $J_{2,3-CH3}$ = 1.3 Hz), 2.02 (d, 3H, *H*8, $J_{6,8}$ = 1.1 Hz). Anal. ($C_{12}H_{13}NO_3$) C, H, N.

Mouse-Skin Anti-Papilloma Assay. The procedure of Verma and Boutwell[22,23] for determining the effect of retinoids in preventing the development of papillomas on the skin of mice was modified as described previously by Lin et al.[27]

Assays for Binding to Cellular Retinoic-Acid Binding Protein and to Nuclear Retinoic Acid Receptor. The procedure for evaluating retinoic acid analogues for binding to CRABP I was described previously,[28] and the procedure for determining binding to RARα was also described previously.[29,30]

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