Dihydroretinoic Acids and Their Derivatives. Synthesis and Biological Activity

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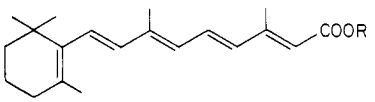
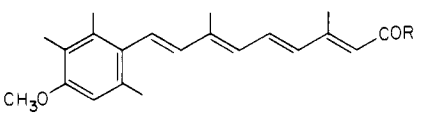
The syntheses of the ring and four side-chain dihydroretinoic acids and/or their esters, **3**–**7**, are described. The syntheses of several other retinoids containing a substituted aromatic ring are also included. The biological activity of the compounds was evaluated in vivo in a chemically induced mouse skin papilloma test and in vitro in two vitamin A deficient assays. The activity observed for **1a**, **1c**, and **2a** in the former test was partially retained in the dihydro derivatives **4b**, **4c**, and **6b**. Similar results were found in the in vitro assays.

The effectiveness of retinol, retinoic acid (**1a**), and some of their analogues (retinoids) in preventing and inhibiting the growth of epithelial tumors has been reviewed.[1,2] Systemic administration of retinoic acid has also been shown to cause regression of carcinogen-induced papillomas on the skin of mice[3a,b] and to have a prophylactic effect when administered during the promotion phase of carcinogenesis.[3c] Further, topical or systemic administration of retinoic acid has been shown to have some effect on precancerous conditions in man.[4–6] Evidence has also been obtained for the presence of partially hydrogenated compounds as products of retinoic acid metabolism; tetrahydroretinoic acid derivatives have been reported as urinary metabolites of retinoic acid.[7]

These findings prompted a chemical effort to synthesize the simple dihydroretinoic acids and their derivatives containing a substituted aromatic ring, shown in Scheme I, for biological evaluation. The effectiveness of the aromatic retinoic acid analogues **2b** and **2c** on chemically induced skin papillomas and carcinomas of mice has previously been reported.[8]



1a, R = -H
b, R = $-CH_3$
c, R = $-CH_2CH_3$



2a, R = -OH
b, R = $-OCH_2CH_3$
c, R = $-NHCH_2CH_3$

Chemistry. When this work was started, the only compound previously reported[9] in the literature was the acid **3**, which was an intermediate in the synthesis of *all-trans*-5,6-dihydroretinal.[9,10] Subsequently during the course of this work, the synthesis of an isomeric mixture containing **4a** and **4b** was also described.[11]

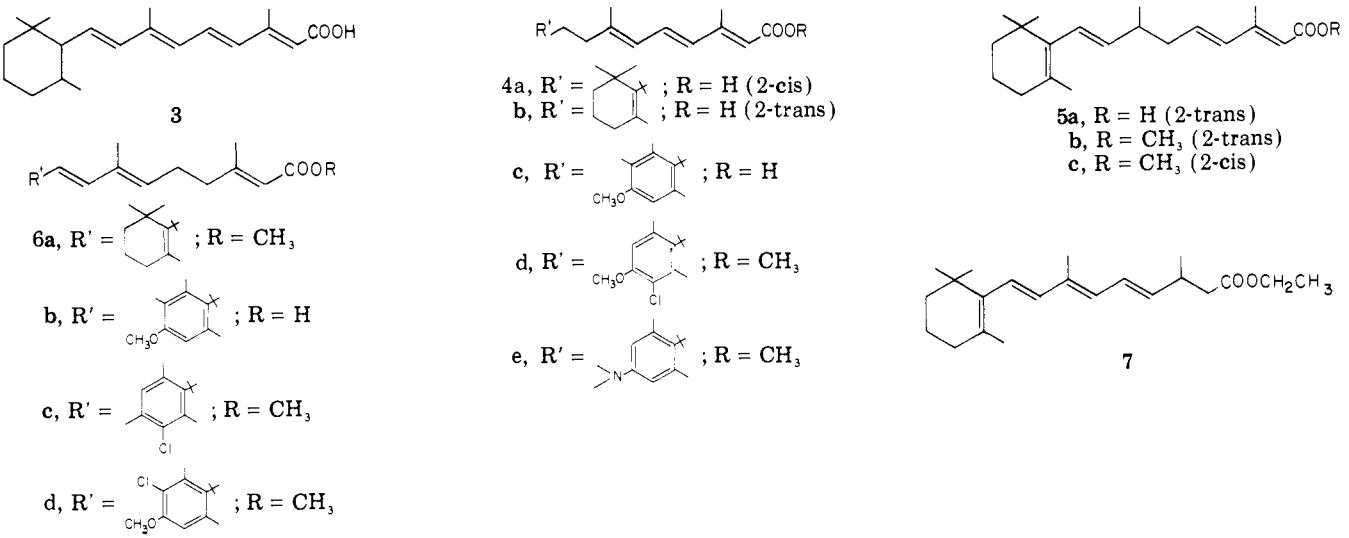
The synthesis of **3** started from dihydrocyclocitral[12] (**8**) as shown by eq 1 (Scheme II). Condensation of **8** with methyl β,β-dimethylacrylate with potassium or sodium amide as previously described[9] afforded in our hands predominantly the cis isomer of acid **11**. However, Horner reaction of the phosphonate[13,14] **9a** with **8** yielded *trans*-**10** as the major isomer. Saponification and crystallization gave the all-trans acid **11**,[9] as one diastereoisomer, which was presumed to have the trans-diequatorial configuration. Reduction of the acid **11** gave the alcohol **12** which upon oxidation with manganese dioxide afforded the aldehyde **13**. Condensation of **13** with the phosphonate **9a** gave the 2-cis/trans ester mixture corresponding to **3**. Separation of the isomers by chromatography and hydrolysis of the 2-trans isomer gave the crystalline acid **3**, mp 157 °C.[9]

The synthesis of acids **4a** and **4b** paralleled that previously reported,[11] utilizing dihydro-β-ionone (**14**) as starting material (eq 2, Scheme II). Separation of the cis and trans isomers formed in the first Horner reaction was achieved by hydrolysis; crystallization of the resulting acid mixture gave the trans acid **15**.[15] The final Horner reaction of the aldehyde **16**, obtained by reduction of **15** and oxidation of the resulting alcohol with manganese dioxide, and the phosphonate **9a** afforded the 2-cis/trans ester mixture corresponding to **4a**,**b**, which was separated by repeated column chromatography. Hydrolysis of the individual esters afforded the respective acids **4a**, mp 135 °C, and **4b**, mp 147 °C.

The "Milas" aldehyde **17**, prepared as described previously[16] by the modified Darzens condensation, served as the starting material for the synthesis of acid **5a** (eq 3, Scheme II). Best results were obtained when the reduction to the alcohol **18** was carried out on the crude reaction product; distillation only led to increased amounts of the α,β-δ,ϵ-dienal in the product. The alcohol was converted to the nitrile **19** and reduced with diisobutylaluminum hydride to give the aldehyde **20**. Horner reaction of **20** with the phosphonate **9b** afforded the 2-cis/trans ester mixture. Separation of the isomers by column chromatography gave the pure 2-cis ester **5c** and the all-trans ester **5b**. Hydrolysis of **5b** afforded the trans acid **5a**, mp 92–93 °C.

The preparation of **6a** succeeded only in low overall yield, as shown in eq 4 (Scheme II), starting with the C_{15} chloride[17] **21**. Alkylation of ethyl acetoacetate with **21** afforded, after hydrolysis and decarboxylation, a mixture containing the desired ketone **22**. Attempts to purify the ketone by chromatography were unsuccessful due to the lability of the double bonds. The best method found for obtaining the desired ketone in a reasonably pure state was to distill the product after decarboxylation and to convert

Scheme I. Dihydroretinoic Acids and Derivatives



the fractions enriched in the desired ketone (40–65% by GC analysis) into the semicarbazone. Recrystallization of the semicarbazone and regeneration of the ketone by refluxing with acetylacetone[18] gave **22** of approximately 90% purity according to GC chromatography. Distillation of **22** at this stage only led to isomerization. The ketone obtained from the semicarbazone was further transformed by reaction with trimethyl phosphonoacetate into the cis and trans ester mixture, from which the all-trans ester **6a** was obtained by chromatography.

The 2,3-dihydro ester **7** was prepared from the Wittig reaction of the C_{15} phosphonium salt[19] **21** ($X = P^+Ph_3Cl^-$) and the saturated aldehyde ester **23**[20] (eq 5, Scheme II). The ester **7** was shown by gas chromatography to be a mixture of four stereoisomers, of which 50% was the all-trans ester, based upon its gas chromatographic properties. The ester mixture, which could be purified but not separated by column chromatography, decomposed on distillation.

The acid **4c**, containing a substituted aromatic ring, was prepared in an analogous manner to **4b** starting from the ketone **24**, obtained by Raney nickel catalyzed hydrogenation of the corresponding unsaturated ketone,[21] via the aldehyde **27** (eq 2, Scheme II). Chromatography of the ester mixture corresponding to **4c** afforded the 2-trans isomer, which upon hydrolysis and recrystallization gave the acid **4c**.

Similarly, 2,6-dimethyl-4-dimethylaminobenzaldehyde[22] was converted via the saturated ketone **28** and the aldehyde **29** to the crystalline ester **4e** (eq 2, Scheme II).

3-Chloro-2,6-dimethyl-4-methoxybenzyl chloride (**30**) served as the starting material for the synthesis of the ester **4d**. Alkylation of ethyl acetoacetate with **30** gave, after hydrolysis and decarboxylation, the ketone **31** (eq 2, Scheme II). Horner condensation of **31** with triethyl phosphonoacetate gave, after chromatographic separation, the trans ester **32**. Conversion of **32** to the aldehyde **34** in the usual manner and reaction with **9b** afforded the desired ester **4d**.

The additional derivatives **6b** and **6c** were prepared in a manner analogous to **6a** (eq 4, Scheme II) from the corresponding halides **35** and **36**, respectively. Alkylation of ethyl acetoacetate, hydrolysis, and decarboxylation gave the corresponding ketones **37** and **38**, respectively. Horner reaction of **38** with trimethyl phosphonoacetate gave the ester **6c**. The acid **6b** was obtained by hydrolysis of the corresponding methyl ester, obtained by Horner reaction of **37** and trimethyl phosphonoacetate.

Table I. Effect of Dihydroretinoic Acid Analogues against Chemically Induced Papillomas in Mice

Retinoid	Dose, mg/kg/day	% change in total papilloma diameter	Activity
1a	40	−55	+
1c[a]	80[a]	−47	+
2a	10	−43	+
3[b]	10[b]	−9	−
4b	80	−11, −33	+
4a	80	−28	−
4c	80	−24, −35	+
4d	80	+1	−
4e	80	+20	−
6a	80	−13	−
6b	160	−66	+
	80	−17	+
6c	80	−29	−
6d	80	+29	−
7	160	−14	−

[a] This result was obtained as described in ref 8. A single dose of 400 mg/kg was given once weekly for 2 weeks. [b] This result was obtained as described in ref 8. A single dose of 50 mg/kg was given once weekly for 2 weeks.

The ester **6d** was prepared by Wittig condensation of the phosphonium salt **39** and the aldehyde **40**[23] (eq 6, Scheme II), hydrolysis of the resulting acetate, oxidation, and esterification.

Biological Results. In Vivo Studies. The compounds described in this study were tested for their therapeutic effect on chemically induced skin papillomas in mice as described by Bollag[8] with the following differences. Royal Hart Swiss albino female mice were fed Charles River Laboratory Chow with a stated content of 24 000 IU of vitamin A per kilogram of food throughout the experiment. Groups of ten rather than four mice were used at each dose level and compounds were administered ip daily, five times per week for 2 weeks, as suspensions in a 0.1% aqueous solution of carboxymethylcellulose. Animals were photographed at the beginning and end of the therapeutic trial period (14 days) and all measurements of papilloma diameters were made from the photographs. Statistical significance levels were determined using a two-tailed t test on the net changes in papilloma diameter per animal. When the probability (p) that the differences between the means of those net changes for control and treated groups could have arisen by chance is <0.01 the compound is considered active. If p is >0.01, the compound is inactive. The results of these tests are shown in Table I.

Scheme II. Syntheses of Dihydroretinoic Acids and Derivatives

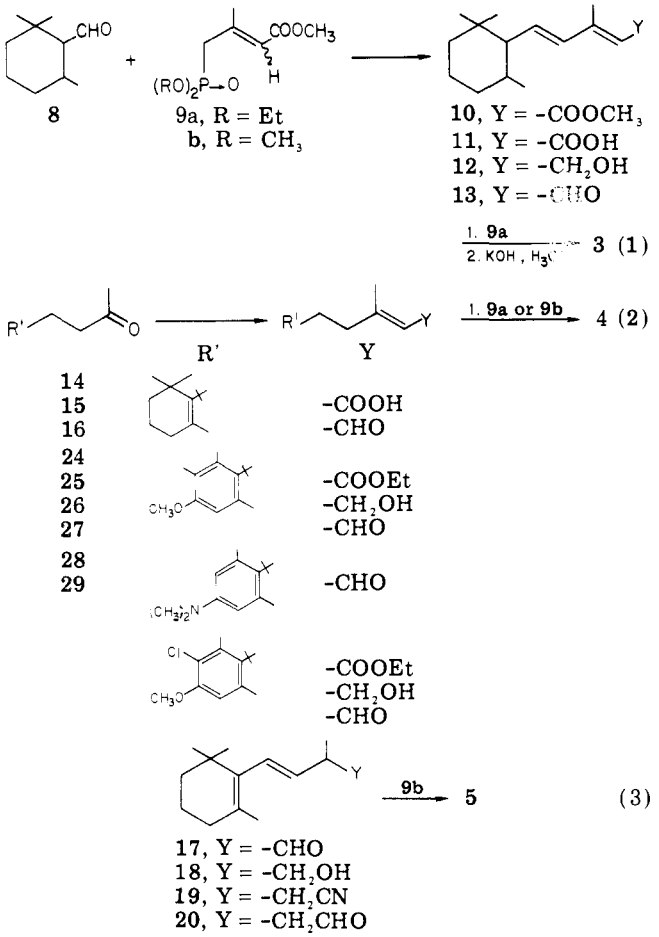


Table II. Reversal of Keratinized Lesions of Vitamin A Deficiency in Tracheal Organ Cultures Treated with Dihydro Analogues of Retinoic Acid

Compd	Treatment of cultures, concn, M (no. of cultures)	% of cultures with keratin and keratohyaline granules
	No retinoid, harvested day 6 (55)	78
	No retinoid, harvested day 13 (56)	93
1a	10^{-7} (16)	**0**
	10^{-8} (33)	3
	10^{-9} (6)	0
1b	10^{-7} (29)	**0**
	10^{-8} (18)	0
1c	10^{-7} (17)	0
	10^{-8} (10)	0
3	10^{-7} (15)	0
	10^{-8} (12)	0
	10^{-9} (18)	6
4b	10^{-7} (22)	0
	10^{-8} (24)	25
5a	10^{-7} (23)	52
	10^{-8} (23)	96
6a	10^{-7} (17)	24
	10^{-8} (23)	83
7	10^{-7} (17)	12
	10^{-8} (24)	83
2a	10^{-6} (15)	0
	10^{-7} (24)	0
	10^{-8} (20)	35
	10^{-9} (11)	100
4c	10^{-6} (9)	**0**
	10^{-7} (19)	63
	10^{-8} (27)	85
6b	10^{-6} (13)	0
	10^{-7} (25)	44
	10^{-8} (29)	86

Of the compounds tested, activity in the papilloma test was retained only in the case of the 8,9-dihydro derivatives **4b** and **4c** and the 4,5-dihydro derivative **6b**. The activity of these three compounds appeared, based on the limited test results, not to be as pronounced as observed for the original molecules **1a** and **2a**. A similar reduction in activity on saturation of a double bond was also found in in vitro studies (see below).

In Vitro Studies. Tracheal organ cultures from vitamin A deficient hamsters[24,25] and epidermal cell cultures from mouse skin[26] were also used to evaluate the biological activity of the various dihydro retinoids. The tracheal assay measures the reversal of squamous metaplasia and the disappearance of keratin from the vitamin A deficient organ which occurs upon incubation with active retinoids, while the epidermal assay measures the increase in RNA, DNA, and protein caused by addition of retinoids to the culture medium. Table II shows that retinoic acid, retinoic acid methyl ester (**1b**), and retinoic acid ethyl ester (**1c**) all had approximately equal activity in the tracheal organ culture assay. Saturation of any of the four double bonds in the side chain causes loss of activity, with the 8,9-dihydro derivative **4b** retaining significantly more activity than the other three respective dihydro analogues **5a**, **6a**, and **7**. Compounds **4c** and **6b**, dihydro analogues of the aromatic acid **2a**, showed appreciable activity only at a higher concentration (10^{-6} M) but were not as active as **2a**. However, compound **3**, in which the ring double bond had been saturated, had almost the same activity as retinoic acid (**1**) in this test.

Assay in epidermal cultures (Table III) confirmed the findings observed for the side-chain hydrogenated retinoids. All of these dihydro analogues were found to be less

Table III. Percentage Increase in RNA in Epidermal Cell Cultures Treated with Analogues of Retinoic Acid[a]

Compd	Concn, M (no. of cultures)	RNA
1a	10^{-6} (6)	115
	10^{-7} (6)	109
	10^{-8} (6)	100
	10^{-9} (6)	68
	10^{-10} (6)	19
1b	10^{-6} (6)	126
	10^{-7} (6)	75
	10^{-8} (6)	25
	10^{-9} (6)	8
1c	10^{-6} (6)	123
	10^{-7} (6)	102
	10^{-8} (6)	57
	10^{-9} (6)	23
4b	10^{-6} (8)	106
	10^{-7} (8)	115
	10^{-8} (8)	82
	10^{-9} (8)	1
5a	10^{-6} (8)	118
	10^{-7} (8)	29
	10^{-8} (8)	28
	10^{-9} (8)	3
6a	10^{-6} (7)	21
	10^{-7} (7)	5
	10^{-8} (7)	9
	10^{-9} (7)	3
7	10^{-6} (9)	69
	10^{-7} (9)	13
	10^{-8} (9)	12
	10^{-9} (8)	12

[a] Epidermal cells were grown for 3 days with the above retinoids. Values are expressed as the percentage increase in RNA in the cultures, compared with control cultures which received no retinoid.

active than their respective parent compound in causing an increase in RNA, DNA, and protein. **4b** was again the most active of the four dihydro analogues. Retinoic acid methyl and ethyl esters were less potent than retinoic acid in the epidermal cell culture assay, presumably because of low esterase activity in the epidermal cells.

Experimental Section

General. Spectra and analyses were made by the Physical Chemistry Departments of F. Hoffmann-La Roche, Basle, Switzerland, and Hoffmann-La Roche Inc., Nutley, N.J. Microanalyses are within ±0.4% of theory except where otherwise noted.

Reagents. All solvents were ACS grade and were not further purified unless otherwise noted. Dry tetrahydrofuran (THF) and dimethoxyethane (DME) were obtained by passing the solvent through a column of Woelm neutral alumina activity I. The sodium hydride suspension in mineral oil was washed three times with low-boiling petroleum ether or pentane and the residual solvent was removed in vacuo prior to suspension in the reaction solvent. Unless otherwise indicated, reaction mixtures were partitioned between water and ether and the aqueous layer was washed twice with ether. The ether extracts were washed successively with saturated aqueous NaCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl to neutrality and dried with anhydrous sodium sulfate. For reactions carried out in an argon atmosphere, the apparatus was evacuated and filled with argon at least three times.

Experimental Methods for Organ and Cell Culture. Methods for organ culture[24,25] and cell culture[26] have been published in detail. Retinoids were dissolved in dimethyl sulfoxide (Me_2SO) and stored in a liquid nitrogen refrigerator. Final Me_2SO concentration in epidermal cell cultures and tracheal organ cultures did not exceed 1.0 and 0.3%, respectively. All control cultures were treated with an equivalent amount of Me_2SO. In the case of tracheal organ cultures, all tracheas were grown for the first 6 days in medium without retinoid. At this time, some tracheas were harvested, while the rest were grown for 7 more days in medium containing either no retinoid or added retinoid at the concentrations shown. The grading system has been reported previously.[25]

3-Methyl-5-(2,2,6-trimethylcyclohexyl)-2,4-pentadienoic Acid (11). Sodium hydride (18.3 g, 0.38 mol, 50% suspension in mineral oil) was suspended in 350 mL of absolute benzene. To the stirred suspension in an argon atmosphere, 108 g (0.43 mol) of the phosphonate **9a** was added dropwise. During the addition the temperature increased to 35 °C and the mixture was stirred at this temperature for 2 h. At the end of this time 49 g (0.38 mol) of dihydrocyclocitral[12] (**8**) was added dropwise at 35 °C. The mixture was stirred for 3 h and then worked up as described above to give 65 g of a mixture of esters **10**, which was hydrolyzed directly with methanolic potassium hydroxide. The acid 11 obtained was crystallized from pentane to give 20.7 g (23%), mp 112–114 °C (lit.[9] mp 110–111 °C), which was shown by gas chromatography to be 96.5% of one isomer. A sample of 11 was recrystallized from low-boiling petroleum ether to a constant melting point, mp 120–121 °C. Gas chromatographic analysis of this material showed it to be 100% of one isomer.

3,7-Dimethyl-9-(2,2,6-trimethylcyclohexyl)-2,4,6,8-nonatetraenoic Acid (3). Sodium hydride (2.76 g, 57.7 mmol, 50% suspension in mineral oil) was suspended in 50 mL of benzene in an argon atmosphere. The phosphonate **9a** (18.6 g, 74.5 mmol) was added dropwise and the mixture was stirred for 2 h. An additional 50 mL of benzene was added and then a solution of 11.7 g (53.3 mmol) of aldehyde **13**, prepared from acid 11 as previously described,[9] in 50 mL of benzene was added dropwise. The reaction was heated to the reflux overnight. After work-up, the ether extract was washed with sodium chloride solution until neutral and dried with sodium sulfate. Evaporation of the solvent afforded 21.5 g of a mixture of esters corresponding to **3**, which was chromatographed on silica gel to give 5.5 g (33%) of the all-trans isomer.

Ester from several preparations (18 g, 57 mmol) was hydrolyzed with 18 g of potassium hydroxide in 250 mL of methanol. Work-up afforded 16 g of crude acid, which after recrystallization from hexane gave 4.8 g (28%) of **3**, as yellow crystals, mp 157 °C (lit.[9] mp 157–158 °C).

***all-trans*-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6-nonatrienoic Acid (4b).** Sodium hydride (3.72 g, 0.085 mol, 55% in mineral oil) was suspended in 50 mL of dry benzene. A solution of 25.05 g (0.1 mol) of phosphonate **9a** in 200 mL of benzene was added and the mixture was heated at 40–45 °C for 6 h.[27] The resulting dark red solution was then cooled to 15–20 °C and a solution of 15.75 g (0.072 mol) of aldehyde **16** in 50 mL of benzene was added and the reaction mixture was stirred overnight. The usual work-up afforded 31.5 g of crude material. Repeated chromatography on silica gel and elution with methylene chloride–hexane (4:1) gave a separation of the 2-cis/trans mixture corresponding to **4a,b**. Saponification of 44 g (0.14 mol) of the 2-trans ester, prepared as described above, in 500 mL of methanol with a solution of 44 g of potassium hydroxide in 50 mL of water for 2.5 h at reflux in an argon atmosphere afforded, after acidification and extraction, 42 g of yellow crystalline material which was recrystallized three times from 9:1 hexane–methylene chloride to give 16.9 g (40%) of **4b**, mp 149 °C. Anal. ($C_{20}H_{30}O_2$) C, H.

In a similar manner, the 2-cis ester (16 g, 0.051 mol) was hydrolyzed to give, after three recrystallizations from 9:1 hexane–methylene chloride, 7 g (46%) of pure **4a**, mp 135–137 °C. Anal. ($C_{20}H_{30}O_2$) C, H.

***rac-trans*-3-Methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-4-pentenenitrile (19).** The aldehyde **17** was prepared as described[16] and, without any purification or distillation, was reduced with sodium dihydrobis(2-methoxyethoxy)aluminate to the alcohol **18**.[28] A solution of 33 g (0.16 mol) of **18** in 100 mL of pyridine was cooled to 5 °C and 46 g (0.24 mol) of *p*-toluenesulfonyl chloride was added. After stirring for 3 h at 5 °C, the reaction mixture was poured onto ice water and extracted with ether. The organic phase was washed with 3 N hydrochloric acid and then treated as previously described. Evaporation of the solvent afforded 48.5 g of an oily tosylate, which was dissolved in 135 mL of dimethyl sulfoxide. Pulverized sodium cyanide (9.85 g, 0.2 mol) was added and the mixture was stirred for 20 h at 60 °C in an argon atmosphere. The reaction mixture was worked

up by extraction with methylene chloride. The methylene chloride solution was washed repeatedly with water and dried with sodium sulfate. Evaporation of the solvent afforded 26.2 g of an amber-colored oil which was chromatographed on silica gel. Elution with hexane containing 5% ether afforded 19.6 g (56%) of pure **19**. Anal. ($C_{15}H_{23}N$) C, H, N.

rac-trans-**3-Methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-4-pentenal (20).** The nitrile **19** (10.85 g, 0.05 mol) was dissolved in 150 mL of toluene and was cooled to −70 °C with stirring in an atmosphere of argon. A 20% solution of diisobutylaluminum hydride (80 mL, 0.09 mol) was added dropwise at −70 °C. The mixture was allowed to stir at this temperature for 10 min and then slowly allowed to warm to room temperature with stirring for 5 h. To the cooled reaction mixture (5 °C), 25 mL of ethyl formate was added dropwise and the resulting mixture was stirred 1 h at room temperature. The reaction mixture was again cooled to 0 °C and 185 mL of concentrated ammonium chloride solution was added and the mixture was stirred at room temperature for 20 min. Ether (750 mL) was added and then, with cooling in an ice bath, 185 mL of 3 N sulfuric acid was added. The mixture was stirred at room temperature overnight. The usual work-up afforded 11.7 g of an oil which was purified by chromatography on silica gel and elution with hexane containing 10% benzene to give 8.8 g (91%) of aldehyde **20**, which had bp 76–81 °C (0.3 mm). Anal. ($C_{15}H_{24}O$) C, H.

Methyl *rac-all-trans*- **and 2-*cis*-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,8-nonatrienoate (5b and 5c).** In a four-necked flask fitted with a mechanical stirrer, dropping funnel, and thermometer, 6.98 g (0.145 mol) of sodium hydride (50% suspension in mineral oil) was stirred with 170 mL of dimethylformamide (DMF). The phosphonate **9b**[13,14] (40 g, 0.16 mol) was added dropwise. After the addition was complete, the mixture was stirred for 0.5 h at room temperature and then cooled to 0–5 °C. A solution of the aldehyde **20** (27 g, 0.12 mol) in 170 mL of DMF was added and the mixture was allowed to stir overnight. After extractive work-up, evaporation of the solvent afforded an oil which was purified by chromatography on silica gel; elution with methylene chloride–hexane (1:1) gave 14.7 g (39%) of the 2-trans ester **5b**, 9.0 g of the 2-cis ester **5c** (24%), and 3.9 g (10%) of a mixture. The 2-trans ester was distilled at 150 °C (oil bath temperature) and 0.4 mm for spectral analysis. Anal. ($C_{21}H_{32}O_2$) C, H. The 2-cis ester **5c** was distilled at 135 °C (0.5 mm). Anal. ($C_{21}H_{32}O_2$) C, H.

rac-all-trans-**3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,8-nonatrienoic Acid (5a).** The ester **5b** (14.7 g, 46 mmol) was hydrolyzed with 29.3 g of potassium hydroxide in 100 mL of ethanol at 60 °C for 1 h. After work-up, acidification, and extraction of the acid-insoluble material with ether, the residue afforded 13.9 g of yellow crystalline material which was recrystallized three times from pentane to give 5.9 g (42%) of yellow crystals, mp 92–93 °C. Anal. ($C_{20}H_{30}O_2$) C, H.

6-Methyl-8-(2,6,6-trimethyl-1-cyclohexen-1-yl)octa-5,7-dien-2-one (22). Sodium hydride (4.6 g, 95.5 mmol) was stirred with 100 mL of anhydrous DME. Ethyl acetoacetate (65 g, 0.5 mol) was added dropwise and the mixture was heated to reflux for 1 h after the addition. The resulting anion was cooled to 0–10 °C and then a solution of 120 g (0.5 mol) of 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-methyl-5-chloropentadiene[17] (**21**, X = Cl) in 40 mL of dry DME was added over a 30–45-min period. The mixture was heated to reflux overnight. The cooled reaction mixture was filtered and the filtrate was diluted with ether, washed with saturated sodium chloride solution until neutral, and dried with sodium sulfate. The crude keto ester (147 g) was treated with 500 mL of 70% ethanol and 140 g of potassium hydroxide was added. The mixture was refluxed 1 h on the steam bath, cooled, and adjusted to pH 2 with concentrated hydrochloric acid and then was heated again for 30 min on the steam bath to remove all carbon dioxide. Work-up gave 115 g of ketone which was distilled at reduced pressure. The fraction boiling between 120 and 135 °C (0.1 mm) was collected to give 40 g of crude product which contained 65% of the desired ketone by gas chromatography.

Semicarbazide hydrochloride (43.8 g) and 60 g of sodium acetate trihydrate were pulverized well in a mortar. The resulting paste was filtered and washed with methanol. The filtrate was added to the ketone **22** together with 100 mL of methanol and the mixture was stirred at room temperature for 3 h. The crystalline semicarbazone was filtered and recrystallized from 350 mL of hot methanol to give 15 g of colorless crystals, mp 152 °C. Anal. ($C_{19}H_{31}N_3O$) C, H, N.

The semicarbazone was heated with acetylacetone (10 mL per gram of semicarbazone) at reflux overnight. After extraction of the cooled reaction mixture, the ether solution was washed twice with 3 N hydrochloric acid and then with water until neutral and dried with sodium sulfate. Evaporation of the solvent, finally at high vacuum to remove the last traces of acetylacetone, afforded 18 g of an orange brown oil, which was reacted further without distillation. Analysis of the product by gas chromatography showed that it contained 88% of the desired ketone **22**.

Methyl *all-trans*-**3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,6,8-nonatrienoate (6a).** Sodium hydride (2.85 g) was suspended in 400 mL of anhydrous THF in an inert atmosphere. Trimethyl phosphonoacetate was added dropwise and the mixture was heated for 2 h at 40–50 °C. The mixture was cooled to 5–10 °C and 12.9 g (0.044 mol) of ketone **22** in 50 mL of anhydrous THF was added. The mixture was heated to reflux overnight, cooled, and extracted. Chromatography of the crude product on silica gel afforded 10 g of trans ester which was distilled at 125 °C (0.05 mm) to give 8.4 g (60%) of pure ester **6a**, which contained approximately 8% of the 6-cis isomer according to gas chromatographic analysis. Anal. ($C_{21}H_{32}O_2$) C, H.

Ethyl 3,7-Dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)-nona-4,6,8-trienoate (7). To a suspension of 69 g (0.138 mol) of phosphonium salt **21** (X = $P^+Ph_3Cl^-$)[19] in 600 mL of THF, 17.5 g (0.137 mol) of ethyl 3-formylbutanoate[20] (**23**) was added. The mixture was cooled to 10 °C and sodium ethoxide (prepared from 3.02 g of sodium and 80 mL of ethanol) was added dropwise. The reaction was allowed to stir overnight and then was extracted with ether. The residue, which contained triphenylphosphine oxide, was triturated with six 150-mL portions of hot hexane and filtered. The hexane extracts were concentrated to give 41 g of crude product. Chromatography on silica gel and elution with methylene chloride–hexane (4:1) afforded 26.5 g (61%) of ester **7** which was shown by gas chromatography to be a mixture of four stereoisomers, of which 50% was the all-trans isomer based on retention properties. Attempted distillation led to decomposition. Anal. ($C_{22}H_{34}O_2$) H; C: calcd, 79.95; found, 79.28.

4-(4-Methoxy-2,3,6-trimethylphenyl)-2-butanone (24). 4-(4-Methoxy-2,3,6-trimethylphenyl)-3-buten-2-one[21] (5.45 g, 0.025 mol) was dissolved in 100 mL of ethanol; 5 mL of Raney nickel, which had been washed several times with ethanol, was added together with 50 mL of ethanol. The mixture was shaken with hydrogen at atmospheric pressure. After the theoretical amount of hydrogen had been taken up, the catalyst was removed by filtration and the filtrate was concentrated. Recrystallization of the residue twice with 125-mL portions of hexane afforded 4.6 g (84%) of **24**, mp 87 °C. Anal. ($C_{14}H_{20}O_2$) C, H.

Ethyl *trans*-**5-(4-Methoxy-2,3,6-trimethylphenyl)-3-methyl-2-pentenoate (25).** Sodium hydride (30.6 g, 55% in oil, 0.7 mol) was suspended in 750 mL of benzene in an argon atmosphere. Triethyl phosphonoacetate (171.5 g, 0.77 mol) was added dropwise without cooling. The temperature rose to 35 °C; after the addition was complete, the mixture was stirred 1 h at 30 °C. A solution of 141 g (0.64 mol) of the ketone **24** in 400 mL of benzene was added and the mixture was stirred overnight. After extractive work-up, the crude product was recrystallized from petroleum ether (bp 30–60 °C) to afford 110 g (59%) of colorless ester **25**, mp 45 °C. Anal. ($C_{18}H_{26}O_3$) C, H.

trans-**5-(4-Methoxy-2,3,6-trimethylphenyl)-3-methyl-2-penten-1-ol (26).** The ester **25** (25.3 g, 87.5 mmol) was dissolved in 100 mL of anhydrous ether and added dropwise to a solution of 34 mL (122 mmol) of sodium dihydrobis(2-methoxyethoxy)aluminate (70% solution in benzene) in 50 mL of anhydrous ether at 5 °C. The mixture was stirred for 4 h at 35 °C and then cooled to 0 °C; 300 mL of 20% aqueous sodium hydroxide was added dropwise until two clear phases were obtained. Evaporation of the solvent following extractive work-up gave 23 g of crude alcohol **26**, which after recrystallization from ethyl acetate yielded 16 g (74%) of product, mp 80 °C. Anal. ($C_{16}H_{24}O_2$) C, H.

trans-5-(4-Methoxy-2,3,6-trimethylphenyl)-3-methyl-2-pentenal (27). The alcohol **26** (16.5 g, 66.5 mmol) was dissolved in 350 mL of methylene chloride and was stirred for 15 h at room temperature with 115.7 g of active manganese dioxide. At the end of this time the mixture was filtered and the filtrate was concentrated to give 14.9 g of crude aldehyde, which after recrystallization from ethyl acetate yielded 14 g (85%) of **27**, mp 66 °C. Anal. ($C_{16}H_{22}O_2$) C, H.

trans-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6-nonatrienoic Acid (4c). To a suspension of 22.4 g (0.466 mol, 50% in mineral oil) of sodium hydride in 800 mL of benzene, a solution of 134 g (0.535 mol) of phosphonate **9b** was added dropwise at room temperature. The mixture was then stirred at 35–45 °C for 5 h. To the cooled (5–10 °C) reaction mixture, 96.7 g (0.39 mol) of aldehyde **27** in 300 mL of benzene was added dropwise. The mixture was heated to the reflux overnight, cooled, and extracted. Concentration of the solution gave 146 g of the 2-cis/trans ester mixture corresponding to **4c**, which was separated by repeated chromatography on silica gel and elution with methylene chloride–hexane (4:1) to give 63 g (47%) of pure 2-trans ester. The above ester (40 g, 0.117 mol) was treated with a solution of 40 g of potassium hydroxide in 400 mL of methanol and the mixture was stirred 15 h at 50 °C. Water was added to the cooled reaction mixture and the aqueous phase was extracted once with methylene chloride. The aqueous phase was then made acidic with concentrated hydrochloric acid and extracted with methylene chloride. The methylene chloride solution was washed with water, dried, and evaporated to give 37.5 g of yellow crystalline product. Recrystallization three times from ethyl acetate afforded 9 g (23%) of pure colorless crystalline acid **4c**, mp 200–201 °C. Anal. ($C_{21}H_{28}O_3$) C, H.

4-(2,6-Dimethyl-4-dimethylaminophenyl)butan-2-one (28). 4-Dimethylamino-2,6-dimethylbenzaldehyde[22] (32.6 g, 0.184 mol), 440 mL of acetone, and 190 mL of water were cooled to 0 °C with stirring and 91.5 mL of 10% aqueous sodium hydroxide solution was added dropwise. After the addition was complete, stirring was continued for 3 days. The acetone was evaporated and the aqueous solution was worked up by extraction. Evaporation of solvent gave 51 g of crude product which was recrystallized from hexane to give 34.4 g (86% yield) of 4-(2,6-dimethyl-4-dimethylaminophenyl)buten-2-one as bright yellow crystals, mp 93–95 °C. Anal. ($C_{14}H_{19}NO$) C, H, N.

A mixture of 20 g (0.092 mol) of the above butenone, 10 mL of Raney nickel, which had been washed repeatedly with ethanol, and 800 mL of ethanol was stirred with hydrogen at atmospheric pressure until the theoretical amount of hydrogen (~2.3 L) had been absorbed. The catalyst was removed by filtration and the solvent was evaporated on the rotary evaporator. Purification by recrystallization from hexane gave 16 g (79%) of **28**, mp 42–53 °C. An analytically pure sample obtained by recrystallization from hexane had mp 57–58 °C. Anal. ($C_{14}H_{21}NO$) C, H, N.

trans-3-Methyl-5-(2,6-dimethyl-4-dimethylaminophenyl)-2-pentenal (29). The ketone **28** (2.19 g, 0.01 mol) in benzene solution was converted to the corresponding α,β-unsaturated ester with triethyl phosphonoacetate and sodium hydride as described above for the preparation of **25**. Extractive work-up afforded 2.8 g of an oil which was purified by chromatography on 100 g of silica gel. Elution with ethyl acetate–hexane (1:9) afforded 2.5 g (87%) of ethyl *trans*-3-methyl-5-(2,6-dimethyl-4-dimethylaminophenyl)-2-pentenoate as a light yellow oil, bp 182–187 °C (0.15 mm). Anal. ($C_{18}H_{27}NO_2$) C, H, N.

The above ester (2.89 g, 0.01 mol) in ether solution was reduced to the corresponding alcohol with sodium dihydrobis(2-methoxyethoxy)aluminate as described above for **26**. Recrystallization of the product obtained from hexane gave 1.6 g (65%) of *trans*-3-methyl-5-(2,6-dimethyl-4-dimethylaminophenyl)-2-penten-1-ol, mp 73–75 °C. Anal. ($C_{16}H_{25}NO$) C, H, N.

A mixture of 14.65 g (0.059 mol) of the above pentenol, 43.5 g (0.116 mol) of silver carbonate on calcium carbonate,[29] and 500 mL of petroleum ether (bp 60–90 °C) was heated to reflux for 7.5 h with a Dean-Stark trap. The reaction mixture was then cooled and filtered with suction and the residue was washed with petroleum ether. Evaporation of the filtrate afforded 13.8 g of an oil which was purified by chromatography on 400 g of silica gel. Elution with ethyl acetate–hexane (1:19), gradually increasing the concentration of ethyl acetate, afforded 8.4 g (58%) of the aldehyde **29** as a yellow oil, bp 140–141 °C (0.1 mm). Anal. ($C_{16}H_{23}NO$) C, H, N.

Methyl *trans*-3,7-Dimethyl-9-(2,6-dimethyl-4-dimethylaminophenyl)-2,4,6-nonatrienoate (4e). The phosphonate **9b** was converted to its anion with sodium hydride (0.2 g, 4.9 mmol) and allowed to react with the aldehyde **29** (1.0 g, 4.1 mmol) as described above for **4c**. Work-up afforded 1.9 g of a yellow oil which was purified by chromatography on 90 g of silica gel. Elution with ethyl acetate in hexane, gradually increasing from 2 to 4% of ethyl acetate, afforded 0.15 g (11%) of the ester **4e**. An analytically pure sample had mp 110–111 °C after three recrystallizations from pentane. Anal. ($C_{22}H_{31}NO_2$) C, H, N.

3-Chloro-2,6-dimethyl-4-methoxybenzyl Chloride (30). 2-Chloro-3,5-dimethylphenol (160.6 g, 1.03 mol) was added to a solution of 62.8 g (1.54 mol) of sodium hydroxide in 1 L of water. Dimethyl sulfate (206 g, 1.69 mol) was added dropwise over a period of 1 h. The resulting mixture was stirred for 3 h and then extracted with hexane three times. The hexane solutions were washed repeatedly with 3 N sodium hydroxide and then with water to neutrality. The dried organic extracts were concentrated on the rotary evaporator and finally distilled at reduced pressure. The anisole had bp 112–113 °C (17 mm). Anal. ($C_9H_{11}ClO$) C, H, Cl.

The anisole (87 g, 0.51 mol) was combined with 201 mL of acetic acid and 376 mL of hydrochloric acid. To this mixture, 46 mL of a 35% solution of aqueous formaldehyde was added rapidly through a dropping funnel. The mixture was heated to 70 °C for 5 h, cooled, and extracted. Evaporation of the solvent afforded a crystalline residue which was recrystallized from petroleum ether (bp 40–60 °C) to give 72% of the benzyl chloride **30**, mp 57–60 °C. Anal. ($C_{10}H_{12}Cl_2O$) C, H, Cl.

4-(3-Chloro-4-methoxy-2,6-dimethylphenyl)-2-butanone (31). Ethyl acetoacetate (42.5 g, 0.327 mol) was converted to the anion with 15.7 g (0.327 mol) of sodium hydride as described above in the preparation of **22** and then added dropwise to a cooled solution of 72 g (0.329 mol) of **30** in 300 mL of dry DME over a period of 30 min. Work-up as described previously afforded 102 g of the crude keto ester which was hydrolyzed with 100 g of potassium hydroxide in 500 mL of 70% ethanol and decarboxylated as before. The cooled reaction mixture was extracted with methylene chloride. The methylene chloride extracts were washed with water until neutral and dried. The solvent was evaporated to give a crystalline residue, which was recrystallized from hexane–ethyl acetate (4:1) to give 56 g (71%) of the ketone **31**, mp 106 °C. Anal. ($C_{13}H_{17}ClO_2$) C, H.

trans-3-Methyl-5-(3-chloro-4-methoxy-2,6-dimethylphenyl)-2-pentenal (34). Triethyl phosphonoacetate (40 g, 0.178 mol) was converted to its anion with 7.2 g (0.15 mol) of sodium hydride, as described above for the preparation of **25**, and allowed to react with a benzene solution of 36 g (0.15 mol) of **31** as above. Work-up afforded 52 g of an oil which was purified by chromatography on silica gel. Elution with methylene chloride–hexane afforded 25 g (45%) of ester **32** which, after recrystallization from hexane, had mp 42–44 °C. Anal. ($C_{17}H_{23}ClO_3$) C, H, Cl.

The trans ester **32** (31 g, 0.1 mol) was converted to the corresponding alcohol as described above for **26**. Crystallization of the oily residue obtained after work-up from ethyl acetate–hexane (1:2) afforded 25.3 g (94%) of the alcohol **33** as colorless crystals, mp 72–73 °C. Anal. ($C_{15}H_{21}ClO_2$) C, H, Cl.

A mixture of 6.5 g (0.024 mol) of the alcohol **33** in 250 mL of methylene chloride was converted to the aldehyde **34** as described above for the preparation of **27**. Recrystallization of the product from ethyl acetate–hexane gave 5.1 g (80%) of **34**, mp 96–98 °C. Anal. ($C_{15}H_{19}ClO_2$) C, H, Cl.

Methyl *trans*-3,7-Dimethyl-9-(3-chloro-2,6-dimethyl-4-methoxyphenyl)-2,4,6-nonatrienoate (4d). The phosphonate **9b** (20.0 g, 0.09 mol) in 75 mL of dry THF was converted to the anion with sodium hydride as described above for the preparation of **4c**. The mixture was stirred for 30 min and a solution of 16.0 g (0.06 mol) of the aldehyde **34** in 75 mL of THF was added at 10 °C. The mixture was stirred for an additional hour at room temperature and then poured onto ice and water. Extractive work-up afforded the all-trans ester 4d which was purified by crystallization from hexane–ethyl acetate (3:1). Three recrystallizations afforded 1.75 g (8%) of colorless crystals, mp 122–123 °C. Anal. ($C_{21}H_{27}ClO_3$) C, H, Cl.

1-Chloro-3-methyl-5-(4-methoxy-2,3,6-trimethylphenyl)-2,4-pentadiene (35). A solution of 12.3 g of 3-hydroxy-3-methyl-5-(4-methoxy-2,3,6-trimethylphenyl)-1,4-pentadiene[21] in 150 mL of absolute ether was cooled to −60 °C with stirring in an argon atmosphere. Ethereal hydrochloric acid (39 mL, 10.3 g of HCl gas/100 mL) was added over a period of 5–10 min at −60 °C. The reaction was then allowed to come to 10 °C and stirred for 5 min at this temperature. After extractive work-up, evaporation of the solvent afforded 17 g of crude **35**, which was used for the alkylation without further purification. A 1-g sample of the crude product, purified for spectral purposes by recrystallization from hexane, gave yellow crystals: mp 60–61 °C; mass spectrum *m/e* 264 (M^+), 229, 228, and 213 (base peak).

6-Methyl-8-(4-methoxy-2,3,6-trimethylphenyl)-5,7-octadien-2-one (37). Ethyl acetoacetate (8.35 g, 64 mmol) was treated with sodium hydride as described for the preparation of **22**. To the cooled (0 °C) solution of the anion, 17 g (64 mmol) of the chloro compound **35** in 60 mL of dry DMF was added dropwise over a period of 20 min at 0–5 °C. The reaction mixture was heated to reflux overnight, then cooled, and worked up as described above. Evaporation of the solvent afforded 22 g of crude keto ester which was hydrolyzed and decarboxylated in the usual manner to give 17.5 g (96%) of crude ketone. A 7.5-g sample was purified by chromatography on 500 g of silica gel and elution with hexane–ethyl acetate (4:1). The trans ketone **37** obtained was crystallized three times from hexane to give 0.7 g of material having mp 89 °C. Anal. ($C_{19}H_{26}O_2$) C, H.

***all-trans*-3,7-Dimethyl-9-(4-methoxy-2,3,6-trimethylphenyl)-2,6,8-nonatrienoic Acid (6b).** The anion of triethyl phosphonoacetate, prepared as described above for the ester **6a** from 9.6 g (43.8 mmol) of the acetate and 1.8 g (37.5 mmol) of sodium hydride, was allowed to react with a solution of 9 g (31 mmol) of ketone **37** in 50 mL of THF. Work-up afforded 12.7 g of material which was purified by chromatography on silica gel and eluted with hexane–methylene chloride.

Hydrolysis of 23 g (67 mmol) of trans ester with methanolic potassium hydroxide in an argon atmosphere afforded 20 g of crude acid which was recrystallized once from ethyl acetate–hexane (1:1) and then from ethyl acetate to give 4.8 g (22%) of **6b**, mp 133 °C. Gas chromatographic analysis showed the product consisted of 99% of one isomer. Anal. ($C_{21}H_{28}O_3$) C, H.

6-Methyl-8-(3-chloro-2,4,6-trimethylphenyl)-5,7-octadien-2-one (38). 3-Chloro-2,4,6-trimethylbenzyltriphenylphosphonium chloride[21] (23.25 g, 0.05 mol) and 7.1 g (0.05 mol) of ethyl 3-formyl-2-butenoate[30] were combined with 25 mL of toluene and 25 mL of 1,2-epoxybutane and heated with stirring for 18 h at 80–90 °C. The toluene was removed on the rotary evaporator, 800 mL of hexane was added, and the mixture was allowed to cool and then was filtered. Concentration of the filtrate gave an oil, which was purified by chromatography on silica gel. Elution with methylene chloride gave 14.2 g of ethyl 3-methyl-5-(3-chloro-2,4,6-trimethylphenyl)-2,4-pentadienoate, bp 175 °C (bath temperature) (0.7 mm). Anal. ($C_{17}H_{21}ClO_2$) C, H; Cl: calcd, 12.11; found, 12.59.

In a 200-mL, four-necked flask fitted with a thermometer and magnetic stirrer and protected by an inert atmosphere, 925 mg (5.1 mmol) of lithium aluminum hydride was suspended in 25 mL of anhydrous ether, and the mixture was cooled to 0 °C. A solution of 5 g (3.4 mmol) of the above ester in 50 mL of anhydrous ether was added dropwise at 0–5 °C. The mixture was stirred at 5 °C for 1 h and then 10 mL of ethyl acetate was added cautiously at 0–5 °C followed by 50 mL of saturated sodium sulfate solution. The usual work-up gave 3.8 g (45%) of 3-methyl-5-(3-chloro-2,4,6-trimethylphenyl)-2,4-pentadien-1-ol.

To a solution of the alcohol (2.6 g, 10.4 mmol) in 18 mL of anhydrous ether, 0.155 mL of anhydrous pyridine was added. The mixture was cooled to −20 °C and a solution of 1.04 g (0.358 mL, 3.84 mmol) of phosphorus tribromide in 10 mL of anhydrous ether was added slowly over a 30-min period. The mixture was allowed to stir for 2 h without cooling and then was worked up by extraction. Concentration of the solution gave 2.4 g of bromo compound **36**, which was used in the alkylation without further purification.

Ethyl acetoacetate (1.0 g, 7.7 mmol) was alkylated in the manner described previously with **2.4** g (7.6 mmol) of **36** in 10 mL of anhydrous DME. After work-up, 2.4 g of β-keto ester was obtained which was hydrolyzed and decarboxylated as described above to give 2.2 g of an oil. Purification by chromatography on 100 g of silica gel and elution with methylene chloride–hexane (1:1) afforded 500 mg (23%) of **38**, mp 65–67 °C, after three recrystallizations from hexane. Anal. ($C_{18}H_{23}ClO$) C, H, Cl.

Methyl 3,7-Dimethyl-9-(3-chloro-2,4,6-trimethylphenyl)-2,6,8-nonatrienoate (6c). Trimethyl phosphonoacetate (504 mg, 2.8 mmol) was converted to its anion with 106.4 mg (2.48 mmol) of sodium hydride in 5 mL of THF as described previously. A solution of 300 mg (1.03 mmol) of **38** in 5 mL of anhydrous THF was added and the mixture was heated to reflux overnight. Extractive work-up afforded 400 mg of crude product which was purified by chromatography on 40 g of silica gel. Elution with methylene chloride–hexane (1:1) gave 55 mg (15%) of the ester **6c**. Anal. ($C_{21}H_{27}ClO_2$) C, H, Cl.

Methyl 3,7-Dimethyl-9-(3-chloro-2,6-dimethyl-4-methoxyphenyl)-2,6,8-nonatrienoate (6d). To a cool (15 °C) suspension of 2.72 g (64.4 mmol) of sodium hydride in 100 mL of DMF, 31 g (64.4 mmol) of the phosphonium salt **39**, prepared from **30** and triphenylphosphine, was added. The mixture was allowed to stir at room temperature for 15 min and then a solution of 12.3 g (58.5 mmol) of the aldehyde acetate[23] **40** in 30 mL of DMF was added slowly. The reaction mixture, which gradually became a clear solution over a period of 1 h, was allowed to stir at room temperature for an additional 4 h. Extractive work-up with ethyl acetate and concentration of the neutral extract afforded an oil which was triturated with three 500-mL portions of hexane and then 350 mL of carbon tetrachloride–hexane (1:1). These extracts were combined and concentrated in vacuo to give 21.5 g of an oil, which was purified by chromatography on 600 g of silica gel in hexane. Elution with hexane–ethyl acetate, gradually increasing from 1–3% ethyl acetate, gave 8.55 g (38%) of 3,7-dimethyl-9-(3-chloro-2,6-dimethyl-4-methoxyphenyl)-2,6,8-nonatrien-1-ol acetate.

The acetate (10.9 g, 29 mmol) in 100 mL of methanol was added slowly to a cool (20 °C) mixture of 23.6 g (0.14 mol) of silver nitrate, 11.2 g (0.28 mol) of sodium hydroxide, 33 mL of water, and 100 mL of methanol. The mixture was allowed to stir at room temperature for 5 h and then heated to 50–55 °C for 1 h.

The mixture was filtered and washed well with methanol and water. The combined filtrate was evaporated on the rotary evaporator to remove the methanol and the aqueous solution was extracted once with 300 mL of ethyl acetate. Chloroform (150 mL) was added to the aqueous phase which was then acidified with 10 mL of 85% phosphoric acid. Extraction with chloroform afforded 6.2 g (17.8 mmol) of crude acid. This material was mixed with 25.4 g (11.1 mL, 0.178 mol) of methyl iodide, 5.42 g (39.2 mmol) of potassium carbonate, and 100 mL of methyl ethyl ketone and heated to the reflux for 3 h. Extractive work-up afforded 4.2 g of crude product **6d**. This material, together with 1.2 g from another preparation, was purified by chromatography on 150 g of silica gel in hexane. Elution with 2% ethyl acetate in hexane gave 800 mg of **6d**. Anal. ($C_{21}H_{27}ClO_3$) C, H, Cl.

Supplementary Material Available: A listing of the spectral data for all new compounds reported (11 pages). Ordering information is given on any current masthead page.

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Methotrexate Analogues. 8. Synthesis and Biological Evaluation of Bisamide Derivatives as Potential Prodrugs

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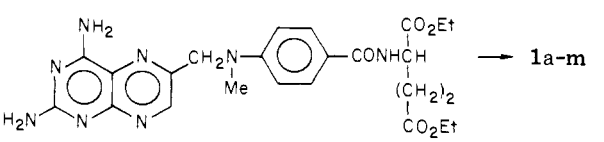
A series of heretofore unknown lipophilic bisamide derivatives (**1a–m**) of the antitumor agent methotrexate (MTX) was synthesized from MTX diethyl ester (**2**) by reaction with various amines. The amines were used in large excess, generally without solvent, at temperatures ranging from 50 to 100 °C and for periods of 24–72 h. Yields were in excess of 60% in most instances, and the products were stable and easily purified. The MTX bisamides proved significantly less active than MTX or MTX esters against human lymphoblastic leukemia (CCRF-CEM) cells in vitro (ID_{50} > 1.0 μg/mL vs. <0.05 μg/mL). However, some enhancement of activity was observed in two instances against rat basophilic leukemia (RBL) cells, which are myeloid rather than lymphoid in character. The bis(*n*-propylamide) **1b** was inactive in vivo against L1210 mouse leukemia even at doses of 525 mg/kg (q3d 1, 4, 7), but the bis(benzylamide) **1h** gave a +77% increase in median survival at 100 mg/kg (q3d 1, 4, 7). Neither compound was cleaved even after 24 h of incubation in whole rat serum at 37 °C, but the bis(benzylamide) **1h** was cleaved substantially in vivo, as evidenced by the detection of free MTX in the liver and plasma of mice 6 and 24 h after treatment with a single 100 mg/kg dose of "prodrug". These results suggest that MTX bis(benzylamide) (**1h**) may owe its in vivo activity against L1210 leukemia to the release of free MTX at sites other than the serum.

The pharmacologic and experimental therapeutic properties of esters of methotrexate (4-amino-4-deoxy-N^{10}-methylpteroyl-L-glutamate, MTX) have been studied in several laboratories.[1-10] While the mode of action of MTX esters is not yet established with certainty, they are believed to act as latent forms of MTX, i.e., "prodrugs", from which the parent acid is released on hydrolysis by extracellular and/or intracellular nonspecific esterases, whose levels are known in general to be species-variable.[11-13] Notwithstanding the fact that serum esterase levels in man are considerably lower than in rodents,[11-13] experimental trials with MTX esters have been confined thus far to mice[1,2,6,9] and, in a more limited way, to the dog.[2] Recent work in this laboratory[7] has shown that MTX esters cause marked inhibition of [^{3}H]-TdR incorporation into the DNA of mouse and human leukemic cells in serum-free short-term culture and that this effect is only partially prevented by leucovorin. Since MTX itself inhibits [^{3}H]-UdR but not [^{3}H]-TdR incorporation under these conditions, it was suggested that the esters may have a somewhat different mode of action than the parent acid at the biochemical level. As a logical extension of our overall program on lipophilic MTX derivatives, we became interested in MTX bisamides, a class of compounds about which very little could be found in the published literature.[14] We reported our first example of an MTX bisamide in 1975, in the form of the bis(*n*-propyl) derivative **1b**.[15] In this paper we wish to describe in detail the synthesis of this and 12 other MTX bisamides (**1a–m**) and to present data from some preliminary biological investigations. Structures of these heretofore unknown MTX bisamides are shown in Table I, along with other physical constants.

The ready availability of MTX diesters by direct HCl-catalyzed esterification[6] offered an attractive route to the desired bisamides, since amide groups could be introduced by nucleophilic displacement. The diethyl ester **2** was employed in most instances, although satisfactory results could also be achieved with the dimethyl analogue.



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Ester **2** was heated with a variety of amines, generally for periods of 24–72 h, at temperatures ranging from 50 to 100 °C (Table I). Whenever the amine was used in