phase was diluted with 100 ml of dry benzene and evaporated to dryness. Chromatography (completely analogous to A) afforded 36 mg (36%) of the TLC-pure aflatoxin $B_1$ (1) and 32 mg (30%) of TLC-pure mixture of epimeric aflatoxins $Q_1$ (2).

**Separation of Epimeric Aflatoxins $Q_1$ (2).** A mixture of epimeric aflatoxins $Q_1$ (2, 341 mg, obtained mainly by procedure A) was dissolved in methylene chloride–methanol–hexane. Upon concentration 141 mg (41%) of the natural epimer crystallized as very fine needles in two crops. A third crystalline crop (98 mg, 29%) obtained from the same solvent mixture turned out to be a mixture (mainly unnatural epimer) of the two epimers as judged by the NMR spectrum in $Me_2SO$-$d_6$. The remaining mother liquor was rechromatographed twice [1. chromatography: 2 20 × 20 × 0.1 cm Analtech silica gel chromatoplates, eluted with chloroform–ethanol–hexane (10:2:1 v/v/v); 2. chromatography 2 20 × 20 × 0.1 cm Analtech silica gel chromatoplates, eluted with methylene chloride–ethyl acetate (2:1 v/v), $R_f$ 0.15] to yield 55 mg of solid residue from which 38 mg (11%) of pure (as judged by the NMR in $Me_2SO$-$d_6$) unnatural epimer of aflatoxin $Q_1$ (2) crystallized from the solvent mixture used above. The two epimers showed the following physical properties.

Aflatoxin $Q_1$: mp 265° dec; uv max (100% $C_2H_5OH$) 223, 242 (sh), 267, 366 nm ($\epsilon$ 22250, 10800, 11800, 18700); uv min (100% $C_2H_5OH$) 252, 286 nm ($\epsilon$ 8600, 1000); ir ($CHCl_3$) 3600, 1780, 1710, 1640, 1610, 1575 $cm^{-1}$; MS (70 eV) *m/e* (rel intensity) 329 (23), 328 (100, $M^+$), 313 (7), 312 (15), 310 (7), 299 (9); NMR, 90 MHz ($Me_2SO$-$d_6$) $\delta$ 2.21 (A part of ABX, 1, $J_{AB}$ = 18, $J_{AX} \approx$ 1.5 Hz), 2.77 (B part of ABX, 1, $J_{AB}$ = 18, $J_{BX}$ = 6.5 Hz), 3.19 (s, $H_2O$ from the solvent, exchanging with ROH of the substance), 3.85 (s, 3), 4.69 (d, 1, $J$ = 7 Hz, of t, $J$ = 2 Hz), 5.29 (t, 1, $J$ = 2 Hz) overlapping with 5.38 (doubletoid m, X part of ABX, 1), 6.62 (t, 1, $J$ = 2 Hz) overlapping with 6.65 (s, 1), 6.84 (d, 1, $J$ = 7 Hz); CD (100% $C_2H_5OH$) 222, 236, 244, 255, 269, 290, 347 nm ($\theta$ −72200, −9500, −15300, −4100, − 10200, ±0, −26700), estimated absolute error in $\theta$ ±2500 deg $cm^2$/dmol.

Epiaflatoxin $Q_1$: mp 235° dec; uv max (100% $C_2H_5OH$) 224, 243 (sh), 267, 366 nm ($\epsilon$ 18200, 8400, 10000, 16500); uv min (100% $C_2H_5OH$) 252, 286 nm ($\epsilon$ 7300, 1200); ir ($CHCl_3$) 3600, 1775, 1710, 1635, 1605, 1575 $cm^{-1}$; MS (70 eV) *m/e* (rel intensity) 329 (25), 328 (100, $M^+$), 326 (24), 313 (9), 312 (21), 310 (7), 299 (10); NMR, 90 MHz ($Me_2SO$-$d_6$) $\delta$ 2.24 (A part of ABX, 1, $J_{AB}$ = 18, $J_{AX}$ = 1.5 Hz), 2.80 (B part of ABX, 1, $J_{AB}$ = 18, $J_{BX}$ = 7 Hz), 3.23 (s, $H_2O$ from solvent, exchanging with ROH of the substance), 3.86 (s, 3), 4.71 (d, 1, $J$ = 7 Hz, of t, $J$ = 2 Hz), 5.34 (t, 1, $J$ = 2 Hz) overlapping with 5.41 (doubletoid m, X part of ABX, 1), 6.65 (t, 1, $J$ = 2 Hz) overlapping with 6.68 (s, 1), 6.85 (d, 1, $J$ = 7 Hz); CD (100% $C_2H_5OH$) 222, 244, 257, 292, 321, 345, 375 nm ($\theta$ −10600, −7200, −15500, ±0, −9600, −3000, −21500), estimated absolute error in $\theta$ ±2500 deg $cm^2$/dmol.

**11-Hydroxy-5,7-dimethoxycyclopenteno[2,3-*c*]coumarin (5) (Silver(I) Oxide–Sodium Hydroxide Reaction).** To a stirred solution of 3 (26 mg, 0.1 mmol) in dichloromethane–methanol–water (11 ml, 4:4:1 v/v/v) in an ice bath was added in succession silver(I) oxide (70 mg, 0.3 mmol) and aqueous sodium hydroxide (0.5 ml, 0.4 *N*, 0.2 mmol). The reaction mixture was filtered through a silica gel column (6 g) after 2.5 hr, and the column washed with 250 ml of chloroform–methanol (3:1 v/v). The resulting solution was diluted with 500 ml of chloroform and washed once with 4 *N* aqueous ammonium chloride (300 ml) and once with distilled water (300 ml). The resulting organic phase was diluted with benzene (100 ml) and evaporated to dryness under reduced pressure. The residue was chromatographed (one 20 × 20 × 0.05 cm Analtech silica gel chromatoplate, chloroform–ethanol–hexanes, 10:2:1 v/v/v) to afford 3 (4.4 mg of a solid, $R_f$ 0.73, 17% recovered) and 5 (10.2 mg of a solid, $R_f$ 0.59, 46% based on amount of 3 reacted). Recrystallization of 5 from chloroform–ethanol–hexane gave pale yellow needles: mp 217–218°; ir (KBr) 3460, 2956, 1750, 1680, 1610, 1065 $cm^{-1}$; MS (70 eV) *m/e* (rel intensity) 276 ($M^+$, 100), 260 (24), 245 (16), 233 (39), 205 (16), 69 (19); NMR ($Me_2SO$-$d_6$) $\delta$ 6.6 (AB pattern, 2, protons on C-6 and C-8), 5.45 (m, 1, proton on C-11), 3.96, 3.92 (two s, 6, $-OCH_3$), 3.32 (broad s, 1, –OH), 2.90 (d of d, 1, $J$ = 6 and 18 Hz, C-9), 2.30 (d of d, 1, $J$ = 2 and 18 Hz, C-9); uv max (100% $C_2H_5OH$) 216, 240 (sh), 248 (sh), 258 (sh), 357 nm ($\epsilon$ 23600, 13960, 13250, 11300, 27000).

**11-Hydroxy-5,7-dimethoxycyclopenteno[2,3-*c*]coumarin (5) (Thallous Ethoxide–Hydrogen Peroxide Reaction).** To a solution of 3 (24.3 mg, 0.09 mmol) in dichloromethane (8 ml) in an ice bath was added a solution of thallous ethoxide (27.3 mg, 0.11 mmol) in dichloromethane (2 ml) and a solution of hydrogen peroxide (98%, 16.8 mg, 0.48 mmol) in methanol (3 ml). After 72 hr the reaction mixture was filtered through a silica gel column (6 g), and the column was washed with 300 ml of chloroform–methanol mixture (3:1 v/v). The resulting solution was evaporated and the residue chromatographed (one 20 × 20 × 0.05 cm Analtech silica gel chromatoplate, chloroform–ethanol–hexanes, 10:2:1 v/v/v) to afford 3 (13.6 mg, $R_f$ 0.73, 56% recovered) and 5 (8.1 mg, $R_f$ 0.59, 71% based on amount of 3 reacted).

**Hydrogenation of 3.** Compound 3 (4.8 mg, 0.0185 mmol) in absolute ethanol (6 ml) was hydrogenated in a Hösli microhydrogenator using a 10% Pd/C catalyst (30 mg) at 22° (730 mm). Hydrogen absorption was complete after an uptake of 0.91 ml (270 min). The catalyst was collected on a Celite filter pad and washed with chloroform. The combined filtrates were evaporated to dryness and the residue was chromatographed using a 0.25 mm silica gel thin layer chromatoplate (Analtech Co.) and chloroform–ethyl acetate (2:1 v/v) as the solvent. The major product, 5,7-dimethoxycyclopenteno[2,3-*c*]coumarin (4, 3.5 mg, 77%), was located by visualizing under long wavelength ultraviolet light (pale blue fluorescence), and eluted off the silica gel with chloroform–methanol (3:1 v/v). Ir, uv, and melting point were identical with those of an authentic sample; MS (70 eV) *m/e* 246 (molecular ion); mp 183–184° after one recrystallization from ethanol. A mixture melting point with an authentic sample showed no depression. Uv max ($C_2H_5OH$) 248, 257, and 325 nm ($\epsilon$ 7700, 7000, 16100); ir ($CHCl_3$) 1706, 1608, and 1567 $cm^{-1}$.

**Hydrogenation and Hydrogenolysis of 5.** Compound 5 (4.6 mg, 0.016 mmol) in absolute ethanol (6 ml) was hydrogenated in a Hösli microhydrogenator using a 10% Pd/C catalyst (30 mg) at 22° (730 mm). Hydrogen absorption became very slow after an uptake of 1.0 ml (4.5 hr, 80% of theoretical value). The 5,7-dimethoxycyclopenteno[2,3-*c*]coumarin (4) was isolated as described for the hydrogenation of 3, yield 3.2 mg (78%); ir, uv, and melting point identical with those of the authentic sample.

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**Registry No.**—1, 1162-65-8; 2 natural, 52819-96-2; 2 epimer, 56648-94-3; 3, 1150-42-1; 4, 1146-71-0; 5, 56599-31-6; thallium(I) ethoxide, 20398-06-5; silver(I) oxide, 1301-96-8.

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# Geometric Isomers of 11,12-Dehydro-15-demethyl-β-axerophtene. New Geometric Isomers of Vitamin A and Carotenoids III[1]

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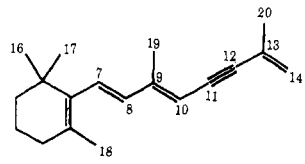
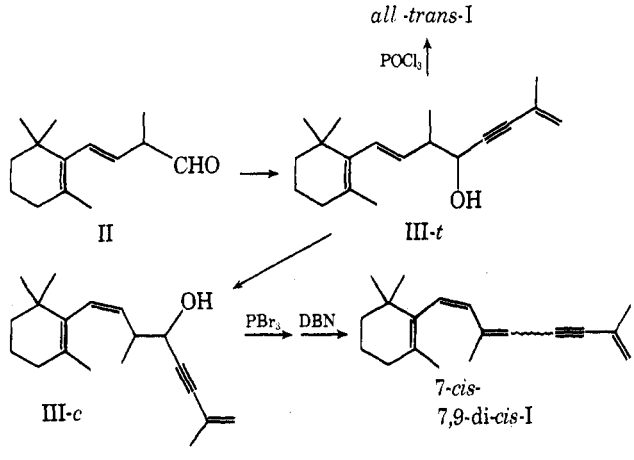
In connection with the photochemical studies of the polyenes in the vitamin A series,[3] we were in need of a complete set of geometric isomers of such a pentaene. The compound 11,12-dehydro-15-demethyl-β-axerophtene (I) was chosen because it has only four isomers and a procedure to the all-trans isomer is in the literature.[4] A modification of



I

this procedure may lead to the other isomers. The preparation and spectroscopic properties of all four isomers of I including the two sterically crowded 7-cis isomers, are now described here.

Aldehyde II, known as the iso-$C_{14}$ aldehyde, was prepared according to the literature procedure.[5] Reaction of II, instead of the conjugated $C_{14}$ aldehyde,[4] with the Grignard of 3-methyl-3-buten-1-yne gave the $C_{19}$ alcohol III-*t* as the only product in high yield [ir 3400 (OH) and 960 $cm^{-1}$ (trans C=C); NMR $J_{7,8}$ = 16 Hz (trans)]. Because of the introduction of a second asymmetric carbon through the Grignard reaction, the presence of diastereomers in III-*t* was expected. This is clearly shown by its NMR spectrum, in which H-8, H-10, and $CH_3$-19 all appear as doublets (in addition to splittings due to spin–spin coupling).




Dehydration of III-*t* with $POCl_3$–pyridine gave a yellow oil. Similar to the reaction from an isomer of III-*t* from $C_{14}$ aldehyde,[4] this reaction appears to give only *all-trans*-I [ir 2200 (C≡C), 970 (trans C=C), 820 $cm^{-1}$ (trisubstituted double bond); NMR (Table I)]. The absence of detectable amounts of the 9-cis isomer in the dehydration reaction is somewhat surprising but appears often in vitamin A synthesis.[6]

One-way sensitized irradiation[3] of the $C_{19}$ alcohol (III-*t*) with β-acetonaphthone gave the 7-cis isomer (III-*c*) in quantitative yield. The cis geometry is revealed by the magnitude of the vinyl coupling constant ($J_{7,8}$ = 12.0 Hz) in NMR and the 730-$cm^{-1}$ band in ir. Alternatively, the compound was also obtained by reaction of 7-cis-iso-$C_{14}$ aldehyde (II-*c*) with the Grignard from 3-methyl-3-buten-1-yne.

Dehydration of the more hindered III-*c* presented some difficulties. With reagents such as proton acids or $POCl_3$ molecular rearrangements occurred, giving complex mixtures. Therefore, instead, dehydration was accomplished in two steps via the intermediacy of the corresponding bromide which was prepared by reaction of III-*c* with $PBr_3$. To avoid possible electrocyclization of the product at elevated temperatures, the mild dehydrobrominating reagent diazabicyclo[4.3.0]nonene (DBN)[7] was used.

The mass spectrum of the product (*m/e* for M = 254) agrees with that of a $C_{19}H_{26}$ hydrocarbon. Its ir spectrum indicates the retention of the 7-cis geometry (720 $cm^{-1}$) with no noticeable absorption between 960 and 980 $cm^{-1}$ for 7-trans products. Its NMR spectrum is also in agreement with the presence of the 7-cis geometry only. However, it also shows the presence of two isomers in a relative ratio of 3:2. Although the isomers have not yet been isolated, the key NMR signals, even in a mixture, are sufficiently well resolved for assignments (Table I). The major product was assigned with the 7,9-di-cis geometry. The 7-cis geometry is evident from the coupling constant and the high field shift of $CH_3$-18.[8] The assignment of the 9-cis geometry was based on the low-field H-8 signal as a result of deshielding by steric polarization by the cis ethynyl group.[9] This downfield shift is in fact characteristic of all 9-cis isomers in the vitamin A series.[9,10] The minor isomer with a higher field H-8 signal therefore must have the 7-cis geometry.

Table I
NMR Spectral Characteristics of Geometric Isomers of 11,12-Dehydro-15-demethyl-β-axerophtene[a]

| Isomer | $H_7$ | $H_8$ | $H_{10}$ | $CH_3$-20 | $CH_3$-19 | $CH_3$-18[b] | $J_{7,8}$[c] |
|---|---|---|---|---|---|---|---|
| *all-trans* | 6.20 | 6.08 | 5.51 | 2.04 | 1.86 | 1.68 | 14.5 |
| 7-*cis* | 5.97 | 6.07 | 5.64 | 1.99 | 1.93 | 1.54 | 12.0 |
| 9-*cis* | 6.21 | 6.73 | 5.32 | 1.86 | 1.84 | 1.68 | 15.0 |
| 7,9-Di-*cis* | 5.95 | 6.74 | 5.46 | 1.95 | 1.76 | 1.54 | 12.2 |

[a] HA-100, solvent $CDCl_3$–$Me_4Si$. [b] Chemical shifts in parts per million. [c] Coupling constants in hertz.

These two isomers exhibit expected photochemical properties for polyenes in this series[3b] in that upon photosensitized irradiation (with benzanthrone or benzo[*a*]pyrene and light >400 nm) they were completely converted to the less hindered 7-trans isomers. The major product in the stationary state mixture (~55% by NMR) is *all-trans*-I. The minor product (~45%) shows NMR characteristics (Table I) only consistent with 9-*cis*-I, i.e., low-field H-8 (9-cis) and a large $J_{7,8}$ (7-trans).

The reaction sequence described above for 7-cis isomers of I suggests a promising route to the unknown 7,11-di-cis isomers of vitamin A[11] by replacing the $C_5$ enyne with isomers of 3-methyl-2-penten-4-yn-1-ol (cis/trans "pentol"). This and other routes to such new isomers are actively being studied in our laboratory.

## Experimental Section

All NMR spectra were recorded on a Varian HA-100 spectrometer with $CDCl_3$ as solvent and $Me_4Si$ as internal standard. Ir spectra were recorded on a Beckman IR-10 and MS on a Hitachi Perkin-Elmer RMU-6D unit.

**Preparation of Iso-$C_{14}$-aldehyde II.** The trans isomer was prepared from β-ionone by a modified procedure[3b] of that of Oediger and Eiter.[5] The cis isomer was obtained by sensitized irradiation as reported earlier.[3b]

**Preparation of $C_{19}$ Alcohol III-*t*.** To an ether solution of ethylmagnesium bromide, prepared from 30 g of ethyl bromide and 6 g of Mg, was added 20 g of 3-methyl-3-buten-1-yne diluted with 30 ml of ether. After 4 hr, 30 g of II-*t* was added dropwise. After completion the solution was heated to 50°C for 1 hr and then allowed to stand for 8 hr. The mixture was worked up with a saturated $NH_4Cl$ solution. Extraction with ether, drying over $MgSO_4$, and evaporation of ether gave the $C_{19}$ alcohol III-*t* [ir 3400 (OH), 960 (trans C=C), 895 $cm^{-1}$ (exo $CH_2$); NMR 1.02 (s, $CH_3$-16, 17), 1.15, 1.16 (s, d, $CH_3$-19, diastereomers), 1.68 (s, $CH_3$-18), 5.39, 5.31 (d, d, $J$ = 16.0 Hz, H-8), 5.97 ppm (d, $J$ = 16.0 Hz, H-7)].

**Preparation of III-*c*.** It was prepared either by sensitized irradiation of III-*t*[3b] or by a similar procedure as described above for III-*t* except using II-*c* instead. [III-*c*: ir 3400 (OH), 895 (exo $CH_2$), 730 $cm^{-1}$ (cis C=C); NMR 1.02 (s, $CH_3$-16, 17), 1.10 (s, $CH_3$-19), 1.60 (s, $CH_3$-18), 5.58, 5.45 (d, d, $J$ = 11.5, H-8), 5.67 ppm (d, $J$ = 11.5 Hz, H-7)].

**Preparation of *all-trans*-I.** A solution of 2 g of III-*t* in 6 ml of toluene was added to 1.2 g of $POCl_3$ in 4 ml of pyridine and 10 ml of toluene. The mixture was refluxed for 45 min, cooled, and

poured over ice, then extracted with ether. The ether solution was dried over $MgSO_4$ and solvent evaporated to give crude *all-trans*-I. The product was purified by passing through a short silica gel column using benzene–hexane solvent mixture [ir 2200 (C≡C), 1620 (conjugated double bonds), 970 (trans C=C), 895 (exo $CH_2$), and 820 $cm^{-1}$ (trisubstituted double bond); NMR (Table I)]. These data are in agreement with those published for I-*t*.[5]

**Preparation of 7-Cis Isomers of I.** To 2 g of III-*c* in 20 ml of ether was added at 0° a solution of 2 g of $PBr_3$ in 10 ml of ether. The mixture was stirred at room temperature for 2 hr, then poured over ice and extracted with ether. Upon evaporation of solvent, the crude bromide was obtained [MS *m/e* (M) 335, ir no OH].

Without further purification the bromide was taken in 20 ml of benzene, previously distilled over Na. To this was added 3 ml of DBN in 10 ml of benzene. The mixture was stirred at 50°C for 1 hr followed by 12 hr at room temperature. Then the mixture was poured over ice and extracted with ether. Upon evaporation of ether, a yellow oil was obtained. It was chromatographed over silica gel using petroleum ether (bp 30–60°) as solvent. The early fractions contained mixtures of 7-*cis*- and 7,9-di-*cis*-I [ir 2200 (C≡C), 895 (exo $CH_2$), 820 (trisubstituted C=C), and 720 $cm^{-1}$ (cis C=C); NMR (Table I)].

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**Registry No.**—*all-trans*-I, 56744-00-4; 7-*cis*-I, 56744-01-5; 9-*cis*-I, 56744-02-6; 7,9-di-*cis*-I, 56744-03-7; *cis*-II, 56013-06-0; *trans*-II, 56013-05-9; III NS isomer, 56744-04-8; 3-methyl-3-buten-1-yne, 78-80-8.

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