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Synthesis and spectroscopic characterization of the doubly locked 9*E*,11*Z* retinal model systems 7*E*,13*E*-11,19-10,20-dimethanoretinal and its 13*Z* isomer

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Abstract. 7*E*,13*E*-11,19-10,20-dimethanoretinal (**1**) and its 13*Z* isomer **2** were prepared from β-ionone, using the novel synthon 2-(diethoxyphosphinyl)-5,5-dimethoxyhexanenitrile. This synthon was also used to prepare 7*E*,9*E*,13*E*-10,20-methanoretinal (**3**) and its 13*Z* isomer **4** in high yield, starting from β-ionone. Spectroscopic analysis (mass, ^{1}H and ^{13}C NMR and UV/Vis) of these compounds is discussed. The introduction of the methano bridges leads to minimal steric and electronic changes. The photostationary state reached from **1** and **2** has the 13*Z* form **2** as the main constituent. This is one of the very few with a *Z* form as the main constituent of the photostationary state. **1** and **2** are very sensitive to acid-catalyzed isomerization of the 13-C=14-C double bond. The presence of the 11,19-methano bridge is responsible for this efficient *Z*–*E* isomerization.

Introduction

Visual pigments are intrinsic membrane proteins that play a central role in the transduction of light information into nerve action that allows the sense of vision[1,2]. As a typical example, bovine rhodopsin has an 11*Z* retinylidene chromophore bound as a protonated Schiff base to lysine-296 in the active site[3,4]. Rhodopsin is very light-sensitive, since its photoreaction is very efficient (quantum yield 0.67)[5].

The main difference between the primary photoproduct bathorhodopsin and rhodopsin lies in the fact that the batho chromophore has a strained all-*E* retinylidene configuration[6], as a result of light-induced 11*Z* → 11*E* isomerization. Bathorhodopsin has a 32 kcal/mol higher energy content than rhodopsin[7,8]. It decays via several intermediates into the apoprotein opsin and free all-*E* retinal[9,10].

Since visual pigments represent a class of G-protein-connected receptor proteins[11], detailed information on their structure and molecular dynamics is of utmost importance not only to the field of vision but also to the understanding of certain patterns of signal transduction in general. The primary structures of several visual pigments have been established[12], but only indirect information exists about their secondary and tertiary structure. The best approach would be to analyse single crystals by X-ray diffraction; however, membrane proteins such as rhodopsin do not crystallize easily. Recently, great progress has been made in crystallization of membrane complexes, such as the photosynthetic reaction center and porins, and exquisite structural information has become available from X-ray investigations of the crystallized reaction centers[13,14]. In the case of rhodopsin, its extreme light sensitivity is one of the factors that seriously hampers crystallization attempts[15].

Recently, we have started a research program to prepare rhodopsins with a chemically modified chromophore that will lead to much lower or no light sensitivity. 10,20-methanoretinal was prepared, in which 11*Z*–11*E* isomerization is prevented by the presence of a six-membered ring[16,17] (Figure 1, compound **3**). This retinal analogue binds efficiently to opsin to form 10,20-methanorhodopsin, which is very similar to native rhodopsin except that it is about 100 times less sensitive to light. The increase in light stability was not sufficient to allow crystallization, as the 10,20-methanorhodopsin is still light-sensitive, even though 11*Z*–11*E* isomerization is blocked. We think that this photoreaction is caused by double-bond *Z*–*E* isomerization, presumably around the 9-C=10-C double bond. Introduction of the 11,19-methano bridge will prevent this *E*–*Z* isomerization. 7*E*,13*E*-10,20-11,19-Dimethanoretinal (**1** and **2**, Figure 1) will be the retinal of choice in which both

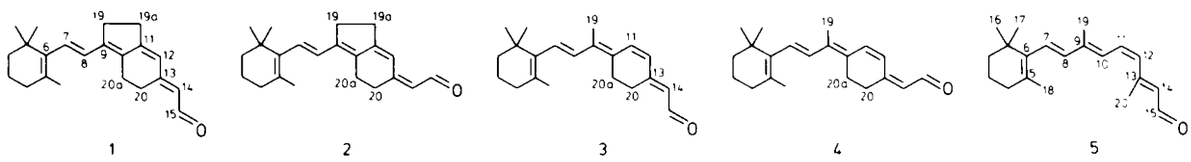


Figure 1. Structure and numbering of **1**, **2**, **3**, **4** *and* **5**.

the isomerizations of the 9-C=10-C and the 11-C=12-C double bond are blocked. The methano bridges lock the chromophore in the native 10-*s-trans*, 12-*s-trans* conformation and 9-*E*,11-*Z* configuration. The introduction of methano bridges induces only minimal steric and electronic changes compared to the native system.

In this paper, we describe an efficient synthesis for 7*E*,13*E*-10,20-11,19-dimethanoretinal (**1**) and its 13*Z* isomer **2** and an improved synthesis of 7*E*,9*E*,13*E*-10,20--dimethanoretinal (**3**) and its 13*Z* isomer **4**. The spectroscopic characterization of **1**, **2**, **3** and **4** is described. The preparation and study of 10,20-11,19-dimethanorhodopsin will be the subject of a future publication.

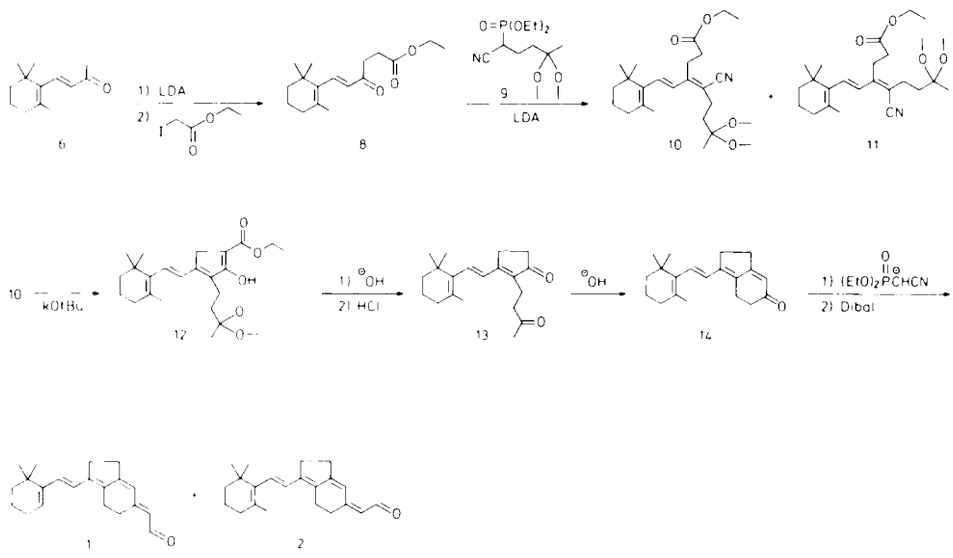
Synthesis

For the synthesis of **1** and **2**, we used the approach given in Scheme 1. Recently, we have found that β-ionone (**6**) can be selectively mono-alkylated at the methyl-ketone position[18]. The anion of β-ionone was prepared by treating **6** in THF with LDA. Treating this anion with 1.0 eq. of ethyl iodoacetate gave the γ-keto ester **8** as the main product, mixed with some dialkylated material and some starting β-ionone. SiO_2 column chromatography gave the pure γ-keto ester in 57% yield. For the next step, the novel synthon 2-(diethoxyphosphinyl)-5,5-dimethoxyhexanenitrile (**9**) was developed *(vide infra)*. 5,5-Dimethoxyhexanenitrile (**17**) was added to a solution of 2.2 eq. of lithium diisopropylamide (LDA). One equivalent of LDA reacts to form the anion of the nitrile **17**. Diethyl chlorophosphate (1.0 eq.) is then added. In an S_N2 reaction, the phosphonate **9** is formed. The second equivalent of LDA reacts with **9** to form its anion. γ-Keto ester **8** is then added. After this Horner–Emmons condensation, the isomeric nitriles **10** and **11** (formed in a ration of 4:1) were obtained in high yield (70% based on **8**). The required *E* isomer **10** could be separated from **11** by SiO_2 column chromatography.

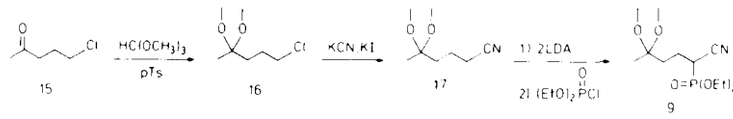
Compound **10** has the right functionalities and stereochemistry for conversion into the unsaturated hydroindenone derivative **14**. First, the five-membered ring was formed via a Ziegler–Thorpe-type condensation by treating **10** with KtBuO. Treating this product with a slurry of SiO_2 and water resulted in hydrolysis of the cyclic imine to the corresponding β-keto ester **12** in the enol state. Saponification and decarboxylation of the β-keto ester was effected by treating **12** with excess KOH in refluxing methanol. The resulting ketone ketal was deprotected, giving diketone **13** which underwent aldol condensation in mild basic environment to form the required unsaturated ketone **14**. This ketone is converted via a two-step procedure into a 1:1 mixture of 7*E*,13*E*-11,19-10,20-dimethanoretinal (**1**) and its 13*Z* isomer **2**. The first step is a Horner–Emmons reaction of **14** with (diethoxyphosphinyl)acetonitrile; the second step is DiBAl reduction of the nitrile to give **1** and **2**. The isomeric mixture was separated into the pure **1** and **2** by HPLC. Each of the isomers was obtained in 2% overall yield based on β-ionone.

The phosphonate nitrile synthon **9** was prepared from commercial 5-chloro-2-pentanone (**15**) (see Scheme 2). **15** was first treated with trimethoxymethane in methanol with a catalytic amount of 4-toluenesulfonic acid to give the ketal chloride **16**. **16** was treated with KCN in the presence of KI to give a clean substitution to the nitrile ketal **17**. After distillation, pure **17** was obtained in 62% yield from **15**. For conversion to the phosphonate nitrile **9**, the nitrile **17** was dissolved in THF and treated with 2 equivalents of LDA followed by addition of 1 equivalent of diethyl chlorophosphate. This solution can be used directly for the Horner–Emmons reaction or after mild acidic work-up the phosphonate nitrile **9** is obtained.

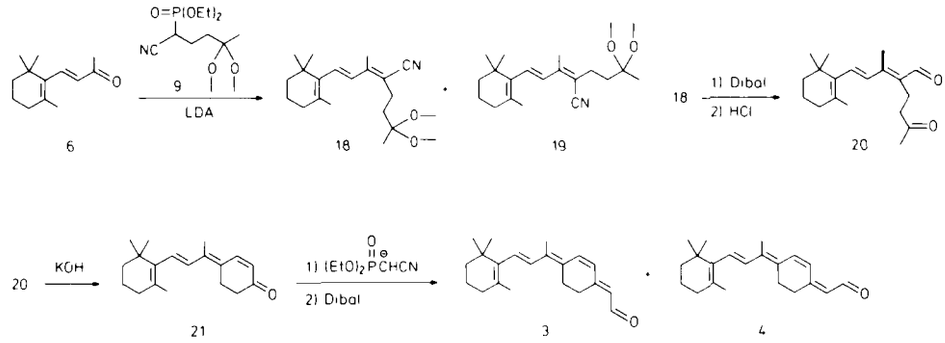
It is clear that Horner–Emmons condensation with phosphonate **9** is the central reaction during the synthesis of **1** and **2** via Scheme 1. We realized that condensation of **9** with β-ionone could play a similar role in the preparation of 10,20-methanoretinals **3** and **4**, whose preparation via a complicated procedure we have described before[17]. Addition of β-ionone to the solution of the anion of **9** in THF gave the isomeric nitriles **18** and **19** (Scheme 3). The all-*E* isomer **18** was separated from the 9-*Z* isomer **19** by SiO_2 column chromatography. DiBAl reduction of **18** converted the nitrile to the aldehyde function. The ketal protection was removed by acid treatment giving the all-*E* aldehyde ketone **20**. Internal aldol condensation of **20** by KOH gave the ketone **21**. Ketone **21** is obtained in the all-*E* form only, which means that, during the reactions starting with **18**, no *E*–*Z* isomerization has taken place. To convert ketone **21** into the required aldehydes **3** and **4**, **21** was submitted to a Horner–Emmons reaction with (diethoxyphosphinyl)acetonitrile and subsequent DiBAl reduction of the resulting



Scheme 1. Synthesis of 7E,13E-*11,19-10,20-dimethanoretinal* (**1**) *and its 13Z isomer* **2**.



Scheme 2. Synthesis of phosphonate nitrile **9**.



Scheme 3. Synthesis of 7E,9E,13E-10,20-methanoretinal (**3**) *and its 13Z isomer* **4**.

nitriles. In this way, 7*E*,9*E*,13*E*-10,20-methanoretinal (**3**) and its 13*Z* isomer **4** were obtained in a ratio of 4:1. The aldehydes were separated via HPLC into their pure states. **3** and **4** were obtained in 34% and 9%, respectively, based on β-ionone.

Spectroscopic characterization

Mass spectroscopy

The double-focus mass spectra (EI at 70 eV) of **1** and **2** show the molecular ion as a parent peak at *m/z* 308.2143 and *m/z* 308.2137, respectively (calcd. for $C_{22}H_{28}O$: 308.2140) and the spectra of **3** and **4** show the molecular ion as a parent peak at *m/z* 296.2147 and *m/z* 296.2142, respectively (calcd. for $C_{21}H_{28}O$ 296.2140).

The single-focus mass spectra of **1** and **2** (EI at 70 eV) did not differ significantly. The molecular ion at *m/z* 308 appeared as the base peak, a common feature in mass spectra of retinals[19]. Major peaks appeared at *m/z* 293, 252, 185, 172, 159 and 91. The fragment at *m/z* 91 may well arise from the tropylium ion. Many retinals show a fragment at $M^{\cdot} - 29$, corresponding to loss of the CHO group. In the spectrum of **1** and **2**, this fragment is not present. The single-focus mass spectra of **3** and **4** also did not differ significantly. Major peaks appear at *m/z* 279, 256, 177, 167, 165, 149, 147, 123, 121, 111, 109, 107, 105, 95 and 91. Again, no fragment at $M^{\cdot} - 29$ is observed.

^1H *NMR*

The 400-MHz ^{1}H NMR spectrum of **1** (in $CDCl_3$), shown in Figure 2, is in complete agreement with its structure. In the high-field region of the spectrum, the signals of the trimethylcyclohexenyl moiety are present, as well as the signals of two AA'BB' sub-spectra. The AA'BB' sub-spectrum with δ 2.69 and 2.73 ppm was assigned to the 19-CH_2 and 19a-CH_2 signals. The AA'BB' sub-spectrum of 20-CH_2 and 20a-CH_2 appears at δ 2.97 and 2.60 ppm, respectively. In the low-field region, one AX and one AB pattern, and one broad singlet are present. 7-H and 8-H are assigned to the AB pattern of a *trans* ethene fragment (δ_{H7} 6.34, δ_{H8} 6.45 ppm, *J* 16.5 Hz), 14-H and 15-H give the AX pattern (δ_{H14} 5.81, δ_{H15} 10.02 ppm, *J* 8.2 Hz). 12-H appears as the broad singlet at δ 6.08 ppm. Irradiation at the 15-H doublet gives a NOE effect at the signal at δ 2.97, confirming its assignment to 20-CH_2 and confirming the

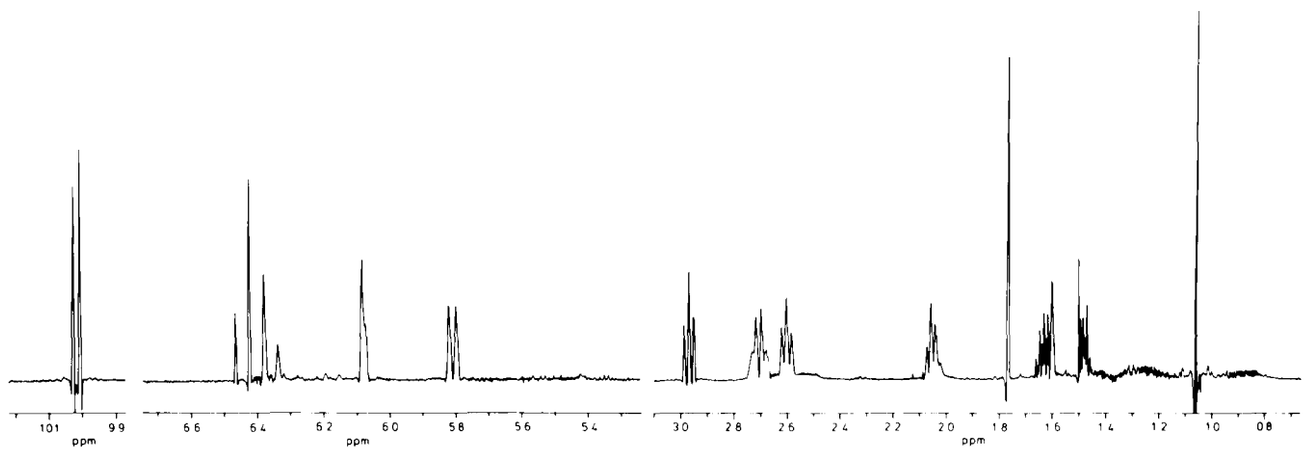


Figure 2. 400-MHz ^1H NMR spectrum of **2**.

Table I ^1H *NMR chemical-shift values of* **1** *and* **2**.

H	1	2
2	1.50	1.50
3	1.63	1.63
4	2.06	2.05
7	6.34	6.34
8	6.45	6.43
12	6.08	6.95
14	5.81	5.67
15	10.02	10.12
16/17	1.06	1.05
18	1.77	1.76
19	2.69	2.67
19a	2.73	2.73
20	2.97	2.57
20a	2.60	2.57

13*E* structure. In Table I, the 1H NMR chemical-shift values of **1** are collected.

The 400-MHz ^1H NMR spectrum of **2** shows that it is the 13*Z* isomer of **1**. 14-H and 15-H form an AX pattern (δ_{H14} 5.67, δ_{H15} 10.12 ppm, J 8.2 Hz). Compared to the spectrum of **1**, this is a 0.10 ppm downfield shift for 15-H and a 0.14 ppm upfield shift for 14-H, confirming the 13*Z* structure. 12-H appears as a broad singlet at δ 6.95 ppm, 0.87 ppm downfield compared to the 12-H resonance in the spectrum of **1**, in agreement with the close proximity of the aldehyde function to 12-H in **2**, again confirming the 13*Z* structure. The signals of 20-CH_2 and 20a-CH_2 give rise to a AA'BB' sub-spectrum at δ 2.57 ppm: this means that the 20-CH_2 resonance is 0.40 ppm upfield compared to the spectrum of **1**, which agrees with the longer distance between 20-CH_2 and the aldehyde function in **2**. 19-CH_2 and 19a-CH_2 appear as a AA'BB' spectrum, at δ 2.67 and δ 2.73, respectively. The signals of the cyclohexenyl end of **2** resemble the spectrum of **1** within 0.02 ppm. The ^1H NMR chemical-shift values of **2** are collected in Table I.

The ^1H NMR spectra of **3** and **4** are in complete agreement with their structure and identical to the spectra published earlier[16]. When **1** is compared to **3**, major chemical-shift differences can be seen at 19-CH_2 (Δ 0.66 ppm), as a result of alkylation at the 19-methyl[18]. 7-H, 8-H and 20a-CH_2 show an upfield shift (Δ −0.06, −0.16 and −0.09 ppm, respectively) indicating relief from steric interaction. At 12-H, 14-H and 15-H, upfield shifts (Δ −0.17, −0.07 and −0.04, respectively) can also be seen. The other signals are the same within 0.02 ppm. Comparing **2** to **4**, similar chemical-shift differences are observed.

^{13}C *NMR*

In Table II, the ^{13}C NMR chemical-shift values of **1**, **2**, **3** and **4** and their assignments are given. The 100-MHz ^1H-noise-decoupled ^{13}C NMR spectrum of **1** shows 21 signals, in agreement with the 21 chemically different carbon atoms. In the sp^3 region, the expected ten signals are present. These signals were easily assigned by comparison with both the $^1H-^{13}C$ correlated spectrum and the spectrum of 11*Z*-retinal[20]. In the sp^2 region, the 11 vinylic carbon signals are present. 7-C, 8-C, 12-C, 14-C and 15-C were assigned by comparison with the $^1H-^{13}C$ correlated spectrum. 5-C and 6-C were assigned by comparison of their values with those of 11*Z*-retinal. The remaining four signals of the quaternary carbons 9-C, 10-C, 11-C and 13-C were assigned by comparison of their values with those of **3**, the major shift to be expected on 11-C (α effect[21]). For 10-C and 13-C, no substancial differences are to be expected between the chemical-shift values of **1** and **3**.

Table II ^{13}C *NMR chemical-shift values of* **1**, **2**, **3** *and* **4**.

C	1	2	3	4
1	34.2	34.2	34.2	34.2
2	39.7	39.6	39.6	39.6
3	19.1	19.1	19.2	19.2
4	33.3	33.3	33.2	33.1
5	131.6	131.5	130.4	130.3
6	137.7	137.7	138.2	138.2
7	132.5	132.4	130.9	130.9
8	126.6	126.5	131.4	131.3
9	136.3	136.3	134.8	134.7
10	133.6	133.6	130.2	130.7
11	146.6	146.5	135.6	135.3
12	119.0	111.8	128.6	121.0
13	157.7	157.7	156.1	156.1
14	123.4	121.1	125.4	123.8
15	190.3	190.0	190.6	189.8
16/17	29.0	29.0	29.0	29.0
18	21.8	21.8	21.9	21.9
19	28.2	28.2	13.7	13.7
19a	30.1	30.2	–	–
20	23.8	31.6	24.8	32.0
20a	21.0	21.5	24.7	25.2

The 100-MHz ^1H-noise-decoupled ^{13}C NMR spectrum of **2** shows 21 signals, 10 signals in the sp^3 region and 11 in the sp^2 region. The ^{13}C NMR spectrum of **2** resembles the spectrum of **1** very closely (within 0.5 ppm), except for the signals from 12-C, 14-C and 20-C, their $\Delta\delta$ being −7.2, −2.3 and 7.8 ppm, respectively.

The 100-MHz ^1H-noise-decoupled ^{13}C NMR spectrum of **3** shows 20 signals, in agreement with the 20 different carbon atoms, 9 signals in the sp^3 region and 11 signals in the sp^2 region. The signals of the ^1H-bearing carbon atoms were identified by comparison with the $^1H-^{13}C$ COSY spectrum. The signals of the quaternary 1-C, 5-C, 6-C and 13-C carbons were identified by comparison to those of 11*Z*-retinal. The two remaining signals at 134.8 (9-C) and 130.2 ppm (10-C) were assigned by comparison to 11*Z*-retinal and 10,20-methanoretinoyl fluoride[16].

The 100-MHz ^1H-noise-decoupled ^{13}C NMR spectrum of **4** shows 20 signals, 9 signals in the sp^3 region and 11 signals in the sp^2 region. The signals were easily identified by comparison to the $^1H-^{13}C$ COSY spectrum to the spectrum of **3**. The spectra of **3** and **4** resemble each other within 0.8 ppm, the only deviations being the signals of 12-C, 14-C and 20-C, $\Delta\delta$ being −7.6, −1.6 and 7.2 ppm, respectively.

UV/Vis spectroscopy

The electronic spectrum of **1** shows a broad Gaussian-curve-shaped absorption without vibrational fine-structure, with λ_{max} (EtOH) 397 nm and λ_{max} (*n*-hexane) 374 nm. The electronic spectrum of **2** is also a broad Gaussian-curve-shaped absorption without vibrational fine-structure, with λ_{max} (EtOH) 389 nm and λ_{max} (*n*-hexane) 368 nm. The electronic absorption spectra of **3** and **4** are identical to the data published earlier[16]. Comparing the λ_{max} of **1** and **3** and the λ_{max} of **2** and **4** in both cases shows a bathochromic shift of 5 nm in ethanol and no observable shift in *n*-hexane. This result indicates no major influence of the additional five-membered ring on the electronic absorption.

Photochemistry

The synthesis method gives an 1:1 mixture of 7*E*,13*E*-11,19--10,20-dimethanoretinal (**1**) and its 13*Z* isomer **2**. Irradia-

tion of pure **1** or **2** in acetonitrile with a 150-W tungsten lamp leads to the same photostationary state consisting of 7 10 of **2** and 3/10 of **1**, whereas irradiation of **3** or **4** leads to a photostationary state with about equal amounts of **3**, 9*Z*-**3**, **4** and 9*Z*-**4**. This shows that the presence of the additional five-membered ring has a profound effect on the photochemistry of the retinal. In retinal itself, the all-*E* isomer is the main constituent in the photostationary state[22,23] with appreciable amounts of the mono *cis* isomers (7*Z*,9*Z*,11*Z*,13*Z*). The central six-membered ring leads to a photostationary state with the all-*E* (**3**), 9*Z* (9*Z*-**3**), 13*Z* (**4**) and the 9*Z*,13*Z* (9*Z*-**4**) present. No formation of 7*Z* was observed. Introduction of the five-membered ring restricts the number of *cis* isomers. No 7*Z* was observed. In this system, the 13*Z* (**2**) contributes for 7/10 to the photostationary state. Only two other retinals are known in which a *Z* isomer is the main constituent of the photostationary state. One of these is 12,19-methanoretinal, described previously[18]; others have reported on its aromatic analogue[24].

Discussion

For the introduction of the hydroindenone system, we have developed the novel synthon 2-(diethoxyphosphinyl)-5,5--dimethoxyhexanenitrile (**9**). The Horner–Emmons reaction of this synthon with the alkylated β-ionone derivative **8** gave the compound that was easily converted into hydroindenone derivative **14**. First, ring closure of **9** was effected by a Ziegler–Thorpe-type condensation to a cyclopentenone ester. The resistance of the β-keto ester to the usual mild saponification and decarboxylation methods[25,26,27,28,29] is presumably due to the stability of the enol and enolate forms of this five-membered ring β-keto ester. Treatment with excess base in methanol/water led to efficient saponification and decarboxylation. Removal of the ketal protection gave a diketone that was cyclized efficiently to the hydroindenone system **14**. It is to be expected that **9** will be extremely useful for the construction of many other not easily accessible cyclohexenone derivatives.

Comparison of **1** and **2** on the one hand, and **3** and **4** on the other, showed that the introduction of an additional five-membered ring leads to only minor changes in the steric and electronic properties. **1** and **2** are sensitive to acid-catalysed isomerization; even traces of acid can effect their interconversion. This increased sensitivity can be explained by the presence of an electron-donating methylene group at 11-C in **1** and **2**, which will stabilise the protonated form of **1** and **2**, and this extra stabilization is not present in **3** and **4**. The presence of the additional five-membered ring also has a profound influence on the photochemistry. In the photostationary state, the 13*Z* form **2** contributes much more than the all-*E* form **1**.

The ^{13}C NMR spectra of **1** and **2** show only a small difference to those of **3** and **4**, except for the signal of 11-C, which is the site of alkylation in **1** and **2**. The UV/Vis spectra of **1** and **2** also show only a small difference to those of **3** and **4**. These facts clearly indicate that the novel retinals **1** and **2** suffer from only minimal electronic and steric change as compared to **3** and **4**.

Experimental

The following solvents were distilled prior to use: triethylamine (from CaH_2), tetrahydrofuran (THF; from $LiAlH_4$), diethyl ether (from P_2O_5), petroleum ether (b.p. 40–60°C; from P_2O_5). Reactions were generally carried out in a nitrogen atmosphere. Diisobutylaluminum hydride (DiBAl) was used as a 1.0M solution in hexanes, *n*-butyllithium (BuLi) as an 1.6M solution in hexanes. Chemicals were bought from Janssen Chimica (Belgium) or Aldrich (USA).

NMR spectra were run in $CDCl_3$ [with tetramethylsilane (δ 0 ppm) as internal standard] at a Jeol FX-200, a Bruker WM-300 or a Bruker MSL-400 (operating at 199.5 MHz, 300.1 and 400.1 MHz for ^1H and 50.1 MHz, 75.4 MHz and 100.4 MHz for ^{13}C, respectively). UV/Vis spectra were run on a Varian DMS-200, using ethanol or *n*-hexane as solvent.

The electron-impact (EI) mass spectra were recorded at 70 eV and 15 eV on a V.G. Micromass ZAB-2HF mass spectrometer, an instrument with reverse geometry, fitted with a high-field magnet and coupled to a V.G. 1/250 data system. The samples were introduced via a direct insertion probe into the ion source. The ion-source temperature was generally 150°C. During the high-resolution EIMS measurements, a resolving power of 20000 (10% valley definition) was used.

Evaporation of solvents was performed *in vacuo* (20 mmHg). Purification was performed by flash column SiO_2 chromatography, using ether/petroleum ether as eluent, unless stated otherwise. Straight-phase isocratic HPLC was performed on a Pharmacia LKB 2150/2151, with a Zorbax SiO_2 column (21.1 mm × 25 cm; Dupont), using 25% ether/petroleum ether as eluent for the mixture of **1** and **2**, and 10% ether/petroleum ether for **3** and **4**.

1-Chloro-4,4-dimethoxypentane (**16**)

5-Chloro-2-pentanone (**15**, 50 g, 0.42 mol), MeOH (11 g, 0.42 mol), 4-toluenesulfonic acid hydrate (1 g) and trimethoxymethane (48.0 g, 0.45 mol) were stirred at room temperature for 6 h. Triethylamine (15 ml) was then added and the solids were filtered off. After evaporation of the solvents, a yield of 67.4 g (98%) was obtained. ^1H NMR (200 MHz): δ 1.28 ppm, s (5-CH_3); 1.68–1.84, m (2-CH_2 + 3-CH_2); 3.16, s (OCH_3); 3.56, t, *J* 7 Hz (1-CH_2).

5,5-Dimethoxyhexanenitrile (**17**)

16 (67.4 g, 0.40 mol), 53 g (0.79 mol) of KCN and 7.0 g (41 mmol) of KI in 150 ml of ethanol and 75 ml of water were refluxed for 72 h. The mixture was then extracted with petroleum ether. The combined organic layers were washed with brine and dried over $MgSO_4$ and the solvents were evaporated. Yield after distillation 40 g (63%); b.p. 50–51°C at 0.15 mmHg. ^1H NMR (200 MHz): δ 3.18 ppm, s (OCH_3); 2.39, t, *J* 6.7 Hz (2-CH_2); 1.74, m (3-CH_2 + 4-CH_2); 1.28, s (6-CH_3). ^{13}C NMR (50 MHz): δ 119.1 ppm (1-C); 100.4 (2-C); 47.5 (OCH_3); 35.0 (5-C); 20.4 (6-C), 20.2 (3-C); 16.7 (4-C). IR $\tilde{\nu}$ 2830 (OCO stretch), 2250 (CN stretch), 1050 cm^{-1} (CO stretch).

Ethyl iodoacetate (**7**)

Dry sodium iodide (47 g, 0.32 mol) and 50 g (0.30 mol) of ethyl bromoacetate were dissolved in acetone and stirred overnight. The solids were filtered off and the solvent was evaporated. Crude yield 62.0 g (97%). ^1H NMR (200 MHz): δ 3.69 ppm, s (CH_2I); 4.20, q, *J* 7.2 Hz (OCH_2); 1.28, t, *J* 6.9 Hz (CH_3).

Ethyl 4-oxo-6-(2,6,6-trimethyl-1-cyclohexenyl)-5-hexenoate (**8**)

β-Ionone (**6**, 6.9 g, 36 mmol) in 10 ml of THF was added dropwise to 37 mmol of lithium diisopropylamide (prepared from 3.84 g of diisopropylamine and 37 mmol of BuLi) in 50 ml of THF at −70°C. After stirring for 30 min, 8.0 g (37 mmol) of **7** in 50 ml of THF were added dropwise at −70°C. The mixture was then allowed to warm to room temperature. After stirring for 3 h, the reaction was quenched with water. The water layer was extracted three times with ether and the combined organic layers were washed with brine and dried over $MgSO_4$ and the solvents were evaporated. The product was purified. Yield 2.04 g of β-ionone (recovery: 23%) 4.54 g of mono product **8** (57% at 77% conversion), 3.36 g of di-alkylated product (25%).

8. ^1H NMR (200 MHz): δ 7.35 ppm, d, *J* 16.4 Hz (7-CH)*; 6.16, d, *J* 16.4 Hz (8-CH); 4.15, q, *J* 7.2 Hz (OCH_2); 2.93, t, *J* 6.4 Hz (19-CH_2); 2.65, t, *J* 6.9 Hz (19a-CH_2); 2.07–2.03, m (4-CH_2); 1.77,

* Retinoid numbering for the sake of comparison.

s (18-CH_3); 1.66–1.59, m (3-CH_2); 1.50–1.45, m (2-CH_2); 1.27, t, *J* 6.9 Hz ($OCCH_3$); 1.07, s (16/17-CH_3). ^{13}C NMR (50 MHz): δ 197.4; 172.3; 141.7; 135.7; 135.5; 129.7; 59.9; 39.4; 34.6; 33.6; 33.1; 28.4; 27.8; 21.3; 18.5; 13.8. IR ν̃ 1730 (C=O stretch ester), 1660 (C=O stretch α,β-unsat. ketone), 1600 (C=C stretch), 1200, 1160 (C–O stretch) cm^{-1}.

Dialkylated product. ^{1}H NMR (200 MHz): δ 7.49 ppm, d, *J* 16.4 Hz (7-CH); 6.28, d, *J* 16.4 Hz (8-CH); 4.14, q, *J* 8 Hz (2 × OCH_2); 3.67, q, *J* 7.9 Hz (19-CH); 2.46 + 2.78, dd, ^2J 16.0, ^3J 7.9 Hz (2 × 19a-CH_2); 2.07–2.03, m (4-CH_2); 1.80, s (18-CH_3); 1.66–1.59, m (3-CH_2); 1.50–1.45, m (2-CH_2); 1.25, t, *J* 8 Hz (2 × $OCCH_3$); 1.09, s (16,17-CH_3).

*Ethyl (4*E*)-(**10**) and (4*Z*)-(**11**)-5-cyano-8,8-dimethoxy-4-[(*E*)-2-(2,6,6-trimethyl-1-cyclohexenyl)ethenyl]-4-nonenoate*

Nitrile **17** (2.71 g, 17.3 mmol) was added dropwise at −50 °C to 36 mmol of lithium diisopropylamide (from 3.94 g of diisopropylamine and 22.5 ml of BuLi) in 50 ml of THF. After stirring for 20 min, 2.97 g (17.3 mmol) of diethyl chlorophosphate in 5 ml of THF were added dropwise at −50 °C. After stirring for 30 min, 4.00 g (14.4 mmol) of ketone **8** in 10 ml of THF were added at 0 °C. The mixture was then stirred for 2 h at room temperature. Water was then added and the water layer was extracted three times with ether. The combined organic layers were washed with brine and dried over $MgSO_4$ and the solvents were evaporated. The product was purified. Yield 2.85 g **10** (57%) and 0.67 g **11** (13%).

10. ^{1}H NMR (300 MHz): δ 6.59 ppm, d, *J* 16.3 Hz (7-CH); 6.31, d, *J* 16.3 Hz (8-CH); 4.16, q, *J* 7.1 Hz (OCH_2); 3.18, s (2 × OCH_3); 2.97–2.93, 2.53–2.49, 2.45–2.33 and 1.85–1.80, 4 × m, (19/19a/10a/10b-CH_2); 2.06, m (4-CH_2); 1.85–1.80, m (CH_2); 1.72, s (18-CH_3); 1.63, m (2-CH_2); 1.52, m (3-CH_2); 1.30, s (O_2CCH_3); 1.27, t, *J* 7.1 Hz ($OCCH_3$); 1.04, s (16,17-CH_3).

11. ^{1}H NMR (300 MHz): δ 6.62 ppm, d, *J* 16.3 Hz (8-CH); 6.51, d, *J* 16.3 Hz (7-CH); 4.16, q, *J* 7.1 Hz (OCH_2); 3.20, s (2 × OCH_3); 2.79–2.74, m (CH_2); 2.46–2.34, m (2 × CH_2); 2.06, m (4-CH_2); 1.90–1.84, m (CH_2); 1.74, s (18-CH_3); 1.63, m (2-CH_2); 1.52, m (3-CH_2); 1.32, s (O_2CCH_3); 1.27, t, *J* 7.1 Hz ($OCCH_3$); 1.04, s (16,17-CH_3).

Ethyl 3-(3,3-dimethoxybutyl)-2-hydroxy-4-[(E)-2-(2,6,6-trimethyl-1-cyclohexenyl)ethenyl]-1,3-cyclopentadiene-1-carboxylate (**12**)

t-BuOK (2.1 g, 19 mmol) was dissolved in 100 ml of *t*-BuOH at 30 °C. After stirring for 30 min, 2.50 g (6.0 mmol) of **10** in 10 ml of *t*-BuOH were added dropwise. After stirring for 40 min, a slurry of 12 g of SiO_2 and 3 g of H_2O was added. The mixture was stirred for 1 h, then $MgSO_4$ was added. The solids were filtered off and the product was purified to yield 2.12 g (80%) of **12**. ^{1}H NMR (300 MHz): δ 6.51 ppm, d, *J* 15.9 Hz (7-CH); 6.37, d, *J* 16.2 Hz (8-CH); 5.8–6.0, br. s (OH); 4.22, q, *J* 6.1 Hz (OCH_2); 3.34, s (19-CH_2); 3.17, s (2 × OCH_3); 2.36, m (10a-CH_2); 2.03, m (4-CH_2); 1.7–1.8, m (10b-CH_2); 1.73, s (18-CH_3); 1.71–1.55, m (2-CH_2); 1.52, m (3-CH_2); 1.33, s (CH_3); 1.02, s (16,17-CH_3). ^{13}C NMR (75 MHz): δ 14.8 ppm (2′-C), 15.2 (1-C), 18.7 (10a-C), 19.1 (3-C), 20.8 (10d-C), 21.8 (18-C), 28.9 (16-C + 17-C), 34.2 (10c-C), 35.2 (19-C), 36.0 (10b-C), 39.6 (2-C), 48.2 (OCH_3), 58.5 (1′-C), 125.7 (8-C), 130.1 (7-C), 166.7 (19b-C), 125.6, 130.6, 135.4, 137.7, 145.7 (5-C, 6-C, 9-C, 10-C, 11-C). UV/Vis: λ_{max} (EtOH): 1368, 261 nm. IR: ν̃ 3340 (OH-stretch, free hydroxyl), 3340 (OH stretch, hydrogen-bridged hydroxyl), 1650 (C=O stretch) cm^{-1}. Mass spectrum (LCMS, 20% MeOH/80% water, discharge on 1 kV): *m/z* 403 (M − CH_3), 390 (McLafferty), 389 (M − C_2H_5), 387 (M − OCH_3), 373 (M − OC_2H_5), 368, 354 (base peak), 352, 308, 282, 280.

2-(3-Oxobutyl)-3-[(E)-2-(2,6,6-trimethyl-1-cyclohexenyl)ethenyl]-2-cyclopenten-1-one (**13**)

12 (920 mg, 2.21 mmol) was refluxed for 24 h in a 2:1 mixture of MeOH/H_2O to which 0.37 g (7 mmol) of KOH was added. The product was extracted with ether. After evaporation of the solvents, the residue was purified to give 343 mg of ketal (45%). The 343 mg ketal were added to 6 ml of MeOH, acidified with 1N HCl to pH 3, and stirred for 1 h to give, after purification, 273 mg of diketone **13** (42% based on **12**). ^{1}H NMR (300 MHz): δ 6.77 ppm, d, *J* 16.2 Hz (7-CH); 6.68, d, *J* 16.4 Hz (8-CH); 2.73–2.41, 4 × m (19/19a/10a/10b-CH_2); 2.13, s ($OCCH_3$); 2.08, m (4-CH_2); 1.79, s (18-CH_3); 1.62, m (3-CH_2); 1.52, m (2-CH_2); 1.08, s (16,17-CH_3). IR: ν̃ 1710, 1690 (C=O stretch), 1620 cm^{-1} (C=C stretch). UV/Vis: λ_{max} (EtOH): 321 nm.

2,3,6,7-Tetrahydro-1-[(E)-2-(2,6,6-trimethyl-1-cyclohexenyl)ethenyl]-5H-inden-5-one (**14**)

13 (270 mg, 0.90 mmol) was dissolved in 10 ml MeOH and 0.15 g (2.7 mmol) of KOH was added and stirred at room temperature for 3 h. After extraction with ether, the combined organic layers were washed with brine and dried over $MgSO_4$. After purification, 183 mg (72%) of **14** were obtained. ^{1}H NMR (300 MHz): δ 6.44 ppm, s (7 + 8-CH); 5.84, s (12-CH); 2.77, m (19, 19a, 20a-CH_2); 2.49, t, *J* 7.3 Hz (20-CH_2); 2.06, m (4-CH_2); 1.77, s (18-CH_3); 1.60, m (3-CH_2); 1.51, m (2-CH_2); 1.06, s (16,17-CH_3). ^{13}C NMR (50 MHz): δ 19.0 ppm, 20.6, 21.8, 28.1, 28.9, 30.3, 33.2, 34.1, 35.4, 39.5, 117.2, 126.2, 132.0, 134.0, 134.9, 137.4, 149.0, 171.0, 198.8. IR: ν̃ 1650 (C=O stretch), 1585 cm^{-1} (C=C stretch). UV/Vis: λ_{max} (EtOH): 344 nm.

11,19-10,20-Dimethanoretinal (**1** + **2**)*

BuLi (0.30 mmol) was added to a solution of 108 mg (0.36 mmol) of (diethoxyphosphinyl)acetonitrile in 10 ml of THF at 0 °C. After stirring for 30 min, 40 mg (0.14 mmol) of ketone **14** in 15 ml of THF was added dropwise. After stirring for 1½ h, the mixture was poured into saturated NH_4Cl solution, the organic layer was separated and the water layer extracted three times with ether. The combined ethereal layers were washed with brine and dried over $MgSO_4$. The solids were filtered off, the solvents evaporated and the residue was purified, yielding 40 mg (93%) of a 13*E*/*Z* mixture of nitriles. ^{1}H NMR (200 MHz): δ 6.45 and 6.02 ppm, 2 × br. s (12-CH); 6.39–6.30, m (7 + 8-CH); 4.82 and 4.92, 2 × br. s (14-CH); 2.71–2.51, m (19/19a/20/20a-CH_2); 2.05, m 4-CH_2); 1.76, s (18-CH_3); 1.60, m (3-CH_2); 1.50, m (2-CH_2); 1.05, s (16,17-CH_3). UV/Vis: λ_{max} 364 nm.

At −60 °C, 0.6 mmol of DiBAl was added via a syringe to a solution of 40 mg (0.13 mmol) of the nitriles in petroleum ether. After stirring of 30 min, a slurry of 0.4 g of SiO_2 and 0.11 g of water in diethyl ether was added. The mixture was stirred for 1 h at 0 °C, then $MgSO_4$ was added. The solids were filtered off and the solvents were evaporated. After purification, 34 mg (85%) of an *E*/*Z* mixture was obtained, λ_{max} 294 nm. The isomers were separated using HPLC, yielding 17 mg of **1** and 17 mg of **2**. The ^{1}H- and ^{13}C-NMR characteristics are presented in Tables I and II. The UV/Vis and mass spectra are described in the section *spectroscopic characterization*.

(2E,4E)-2-(3,3-Dimethoxybutyl)-3-methyl-5-(2,6,6-trimethyl-1-cyclohexenyl)-2,4-pentadienenitrile (**18**)

17 (1.95 g, 12.5 mmol) was added dropwise to 27 mmol of lithium diisopropylamide (from 3.0 g of diisopropylamine and 17 ml of BuLi) in 20 ml of THF at −50 °C. After stirring for 20 min, 2.42 g (14 mmol) of diethyl chlorophosphate in 5 ml of THF were added dropwise at −50 °C. After stirring for 30 min, 2.00 g (10.4 mmol) of β-ionone (**6**) in 10 ml of THF were added at 0 °C. The mixture was then stirred for 2 h at room temperature. Water was then added and the water layer was extracted three times with ether. The combined organic layers were washed with brine and dried over $MgSO_4$ and the solvents were evaporated. The product was purified and the isomers were separated using SiO_2 column chromatography. Yield 3.0 g (87%) of **18**. ^{1}H NMR (200 MHz): δ 6.56 ppm, d, *J* 16.5 Hz (7-CH); 6.43, d, *J* 15.9 Hz (8-CH); 3.19, s (2 × OCH_3); 2.41–2.30, m (10a-CH_2); 2.21, s (19-CH_3); 2.05, m (4-CH_2); 1.87–1.79, m (10b-CH_2); 1.72, s (18-CH_3); 1.63, m (2-CH_2); 1.51, m (3-CH_2); 1.31, s (O_2CCH_3); 1.04, s (16,17-CH_3). IR: ν̃ 2200 (C≡N stretch), 1650, 1600 (C=C stretch), 1050 (C–O stretch), 980 cm^{-1} (RHC=CHR wagging o.o.p.).

(2E,4E)-3-Methyl-2-(3-oxobutyl)-5-(2,6,6-trimethyl-1-cyclohexenyl)-2,4-pentadienal (**20**)

DiBAl (14 mmol) was added dropwise to 3.0 g (9.0 mmol) of **18** in 20 ml petroleum ether at −60 °C. After stirring for 1 h, a slurry of

* IUPAC name: (*E*)- (**1**) and (*Z*)- (**2**) -[2,3,6,7-tetrahydro-1-[(*E*)-2-(2,6,6-trimethyl-1-cyclohexenyl)ethenyl]-5*H*-inden-5-ylidene]-acetaldehyde.

18 g silica gel and 4.5 g of water in ether/pet.-ether (1:1) was added and the mixture was stirred at 0°C for 1 h. After drying over $MgSO_4$ the solids were filtered off. Evaporation of the solvents yielded 2.68 g (89%) of the crude aldehyde. The aldehyde (2.68 g; 8.3 mmol) in 15 ml ethanol was brought to pH 3 with 1.3N HCl and stirred for 5 h at room temperature. Water was then added and the mixture was extracted three times with ether. The combined organic layers were washed with brine and dried over MgSO. After evaporation of the solvents, the crude product was purified, yielding 2.3 g (88%) of **20**. ^{1}H NMR (200 MHz): δ 10.23 ppm, s (CH=O); 6.78, d, *J* 15.1 Hz (7-CH); 6.60, d, *J* 16.5 Hz (8-CH); 2.70–2.50, t, *J* 8.0 Hz (10a-CH_2); 2.50–2.43, t, *J* 8.0 Hz (10b-CH_2); 2.33, s (19-CH_3); 2.14, s (O=CCH_3); 2.08–2.02, m (4-CH_2); 1.73, s (18-CH_3); 1.64–1.60, m (3-CH_2); 1.51–1.46, m (2-CH_2); 1.05, s (16,17-CH_3). ^{13}C NMR (50 MHz): δ 207.3 ppm; 190.7; 149.6; 137.0; 135.2; 134.8; 131.7; 130.9; 42.7; 38.8; 33.6; 32.5; 29.0; 28.3; 21.2; 18.8; 18.4; 12.1.

(E)-4-[(E)-1-Methyl-3-(2,6,6-trimethyl-1-cyclohexenyl)-2-propenylidene]-2-cyclohexen-1-one (**21**)

0.3 g (5 mmol) of KOH was added to a solution of 270 mg (1.0 mmol) of **20** in 10 ml of methanol. The mixture was stirred for 40 minutes at room temperature and then extracted three times with ether. The combined organic layers were washed with brine and dried over $MgSO_4$. Evaporation of the solvents and purification yielded 210 mg (83%) of **21**. The product was spectroscopically identical to the material described earlier[16].

10,20-Methanoretinal (**3** + **4**)*

BuLi (0.32 ml, 0.51 mmol) was added to a solution of 100 mg (0.56 mmol) of (diethoxyphosphinyl)acetonitrile in 10 ml of THF at 0°C. After stirring for 30 min, 100 mg (0.37 mmol) of **21** in THF were added dropwise. After stirring for 1 h at room temperature, water was added and the product was extracted with ether. The combined organic layers were washed with brine and dried over $MgSO_4$ and the solvents were evaporated. After purification, 90 mg (83%) of the retinonitrile was obtained as a mixture of isomers.

90 mg (0.3 mmol) of the nitriles were dissolved in petroleum ether at −60°C and 0.6 ml of DiBAl was added dropwise. After stirring for 1 h, a slurry of 1 g silica gel and 0.2 g water in ether was added and the mixture was stirred for 1 h. After drying over $MgSO_4$, the solids were filtered off. After evaporation of the solvents and purification, 80 mg (88%) of 10,20-methanoretinal was obtained as a 4:1 mixture of isomers. **3** and **4** were separated into their isomerically pure states by HPLC, yielding 63 mg of **3** (69%) and 17 mg of **4** (19%). **3** and **4** were spectroscopically identical to the material described earlier[16]. The ^{13}C chemical-shift parameters are collected in Table II. The mass spectra are described in the section *spectroscopic characterization*.

* IUPAC name: (*E*)- (**3**) and (*Z*)- (**4**) -[(*E*)-4-[(*E*)-1-methyl-3--(2,6,6-trimethyl-1-cyclohexenyl)-2-propenylidene]-2-cyclohexenylidene]acetaldehyde.

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