254. Isolation by HPLC. and Identification by NMR. Spectroscopy of 11 mono-, di- and tri-*cis* Isomers of an Aromatic Analogue of Retinoic Acid, Ethyl all-*trans*-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-nona-2,4,6,8-tetraenoate

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(24.VII.78)

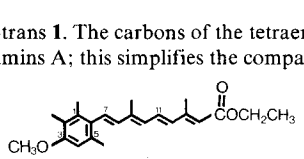
Summary

Photoisomerization of an aromatic analogue of retinoic acid, ethyl all-*trans*-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-nona-2,4,6,8-tetraenoate **1** in dilute solutions of hexane, benzene, and ethanol yielded multi-component mixtures of *cis* isomers which were separated by HPLC. FT-^{1}H-NMR. at 270 MHz and, in some cases, homonuclear decoupling and *Overhauser* experiments as well as ^{13}C-NMR. were applied to establish the structures of 4 mono-*cis*, 4 (of 6 possible) di-*cis*, and 3 (of 4 possible) tri-*cis* isomers. The structures of 3 isomeric esters, namely (2*Z*,4*E*,6*E*,8*E*) **6**, (2*Z*,4*Z*,6*E*,8*E*) **9**, and (2*Z*,4*Z*,6*Z*,8*E*) **7** were independently confirmed by direct syntheses. The ^{1}H-NMR. data of all these compounds and the ^{13}C-NMR. data of the all-*trans* and of 6 *cis* isomers available in sufficiently large quantities are discussed.

Introduction. – In recent years considerable experimental and theoretical efforts have been focussed on the study of the primary photochemical step and the subsequent chemical events following the absorption of light by the visual protein rhodopsin. For better understanding of the physical and chemical factors influencing the photochemically initiated isomerization process several groups have studied extensively the photoisomerization of vitamin A and related polyenes in solution. In the course of these studies, several new *cis* isomeric photo-products have been isolated and identified by spectroscopic methods ([1] and ref. therein).

In the following we report on the photoisomerization of an aromatic analogue of retinoic acid, ethyl all-*trans*-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-nona-2,4,6,8-tetraenoate **1** *(Scheme 1)*. As expected, irradiation of dilute solutions of **1**

Scheme 1. *Chemical structure of all*-trans **1**. The carbons of the tetraene are numbered in accordance with the system adopted for the vitamins A; this simplifies the comparison of the spectroscopic data.



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with a xenon high-pressure light source yielded complex mixtures of photo-products (High Pressure Liquid Chromatography, HPLC.). According to mass spectrometric analysis all the photo-products were isomers of the all-*trans* compound **1**, *i.e.* no products with altered chemical structure were detectable. We elucidated the structure of the different *cis* isomers mainly by application of 270 MHz FT-^{1}H-NMR. spectroscopy. In several cases only µg-amounts of the isomers were available. In other cases where larger quantities could be isolated or prepared synthetically, homonuclear decoupling and homonuclear *Overhauser* experiments as well as ^{13}C-NMR. were included to confirm the derived structures. Since the spectroscopic data presented here are intended to serve as a reference basis for the interpretation of the NMR. spectra of the *cis* isomeric vitamins A and carotenoids we prefer to apply the numbering system *(Scheme 1)* of this class of compounds rather than the IUPAC-system. This greatly facilitates a direct comparison of the spectra and a discussion of the structural origins of the observed chemical shift changes.

Results. – 1. *^{1}H-NMR. data.* In all the products isolated, only the configuration of the different double bonds of the tetraene side-chain was altered compared to the all-*cis* **1** (MS., UV.). The ^{1}H-NMR. spectra fully confirmed this finding. First, we should like to make some remarks about the ^{1}H-NMR. spectrum of the all-*trans* compound which will serve as the basis for the interpretation of all the other spectra.

The assignment of most of the ^{1}H-NMR. signals of the all-*trans* compound has already been given [2]. Decoupling and homonuclear *Overhauser* (NO.) experiments fully confirmed and extended these findings *(Table 1)*. Thus the signal at 2.299 ppm must be assigned to $H_3C-C(5)$ since irradiation of this signal resulted in an NO.-enhancement of 12% of the signal of the aromatic proton H–C(4) at 6.603 ppm. Irradiation of the methyl signal at 2.369 ppm (*d*, 3 H, $H_3C-C(13)$) yielded a 45% increase of the peak height of the signal at 5.785 ppm (H–C(14)). However, as expected the *trans* configuration gave no NO.-enhancement in this experiment at the signal of H–C(14) but a *ca.* 12% increase of the integral of H–C(11) was observed.

Irradiation of the signal at 2.107 ppm ($H_3C-C(9)$) gave an NO.-enhancement of 9% each at the signals of H–C(11) and H–C(7) which were sufficiently separated from the other signals for accurate measurement. These findings point to the strong steric interactions of these methyl and olefinic protons as expected for a planar tetraene chain in analogy to the findings with retinal and its isomers [3–5].

As in the spectra of most of the other isomers, the proton signals of $H_3C-C(9)$ and $H_3C-C(13)$ could be distinguished also from their appearance as a broadened singlet and as a more or less pronounced doublet respectively. Similarly, the differentiation between the doublets of H–C(7) and H–C(8) was in most cases possible by inspection of the line-width of these signals which was slightly larger in the former than in the latter case owing to unresolved long-range couplings.

There remains the assignment of the signals of $H_3C-C(1)$ and $H_3C-C(2)$. We tentatively assigned the signal at 2.238 ppm to $H_3C-C(1)$ since its chemical shift was expected to be close to that of the $H_3C-C(5)$. The $H_3C-C(2)$ must then give rise to the remaining signal at 2.107 ppm. The relatively high shielding is explained by the neighbourhood of $H_3CO-C(3)$. These assignments were corroborated by the observation (see below) that the signals of the less shielded

Table 1. *^{1}H-NMR. data (δ in ppm; couplings in Hz) of the all-*trans *compound* **1** *and of its mono-*cis *isomers.* Significant chemical shift differences $\Delta = \delta_{cis} - \delta_{trans} \geq 0.03$ ppm. Solvent: $CDCl_3$

Protons	Peak VIII all-*trans*	Peak VI 7-*cis*		Peak VII 9-*cis*		Peak VA 11-*cis*		Peak V 13-*cis*	
	δ	δ	Δ	δ	Δ	δ	Δ	δ	Δ
H–C(4)	6.603	6.563	−0.04	6.625	–	~6.60	–	6.605	–
H–C(7)	6.685	6.370	−0.32	6.700	–	6.685	–	6.677	–
H–C(8)	6.247	6.310	0.06	6.795	0.55	6.243	–	6.255	–
H–C(10)	6.196	6.173	–	6.133	−0.06	~6.54	~0.34	6.300	0.10
H–C(11)	7.022	6.845	−0.18	7.066	0.04	~6.62	~−0.40	7.006	–
H–C(12)	6.316	6.248	−0.07	6.240	−0.08	~5.94	~−0.38	7.820	1.50
H–C(14)	5.785	5.766	–	5.767	–	5.851	0.07	5.659	−0.13
H_3C–C(1)	2.238	2.136	[b]	2.254	–	2.241	–	2.237	–
H_3C–C(2)	2.150	2.149	[b]	2.162	–	2.148	–	2.152	–
H_3C–C(5)	2.299	2.175	[b]	2.315	–	2.301	–	2.298	–
H_3C–O	3.816	3.816	–	3.823	–	3.816	–	3.815	–
H_3C–C(9)	2.107	1.462	−0.65	2.107	–	2.068	−0.04	2.100	–
H_3C–C(13)	2.369	2.306	−0.06	2.291	−0.08	2.341	−0.03	2.083	−0.29
O–CH_2	4.176	4.165	–	4.160	–	4.164	–	4.165	–
CH_3	1.292	1.286	–	1.281	–	1.280	–	1.285	–
$J_{7,8}$	16.3 Hz	~12 Hz		16.2 Hz		16.2 Hz		16.3 Hz	
$J_{10,11}$	11.4 Hz	~11 Hz		11.3 Hz		(~12 Hz)[a]		11.6 Hz	
$J_{11,12}$	15.1 Hz	~15 Hz		15.0 Hz		(~12 Hz)[a]		15.3 Hz	

[a] Estimated from C_6D_6 solution.

[b] Assignments not clear. Upfield shifts of the signals of H_3C–C(1) and H_3C–C(5) between −0.06 and −0.16 ppm are likely.

H_3C–C(1) and H_3C–C(5) were shifted upfield in the spectra of all the isomers with the 7-*cis* configuration *(Tables 1* and *2).*

The assignment of the structures of the mono-*cis* compounds was now straightforward and mainly based on the values of the observed couplings $J_{7,8}$ and $J_{11,12}$ as well as on the chemical shift differences $\Delta = \delta_{cis} - \delta_{trans}$ (in ppm) between the *cis* and *trans* isomers. The ^{1}H-NMR. data of all the compounds investigated here are presented in *Tables 1* and *2*.

The structure of the 7-*cis* isomer was easily derived from the value of $J_{7,8} \sim 12$ Hz and the significant upfield shift of *ca.* 0.65 ppm of the signal of H_3C–C(9). The unexpectedly high shielding compared to other 7-*cis* retinoids [6] was observed in all 5 7-*cis* compounds. It is explained by the assumption that in these compounds the methyl protons are situated in the shielding zone above or below the aromatic ring. This must thus be caused by an exceptionally strong steric interaction of the hydrogens of the methyl groups at C(1), C(5) and C(9) enforcing a non-planar arrangement of the aromatic ring and the tetraene side-chain.

The proof of the structure of the 9-*cis* isomer was mainly based on the observed strong downfield shift of the signal of H–C(8) caused by the steric interaction with H–C(11). The value of $\Delta \sim 0.5$ ppm was also measured in the spectra of the 4 other

Table 2. *^{1}H-NMR. data (δ in ppm; couplings in Hz) of the di-*cis *and tri-*cis *isomers of* **1**.
Significant chemical shift differences $\Delta = \delta_{cis} - \delta_{trans} \geq 0.03$ ppm. Solvent: $CDCl_3$

Protons	Peak IV 7,9-di-*cis*		Peak I 7,13-di-*cis*		Peak III 9,13-di-*cis*		Peak X 11,13-di-*cis*		Peak II 7,9,13-tri-*cis*		Peak IX 7,11,13-tri-*cis*		Peak XI 9,11,13-tri-*cis*	
	δ	Δ	δ	Δ	δ	Δ	δ	Δ	δ	Δ	δ	Δ	δ	Δ
H–C(4)	6.551	−0.05	6.558	−0.05	6.623	–	6.594	–	6.548	−0.06	6.553	−0.05	6.608	–
H–C(7)	6.507	−0.18	6.360	−0.33	6.685	–	6.675	–	6.490	−0.20	~6.37	~−0.32	6.680	–
H–C(8)	6.752	0.51	6.330	0.08	6.790	0.54	6.213	−0.03	6.753	0.51	~6.41	~0.16	6.805	0.56
H–C(10)	5.946	−0.25	6.280	0.08	6.236	0.04	6.435	0.24	6.056	−0.14	6.223	0.03	6.356	0.16
H–C(11)	6.991	−0.03	6.830	−0.19	7.052	0.03	6.638	−0.38	6.981	−0.04	~6.38	−0.64	6.673	−0.35
H–C(12)	6.170	−0.15	7.760	1.44	7.743	1.43	6.990	0.67	7.672	1.36	n.o.	?	6.857	0.54
H–C(14)	5.759	−0.03	5.638	−0.15	5.635	−0.15	5.705	−0.08	5.645	−0.14	5.620	−0.17	5.708	−0.08
H_3C–C(1)	2.122	[a]	2.133	[a]	2.252	–	2.229	–	2.123	[a]	2.119	[a]	2.243	–
H_3C–C(2)	2.146	[a]	2.149	[a]	2.162	–	2.143	–	2.145	[a]	2.141	[a]	2.154	–
H_3C–C(5)	2.178	[a]	2.174	[a]	2.312	–	2.289	–	2.172	[a]	2.162	[a]	2.307	–
H_3C–O	3.800	–	3.811	–	3.824	–	3.811	–	3.801	–	3.801	–	3.816	–
H_3C–C(9)	1.450	−0.66	1.439	−0.67	2.110	–	2.062	−0.05	1.445	−0.66	~1.45	~−0.66	2.090	–
H_3C–C(13)	2.331	−0.04	2.022	−0.35	2.001	−0.37	2.151	−0.22	2.049	−0.32	1.893	−0.48	2.167	−0.20
O–CH_2	4.172	–	4.167	–	4.166	–	4.140	−0.04	4.158	–	4.120	−0.06	4.134	−0.04
CH_3	1.290	–	1.287	–	1.286	–	1.260	−0.03	1.282	–	1.282	–	1.257	−0.04
$J_{7,8}$	12.3 Hz		~12 Hz		16.3 Hz		16.2 Hz		12.3 Hz		~12 Hz		16.3 Hz	
$J_{10,11}$	11.5 Hz		11.4 Hz		11.5 Hz		12.2 Hz		11.7 Hz		~12 Hz		12.3 Hz	
$J_{11,12}$	15.0 Hz		15.3 Hz		15.4 Hz		12.0 Hz		15.2 Hz		~12 Hz ?		~12 Hz	

[a]) Assignments not given. Upfield shifts of the signals of H_3C–C(1) and H_3C–C(5) between −0.06 and −0.18 ppm are likely. n.o. signal not detected, possibly overlapped by strong solvent signal.

isomers with 9-*cis* partial structures *(Table 2)*. The assignments of the signals of $H_3C-C(9)$ and of $H-C(10)$ as well as of $H_3C-C(13)$ and of $H-C(14)$ were confirmed by decoupling experiments.

The assumed 11-*cis* structure was in compliance with the value of $J_{11,12} \sim 12$ Hz measured in C_6D_6-solution where better separation of the different signals was achieved. This was additionally supported by the fact that no significant chemical shift effects at $H-C(8)$ and the 2 in-chain methyl groups were detected. In addition, the Δ-values of the protons at C(10), C(11) and C(12) were in close agreement with those for other 11-*cis* retinoids [5].

The 13-*cis* structure was evident from the very strong downfield shift (*ca.* 1.5 ppm) of the signal of $H-C(12)$. In the spectra of the 11,13-di-*cis* and 9,11,13-tri-*cis* isomers this downfield shift is, however, considerably weaker ($\Delta = 0.67$ and 0.54 ppm). This might be caused by a distorted planar structure of the tetraene chain in these highly hindered isomers [3] [4].

The ^{1}H-NMR. data of the di-*cis* and tri-*cis* isomers are given in *Table 2*. Again, the assignment of the structures was based on the observed couplings, the chemical shift effects, and in some cases, on the measured NO.-effects. The Δ-values of some of the isomers, especially those with separated *cis* bonds, can be fairly well predicted from the Δ-values of the mono-*cis* compounds. As already mentioned, significant deviations from additivity were observed for the compounds with 11,13-di-*cis* structure. This is generally to be expected for compounds with consecutive *cis* bonds, *i.e.* also for 7,9-di-*cis*. A few additional remarks must therefore be made with respect to the derivation of the di-*cis* and tri-*cis* structures.

Although the 13-*cis* partial structures of the 7,13-, 9,13- and 11,13-di-*cis* isomers were clearly visible from the strong downfield shift of $H-C(12)$, an independent proof for this was easily derived by NO.-experiments. Irradiation of the signal of $H_3C-C(13)$ in all 3 cases gave NO.-enhancements at $H-C(14)$ between 20 and 30% in agreement with the 13-*cis* partial structures. Similar experiments with the 9,11,13-tri-*cis* isomer likewise resulted in a 15% NO.-enhancement at $H-C(14)$ and a 13% increase at $H-C(10)$. Irradiation of the signal of $H_3C-C(9)$ gave *ca.* 10% enhancements at the signals of $H-C(7)$ and $H-C(10)$ in accordance with the assumed structure.

The elucidation of the structure of the 7,9,13- and 7,11,13-tri-*cis* isomers was less easily accomplished on the basis of chemical shift considerations alone. Here, the chemical shifts were expected to be completely non-additive and furthermore, only *ca.* 10 µg of these isomers were available. As an example, we present *(Fig. 1)* the olefinic portion of the 270 MHz ^{1}H-FT-NMR. spectrum of the 7,9,13-tri-*cis* isomer (*ca.* 10 µg, 18 h of accumulation). From $J_{7,8} \sim 12$ Hz together with the proton chemical shift of $H_3C-C(9)$ *(Table 2)* a 7-*cis* partial structure is evident. The low-field shifts of $H-C(8)$ and $H-C(12)$ and the observed coupling $J_{11,12} = 15$ Hz unequivocally prove the assumed 7,9,13-tri-*cis* structure. It should be pointed out that the observed Δ-values *(Table 2)* are in accordance with those calculated from the chemical shifts of the 7,9,13-tri-*cis* isomer of methyl retinoate reported by *Asato & Liu* [6].

The ^{1}H-NMR. spectrum of the 7,11,13-tri-*cis* isomer (*ca.* 10 µg) was more difficult to understand since 3 of the olefinic protons showed very similar shifts and

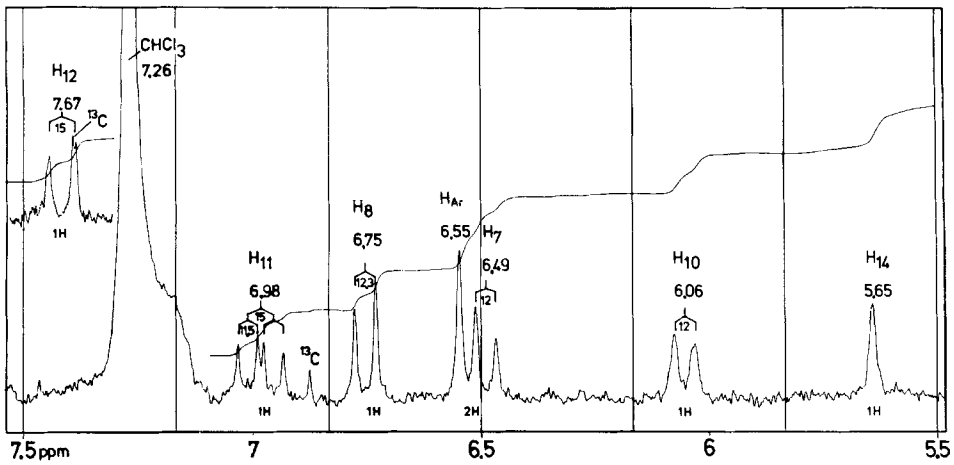


Fig. 1. *270 MHz FT-^{1}H-NMR. partial spectrum of the 7,9,13-tri-*cis *isomer of* **1**: *ca.* 10 µg, *ca.* 18 h of accumulation; solvent: 100% D-$CDCl_3$

the signal of H–C(12) was most likely hidden underneath the very intense solvent signal. Furthermore, the coupling $J_{11,12}$ was not unequivocally measurable and during the long accumulation time of the spectrum a partial isomerization of the sample occurred. The assumed structure was based on the following arguments. From the chemical shifts of the methyl groups at C(1), C(5) and C(9) as well as of H–C(8) it was obvious that a 7-*cis*-9-*trans* partial structure was present. From the remaining 2 alternatives, namely 7,11-di-*cis* and 7,11,13-tri-*cis*, the former could be discarded since it could not explain the high-field shift of the signal of H_3C–C(13) by 0.5 ppm.

Some of the structures derived so far were confirmed independently from the analysis of their ^{13}C-NMR. spectra.

2. *^{13}C-NMR. data.* In addition to the all-*trans* compound 5 isomers were available in quantities of at least 5 mg and could thus be additionally investigated by ^{13}C-NMR. The observed chemical shifts and our assignments are compiled in *Table 3*. The different techniques used for the assignments are specified. The calculated chemical shift differences $\Delta = \delta_{cis} - \delta_{trans} \geqslant 0.3$ ppm are also given which are to be compared to those previously reported for a number of *cis* isomeric retinoids [7]. In order to allow an independent derivation of the structures of the different *cis* isomers the assignments were as far as possible based on a series of measurements with increasing quantities of the shift reagent Yb(dpm)$_3$. Recently this method was successfully utilized for the assignments of the ^{13}C-NMR. spectra of numerous retinoids and carotenoids [7].

As expected, the complexation of the all-*trans* and the different *cis* isomers mainly occurs at the ester moiety as evidenced by the strong lanthanide induced downfield shifts (LIS.-values, in ppm) of the signals of the carbons close to this site. A second, much weaker complexation takes place at the OCH_3-group, as seen from very small LIS.-values of this signal and, in some cases, of the signals of C(3), C(2) and C(1). This effect also allowed us to distinguish between the signals of H_3C–C(1) and H_3C–C(2).

Table 3. *^{13}C-NMR. chemical shifts δ in ppm of all-*trans **1** *and of 6* cis *isomers.*
Significant chemical shift differences $\Delta = \delta_{cis} - \delta_{trans} \geq 0.3$ ppm. Solvent: $CDCl_3$

Carbon	all-*trans*	9-*cis*		13-*cis*		7,13-di-*cis*		9,13-di-*cis*		11,13-di-*cis*		9,11,13-tri-*cis*	
	δ	δ	Δ	δ	Δ	δ	Δ	δ	Δ	δ	Δ	δ	Δ
C(1)	135.82	135.97	–	135.91	–	135.43	−0.4	135.99	–	135.84	–	135.92	–
C(2)	122.84	123.04	–	122.87	–	122.24	−0.6	123.00	–	122.79	–	122.92	–
C(3)	156.23	156.48	–	156.45	–	156.54	0.3	156.51	–	156.37	–	156.45	–
C(4)	110.24	110.28	–	110.26	–	109.57	−0.7	110.22	–	110.18	–	110.26	–
C(5) }	133.85	133.94	–	133.92	–	133.41	−0.4	133.96	–	133.85	–	133.89	–
C(6) }	130.02	130.26	–	130.07	–	130.63	0.6	130.36	−0.3	130.11	–	130.23	–
C(7)	128.64	130.13	1.5	128.46	–	132.54[e])	(3.9)	129.89[e])	(1.3)	128.40	–	130.14	1.5
C(8)	138.18	130.70	−7.5	138.38	–	135.37[e])(	−2.8)	130.82[e])(	−7.4)	138.63	0.5	130.23	−8.0
C(9)	138.95	137.84	−1.1	139.20	–	140.30	1.4	138.09	−0.9	138.86	–	137.25	−1.7
C(10)	130.41	128.90	−1.5	131.26	0.9	130.13	−0.3	129.79	−0.6	127.22	−3.2	125.45	−5.0
C(11)	130.53	129.51	−1.0	131.77	1.2	131.68	1.2	130.70	–	128.74	−1.8	127.47	−3.1
C(12)	135.87	135.07	−0.8	130.16	−5.7	130.13	−5.7	129.31	−6.6	127.63	−8.2	126.91	−9.0
C(13)	152.28	152.43	–	150.73	−1.6	150.75	−1.5	150.88	−1.4	151.98	−0.3	152.04	–
C(14)	119.11	119.04	–	117.15	−2.0	117.17	−1.9	117.07	−2.0	119.08	–	118.99	–
C=O	166.85	167.08	–	166.24	−0.6	166.36	−0.5	166.39	−0.5	165.90	−1.0	166.02	−0.8
OCH_2	59.50	59.64	–	59.56	–	59.62	–	59.64	–	59.65	–	59.69	–
|													
CH_3	14.40	14.38	–	14.40	–	14.37	–	14.38	–	14.33	–	14.29	–
CH_3 at													
C(1)	17.36	17.49	–	17.37	–	17.22	–	17.49	–	17.38	–	17.44	–
C(2)	11.82	11.82	–	11.82	–	11.73	–	11.82	–	11.81	–	11.80	–
−OC(3)	55.41	55.64	–	55.51	–	55.65	–	55.65	–	55.52	–	55.61	–
C(5)	21.36	21.40	–	21.37	–	20.92	−0.4	21.38	–	21.32	–	21.40	–
C(9)	12.86	20.79	7.9	12.89	–	14.73	1.9	20.81	8.0	12.30	−0.6	21.13	8.3
C(13)	13.84	13.91	–	20.78	6.9	20.83	7.0	20.89	7.1	25.38	11.5	25.48	11.6
Remarks	[a])[b])[c])[d])	[a])[c])		[a])[b])[c])		[a])		[a])[c])		[a])[b])[c])		[a])[c])	

[a]) ^{1}H-CW. offset decoupling. [b]) ^{1}H-coupled spectrum. [c]) $Yb(dpm)_3$ experiments. [d]) Selective decoupling. [e]) Corresponding assignments may be interchanged.

The quaternary carbons C(9) and C(13) were identified from the CW.-offset decoupled or from the ^{1}H-coupled spectra and distinguished from each other by their LIS.-values. Similarly, the assignment of the signals of the methine carbons C(14), C(12), C(11) and C(10) was based on the assumption that the relative magnitude of the LIS.-values should decrease in the same order. The signals of H_3C–C(13) and H_3C–C(9) were correspondingly distinguished. The signals of C(7) and C(8) could not be assigned in this way since their LIS.-values were too small. In analogy to several other retinoids [7] the signal of C(8) in compounds with Δ^9-*trans* should be at *ca.* 138 ppm and that of C(7) at *ca.* 130 ppm as was observed experimentally. In the case of the all-*trans* and the 11,13-di-*cis* compounds the assignments of C(7) and C(11) were confirmed by the ^{1}H-coupled spectra. Here, only these 2 signals were observed as pure doublets, *i.e.* no couplings were resolved other than the $^1J_{CH}$-coupling to the directly attached proton.

The ^{1}H-coupled spectra also made possible the identification of H_3C–C(5) the signal of which appeared as a quartet ($^1J_{CH}$) of doublets ($^3J_{CH}$) in contrast to the pure quartets observed for H_3C–C(1) and H_3C–C(2).

Thus, the assignments given in *Table 3* are generally unambiguous. A few cases where the assignments could, in principle, be reversed are specifically marked although the conclusions concerning the structures derived will not be affected by these uncertainties.

The ^{13}C-NMR. data contained in *Table 3* were used to derive independently the structures of the 6 *cis* isomers from the observed changes Δ of the chemical shifts are compared to the all-*trans* compound. These values directly reflect the site of the *cis* double bond in the tetraene chain [7]. Thus, the given Δ-values can be qualitatively understood by the assumption that any increase (decrease) of steric interaction in the *cis* compounds gives rise to upfield (downfield) shifts of the corresponding signals. A 9-*cis* configuration will thus result in a downfield shift of the signal of H_3C–C(9) ($\Delta \sim 8$ ppm) and an upfield shift of C(8) (-7.5 ppm). Similar shifts were observed in the spectrum of the 13-*cis* isomer, namely for the signals of H_3C–C(13) and for C(12).

In the spectrum of the compounds with 2 consecutive *cis* bonds (11,13-di-*cis*) the signal of H_3C–C(13) was assigned even further downfield (11.6 ppm) compared to the all-*trans* isomer. The Δ-values of the 9,13-di-*cis* isomer can be fairly accurately predicted from the values of the 2 mono-*cis* isomers. This is, however, again not the case for isomers with consecutive *cis* double bonds, *e.g.* the 11,13-di-*cis* and 9,11,13-tri-*cis* isomers, as is seen if the previously reported Δ-values for 11-*cis* retinoids [7] are combined with those of the 13-*cis* isomer reported here.

Conclusion. – In this study the 270 MHz ^{1}H-NMR. spectra of 11 different mono-, di- and tri-*cis* isomers of an aromatic analogue of retinoic acid, ethyl 9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-nona-2,4,6,8-tetraenoate were analysed. These data together with the results of several nuclear *Overhauser* experiments were utilized to derive the structures of 4 mono-*cis*, 4 di-*cis* and 3 tri-*cis* isomers.

The ^{13}C-NMR. spectra of the all-*trans* and of 6 *cis* isomers available in sufficiently large quantities fully confirmed the results obtained by ^{1}H-NMR. The

chemical shift differences of the *cis* isomers and the all-*trans* compound are of general use for the elucidation of the structure of similar *cis* isomeric structures in the field of retinoids and carotenoids.

The authors are indebted to Messrs. *W. Grunauer, J. Schlegel* and *M. Turtschi* for their skilful technical assistance.

Experimental Part

1. Photoisomerization and isolation. – *Solvents.* Hexane, free of fluorescent compounds, was filtered through a column of Al_2O_3. Benzene p.a. was purchased from *Merck.* Ethanol, 94% *w/w*, was distilled over KOH before use.

Solutions. For semi-preparative purposes 500 mg all-*trans* **1** were dissolved in 2 ml of benzene (25% *w/v*) and transferred into a 2 ml ampoule of colourless glass and stoppered. For analytical purposes, 5 ml of a 0.25% *w/v* solution in hexane, benzene or ethanol were filled into 5 ml ampoules, gassed with argon and sealed.

Irradiations. Light source: Spectrotest apparatus (Original *Hanau*) equipped with a high-pressure xenon lamp, type NXe 900 generating a continuous spectrum from 300–1000 nm. The distance between the light source and the ampoules was 23 cm. The measured illumination intensity was *ca.* 10^5 lux. The photoisomerization of **1** was carried out at *ca.* 30°.

The equilibrium concentrations of the main isomers were reached within 1 h of irradiation, after which there was only a small increase of the minor components and a slow decrease of the all-*trans* compound. A typical analytical chromatogram obtained after irradiation of a 0.25% solution of **1** in benzene for 4 h is shown in *Figure 2.* Main peaks were attributed to the 9,13-di-*cis* isomer (peak III, *ca.* 20%), the 13- and 11-*cis* isomers (here unresolved peaks V and VA, *ca.* 19%), the 11,13-di-*cis* isomer (peak X, *ca.* 15%), the all-*trans* compound **1** (peak VIII, *ca.* 12%), the 9-*cis* (peak VII, *ca.* 10%), the 7,13-di-*cis* (peak I, *ca.* 10%) and the 7-*cis* isomer (peak VI, *ca.* 7%). The remaining 7% were distributed among 4 other isomers. The 3 tri-*cis* isomers (peak II, IX and XI) were detectable only after at least 15 min of irradiation. Since the molar absorptivities of the various isomers are mostly unknown, this estimation of the relative concentrations is based on the (incorrect) assumption of an identical absorptivity at 350 nm.

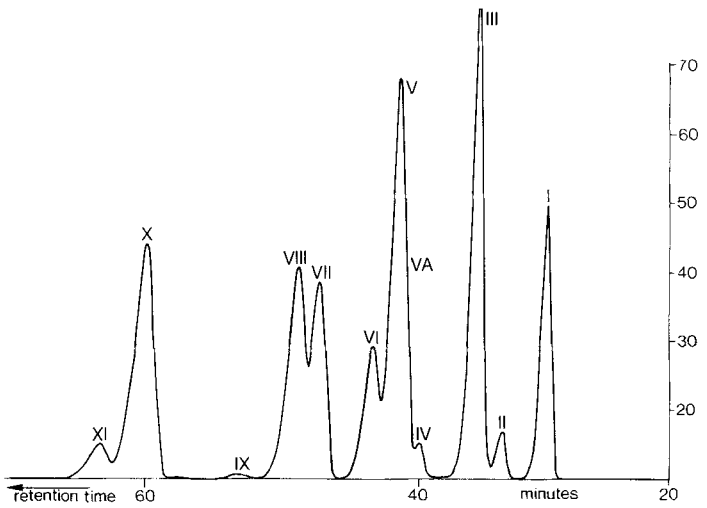


Fig. 2. *Analytical HPLC.-chromatogram of a mixture of all-*trans **1** *(peak VIII) and of its* cis *isomers obtained after 4 h of irradiation of* **1** *in benzene.* Eluent: 1.5% diisopropylether in hexane.

Irradiation in hexane or ethanol gave somewhat different isomer distributions. Thus, the 9,11,13-tri-*cis* isomer (peak XI) could be obtained in quantities of up to 9% after irradiation in hexane for 16 h.

Isolation and separation by HPLC. 20 samples with 25 µl each of irradiated solution were injected into the HPLC.-column and the corresponding eluate fractions combined. The solvent was evaporated in a *Büchi* rotatory evaporator.

Semi-preparative HPLC. Column: steel, 25 cm, i.d. 10 mm. Stationary phase: LiChrosorb SI 60, particle size 5 µm. Mobile phase: 0.25–0.5% ethyl acetate in hexane. Flow rate 2 ml/min., 35 atm. UV. detector *Beckman* model 24, wavelength 350 nm; sample injection 25 µl.

Analytical HPLC. Column: steel, 50 cm, i.d. 3 mm, LiChrosorb SI 60, particle size 5 µm. Mobile phase: 1.5% diisopropylether in hexane (see *Fig. 2*). For separation of components V and VA 0.5% ethyl acetate in hexane was used. Pressure 70 atm. Sample injection 5 µl.

2. UV. data. – The spectra were directly measured in the flow cell of a *Beckman* UV./VIS. Spectrophotometer model 24. Solvent 1.5% diisopropylether in hexane (eluent).

HPLC.-Peak	isomer	λ_{max}	HPLC.-Peak	isomer	λ_{max}
I	7,13-di-*cis*	340 nm	VI	7-*cis*	335 nm
II	7,9,13-tri-*cis*	335 nm	VII	9-*cis*	345 nm
III	9,13-di-*cis*	350 nm	VIII	all-*trans*	351 nm
IV	7,9-di-*cis*	330 nm	IX	7,11,13-tri-*cis*	~ 335 nm
V }	13-*cis*	~ 355 nm	X	11,13-di-*cis*	348 nm
VA }	11-*cis*		XI	9,11,13-tri-*cis*	338 nm

3. ^{1}H- and ^{13}C-NMR. – The ^{1}H-NMR. spectra were run in the FT-mode at 270 MHz on a *Bruker* HX-270 instrument with BNC-80 computer (40 K memory). The ^{13}C-NMR. spectra were recorded on the same instrument at 68 MHz or at 22.6 MHz on a *Bruker* HX-90/15 spectrometer with *Nicolet* 1083 computer (12 K memory). Throughout this study $CDCl_3$ was used as the solvent and tetramethylsilane as internal standard.

Where only small sample quantities were available long accumulations of up to 63 h were used. In these cases, cylindrical NMR. micro cells (*ca.* 0.2 ml; *Wilmad* Glass Company) were employed and the ^{1}H-NMR. spectra were then obtained in so-called 100% D-$CDCl_3$ (CEA-France or *Stohler* Isotope Chemicals).

The experiments with Yb $(dpm)_3$ (*Stohler* Isotope Chemicals) were accomplished by adding weighed amounts of shift reagent to the $CDCl_3$ solutions. Normally 4 concentration ratios of substrate to shift reagent, in general 10/1, 5/1, 2.5/1, and 1.25/1, were used to make possible a reliable assignment of the signals [7].

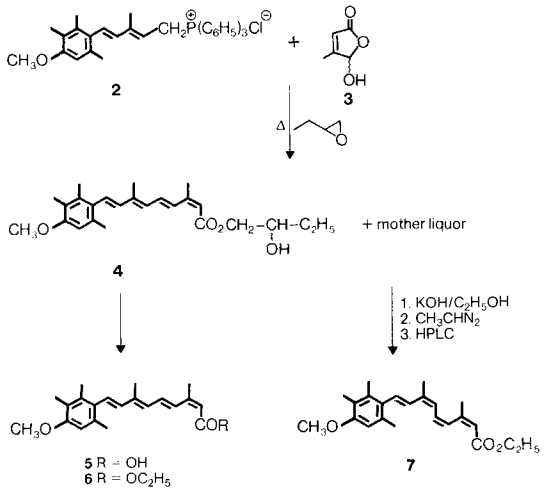
Homonuclear *Overhauser* experiments were performed on carefully degassed solutions. Usual homodecoupling or homogated decoupling FT-techniques were applied to measure the enhancement of the integral intensities.

4. Synthesis of 13-*cis* ester 6, 9,11,13-tri-*cis* ester 7 (Scheme 2) and 11,13-di-*cis* ester 9 (Scheme 3). – a. *13*-cis *ester* **4**. To a suspension of 52.6 g (100 mmol) of C_{15}-phosphonium chloride **2** [8] in 200 ml of butene oxide were added 11.4 g (100 mmol) of lactol **3** [9]. The mixture was heated to 80° under argon for 3.5 h. The cooled solution was poured into 1000 ml of a methanol/water mixture (6:4) and extracted 3 times with water, dried (Na_2SO_4) and evaporated. The oily residue was crystallized from ethyl acetate to give 35 g of an oily mother liquor and 12.1 g (30.4%) of yellow 13-*cis*-hydroxy-ester **4**, m.p. 142–144°. – UV. (ethanol, λ_{max} (nm), ε): 363 (35000), 244 (11400). – IR. (KBr, cm^{-1}): 3470, 1714, 1620, 1244, 1194, 1123, 976. – MS. (*m/e*, %): 398 (M^+, 55), 383 (15), 281 (83), 203 (68), 163 (100), 150 (92).

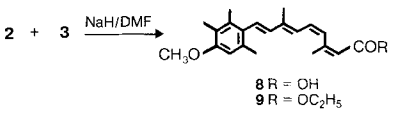
$C_{25}H_{34}O_4$ (398.54) Calc. C 75.34 H 8.60% Found C 74.97 H 8.56%

b. *13*-cis *acid* **5**. To a suspension of 11.7 g (29.4 mmol) of 13-*cis*-hydroxyester **4** in 75 ml of ethanol was added a solution of 5.6 g (100 mmol) of KOH in 25 ml of water. The mixture was stirred at 50° for 4 h under argon. The clear solution was poured onto ice, acidified with 2 N HCl and extracted with ethyl acetate. The organic phase was washed twice with water, dried (Na_2SO_4) and

Scheme 2



Scheme 3



evaporated. Recrystallization of the residue from ethyl acetate yielded 7.6 g (80%) of yellow 13-*cis* acid **5**, m.p. 206–208°. – UV. (ethanol, λ_{max} (nm), ε): 354 (28900), 243 (8100). – IR. (KBr, cm^{-1}): 2668, 1680, 1580, 1253, 977. – MS. (*m/e*, %): 326 (M^+, 68), 282 (72), 267 (59), 201 (52), 163 (100), 150 (80).

$C_{21}H_{26}O_3$ (326.43) Calc. C 77.27 H 8.03% Found C 77.40 H 8.15%

c. *13*-cis *Ethyl ester* **6**. To a suspension of 4.4 g (13.5 mmol) of 13-*cis* acid **5** in 50 ml of ethanol were added slowly with stirring 250 ml of a diazoethane solution in ether [10]. After 15 min the clear solution was evaporated and the residue recrystallized from hexane to give 4.0 g (84%) of yellow 13-*cis* ethyl ester **6**, m.p. 97–98°. – UV. (ethanol, λ_{max} (nm), ε): 362 (39000), 244 (10900). – IR. (KBr, cm^{-1}): 1705, 1588, 1243, 1161, 977. – MS. (*m/e*, %): 354 (M^+, 75), 339 (30), 281 (82), 251 (55), 203 (68), 163 (100), 150 (97).

$C_{23}H_{30}O_3$ (354.49) Calc. C 77.93 H 8.53% Found C 77.80 H 8.75%

d. *9,11,13-Tri*-cis *ester* **7**. The mother liquor of 4a was hydrolyzed as described in 4b to give, after recrystallization from ethyl acetate, 17.3 g of a mixture of *cis/trans* isomeric acids. The corresponding mixture of ethyl esters obtained by esterification with diazoethane, as in 4c, consisted, according to HPLC., of 45% 11,13-di-*cis*, 40% 9,11,13-tri-*cis*, 10% 9,13-di-*cis* and 5% 13-*cis* ethyl ester. Fractionated crystallization of the mixture from ether/hexane yielded the 9,11,13-tri-*cis* isomer **7** in pure crystalline form, m.p. 93–94°. – UV. (ethanol, λ_{max} (nm), ε): 348 (26400), 249 (11900). – IR. (KBr, cm^{-1}): 1707, 1594, 1541, 1305, 1161, 996. – MS. (*m/e*, %): 354 (M^+, 45), 339 (20), 281 (63), 251 (38), 203 (60), 163 (90), 150 (100).

$C_{23}H_{30}O_3$ (354.49) Calc. C 77.93 H 8.53% Found C 77.87 H 8.50%

e. *11,13-Di*-cis *ethyl ester* **9**. Sodium hydride (3.84 g, 80 mmol, 50% in mineral oil) was washed with pentane to remove the mineral oil and suspended in 40 ml of dry DMF. At 0° a solution of 21.0 g (40 mmol) of C_{15}-phosphonium chloride **2** in 100 ml of dry DMF was added dropwise. The dark-red

solution was stirred at 0° for 30 min. A solution of 4.6 g (40 mmol) of lactol **3** in 20 ml of dry DMF was added slowly. The mixture was then stirred at RT. for 3 h, poured onto ice, acidified with 2 N HCl and extracted with ethyl acetate. The organic phase was washed twice with water, dried (Na_2SO_4) and evaporated. To remove most of the triphenylphosphine oxide, the oily residue was dissolved in ethyl acetate and cooled in an ice bath. The precipitated triphenylphosphine oxide was filtered off, the filtrate evaporated and filtered through a short column of silica gel (hexane/ethyl acetate 1:1). The eluate was crystallized from ethyl acetate to give 3.3 g (25%) of yellow 11,13-di-*cis* acid **8**, m.p. 163–165°. – UV. (ethanol, λ_{max} (nm), ε): 351 (30400), 248 (10800). – IR. (KBr, cm^{-1}): 2628, 1682, 1584, 1250, 980, 936. – MS. (*m/e*, %): 326 (M^+, 72), 311 (30), 281 (68), 267 (31), 201 (65), 163 (100), 150 (85).

$C_{21}H_{26}O_3$ (326.43) Calc. C 77.27 H 8.03% Found C 77.03 H 8.11%

f. *Esterification of the acid* **8** (3.2 g) with diazoethane gave after recrystallization from hexane 2.4 g (92%) of 11,13-di-*cis* ethyl ester **9**, m.p. 68–70°. – UV. (ethanol, λ_{max} (nm), ε): 356 (30200), 250 (11900). – IR. (KBr, cm^{-1}): 1697, 1588, 1303, 1166, 970. – MS. (*m/e*, %): 354 (M^+, 75), 339 (35), 291 (94), 251 (60), 203 (78), 163 (97), 150 (100).

$C_{23}H_{30}O_3$ (354.49) Calc. C 77.93 H 8.53% Found C 78.00 H 8.59%

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