Reference Data

(12) (m.p. 131–133 °C, $CHCl_3$) were synthesized from 7-hydroxycoumarin (Merck- Schuchardt) as reported previously.[8,9]

Acknowledgements

The authors are grateful to the Comisión Asesora de Investigación Científica y Técnica (CAICYT, No. PR84-0247) for support of this research. F.A.M. acknowledges financial support from the US–Spain Joint Committee for Scientific and Technological Cooperation (Project No. IPB-8509011) for a Visiting Professorship at Louisiana State University.

References

1. M. Gosálvez, R. G. Cañero and M. Blanco, *Eur. J. Cancer* **12**, 1003 (1976).
2. A. G. González, V. Darias, G. Alonso, J. N. Boada and F. Rodríguez-Luis, *Planta Med.* **31**, 351 (1977).
3. G. C. Levy, R. L. Lichter and G. L. Nelson, *Carbon-13 Nuclear Magnetic Resonance Spectroscopy*. Wiley, New York (1980).
4. A. Patra and A. K. Mitra, *Org. Magn. Reson.* **17**, 222 (1981).
5. M. H. A. Elgamal, N. H. Elewa, E. A. M. Elkhrisy and H. Duddeck, *Phytochemistry* **18**, 139 (1979).
6. H. Duddeck and M. Kaiser, *Org. Magn. Reson.* **20**, 55 (1982).
7. A. G. González, E. Díaz Chico, H. López Dorta, J. R. Luis and F. Rodríguez-Luis, *An. Quim.* **73**, 430 (1977).
8. G. M. Massanet, E. Pando, F. Rodríguez-Luis and J. Salvá, *Heterocycles* **26**, 1541 (1987).
9. R. Hernández Galán, G. M. Massanet, E. Pando, F. Rodríguez-Luis and J. Salvá, *Heterocycles* **27**, 775 (1988).

Received 19 October 1988; accepted (revised) 9 January 1989

Assignment of ^1H and ^{13}C NMR Spectra of Retinoic Acid Ester Isomers Observed in the Long-Wavelength UV-Induced Photostationary State

ROBERT W. CURLEY, JR. (to whom correspondence should be addressed) and JOHN W. FOWBLE
Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy,
Ohio State University,
Columbus,
43210,
USA

^1H and ^{13}C chemical shift assignments, obtained with the aid of two-dimensional heteronuclear shift correlation and double-quantum coherence ^{13}C-INADEQUATE experiments, are reported for four retinoic acid ester isomers observed at the photostationary state. Also detailed are ^1H assignments for three additional isomers obtained in insufficient amounts for ^{13}C measurements.

KEY WORDS Retinoic acid esters Isomer identification ^1H–^{13}C correlation spectra ^{13}C-INADEQUATE experiment

INTRODUCTION

Recently we have conducted studies of factors influencing the isomer distribution of retinoic acid (**1**) (Fig. 1) upon photoisomerization.[1] The biological activity of this molecule in inducing differentiation and acting as a possible cancer chemopreventive agent can be strongly influenced by the side-chain isomeric composition.[2,3] Because of its sensitivity to changes in olefin stereochemistry, we chose to employ NMR spectroscopy in structural assignments of the retinoid geometric isomers resulting at the long-wavelength UV light-induced photostationary state.

Englert[4] has suggested that ^{13}C NMR can be employed to assign retinoate isomers through the use of repetitive titrations with lanthanide shift reagents; however, some doubts remain when using these techniques. In earlier work on retinoic acid photoisomerates the stereochemistry of side-chain isomers was assigned using ^1H NMR spectroscopy.[5] Unfortunately, these assignments relied on relatively low-field spectra obtained with minimal sample amounts and required computer simulations to make certain proton assignments. Recently, the high-field ^1H NMR spectra of a limited number of retinoate isomers dissolved in DMSO-d_6 have been determined.[6]

As it should prove useful as a reference source, we report here results derived from high-field NMR experiments, relying extensively on correlation spectroscopic techniques, to clarify certain resonance assignments and conclusively identify the seven photoisomers of methyl-**1** which we observed.

EXPERIMENTAL

All-*trans*-retinoic acid was obtained from Aldrich (Milwaukee, WI, USA). 11-*cis*-Retinal, 13-*cis*,9-*cis*- and 11,13-di-*cis*-retinoic acid were generously provided by P. F. Sorter of Hoffman-LaRoche (Nutley, NJ, USA). Methyl retinoates were prepared by reaction with diazomethane generated in the usual manner from Diazald (Aldrich). Methyl 11-*cis*-retinoate was prepared from 11-*cis*-retinal as described previously.[1] 9,13-Di-*cis*- and 9,11,13-tri-*cis*-retinoic acid were isolated by high-performance liquid chromatography as reported.[1]

Fourier transformed ^1H NMR spectra were obtained at ambient temperature on samples dissolved in $CDCl_3$ (1–10 mM) in amber-glass 175 × 5 mm sample tubes (Wilmad Glass, Buena, NJ, USA). ^1H NMR spectra were obtained on IBM AF-270 or Bruker AM-500 spectrometers operating at 270.13 and 500.13 MHz, respectively. ^{13}C NMR and ^1H–^{13}C correlation spectra[7,8] were determined on an IBM AF-270 spectrometer operating at 67.9 MHz for ^{13}C measurements. A ^{13}C-INADEQUATE study[9] was performed on a Bruker MSL-300 spectrometer at 75.4 MHz. Two-dimensional heteronuclear shift correlation, double-quantum coherence ^{13}C-INADEQUATE and nuclear Overhauser effect (NOE) difference experiments were performed using standard Bruker Instruments software. All spectrometers were equipped with the Aspect 3000 data system.

Typical 1D ^1H NMR spectra were obtained using a spectral width of 2500 Hz over 16K data points, no delay, a pulse width of 2 μs (35°) and an acquisition time of approximately 3.5 s, leading to a digital resolution of 0.2–0.3 Hz per point. For 1D ^{13}C NMR measurements a sweep width of 17 000 Hz over 32K data points, a 2.1–2.5 s delay, a 2 μs (33°) pulse width and an acquisition time of 0.95 s (digital resolution 1.05 Hz per point) were employed.

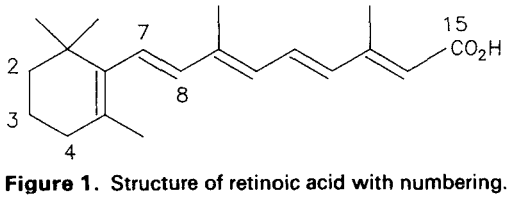

Figure 1. Structure of retinoic acid with numbering.

Table 1. ^{13}C NMR chemical shifts of methyl retinoate isomers in $CDCl_3$[a]

Isomer	Carbon number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	$(CH_3)_2$	5-CH_3	9-CH_3	13-CH_3	OCH_3
All-*trans*(1)	34.19	39.62	19.19	33.05	129.85	137.67	128.59	137.22	139.45	129.42	130.93	135.05	152.79	118.05	167.38	28.88	21.61	12.78	13.76	50.74
13-*cis*	34.18	39.58	19.17	33.05	129.91	137.62	128.44	137.37	139.68	130.25	132.15	129.24	151.17	116.03	166.67	28.88	21.62	12.77	20.83	50.70
9-*cis*	34.28	39.63	19.27	33.11	130.11	138.06	130.28	129.55	138.46	127.96	129.91	134.39	152.99	118.07	167.56	29.01	21.08	20.85	13.95	50.67
11-*cis*	34.30	39.60	19.25	33.07	129.82	137.75	128.64	137.62	139.93	125.76	129.06	131.29	153.72	118.78	167.39	28.97	21.75	12.39	19.25	50.94

[a] Relative to $CDCl_3$ = 77.00 ppm as internal standard. Numbering as in Fig. 1.

Table 2. ^{1}H NMR vinyl and methyl proton chemical shifts of methyl retinoate isomers in $CDCl_3$[a]

Isomer	Methyl protons				Vinyl protons						Coupling constant (Hz)		
	1	5	9	13	7	8	10	11	12	14	7,8	10,11	11,12
All-*trans*-methyl-**1**	1.00	1.68	1.96	2.32	6.26	6.11	6.15	6.98	6.27	5.76	16.0	11.4	15.0
9-*cis*	1.02	1.73	1.98	2.32	6.27	6.65	6.05	7.08	6.23	5.78	16.2	11.4	15.0
11-*cis*	1.00	1.69	1.94	2.32	6.25	6.10	6.52	6.54	5.86	5.84	16.2	12.7	12.6
13-*cis*	0.99	1.68	1.96	2.03	6.25	6.13	6.23	6.97	7.76	5.62	16.2	11.4	15.4
9,13-Di-*cis*	1.01	1.71	1.97	2.03	6.23	6.61	6.13	7.05	7.66	5.60	16.0	10.9	15.4
9,11,13-Tri-*cis*	1.00	1.69	1.95	2.14	6.24	6.64	6.25	6.67	6.84	5.66	15.7	11.8	11.0
11,13-Di-*cis*	1.02	1.71	1.96	2.17	6.26	6.10	6.37	6.59	6.93	5.67	16.2	12.3	12.3

[a] All shifts relative to $CHCl_3$ = 7.24 ppm as internal standard. Numbering as in Fig. 1.

Reference Data

RESULTS

Complete ^{13}C NMR assignments were made for four of the esterified isomers of **1** (Table 1). A ^{13}C-INADEQUATE study of methyl-**1** was employed to assign unambiguously the carbon atoms in the parent isomer, thereby facilitating the use of ^{1}H–^{13}C correlation spectra to assign the carbon atoms of the three other abundant isomers.

The ^{1}H NMR chemical shift data for the important methyl and vinyl proton resonances of the seven observed isomers of methyl-**1** are given in Table 2. For three of these isomers insufficient sample amounts prevented ^{13}C NMR studies. In these instances, isomer identification and proton resonance assignments were made using the necessary decoupling and NOE experiments where any ambiguities existed.

DISCUSSION

Surprisingly, complete assignment of the spectra of the parent isomer of methyl-**1**, using the indicated experiments, proved to be the most challenging. In particular, the vinyl proton resonances of methyl-**1**, at 270 MHz, are poorly resolved and not completely first order. Additionally, the ^{1}H–^{13}C correlation experiment was unable to assist in clearly identifying these resonances in the absence of other information. Therefore, the double-quantum coherence ^{13}C-INADEQUATE experiment was employed to establish the carbon connectivity of methyl-**1** and to allow unambiguous assignment of the difficult ring methylene and 7-, 8-, and 10-position carbon atoms, and their associated protons, by ^{1}H–^{13}C correlation.

^{13}C NMR

Although only four side-chain isomers of methyl-**1** were abundant enough for ^{13}C NMR work, these were valuable in permitting ^{1}H–^{13}C correlation spectroscopy, thereby facilitating proton assignments in abundant and minor isomers. Even with the relatively few ^{13}C NMR spectra available, a number of patterns emerge which can be helpful in assigning isomer structure. Most notably, relative to methyl-**1**, in the 13-*cis* isomer there is a marked downfield shift in the 13-methyl resonance and a corresponding upfield shift in the signal of C-12. Similarly, in the 9-*cis* isomer there is a downfield shift of the 9-methyl carbon resonance and an upfield shift of that of C-8. Methyl 11-*cis*-retinoate, an isomer whose ^{13}C NMR spectrum to our knowledge has not previously been reported, shows an upfield shift for the C-10 signal and a downfield 13-methyl resonance position, presumably due to steric effects between the 10-proton and the 13-methyl group. Additionally, by unambiguously assigning the ^{13}C resonances for the 2- and 3-carbon atoms in methyl-**1** via the ^{13}C-INADEQUATE experiment, we have confirmed the correctness of earlier uncertain assignments of the relative resonance positions of these two carbon atoms in retinoids.[10]

^{1}H NMR

The more extensive family of ^{1}H NMR spectra allows a number of generalizations which can be useful in retinoic acid isomer assignment. In 13-*cis* isomers an upfield shift occurs for the 13-methyl proton signals with a corresponding downfield shift in the 12-proton doublet, while in 9-*cis* isomers both the 8- and 10-proton signals experience a strong downfield shift. In these 9- and 13-*cis* isomers, in which side-chain methyl groups and adjacent vinyl protons bear a fixed *cis* stereochemical relationship to each other (e.g. the 13-methyl and 14-proton), there is a measurable NOE between these protons, further confirming the *cis* stereochemistry. The presence of 11-*cis* stereochemistry in retinoates is readily determined by the collapse of the 11-proton doublet of doublets to an apparent triplet, because the magnitude of the coupling constant between the now *cis* 11- and 12-protons decreases to become nearly equivalent to that between the 10- and 11-protons.[11]

As described earlier,[1] we have found that the previously assigned[5] resonance positions for the 7- and 12-protons of methyl 9-*cis*-retinoate are in error and should be reversed. We believe the accurately determined information presented here should prove useful for others engaged in retinoate isomer identification by providing a reasonable basis for relating patterns of frequency shifts to isomeric composition. Further, these results may also aid in assigning isomer structures to closely related metabolities and analogues of **1**, currently active acreas of study.

Acknowledgement

Financial support of this work by a grant from the National Cancer Institute (CA 40967) is gratefully acknowledged. We also appreciate the assistance of Dr Charles E. Cottrell of the Ohio State University Campus Chemical Instrument Center in obtaining some NMR spectra. We thank Ms Pam Smith for the preparation of the manuscript.

References

1. R. W. Curley, Jr, and J. W. Fowble, *Photochem. Photobiol.* **47**, 831 (1988).
2. R. W. Curley, Jr, D. P. Sivla, Jr, and H. F. DeLuca, *Arch. Biochem. Biophys.* **238**, 484 (1985).
3. A. Yen, V. Powers and J. Fishbaugh, *Leukemia Res.* **10**, 619 (1986).
4. G. Englert, *Helv. Chim. Acta* **58**, 2367 (1975).
5. B. A. Bailey and E. C. Nelson, *J. Chromatogr.* **175**, 113 (1979).
6. B. Perly, G. C. Pappalardo, M. Klaus and E. Montoneri, *Z. Naturforsch. Teil B* **43**, 1072 (1988).
7. A. Bax, *J. Magn. Reson.* **53**, 517 (1983).
8. J. A. Wilde and P. H. Bolton, *J. Magn. Reson.* **59**, 343 (1984).
9. A. Bax, R. Freeman and S. P. Kempsell, *J. Am. Chem. Soc.* **102**, 4849 (1980).
10. R. Rowan, III, and B. D. Sykes, *J. Am. Chem. Soc.* **96**, 7000 (1974).
11. D. J. Patel, *Nature (London)* **221**, 825 (1969).

Received 18 November 1988; accepted 19 January 1989