Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 9, pp. 953-958, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00151-4

CONFORMATIONALLY CONSTRAINED PRECURSORS TO RETINOIC ACID ANALOGS WHICH STABILIZE THE 9Z-CONFIGURATION

Cynthia Y. Robinson, D. Vincent Waterhous, Donald D. Muccio,* and Wayne J. Brouillette*

Department of Chemistry, University of Alabama at Birmingham, Birmingham, Alabama 35294

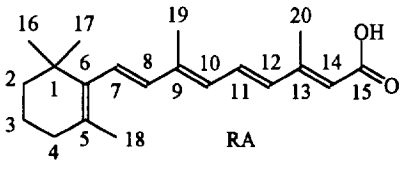
Abstract. The iodine-catalyzed isomerization (dark) of (2*Z*,4*E*)-4-(2'-isopropylidenecyclohexylidene)-3-methyl-2,4-butadienal and (2*Z*,4*E*)-4-(2'-isopropyl-3'-methyl-2'-cyclohexen-1'-ylidene)-3-methyl-2-butenal, precursors to conformationally defined 6-s-*cis* and 6-s-*trans* retinoic acid analogs, resulted in the formation of isomer mixtures for which the 9*Z*-configuration [using retinoic acid numbering] is most stable.

The vitamin A family of compounds, particularly retinoic acid (RA) and its synthetic analogs, exhibit potent biological effects on cellular proliferation, differentiation, and morphogenesis (see reference 1 for a review). Many of these agents have shown exciting experimental and clinical activity in dermatological disorders and oncology, including cancer chemoprevention, but toxicity (hypervitaminosis A) and teratogenicity limit their utility. Such side effects may result from the ability of an individual retinoid to activate multiple retinoid receptors. The design of agents that specifically activate individual retinoid receptors may provide compounds with improved therapeutic ratios and increased clinical utility.

Two families of nuclear retinoid receptors have recently been identified. These function as ligand-modulated transcription factors which control the expression of specific genes and include the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). Each family contains three subclasses, designated as α, β, and γ. The RARs are activated by both (*all-E*)-RA and (9*Z*)-RA. In contrast, only (9*Z*)-RA activates the RXRs, which may transactivate genes and have corresponding biological actions that are different from those of the RARs.[2-4]

(*All-E*)-RA is the most stable geometrical isomer. Recent equilibration studies reveal that bovine liver membranes or certain thiol-containing chemical catalysts isomerize pure *E,Z*-isomers to produce the equilibrium mixture described below.[5] This suggests that efficient isomerization may also occur *in vivo*. The observed

therapeutic effects and/or toxicities of individual *E,Z*-isomers of retinoids may thus reflect activities of an isomeric mixture that is produced *in vivo*. Presumably, this activity could be modified by changing the relative isomer abundance. This situation is of particular concern for (9*Z*)-RA, since *in vivo* isomerization (if present) would primarily produce the *all-E*-isomer. In order to minimize this complication, there is a need to obtain biologically active 9*Z*-analogs of RA which are more stable than other isomeric forms.



RA

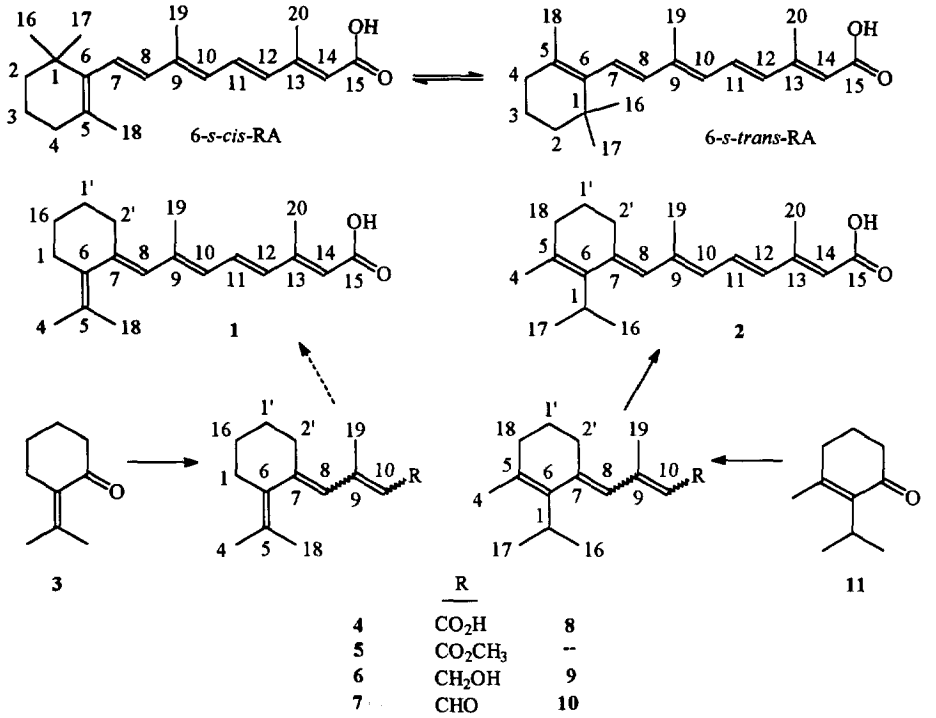
RA Isomer	Equilibrium Percentage[5]
(*All-E*)	61.4
(13*Z*)	17.2
(9*Z*)	14.8
(9*Z*,13*Z*)	6.7

As a possible approach to receptor-selective retinoic acid analogs, we proposed the synthesis of conformationally constrained retinoic acid analogs 6-s-*cis*-**1** and 6-s-*trans*-**2**. These analogs utilize a dimethylene bridge (indicated as atoms 1' and 2') to form a cyclohexane ring in a region of space different than that for (*all-E*)-RA. Here we describe isomerization studies showing that synthetic precursors to **1** and **2**, aldehydes **7** and **10**, equilibrate to favor the 9*Z* over the *all-E*-geometry.

We previously reported[6] the synthesis of aldehyde **10** from ketone **11** and the subsequent conversion of **10** to **2**. In this procedure enone **11** was reacted with Zn and ethyl 4-bromo-3-methylbut-2-enoate under Reformatsky conditions to give a spiro δ-lactone, which subsequently underwent eliminative ring opening to exclusively provide acid (7*E*,9*Z*)-**8**. This methodology thus provides a stereospecific synthetic approach to (9*Z*)-RA analogs. Intermediate (7*E*,9*Z*)-**8** was reduced with $LiAlH_4$ to give alcohol (7*E*,9*Z*)-**9**, which was oxidized with MnO_2 to provide aldehyde (7*E*,9*Z*)-**10**. Similarly, we previously described[7] the reaction of ketone **3** under identical Reformatsky conditions to exclusively provide (7*E*,9*Z*)-**4**. This reaction presumably occurred via a spiro δ-lactone intermediate, as in the formation of (7*E*,9*Z*)-**8**, since this lactone could be isolated under different conditons. For the present study we reacted **4** with diazomethane/ether which, after flash chromatography on silica (R_f 0.86, 10% acetone/hexane), provided ester (7*E*,9*Z*)-**5** in 96% yield. Ester **5** was reduced with $LiAlH_4$/ether at -78 °C to give alcohol (7*E*,9*Z*)-**6** (R_f 0.21, silica, 10% acetone/hexane) in 88% yield. This alcohol was immediately oxidized with MnO_2/CH_2Cl_2 at 0 °C to give aldehyde (7*E*,9*Z*)-**7**, which was purified on a preparative silica gel plate (R_f 0.52, 10% acetone/hexane) to provide a 61% yield. The ^1H NMR assignments for the 7*E*,9*Z*-isomers of **4**-**7** are provided in Table I.

The iodine-catalyzed isomerization (in the dark) of polyenes containing *Z*-configurations to generate the more thermodynamically favored polyene is well documented in the literature.[8,9] This approach most commonly produces the *all-E*-configuration, as observed for RA. Similarly, *Z*-retinoids have been isomerized to the corresponding *E*-compounds,[10,11] and an equilibrium mixture of retinal isomers has been generated from (*all-E*)-

retinal[12] by catalysis with iodine, either in the dark or with light (the latter may favor formation of less stable isomers).



The treatment of acid (7*E*,9*Z*)-**8** with 0.1% I_2 in $CDCl_3$ at room temperature, in the dark or in the presence of light (100 W tungsten), produced no detectable isomerization, suggesting that the starting geometry is highly favored or that the activation energy for isomerization is high. In contrast, as we previously described,[6] the treatment of aldehyde (7*E*,9*Z*)-**10** with I_2 in $CDCl_3$ or benzene/hexane, in the dark at room temperature, produced a 2:1 mixture of (7*E*,9*Z*)-**10** and (7*E*,9*E*)-**10** within one hour. This result reveals that the 7*E*,9*Z*-configuration is thermodynamically favored in this system.

Similarly, attempts to isomerize acid (7*E*,9*Z*)-**4** (performed in $CDCl_3$ in an NMR tube and followed by 300 MHz ^1H NMR) in the presence of I_2 (dark) did not produce any change during two hours, even at 40 °C. However, the treatment of acid (7*E*,9*Z*)-**4** with I_2 and light (100 W tungsten) at room temperature rapidly produced a mixture containing one new isomer and starting material in a 9:1 ratio. NOE studies established the 7*Z*,9*Z*-configuration for this new isomer. A large NOE (28%) was observed for H10 when the H19 methyl protons were irradiated [similar to that observed previously[7] for (7*E*,9*Z*)-**4**], and little NOE (3%) was observed for H8 when the H18 protons were irradiated [unlike that observed previously[6] for (7*E*,9*Z*)-**4**].

Table I. ^{1}H NMR Assignments for **4-7** in $CDCl_3$ (Chemical Shifts in ppm Relative to TMS).

Proton	(7*E*,9*Z*)-4	(7*E*,9*Z*)-5	(7*E*,9*Z*)-6	(7*E*,9*Z*)-7	(7*E*,9*E*)-7	(7*Z*,9*Z*)-7	(7*Z*,9*E*)-7
H1'	1.58	1.62	1.48	1.61	1.63	1.26	1.26
H2'	2.17	2.19	2.16	2.19	2.42	2.36	2.36
H1	2.26	2.29	1.93	2.30	2.29	2.21	2.31
H4	1.69	1.71	1.60	1.73	1.72	1.65	1.68
H8	6.01	6.07	5.35	5.71	5.66	6.39	5.91
H10	5.72	5.72	5.37	5.95	5.94	5.75	5.92
H16	1.58	1.59	1.48	1.61	1.63	1.65	1.63
H18	1.79	1.86	1.67	1.79	1.74	1.51	1.52
H19	2.02	2.02	1.69	2.03	2.27	1.96	2.16
H(R)	--	3.66	3.98	9.72	10.05	9.91	10.04

The iodine-catalyzed isomerization of a $4x10^{-4}$ M solution of (7*E*,9*Z*)-**7** in $CDCl_3$ at 40°C was then performed in the dark and was monitored by ^{1}H NMR. The isomer composition rapidly changed during the first 20 minutes and then remained essentially constant for 51 hours. The aldehydic region clearly showed that a mixture of four isomers was produced. A repeat of the isomerization experiment beginning with pure (7*E*,9*E*)-**7** yielded a nearly identical mixture, suggesting that equilibrium was obtained. The individual aldehyde isomers were then separated by HPLC [Whatman Partisil 10 M9/50 silica column, 5% ether/hexane, 2 mL/min, retention times for the isomers of **7**: 31.7 min (7*Z*,9*E*); 29.2 min (7*E*,9*E*); 27.7 min (7*Z*,9*Z*); 22.0 min (7*E*,9*Z*)].

The configurations for these pure isomers were assigned by NOE studies, where the steady-state enhancements were calculated from the ratio of the integrated areas of the peaks in the difference spectrum to that in the off-resonance spectrum (Table II). Upon irradiation of the H19 methyl hydrogens, the 9*Z*-isomers were identified by a large NOE to H10, while the 9*E*-configuration was characterized by a large NOE to the aldehydic proton [H(R)]. (Similar enhancements were observed with retinal.[13]) The configuration at C7 was similarly assigned as *E* when irradiation of the H18 methyl protons produced a large NOE to H8. In addition to the NOE data, the configurational assignments at C7 were supported by chemical shift evidence. An upfield shift was observed for the H18 methyl protons of the 7*Z*-isomers as compared to the 7*E*-configuration (Table I). This is consistent with the shifts observed for the comparable 7*Z*-isomers in the C_{15} aldehydic precursor to retinal.[14] Furthermore, the large downfield shift for H8 ($\Delta\delta$ =0.48 ppm) in (7*Z*,9*Z*)-**7** relative to (7*E*,9*E*)-**7** is in agreement with the downfield shift experienced by H8 for (9*Z*)-retinal relative to (*all-E*)-retinal.[15] Additionally, the chemical shifts for comparable protons [H1', H2', H4, H10, H19, and H(R)] in **8-10**,[16] with the exception of H8 (which experiences extra steric effects from H18), are within 0.15 ppm of those in **4-7**.

Also of interest was the observation that reisomerization of the isolated isomers occurred most rapidly

about the C7 double bond. Thus, when (7*E*,9*Z*)-**7** and (7*Z*,9*Z*)-**7** were isomerized individually, they quickly interconverted, while the other two isomers were formed more slowly. A similar rapid interconversion between (7*E*,9*E*)-**7** and (7*Z*,9*E*)-**7** was also observed.

The percent composition for the isomeric mixture produced by the iodine-catalyzed isomerization of (7*E*,9*Z*)-**7** was calculated by integrating the aldehydic doublets [H(R)] in the ^{1}H NMR spectrum. The observed populations for the isomers of **7** were: 32% (7*E*,9*Z*), 28% (7*Z*,9*Z*), 26% (7*Z*,9*E*), and 13% (7*E*,9*E*). Thus the least stable is the (7*E*,9*E*)-isomer. As for aldehyde **10**, this is in contrast to the isomers of retinal or retinoic acid for which the *all-E*-isomer is most stable.

Table II. Steady-State NOE (%) for the Isomers of **7**.

Irradiated Protons	Observed Proton	(7*E*,9*E*)	(7*E*,9*Z*)	(7*Z*,9*Z*)	(7*Z*,9*E*)
H18 methyl	H8	14	16	2	2
H19 methyl	H8	9	10	8	3
H19 methyl	H10	0	19	12	0
H19 methyl	H(R)	19	0	0	19

These results reveal that conservative structural changes near the ring end of RA may dramatically modify the equilibrium population of *E,Z*-isomers as compared to RA. For our constrained 6-*s-trans*-retinoid precursor, the 9*Z*-isomer was significantly more stable than the *all-E*-isomer, and the other two possible *E,Z*-isomers were not produced in detectable amounts. For the analogous 6-*s-cis*-precursor, the 9*Z*-isomer was more stable than the *all-E*-isomer, although the 13*Z*- and 9*Z*,13*Z*-isomers were also stabilized. The ability to stabilize individual *E,Z*-isomers may be useful in designing nuclear receptor-selective retinoids, which may provide agents with greater therapeutic ratios. In particular, some of the 9*Z*-retinoids proposed here may form the basis for designing RXR-selective retinoids which do not readily convert to other isomeric forms.

Acknowledgement. These studies were supported by grant P01 CA34968 from the National Institutes of Health.

References and Notes

1. Sporn, M. B.; Roberts, A. B.; Goodman, D. S., Eds. *The Retinoids. Biology, Chemistry, and Medicine*; 2nd edition, Raven Press: New York, 1994.

2. Lehman, J. M.; Jong, L.; Fanjul, A.; Cameron, J. F.; Lu, X. P.; Haefner, P.; Dawson, M. I.; Pfahl, M. *Science* **1992**, *258*, 1944.

3. Allenby, G.; Bocquel, M. T.; Saunders, M.; Kazmer, S.; Speck, J.; Rosenburger, M.; Lovey, A.; Kastner, P.; Grippo, J. F.; Chambon, P.; Levin, A. A. *Proc. Natl. Acad. Sci. U.S.A* **1993**, *90*, 30.

4. Allenby, G.; Janocha, R.; Kazmer, S.; Speck, J.; Grippo, J. F.; Levin, A. A. *J. Biol. Chem.* **1994**, *269*, 16689-16695.

5. Urbach, J.; Rando. R. R. *Biochem. J.* **1994**, *299*, 459.

6. Vaezi, M. F.; Alam, M.; Sani, B. P.; Rogers, T. S.; Simpson-Herren, L.; Wille, J. J.; Hill, D. L.; Doran, T. I.; Brouillette, W. J.; Muccio, D. D. *J. Med. Chem.* **1994**, *37*, 4499.

7. Robinson, C. Y.; Brouillette, W.J.; Muccio, D. D. *J. Org. Chem.* **1989**, *54*, 1992.

8. Zechmeister, L.; Lemmon, R. M. *J. Am. Chem. Soc.* **1944**, *66*, 186.

9. Pattenden, G.; Weedon, B. C. L. *J. Chem. Soc. (C)* **1968**, 1997.

10. Cainelli, G.; Cardillo, G. *Acc. Chem. Res.* **1981**, *14*, 89.

11. Lewin, A. H.; Whaley, G. W.; Parker, S. R.; Carroll, F. I. *J. Org. Chem.* **1982**, *47*, 1799.

12. Rando, R. R.; Chang, A. *J. Am. Chem. Soc.* **1983**, *105*, 2979.

13. Rowan III, R.; Sykes, B. D. *J. Am. Chem. Soc.* **1975**, *97*, 1023.

14. Ramamurthy, V.; Tustin, G.; Yau, C. C.; Liu, R. S. H. *Tetrahedron* **1975**, *31*, 193.

15. Patel, D. J. *Nature* **1969**, *221*, 825.

16. Vaezi, M. F.; Brouillette, W. J.; Muccio, D. D. *Magnet. Res. Chem.* **1995**, *in press*.

(*Received in USA* 17 *January* 1995; *accepted* 20 *March* 1995)