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Reaction of Geometrical Isomers of Retinoic Acid with 1,2,4-Triazoline-3,5-dione Having Fluorescent Chromophore

Masato Shimizu, Kyoko Yaguchi, Yukiko Iwasaki and Sachiko Yamada*

Institute for Medical and Dental Engineering, Tokyo Medical and Dental University
2-3-10 Surugadai, Kanda, Chiyoda-ku, Tokyo 101, Japan

Abstract: Reactions of 1,2,4-triazoline-3,5-dione having fluorescent chromophore (DMEQ-TAD) with 9*Z*-, 11*Z*-, 13*Z*-, and 11*Z*,13*Z*-isomers of retinoic acid were studied. Except for the 9*Z*-isomer, the reactions gave the 7,10-adducts in high selectivity while 9*Z*-isomer gave only the 5,8,11,14-bis adducts. Interesting self-sensitized photochemical isomerizations of these adducts and the mechanism of the addition reactions are discussed.

Retinoic acid (**1a**) modulates proliferation and differentiation of a variety of cell types.[1] In addition to the all-*trans* compound, some of its geometrical isomers, such as 9-*cis* and 13-*cis* isomers (**1e** and **1b**), are present in biological fluid.[2] Recent finding that 9-*cis* isomer (**1e**) plays an important role as a ligand of nuclear transcription factor (RXR) has stirred much interest among those working on these fields.[3]

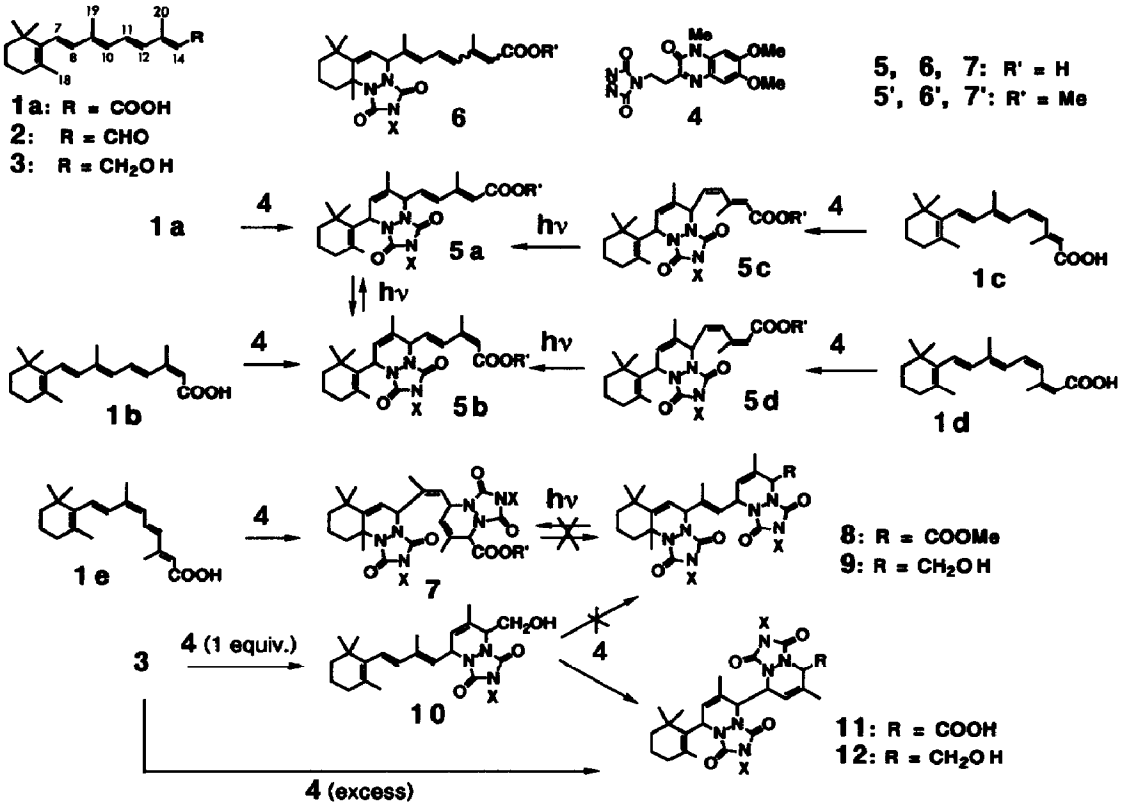
In the course of studying fluorescence-labeling reaction with fluorescent TAD (DMEQ-TAD, **4**) of conjugated dienes in biological fluid,[4] we became interested in the reaction of TADs with more complicated polyene systems. As we already reported,[5] retinol (**3**) and its metabolites (**1a** and **2**) reacted with 4-phenyl triazolinedione (PTAD) and DMEQ-TAD (**4**) with remarkable regioselectivity (>90%): Retinol (**3**) yielded exclusively the 11,14-adduct (**10**), while retinoic acid (**1a**) and retinal (**2**) gave predominantly (90-95% selectivity) the 7,10-adduct (such as **5a**) along with a minor 5,8-adduct (**6**). These results indicate that TADs add to vitamin A pentaene system with regioselectivity completely different from that of singlet oxygen[6] and provided intriguing evidence for the mechamism of the reaction of TADs. In this paper we report characteristic reactivity of geometrical isomers of retinoic acids towards TAD. We also report interesting sensitized photochemical reactions of the 7,10-DMEQ-TAD adducts (**5**) caused by the chromophore attached on their molecules.

The reactions of (13*Z*)-, (11*Z*)-, and (11*Z*,13*Z*)-retinoic acids (**1b**, **1c** and **1d**)[7] with DMEQ-TAD (**4**) were similar to that of the all-*trans* isomer (**1a**). These geometrical isomers (**1b**, **1c** and **1d**) reacted with DMEQ-TAD (**4**) (0°C, CH_2Cl_2, 1 hr) yielded 7,10-adducts (**5b**, **5c** and **5d**) as major products together with minor 5,8-adducts (**6b**, **6c** and **6d**) in about 9 : 1 ratio (ca. 70% yield). Introduction of *cis* geometry at the 11- and 13-positions of the conjugated pentaene system of vitamin A did not appreciably change the regiochemistry of the addition reaction. On the other hand introduction of *cis* stereochemistry at the 9-position drastically changed the reactivity of the pentaene system. When the 9*Z*-isomer (**1e**)[7] was treated with 1 equivalent of DMEQ-TAD (**4**) under usual conditions (0°C, CH_2Cl_2, 1 hr) only bis adducts **7** (as a mixture of diastereomers, >95 : 5 ratio), where the reagent added at the 5,8- and 11,14-positions, were obtained (ca. 60% yield based on **4**)[8]. It is

interesting to note that no mono adduct was obtained even the reaction was carried out under controlled conditions using a half equivalent of the reagent **4**.

The structures of the 7,10-adducts (**5b**, **5c** and **5d**) and 5,8-adducts (**6b**, **6c** and **6d**) were determined by comparing their spectral properties to those of **5a** and **6a**.[5,9] Photochemical interconversion of these isomeric 7,10-adducts (**5a**, **5b**, **5c** and **5d**) further supports the structures.

Since 7,10-adducts (**5** and **5'**) possess a chromophore that absorbs visible light (367 nm), they are sensitive to light and easily undergo photochemical isomerization even on exposure to desk lamp. Adducts **5a'** and **5b'** isomerized only at the 13-double bond[10]. Upon irradiation of visible light (365 nm) either **5a'** or **5b'** gave a same photostationary state mixture of **5a'** and **5b'** (ca. 1:1). The adducts (**5c'** and **5d'**) with 11*Z* structure are even more sensitive to light. By light irradiation, both **5c'** and **5d'** gave a mixture of 11*E*,13*E*- and 11*E*,13*Z*-isomers (**5a'** and **5b'**). No interconversion between **5c'** and **5d'** was observed. Thus it is clear in these adducts that first rapid one-way *Z* to *E* isomerization occurred at the 11-position and then reversible isomerization at the 13-position. All these results confirmed the regiochemistry of these 7,10-adducts.



The structure of the bis adduct (**7**) was based on the spectral properties[9] and on some related reactions. The mass and the ^{1}H NMR spectra indicate the incorporation of two DMEQ-TAD per molecule. In the ^{1}H NMR spectra, the 5,8-adduct formation is shown by the H-7 resonance appearing lower field (δ 5.63, d, *J*=4.3 Hz) and considerably deshielded H-4 resonance (δ 2.90) owing to the anisotropic effect of the TAD moiety; and the 11,14-adduct formation is indicated by a characteristic singlet of H-14 resonance (δ 4.69) that appears higher field. To comfirm the structure and to clear the mechanism of the bis adduct formation, we tried to synthesize bis

adducts from mono adducts, **5a** and **6a**. The reaction of (11*E*,13*E*)-5,8-adduct (**6a'**)[10] with DMEQ-TAD gave only 5,8,11,14-bis adduct **8** as a mixture of diastereomers (5:1). Spectral properties of **8** were similar but not identical with **7'**.[9] Contrary to the mono adducts, the bis adducts **7'** and **8** were stable for visible light irradiation. So **7'** and **8** were not interconverted by visible light irradiation. Interestingly the 7,10-adduct (**5a**) and the (11*E*,13*Z*)-5,8-adduct (**6b'**) did not react with excess DMEQ-TAD even under forced conditions (prolonged reaction at room temperature). It should be noted that retinol (**3**) gave no 5,8,11,14-bis adducts (**9**) but yielded only 7,10,11,14-bis adducts (**12**, ca. 1:1 mixture of diastereomers)[9] directly on treatment with excess DMEQ-TAD or via the 11,14-mono adduct (**10**).

Previously we proposed a mechanism of the addition of TAD in the reaction with all-*trans*-retinoic acid (**1a**): TAD undergoes preferably concerted 1,4-addition at the 7,10-position and a stepwise addition via 5,6-aziridinium ion at 5,8-position as a side reaction on the basis of its electronic structure calculated by MNDO method.[5] From the present reactions it is clear that as far as the 7,10-position holds the *E,E*-stereochemistry, 7,10-adddition is still the major pathway. As expected, the *Z*-stereochemistry introduced at the reactive position significantly changed the reactivity. We explain the reason why only the bis adduct (**7**) was formed from the 9*Z*-isomer (**1e**) as follows: Since the introduction of *cis* geometry blocks the reaction at the 7,10-position, the reagent attacks the second reactive position, 11,14-bond, first. The 11,14-adduct formation eliminates the effect of the electron withdrawing carboxyl group so that the electron density of the remaining triene system is raised and in turn the reactivity is increased. Thus the 5,8-position becomes much more reactive than in the starting pentaene system to yield the bis adduct with no mono adducts remained.

In the present study we showed intriguing reactivity of geometrical isomers of retinoic acid towards DMEQ-TAD, and interesting photochemical isomerization of fluorescent-TAD adducts. It should be noted that in the reaction with DMEQ-TAD both biologically important retinoic acid isomers, all-*trans*- and 9*Z*-isomers (**1a** and **1e**), gave **5a** and **7** as major fluorescence-labeled products even in the presence of excess reagent. Thus DMEQ-TAD might be useful as a fluorescence labeling reagent for retinoic acids in biological fluid.

REFERENCES AND NOTES

1. Breitman, T. R.; Selonick, S. E.; Collins, S. J. *Proc. Natl. Acad. Sci.* USA **1980**, 77, 2936-2940.
2. Tang, G.; Russel, R. M. *J. Lipid Res.* **1990**, 31, 175-182.
3. a) Carlberg, C.; Bendik, I.; Wyss, A.; Meier, E.; Sturzenbecker, L. J.; Grippo, J. F.; Hunziker, W. *Nature* **1993**, 361, 657-660. b) Levin, A. A.; Sturzenbecker, L. J.; Kazmer, S.; Bosakowski, T.; Huselton, C.; Allenby, G.; Speck, J.; Kratzeisen, C.; Rosenberger, M.; Lovey, A.; Grippo, J. F. *Nature* **1992**, 355, 359-361. c) Heyman, R.; Mangelsdorf, D. J.; Dyck, J. A.; Stein, R. B.; Eichele, G.; Evans, R. M.; Thaller, C. *Cell* **1992**, 68, 397-406.
4. 4-[2-(6,7-Dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalyl)ethyl]-1,2-4-triazoline-3,5-dione (DMEQ-TAD, **4**) is a highly sensitive fluorescent dienophile and was developed as a fluorescence-labeling reagent for assaying vitamin D metabolites in biological fluid. a) Shimizu, M.; Takahashi, T.; Uratsuka, S.; Yamada, S. *J. Chem. Soc., Chem. Commun.* **1990**, 1416-1417. b) Shimizu, M.; Kamachi, S.; Nishii, Y.; Yamada, S. *Anal. Biochem.* **1991**, 194, 77-81. c) Shimizu, M.; Gao, Y.; Aso, Y.; Nakatsu, K.; Yamada, S. *Anal. Biochem.* **1992**, 204, 258-264. d) Shimizu, M.; Yamazaki, T.; Yamada, S. *BioMed. Chem. Lett.* **1993**, 3, 1809-1814.

5. Yamada, S.; Hamano, K.; Shimizu, M.; Ichikawa, H. *Tetrahedron Lett.* **1991**, 32, 2379-2382.
6. a) Lerner, D. -A.; Mani, J. -C.; Mousseron-Canet, M. *Bull. Soc. Chim.* France **1970**, 1968-1974. b) Pfoertner, K. -H. *Helv. Chim. Acta* **1975**, 58, 833-839.
7. The *cis*-isomers (**1c**, **1d** and **1e**) were prepared by photoisomerization of all-*trans*-retinoic acid with halogen lamp followed by purification by HPLC. a) Halley, B. A.; Nelson, E. C. *J. Chromatogr.* **1979**, 175, 113-123. b) Liu, R. S. H.; Asato, A. E. *Tetrahedron* **1984**, 40, 1931-1965.
8. No other isolable products were obtained.
9. The structrures of new compounds were comfirmed by ^{1}H NMR, mass, IR and UV spectra after being methylated.
5b': ^{1}H NMR ($CDCl_3$) δ 1.59 (3 H, s, H-18), 1.81 (3 H, s, H-19), 1.91 (3 H, d, *J*=1.3 Hz. H-20), 3.69 (3 H, s, COOMe), 3.93 and 4.01 (each 3 H, s, OMe), 4.61 (1 H, br.s, H-7), 4.78 (1 H, d, *J*=7.6 Hz, H-10), 5.36 (1 H, d, *J*=1.3 Hz, H-8) 5.70 (1 H, s, H-14), 5.92 (1 H, dd, *J*=7.6 & 16.2 Hz, H-11), 7.78 (1 H, d, *J*=16,2 Hz, H-12). MS *m/z* (%) 659 (M^+, 100), 413 (6), 347 (8), 313 (13), 246 (62). UV (95% EtOH) 244, 367 nm. **6b'**: ^{1}H NMR ($CDCl_3$) δ 1.42 (3 H, s, H-18), 1.64 (3 H, d, *J*=0.7 Hz, H-19), 2.02 (3 H, d, *J*=1.3 Hz, H-20), 2.94 (1 H, m, H-4), 3.70 (3 H, s, COOMe), 3.92 and 4.00 (each 3 H, s, OMe), 4.67 (1 H, d, *J*=4.1 Hz, H-8), 5.40 (1 H, d, *J*=4.1 Hz, H-7), 5.66 (1 H, s, H-14), 6.26 (1 H, d, *J*=10.9 Hz, H-10), 6.68 (1 H, dd, *J*=10.9 & 15.5 Hz, H-11), 7.71 (1 H, d, *J*=15.5 Hz, H-12). UV (95% EtOH) 308, 367 nm. **5c'**: ^{1}H NMR ($CDCl_3$) δ 1.64 (3 H, s, H-18), 1.70 (3 H, s, H-19), 2.24 (3 H, d, *J*=0.7 Hz, H-20), 3.72 (3 H, s, COOMe), 3.39 and 4.00 (each 3 H, s, OMe), 4.53 (1 H, br.s, H-7), 5.09 (1 H, d, *J*=9.9 Hz, H-10), 5.30 (1 H, dd, *J*=9.9 & 11.5 Hz, H-11), 5.32 (1 H, d, *J*=1.6 Hz, H-8), 5.90 (1 H, d, *J*=11.5 H-20), 6.18 (1 H, s, H-14). **5d'**: ^{1}H NMR ($CDCl_3$) δ 1.62 and 1.65 (each 3H, s, H-18, 19), 2.30 (3 H, s, H-20), 3.67 (3 H, s, COOMe), 3.94 and 4.00 (each 3 H, s, OMe), 4.53 (1 H, br.s, H-7), 4.93 (1 H, d, *J*=10.2 Hz, H-10), 5.30 (1 H, d, *J*=1.6 Hz, H-8), 5.42 (1 H, dd, *J*=10.2 & 11.8 Hz, H-11), 5.79 (1 H, br.s, H-14), 6.92 (1 H, d, *J*=11.,8 Hz, H-12). **7'**: ^{1}H NMR ($CDCl_3$) δ 1.39 (3 H, s, H–18), 1.60 (3 H, s, H–19), 1.93 (3 H, s, H-20), 2.90 (1 H, m, H-4), 3.75 (3 H, s, COOMe), 3.92, 3.95, 4.00 and 4.01 (each 3 H, s, OMe), 4.69 (1 H, s, H-14), 5.12 (1 H, d, *J*=9.2 Hz, H-10), 5.30 (1 H, dm, *J*=9.2 Hz, H-11), 5.35 (1 H, d, *J*=4.3 Hz, H-8), 5.63 (1 H, d, *J*=4.3 Hz, H-7), 5.74 (1 H, m, H-12). MS/MS *m/z* (%) 1004 (M^+, 29), 758 (16), 658 (39), 534(100), 494 (46), 247 (49). **8**: ^{1}H NMR ($CDCl_3$) δ 1.40 (3 H, s, H-18), 1.61 (3 H, s, H-19), 1.94 (3 H, s, H-20), 2.91 (1 H, m, H-4), 3.73 (3 H, s, COOMe), 3.92, 3.94, 4.00 and 4.01 (each 3 H, s, OMe), 4.70 (1 H, s, H-14), 4.71 (1 H, d, *J*=4.0 Hz, H-8), 4.81 (1 H, m, H-11), 5.40 (1 H, m), 5.42 (1 H, d), 5.45 (1 H, d, *J*=4.0 Hz, H-7). **8'**(diastereomer of **8**): ^{1}H NMR ($CDCl_3$) δ 1.40 (3 H, s, H-18), 1.65 (3 H, s, H-19), 1.91 (3 H, s, H-20), 2.91 (1 H, m, H-4), 3.75 (3 H, s, COOMe), 3.91 and 3.93 (each 3 H, s, OMe), 3.98 (6 H, s, OMe), 4.68 (1 H, d, *J*=4.0 Hz, H-8), 4.685 (1 H, s, H-14), 4.83 (1 H, m, H-11), 5.42 (1 H, m), 5.44 (1 H, m), 5.45 (1 H, d, *J*=4.0 Hz, H-7). **12**: ^{1}H NMR ($CDCl_3$) δ 1.70, 1.78 and 1.88 (each 3 H, s, H-18, 19, 20), 3.92 and 3.94 (each 3 H, s, OMe), 4.01 (6 H, s, OMe), 4.42 (1 H, m), 4.56 (1 H, m), 4.76 (1 H, m), 5.19 (1 H, m), 5.45 (1 H, m), 5.68 (1 H, d, *J*=4.3 Hz). **12'**(diastereomer of **12**): ^{1}H NMR ($CDCl_3$) δ 1.78, 1.83 and 1.88 (each 3 H, s, H-18, 19, 20), 3.91, 3.93, 3.99 and 4.01 (each 3 H, s, OMe), 4.56 (1 H, m), 4.65 (1 H, m), 4.87 (1 H, m), 4.95 (1 H, d, *J*=7.6 Hz), 5.45 (1 H, m), 5.79 (1 H, d, *J*=4.9 Hz).
10. Photochemical interconversions and bis-adduct productions from the mono adduct were carried out using the methyl esters because of ease of handling and were monitored by HPLC.

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