SPECIFIC OXIDATION OF RETINOIC ACID TO 4-*OXO*-RETINOIC ACID IN DILUTED ACID SOLUTIONS

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Treatment of retinoic acid (**1a**) or its methyl ester (**2a**) with hydrochloric acid in methanol gave methyl 4-*oxo*-retinoates (**4a** - **c**). Similar oxidation proceeded when **2a** was treated with trifluoromethanesulfonic acid in the presence of lithium chloride in methanol.

KEY WORDS vitamin A; retinoic acid; allylic oxidation; trifluoromethanesulfonic acid

Retinoic acid (vitamin A acid, **1a**, all-*E*) has recently attracted much attention because of its key roles in cell differentiation and proliferation,[1] and in morphogenesis of vertebrates.[2] Biological investigations have shown that retinoic acid (**1a**) elicits various specific activities by the direct control of gene expressions through binding to the specific nuclear receptor(s).[1,3] Therefore, retinoic acid (**1a**) should be classified as a "hormone", similar to the steroid hormones, thyroid hormones, and activated vitamin D_3, although the precise mechanisms of its actions are still unclear. Recently, several metabolites of **1a**, such as 9-*cis*-retinoic acid[4] and 4-*oxo*-retinoic acid,[5] have been proven to participate in or to exhibit specific activities of retinoic acid (**1a**). Structurally, retinoic acid (**1a**) consists of a conjugated pentaene system which seems to be very prone to isomerization or oxidation, either enzymatically or chemically. An understanding of the fundamental chemical behaviors of retinoic acid (**1a**) is important for biological investigations on retinoic acid (**1a**) and its metabolites, and for clinical applications in the fields of dermatology and oncology.[6] Previously, we reported that retinoic acid (**1a**) easily decomposed in various acidic conditions to give a mixture of cyclized isomers.[7] The reactions strongly depended on the acidity of the solution. In the course of our investigations on the acid-catalyzed isomerization of **1a**, we found an unusual, specific oxidation of **1a** at the 4 position. In this paper, the allylic oxidations of **1a** and its methyl ester (**2a**) in dilute acids are discussed, in comparison with the *retro*-isomer formation.

Treatment of **1a** with gaseous hydrogen chloride (1 M solution, 60 eq to **1a**) in anhydrous ether at 18°C for 24 h gave 4,14-*retro*-retinoic acids (**3a** - **d**, 12 %), which consist of four geometrical isomers at the 6 and 12 positions, besides the recovered **1a** (30 %) and its 13Z-isomer (**1b**, 17 %). In this reaction condition, no cyclized compound, such as can be formed by treating **1a** with strong acid,[7] was not detected. The structures of **3a** - **d** were determined from ^{1}H-NMR chemical shifts,[8,9] after esterification with CH_2N_2. On the other hand, when **1a** was treated with hydrochloric acid in methanol (1 M solution, 60 equiv to **1a**) at 18°C for 24 h under argon,[10] the *retro*-isomers were not formed and three other neutral products **4a** - **c** were obtained in 24 %, 5 % and 16 % yields, respectively. The major product (**4a**, pale yellow prisms from *n*-hexane, mp 94.5 -

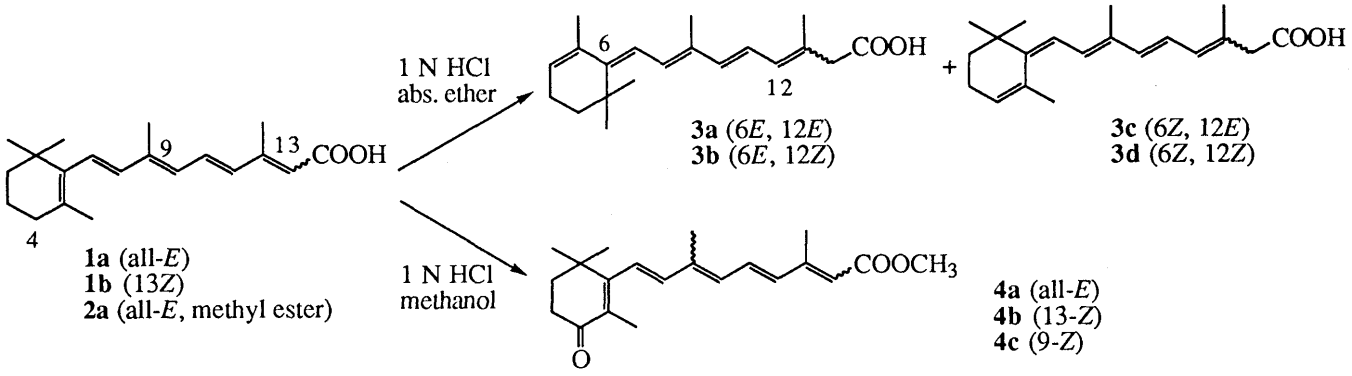

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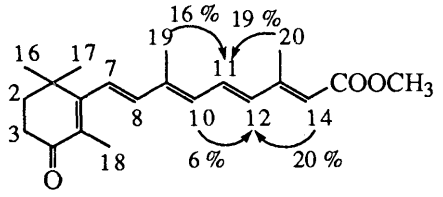
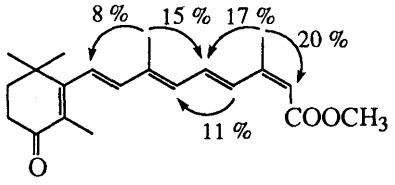
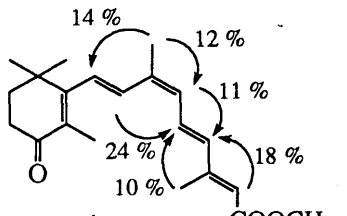
Table I. ^{1}H NMR Chemical Shifts[a] and NOE of Methyl 4-*oxo*-Retinoates **4a** - **c**



4a



4b



4c

	H_2	H_3	H_7	H_8	H_{10}	H_{11}	H_{12}	H_{14}	$H_{16,17}$	H_{18}	H_{19}	H_{20}	OCH_3
4a	1.86	2.51	6.33	6.33	6.26	6.99	6.37	5.82	1.19	1.86	2.04	2.36	3.72
4b	1.86	2.51	6.31	6.36	6.37	6.96	7.84	5.70	1.19	1.85	2.02	2.08	3.71
4c	1.88	2.53	6.33	6.84	6.20	7.01	6.28	5.80	1.20	1.89	2.03	2.32	3.72

[a] Chemical shifts are expressed in ppm relative to Me_4Si in $CDCl_3$.

95°C) has the molecular formula $C_{21}H_{28}O_3$, with two carbonyl groups (IR: 1665 cm^{-1} due to a cyclic conjugated ketone and 1714 cm^{-1} due to an ester) in the structure. From the ^{1}H-NMR spectrum, **4a** has no protons at the C_4 position and the signal of the H_3 protons is shifted to lower field (2.51 ppm) than that of **1a** (2.03 ppm). Therefore, **4a** is methyl 4-*oxo*-retinoate and the stereochemistry of the side chain was determined as all-*E* form from the chemical shifts (Table I) and NOE experiments.[11] Two minor compounds, **4b** and **4c**, were isolated by HPLC (ODS; eluent: 20% H_2O-CH_3CN) and are also derivatives of methyl 4-*oxo*-retinoate. Comparisons of ^{1}H-NMR chemical shifts (Table I) and NOE experiments indicated that **4b** and **4c** are the 13*Z*- and 9*Z*-isomer of **4a**, respectively. This non-enzymatic oxidation is important, because 4-*oxo*-retinoic acid is believed to be one of the major metabolites of **1a**.

For eliminating the esterification process, the reactions of methyl retinoate (**2a**) were examined (Table II). The selective oxidation of **2a** at the 4 position proceeded under the same condition as for **1a** (entry #1). In HCl-anhydrous ether, methyl 4,14-*retro*-retinoate (**5a** - **d**) with **2a** and methyl 13-*cis*-retinoate (**2b**) were obtained, but the 4-*oxo* compounds were not detected. When methanol (10 % to ether) was added after 24 h to the reaction mixture (#2), containing **2** and **5**, methyl 4-*oxo*-retinoates (**4**) were formed in 29 % yield (#3). Treatment of **2a** with trifluoromethanesulfonic acid (TFSA, 80 eq) in methanol gave only methyl 4,14-*retro*-retinoates (**5**) in 24 % yield (#4). Interestingly, the addition of LiCl (60 eq) caused a dramatic change in the reaction, and methyl 4-*oxo*-retinoates (**4**) were obtained in 52 % yield (#5). Addition of KBr (60 eq) also oxidized **2a**, although the reaction was slower than #5, probably due to the low solubility of KBr in methanol. From these observations, the formation of methyl 4-*oxo*-retinoates (**4**) from **2a** seems to be influenced by three factors, acid, halide, and solvent. Using trifluoroacetic acid (TFA), which is a much weaker acid than TFSA or HCl, 93% of **2a** (with the isomer **2b**) was recovered after 24 h at 23°C.[12] This result indicates that the 4-*oxo* formation requires sufficient acidity to isomerize **2a** to the methyl 4,14-*retro*-retinoate (**5**).

Table II. Reaction of Methyl Retinoate (**2a**) under Various Acidic Conditions (23°C)

	Acid	Additive	Solvent	Time	Yield[a]		
					2	**4** (4-*oxo*)	**5** (4,14-*retro*)
#1	1 N HCl (60 eq)	none	methanol	24 h	4 %	49 %	-
#2	1 N HCl (60 eq)	none	abs. ether	24 h	47 %	-	24 %
#3	1 N HCl (60 eq)	methanol[b]	abs. ether	46 h	15 %	29 %	9 %
#4	TFSA (80 eq)	none	methanol	24 h	21 %	-	24 %
#5	TFSA (60 eq)	LiCl (60 eq)	methanol	24 h	9 %	52 %	-

[a] Yields were determined from ^{1}H-NMR integrations and are the sum of geometrical isomers.

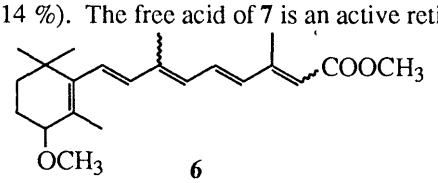
[b] Methanol (10 % to abs. ether) was added to the reaction mixture of **2a** in abs. ether after 24 h.

Several oxidations of retinoic acid (**1a**) or its derivatives have been reported.[1,11] In the present oxidation reaction of retinoic acid, a radical scavenger, such as hydroquinone or 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH), decreased the yield of 4-*oxo* derivatives, and oxygen also inhibited the reaction. Therefore, the oxidation is considered to proceed through a radical pathway, although the detailed mechanism is unclear. The reactivity of several conjugated unsaturated esters in TFSA-LiCl at room temperature was examined. Methyl 1-cyclohexenylcarboxylate and methyl 3-(2,6,6-trimethylcyclohexen-1-yl)propenoate did not react under this condition. Methyl 3-methyl-5-(2,6,6-trimethylcyclohexen-1-yl)pentadienoate afforded only the *retro*-isomers even in the presence of LiCl. β-Ionone, a synthon for vitamin A or carotenoids, was poorly oxidized at the 4 position with TFSA (60 equiv)-LiCl (60 eq), although the reaction afforded a complex mixture of compounds containing many acid-catalyzed aldol condensation products.[13] Thus, the oxidation by HCl-CH_3OH or TFSA-LiCl-CH_3OH seems to be very specific to retinoic acids or *retro*-retinoic acids.

In conclusion, retinoic acid is oxidized in diluted methanolic hydrochloric acid or in a similar acidic condition (methanolic TFSA-LiCl) to 4-*oxo* derivatives (**4**) in moderate yields. Since the resulting 4-*oxo*-retinoic acid is an important metabolite of retinoic acid (**1a**) and is biologically active,[5] this easy chemical conversion should be taken into account in experiments on and discussions of retinoic acid chemistry and biology.

REFERENCES AND NOTES

1) M. B. Sporn, A. B. Roberts (ed.), "The Retinoids," 2nd ed., Academic Press, Inc., Orlando, FL, 1993.
2) C. Thaller, G. Eichele, *Nature (London)*, **327**, 625 (1987).; V. Giguere, E. S. Ong, R. M. Evans, C. J. Tabin, *Nature (London)*, **337**, 566 (1989).
3) Y. Hashimoto, K. Shudo, *Cell Biol. Rev.*, **25**, 209 (1991).; M. Petkovich, *Ann. Rev. Nutr.*, **12**, 443 (1992).; E. Linney, *Curr. Top. Dev. Biol.*, **27**, 309 (1992).
4) D. J. Mangelsdorf, E. S. Ong, J. A. Dyck, R. M. Evans, *Nature (London)*, **345**, 224 (1990).; R. A. Heyman, D. J. Mangelsdorf, J. A. Dyck, R. B. Stein, G. Eichele, R. M. Evans, C. Thaller, *Cell*, **68**, 397 (1992).
5) W. W. M. Pijnappel, H. F. J. Hendricks, G. E. Folkers, C. E. van den Brink, E. J. Dekker, C. Edelenbosch, P. T. van der Saag, A. J. Durston, *Nature (London)*, **366**, 340 (1993).
6) W. J. Cunliff, A. J. Miller, (ed.), "Retinoids Therapy," MTP Press Limited, Lancaster, 1984.; W. Bollag, E. Holdener, *Ann. Oncol.*, **3**, 513 (1992).; D. L. Hill, C. J. Grubbs, *Ann. Rev. Nutr.*, **12**, 161 (1992).
7) H. Tanaka, H. Kagechika, K. Sugita, Y. Hashimoto, A. Itai, K. Shudo, *Tetrahedron Lett.*, **29**, 6279 (1988).
8) G. Englert, "Carotenoid Chemistry & Biochemistry," Pergamon Press, 1982, pp 107.
9) ^{1}H-NMR (400 MHz, ppm in $CDCl_3$) **5a** (methyl ester of **3a**, 6*E*, 12*E*): δ 6.73 (H_8), 6.44 (H_{11}), 6.37 (H_7), 6.34 (H_{12}), 6.04 (H_{10}), 5.77 (H_4), 3.69 (OCH_3), 3.10 (H_{14}), 2.13 (H_3), 1.92 (H_{20}), 1.91 (H_{19}), 1.89 (H_{18}), 1.51(H_2), 1.29 ($H_{16,17}$).; **5c** (methyl ester of **3c**, 6*Z*, 12*E*): δ 6.64 (H_8), 6.44 (H_{11}), 6.37 (H_7), 6.29 (H_{12}), 6.02 (H_{10}), 5.62 (H_4), 3.69 (OCH_3), 3.09 (H_{14}), 2.13 (H_3), 2.07 (H_{18}), 1.92 (H_{20}), 1.91 (H_{19}), 1.51(H_2), 1.12 ($H_{16,17}$).
10) Argon was deoxygenated through basic pyrogallol solution and then concentrated sulfuric acid. Some experiments were performed under freeze-degassed conditions.
11) Methyl 4-*oxo*-retinoates can be formed from **2a** by treatment with manganese dioxide,[a] or photochemically by iodine.[b] a) A. B. Barua, M. C. Ghosh, *Tetrahedron Lett.*, 1823 (1972).; M. S. S. Rao, J. John, H. R. Cama, *Int.J.Vitam.Nutr.Res.*, **368**, 42 (1972).; b) R. M. McKenzie, D. M. Hellwege, M. L. McGregor, E. C. Nelson, *Lipids*, **14**, 714 (1979).
12) Compound **4** was formed in 12 % yield when **2a** was kept in methanolic TFA (60 eq)-LiCl (30 eq) for 10 days at 23°C. However, the reaction afforded various products, such as methyl 4-methoxyretinoates (**6**, 26 %) and methyl 5-acetyl-4,18-*dinor*-retinoates (**7**, 14 %). The free acid of **7** is an active retinoid.



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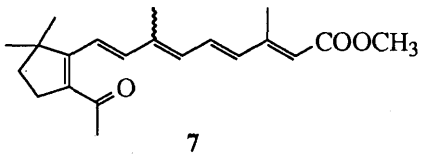

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13) Oxidation of β-ionone to 4-alkoxy-β-ionone by electron-transfer reaction was reported. V. Calo, L. Lopez, L. Troisi, *J. Chem. Soc., Chem. Commun.*, 25 (1989).

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