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Stability of Retinol Analogs. VIII.[1] Pyrolysis and Photolysis of Retinoic Acid in Aqueous Ethanolic Solution

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In order to study the decomposition of retionic acid in aqueous solution without surface-active agent, retinoic acid was dissolved in aqueous ethanolic solutions which contained various amount of water, and its thermal decomposition and photodecomposition was investigated.

(1) In pyrolysis, retinoic acid was more stable than retinol or retinal. When water content was increased, the stability of retionic acid did not change as that of retinol and retinal. In photolysis, retinoic acid was decomposed rapidly, and its stability was not largely affected by N_2 gas exchange and increasing water content.

(2) Two decomposition products were recongnized, as the common decomposition products of retionic acid in pyrolysis and photolysis. The one seemed to be decarboxylated retinoic acid and the other seemed to be formed by the addition of a decomposition product to retinoic acid. In photolysis, additionally three other substances were recognized, the one was ethyl retionate and the two other substances seemed to be isomers of retinoic acid.

(3) In photolysis, all-*trans*-retinoic acid was isomerized into 13-*cis*- and 9,13-di-*cis*-isomers, but isomerization did not occur in pyrolysis.

Keywords——retinoic acid; stability constant; isomerization; thermal and photochemical degradation; aqueous ethanolic solution

Retinoic acid has a carboxyl group in its terminal part and it is not converted into retinal *in vivo*,[3] so that it does not act as vitamin A in vision, but it has other vitamin A effects of promoting growth and preserving the biomembrane in a good state in various vitamin A-deficient animals. Retinoic acid is more water soluble than other retinol analogs, and its use as a drug is being attempted. In recent years, retinoic acid has been applied to skin in dermatological studies and has been noted in biochemistry as having an antitumor activity. Several physiological and biochemical studies on retinoic acid have been reported, but the reports on physicochemical studies are few. The effect of metal ion on the stability of retinoic acid in buffer solution was reported only in pyrolysis condition,[4] but in another condition, further detailed investigations on the thermal stability and decomposition products have not been reported. In photolysis, the stability of retinoic acid was investigated in some organic solvents by Sherman,[5] and photochemical stability of retinoic acid in powdered state was reported by Tabata *et al.*[6] In these reports, stability of retinoic acid was determined mainly by ultraviolet (UV) absorption, and the examination of its isomerization and decomposition products has not been made.

1) Part VII: Y. Takashima, M. Furuya, M. Ikuta, M. Washitake, I. Tanaka, and T. Tabata, *Bitamin*, **48**, 9 (1974).
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3) A.B. Roberts and H.F. DeLuca, *Biochem. J.*, **102**, 600 (1967); J.E. Dowling and G. Wald, *Proc. Nat. Acad. Sci. U.S.A.*, **46**, 587 (1960).
4) T. Kawasaki, Y. Ito, and M. Ogawa, *Bitamin*, **37**, 204 (1968).
5) B.S. Sherman, *Clin. Chem.*, **13**, 1039 (1967).
6) T. Tabata, T. Okuma, and Y. Ito, *Bitamin*, **42**, 46 (1970).

In order to elucidate the stability of retinoic acid in aqueous preparations, which is applied externally, retinoic acid was dissolved in aqueous ethanolic solution, and decomposed by pyrolysis and photolysis. The stability, isomerization of retinoic acid, and the structure of decomposition products in these conditions were investigated. In the previous studies,[1] the effect of water contents on the stability of retinol analogs, physical properties of these decomposition products, and isomerization rates were investigated. In the present work, similar examinations were made by pyrolysis of retinoic acid, and these results compared with those of retinol[7] and retinal.[8] The photodecomposition, isomerization of retinoic acid, and the structure of photodecomposition products were also examined.

Experimental

Preparation of Test Solution——All-*trans*-retinoic acid of pure crystals was obtained from Hoffmann-La Roche Co., Ltd., Basel. All-*trans*-retinoic acid was dissolved in absolute EtOH, 80%, and 60% (v/v) aqueous EtOH in a concentration of 10 mg/100 ml. For the pyrolysis study, these solutions were poured into amber ampules and stored at 100°, 50°, or 35°. For the photolysis study, these solutions were poured into colorless ampules filled with N_2 gas or with air, and irradiated with a xenon fade-meter (Model XF-1, Toyo Rika Instrument Inc., Tokyo) for 5 hr. The intensity of the xenon ray was measured by an integrating actinometer (Model PH-11, Toyo Rika Instrument Inc., Tokyo). This value was 350 counts/hr. Isomerization of retinoic acid and structural examination of the decomposition products were investigated in absolute ethanolic solution in a concentration of 500 mg/100 ml. For the pyrolysis study, the solution was poured into 20-ml amber ampules and stored at 100° for 300 hr. The residual rate of this sample was about 85%. For the photolysis study, this solution was poured into colorless 20 ml ampules and irradiated with sunlight. The structural examination of the photodecomposition products of retinoic acid was carried out on about 50% decomposed sample. Isomerization of retinoic acid was examined by irradiation with sunlight for 20000 counts.

UV Spectrophotometric Determination of Retinoic Acid——One ml of the test solutions was diluted with absolute EtOH to 25 ml and the UV spectrum was obtained by a Hitachi EPS-2U spectrophotometer. The residual rate of retinoic acid was measured by the UV absorbance at 350 nm. The instrument for this determination was a Hitachi Perkin-Elmer 319 spectrophotometer.

Procedure for Thin-layer Chromatography (TLC)——The decomposition products were examined by TLC with plates of 250 μm Kieselgel G (Merck and Co., Inc., Darmstadt) activated at 120° for 1 hr. Coloration reagent was $SbCl_3$–$CHCl_3$ solution, and developing solvent was ether–cyclohexane (50: 50). In order to detect and examine the decomposition products of retinoic acid, 50—100 μl of the sample solutions which were stored under various conditions were applied to this TLC plates.

Nuclear Magnetic Resonance (NMR) Analysis for Evaluation of Isomerization Ratio——Ten ml of the above-mentioned test samples was evaporated in vacuum, dissolved in $CDCl_3$, added tetramethylsilane (TMS) as an internal standard, and NMR spectrum was measured at 60 MHz with a Hitachi Perkin–Elmer Model R-20 High Resolution NMR spectrometer. In the NMR spectra, 13-methyl signals of all-*trans*- and 13-*cis*-retinoic acid appeared at 2.30—2.40 and 2.05—2.15 ppm, respectively. Used 13-*cis*-retinoic acid for this

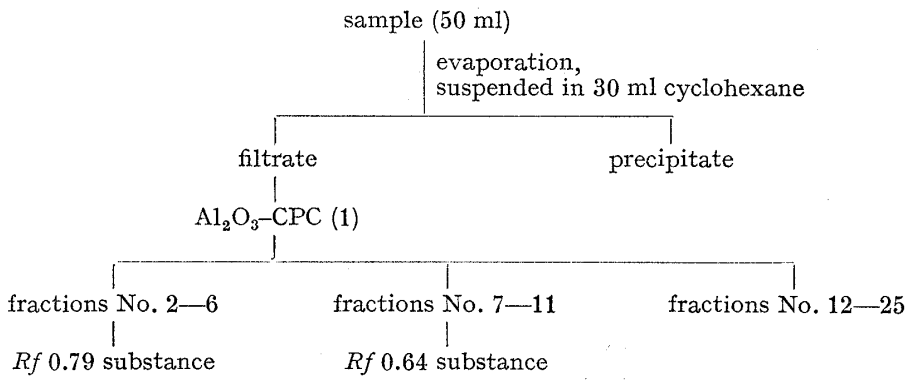


Chart 1. Separation of Thermal Decomposition Products from Retinoic Acid Solution

7) T. Anmo, M. Washitake, Y. Takashima, M. Isohata, M. Furuya, and K. Koike, *Bitamin*, **46**, 193 (1972).
8) T. Anmo, M. Washitake, Y. Takashima, M. Furuya, and K. Koike, *Bitamin*, **47**, 341 (1973).

experiment was obtained as pure crystals from Hoffmann–La Roche Co., Ltd., Basel, and the purity was confirmed by NMR and UV spectrum. Moreover, in the NMR spectrum of photodecomposed sample, another signal was recognized at 2.20—2.25 ppm and it was assigned to 13-methyl signal of 9,13-di-*cis*-retinoic acid from literature.[9] The integrated area ratio of 13-methyl signals of *cis*-isomer to all-*trans*-retinoic acid was proportional to the weight ratio of them, so that the isomerization ratio was examined with this relation.

Examination of Decomposition Products of Retinoic Acid——Separation and purification procedures for the thermal decomposition products are shown in Chart 1. Retinoic acid was so stable in this pyrolysis condition that the test solutions were evaporated in vacuum, added cyclohexane, and then the precipitated unchanged retinoic acid was removed by filtration and ratios of decomposition products were elevated. The concentrated mother liquor was applied to Al_2O_3 (Woelm, Eschwege) column which was eluted with ether–cyclohexane solution, and thermal decomposition products were separated.

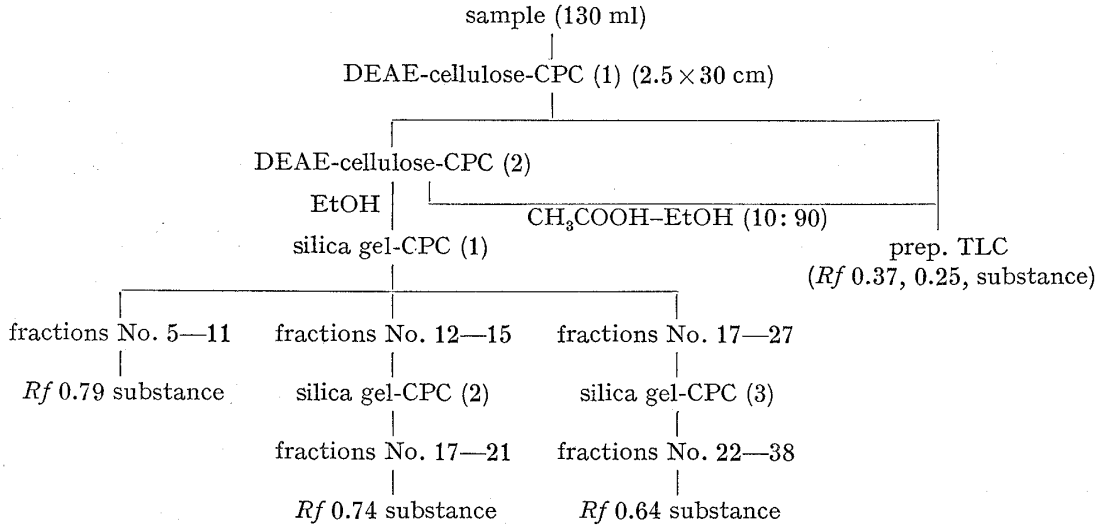


Chart 2. Separation of Photodecomposition Products from Retinoic Acid Solution

The separation and purification of photodecomposition products are shown in Chart 2. DEAE-cellulose column partition chromatography (CPC), silica gel-CPC, and preparative TLC were used for the separation of photodecomposition products. DEAE-cellulose (Seikagaku Kogyo Co., Tokyo) with an exchange capacity of 0.86 meq/g, was thoroughly washed with EtOH. EtOH or CH_3COOH–EtOH (10: 90) was used as the eluting solvent for this CPC. Silica gel No. 950, 60—200 mesh (Wako Pure Chemical Industries, Ltd., Osaka) was deactivated by the addition of water (2—3%). Ether–cyclohexane was used as the eluting solvent for these CPC. The preparative TLC was carried out with the plate of 400 μm Kieselgel G by above-mentioned procedures.

Results and Discussion

UV Spectral Change of Test Solutions with Time

UV spectral change of the test samples, prepared in absolute EtOH, 80%, or 60% aqueous EtOH at 100° by pyrolysis, was examined. Initially, the absorption maximum of retinoic acid was at 350 nm in its UV spectrum and after storage, the absorption maximum of this solution slightly shifted into shorter wavelength, although spectral pattern did not change. After storage at 50°, 35°, the shift of these UV maxima was almost the same as that seen after at 100° storage. In the photolysis condition, UV spectral changes of the test samples without N_2 gas are shown in Fig. 1. The absorbance at 350 nm decreased with passage of time while the absorbance at 265 nm increased gradually. In 60% aqueous ethanolic solution without N_2 gas exchange, the increase of the absorbance at 265 nm was remarkable.

9) D.J. Patel, *Nature* (London), **221**, 825 (1969).

Stability of Retinoic Acid

Thermal Stability of Retinoic Acid——The residual rates of retinoic acid were obtained directly by UV absorbance at 350 nm because thermal decomposition products did not have the absorbance at 350 nm and the isomerization did not occurred. The residual rates of retinoic acid stored at 100°, 50°, or 35° are shown in Fig. 2. With increasing water content, the decomposition rates were slightly accelerated. When the residual rate of retinoic acid was plotted as a function of time on a semilogarithmic graph paper, a straight line was obtained. Therefore this reaction was considered to be an apparent first-order reaction and the rate constants were calculated by the least squares method, and from these data half-lives periods were obtained. These results are shown in Table I. Comparison of these data with previously reported rate constants of retinol[7] ($k=1.42\times10^{-3}$ hr^{-1} in absolute EtOH, $k=6.74\times10^{-3}$ hr^{-1} in 60% aqueous EtOH at 50°) and retinal[8] ($k=1.81\times10^{-3}$ hr^{-1} in absolute EtOH, $k=4.01\times10^{-4}$ hr^{-1} in 60% aqueous EtOH at 50°) shows that retinoic acid is more stable and the effect of water content on these decomposition is not so great.

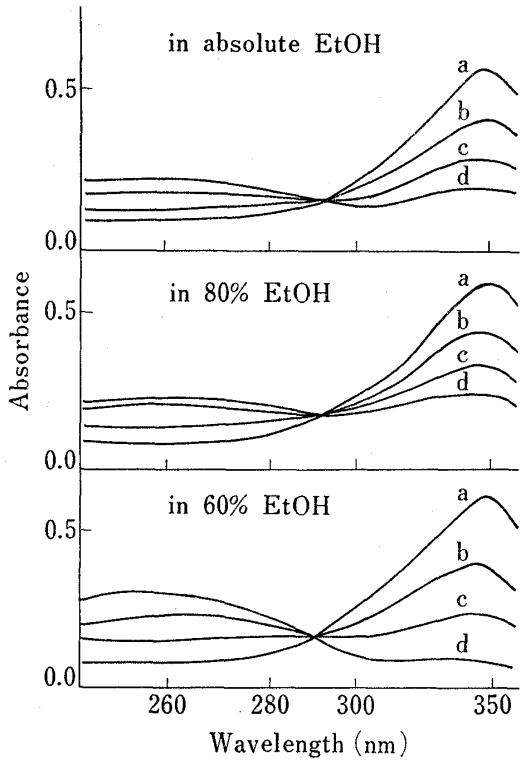


Fig. 1. UV Spectra of Retinoic Acid Solutions irradiated with Xenon Ray

a, initial; b, 1 hr; c, 3 hr; d, 5 hr.

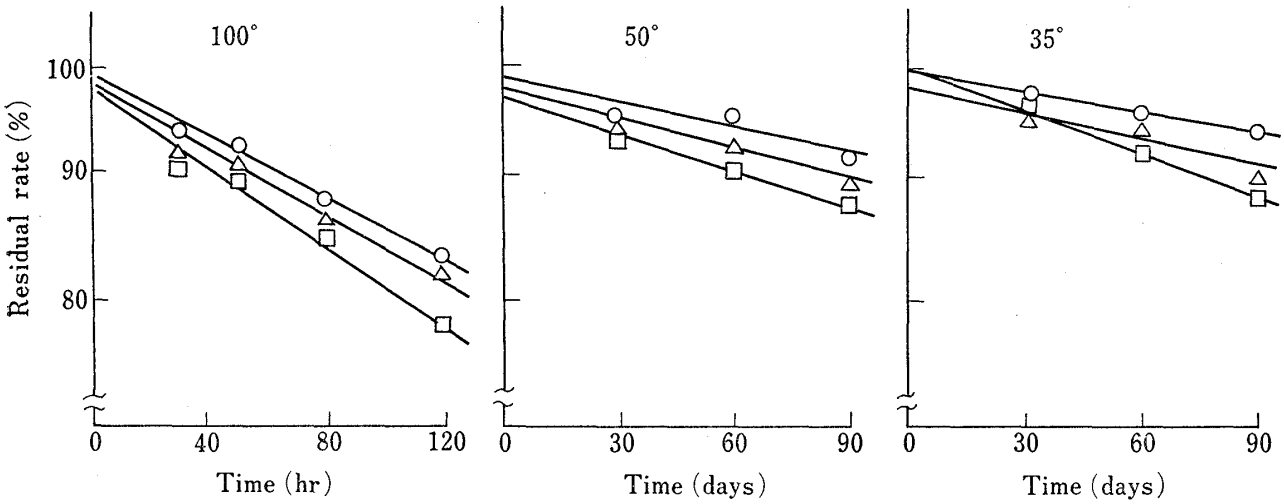


Fig. 2. Residual Rates of Retinoic Acid stored in Ethanolic Solutions at 100°, 50°, and 35°

TABLE I. Rate Constants and Half-lives of Retinoic Acid in Aqueous Ethanolic Solutions stored at 100°, 50°, and 35°

EtOH	at 100°		at 50°		at 35°	
	k (hr^{-1})	τ (hr)	k (hr^{-1})	τ (hr)	k (hr^{-1})	τ (hr)
Absolute	1.46×10^{-3}	4.75×10^{2}	3.50×10^{-5}	1.98×10^{4}	2.58×10^{-5}	2.69×10^{4}
80%	1.61×10^{-3}	4.30×10^{2}	4.86×10^{-5}	1.43×10^{4}	4.42×10^{-5}	1.57×10^{4}
60%	1.94×10^{-3}	3.57×10^{2}	5.73×13^{-5}	1.21×10^{4}	5.64×10^{-5}	1.23×10^{4}

Photochemical Stability of Retinoic Acid——The residual rates of retinoic acid irradiated with xenon ray are shown in Fig. 3. In this condition, all-*trans*-retinoic acid was partly changed into *cis*-isomers which had similar absorbance at 350 nm, so these data showed the residual rates of total retinoic acid. The isomerization ratios were discussed later. In the photolysis condition, retinoic acid is less stable than in the pyrolysis condition. With increasing water content, photodecomposition was also slightly accelerated but exchanging of air of the test solution with N_2 gas resulted in slight stabilization of retinoic acid.

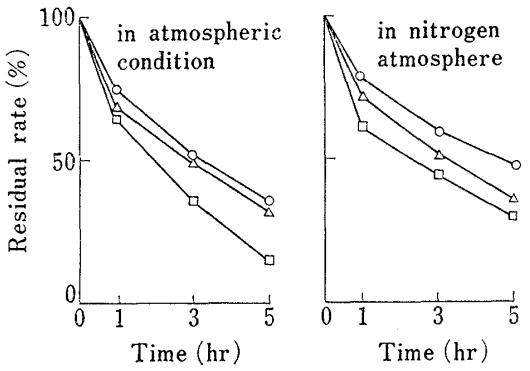


Fig. 3. Residual Rates of Retinoic Acid Solutions irradiated with Xenon Ray

○, absolute EtOH; △, 80% EtOH; □, 60% EtOH.

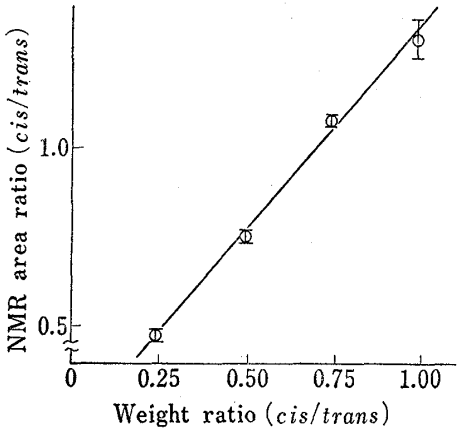


Fig. 4. Calibration Curve for the Ratio of 13-*cis* to All-*trans* Retinoic Acid by NMR

Φ: mean ± S.D., $n=3$.

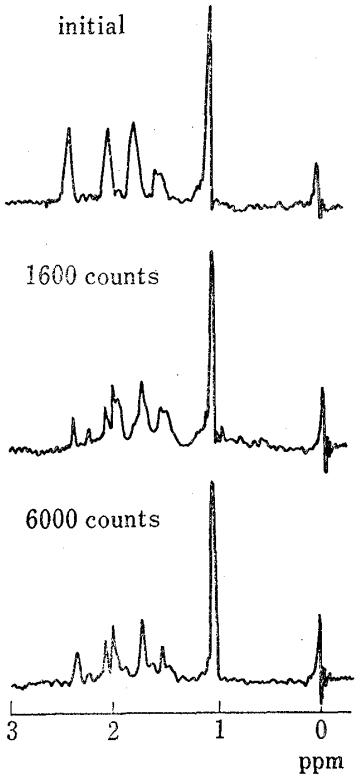


Fig. 5. NMR Spectra of Retinoic Acid Solutions irradiated with Sunlight

Conversion Rate of All-*trans*-Retinoic Acid into *cis*-Isomer Determined with NMR

In order to obtain the calibration curve, various amounts (5, 10, 15, 20 mg) of 13-*cis*-isomer were mixed with 20 mg of all-*trans*-retinoic acid, and NMR spectra of these samples were determined. The plots of the area ratios of 13-methyl signal of 13-*cis*-isomer to all-*trans*-retinoic acid against the weight ratios of them were found to be linear, as shown in Fig. 4. This relation was also recognized in the case of previously reported retinal,[8] and same relation is assumed to be present between 9,13-di-*cis*-isomer to all-*trans*-retinoic acid.

Isomerization in Thermal Condition——NMR spectra of the retinoic acid solution stored at 100° for 100—300 hr were examined. The 13-methyl signal was detected only at 2.30—2.40 ppm, so that all-*trans*-retinoic acid was not changed into the *cis*-isomer in the thermal decomposition in this aqueous ethanolic solution.

Isomerization in Photolysis Condition——NMR spectra of the retinoic acid solution irradiated for 6000 counts are shown in Fig. 5. The signals which resulted from isomerization of 13-*cis*-isomer and 9,13-di-*cis*-isomer are recognized. Other signals are scarcely recognized. The isomerization ratios of 13-*cis*-isomer or 9,13-di-*cis*-isomer to all-*trans*-retinoic acid were investigated for 20000

counts. These data and residual rates of total retinoic acid which were determined by UV absorbance are shown in Fig. 6. When irradiated for only 800 counts, ratio of 13-*cis*-isomer to all-*trans*-retinoic acid already reached 0.87. This isomerization into 13-*cis*-isomer occurred rapidly and the ratio of 13-*cis*-isomer to all-*trans*-retinoic acid increased as the photolysis proceeded. On the other hand, ratio of 9,13-di-*cis*-isomer to all-*trans*- retinoic acid increased gradually for 6000 counts and at that time, this ratio reached 0.49. Thereafter this ratio did not increase with progress of photolysis.

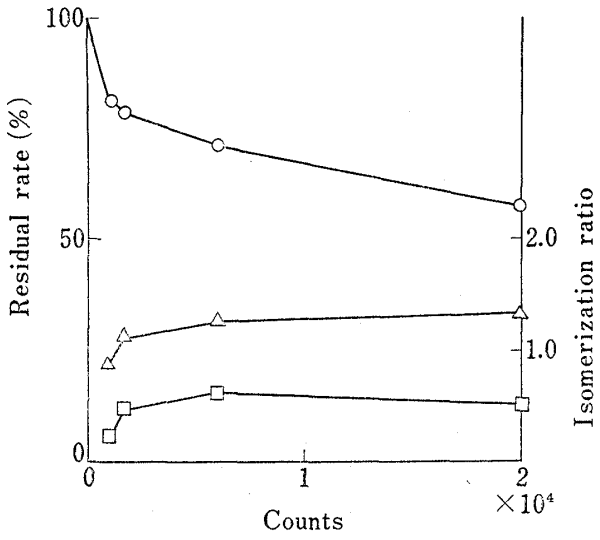


Fig. 6. Residual Rates and Isomerization Ratios of Retinoic Acid Solutions irradiated with Sunlight

○, residual rates; △, 13-*cis*/all-*trans*; □, 9,13-di-*cis*/all-*trans*.

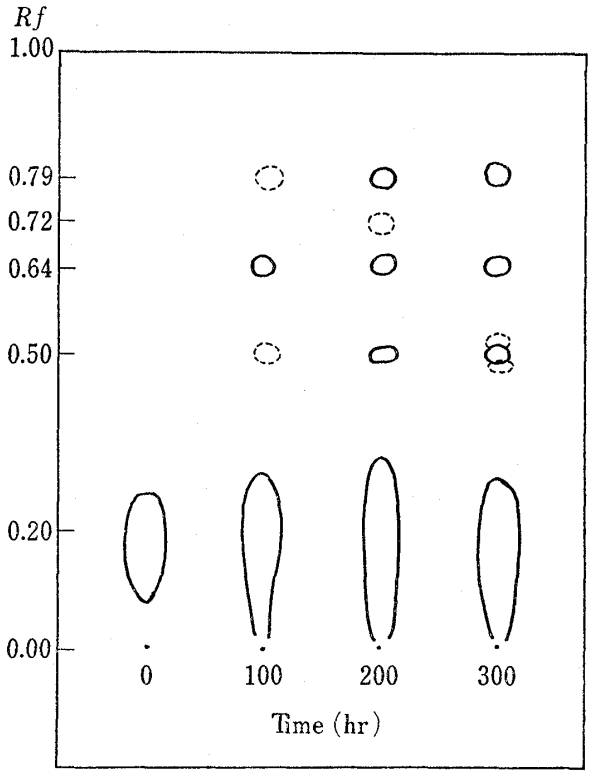


Fig. 7. TLC of Retinoic Acid stored in Absolute Ethanolic Solutions at 100°

Separation and Structural Examination of the Decomposition Products

In the thermal decomposition, the amount of decomposition products was small, so mainly the photodecomposition products were separated, and the structural examination of each substance was carried out. Thermal decomposition products were separated in a small scale and were compared qualitatively with the photodecomposition products.

Thermal Decomposition Products——TLC Results of Decomposition Products: When 100 μl of the stored sample (initial retinoic acid content, 10 mg/100 ml) was applied directly to the TLC plate, the residual retinoic acid was so much and the decomposition products were so small that they were not clearly detected by TLC. Then the concentrated sample (500 mg/100 ml) was prepared and stored at 100° for 300 hr, and 50 μl of this stored sample was taken at time intervals and decomposition products were examined by TLC. These are shown in Fig. 7. Initially, only retinoic acid in this sample was detected at *Rf* 0.20 and other spots were not detected. After 100 hr, the decomposition products were detected at *Rf* 0.79 and 0.64, and a trace at 0.50. After 200 hr, an additional spot was detected at *Rf* 0.72 but, after 300 hr, this spot was detected. Prolongation of the storage period resulted in the tailing on the TLC plate from the application point to *Rf* 0.30 and after 300 hr, a few substances seemed to overlap at *Rf* 0.50.

Pretreatment: In pyrolysis, retinoic acid was not so much decomposed (residual rate, 85% when stored at 100° for 300 hr), therefore unchanged retinoic acid was separated

beforehand. As shown in Chart 1, 50 ml of the test solution stored at 100° for 300 hr was evaporated to 5 ml and 30 ml of cyclohexane was added and the mixture was warmed to 50°, and stirred to effect dissolution of decomposition products. This solution was stored in a refrigerator for 3 hr and the precipitated retinoic acid was filtered. Examination by TLC showed that this precipitate seemed to be only retinoic acid, and other decomposition products were not recognized. The filtrate which contained the decomposition products was evaporated almost to dryness, dissolved in 3 ml of cyclohexane, and applied to the following column chromatography.

Alumina–CPC(1): Alumina adsorbent which contained 5% of H_2O was packed into a column (1.8×26 cm) by the slurry packing technique and the pretreated sample was applied. The chromatography was developed as follows: Each fraction weighed 15 g, and fractions No. 1—3 were eluted with cyclohexane, No. 4—8 with ether–cyclohexane (3: 97), No. 9—14 with ether–cyclohexane (5: 95), No. 15—23 with ether–cyclohexane (10: 90), and thereafter with ether. In fractions No. 2—6, a spot was detected at *Rf* 0.79, in fraction No. 10, a spot at *Rf* 0.64. In other fractions, trace spots were recognized at *Rf* 0.72, 0.59, 0.53, 0.46, and 0.36 but the amount of the these substances was so small that purification and structural examination were carried out only on the substances of *Rf* 0.79 and 0.64. In the pyrolysis condition, a spot at *Rf* 0.74 which was recognized in the photolysis condition was not recognized in any fraction.

Alumina–CPC(2). Purification of *Rf* 0.79 Substance: The substance at *Rf* 0.79 was eluted almost as one spot in fractions No. 2—6 of the above-described alumina-CPC(1). In order to examine the purity of these fractions, this part was collected and rechromatographed over alumina which contained 3% of H_2O packed into a column (1.2×25 cm) by the slurry packing technique. The sample solution (fractions No. 2—6) was concentrated to about 1 ml and applied to this column, which was developed with cyclohexane. After 40 g of cyclohexane was eluted, the fractionation was started. One fraction weighed 3 g. In fractions No. 2—5, a spot was recognized at *Rf* 0.79 which showed UV maximum at 324 nm, and a spot about *Rf* 0.79 was recognized in fractions No. 7—9 but its UV maximum was at 354, 372, and 394 nm. Fractions No. 2—5 were collected, its mass spectrum (MS) was determined, and the parent peak of this substance was recognized at *m/e* 256 (M^+). The amount of this substance was so small that NMR and IR spectra could not be measured but other data of this substance agreed very closely with the substance formed by decarboxylation of retinoic acid in the photolysis condition. The amount of other substance at about *Rf* 0.79 eluted in fractions No. 7—9 was so small that its properties could not be determined.

Alumina–CPC(3). Purification of *Rf* 0.64 Substance: In the above-described alumina–CPC(1), the substance at *Rf* 0.64 was present in fractions No. 7—11. In order to examine the purity of these fractions, this part was rechromatographed over alumina which contained 3% of H_2O packed into a column (1.2×25 cm) by the usual technique. The solution (fractions No. 7—11 of CPC(1)) was evaporated to about 1 ml and applied to this column, one fraction weighed 3 g, and fractions No. 1—6 were eluted with cyclohexane, No. 7—32 with ether-cyclohexane (3: 97) and No. 33—39 with ether-cyclohexane (5: 95). The spot at *Rf* 0.64 was eluted in fractions No. 30—38, fractions No. 31—36 were collected, and its UV and MS were determined. UV maximum appeared at 329 nm, and the parent peak in its MS was recognized at *m/e* 382 (M^+). The amount of this substance at *Rf* 0.64 was so small that its NMR spectrum could not be determined, but other properties of this substance, *Rf* value, UV spectrum, and MS agreed with the properties of the substance at *Rf* 0.64 obtained in the photolysis condition. This substance seemed to be formed by the addition of a decomposition product to retinoic acid.

Photodecomposition Products——As shown in Chart 2, separation and purification of the photodecomposition products were carried out.

TLC Results of Photodecomposition Products: When 100 μl of the photodecomposed sample (initial retinoic acid content, 10 mg/100 ml) was applied to the TLC plate directly, some spots close to *Rf* 0.20, which seemed to be isomers of retinoic acid and a spot at *Rf* 0.79 were recognized. In this sample, the amount of the decomposition products was so small that they were not clearly detected. Then the concentrated solution (500 mg/100 ml) was decomposed with sunlight until residual rate reached about 50% and 50 μl of this sample was applied to the TLC plate. In this condition, in addition to the above-mentioned spots, several spots were appeared at *Rf* 0.74, 0.64, 0.37, and 0.25. The spot at *Rf* 0.74 was not recognized in the thermal decomposition products. Tailing was recognized from application point to *Rf* 0.20 and several spots seemed to overlap in this place. These parts could not be separated.

DEAE-cellulose–CPC(1): By DEAE-cellulose–CPC(1), retinoic acid and the decomposition products which contained a carboxyl group, were separated from the decarboxylated substances. The test solution (130 ml) was applied to the DEAE-cellulose column (2.5×30 cm), by which retinoic acid and decomposition products which contain carboxyl group were adsorbed and other decomposition products were eluted. After almost all the decarboxylated products were eluted, the decomposition products which contain the carboxyl group were eluted with 100 ml of CH_3COOH–EtOH (10: 90). These operations were repeated three times and totally 390 ml of the test sample was treated.

DEAE-cellulose–CPC(2): In order to separate the substances which contain a carboxyl group, 100 ml of ethanolic fraction which was eluted by DEAE-cellulose–CPC(1) was rechromatographed over DEAE-cellulose–CPC. This procedure for separation was the same as that of DEAE-cellulose–CPC(1). A total of 300 ml of the eluent, 100 ml of DEAE-cellulose–CPC(1) fractions collected three times, was submitted to this rechromatography. Without evaporation, 150 ml of this solution was applied to DEAE-cellulose column (1.8×60 cm) and fractionated with 99.5% EtOH. One fraction weighed 15 g. In fractions No. 7—20, the decarboxylated substances were eluted, and in these fractions, retinoic acid and the decomposition products which have a carboxyl group were not recognized by TLC. Thereafter, the adsorbed substances were eluted with 100 ml of CH_3COOH–EtOH (10: 90) and these fractions which contained retinoic acid were collected and was added to later fractions of DEAE-cellulose–CPC(1), which was used for preparative TLC(1).

Silica gel–CPC(1). Separation and Purification of Decarboxylated Products: Fractions No. 7—20 on DEAE-cellulose–CPC(2) were evaporated to dryness, dissolved in 20 ml of cyclohexane, and this solution was applied to the silica gel containing 3% of H_2O, was packed

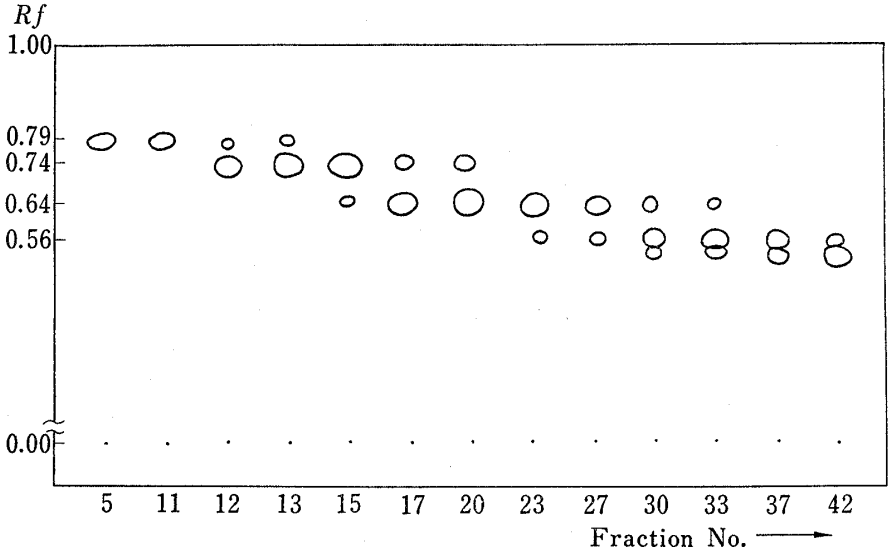
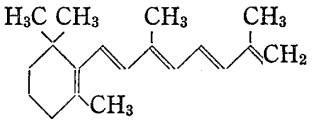
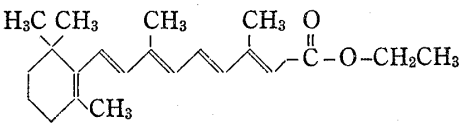


Fig. 8. TLC of Photodecomposition Products from Retinoic Acid Solutions separated by Silica gel-CPC(1)

into a column (1.2×25 cm) by the usual method. The fractionation was made with ether–cyclohexane (3: 97). One fraction weighed each 10 g, and the elution was continued until 85 fractions were obtained. The degree of purification of each fraction was examined by TLC and these results are shown in Fig. 8. A substance at *Rf* 0.79 was eluted in fractions No. 5—11 as a single spot, and its UV, NMR spectra, and MS were examined. These results are shown in Table II. As shown in Fig. 8, substances at *Rf* 0.74 and 0.64 were recognized in these fractions and these substances were purified as shown in following silica gel–CPC(2) and (3) procedures.

Table II. Physical Properties of Photodecomposition Products from Retinoic Acid Solution

Decomposition product	*Rf* value	UV λ_{max}^{EtOH} (nm)	MS (M^+)	IR (cm^{-1})	NMR (ppm)	Structure of decomposition product
I	0.79	324	256	—	1.01 1.22 1.58	
II	0.74	328	328 299 282 255	1700 1200 1150	1.10 1.50 1.69 1.83 1.93 3.40 5.10	
III	0.64	329	382 297 255	—	1.00 1.10 1.22 1.52 2.08	—
IV	0.37	281	300 285 267	—	0.95 1.03 1.24	Isomer
V	0.25	261	300 285 255	—	1.23	Isomer

Silica gel–CPC(2). Purification of *Rf* 0.74 Substance: In the above-described silica gel–CPC(1), a substance at *Rf* 0.74 was present almost wholly in fractions No. 12—15, which were collected and this part was rechromatographed over silica gel containing 2% of H_2O packed into a column (1.5×40 cm). The sample solution (fractions No. 12—15 of silica gel–CPC(1)) was evaporated, dissolved in about 2 ml cyclohexane, and applied to this column, fractionated with ether–cyclohexane (3: 97). One fraction weighed 5 g. In fractions No. 17—21, the substance at *Rf* 0.74 was eluted as one spot, and its IR and NMR spectra were determined. These results are shown in Table II.

Silica gel–CPC(3). Purification of *Rf* 0.64 Substance: In the above-described silica gel–CPC(1), the substance at *Rf* 0.64 was present in fractions No. 17—27 which were collected, evaporated, and dissolved in about 2 ml of cyclohexane. This solution was applied to a column (1.5×45 cm) packed with the silica gel, and fractionated with ether–cyclohexane (2: 98). One fraction weighed 10 g. The fractionation was continued until 33 fractions were obtained and, thereafter, fractionation was carried out with ether–cyclohexane (3: 97). In fractions No. 22—38, a substance at *Rf* 0.64 was eluted as one spot and its UV, IR, NMR spectra, and MS were determined. These results are shown in Table II.

Preparative TLC(1): The fraction eluted with CH_3COOH–EtOH (10: 90) contained retinoic acid and decomposition products which have a carboxyl group. These substances were strongly adsorbed on the column and their *Rf* values in TLC were low. In these fractions,

two decomposition products were mainly recognized at *Rf* 0.37 and 0.25. Attempts to separate these substances by CPC from these fractions were unsuccessful, because of the presence of much retinoic acid in these fractions. When the solution was applied to TLC plate in a large amount as a streak and the plate was developed, two substances were separated. These procedures are shown as follows. The decomposition products with a carboxyl group were collected from DEAE-cellulose–CPC(1) and (2), evaporated to dryness, and dissolved in 4 ml of EtOH. Fifty μl this solution was applied to the TLC plate as a streak, the plate was developed with ether–cyclohexane (50: 50), and the part of *Rf* 0.25—0.40 was collected from these developed plates. These procedures were carried out with 80 sheets of these TLC plates. These slurries were collected and extracted with EtOH. This extracted solution did not contain retinoic acid, and substances giving spots at *Rf* 0.25 and 0.37 were concentrated.

Preparative TLC(2): The above extracted solution was evaporated to about 1 ml and this sample was applied to TLC plate as a streak, and developed with ether–cyclohexane (50: 50). The parts corresponding to *Rf* 0.25 and 0.37 were collected separately, and extracted with EtOH. These procedures were carried out with 20 sheets of this TLC plate. The substance corresponding to *Rf* 0.25 was recognized as almost a single spot, but the substance corresponding to *Rf* 0.37 was not clearly recognized as one spot and the slight tailing was recognized. Properties of these substances are shown in Table II.

Consideration of Decomposition Products——i) *Rf* 0.79 Substance: As this substance was not so strongly adsorbed on TLC, terminal part of isoprenoid chain of this substance seems to be $=CH_2$, same as anhydrovitamin A. UV spectrum of this substance was different from that of anhydrovitamin A and its absorption maximum was at 324 nm. The parent peak in its MS was recognized at *m/e* 256 (M^+), which agreed with the value of decarboxylated retinoic acid. From these results, this substance at *Rf* 0.79 seems to have been formed by decarboxylation of retinoic acid. This structure is the same as loss of CH_2 from the terminal part of anhydrovitamin A as shown in Table II. The signals in its NMR spectrum did not conflict with this structure. This substance was not recognized in other vitamin A analogs decomposition and was newly recognized in thermal decomposition and photodecomposition of retinoic acid.

ii) *Rf* 0.74 Substance: As shown in Table II, the substance at *Rf* 0.74 seems to be ethyl retinoate. The parent peak in its MS was at *m/e* 328 (M^+) and its fragment peaks agreed with that of methyl retinoate.[10] In its NMR spectrum, all methyl signals of retinoic acid were recognized and its methylene (3.40 ppm) and its methyl (1.17 ppm) signals were recognized additionally for the introduction of an ethyl group. Its IR spectrum exhibited absorption bands due to the ester bond at 1700, 1200, and 1150 cm^{-1}. The substance of *Rf* 0.74 was identified as ethyl retinoate prepared by reported method.[11] This substance was only recognized in photodecomposition but was not recognized in thermal decomposition.

iii) *Rf* 0.64 Substance: This substance was easily separated as a precipitate but its amount was so small that only its UV, NMR spectra, and MS were examined. UV spectrum of this substance showed absorption maximum at 329 nm, and the parent peak in its MS was recognized at *m/e* 382 (M^+). In its NMR spectrum, all methyl signals of retinoic acid were recognized so that substance seems to be formed by the addition of a decomposition product to retinoic acid. This substance was recognized commonly in thermal decomposition and photodecomposition of retinoic acid but not recognized in thermal decomposition of retinal.

iv) *Rf* 0.37 Substance: The UV spectrum of this substance exhibited absorption maximum at 281 nm, and this substance seems to be the source substance which increases shorter

10) R.L. Lin, G.R. Waller, E.D. Mitchell, K.S. Yang, and E.C. Nelson, *Anal. Biochem.*, **35**, 435 (1970); B. Morgan and J.N. Thompson, *Biochem. J.*, **101**, 835 (1966).
11) S. Saijo and K. Oizumi, Japan Patent 36-17175 (1961).

wavelength of UV spectra in photodecomposition. The MS of this substance resembled that of retinoic acid and this substance seems to be an isomer of retinoic acid. An isomer of retinoic acid which have absorption maximum at 281 nm has not been reported[12] and isomerized part of this substance was not elucidated.

v) *Rf* 0.25 Substance: UV spectrum of this substance exhibited absorption maximum at 261 nm, so this substance also seems to be source substance which increases shorter wavelength of UV spectra in photodecomposition. The MS of this substance resembled that of retinoic acid and this substance seems to be an isomer of retinoic acid. The isomerized part of this substance also was not elucidated. These two substances differed from 13-*cis*-isomer (λ_{max}^{EtOH}: 354 nm, *Rf* 0.20) and 9,13-di-*cis*-isomer (λ_{max}^{EtOH}: 346 nm, *Rf* 0.20) which were examined previously in Fig. 6 with NMR.

In conclusion, in pyrolysis, retinoic acid was more stable than other vitamin A analogs and isomerization did not arise, but in photolysis, retinoic acid was decomposed and isomerization arose rapidly.

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12) C. von Planta, U. Shwieter, L. Chopard-did-Jean, R. Ruegg, M. Kofler, and O. Isler, *Helv. Chim. Acta*, **45**, 548 (1962).