Tetrahedron Vol. 45, No. 24, pp. 7679 to 7694, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00

CHARACTERIZATION OF AUTOXIDATION PRODUCTS OF RETINOIC ACID

A. R. Oyler,* M. G. Motto, R. E. Naldi, K. L. Facchine,
P. F. Hamburg, D. J. Burinsky, R. Dunphy, and M. L. Cotter

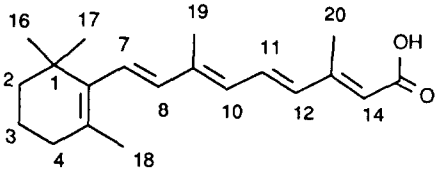
R. W. Johnson Pharmaceutical Research Institute
at Ortho Pharmaceutical Corporation
Raritan, New Jersey 08869-0602

(*Received in USA 5 July* 1989)

Abstract - Retinoic acid underwent autoxidation in 90% ethanol at 25-85.5 °C to give epoxides, dioxetanes, an endoperoxide, and double-bond cleavage products. The majority of these products appear to have resulted from the initial direct oxidation of the olefinic carbons rather than from the expected allylic (α) oxidation process.

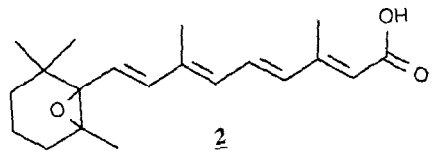
INTRODUCTION

Retinoic acid (**1**, RA) is a member of an important class of biomolecules and has various therapeutic uses including the treatment of acne.[1,2] Retinoic acid and related retinoids (*e.g.* retinal, retinol) are known to undergo autoxidation,[3] but the resulting product mixtures have not been well characterized. However, the 5,6-epoxide is known to be a product of autoxidation of compounds such as retinoic acid[3g,k] and β-ionone.[3l] In spite of the lack of reports on product distributions, allylic (α) oxidation[4] is usually assumed to be involved in retinoid autoxidation.[3c-f]

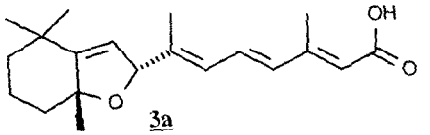


1

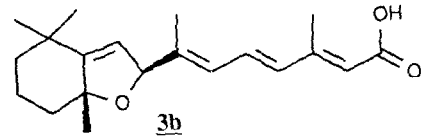
We recently characterized the more predominant products (**2**-**12**) that were formed during the autoxidation of retinoic acid in 90% ethanol at 25-85.5 °C. These products included epoxides (**2**, **8a**, **8b**), dioxetanes (**7a**, **7b**), an endoperoxide (**6**), and double-bond cleavage products (**9**-**12**). At least some of these products, such as the peroxide, dioxetane, and double-bond cleavage compounds, appear to have resulted from initial direct oxidation of the olefinic carbons rather than from the expected allylic oxidation process. The formation of these unusual products with triplet oxygen has been reported only for a few highly strained[5a-c]



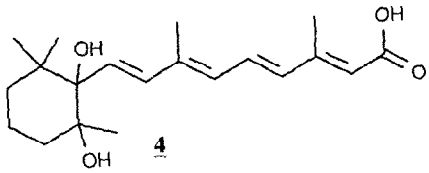
2



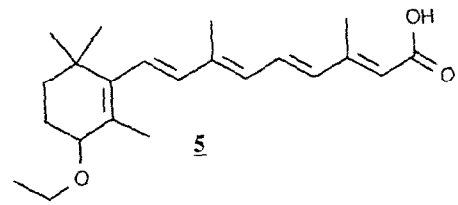
3a



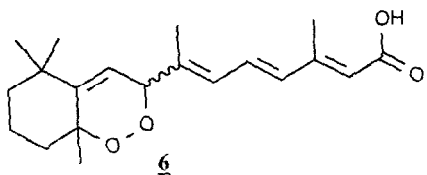
3b



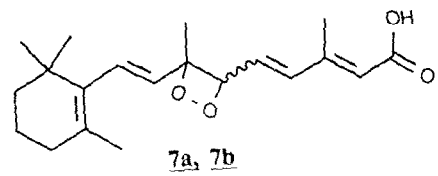
4



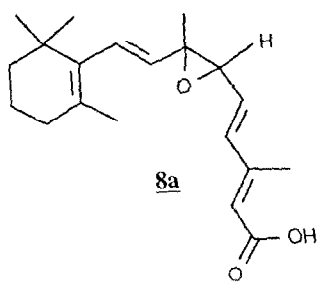
5



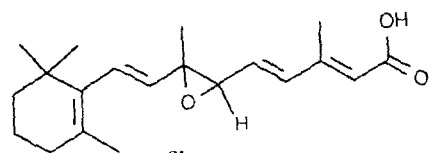
6



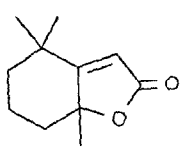
7a, 7b



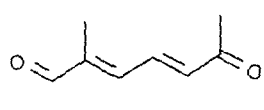
8a



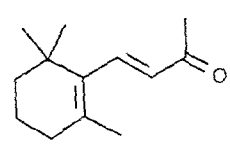
8b



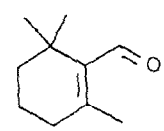
9



10



11



12

or electron rich[5d-f] substrates (normally with high temperatures) and for reactions of more general substrates that were conducted with high temperatures and high oxygen pressures,[4e] or in the presence of radical cation[5g-i] or Lewis acid[5j] catalysts. In contrast to these literature reports, the non-allylic autoxidation products of retinoic acid were formed even under ambient conditions (room temperature/air).

RESULTS AND DISCUSSION

Reactions were conducted with 3.3 x 10^{-3} M retinoic acid in 90:10 ethanol:water under one atmosphere of air at 25 °C or under one atmosphere of oxygen at 70.7-85.5 °C (Table 1). These reactions were monitored by gradient reversed-phase HPLC (Figures 1 and 2). The autoxidation reactions of retinoic acid showed increasing induction periods with decreasing temperature (Table 1). Similarly, an induction period was also reported for the autoxidation of methyl retinoate.[3d] The retinoic acid reactions displayed pseudo-first order kinetics during the propagation phase since plots of ln $\{([RA]_{t=0})/([RA]_{t=t})\}$ versus time were linear. The corresponding rate constants, k_{obs}, are shown in Table 1. An Arrhenius plot of ln (k_{obs}) versus 1/T for the retinoic acid reactions which were conducted under one atmosphere of oxygen in 90% ethanol was reasonably linear and corresponded to an activation energy of 25 ± 9 kcal/mole. This value was comparable to activation energies that were reported for other autoxidation reactions.[5e,6]

Table 1

Rate Data for Autoxidation of Retinoic Acid in 90% Ethanol

Oxygen Atm	Temp. °C	Induction Period[a] Days	k_{obs}[b], s^{-1} x 10^6
0.2	25	30	0.102 ± 0.002
1	70.7	2.0	0.22 ± 0.01
1	75.7	2.6	0.36 ± 0.02
1	81.2	0.6	1.11 ± 0.04
1	85.5	0.6	0.77 ± 0.02

[a]Estimated from a plot of $\ln\{([RA]_{t=0})/([RA]_{t=t})\}$ versus time during the propagation phase. [b]From a linear regression analysis of plots of $\ln\{([RA]_{t=0})/([RA]_{t=t})\}$ versus time.

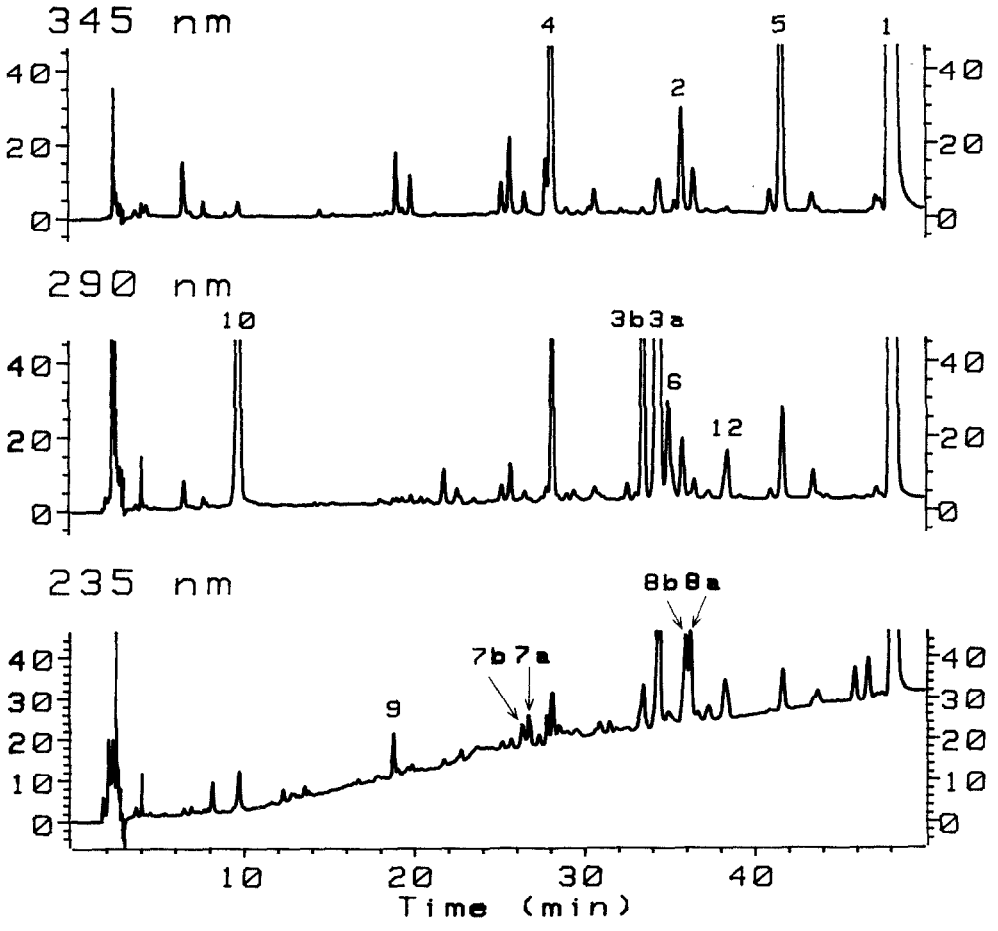


Figure 1. HPLC of autoxidation product mixture that was obtained in 90% ethanol at 25 °C under air atmosphere. The amount of unreacted retinoic acid was 61 mole %.

The predominant products (**2**-**10**, Table 2, Figure 2) of autoxidation in 90% ethanol were isolated by preparative HPLC and characterized by UV, MS, and ^{1}H NMR spectroscopy. The structural assignments of compounds **2**, **3a**, and **3b** were also confirmed by comparison of

spectral and chromatographic data for the isolated materials with data for authentic synthetic samples. The presence of β-ionone (**11**) was determined by comparison of HPLC-UV (Figure 1), GC-MS, and HPLC-MS (Figure 3) data which was obtained for autoxidation product mixtures with the corresponding data for an authentic sample. The presence of β-cyclocitral (**12**) was determined by GC-MS. The presence of the isolated compounds in the original autoxidation product mixtures was verified by HPLC-UV and HPLC-MS (Figure 3).

Table 2

Selected Product Distribution Data for Autoxidation Reactions

	Mole % of Total Product Material[a]		
Compound	25°C/90%EtOH[b]	85.5°C/90%EtOH[c]	82°C/Benzene[d]
2	1.3	trace	23
3a	21	8.4	
3b	5.1	3.4	
4	5.0	0.7	
5	3.4	6.9	
6	1.5	1.6	
7a	0.8	0.5	
7b	0.9	0.5	
8a	3.7	0.8	
8b	3.5	0.5	
9	4.7	13	
10	10	24	7.9
11	3.3	1.9	
12	0.6	0.5	
Sum	65	63	31

[a]All values were determined by HPLC except the value for β-cyclocitral which was determined by GC. % of total product material = (Mole % individual product x 100)/(100 - Mole % retinoic acid). [b]One atmosphere air, 90 days, 61 mole % of retinoic acid remained. [c]One atmosphere of oxygen, 8 days, 64 mole % of retinoic acid remained. [d]One atmosphere of oxygen, 2.75 hours, 60 mole % of retinoic acid remained.

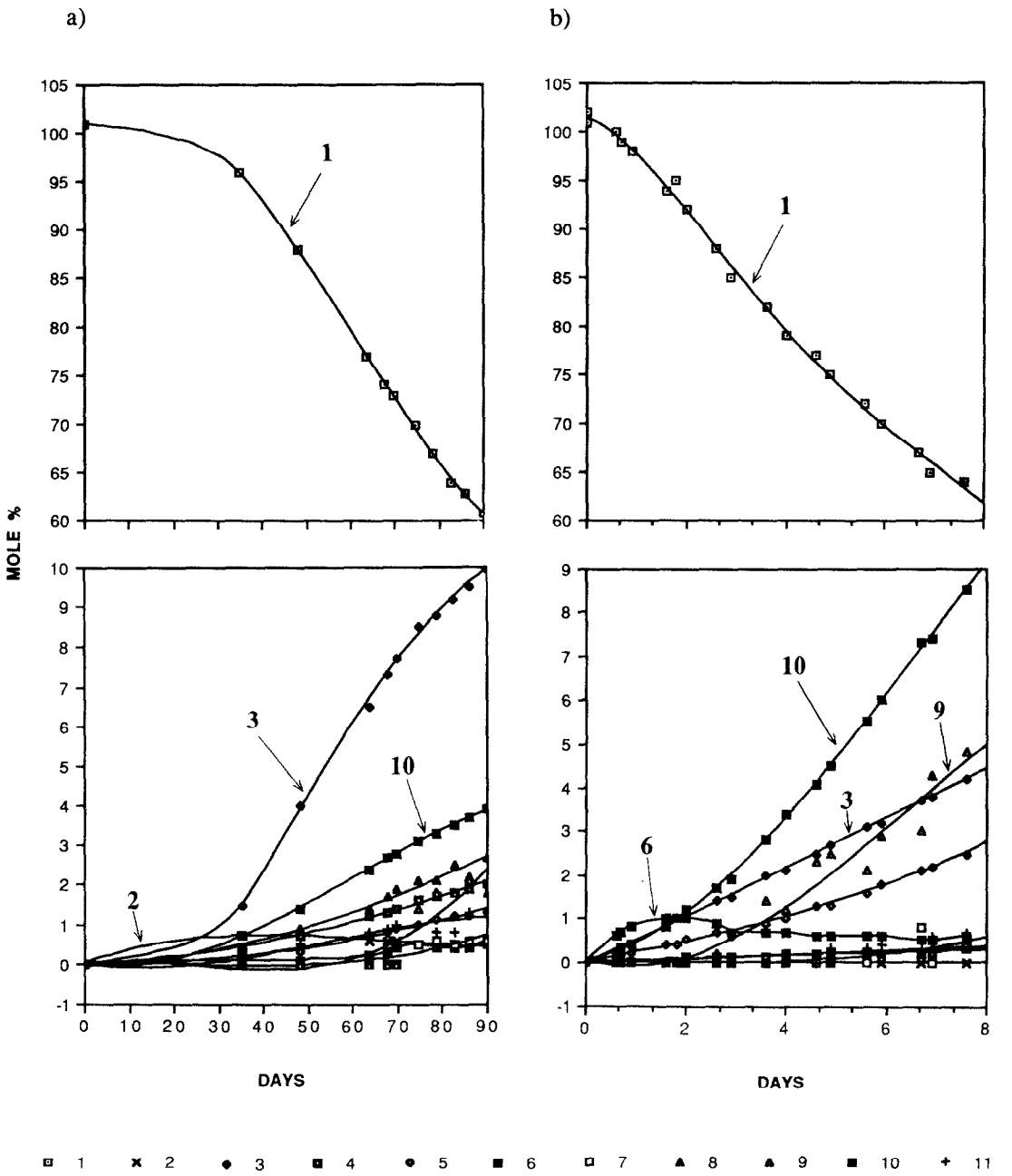


Figure 2. Variation of autoxidation products with respect to time for reactions at 25 °C (**a**) and 85.5 °C (**b**).

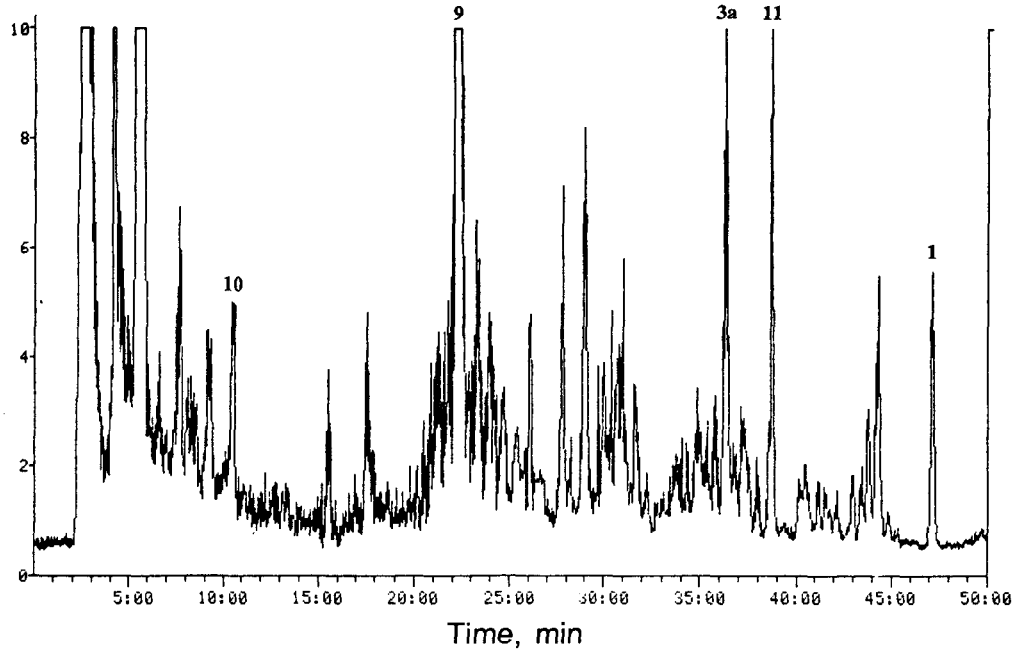


Figure 3. Reconstructed ion chromatogram (HPLC-MS) of autoxidation product mixture that was obtained in 90% ethanol at 25 °C under air atmosphere. The amount of unreacted retinoic acid was 11%. The peak areas do not necessarily reflect the relative amounts of products since the ionization efficiencies are structure dependent.

Authentic samples of **3a** and **3b** were synthesized for comparison with the corresponding isolated autoxidation products and for stereochemical analysis. These furanoid compounds were previously prepared from the epoxide **2** by an acid-catalyzed rearrangement reaction, but they were not separated.[7a] We prepared a mixture of **3a** and **3b** by the same route and separated the isomers by preparative HPLC. The ^{1}H NMR spectra of **3a** and **3b** were very similar to the spectra which were reported[7b] for the two corresponding derivatives of retinal. Assignment of the stereochemistry about the 5 and 8 positions in these types of furanoid compounds has been based on the chemical shifts and coupling constants of the H_7 and H_8 protons.[7b] We confirmed this approach to the stereochemical assignments by X-ray analysis of the synthesized sample of **3a** (Figure 4). The X-ray data showed the C_5 methyl and the trienoic acid groups of **3a** to have a *trans* relationship as predicted[7b] by the ^{1}H NMR data.

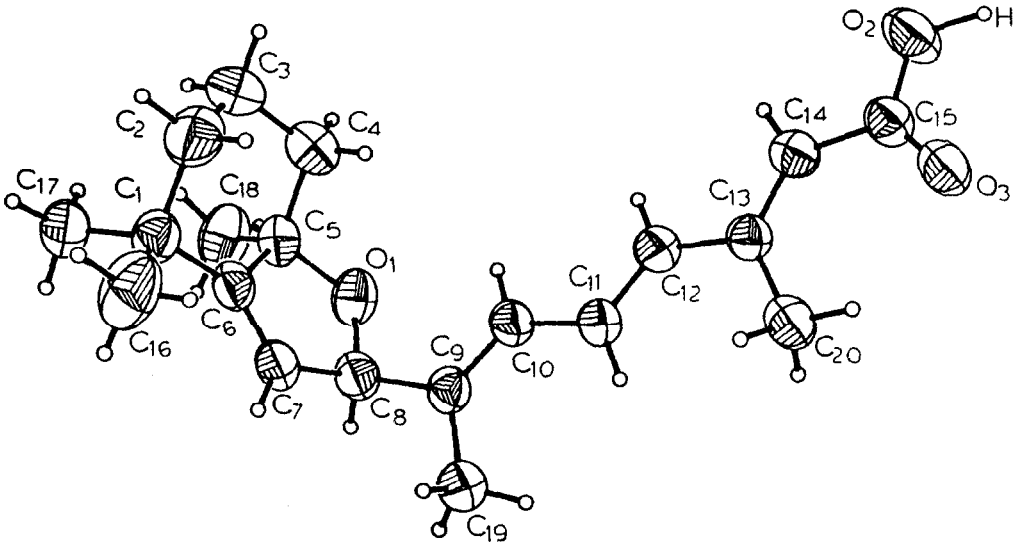


Figure 4. X-ray structure of furanoid 3a.

The 5,6-diol **4** is a known compound but the stereochemistry and spectral data have not been reported.[8a] Spectral data, however, have been reported for the 5,6-*cis* and 5,6-*trans* isomers of the methyl ester of **4**.[8b] The ^{1}H NMR data for the isolated autoxidation product (**4**) was very similar to the data reported[8b] for these two esters. Unfortunately, the similarity of the ^{1}H NMR spectra of the two isomeric esters[8b] precluded the assignment of the stereochemistry of the isolated diol. However, the formation of the *trans* diol by hydrolysis of the 5,6-epoxide **2** is expected.[8c]

4-Ethoxyretinoic acid **5** has not been reported previously, but the methyl ester of **5** is known.[9] However, no spectral data have been reported for comparison with the corresponding isolated autoxidation product.

The endoperoxide **6** has also not been reported, while the corresponding ethyl ester has been prepared with singlet oxygen.[10] The ^{1}H NMR spectrum[10] of this ester was nearly identical to the spectrum of the sample of **6** which was isolated from the autoxidation product mixture. The stereochemistry about the 5 and 8 positions in these samples was not determined. Additional support for the structural assignment of the isolated autoxidation product as the endoperoxide **6** was obtained by observation of the thermal degradation of this compound to dihydroactinidiolide (**9**) in a GC inlet (225 °C). Dihydroactinidiolide (**9**) was also observed as a degradation product of the corresponding endoperoxide derivative of retinal.[11]

The isomeric dioxetanes **7a** and **7b** have not been reported previously. The stereochemistry about the 9 and 10 positions in the isolated samples of **7a** and **7b** was not determined. In addition to spectral data, support for the structural assignments of the dioxetanes **7a** and **7b** was obtained by observation of the thermal degradation of the isolated samples of these compounds to the expected[12] β-ionone (**11**) in a GC inlet (225°C).

The *cis* and *trans* epoxides **8a** and **8b** also have not been reported previously. Nuclear Overhauser effect (nOe) experiments were conducted on the two isolated isomers to determine the stereochemistry about the 9,10-epoxide groups. Irradiation of the C_9 methyl in both cases gave an enhancement for the H_7 and H_8 protons. In compound **8a**, however, an additional enhancement was observed for the H_{10} proton upon irradiation of the C_{19} methyl protons. This nOe effect suggested a *cis* orientation for the H_{10} proton and the C_{19} methyl group as in **8a**.

The isolated sample of dihydroactinidiolide (**9**) showed the same UV and ^1H NMR data as the known compound.[11]

The ketoaldehyde **10** is unknown although an isomer ((2*E*,4*Z*)-2-methyl-6-oxo-2,4-heptadienal) has been reported.[13]

The products of autoxidation in 90% ethanol were identical over the temperature range of 25-85.5 °C, although the relative amounts of individual products differed somewhat (Table 2, Figure 2). These differences were likely related to temperature-dependent variations in the rates of decomposition of initially formed products. The 5,6-epoxide **2**, the 5,8-endoperoxide **6**, the 9,10-dioxetanes **7a** and **7b**, and the 9,10-epoxides **8a** and **8b** were products that reached steady state concentrations (see Figure 2) since they subsequently decomposed to secondary products. In a separate experiment, the 5,6-epoxide **2** was shown to undergo rearrangement in 90% ethanol under argon to give the furanoids **3a** and **3b**[7], hydrolysis to give the 5,6-diol **4**[8b], and conversion to 4-ethoxyretinoic acid (**5**). The furanoid products **3a** and **3b** may have been further degraded to dihydroactinidiolide (**9**) in the autoxidation reaction medium.[14] The endoperoxide **6** probably decomposed to dihydroactinidiolide[11] (**9**) and other compounds.[15] The dioxetanes **7a** and **7b** most likely decomposed to carbonyl compounds[12] (e.g. **11**). The presence of the ketoaldehyde **10** and β-cyclocitral (**12**) suggested that dioxetane formation also occurred at the 7,8 and 13,14-positions.[12]

The identified products amounted to approximately 64 mole % of the available retinoic acid that was degraded in 90% ethanol (Table 2). The remaining product material was represented by the numerous small peaks that were present in the HPLC-UV and HPLC-MS

chromatograms (Figures 1, 3). Reversed-phase TLC of the autoxidation product mixture (90% ethanol) with the same mobile phase as HPLC (final conditions) showed that the product material eluted faster than retinoic acid. Therefore all of the product material that was obtained in 90% ethanol was assumed to elute from the HPLC column (Figure 1).

The 5,6-epoxide **2** was one of the major primary products in the autoxidation reactions.[3g,k] In 90% ethanol, this compound and its secondary decomposition products (**3**-**5**) amounted to approximately 35 and 19 mole % of the reacted retinoic acid at 25 and 85.5 °C, respectively (Table 2). The epoxide **2** was a major constituent (23 mole % of reacted retinoic acid) of the product mixture that was obtained in benzene at 82 °C (Table 2), since secondary decomposition reactions of **2** were minimized in this aprotic solvent. The ketoaldehyde **10** was also a significant constituent of the product mixtures that were obtained in 90% ethanol and benzene (Table 2).

The formation of the endoperoxide **6**, the dioxetanes **7a** and **7b**, and the double bond cleavage products **10**-**12** was indicative of reaction processes that differed from the usual allylic autoxidation process and resulted in the direct oxidation of the olefinic carbons.[4e,5] These non-allylic autoxidation processes may involve charge transfer complexes[5c-f] and radical cation chain mechanisms[4e,5g,i] in comparison to allylic oxidation which involves neutral radical chain mechanisms.[4] The electron rich nature of retinoic acid seemed to favor the formation of the non-allylic products (*i.e.* **6**, **7**, **10**-**12**) without the need for catalysts,[5g-j] elevated temperatures,[5a-e] or elevated oxygen pressures.[4e]

The epoxide products (**2**, **8a**, **8b**) may have resulted from an allylic oxidation process,[4] from alternate autoxidation processes,[4e,5f] or from a combination of these processes. However, if the epoxides did result from allylic oxidation, then the formation of other allylic oxidation products (*e.g.* allylic alcohols) might have been expected.[4c] The lack of major products with oxidized allylic carbons (other than **5**) suggested that the epoxide products may not have resulted solely from an allylic oxidation process.

CONCLUSIONS

The autoxidation of retinoic acid was previously assumed to proceed by allylic oxidation with neutral free radical chain mechanisms.[3e,f] The present investigation has identified some products (*e. g.* **6**, **7**, **10**-**12**) that probably result from alternative processes which may involve radical cation chain mechanisms. The epoxide products (**2**, **7**) and the remaining unidentified products may have resulted from some combination of allylic and non-allylic oxidation

processes. The operation of multiple types of autoxidation processes has been noted for other electron rich molecules such as azulenes.[5d]

EXPERIMENTAL DETAILS

Retinoic acid was obtained from Eastman Kodak. Water was obtained from a Millipore water purification system and ethanol was from Pharmaco. The solvents used for HPLC were from Burdick and Jackson. β-Ionone was obtained from Aldrich.

Melting points were recorded on a Thomas Hoover melting point apparatus in open capillaries and are uncorrected.

^{1}H NMR spectra were obtained on a Varian XL-400 (400 MHz) spectrometer in $CDCl_3$ or CD_3COCD_3 with tetramethylsilane as internal reference. ^{13}C NMR were obtained on a GE QE-300 spectrometer at 75 MHz. Mass spectra were obtained on a Finnigan MAT 8230 mass spectrometer by desorption electron ionization (70 eV, DEI) or desorption chemical ionization (DCI). DCI spectra were obtained with isobutane as reagent gas. High resolution mass spectra were also obtained on a Finnigan MAT 8230 with perfluorokerosine as reference. UV spectra, unless stated otherwise, were recorded on a Hewlett-Packard 1040A diode array HPLC detector (HPLC-UV) as the compounds eluted from the HPLC column (retinoic acid gave a λ_{max} of 339 nm). UV spectra were also recorded or on a Hewlett-Packard 8450A UV spectrometer in methanol or ethanol (retinoic acid gave a λ_{max} of 354 nm in ethanol).

Analytical HPLC (HPLC-UV) was performed on a Perkin-Elmer Series 4 instrument with a Hewlett-Packard 1040A diode array detector which was set to monitor 345, 290, and 235 nm. The detector contained a multi-channel DPU board for peak integration. Complete UV spectra were collected at the peak apices. Injections (20 μL) were made with a Rheodyne 7126 injector valve. A Rainin Dynamax 5 μm C-18 column (4.6 x 250 mm) was used. The method was a binary gradient from 90% A/10% B to 20% A/80% B over 60 min (convex curve 0.2) with a flow rate of 1.2 mL/min and a column temperature of 30 °C. Mobile phase A was 0.1 M ammonium acetate in water and mobile phase B was 0.0625 M ammonium acetate in 5:3 (v/v) methanol/tetrahydrofuran. Calibration curves were prepared for retinoic acid and compounds **2**, **3a**, and **3b**. Response factors were determined for the remaining products.

HPLC-MS data was obtained with a Finnigan TSQ 70 triple stage quadrupole mass spectrometer that was equipped with a Finnigan thermospray ionization interface. The instrument was scanned from 130-143, 147-160, 164-299, and 303-400 mass units in a total scan time of 1 s (windows allowed exclusion of major mobile phase reagent ions). The auxiliary ionization (corona discharge) was employed and the ion source block was set to 270 °C. The thermospray vaporizer temperature was varied from 110 to 95 °C (linear) while the HPLC solvent gradient was varied from 90% A/10% B to 90% A/10% B over 50 min (linear). The flow rate was 1.2 mL/min with the same solvents and column temperature as above.

TLC was accomplished with Whatman KC 18F 200 μm 20 x 20 cm reversed-phase plates and mobile phase B (see above).

GC was performed on a Hewlett-Packard 5890A instrument that was equipped with a Chrompack CP-Sil-5 CB WCOT fused silica capillary column (0.32 mm x 25 m) and a flame ionization detector. The inlet temperature was 225 °C and the detector temperature was 250 °C. A temperature program was used with an initial hold for one min at 70 °C, an increase from 70 to 240 °C at 10 °C/min, and a final hold at 240 °C for 12 min.

GC-MS data was obtained with a Finnigan-MAT 8230 mass spectrometer and a Varian 3400 GC with a Restek dimethyl polysiloxane column (0.32 mm x 30 m). The injector and

interface temperatures were 250 °C. The temperature was programmed from 70 to 270 °C at 10 °C/min.

All laboratory work was conducted under gold fluorescent light (GE F40GO).

Preparation of 5,6-Epoxy-5,6-dihydroretinoic Acid (2). This compound was prepared according to a modified literature[7a] procedure with retinoic acid instead of the methyl ester and *m*-chloroperoxybenzoic acid instead of monoperphthalic acid. **2**: mp 153-155 °C (lit.[7a] mp 153 °C); ^{1}H NMR δ (CD_3COCD_3) 0.910 (3 H, s,C_{16}-$CH_3^{\dagger}$), 1.04-1.12 (1 H, m, $H_2^{\ddagger}$), 1.114 (3 H, s, C_{17}-$CH_3^{\dagger}$), 1.134 (3 H, s, C_{18}-$CH_3^{\dagger}$), 1.38-1.47 (1 H, m, $H_2^{\ddagger}$), 1.38-1.47 (2 H, m, $H_3^{\ddagger}$), 1.76-1.84 (2 H, m, $H_4^{\ddagger}$), 2.008 (3 H, br s, C_{19}-CH_3), 2.343 (3 H, d, J = 1.1 Hz, C_{20}-CH_3), 5.850 (1 H, br s, H_{14}), 6.131 (1 H, d, J = 15.6 Hz, H_8), 6.263 (1 H, d, J = 11.4 Hz, H_{10}), 6.311 (1 H, d, J = 15.6 Hz, H_7), 6.455 (1 H, d, J = 15.1, H_{12}), 7.105 (1 H, dd, J = 15.1, 11.4 Hz, H_{11}) (†,‡assignments may be reversed); UV λ_{max} 325; UV (methanol) λ_{max} 342 (ε 47,000); MS (DEI) m/z (relative intensity) 316 ($M^{+\cdot}$, 100), 301 (34), 273 (12), 271 (40); MS (DCI) m/z (relative intensity) 317 ($M+H^+$, 100), 299 ($M+H^+-H_2O$, 18). Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.86; H, 8.83.

Preparation of 5,8-Epoxy-5,8-dihydroretinoic Acid Isomers (3a) and (3b). A 4:1 mixture (3.5 g) of **3a** and **3b** was prepared from the epoxide **2** with ethanolic HCl as reported previously.[7a] The two diastereomers were then separated by reversed-phase HPLC with a Rainin preparative HPLC system, a Rainin Dynamax C-18 column (8 μm, 25 cm x 41.4 mm), a mobile phase of 45% A/55% B (same mobile phases as above), and a flow rate of 30 mL/min. Seven injections were made with 0.5 g of the mixture in 3 mL of tetrahydrofuran per injection. Appropriate fractions were combined and rechromatographed as necessary to obtain pure material. The furans were removed from the HPLC fractions by extraction with ether. The crude samples of each isomer were recrystallized from petroleum ether (bp 37-53 °C). **3a**: 500 mg; mp 167-169 °C; ^{1}H NMR δ ($CDCl_3$) 1.108 (3 H, s, C_{16}-$CH_3^{\dagger}$), 1.15-1.27 (1 H, m, H_2), 1.155 (3 H, s, C_{18}-$CH_3^{\dagger}$), 1.436 (3 H, s, C_{17}-$CH_3^{\dagger}$), 1.46-1.68 (1 H, m, H_2), 1.46-1.68 (1 H, m, H_3), 1.46-1.68 (1 H, m, H_3), 1.46-1.68 (1 H, m, H_4), 1.788 (3 H, d, J = 1.1 Hz, C_{19}-CH_3), 1.980 (1 H, br d, H_4), 2.338 (3 H, d, J = 1.0 Hz, C_{20}-CH_3), 5.163 (1 H, br s, H_8), 5.181 (1 H, br s, H_7), 5.795 (1 H, br s, H_{14}), 6.228 (1 H, dd, J = 11.1, 1.1 Hz, H_{10}), 6.287 (1 H, d, J = 15.2 Hz, H_{12}), 6.899 (1 H, dd, J = 15.2, 11.1 Hz, H_{11}) (†assignments may be reversed); ^{13}C NMR δ ($CDCl_3$) 13.06 (q, C_{19}), 14.05 (q, C_{20}), 20.37 (t, C_3), 25.99 (q, $C_{16}^{\dagger}$), 25.99 (q, $C_{17}^{\dagger}$), 30.69 (q, $C_{18}^{\dagger}$), 34.66 (s, C_1), 41.28 (t, C_4), 41.42 (C_2), 87.19 (d, C_8), 87.93 (s, C_5), 117.82 (d, C_{14}), 118.4 (d, C_7), 125.61 (d, C_{10}), 131.31 (d, C_{11}), 135.32 (d, C_{12}), 143.39 (s, C_9), 154.92 (s, C_6), 155.14 (s, C_{13}), 171.64 (s, C_{15}) (†assignments may be reversed); UV λ_{max} 295; UV (ethanol) λ_{max} 308 (ε 36,000); MS (DEI) m/z (relative intensity) 316 ($M^{+\cdot}$, 100), 301 (44), 271 (30); MS (DCI) m/z (relative intensity) 317 ($M+H^+$, 100), 299 ($M+H^+-H_2O$, 8). Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.87; H, 9.30. Crystal data for **3a**:[16] Single crystals (recrystallized from ethanol) are, at 20 ± 1 °C, triclinic, space group P1 - C_i^1 (No. 2) with a = 6.784 (2) Å, b = 12.539 (3) Å, c = 13.150 (3) Å, α = 118.54(2)°, β = 96.03(2)°, γ = 103.63(2)°, V = 923.5(4) Å^3 and z = 2 (d_{calcd} = 1.138 gcm^{-3}; μ_a(Mo K_α) = 0.07 mm^{-1}). A total of 4238 independent reflections having 2Θ < 55° were collected on a computer-controlled four-circle Nicolet autodiffractometer using 0.90°-wide ω scans and graphite-monochromated Mo K_α radiation. The structure was solved using direct methods techniques with the Nicolet SHELXTL software package as modified at Crystalytics Company. A structural model which utilized anisotropic thermal parameters for all C and O atoms and isotropic thermal parameters for all H atoms has been refined to convergence [R_1 (unweighted, based on F) = 0.051 for 2332 independent reflections having 2Θ < 55° and I > 3σ(I)] using counter-weighted cascade block diagonal least-squares techniques. The five methyl groups were

refined as idealized rigid rotors and gave C-C-H angles which ranged from 105° to 115°; the acidic proton was refined as an independent isotropic atom. All other hydrogen atoms were included in the structure factor calculations at idealized positions.

3b: 20 mg; mp 178-180 °C; ^{1}H NMR δ ($CDCl_3$) 1.111 (3 H, s, C_{17}-$CH_3^{\dagger}$), 1.14-1.24 (1 H, m, H_2), 1.180 (3 H, s, C_{18}-$CH_3^{\dagger}$), 1.43-1.67 (1 H, m, H_2), 1.43-1.67 (2 H, m, H_3), 1.43-1.67 (1 H, m, H_4), 1.464 (3 H, s, C_{16}-$CH_3^{\dagger}$), 1.838 (3 H, d, J = 1.0 Hz, C_{19}-CH_3), 1.935 (1 H, br d, H_4), 2.341 (3 H, d, J = 1.0 Hz, C_{20}-CH_3), 5.077 (1 H, br s, H_8), 5.254 (1 H, br s, H_7), 5.796 (1 H, br s, H_{14}), 6.230 (1 H, br d, J = 11.2 Hz, H_{10}), 6.285 (1 H, d, J = 15.2 Hz, H_{12}), 6.903 (1 H, dd, J = 15.2, 11.2 Hz, H_{11}) (†assignments may be reversed); ^{13}C NMR δ ($CDCl_3$) 13.70 (q, C_{19}), 14.04 (q, C_{20}), 20.72 (t, C_3), 25.56 (q, $C_{16}^{\dagger}$), 27.55 (q, $C_{17}^{\dagger}$), 30.64 (q, $C_{18}^{\dagger}$), 35.09 (s, C_1), 41.44 (t, $C_2^{\ddagger}$), 42.05 ($C_4^{\ddagger}$), 87.61 (d, C_8), 88.26 (s, C_5), 117.32 (d, $C_{14}^{\dagger\dagger}$), 117.53 (d, $C_7^{\dagger\dagger}$), 124.38 (d, C_{10}), 131.44 (d, C_{11}), 135.09 (d, C_{12}), 144.03 (s, C_9), 154.13 (s, $C_6^{\ddagger\ddagger}$), 155.21 (s, $C_{13}^{\ddagger\ddagger}$), 171.16 (s, C_{15}) (†,‡,††,‡‡assignments may be reversed); UV λ_{max} 295; UV (ethanol) λ_{max} 307 (ε 31,000); MS (DEI) m/z (relative intensity) 316 ($M^{+\cdot}$, 100), 301 (52), 271 (34); MS (DCI) m/z (relative intensity) 317 ($M+H^+$, 100), 299 ($M+H^+-H_2O$, 14); HRMS calcd for $C_{20}H_{28}O_3$ 316.2038, found 316.2022.

Kinetics Measurements. Approximately 0.5 g of retinoic acid was accurately weighed into a 500 mL volumetric flask and diluted to the mark with 90% ethanol (3.3×10^{-3} M). The solution (400 mL) was then placed into a 600 mL stainless steel reactor (Parr Instruments). The reactor was purged with oxygen and placed into a constant temperature bath (± 0.2 °C). The internal oxygen pressure was maintained at the vapor pressure of the solvent at the temperature which was employed and therefore the oxygen partial pressure was 1 atm. Samples were periodically removed and analyzed by HPLC. Reactions were also conducted by storing approximately 100 mL of the reaction solution in a stoppered 500 mL flask (air atmosphere) in the dark at room temperature.

Preparative-scale Autoxidation Reactions: Isolation and Identification of Products. A typical reaction and isolation sequence is described. Multiple reactions and isolation procedures were performed. Retinoic acid (1.07 g) was dissolved in 400 mL of 90% ethanol (8.3×10^{-3} M) and the solution was placed into a 600 mL stainless steel reactor. The reactor was maintained at 86 °C for three days with an oxygen pressure of 1 atm. The amount of unreacted retinoic acid was approximately 9 mole %. The reaction solution was then concentrated to 40 mL and fractionated by reversed-phase HPLC (Rainin Instruments). Twenty mL of this concentrated solution was injected in each of two separations and fractions were collected. The reversed-phase column was 25 cm x 21.4 mm (Rainin Dynamax, 8 μm) and was typically eluted with a linear gradient from 100% A/ 0% B to 44% A/56% B over 30 min and then a linear gradient to 10% A/90% B over 20 min. The flow rate was 15 mL/min. Appropriate fractions were combined and extracted with ether. The isolated material was rechromatographed as necessary to obtain pure products. The following products were isolated:

2, **3a**, **3b**: The chromatographic retention time and UV, MS, and ^{1}H NMR spectral data for the isolated sample were identical to the data for the authentic synthesized compound.

5,6-Dihydro-5,6-dihydroxyretinoic Acid (4): ^{1}H NMR δ (CD_3COCD_3) 0.827 (3 H, s, C_{16}-$CH_3^{\dagger}$), 1.124 (3 H,s, C_{17}-$CH_3^{\dagger}$), 1.163 (3 H, s, C_{18}-$CH_3^{\dagger}$), 2.012 (3 H, d, J = 1.2 Hz, C_{19}-CH_3), 2.346 (3 H, d, J = 1.2 Hz, C_{20}-CH_3), 5.838 (1 H, br s, H_{14}), 6.149 (1 H, d, J = 15.6 Hz, H_8), 6.236 (1 H, d, J = 11.3 Hz, H_{10}), 6.433 (1 H, d, J = 15.0 Hz, H_{12}), 6.530 (1 H, dd, J = 15.6, 0.7 Hz, H_7), 7.118 (1 H, dd, J = 15.0, 11.3 Hz, H_{11}) (†assignments may be reversed; these spectral data were similar to the data for the 5,6-*trans* (and *cis*) isomer of the methyl

ester of **4**.[8b]); UV λ_{max} 327; MS (DEI) m/z (relative intensity) 334 ($M^{+\cdot}$,11), 316 ($M^{+\cdot}$-H_2O, 14), 109 (100); MS (DCI) m/z (relative intensity) 335 ($M+H^+$, 38), 317 ($M+H^+$-H_2O, 68), 168 (100); HRMS calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2145.

4-Ethoxyretinoic Acid (5): ^{1}H NMR δ (CD_3COCD_3) 1.022 (3 H, s, C_{16}-$CH_3^{\dagger}$), 1.034 (3 H, s, C_{17}-$CH_3^{\dagger}$), 1.160 (3 H, t, J = 7.0 Hz, -$CH_2\underline{CH_3}$), 1.367 (2 H,m, H_2), 1.671 (2 H, m, H_3), 1.775 (3 H, dd, J = 0.9, 0.8 Hz, C_{18}-CH_3), 2.045 (3 H, d, J = 1.3 Hz, C_{19}-CH_3), 2.354 (3 H, d, J = 1.2 Hz, C_{20}-CH_3), 3.418 (1 H, dq, J = 9.2, 7.0 Hz, -$C\underline{H_2}CH_3$), 3.621 (1 H, br t, J = 4.4 Hz, H_4), 3.639 (1 H, dq J = 9.2, 7.0 Hz, -$C\underline{H_2}CH_3$), 5.849 (1 H, br s, H_{14}), 6.23 (1 H, dd, J = 16.1, 0.6 Hz, H_8), 6.285(1 H, br d, J = 11.2 Hz, H_{10}), 6.31 (1 H, br d, J = 16.1 Hz, H_7), 6.444 (1 H, d, J = 15.1 Hz, H_{12}), 7.140 (1 H, dd, J = 15.1, 11.2 Hz, H_{11}) (†assignments may be reversed); UV λ_{max} 237; MS (DEI) m/z (relative intensity) 344 ($M^{+\cdot}$, 100), 329 (18), 298 ($M^{+\cdot}$-CH_3CH_2OH, 48); MS (DCI) m/z (relative intensity) 345 ($M+H^+$, 28), 299 ($M+H^+$-CH_3CH_2OH, 100); HRMS calcd for $C_{22}H_{32}O_3$ 344.2351, found 344.2330.

5,8-Epidioxy-5,8-dihydroretinoic Acid (6): ^{1}H NMR δ (CD_3COCD_3) 1.139 (3 H, s, C_{16}-$CH_3^{\dagger}$), 1.185 (3 H, s, C_{17}-$CH_3^{\dagger}$), 1.372 (3 H, d, J = 1.1 Hz, C_{18}-CH_3), 1.951 (3 H, d, J = 1.4 Hz, C_{19}-CH_3), 2.336 (3 H, d, J = 1.2 Hz, C_{19}-CH_3), 4.716 (1 H, d, J = 4.0 Hz, H_8), 5.484 (1 H, br s, H_{14}), 5.659 (1 H, d, J = 4.0 Hz, H_7), 6.225 (1 H, br d, J = 11.0 Hz, H_{10}), 6.416 (1 H, d J = 15.3 Hz, H_{12}), 6.997 (1 H, dd, J = 15.3, 11.0 Hz, H_{11}) (†assignments may be reversed; these spectral data were nearly identical to the data for the ethyl ester of **4**[10]); UV λ_{max} 297; MS (DEI) m/z 332 ($M^{+\cdot}$), 316, 300, 163; MS (DCI) m/z (relative intensity) 333 ($M+H^+$, 28), 317 (27), 307 (76), 305 (100); HRMS (DCI) calcd for $C_{20}H_{29}O_4$ 333.2066 ($M+H^+$), found 333.2016 ($M+H^+$).

9-*cis*(or *trans*)-9,10-Epidioxyretinoic Acid (7a): ^{1}H NMR δ ($CDCl_3$) 0.972 (3 H, s, C_{16}-CH_3), 0.972 (3 H, s, C_{17}-CH_3), 1.396 (3 H, s, C_{19}-CH_3), 1.444 (2 H, m, H_2), 1.599 (2 H, m, H_3), 1.638 (3 H, dt, J = 0.9, 0.9 Hz, C_{18}-CH_3), 1.965 (2 H, m, H_4), 2.265 (3 H, d, J = 1.2 Hz, C_{20}-CH_3), 4.145 (1 H, br d, J = 6.0 Hz, H_{10}), 5.449 (1 H, d, J = 16.2 Hz, H_8), 5.82 (1 H, br s, H_{14}), 6.152 (1 H, dtq, J = 16.2 Hz, H_7), 6.207 (1 H, ddd, J = 15.8, 6.0, 0.5 Hz, H_{11}), 6.402 (1 H, ddd, J = 15.8, 1.3, 0.8 Hz, H_{12}); UV λ_{max} 252; MS (DEI) m/z (relative intensity) 332 ($M^{+\cdot}$, 2.5), 316 (10), 219 (40), 193 (100); MS (DCI) m/z (relative intensity) 333 ($M+H^+$, <2), 317 (70), 299 (33), 193 (100); HRMS (DCI) calcd for $C_{20}H_{29}O_4$ 333.2066 ($M+H^+$), found 333.2080 ($M+H^+$).

9-*trans*(or *cis*)-9,10-Epidioxyretinoic Acid (7b): ^{1}H NMR δ ($CDCl_3$) 0.986 (3 H, s, C_{16}-$CH_3^{\dagger}$), 0.989 (3 H, s, C_{17}-$CH_3^{\dagger}$), 1.297 (3 H, s, C_{19}-CH_3), 1.452 (2 H, m, H_2), 1.61 (2 H, m, H_3), 1.661 (3 H, br d, J = 0.6 Hz, C_{18}-CH_3), 1.981 (2 H, m, H_4), 2.285 (3 H, d, J = 1.1 Hz, C_{20}-CH_3), 4.146 (1 H, m, H_{10}), 5.491 (1 H, d, J = 16.0 Hz, H_8), 5.828 (1 H, br s, H_{14}), 6.181 (1 H, d, J = 15.8 Hz, H_{11}), 6.197 (1 H, br d, J = 16.0 Hz, H_7), 6.422 (1 H, d, J = 15.8 Hz, H_{12}) (†assignments may be reversed); UV λ_{max} 252; MS (DEI) m/z (relative intensity) 332 ($M^{+\cdot}$, 9), 316 (100), 301(53), 289 (58); MS (DCI) m/z (relative intensity) 333 ($M+H^+$, 4), 317 (90), 299 (36), 193 (100).

9-*cis*-9,10-Epoxyretinoic Acid (8a): ^{1}H NMR δ ($CDCl_3$) 0.995 (3 H, s, C_{16}-$CH_3^{\dagger}$), 0.998 (3 H, s, C_{17}-$CH_3^{\dagger}$), 1.316 (3 H, s, J = 0.7 Hz, C_{19}-CH_3), 1.458 (2 H, m, H_2), 1.613 (2 H, m, H_3), 1.668 (3 H, dt, J = 0.9, 0.9 Hz, C_{18}-CH_3), 1.978 (2 H, m, H_4), 2.250 (3 H, br s, C_{20}-CH_3), 4.150 (1 h, dd, J = 5.7, 1.4 Hz, H_{10}), 5.469 (1 H, d, J = 16.6 Hz, H_8), 5.82 (1 H, br s, H_{14}),

6.067 (1 H, dtq, J = 16.6 Hz, H_7), 6.263 (1 H, dd, J = 15.8, 5.7 Hz, H_{11}), 6.445 (1 H, dd, J = 15.8, 1.4 Hz, H_{12}) (†assignments may be reversed); UV λ_{max} 260; MS (DEI) m/z (relative intensity) 316 ($M^{+\cdot}$, 2), 221 (100); MS (DCI) m/z (relative intensity) 317 ($M+H^+$, 32), 299 ($M+H^+-H_2O$, 16), 223(30), 221 (100); HRMS calcd for $C_{20}H_{28}O_3$ 316.2038, found 316.2007.

9-*trans*-9,10-Epidioxyretinoic Acid (8b): ^{1}H NMR δ ($CDCl_3$) 1.002 (3 H, s, C_{16}-CH_3†), 1.003 (3 H, s, C_{17}-CH_3†), 1.300 (3 H, s, C_{19}-CH_3), 1.463 (2 H, m, H_2), 1.616 (2 H, m, H_3), 1.674 (3 H, dt, J = 1.0, 0.9 Hz, C_{18}-CH_3), 1.984 (2 H, m, H_4), 2.244 (3 H, d, J = 1.2 Hz, C_{20}-CH_3), 4.190 (1 H, dd, J = 5.0, 1.7 Hz, H_{10}), 5.355 (1 H, d, J = 16.4 Hz, H_8), 5.81 (1 H, br s, H_{14}), 6.099 (1 H, dtq, J = 16.4 Hz, H_7), 6.267 (1 H, ddd, J = 15.8, 5.0, 0.5 Hz, H_{11}), 6.477 (1 H, ddd, J = 15.8, 1.7, 0.7 Hz, H_{12}) (†assignments may be reversed); UV λ_{max} 261; MS (DEI) m/z (relative intensity) 316 ($M^{+\cdot}$, 0.4), 221 (100); MS (DCI) m/z (relative intensity) 317 ($M+H^+$, 2), 223 (62), 221 (100); HRMS calcd for $C_{20}H_{28}O_3$ 316.2038, found 316.2019.

5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone (9): ^{1}H NMR δ ($CDCl_3$) 1.20-1.51 (2 H, m, H_5*), 1.224 (3H, s, 4-CH_3†), 1.272 (3 H, s, 4-CH_3†), 1.554 (3 H, d, J = 0.9 Hz, 7a-CH_3), 1.64-1.79 (2 H, m, H_6‡), 1.64-1.79 (1 H, m, H_7‡), 2.237 (1 H, dddt, J = 12.5, 3.2, 2.0, 0.5 Hz, H_7), 5.643 (1 H, d, J = 0.5 Hz, H_3) (†,‡assignments may be reversed; these spectral data were nearly identical to data for the known compound[11]); UV λ_{max} 227; MS (DEI) m/z (relative intensity) 180 ($M^{+\cdot}$, 84), 137 (58), 111 (100); MS (DCI) m/z 181 ($M+H^+$).

(*E*,*E*)-2-Methyl-6-oxo-2,4-heptadienal (10): ^{1}H NMR δ (CD_3COCD_3) 1.951 (3 H, dd, J = 1.5, 0.5 Hz, C_2-CH_3), 2.351 (3 H, s, H_7), 6.574 (1 H, br d, J = 15.6 Hz, H_5), 7.167 (1 H, ddq, J = 11.4, 1.5, 0.8 Hz, H_3), 7.666 (1 H, dd, J = 15.6, 11.4 Hz, H_4), 9.589 (1 H, s, RCHO); UV λ_{max} 291; MS (DEI) m/z (relative intensity) 138 ($M^{+\cdot}$, 100), 123 (22), 109 (14); MS (DCI) m/z 139 ($M+H^+$); HRMS calcd for $C_8H_{10}O_2$ 138.0681, found 138.0686.

Acknowledgement - The crystallographic analysis was performed by Crystalytics Company, Lincoln, Nebraska.

REFERENCES AND NOTES

1. Sporn, M.; Roberts, A.; Goodman, D., Eds. "The Retinoids", Academic Press: London, **1984**.
2. a) Mandy, S.; Thorne, E. *Adv. Ther.* **1984**, *1*, 404. b) Weiss, J.; Ellis, C.; Headington, J.; Tincoff, T.; Hamilton, T.; Voorhees, J. *J. Am. Med. Assoc.* **1988**, *259*, 527.
3. a) Takashima, Y.; Nakajima, T.; Washitake, M.; Anmo, T.; Sugiura, S.; Matsumaru, H. *Chem. Pharm. Bull.* **1979**, *27*(1), 12. b) Finkelshtein, E.; Dolotov, M.; Kozlov, E. *Zhurn. Org. Khim.* **1978**, *14*(3), 486. c) Finkelshtein, E.; Dolotov, M.; Kozlov, E. *Zhurn. Org. Khim.* **1978**, *14*(3), 525. d) Finkelshtein, E.; Rubchinskaya, Y.; Kozlov, E. *Int. J. Chem. Kin.* **1984**, *16*, 513. e) Finkelshtein, E.; Rubchinskaya, Y.; Kozlov, E. *Zhurn. Org. Khim.* **1981**, *17*(5), 936. f) Finkelshtein, E.; Bulenkov, M.; Arzamastsev, A. *Zhurn. Org. Khim.* **1984**, *20*(5), 941. g) McKenzie, R.; McGregor, M.; Nelson, E. *J. Label. Compd. and Radiopharm.* **1978**, XV, 265. h) Parizkova, H.; Blattina, J. *J. Chromatogr.* **1980**, *191*, 301. i) Parizkova, H.; Blattina, J. Mitt. *Gebiete Lebensm. Hyg.* **1982**, *73*, 166. j) Ogata, Y.; Kosugi, Y.; Tomizawa, K. *Tetrahedron* **1970**, *26*, 5939. k) Iwahashi, H.; Ikeda, A.; Negoro, Y.; Kido, R.

Biochem. J. **1986**, *236*, 509. l) Hart, H.; Lavrik, P. *J. Org. Chem.* **1974**, *39*, 1793.
4. a) Howard, J.; "The Chemistry of Peroxides", Patai, S., Ed; John Wiley & Sons: NY, **1983**, Chapt. 8. b) Simic, M. *J. Chem. Ed.* **1981**, *58*(2), 125. c) Ingold, K. *Acc. Chem. Res.* **1969**, *2*(1), 1. d) Porter, N. *Acc. Chem. Res.* **1986**, *19*, 262. e) Correa, P.; Hardy, G.; Riley, D. *J. Org. Chem.* **1988**, *53*, 1695. f) Mayo, F. *Acc. Chem. Res.* **1968**, *1*, 193.
5. a) Meijer, E.; Wynberg, H. *Tetrahedron Lett.* **1981**, 22, 785. b) Krebs, A.; Schmalstieg, H.; Jarchow, O.; Klaska, K. *Tetrahedron Lett.* **1980**, *21*, 3171. c) Turro, N.; Ramamurthy, V.; Liu, R.; Krebs, A.; Kemper, R. *J. Am. Chem. Soc.* **1976**, *98*(21), 6758. d) Takekuma, S.; Matsubara, Y.; Yamamoto, H.; Nozoe, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 3721. e) Beaver, B.; Cooney, J.; Watkins, Jr., J. *J. Heterocyclic Chem.* **1986**, *23*, 1095. f) Turro, N.; Chow, M.; Ito, Y. *J. Am. Chem. Soc.* **1978**, *100*, 5580. g) Nelsen, S.; Teasley, M.; Kapp, D. *J. Am. Chem. Soc.* **1986**, *108*, 5503. h) Nelsen, S.; Teasley, M.; *J. Org. Chem.* **1986**, *51*, 3221. i) Tang, R.; Yue, H.; Wolf, J.; Mares, F. *J. Am. Chem. Soc.* **1978**, *100*, 5248. j) Haynes, R.; Probert, M.; Wilmot, I. *Aust. J. Chem.* **1978**, *31*, 1737.
6. Chen, T.; Chafetz, L. *J. Pharm. Sci.* **1987** *76*, 703.
7. a) John, K.; Lakshmanan, M.; Cama, H. *Biochem. J.* **1967**, *103*, 539. b) Ito, M.; Yamane, T.; Tsukida, K. *J. Chromatogr.* **1982**, *253*, 113.
8. a) Trown, P.; Palleroni, A.; Bohoslawec, O.; Richelo, B.; Halpern, J.; Gizzi, N.; Geiger, R.; Lewinski, C.; Machlin, L.; Jetten, A.; Jetten, M. *Cancer Research* **1980**, *40*, 212. b) Akhtar, M.; Faruk, A.; Harris, C.; Moss, G.; Russell, S.; Weedon, B. *J. Chem. Soc., Perkin I* **1978**, 1511. c) House, H. O. "Modern Synthetic Reactions," Benjamin Inc.: Menlo Park, California, **1972**, 2nd Ed., p. 301.
9. Smith, J.; Borek, C.; Gawinowicz, M.; Goodman, D. *Arch. Biochem. Biophys.* **1985**, *238*, 1.
10. Pfoertner, K. *Helvitica Chimica Acta* **1975**, *58*, 833.
11. Lerner, D.; Mani, J.; Mousseron-Canet, M. *Bull. Chim. Soc. Fr.* **1970**, 1968.
12. a) Batt, L.; Liu, M. "The Chemistry of Peroxides", Patai, S., Ed; John Wiley & Sons: NY, **1983**, pp. 704-708. b) Baumstark, A.; Retter, C.; Tehrani, K.; Kellogg, C. *J. Org. Chem.* **1987**, *52*, 3308.
13. Brouwer, M.; Hulkenberg, A.; van Moorselaar, R.; Overbeek, W.; Wesselman, P. *Recl. Trav. Chim. Pays-Bas* **1979**, *98*, 316.
14. Schwieter, U.; Arnold, W.; Oberhansli, W.; Rigassi, N.; Vetter, W. *Helv. Chim. Acta* **1971**, *54*, 2447.
15. Balci, M. *Chem. Rev.* **1981**, *81*, 91.
16. Detailed crystallographic data has been deposited at the Cambridge Crystallographic Data Centre.