145. Oxidation Products of Retinylidene Dimedone

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Dedicated to Dr. *Otto Isler* on the occasion of his 70th birthday

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Summary

Several oxidation products of the retinoid retinylidene dimedone (RD) have been synthesized and characterized for biological testing in the expectation that they may be found as metabolites of RD.

Condensation products of all-*trans*-retinal with aliphatic and alicyclic 1,3-diketones represent a new lead in the chemotherapy of cancer prevention, and all-*trans*-retinylidene dimedone (RD) emerged as a candidate for further investigation [1] [2]. The current interest in this diketone as an experimental non-toxic retinoid [3], and the likelihood that its metabolism might parallel that of classical retinoids, suggested the preparation of well characterized potential metabolites for biological evaluation and for identification purposes. Metabolism of all-*trans*-retinoic acid primarily involves oxidation at C(4) to give 4-hydroxyretinoic acid and 4-oxoretinoic acid [4] [5]. In addition, it has been shown that the double bond in ring A is epoxidized, and that 5,6-epoxy-5,6-dihydroretinoic acid undergoes facile acid catalyzed ring expansion to a 5,8-dihydrofuran [5].

With this information, we prepared the five oxygenated RD derivatives I–V shown in *Scheme 1*, as well as the tetraketone VI, which was obtained by cleavage of the 5′,6′-double bond.

Epoxy-RD (I), λ_{max} 470 nm, was prepared from RD by perphthalic or *m*-chloroperbenzoic acid oxidation. The acid catalyzed rearrangement of I (methylene chloride/conc. sulfuric acid) afforded, as expected [6], the yellow dihydrofuran II, λ_{max} 435 nm. The acetate III, λ_{max} 480 nm, and the alcohol IV were prepared by condensation of 4-acetoxy-[7] and of 4-hydroxyretinal[1]) with dimedone. In our hands, purification of these oxygenated retinal derivatives proved difficult, and

[1]) 4-Hydroxyretinal has been prepared by selenium dioxide oxidation of the enol acetate of retinal [8]. *Henbest* [9] has obtained 4-hydroxyretinal as an oil by 'precipitated MnO_2' oxidation of retinal.

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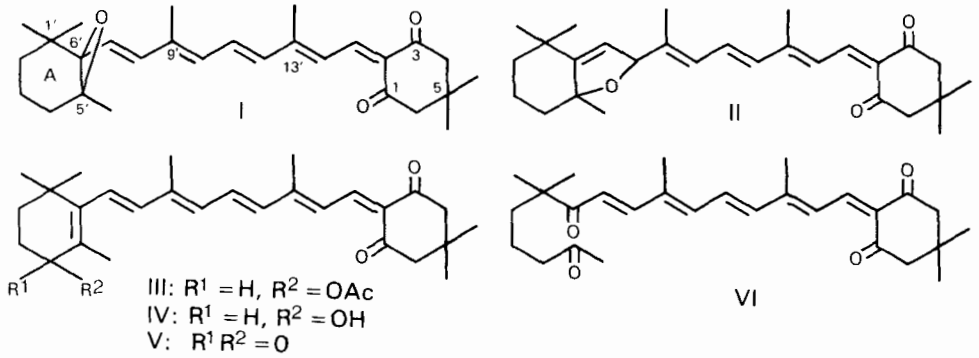
Scheme 1

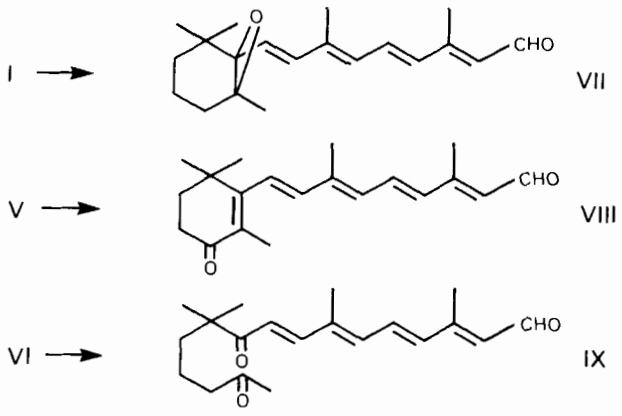


extensive purification was therefore deferred until the final dimedone derivative had been prepared. The triketone V was formed on pyridinium dichromate oxidation of the alcohol IV, whereas oxidation of RD with the same reagent, but used in excess for a longer period of time, gave the expected tetraketone VI, λ_{max} 455 nm [10]. Attempted cyclization of tetraketone VI (using either acid or base) was unsuccessful [11].

We recently reported that RD and other retinylidene 1,3-diketones, when dissolved in aprotic solvents and treated with aqueous methylamine, undergo a retro-aldol reaction to retinal and dimedone or other diketones [12]. Some of the oxygenated RD derivatives prepared here behave similarly, thus allowing the preparation of 5,6-epoxy-5,6-dihydroretinal (VII) and of 4-oxoretinal (VIII) [9] by this alternate pathway, as well as the preparation of the novel diketoaldehyde IX *(Scheme 2)*.

Retinylidene dimedone and analogs can be considered to be protected retinal, and chemical transformation can therefore be carried out before cleavage to the polyene aldehyde. This cleavage occurs under very mild alcaline conditions. Protection of polyene aldehydes by condensation with 1,3-diketones might thus be a general and useful procedure for preparing a variety of substituted polyenes otherwise difficult to obtain.

Scheme 2



All oxygenated RD derivatives were tested in the hamster tracheal organ culture system of *Sporn* [13]. None of the compounds showed activity at less than 10^{-8} M, whereas RD itself is active at 10^{-9} to 10^{-10} M levels [1]. It seems oxidation in the A ring of RD lowers biological activity, at least in this tracheal organ culture system. This result parallels that found for retinoic acid oxidation products [4].

We are grateful to Drs. *W. Leimgruber* and *G. Saucy* of *Hoffmann-La Roche, Inc.*, Nutley, New Jersey, USA, for generous gifts of retinal and to *Dianne Newton* and Dr. *Michael Sporn* of the *National Cancer Institute* for the results of the tracheal organ culture assays.

Experimental Part

Melting points (m.p.) were determined on a *Thomas Hoover* capillary apparatus and are uncorrected. Chromatographies utilized *Merck* Silica Gel 60, 70–230 mesh. Elemental analyses were performed by our Microanalytical Laboratory. UV./VIS. spectra (λ_{max}: nm (ε)) were measured in EtOH using a *Beckman* DB-G grating spectrophotometer. A *Beckman* IR 4320 spectrophotometer was used for IR. spectra which are reported in cm^{-1} and are in CCl_4 solution. MS. were determined on a *Finnegan* 1015 D instrument (chemical ionization). ^{1}H-NMR. spectra (220 MHz) were obtained with a *Varian* HR 220 spectrometer and are in $CDCl_3$ solution. Abbreviations: RT. = room temperature.

2-(5′,6′-Epoxy-5′,6′-dihydroretinylidene)-dimedone (I). Retinylidene dimedone (423 mg, 1.04 mmol) was dissolved in 50 ml of ether, and 4 ml of 0.3 M perphthalic acid in ether [14] added during 4 min. After 1 h at RT., an additional 4 ml of peracid solution was added, and stirring continued for 1 h. The ether solution was washed with 5% $NaHCO_3$-solution, then water, dried (Na_2SO_4), and the solvent stripped. The product was chromatographed on 150 g of silica gel (hexane/ether 3:1), and the second band collected and crystallized from petroleum ether to yield 197 mg (45%) of bronze colored crystals, m.p. 110–112.5°. – UV.: 470 (50,300), 290 (11,950). – IR.: C=O: 1691*w*, 1653*sh*, 1650*s*; epoxy: 1240, 960; C=C: 1512, 971. – ^{1}H-NMR.: 0.94 (*s*, 3 H, CH_3–C(5′)); 1.08 (*s*, 6 H, 2 CH_3–C(5)); 1.10 (*s*, 3 H) and 1.16 (*s*, 3 H, 2 CH_3–C(1′)); 1.44 (*m*, 4 H, 2 H–C(2′), 2 H–C(3′)); 1.77 (*m*, 2 H, 2 H–C(4′)); 2.00 (*s*, 3 H, CH_3–C(9′)); 2.23 (*s*, 3 H, CH_3–C(13′)); 2.52 (*s*, 4 H, 2 H–C(4) and 2 H–C(6)); 6.06 (*d*, J = 15.5, 1 H, H–C(7′))[2]; 6.26 (*d*, J = 12, 1 H, H–C(10′)); 6.35 (*d*, J = 15.5, 1 H, H–C(8′))[2]; 6.59 (*d*, J = 15, 1 H, H–C(12′)); 7.09 (*d* × *d*, J = 15, J' = 12, 1 H, H–C(11′)); 7.80 (*d*, J = 13.5, 1 H, H–C(14′)); 8.16 (*d*, J = 13.5, 1 H, H–C(15′)). – MS. (CI, NO–N_2): 422.

$C_{28}H_{38}O_3$ (422.61) Calc. C 79.58 H 9.06% Found C 79.74 H 9.11%

Oxidation using *m*-chloroperbenzoic acid in methylene chloride afforded the 5′,6′-epoxide in comparable yield.

2-(5′,8′-Epoxy-5′,8′-dihydroretinylidene)-dimedone (II). A solution of 359 mg (0.85 mmol) of I in 80 ml of methylene chloride was treated with *ca.* 2 µl of conc. sulfuric acid. After 20 min at RT., 600 mg of solid $NaHCO_3$ was added, and the mixture stirred for 15 min. It was then filtered, concentrated, and passed through silica gel (50 g, ether/hexane 1:1). After standing for several weeks at 0°, a petroleum ether solution of the product deposited 151 mg (42%) of crystalline material, m.p. 115–124°. – UV.: 435 (43,100). – IR.: C=O: 1692*w*, 1650*s*; C=C: 1515*s*, 960*m*. – ^{1}H-NMR.: 1.07 (*s*, 6 H, 2 CH_3–C(5)); 1.11 (*s*, 3 H) and 1.16 (*s*, 3 H, 2 CH_3–C(1′)); 1.43 (*s*, 3 H, CH_3–C(5′)); 1.64 (*m*, 4 H, 2 H–C(2′) and 2 H–C(3′)); 1.82 (*s*, 3 H, CH_3–C(9′)); 1.99 (*m*, 2 H, 2 H–C(4′)); 2.22 (*s*, 3 H, CH_3–C(13′)); 2.51 (*s*, 4 H, 2 H–C(4), 2 H–C(6)); 5.18 (*s*, 2 H, H–C(7′) and H–C(8′)); 6.30 (*d*, J = 11.5, 1 H, H–C(10′)); 6.55 (*d*, J = 15, 1 H, H–C(12′)); 7.00 (*d* × *d*, J = 15, J' = 11.5, 1 H, H–C(11′)); 7.77 (*d*, J = 13, 1 H, H–C(14′)); 8.18 (*d*, J = 13, 1 H, H–C(15′)). – MS. (CI, NO–N_2): 422.

$C_{28}H_{38}O_3$ (422.61) Calc. C 79.58 H 9.06% Found C 79.50 H 9.02%

5,6-Epoxy-5,6-dihydroretinal (VII). A solution of 135 mg (0.32 mmol) of I in 5 ml of benzene was stirred with 5 ml of 40% aqueous methylamine for 5 min. The aqueous layer was extracted with benzene, and the organic solution washed with 0.1 N NaOH, then water. The solution was dried (Na_2SO_4), concentrated, and passed through silica gel (75 g, ether/hexane 1:1). The major yellow

[2]) Assignments may be reversed.

band formed crystals (34 mg, 35%) from cold petroleum ether, m.p. 104–106° (lit. [15]): 101–102°), and was identified spectroscopically (IR., ^{1}H-NMR., MS.).

2-(4'-Acetoxyretinylidene)-dimedone (III). 4-Acetoxyretinal was prepared from retinal and *N*-bromosuccinimide in acetic acid/methylene chloride according to the procedure of *Surmatis et al.* [7]. In our hands, the product from this procedure contained a considerable amount of unreacted starting material and/or other impurities. Rapid silica gel chromatography removed some, but not all, of these impurities. Crude 4-acetoxyretinal (590 mg) and dimedone (255 mg) in 10 ml benzene + 2 drops of piperidine was stirred for 3.5 h at RT. Silica gel chromatography (150 g, ether/hexane 1:1) and crystallization of the major band from cold petroleum ether afforded 195 mg of orange product, m.p. 118–119°. – UV.: 480 (51,200), 285 (12,200), 265 (13,000). – IR.: acetate: 1740*s*, 1240*s*; C=O: 1690*w*, 1650*s*; C=C: 1510*s*, 965*m*. – ^{1}H-NMR.: 1.03 (*s*, 3 H), 1.07 (*s*, 3 H) and 1.08 (*s*, 6 H, 2 CH_3–C(1') and 2 CH_3–C(5)); 1.45 (*m*, 2 H, 2 H–C(2')); 1.73 (*s*, 3 H, CH_3–C(5')); 1.86 (*m*, 2 H, 2 H–C(3')); 2.04 (*s*, 3 H, CH_3–CO)[2]); 2.07 (*s*, 3 H, CH_3–C(9'))[2]); 2.24 (*s*, 3 H, CH_3–C(13')); 2.52 (*s*, 4 H, 2 H–C(4), 2 H–C(6)); 5.23 (*t*, J=4.5, 1 H, H–C(4')); *ca.* 6.25 (*m*, 3 H, H–C(7'), H–C(8'), H–C(10')); 6.57 (*d*, J=15, 1 H, H–C(12')); 7.12 (*d*×*d*, J=15, J'=12, 1 H, H–C(11')); 7.80 (*d*, J=13, 1 H, H–C(14')); 8.16 (*d*, J=13, 1 H, H–C(15')). – MS. (CI, NH_3): 404 (M–HOAc).

$C_{30}H_{40}O_4$ (464.65) Calc. C 77.55 H 8.68% Found C 77.48 H 8.87%

2-(4'-Hydroxyretinylidene)-dimedone (IV). Crude 4-acetoxyretinal prepared from 6 g of retinal [7] was dissolved in 100 ml of methanol and 10 ml of 10% KOH-solution in methanol added. After 2 h at RT., the solution was concentrated, taken up in ether, washed with water (3 times), brine (once), dried ($MgSO_4$), concentrated, and passed through silica gel (175 g). Chloroform eluted nonpolar impurities. Chloroform/methanol 10:1 eluted the 4-hydroxyretinal contaminated with polar impurities (*ca.* 4 g). This crude material (*ca.* 75% 4-hydroxyretinal by HPLC.)[3]) and dimedone (1.87 g, 13.3 mmol) in 40 ml of benzene and 6 drops of piperidine was stirred at RT. for 4 h, concentrated, and passed through silica gel (175 g). Ether/hexane 1:1 eluted nonpolar impurities. Ether eluted the product (2.8 g) as a solid foam. Rechromatography of a 200 mg sample on a *Merck* Lobar B silica gel column ($CHCl_3$/EtOAc 9:1, 2 ml/min) gave (4'-hydroxyretinylidene)-dimedone as a red foam (*ca.* 120 mg), m.p. 80–90°, which appeared homogeneous by TLC., by HPLC. (μ-porasil, $CHCl_3$/EtOAc 9:1) and by ^{1}H-NMR. – UV.: 480 (47,890). – ^{1}H-NMR.: 1.02 (*s*, 3 H), 1.07 (*s*, 3 H) and 1.08 (*s*, 6 H) (2 CH_3–C(1') and 2 CH_3–C(5)); 1.86 and 1.6–2.0 (*s* superimposed on *m*, 8 H, 2 H–C(2'), 2 H–C(3'), OH and CH_3–C(5')); 2.04 (*s*, 3 H, CH_3–C(9')); 2.25 (*s*, 3 H, CH_3–C(13')); 2.52 (*s*, 4 H, 2 H–C(4) and 2 H–C(6)); 4.04 (*t*, J=4.5, 1 H, H–C(4')); 6.20 (*d*, J=16, 1 H, H–C(7'))[2]); 6.23 (*d*, J=11.5, 1 H, H–C(10')); 6.32 (*d*, J=16, 1 H, H–C(8'))[2]); 6.59 (*d*, J=14.5, 1 H, H–C(12')); 7.13 (*d*×*d*, J=14.5, J'=11.5, 1 H, H–C(11')); 7.82 (*d*, J=13, 1 H, H–C(14')); 8.18 (*d*, J=13, 1 H, H–C(15')). – MS. (CI, NH_3): 404 (M–H_2O).

2-(4'-Oxoretinylidene)-dimedone (V). (4'-Hydroxyretinylidene)-dimedone (IV) (1.5 g, 3.5 mmol), pyridinium dichromate [16] (2.2 g, 6.8 mmol), and pyridinium trifluoroacetate (270 mg, 1.3 mmol) in 30 ml of methylene chloride was stirred for 2 h at RT. It was then diluted with 30 ml of ether/hexane 1:1, filtered, and the filter cake washed with ether. The filtrate was concentrated, passed through silica gel (175 g, ether/hexane 1:1), and the product crystallized from cold ether/petroleum ether to afford 256 mg (17%) of dark red crystals, m.p. 150–151°. – UV.: 470 (52,600). – IR.: 1685, 1670, 1650 (C=O). – ^{1}H-NMR.: 1.09 (*s*, 6 H) and 1.20 (*s*, 6 H, 2 CH_3–C(5) and 2 CH_3–C(1')); 1.87 (*s* superimposed on *m*, CH_3–C(5') and 2 H–C(2')); 2.07 (*s*, 3 H, CH_3–C(9')); 2.25 (*s*, 3 H, CH_3–C(13')); 2.55 (*s* superimposed on *m*, 6 H, 2 H–C(4), 2 H–C(6) and 2 H–C(3')); 6.12 (*d*, J=11.5, 1 H, H–C(10')); 6.40 (*s*, 2 H, H–C(7') and H–C(8')); 6.43 (*d*, J=15, 1 H, H–C(12')); 7.12 (*d*×*d*, J=15, J'=11.5, 1 H, H–C(11')); 7.82 (*d*, J=13, 1 H, H–C(14')); 8.18 (*d*, J=13, 1 H, H–C(15')). – MS. (CI, NH_3): 420.

$C_{28}H_{36}O_3$ (420.60) Calc. C 79.96 H 8.63% Found C 80.03 H 8.66%

4-Oxoretinal (VIII). A mixture of triketone V (98 mg, 0.23 mmol) in 5 ml of benzene and 2 ml of 40% aqueous methylamine was stirred for 5 min at RT. The aqueous layer was extracted with benzene, and the combined benzene extracts washed with 1N NaOH, brine, dried (Na_2SO_4), concentrated, and passed through silica gel (20 g, ether/hexane 1:1). Crystallization from cold ether/petroleum ether afforded 34 mg (50%) of VIII, m.p. 114–117° (lit. [9]: m.p. 117–118°). – UV.: 375 (50,930). – IR.: 1665 (C=O). – ^{1}H-NMR.: 1.18 (*s*, 6 H, 2 CH_3–C(1)); 1.86 (*s* superimposed

[3]) 4-Hydroxyretinal prepared according to [8] was equally impure.

on *t*, J=6.5, 5 H, CH_3–C(9) and 2 H–C(2)); 2.08 (*s*, 3 H, CH_3–C(13)); 2.35 (*s*, 3 H, CH_3–C(5)); 2.52 (*t*, J=6.5, 2 H, 2 H–C(3)); 6.00 (*d*, J=8, 1 H, H–C(14)); 6.30 (*d*, J=11.5, 1 H, H–C(10)); 6.35 (*s*) and 6.36 (*s*) (2 H, H–C(7) and H–C(8)); 6.45 (*d*, J=15, 1 H, H–C(12)); 7.11 (*d*×*d*, J=15, J'=11.5, 1 H, H–C(11)); 10.14 (*d*, J=8, 1 H, CHO). – MS. (CI, NH_3): 298.

$C_{20}H_{26}O_2$ (298.43) Calc. C 80.49 H 8.78% Found C 80.67 H 8.90%

2-(5',6'-Dioxo-5',6'-secoretinylidene)-dimedone (VI). A mixture of RD (4.0 g, 9.85 mmol) and pyridinium dichromate [16] (32 g, pulverized in a mortar) in 250 ml of methylene chloride was stirred at RT. for 2 days. The mixture was diluted with 200 ml of petroleum ether, filtered, and the filter cake washed with ether. The filtrate was concentrated and chromatographed on silica gel (350 g) eluting first with hexane/ether 1:1, then with ether. Evaporation of the major red band afforded 1.47 g (34%) of tetraketone VI, m.p. 90–93°. – UV.: 455 (60,000), 305 (15,250). – IR.: 1722, 1680, 1655 (C=O). – ^{1}H-NMR.: 1.09 (*s*, 6 H) and 1.18 (*s*, 6 H, 2 CH_3–C(5) and 2 CH_3–C(1')); 1.52 (*m*, 4 H, 2 H–C(2') and 2 H–C(3')); 2.07 (*s*, 3 H, CH_3–C(9'))^2); 2.12 (*s*, 3 H, CH_3–C(5'))^2); 2.24 (*s*, 3 H, CH_3–C(13')); 2.41 (*t*, J=7, 2 H, 2 H–C(4')); 2.53 (*s*, 4 H, 2 H–C(4) and 2 H–C(6)); 6.60 (*d*, J=11.5, 1 H, H–C(10')); 6.62 (*d*, J=15, 1 H, H–C(8'))^2); 6.70 (*d*, J=15, 1 H, H–C(12'))^2); 7.05 (*d*×*d*, J=15, J'=11.5, 1 H, H–C(11')); 7.40 (*d*, J=15, 1 H, H–C(7')); 7.82 (*d*, J=13, 1 H, H–C(14')); 8.12 (*d*, J=13, 1 H, H–C(15')). – MS. (CI, NH_3): 438.

$C_{28}H_{38}O_4$ (438.61) Calc. C 76.67 H 8.73% Found C 76.44 H 8.93%

3,7,11,11-Tetramethyl-10,15-dioxo-2,4,6,8-hexadecatetraenal (IX). Tetraketone VI (333 mg, 0.76 mmol) in 5 ml of benzene and 3 ml of 40% aqueous methylamine was stirred for 5 min at RT. The aqueous layer was extracted with benzene, and the combined benzene extracts washed with 1N NaOH, brine, dried (Na_2SO_4), concentrated and passed through silica gel (20 g, ether). Crystallization from cold ether/petroleum ether afforded 90 mg (37%) of yellow-brown crystals, m.p. 77–79°. – UV.: 370 (58,395). – IR.: 1720, 1670 (C=O); 1595, 970 (C=C). – ^{1}H-NMR.: 1.17 (*s*, 6 H, 2 CH_3–C(1)); 1.58 (*m*, 4 H, 2 H–C(2) and 2 H–C(3)); 2.06 (*s*, 3 H, CH_3–C(9))^2); 2.13 (*s*, 3 H, CH_3–C(13))^2); 2.34 (*s*, 3 H, CH_3–C(5)); 2.42 (*t*, J=6.5, 2 H, 2 H–C(4)); 6.03 (*d*, J=8, 1 H, H–C(14)); 6.55 (*d*, J=15, 1 H, H–C(8))^2); 6.57 (*d*, J=11.5, 1 H, H–C(10)); 6.66 (*d*, J=15, 1 H, H–C(12))^2); 7.10 (*d*×*d*, J=15, J'=11.5, 1 H, H–C(11)); 7.38 (*d*, J=15, 1 H, H–C(7)); 10.16 (*d*, J=8, 1 H, CHO). – MS. (CI, NH_3): 316.

$C_{20}H_{28}O_3$ (316.44) Calc. C 75.91 H 8.92% Found C 76.10 H 8.83%

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