146. *Retro*-Aldol Reaction of Retinylidene-1,3-Diketones; Correlation with Biological Activity

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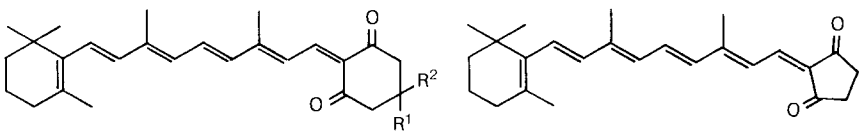
Summary

Retro-Aldol reaction of retinylidene-dimedone is reported.

We recently reported the preparation, characterization, and biological activity of a number of 2-retinylidene-1,3-diketones [1]. Many of these compounds, especially those derived from 1,3-cyclohexanediones (**1**, *Scheme 1*) show considerable activity in the hamster tracheal organ culture (TOC.) assay. This activity, combined with their low toxicity, has made some of these retinoids excellent materials for *in vitro* anti-tumor experiments [2]. However, retinoids derived from 1,3-cyclopentanediones are markedly less active, 2-retinylidene-1,3-cyclopentanedione (**2**, *Scheme 1*) itself having virtually no activity in the TOC. test. We now report a procedure for effecting a *retro*-aldol reaction of some of these retinoids which provides a chemical parallel to the observed variations in biological activities.

Scheme 1



1 R^1 and/or R^2 = H, alkyl, aryl **2**

Retinylidene-1,3-diketones can be considered to be 'cryptic' *Lewis* acids similar to benzylidene *Meldrum*'s acid and related compounds whose reaction with nucleophiles has been studied by *Shuster et al.* [3] and by *Bernasconi & Leonarduzzi* [4]. Benzylidene *Meldrum*'s acid contains a C,C double bond which is extremely susceptible to nucleophilic attack, and it ultimately fragments to give the products

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of a *retro*-aldol reaction, benzaldehyde and *Meldrum*'s acid. The process consists of two kinetically important steps represented in *Scheme 2* [4].

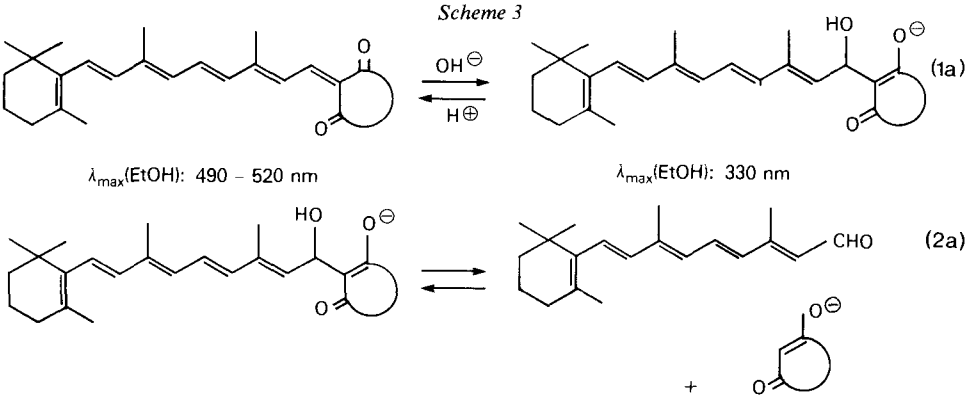
Scheme 2

$$\text{Substrate S} + \text{Nucleophile B} \rightleftharpoons \text{SB} \quad (1)$$

$$\text{SB} + \text{Electrophile E} \rightleftharpoons \text{Product P} \quad (2)$$

In the case of benzylidene *Meldrum*'s acid, reaction 1 *(Scheme 2),* where B is an amine, water, or phenoxide [3] [4], is extremely fast, requiring stopped flow or temperature jump relaxation methods for measurement. The fact that many 2-retinylidene-1,3-diketones (including **2**) undergo an instantaneous bleaching reaction when dissolved in ethanol/water 95:5 and treated with 0.1 N NaOH, indicates that reaction 1 is also rapid for these compounds (*Scheme 3*, equation 1a) [1]. However, chemical differences in the 2-retinylidene-1,3-diketones become apparent in the slow rate determining reaction 2 of the *retro*-aldol process, which in the retinoid case involves fragmentation of the retinylidene-diketone to retinal and the 1,3-diketone (*Scheme 3*, equation 2a). Thus we have found experimental conditions under which the biologically active cyclohexane-1,3-dione derivatives (**1**) fragment rapidly and in high yield to retinal while the biologically inactive cyclopentane-1,3-dione derivative (**2**) does not.

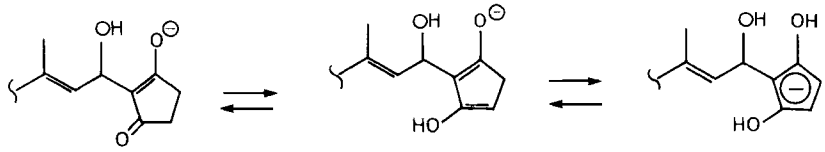
Scheme 3



The best experimental conditions for effecting this *retro-Knoevenagel* fragmentation involve a two phase system consisting of benzene and aqueous methylamine. When 2-retinylidene-5,5-dimethyl-1,3-cyclohexanedione (retinylidene-dimedone (**1**), $R^1 = R^2 = CH_3$) [1] was dissolved in benzene and stirred at room temperature with 40% aqueous methylamine, the initially deep red color of the starting material rapidly changed to yellow. Work-up after 15 minutes allowed isolation of *all-trans*-retinal from the benzene layer in 90% yield and of dimedone from the aqueous layer. Treatment of 2-retinylidene-1,3-cyclopentanedione (**2**) under identical conditions resulted in a colorless two phase mixture. No product was found in the organic phase, but acidification of the aqueous phase led to recovery of the starting material in 88% yield. This difference in reactivity is presumably

attributable to differences in the stability of the initially formed intermediates SB of *Scheme 1*, the intermediate from **2** being sufficiently stable to resist fragmentation. Although many factors may be responsible for the failure of **2** to react (*e.g.* more favorable intramolecular hydrogen bonding between the enolate and the proton(s) of the entering nucleophile, solvation differences *etc.*), the overriding factor may be the resonance stabilization contributed by a tautomeric form of the enolate which contains a 6-π-electron cyclopentadienyl anion *(Scheme 4)*.

Scheme 4



The *Table* summarizes the yields of retinal obtained from several 2-retinylidene-1,3-diketones and their previously reported biological activities. Other conditions that gave less favorable fragmentation of retinylidene-dimedone are listed in the Experimental Section.

These results raise the possibility that biologically active 2-retinylidene-1,3-diketones are simply prodrugs of retinal and/or metabolically derived retinol or retinoic acid. Alternatively, reactivity in the *retro-Knoevenagel* reaction may parallel reactivity in an unknown process by which these retinoids exert their *in vitro* anti-tumor properties. Moreover, the facility with which retinylidene-dimedone and analogs can be converted back into retinal makes these compounds aldehyde-protected forms of retinal capable of chemical transformation elsewhere in the molecule before regeneration of the derived retinal by treatment with methylamine. Thus, retinylidene-dimedone can be oxidized to the 5′,6′-epoxyretinylidene-dimedone, 4′-oxo-retinylidene-dimedone, *etc.*, and the ring-oxidized aldehyde obtained by treatment with aqueous methylamine [5]. The procedure works for 9-*cis*-retinylidene-dimedone as well. Treatment of 9-*cis*-retinylidene-dimedone [1] with methylamine regenerates the aldehyde (85% 9-*cis*) in 83% yield.

Table. Retro-*aldol reaction and biological activities of retinylidene-1,3-diketones*

1,3-Diketo Moiety	Yield[a]) after (min)	Biological Activity[b])
Dimedone	91 (15)	10^{-10}
1,3-Cyclohexanedione	74 (15)	10^{-10}
Acetylacetone	92 (30)	10^{-9}
1,3-Cycloheptanedione	74 (15)	10^{-10}
1,3-Cyclooctanedione	93 (20)	10^{-10}
4,6-Di-*t*-butyl-1,3-cyclohexanedione	27 (24 h)[c])	53% Inactive (10^{-8} M)[c])
1,3-Cyclopentanedione	0 (20)	94% Inactive (10^{-8} M)

[a]) Yields are based on isolated crystalline retinal.

[b]) Reported as ED_{50}'s in the hamster tracheal organ culture assay except for inactive compounds which are reported as the percent of cultures found to be inactive at a particular concentration. See [1].

[c]) The low yield and reactivity are presumably steric in origin.

Experimental Part

Fragmentation of all-trans-*retinylidene-dimedone.* A solution of I ($R^1 = R^2 = CH_3$; 1.06 g, 2.6 mmol) in 25 ml benzene was stirred at RT. for 15 min with 25 ml of 40% aq. $MeNH_2$-solution. The aqueous layer was extracted with benzene, and the light yellow benzene solution washed with 1N NaOH, then brine, dried (Na_2SO_4), concentrated, and passed through silica gel (35 g, ether/hexane 1:1). A solution in petroleum ether (10 ml) cooled to −70° for several hours afforded 700 mg (91%) of *all-trans*-retinal, m.p. 57–61°. Recrystallization from petroleum ether at 0° provided *all-trans*-retinal as large prisms (570 mg), m.p. 61–63° (lit. [6] 61–62°), identical with authentic material by mixed m.p., UV., IR. and 220-MHz-^{1}H-NMR. The aqueous layer was combined with the NaOH-solution washings, extracted with ether, evaporated nearly to dryness, the residue dissolved in water, and acidified with 10% aq. HCl-solution. After standing overnight, crystalline dimedone (222 mg, 61%) was deposited, m.p. 147–148° (lit. [7]: 150°), spectrally identical with an authentic sample. Addition of excess formaldehyde to the dimedone mother liquor provided 54 mg of the dimedone-formaldehyde condensation product, m.p. 185–188° (lit. [8]: 189°), thus raising the total yield of dimedone to 75%.

Attempted fragmentation of **2**. 2-Retinylidene-1,3-cyclopentanedione (121 mg) in 6 ml of benzene and 4.6 ml of 40% aq. methylamine-solution was stirred at RT. for 20 min. The nearly colorless benzene layer was separated from the colorless emulsion-containing aqueous layer and washed with 1N NaOH, then with brine. After drying (Na_2SO_4), removal of solvent left 9 mg of brown residue containing only a trace, at most, of retinal. The original aqueous layer was washed with ether, then acidified with hydrochloric acid to regenerate the deep purple-red color of the starting material. It was extracted three times with chloroform, washed with brine, dried (Na_2SO_4), stripped, redissolved in ether, filtered and stripped to afford 107 mg (88%) of recovered starting material.

*Fragmentation of 2-(9-*cis-*retinylidene)-dimedone.* 2-(9-*cis*-retinylidene)-dimedone (100 mg, 0.246 mmol) was allowed to react for 10 min in 5 ml of benzene and 3 ml of 40% aq. methylamine-solution. Work-up and chromatography as described for the *trans*-isomer, followed by crystallization from petroleum ether at −70° afforded 58 mg (83%) of retinal, m.p. 59.5–62° (lit. [9]: 64.5°), identical with 9-*cis*-retinal by IR. and 220-MHz-^{1}H-NMR. HPLC. analysis (μ-porasil, 1% 2-propanol in trimethylpentane) indicated a mixture consisting of 85% 9-*cis*-derivative and two other isomeric products.

Experimental conditions giving less favorable or no fragmentation of retinylidene dimedone: 1) 40% Aqueous dimethylamine-solution, benzene, RT., 35 min: low yield of impure retinal(s) as a red oil; 2) 25% Aqueous trimethylamine-solution, benzene, RT. overnight: no retinal, some decomposition, but largely recovered starting material; 3) Conc. NH_4OH-solution, benzene, RT., overnight: no reaction; 4) 0.2N Aqueous KOH, methanol, 48°, 30 min: no reaction; 5) 0.2N Aqueous KOH, methanol, 48°, overnight: trace of retinal and several other unidentified products; 6) 5% Aqueous $NaHCO_3$-solution, methanol, RT., overnight: no reaction; 7) 20% Aqueous NaOH-solution, benzene, RT., 25 h: no reaction; 8) 40% Aqueous triethylamine-solution, RT., 1 h: no reaction; 9) Conc. HCl-solution, HOAc, RT., 30 min: trace of retinal and extensive decomposition.

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