trofluorometer equipped with a cooling fan to minimize fluctuations in the xenon lamp source. Wavelength calibration was performed as described in the manual for the instrument. One-centimeter-square cuvettes were used. The excitation wavelength was 525 nm and the emission wavelength was 600 nm. The 100× scale of medium sensitivity was generally used, and water was circulated between the cell compartment and a thermally regulated bath at 22 °C. The fluorometric method to detect CLC (covalently linked complementary) sequences in λ-DNA has been described. Cross-linking of DNA creates a nucleation site which allows renaturation of λ-DNA after heat denaturation (96 °C/3 min) and rapid cooling and thus provides intercalation sites for ethidium.[33,34] That this assay procedure detects the formation of CLC-DNA as a result of a chemical cross-linking event has been confirmed by experiments with the enzyme $S_1$-endonuclease.[34] This enzyme specifically cleaves single-stranded DNA and is essentially inactive on duplex DNA; therefore, it distinguishes DNA which is renaturable by virtue of a chemical cross-link and DNA which separates into single strands on heating. A 20-μL aliquot was taken at intervals from the reaction mixture (50 mM potassium phosphate, pH 7.2; 1.0 $A_{260}$ unit of λ-DNA; 5 mM nitrosourea; total volume 200 μL) at 37 °C and added to the standard assay mixture (which was 20 mM potassium phosphate, pH 11.8; 0.4 mM EDTA; and 0.5 μg/mL of ethidium). The fluorescence after the heating and cooling cycle compared with control times 100 gives the percentage of CLC-DNA, i.e., DNA molecules containing at least one cross-link in a sample. For a standard set of conditions (i.e., type and concentration of DNA, pH, ionic strength, and the temperature), the accuracy of the CLC assay is determined by the precision of the fluorescence readings. Overall accuracy of the CLC assay is estimated at ±2%.

**Fluorescence Determination of Alkylation of PM2-CCC-DNA by Nitrosoureas.** A 20-μL aliquot was taken at intervals from the reaction mixture [50 mM potassium phosphate, pH 7.2; 1.2 $A_{260}$ units of PM2-CCC-DNA (90% CCC); 5 mM nitrosourea or other alkylating agent in a total volume of 200 μL at 37 °C] and added to the standard assay mixture (which was 20 mM potassium phosphate, pH 11.8; 0.4 mM EDTA; and 0.5 μg/mL of ethidium). The fluorescence after heating at 96 °C/4 min, followed by rapid cooling, was compared with the initial value.

Under these conditions, unreacted PM2-CCC-DNA returns to register after heat denaturation because of topological constraints. Alkylated PM2-CCC-DNA shows a decrease in fluorescence because of thermally induced depurination followed by alkaline strand scission of the apurinic site in the assay medium.[35] The ratio of the decrease in fluorescence (after the heating and cooling cycle) to that of the control is a measure of the extent of alkylation. In a control experiment it was shown that none of the components interfered with the ethidium fluorescence. Provided no single-strand cleavage of PM2-CCC-DNA is observed (detected by the characteristic rise in fluorescence before the heating/cooling cycle), this technique can be used to measure levels of alkylation not readily observed using λ-DNA.

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# Potential Prophylactic Antitumor Activity of Retinylidene 1,3-Diketones

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Treatment of *all-trans*-retinal with a series of 1,3-diketones using Knoevenagel conditions gave the expected condensation products. These retinylidene 1,3-diketones were characterized and their biological activities in the hamster tracheal organ culture test measured. It was found that the cyclohexane-1,3-dione derivatives are highly active in this in vitro assay, while other 1,3-diketones are less active. Retinylidenedimedone has been chosen for further evaluation.

The search for retinoids with improved systemic tolerance over that of retinal, retinol, or retinoic acid has focused attention on derivatives of retionic acid with a modified end group.[1,3] The finding that the known condensation product of retinal with acetylacetone[2] had substantial activity in the tracheal organ culture test[3] prompted a chemical effort to synthesize a variety of condensation products of retinal with aliphatic and alicyclic 1,3-diketones. The preparation of these retinylidene diketones,[4] their physical properties, and their biological

Scheme I

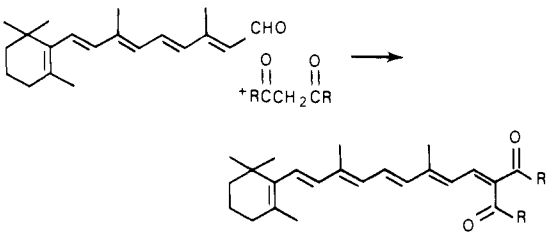


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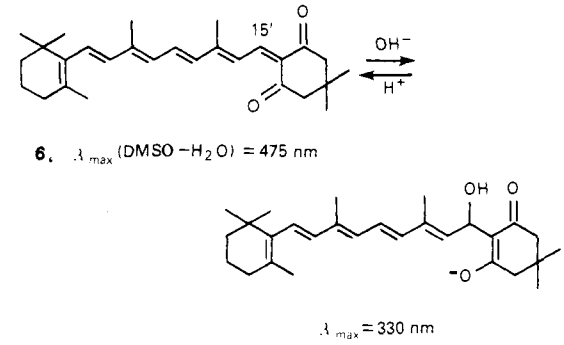
Table I.[a]

| no. | ketone[b] | mmol of retinal | catalyst[c] | yield, % | mp, °C | UV-Vis solvent | UV-Vis $\lambda_{max}$ ($\epsilon$) | %$A_1$ 3 h[d] | formula | anal. | $\Delta\nu$[e] | TOC $ED_{50}$, M (no. of cultures) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | acetylacetone | 36.6 | P A | 48–55 | 96.5–97[f] | EtOH | 423 (46 700)<br>270 (7 760) | 85 | $C_{25}H_{34}O_2$ | C, H | 246 | $1.8 \times 10^{-9}$ (86) |
| | | | | | | $CH_3CN$ | 414 (55 500)<br>275 (8 820) | | | | | |
| | | | | | | hexane | 414 (59 700)<br>275 (7 770) | | | | | |
| 2 | heptane-3,5-dione | 8.1 | P A | 10 | 79.5–81.5 | EtOH | 420 (45 400)<br>270 (7 950) | 85 | $C_{27}H_{38}O_2$ | C, H | 250 | act., 4/5, $10^{-8}$ M<br>inact, 7/7, $10^{-9}$ M (12) |
| 3 | nonane-4,6-dione | 12.1 | P A | 23 | 98.5–100 | EtOH | 418 (48 400)<br>275 (10 550) | 85 | $C_{29}H_{42}O_2$ | C, H | 247 | inact, 6/7, $10^{-8}$ M (14) |
| 4 | cyclopentane-1,3-dione | 3.8 | P A | 49 | 148–149 | EtOH | 520 (35 300)<br>265 (9 180) | 0 | $C_{25}H_{32}O_2$ | C, H | 0 | inact, 16/17, $10^{-8}$ M (29) |
| | | | | | | $CH_3CN$ | 500 (42 200)<br>275 (9 180) | | | | | |
| | | | | | | hexane | 484 (55 500)<br>280 (11 480) | | | | | |
| 5 | cyclohexane-1,3-dione | 35.2 | P | 89 | 114–115 | EtOH | 489 (45 000)<br>260 (12 850) | 0 | $C_{26}H_{34}O_2$ | C, H | 90 | $1.1 \times 10^{-10}$ (107) |
| | | | | | | $CH_3CN$ | 475 (49 800)<br>280 (15 480) | | | | | |
| | | | | | | hexane | 465 (54 700)<br>275 (14 330) | | | | | |
| 6 | dimedone | 35 | P | 85 | 122.5–124.5 | EtOH | 490 (49 800)<br>270 (12 680) | 0 | $C_{28}H_{38}O_2$ | C, H | 79 | $2.4 \times 10^{-10}$ (150) |
| 7 | 5-methylcyclohexane-1,3-dione | 3.7 | P | 20 | 108–109 | EtOH | 492 (47 300)<br>270 (10 380) | 0 | $C_{27}H_{36}O_2$ | C, H | 83 | $8.0 \times 10^{-10}$ (25) |
| 8 | 5-ethylcyclohexane-1,3-dione | 7.2 | P | 50 | oil | EtOH | 495 (34 100)<br>280 (13 000) | 0 | oil | not analyzed | 83 | $3.6 \times 10^{-10}$ (27) |
| 9 | 5-isopropylcyclohexane-1,3-dione | 3.6 | P | 21 | 88–90 | EtOH | 492 (46 000)<br>270 (11 710) | 0 | $C_{29}H_{40}O_2$ | C, H | 86 | $2.1 \times 10^{-10}$ (28) |
| 10 | 5-phenylcyclohexane-1,3-dione | 3.6 | P | 35 | 124–126 | EtOH | 495 (46 500)<br>270 (10 530) | 0 | $C_{32}H_{38}O_2$ | C, H | 85 | $1.0 \times 10^{-10}$ (39) |
| 11 | 5-[*p*-(trifluoromethyl)-phenyl]cyclohexane-1,3-dione | 3.9 | P | 43 | 157–158 | EtOH | 495 (42 500)<br>265 (10 720) | 0 | $C_{33}H_{37}O_2$ | C, H, F | 88 | $1.0 \times 10^{-10}$ (27) |
| 12 | 5-*p*-anisylcyclohexane-1,3-dione | 3.6 | P | 35 | 127–129 | EtOH | 495 (48 100)<br>270 (12 650) | 0 | $C_{33}H_{40}O_3$ | C, H | 85 | $1.8 \times 10^{-10}$ (26) |
| 13 | 4,6-di-*tert*-butylcyclohexane-1,3-dione | 3.6 | P | 53 | 143.5–144 | EtOH | 467 (52 000)<br>283 (23 400) | 89 | $C_{34}H_{50}O_2$ | C, H | 135 | inact, 8/15, $10^{-9}$ M<br>inact, 9/12, $10^{-10}$ M (25) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | indan-1,3-dione | 3.5 | P | 70 | 175–176 | EtOH | 507 (55 300)<br>275 (18 500) | 73 | $C_{29}H_{32}O_2$ | C, H | inact, 9/12, $10^{-8}$ M (22) |
| 15 | cyclopentene-1,3-dione | 3.5 | P | 34 | 177–178 | EtOH | 465 (48 600)<br>275 (11 590) | 35 | $C_{25}H_{30}O_2$ | C, H | 23 | inact, 3/4, $10^{-9}$ M (10) |
| 16 | cycloheptane-1,3-dione | 3.8 | P<br>A | 64 | 90–91 | EtOH | 450 (46 700)<br>275 (12 100) | 78 | $C_{27}H_{36}O_2$ | C, H | 217 | $3.8 \times 10^{-10}$ (51) |
| 17 | cyclooctane-1,3-dione | 3.9 | P<br>A | 56 | 131.5–132.5 | EtOH | 432 (49 800)<br>280 (10 740) | 82 | $C_{28}H_{38}O_2$ | C, H | 283 | $2.2 \times 10^{-10}$ (37) |
| 18 | cyclononane-1,3-dione | 4.0 | P<br>A | 55 | 108.5–110.5 | EtOH | 428 (47 000)<br>277 (10 500) | 85 | $C_{29}H_{40}O_2$ | C, H | 277 | $2.4 \times 10^{-10}$ (36) |
| 19 | cyclotetradecane-1,3-dione | 2.3 | P<br>A | 3 | 96–100 | EtOH | 425 (51 500)<br>275 (10 650) | 86 | $C_{34}H_{50}O_2$ | C, H | ~260 [h] | inact, 12/16, $10^{-9}$ (32) |
| 20 | [3]ferrocenophane-1,3-dione | 3.0 | P | 52 | 141–143 | EtOH | 460 (29 800)<br>300 (21 100) | 60 | $C_{33}H_{36}FeO_2$ | C, H, Fe | 224 | inact, 14/16, $10^{-9}$ (32) |

[a] All reactions were run at room temperature under argon in either benzene or toluene for ca. 18–20 h. In small-scale experiments, no attempt was made to maximize yields. [b] Ketone was used in about 10% excess. [c] P = piperidine; A = acetic acid. About 50 μL of each component was used per gram of retinal. [d] Percent of initial absorption at $\lambda_{max}$ after 3-h exposure to daylight of a $10^{-5}$ M solution in ethanol. [e] Reference 13; $\Delta\nu$ in hertz. [f] Lit. 99–101 °C (ref 2). [g] The AB quartet is obscured by the aromatic protons. [h] The H-14' absorption is not resolved from the remaining olefinic protons.

Scheme II




activities will now be reported.[5]

**Chemistry.** Retinylideneacetylacetone (1) was obtained in 36% yield by Haeck, Kralt, and van Leeuwen[2] who condensed retinal with acetylacetone in benzene as solvent and using piperidine as the Knoevenagel catalyst. We have used this general procedure (Scheme I) with one modification to prepare a series of 20 retinylidene 1,3-diketones in moderate to good yields.

In our hands, the yield of retinylideneacetylacetone (1) was considerably lower than that reported, especially when the reaction was run on a large (10 g of retinal) scale. We found that addition of acetic acid to the mixture increased the yield to about 50% (Table I). Therefore, we used in situ generated piperidinium acetate for preparations of retinylidene derivatives of diketones of relatively low acidity, i.e., for the acyclic diketones and for the 7-, 8-, 9-, and 14-membered cyclic diketones. Using piperidine alone in the reaction of retinal with heptane-1,3-dione or with nonane-4,6-dione gave mixtures from which no clean product could be isolated. Changing the catalyst to piperidinium acetate allowed isolation of modest yields of clean products (Table I). Cyclohexane-1,3-dione and cyclopentane-1,3-dione derivatives gave good yields of condensation products using piperidine alone.[6-8]

The preparation of retinylidenedimedone (6),[9] the most extensively studied derivative in this series, is used to illustrate our procedure (see Experimental Section). Treating retinal with a slight excess of dimedone in toluene solution using piperidine as catalyst gave a 75–85% yield of product after purification by chromatography on silica gel and recrystallization from an ether–petroleum ether mixture. This dark red compound of mp 122.5–124.5 °C, with six conjugated double bonds in its nonenolized form, showed UV maxima in 95% ethanol at 490 (ε 49 800) and at 270 nm (12 600). The electronic spectrum of this compound undergoes a large hypsochromic shift on treatment with base. A sample dissolved in dimethyl sulfoxide and then treated with 0.1 N NaOH exhibits a change in $\lambda_{max}$ from 475 to 330 nm. This shift of $\lambda_{max}$ is reversible, since

(4) The term retinal or retinylidene refers to all-trans compounds unless otherwise specified.

(5) A preliminary account of this study was presented by N.A. (NIAMDD) and D.L.N. (NCI) at a workshop held at the National Institutes of Health, Bethesda, Md., on Oct 25–26, 1978.

(6) The $pK_a$ of acetylacetone is 9.0. That of cycloheptane-1,3-dione is 6.5, while that of cyclohexane-1,3-dione is 5.1.[7] For comparison, the $pK_a$ of acetic acid is 4.7.[8]

(7) Eistert, B.; Haupter, F.; Schank, K. *Justus Liebigs Ann. Chem.* **1963**, *665*, 55.

(8) Gordon, A. J.; Ford, R. A. "The Chemist's Companion"; Wiley: New York, 1972; p 58.

(9) 2-Retinylidene-5,5-dimethyl-1,3-cyclohexanedione; IUPAC: (*all-E*)-2-[3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenylidene]-5,5-dimethyl-1,3-cyclohexanedione.

acidification regenerates the 475-nm absorption. An absorption at 330 nm in the retinoid system indicates a chromophore with five conjugated double bonds,[10,11] and we believe that this reversible bleaching is the result of attack of hydroxide at C-15′ (Scheme II). This behavior is shared by the other six-membered cyclic diketones (except **13** in which the bulky *tert*-butyl groups may hinder attack by hydroxide) and by the cyclopentane-1,3-dione derivative, but not the acyclic or 7-, 8-, 9-, or 14-membered cyclic diketone derivatives.

In common with other retinoids, retinylidenedimedone is relatively unstable in light, especially when in dilute polar solutions. A $10^{-5}$ M solution of **6** in 95% ethanol left in daylight will bleach entirely within about 3 h. This decomposition results in a shift in the absorption maximum to 250 nm, indicating an extensive disruption of the chromophore. Fragmentation is also indicated by high-pressure liquid chromatography, which shows several components for this photodecomposed solution. This photosensitivity is shared by the other retinylidene diketones, but the decomposition is very slow (several days) for the acyclic derivatives and for the 7-, 8-, 9-, and 14-membered cyclic derivatives. Of the six-membered cyclic diketones, the di-*tert*-butyl derivative (**13**) is relatively stable toward photochemical degradation: a $10^{-5}$ M solution left in daylight for 6 days still retained about 40% of the long-wavelength absorption. Derivatives **14** and **15** are also relatively light stable.

Nuclear magnetic resonance spectra for all derivatives are in accord with the expected structures. The $^1H$ and $^{13}C$ NMR spectra for retinylidenedimedone (**6**) are reported in detail under Experimental Section. In the proton spectra for this series of compounds, all upfield retinylidene resonances are virtually identical with reported absorptions for other retinoid derivatives.[12] The olefinic regions of the $^1H$ NMR spectra are similar, except for the spacing between the two halves of the AB quartet ($J$ = 12.5–13.5 Hz for all derivatives) associated with H-14′ and H-15′. The values for $\Delta\nu$[13] are listed in Table I. These values show a linear relationship with the $\lambda_{max}$ values[14] and presumably reflect the extent to which ring constraints ensure overlap of the 1,3-diketone chromophore with the extended conjugated double bond system.

This synthetic procedure is not applicable to the preparation of derivatives of 13-*cis*-retinal. Treating 13-*cis*-retinal with acetylacetone using either piperidine or piperidinium acetate as catalyst resulted in conversion of the 13-*cis*-retinal to *all-trans*-retinal (monitored by thin-layer chromatography) and the final isolation of the all-trans product (**1**) in 28% yield. However, piperidine does not readily isomerize 9-*cis*-retinal to the all-trans derivative. Treatment of 9-*cis*-retinal with dimedone under the usual condensation conditions led to the formation of 9-*cis*-retinylidenedimedone, mp 99–104 °C. Although this material had the same $R_f$ as *all-trans*-retinylidenedimedone (TLC: silica, 3:1 hexane–ether; high-pressure LC: $\mu$-Porasil, 1% isopropyl alcohol in trimethylpentane), the $^1H$ NMR spectrum confirms that it is the 9-cis derivative. The absorption for H-8′ appears at 6.70 ppm vs. 6.25 for the all-trans isomer. This shift in the position of H-8 is comparable to the shift observed for the retinal isomers: *all-trans*-retinal has H-8 at 6.15 ppm, whereas H-8 is at 6.67 ppm in 9-*cis*-retinal.

**Biology.** The hamster tracheal organ culture assay measures the intrinsic ability of retinoids to control epithelial cell differentiation and is believed to have significant predictive value for the potential use of a new retinoid for the prevention of epithelial cancer. The procedure has been described previously.[15–17] Table I contains the tracheal organ culture test results for these compounds. Results are reported as $ED_{50}$ values, except in cases in which the compound was considered to be inactive. In these cases the results are reported as the number of cultures which were active or inactive compared with the total number of cultures. Activities were measured at $10^{-8}$, $10^{-9}$, and $10^{-10}$ M concentrations. For comparison, *all-trans*-retinoic acid, the most active compound found thus far in the tracheal organ culture system, has an $ED_{50}$ of $1 \times 10^{-10}$ (>1000 cultures).

The cyclohexane-1,3-dione derivatives (except for the sterically hindered **13**) are all highly active in this in vitro test system, some with activities approaching that of retinoic acid. The seven-, eight-, and nine-membered ring diketones (**16–18**) also have high activities, while the cyclopentane-1,3-dione derivatives (**4**, **14**, and **15**) are inactive at the concentrations used in this study. Although the acetylacetone derivative (**1**) has a relatively high activity, its two homologues (**2** and **3**) are much less active.

The reason for the observed variations in in vitro activity is not clear. However, the high activities of some of these derivatives, coupled with the previously demonstrated low toxicities of two of them,[3] make these compounds interesting candidates for further antitumor investigations. We are currently conducting additional toxicity tests for some of these derivatives (**6**, **16**, and **17**) as well as some in vivo antitumor tests. These results will be reported at a later time.

## Experimental Section

**Technical.** Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory of NIAMDD, NIH. Proton NMR spectra (220 MHz) were obtained with a Varian HR 220 spectrometer and are in $CDCl_3$ solution unless otherwise indicated. A Beckman IR 4230 spectrophotometer was used to obtain infrared spectra, which are reported in reciprocal centimeters and in $CHCl_3$ solution unless otherwise indicated. Mass spectra were determined on a Finnegan 1015 D instrument (chemical ionization) or on a Perkin-Elmer Hitachi RMU 6D (electron impact). Ultraviolet–visible spectra were measured using a Beckman DB-G grating spectrophotometer. Chromatographies utilized Merck silica gel 60, 70–230 mesh.

**Chemicals.** *all-trans*-Retinal was obtained from Eastman Organic Chemical Co. Diketones required for the preparation of 1–6, 14, and 15 were commercial samples (Aldrich Chemical Co.). Diketones required for the preparation of **9–12** were gifts from Dr. W. Dürckheimer of Hoechst, Germany.[18] Cyclotetradecane-1,3-dione was a gift from Dr. Anton Stütz, Sandoz, Forschungsinstitut, Vienna, Austria.[19] Dr. Fritz Frickel, BASF Aktiengesellschaft, Ludwigshafen, Germany, provided a generous sample of 9-*cis*-retinal.

(10) *all-trans*-Retinol absorbs at 325 nm in ethanol.[11]
(11) Isler, O. In "Carotenoids"; Isler, O., Ed.; Birkhäuser Verlag: Basel, 1971; p 18.
(12) For example, see Vetter, W.; Englert, G.; Rigassi, N.; Schwieter, U. ref 11; p 214.
(13) $\Delta\nu$ = [(line 1 − line 4)(line 2 − line 3)]$^{1/2}$: Gordon, A. J.; Ford, R. A. ref 8; p 307.
(14) Slope = −2.54 ± 0.31, 95% confidence level.
(15) Clamon, G. H.; Sporn, M. B.; Smith, J. M.; Saffiotti, U. *Nature (London)* **1974**, *250*, 64.
(16) Sporn, M. B.; Clamon, G. H.; Dunlop, N. M.; Newton, D. L.; Smith, J. M.; Saffiotti, U. *Nature (London)* **1975**, *253*, 47.
(17) Sporn, M. B.; Dunlop, N. M.; Yuspa, S. H. *Science* **1973**, *182*, 722.
(18) We are grateful to Dr. Jürgen Reden of Hoechst, Germany, for obtaining these diketones for us.
(19) Stütz, A.; Reinshagen, H. *Tetrahedron Lett.* **1978**, 2821. Fliri, H. G.; Scholz, D.; Stütz, A. *Monatsh. Chem.* **1979**, *110*, 245.

5-Methylcyclohexane-1,3-dione and 5-ethylcyclohexane-1,3-dione were made by condensing diethyl malonate with 3-penten-2-one and with 3-hexen-2-one, respectively.[20] Cycloheptane-1,3-dione, cyclooctane-1,3-dione, and cyclononane-1,3-dione were prepared by a procedure adumbrated by Y. Ito et al.[21] [3]Ferrocenophane-1,3-dione was prepared according to M. Sališová et al.[22]

**Syntheses. General Procedure for Preparing Retinylidene Diketones.** Retinal (1.0 g, 3.5 mmol) and the diketone (ca. 3.8 mmol) were dissolved in 30 mL of benzene or toluene, catalyst was added, and the mixture was stirred overnight under argon at room temperature in the dark. After the solvent was removed on a rotary evaporator, the residue was passed through silica gel (150 g), eluting with 3:1 hexane ether. A heart cut of the major colored band was collected, stripped, and recrystallized from an ether-petroleum ether (30-60 °C) mixture.

**2-Retinylidene-5,5-dimethyl-1,3-cyclohexanedione (6).** *all-trans*-Retinal (10 g, 35 mmol) was dissolved in 200 mL of toluene. Dimedone (5.5 g, 39 mmol) was added, followed by 8 drops of piperidine. After stirring at room temperature under argon for 18 h, the dark red solution was stripped, passed through silica gel (350 g) eluting with 3:1 hexane-ether, and recrystallized from ether-petroleum ether to yield 10.8 g (75%) of product: mp 119-121 °C; $^1$H NMR $\delta$ 1.05 and 1.07 [s, 12 H, 1′,1′-$(CH_3)_2$ and 5,5-$(CH_3)_2$], 1.50 (m, 2.27 H, H-2′), 1.63 (m, 1.96 H, H-3′), 1.74 (s, 3.14 H, 5′-$CH_3$), 2.05 (s superimposed on m, 4.93 H, 9′-$CH_3$ and H-4′), 2.24 (s, 2.97 H, 13′-$CH_3$), 2.52 (s, 3.91 H, H-4 and H-6), 6.25 (d, $J$ = 16 Hz, 0.98 H, H-8′), 6.27 (d, $J$ = 12 Hz, 0.98 H, H-10′), 6.40 (d, $J$ = 16 Hz, 1.09 H, H-7′), 6.62 (d, $J$ = 15 Hz, 0.94 H, H-12′), 7.18 (d of d, $J$ = 15 Hz, $J'$ = 12 Hz, 1.02 H, H-11′), 7.84 (d, $J$ = 13 Hz, 0.94 H, H-14′), 8.21 (d, $J$ = 13 Hz, 0.94 H, H-15′); IR ($CCl_4$) 2950 (m), 2920 (w), 2862 (w), 2820 (w), 1688 (w), 1647 (m), 1510 (s), 1443 (w), 1388 (w), 1372 (m), 1335 (w), 1292 (w), 1227 (w), 1268 (w), 1240 (m), 1175 (m), 1157 (w), 1136 (w), 1110 (w), 965 cm$^{-1}$ (m); EIMS (70 eV) M$^+$ 406 (44%); $^{13}$C NMR ($CDCl_3$)[23] $\delta$ 12.92, 13.04 (9′- and 13′-$CH_3$), 19.07 (C-3′), 21.68 (5′-$CH_3$), 28.39 and 28.86 [1′,1′-$(CH_3)_2$ and 5,5-$(CH_3)_2$], 29.88 (C-5), 33.05 (C-4′), 34.07 (C-1′), 39.46 (C-2′), 52.06 and 53.88 (C-4 and C-6), 126.36, 128.17, 129.49, 130.04, 130.25, 132.74, 136.29, 136.81, 137.23, 141.30, 144.61, and 157.76 (olefinic carbons), 197.19 and 198.24 (carbonyl carbons). Anal. ($C_{28}H_{38}O_2$) C, H.

**9-*cis*-Retinylidenedimedone.** A benzene solution (30 mL) of 9-*cis*-retinal (521 mg, 1.84 mmol), dimedone (260 mg, 1.85 mmol), and piperidine (3 drops) was stirred at room temperature for 22 h. After chromatography (150 g of silica gel, 1:1 ether-hexane) and crystallization from cold petroleum ether, 190 mg (25%) of product was obtained, mp 99-104 °C; $^1$H NMR $\delta$ 1.07 and 1.09 [2s, 11.20 H, 1′,1′- and 5,5-$(CH_3)_2$], 1.50 (m, 2.16 H, H-2′), 1.64 (m, 2.07 H, H-3′), 1.77 (s, 3.06 H, 5′-$CH_3$), 2.06 (s superimposed on m, 5.32 H, 9′-$CH_3$ and 4′-$CH_2$), 2.24 (s, 2.89 H, 13′-$CH_3$), 2.53 (s, 3.88 H, H-4 and H-6), 6.15 (d, $J$ = 11.5 Hz, 0.90 H, H-10′), 6,36 (d, $J$ = 15.5 Hz, 1.08 H, H-7′), 6.52 (d, $J$ = 15.0 Hz, 0.90 H, H-12′), 6.70 (d, $J$ = 15.5 Hz, 0.99 H, H-8′), 7.23 (d of d, $J$ = 15.0 Hz, $J'$ = 11.5 Hz, 0.99 H, H-11′), 7.80 (d, $J$ = 13 Hz, 0.90 H, H-14′), 8.20 (d, $J$ = 13 Hz, 0.99 H, H-15′); IR ($CCl_4$) 2960 (m), 2940 (m), 2890 (w), 2880 (w), 2835 (w), 1693 (w), 1652 (s), 1512 (s), 1468 (w), 1448 (w), 1388 (m), 1375 (m), 1335 (m), 1295 (w), 1278 (w), 1260 (w), 1240 (m), 1200 (w), 1172 (m), 1145 (m), 1115 (w), 1027 (w), 964 cm$^{-1}$ (m); UV $\lambda_{max}$ (EtOH) 483 nm ($\epsilon$ 41 500), 255 (12 400); MS (CI, $NH_3$) M$^+$ 407.[24]

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(24) **Note Added in Proof:** TOC assay shows the 9-cis derivative to be inactive in six out of eight cultures at $10^{-9}$ M.

# *Notes*

## Simple β-Lactam Compounds Derived from 6-Aminopenicillanic Acid

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As part of a general program of structural modification in β-lactam antibiotics, we have synthesized several simple penicillins from 6-aminopenicillanic acid where the C-3 carboxyl group has been replaced by a hydroxy or an acetoxy group and the C-6 side chain has been substituted by bromine or hydrogen. Some of the compounds exhibit mild activity against the Gram-positive strain *Bacillus subtilis*.

Structural modifications at the C-3 position of penicillin and C-4 position of cephalosporin seem to indicate that the acid group at these positions is necessary for biological activity. Variations at the C-3 carboxyl group resulting in penicillin compounds with improved or diminished biological activity have been discussed in recent reviews.[1,2] We have synthesized several simple penicillins where the C-3 carboxyl group has been replaced by a hydroxy or an acetoxy group and the C-6 side chain has been substituted by bromine or hydrogen. Interest in bromine and hydrogen as the C-6 substituents arises from recent reports[3,4]

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