π-Complexes with Biologically Significant Materials. III.[1] Vitamin A Acetate Iron Tricarbonyl

AKIRA NAKAMURA AND MINORU TSUTSUI

New York University, Research Division, Department of Chemical Engineering, New York 53, New York

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The first isolation of an organometallic π-complex of vitamin A acetate is reported. The reaction of vitamin A acetate with triiron dodecacarbonyl followed by chromatography and sublimation gave vitamin A acetate iron tricarbonyl (I) as an orange semisolid in *ca.* 20% yield. Deacetoxylation of vitamin A acetate occurred at the same time to the extent of 60%. Iron tricarbonyl π-complexes of the deacetoxylated products, namely anhydro vitamin A and its dimer, were also obtained as reaction products.

In the previous reports of this series the formation of organometallic π-complexes with biologically significant materials such as estrone[1] and acetylergosterol[2] has been reported. The objective of this research is to investigate the possible occurrence of π-bonding in biological media. The significance of biological π-bonding has been pointed out by one of us previously.[3] A transition metal–alkyl bond, which is closely related to an olefin π-complex, has been postulated recently as intermediate in an enzymatic reaction involving a coenzyme.[4] It is also possible to explain some enzymatic reactions by means of a π-complex mechanism. Vitamin A acetate, in addition to being significant in biological π-complex formation, is an interesting ligand from the organometallic point of view.

Although a number of iron carbonyl complexes[5] have been prepared with cyclic conjugated polyene systems such as cyclooctatetraene, linear conjugated polyene systems have been studied only up to 1,3,5-hexatriene. Linear hydrocarbons containing more than four conjugated double bonds have not been investigated for the formation of π-complexes. The reaction of vitamin A acetate, which contains five conjugated double bonds, with iron carbonyl compounds is thus of interest.

Vitamin A acetate was allowed to react with triiron dodecacarbonyl in a molar ratio of 1:1.1 in refluxing benzene under nitrogen for 6 hr. During the course of the reaction some gas evolution and precipitation of a brown solid were observed. The brown precipitate was removed by filtration and identified as ferrous acetate. The yellow filtrate was evaporated to give a yellow semisolid. Alumina chromatography of the semisolid gave two major fractions. One fraction gave an orange semisolid in *ca.* 20% yield on short pass sublimation *in vacuo* at 120° and was found to have the composition (vitamin A acetate)·$Fe(CO)_3$ (I). The infrared spectrum of I showed strong peaks at 1980 and 2040 $cm.^{-1}$ due to the presence of an iron tricarbonyl group[5] π-bonded to the vitamin A acetate. The presence of peaks at 1745 and 1232 $cm.^{-1}$ indicated the presence of an acetoxy group in the complex. The position of the C=O and C—O stretching frequencies in the complex is almost identical with those of vitamin A acetate itself, *viz.*, 1743 and 1230 $cm.^{-1}$. The ultraviolet spectrum had absorption maxima at 332 and 288 mμ. These values for the maxima may be compared with that of vitamin A acetate, 327 mμ. Ultraviolet absorption maxima of conjugated dienes have been shown to shift towards shorter wave lengths on π-complex formation with the iron tricarbonyl group.[2,6] The splitting of the maximum at 327 mμ of vitamin A acetate into two peaks on complex formation may be due to a "π-complex effect." The n.m.r. spectrum of the compound was found to be very complex. However, it was possible to assign almost all strong peaks to the proposed structure by a comparison with the n.m.r. spectrum of vitamin A acetate itself.[7] Peaks due to the protons around the six membered ring of the vitamin A structure were observed in the n.m.r. spectrum of the π-complex. Therefore the six-membered ring may not participate in π-complex formation with the iron tricarbonyl group. However, the exact location of π-bonding in the vitamin A structure could not be determined unequivocally by the n.m.r. evidence alone.

Considering the structure of I, at least four possible isomers are expected because iron tricarbonyl complexes have been reported to form through bonding with two adjacent conjugated double bonds in the cisoid form,[5] and vitamin A acetate has five conjugated double bonds, $\Delta^{5,7,9,11,13}$ in a chain. However, crystalline vitamin A acetate has its double bonds all in the transoid form. Bonding through the double bonds at the 5- and 7-positions (Ia) is not likely because of steric hindrance resulting from the proximity of the *gem*-dimethyl group in the 1-position. If structure Ia is correct, the *gem*-dimethyl group should show a splitting in the n.m.r. spectrum since one methyl group is in close proximity to the iron. However, a very sharp n.m.r. peak of I showed this is not the case. π-Complex formation through the double bonds at the 11- and 13-positions (Id) would probably lead to deacetoxylation since allyl acetate has been reported[8] to react with nickel tetracarbonyl giving a deacetoxylated product, diallyl.

$$CH_2{=}CHCH_2OAc \xrightarrow{Ni(CO)_4} (CH_2{=}CHCH_2{-})_2$$

Bonding through the double bonds at positions 7, 9

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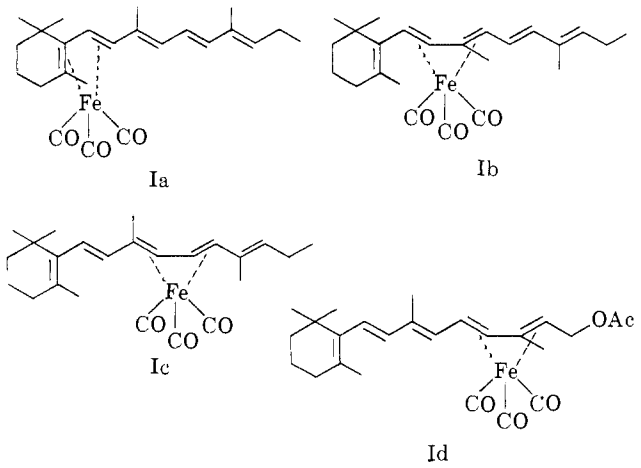
(6) B. F. Hallam and P. L. Pauson, *J. Chem. Soc.*, 642 (1958).

(7) Description of these n.m.r. spectra will be found in the Experimental section.

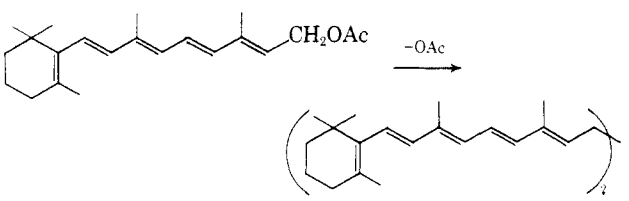
(8) N. L. Bauld, *Tetrahedron Letters*, **No. 19**, 859 (1962).



(Ib) and 9, 11 (Ic), respectively, is most probable for I. Compound I may consist of a mixture of isomers, Ib and Ic. However, the ease with which the iron tricarbonyl group migrates along the conjugated polyene system might finally result in only one isomer, Ic or Id. In isomer Ic the methyl group gives some steric compression and this isomer may be less stable than Ib for this reason. Structure Ib is preferred therefore for I.

The other chromatographic fraction of the reaction mixture was sublimed at 100° (0.3 mm.). A small amount of a yellow oil (II) was obtained as sublimate. This oil showed strong peaks due to the $Fe(CO)_3$ group in the infrared and an absence of peaks due to an acetoxy group. In the ultraviolet region II showed a maximum at 324 mμ. The elemental analysis gave an approximate composition, $C_{20}H_{28}\cdot Fe(CO)_3$ or $C_{20}H_{30}\cdot Fe(CO)_3$. Following removal of II by sublimation, a yellow solid (III) was obtained as sublimation residue. This solid was further purified by chromatography. The infrared spectrum of III indicated the presence of an $Fe(CO)_3$ group and the absence of an acetoxy group. An absorption maximum at 327 mμ and a shoulder at 318 mμ were observed in the ultraviolet region. The elemental analysis and molecular weight determination indicated that III is possibly a mixture of π-complexes and has a composition near $2(C_{40}H_{56})Fe(CO)_3\cdot(C_{40}H_{56})\text{-}Fe_2(CO)_6$.

The formation of II and III which contain no acetoxy groups indicates that deacetoxylation of vitamin A acetate occurred during the reaction with triiron dodecacarbonyl. Since ferrous acetate was found in 60% yield from the reaction, deacetoxylation is estimated to occur to the extent of 60%. As mentioned previously, allyl acetate gives nickel acetate and diallyl on reaction with nickel carbonyl. Since vitamin A acetate is a derivative of allyl acetate, the reaction with triiron dodecacarbonyl may give ferrous acetate and a dimer of anhydrovitamin A in a similar manner. Complex



formation of the dimer with the iron tricarbonyl group could lead to III. The position of the bonding with the iron tricarbonyl group is not clear at present and awaits further study.

The iron carbonyl complexes, I, II, and III, are all unstable in air at room temperature. However, the complexes are stable under nitrogen at −20°. Attempts to crystallize these complexes have been unsuccessful so far.

The reaction of vitamin A acetate with iron pentacarbonyl in ethylcyclohexane proceeded less readily than with the triiron compound even when the temperature was high (140°). The product in both cases were essentially identical. The yield of II increased to *ca.* 5% with iron pentacarbonyl. The reaction with nickel carbonyl gave also deacetoxylated products without formation of π-complexes. Anhydrovitamin A was identified from the products. The reaction with chromium hexacarbonyl in di-*n*-butyl ether gave deacetoxylated products in 20% yield but the reaction was rather sluggish even at 140° for 18 hr.

Experimental

The crystalline vitamin A acetate used was a product of Hoffmann-LaRoche or the U. S. Vitamin Co. Iron pentacarbonyl, chromium hexacarbonyl, nickel tetracarbonyl, and organic solvents used in the experiments were commercial products. Triiron dodecacarbonyl was prepared by a method essentially the same as that described in the literature.[9] Merck aluminum oxide, acid washed, was deactivated by addition of a small amount (1%) of glacial acetic acid for use in chromatography.

Infrared spectra were measured by the Perkin-Elmer Infracord 137B. Ultraviolet spectra were determined using a Beckman DK-2 and a Bausch & Lomb Spectronic 505 in spectrograde heptane. N.m.r. spectra were taken on a Varian A-60 in carbon tetrachloride using tetramethylsilane as an internal standard. Chemical shifts were measured in τ-values.

Reaction of Vitamin A Acetate with Triiron Dodecacarbonyl.—Vitamin A acetate (2.0 g., 6.02 mmoles) and triiron dodecacarbonyl (3.33 g., 6.6 moles) were heated at gentle reflux under nitrogen in 20 ml. of benzene for 6 hr. During the course of the reaction *ca.* 200 ml. (8 mmoles) of a gas was evolved. The initial deep green color of the reaction mixture faded as the reaction proceeded and finally turned to brown. At the same time a brown precipitate formed. It was removed by filtration and the yellow filtrate was evaporated under reduced pressure below room temperature to give a yellow semisolid. The semisolid was separated quickly by chromatography on deactivated alumina in air. Elution with hexane and with hexane–benzene mixture (5:1 to 1:1) gave, on evaporation *in vacuo* below room temperature, a yellow semisolid. Sublimation of the semisolid at 120° (0.3 mm.) yielded a yellow viscous liquid as the sublimate (0.02 g.). This was further purified by chromatography and gave II.

Anal. Calcd. for $C_{20}H_{28}Fe(CO)_3$: C, 67.65; H, 6.91; Fe, 13.68. Found: C, 67.62; H, 6.70; Fe, 13.40.

The residue (1.5 g.) from the sublimation was purified by rechromatography and gave III, on evaporation of the solvent *in vacuo*, as a yellow solid.

Anal. Calcd. for $^2/_3(C_{20}H_{28})_2Fe(CO)_3\cdot{}^1/_3(C_{20}H_{28})_2Fe_2(CO)_6$: C, 73.42; H, 7.86; Fe, 10.06. Found: C, 73.70; H, 8.26; Fe, 9.70.

Elution with benzene gave a yellow solution which was evaporated *in vacuo* under nitrogen to give an orange semisolid. Sublimation of the semisolid at 120° (0.3 mm.) in a short-pass apparatus furnished I as an orange semisolid (0.1 g.).

Anal. Calcd. for $C_{25}H_{32}FeO_3$: C, 64.11; H, 6.88; Fe, 11.90; mol. wt., 468. Found: C, 64.59; H, 6.88; Fe, 11.30; mol. wt. (cryoscopic in CH_2Br_2), 440.

Reaction of Vitamin A Acetate with Nickel Carbonyl.—Vitamin A acetate (3.0 g.) was allowed to react with nickel carbonyl (10 ml.) in 30 ml. of methanol under nitrogen at reflux for 0.5 hr. Then 10 ml. of nickel carbonyl was added and the reaction was continued for 15 min. During the reaction, a nickel mirror was produced on the wall of the flask. The reaction mixture was poured into water and the product was extracted twice with

(9) *Inorg. Syn.*, **7**, 193 (1963).

hexane. Evaporation of the extract followed by chromatography on alumina gave several fractions. The hexane eluate was evaporated *in vacuo* under nitrogen to give a yellow oil. The oil was sublimed at 120° (0.5 mm.) giving a yellow oil (*ca.* 0.1 g.), which was identified as anhydrovitamin A by ultraviolet absorption maxima (351, 368, and 389 mμ) and by its infrared spectrum.

Anal. Calcd. for $C_{20}H_{28}$: C, 89.49; H, 10.51. Found: C, 89.08; H, 10.67.

The sublimation residue was a yellow solid which gave yellow crystals, m.p. 120–125°. This compound showed an ultraviolet maximum at 323 mμ and showed the presence of an acetoxy group by the infrared spectrum.

Anal. Found: C, 74.25; H, 9.35; mol. wt. in CH_2Br_2, 713.

Reaction of Vitamin A Acetate with Chromium Hexacarbonyl.—Vitamin A acetate (1.0 g., 3.01 mmoles) was allowed to react with chromium hexacarbonyl (1.0 g., 4.55 mmoles) in 7 ml. of *n*-butyl ether and 3 ml. of hexane. The addition of hexane to the reaction mixture was to prevent sublimation of chromium hexacarbonyl during the reaction. The reaction was continued for 18 hr. under nitrogen at gentle reflux. During the reaction 250 ml. of a gas was evolved. Greenish brown precipitates were formed and the solution was yellow. The solution was decanted and the solvent was removed *in vacuo*. The greenish yellow oily residue was separated by chromatography on deactivated alumina. Elution with hexane gave a yellow semisolid which on sublimation at 110° (0.5 mm.) gave *ca.* 0.2 g. of a yellow liquid. This was found to have a composition near $C_{20}H_{30}$ as indicated by elemental analysis.

Anal. Calcd. for $C_{20}H_{30}$: C, 88.81; H, 11.19. Found: C, 88.09; H, 11.13.

The infrared spectrum showed neither terminal vinyl nor vinylidene groups. The ultraviolet spectrum exhibited a maximum at about 324 mμ, showing that 5 conjugated double bonds are present as in vitamin A acetate (absorption maximum at 327 mμ). The greenish brown precipitate from the reaction mixture turned to a deep brown color on exposure to air. This precipitate was washed with acetone and the color changed again to yield a mixed green and red precipitate. The mixed precipitate was found to be soluble in water resulting in the formation of a blue-green aqueous solution. On the addition of aqueous alkali, the solution gave a blue precipitate which was probably chromic hydroxide. Therefore the original greenish brown precipitate from the reaction mixture may contain chromous acetate.

The benzene eluate of the chromatography yielded a yellow semisolid on evaporation. In the ultraviolet region it had maxima at 313 and 300 mμ and in the infrared it showed absorption due to an acetoxy group. The infrared spectrum indicated similarity of its structure to that of vitamin A acetate, but the ultraviolet maxima are different from those of vitamin A acetate.

Nuclear Magnetic Resonance Results.—Table I shows the peak position, splitting, relative ratio of the areas, coupling constants, and assignment of protons to vitamin A acetate and its iron tricarbonyl π-complex (I).

TABLE I

Peak position (τ)	Splitting	Relative ratio of area	Coupling const., *J* (c.p.s.)	Assignment
		Vitamin A Acetate		
3.68	d	1	13	=CH—
3.95	s	2		=CH—
4.50	d	1	13	=CH—
5.35	d	2	14	$—CH_2—O—$
8.02	s	3		$CH_3—CO—$
8.10	s	6		$CH_3—C$
8.14	s	3		$CH_3—C$
8.30	s	4		$—CH_2—$
8.5	multi.	2		$—CH_2—$
9.00	s	6		$(CH_3)_2C$
		Vitamin A Acetate Iron Tricarbonyl		
4.0	multi.	2	?	=CH—
5.75	multi.	4	?	—CH—
7.8	s	3		$CH_3—C=$
8.0	s	6		$CH_3—CO$ and $CH_3—C$
8.2	s	3		$CH_3—C$
8.32	multi.	4		$—CH_2—$
8.42	multi.	2		
9.0	s	6		$(CH_3)_2C$

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Potential Anticancer Agents. I. Schiff Bases and Hydrazone Derivatives of Pyrimidine-4-carboxaldehydes[1]

CLAUDE PIANTADOSI, VILHJALMUR G. SKULASON, J. LOGAN IRVIN, J. MEYERS POWELL, AND LYNOUS HALL

Departments of Pharmaceutical Chemistry and Biochemistry, University of North Carolina, Chapel Hill, North Carolina

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A series of hydrazone derivatives and Schiff bases have been prepared by the interaction of the appropriate amine, hydrazine, or hydrazide with substituted and unsubstituted 6-hydroxy-2-thiopyrimidine-4-carboxaldehydes. Several 5- and N-1-substituted thiopyrimidine-4-carboxaldehydes have also been prepared, and in addition the ultraviolet spectra of the N-methylpyrimidine acetals have been determined. These compounds were tested *vs.* the Ehrlich ascites carcinoma in mice.

The interesting biological properties of pyrimidine derivatives, notably as antimetabolites and as potential inhibitors of cancerous growth, have resulted in the synthesis of a large number of related compounds.[2]

The present investigation was prompted by the possibility that examination of a wider spectrum of pyrimidine-4-carboxaldehyde derivatives might lead to more potent inhibitors of nucleic acid metabolism of the cells. Consequently, a series of Schiff bases and hy-

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