the yellow granular solid (the sodium salt of the 21-oxalyl enolate) weighed 3.85 g. The ester enolate salt (3.5 g.) was saponified with 5 g. of KOH in 100 ml. of methanol at room temperature for 3 hr. The reaction mixture was then diluted with 2 ml. of ether to give a light yellow precipitate (1 g.). This salt was dissolved in a small amount of water and acidified with N acetic acid to give 312 mg. of material, m.p. 222–228°. The product gave a red-brown ferric chloride test and its infrared spectrum (Nujol) showed broad bonded hydroxyl absorption at 3.8–4.6, carbonyl bands at 5.79, 5.92 and additional bands (enol?) at 6.12 with a shoulder at 6.25 mμ.

21-Carboxypregnan-3α-ol-11,20-dione.—The oxalyl derivative (2 g.) was added to a solution of 800 mg. of potassium bicarbonate in 20 ml. of water. On cooling in ice a thick gel formed. To this gel was added with stirring 3 ml. of 30% hydrogen peroxide in three portions over a 10-min. period. After an additional 10 min. of stirring in the ice bath, when gas evolution had ceased, the reaction mixture was extracted well with several portions of ether to remove any neutral material. Then the aqueous layer from the extraction was made acid with 2 N sulfuric acid and again extracted with ether to separate the acidic fraction. This was reextracted into 800 mg. of potassium bicarbonate, dissolved in 10 ml. of cold water, acidified with 2 N sulfuric acid, and reextracted into ether. The ether extract was dried with magnesium sulfate, filtered, and concentrated in a dry stream of nitrogen at 20°. A first group of crystals (285 mg.) was obtained from a small residual volume of ether, m.p. 140–145° (CO_2 evolution). The Kofler block was slowly heated above the melting point, and crystallization occurred on the microscope slide with a second m.p. of 176–178°. This second melting point was not depressed on admixture with authentic 11-ketopregnanolone. Although good analytical data were difficult to obtain because of facile decarboxylation, analysis of the material agreed reasonably well with a hemihydrate.

Anal. Calcd. for $C_{22}H_{32}O_5 \cdot 0.5H_2O$: C, 68.6; H, 8.7. Found: C, 68.93; H, 8.53.

The infrared spectrum (Nujol) showed bands at 2.98 (hydroxyl), 3.8 (broad bonded hydroxyl), 5.77 (moderate), 5.92 (strong), and 6.1 (very strong) μ; ($[\alpha]^{25}D$ +103 (acetone)).

Pregnan-3α-ol-20-one-3-phosphate Ester.—Pregnanolone (0.5 g.) in 5 ml. of pyridine was added dropwise to a cool stirred solution of 1 ml. of phosphorus oxychloride in 10 ml. of pyridine. The ice in the bath was allowed to melt, and the reaction mixture was stirred overnight at room temperature. Then the reaction mixture was slowly poured with stirring into an acidified medium of cracked ice (approximately N HCl). This reaction mixture was heated on a steam bath for approximately 1 hr. to complete hydrolysis of the residual chlorophosphate ester. The hydrolysate was then cooled in ice and extracted into ethyl acetate. The extract was washed with N HCl, and dried over magnesium sulfate. Following concentration of the solution, 325 mg. of crystalline material was obtained, melting at 198–203°. Recrystallization from ethyl acetate sharpened the melting point to 196–198°. The infrared spectrum (Nujol) showed a broad band at 3.5–4.5 and a carbonyl band at 5.84 μ.

Anal. Calcd. for $C_{21}H_{33}O_5P$: P, 7.77. Found: P, 7.40.

Potentiometric titration in 20% methanol–water showed $pH_{1/2}$ 3.6 and pH 7.8 (equiv. for the former, 4.69, and the latter 4.32; theory 3.98). For comparison the $pH_{1/2}$ values for cortisone-21-phosphate are 3.0 and 7.1.

π-Complexes with Biologically Significant Materials. II.[1] Acetylergosterol Iron Tricarbonyl

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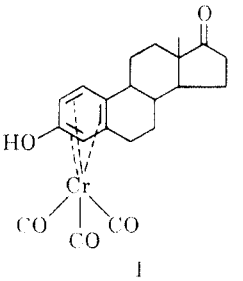
It is generally accepted that a trace amount of transition metals is essential in many biological systems. Many important enzymes contain certain transition metals. The problem of bonding of these metals to the enzymes or other biologically significant materials has attracted much interest. Recently, the possible significance of organometallic π-complexes in biological media has been pointed out by one of us.[2]

π-Complexes with transition metals sometimes are very stable and quite effective in certain reactions as catalysts. Therefore, the trace amount of transition metals in biological systems might be in the form of π-complexes. As the first step in the elucidation of the nature of bonding of transition metals in biological systems, we have prepared several organometallic π-complexes with biologically significant materials, for example, vitamins and hormones.[1]

We have reported the synthesis of a π-complex of the aromatic steroid hormone,[1] estrone, with a chromium tricarbonyl group I. The three double bonds in the A ring of estrone are thought to be coordinated to the chromium forming a stable bond. On the other hand,



I

conjugated diene systems have been found to form stable π-complexes with the iron tricarbonyl group. Therefore, the conjugated diene part of ergosterol can likewise form a stable π-complex with the iron tricarbonyl group. This paper describes a detailed account of the preparation of acetylergosterol iron tricarbonyl (II).

The reaction of acetylergosterol with triiron dodecacarbonyl in refluxing benzene gave a mixture of products, which was then separated by chromatography on alumina into two major fractions. The one fraction eluted with hexane gave colorless crystals of III in small yield. The crystals III were identified as 3,5-cyclo-$\Delta^{6,8(14),22}$-ergostatriene by a comparison of the infrared and ultraviolet spectra with those of an authentic sample.[3] The other fraction eluted with benzene gave a yellow solid. The solid was dissolved partly in methanol forming a yellow solution. A methanol-insoluble portion was almost colorless and found to be unchanged acetylergosterol by its infrared spectrum. The yellow methanol solution was evaporated to give yellow crystals II in 16% yield. Compound II was identified as acetylergosterol iron tricarbonyl by elemental analysis and by spectral evidence explained later.

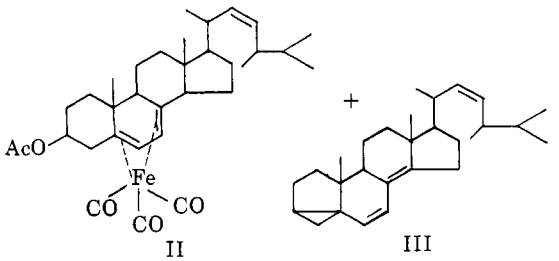
The infrared spectrum of II has strong absorption peaks at 1950 and 2030 cm.$^{-1}$ due to the iron carbonyl group and a peak at 1730 cm.$^{-1}$ due to the acetyl group. The peaks due to the iron carbonyl group fall into regions generally known to be due to diene iron tricarbonyl complexes, near 1950 and 2030 cm.$^{-1}$.

(1) Part I: A. Nakamura and M. Tsutsui, *Z. Naturforsch.*, **18b**, in press.

(2) (a) M. Tsutsui, *Chem. Eng. News*, **39**, No. 16, 42 (1961); (b) M. Tsutsui, *Z. Chem.*, **2**, 214 (1962).

(3) M. Fieser, W. E. Rosen, and L. F. Fieser, *J. Am. Chem. Soc.*, **74**, 5397 (1952).

Acetylergosterol $\xrightarrow{Fe_3(CO)_{12}}$

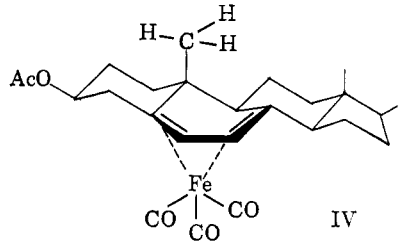


II III

In the ultraviolet region, II had an absorption maximum at 235 mμ (log ε 3.86) and a shoulder at 295 mμ (log ε 2.89). Comparison with the ultraviolet spectrum of acetylergosterol, which has maxima at 272 mμ (log ε 3.78), 283 mμ (log ε 3.78), and 295 mμ (log ε 3.59), shows clearly that a shift toward shorter wave lengths has occurred on π-complex formation. The same kind of shift has been observed with other dienes,[4] and can be due to a "π-complex effect."

Isomerization of the double bonds has been observed in some cases on reaction with iron carbonyl compounds.[5] However, no isomerization was observed in this case. The starting material was recovered unchanged in 28% yield. This fact suggests that the position of the double bonds in ergosterol is a stable one. Compound II was found to have optical activity, $[\alpha]^{22}D$ −77.2° (AcOEt); the value may be compared with that of ergosterol, $[\alpha]^{20}D$ −135°. The value of optical activity decreased on π-complex formation. This change may be indicative of a change in electronic structure around the asymmetric centers of ergosterol through bonding with the iron tricarbonyl group.

It is very difficult to estimate the magnitude of the effect due to π-complex formation, since this is the first example of a change in optical rotation on π-complex formation. Therefore the stereochemical problem of the attachment of the iron tricarbonyl group cannot be discussed from the observed rotation. Consideration of a molecular model shows that the α side of the B ring has considerable steric hindrance for the attachment of the iron tricarbonyl group, owing to the proximity of the angular methyl group at the 10-position. Therefore the iron tricarbonyl group probably attaches on the β side of the B ring. The structure shown as IV is proposed for compound II based on the data mentioned earlier.



IV

It is well known that ergosterol yields vitamin D_2 on ultraviolet irradiation.[6] Compound II was irradiated by an ultraviolet lamp in order to see how the attachment of the iron carbonyl group affects the reactivity of ergosterol. Although some color change and some decomposition occurred, the original compound was recovered unchanged as evidenced by the infrared spectrum of the irradiated product.

The formation of III is of interest, because this homoallylic elimination with the formation of a three-membered ring is the first example observed upon reaction with metal carbonyl. A similar allylic system, allyl acetate, has been reported to react with nickel tetracarbonyl forming the coupled product, diallyl, and nickel acetate.[7] Cholesteryl acetate was allowed to react with tri-iron dodecacarbonyl in order to investigate the possibility of the occurrence of homoallylic elimination. However, after refluxing in benzene for 6 hr., followed by chromatographic separation, cholesteryl acetate was recovered as the main product and only a very small amount of deacetoxylated product was found. From this result it is conceivable that more than two conjugated double bonds are necessary for the homoallylic elimination by iron carbonyl to occur to an appreciable extent. This conclusion may be important because we have observed similar deacetoxylation in the reaction of vitamin A acetate with tri-iron dodecacarbonyl to the extent of 60%.[8] However, a similar reaction involving allyl acetate and iron carbonyl did not effect any appreciable deacetoxylation under the same conditions.

The reaction of ergosterol and iron carbonyls, such as iron pentacarbonyl and triiron dodecacarbonyl, has so far yielded only an iron containing brown solid, which is most likely iron ergosteroxide, since it has been reported that reaction of methanol with iron pentacarbonyl yields iron methoxide.[9] The reaction of cholesterol and cyclohexanol, respectively, with iron pentacarbonyl similarly gave brown solids. Therefore it is obvious that an hydroxyl group interferes with π-complex formation between double bonds and an iron tricarbonyl group. Vitamin D_2 is structurally very closely related to ergosterol and has three conjugated double bonds. Vitamin D_2 and acetylvitamin D_2 were examined for the formation of a π-complex with an iron tricarbonyl group. Although the infrared spectrum of the reaction product exhibited strong peaks apparently due to a π-bonded iron tricarbonyl group, no well defined compounds could be isolated in either case. The failure to prepare pure π-complexes of vitamin D_2 is mainly due to instability of the position of the double bonds and also to thermal instability as compared with ergosterol.

Experimental[10]

Infrared spectra were measured by the Perkin–Elmer Infracord 137B. Ultraviolet spectra were determined using a Beckman DK-2 and Bausch & Lomb Spectronic 505 in spectrograde heptane solution.

Ergosterol, cholesterol, and cyclohexanol were commercial samples. Acetylergosterol, m.p. 172–174°, was prepared by acetylation of ergosterol.[11] Triiron dodecacarbonyl was prepared essentially from iron pentacarbonyl by the method of

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

(5) (a) J. E. Arnett and R. Pettit, *J. Am. Chem. Soc.*, **83**, 2954 (1961); (b) T. A. Manuel, *J. Org. Chem.*, **27**, 3941 (1962).

(6) K. Dimroth and A. Windaus, *Ber.*, **70**, 576, 1631 (1937).

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

(8) A. Nakamura and M. Tsutsui, unpublished work.

(9) W. Hieber, *Ber.*, **63**, 1417 (1930).

(10) All melting points are uncorrected. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside 77, N. Y. Measurement of $[\alpha]D$ values was done by Mr. Arnold Gordon, to whom our thanks are due.

(11) H. A. Stanbury, Jr., *J. Am. Chem. Soc.*, **65**, 1243 (1943).

Hieber.[12] Alumina used for chromatography was Merck acid washed aluminum oxide.

Acetylergosterol Iron Tricarbonyl (II).—Acetylergosterol (0.9 g., 2.0 mmoles) and triiron dodecacarbonyl (1.1 g., 2.1 mmoles) were heated at reflux under nitrogen in 10 ml. of benzene for 18 hr. The black precipitates[13] formed in the reaction were removed by filtration and the deep brown filtrate was evaporated *in vacuo* to give a yellowish brown solid. Separation of products was carried out by alumina chromatography. From one fraction, III (0.02 g.) was isolated. The other fraction, a yellow solid, was further purified by digestion with methanol. The methanol-insoluble portion was found to be the starting material. The yellow methanol-soluble portion crystallized on concentration and standing. This material was recrystallized from methanol to give pure II (0.2 g.), m.p. 95–99° (sintering at 90°).

Anal. Calcd. for $C_{33}H_{46}FeO_5$: C, 68.50; H, 8.02; Fe, 9.65. Found: C, 69.00; H, 8.08; Fe, 10.97.

Reaction of Ergosterol with Iron Carbonyls.—Ergosterol (1.0 g., 2.2 mmoles) was treated with triiron dodecacarbonyl (0.3 g., 0.8 mmole) in 20 ml. of benzene under nitrogen by heating at 80–90° for 18 hr. The color changed to deep brownish red. Evaporation of the reaction mixture gave a mixture of colorless and brown solids. These solids were soluble in benzene. Chromatographic separation of the solids caused decomposition of the brown compound on the column and only ergosterol was recovered as colorless crystals. An attempt was made to recrystallize the brown solid from benzene–methanol solution by evaporation *in vacuo*. However, crystalline products were not obtained and the brown solid (m.p. 230°, dec. at 200–220°) obtained was found to contain iron on burning and on dissolution in dilute hydrochloric acid. A similar reaction using iron pentacarbonyl and ergosterol with irradiation by an ultraviolet lamp under nitrogen gave a brown solid which had properties similar to that obtained by the thermal reaction. These brown solids could not be purified to a well defined compound. The infrared spectrum of the solids revealed the presence of a very small amount of π-complex as evidenced by absorption near 2000 cm.$^{-1}$.

Irradiation of Acetylergosterol Iron Tricarbonyl (II).—A solution of 0.1 g. of II in benzene was irradiated by a 100-w. ultraviolet lamp for 18 hr. under nitrogen. The color changed to brown and a brown semisolid was obtained on evaporation of the solvent. The infrared spectrum showed essentially no change occurred during the irradiation.

Acknowledgment.—We gratefully acknowledge financial support from the National Institutes of Health, United States Public Health Service, Grant RG-8921.

(12) W. Hieber, *Z. anorg. allgem. Chem.*, **204**, 165 (1932); R. B. King, T. A. Manuel, and F. G. A. Stone, *J. Inorg. Nucl. Chem.*, **16**, 233 (1961).

(13) The precipitates ignited spontaneously in air and gave ferric oxide.

Synthesis of 16-Chlorinated Pregnenes[1]

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Substitution at the 16-position of steroids often results in significant enhancement of biological activities. Among the groups introduced at position 16 which produce favorable effects are the methyl,[2] hydroxy,[3] fluoro,[4] 16β-chloro,[5] and methoxy[6] groups. Most of the known 16-substituted steroids are in the glucocorticoid class. It is, therefore, of considerable interest to investigate the effect of 16-substitution on the progestational activity of known precursors.

Recently, Hoffman, *et al.*,[7] reported that 16-substituted progesterone derivatives such as 16α-methylthioprogesterone and 16α-methylsulfinylprogesterone appear to be less active than progesterone on subcutaneous assay by the Clauberg–McPhail procedure.[8,9] We have also found that 16α-chloro-6α-methylprogesterone (IIb) is approximately equivalent to progesterone at a total dose level of 1 mg. per rabbit.[9] At the same levels, 16α-chloroprogesterone (IIa) and 16α-chloro-6-methyl-6-dehydroprogesterone (III) had about one-sixth the activity of progesterone as shown in Table I.

TABLE I

Compound	Total dose, mg./rabbit	Response
16α-Chloroprogesterone (IIa)	1.0	+0.5
	10.0	+3.0
16α-Chloro-6α-methylprogesterone (IIb)	1.0	+2.8
	10.0	+4.0
16α-Chloro-6-methyl-6-dehydroprogesterone (III)	1.0	+0.5
	10.0	+3.8
Progesterone	0.8	+2.0
	1.0	+3.0
	1.5	+4.0

The addition of hydrogen chloride (Chart 1) to 16-dehydroprogesterone (Ia) and 6α-methyl-16-dehydroprogesterone (Ib) furnished the corresponding 16α-chloro derivatives. The α-configuration of 16-chlorine has been assigned on the grounds previously reviewed by Gould and co-workers[10]; namely by the negative rotatory contribution.[7]

6-Methyl-16α,17α-epoxypregnenolone acetate (IV) was converted to the chlorohydrin V by opening the epoxide with hydrochloric acid.[11] Saponification of V to 6-methyl-16β-chloro-5-pregnene-3β,17α-diol-20-one (VI) and subsequent oxidation gave rise to 6α-methyl-16β-chloro-4-pregnen-17α-ol-3,20-dione (VII).

The mass spectroscopy studies[12] carried out in these Laboratories on compounds Ia, Ib, IIa, IIb, IV, V, VI, and VII support the specific structures herein pre-

(1) GMI Journal Series No. 335.

(2) G. E. Arth, D. B. R. Johnston, J. Fried, W. W. Spooncer, D. R. Hoff, and L. H. Sarett, *J. Am. Chem. Soc.*, **80**, 3160 (1958); **80**, 3161 (1958); E. P. Oliveto, R. Rausser, A. L. Nussbaum, W. Gebert, E. B. Hershberg, S. Talksdorf, M. Eisler, P. L. Perlman, and M. M. Pechet, *ibid.*, **80**, 4428 (1958); **80**, 4431 (1958); D. Taub, R. H. Hoffsommer, H. L. Slates, and N. L. Wendler, *ibid.*, **80**, 4435 (1958); **82**, 4012 (1960); E. P. Oliveto, R. Rausser, H. O. Herzog, E. B. Hershberg, S. Talksdorf, M. Eisler, P. L. Perlman, and M. M. Pechet, *ibid.*, **80**, 6687 (1958).

(3) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, G. M. Stolar, L. I. Feldman, and R. H. Blank, *ibid.*, **78**, 5693 (1956); **81**, 1689 (1959).

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(6) S. Bernstein, M. Heller, and S. M. Stolar, *Chem. Ind.* (London), 516 (1961); G. R. Allen Jr., and N. A. Austin, *J. Org. Chem.*, **26**, 4574 (1961).

(7) A. S. Hoffman, H. M. Kissman, and M. J. Weiss, *J. Med. Pharm. Chem.*, **5**, 962 (1962).

(8) M. K. McPhail, *J. Physiol.* (London), **83**, 145 (1934).

(9) Subcutaneous (diluent sesame oil) Clauberg–McPhail assay, carried out by Endocrine Laboratories, Madison, Wisconsin.

(10) D. Gould, F. Gruen, and E. B. Hershberg, *J. Am. Chem. Soc.*, **75**, 2510 (1953).

(11) P. Crabbe, H. J. Ringold, and J. A. Zderic, *Bull. Soc. Chim. Belges*, **70**, 271 (1961); *Chem. Abstr.*, **55**, 27417 (1961).

(12) The author is grateful to Dr. L. E. Peterson and Mr. L. G. Hickman for the measurement and interpretation of mass spectra. See also L. E. Peterson, *Anal. Chem.*, **34**, 1781 (1962).