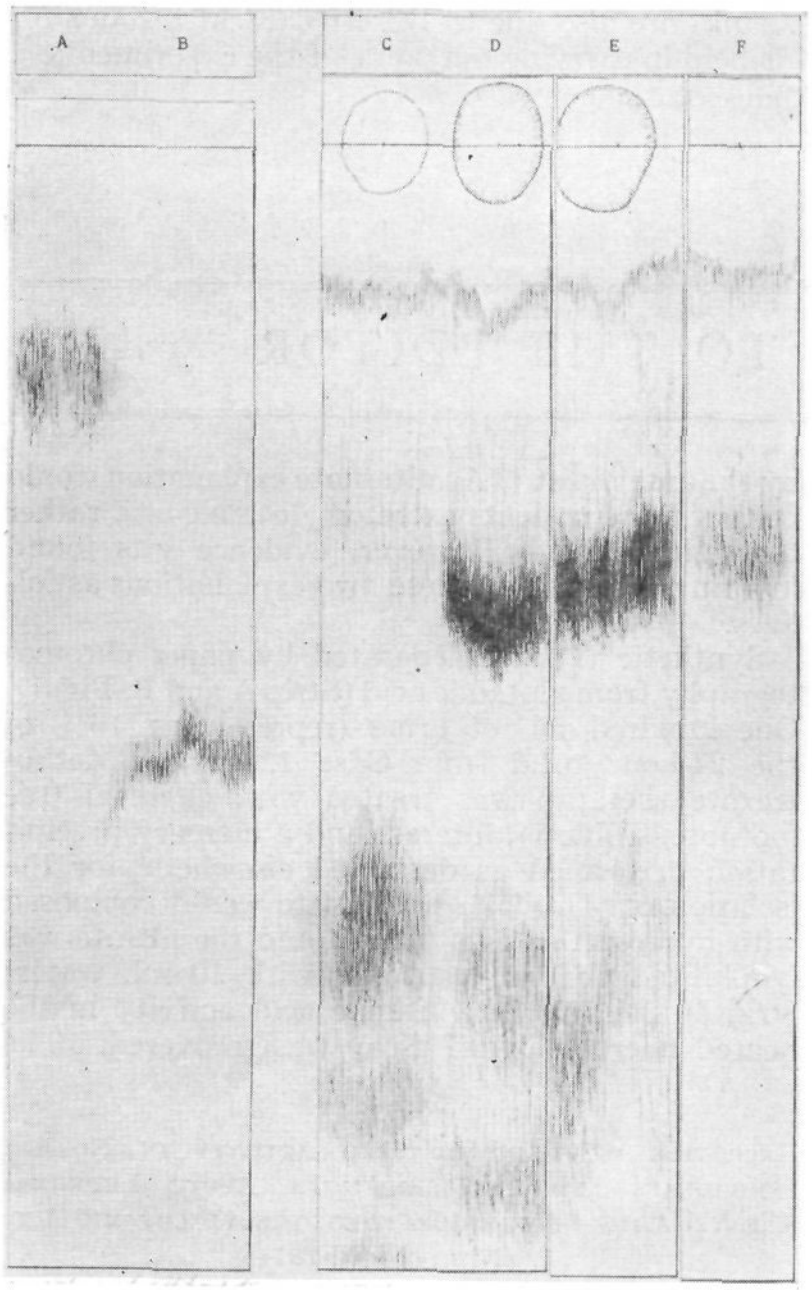

Fig. 1.—Chromatographic evidence for the presence of formiminoglutamic acid in urine following folic acid antagonist therapy in acute leukemia: solvent system: 100 g. phenol + 20 ml. water containing 6.3% sodium citrate, 3.7% monobasic potassium phosphate, and 0.5% ascorbic acid. Chromatographic conditions: descending technique, 36 hr., 25–30°; development with ninhydrin: Strip A, 20 γ glutamic acid; Strip B, 40 γ formiminoglutamic acid (strip first exposed to ammonia vapor, 1 hr., then aerated, then sprayed with ninhydrin); Strip C, 0.04 ml. of urine eluate (see text); Strip D, 0.04 ml. urine eluate autoclaved 10 min., 15 lb. pressure, *p*H 7.5, then chromatographed; Strip E, urine eluate adjusted to *p*H 12 and held 24 hr. at 25°, then chromatographed; Strip F, 20 γ glutamic acid, control for chromatograms, C, D, and E.

concentrate (0.7 ml.) was next applied in 0.02 ml. amounts across a sheet of Whatman #1 paper (18.5 × 11.5) and developed in the solvent system described in Fig. 1 for 36 hours. Separate chromatograms of glutamic acid and (I) served as controls which were visualized by spraying with ninhydrin, and that area of the urine chromatogram coincident with (I) was cut out and eluted *with water (final volume, 0.5 ml.). The eluate* was devoid of microbiological activity for glutamic acid unless autoclaved and contained glutamic acid activity equivalent to 200 γ/ml. (see Table I). When the eluate was either autoclaved or let stand for 24 hours at 25° at *p*H 12, the concomitant formation of glutamic acid could be demonstrated by paper chromatography (Fig. 1, Strips C, D, E, and F). The properties of the glutamic acid derivative present in the urine eluate were shown by (I). Comparable treatment of normal urine did not yield any material containing alkali- or heat-labile glutamic acid activity. Work on the isolation of the glutamic acid precursor from appropriate urine specimens is in progress to confirm the above findings.

Acknowledgments.—The author wishes to thank Dr. A. L. Luhby for his collaboration and advice on clinical aspects of this problem; Dr. Lois Murphy for obtaining pathological urine specimens; and Dr. Heinrich Waelsch and Dr. Herbert Tabor for gifts of authentic formiminoglutamic acid.

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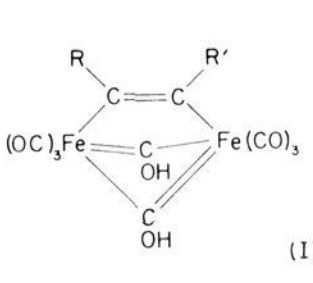
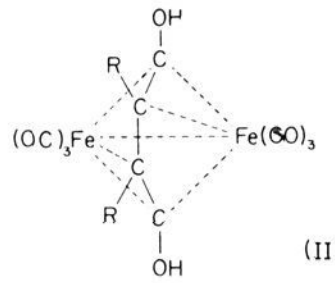
THE STRUCTURE OF THE BRIDGED IRON COMPLEXES FROM IRON HYDROCARBONYL AND ACETYLENES

Sir:

The complex $Fe_2C_{10}H_4O_8$,[1] obtained from acetylene and the monoanion of iron carbonyl hydride, has been discussed recently and tentatively depicted as (I, R = R′ = H).[2] The propyne and



(I)



(II)

hex-1-yne analogs were described in 1955,[3] but analytical difficulties prevented early publication of our results. We now present evidence for an alternative formula (II). Analogous complexes can be obtained from acetylene, propyne, and but-2-yne by essentially similar methods; they have the same composition $H_2[RC{\equiv}CRFe_2(CO)_8]$, and show similar chemical and spectroscopic properties. However, mild acetylation converts the acetylene complex into a diacetate, while the propyne complex forms a monoacetate and the but-2-yne complex is unchanged. All yield diacetates under more vigorous conditions; that from propyne is converted by hot methanol into an isomeric monoacetate. Chloroacetic anhydride converts the two monoacetates into different acetyl-chloroacetyl derivatives, m.ps. 107–108° and 89–90°, respectively. We conclude (a) that in the propyne complex the two hydroxyl groups are not placed symmetrically with respect to the propyne molecule [as they would be in (I)]; and (b) that the hydroxyl groups are sufficiently close to the acetylene residue to suffer appreciable steric hindrance from even a methyl substituent on the latter.

(1) W. Reppe and H. Vetter, *Ann.*, **582**, 133 (1953).

(2) I. Wender, R. A. Friedel, R. Markby and H. W. Sternberg, THIS JOURNAL, **77**, 4946 (1955); **78**, 3621 (1956).

(3) M. C. Whiting, Symposium at Manchester, April 21, 1955; see J. Chatt, *Nature*, **176**, 59 (1955).

In the acetylene cobalt hexacarbonyls, each acetylene molecule replaces two carbonyl groups,[4] *i.e.*, contributes its four π-electrons to the π-d bonding of the complex; similarly the C—OH groups should each contribute three electrons.[2] In the present complex, however, the [RC≡CR'2C(OH)] grouping as a whole is required to contribute only eight or six (Fe–Fe bond assumed) electrons. It therefore seemed probable that four electrons are utilised in σ-bonds, presumably between the C—OH groups and the acetylene residue. This would also explain the steric effects described. Formula (II) was thus obtained. In conformity with this structure, the but-2-yne complex was found to be rapidly attacked by potassium ferricyanide, with the formation of dimethylmaleic anhydride; this evident *in itself* would not have been enough to establish the postulated σ-bonding, since these links might have been formed during the oxidative breakdown.

In formula (II) full bonds have been used for the π-electron framework, and dashed bonds to indicate the π- and d-electron bonding which confers upon the complex its aromatic character.[2] In view of the lack of agreement on the description of even so simple a compound as ferrocene,[5] which similarly possesses the necessary number of electrons for an inert-gas completed shell and shows "aromatic" stability,[6] the precise bonding in (II) is not specified, although a close relationship to $Fe_2(CO)_9$ seems probable. It is implied in (II) that the two iron atoms lie on either side of the (approximate) plane of the four central carbon atoms. The steric hindrance observed when R = Me requires an HO–C–CR angle of about 120°, rather than 180°, and suggests sp^2 hydrisation for the C–C and C–O bonds of the entral grouping.

Formation of hydroquinone[1] by the action of a second molecule of an acetylene on (II) can readily be envisioned.

Acknowledgments.—We are indebted to the Commonweath Scientific and Industrial Research Organisation (Australia) and to the British Oxygen Company, Ltd., for fellowships held by P. C. W., and R. C., respectively.

(4) R. A. Friedel, I. Wender, S. L. Shufler and H. W. Sternberg, THIS JOURNAL, **77**, 3951 (1955).

(5) See *inter alia* B. F. Hallam and P. L. Pauson, *J. Chem. Soc.*, 3030 (1956).

(6) G. Wilkinson, M. Rosenblum, M. C. Whiting and R. B. Woodward, THIS JOURNAL, **74**, 2125 (1952).

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RECEIVED OCTOBER 8, 1956

THE PREPARATION AND PROPERTIES OF COMPLETELY DISPERSED THYMUS NUCLEOPROTEIN

Sir:

The preparation and characterization of deoxyribonucleic acids (DNA) from a variety of sources have now reached a level of development where there is semi-quantitative reproducibility and widespread agreement. In contrast the parent nucleoprotein particles have not been isolated in dispersed form. We wish to report on the preparation of such nucleoprotein particles and a study of their properties which shows that they consist of one DNA molecule and a protein complement of approximately equal weight.

The preparative procedures commonly used for the isolation of nucleoprotein material[1,2,3] generally consist of mincing and multiple washings of tissue in cold 0.15 *M* NaCl, followed by repeated suspension and centrifugation in water and finally homogenization in water to produce a viscous solution of about 0.5 g./dl. concentration which exhibits pronounced gel-like character and is admittedly incompletely dispersed.[1] We have found that irreversible gel formation was caused by (1) maintaining nucleoprotein at concentrations above 0.08 g./dl., (2) precipitating nucleoprotein with 0.15 *M* NaCl or alcohol, or (3) allowing the salt-washed sediment to stand too long before homogenizing in water and by carrying out this homogenization too slowly. This led to the following procedure for isolating DNP in high yield free of gel.

Working in a cold room, about 20 g. of fresh, frozen thymus glands are chopped and homogenized in a Waring Blendor[4] with 200 cc. of a saline-Versene (0.075 *M* NaCl and 0.024 *M* Versene, *p*H 8) solution containing a few drops of capryl alcohol. The homogenate is strained through cheesecloth and the filtrate is centrifuged at 2000 r.p.m. for ten minutes. The sediment is again homogenized in the Blendor (at slow speed for three minutes) in the same volume. The homogenate is centrifuged as above and the supernatant is decanted. This washing procedure is repeated six more times. The final sediment is dissolved by rapid mixing with water in the Blendor starting with 50 cc. of water and increasing this to 1 liter in a few seconds. The mixture is quickly transferred to a beaker and stirred for an hour and then dialyzed against 0.0007 *M* potassium phosphate buffer at *p*H 6.8. This solvent was employed for all the physical measurements.

Physical measurements on several samples of DNP are summarized in the table. From these data it appears that the nucleoprotein particle is an irregularly shaped coil somewhat less extended than DNA itself, that is, the average separation of the ends is about 4200 Å. for DNP compared with about 7100 Å. for DNA. DNA was prepared directly from the DNP and was found to have the same physical properties as samples carefully prepared by Simmons' method. These results are given in the table for comparison.

In making the physical measurements the concentration was determined by optical density measurements using $E^{1\%}_{259} = 106 \pm 5$. The amount of DNA in the nucleoprotein was found to be 49 ± 2%. Other constants established for the DNP

(1) K. V. Shooter, P. F. Davidson and J. A. V. Butler, *Biochem. et Biophys. Acta*, **13**, 192 (1954).

(2) P. F. Davidson, B. E. Conway and J. A. V. Butler, in "Progress in Biophysics and Biophysical Chemistry," Vol. 4, Academic Press, New York, 1954, p. 148.

(3) C. F. Crampton, R. Lipshitz and E. Chargaff, *J. Biol. Chem.*, **206**, 499 (1954).

(4) The Blendor is operated at about half its maximum speed for 1 minute and then at a very slow speed, just sufficient to agitate the mixture, for 3 minutes.