UCRL 8457

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

BIO-ORGANIC CHEMISTRY QUARTERLY REPORT
June, July, August 1958

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BIO-ORGANIC CHEMISTRY QUARTERLY REPORT

June, July, August 1958

M. Calvin, Director

Edited by James A. Bassham

Radiation Laboratory and Department of Chemistry
University of California, Berkeley, California

September 18, 1958

FOUR SPECIAL IONIZATION CHAMBERS

Bert M. Tolbert

An ionization chamber in combination with a vibrating-reed electrometer and a recorder provides precise and sensitive measurement of radioactivity. In order to realize further the possibilities of this method, four special-purpose ionization chambers have been designed, built, and partially tested.

The first is a general-purpose gas-measuring chamber designed for C^{14}, tritium, and other weak beta emitters. It is shown in exploded view in Fig. 1. This chamber is constructed from a 3-1/2-inch stainless steel float equipped with 1/8-inch pipe connection (Schnitzer Alloy Products, 325 Pine Street, Elizabeth 1, New Jersey, via Electric Steel Foundry Co., Emeryville, California). The mode of connecting the chamber to the collecting electrode is through a rubber electrical grommet, and provides a blind closure that is inexpensive, simple, and vacuumtight. This chamber has a low background and excellent settling characteristics, and should have good efficiency, since the spherical geometry is optimum.

The limiting sensitivity of an ion chamber is dependent on the background current and the precision with which this current may be measured. In order to improve this sensitivity, a zero-background ion chamber has been constructed; it is shown in Fig. 2 in an exploded view. This chamber has two cavities of equal size and similar construction. In operation, a negative collection voltage is connected to the upper-chamber shell, which is the active volume for samples. A positive collection voltage is connected to the lower-chamber shell, and the center electrode guard is connected to the ground as is usual. Background current due to cosmic radiation and construction material arising in the upper chamber is offset by an equal current of opposite polarity arising (from the same sources) in the lower chamber and collected on the same electrode. In addition, this chamber has been constructed of Lucite coated with evaporated chromium in an attempt to reduce alpha-particle background. This chamber shows a very low background, limited by insulator current, and an alpha-particle background of about 1 per 30 minutes.

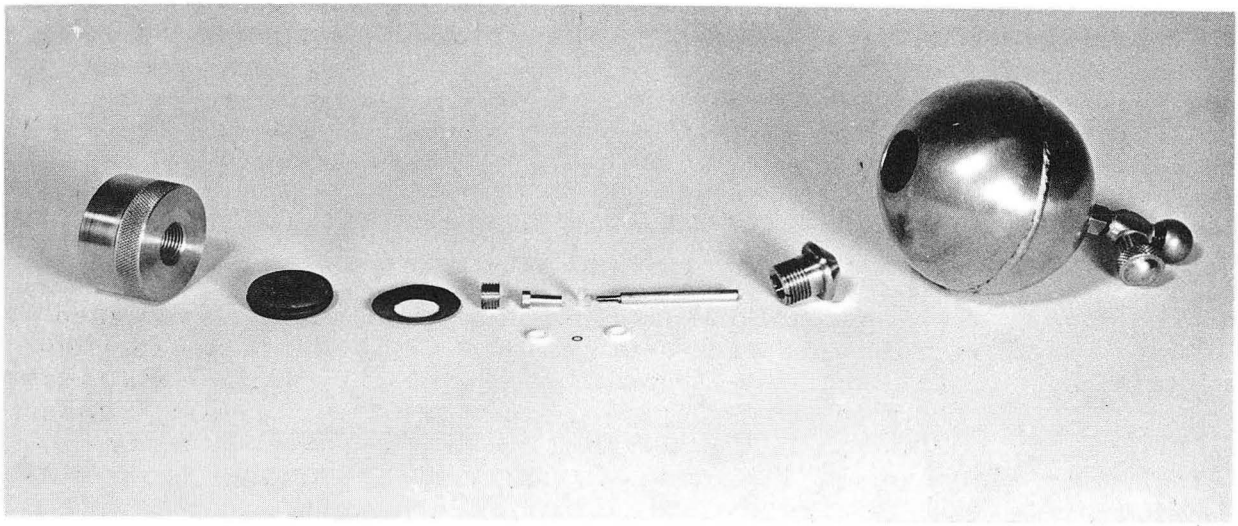

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Fig. 1. General-purpose gas-measuring ionization chamber for C^{14}, tritium, and other weak beta emitters.

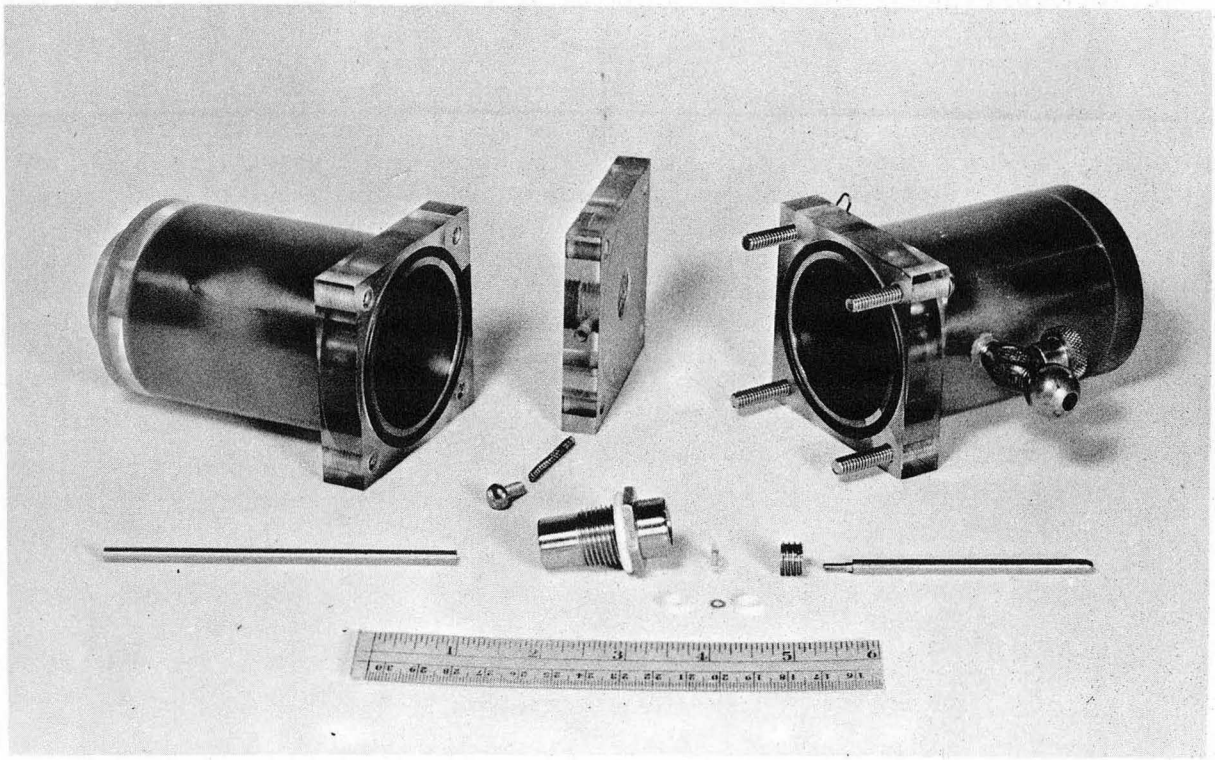

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Fig. 2. Zero-background ionization chamber.

Sensitive monitoring of cosmic-ray and gamma-ray backgrounds may be desirable in areas near nuclear reactors, cyclotrons, and other particle accelerators. A 15-liter spherical radiation-monitoring ionization chamber has been constructed of a 12-inch stainless steel float purchased from the same firm as the 3-1/2-inch floats. This chamber is shown in Fig. 3. The background current for this chamber is 1.2×10^{-14} amp. Local cosmic-ray showers are easily observed. Cyclotron operation a block away is also readily observed. The electrometer is operated with a 5×10^{11}- or a 10^{12}-ohm resistor. The alpha-particle background of the chamber is about 5 per 10 minutes.

In vapor-phase chromatography of labeled compounds, it is convenient to measure continuously radioactivity as well as the organic vapor in the effluent gas stream. A small, high-temperature ion chamber has been designed to operate in a Wilkens Instrument Company gas chromatography analyzer ("Aerograph"). A preliminary design has been constructed and tested. The unit operates satisfactorily at temperatures up to 225°C, but testing of sensitivity yet remains. Response times and versatility are good, but these problems remain: (a) Spurious signals are obtained when non-radioactive samples are introduced. An ion trap should help to eliminate this problem. (b) Deformation of some Teflon insulators has caused leaks. Such insulators must be redesigned. (c) Some kind of an electrical malfunction yet exists, derived from the ion-chamber–electrometer combination, in which a reverse ion-chamber signal is produced. The ionization chamber is being operated as follows: Collection voltage, 90 v; 10^{12}-ohm leak resistor; 10-mv full-scale deflection; 40-cc/min gas flow.

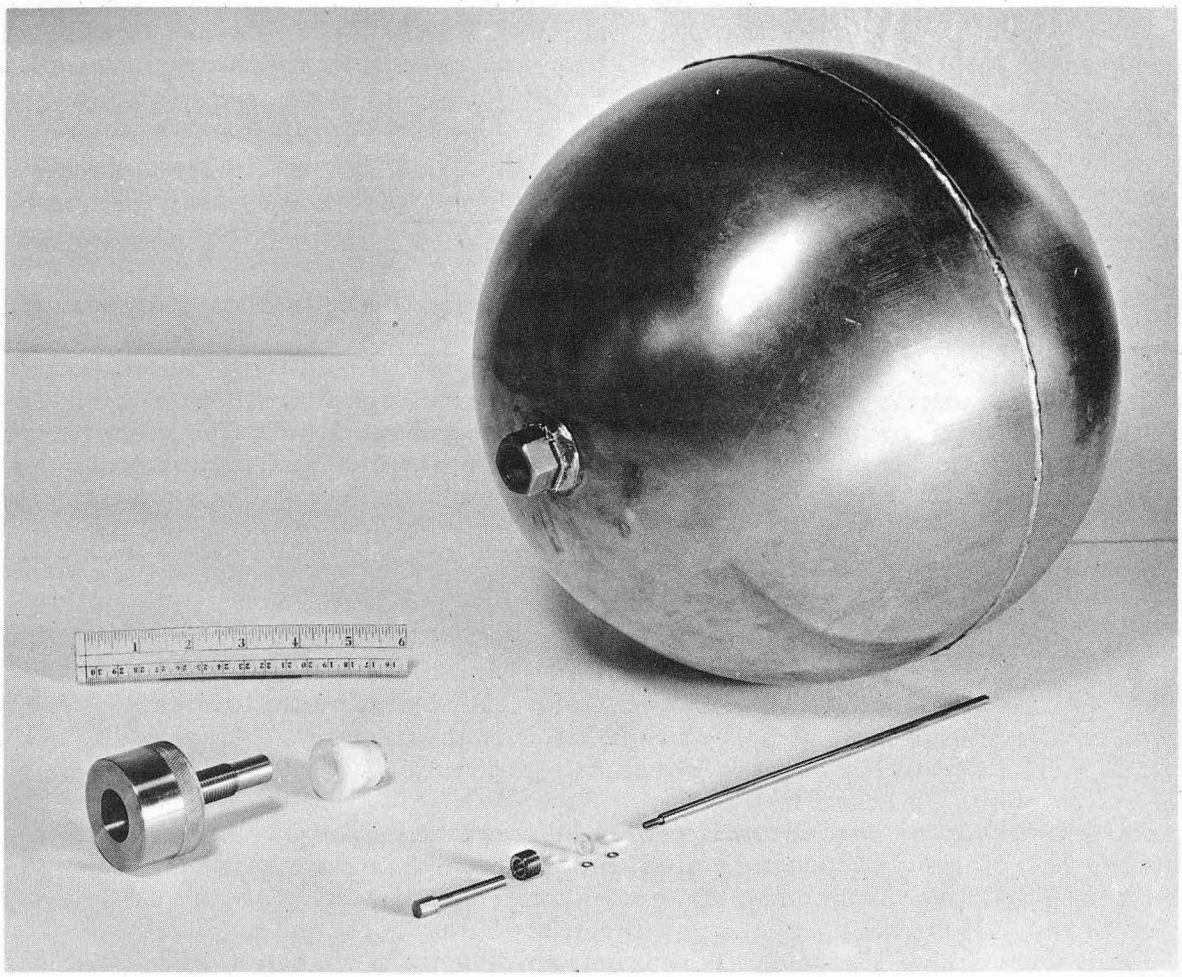

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Fig. 3. 15-liter spherical radiation-monitoring ionization chamber.

PARAMAGNETISM IN CHARGE-TRANSFER COMPLEXES

Walter Strohmeier and Richard M. Lemmon

It was recently shown that electron-spin resonance (ESR) signals could be detected in some charge-transfer complexes, while other such complexes seemed to be diamagnetic.[1] In a charge-transfer complex between a donor (D) and an acceptor (A), only the polar structure (A^-D^+) can be paramagnetic. The detectability of the paramagnetism depends therefore on the percentage of polar structure in the molecular complex (A:D). Of course, the electron transferred to the acceptor must be present as an odd, uncoupled electron:

$$A + :D \rightleftharpoons A:D \longleftrightarrow (A^-D^+) \ .$$

It is of interest to determine whether the paramagnetism in charge-transfer complexes depends on the donor, the acceptor, or both. As chloranil (tetrachloroquinone) forms paramagnetic charge-transfer complexes, we examined the chloranil-hexamethylbenzene complex for paramagnetism. For the molecular complex compound between chloranil and hexamethylbenzene the percentage of the polar structure in the ground state has been found to be 5%.[2] A few milligrams of this complex should be enough to detect the paramagnetism.

Experimental

Chloranil was prepared by oxidation of $C_6Cl_4(OH)_2$ with nitric acid,[3] and then recrystallized from CCl_4 (elemental analysis: C = 29.28%, Cl = 57.7%; found: C = 29.16%, Cl = 57.8%). The hexamethylbenzene (Eastman "White Label") was recrystallized from carbon tetrachloride. The chloranil (0.0119 mmole) and the hexamethylbenzene (0.0199 mmole) were dissolved in about 20 ml of hot CCl_4. After the solution was cooled, $C_6Cl_4O_2$ crystals appeared, as the solubility of this compound in CCl_4 is low.[2] The remaining solution had a purple color and contained an excess of $C_6(CH_3)_6$. As the equilibrium constant for the complex is 0.1 in CCl_4 at 20°,[2] most of the $C_6Cl_4O_2$ was present as the molecular compound. This solution was tested with the ESR for paramagnetism. A part of this solution was evaporated to dryness at room temperature. Purple crystals of the molecular compound, mixed with $C_6(CH_3)_6$, were obtained. This solid residue was also tested for paramagnetism.

The results of these experiments were that no signal could be detected either for the solution or for the solid residue, even though the magnetic field strength was varied from approximately 2,000 to 5,000 oersteds.

[1] Buck, Lupinski, and Oosterhoof, Molecular Phys. 1, 196 (1958).

[2] G. Briegleb and J. Czekalla, Z. Elektrochem. 58, 249 (1954).

[3] C. Graebe, Ann. 146, 1 (1868).

The charge-transfer effect in this molecular compound is well established by the charge-transfer absorption band in the visible region and by dipole moment measurements.[2] We therefore presume that the electron on the acceptor ($C_6Cl_4O_2$) is not a true free electron. The electron is not in a triplet state and its spin is still coupled with the odd electron on the donor [$C_6(CH_3)_6$].

It is of interest that at present no paramagnetism has been reported in charge-transfer complexes when the donor component is of the "π" type. Whenever paramagnetism has been found in such complexes, the donor has been of the "n" (onium) type, that is, the donor has either been an oxygen, nitrogen, or phosphorus compound (see References 1, 2, and 4), or has been a metal, as in chloranil, which forms a stable, paramagnetic salt with sodium,[5] namely, $(C_6Cl_4O_2)^-Na^+$, formed by the reaction:[6]

$$2\ C_6Cl_4O_2 + 2\ NaI \rightleftharpoons 2(C_6Cl_4O_2Na) + I_2\ .$$

[4] F. Ramirez and S. Dershowitz, J. Org. Chem. 22, 856 (1957).

[5] Kainer, Bijl, and Rose-Innes, Nature 178, 1462 (1956).

[6] H. A. Torrey and W. H. Hunter, J. Am. Chem. Soc. 34, 702 (1912).

PROTON-RESONANCE SPECTRA OF CYCLOPENTADIENE, METHYLCYCLOPENTADIENE, CYCLOHEPTATRIENE, AND TRIMETHYLSILICON CHLORIDE

Walter Strohmeier, Erminio Lombardi, and Richard M. Lemmon

An earlier report from this laboratory described the use of nuclear magnetic-resonance (NMR) spectra in determining the chemical shift in organic compounds containing nitrogen.[1] In this work we have undertaken to determine the NMR spectra occasioned by hydrogen atoms in different molecular environments. Such spectra are of value for several reasons: (a) They provide future identification of these same compounds, and of chemically similar compounds, (b) they tell us whether the different hydrogen atoms of a particular compound are in the same, or different, internal magnetic fields, and (c) they tell us how far the hydrogen-atom (or proton) resonance is shifted by the particular environment of a given hydrogen atom, i.e., by the position of the hydrogen atom in a particular chemical compound.

Experimental Procedure

Methods and Equipment

The NMR spectra were determined with a Varian High-Resolution NMR Spectrometer (V4300B) at 40 Mc. The chemical shift is defined by the equation

$$\delta = 10^6 (H_{H_2O} - Hc)/H_{H_2O},$$

in which H_{H_2O} is the magnetic field strength at which the proton resonance for water appears, and Hc is the field strength for the particular proton resonance in the compound being observed. A value of 1 for the chemical shift means that there is a difference of 1 part in a million in the field strengths at which the proton resonances appear. Chemical shifts are therefore usually expressed as ppm.

Ethyl alcohol was employed for the calibration of the magnetic field. The separation of the CH_3 and OH frequencies is 125 cycles/sec at 30 Mc.[2] This is equivalent to a 4.2-ppm separation of the resonance lines.

Most of the NMR spectra were determined on the compounds dissolved in cyclohexane. The proton resonance of the cyclohexane hydrogen atoms therefore served as an "inner" standard. The value of −3.9 ppm was employed for the chemical shift of the cyclohexane CH_2 group.[3] For the determination of the proton resonance of pure compounds, or for compounds dissolved in

[1] Erminio Lombardi and Power B. Sogo, Proton Magnetic Resonance of Irradiated Choline Chloride and Its Analogs, in Bio-Organic Chemistry Quarterly Report, UCRL-8204, March 1958, p. 3.

[2] J. T. Arnold, Phys. Rev. 102, 136 (1956).

[3] Meyer, Seika, and Gutowsky, J. Am. Chem. Soc. 75, 4567 (1953).

benzene, cyclohexane was employed as an "outer" standard. The values so obtained were related empirically to the "inner" standard.

Purification of Compounds

Dimeric cyclopentadiene, a sample of which was kindly given to us by the Ethyl Corporation, was twice distilled at 50 mm pressure and the fraction boiling at 82° was retained. This fraction had a melting point of 33°.[4]

Cyclopentadiene was prepared by the thermal splitting of the dimer.[5] At the top of our column we employed a reflux condenser through which was passed water at 43°. The receiver was maintained at -80°. The distillate was purified by gas chromatography (Wilkens "Aerograph"). Through the employment of five different substrates (paraffin, "Ucon," "Carbowax," silicone, and triheptylamine) the purity of the cyclopentadiene was demonstrated.

Dimeric methylcyclopentadiene (also a gift of the Ethyl Corporation) was twice distilled at 30 mm pressure and the fraction boiling at 98° was used.[6]

Methylcyclopentadiene was prepared similarly to the cyclopentadiene; the only difference was a reflux-condenser temperature of 75°. Gas chromatography was similarly employed to demonstrate the product's purity.

Cycloheptatriene was prepared from benzene and diazomethane, and was purified in the manner previously described.[7]

The trimethylsilicon chloride that we used was prepared by the Anderson Chemical Company, Weston, Michigan. It was purified of traces of HCl by gas chromatography.

Results and Discussion

The proton resonance spectra of cyclopentadiene, methylcyclopentadiene, cycloheptatriene, and trimethylsilicon chloride are shown in Fig. 4.

Cyclopentadiene shows a sharp CH resonance peak with $\delta_{CH} = +1.1$ ppm, (Fig. 4, a). The chemical shift for the CH_2 protons is -2.5 ppm. So far as response to the magnetic fields of neighboring atoms is concerned, the four CH protons of cyclopentadiene are equivalent. Under the conditions of our experiment no hyperfine structure could be seen.

The proton resonance spectrum of methylcyclopentadiene consists of three groups of lines that arise from CH, CH_2, and CH_3 protons (Fig. 4, b). The principal peaks of the chemical shifts are: $\delta_{CH} \cong +1.1$ ppm and

[4] Uhrig, Lynch, and Becker, Ind. Eng. Chem. Anal. Ed. 18, 550 (1946).

[5] C.R. Gardner, Anal. Chem. 20, 331 (1948).

[6] Edson, Powell, and Fisher, Ind. Eng. Chem. 40, 526 (1948).

[7] Ann Coombs and R.M. Lemmon, in Chemistry Division Quarterly Report, UCRL-3836, June 1957, p. 16.

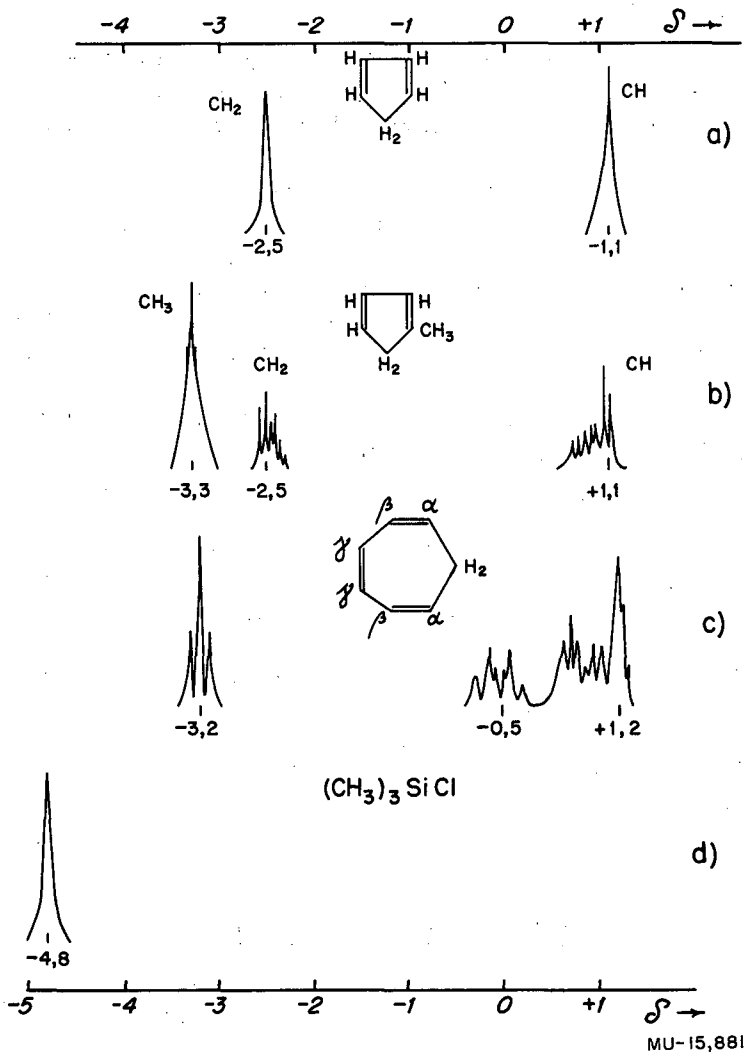

Fig. 4. The proton resonance spectra of (a) cyclopentadiene, (b) methylcyclopentadiene, (c) cycloheptatriene, and (d) trimethylsilicon chloride. The compounds were dissolved in cyclohexane at a mole fraction of 0.8.

$\delta_{CH_2} \simeq -2.5$ ppm. Since the individual lines of the CH and CH_2 resonances are partially superimposed, no assignment can be made at present as to which peaks arise from spin coupling, which from second-order effects, and which from nonequivalent protons. A determination of the spectrum at 60 Mc might permit a definite assignment. The possibility that our compound is a mixture of 1-methyl- and 2-methylcyclopentadiene can be definitely excluded; the use of gas chromatography, with five different substrates, failed to show anything but a single compound. In no chromatogram could even a suggestion of a "shoulder" be seen.

The CH_3 protons of methylcyclopentadiene showed a chemical shift of -3.3 ppm. The spectrum has some barely discernible fine structure. However, since we do not know whether we have 1-methyl- or 2-methyl-cyclopentadiene, a definite interpretation cannot be made.

The proton resonance spectrum of cycloheptatriene (Fig. 1, c) shows a δ CH_3 = -3.2 ppm, while the CH protons exhibit a group of complex and overlapping peaks. The four resonance lines at -0.5 ppm can be ascribed to the two protons in the α position. Through spin coupling with the protons of the CH_2 group and the proton in the adjacent β position, the α-proton resonance is split into four peaks. The sharp triplet of the CH_2 resonance is proof that a spin coupling occurs between the CH_2 and the α-CH protons. A definitive interpretation of the rest of the lines of the β and γ protons is not possible because of the overlapping. The principal peak of the chemical shift of the β and γ protons lies in the region of the CH resonance of cyclopentadiene and methylcyclopentadiene.

Trimethylsilicon chloride shows a single sharp resonance peak at δ_{CH_3} = -4.9 ppm. In tetramethylsilicon the chemical shift (for the pure compound) has been found at -5.15 ppm.[8] Under the influence of the electronegative chlorine atom, electrons are withdrawn from the silicon. Thus the "chemical shielding" of the protons is diminished and the δ_{CH_3} peak is displaced in the direction of a decreased external field strength.[3]

The resonance spectra for the pure compounds were identical to those found in both cyclohexane and benzene solutions (at mole fractions of 0.8, 0.45, and 0.3).

The proton-resonance spectra of dimeric cyclopentadiene and dimeric methylcyclopentadiene are shown in Figs. 5 and 6. In the dimeric cyclopentadiene (Fig. 5, d) the CH protons show sharp peaks with δ_{CH} = + 0.3 and + 0.7. The CH protons are no longer equivalent (as in the monomer), and this is to be expected from the structure of the dimer.[9] The same holds for the CH_2 protons; they also are no longer equivalent. Of course, the peak of one of the CH_2 groups appears at the same place as in the spectrum of the monomer. The circumstances are similar for dimeric methylcyclopentadiene.

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[8] A. L. Allred and E. G. Rochow, J. Inorg. and Nuclear Chem. 5, 269 (1958).

[9] P. J. Wilson and J. H. Wells, Chem. Revs. 34, 1 (1944).

[10] K. C. Edson, J. S. Powell, and E. L. Fisher, Ind. Eng. Chem. 40, 1526 (1948).

Its spectrum (Fig. 6, d) shows three groups of peaks corresponding to the CH, CH_2, and CH_3 protons. In comparison with the monomer, fine structure is almost completely lost in dimeric methylcyclopentadiene, and a relatively broad resonance curve is observed. It is probable that our dimeric cyclopentadiene is actually a mixture of isomers.

Since cyclopentadiene and methylcyclopentadiene dimerize with a measurable rate,[10] we measured their proton resonance spectra as a function of time from 22 to 96 hours after the start of dimerization. As Figs. 5 and 6 show, the NMR spectra can be used to advantage in studying dimerization and polymerization. The spectra of the dimers obtained through dimerization of the monomers at room temperature were identical with the dimer spectra obtained after distillation from the starting dimer at 30 or 50 mm pressure.

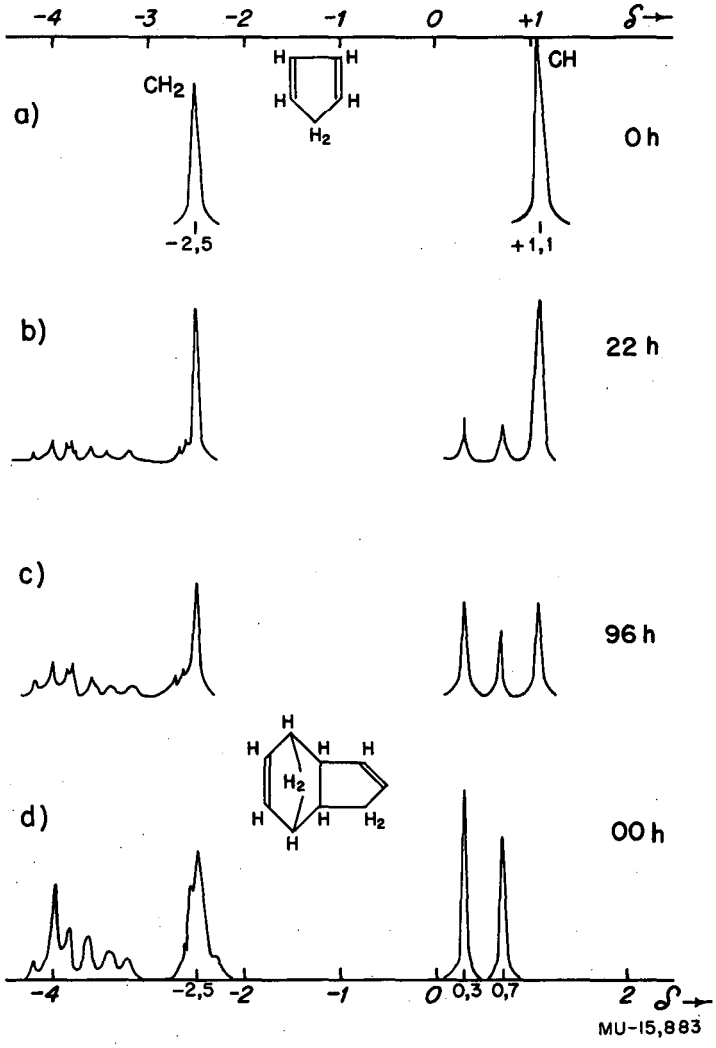

Fig. 5. Proton resonance spectrum of cyclopentadiene as a function of time at 20°
(a) Directly after preparation
(b) After 22 hours
(c) After 96 hours
(d) The dimer

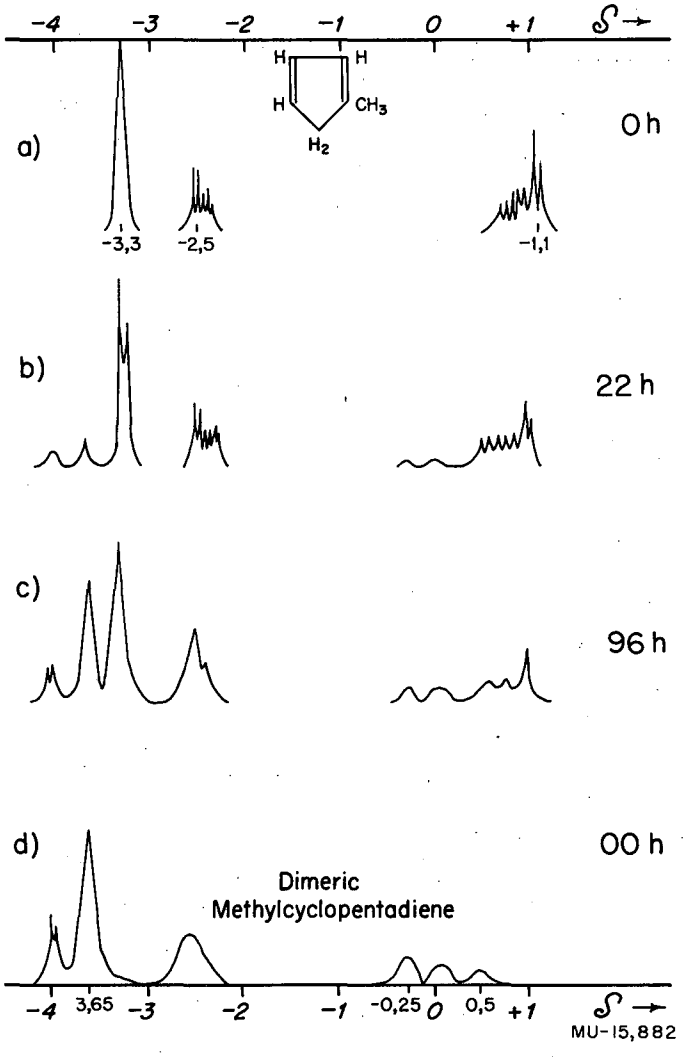

Fig. 6. Proton resonance spectrum of methylcyclopentadiene as a function of time at 20°
(a) Directly after preparation
(b) After 22 hours
(c) After 96 hours
(d) The dimer

THE DIFFERENCE BETWEEN THE NMR SPECTRA OF CYCLOPENTADIENYL COMPOUNDS WITH A "SANDWICH" STRUCTURE AND THOSE WITH LOCALIZED METAL-CARBON BONDS

Walter Strohmeier and Richard M. Lemmon

Proton resonance spectra, the nuclear magnetic-resonance (NMR) spectra arising from proton-magnetic field interactions, depend (among other things) on the electron distribution in the proton's environment. Thus, the resonance of a particular proton attached to a particular carbon atom is affected by the type of bonding that exists between that carbon atom and other atoms of groups. In this work we have used NMR spectra to investigate the kinds of metal-carbon bonds existing between a metal atom and cyclopentadienyl rings. For such compounds two extreme types of metal-carbon bonds are possible. One occurs in the so-called "sandwich" structures, in which, for example, a metal atom may be sandwiched in between two cyclopentadiene rings. In this type of compound the metal atom is bonded equally to all ten carbon atoms of the two rings. In the second type of bond, the metal atom is bonded specifically to certain carbon atoms only. The proton resonance spectra provide an excellent tool for deciding which type of bonding exists in various metal-organic compounds, and they have been used for that purpose in this investigation.

Experimental Procedure

The NMR spectra were taken with a Varian High-Resolution NMR spectrometer, Model V4300B, at 4.0 Mc. Cyclohexane was used as the solvent. The chemical shift of aromatic compounds in cyclohexane is nearly independent of the concentration.[1] The spectra of all compounds were also measured in benzene and CCl_4 in order to eliminate the masking of CH_2 protons by the CH_2 groups in cyclohexane. The chemical shift for the cyclohexane CH_2 protons was taken as -3.9 ppm ("inner standard").[2] The relative positions for the chemical shifts were measured to within ± 0.05 ppm. The absolute values were determined to within ± 0.1 ppm.

Cyclopentadienyl manganese tricarbonyl, $MnC_6H_7(CO)_3$, and methylcyclopentadienyl manganese tricarbonyl, $MnC_6H_7(CO)_3$, were provided by the Ethyl Corporation. Ferrocene and 1, 1'-dimethylferrocene were provided by Professor K.L. Rinehart of the University of Illinois, and were purified by vacuum sublimation. Dicyclopentadienyl mercury was prepared as previously described.[3] Its elemental analysis was C = 36.44%, H = 3.2% (calculated: C = 36.3%, H = 3.1%). Cyclopentadienyltrimethyl silicon was prepared according to the directions of Frisch.[4] It was purified by gas chromatography ("Aerograph," "Ucon" substrate). Its elemental analysis was C = 69.62%, H = 10.42%

[1] P.L. Cario and B.P. Daily, J. Am. Chem. Soc. 78, 3043 (1956).

[2] Meyer, Seika, and Gutowsky, J. Am. Chem. Soc. 75, 4567(1953).

[3] G. Wilkinson and T.S. Piper, J. Inorg. and Nuclear Chem. 2, 32 (1955).

[4] K.C. Frisch, J. Am. Chem. Soc., 75, 6050 (1953).

(calculated: C = 69.49%, H = 10.2%).

Results and Discussion

The chemical shifts determined in the course of this work are presented in Figs. 7 and 8.

The CH proton resonance in ferrocene (Fig. 7a) has the value of -1.2 ppm, which contrasts with the value of +1.1 ppm found for the same resonance in cyclopentadiene.[5] It thus appears that ferrocene has the sandwich structure. The same holds for dimethylferrocene (Fig. 7b), whose proton resonances are $\delta_{CH} = -1.40$ ppm and $\delta_{CH_3} = -3.35$ ppm. Whereas the CH resonance of sandwich-structure cyclopentadiene compounds is strongly shifted away from its value in cyclopentadiene itself, the CH_3 resonance remains (within experimental accuracy) at the same position, namely, -3.3 ppm. In contrast, the spectrum for dicyclopentadienyl mercury (Fig. 7d) shows $\delta_{CH} = +0.7$, and this is in the neighborhood of the +1.1-ppm value found for pure cyclopentadiene.

The spectra for dicylopentadienyl mercury were also determined in solutions in benzene, CCl_4, and CS_2. In each case only a single, sharp resonance line was obtained. Since for this compound only a localized metal-carbon bond is assumed,[3] we should have two resonance lines with an intensity ratio of 4:1. These two lines would correspond, respectively, to the four protons (in each ring) on the two double bonds and the one proton on the carbon that is bound to the mercury. The failure of a second proton resonance to appear makes it doubtful that dicyclopentadienyl mercury really possesses localized metal-carbon bonds.

In order to clarify this situation, we investigated the NMR spectrum of cyclopentadienyltrimethyl silicon. For a variety of reaons, this latter compound is known to contain only a localized bond between silicon and the ring.[3] In fact, we found for cyclopentadienyltrimethyl silicon (Fig. 8a) two CH proton resonances with the expected intensity ratio of 4:1. The chemical shift for the four protons on the double bonds occurred at +1.3 ppm, just as for cyclopentadiene. The resonance for the other ring protons comes at -2.7 ppm. Therefore, for cyclopentadienyltrimethyl silicon, the NMR spectrum confirms the hypothesis of a localized metal-earbon bond. The proton resonance of the three CH_3 groups lies at approximately -5.3 ppm; it exhibits two peaks with an intensity ratio of about 2:1. Since gas chromatography strongly indicates that we have a single, pure compound, the two peaks show that the CH_3 groups are not equivalent. A molecular model of the compound shows that the proton on carbon No. 1 of the ring lies between two methyl groups. Therefore the electron distribution of these two groups is slightly different from that of the third methyl group, and the 2:1 splitting of the CH_3 proton resonances is thus understandable.

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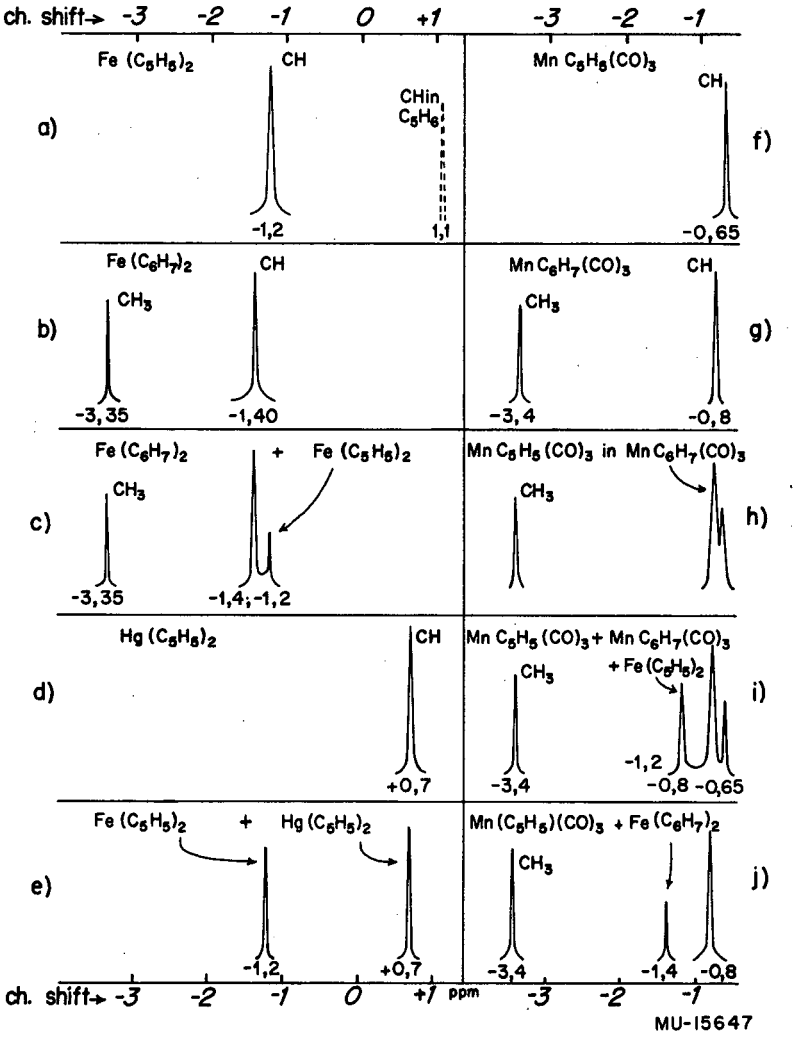
[5] W. Strohmeier, E. Lombardi, and R.M. Lemmon, This report, p. 10.


ch. shift→ -3 -2 -1 0 +1
-3 -2 -1
a)
Fe (C5H5)2
CH
CHin C5H6
-1,2
1,1
f)
Mn C5H5(CO)3
CH
-0,65
b)
Fe (C6H7)2
CH3
CH
-3,35
-1,40
g)
Mn C6H7(CO)3
CH3
CH
-3,4
-0,8
c)
Fe (C6H7)2
+
Fe (C5H5)2
CH3
-3,35
-1,4; -1,2
h)
Mn C5H5 (CO)3 in Mn C6H7(CO)3
CH3
d)
Hg (C5H5)2
CH
+0,7
i)
Mn C5H5 (CO)3 + Mn C6H7(CO)3
+ Fe (C5H5)2
CH3
-3,4
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-0,8 -0,65
e)
Fe (C5H5)2
+
Hg (C5H5)2
-1,2
+0,7
j)
Mn (C5H5)(CO)3 + Fe (C6H7)2
CH3
-3,4
-1,4
-0,8
ch. shift→ -3 -2 -1 0 +1 ppm -3 -2 -1
MU-15647

Fig. 7. The proton resonance spectra of cyclopentadienyl-metal compounds at 25° in cyclohexane and benzene
(a) Ferrocene (0.03)* in C_6H_{12}
(b) Dimethylferrocene (0.046) in C_6H_{12}
(c) Ferrocene (0.02) and dimethylferrocene (0.06) in C_6H_{12}
(d) Dicyclopentadienyl mercury (0.07) in C_6H_6**
(e) Dicyclopentadienyl mercury (0.07) and ferrocene (0.04) in C_6H_6**
(f) Cyclopentadienyl manganese tricarbonyl (0.046) in C_6H_{12}
(g) Methylcyclopentadienyl manganese tricarbonyl (0.16) in C_6H_{12}
(h) $MnC_5H_5(CO)_3$, (0.026), dissolved in $MnC_6H_7(CO)_3$
(i) $MnC_5H_5(CO)_3$, (0.05), $MnC_6H_7(CO)_3$, (0.19), and ferrocene (0.05) in C_6H_{12}
(j) $MnC_6H_7(CO)_3$, (0.32), and $Fe(C_6H_7)_2$, (0.06) in C_6H_{12}

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*The values given parenthetically are mole fractions.

**Benzene was used because of the greater solubility. The chemical shift was determined through the help of an "outer" standard of cyclohexane.

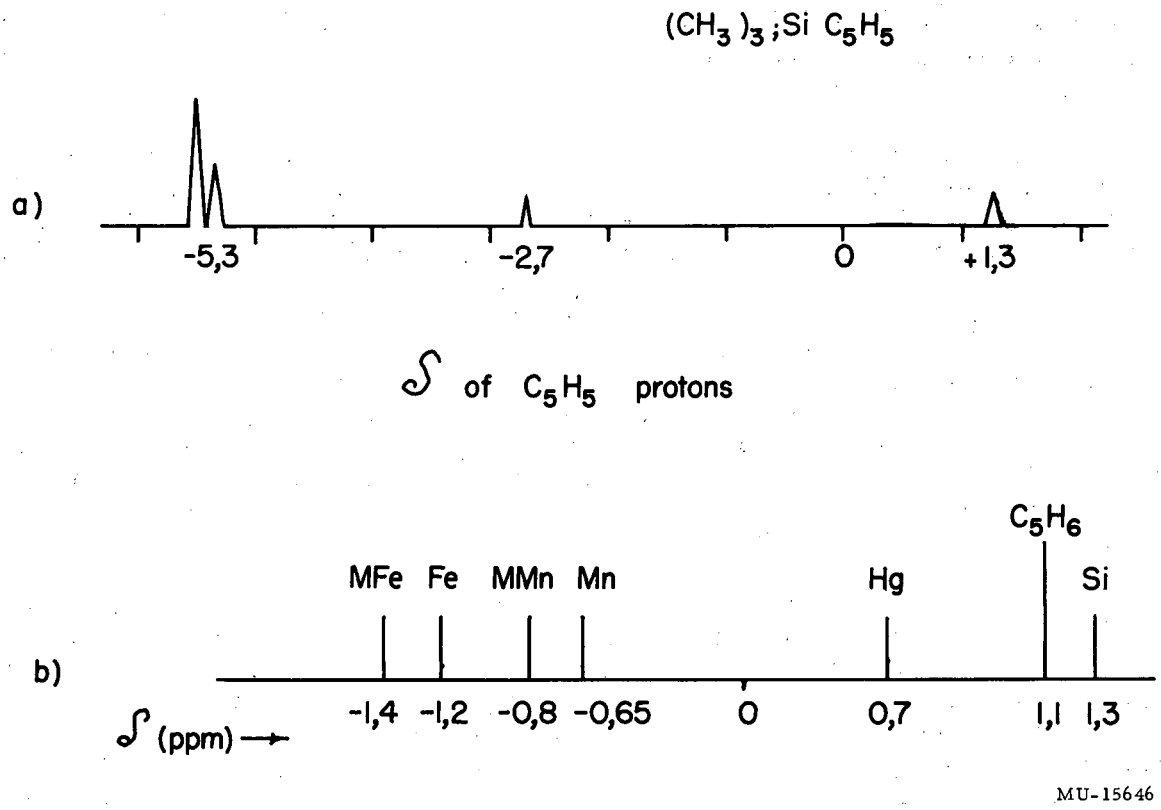

Fig. 8. (a) The proton resonance spectrum of cyclopentadienyltrimethyl silicon

(b) The positions of the chemical shift of the CH ring protons in various compounds. MFe = 1, 1'-dimethylferrocene, Fe = ferrocene, MMn = methylcyclopentadienyl manganese tricarbonyl, M = cyclopentadienyl manganese tricarbonyl, Hg = dicyclopentadienyl mercury, C_5H_6 = cyclopentadiene, Si = cyclopentadienyltrimethyl silicon.

Other investigators have found that cyclopentadienyl sodium, dissolved in tetrahydrofuran, shows a single proton resonance at +0.62 ppm.[6] This is very near the position we have found for the same resonance in dicyclopentadienyl mercury. There is no doubt that the sodium compound possesses a heteropolar ring-metal bond.

In cyclopentadienyl manganese tricarbonyl (Fig. 7f; $\delta_{CH} = -0.65$ ppm) and methylcyclopentadienyl manganese tricarbonyl (Fig. 7g; $\delta_{CH} = -0.8$ ppm), the CH resonances have negative values, as is the case for ferrocene. The CH_3 resonances come at −3.4 ppm, at the same position as the methyl proton resonances in methylferrocene.

The differences in the chemical shifts of the compounds reported here make possible--as Figs. 7c, 7e, 7h, 7i, and 7j show--the use of NMR spectra of mixtures for analyses. In cyclohexane solutions the position of the chemical shift appears independent of concentrations in the mole-fraction range of 0.2 to 0.4. Cyclohexane is therefore the solvent of choice, since the results at different concentrations may be compared with one another. It must be emphasized that only for diamagnetic cyclopentadienyl-metal compounds does there exist the possibility to distinguish between a sandwich structure and a localized metal-carbon bond. In paramagnetic compounds, such as dicyclopentadienyl nickel, the proton resonance peak is greatly broadened and shifted by about 1.1 gauss toward a higher field strength.[7] For the compounds we have reported here, the shift in the position of the proton resonances is on the order of only about 10 milligauss.

In Fig. 8b are summarized the chemical shifts of the CH protons in the compounds we have investigated. The compounds in which the cyclopentadienyl ring possesses aromatic character (e.g., in ferrocene) exhibit CH resonances shifted towards negative values in comparison with the shifts found for compounds with less aromatic character (e.g., dicyclopentadienyl mercury). It was also observed that the compounds with positive values for the CH shift exhibit greater reactivity toward maleic anhydride. Whether this observation may be extended to become a general rule can only be decided after the NMR spectra of further, known diamagnetic cyclopentadienyl-metal compounds are investigated.

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THE PHOTOVOLTAIC EFFECT AND PHOTOCONDUCTIVITY IN LAMINATED ORGANIC SYSTEMS*

David Kearns and Melvin Calvin

As a result of a wide variety of studies on photosynthesis in living plants and plant fragments, together with the development of photosensitive photovoltaic junctions in inorganic crystals[1] and the discovery and exploration of semiconduction in organic molecular substances,[2-7] a suggestion has been made[8-13] that the primary quantum conversion process in photosynthetic tissues involves the creation and separation of charge to opposite sides of an asymmetrically constructed lamina, followed by the trapping of both the electrons and the holes; the trapping then leads to the respective chemical processes of the electrons and holes, namely, reduction of carbon dioxide and oxidation of the water to oxygen. This has led us to study model systems[1] as semiconductors with a view to creating an organic photovoltaic junction.[14]

We have found that discs of magnesium phthalocyanine (MgPh) coated with a thin film of air-oxidized tetramethyl p-phenylenediamine (TMϕD) are organic systems that do show the photovoltaic effect. The maximum voltage as yet developed by one of these discs was 200 mv. The rise and decay is instrument-limited (~ 1 sec). The maximum power output was only 3×10^{-12} watt. The power output appears to be limited by an internal cell resistance of 10^8 ohms and by the maximum radiant energy incident on the junction.

Discs 1.5 cm in diameter and 0.1 cm thick were formed by pressing powdered MgPh in a die. These discs were then made photovoltaic when coated with a film of air-oxidized TMϕD by evaporation of an acetone solution

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*This article has been submitted to J. Chem. Phys.

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of the amine. The coated sides of the discs were pressed in contact with a conducting glass electrode by a stainless steel piston applied to the back of the disc. This formed the photovoltaic cell, which was placed in a vacuum chamber along with suitable electrical leads in insulation. During measurements a mechanical pressure of 9 kg/cm^2 held the disc against the glass and a gas pressure of 10^{-4} mm Hg was maintained. The junction was illuminated by passing light from a 500-watt projection lamp through a window in the chamber and through the conducting glass electrodes. A recording vibrating-reed electrometer was used to measure the photovoltage, photoconductivity, and resistance of the cells. Action spectra for the photovoltaic effect and the photoconductivity were obtained by use of a Bausch and Lomb monochromator.

In all cases the MgPh layer was at a positive potential with respect to the oxidized TMϕD layer, indicating electron transfer from the MgPh to the TMϕD. For several junctions made with MgPh and TMϕD precautions were taken to prevent air oxidation of the amine. These junctions produced little or no detectable photovoltage, indicating that the oxidized TMϕD is required. Space-charge effects were present only when there was a visible amount of white unoxidized TMϕD on the coated MgPh discs. The MgPh alone exhibited no photovoltaic effect. The oxidized TMϕD alone gave a very small photovoltaic effect, appearing slightly negative with respect to the stannic oxide layer of the conducting glass. The action spectra of the photovoltaic effect and the photoconductivity of a representative cell are shown in Fig. 9.

The action spectrum, although resembling that of the absorption of solid magnesium phthalocyanine, does not coincide with it. This is to be expected, since the action spectrum should be that of the junction layer rather than of the magnesium phthalocyanine itself, and perhaps has more in common with Wurster's blue than with the phthalocyanine derivative.

A completely different system has also been investigated in a preliminary way. A matrix of coronene was coated with a thin layer of o-chloranil as an electron acceptor. In this case the internal resistance of the cell was extremely high (10^{11} ohms), but a photovoltaic effect was easily measured in which the coronene was positive and the chloranil layer was negative. These and other systems involving matrices of much better conductivity and both electron-donor and electron-acceptor layers are being investigated in greater detail.

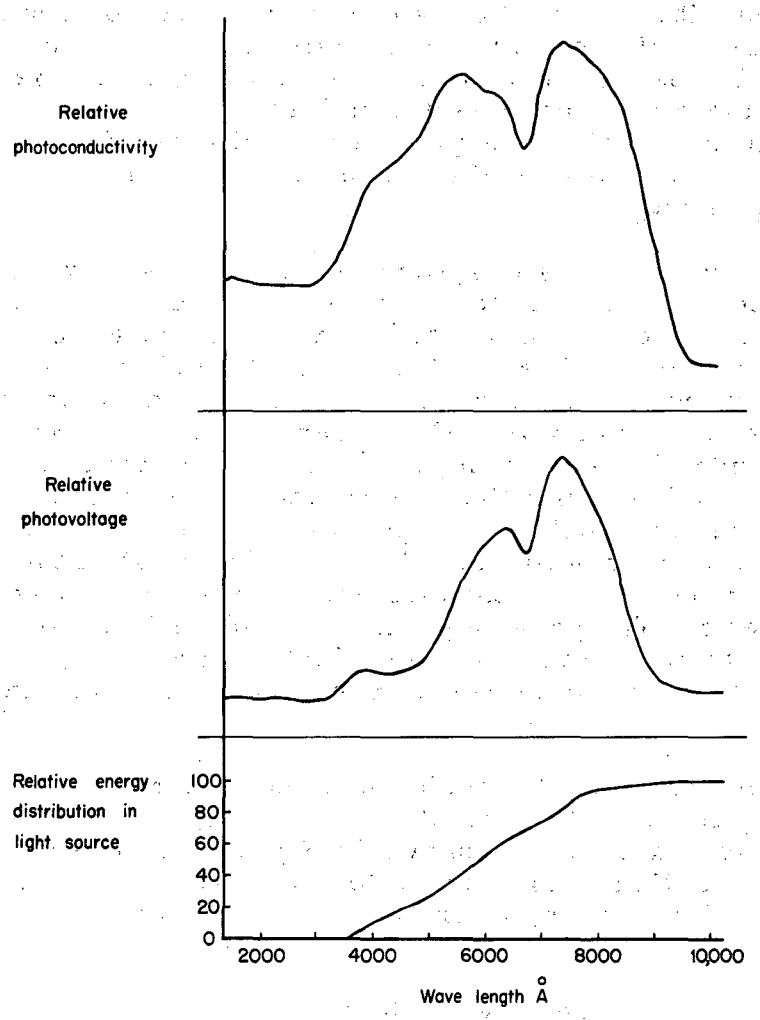

Figure 1. PHOTOCONDUCTIVITY AND PHOTOVOLTAIC EFFECT AT Mg PHTHALOCYANINE-OXIDIZED TMΦD JUNCTION

MU-15553

Fig. 9. Photoconductivity and photovoltaic effect at Mg phthalocyanine-oxidized TMΦD junction.

THE ELECTRICAL PROPERTIES OF PURE AND "DOPED" ORGANIC SEMICONDUCTORS

David Kearns and Melvin Calvin

The temperature variation of the electrical resistance of organic semiconductors can be described by the well-known semiconductor equation $\rho = \rho_0 e^{\Delta E/2kT}$, where ΔE is the thermal activation energy for conduction and ρ_0 is the specific resistance at infinite temperature. In this and other ways semiconductor theory has been useful in describing the electrical behavior of pure organic compounds. We have qualitatively attempted to extend these ideas to the description of mixtures of an organic semiconductor and an oxidizing or reducing agent.

The electrical conductivity of a semiconductor arises from excitation of electrons in a filled valence band to an unfilled conduction band. This is shown in Fig. 10a. If we now incorporate into the semiconductor crystal, by means of impurity molecules, acceptor levels that lie below the top of the valence band, electrons will be lost from the valence band to the acceptor levels. This will produce a partially filled valence band which may conduct electricity by hole conduction as shown in Fig. 10b. The conductivity in Case b would be essentially metallic, with little or no activation energy ΔE. Any activation energy would presumably be an activation energy for mobility, as pointed out by Schneider and co-workers.[1] A second result of the charge transfer would be the production of paramagnetic ions in compounds that are individually diamagnetic. The paramagnetism should be detectable by electron-spin resonance. The temperature variation of the paramagnetism could be used to demonstrate the relative energies of the acceptor level and the valence band.

Essentially the same results could be expected for a semiconductor doped with a reducing agent, with a filled electronic level above that of the bottom of the conduction band (this is shown in Fig. 10c). The only difference would be that electron motion in the conduction band would be studied instead of hole motion in the valence band, as in Case b.

Experimental Results

To test the above hypothesis the organic semiconductors, metal-free phthalocyanine (Ph) and coronene, and the oxidizing agent, ortho-chloranil, were studied. All the conductivity measurements to be described were made with packed polycrystalline samples of the compounds and mixtures. The experimental techniques are briefly described in an earlier report.[2]

Coronene

High-purity coronene from Badlische Anilin and Soda Fabrik was used without further purification. No detectable paramagnetism was found in this material. Owing to the high resistance of the coronene ($\sim 3 \times 10^{14}$ Ω-cm) it

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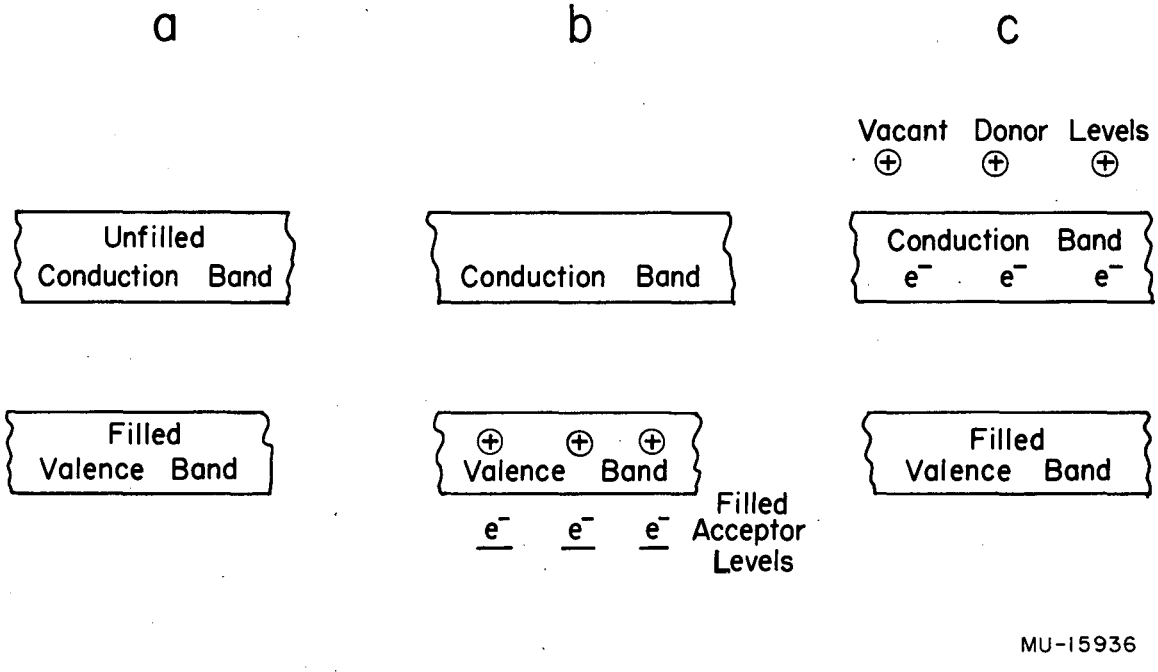

Fig. 10. Electronic bond structure of a pure and a "doped" semiconductor.

was difficult to obtain an accurate value for the thermal activation energy. It has been reported by others[3] as 1.15 ev.

Ortho-chloranil

Recrystallized ortho-chloranil was found to have no paramagnetism. Again, as with coronene, the high electrical resistance (10^{15} Ω-cm) prevented an accurate determination of ΔE. Ortho-chloranil, however, does appear to be a semiconductor, and an approximate value of ΔE = 3.4 ev was found.

Metal-Free Phthalocyanine

Dupont metal-free phthalocyanine (Ph) was found to have a large free-radical content, which had to be removed before semiconductivity measurements were made. This was accomplished in the following manner. Crude Ph was washed with hot dimethyl formamide (DMF) and then dissolved in a boiling solution of DMF containing about 15% benzyltrimethyl ammonium hydroxide. The metal phthalocyanines are insoluble in this solution. The Ph was precipitated from the solution by dilution with acetone or water. The Ph precipitate was then collected and a quinoline Soxhlet extraction was performed on it. Small crystals were collected from the quinoline and were found to have essentially no free-radical content. The semiconductivity of this material is shown in Fig. 11. The thermal activation energy for this polycrystalline material was found to be 1.73 ev. This is in very good agreement with the value 1.71 ev reported for single-crystal phthalocyanine measurements.[4]

After the behavior of the pure compounds was established we next investigated the electrical behavior of the Ph and coronene "doped" with o-chloranil. The doping was done as follows: a benzene solution of o-chloranil was added to a benzene suspension of coronene or Ph. The result was that the red o-chloranil color disappeared from the solution and the suspended material became coated with a red film. The decolorized benzene solution was then pumped off under reduced pressure. In general, about 10 moles of Ph or coronene per mole of o-chloranil were used, and electron-spin resonance measurements indicated a free-radical content that would correspond to a 5 to 10% conversion of the o-chloranil into free radicals. Free radicals could also be produced by grinding together the compounds in the solid state; this indicates a fairly high degree of interaction between the molecules of the semiconductor and the o-chloranil. The free-radical concentration increased very slightly as the temperature of the sample decreased. This indicated that the o- chloranil acceptor level lay below the top of the valence band in both cases. The semiconductivity of o-chloranil-doped coronene is shown in Fig. 12 and that of o-chloranil-doped Ph in Fig. 13. The results of measurements on the pure and doped compounds are summarized in Table I. As expected, large increases in conductivity were produced by doping the two semiconductors with an electron acceptor. Doping increased the conductivity of Ph by a factor of 10^7, and that of coronene by a factor of 10^5. The activation energy for conduction in Ph dropped from 1.73 ev to about 0.25 ev as a result of doping. The results on coronene were inconclusive.

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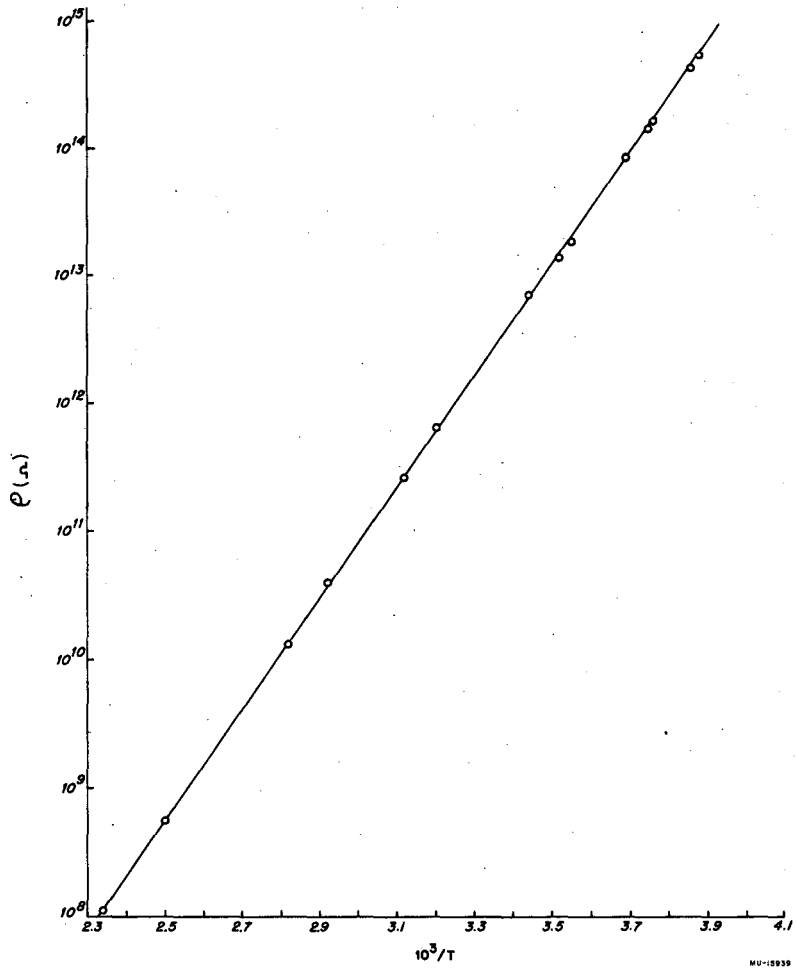

Fig. 11. The resistance of metal-free phthalocyanine as a function of temperature.

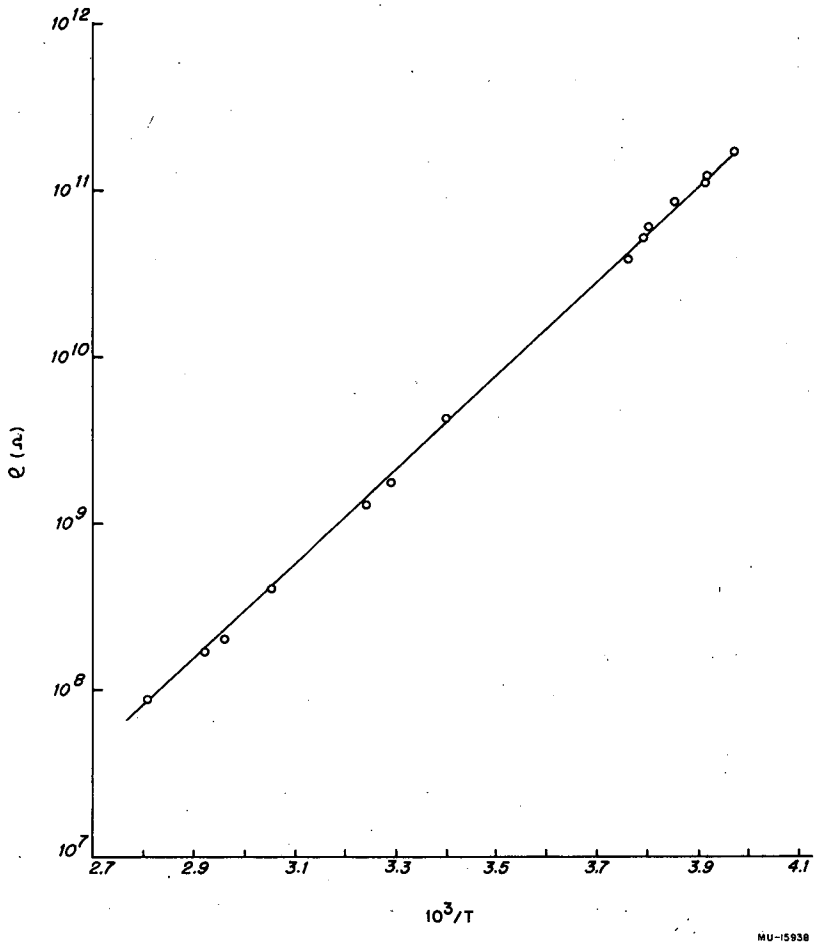

Fig. 12. The resistance of ortho-chloranil- "doped" coronene as a function of temperature.

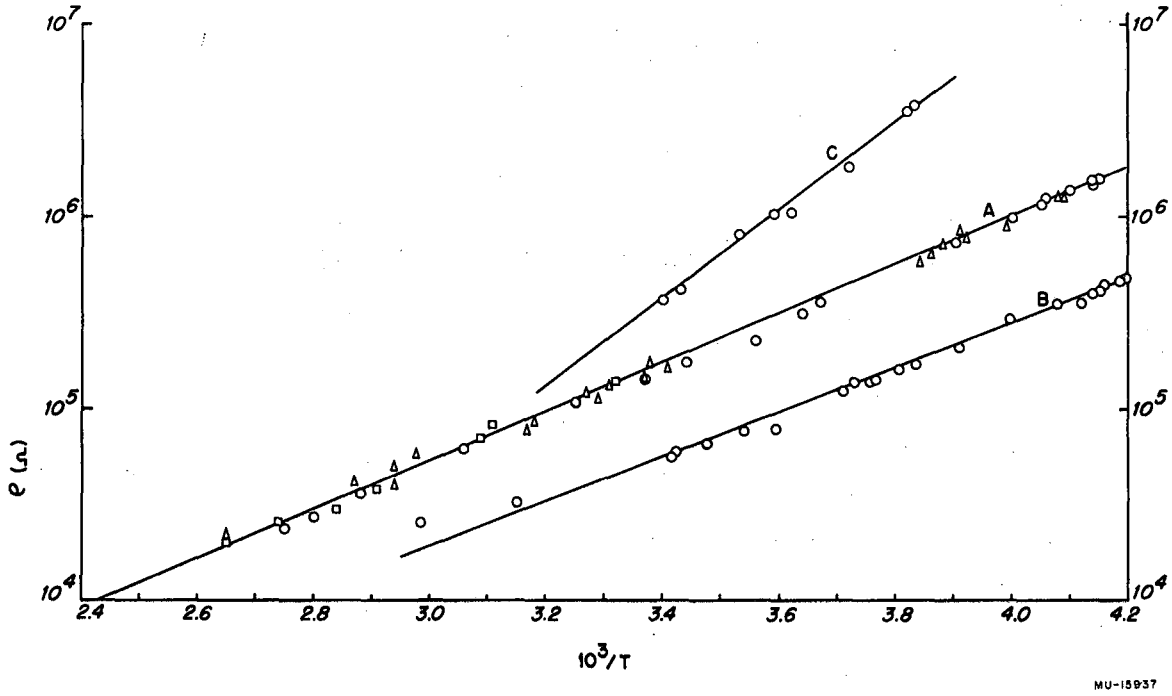

Fig. 13. The resistance of ortho-chloranil-"doped" phthalocyanine as a function of temperature.

The specific conductivity is a product of the carrier mobility, μ, and the number of sites N_0 that may be activated to produce charge carriers. At present it has not been possible to measure carrier mobilities in organic semiconductors, but rather they can be inferred from an estimate of N_0. Accurate measurements of free-radical concentrations will be made to relate quantitative conductivity increases to free-radical concentrations. It is hoped that the technique of injection of a known number of holes or electrons into a semiconductor will allow one to calculate carrier mobilities from a knowledge of resistivities. This will require single-crystal work, which is planned for the future.

In conjunction with the work reported an attempt has been made to find a suitable organic-semiconductor—doping-agent system in which electrons will be injected into the conduction band of the semiconductor. So far, this search has been unsuccessful.

Table I

Properties of pure and "doped" organic semiconductors

Material		ΔE (ev)	ρ Room temp. (Ω-cm)	$\frac{\rho\ RT(\text{pure})}{\rho\ RT(\text{doped})}$
Metal-free phthalocyanine		1.73	10^{11}	
Coronene		--	5×10^{13}	
O-Chloranil		3.4	10^{15}	
Phthalocyanine doped with o-chloranil	a	0.25	5×10^{3}	2×10^{7}
	b	0.23	2×10^{3}	5×10^{7}
	c	0.4	10^{4}	10^{7}
Coronene doped with o-chloranil		--	5×10^{8}	10^{5}

THE LIGHT-DARK EFFECT IN CHLORELLA AND IN SCENEDESMUS

Gabriel Gingras and James A. Bassham

When some plants (Chlorella, Scenedesmus, Crassulaceans[1]) are allowed to photosynthesize for a few minutes in the presence of $C^{14}O_2$ and then the light is turned off, some interesting phenomena may be observed. One of these is the sudden increase in the labeling of certain intermediates (citric and glutamic acids) related to the Krebs cycle.[2,3,4] This effect was interpreted, at first, as the result of a photoinhibition of respiration.[3,5] The studies by Brown,[6] however, indicated that total respiration was not inhibited by light, since the rate of O_2^{18} consumption by Chlorella remained unchanged through consecutive periods of light and dark. Brown did find under some conditions a sudden increase in respiration when the light was turned off.[6]

The light-dark effect following a period of steady-state photosynthesis was studied in this laboratory,[4] and it was concluded that the evidence gave support to the earlier hypothesis that there is a photoinhibition of the decarboxylation of pyruvic acid to acetyl-coenzyme A (acetyl-CoA) through a reduction of lipoic acid.[3] More recently, Biswas and Sen found that the addition of lipoic acid in its oxidized form to Chlorella during photosynthesis in the presence of $C^{14}O_2$ does increase the amount of labeling of intermediates in the tricarboxylic acid cycle.[7] An additional factor is the increase caused by ammonia or nitrate in the labeling of glutamic acid and of carboxylic acids related to the Krebs cycle, during periods of photosynthesis in the presence of $C^{14}O_2$.[8]

As an attempt at further investigation of this problem we decided to look at the synthesis of fatty acids during conditions under which the "light-dark effect" could be observed. On the assumption that fatty acid synthesis proceeds from acetyl-CoA as its main building block, a photoinhibition of the pathway producing acetyl-CoA would be expected to affect not only the Krebs cycle but fatty acid synthesis as well. However, fatty acid synthesis is closely dependent on the level of reduced triphosphopyridinenucleotide,[9] one of the electron carriers thought to be formed in photosynthesis. If, nevertheless, fatty acid synthesis were found to proceed at a slower rate in the light, it would be a strong argument in favor of a blocking in the sequence of reactions from phosphoglyceric acid to acetyl-CoA.

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Experimental Procedure

Photosynthesis

Ten-ml samples of 1% suspension of Chlorella pyrenoidosa or of Scenedesmus obliquus in 5 x 10^{-4} M $NH_4H_2PO_4$:$(NH_4)_2HPO_4$(3:2 vv) were pipetted into flattened tubes and exposed to light with 1% CO_2-in-air bubbling through. The light source consisted of one General Electric RSP2 photospot at a distance of 15 cm from the tube. A water-cooled infrared filter served to absorb the excess heat evolved by the photospot. After a preillumination period of 4 min, the flow of CO_2 in air was stopped and 1 ml of $NaHC^{14}O_3$ (13 micromoles and 400 microcuries) was injected into the suspension. The tube was stoppered and shaken by hand for periods of 1 to 6 min. The dark experiments were done in total darkness. The algae were killed in a volume of absolute ethanol sufficient to make a final concentration of 80% ethanol.

Extraction Procedure

The alcoholic suspension was brought to about 60°C on a steam bath, filtered on a suction funnel, and extracted with another 60 ml of 80% ethanol, then with 50 ml of acetone. The residue was resuspended in 40 ml of 20% ethanol, where it was left for 3 hours to liberate the lipids from lipoproteins. After another filtration, the residue was extracted with 60 ml of 20% ethanol, 50 ml of acetone, 30 ml of petroleum ether, and finally with 30 ml of water.

After evaporation in vacuo, the extracts were resuspended in 1 ml of water in glass-stoppered conical centrifuge tubes, and the scheme indicated in Fig. 14 was followed. This suspension was extracted six times with 2 ml of petroleum ether. The sixth extract was found to contain but little radioactivity. The petroleum ether extracts were combined in a glass-stoppered conical centrifuge tube. After concentration to a volume of 1 ml under a gentle nitrogen stream, the solution was washed three times with 2 ml of water to remove the contaminants extracted along with the lipids. These contaminants were found, by chromatography, to be amino acids, sugar phosphates, and malic, fumaric, and citric acids. They accounted for 2 to 3% of the radioactivity found in the lipid material.

The petroleum ether was then completely evaporated under a nitrogen stream and the tubes were stored overnight in a desiccator in vacuo over "Drierite." Exactly 1 ml of petroleum ether was pipetted into the tubes and the extracts carefully redissolved. Two suitable aliquot portions from each tube were plated and counted for radioactivity. This will subsequently be designated as "total lipids."

Saponification

After another evaporation under a nitrogen stream, the total lipids were redissolved in 2 ml of ethanol-ether (3:1 vv). One ml of 1 N sodium ethanolate was added along with a small carborundum boiling chip. Each tube was covered with a burette funnel, placed in a small electrical water bath, and boiled for 40 minutes. Two ml of water was then added and the solution was heated for an additional 15 min. The volume was reduced to 1/2 ml under a nitrogen stream to evaporate the alcohol. The aqueous solution was readjusted to a

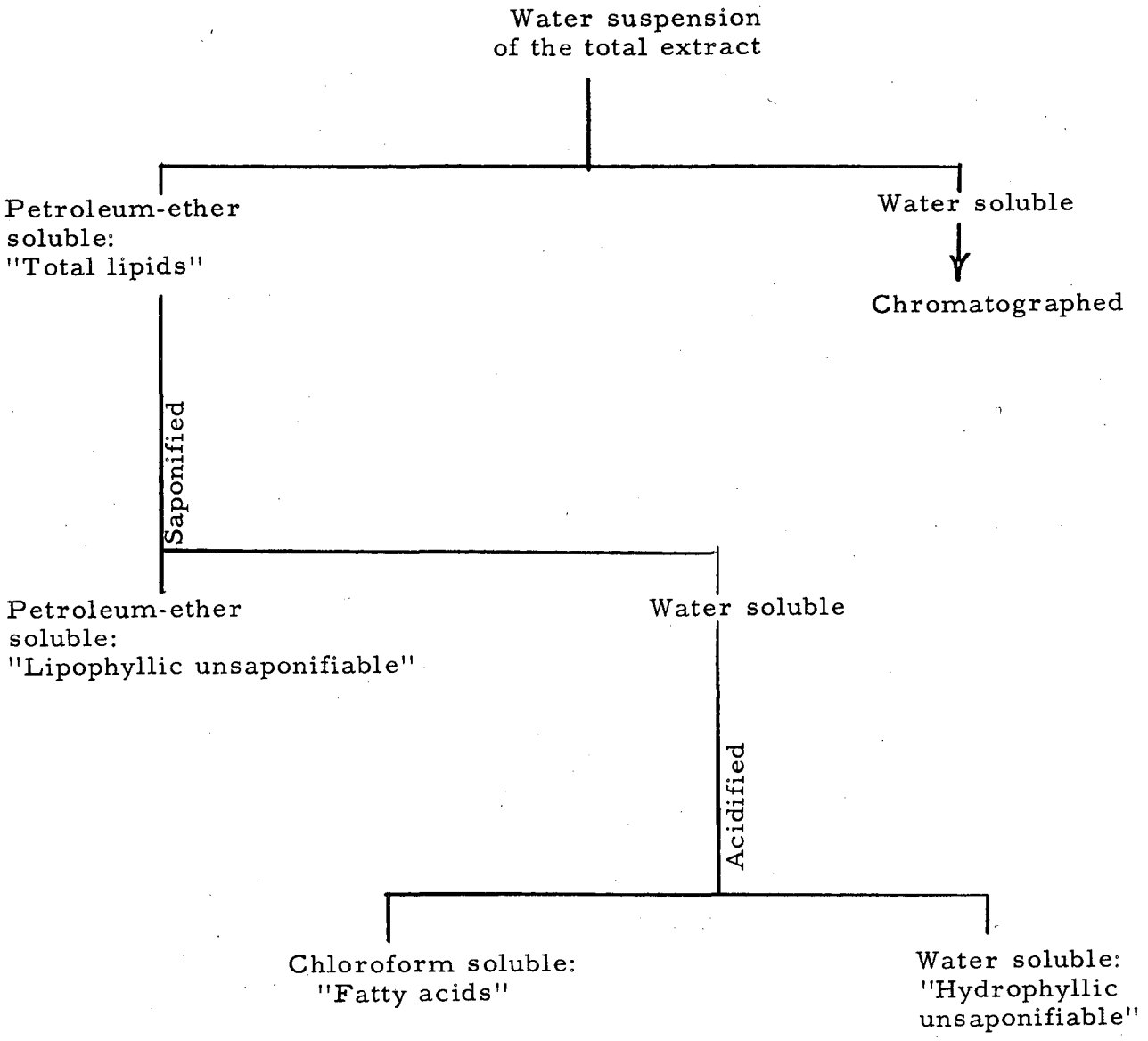

Fig. 14. Separation scheme for extracts from algae.

volume of 1 ml and extracted six times with 2-ml volumes of petroleum ether to remove the lipophyllic unsaponifiable material. The aqueous solution was then acidified with 5 N sulfuric acid and exactly 1 ml of chloroform was pipetted into each tube. The tubes were stoppered, vigorously shaken for 2 min, and centrifuged at 2500 rpm during 15 min to insure separation of the aqueous and organic layers. An aliquot portion of the organic phase was withdrawn and its contents spread on an aluminum planchet, after which its radioactivity was determined.

Chromatography

A suitable aliquot portion of the nonlipidic part of the soluble extract was chromatographed two-dimensionally by the usual procedure. The radioactive substances were detected by radioautography and counted directly on the paper. All the spots were counted and their radioactivity totaled. The percentage of each substance with respect to this total was corrected for the radioactivity present in the lipids and is here given with respect to the total soluble activity (Table II).

It was shown that the previous extraction with petroleum ether did not introduce any measurable error in these determinations. This was demonstrated by measuring the ratios of the radioactivities of the individual spots to the total soluble radioactivity, before and after extraction with petroleum ether.

Results

It is seen in Table II that under similar conditions the dark effect does not manifest itself in the same way in Chlorella and in Scenedesmus. In Chlorella the turning off of the light causes an increase in the quantity of radiocarbon in citric acid (6 times) and in glutamic acid (1.6 times). In Scenedesmus the presence of ammonia in the medium shifts the increase to glutamic acid (2.3 times) and to aspartic acid (1.6 times), whereas the radioactivity decreases in malic (2.5 times) and fumaric acids.

Although it is of a preliminary nature, our study of the rate of radiocarbon incorporation into fatty acids points out a few important facts, (see Table III).

(a) In conditions suitable for observation of the dark effect the rate of incorporation of radiocarbon into fatty acids is not accelerated by shutting off the light. On the contrary, it is decreased during the first minute of darkness (Experiments 1 and 4), and fails to show any stimulation during longer periods of darkness (Experiments 3 and 5).

(b) The distribution of the radiocarbon in the lipids differs very much between Chlorella and Scenedesmus. In Chlorella about 20% of the lipidic radioactivity in the light is accounted for by the fatty acids, as compared with 80% in Scenedesmus.

(c) On the turning off of the light the rate of entry of the radiocarbon into the hydrophyllic unsaponifiable fraction not only is not decreased but also seems to be much faster during the first minute of darkness (see Experiments 1 and 4).

Table II

Percentages of the radioactivity in some substances compared to the total soluble fixation

Substance	Experiment No.	3 min light	2 min light	2 min light, 1 min dark	3 min light, 1 min dark
Malic acid	1	9.44	11.97	9.07	----
	4	15.99	-----	----	6.14
Citric acid	1	0.645	0.573	3.87	----
	4	1.24	-----	----	1.22
Fumaric acid	1	2.32	2.61	2.05	----
	4	0.649	-----	----	~0
Glutamic acid	1	5.10	2.65	8.29	----
	4	5.46	-----	----	12.81
Aspartic acid	1	6.57	5.22	5.45	----
	4	7.81	-----	----	12.42
Alanine	1	18.38	14.80	18.27	----
	4	2.88	-----	----	3.13
Fatty acids	1	1.46	1.18	1.50	----
	4	15.68	-----	----	17.71
Total[a] soluble fixation	1	30.10	24.05	24.27	----
	4	32.00	-----	----	29.36

Note: Experiment 1 was done on Chlorella pyrenoidosa,
Experiment 4 on Scenedesmus obliquus.
[a]Expressed in millions of counts.

Table III

Distribution of the radioactivity in lipids during short periods of $C^{14}O_2$ fixation in light and in dark

(Radioactivities expressed in counts per minute x 10^{-3})

Experiment No.	Minutes of light	Minutes of dark	Total lipids	Fatty acids	Incorporation of radioactivity in fatty acids per minute		Unsaponifiable	
					Light	Dark	Hydro-phyllic	Lipo-phyllic
1	3	0	2704	440	147	--	---	---
	2	0	1701	285	142	--	---	---
	2	1	1879	366	142	81	---	---
2	6	0	4286	1742	291	--	2364	180
	1	0	512	116	116	--	391	4.6
	1	5	1068	170	116	11	839	59
3	1	0	1576	467	467	--	1097	12.3
	1	5	1696	633	467	33.3	1021	42.4
4	3	0	5797	5018	1672	--	169	610
	3	1	7400	5201	1672	183	1584	615

Note: Experiments 1, 2 and 3 have been done on *Chlorella pyrenoidosa*, Experiment 4 on *Scenedesmus obliquus*.

Discussion

The labeling of fatty acids does not appear to be faster in the dark than in the light. This result is especially significant for the first minute of darkness: it can be assumed that the specific activity of acetyl-CoA is about the same then as when the light was turned off. Although this finding does not corroborate the proposal of a direct photoinhibition of the decarboxylation of pyruvic acid, it cannot be taken as evidence against such a hypothesis. It suggests, however, that the site of fatty acid synthesis may be closely related to the photosynthetic system that reduces pyridine nucleotides, since light has an immediate effect on fatty acid synthesis. Langdon has shown the close dependence of the rate of fatty acid synthesis to the concentration of TPNH in liver tissue.[9]

Although we have no evidence against the mechanism proposed earlier (photoinhibition of pyruvic acid oxidation),[3] we would like to suggest another possible explanation (not mutally exclusive). When the light is turned off, the flow of acetyl-CoA going to synthetic processes, such as fat synthesis, which require reduced pyridine nucleotides is diminished. As a result, a higher concentration of acetyl-CoA is available to the condensing enzyme to make citric acid. In the presence of ammonia in the medium the conversion of citrate to α-ketoglutarate and thence to glutamate via amination would be accelerated. This would explain the build-up of radioactive glutamate while radioactive citrate remains constant in Scenedesmus. A diversion of the acetyl-CoA flow from synthetic processes to oxidative ones can also be inferred from the work of Milhaud, Benson, and Calvin.[10] These authors fed pyruvic acid-2-C^{14} to Scenedesmus in the light and in the dark and did a chromatographic analysis of the radioactive compounds. In the light most of the radioactivity was found in the lipids and relatively little in the Krebs-cycle intermediates. In the dark, the reverse was true: little radioactivity in the lipids and most of it in the Krebs-cycle intermediates and in alanine.

Investigations are now in progress on the effect of cellular oxidants on fatty acid synthesis and on the Krebs-cycle intermediates.

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[10] Milhaud, Benson, and Calvin, J. Biol. Chem. 218, 599 (1956).

ATTEMPTS TO PREPARE ACTIVE CARBON DIOXIDE COMPOUNDS

Ning G. Pon and M. Calvin

INTRODUCTION

When ribulose-1,5-diphosphate is enzymatically carboxylated in the presence of bicarbonate, 3-phosphoglyceric acid (PGA) is formed. The enzyme which performs this carboxylation (carboxydismutase) has a turnover number of 1500 moles of PGA per minute per mole of enzyme (assuming that the latter has a molecular weight of 300,000) and has a Michaelis constant for bicarbonate ion of 1.1×10^{-2} M.[1] Applying the Michaelis-Menten equation and substituting the numbers given above, one can calculate the rate of CO_2 fixation at any concentration of bicarbonate. Thus, for a bicarbonate concentration of 1.3×10^{-3} M, the amount of CO_2 fixed per minute per mg protein is 0.27μmole. This concentration of bicarbonate corresponds to 0.4% CO_2 in equilibrium with a pH 7.3 buffer. Since the rate of CO_2 fixation is not known for spinach, it is not possible to make a direct comparison of this fixation rate in the intact leaf with that of the enzyme derived from it. It would still be of interest, however, to make a comparison of the latter rate with that of intact Chlorella, even though it is not strictly correct.

It was found that the maximum rate of photosynthesis (both CO_2 uptake and O_2 evolution) can be maintained (in Chlorella) at pH 7.3 with a minimum of 0.4% CO_2 in light of 2,000 ft-c intensity.[2] On this basis, calculations show that 0.4 μmole of CO_2 is fixed per minute per mg protein. It must be emphasized here that these calculations assume that all the protein within the cell is made up of the carboxylation enzyme. In addition, the following assumptions were made: (a) 20% of the fresh weight of the algae represents the dry weight, and (b) 50% of the dry weight is the weight of total protein. It appears, therefore, that the in vitro rate of CO_2 fixation is comparable to the in vivo rate. If, however, 5 to 10% of the soluble protein is carboxydismutase, then only a maximum of 7% of the in vivo rate can be attained by the carboxydismutase system. It becomes necessary, therefore, to explain this discrepancy in terms of the denaturation of the enzyme, the lack of a necessary cofactor(s) and (or) the possibility of another type of CO_2 substrate. In this last regard, the possibility that an "active" CO_2 is a natural substrate for the carboxydismutase reaction has been considered,[3] for it could account for the low rate of CO_2 fixation when bicarbonate is used as a substrate. Attempts to test this possibility have now been made.

The "active" CO_2 system has been postulated as an intermediate in at least two enzymatically catalyzed reactions. These reactions are (a) the carboxylation of propionyl-CoA in the presence of CO_2 and ATP to form succinyl-CoA,[4] and (b) the carboxylation of hydroxyisovaleryl-CoA in the

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[1] Weissbach, Horecker, and Hurwitz, J. Biol. Chem. 218, 795 (1956).

[2] James A. Bassham, UCRL, private communcation.

[3] Metzner, Metzner, and Calvin, Proc. Natl. Acad. Sci. U.S. 44, 205 (1958).

[4] Flavin, Castro-Mendoza, and Ochoa, Biochim. Biophys. Acta 20, 591 (1956).

presence of CO_2 and ATP to form β-hydroxymethyl-β-methylglutaryl-CoA.[5] In the former reaction, the proposed "active" form of CO_2 is phosphoryl carbonate, whereas in the latter reaction the proposed form is adenosine phosphoryl carbonate. A compound with a tentative structure of adenosine monophosphoryl carbonate ethyl ester has been synthesized, tested with hydroxisovaleryl-CoA carboxylase, and found to be active as a substrate.[5] This ethyl ester was synthesized by reacting the monosilver salt of adenosine monophosphate with ethyl chloroformate.[6] The phosphoryl carbonate, however, has not been synthesized. It is the purpose of this report to describe attempts to prepare these compounds.

Experimental Procedure

Attempted synthesis of the disilver salt of phosphoryl carbonate ethyl ester (Compound I). This method is similar to the procedure used by Lipmann and Tuttle for the synthesis of acetyl phosphate.[7] Freshly prepared silver phosphate, 3.2 g (Ag_3PO_4, 7.6 mmole) was mixed with 1.2 ml of 85% H_3PO_4 (17.6 mmole). The powder turned from bright yellow to a brown-gray paste. Ethyl ether, 2.5 ml, was added to evenly distribute the material. The mixture was cooled in an ice bath and 2.3 ml of ethyl chloroformate (24 mmole) diluted with an equal volume of ether (cooled) was added slowly. After 24 hours at 0°C, with occasional stirring, the pH of the mixture was adjusted to 3.5 with 1 M Na_2CO_3. (Approximately 8 ml was required.) The grayish-yellow precipitate was removed by centrifugation at 5°C and the supernatant liquid was further neutralized with 8 N NaOH until the pH was 5.7. A thick mass of yellowish-white crystals was formed. These crystals were removed by suction filtration (accomplished in the cold room with all apparatus precooled). More white crystals of sodium phosphate were removed by further adjustment of the pH of the filtrate to 6.8 followed by suction filtration. At this point the filtrate was subjected to inorganic phosphate and chloride analysis (see below). A 10% excess of the calculated volume of 1.47 M $AgNO_3$ required to precipitate all the inorganic phosphate and chloride was added. The yellowish-white precipitate was removed by centrifugation and $AgNO_3$ solution was added drop by drop until no more precipitate was obtained. The silky white precipitate was washed once with ice water, twice with 33% ethanol, twice with absolute ethanol, and, finally, twice with diethyl ether. The material was dried under reduced pressure over P_2O_5. Yield: 260 mg.

Preparation of the monosilver salt of adenosine monophosphate (AMP): Adenylic acid, 383 mg (AMP·$2H_2O$, 1 mmole) was suspended in 5 ml of water. The pH of this suspension was adjusted to 6.0 with 1 M KOH. (All the material dissolved at this point.) $AgNO_3$ solution, 0.68 ml (1.47 M, 1 mmole) was added slowly, accompanied by constant stirring. The gelatinous precipitate could not be collected without forming a solid gel. Hence, the precipitate was mixed with two volumes of 80% ethanol and then centrifuged. The precipitate was washed an additional two times with 80% ethanol, three times with acetone, and, finally, three times with diethyl ether. The white powdery residue was allowed to dry overnight at room temperature in air and in the dark. Yield: 431 mg. Theoret for monosilver AMP: 454 mg; therefore yield is 95%.

[5] Bachhawat, Woessner, and Coon, Federation Proc. 15, 214 (1956).
[6] M. J. Coon, University of Mich. Med. School, unpublished communication.
[7] F. Lipmann and L. C. Tuttle, J. Biol. Chem. 153, 571 (1944).

Attempted synthesis of monosilver adenosine monophosphoryl carbonate ethyl ester (Compound II). The monosilver salt of AMP, 215 mg, was suspended in about 3 ml of diethyl ether. Ethyl chloroformate, 0.4 ml, was added and the mixture was stirred vigorously overnight (8 hours) at room temperature. About 2 ml of water was added and the pH of the mixture was adjusted to approximately 6.3 with 1 M Na_2CO_3. The excess ethyl chloroformate was extracted three times with ether. The precipitate was removed by centrifugation and the supernatant liquid was subjected to chloride and inorganic phosphate analyses. Silver nitrate solution (1.47 M) was added in sufficient amount to precipitate all the chloride ion plus a 10% excess (no inorganic phosphate was detected). After removal of the AgCl by centrifugation the slightly turbid supernatant liquid was mixed with an additional 0.2 ml of 1.47 M $AgNO_3$. A chalky white precipitate was obtained. It was washed three times with water, three times with 50% ethanol, twice with absolute ethanol, twice with acetone, and, finally, three times with ether. The powder was allowed to stand overnight at room temperature in the dark to permit the evaporation of the ether. Yield: 90 mg.

A small amount of each of the above samples was subjected to several of the following analyses:

Acyl phosphate by the hydroxamate-$FeCl_3$ colorimetric test.[8]
Silver as AgCl, gravimetrically.
CO_2, after acidification with H_2SO_4, manometrically.
Inorganic phosphate at pH 4 by reaction with molybdate, colorimetrically.[9]
Total phosphate, after digestion in $HClO_4$, by reaction with molybdate, colorimetrically.[10]
C, H, and C-CH_3 analyses.
Infrared analysis mounted in a KBr pellet.

Results

The results of the analyses are given in Tables IV and V.

Spectral analysis of Compound I in the infrared region shows the existence of a small absorption band in the 6.1-μ region. This may, or may not be, a carbonyl band. The absorption bands associated with the phosphate group are also present although the assignments of these bands are not definite. All the absorption bands are broadened by the mottled appearance of the KBr pellet. This appearance is probably caused by AgBr formed when the silver salt was pressed with the KBr to form the pellet.

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[8] F. Lipmann and L.C. Tuttle, J. Biol. Chem. 159, 21 (1945).

[9] O.H. Lowry and J.A. Lopez, J. Biol. Chem. 162, 421 (1946).

[10] R.J.L. Allen, Biochem. J. 34, 858 (1940).

Table IV

Analyses of Compound I

State of the compound	Storage conditions	Pretreatment	Analysis	Percent of theoret.[a]
Silver salt, solid	------	Converted to sodium salt	Silver	110
Sodium salt, solution	------	------	Acyl phosphate	neg.
Sodium salt, solution	------	In 0.83 N H_2SO_4 at 38°C	CO_2	9.3-17
Sodium salt, solution	------	------	Inorg. phosphate	0.84
Sodium salt, solution	------	In 8.7 N $HClO_4$ for 12 hr, at room temp. and 1 hr at 90°C	Liberated phosphate	7.3
Sodium salt, solution	------	------	Total phosphate	92
Sodium salt, solution	10 days at -15°C	In 0.05 N NaOH for 6 hr at 95°C	CO_2	0.0
Sodium salt, solution	11 days at -15°C	In 0.05 N NaOH for 6 hr at 95°C	Inorg. phosphate	7.1
Sodium salt, solution	11 days at -15°C	In 0.05 N NaOH for 6 hr at 95°C	Total phosphate	120
Silver salt, solid	12 days, room temp. over P_2O_5	2.0 N H_2SO_4 added to dry powder at 25°C	CO_2	0.0
Silver salt, solid	19 days, room temp. over P_2O_5	[illegible]	C-CH_3	90
Silver salt, solid	19 days, room temp. over P_2O_5	------	H	121
Silver salt, solid	19 days, room temp. over P_2O_5	------	C	66

[a]Percent of the theoretical value assuming that the compound is disilver phosphoryl carbonate ethyl ester.

Table V

Analysis of Compound II

State of the compound	Storage conditions	Pretreatment	Analysis	Percent of theoret.[a]
Silver salt, solid	-----	Converted to the sodium salt	Adenylic acid[b]	86
Sodium salt, solution	Several days at -15°C	In 0.05 N NaOH for 12 hr at 37°C and for 6 hr at 95°C	CO_2	32
Silver salt, solid	Several days, room temp.	2 N H_2SO_4 added to dry powder at 25°C	CO_2	0
Silver salt, solid	1.5 weeks, room temp.	2 N H_2SO_4 added to dry powder at 25°C	C-CH_3	3.8
Silver salt, solid	3 weeks, room temp.	Converted to the sodium salt	Acyl phosphate	neg.

[a]Percent of the theoretical value assuming that Compound II is the monosilver salt of AMP-CO_2 ethyl ester.

[b]Determined spectrophotometrically at 260 mμ.

Discussion

From the data in Table IV it is apparent that Compound I is not the disilver salt of phosphoryl carbonate ethyl ester. This conclusion is substantiated by the unusual stability of the compound towards both acid and alkaline hydrolysis. The liberation of phosphate only after digestion in concentrated perchloric acid indicates that the compound is an organic phosphate. Whether an "active" CO_2 or free CO_2 is present in the compound remains a question. To further confuse the picture, infrared analysis shows the presence of possibly a carbonyl absorption band. Both the C analysis and the acyl phosphate test are unreliable. The former is unsatisfactory on the grounds that some residue remained after combustion. This residue tends to give a low result, so that the C content can only be a minimum value. The acyl phosphate test, on the other hand, does not give a positive test for carbamyl phosphate. On the basis of most of the evidence, the following conclusion is reached: the compound is now the disilver salt of monoethyl phosphate. Whether or not it was this compound initially remains a mystery.

The data of Table V provide some evidence that Compound II is probably the disilver salt of AMP. Thus, both the absence of $C\text{-}CH_3$ and the correct adenylic acid percentage when calculated on the basis that the compound is disilver AMP (94%) supports this contention. Again, as in the previous case, the presence or absence of CO_2 in the preparation is uncertain. The acyl phosphate test was unreliable for the reason given above. It is interesting to note that a compound with a tentatively assigned structure of adenosine phosphoryl carbonate ethyl ester has been synthesized (see Introduction).[6] This synthesis was carried out in water solution and the excess ethyl chloroformate was removed by reaction with excess bicarbonate (instead of extraction with ether). In this case, hydroxylamine has been shown to convert the product to a heat-labile hydroxamic acid (identified by chromatography with the hydroxamic acid derived from the reaction of ethyl chloroformate with hydroxylamine).[11] If, indeed, the hydroxamate is formed, then either the negative results in the acyl phosphate test must stem from the formation of an uncolored $FeCl_3$ complex, or no reaction took place between the hydroxamic acid and $FeCl_3$. Whatever is the reason remains undecided.

In summary, attempts have been made to synthesize two "active" CO_2 systems. These attempts were unsuccessful. Instead of the expected products, the disilver salt of monoethyl phosphate and the disilver salt of AMP were formed. The chemistry of these compounds is still unexplainable in many ways.

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[11] B.K. Bachhawat and M.J. Coon, J.Am.Chem.Soc. 79, 1505 (1957).

SALICYLATES AND CARBOHYDRATE METABOLISM

M. J. H. Smith

Salicylate drugs, such as sodium salicylate and aspirin, can produce various disturbances of carbohydrate metabolism. Some of these have been observed in man, either in patients with rheumatic disorders receiving salicylate therapy or when the drugs have been taken for suicidal purposes, and they may be duplicated in experimental animals. The most frequent effects are severe depletion of liver glycogen, hyperglycemia, and glycosuria. It seems probable that an important factor in their causation is stimulation of the adrenal medullae by the salicylates. The subsequent course of events involves an increased secretion of adrenaline, leading to an increased rate of hepatic glycogenolysis, which in turn produces hyperglycemia and glycosuria.[1]

A second group of effects is observed in animal preparations with endocrine imbalance, in which the glycogen stores are low and the regulatory mechanisms for the maintenance of blood-sugar levels are relatively labile. Here the most important action of salicylate is the production of a rapid hypoglycemia. This is particularly evident in hypophysectomized and adrenalectomized rats,[2] and also occurs in experimental diabetes in animals[3] and diabetes mellitus in man.[4] Before the advent of insulin there were numerous reports in the clinical literature[5] that salicylates were useful in the management of diabetic patients because they reduced diabetic glycosuria.

The mechanism of this hypoglycemia-producing action of salicylate has been studied in recent years by experiments on isolated tissues and subcellular particulate preparations. Salicylates have been found to uncouple oxidative phosphorylation reactions in respiring mitochondrial suspensions, thus preventing the formation of ATP, which is a key substance in a large number of biological synthetic mechanisms. It has been suggested[6] that many of the actions of salicylate on carbohydrate metabolism, such as the hypoglycemia effect described above, may be due to defective carbohydrate synthesis as a result of the impaired production of ATP. This concept has remained tentative in the absence of direct evidence from whole-animal preparations. The work presented here was undertaken in an attempt to provide direct evidence for or against the above hypothesis.

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[1] M. J. H. Smith, Brit. J. Pharmacol. 10, 110 (1955).

[2] Smith, Meade, and Bornstein, Biochem. J. 51, 18 (1952).

[3] M. D. Dell'Aquila and D. Angarano, Folia endocrinol. (Pisa) 7, 5 (1954): Reid, MacDougall, and Andrews, Brit. Med. J. 2, 1071 (1957).

[4] M. Gross and L. Greenberg, The Salicylates (Hillhouse, New Haven, (1948).

[5] T. M. Brody, J. Pharmacol. 117, 39 (1956). Penniall, Kalnitsky, and Routh, Arch. Biochem. Biophys. 64, 390 (1956).

[6] M. J. H. Smith and S. W. Jeffrey, Biochem. J. 64, 589 (1956).

In the first series of experiments, rats--fasted for 24 hours previously--were divided into two subgroups. One subgroup received 1 mg of acetate-2-C^{14}, containing about 10 μC of C^{14}, by intraperitoneal injection, 1 hour after the administration of 3 mmoles of sodium lactate by stomach tube. The other subgroup was treated similarly except that they received an intraperitoneal injection of sodium salicylate, in dose of either 250 mg or 500 mg per kg body weight, 30 min before the administration of the acetate. The respiratory excretion of total CO_2 and $C^{14}O_2$ was followed in each animal for 1 hour after the acetate injection. The rat was then killed by an intraperitoneal injection of nembutal, the liver excised, and the liver glycogen isolated and its radioactivity determined. The results are shown in Table VI.

The general procedure was similar in the second series of experiments except that nonfasted animals were used, the lactate was omitted, and total fatty acids instead of glycogen were isolated from the liver. The results are given in Table VIII.

It is seen that in the rat injected with acetate-2-C^{14}, salicylate increased the respiratory excretion of total CO_2 and $C^{14}O_2$ and reduced the incorporation of the C^{14} into the glycogen and fatty acids of the liver. Salicylate therefore increased the oxidation of the acetate carbons and reduced their eventual participation in the synthetic reactions of glycogen formation and lipogenesis of the liver. These results provide direct support of the view that salicylate inhibits the synthesis of carbohydrate in the whole animal.

The increased respiratory output of total CO_2 suggests a general effect of salicylate on substrate oxidation in the tissues, since it has been demonstrated that the respiratory response to salicylate in the intact animal is a summation of both a direct central stimulant action and an increased metabolic production of CO_2.[7] The increased oxygen uptake of the intact rat given salicylate[8] is also evidence in support of this conclusion. If salicylate interferes with oxidative phosphorylation reactions in the tissues, then the increased oxidation of metabolic intermediates, including the introduced labeled acetate, may indicate an increased substrate breakdown to provide energy for inefficient phosphorylation mechanisms. In this way there is a decreased availability of C^{14} for the synthetic processes of glycogenesis and lipogenesis. However, the liver glycogen from the salicylate-treated rats was virtually free from radioactivity, whereas the fatty acids still contained about one-third as much as found in the corresponding control animals. This difference may be ascribed to the necessity for additional energy-rich phosphate bonds in the pathway between acetate and liver glycogen and not in the reactions involving lipogenesis from acetate. The major route by which acetate carbons are incorporated into liver glycogen is via acetylcoenzyme-A, the Krebs cycle, decarboxylation of oxaloacetate to give phosphopyruvate, and modified reversal of the Embden-Meyerhof scheme of glycolysis. ATP is necessary in at least two of these inter-

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[7] S. M. Tenny and R. M. Miller, Am. J. Med., 19, 498 (1955).

[8] B. W. Meade, Ann. Rheum. Dis., 13, 60 (1954).

Table VI

Effect of salicylate on the respiratory excretion of total CO_2 and $C^{14}O_2$ and on the incorporation of C^{14} into the liver glycogen of fasted rats. Results expressed as means ± standard deviations.[a]

Group	Number of rats	Total CO_2, (mg/min) 0-60 min	$C^{14}O_2$ cumulative % of injected dose, 0-60 min	Liver glycogen (dpm/mg)
Control	6	7.32 ± 0.45	32.94 ± 2.01	5193 ± 1640
Salicylate, 250 mg/kg	4	9.25 ± 0.40 (P = 0.02)	48.22 ± 4.33 (P = 0.02)	60 ± 18 (P = 0.01)
Salicylate, 500 mg/kg	4	14.70 ± 1.52 (P = 0.01)	52.27 ± 3.93 (P = 0.01)	20 ± 12.5 (P = 0.01)

[a]In both this Table and Table VII the significances of the differences of the means between the salicylate and corresponding control group have been analyzed by Student's t-test, and values of P are included. The minimum acceptable level of significance has been taken as P = 0.02.

Table VII

Effect of salicylate on respiratory excretion of total CO_2 and $C^{14}O_2$ and on the incorporation of C^{14} into the liver fatty acids of nonfasted rats. Results given as means ± standard deviations

Group	Number of rats	Total CO_2 (mg/min)			$C^{14}O_2$ cumulative, % dose, 0-60 min	% isolated w/w wet wt liver	Radio-activity % digested
		10-20	20-40	40-60			
Control	6	11.46±1.39	10.58±1.83	8.71±1.55	37.42±4.09	3.88±0.45	0.436±0.180
Salicylate, 500 mg/kg	6	21.51±3.63 P = 0.01	22.81±3.29 P = 0.01	22.75±4.32 P = 0.01	58.13±8.00 P = 0.01	4.16±0.33 P = 0.3	0.159±0.051 P = 0.01

mediate stages, whereas fatty acid synthesis from acetate is essentially dependent on high ratios of acetylcoenzyme-A to coenzyme-A and of reduced flavoprotein and disphosphopyridine nucleotides to the corresponding oxidized forms, rather than on ATP.

The demonstration that salicylate inhibits liver glycogen synthesis "in vivo" from small molecules, such as acetate, is relevant to other reported effects of salicylate on carbohydrate metabolism. Thus, although an increased rate of glycogenolysis is an important factor in the depletion of liver glycogen caused by salicylate in the intact rat, impaired synthesis of glycogen may also be a contributory factor, particularly as salicylates diminish glycogen formation in surviving rat-liver slices.[9] Again, the hypoglycemic effect of salicylate in the fasting alloxan-diabetic rat[2] is explicable on the same basis because in such an animal all the blood glucose must come from synthesis, the increased liver glucose-6-phosphatase activity present[10] acting to maintain the hyperglycemia rather than producing deposition of liver glycogen. This mechanism may also be extended to include the reduction of glycosuria and hypoglycemia caused by salicylates in patients with diabetes mellitus.

Appendix: Experimental Methods

The respiratory excretion of total CO_2 and $C^{14}O_2$ were measured by continuous-recording equipment described by Tolbert, Kirk, and Baker.[11] Liver glycogen was isolated by the method of Marks and Feigelson[12] and purified to constant specific activity according to the directions of Stetten and Boxer.[13] Its radioactivity was determined by dissolving in water, plating directly onto platinum dishes 3-1/2 cm^2 in area, and counting in a windowless Nuclear Chicago D-47 Flow Counter. Liver fatty acids were isolated by the procedure of Masoro, Chaikoff, Chernick, and Felts,[14] and their radioactivity was measured by dissolving in toluene containing 0.5% w/v 2.5 diphenyloxazole and counting in an internal standard liquid scintillation counter.

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[7] S. M. Tenny and R. M. Miller, Am. J. Med., 19, 498 (1955).

[8] B. W. Meade, Ann. Rheum. Dis., 13, 60 (1954).

[9] M. J. H. Smith, Biochem. J. 59, 52 (1955).

[10] R. G. Langdon and D. R. Weakley, J. Biol. Chem. 214, 167 (1955).

[11] Tolbert, Kirk, and Baker, Am. J. Physiol. 185, 269 (1956).

[12] P. A. Marks and P. Feigelson, J. Biol. Chem. 226, 1001 (1957).

[13] D. Stetten and G. E. Boxer, J. Biol. Chem. 155, 231 (1944).

[14] Masaro, Chaikoff, Chernick, and Felts, J. Biol. Chem., 185, 845 (1950).

FURTHER STUDIES ON D_2O-INDUCED STERILITY

Ann M. Hughes

The production of sterility in mice by D_2O has been reported.[1] In an effort to determine more exactly the length of treatment necessary to produce sterility, the experiment has been expanded.

Ten C_{57}-strain male mice were given 30% D_2O in their drinking water for 1 week. Then, while the D_2O treatment was continued, each mouse was placed in a separate cage with a female mouse. At the end of one week the female was replaced by a new female. This procedure was repeated weekly for 6 weeks. The females were individually caged until either (a) their litters were 2 weeks old, at which time the young were counted and their sex determined, or (b) it was definitely established that the female was not pregnant. The results are shown in Table VIII.

Experiments are now in progress in which the males are treated with D_2O for 1, 2, or 3 weeks, and then mated on successive weeks as in the experiment described above.

[1] Ann M. Hughes, in Chemistry Division Quarterly Report, UCRL-8141, Dec. 1957, p. 66.

Table VIII

The effect of D_2O on the fertility of male C_{57} mice. Number of days after female was put with male until litter was born; number and sex of offspring. ("0" indicates no pregnancy.)

Days of D_2O treatment before mating	Mouse Number									
	1	2	3	4	5	6	7	8	9	10
7	20; all died	20; 2M, 5F	21; 4M, 2F	25; all died	0	21; 4M, 3F	25; 3M, 6F	22; 2M, 1F	24; all died	24; 2M, 3F
14	20; 5M, 1F	0	23; 4M, 4F	21; 3M	0	0	23; 5M, 2F	21; 2M, 1F	0	26; 3M, 2F
21	22; 4M, 4F	23; 4M, 3F	21; 2M, 5F	21; all died	20; 1M, 4F	22; 5M, 3F	0	20; 1M, 2F	20; 3M,4F	20; all died
28	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0

THE TOXICITY OF D_2O FOR DROSOPHILA MELANOGASTER

Ann M. Hughes

That D_2O can produce sterility in mice has been established.[1] In consideration of the mechanism involved, it seems very possible that D_2O has some effects on the sperm, one of which may be the production of lethal mutations.

For the investigation of the production of lethal mutations, Drosophila provide an ideal biological system. As a preliminary to such a study, the toxicity of D_2O for Drosophila has been determined.

The basic media for growing Drosophila contained

Water	50 oz
Agar	10 g
Brewer's yeast	28 g
Molasses	4 oz
Corn meal	8 oz
Tegosept	2 tsp

Media was made in which 0 to 100% (in 10% intervals) of the water was replaced by D_2O. Bottles of such media were then inoculated with Canton^{++} (wild type) Drosophila, two pairs per bottle. On media containing 70 to 100% D_2O, adult flies died within 3 days. At a concentration of 40% or greater, there were no larvae. Beginning on the first day of eclosion, fly counts were made for 6 days on bottles containing media with 0 to 30% D_2O. Results are shown in Table IX.

[1] Ann M. Hughes, in Chemistry Division Quarterly Report, UCRL-8141, Dec. 1957, p. 66

Table IX

Fly counts (numbers of males and females) on media containing D_2O

Days after eclosion	% D_2O in media							
	0		10		20		30	
	M	F	M	F	M	F	M	F
1	18	21	1	6	0	0	0	0
	14	17	2	3				
2	17	20	14	12	8	6	0	0
	19	10	12	14	5	6		
3	10	7	19	15	8	5	0	0
	17	16	15	11	8	8		
4	7	8	7	10	4	7	0	0
	7	7	5	5	5	4		
5	18	14	6	13	5	3	0	1
	19	14	5	14	4	7		
6	8	7	4	3	6	0	0	0
	6	8	7	9	0	3		
Totals	160	149	97	115	53	49	0	1

PAPERS SUBMITTED FOR PUBLICATION

Several topics of investigation in plant biochemistry were brought to the stage of publication during this quarter, and papers on them have been submitted to journals, as follows.

V. Moses and M. Calvin, Photosynthetic Studies with Tritiated Water (submitted to Biochem. et Biophys. Acta).

V. Moses and M. Calvin, The Metabolism of 2-Carboxy-4-Ketopentitol Diphosphate (submitted to Biochem. et Biophys. Acta). The paper describes experiments in which this keto acid, previously isolated from photosynthesizing plants, was administered first to whole Chlorella cells (it apparently did not penetrate) and then to spinach chloroplasts. In the latter, the keto acid was converted principally to another six-carbon acid, identified as a saccharic or a saccharinic acid. It is not known whether either the keto acid or its conversion product has any physiological significance.

Osmund Holm-Hansen, Kojiro Nishida, V. Moses, and M. Calvin, Effects of Mineral Salts on Short-Term Incorporation of Carbon Dioxide in Chlorella (submitted to J. Exptl. Botany). Both nitrate and phosphate were found to produce differences for as long as half an hour in the carbon-labeling pattern of Chlorella that had been centrifuged from its medium and resuspended in distilled water. In particular, the level of amino acids is greatly increased in the presence of ammonium salts.

V. Moses, Osmund Holm-Hansen, James A. Bassham, and M. Calvin, The Relationship Between the Metabolic Pools of Photosynthetic and Respiratory Intermediates (to appear in J. Molecular Biol. 1 (1959)). These studies included administration of various combinations of labeled and unlabeled CO_2, glucose, and acetate to Chlorella during periods of light and dark. It was concluded that many of the metabolic pools, particularly those of phosphate esters, are separate for photosynthesis and respiration but that intermediates of photosynthesis may be converted to intermediates of respiration by certain prescribed pathways.

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