oxidation and from by-products obtained on hydrolysis with crystalline β-amylase. Though it has not been possible to crystallize higher members of the maltoöligosaccharide series, the acetylated glycitols have been obtained crystalline. However, reduction of maltohexaose followed by acetylation failed to yield a crystalline glycitol acetate.

Experimental

Isolation of Maltohexaose.—Approximately 20 g. of corn sirup[8] was placed on a carbon column[5] 75 × 750 mm. and eluted with 4 l. of 16% aqueous ethanol to remove D-glucose and malto-homologs below D.P. 6. Subsequent elution with 4 l. of 20% aqueous ethanol removed maltohexaose contaminated with homologs of similar D.P. On evaporation of this eluate approximately 2 g. of sirup was obtained. This residue with others similarly obtained to make 7 g. was placed on a cellulose[6] (Whatman Standard Grade W) column, 75 × 1050 mm., and the column eluted successively with 8 l. of 85% ethanol, 8 l. of 82% ethanol and finally with 80.5% ethanol. The 80.5% ethanol eluate was collected in a fraction collector[9] and fractions were tested by paper chromatography to determine which principally contained maltohexaose. These fractions were combined, concentrated to a sirup and again chromatographed on a cellulose column as described. The eluate containing maltohexaose from this column was free from other carbohydrates as evidenced by paper chromatography using ethyl acetate, pyridine and water (10:4:3 v./v.). Evaporation gave a white amorphous powder, $[\alpha]^{25}D$ +182° (*c* 1.0, water).

Anal. Calcd. for $C_{36}H_{62}O_{31}$: C, 43.6; H, 6.3. Found: C, 43.6; H, 6.5.

Characterization of Maltohexaose.—Reducing power was determined before and after hydrolysis according to the procedure previously used.[2] Hydrolysis caused the reducing power to be increased by a factor of 5.84 which is near the expected factor of 6. Under similar conditions hydrolysis increased the reducing power of maltose by a factor of 1.87.

Periodate oxidation by the method previously described[2] gave the results shown in Table I.

TABLE I

PERIODATE OXIDATION OF MALTOHEXAOSE

Determination	Mole equiv. of substance Calcd.	Found
Periodate consumed	9	8.78
Formic acid produced	3	2.92
Formaldehyde produced	1	1.15

β-Amylolysis of maltohexaose was performed as described by Whistler and Hickson[2] for maltotetraose. The products from cellulose column separation are given in Table II.

TABLE II

Fraction	Mg.	Yield, %	Substance by paper chromatography
A	510	76	Maltose
B	80	12	Maltotetraose
C	50	7.5	Maltohexaose

Maltose from the β-amylolysis was acetylated to produce crystalline octa-*O*-acetyl-β-maltose, m.p. 159°.

Maltohexaitol.—Two grams of maltohexaose in 75 ml. of water was hydrogenated at 50 lb. pressure with 0.7 g. of platinum oxide as catalyst.[10] After 7 days 0.3 g. of fresh catalyst was added and the hydrogenation continued for a further 4 days. At the end of this time the solution had no reducing power.

The catalyst was filtered off and the solution was evaporated under reduced pressure at 50° to a white amorphous powder; yield 1.95 g., $[\alpha]^{25}D$ +163° (*c* 1.0, water).

One-half gram of maltohexaitol was acetylated with 5.0 ml. of acetic anhydride and 0.2 g. of sodium acetate by the previously used procedure. Evaporation of the chloroform extract under reduced pressure gave a pale yellow solid. The crude maltohexaitol acetate, obtained in 0.7 g. yield, was dissolved in 30 ml. of benzene and placed on a silene EF column.[11] The column was developed with 12 l. of a mixture of benzene and *t*-butyl alcohol (75:1 v./v.). The extruded column was streaked with alkaline potassium permanganate. The acetate zone which was in the middle section of the column was extracted with acetone. On evaporation of the acetone there was obtained a pale yellow powder. The acetate was dissolved in 99.5% ethanol and purified with charcoal, $[\alpha]^{25}D$ +133.4° (*c* 0.9, chloroform). The maltohexaitol acetate did not crystallize.

(8) Corn sirup unmixed, 42 D. E., Corn Products Refining Company, Argo, Ill.

(9) J. L. Hickson and R. L. Whistler, *Anal. Chem.*, **25**, 1425 (1953).

(10) V. Voorhees, R. Adams and R. L. Shriner, "Organic Syntheses," H. Gilman, Editor, Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1932, p. 452.

(11) A. Thompson and M. L. Wolfrom, THIS JOURNAL, **73**, 5849 (1951).

DEPARTMENT OF BIOCHEMISTRY
PURDUE UNIVERSITY
LAFAYETTE, INDIANA

COMMUNICATIONS TO THE EDITOR

THE BONDING OF THE HYDROGEN ATOM IN $Co(CO)_4H$[1]

Sir:

Cobalt carbonyl hydride is perhaps the best known example of an unusual class of substances. Its intriguing chemical and physical properties have led to much speculation about its structure, particularly in the location and the nature of the bonding of the hydrogen atom. Edgell, Magee and Gallup[2] have shown that their infrared spectra are consistent with the following structure: the four CO groups tetrahedrally arranged about the cobalt atom with the hydrogen atom embedded in one face of the tetrahedron (on the figure axis) to form a *bridge between the three CO groups*. We have examined such a structure with the aid of molecular orbital theory and find that it is plausible. Moreover the details of the bonding give a picture of the molecule which is in agreement with the substantiated experimental facts.

The starting point of the analysis is the assumed C_{3v} symmetry of the molecule which, while not proven by the infrared[2-4] and electron diffraction[5] data, is in harmony with them. With the localiza-

(1) This work was sponsored by the Atomic Energy Commission.

(2) Walter F. Edgell, C. Magee and G. Gallup, to be published; presented before the 127th meeting of the American Chemical Society, Cincinnati April, 1955.

(3) H. W. Sternberg, I. Wender, R. A. Friedel and M. Orchin, THIS JOURNAL, **75**, 2717 (1953).

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

(5) R. V. G. Ewens and M. W. Lister, *Trans. Faraday Soc.*, **35**, 681 (1939).

tion of the σ bonds, four orbitals have the required symmetry (A_1) to participate in the bonding of the hydrogen atom. Besides the 1s orbital of hydrogen (ϕ_H) and the $3d_{z^2}$ orbital of the cobalt (ϕ_{Co}), one has a molecular orbital formed from a properly oriented p orbital of each of the three symmetrically equivalent carbon atoms plus a similar orbital involving the oxygen atoms. In the LCAO approximation these latter are:

$$\phi_C = \frac{1}{\sqrt{3}}\{p_C(1) + p_C(2) + p_C(3)\}$$

$$\phi_O = \frac{1}{\sqrt{3}}\{p_O(1) + p_O(2) + p_O(3)\}$$

If we place one electron in each of these four orbitals, there are just enough electrons left over to fill the remaining bonding and atomic orbitals. The problem then is how the orbitals ϕ_H, ϕ_{Co}, ϕ_C and ϕ_O combine to form bonds. The overlap integrals suggest that the cobalt–hydrogen distance is just under 2A, and m.o. calculations of the approximate energies and the approximate form of the bonding and antibonding orbitals were made for this location. The resultant orbitals are classified on the basis of relative energies as two which are bonding and two which are antibonding—leading nicely to the formation of a diamagnetic molecule.

The bonding orbitals, in the order of decreasing stability, are

$$\Psi_I = 0.612\phi_H + 0.416\phi_C + 0.396\phi_O + 0.005\phi_{Co}$$

$$\Psi_{II} = 0.625\phi_H + 0.005\phi_C - 1.000\phi_O - 0.001\phi_{Co}$$

The electron pair in Ψ_I is shared almost exclusively between the hydrogen, carbon and oxygen atoms while the pair in Ψ_{II} is concentrated on the hydrogen and the oxygen atoms. The charge on the hydrogen atom due to both Ψ_I and Ψ_{II} amounts to 1.6 electrons. Thus, the hydrogen is immersed in a sheath of negative charge in excellent agreement with the findings from the proton magnetic resonance spectrum (H. S. Gutowsky, private communication). The reason ϕ_{Co} participates to only a small extent in the bonding orbitals is its unfavorable energy position above ϕ_C, ϕ_O and ϕ_H. These results indicate that the bridge structure is plausible but do not prove its existence.

CHEMISTRY DEPARTMENT
PURDUE UNIVERSITY WALTER F. EDGELL
LAFAYETTE, INDIANA GORDON GALLUP

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THE ROLE OF A POLYNUCLEOTIDE IN OXIDATIVE PHOSPHORYLATION[1]

Sir:

As previously shown, the enzyme system that catalyses phosphorylation linked to the oxidation of DPNH in extracts of *Alcaligenes faecalis* can be separated into three components.[2] These are a particulate DPNH oxidase, a soluble heat labile component, and a soluble heat stable factor. The tentative characterization of the latter as a polynucleotide is the subject of this communication.

The activity of the heat stable factor was assayed in a phosphorylating system containing the other two components isolated as previously described. The energy rich phosphate formed during DPNH oxidation was trapped as glucose-6-phosphate, which was determined enzymatically.[3] Heat stable factor was prepared by centrifuging out the DPNH oxidase from crude extracts at 105,000 × *g*. The precipitate was washed by centrifugation, taken up in water and heated to 100° for 6 minutes. The supernatant solution following centrifugation was used as a source of the factor. Boiled extracts so prepared were active in phosphorylation. They had a nucleotide absorption spectrum with a single peak at 260 mμ, and a minimum at 230. The 280/260 ratio was 0.50. Active heat stable factor could be precipitated with 4% trichloroacetic acid, and redissolved in water at slightly alkaline *p*H. The activity could also be recovered by precipitation with ammonium sulfate or with two volumes of alcohol in the presence of 0.1 *M* $MgCl_2$ at a *p*H of 5.5. Activity was not lost on prolonged dialysis against water, but it was completely destroyed by incubation with 1 *N* HCl at 100° for 20 minutes or with 0.3 *N* KOH at 37° for 15 hours.

Incubation of the heat stable factor with an excess of crystalline RNAase and DNAase (Worthington), in the presence 0.01 *M* $MgCl_2$, in phosphate buffer at *p*H 7.4 for 2.5 hours did not decrease its activity. Following enzymatic treatment the factor was dialyzed, precipitated with alcohol and again dialyzed. The effect of such partially purified factor on oxidative phosphorylation is shown in Table I.

TABLE I

OXIDATIVE PHOSPHORYLATION DEPENDENT ON THE ADDITION OF PARTIALLY PURIFIED HEAT STABLE FACTOR

The reactions were carried out in a Warburg apparatus under general conditions outlined in reference 1. DPNH was formed in the experimental vessels by tipping 0.8 μM. of DPN and 100 μM. of alcohol into the main space which already contained excess crystalline alcohol dehydrogenase as well as the phosphorylating system. The DPN control vessels lacked alcohol and alcohol dehydrogenase. 0.4 μM. of ATP was present in each vessel. The amount of heat stable factor used was determined spectrophotometrically and expressed as μM. of ADP giving the same optical density at 260 mμ. Phosphorylation due to the factor is the increment of glucose-6-phosphate found in the DPNH vessel over the DPN control in the presence of added factor minus this increment in the absence of added factor.

Heat stable factor added	μM Glucose-6-phosphate formed DPN	DPNH	μM. phosphorylation due to factor
None	0.7	0.8	...
Crude equivalent to 3.15 μM. ADP	0.6	1.2	0.5
Partially purified equivalent to 1.14 μM. ADP	0.5	1.2	0.6
Partially purified equivalent to 0.25 μM. ADP	0.7	1.1	0.3

A sample of similarly prepared factor was chromatographed by the ascending paper technique with isoamyl alcohol layered over 2% disodium phosphate.[4] A single ultraviolet light absorbing spot

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(2) G. B. Pinchot, *J. Biol. Chem.*, **205**, 65 (1953).

(3) E. Racker, *ibid.*, **167**, 843 (1947).

(4) C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).