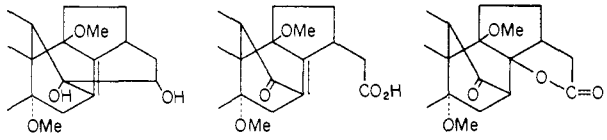
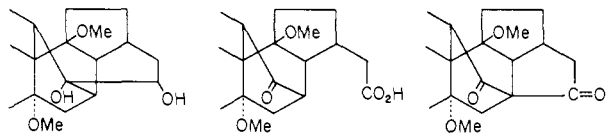
exposes a tertiary hydroxyl.[12] Pyrolytic loss of acetic acid furnishes unsaturated pyro compounds which in certain cases can be hydrogenated[9] and isomerized. Thus pyro-α-oxodelphinine ($\Delta^{8,14}$) isomerizes to isopyro-α-oxodelphinine ($\Delta^{9,14}$). Saponification of its benzoyl group gives isopyro-α-oxodelphonine (II), exposing a secondary hydroxyl vicinal to the free tertiary hydroxyl as shown by subsequent oxidation to an unsaturated keto acid $C_{24}H_{33}NO_8$ (III).[13]

Isomerization of the latter to the keto-γ-lactone



(IV) (ν^{CHCl_3} 1783, 1706 cm.$^{-1}$) can be explained by lactonization upon the $\Delta^{9,14}$ bond. Placing the benzoyloxy group at C-18 and the tertiary hydroxyl at C-19 satisfies these observations. Dihydroisopyro-α-oxodelphonine (V) on oxidation gives a ketoacid, $C_{24}H_{35}NO_8$ (VI) [ν^{Nujol}, (six-membered ketone), 1700 cm.$^{-1}$; methyl ester: ν^{Nujol} (CO_2Me), 1743 cm.$^{-1}$; (six-membered ketone), 1705 cm.$^{-1}$] which can be cyclized to a β-diketone,[14] $C_{24}H_{33}NO_7$ (VII) (ν^{KBr} 1766, 1720 cm.$^{-1}$). This may be reopened to the original acid. On catalytic reduction VII affords a hydroxy pentanone. (ν^{Nujol} 3409, 1746 cm.$^{-1}$).



For aconitine, which has two more hydroxyls than delphinine but bears an N-ethyl group, we envision the same basic skeleton since much of its chemistry parallels that of delphinine.

(13) W. A. Jacobs and Y. Sato, *J. Biol. Chem.*, **180**, 479 (1949).

(14) Neither the lycoctonine skeleton of Przybylska and Marion [*Canad. J. Chem.*, **34**, 185 (1956)] nor the related skeleton, derived from veatchine by the biogenetic pathway used by Valenta and Wiesner [*Chem. and Ind.*, 354 (1956)] to relate lycoctonine to atisine, accommodate all of our data.

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RECEIVED JUNE 11, 1956

FREQUENCY SHIFT OF CARBON-CHLORINE BOND VIBRATION IN METAL DERIVATIVES OF TETRA-(*p*-CHLOROPHENYL)-PORPHINE

Sir:

In a study of the infrared spectra of tetra-(*p*-chlorophenyl)-porphine and its metal derivatives,[1] we find (Table I) that the absorption band due to stretching of the C–Cl linkage will shift position depending upon whether the free porphine or a particular metal complex is under consideration.

Although Thomas and Martell[2] recently suggested that the C–Cl stretching frequency in these compounds might occur at 941 cm.$^{-1}$, the reference[3] upon which this suggestion was based indicates to us that this type of absorption would be expected to occur below 650 cm.$^{-1}$, and the higher value quoted by these authors is undoubtedly the first overtone of the stretching vibration.

TABLE I[a]

Porphyrin	ν_{C-Cl} (cm.$^{-1}$)
Free tetra-(*p*-chlorophenyl) porphine	491.0
Vanadyl salt	498.3
Copper salt	502.0
Cobalt salt	503.5
Nickel salt	504.5
Zinc salt	497.5

[a] Measured as KBr discs on a P. E. Model 12c Infrared Spectrophotometer equipped with KBr prism.

The structural formula of tetra-(*p*-chlorophenyl)-porphine (Fig. 1) indicates that the C–Cl linkages

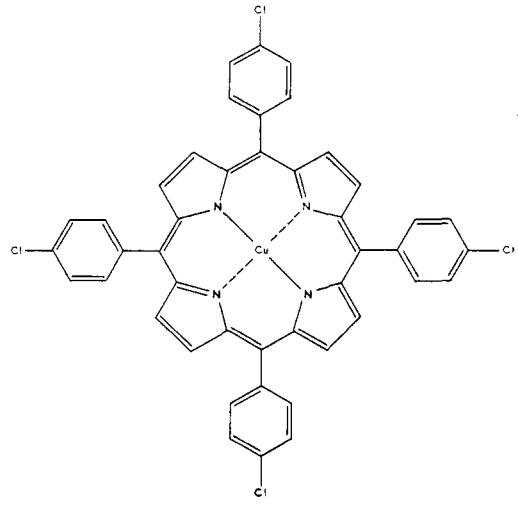

Fig. 1.

are relatively distant from the center of the porphine ring. Furthermore, the suggestion of these authors[2] that "the four benzene rings have partial rotation which cannot bring them 60° of being coplanar with the resonating porphine system" would indicate that the benzene rings themselves could not participate in resonance contributing forms of the porphine ring. Therefore, one might conclude that the C–Cl bond should not be affected by substituting a metal into the center of the molecule in place of the two hydrogen atoms of the free porphyrins. For these reasons, the observed frequency shifts of the C–Cl vibration seem somewhat contrary to expectation and may offer direct experimental evidence that the benzene rings are in fact in conjugation with the porphine nucleus. The higher vibrational frequencies of the metal derivatives seem indicative of more double bond character of the C–Cl linkage in these cases and, with this in mind, a great number of resonance structures can be postulated of which Fig. 2 may be taken as an example.

The hypothesis that the C–Cl bond character is altered by the introduction of a metal into the por-

(1) Prepared by method of P. Rothemund and A. R. Menotti, THIS JOURNAL, **63**, 268 (1941), and **70**, 1809 (1948). The vanadyl salt was prepared by method of J. T. Horeczy, *et al.*, *Anal. Chem.*, **27**, 1899 (1955).

(2) D. W. Thomas and A. E. Martell, THIS JOURNAL, **78**, 1338 (1956).

(3) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1952, p. 271.

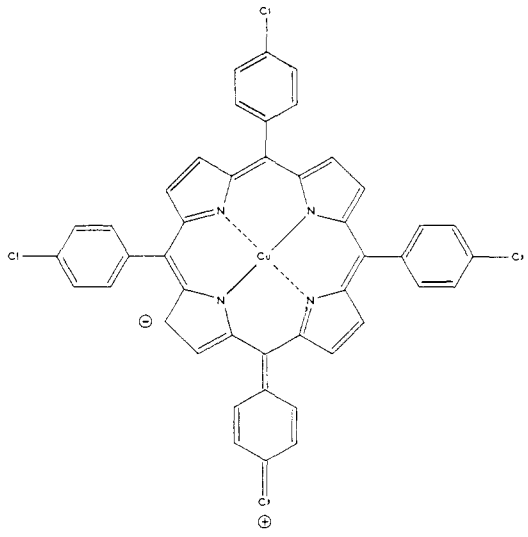

Fig. 2.

phine ring system finds additional support in a recent comparison of the paramagnetic resonance absorption spectra of copper tetraphenylporphyrin and the *p*-chloro derivative. This study suggests that the unpaired electron of the copper atoms can move out to the peripheral Cl- atoms via π orbitals of the conjugated resonating system.[4]

(4) D. J. E. Ingram, J. E. Bennett, P. George and J. M. Goldstein, THIS JOURNAL, **78**, 3545 (1956).

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RECEIVED MAY 9, 1956

AN ENZYMATIC PATHWAY FROM L-XYLULOSE TO D-XYLULOSE[1]

Sir:

The detection of L-xylulose in the urine of "non-pentosuric" humans,[2,3,4] guinea pigs,[3] and rats,[4] together with the discovery of the presence, in guinea pig liver mitochondria, of a highly active enzymatic system for the reduction of L-xylulose to xylitol,[5] indicates that this ketopentose may be a normal metabolite. Recent work[6,7] has shown that D-xylulose-5-phosphate, rather than D-ribulose-5-phosphate, is the ketopentose substrate of transketolase (TK) in the 6-phosphogluconate pathway of carbohydrate metabolism. We wish to describe studies on new mitochondrial enzymes which link metabolically the enantiomorphic forms of xylulose.

A stable, soluble enzyme preparation has been obtained by a butanol treatment of the insoluble portion of ruptured guinea pig liver mitochondria. It contains two enzymes which would make possible the interconversion of L-xylulose and D-xylulose. One enzyme, requiring triphosphopyridine nucleotide (TPN) as coenzyme, uses only L-xylulose and xylitol as substrates. The second enzyme, requiring diphosphopyridine nucleotide (DPN), has D-xylulose and xylitol as its preferred substrates.

The TPN-dependent enzyme catalyzes the dehydrogenation of xylitol but has practically no action on erythritol, ribitol, L-arabitol, D-sorbitol, D-talitol,[8] D-gulitol,[8] or D-mannitol. Oxidation of reduced TPN is effected by L-xylulose, whereas D-xylulose, L-erythrulose,[9] D-ribulose,[10] D-fructose, D-sorbose,[8] and L-sorbose are essentially inactive. Since the L-xylulose-xylitol enzyme has a marked preference for TPN over DPN, it appears to be unique among polyol dehydrogenases (ketose reductases) thus far reported, all of which are DPN-dependent.

The DPN-dependent enzyme catalyzes the oxidation and reduction of xylitol and D-xylulose, respectively. The DPN system acts more slowly on ribitol and D-ribulose but has little or no action on erythritol, L-arabitol, D-talitol, D-gulitol, D-mannitol, L-xylulose, D-fructose, D-sorbose, or L-sorbose.[11]

Figure 1 presents evidence for the existence of two distinct xylitol dehydrogenases. The TPN-dependent enzyme, unlike the DPN-enzyme, loses all or most of its activity by dialysis against water or by preliminary washing of the mitochondrial residue. Table I shows that both dehydrogenases

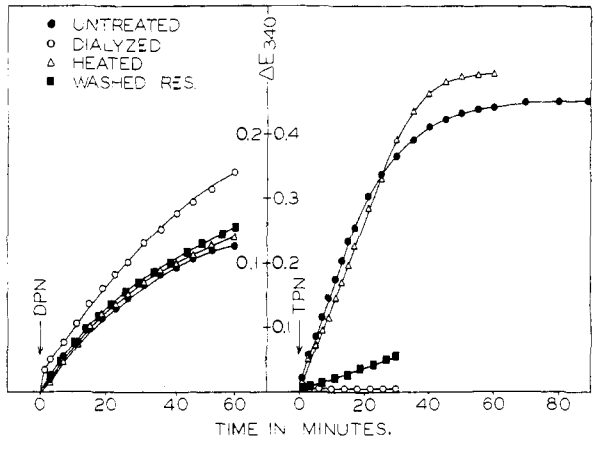

Fig. 1.—Comparison of the two mitochondrial xylitol dehydrogenases: flask contents, 19.7 μM. xylitol, 0.39 μM. coenzyme, 0.3 ml. enzyme, in 3.0 ml. of solution 0.05 *M* to "tris" buffer (*p*H 8.0) and 0.008 *M* to $MgCl_2$; temperature, 23.5°. Dialysis was against water overnight. Heat treatment of enzyme was at 50° for 20 min. "Washed residue" indicates enzyme prepared from insoluble mitochondrial residue which had been washed three times with water before butanol treatment.

(1) Supported in part by a grant from the National Science Foundation.

(2) O. Touster, R. M. Hutcheson and V. H. Reynolds, THIS JOURNAL, **76**, 5005 (1954).

(3) O. Touster, R. M. Hutcheson and L. Rice, *J. Biol. Chem.*, **215**, 677 (1955).

(4) S. Futterman and J. H. Roe, *ibid.*, **215**, 257 (1955).

(5) O. Touster, V. H. Reynolds and R. M. Hutcheson, *J. Biol. Chem.*, in press.

(6) P. A. Srere, J. R. Cooper, V. Klybas and E. Racker, *Arch. Biochem. Biophys.*, **59**, 535 (1955).

(7) B. L. Horecker, J. Hurwitz and P. Z. Smyrniotis, THIS JOURNAL, **78**, 692 (1956).

(8) Kindly supplied by Dr. N. K. Richtmyer.

(9) Kindly supplied by Dr. G. C. Mueller.

(10) Kindly supplied, as the *o*-nitrophenylhydrazone, by Dr. B. L. Horecker.

(11) The extract catalyzes the reduction of DPN in the presence of sorbitol. This reaction is probably due to a third enzyme, since L-sorbose and D-fructose, the expected dehydrogenation products, are inactive. The product may be a 3-ketopolyol.