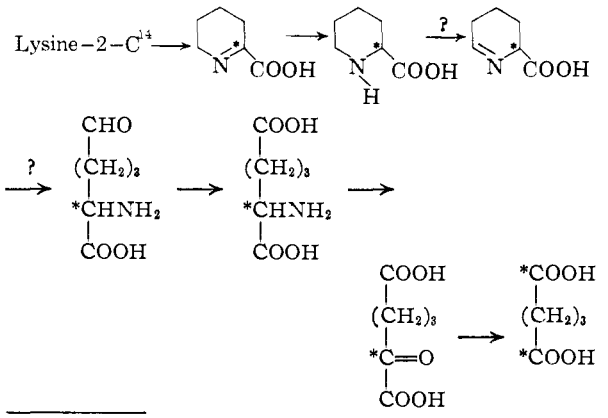
A major problem in the isolation of α-aminoadipic acid is the removal of traces of contaminating glutamic acid. The authors feel that this was not ruled out in the original isolation of this compound[5] since, in the present work, the criterion of constant specific activity by recrystallization was found not to be a guarantee of radiopurity. Therefore, isolation was undertaken. The combined fractions containing α-aminoadipic acid were run on six papers (Whatman no. 1) for 1.5 hours at *p*H 6.4 on a high voltage electrophoresis apparatus[7] under toluene (2000 v.). The α-aminoadipate (2500 c./m.) was located, eluted from the paper and converted to ornithine by the Schmidt reaction. Part of the product was treated on paper as above. The ornithine was eluted and then run on paper in butanol–pyridine–water (1:1:1) for two days (descending), located and eluted. A small amount of α,γ-diaminobutyric acid resulting from the Schmidt reaction with contaminating glutamic acid was found on the paper 2 inches below the ornithine. From approximately 250 c./m. put on the final paper, 145 c./m. was obtained after elution of the ornithine. There thus can be little doubt that α-aminoadipic acid is a product of pipecolic acid breakdown in rat liver mitochondria.

As additional evidence supporting the position assigned to pipecolic acid, we have ascertained that DL-pipecolic acid-2-C^{14} is converted to carboxy-labeled glutaric acid both by rat liver mitochondria and the intact rat.

Taken together these data lend strong support to the degradative pathway for lysine outlined here



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RECEIVED JULY 9, 1959

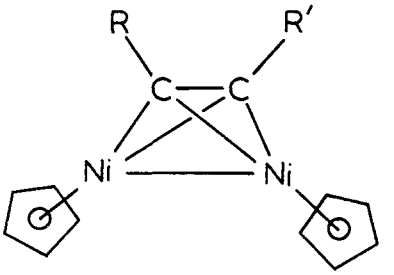
CYCLOPENTADIENYLNICKEL–ACETYLENE COMPLEXES

Sir:

In a recent communication[1] it was suggested that the bridging groups in dicobalt octacarbonyl are not coplanar with the two metal atoms, but occupy two of the octahedral sites about each metal atom, whilst three others are occupied by terminal carbonyl groups and the sixth is unoccupied. The same arrangement would be expected for the isoelectronic di-iron octacarbonyl anion. Both these substances are noted for their catalytic activity[2] and for the ease with which they react with acetylenes.[3] These properties may be explained by the suggested geometry as the unoccupied octahedral position should be readily attacked by the substrate.

An infrared spectral study of the neutral isoelectronic dicyclopentadienyldinickel dicarbonyl (I), first prepared by Fischer and Palm,[4] suggests that it also belongs to the same group. Thus (I) exhibits two carbonyl stretching frequencies in the bridging carbonyl region (at 1879(m) and 1838(s) $cm.^{-1}$), in contrast to the related dicyclopentadienyldi-iron tetracarbonyl in which the two bridging carbonyl groups have been shown to lie in a plane which contains the metal atoms which are essentially fully octahedrally coördinated.[5] These considerations led us to study the reactions of (I) with various acetylenes. The components react smoothly on heating in toluene and in every case both carbonyl groups are replaced by one molecule of acetylene. Tolan yields the complex (II, R = R′ = C_6H_5) as black crystals, m.p. 149–150° (Found: C, 68.0; H, 5.2; M, 409. $C_{24}H_{20}Ni_2$ requires: C, 67.7; H, 4.7; M, 426). Phenylacetylene similarly affords (II, R = C_6H_5, R′ = H) as black needles m.p. 132–133° (Found: C, 62.1; H, 4.3; $C_{18}H_{16}Ni_2$ requires: C, 61.8; H, 4.6) whilst hexyne-3 gives a dark green oil (II, R = R′ = C_2H_5) (Found: C, 58.3; H, 6.3. $C_{16}H_{20}Ni_2$ requires: C, 58.3; H, 6.1). Complexes of the type (II) form black crystals or dark green oils, and the solids are stable in air. The oils, or solutions of solid complexes in hydrocarbon solvents, are, however, slowly decomposed in air and solutions in ethanol or acetic acid are rapidly oxidized. Reduction of (II, R = R′ = C_6H_5) with sodium and alcohol in liquid ammonia yields dibenzyl showing that the diphenylacetylene residue is bonded only to the nickel atoms.

This reduction together with the formal analogy of the reactions of (I) and of cobalt octacarbonyl with acetylenes leads us to propose structure (II) for our products, analogous to that of the cobalt complexes.[6]



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RECEIVED JULY 20, 1959

A NEW ENZYMATIC SYNTHESIS OF HEXOSE PHOSPHATES[1]

Sir:

An enzyme which catalyses the formation of hexose-6-phosphate and ammonia from potassium phosphoramidate (PNH_2) and hexose (reaction 1) has been obtained from extracts of succinate grown *E. coli*. The enzyme, presently named phosphoramidic hexose transphosphorylase (PHT), has been purified about 60-fold by fractionation with protamine sulfate, ammonium sulfate and diethylaminoethyl cellulose. The formation of P^{32} labeled organic phosphate esters from the free sugar and labeled PNH_2 was used as an enzyme rate assay. The results shown in Table I indicate that PHT catalyzed a phosphoryl transfer from PNH_2 to several hexoses, although the rate differed considerably with different hexoses. Neither pentoses nor nucleosides were phosphorylated by partially purified PHT.

TABLE I

FORMATION OF HEXOSE PHOSPHATES FROM PHOSPHORAMIDATE

The reaction mixture contained in 1 ml. 2-amino-2-methyl-1,3-propanediol buffer, *p*H 8.0, 100 μmoles; PNH_2, 9 μmoles (2160 c.p.m. per μmole); carbohydrate, as shown, 10 μmoles; enzyme *ca.* 0.9 mg. protein (obtained from a protamine treated extract of *E. coli* by precipitation with $(NH_4)_2SO_4$ at 68 to 78% saturation). Reaction was incubated at 37°, and stopped by addition of 0.5 ml. of 12% trichloroacetic acid and boiled for two minutes to hydrolyze remaining PNH_2.

Organic substrate	Total incorporation[a] c.p.m./15 min.	PNH_2 utilization[b] μmoles/15 min.
D-Fructose	3810	1.85
D-Fructose[c]	3030	1.40
L-Sorbose	2480	1.15
D-Glucose	1800	0.80
D-Glucosamine	980	0.45
D-Galactose	370	0.20

[a] Organic and inorganic phosphates separated by method of S. O. Nielson and A. L. Lehninger, *J. Biol. Chem.*, **215**, 555 (1955). [b] Measured as inorganic phosphate by method of C. H. Fiske and Y. SubbarRow, *ibid.*, **66**, 375 (1925). [c] Enzyme pretreated with charcoal at *p*H 5.5, 0.7 mg. of protein in assay.

The PHT reaction could be coupled to the reaction catalyzed by glucose-6-phosphate dehydrogenase, when glucose was used as the phosphate acceptor. Thus, the rate of the over-all reaction could be followed by reduced triphosphopyridine nucleotide (TPNH) formation and furthermore glucose-6-phosphate could be assumed as the product of transphosphorylation reaction. When individual hexoses were used as phosphate acceptors, with PHT and PNH_2, the corresponding hexose-6-phosphate was isolated and identified chromatographically using the solvent systems of Mortimer.[2]

PHT showed neither a divalent metal requirement nor a dependence on added nucleoside diphosphates and treatment of the partially purified enzyme with charcoal (*p*H 5.5), or with Dowex-I or with Versene (*p*H 7.5) failed to remove any cofactors participating in the reaction. Furthermore, when PHT was coupled with glucose-6-phosphate dehydrogenase no TPNH formation was observed if PNH_2 was replaced by adenosine triphosphate (ATP). When crystalline yeast hexokinase replaced PHT in the coupled system TPNH formation was observed only if ATP was added.

The enzyme preparation used in these studies was contaminated with PNH_2 hydrolase activity,[3] making stoichiometric measurements of NH_3 in reaction 1 unreliable. Further work on the purification and characterization of PHT is in progress.

(1) This investigation was supported in part by grants from the Williams-Waterman Fund and the United States Public Health Service.

(2) D. C. Mortimer, *Can. J. Chem.*, **30**, 653 (1952).

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THE EFFECT OF PRESSURE ON SEDIMENTATION RATE

Sir:

Recently, Fujita[1] has derived relationships relating the boundary position to time for a monodisperse species in a sector-shaped cell, when the sedimentation coefficient depends on both pressure and concentration.

The boundary position $y_* = (r/r_0)^2$, the dilution (or concentration) factor $\theta_* = C/C_0$ and the reduced time, $\tau = 2\omega^2 S_0 t$, are related as shown in eq. (1).

$$\frac{dy_*}{d\tau} = \frac{y_*}{1 + \alpha\theta_*}(1 - m(y_* - 1)) \qquad (1)$$

where m is a pressure dependence parameter[1] and α is a concentration dependence parameter.[1]

We have solved Fujita's system of Equations (69) through (72), p. 3603 of reference (1), by the use of Runge-Kutta integration combined with trial and error iteration, using a Bendix G-15D digital computer programmed in pseudocode. A range of α from 0.1 to 1.0 was covered, and of m from 0.1 to 0.9. For flotation, negative values of τ were used. In the case of flotation, the reference pressure is the pressure at the cell bottom (see reference (1)) for definition of symbols.

It is the purpose of this communication to show that a simple relation between boundary position and reduced time can be developed, which fits the exact solution of this system of equations to a very good approximation. Oth and Desreux[2] developed a relationship which is essentially equivalent to letting $\theta_* = 1/y_*$ in Equation (1).

(1) H. Fujita, THIS JOURNAL, **78**, 3598 (1956).

(2) J. Oth and V. Desreux, *Bull. soc. Chim. Belges*, **63**, 133 (1954).