precisely to the experimentally obtained trace (Fig. 2a,b).

While the P^{31} multiplet pattern clearly pointed to the non-equivalence of the methylenic protons, final corroboration was effected by returning to the proton resonance pattern. The coupling constant (10.7 cps.) obtained from the P^{31} trace was found to correspond with acceptable precision to the mean of the experimentally measured coupling constants from the H^1 methylenic trace, on the assumption of two similar, but chemical-shifted, methylenic multiplets with slightly different coupling constants (J_{P,CH_2} = 10.7 cps., $J_{P,C'H_2}$ = 10.3 cps.). The detailed pattern of each of the multiplets could not, however, be clearly established because of some overlap of fine structure. In order to obviate this difficulty, the magnetic anisotropy of benzene[6] was utilized in an ancillary fashion to increase the chemical shift separation of the two overlapping multiplets.

Several solutions of the phosphothionate ester of varying concentration in benzene were prepared. As expected, the difference in relative shieldings of the non-equivalent methylenic protons showed a concentration dependence, and degeneracies were removed at a sample concentration of 35 volume % in benzene solution (Fig. 1b). The emergent pattern became that of two similar multiplets, the fine structure of each corresponding to the predicted second order perturbation treatment of the energy levels for this type of system.

The non-equivalence of the two ethoxy groups in the molecule therefore has been unequivocally established by both the H^1 and P^{31} spectra. In order better to evaluate the role of restricted internal rotation in the phenomenon, studies of the temperature dependence of the spectrum were conducted with the induction probe as modified by a variable temperature sample cavity. The critical n.m.r. parameters remained invariant between 300–500°K., indicating a rather striking stability of structure associated with the non-equivalent methylenic groups. This can be explained in terms of preferred ethoxy group orientations about P–O(R) single bonds. However, on the basis of chemical reactivity, the author prefers, and proposes to elaborate elsewhere, an explanation based on unequal P–O(R) bond orders in some molecular systems containing p,d pi bonds. The net effect can be likened to a resonance stabilization of the molecule in which the anti-bonding electrons of only one of the oxygen atoms contribute to canonical structures involving a conjugated P=S bond.

A final feature may be noted. The molecular asymmetry as demonstrated by the nuclear resonance evidence can lead to the prediction of possible optical activity for this compound.

The author thanks R. Schumm and E. J. Prosen for their coöperation in making available the purified sample used in this study.

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(6) A. A. Bothner-By and R. E. Glick, *J. Chem. Phys.*, **26**, 1651 (1957).

ON THE MECHANISM OF THE COBAMIDE COENZYME DEPENDENT ISOMERIZATION OF METHYLMALONYL CoA TO SUCCINYL CoA

Sir:

It has been established that the isomerization of methylmalonyl CoA[1] to succinyl CoA is a key step in the metabolism of propionate[2] and that a cobamide coenzyme[3] is an obligatory co-catalyst for this isomerization.[4,5] The reaction mechanism now has been investigated using 2-C^{14}-methylmalonyl CoA.[6] With this substance, migration of the carboxyl group to the beta methyl carbon would yield 2-C^{14}-succinyl CoA, whereas movement of the thiolester group would yield 3-C^{14}-succinyl CoA.

$$\begin{array}{l} HO_2CCH_2\overset{*}{C}H_2COSCoA \\ \quad\uparrow \text{ } CO_2H \text{ migration} \\ \qquad CH_3 \\ \qquad | \\ HO_2C\overset{*}{C}HCOSCoA \\ \quad\downarrow \text{ COSCoA migration} \\ HO_2C\overset{*}{C}H_2CH_2COSCoA \end{array}$$

In the experiment described in Table I, the 2-C^{14}-methylmalonyl CoA was isomerized by incubation with an enzyme preparation from *Propionibacterium shermanii*. The acyl-CoA compounds thus formed were converted to their acid amide derivatives by treatment with concentrated ammonium hydroxide and, after the addition of 550 mg. of carrier succinic acid amide, the C^{14}-succinic acid amide was crystallized to constant specific activity[7] and was degraded to determine the distribution of the isotope. To carry out the degradation (1) the succinic acid amide was converted to β-alanine and carbon dioxide by a Hofmann degradation.[8] The carbon dioxide is derived from carbon 1 of the succinyl CoA. (2) The β-alanine was converted to acrylic acid by exhaustive methylation with dimethyl sulfate.[9] The acrylic acid was reduced to propionic acid which was degraded stepwise to carbon dioxide and methylamine by the Schmidt reaction as modified by Phares.[10] Carbons 1, 2, and 3 of the propionic acid thus correspond to carbons 4, 3 and 2, respectively, of the succinyl CoA. The data summarized in Table I show that 80% of the isotope was in carbon 3 of succinyl CoA.[11] It is thus

(1) Coenzyme A is abbreviated as CoA.

(2) M. Flavin and S. Ochoa, *J. Biol. Chem.*, **229**, 965 (1957); R. E. Swick and H. G. Wood, *Proc. Natl. Acad. Sci. U. S.*, **46**, 28 (1960).

(3) H. Weissbach, J. Toohey and H. A. Barker, *ibid.*, **45**, 521 (1959).

(4) E. R. Stadtman, P. Overath, H. Eggerer and F. Lynen, *Biochem. Biophys. Research Comm.*, **2**, 1 (1960).

(5) J. R. Stern and D. L. Friedman, *ibid.*, **2**, 82 (1960).

(6) The 2-C^{14}-methylmalonyl-CoA was prepared by the enzyme catalyzed transcarboxylation reaction: methylmalonyl CoA + 2-C^{14}-propionyl CoA ⇆ 2-C^{14}-methylmalonyl CoA + propionyl CoA (4).

(7) The succinic acid amide contained no 2-C^{14}-methylmalonic acid amide.

(8) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, New York, N. Y., 1943, p. 19.

(9) R. Willstätter, *Chem. Ber.*, **35**, 584 (1902).

(10) E. F. Phares, *Archiv. Biochem. Biophys.*, **33**, 173 (1951).

(11) The small amount of isotope found in the number 2 carbon atom probably is due to the enzyme (CoA-transphorase) catalyzed equilibration of 3-C^{14}-succinyl CoA with its symmetrical hydrolysis product, 2,3-C^{14}-succinate, which is produced in trace amounts under the experimental conditions. Evidence that such equilibration can account

TABLE I

ISOTOPE DISTRIBUTION IN SUCCINYL CoA DERIVED FROM 2-C^{14}-METHYLMALONYL CoA

Carbon		Cpm./μatom
1	COSCoA	0
2	CH_2	14
3	CH_2	54
4	COOH	0

Ten ml. of reaction mixture containing 0.4 μmole of 2-C^{14}-methylmalonyl CoA (1.9×10^5 cpm.), 0.035 μmole of dimethylbenzimidazole—cobamide-coenzyme (supplied by H. A. Barker), 2.6 units of avidin,[12] 200 μmoles of histidine-HCl buffer (*p*H 6.2), and an isomerase preparation from *P. shermanii* (700 μg. protein) were incubated at 25° for 10 minutes. The mixture was then treated with concentrated NH_4OH to convert the acyl CoA compounds to their acid amide derivatives. This and three similar reaction mixtures containing a total of 1.8 μmoles of 2-C^{14} D,L-methylmalonyl CoA (7.3×10^5 cpm.) were pooled and the succinic acid amide was isolated and degraded as described in the text.

established that isomerization involves rearrangement of the thiolester group.

Although the data do not eliminate the possibility that isomerization occurs by an intermolecular transfer of the thiolester group, a much more reasonable mechanism is suggested by analogy to the intramolecular rearrangement of phenylneopentyl radical discovered by Urry and Kharasch.[13] In the proposed mechanism (Fig. 1)

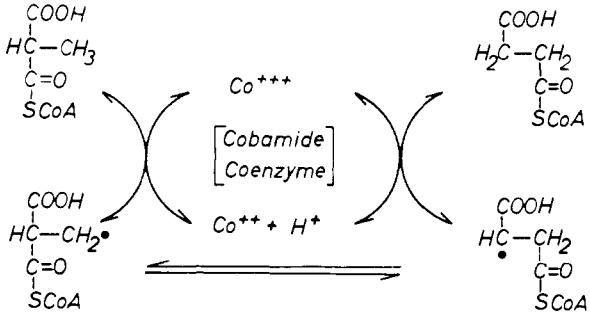

Fig. 1.

the role of the cobamide coenzyme is to produce a radical by the one electron oxidation of methylmalonyl-CoA. The radical then could isomerize by the mechanism of Urry and Kharasch and the succinic acid derivative could be stabilized by interaction with reduced cobamide coenzyme.

In view of the fact that the cobalt in cobamide coenzyme has a valence of +3 it is of further significance for the proposed mechanism, that Co^{+3} is known to produce radicals by reaction with a variety of organic compounds.[14]

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for randomization of the carbon atoms was obtained by showing that, under otherwise identical conditions, the isomerization of unlabeled methylmalonyl CoA in the presence of added trace amounts of 1,4-C^{14}-succinate led to the formation of C^{14}-succinyl CoA which was isolated as the succinic amide derivative.

(12) Avidin was added to prevent the decarboxylation of methylmalonyl CoA to propionyl CoA (4).

(13) W. H. Urry and M. S. Kharasch, THIS JOURNAL, **66**, 1438 (1944).

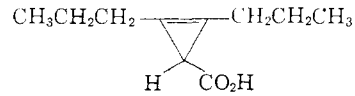
(14) A. F. Trotman-Dickenson, "Free Radicals," John Wiley and Sons, New York, N. Y., 1959, p. 10.

THE SYNTHESIS OF THE DIPROPYLCYCLOPROPENIUM ION

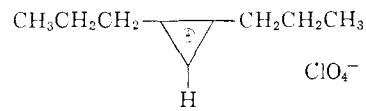
Sir:

Although the properties of the triphenylcyclopropenium ion[1] strongly support the idea that the cyclopropenyl cation is a fundamental aromatic system, it seemed desirable to prepare a derivative free of phenyl groups. The properties of the diphenylcyclopropenyl cation[2,3] suggested that a simple alkylated cation might well be stable, and we wish to report that this is indeed the case.

Reaction of dipropylcyclopropene carboxylic acid (I)[4] with acetyl perchlorate in acetic anhydride,[3,5] and then ether precipitation, led to dipropylcyclopropenium perchlorate (II), m.p. ~80° (dec.), which was recrystallized from ethyl acetate and ether. Found: C, 48.7; H, 6.6. The compound is insoluble in ether, hexane, or other nonpolar solvents, but is soluble in acetone and other polar organic solvents and in 1 *N* HCl. It gives a positive perchlorate test with potassium nitrate, and the infrared spectrum also reveals the presence of perchlorate ion. In strong acid the compound has only end absorption in the ultraviolet.



I



II

The n.m.r. spectrum, in 50% aqueous sulfuric acid, is as expected, with a sharp band at −4.15 p.p.m., shifted to very low field for the proton on a positive carbon, and the characteristic pattern of two equivalent propyl groups also strongly shifted, with a 4 proton triplet centered at +3.12, a four proton sextuplet at +4.43, and a six proton triplet at +5.30 p.p.m. relative to a benzene capillary; the absence of any further bands was established by dilution of the solvent, with consequent shift of its absorption.

Although the cation is soluble and stable in 1 *N* acid, its solution in 0.1 *N* acid or water becomes turbid. This reaction of the carbonium ion with water gives a complex mixture of products, but at least some of the components are cyclopropene derivatives, as evidenced by their characteristic double-bond stretching absorption in the infrared at 5.3μ. From this it can be estimated that the pK_a of the cation is greater than 0 and probably is less than 1. For comparison, the diphenylcyclopropenyl cation has a pK_a of 0.3.[2] That these two are so close is certainly a chance coincidence,

(1) R. Breslow and C. Yuan, THIS JOURNAL, **80**, 5991 (1958).

(2) The diphenylcyclopropenyl cation has been prepared by Dr. Joyce Lockhart, unpublished work, by the reaction of phenylchlorocarbene with phenylacetylene.

(3) Prepared independently by Dr. Donald Farnum by the use of acetyl perchlorate on the appropriate acid. We wish to thank Dr. Farnum for making his results available prior to publication.

(4) I. A. Dyakonov, *et al.*, *Zhur. Obshchei Khim.*, **29**, 3848 (1959).

(5) A similar reaction with acetyl fluoroborate, to form the tropylium ion, has been reported by M. Dewar and C. Ganellin, *J. Chem. Soc.*, 2438 (1959).