position would not be accommodated easily, and that is actually what is observed.

The Role of Water of Hydration of the Annular Nitrogen Group.—In the work reported here and previously, it is apparent that the ring nitrogen acts as though it were a large substituent on an aromatic ring. This effect is attributed to the presence of water of hydration which modifies the steric configuration of the hapten and affects its combination with antibodies.

That pyridine is hydrated in aqueous solution is apparent from the fact that it is miscible with water, whereas benzene is only slightly soluble. Moreover, the heat of hydration of pyridine has been measured by Briegleb[15] and has been found to be 12 kcal. per mole.

Carsten and Eisen[16] reported the heat of reaction of a hapten (ϵ-DNP-lysine) with its specific antibody to be about 1 kcal. Since the heat of hydration of pyridine is much greater than this value, further evidence points to the fact that combination of the pyridine and quinoline carboxylates with antibody, in the systems studied here, takes place with the water of hydration still attached to the nitrogen atom. Otherwise, the combination would be very weak if energy were required to break the water away first.

For the same reasons, biological reactions in general involving similar annular nitrogen compounds may specifically require the hydrated form for combination to take place.

(15) G. Briegleb, *Z. Elektrochem.*, **53**, 350 (1949).

(16) M. Carsten and H. Eisen, THIS JOURNAL, **77**, 1273 (1955).

BUFFALO 3, NEW YORK

COMMUNICATIONS TO THE EDITOR

ORGANIC FREE RADICALS IN THE SOLID STATE[1,2]

Sir:

Solid solutions of triphenylmethyl in fluorene, phenanthrene, and triphenylamine, respectively, of unknown composition have been prepared. The solid solutions were the characteristic corn color of the solutions in liquid benzene, in all three cases. They were prepared by dissolving chosen amounts of hexaphenylethane and the other component in benzene and evaporating the benzene at room temperature in a pure nitrogen atmosphere in a dry box.

It is to be assumed that all the hexaphenylethane that dissolved in the second component was dissociated into free radicals since a planar structure would be required in order that the crystal lattice of the planar fluorene or phenanthrene could accommodate it. Triphenylamine is also sufficiently planar that the hexaphenylethane could hardly dissolve in it as such. Thus the susceptibility gave a measure of the concentration in the solid solution.

Susceptibilities were measured with a Gouy balance at room temperature and the amount of free radical in the sample tube calculated by the method of Roy and Marvel.[3] The mole fractions of triphenylmethyl in solid solution in fluorene, triphenylamine, and phenanthrene were found to be approximately 0.24, 0.22, and 0.17, respectively, assuming complete dissociation in the solid solution. If all the hexaphenylethane were in solid solution, the dissociation in each case would be 17, 14, and 10%, respectively.

In all cases the resultant diamagnetic susceptibility was somewhat less than half that calculated if there were no dissociation to free radicals. If all samples obeyed the Curie–Weiss law, the same amount of free radical would correspond to a considerable paramagnetism at helium temperatures.

The susceptibility was therefore studied by an inductance method down to 1.6°K.; the method was sufficiently sensitive to detect one per cent. of the hexaphenylethane present if it were in the form of free radical active as a perfect paramagnetic substance (due to spin only). No paramagnetism was detected.

However, it was found that under similar conditions hexabiphenylethane, which is completely dissociated into free radicals in the solid at room temperature also gave no paramagnetism. Hexabiphenylethane roughly follows the Curie–Weiss law down to liquid nitrogen temperatures.[4] We found that the susceptibility measured by the inductance method was also essentially zero at 20°K. The "paramagnetism" measured in the Gouy balance in this laboratory at room temperature corresponded to complete dissociation. The solid solutions are being investigated spectroscopically and the transition below 70°K. responsible for loss of paramagnetism of tribiphenylmethyl is being investigated.

We wish to thank Dr. C. Haas for the opportunity to use his Gouy balance.

(1) Publication No. 97 of the Cryogenic Laboratory of the College of Chemistry and Physics, The Pennsylvania State University, University Park, State College, Pennsylvania.

(2) Supported by Contract No. NSF-G1611 with the National Science Foundation.

(3) M. L. Roy and C. S. Marvel, *J. Am. Chem. Soc.*, **69**, 2622 (1937).

(4) E. Müller, I. Müller and W. Bunge, *Ann.*, **520**, 235 (1935).

(5) On leave from the University of Osaka, Japan.

COLLEGE OF CHEMISTRY AND PHYSICS
THE PENNSYLVANIA STATE UNIVERSITY
UNIVERSITY PARK, STATE COLLEGE, PENNA.
J. G. ASTON
J. J. FRITZ
S. SEKI[5]

RECEIVED JANUARY 25, 1957

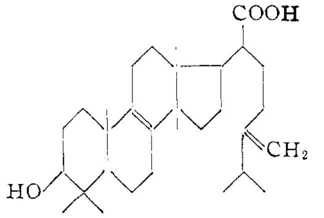
THE BIOSYNTHETIC PRECURSOR OF THE EXTRA CARBON ATOM IN THE SIDE-CHAIN OF STEROIDS[1]

Sir:

The concept of the biosynthesis of steroids from acetate through squalene accounts for the source of all the carbon atoms of C_{27} and C_{30} compounds

(1) This work was supported, in part, by grant No. AT(11-1)-34, Project No. 16, U. S. Atomic Energy Commission.

of this series.[2] This concept, however, does not account for the source of the extra carbon atoms on the side-chains of various C_{28}, C_{29} and C_{31} steroids. Recently,[3] it has been postulated that such ancillary carbon atoms also find their source in acetate but subsequently it has been shown[4] that the extra carbon atom, C_{28}, of the side-chain of the C_{31}-steroid, eburicoic acid (I), is not derived from acetate. We now wish to report that formate is the precursor of this extra carbon atom.



I

When the fungus, *P. sulfureus*, is allowed to grow on a standard medium containing sodium formate-C^{14}, 1.7% of the initial radioactivity is found in the eburicoic acid produced. Ozonolysis of the steroid yields C_{28} as formaldehyde and oxidation of the steroidal residue gives rise to 3-hydroxy-28-noreburic-7,11,24-trione-8-ene-21-oic acid. The specific activities of the materials were: eburicoic acid, 4250 dis./min./mg. C; formaldehyde, 78,500 dis./min./mg. C; trione, 1640 dis./min./mg. C. Thus, 60% of the C^{14} resides in the extra carbon atom, C_{28}. In order to establish that the remainder of the C^{14} is distributed equally throughout the rest of the molecule, the eburicoic acid was diluted with unlabeled carrier and ring A was degraded in the usual fashion.[4] The specific activities were as follow: 3-(2-hydroxy-2-propyl)-3-hydroxy-eburic-A-nor-8-ene-21-oic acid, 376 dis./min./mg. C; eburic-A-nor-3-one-8-ene-21-oic acid, 400 dis./min./mg. C; acetone, 150 dis./min./mg. C; iodoform, 150 dis./min./mg. C. These results establish that all the carbon atoms of the C_{30} steroidal molecule are labeled equally and that the specific activity of C_{28} is 48 times that of any of the other carbon atoms of eburicoic acid.

The finding of a slight activity in the carbon atoms which are known to be derived from acetate shows that this fungus is capable of utilizing a one carbon source for the building of randomly labeled acetate and is in line with other experiments which show that formate can serve as a precursor of acetate.[5]

---

(2) R. B. Woodward and K. Bloch, THIS JOURNAL, **75**, 2023 (1953); W. G. Dauben, S. Abraham, S. Hotta, I. L. Chaikoff, H. L. Bradlow and A. H. Soloway, *ibid.*, **75**, 3038 (1953); A. Eschenmoser, L. Ruzicka, O. Jeger and D. Arigoni, *Helv. Chim. Acta*, **38**, 1890 (1955).

(3) K. Bloch in "Currents in Biochemical Research," Edited by D. Green, Interscience Publishers, Inc., New York, N. Y., 1956, p. 474.

(4) W. G. Dauben and J. H. Richards, THIS JOURNAL, **78**, 5329 (1956); W. G. Dauben, Y. Ban and J. H. Richards, *ibid.*, **79**, 968 (1957).

(5) R. L. Kisliuk and W. Sakami, *J. Biol. Chem.*, **214**, 47 (1955); J. C. Rabinowitz and H. A. Barker, *ibid.*, **218**, 147 (1956), and reference cited therein.

(6) National Science Foundation Predoctoral Fellow, 1956–1957.

CHEMICAL LABORATORY WILLIAM G. DAUBEN
UNIVERSITY OF CALIFORNIA GERHARD J. FONKEN
BERKELEY 4, CALIFORNIA GEORGE A. BOSWELL[6]

RECEIVED JANUARY 12, 1957

THE EXCHANGE OF F^{18} BETWEEN METALLIC FLUORIDES AND GASEOUS FLUORINE COMPOUNDS[1]

Sir:

The fluorocarbons and their derivatives are known for their high thermal stability and low reactivity. Non-exchange of F^{18} between hydrogen fluoride and fluorinated methanes has been noted.[2] On the assumption that catalysts for fluorocarbon reactions may be found among various metallic fluorides, some exchange reactions between fluorocarbons and their derivatives with various metallic fluorides are under study. Exchange at moderate temperatures has been observed in a number of systems such as

$$CF_3CF{=}CF_2 + RbF^{18} \rightleftarrows C_3F_6{}^{18} + RbF$$

The fraction exchanged was calculated by the equation:

$$\text{Fraction exchanged} = \frac{(F^{18}{}_{gas}/F^{18}{}_{total})}{(F_{gas}/F_{total})}$$

where

$F^{18}{}_{gas}$ = F^{18} counting rate, gas phase
$F^{18}{}_{total}$ = sum of F^{18} counting rates, gas and solid phases
F_{gas} = weight of fluorine, gas phase
F_{total} = sum of weights of fluorine, gas and solid phases.

The counting efficiency of a well type scintillation counter in the gas system relative to an external well type scintillation counter was determined by counting radioactive C_3F_6 in the system, then condensing all of the C_3F_6 into a small bulb and measuring the counting rate in the external counter. The fluoride salts were counted in the external counter as aqueous solutions at the same geometry as the condensed C_3F_6 in the counting efficiency comparison experiment. In the exchange reaction described above, C_3F_6 was circulated in a closed system over RbF and through the counter. The salt was heated at a rate of 5°/minute. The results of this experiment are shown in Fig. 1. At the end of the experiment, the F^{18} activity in the gas phase was 5.2×10^9 cpm. per mole of C_3F_6. When the gas was removed from the system, the counting rate returned to a normal background rate. In a similar run, the gas was removed from the apparatus, and its gamma ray spectrum and half-life determined. These measurements indicated the presence of F^{18} radiations only.

The fraction exchanged, in experiments similar to those shown in Fig. 1, increased rapidly at temperatures higher than those shown in the figure. The data shown in Fig. 1 do not represent equilibrium conditions; when the temperature was held constant the fraction exchanged increased with time. In a survey of the relative rates of exchange of fluorine atoms between alkali fluorides and fluorocarbons, conducted similarly to the experiment described above, the order of reactivity

---

(1) This research was performed at the Oak Ridge National Laboratory which is operated by Union Carbide Nuclear Company for the Atomic Energy Commission. The Chemistry Branch of the Office of Naval Research contributed to the work through its contract with the University of Florida. This publication may be reproduced in part or in whole for the benefit of the United States Government.

(2) J. E. Boggs, E. R. Van Artsdalen and A. R. Brosi, THIS JOURNAL, **77**, 6505 (1955).