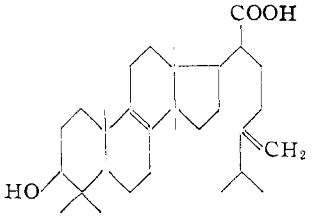
of this series.[2] This concept, however, does not account for the source of the extra carbon atoms on the side-chains of various $C_{28}$, $C_{29}$ and $C_{31}$ steroids. Recently,[3] it has been postulated that such ancillary carbon atoms also find their source in acetate but subsequently it has been 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
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

WILLIAM G. DAUBEN
GERHARD J. FONKEN
GEORGE A. BOSWELL[6]

RECEIVED JANUARY 12, 1957

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## 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 $5.2 \times 10^9$ 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).

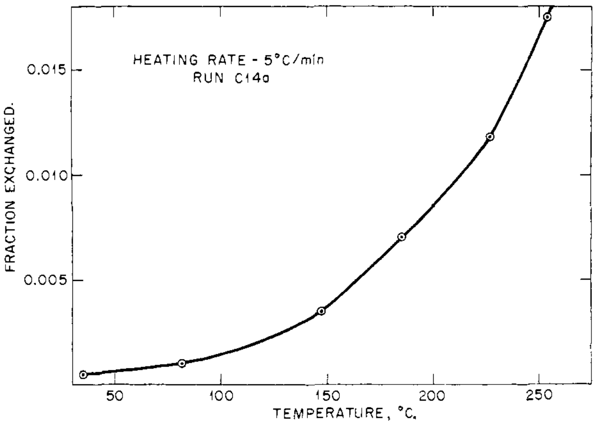

Fig. 1.—The Exchange of $F^{18}$ between $C_3F_6$ and RbF as a Function of Temperature.

per square meter of surface was observed to be Cs > Rb > K, Na, Li.[3]

Perfluoropropene with any single salt exchanged more readily than $CF_4$, $C_4F_{10}$ and $(C_2F_5)_2O$, although appreciable exchange has been observed in all cases. Sulfur hexafluoride failed to exchange with any of these salts below 300°. Above that temperature, complicated reactions set in. Silicon tetrafluoride exchanged rapidly at room temperature with the alkali fluorides but manometric measurements showed complex fluoride formation at higher temperatures. Currently, a survey of exchange reactions in other fluorocarbon-fluoride systems is under way.

[3] Surface areas were determined by the BET method on representative alkali fluorides using krypton. We are indebted to P. K. Melroy of the Special Analytical Services Department of the Oak Ridge Gaseous Diffusion Plant for these measurements.

[4] Oak Ridge Institute of Nuclear Studies Fellow, 1955–57.

[5] National Carbon Company, A Division of Union Carbide and Carbon Corporation, Cleveland, Ohio.

DEPARTMENT OF CHEMISTRY AND OF CHEMICAL ENG.
UNIVERSITY OF FLORIDA T. A. GENS
GAINESVILLE, FLORIDA JOHN A. WETHINGTON, JR
CHEMISTRY DIVISION A. R. BROSI
OAK RIDGE NATIONAL LABORATORY
OAK RIDGE, TENNESSEE E. R. VAN ARTSDALEN[5]

RECEIVED NOVEMBER 23, 1956

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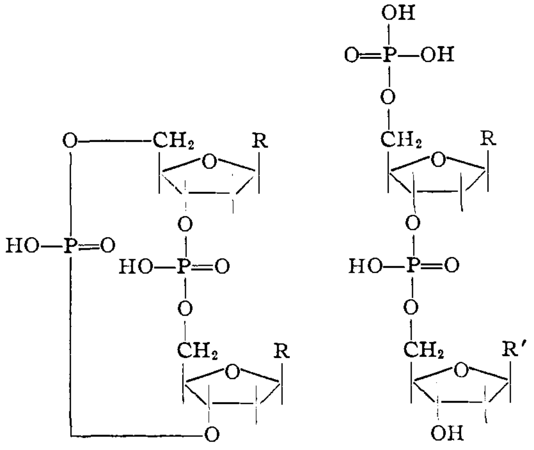
## SYNTHESES OF DIDEOXYRIBONUCLEOTIDES

*Sir:*

A new method for the synthesis of diesters of phosphoric acid recently has been reported from this Laboratory.[1] This approach involved the phosphorylation of a hydroxylic compound by a *mono*ester of phosphoric acid in the presence of *p*-toluenesulfonyl chloride. We now wish to report that dicyclohexylcarbodiimide (DCC) can not only replace *p*-toluenesulfonyl chloride but is, in fact, the preferred reagent. The method has now been applied successfully to syntheses of dideoxyribonucleotides containing 5′ to 3′ phosphodiester linkages and bearing 5′-phosphate end-groups (I).

Thymidine 5′-phosphate was treated with acetic anhydride in pyridine to give the 3′-acetyl derivative in quantitative yield. The latter (1 mole) was brought into reaction in anhydrous pyridine with thymidine 5′-dibenzyl phosphate[2] (1.15 mole) in the presence of DCC (5 moles). Water was added after a reaction period of 24 hours at room temperature and the solvent removed by evaporation. The benzyl groups were then removed by hydrogenation in 10% acetic acid using palladium oxide on barium sulfate[3] as catalyst and the acetyl group by mild alkaline treatment. The dinucleotide (I, R = R′ = thymine) was purified by preparative paper chromatography using isopropyl alcohol–ammonia–water[4] (7–1–2) (Solvent A) and was finally obtained as the highly crystalline trisodium salt in 40% yield.[5] A sample was recrystallized from aqueous ethyl alcohol and dried at 110° in a high vacuum for 12 hours; $[\alpha]^{20}D$ + 10.9° (Michelson and Todd report $[\alpha]^{20}D$ + 7.0° for the hydrated calcium salt).[2] *Anal.* Calcd. for $C_{20}H_{25}O_{15}N_4P_2Na_3$; P, 8.95. Found.[6] P, 8.80. The ratio of total phosphorus to thymidine, using a figure of 9700 for $E_{max}$ at 267 mμ for thymidine, was 1.054.



II I

Hydrolysis with 1 *N* hydrochloric acid required 2 hours at 100°, the products being thymidine 3′,5′-diphosphate, thymidine and inorganic phosphate. An electrometric titration of the free acid, obtained by passing the sodium salt through Dowex-50($H^+$) resin, showed that the ratio of primary (*p*H range 1–3.5) to secondary (*p*H range 5.5–8) phosphoryl dissociations was 2.

Incubation of the dinucleotide in tris-(hydroxymethyl)-aminomethane buffer (*p*H 8.8) with unfractionated snake venom[7] resulted in quantitative degradation to thymidine and inorganic phosphate as shown by paper chromatography in Solvent A, whereas a purified phosphodiesterase fraction[8] gave thymidine 5′-phosphate (chromatography in *n*-butyl alcohol–acetic acid–water (5–2–3) (Solvent

[1] H. G. Khorana, G. M. Tener, J. G. Moffatt and E. H. Pol, *Chemistry and Industry*, 1523 (1956).

[2] Prepared in quantitative yield from 3′-acetyl thymidine by a modification of the procedure of Michelson and Todd, (*J. Chem. Soc.*, 2632 (1955).

[3] R. Kuhn and H. J. Haas, *Angew. Chem.*, **67**, 785 (1955).

[4] D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 2040 (1953).

[5] The yield can probably be greatly improved since most of the coproduct is thymidine 5′-phosphate.

[6] E. J. King, *Biochem. J.*, **26**, 292 (1932).

[7] *Crotalus adamenteus*, purchased from Ross Allen's Reptile Farm, Florida.

[8] The procedure used to purify the diesterase involves fractional acetone precipitation in the cold (unpublished work of Dr. R. L. Sinsheimer).