lyzes the incorporation of serine-C^{14} into sphingosine and to identify some of the cofactors required for this conversion. The incorporation of radioactivity from acetate-1-C^{14} and L-serine-3-C^{14} into sphingosine has been observed *in vivo*.[1,2]

Brain tissue obtained from 10–14 day old rats was homogenized with 3 volumes of isotonic sucrose or 0.1 *M* potassium phosphate buffer (*p*H 7.8), and differential centrifugation was accomplished according to Brody and Bain.[3] The enzymes required for the conversion of L-serine to sphingosine were found in the supernatant solution obtained after sedimenting the particulate matter by centrifuging at 25,000 × *g* for 30 minutes. The enzyme preparation was dialyzed for 4 hours against 100 volumes of a solution containing 0.01 *M* potassium phosphate buffer (*p*H 7.0), 0.001 *M* cysteine hydrochloride and 0.001 *M* disodium ethylenediaminetetraacetic acid.

Following incubation, the lipids were quantitatively extracted with *n*-butanol and 18 μmoles of sphingosine sulfate were added to each sample as carrier. The mixtures were refluxed for 6 hours in 85% methanol made 1.2 *M* with respect to H_2SO_4, and subsequently extracted with petroleum ether which was back-washed two times with dilute acid. The methanolic solution containing the sphingosine sulfate was concentrated under vacuum, and the sphingosine base was recovered from salt-saturated alkaline aqueous phase by extracting with 2% isoamyl alcohol in heptane.[4] The solvents were evaporated under reduced pressure and the sphingosine was recrystallized two times from petroleum ether. Approximately 80% of the carrier sphingosine was recovered with the use of this procedure. The twice recrystallized sphingosine was found to have constant specific radioactivity.

TABLE I

ENZYMATIC CONVERSION OF SERINE-3-C^{14} TO SPHINGOSINE

Except as noted, the incubation mixtures contained 1.5 ml. of dialyzed supernatant solution obtained by centrifuging a 1:3 (w./v.) cell-free preparation of rat brain tissue at 25,000 × *g* for 30 minutes, 150 μmoles of potassium phosphate buffer (*p*H 7.8), 4 μmoles of DL-serine-3-C^{14} (4 μC.), 1 μmole of pyridoxal phosphate, 5 μmoles of $MgCl_2$, 0.3 μmole of triphosphopyridine nucleotide (TPN), 0.3 μmole of diphosphopyridine nucleotide (DPN), 20 μmoles of nicotinamide, 2μmoles of uridine triphosphate (UTP), 0.4 μmole of cytidine triphosphate (CTP), 5 μmoles of adenosine triphosphate (ATP), and 0.1 ml. of liver kochsaft in a total volume of 2.0 ml. Incubation time, 3 hours at 38° in air.

Reactant omitted	Distribution of radioactivity in recovered sphingosine: Sphingosine, c./m./μmole	Residue after periodate degradation, c./m./μmole
None	155	26
None, heated at 100° for 10 min.	3	..
Kochsaft	11	..
Pyridoxal phosphate	35	23
$MgCl_2$	71	20
TPN	90	20
DPN	93	22
Nicotinamide	110	25
UTP	140	26
CTP	165	25
ATP	170	28

As shown in the table, the conversion of serine-3-C^{14} to sphingosine requires the presence of pyridoxal phosphate, magnesium chloride, triphosphopyridine nucleotide, diphosphopyridine nucleotide, and an as yet unidentified substance present in a kochsaft obtained from rat liver tissue. The recovered sphingosine was degraded with sodium periodate,[4,5] and the radioactivity of the residual aliphatic aldehyde was determined. It is apparent from these data that the radioactivity from the serine-C^{14} is preferentially localized in positions 1 and 2 of sphingosine and that the reaction does not represent random incorporation of radioactivity via an active one or two carbon fragment. These findings have been confirmed with the use of L-serine-U-C^{14} as substrate in this system.

(1) I. Zabin and J. F. Mead, *J. Biol. Chem.*, **205**, 271 (1953).
(2) D. B. Sprinson and A. Coulon, *ibid.*, **207**, 585 (1954).
(3) T. M. Brody and J. A. Bain, *ibid.*, **195**, 685 (1952).
(4) R. O. Brady and R. M. Burton, *J. Neurochem.*, **1**, 18 (1956).
(5) H. E. Carter, F. J. Glick, W. P. Norris and G. E. Phillips, *J. Biol. Chem.*, **170**, 285 (1947).

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE ROSCOE O. BRADY
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
BETHESDA, MARYLAND GEORGE J. KOVAL

RECEIVED APRIL 1, 1957

A NOVEL ORGANO-CHROMIUM COMPOUND

Sir:

The known organo-chromium compounds, apart from the anionic cyanide and ethynyl complexes[1] of chromium(III) are compounds of chromium(0), chromium(I) or chromium(II)[2] which generally do not contain simple carbon–chromium bonds. We wish to report the first preparation (in solution) of a simple organo-chromium compound of the type $Cr(H_2O)_5R^{++}$ where R is benzyl. The compound has not yet been obtained crystalline, but solutions of the pure perchlorate in dilute perchloric acid solution have been obtained and are fairly stable (half-life in absence of oxygen at room temperature of about 1.5 days). The structure of the compound, benzylpentaaquochromium(III) perchlorate, can be regarded as analogous to that of the chloride complex, $Cr(H_2O)_5Cl^{++}$, of chromium(III); *i.e.*, the compound is a complex of Cr(III) with the *benzyl anion*. The remarkable stability of this organo-metallic compound in acid solution is undoubtedly related to the inertness of Cr(III) complexes to substitution.[3]

The benzylchromium compound was prepared by reducing benzyl chloride (or bromide or iodide) with aqueous 1 *M* chromous perchlorate in 1 *M* perchloric acid, either heterogeneously or in presence of alcohol or other suitable organic solvents. Countercurrent distribution of the product at 5° with the solvent system 0.01 *M* perchloric acid–butanol gave, after 70 transfers, a separation of the organo-chromium compound (partition coefficient

(1) R. Nast and E. Sirtl, *Chem. Ber.*, **11**, 1723 (1955).
(2) F. A. Cotton, *Chem. Revs.*, **55**, 551 (1955); H. H. Zeiss and W. Herwig, THIS JOURNAL, **78**, 5959 (1956); E. O. Fischer and W. Hafner, *Z. anorg. Chem.*, **286**, 146 (1956); G. Wilkinson, THIS JOURNAL, **76**, 209 (1954); T. S. Piper and G. Wilkinson, *J. Inorg. Nuclear Chem.*, **3**, 104 (1956).
(3) H. Taube, *Chem. Revs.*, **50**, 69 (1952).

0.35) from $Cr(H_2O)_6^{+++}$ (mainly) and $Cr(H_2O)_5Cl^{++}$ (partition coefficient 0.13). Separation was also effected by ion-exchange chromatography on Dowex 50-X4 resin and elution with 1 *M* perchloric acid. The ion $Cr(H_2O)_5Cl^{++}$ (green) was eluted first, followed by $Cr(H_2O)_5CH_2Ph^{++}$ (yellow to orange-yellow) whilst $Cr(H_2O)_6^{+++}$ remained on the column. All the above operations were carried out in an atmosphere of oxygen-free nitrogen and as rapidly as possible.

Solutions of the benzylchromium compound were yellow to brownish-red depending on the concentration, and the spectrum of the solution had a low intensity maximum at 540 mμ and a high intensity maximum at 358 mμ. Decomposition of the complex in the absence of oxygen by heating or by keeping for several days gave bibenzyl; in the presence of oxygen benzaldehyde was the main product. The compound was decomposed very rapidly by sulfur dioxide but was stable to carbon dioxide. Hydrogenation in presence of a palladium catalyst gave toluene. Solutions of the compound reacted very quickly with aqueous mercuric chloride with *no change in pH of the solution* to give benzylmercuric chloride and $Cr(H_2O)_6^{+++}$ (not $Cr(H_2O)_5Cl^{++}$) in equimolecular amounts. Therefore the compound must contain one benzyl group per chromium atom and two positive charges. Hence it is a Cr(III) complex and its stability is best rationalized by the normal octahedral complex structure mentioned before. It differs, as might be expected, from the chloride complex in having a tendency to dissociate in a homolytic fashion to give benzyl radicals and chromous ion. An alternate structure which can be considered for the compound is a complex of the tropylium ion and Cr(I) in the manner[2] of dibenzene–Cr(I) but with only one ring involved per chromium atom, although this is somewhat unlikely on the above evidence. However, such a structure can be excluded because all the carbon atoms would then become equivalent and it was found that α-methyl, *o*-methyl- and *p*-methylbenzyl halides gave different compounds on reduction with chromous perchlorate.

Reduction of benzyl chloride with chromous chloride in hydrochloric acid gave toluene rather than an organo-metallic compound. This can be understood from the work of Taube[4] as the initially formed complex, $Cr(H_2O)_4Cl{\cdot}CH_2Ph^+$, will have chloride ion as well as water molecules in the coördination sphere and such a chlorine atom favors reduction by bridging[4] with the reducing agent. The resultant Cr(II) complex is then no longer substitution-inert and dissociates to benzyl anions which react with the solvent to give toluene. Therefore, the isolation of $Cr(H_2O)_4CH_2Ph^{++}$ in the chromous perchlorate reduction is due to the fact that this compound (like $Cr(H_2O)_6^{+++}$ but unlike $Cr(H_2O)_5Cl^{++}$ and $Cr(H_2O)_4Cl_2^+$) is reduced by Cr^{++} extremely slowly, as well as to the substitution-inertness of dipositive complexes of Cr(III).

Allyl chloride and phenacyl chloride were readily reduced by chromous perchlorate, but without observable formation of organo-metallic compounds. Work on the reduction of halogen compounds by chromous salts is continuing.[5]

(4) H. Taube and E. L. King, THIS JOURNAL, **76**, 4053 (1954); H. Taube and H. Myers, *ibid.*, **76**, 2103 (1954).

(5) This work was supported by a grant from the National Research Council of Canada.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF OTTAWA F. A. L. ANET
OTTAWA, ONTARIO E. LEBLANC

RECEIVED APRIL 15, 1957

THE BIOSYNTHESIS OF SQUALENE FROM MEVALONIC ACID

Sir:

Tavormina, *et al.*,[1] have reported the incorporation of 2-C^{14}-labeled mevalonic acid (3,5-dihydroxy-3-methylvaleric acid) into cholesterol by homogenates of rat liver. These investigators have also provided evidence that the carboxyl carbon of the substrate is almost completely converted to carbon dioxide, and therefore not incorporated into cholesterol.[2]

We have confirmed the finding that 2-C^{14}-mevalonic acid[3] is converted to cholesterol, and in addition have demonstrated that it is rapidly converted to squalene by homogenates as well as by the supernatant fluid obtained from such preparations following high speed centrifugation.[4]

Employing homogenates of rat liver and 2-C^{14}-mevalonic acid, we have repeatedly isolated C^{14}-squalene into which has been incorporated 10 to 20% of the substrate. Squalene recovered from several of the experiments was combined and purified by chromatography on alumina.[4] It was subsequently chromatographed on silicic acid, eluting with an increasing gradient of benzene in petroleum ether. The latter process yields squalene as a single radioactive peak.[5]

In contrast to cruder preparations of squalene, this product is completely degraded by ozonolysis to acetone, levulinic acid and succinic acid. These substances were separated and further degraded. Acetone was converted to iodoform and acetic acid, and the acetic acid degraded by the Schmidt method.[6] The levulinic acid was converted to iodoform and succinic acid. The succinic acid was degraded by the Schmidt technique to carbon dioxide and ethylenediamine. All of the degradation products were converted to carbon dioxide and counted as barium carbonate.

The radio-analyses (Table I) demonstrated that approximately 80% of the isotopic carbon of the squalene is present in the methyl carbons of acetone and in carbon 3 of levulinic acid. The succinic acid sample, representing the central four carbons of squalene, contained only a small amount of isotope. It is clear that only one of the methylene groups of the levulinic acid was appreciably labeled,

(1) P. A. Tavormina, M. H. Gibbs and J. W. Huff, THIS JOURNAL, **78**, 4498 (1956).

(2) P. A. Tavormina and M. H. Gibbs, *ibid.*, **78**, 6210 (1956).

(3) The authors express their appreciation to Merck Sharp and Dohme for a generous supply of labeled mevalonic acid used in these experiments.

(4) F. Dituri, F. A. Cobey, J. V. B. Warms and S. Gurin, *J. Biol. Chem.*, **221**, 181 (1956).

(5) F. Dituri, J. L. Rabinowitz and S. Gurin, in preparation.

(6) E. F. Phares and M. V. Long, THIS JOURNAL, **77**, 2556 (1955).