TABLE I

TYPICAL CONDITIONS FOR $Mo(CN)_8^{-3}$–$Mo(CN)_8^{-4}$ EXCHANGE

Mo(IV), *f*	Mo(V), *f*	Other species, *f*	*p*H	Separation agent	Extent of exchange, %
5.0×10^{-2}	1.5×10^{-2}[a]	ClO_4^-, 2×10^{-2}; NH_4^+, 1×10^{-2}	2	C_2H_5OH	94
1.7×10^{-3}	1.2×10^{-4}[a]	ClO_4^-, 4×10^{-4}; NH_4^+, 1×10^{-4}	4–5	$Cd(NO_3)_2$ 0.1*f*	100
5.0×10^{-5}[a]	1.5×10^{-4}	Cl^-, 7×10^{-4}; NH_4^+, 3×10^{-3}	10–11	$(C_6H_5)_4AsCl$ 0.2*f*	100
9.6×10^{-4}[a]	1.1×10^{-3}	Cl^-, 1×10^{-2}; NH_4^+, 1×10^{-3}	1–2	$(C_6H_5)_4AsCl$ 0.2*f*	106
5.0×10^{-5}	4.0×10^{-5}[a]	NO_3^-, 1×10^{-4}; NH_4^+, 3×10^{-5}	6–8	$(C_6H_5)_4AsCl$ 0.2*f*	116

[a] Denotes initially active species.

electron.[6] This implies that both complexes have d^4sp^3 binding and should thus have a very similar configuration and internuclear spacing. It follows that the Franck–Condon principle should not impose any considerable barrier to electron transfer —a prediction which is in accord with the experimental findings. The results, furthermore fit well into an empirical correlation proposed by Adamson,[7] according to which exchange is rapid between species both having a low magnetic susceptibility.

The author greatly appreciates the helpful discussions of Dr. R. W. Dodson who first pointed out to him the interest which might be attached to this research. The advice of Dr. Joan Welker on the method of preparation and the kindness of Dr. A. W. Adamson in supplying the author with a sample of $K_4Mo(CN)_8 \cdot 2H_2O$ are gratefully acknowledged.

(6) P. W. Selwood, "Magnetochemistry," Interscience Publishers, Inc., New York, N. Y., 1943, p. 150.

(7) A. W. Adamson, *J. Phys. Chem.*, in press (1952).

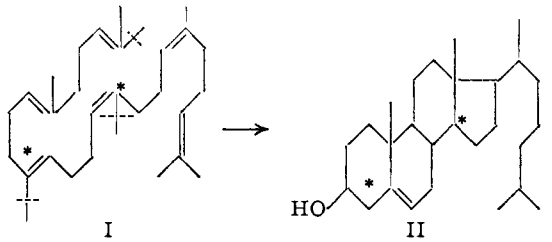
CHEMISTRY DEPARTMENT
BROOKHAVEN NATIONAL LABORATORY
UPTON, LONG ISLAND, NEW YORK

Synthetic C^{14}-"Squalene": Concerning its Incorporation into Cholesterol by Liver[1]

BY G. M. TOMKINS,[2] I. L. CHAIKOFF, W. G. DAUBEN, H. L. BRADLOW AND P. A. SRERE

RECEIVED JUNE 24, 1952

In 1926 and again in 1937, Channon[3] reported that the feeding of the hydrocarbon squalene (I) to rats resulted in an increase in the cholesterol (II) contents of their livers. In 1934, Robinson[4] suggested that by the following cyclization of the dihydrotriterpene, this compound might serve as a direct precursor of cholesterol.



I II

Subsequent work utilizing labeled acetate[5] has yielded results which can readily be interpreted in terms of this hypothesis[6] and, indeed, recently Langdon and Bloch[7] demonstrated the conversion of labeled squalene to this sterol.

Independently, we had undertaken[8] to study this conversion and should like to report our results employing specifically labeled, synthetic "squalene."[9] The metabolic fate of this compound was studied both *in vitro* and *in vivo*. The former type of experiment was conducted in two distinct fashions, (1) the compound, dispersed in saline with the aid of Tween 80, was incubated with liver slices and (2) liver slices of animals previously injected with the labeled material were incubated. In the *in vivo* experiment, the "squalene" was introduced by stomach tube into fasted rats. The results of these experiments are given in Tables I, II and III.

TABLE I

Liver slices incubated in the presence of labeled substrate for 3 hours at 37.5°

Expt.	Wt. of slices, g.	Contents of flask: Substrate	μM.	C^{14}, c.p.m.	Per cent. of added C^{14} recovered as CO_2	Cholesterol
I	1	Acetate-1-C^{14}	5	6.85×10^5	31.0	0.82
	1	Squalene-C^{14}		7.5×10^5	0.40	0
II	1	Acetate-1-C^{14}	5	6.85×10^5	36.0	0.68
	1	Squalene-C^{14}		7.5×10^5	0.62	0
III	1	Acetate-1-C^{14}	5	6.85×10^5	34.0	0.62
	1	Squalene-C^{14}		7.5×10^5	0.46	0

TABLE II

The labeled substrate was injected into the portal vein at time of sacrifice, and liver slices were incubated for 3 hours at 37.5°

Activity injected per 2 g. of liver, c.p.m.	Weight of liver slices per flask, g.	Per cent. of added C^{14} recovered as CO_2	Cholesterol
5.0×10^5	2	0.11	0.0
	2	.20	.0

TABLE III

The labeled substrate was administered enterally

Animal	Total activity administered, c.p.m.	Total activity in liver at end of 24 hr., c.p.m.	Per cent. C^{14} administered recovered as cholesterol
1	9.0×10^5	2.4×10^4	0.0
2	1.7×10^6	5.7×10^4	.0

(1) Aided by a grant from the U. S. Public Health Service and the Life Insurance Medical Research Fund.

(2) Life Insurance Medical Research Fellow.

(3) H. J. Channon, *Biochem. J.*, **20**, 400 (1926); H. J. Channon and B. R. Tristam, *ibid.*, **31**, 738 (1937).

(4) R. Robinson, *J. Soc. Chem. Ind.*, **53**, 1062 (1934).

(5) H. N. Little and K. Bloch, *J. Biol. Chem.*, **183**, 33 (1950), and earlier papers.

(6) P. Srere (Dissertation, Univ. of California, 1951), in this Laboratory, has demonstrated that livers from animals kept for 30 days on a 1% squalene diet, show a decreased ability to incorporate C^{14}-labeled acetate into cholesterol but not into carbon dioxide. Such a result is compatible with the concept that an intermediate metabolic pool in the conversion acetate to cholesterol has been diluted or that squalene might act as a specific precursor of the sterol.

(7) R. G. Langdon and K. Bloch, THIS JOURNAL, **74**, 1869 (1952).

(8) "Ciba Foundation Conference on Isotopes in Biochemistry," J. and A. Churchill, Ltd., London, W. 1, England, p. 24 ff.

(9) The term "squalene" is used to indicate that the synthetic product is a dihydrotriterpene with six double bonds and is a mixture of double bond isomers (see W. G. Dauben and H. L. Bradlow, THIS JOURNAL, **74**, in press (1952)).

The data show that synthetically prepared squalene fails to be incorporated into cholesterol under all three conditions. Since in the *in vitro* experiment, the "squalene" was dispersed in Tween 80, it was essential to verify that this agent had not interfered with the biological synthesis of cholesterol. This was tested by incubating slices from the same liver with C^{14}-labeled acetate and amounts of Tween 80 equal to that employed in the "squalene" study. It was found that 0.6–0.8% of the acetate activity was incorporated into cholesterol and from 31–36% was oxidized to carbon dioxide. It is therefore clear that the failure in the incorporation of the labeled "squalene" under our experimental conditions cannot be due to interference by the Tween 80. It is of interest that the C^{14}-squalene was oxidized to carbon dioxide by liver slices. The recovery of radioactivity in the crude non-saponifiable fraction of the liver of the rats fed the labeled material indicates that absorption of the unchanged "squalene" had occurred.

The results employing synthetic squalene are in contrast to those reported by Langdon and Bloch[7] in which it was demonstrated that *biologically* prepared squalene is a more active precursor of cholesterol than acetate. The failure of the synthetic material to be incorporated into the sterol could be due to the fact that the labeled "squalene," which is a mixture of double bond isomers, contains no molecules of the identical natural configuration. It should be recalled, however, that this synthetic material is apparently identical with the squalene obtained by the purification of the natural triterpene through its solid hexahydrochloride.[9] The high degree of stereospecificity of the conversion reported by Langdon and Bloch[8] is of interest since Hubbard and Wald[10] have shown that in the intact animal, inactive isomers of vitamin A are readily isomerized to the biological active form. Another possible explanation of this failure to be incorporated into cholesterol is that the presence of non-natural squalene molecules might block the conversion of any natural material which might have been present in the synthetic material.

Experimental

C^{14}-Labeled "Squalene."—The material was prepared as described in reference 9 and was labeled with C^{14} in the carbon atoms indicated by the asterisks in I.

In Vitro **Experiments.**—Two types of *in vitro* experiments were conducted. In one, liver slices approximately 0.5 mm. thick were incubated in a bicarbonate buffer containing the labeled "squalene." The "squalene" was dispersed in 1 ml. of 0.85% sodium chloride solution with the aid of 3–4 drops of Tween 80, and this solution was added to the medium. In the second type of experiment the labeled hydrocarbon, dispersed in saline and Tween 80 as described above, was injected directly into the portal vein of rats immediately after they had been stunned by a blow on the head. This injection procedure ensured entrance of the radioactive "squalene" into the liver. The livers were excised and sliced, and weighed portions of slices were incubated in the bicarbonate buffer medium as previously described. Incubation lasted three hours in both types of experiments. At the end of the run, the carbon dioxide was collected and its C^{14} content determined as described elsewhere.[11] Cholesterol was isolated from the contents of the flask and its C^{14} content measured in the manner reported earlier.[11]

In Vivo **Experiment.**—The labeled triterpene was administered as a Tween dispersion by stomach tube to two rats that had been fasted for 10 hours. They were allowed to eat a stock diet *ad libitum* for 24 hours and then were sacrificed. The livers of these rats were removed and hydrolyzed overnight with alcoholic potassium hydroxide. The alkaline solution so obtained was extracted repeatedly with petroleum ether until radioactivity was no longer obtained. The residual hydrolysate was then acidified and re-extracted in the same manner with petroleum ether. The lipid-free residue was finally extracted with warm water. The C^{14} content of the non-saponifiable fraction, the saponifiable fraction and the aqueous extract of the residue was then determined. Measurable amounts of radioactivity were found only in the non-saponifiable fraction. The cholesterol was isolated from this latter fraction and its radioactivity determined as described elsewhere.[11]

DIVISION OF PHYSIOLOGY
SCHOOL OF MEDICINE AND THE
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

(10) R. Hubbard and G. Wald, *Science*, **115**, 60 (1952).

(11) G. M. Tomkins and I. L. Chaikoff, *J. Biol. Chem.*, **196**, 569 (1952).

The Preparation and Some Properties of Ruthenocene and Ruthenicinium Salts

BY GEOFFREY WILKINSON

RECEIVED JUNE 12, 1952

A structure for the compound bis-cyclopentadienyliron $(C_5H_5)_2Fe$[1] has been suggested,[2] in which the central metal atom is symmetrically placed between the planes of two cyclopentadienyl rings, the π-electrons of which are involved in the filling of the 3d orbitals of the metal. With this scheme, it can be expected that ruthenium and osmium, both of which, like iron, have electronic structures for the atom ten electrons short of that of the next inert gas, will form π-complexes analogous to $(C_5H_5)_2Fe$.

The ruthenium compound, $(C_5H_5)_2Ru$, has now been made. Since the iron compound bis-cyclopentadienyliron has been named ferrocene[3] on account of its chemical behavior as an aromatic system, the ruthenium analog may be referred to as ruthenocene. Its systematic name is bis-cyclopentadienylruthenium. The unipositive ions $[(C_5H_5)_2Fe]^+$ and $[(C_5H_5)_2Ru]^+$ which are formed on oxidation of the neutral compounds, are, respectively, designated the ferricinium and the ruthenicinium ions.

Experimental

Ruthenocene has been prepared by the reaction of ruthenium(III) acetylacetonate with a fivefold excess of cyclopentadienylmagnesium bromide. The acetylacetonate was made by heating ruthenium chloride with acetylacetone in potassium bicarbonate solution[4]; the complex was extracted with benzene and purified by crystallization from benzene. Although the reaction mixture was held at 80° for 24 hours, subsequent experiments suggest that this procedure is unnecessary and that the reaction is almost immediate. After the reaction period, the Grignard mixture was decomposed with ice-water, and the product extracted with ether. The solvent was then removed and the residue extracted with petroleum ether, which in turn was removed. This residue

(1) First reported by T. J. Kealy and P. L. Pauson, *Nature*, **168**, 1039 (1951). See also Miller, Tebboth and Tremaine, *J. Chem. Soc.*, 632 (1952).

(2) G. Wilkinson, M. Rosenblum, M. C. Whiting and R. B. Woodward, THIS JOURNAL, **74**, 2125 (1952).

(3) M. Rosenblum, M. C. Whiting and R. B. Woodward, *ibid.*, **74**, 3458 (1952).

(4) G. A. Barbieri, *Atti accad. Lincei*, **23**, [5] 336 (1914).