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 nonsaponifiable 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 nonnatural 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 reextracted 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]

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The Preparation and Some Properties of Ruthenocene and Ruthenicinium Salts

BY GEOFFREY WILKINSON

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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

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[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).

was sublimed at 120° in high vacuum, and the sublimate, which contained yellowish crystals in addition to gummy hydrocarbon polymers, was dissolved in ether and crystallized. The product was resublimed and was finally repeatedly crystallized from carbon tetrachloride. The yield of ruthenocene was about 20% of the theoretical yield to be expected when based on ruthenium(III) acetylacetonate.

Ruthenocene crystallizes in transparent, plate-like crystals, quite similar to those formed by ferrocene; the compound has only a very slight yellow color. The melting point is 195.5°, but the compound will sublime at lower temperatures. Analyses gave C, 51.68; H, 4.36, Ru, 43.8 (calcd. for $(C_5H_5)_2Ru$: C, 51.83; H, 4.31; Ru, 43.5). The ruthenium was determined by destruction of the compound by repeated evaporation with strong nitric acid, followed by careful ignition and reduction by hydrogen of the blue ruthenium dioxide thus obtained, to metallic ruthenium. Check analysis was made by the thiourea colorimetric method[5] using a ruthenium chloride reference solution; this in turn was standardized by conversion of an aliquot to the metal. The molecular weight determined by camphor melting point depression micromethod is 237 ± 5 (calcd. for $(C_5H_5)_2Ru$, 231.88). Ruthenocene closely resembles ferrocene in its general properties; it is soluble in organic solvents, sublimes readily, is unaffected by bases and by sulfuric and hydrochloric acids in absence of oxygen. The infrared absorption spectrum (Fig. 1), measured both in carbon tetrachloride and in carbon disulfide solution, has a remarkable resemblance to that of ferrocene, showing only the single sharp band at 3.23 μ attributable to C–H stretching and indicating the presence of only one type C–H bond. The ultraviolet absorption spectrum (Fig. 2, curve A) is again rather similar to that of ferrocene except for the non-existence of the second peak which is found in the ferrocene spectrum at 440 mμ.

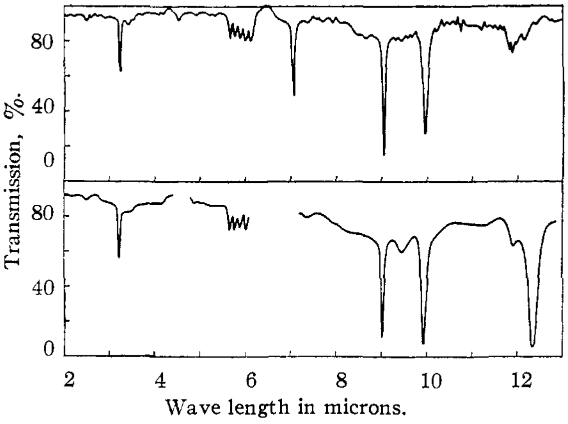

Fig. 1.—Infrared absorption spectrum of ruthenocene: top, carbon tetrachloride solution; bottom, carbon disulfide solution; Baird recording spectrophotometer, NaCl cell.

Ruthenocene can be oxidized to a unipositive ion $[(C_5H_5)_2Ru]^+$. Polarographic measurements[6] show that the oxidation of ruthenocene is a one-electron step. Unlike the deep blue color of solutions containing the ferricinium ion, the ruthenicinium salts give pale yellow aqueous solutions. The ultraviolet absorption spectrum of ruthenicinium perchlorate in 0.01 *N* perchloric acid solution is shown in Fig. 2, curve B.

Ruthenicinium perchlorate $[(C_5H_5)_2Ru]ClO_4$ has been prepared as a yellow crystalline solid, by controlled potential anodic oxidation of ruthenocene in 90% alcohol solution containing perchloric acid. On oxidation, the perchlorate separates and can be centrifuged and dried with absolute alcohol. Analysis for ruthenium gave 30.62%, calculated 30.69%. The salt dissolves in water to give a yellow solution; the only moderate solubility in water (~ 1 mg./ml.) and insolubility in alcohol is in contrast to the much greater solubility of ferricinium and cobalticinium[7] perchlorates in water. Measurement of the ultraviolet absorption spectrum of ruthenicinium perchlorate shows that the solutions of the salt in dilute perchloric acid are stable for an hour or two, but that partial decomposition of neutral solutions occurs fairly rapidly within half an hour with the formation of some ruthenocene; this behavior is similar to that of the ferricinium solutions which partially decompose within a few minutes to give some ferrocene, ferric hydroxide and unidentified organic hydrolysis products.

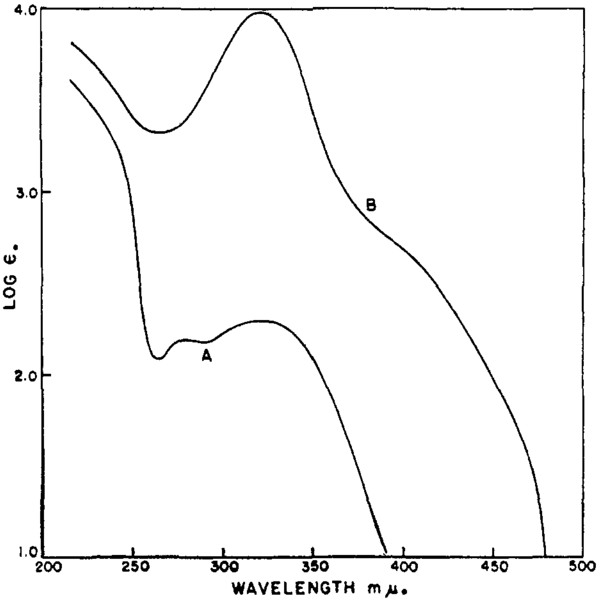

Fig. 2.—Ultraviolet spectra: A, ruthenocene in cyclohexane solution; B, ruthenicinium perchlorate in 0.01 *N* perchloric acid; Beckman spectrophotometer, silica cells, 25°.

The solution reactions of the ruthenicinium ion are those that might be expected for a large unipositive ion. From hydrochloric acid solutions silicotungstic acid precipitates a pale yellow silicotungstate. On addition of saturated solutions of sodium picrate, sodium 2,4-dinitrophenolate, sodium 7-iodo-8-hydroxyquinoline-5-sulfonate or sodium 6-chloro-5-nitrotoluene-3-sulfonate, to neutral solutions containing ~ 0.8 mg./ml. of ruthenicinium perchlorate, yellow colored precipitates are obtained. Analysis of the recrystallized picrate gave 21.95% Ru, calculated 22.12%. Ruthenicinium triiodide $[(C_5H_5)_2Ru]I_3$, is precipitated as a reddish solid on mixing solutions of iodine and ruthenocene in carbon tetrachloride. An excess (50 ml.) of a solution of iodine in carbon tetrachloride (equivalent to 26.10 ml. of 0.0153 *N* thiosulfate solution) was added to 21.10 mg. of ruthenocene in carbon tetrachloride; after centrifugation of the ruthenicinium triiodide and washing, the excess iodine was titrated with thiosulfate, 8.30 ml. being required. The iodine consumed in the reaction was thus in accord with the formation of ruthenicinium triiodide. Direct analysis of the compound gave Ru, 16.52; I, 62.23 (calcd. Ru, 16.60; I, 62.15). The compound is insoluble in water and most organic solvents; it is soluble in acetonitrile, alcohol and acetone. The behavior of ruthenocene with iodine closely resembles that of ferrocene, and the two triiodides have very similar properties.

Like ferrocene, ruthenocene can be oxidized with various chemical reagents such as bromine water and aqueous silver sulfate, but dilute nitric acid and stronger oxidizing agents destroy the ruthenicinium ion first formed, giving red solutions of tri- or tetravalent ruthenium.

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[5] E. B. Sandell, "Colorimetric Determination of Traces of Metals," 2nd Ed., Interscience Publishers, Inc., New York, N. Y., 1950.

[6] J. A. Page and G. Wilkinson, THIS JOURNAL, **74**, 6149 (1952).

[7] G. Wilkinson, *ibid.*, **74**, 6148 (1952).