tions of the δ-lactones of gluconic (13), mannonic (11) and rhamnonic acids (12) are relatively high initial specific rotations which show rapid diminution in value during the first few hours after solution. In comparison with the parent lactones, the acetyl derivatives of mannonic (11a) and rhamnonic (12a) do not show as rapid diminution of specific rotation. The behavior of acetyl δ-gluconic lactone, however, parallels very closely that of the parent lactone. In comparison with the corresponding acetyl γ-lactone derivatives these acetylated δ-lactones all show a much more rapid rate of mutarotation and the initial rotations are all very close to those of the parent lactones. Thus the acetylated δ-mannonic lactone (2–11a) changes in rotation from +97 to +63° in twenty-four hours, whereas the corresponding gamma derivative (1–9a) has an initial specific rotation of +60° which shows no change even after ten days. In like manner the rotation of acetylated γ-rhamnonic lactone (2–12a) has an initial value of −114° which changes to −94° in twenty-four hours, while the acetyl derivative of the corresponding γ-lactone (1–3a) exhibits an initial specific rotation of −60° which shows no change even after five days. These acetylated δ-lactones are of special interest since they are the first derivatives to be prepared directly from the delta lactones themselves.

Summary

1. Thirteen acetylated monobasic sugar acid lactones have been prepared, ten of the γ-variety, and three of the δ-configuration.

2. The change in specific rotation with time at 25° has been determined.

3. It has been shown that the acetylated lactones parallel rather closely the parent lactones as regards change of rotations with time.

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[A CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Deuterium Abundance Ratios in Organic Compounds. III. Cholesterol

BY MALCOLM DOLE AND ROBERT B. GIBNEY

The interesting possibility of variations in the isotopic abundance ratios of deuterium in naturally occurring organic compounds as compared to the value of this ratio in normal water has been realized and investigated by a number of workers,[1] but there has not yet been published definite proof of an abnormal deuterium content in any organic compound. The hydrogen of benzene has been analyzed by one of us using a method presumably free of isotopic error[1] but its deuterium content is practically normal. In this paper we report data for the abundance of deuterium in cholesterol which appear to demonstrate the existence of a compound containing a slightly low abundance of deuterium although the observed effect is scarcely greater than the experimental error.[2] We chose cholesterol for this study because it is definitely an animal product,[3] and because its high molecular weight and complexity of structure led us to believe that if any animal product would be abnormal in its deuterium content, cholesterol would be that compound. Cholesterol is also interesting because of its apparently important relations to the hormones and other physiologically active substances. As compared to benzene cholesterol has a relatively high percentage of hydrogen; the small amount of oxygen present introduces a negligible error.

(1) See M. Dole, THIS JOURNAL, 58, 580 (1936), for a review of all previous work and a critical discussion of existing data.

(2) See M. Dole, *Science*, 83, 31 (1936), for calculations showing that the work of Washburn and Smith apparently indicated a smaller than normal abundance of deuterium in dry wood of the willow tree after proper allowance had been made for the difference in atomic weights of oxygen of the air and of water. However, the isotopic composition of the oxygen in the willow tree water is still uncertain even after this correction has been made.

(3) L. F. Fieser, "The Chemistry of Natural Products Related to Phenanthrene," Reinhold Publishing Corporation, New York, 1936, states, p. 122: "Although the evidence is conclusive that the cholesterol present in higher animals is synthesized in the animal organism, the site and mechanism of cholesterol formation are unknown."

Experimental

Combustion of the Cholesterol.—The method of determining the deuterium content previously used was followed here. The source of the cholesterol was the Wilson Laboratories, Chicago, highest grade "Cholesterin" which comes 95% from the spinal fluid of cattle and 5% from the brains of pigs. The purity of this product was claimed by the manufacturers to be 99.9%, but Fieser states[3] (p. 113) that commercial cholesterol contains small amounts of closely related substances which cannot be eliminated by repeated crystallization, to the extent perhaps of 1 to 2%. Since these related compounds would probably contain similar isotopic proportions of deuterium, this small impurity can be neglected. The melting point temperature of the cholesterol was 149°. The combustion of the cholesterol was carried out using four different methods in order to make it fairly certain that the water obtained contained a representative sample of the hydrogen. Deuterium containing substances apparently burn more slowly than similar compounds made of protium, so that unless all or practically all the cholesterol suffers combustion, the

resulting water might not contain all the deuterium it should contain.

The first combustion of cholesterol was carried out by one of us in 1934 by heating the cholesterol to 350° in a Pyrex flask and leading the vapors over hot copper oxide by means of a stream of air which was passed over the surface of the hot cholesterol.[4] The cholesterol burned on the catalyst and tank oxygen of an unknown source (probably Linde oxygen) was added in relatively small amounts whenever the oxygen in the air was insufficient to keep the catalyst in the oxide form. The burning of the cholesterol was repeated by this method in 1936 using air to sweep the vapors of the cholesterol over the copper oxide. Air was also admitted to the middle portion of the combustion tube in just sufficient quantities to maintain the catalyst in the oxide form. An excess of air was avoided in order to prevent a separation of the isotopes of water by a fractional condensation of its vapors. In the case of this second combustion a large charred residue remaining in the cholesterol flask was removed, ground fine and burned in a modified apparatus using air. The yield of water was approximately 75% of the theoretical.

The third combustion of the cholesterol was carried out in a specially designed apparatus using instead of air Airco oxygen, whose isotopic composition in reference to that of atmospheric oxygen was known. The cholesterol burned at a jet made out of capillary tubing in a chamber 6.4 cm. in diameter, 10.2 cm. long which was sealed onto the combustion tube containing the copper oxide catalyst. Oxygen was supplied through a side arm. The cholesterol was kept in a liquid state in a flask by means of a cottonseed oil-bath heated to 180°. Nitrogen forced the cholesterol from the flask into the combustion system through a glass tube while more nitrogen entering into the tube halfway to the combustion chamber, broke the stream of cholesterol into bubbles and blew the cholesterol vapor which was now formed from the heat of the combustion furnace through the jet where the cholesterol burned in a flame steadily and brilliantly. Sometimes it was found unnecessary to add nitrogen at the half-way point. Oxygen was added just rapidly enough to keep the copper oxide catalyst in the oxide form. The yield of water was about 75%. Some of the cholesterol was occasionally blown through the apparatus unburnt due to slight explosions which occurred when the flame went out. Considerable attention was required to maintain a flame at the jet; the flame was lighted by explosions of the oxygen–cholesterol vapor mixture traveling back to the capillary tip.

The fourth method of burning the cholesterol (number III of Table I) consisted in dropping liquid cholesterol onto a copper oxide catalyst heated to about 500°. Air was admitted into the tube to keep the copper catalyst oxidized, and the water formed in the combustion was partially condensed in a water condenser, and the uncondensed residue completely frozen out in a dry ice–acetone trap. This trap was quite necessary as the condensing water was so warm due to the fact that the experiment was done in the summer that only 66% of the water was collected in the water condenser. This method of combustion produced about 75–80% of the theoretical yield.

(4) M. Dole, *J. Chem. Phys.*, **2**, 548 (1934).

Measurement of the Density.—The water was distilled from alkaline permanganate over hot copper oxide until all odor disappeared. It was then purified according to our previous method. The method of measuring the density was also the same as previously described except that the apparatus was rebuilt to accommodate smaller volumes of water, a smaller float was made and the motion of the float was observed with the aid of a cathetometer. The temperature measurements and corresponding values of γ are given in Table I where γ is the density of the water under investigation less the density of normal water at the same temperature, the difference expressed in parts per million.

TABLE I

DENSITY OF WATER OBTAINED IN THE COMBUSTION OF CHOLESTEROL

	Cholesterol + air Δt	γ
I	+0.007	
	+ .004	
	Av. + .005	+1.1
II	+ .005	
	+ .004	
	Av. + .004	+0.9
III	+ .005	
	+ .003	
	Av. + .004	+1.2
	Cholesterol + Airco oxygen Δt	γ
IV	(+0.019)	
	+ .008	
	+ .009	
	Av. + .008	+1.8
Corr. for oxy:	− .002	−0.5
Final result:	+ .006	+1.3

The method of measuring and the value for the correction to Airco oxygen to bring its atomic weight to that of atmospheric oxygen have already been published.[1] Oxygen from the same Airco tank was used in this experiment.

The density measurements for the combustion number III of Table I were made at 29.9° which accounts for the different relation between Δt and γ.

Interpretation of the Results

In interpreting the very slight difference between the density of the cholesterol–water and normal water calculated from the data of the first combustion, one of us[4] originally concluded that the deuterium content of cholesterol was normal, but at that time we were ignorant of the relatively large difference between the atomic weight of atmospheric oxygen and oxygen of water which has since been observed.[5] In order to correct for this effect 6.0 γ must be subtracted from 1.1 γ, the average of the four data from the three combustions, yielding −4.9 γ as the apparently correct value for the difference in density between

(5) M. Dole, *ibid.*, **4**, 268 (1936).

water made from the hydrogen in cholesterol and normal water. However, there is the possibility that the oxygen isotopes may have separated during the combustion of the cholesterol as one of us has already pointed out.[1] We have investigated this source of error by electrolyzing the cholesterol water along with normal water, combining the liberated oxygen with the same tank hydrogen in both cases and comparing the density of these waters. Two independent experiments were carried out, the second series being the more reliable since more measurements were made in determining the density. Much to our surprise we found that the oxygen in the cholesterol which originally came from the air was not as heavy as we had suspected so that instead of subtracting 6.0 γ from the value of 1.1 γ given in Table I we should subtract only 2.5 γ (the average of the results given in Table II, giving double weight to the more accurate value of the second series) which is the extent in p. p. m. to which the density of water made from the oxygen in the cholesterol water exceeds the density of water made from the oxygen in normal water, the hydrogen in the two waters being identical.

TABLE II

DENSITY DATA OBTAINED IN THE ANALYSIS OF THE OXYGEN CONTAINED IN THE CHOLESTEROL WATER

Type of water		Δt	γ
First series (at 23.4°)			
Aqueous oxygen after electrolysis combined with tank hydrogen		−0.045	−10.4
Cholesterol water oxygen after electrolysis combined with tank hydrogen		− .036	− 8.3
Difference		− .009	−2.1
Second series (at 29.9°)			
Aqueous oxygen after electrolysis combined with tank hydrogen	I	− .040	−12.0
		− .042	−12.6
		− .0426	−12.8
	II	− .040	−12.0
		− .039	−11.7
	Average	− .0407	−12.2
Cholesterol water oxygen after electrolysis combined with tank hydrogen		(− .027)	
		− .032	− 9.6
		− .0315	− 9.5
	Average	− .032	− 9.5
	Difference	− .009	− 2.7
	Average of both series		− 2.5

The result of this calculation is −1.4 γ which is the extent in p. p. m. to which the density of water made from the hydrogen in cholesterol is lighter than water made from normal hydrogen, the oxygen in the two waters being identical in isotopic composition. Considering all the corrections which must be made to the measured values in addition to the errors which are inherently present in the measurements themselves, we cannot consider this value of −1.4 γ as proving definitely that the isotopic abundance of deuterium in cholesterol is lower than normal. Indeed, when one considers the complicated nature of cholesterol, it is surprising that the hydrogen is not more abnormal.

We have also burned halibut liver oil residues in the same way that combustion III of Table I was carried out using Airco oxygen, however, instead of air. The water, after correcting for the Airco oxygen as in Table I, was 3.6 γ heavier than normal. The volume of the water obtained, however, was so small because of the limited amount of the oil at our disposal that we were unable to analyze the oxygen in the water with respect to its isotopic composition. Assuming that the oxygen behaved in this combustion similarly to the cholesterol combustion, and subtracting 2.5 γ for the excess density because of the different oxygen present, we obtain only +1.1 γ for the excess density of water made from the hydrogen in the halibut liver oil residues over normal water, the oxygen in each water being of identical isotopic composition. Once again we find the hydrogen to be surprisingly normal in its isotopic composition.

Since we have now analyzed benzene, a plant product, cholesterol, an animal product, and halibut liver oil residues, a fish product, without finding the hydrogen isotopic composition to be definitely abnormal, we are forced to the conclusion that the role of heavy hydrogen in nature cannot be very significant. There is definitely no indication here of the accumulation or rejection of deuterium in the building of these compounds utilizing hydrogen from the ultimate source of water.

In the combustion of the benzene one of us found no separation of the oxygen isotopes,[1] but the benzene burned in a blue flame at a much higher temperature than the temperature at which the cholesterol burned. The cholesterol burned at a catalyst about 500–600°, but even this temperature is so high that one would not expect an appreciable fractionation of the oxygen isotopes, at least from equilibrium theory, since the fractionation factor for the isotopic equilibrium reaction

$$CO_2^{16} + 2H_2O^{18}(g) \rightleftharpoons CO_2^{18} + 2H_2O^{16}(g)$$

falls from 1.054 at 25° to 1.014 at 323°.[6] At 600° it would probably be even smaller. Perhaps an isotopic exchange takes place at lower temperatures before the carbon dioxide and steam are separated, but if such an exchange takes place, it would have to be catalyzed by products formed in the combustion since a simple mixing of carbon dioxide and steam for a short period of time produces no isotopic exchange.[1] The magnitude of the effect, about 4 p. p. m. in terms of water density, is small enough to be accounted for by such an exchange and it is in the direction expected.

Acknowledgments.—We are greatly indebted to Dr. D. L. Tabern of the Abbott Laboratories for supplying the halibut liver oil residues, to Mr. Stanley Cristol for carrying out the combustion of the oil, to Mr. J. L. Gabbard for the loan of the electrolysis cells, and to Mr. B. Z. Wiener for carrying out the second electrolysis mentioned in Table II.

(6) H. C. Urey and Lotti J. Greiff, THIS JOURNAL, 57, 321 (1935).

Summary

Cholesterol has been examined for its deuterium content, but after all the necessary corrections have been applied to the data it appears that the deuterium content of cholesterol is normal. The measurements indicate a slight deficiency of heavy hydrogen, but the magnitude of the deficiency is hardly greater than the experimental error. Halibut liver oil residues have also been analyzed isotopically, but again the hydrogen appears to be normal in its deuterium content.

We have found, however, that there is a marked fractionation of the oxygen isotopes on combustion of the cholesterol.

EVANSTON, ILL. RECEIVED SEPTEMBER 21, 1936

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, THE UNIVERSITY OF LIVERPOOL]

The Reactivity of Hydrogen Peroxide in Bromine–Bromide Solutions

BY ROBERT OWEN GRIFFITH AND ANDREW MCKEOWN

A recent paper by Livingston and Schoeld[1] on the photochemical behavior of H_2O_2–HBr–Br_2 mixtures in aqueous solution prompts us to communicate briefly the results of some experiments which we carried out in 1933–34 on this same system, especially as (a) our conclusions regarding the photochemical reaction are diametrically opposed to those of Livingston and Schoeld, and (b) our observations on the thermal reaction differ in some respects from those of Bray and Livingston.[2]

Dealing first with the thermal reaction, we find that H_2O_2–HBr–Br_2 systems at constant temperature and in the dark never attain "steady state" conditions as defined by Bray and Livingston, that is, the concentration of bromine [ΣBr_2], and correspondingly that of hydrobromic acid, never reach stationary values so long as any hydrogen peroxide remains undecomposed. According to our measurements, if no bromine is originally present in the system, [ΣBr_2] at first rapidly increases toward a pseudo-stationary value, but instead of remaining at this value it then slowly but progressively increases with diminishing concentration of hydrogen peroxide. If excess of bromine is originally present, [ΣBr_2] at first rapidly falls to a pseudo-stationary value, passes through a minimum, and then as before slowly increases. In either case, provided the sum [HBr] + $^1/_2$[ΣBr_2] has the same value and provided enough hydrogen peroxide be present initially, the concentration of bromine becomes after a sufficient lapse of time the same function of the hydrogen peroxide concentration, independent of the previous history of the system.

For our experiments we have employed, with identical results, hydrogen peroxide from the following sources: (1) Merck 30% Perhydrol free from preservatives, (2) a preparation from sodium peroxide following the method of Rice, Rieff and Kirkpatrick,[3] and (3) a sample obtained from an A. R. hydrogen peroxide by distillation under reduced pressure. Our measurements of bromine concentration were carried out (α) analytically by the method described by Bray and Livingston,[2] and (β) spectrophotometrically.

Table I gives the results of four typical experiments. It will be noted that, after the attainment of pseudo-stationary conditions, the con-

(1) Livingston and Schoeld, THIS JOURNAL, 58, 1244 (1936).
(2) Bray and Livingston, *ibid.*, 45, 1264 (1923).
(3) Rice, Rieff and Kirkpatrick, *ibid.*, 48, 3019 (1926).