"O" brand, originally containing 29.4% SiO_2 and 9.3% Na_2O, ratio 3.15, a similar addition was made to the 5% potassium oleate here. This made no influence upon the result, as shown by the three triangles which fall upon the original curve in Fig. 3. In Woo's experiments the oleate was not only solubilizing Yellow AB, but to a still greater extent was suspending and protecting colloidal particles of Yellow AB, and we have since shown that solubilizing and protective or suspending actions are wholly separate processes. The present experiments are designed to exclude the protective factor. The foam in 1 atmosphere of propylene with and without silicate was not very different; possibly the one containing silicate was creamier after standing a short time. The effect of the silicate upon the solubility in water was not measured for lack of time. However, in such dilution it would have been negligible.

The soap is exerting its expected action, namely, definite solubilization, overriding other factors. Electrolytes as such would be expected slightly to reduce the solubility by salting out. An additional small influence in the same direction would be caused by hydration of the soap. This is illustrated by the slight but distinct negative sorption of propylene on cotton thread. One gram of cotton thread added to 50 g. of water (the two square points on Fig. 3, corresponding to the crosses for pure water) caused this small decrease in the apparent solubility of the propylene, showing that the cotton preferentially sorbed the water.

In the previous work with the less volatile hexane and methylcyclopentane the amount taken up from the saturated vapor was approximately one mol of hydrocarbon to one mol of soap. It is quite possible that the solubility of the present hydrocarbons would be of comparable magnitude if measured at similarly high relative vapor pressures. It is thought that if the excess solubility above that in water alone were plotted against relative vapor pressure of hydrocarbon, the general curve would be sigmoid in character. The first part would, as here, be concave downward, and pass through a point of inflection before rising to much higher values. The isobutane and propylene are here studied only at the left-hand part of the curve; the butadiene extends further and indicates a further stage of the S-shaped curve, whereas the previous less volatile hydrocarbons were studied on the right-hand portion near saturation. According to this working hypothesis, the soap itself is strongly modified by the larger amounts of solubilized hydrocarbon.

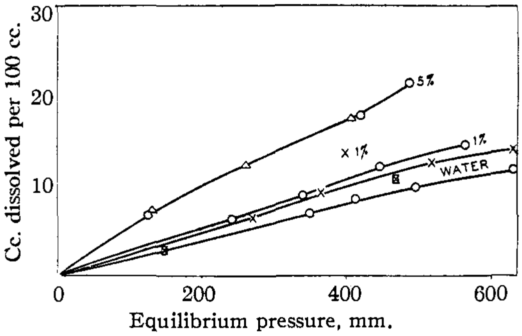

Fig. 3.

Comparing the amount of extra hydrocarbon dissolving in the presence of soap with that dissolving in an equal amount of olein, the only comparable material for which we have data,[3] we find that the potassium oleate dissolves only about one quarter as much as would an equal weight of olein. In general, solubilization appears to involve several factors other than mere solution in hydrocarbon groups, if any.

The most extensive series of studies of the solubility of gases as affected by other dissolved substances are those of Findlay and collaborators, 1910–1915. They found that proteins sometimes increased the solubility of gases such as carbon dioxide, and that polysaccharides did not. They likewise observed departures from Henry's law similar to those found here.

Summing up, the solubility of the hydrocarbons, propylene, isobutane and butadiene, is enhanced by presence of potassium oleate acting as solubilizer. This is one, but only one, of the important factors in detergent action.

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[3] Berthelot, *Ann. chim. phys.*, **43**, 276 (1855).

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Heavy Oxygen (O^{18}) as a Tracer in the Study of Photosynthesis

BY SAMUEL RUBEN, MERLE RANDALL, MARTIN KAMEN AND JAMES LOGAN HYDE

It is generally agreed that the net reaction for green plant photosynthesis can be represented by the equation

$$CO_2 + H_2O + h\nu \xrightarrow{\text{Chlorophyll}} O_2 + (1/n)(C{\cdot}H_2O)n \qquad (1)$$

and also that very little is known about the actual mechanism. It would be of considerable interest to know how and from what substance the oxygen is produced. Using O^{18} as a tracer we have found that the oxygen evolved in photosynthesis comes from water rather than from the carbon dioxide.

The heavy oxygen water used in these experiments was prepared by fractional distillation[1] and was distilled from alkaline permanganate before use. The isotopic oxygen content was determined by the method of Cohn and Urey[2] using carbon dioxide and a mass spectrometer. Heavy oxygen carbonate was prepared by allowing a solution of potassium acid carbonate ($KHCO_3$) in heavy oxygen water to come to approximate isotopic equilibrium, adding a nearly equivalent quantity of potassium hydroxide and distilling off the water, finally drying in an oven at 120°. Isotopic analysis of this carbonate or of the carbonate in a solution, was performed by rendering the solution sufficiently alkaline to prevent exchange[3] and precipitating calcium carbonate. The calcium carbonate after filtering, washing and drying at 120°, was calcined at red heat in an evacuated platinum bulb connected to the gas handling system of the mass spectrometer, and the evolved carbon dioxide analyzed for heavy oxygen.

Young active *Chlorella* cells were suspended in heavy oxygen water (0.85% O^{18}) containing ordinary potassium bicarbonate and carbonate. Under these conditions the oxygen exchange between the water and bicarbonate ion is slow and readily measurable.[3] The isotopic ratio in the evolved oxygen was measured with a mass spectrometer. In other experiments the algae were allowed to carry on photosynthesis in ordinary water and heavy oxygen potassium bicarbonate and carbonate. The results of these experiments are summarized in Table I.

TABLE I

ISOTOPIC RATIO IN OXYGEN EVOLVED IN PHOTOSYNTHESIS BY *Chlorella*[a]

Expt.	Substrate	Time between dissolving $KHCO_3 + K_2CO_3$ and start of O_2 collection, minutes	Time at end of O_2 collection, minutes	Percent. O^{18} in H_2O	Percent. O^{18} in $HCO_3^- + CO_3^=$	Percent. O^{18} in O_2
1	0.09 *M*	0		0.85	0.20	..
	$KHCO_3$	45	110	.85	.41[b]	0.84
	+0.09 *M*	110	225	.85	.55[b]	.85
	K_2CO_3	225	350	.85	.61	.86
2	0.14 *M*	0		.20		..
	$KHCO_3$	40	110	.20	.50	.20
	+0.06 *M*	110	185	.20	.40	.20
	K_2CO_3					
3	0.06 *M*	0		.20	.68	..
	$KHCO_3$	10	50	.20		.21
	+0.14 *M*	50	165	.20	.57	.20
	K_2CO_3					

[a] The volume of evolved oxygen was large compared to the amount of atmospheric oxygen present at the beginning of the experiment. [b] These are calculated values.

It is apparent that the O^{18}/O^{16} ratio of the evolved oxygen is identical with that of the water. Since the oxygen in OH, COOH, O—O, C=O, etc., groups exchanges but very slowly[4] with water at room temperature and moderate *p*H, it seems reasonable to conclude that the oxygen originates solely from the water. While this conclusion makes it possible to reject many of the suggestions proposed in the past[5] it does not enable a choice to be made between the several more recent hypotheses. However it is of interest to note that van Niel[6] has specifically suggested that the oxygen may arise by a dehydrogenation of water.

We have also attempted to ascertain whether the evolution of oxygen was a reversible reaction. The algae were suspended in ordinary potassium bicarbonate and carbonate solution and photosynthesis allowed to proceed in the presence of heavy oxygen. In other experiments the algae evolved heavy oxygen in the presence of light oxygen. The results are shown in Table II.

TABLE II

ISOTROPIC RATIO IN OXYGEN EVOLVED IN PHOTOSYNTHESIS BY *Chlorella* IN PRESENCE OF OXYGEN

O_2 present in gas space at beginning, ml.	O_2 produced in photosynthesis by 200 mm.³ algae, ml.	Per cent. O^{18} at end of experiment Obsd.	Per cent. O^{18} at end of experiment Calcd. for no exchange
2.29 (O^{18} = 0.20%)	1.55 (O^{18} = 0.85%)	0.43	0.46
3.64 (O^{18} = .20%)	1.18 (O^{18} = .85%)	.34	.36
1.44 (O^{18} = .85%)	0.73 (O^{18} = .20%)	.59	.62
4.81 (O^{18} = .85%)	1.22 (O^{18} = .20%)	.69	.71

There is no indication of exchange reactions involving oxygen. The experimental errors are such that an exchange involving less than $5 \cdot 10^{-8}$ mol of oxygen with each cu. mm. of algae would not be detected.

Similar experiments with *Chlorella* and yeast were performed in order to determine whether the oxidation (respiration) reactions utilizing oxygen

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(1) Randall and Webb, *Ind. Eng. Chem.*, **31**, 227 (1939).

(2) Cohn and Urey, THIS JOURNAL, **60**, 679 (1938).

(3) Mills and Urey, *ibid.*, **62**, 1019 (1940).

(4) For a review of oxygen exchange reactions see Reitz, *Z. Elektrochem.*, **45**, 100 (1939).

(5) For an excellent review of this subject up to 1926 see H. A. Spoehr "Photosynthesis," Chem. Cat. Co., New York, N. Y., 1926.

(6) Van Niel, *Cold Spring Harbor Symposia on Quant. Biol.*, **3**, 138 (1935).

were reversible. The results are summarized in Table III.

TABLE III

ISOTOPIC OXYGEN RATIO IN RESPIRATION WITH *Chlorella* AND YEAST

Respiring system	O_2 (O^{18} = 0.85%) present at start of expt., ml.	O_2 utilized in respiration, ml.	Per cent. O^{18} in O_2 at end of experiment Obsd.	For no exchange
Chlorella cells in dark for 90 minutes	0.82	0.11	0.85	0.85
Yeast cells for 60 minutes	4.5	2.4	.84	.85

Here also there is no indication for an exchange reaction involving molecular oxygen.

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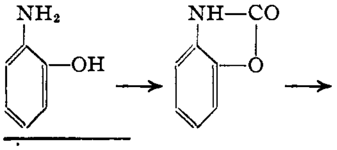
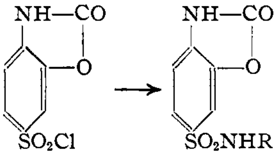
A Stable Derivative of 1-Amino-2-hydroxybenzene-4-sulfonamides

BY JOHN V. SCUDI AND R. P. BUHS

The urinary elimination of oxidation products of the sulfonamide drugs has been definitely established.[1,2] Certain oxidation products have been shown to be of importance in relation to the incidence of acetylsulfapyridine urolithiasis in experimental animals.[3] Since such oxidation products are of interest in connection with theories of the mode of action of these drugs, much attention has been devoted to 1-hydroxylaminobenzene-4-sulfonamide.[4] Another oxidation product which may have physiological significance is the 1-amino-2-hydroxybenzene-4-sulfonamide. The stable benzoxazolone analog of this compound has been prepared as follows





The method appears to be general for the preparation of benzoxazolone-5-sulfonamides.

The benzoxazolone-sulfonamide was administered in aqueous suspension by stomach tube to a series of mice infected with cultures of hemolytic streptococci. Subsequent examination of the urine showed that the oxazolone ring had not been cleaved in passage through the animal. The compound was not effective in protecting the infected mice.

Experimental

Benzoxazolone.—*o*-Hydroxyaniline in dry pyridine was treated with phosgene according to the method of von Meyer.[5] The benzoxazolone, crystallized from acidulated water, melted at 138–139°, cor. It was obtained in 50% yields. In our hands this method failed to give the yields previously reported.

Benzoxazolone-5-sulfonyl Chloride.—One-half mole of the benzoxazolone was added slowly with stirring to 2.5 mole of chlorosulfonic acid at 10–15°. The reaction mixture was stirred for fifteen minutes after all the benzoxazolone was added. The temperature was then raised to 60° and maintained at this temperature for two hours. The reaction mixture was poured onto ice. The product was removed by filtration and dissolved in ether. The ethereal solution was washed free of acid and dried with anhydrous sodium sulfate. The product was precipitated in good crystalline form by the addition of petroleum ether, m. p. 182–183° cor. The yields were 50 to 60%.

Anal. Calcd. for $C_7H_4O_4NSCl$: C, 35.96; H, 1.72. Found: C, 35.79, 35.66; H, 2.07, 2.08.

Benzoxazolone-5-sulfonamide.—9.0 g. of benzoxazolone-5-sulfonylchloride was added to 100 cc. of cold concentrated ammonium hydroxide, and the mixture was warmed to solution. On cooling the ammonium salt of the benzoxazolone-5-sulfonamide separated. This was removed by filtration and converted to the free sulfonamide by crystallization from 300 cc. of acidulated water. The product, melting at 269–270° (decompn.), was obtained in 60% yields.

Anal. Calcd. for $C_7H_6O_4N_2S$: C, 39.23; H, 2.82; N, 13.08. Found: C, 39.33; H, 3.02; N, 13.01, 12.91.

Benzoxazolone-5-sulfonanilide.—150 mg. of the benzoxazolone-5-sulfonyl chloride and 1 cc. of freshly distilled aniline in 3 cc. of dry dioxane were refluxed for fifteen minutes. The reaction mixture was cooled, acidified with hydrochloric acid and diluted to 10 cc. with water. The oil which separated crystallized on standing. The product, obtained in approximately 50% yields, melted at 215–216° cor. after crystallization from water.

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(1) J. V. Scudi, *Science*, **91**, 486 (1940).

(2) W. V. Thorpe and R. T. Williams, *Nature*, **146**, 686 (1940).

(3) J. V. Scudi and H. J. Robinson, *Am. J. Med. Sci.*, in press.

(4) For bibliography see A. C. Bratton, H. J. White and E. K. Marshall, Jr., *Proc. Soc. Exptl. Biol. Med.*, **42**, 847 (1939).

(5) E. von Meyer, *J. prakt. Chem.*, **92**, 256 (1915).