same direction. Thus, in configurationally-related carboxylic acids or esters, identical substitution on the carboxyl groups should produce a shift of rotation in the same direction. Many of these rules were found very useful and results obtained by these methods were substantiated subsequently by direct chemical methods. On the other hand, there have accumulated in our laboratory a number of observations in which these rules, if used as the basis for establishing configurations, would have led to erroneous conclusions.

In order to show the possible error to which a "Rule of Shift" may lead, we present in the table the rotations of five acids and their esters. All the acids are configurationally related.

TABLE I

MAXIMUM MOLECULAR ROTATIONS OF CONFIGURATIONALLY RELATED ACIDS AND THEIR ETHYL AND (*p*-) NITROPHENYL ESTERS ($[M]_D^{25}$ (HOMOGENEOUS))

| | | Free acid | Ethyl ester | *p*-Nitrophenyl ester |
|---|---|---|---|---|
| | Acetic Acid Series | | | |
| (1) | $C_2H_5$—CH($CH_3$)--COOH | −18.0 | −22.9 | −52.5 |
| (2) | $C_4H_9$—CH($CH_3$)--COOH | −24.3 | −30.7 | −65.7 |
| | Propionic Acid Series | | | |
| (3) | $C_2H_5$—CH($CH_3$)--$CH_2$—COOH | −10.4 | −11.5 | −20.0 |
| (4) | $C_3H_7$—CH($CH_3$)--$CH_2$—COOH | + 3.6 | + 0.7 | + 5.0 |
| (5) | $C_5H_{11}$—CH($CH_3$)—$CH_2$—COOH | + 8.1 | + 4.2 | +11.2 |

In the first three acids the substitution of the ethyl group by a *p*-nitrophenyl leads to a shift of the rotation to the left, and in the fourth and fifth, to the right.

In the first two acids, the contribution of the carboxyl is levorotatory and the increase of the rotation due to substitution of the ethyl group by a *p*-nitrophenyl is as expected. However, in the third, fourth and fifth, the contributions of the carboxyl groups are dextrorotatory and yet the fourth and fifth behave differently from the third on an identical substitution.

Thus, another case is presented in which the "Rule of Shift" fails.

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G. M. MEYER

RECEIVED DECEMBER 11, 1933

## THE ALCOHOLIC FERMENTATION OF *d*-GLUCOSE IN DEUTERIUM WATER

*Sir:*

The production of 100% deuterium water ($D_2O$) in this Laboratory has made it possible to study a natural process, the alcoholic fermentation of *d*-glucose by yeast, in this medium. Measurements of the rate of fermentation were made by determining the amount of carbon dioxide formed during the enzymatic reaction in a fermentation saccharimeter after various intervals of time. One half cc. of 100% deuterium water ($d_4^{20}$ 1.105) containing 40 mg. of *d*-glucose and 18 mg. of yeast was employed in the first experiment. The changes in height of the glycerol column in the calibrated tube of the apparatus indicated the volumes ($V_1$) of carbon dioxide. A control experiment was run simultaneously with ordinary distilled water; in this case $V$ indicates the volume of carbon dioxide evolved in the process. In a second experiment 0.4 cc. of $D_2O$ containing 32 mg. of *d*-glucose and 18 mg. of yeast was used. This experiment, too, was at the same time duplicated with ordinary distilled water. No special care was exercised to keep the temperature constant; it varied between 21 and 25.5°, affecting the corresponding experiments with heavy and ordinary water equally. The results obtained are shown in Table I.

TABLE I

| Expt. | Time, hours | $V$, vol. of $CO_2$ in cc. with $H_2O$ | $V_1$, vol. of $CO_2$ in cc. with $D_2O$ | $V/V_1$ |
|---|---|---|---|---|
| 1 | .. | .. | .. | ... |
| | 2 | 0.80 | 0.10 | 8.0 |
| | 12 | 4.40 | .45 | 9.8 |
| | 15 | 5.50 | .60 | 9.1 |
| | 18 | 6.50 | .73 | 9.0 |
| | 21 | 7.25 | .85 | 8.6 |
| | 24 | 7.65 | 1.00 | 7.6 |
| | 45 | .. | 1.70 | ... |
| | 85 | .. | 2.63 | ... |
| | 205 | .. | 8.50 | ... |
| 2 | .. | .. | .. | ... |
| | 2 | 0.90 | 0.10 | 9.0 |
| | 8 | 3.65 | .40 | 9.1 |
| | 12 | 5.00 | .50 | 10.0 |
| | 24 | 6.30 | .70 | 9.0 |
| | 48 | .. | 1.20 | ... |
| | 73 | .. | 1.80 | ... |
| | 96 | .. | 2.35 | ... |

The values of the factor $V/V_1$ clearly indicate that the alcoholic fermentation of glucose in 100% deuterium water is about 9 times slower than in ordinary distilled water. In another set of experiments with 60% heavy water the values for $V/V_1$ were found to be about 1.6. When the fermenting sugar solution with $D_2O$, in the second experiment, became four days old, it was diluted with 0.5 cc. of ordinary distilled water. Subsequently, no significant increase in the daily rate of carbon dioxide formation was experienced.

This unexpected result seems to indicate that the retarding effect of the heavy water on the alcoholic fermentation might be due to a decreased activity of the zymase complex occasioned by an irreversible, partially destructive action of the heavy water on the enzymes. Further experiments with the object of studying the effect of $D_2O$ on extracellular enzymatic reactions are in progress in this Laboratory.

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RECEIVED DECEMBER 14, 1933

## SOME PHARMACOLOGICAL EXPERIMENTS WITH DEUTERIUM

*Sir:*

In consideration of the brief reports concerning physiological effects of deuterium, published by Lewis [THIS JOURNAL, **55,** 3502(1933)], who found it affected the germination of tobacco seeds, and Barnes [*ibid.*, **55,** 4332 (1933)], who noted an inhibitory effect of heavy water on spirogyra, we deemed it desirable to perform a series of pharmacological experiments with heavy water. Agreeing with Barnes that the most valuable practical information would be gleaned from experiments with weak solutions of deuterium, we employed a heavy water containing one part of deuterium to two thousand parts of protium, purchased from the Ohio Chemical and Manufacturing Company, which was of practically the same specific gravity (1.000060).

I. Germination of *Lupinus* seeds, soaked in this solution overnight, was studied by phytopharmacological methods described elsewhere [*Science*, **71,** 302 (1930); *J. Gen. Physiol.*, **4,** 573 (1922)]. It was found that the germination of seedlings in 1:2000 deuterium solution was slightly inhibited as compared with their growth in ordinary, glass-distilled water.

II. Growth of *Lupinus* seedlings in Shive's physiological saline [*Physiological Researches*, **1,** 327 (1915)], prepared with and without deuterium solution, respectively, revealed that the latter solution produced slight inhibition. Comparison of seedlings grown in deuterium saline with the controls showed that this difference, 5 to 10%, easily explainable by variations in the hydrogen-ion concentration, was insignificant.

III. Fermentation experiments with bakers' yeast and 4% cane sugar dissolved in ordinary and deuterium water, showed no difference in their activity.

IV. Mice injected with physiological and sodium chloride solutions, prepared with and without deuterium, respectively, exhibited no toxic action.

V. Goldfish, *Carassius Auratus*, behaved exactly alike when placed in ordinary and deuterium water, respectively.

VI. Surviving pieces of cats' intestines, suspended in oxygenated Locke's solution prepared with ordinary, distilled water and in deuterium water, showed no difference in normal rhythmic contractions of smooth muscle or response to pilocarpine and mono-brom saligenin [*Proc. Soc. Exptl. Biol. Med.*, **30,** 378 (1932)].

VII. Similar experiments with vasa deferentia of white rats showed no difference in contractions of such organs to epinephrine and corpus luteum.

VIII. Similar experiments with surviving segments of guinea pig uteri revealed no difference in the action of normal and deuterium Locke's solution.

IX. Intravenous injection into cats under ether of physiological saline itself, prepared with and without deuterium, revealed no difference in effect on blood pressure and respiration.

X. Identical results were obtained by assay of digitalis tincture on cats with ordinary and deuterium saline, respectively.

Our experiments indicate that when deuterium water is employed in concentrations of 1:2000, or less, no striking physiological or pharmacological effects are noted, except possibly a slight inhibitory influence on germination of some seeds. This does not preclude the possibility of more profound biological changes being produced by pure or very concentrated heavy water. Furthermore, since the plant-physiological preparations employed by us were not the same as those described by Lewis and Barnes, no factual contradiction between our experiments and theirs need be inferred. However, if a speculative *a priori* conjecture may be permitted, we doubt that weak concentrations of deuterium would produce remarkable biological effects, for we are probably all dealing with isotopes of many elements in our physiological, pharmacological and biochemical work.

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