THE EXCHANGE REACTIONS BETWEEN HEAVY WATER AND AMINO ACIDS AND CARBOHYDRATES.

By Eijiro OGAWA.

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I. The Mechanism of the Exchange Reactions. The electronic structures of the H-substituted compounds and D-substituted compounds are the same. The two compounds only differ in the zero point energy content. It is doubtful that the exchange of H and D in one molecule occurs strictly step by step. The author considered following two assumptions.

(a) It is assumed that the exchange occurs perfectly at random. Then the following reactions must be considered in the case of glucose for instance.

$$C_6H_{12}O_6+HDO = C_6H_{11}DO_6+H_2O\ ;$$
$$C_6H_{12}O_6+2HDO = C_6H_{10}D_2O_6+2H_2O\ ;$$
$$C_6H_{12}O_6+3HDO = C_6H_9D_3O_6+3H_2O\ ;$$
$$C_6H_{12}O_6+4HDO = C_6H_8D_4O_6+4H_2O\ ;$$
$$C_6H_{12}O_6+5HDO = C_6H_7D_5O_6+5H_2O.$$

The concentrations of $C_6H_{11}DO_6$, $C_6H_{10}D_2O_6$, $C_6H_9D_3O_6$, $C_6H_8D_4O_6$, and $C_6H_7D_5O_6$ can not be obtained from the experiments. But the above hypothesis equals to the assumption that the exchangeable radicals can react quite independently. The equilibrium constant of the reaction $OH+HDO=OD+H_2O$ is

$$K_a = \frac{[OD][H_2O]}{[OH][HDO]}\ .$$

Generally on the molecule AH_n which contains n different exchangeable radicals, it becomes as follows:

$$AH_n/n+HDO = AD_n/n+H_2O\ ;$$

$$K_m = \frac{[AD_n/n][H_2O]}{[AH_n/n][HDO]}\ .$$

K_m is called the mean equilibrium constant. If the equilibrium constants of each radicals K_1, K_2, ..., K_n are known, then

$$K_m = (K_1+K_2+\ \ldots\ldots\ +K_n)/n\ .$$

(b) If it is assumed that the exchange reactions proceed strictly step by step as in the ordinary chemical reactions, only one of H-atoms of the exchangeable radicals in one molecule is substituted at first. For instance in the case of glucose the equilibrium constant of the reaction $C_6H_{12}O_6 + HDO = C_6H_{11}DO_6 + H_2O$ is

$$K_b = \frac{[C_6H_{11}DO_6][H_2O]}{[C_6H_{12}O_6][HDO]}\ ; \text{ and } K_b \neq K_a\ .$$

For five OH–radicals :

$$5C_6H_{12}O_6 + 5HDO = 5C_6H_{11}DO_6 + 5H_2O\ ;$$

$$K = \frac{[C_6H_{11}DO_6]^5[H_2O]^5}{[C_6H_{12}O_6]^5[HDO]^5}\ ; \text{ and } K = K_b{}^5\ .$$

If one molecule contains n different exchangeable radicals, then

$$nAH_n + nHDO = nAH_{n-1}D + nH_2O\ ;$$

$$K_n = \frac{[AH_{n-1}D]^n[H_2O]^n}{[AH_n]^n[HDO]^n}\ .$$

If Kb_1, Kb_2, ... Kb_n are the equilibrium constants of each radical, then

$$\sqrt[n]{Kb_1\, Kb_2\ \ldots\ Kb_n} = Km_b\ .$$

Km_b is called mean equilibrium constant. The concentration of D-substituted compounds in Km_b is expressed approximately by 1/n of the decrease in concentration of HDO, when the differences between Kb_1, Kb_2, ... Kb_n are not large. The author considered that the mechanism of the exchange reaction is expressed by the assumption (a) approximately and calculated the mean equilibrium constant K_m in the following experiments. Since the concentration of D_2O can not be obtained, it is assumed that all the D in water are in the state of HDO.

II. The Velocity of the Exchange Reactions. Urea was dissolved at 16°C. in water containing hydrogen isotope and after 5 minutes precipitated perfectly as $CO(NH_2)_2 \cdot 2Hg(NO_3)_2$ with the calculated quantity of $Hg(NO_3)_2 \cdot H_2O$. The results were compared with the value at 22°C. at equilibrium in the previously published papers (Table 1).

Table 1.

Temperature	Concentration (molarity)	Concentration of HDO	Decrease of concentration of HDO
22°C.	2.000	0.463	0.047
16°C.	2.000	0.458	0.030

Namely the exchange has already reached at 60% of the equilibrium value.

III. The Influence of Catalyser on the Exchange Reactions. In the previously published papers it was reported that the D-content of nitric acid isolated when $CO(NH_2)_2 \cdot 2Hg(NO_3)_2$ slowly transforms into $CO(NH)_2 \cdot 2HgNO_3$ is considerably high. It is probably due to the catalytic action of Hg-salt in solid phase, and DNO_3, which has lower energy of formation, is first formed.

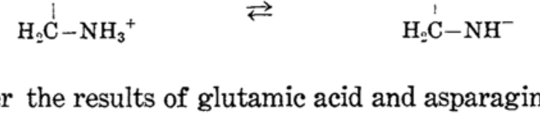
In this paper, in the exchange reaction of glucose the influence of platinum black was examined. The results are shown in Table 2.

Table 2.

Temperature	Pt-black	Conc. of glucose (molarity)	Conc. of HDO (mol)	Decrease in conc. of HDO (mol)	Conc. of H_2O (mol)	K_m
100°C.	0.23 g.	1.110	0.490	0.035	55.054	0.78
100°C.	0	1.459	0.396	0.036	55.149	0.77

K_m values are perfectly coincident, and this indicates that the equilibrium value obtained in the absence of catalyser is the true equilibrium constant.

IV. The Exchange Reactions of Amino Acids. In the previously published paper, in the molecule of glycocoll, it was assumed that COOH and NH_2 in α-position forms internal salt and the following two forms can be in equilibrium:

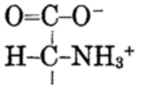
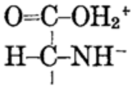


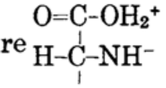
$$\begin{array}{c} O{=}C{-}O^- \\ | \\ H_2C{-}NH_3^+ \end{array} \rightleftarrows \begin{array}{c} O{=}C{-}OH_2^+ \\ | \\ H_2C{-}NH^- \end{array}$$

In this paper the results of glutamic acid and asparagine were examined (Table 3).

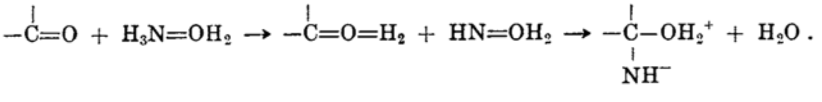
Table 3.

Substance	Tem. (°C.)	Conc. (molarity)	Conc. of HDO (mol)	Decrease in conc. of HDO	Conc. of H_2O	K_m
Glutamic acid (Kahlbaum)	100	0.647	0.490	0.014	55.033	0.64
Asparagine (Kahlbaum)	100	1.560	0.490	0.040	55.059	0.64

The constitution of glutamic acid and asparagine are $HOOC\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH$ and $H_2NOC\cdot CH_2\cdot CH(NH_2)\cdot COOH$ respectively. The exchangeabilities of $=NH$ and $-COOH$ were taken as 0.50 and 0.41, respectively, and then the calculated value of K_m is 0.45 for glutamic acid and 0.48 for asparagine. If the constitution  $\overset{O=C-O^-}{H-\underset{|}{\overset{|}{C}}-NH_3^+}$ is assumed, then the calculated values become 0.48 for glutamic acid and 0.50 for asparagine using 0.50 for $\equiv NH.^+$ If the constitution  $\overset{O=C-OH_2^+}{H-\underset{|}{\overset{|}{C}}-NH^-}$ is assumed, and the value 0.80 is taken for the exchangeability of $=OH^+$ from hydroxylamine hydrochloride, then the calculated values become 0.63 for glutamic acid and 0.62 for asparagine. Both values almost coincide with experimental values.

The electronic structure of N-atom and O- atom are$(1s)^2$ $(2s)^2$ $(2p)^3$ and $(1s)^2$ $(2s)^2$ $(2p)^4$, respectively. When the above-mentioned structure  $\overset{O=C-OH_2^+}{H-\underset{|}{\overset{|}{C}}-NH^-}$ is accepted, it follows that two of electrons of 2p level in oxygen atom become valence electrons before two of electrons of 2s level in nitrogen atom do.

The reactions of aldehyde and ketone furnish several illustrations of reactions of this type. For example, in the case of the reaction of aldehyde or ketone with ammonia in water, it follows



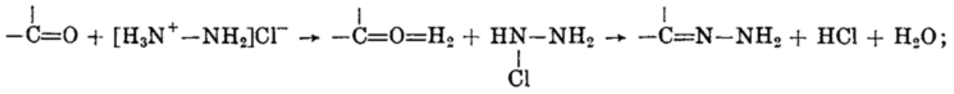
$$-\overset{|}{C}=O + H_3N=OH_2 \rightarrow -\overset{|}{C}=O=H_2 + HN=OH_2 \rightarrow -\underset{\underset{NH^-}{|}}{\overset{|}{C}}-OH_2^+ + H_2O\,.$$

(The structure of ammonioum hydroxide was considered as $H_3N=OH_2$ owing to its low dissociation constant, instead of $[NH_4]OH$.)

The reaction between aldehyde or ketone and hydroxylamine hydrochloride:

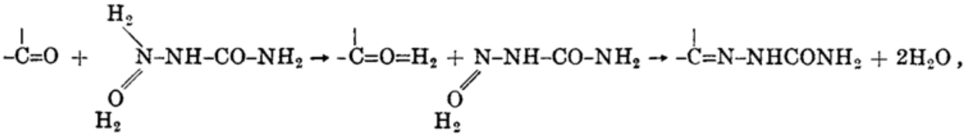
$$-\overset{|}{C}=O + [H_3N=OH]Cl \rightarrow -\overset{|}{C}=O=H_2 + [HN=OH]Cl \rightarrow -\overset{|}{C}=NOH + HCl + H_2O\,;$$

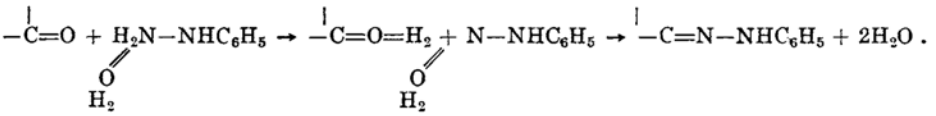
and the reaction between aldehyde or ketone and hydrazine hydrochloride:



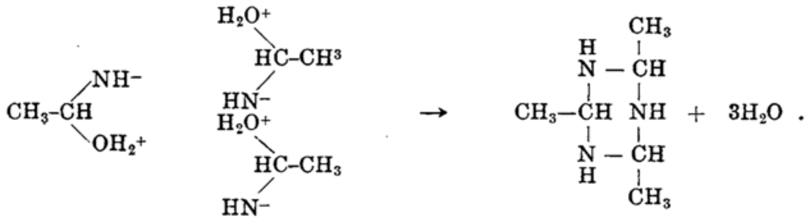
$$-\overset{|}{C}{=}O + [H_3N^+{-}NH_2]Cl^- \rightarrow -\overset{|}{C}{=}O{=}H_2 + \underset{\overset{|}{Cl}}{HN}{-}NH_2 \rightarrow -\overset{|}{C}{=}N{-}NH_2 + HCl + H_2O;$$

and the reaction between aldehyde or ketone and semicarbazide or phenylhydrazine in water:

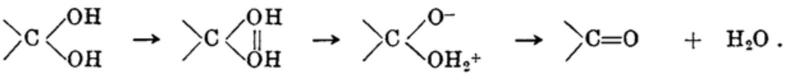


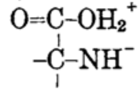


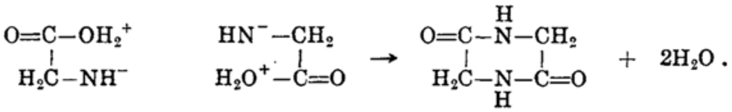
The compound of the constitution of $=OH_2$ easily loses water, so $H_3N{=}OH_2$ decomposes into NH_3 and H_2O and aldehyde ammonia easily polymerizes separating water:



Generally in these examples the oxygen atom takes oxonium structure at first and then separates out as water. This course is probably the actual mechanism of dehydration. For example from two hydroxyl groups one molecule of water separate out in the following manner:

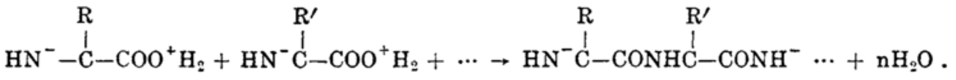


Then the dehydration of amino-acids can be considered easily, assuming the structure of  $\begin{array}{c} O{=}C{-}OH_2^+ \\ -\overset{|}{\underset{|}{C}}{-}NH^- \end{array}$. From two molecules of glycocoll diketopiperazine is obtained:



$$\begin{array}{l} O{=}C{-}OH_2^+ \\ \;\;| \\ H_2C{-}NH^- \end{array} \qquad \begin{array}{r} HN^-{-}CH_2 \\ | \\ H_2O^+{-}C{=}O \end{array} \rightarrow \begin{array}{l} O{=}C{-}\overset{H}{N}{-}CH_2 \\ \;\;|\qquad\;\; | \\ H_2C{-}\underset{H}{N}{-}C{=}O \end{array} + 2H_2O\,.$$

Or from several molecules of amino-acids polypeptide is obtained:



$$\mathrm{HN^-{-}\overset{R}{\overset{|}{C}}{-}COO^+H_2 + HN^-\overset{R'}{\overset{|}{C}}{-}COO^+H_2 + \cdots \rightarrow HN^-\overset{R}{\overset{|}{C}}{-}CONH\overset{R'}{\overset{|}{C}}{-}CONH^- \cdots + nH_2O}\,.$$

V. The Exchange Reactions of the Carbohydrates. The exchangeabilities of fructose, glucose, galactose, maltose, lactose, starch, and inulin were measured. Glucose, galactose, maltose, lactose, and inulin used were the manufactures of Kahlbaum. Starch was a so-called soluble starch. Glucose and galactose were nearly anhydrous. Maltose, lactose, starch, and inulin lost water of 5.0, 1.5, 20.3, and 10.5% of their weights, respectively, when heated at 110°C. for 15 minutes. Glycerin used was 84.73%. D-content of these waters was calculated as 0.020 mol HDO and assumed that this water is miscible completely when solved.

In the case of glycerine the mean value of K_m at 100°C. was 0.46 and at 50°C. 0.64, which correspond to a 40% increase compared with 0.46. The value of fructose was too large compared with other K_m-values. This is probable due to its change of constitution in solution.

Generally the exchangeabilities depend upon polarities, kind and valency states of elements to which exchangeable radicals attach, or molecular structures. In these experiments the constitution of compounds are similar and the exchangeable radical is OH only, so the exchangeability of each OH can be regarded as constant. It becomes more reliable to apply the results of exchangeabilities to the determination of the constitution of these compounds.

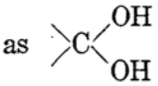
K_m-values of glucose and galactose at 100°C. were 0.73 and 0.67, respectively, assuming that hexose has 5 OH; at 50°C. 0.83 and 0.65, respectively. The values of glucose are somewhat higher, but the values of 100°C. and 50°C. are very near in both cases. In the case of glycerin K_m-values at 100°C. and 50°C. indicate that the exchange reaction of hydroxyl radical is exothermic. K_m-value at 50°C. is about 40% larger than the value at 100°C. It is very natural to assume that the exchange reactions of hydroxyl radicals in glucose and galactose are also exothermic. If assumed that the value at 50°C. in glucose and galactose are about 40% larger than those at 100°C., there is no way but to consider the unclosed structure 100°C. If assumed that glucose and galactose have 7 OH at 100°C., K_m becomes 0.58 for glucose and 0.52 for galactose. In these calculations it was assumed that water attached to hexoses contains H_2O and HDO in the same molecular ratio in the solution. The author considers that aldehyde radical of aldose in water solution at 100°C. has the structure such as  in large amount and,

Table 4.

Substance	Temp. °C.	Conc.	Conc. of HDO	Decrease in conc. of HDO	Conc. of H_2O	K_m
Glycerin	100	6.582	0.355	0.053	55.207	0.49
	100	5.637	0.361	0.042	55.190	0.44
	50	6.146	0.357	0.062	55.214	0.63
	50	4.346	0.369	0.049	55.189	0.65
Fructose	100	1.110	0.490	0.048	55.067	1.08
	50	1.122	0.490	0.051	55.070	1.15
Glucose	100	1.110	0.396	0.028	55.141	0.76 0.60(7OH)
	100	1.110	0.498	0.031	55.032	0.66 0.55(7OH)
	100	1.459	0.396	0.036	55.149	0.76 0.60(7OH)
	50	2.776	0.396	0.067	55.180	0.83
Galactose	100	2.776	0.396	0.057	55.168	0.67 0.52(7OH)
	50	2.776	0.396	0.056	55.169	0.65
Maltose	100	1.354	0.387	0.035	55.157	0.50
	100	0.550	0.485	0.019	55.043	0.51
	100	0.936	0.390	0.025	55.134	0.50
	50	0.550	0.485	0.030	55.054	0.83
Lactose	100	1.357	0.393	0.044	55.160	0.64
	100	1.428	0.393	0.049	55.165	0.69
	50	0.717	0.394	0.031	55.146	0.82
Starch	100		0.361	0.028	55.176	
Inulin	100		0.377	0.032	55.164	

when aldose crystallises, it forms a ring structure containing one molecule of water as water of crystallization.

Maltose is composed from two molecules of glucose losing one molecule of water and lactose is composed from one molecule of galactose and one molecule of glucose. Each molecule has eight hydroxyl radicals. K_m-value of maltose were 0.50 at 100°C. as a mean value and 0.83 at 50°C. K_m-values of lactose were 0.66 at 100°C. as a mean value and 0.82 at 50°C. In these cases the values at 50°C. indicate clearly that the exchange reaction of hydroxyl group are exothermic. There is no need of considering the unclosed structures of disaccharides.

From these values it is concluded that the exchangeabilities of hydroxyl radicals of carbohydrates are about 0.6 at 100°C. and 0.8 at 50°C. Slightly different values are possibly due to the different degrees of dehydration of the compounds used. The exchangeabilities of the carbohydrates are somewhat higher than that of the aliphatic hydroxyl compounds, namely, glycerin. This is probably due to the ring-form structure of the carbohydrates.

Starch is considered to be composed from anhydrides of maltose. Assuming that at 100°C. the molecule is hydrated such that there are 4 OH for each six of carbon atoms, K_m becomes 0.56. If 5 OH assumed for each six of carbon atoms, K_m becomes 0.46.

Inulin is considered to be composed from anhydride of fructose. If assumed that at 100°C. there are 4 OH for each six carbon atoms, then K_m becomes 0.55. If 5 OH assumed, then K_m becomes 0.44.

From these results it must be concluded that starch molecules in water at 100°C. are hydrated to the state of the constitution very near to those of maltose molecules. Similarly, inulin molecules are hydrated to the state of disaccharide instead of the fructose constitution. It is supposed that in the polysaccharide molecules the molecules of disaccharides become the unit of these structures and have the specially firmly combined constitutions.

Chemical Institute, Faculty of Engineering,
Kyushu Imperial University, Fukuoka.