STUDIES ON ASYMMETRIC OXIDATION.

(Preliminary Note.)

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Introduction. The cause of the well known fact that organic substances containing one or more asymmetric carbon atoms in their molecules occur in the nature always in optically active forms, while the same substances synthesised in vitro are of racemic compounds, has long been discussed and various experiments, physical as well as chemical, have been attempted(1)* for the purpose of finding any reasonable theory on this subject. Notwithstanding those efforts, we are not able, up to the present time, to arrive to a conclusion which will definitively explain this fundamental fact in the nature.

On the other hand, we are acquainted with many examples of such facts that certain organic ferments extracted from living bodies act selectively upon racemic compounds, that is to say, either *d*- or *l*-form of the

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(1) Compare Byk, *Z. physik. Chem.*, **49** (1904), 641; *Ber.*, **37** (1904), 696; and Bredig: Ueber absoluter asymmetrische Synthese, *Z. angew. Chem.*, No. 62 (1923).

* The asymmetric syntheses which were attempted on the standpoint of pure synthetic chemistry without any biochemical consideration have often been succeeded by Marckwald, Mckenzie and others.

latter substances is attacked more easily than the other by ferments: for example, pancreas extracts act upon *d*, *l*-tyrosine ethyl ester, decomposing the *l*-form of this compound more quickly than the *d*-form(1); pancreatine hydrolyses only *l*-form of carboethoxyl-glycyl-*d*, *l*-leucine(2) and intestinal erepsine decomposes asymmetrically *d*, *l*-leucyl tyrosine(3) and so on. It appears, therefore, to be quite logical to conclude that the syntheses in living bodies are asymmetric by the reason that they are carried out by ferments which are themselves asymmetrically constructed.

But it will soon be found that this explanation is by no means conclusive, because there is, up to the present time, no experimental evidence as to the assimmetricity of nuclear molecules (or active centre) of ferments.

One of the present authors, Yuji Shibata together with Keita Shibata has found some years ago an interesting phenomenon exhibited by some metallic complex salts: it deals with namely the oxidase-like oxidising actions which differ entirely from the oxidation of the electrochemical nature shown often by stannic- or ferric salts. A series of investigations on this subject executed by Y. and K. Shibata and their collaborators(4) have proved that those oxidation reactions carried out by some metallic complex salts must be regarded to have a catalytic nature and resemble closely to enzyme actions from the point of view of chemical kinetics. Now, among those complex salts which possess the oxiding action, there are some ones, the molecules of which are asymmetrically constructed, such as $[\mathrm{Co}\,\underline{en}_2\,\mathrm{NH_3Cl}^{(1)}_{(2)}]\,\mathrm{X_2}$, $[\mathrm{Co}\,\underline{en}_2\,\mathrm{H_2OCl}^{(1)}_{(2)}]\,\mathrm{X_2}$, $[\mathrm{Co}\,\underline{en}_2\,\mathrm{Cl_2}^{(1)}_{(2)}]\,\mathrm{X}$, &c. When a certain racemic organic compound (preferably polyphenol containing an asymmetric carbon atom in the side chain) is put under the action of an optically active cobalt complex salt, it will be suggested that the oxidation of the former substance proceeds asymmetrically, i.e., one of the *d*- or *l*-form is more easily oxidised than the other by *d*- or *l*-form of cobalt complex salts above mentioned. If this is experimentally realised, it will be of no less interest,

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(1) E. Abderhalden, H. Sickel and H. Ueda, *Fermentforschung*, **7** (1923), 91.

(2) E. Fischer and P. Bergell, *Ber.*, **36** (1903), 2592.

(3) E. Abderhalden and E. Schwab, *Fermentforschung*, **9** (1927), 252.

(4) Yuji Shibata and Keita Shibata: On the Oxydase-like Oxidising Action of Certain Metallic Complex Salts, *Journ. Chem. Soc. Japan*, **41** (1920), 35; *Chem. Abst.*, **14** (1920), 2590. Yuji Shibata and Hideo Kaneko: Chemical Kinetics of Oxidation by the Catalytic Action of Some Complex Salts of Metals, *Journ. Chem. Soc. Japan*, **43** (1922), 833; *Chem. Abst.*, **17** (1923), 2811. Yuji Shibata and Hideo Kaneko: Decomposition of Hydrogen Peroxide by Complex Salts of Metals, *Journ. Chem. Soc. Japan*, **44** (1923), 166; *Chem. Abst.*, **17** (1923), 2812. Hideo Kaneko: On the properties of Aqueous Solutions of Some Cobalt Complex Salts Which Show the Oxidase-like Actions, Part 1 and 2, *Journ. Chem. Soc. Japan*, **48** (1927), 391; **49** (1928), 380. Keita Shibata: The Vital Oxidising Reaction, *Toyo Gakugei-Zasshi*; *Chem. Abst.*, **16** (1922), 1443. Atsushi Watanabe: Über die vitale Oxydation in den Pflanzenzellen mit den Kobaltamminkomplexsalzen, *Japan. Journ. Bot.*, **4** (1928), 31. Atsushi Watanabe: Oxidation of Metallic Iodides by Complex Salts of Metals, *Journ. Chem. Soc. Japan*, **49** (1928), 476.

because the further analogy between the behaviours of enzymes and some metallic complex salts is thus established and, on the other hand, the supposed asymmetrical structure of essential molecules of enzymes will be revealed on an experimental basis.

The present investigation of the authors has, as will be briefly described below, positively proved that the asymmetric oxidation, as the phenomenon is so called by the authors, is really the case, at least in the interaction between the substances now studied, namely racemic 3,4-dioxyphenyl-alanine as the substance to be oxidised and optically active ammonio-chloro-diethylenediamine cobaltic bromide as the oxidiser.

Experimental.*

Racemic ammonio-chloro-diethylenediamine cobaltic bromide was first synthesised and resolved into optically active components by the method of fractional crystallisation of its bromocamphersulfonate(1); the results of the determinations of rotatory powers of the *d*- and *l*-components are as follows:

$$\begin{array}{ll} d\,[\mathrm{Co}\,\underline{en}_2\,\mathrm{NH_3Cl}]\text{-}d\,(\mathrm{C_{10}H_{14}O\,BrSO_3})_2, & [\alpha]_{\mathrm{red}}^{18.5^\circ}=+68.3^\circ \\ l\,[\quad\text{,,}\quad]\text{-}d\,(\quad\text{,,}\quad)_2, & [\alpha]_{\mathrm{red}}^{18.5^\circ}=+32.2^\circ \end{array}$$

The *d*- and *l*-bromocamphersulfonate were then transformed again into the respective bromides by dissolving in hydrobromic acid; the rotatory powers of these bromides were as follows:

$$\begin{array}{ll} d\,[\mathrm{Co}\,\underline{en}_2\,\mathrm{NH_3Cl}]\,\mathrm{Br_2}, & [\alpha]_{\mathrm{red}}^{11^\circ}=+45.8^\circ \\ l\,[\quad\text{,,}\quad]\ \text{,,}\ , & [\alpha]_{\mathrm{red}}^{10^\circ}=-45.5^\circ \end{array}$$

The racemic 3, 4-dioxyphenyl-alanine(2) was, on the other hand, synthesised in starting from glycine anhydride, according to the method given by K. Hirai.(3)

In order to save the materials, the preparations of which are fairly laborious and expensive, the authors have only determined in definite time intervals, the change of rotatory power of dilute solutions containing the reacting substances, without isolating and examining the oxidation products. The experiments were carried out in the following manner:

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(1) A. Werner, *Ber.*, **44** (1911), 1887.

(2) The authors chose at first dioxymandelic acid as the substance to be oxidised, dioxyphenyl-alanine has afterwards been preferred to the former for the sake of its larger yield in the syntheses.

(3) K. Hirai, *Biochem. Zeitsch.*, **114** (1921), 67.

* One of the authors, Y. Shibata with his collaborators is continuing this work using racemic catechin as the substance to be oxidised and at the same time is studying chemical kinetics in the case of interaction between both optically active polyphenol, such as dioxyphenyl-alanine and catechin, and metallic complex salts.

1. To 100 c.c. of a saturated solution of racemic 3, 4-dioxyphenyl-alanine, there were added 25 c.c. of a solution of *l*-ammonio-chloro-diethylene-diamine cobaltic bromide, in which 0.3030 gr. of this complex salt is contained, and 5 c.c. of a buffer solution of phosphate (1/5 mol, PH=6.2). This solution mixture was put in a thermostat of 20° ; 10 c.c. of the solution was then taken out from time to time and its rotatory power was observed in adding about 2 c.c. of acetic acid solution (1 normal), in order to arrest the course of the oxidation. The three-field polariscope of Adam-Hilger, and the monochromater of Leitz as the light source were used ; the observations were made with 10 cm. tube in red light. The results of the determinations are shown in Table 1 and Figure 1 :

Table 1.

Time (hour)	Angle of rotation	Time (hour)	Angle of rotation
0.0	−0.03	6.5	−0.05
0.5	−0.06	8.5	−0.07
1.5	−0.07	10.0	−0.07
2.0	−0.08	13.0	−0.07
2.5	−0.05	17.0	−0.08
3.5	−0.04	26.0	+0.02
4.5	−0.04	29.0	+0.07
5.5	−0.08		

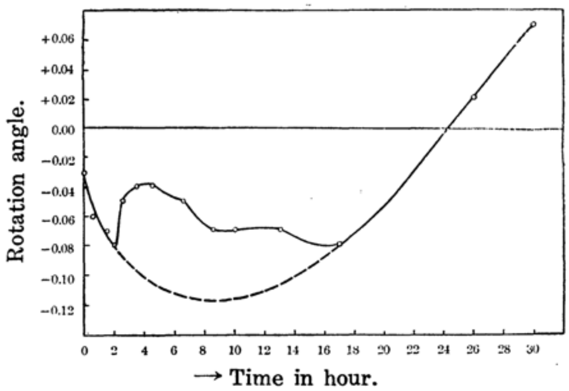


Fig. 1.

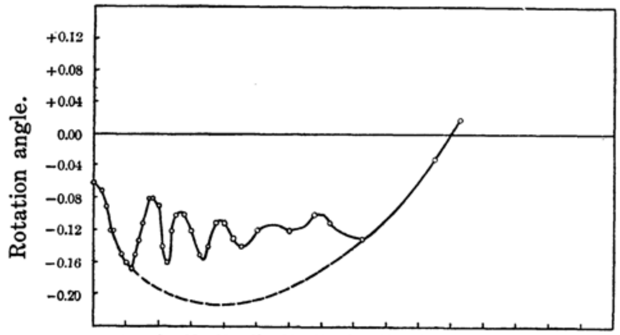
From the above figure, it is easily seen that the solution changes its rotatory power in such a manner that in the early stadium of the oxidation reaction, the *l*-rotation increases by and by, and after tracing a somewhat irregular course for a while, the *l*-rotation begins to decrease in its amount, until it reaches to the zero-point; the sign of the rotatory power is then changed, the *d*-rotation being henceforth again increased.*

2. Racemic 3, 4-dioxyphenyl-alanine: 0.6157 gr. in 200 c.c. water; *l*-ammonio-chloro-diethylenediamine cobaltic bromide: 0.4371 gr. in 50 c.c. water. The other conditions are similar as in the former experiment.

In this second experiment, the time interval of the determination of rotatory power was considerably shortened, for the purpose of studying more closely the part of the time-rotation curve, where there was observed some irregularity in the former experiment. The results of this observation are given in Table 2 and Fig. 2.

Table 2.

Time (hour)	Angle of rotation	Time (hour)	Angle of rotation
0.0	−0.06	5.0	−0.15
1.0	−0.07	5.5	−0.13
1.5	−0.09	6.0	−0.11
2.0	−0.12	6.5	−0.08
2.5	−0.12	7.0	−0.08
3.5	−0.15	8.0	−0.09
4.0	−0.16	8.5	−0.14
4.5	−0.17	9.0	−0.16
9.0	−0.12	18	−0.14
10	−0.10	20	−0.12
11	−0.10	24	−0.12
12	−0.12	27	−0.10
13	−0.15	29	−0.11
14	−0.14	33	−0.13
15	−0.11	42	−0.03
16	−0.11	45	+0.02
17	−0.13		



→ Time in hour.

Fig. 2.

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* A control experiment, in which dioxyphenyl-alanine was omitted, the other condition being the same as the experiment above mentioned, showed that the rotatory power of the solution stayed constant within 320 hours.

By this study, it was shown that the change of the rotatory power of the solution begins to take a zig-zag-formed course several hours after since the reacting substances were mixed; this zig-zag-shape is then gradually flattened until the curve become finally smooth. It appears, anyhow, that the general form of the time-rotation curve which is represented by broken lines in the figures is almost the same in both cases of the experiments (1) and (2).*

Let us now theoretically consider various possible cases of the interaction between the substances in question. The following four figures representing also the time-rotation curve are given to illustrate the courses of those possible reactions: if, for example, the *d*- and *l*-components of a racemic organic compound are oxidised with an equal easiness by an optically active complex salt, say *l*-salt, and the oxidation product thus formed possess no optical activity, the rotation-change of the solution should be represented by the curve in Fig. 3,⁽¹⁾ while if only the *l*-component of the racemic organic compound is attacked by the *l*-complex salt, the *l*-activity of the solution will be gradually diminished and after some times the solution will become dextro-rotatory due to the unattacked *d*-component of

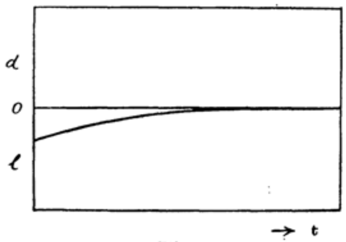

Fig. 3.

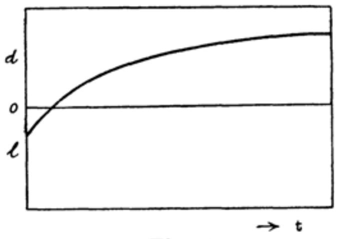

Fig. 4.

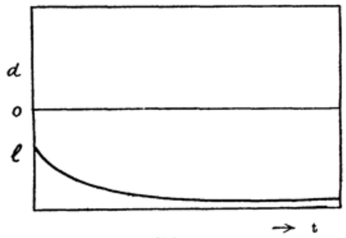

Fig. 5.

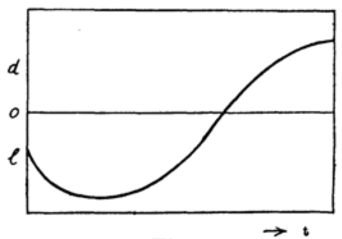

Fig. 6.

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* One more series of observation, similarly arranged as the experiment (2), was carried out by the authors, but the results of this measurement were not described here, for the data thus obtained were proved to be in good accordance with those of the former.

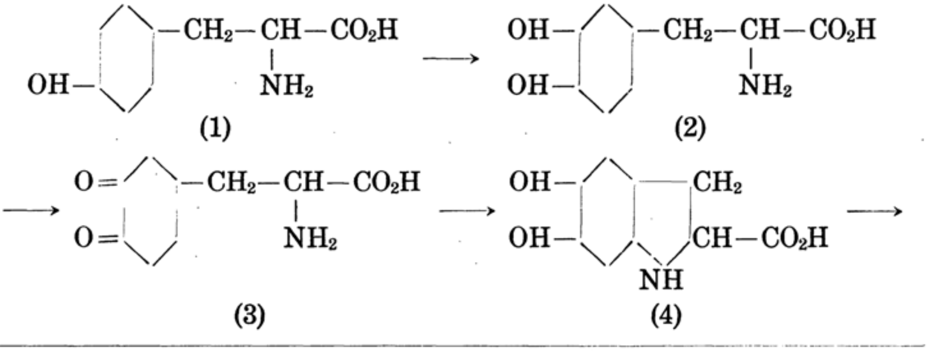
(1) In considering quite theoretically, the curve must be parallel to the zero-line, but, in fact, metallic complex salt itself, after some times, gradually undergo a certain change, losing its optical activity.

the organic compound (curve in Fig. 4), provided that the *d*-rotation of the latter substance is higher than the *l*-rotation of the complex salt. If, in the latter case, a *l*-rotatory compound, a quinone for example, is produced by the oxidation, the *l*-activity of the solution will be increased by degrees, the activity of the oxidation product superposing with that of the oxidising agent. The curve of Fig. 5 represents this sort of reaction. Finally, if the *l*-rotatory quinone, just now supposed to be produced, is then decomposed by the further oxidation and lose its own activity, the solution will become by and by less *l*-rotatory and finally *d*-activity will take its appearance, as is shown in Fig. 6.

Now, if the curves (Fig. 1 and 2) which were obtained by the authors as the results of the present investigation are compared with those above discussed, it may easily be found that the former curves show a close resemblance with that of Fig. 6. From this experimental fact, it might reasonably be concluded that *l*-ammonio-chloro-diethylenediamine cobaltic bromide oxidises the *l*-component of racemic 3, 4-dioxyphenyl-alanine more easily than its *d*-component and a *l*-rotatory substance, probably quinonic compound, is intermediately produced. This latter substance undergo further oxidation and is transformed into an inactive compound (perhaps by a decomposition or polymerisation) thus the solution becomes gradually less *l*-rotatory and finally *d*-rotatory owing to the remaining *d*-dioxyphenyl-alanine.

Consideration on the Reaction Mechanism.

As is well known, dioxyphenyl-alanine is oxidised by tyrosinase,(1) and the same enzyme act upon *l*-tyrosine, giving intermediately *l*-dioxyphenyl-alanine and finally melanine.(2) Raper explained the mechanism of this oxidation reaction as follows :(3)



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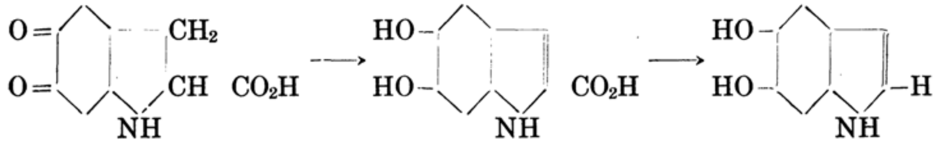
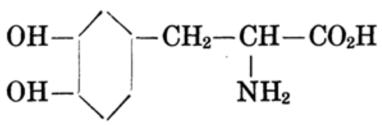
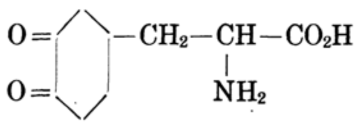
(1) H. Prigbaum, *Arch. Entwickl. Org.*, **48** (1921), 140.

(2) H. S. Raper, *Biochem. Journ.*, **20** (1926), 735.

(3) *Fermentforsch.*, **9** (1927), 206.



(5) (6) (7)

If the oxidation of racemic 3, 4-dioxyphenyl-alanine by *l*-ammonio-chloro-diethylenediamine bromide follows too the reaction mechanism above stated, the solution should contain *d*  and *l*  in the early stage of the reaction; the quinonic compound will then be further oxidised and, passing (4) and (5), lose its activity at (6). The transition of (6)⟶(7) will be accompanied by separation of carbonic acid and consequently the acidity of the solution augments at this moment, resulting temporary retardation of the reaction velocity; the repetition of the latter chemical changes might perhaps be regarded as the cause of giving the zig-zag shape in a part of the time-rotation curve, as was observed by the authors.

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