STUDIES ON THE ASYMMETRIC OXIDATION.
PART II.

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In the preceding preliminary note, one of the present authors (Yuji Shibata), together with Ryutaro Tsuchida, described of the experiments on the asymmetric oxidation,(1) which was accomplished by oxidising synthetic racemic 3,4-dioxy-phenylalanine with optically active *l*-diethylenediamine-monoammonio-monochloro-cobaltic bromide, $[\mathrm{Co}\,\underline{\mathrm{en}}_2\cdot\mathrm{NH_3}\cdot\mathrm{Cl}]\,\mathrm{Br_2}$. In observing, time after time, the rotation changes of the solution containing the substances just mentioned, they came to the conclusion that *l*-dioxy-phenylalanine was oxidised more rapidly than its *d*-form by *l*-cobaltic complex salt.

Now we carried out an analoguous study in using *d*-catechin and *d*-and *l*-form of the same cobaltic complex salt that was employed as oxidiser in the former investigation, for the purpose of determining, if possible, the velocity constants of the oxidising reaction in two systems of (*d*-catechin + *d*-complex salt) and (*d*-catechin + *l*-complex salt). For the reason of complexity of the reaction, in which the oxidation product of catechin suffers very probably further changes such as decomposition or polymerisation, etc., we failed to calculate velocity constants desired. But, in comparing the curves obtained by tracing the successive changes of rotation angles, we could distinctly recognise that *d*-catechin is oxidised with different velocities by *d*- and *l*-complex cobaltic salts, *d*-complex showing to behave more actively than *l*-complex towards *d*-catechin.

Then we tried to carry out an experiment of the same reaction between *d*-catechin and the racemic compound of the same cobaltic complex salt, and thus we could find a very interesting fact: namely, in this case, the oxidation reaction took a course closely resembling to that of the system of (*d*-catechin + *l*-complex), as if there is none of *d*-complex salt in the solution. This somewhat curious result, however, may easily be understood, if we take into consideration the recent work of Willstätter and his collaborators on the asymmetric hydrolysis of mandelic ester by an esterase extracted from liver(2). They found, namely, that if racemic mandelic ester is subjected to the action of the esterase, *d*-ester will be hydrolysed more

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(1) This Bulletin, **4** (1929), 142; compare Y. and K. Shibata: Untersuchungen über die oxydasenartigen Wirkungen gewisser Metallkomplexsalze, *Acta Phytochimica*, **4** (1929) No. 3.

(2) *Ber.* **61** (1928), 886.

quickly than *l*-ester, but the relation is quite reversed, when the isolated *d*- and *l*-ester are separately put under the action of the hydrolytic enzyme: in this latter case, *l*-ester is hydrolysed more rapidly than *d*-ester.

These authors explained this quite unexpected result in considering as follows: the apparent velocity of an enzyme action should involve two factors, that are (1) affinity relation, which take its appearence between enzyme and substrate at first and (2) the reaction velocity proper to that system. In considering, therefore, that the affinity between *d*-ester and esterase is larger than that between *l*-ester and esterase, while the velocity of hydrolysis of *l*-ester by the same esterase surpasses that of *d*-ester, the experimental results obtained by them will readily be explained.

In adopting this theory, our experimental results on asymmetric oxidation may equally be interpreted, because if we consider that the affinity between *d*-catechin and *l*-complex salt is superior to that between *d*-catechin and *d*-complex salt, it should be expected that the oxidising effect of *l*-complex salt on *d*-catechin molecules must prevail over that of *d*-salt, when the solution contains the racemic compound.

Now then, for velocity determination of the oxidation reaction, we took the other method than rotation measurements: we observed namely the quantities of oxygen absorbed in the course of the oxidation reaction, in making use of Warburg's apparatus, which was slightly modified in its shape and dipped entirely, even its capillary manometer, in a thermonstat of 20°. In this way, we were able again to find a distinct difference in oxidation velocities of *d*-catechin respectively by *d*- and *l*-cobaltic complex salts, the results showing a good accordance with the former investigation of the measurement of rotation changes. In this case, too, we could not calculate the velocity constants for the same reason that was above described; anyhow we could here add one more example of asymmetric oxidation and establish the further analogies between the actions of natural enzymes and those of some metallic complex salts.

Experimental.

(1) Measurements of rotation changes of solutions containing *d*-catechin and respectively *d*-, *l*- and *r*-diethylene-diamine-monoammonio-monochloro-cobaltic bromide.

As was shown in our preceding investigations⁽¹⁾, the oxidation performed by some metallic complex salts is strongly influenced by the PH-

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(1) loc. cit.

value of solutions: it will namely be accelerated by OH-ions and retarded by H-ions. In order to find, therefore, the optimum value of PH in carrying out the experiments of this sort, we have preliminarily made a series of measurements, in which the PH-value of solutions were rendered respectively 7, 7.5 and 6 by adding varying quantities of the buffer solution of NaH_2PO_4+NaOH.

Table 1.

Rotation changes of the solutions containg *d*-catechin (0.6%), *d*- $[Co\,\underline{en}_2NH_3Cl]\,Br_2$ (0.3%) and phosphate buffer (0.05 Mol).

PH = 6		PH = 7		PH = 7.5	
Time (hour)	Angle	Time (hour)	Angle	Time (hour)	Angle
0.0	+0.26	0.0	+0.27	0.0	+0.28
5.0	.19	0.5	.26	0.5	.25
9.0	.20	1.0	.19	1.0	.20
12.0	.20	1.5	.13	1.5	.06
22.0	.12	2.0	.08	2.0	−0.05
24.0	.09	2.5	.06	2.5	.13
26.0	.06	3.0	.05	3.0	.17
29.0	.04	4.5	−0.02	4.0	.25
34.0	.00	5.0	.02	4.5	.24
49.0	−0.01	6.0	.02	5.0	.22
54.0	+0.01	7.0	.04	5.5	.22
		8.0	.06	6.0	.20
		10.0	.08	7.0	.16
		12.0	.10	8.0	.16
		25.0	.08	10.0	.17
		30.0	.08	11.0	.14
		53.0	.02	12.0	.14
				13.0	.12
				25.0	.04
				27.0	.04
				30.0	.01
				51.0	.00

From the courses of the reactions shown by the rotation-time curves I and II, it will easily be seen that *d*-catechin in neutral or slightly alkaline solution produces at first a certain substance with laevo-rotatory power in undergoing oxidation by the cobalt complex salt and this latter product loses slowly its optical activity, while, in slightly acidic solution, it appears that the oxidation of *d*-catechin by the cobalt salt does not practically proceed, although the curve III shows that there is observed a slight decrease of dextro-rotation in the solution mixture. The following studies were, therefore, carried out always with the solutions of PH = 7.5.

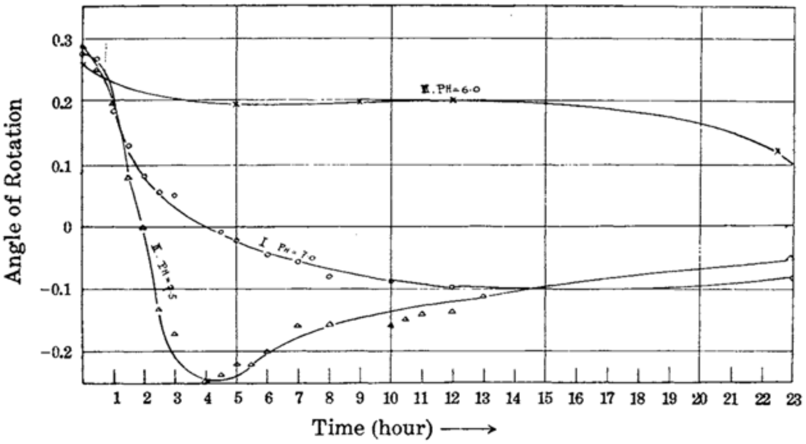

Fig. 1.

Table 2.

Rotation changes of the solutions containing *d*-catechin (0.4%) and respectively *d*-, *l*- and r- [Co $\underline{en}_2NH_3Cl$] Br_2 (0.25%) and phosphate buffer (PH = 7.5).

Time (hour)	Angles of rotation		
	d-complex	*l*-complex	*r*-complex
0.0	+0.19	−0.09	+0.04
0.5	.19	—	—
1.0	.16	.13	−0.01
1.5	.09	.19	—
2.0	.03	.25	.13
2.5	−0.03	.29	—
3.0	.06	.32	.20
3.5	.11	.34	.21
4.0	.16	.37	.23
4.5	.16	—	.24
5.0	.17	.35	.23
5.5	.18	—	—
6.0	—	.32	.21
6.5	.17	—	—
7.0	—	.29	.20
7.5	.15	—	—
8.0	—	.28	—
9.0	—	—	.18
10.0	.12	.25	.17
11.0	—	.23	.16

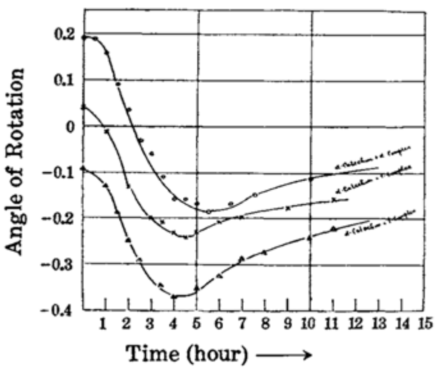

Fig. 2.

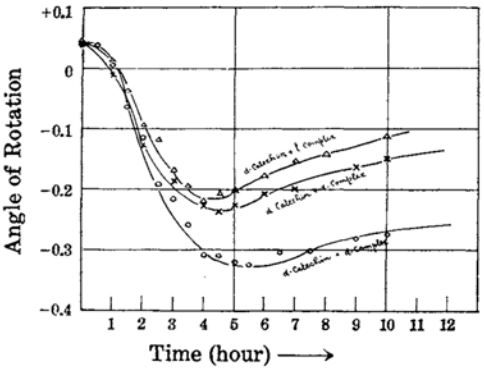

Fig. 3.

The curves shown in Fig. 2 are nothing but the graphical representation of numerical data in Table 2. In order that the amounts of the oxidation product of catechin in these three cases may be compared with readiness, we have reproduced these curves in bringing them at the same origin (Fig. 3). It must only be taken in mind that, if optical activities of *d*- and *l*-complex salts remain constant throughout the reaction, the curves in Fig. 3 may be taken for comparison just as it is; but, in fact, the complex salts used as oxidiser somewhat decompose in the course of reaction, instead of behaving normally as catalyser. Therefore, when it concerns to the true amounts of rotation of the oxidation products, the curves for *d*- and *l*- complex salts should be a little drawn near to each other. At any rate, it will be clear enough to be noticed that the oxidation of *d*-catechin by *d*- and *l*-cobalt complex salts takes place asymmetrically. Further it will readily be recognised that the course of the oxidation of *d*- catechin by *r*-complex salt remarkably accesses to that of the system of (*d*- catechin + *l*-complex), instead of taking its way at the middle position of the other two. With regard to this latter fact, we have already discussed in the introductory part.

(2) Measurement of the oxygen quantities absorbed by the solutions containing *d*-catechin + *d*- [Co $\underline{\text{en}}_2$NH$_3$Cl] Br$_2$ and *d*-catechin + *l*- [Co $\underline{\text{en}}_2$NH$_3$Cl] Br$_2$.

In the measurements of the pressure depression in the Warburg's apparatus, that indicates the quantities of oxygen absorbed in the course of an oxidation reaction, if its capillary manometer will be put outside the thermostat, the pressure-time curve, which represents the reaction course, takes a zigzag shaped path. If the whole apparatus, however, is dipped in the thermostat, as is the case in our actual experiment, the results become well concordant and reproduceable, giving always smooth curves.

We have carried out the measurements with three apparatus of different capacities, catechin solution being put in a main bulb, complex salt solution in a side bulb and alkali liquor* in a small cylinder which is fused at the middle position of the bottom of the main bulb. Their volumes were as follows:

No. 1, solution= 5.0 c.c.; air=ca. 43 c.c.; alkali liquor=2 c.c.

No. 2, solution= 5.0 c.c.; air=ca. 38 c.c.; alkali liquor=2 c.c.

No. 3, solution=10.0 c.c.; air=ca. 97 c.c.; alkali liquor=1 c.c.

In Table 3 and Fig. 4, we mentioned only the result of determination executed with the apparatus No. 2, because the other results were so similar with this that they are not here worth noting.

Table 3.

(Temp. = 20°C)

Time (hour)	Press. depression (cm. of water column)		Time (hour)	Press. depression (cm. of water column)	
	d-complex	*l*-complex		*d*-complex	*l*-complex
0	0.00	0.00	11	4.60	1.72
1	0.08	0.06	12	5.66	2.01
2	0.10	0.06	13	6.50	2.32
3	0.27	0.28	14	6.99	2.75
4	0.41	0.33	16	7.93	3.95
5	0.90	0.31	18	8.60	5.91
6	1.57	0.42	20	9.20	7.00
7	1.60	0.90	22	9.39	8.01
8	2.51	0.91	24	9.60	8.40
9	3.42	1.22	26	9.81	9.58
10	4.41	1.35	29	9.92	9.60

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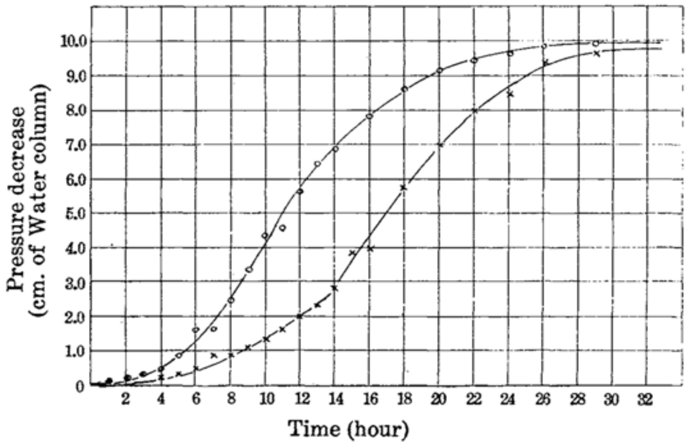
* This concentrated alkali solution is provided for the purpose of absorption of CO_2 produced in the course of oxidation of catechin.



○ *d*-catechin + *d*-complex salt.
× *d*-catechin + *l*-complex salt.

Fig. 4.

Two curves in the above Figure indicate distinctly again that the velocity with which *d*-catechin is oxidised by *d*-cobaltic complex salt is larger than that by *l*-complex salt.

Summary.

The catalytic oxidation of *d*-catechin by *d*- and *l*- [Co $\underline{en}_2NH_3Cl$] Br_2 was studied. The course of these oxidation reactions were observed in two different ways: one of the methods was the measurements of rotation changes of solutions containing *d*-catechin and *d*- or *l*-cobaltic complex salt, and the other was the determinations of oxygen quantities absorbed by those solutions during the oxidation reactions. In both cases we could confirm that the oxidation takes place always asymmetrically, *d*-complex salt oxidising *d*-catechin more rapidly than *l*-complex salt.

Further we studied the oxidation of *d*-catechin by racemic [Co $\underline{en}_2NH_3Cl$] Br_2 and could confirm that the oxidation in this case goes on very similarly with the system of (*d*-catechin + *l*-complex salt), instead of taking an average course of those shown respectively by *d*- and *l*-complex salts. Concerning to the cause of this result, it was discussed from the point of view of natural enzyme actions.

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