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# Catalytic Activities of Pyridoxal Analogues. I. The Catalytic Effects of Pyridoxal, Pyridoxal Methochloride, and Pyridoxal *N*-Oxide on the Racemization of L-Glutamic Acid

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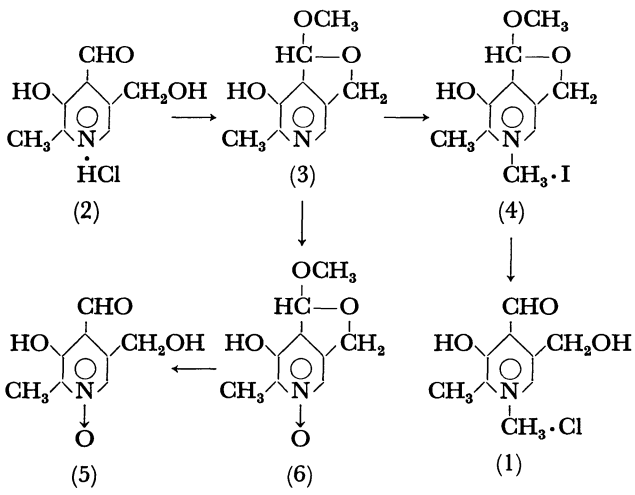
**Synopsis.** An improved method for the syntheses of pyridoxal methochloride (**1**) and pyridoxal *N*-oxide (**5**) was investigated. Further, the catalytic activities of pyridoxal, **1**, and **5** on the racemization of L-glutamic acid in the presence of cupric ions were studied.

In the presence of a metal ion, pyridoxal (PAL) catalyzes many reactions of amino acid, including racemization[1] and transamination.[2] Catalytic activities are also observed for the racemization with salicylaldehyde (SAL) derivatives, the benzene analogue of PAL.[3] In a previous paper, it has been reported that an electron-attracting substituent, introduced on the benzene ring of SAL derivatives, promoted the catalytic activity on the racemization of L-glutamic acid (L-Glu) in the presence of cupric ions at pH 10 and at 80 °C.[4] The catalytic activity may be expected to be more powerful when the PAL derivatives, which have a substituent on the ring nitrogen in order to increase the electron affinity of the pyridine ring, are used than when PAL itself is used.



Scheme 1

Anhydrous PAL methochloride (**1**) had been synthesized from PAL hydrochloride (**2**) in a 21% yield *via* PAL monomethyl acetal (**3**) and then PAL monomethyl acetal methiodide (**4**).[5] When the synthetic conditions were improved, **1** monohydrate was prepared in a 42% yield. PAL *N*-oxide (**5**) had also been obtained from pyridoxine *via* pyridoxine *N*-oxide.[6] In tetrahydrofuran (THF), **3** was oxidized with *m*-chloroperbenzoic acid (CPA) to PAL monomethyl acetal *N*-oxide (**6**),[7] which was then easily hydrolyzed to **5**.

According to the precedents,[3,4,8] the racemization of L-Glu was carried out in the presence of cupric ions at pH 10, where it is known that side reactions, such as transamination, proceed scarcely at all. Theoretically, $(\log \alpha_t - \log \alpha_0)/t$ should be a constant, where $t$ is the time and $\alpha_t$ is the observed rotation at $t$. Table 1 shows that the catalytic activities of **1**, **2**, and **5** were gradually lowered as the reaction proceeded at 80 °C; a similar phenomenon had been observed with **2** by Yoshikawa *et al.*[8] At 25 °C, however, the racemization catalyzed by **2** and **5** was found to obey a psudo-first-order reaction at an early stage of the reaction. Though **1** was unstable even at 25 °C, the most active catalyst was **1**, followed by **5** and **2**; this order agreed with the one expected from their molecular structures. The decrease in the catalytic activity was parallel to that in the absorption maximum of the alkaline racemization mixture at around 380 nm, attributed to the chelation of the Schiff base of Glu with **1**, **2**, or **5** and cupric ion.[9] Therefore, in these cases, the catalytic activity is seemingly lowered by the decomposition of the cayalyst. Fukui *et al.* reported that, in $\beta$-elimination and transamination, the catalytic activity of **5** was inferior to that of **2** at pH 4—5,[10] where **2** forms pyridinium salt; consequently, the electron affinity of **2** is larger than that of **5**. Although the Braunstein-Snell mechanism of the non-enzymatic reactions of amino acid catalyzed by PAL, such as racemization, transamination, and $\beta$-elimination, is generally accepted,[11] the details of the

Table 1. Catalytic activities

| Temp. °C | Time min | $(\log \alpha_0 - \log \alpha_t) \times 10^3/t$ PMC $min^{-1}$ | PAL $min^{-1}$ | PNO $min^{-1}$ |
|---|---|---|---|---|
| 80 | 10 | 8.1 | 11.0 | 11.7 |
| | 20 | 5.8 | 9.9 | 11.0 |
| | 30 | 4.8 | 9.1 | 10.2 |
| | 40 | 4.4 | 8.5 | 9.5 |
| | 50 | 4.3 | 8.4 | 9.3 |
| | 60 | 4.1 | 8.2 | 8.7 |
| | 80 | 4.1 | 7.5 | 8.1 |
| | 100 | 3.8 | 7.1 | 8.1 |
| | 120 | 3.6 | 6.9 | 7.6 |
| 25 | 120 | 0.95 | 0.30 | 0.44 |
| | 240 | 0.79 | 0.31 | 0.44 |
| | 480 | 0.55 | 0.30 | 0.41 |
| | 960 | 0.50 | 0.29 | 0.39 |
| | 1440 | 0.47 | 0.29 | 0.40 |
| | 1920 | 0.47 | 0.27 | 0.36 |
| | 2400 | 0.46 | 0.27 | 0.37 |
| | 2880 | 0.42 | 0.27 | 0.37 |

PMC: pyridoxal methochloride; PAL: pyridoxal hydrochloride; PNO: pyridoxal *N*-oxide.

Table 2. Effect of pH on the racemization yield

| pH | | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|
| 80 °C | PMC | 45% | 43% | 41% | 40% |
| 1 hr | PAL | 31 | 68 | 91 | 97 |
| | PNO | 43 | 70 | 82 | 89 |
| 25 °C | PMC | 25 | 45 | 47 | 49 |
| 8 hr | PAL | 7 | 28 | 58 | 94 |
| | PNO | 11 | 37 | 63 | 83 |

intermediates are still uncertain, and it has not been completely elucidated whether racemization, transamination, and $\beta$-elimination proceed with the same mechanism. As far as the order of the catalytic activity is concerned, the results of Fukui are consistent with those of our racemization.

No optimum pH was found for the racemization system, containing L-Glu, SAL derivatives (0.02 mole) equivalents), and cupric ions (0.02 mole equivalents) at 80 °C.[3,8] On the other hand, an optimum pH was found at around pH 10 for the racemization of L-alanine with **2** (equimolar) in the presence of cupric or aluminum ions (0.1 mole equivalents) at 100 °C for 10 min.[1] Therefore, we investigated the effect of the pH on the racemization of L-Glu under our conditions. As Table 2 shows, in a lower-pH solution, the catalytic activities decreased in the order of **1**, **5**, and **2** as was to be expected from their molecular structures, whereas, in a higher-pH solution, the order was reversed because of the unstabilities of the catalysts. In all the cases tested except the case of **1** at 80 °C, in the higher-pH solutions faster racemizations were observed. Without exception, no optimum pH was found under our conditions, in contrast to Snell's observations.[1]

It was concluded that the more powerful catalytic activities of PAL derivatives, as well as of SAL derivatives, were observed when a larger electron affinity was introduced on the aromatic ring; however, a catalyst for the racemization should be stable in an alkaline solution.

## Experimental

All the mps are uncorrected. The IR spectra were recorded by means of a Shimadzu IR-27G instrument in KBr disks. The absorption spectra of the diluted alkaline racemization mixture were measured by means of a Hitachi 124 spectrophotometer.

*PAL Methochloride (1).* A mixture of a benzene solution of PAL monomethyl acetal (**3**), prepared from PAL hydrochloride (**2**) (8 g),[5] and $CH_3I$ (12 ml) was refluxed for 72 hr while being stirred; during the reaction, additional 3 ml portions of $CH_3I$ were added every 12 hr. Treating the mixture in a way similar to Heyl's method[5] gave a crude PAL monomethyl acetal methiodide (**4**) (6.1 g), which melted at 167—169 °C (decomp.) (lit,[5] mp 178—179 °C (decomp.)). Its IR spectrum showed the presence of a carbonyl group resulting from the partial hydrolysis of acetal. The crude **4** was converted to **1** as has been described in the literature.[5] Drying the final product over silica gel at room temperature under atmospheric pressure afforded 3.9 g of **1** monohydrate (42% based on **2**), which partly melted at around 100 °C and then gradually decomposed above 160 °C, as has been described in the literature.[5] IR 1660 $cm^{-1}$ (C=O).

Found: C, 45.74; H, 5.92; N, 6.07; Cl, 15.25%. Calcd for $C_9H_{14}O_4NCl$: C, 45.86; H, 5.99; N, 5.94; Cl, 15.04%.

*PAL Monomethyl Acetal N-Oxide (6).* To a solution of **3** (5 g) in THF (500 ml), a solution of CPA (5.4 g; 85% purity) in THF (50 ml) was stirred at 0—5 °C over a period of 90 min; soon a precipitate resulted. The mixture was stirred for another 30 min at 0—5 °C and then left to stand overnight at room temperature. The separation of the precipitate gave 3.9 g of **6** (72%); mp>280 °C.

Found: C, 54.88; H, 5.64; N, 6.98%. Calcd for $C_9H_{11}O_4N$: C, 54.82; H, 5.62; N, 7.10%.

*PAL N-Oxide (5).* A solution of **6** (3.9 g) in boiling water containing a few drops of conc. HCl was cooled in an ice-water bath to precipitate 3.2 g of **5** monohydrate (80%), which melted at 120 °C (decomp.) (lit,[6] mp 120 °C (decomp.)).

*Procedure of Racemization.*[3] PAL was used in the form of hydrochloride; **1** and **5** monohydrates were used without drying. In a tightly stoppered test tube, a mixture of a pH-adjusted solution (2 ml) of sodium L-glutamate (2 mmol) and $CuSO_4$ (0.04 mmol) and a pH-adjusted borate buffer solution (2 ml) of the catalyst (0.04 mmol) was kept at 80 °C or 25 °C. After the required time, 6 M HCl (5 ml) was added immediately to the cooled mixture. The optical rotation of the solution was measured by means of a Perkin-Elmer 141 polarimeter; the racemization yield was calculated as follows:

$$100(\alpha_0-\alpha_t)/\alpha_0$$

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