Preparation of 2,4-Dnps-L-Cys(Bzl)-L-Leu-OBzl (10)——10 was prepared from 2,4-Dnps-L-Cys(Bzl)-OH and L-Leu-OBzl by the DCC[8] method. The yield was 62%; mp 140—141°; $[\alpha]_D$ −155.6 (*c*, 0.3 in ethyl acetate); *Anal.* Calcd. for $C_{29}H_{30}N_4O_7S_2$: C, 57.04; H, 4.95; N, 9.17. Found: C, 57.11; H, 5.04; N, 9.15.

Preparation of Sulfenyl Amino Acids[2,3] (Table I)——The amino acid (0.02 mol) was dissolved in a mixture of 2 N NaOH (10 ml) and dioxane (25 ml). During a period of 30 min, sulfenyl chloride (0.022 mol) was added in 10 equal portions as 2 N NaOH (12 ml) was added dropwise, with vigorous shaking. After 1 hr, the solution was diluted with water (300 ml), filtered and acidified at 0° with 1 N sulfuric acid. The product was filtered off, washed with water and dried under high vacuum over P_2O_5. For recrystallization the crude product was dissolved in ethyl acetate or ether and precipitated with petroleum ether.

The sulfenyl amino acids can also be purified as dicyclohexylammonium salt. For this purpose, after acidification with sulfuric acid, the product was redissolved in ethyl acetate. The solution was repeatedly washed with water and then dried over sodium sulfate; upon addition of dicyclohexylamine (4 ml) the corresponding salt separated out, in most cases, in the form of needles.

Measurement of the "Half-life Period" of the Sulfenyl Amino Acids in Acidic Media——A solution of sulfenyl amino acids (0.1 mmol) in 80% acetic acid was kept at 23°. After aliquots (20 μl) of the solution had been diluted with MeOH (10 ml) at regular time intervals, the absorbance of Nps-, 2,4-Dnps- and Nmps-amino acids were measured respectively at 380, 335 and 388 nm. From the time course of a decrease in absorbance, the time required for the absorbance at absorption maximum) of sulfenyl amino acids to reach the middle value ((Di+Df)/2) of the initial (Di) and final (Df) values was determined.

Removal of the 2,4-Dnps Group——To a 0.5 N solution of hydrogen chloride in dioxane (40 ml), 2,4-Dnps-L-Ala–Gly–OBzl (1 g, 2.5 mmol) was added. After the reaction mixture had been stirred for 20 min at room temperature, the solvent was removed *in vacuo*. Dry dioxane was added to the residual oil with handswirling and then ethyl acetate was added to the resulting mixture. After the mixture had been allowed to stand for 4 hr in refrigerator, the crystals separated out were collected by filtration and washed first with ethyl acetate and then with ether. The yield was 0.25 g (84%), mp 176—179°. The product gave single Ninhydrin-positive spot having *Rf* of 0.42 on Silica Gel thin-layers with the solvents of methylene chloride /methanol 5: 1.

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Oxidation with Nickel Peroxide. XI.[1] Oxidation of Aromatic Aldehydes to Carboxylic Acids in Aqueous Alkaline Solution

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Nickel Peroxide (Ni-PO) was shown to be useful oxidizing agent for a preparation of substituted benzoic acids from the corresponding benzaldehydes in aqueous alkaline solution. Some kinds of aromatic carboxylic acids were also effectively obtained from the corresponding aldehydes in a similar way. The mechanism of these oxidations were discussed.

Keywords——nickel peroxide oxidation; aromatic aldehyde; aromatic carboxylic acid; alkaline medium; mechanism

In our previous paper,[3] it was shown that allylic and benzylic alcohols were oxidized by Nickel Peroxide (Ni-PO) in organic solvents to give the corresponding carbonyl compounds. While oxidation of alcohols in aqueous alkaline solution gave carboxylic acids.

In the case of oxidation in aqueous alkaline solution, it seemed that the reaction proceeded *via* the formation of aldehyde as an intermediate follow by further oxidation to give carboxylic acid.

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We report here that the Ni-PO oxidation of aromatic aldehyde in aqueous alkaline solution can be used conveniently to obtain the corresponding carboxylic acid. The oxidation of benzaldehyde was carried out as follows. Ni-PO was added to aqueous alkaline solution of benzaldehyde and the heterogeneous mixture was stirred under nitrogen at 30° for 30—90 minutes. These reaction were conducted by employing Ni-PO 1.2 times as much as the theoretical amount based on the available oxygen-contents which were determined by means of iodometry. After removal of Ni-PO, the filtrate was extracted by ether to remove the unreacted benzaldehyde, and on acidifying, the filtrate deposited pure benzoic acid.

Table I. Oxidation of Benzaldehyde in Alkaline Medium[a)]

Ni–PO		NaOH		Temp. (°C)	Time (min)	Benzoic acid	
g	Ratio[b)]	g	Ratio[c)]			g	Yield (%)
19.3	1.2	2.5	1.2	30	30	5.46	89.5
19.3	1.2	2.5	1.2	30	60	5.72	93.7
19.3	1.2	2.5	1.2	30	90	5.75	94.3
0	0	2.5	1.2	30	60	0.13	2.1
1.61	0.1	2.5	1.2	30	60	0.62	10.2
8.06	0.5	2.5	1.2	30	60	3.02	49.5
19.3	1.2	0.8	0.4	30	60	1.70	27.8
19.3	1.2	0	0	30	60	0.32	5.3
MnO_2[d)]	1.2	2.5	1.2	60	60	0.95	15.6

a) 5.30 g (0.05 mol) of benzaldehyde and 125 ml of H_2O were used.
b) The numbers in the column indicate the ratio of Ni-PO to reactant based on the available oxygen atom.
c) The numbers in the column indicate the mole ratio of NaOH for benzaldehyde.
d) MnO_2 was prepared according to a method of Attenburrow, *J. Chem. Soc.*, **1952**, 1094.

The oxidation of benzaldehyde with Ni-PO at various condition are summarized in Table I. Judging from the comparison of the oxidizing power of Ni-PO with that of active manganese dioxide, it was concluded that the oxidizing power of the former is stronger than that of the latter.

Concerning the mechanism of the formation of benzoic acid, it seemed reasonable that Cannizzaro reaction of benzaldehyde in aqueous alkaline solution took place in first step and Ni-PO was used for oxidation of resulting benzylalcohol to benzaldehyde as Chart 1. But, when benzaldehyde was treated in similar manner as mentioned previously without the addition of Ni-PO, the formation of benzoic acid was quite poor (2.1%). When the reaction was conducted by employing Ni-PO 0.1—0.5 times as much as the theoretical amount based on the available oxygen-contents, the yields of benzoic acid were obtained about 10—50% from benzaldehyde.

$$2C_6H_5CHO \xrightarrow{NaOH} C_6H_5CH_2OH + C_6H_5COONa$$

$$C_6H_5CH_2OH \xrightarrow{Ni\text{–}PO} C_6H_5CHO$$

Chart 1

Judging from these results, it was concluded that the Cannizzaro reaction of benzaldehyde scarcely took place in such a dilute alkaline solution shown in Table I. The formation of benzoic acid was well explained by the behavior of Ni-PO similar to that we previously reported[4)] on the ammonoxidation of some aldehydes showing as Chart 2.

Therefore, the mechanism of the formation of benzoic acid may be described in similar manner as Chart 3. In the previous paper,[5)] we reported that Ni-PO could be generate a

4) K. Nakagawa, H. Onoue, and K. Minami, *Chem. Commun.*, **1966**, 17.

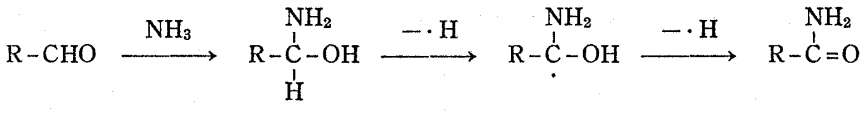

Chart 2

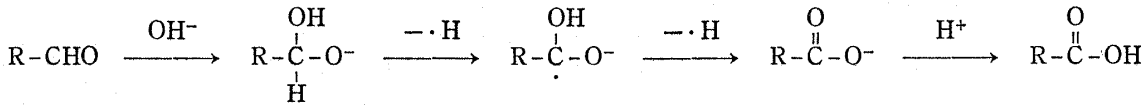

Chart 3

hydroxyl radical and abstracted hydrogen radical from a molecule at the favorable position. Konaka and coworkers[6] have suggested that the Ni-PO oxidation of benzhydrol involves the initial abstraction of the α-hydrogen atom, followed by the hydrogen atom abstraction from the OH group.

Table II summarized the results of the oxidation of several aromatic aldehydes in aqueous alkaline solution. In the case of aldehyde possessing a methyl group in the *p*-position, the methyl group was in part simultaneously oxidized at 60° to give dicarboxylic acid. When the reaction temperature was lowered in such cases, however, the oxidation of aldehyde group proceeded so predominantly that the corresponding monocarboxylic acid was obtained in pure state.

Table II. Oxidation of Aromatic Aldehydes in Alkaline Medium

Aldehyde	Temp. (°C)	Time (min)	Carboxylic acid (%)
o-Nitrobenz-	30	90	99.9
m-Nitrobenz-	30	90	98.9
p-Nitrobenz-	30	90	96.2
o-Chlorobenz-	30	90	97.9
p-Chlorobenz-	60	180	95.7
p-Anisaldehyde	60	180	94.0
3,4-Dimethoxybenz-	60	180	100.0
α-Naphthalene-	30	180	89.0
Furfural	30	90	93.5
2-Thiophene-	60	180	95.7
p-Tolualdehyde	60	180	62.5[a]
			13.7[b]
p-Tolualdehyde	30	180	58.2[a]
			1.2[b]

a) Yield of *p*-toluic acid.
b) Yield of terephthalic acid.

It has been reported[7] that the oxidation of furfural with potassium permanganate in alkaline solution resulted in the opening of the ring. However, in the oxidation with Ni-PO at room temperature, α-furoic acid was readily obtained in a good yield.

A great variety of oxidizing agents for the preparation of carboxylic acids by oxidation of the corresponding aldehydes has been reported, but the oxidation with Ni-PO is a much convenient and economical method because the work-up procedure is very simple and the oxidant can be stored at room temperature without a drop of activity for a long time and the oxidant recovered after the oxidation can be renewed with alkaline hypochlorite solution.

5) K. Nakagawa and T. Tsuji, *Chem. Pharm. Bull.* (Tokyo), **11**, 296 (1963).
6) R. Konaka, S. Terabe, and K. Kuruma, *J. Org. Chem.*, **34**, 1334 (1969).
7) J. Volhard, *Ann.*, **261**, 379 (1891).

Experimental

Ni-PO——Ni-PO was prepared from nickel sulfate and its available oxygen-content was determined by iodometry as reported in the previous paper.[3] Its quantity used in stoichiometric oxidation was calculated on the basis of the available oxygen-content.

Oxidation of Aromatic Aldehydes——Unless otherwise stated, the oxidation of the aromatic aldehydes detailed in Table II were carried out in a following procedure of benzaldehyde.

Oxidation of Benzaldehyde——To a solution of benzaldehyde (5.30 g) and sodium hydroxide (2.5 g) in 125 ml of water was added 19.3 g of Ni-PO (1.2 times the theoretical amount) and stirred for 90 minutes at 30° under nitrogen. The reaction mixture was filtered through a glass filter, Ni-PO was washed with water. The combined filtrate was extracted by ether to remove the unreacted benzaldehyde and acidified with dilute sulfulic acid. The solution deposited 5.75 g (94.3%) of benzoic acid.

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UDC 612.014.1 : 612.734-08

Studies on Isolated Smooth Muscle Cells. II.[1] Potentiation of Calcium Contraction of Isolated Smooth Muscle Cells from *Vas Deferens* of Guinea Pig by Cocaine

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Single smooth muscle cells were isolated from *vas deferens* of guinea pig and effect of cocaine on calcium contraction of the individual cells under partially depolarized condition was examined. Among the isolated cells, a few cells were contracted by 20 mM calcium chloride in medium containing 60 mM potassium chloride. Cocaine increased the ratio of contracted cells. The result suggested that cocaine facilitated calcium contraction of individual cells in the tissue and induced larger contraction of the tissue.

Keywords——cocaine; smooth muscle; smooth muscle cells; isolated cells; guinea pig; *vas deferens*

It is well known that cocaine potentiates various pharmacological responses.[3-7] It was also reported from this laboratory that cocaine potentiated calcium contraction of partially depolarized *vas deferens* of guinea pig.[8] Two mechanisms could be considered for the potentiation: 1) cocaine facilitates propagation of electrical excitation and induces synchronization of contraction of each muscle cell in the tissue which resulted in larger contraction of tissue, or 2) cocaine facilitates contraction of individual cells in the tissue and induces larger contraction. In order to clarify which mechanism worked for the potentiation, effect of cocaine on contraction of individual cells isolated from *vas deferens* of guinea pig was examined.

A male albino guinea pig weighing approximately 300 g was killed with a blow and a pair of *vas deferens* were isolated. The tissue was allowed to stand in calcium-free modified Tyrode solution (2.7 mM KCl, 137 mM NaCl, 1.0 mM $MgCl_2$, 5.6 mM glucose and 6.0 mM $NaHCO_3$) for 90 min at 30°. The medium was gently stirred by aeration and was changed by fresh

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