Studies on the Silk-Palladium Catalyst. II. General Properties

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(Received March 27, 1959)

In the previous paper[1] of this series it was reported that the silk-palladium catalyst possesses a number of excellent characteristics suitable for the purpose of catalytic hydrogenation. In order to obtain further information concerning the fundamental properties of the catalyst and to provide a basis for its practical application, it is intended in the present investigation to study its selectivity, durability and behaviors towards catalytic poisons. The present paper describes the results obtained in these experiments.

Results and Discussion

I. Selectivity of the Catalyst.—The catalytic efficiency of the silk-palladium catalyst was mainly examined in the previous paper[1] on the hydrogenation of nitrobenzene to aniline. In the present study the activity of the catalyst was examined with a variety of unsaturated compounds listed in Table I. The results obtained are also recorded in the same table.

Hydrogenation of C=C Double Bonds.—The C=C double bonds were found to be very easily reduced at low temperatures in the presence of the silk-palladium catalyst. For instance, it was found that mesityl oxide absorbed 70% of the theoretical amount of hydrogen at 30°C for 20 min. if the catalyst (containing ca. 0.08% of palladium metal per substrate) was supplied. This could be compared with the data obtained with 1% (by weight) Raney nickel in the presence of which it took 60 min. at 60°C for mesityl oxide to absorb the same amount of hydrogen. It was further noticed that the catalyst was able to selectively hydrogenate the C=C double bonds in mesityl oxide, crotonaldehyde and acrolein, leaving their carbonyl groups quite unattacked even at higher temperatures.

These results indicate that the silk-palladium catalyst is particularly suitable for the preparation of saturated aliphatic aldehydes and ketones from the corresponding unsaturated compounds. That azlactone and acetaminocinnamic acid are readily hydrogenated by the silk-palladium catalyst was previously reported[2-4]. The catalyst was, however, unable to reduce phenol at all, indicating that the double bonds in benzene rings are inert to the catalytic action.

Hydrogenation of Carbonyl Groups.—As described above, the silk-palladium catalyst was rather inactive in hydrogenating aliphatic carbonyl compounds. This conclusion was further confirmed in an attempt to reduce acetone and methyl ethyl ketone which were not reduced at all. It was, however, found that the hydrogenation of benzaldehyde in the presence of the catalyst readily gave rise to the formation of toluene. It is, therefore, probable that carbonyl groups attached to aromatic rings may be susceptible to the action of the catalyst. The catalyst which was recovered after having been used for the reduction of acetone was found to show no appreciable depression in the activity of hydrogenating nitrobenzene. It therefore seems that the failure in the hydrogenation of acetone (and other aliphatic

1) Y. Izumi, This Bulletin, **32**, 932 (1959).

2) S. Akabori, Y. Izumi, Y. Fujii and S. Sakurai, *Nature*, **178**, 323 (1956).

3) S. Akabori, Y. Izumi, Y. Fujii and S. Sakurai, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **77**, 1374 (1956).

4) S. Akabori, Y. Izumi, Y. Fujii and S. Sakurai, ibid., **78**, 168 (1957).

TABLE I. SPECIFICITY OF CATALYST

Substrate (g.)	Product	Yield (%)	Solvent	Catalyst (mg.)	Temp. (°C)	Reaction time (min.) 70%	Reaction time (min.) 100%
Mesityl oxide 41	Me-isobutyl ketone	86	—	350	30	21.5	60
Crotonaldehyde 21	Butyraldehyde	70	Ether	100	20	21	102
Acroleine 13	Propionaldehyde	100*	Ether	350	20	4	36
Acrylonitrile 50	Ethyl cyanide	79	—	100	6	47	71
Fumaric acid 12	Succinic acid	88	NaOH	350	53	16	28
Phenol 5	—	—	Methanol	350	170	—	—
Acetone 30	—	—	—	350	120	—	—
Methyl ethyl ketone 30	—	—	—	350	120	—	—
Benzaldehyde 11	Toluene	46	Methanol	350	27	41.3	426
Benzyl cyanide 6	Acetylphenethylamine	79	Ac_2O HOAc	350	75	179	276
Benzaldehyde 10.6	Dibenzylamine	65	Methanol NH_4OH	350	80	145	220
α-Ketoglutaric acid 5	Glutamic acid	50	Methanol NH_4OH	350	60	—	60
Nitrobenzene 4	Aniline	90	Methanol	350	20	9.5	23
MDAN** 12.4	MDAA***	80	Methanol	350	60	38	90
ω-Nitrostyrene 15	Phenylacetoaldoxime	35	Methanol	350	20	9.5	23
Diethyl nitro malonate 6.9	Diethyl aceto-aminomalonate	36	Ac_2O HOAc	350	100	31	90
Azobenzene 5.2	Hydrazobenzene	58	Methanol	100	20	95	185
Azobenzene 10	Aniline	88	Methanol	350	70	20	125
Dimethylglyoxime 3	—	—	Methanol	350	90	—	—
Acetophenonoxime 3	—	—	Methanol	350	70	—	—

* Calculated from dinitrophenylhydrazone.

** $(CH_3)_2CH\text{-}CH_2\text{-}CH(N(CH_3)_2)\text{-}CH(O\text{-}COC_6H_4NO_2)\text{-}CH_3 \cdot HCl$

*** $(CH_3)_2CH\text{-}CH_2\text{-}CH(N(CH_3)_2)\text{-}CH(O\text{-}COC_6H_4NH_2)\text{-}CH_3 \cdot HCl$

carbonyl groups) is an intrinsic property of the catalyst and not due to the poisoning by these compounds.

Reduction of Nitrile Groups.—It has already been mentioned that the catalyst was inactive in reducing the nitrile group of acrylonitrile, at least at low temperatures. Using acetic anhydride as a solvent, however, benzyl nitrile was converted to *N*-acetyl-phenethylamine in the presence of hydrogen and the silk-palladium catalyst.

Reductive Amination of α-Ketoglutaric Acid and Benzaldehyde.—The silk-palladium catalyst was able to convert α-ketoglutaric acid to glutamic acid in the presence of ammonia and hydrogen. When benzaldehyde was subjected to reductive amination under similar conditions, the main product obtained was identified as dibenzylamine.

Reduction of Nitro Groups.—In the previous paper[1] the reduction of nitrobenzene to aniline was employed as the test system for comparing the catalytic activity. In the present study, it was further found that 5-methyl-3-dimethyl-aminohexan-2-ol-*p*-nitrobenzoate hydrochloride (MDAN) was also readily hydrogenated by this catalyst to form the corresponding amine hydrochloride (MDAA) in as excellent a yield as in the case of the reduction of nitrobenzene. On the contrary, both ω-nitrostyrene and diethyl nitromalonate were reduced very slowly, yielding phenylacetaldoxime and diethyl acetamidomalonate, respectively; their yields also being low. It may be inferred that the catalyst is able effectively to catalyze the hydrogenation of aromatic nitro groups, but relatively weakly that of aliphatic nitro groups.

Reduction of Azobenzene.—Hydrogenation of azobenzene by means of the silk-palladium catalyst at low temperatures resulted in the formation of hydrazobenzene. At higher temperatures, on the other hand, the hydrogenation proceeded in two steps, as shown in Fig. 1, taking up more than one mole of hydrogen and the main product was aniline.

Reduction of Oximino Groups.—It was already reported[2-4] that the silk-palladium catalyst could catalyze the reduction of

TABLE II. DURABILITY TEST OF CATALYST

Substrate	Catalyst sample (Pd mg.)	Reaction temp. (°C)	Reaction velocity	Temp. at which H_2 uptake starts (°C)
Diethyl maleate 50 g.	Original silk-Pd (9.2)	150	9.5	105
	After 1st recovery	〃	22.0	90
	〃 2nd 〃	〃	18.0	70
	〃 3rd 〃	〃	17.0	50
	〃 4th 〃	〃	25.0	60
〃	Original carbon-Pd (10)	〃	30.0	50
	After 1st recovery	〃	7.4	60
	〃 2nd 〃	〃	9.1	70

benzyldioxime, α-acetoximino-phenylpropionic ethyl ester and glutaric diethyl ester to the corresponding amines. It was, however, found in the present study that both dimethylglyoxime and acetophenone oxime could not be reduced at all.

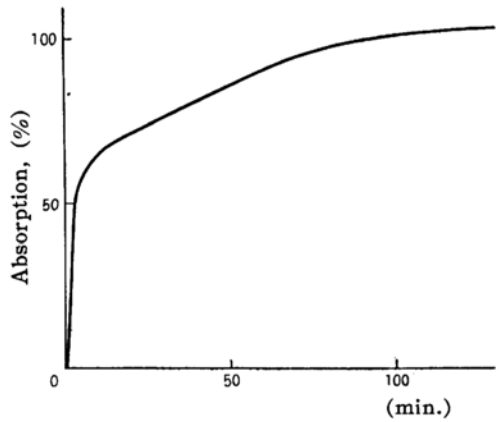

Fig. 1.

II. Durability of Catalyst.—The hydrogenation of diethyl maleate to diethyl succinate was selected as the test system to study the durability of the silk-palladium catalyst. The durability test was conducted in comparison to the carbon-palladium catalyst[5] commonly used as a hydrogenation catalyst.

The results of the test are summarized in Table II. As can be seen from Table II, a very interesting phenomenon was observed concerning the durability of silk-palladium catalyst. When freshly prepared silk-palladium catalyst was used, the temperature at which hydrogen absorption starts was relatively high (105°C) and the reaction proceeded rather slowly. In the hydrogenation using the catalyst recovered from the first run, however, the temperature of initial hydrogen uptake was not only considerably lowered (90°C), but also the reaction itself was accelerated. This elevated activity of pre-used catalyst persisted at least after the fifth run. Such a phenomenon was not observed with the carbon-palladium catalyst which showed the highest activity in the first run and lost it thereafter. It does not seem unreasonable to suppose that such an excellent durability of the silk-palladium catalyst is due to the possibility that the palladium attached to the carrier protein may be sterically rearranged after the first run in favor of the catalytic activity.

In connection with the durability, it might be pointed out that the relatively high ignition point (as compared to the carbon-palladium catalyst) and bulkiness of the fibrous silk-palladium catalyst were very convenient for its handling and recovery.

III. Poisoning of Catalyst.—The silk-palladium catalyst was found to be sensitive to poisonous substances or to various chemical treatments. In Table III are summarized the results of such inhibition studies. In examining the toxic effects, the hydrogenation of either nitrobenzene or diethyl maleate was adopted as the test system and the velocity of hydrogen uptake was calculated and compared as previously described. The carbon-palladium catalyst was also tested as a reference.

Inhibition by Mercaptan.—As can be seen from Table III, the addition of or treatment with ethyl mercaptan markedly decreased the catalytic activity of silk-palladium to hydrogenate both nitrobenzene and diethyl maleate. This poison, which is a well-known inhibitor of palladium catalysts, was also deleterious to the carbon-palladium catalyst. The silk catalyst was, however, much more

5) R. Mozingo, "Organic Syntheses", Vol. 26, John Wiley & Sons., Inc., New York (1947), p. 77, (Procedure D).

TABLE III. EFFECT OF VARIOUS TREATMENTS AND ADDITIONS ON THE ACTIVITY OF SILK AND CARBON-PALLADIUM CATALYSTS

Substrate	Catalyst (Pd mg.)	Addition (mg.)	Reaction temp. (°C)	Reaction velocity
Nitrobenzene 4 g. in 50 ml. MeOH	Silk-Pd (18.4)*	None	50	84
	Carbon-Pd (20)	〃	21	100
	Silk-Pd (18.4)** treated with EtSH	〃	50	22
	Carbon-Pd (20)** treated with EtSH	〃	〃	100
	Silk-Pd (18.4)	EtSH	〃	16.6
	Carbon-Pd (20)	〃	〃	16.6
	Silk-Pd (18.4) acetylated	None	19	1.75
Diethyl maleate 50 ml.	Silk-Pd (18.4)	〃	150	22
	The same after recovery	〃	〃	50
	Silk-Pd (18.4)	EtSH	〃	1.1
	Carbon-Pd (20)	〃	〃	6.25
	Silk-Pd (18.4)	$Fe(NO_3)_3 \cdot 12H_2O$ (40)	〃	1.7
	The same after 1st recovery	〃	〃	22.5
	The same after 2nd recovery	〃	〃	7.4
	The same after 3rd recovery reactivated with EDTA	None	〃	125
	Silk-Pd (18.4)	$Cu(NO_3)_3 \cdot 3H_2O$ (25)	〃	5.3
	The same after 1st recovery	〃	〃	2.7
	The same after 2nd recovery reactivated with EDTA	None	〃	45.0

* Calculated from the activity at 20°C.

** The catalyst was brought in contact with 15 ml. of 0.02% EtSH methanol.

seriously poisoned by the mercaptan than the carbon catalyst. It was, however, noted that no palladium was extracted from the silk catalyst in the presence of ethyl mercaptan, in contrast to the considerable extraction observed in the case of the carbon catalyst. It appears probable that the mercaptan inhibits the catalysts by directly blocking the active palladium.

Inhibition by Metal Ions.—Unlike the carbon palladium and other palladium catalysts, the activity of the silk-palladium catalyst was greatly lowered in the presence of ferric and cupric ions as supplied in the form of nitrates. Cupric ion was somewhat more toxic than ferric ion. It is more likely that these metal ions form chelate linkages with the carrier protein, sometimes three-dimentionally, and thus prevent the reactant from approaching the active palladium atoms which are distributed inside the fibroin micelles.

Reactivation by Ethylenediamine Tetraacetic Acid.—The catalyst poisoned by ferric or cupric ion could be reactivated by treating it with ethylenediamine tetraacetic acid (EDTA). As a matter of especial interest it was revealed that the catalyst thus reactivated exerts very powerful catalytic activity. In fact, EDTA treatments of ferric ion- and cupric ion-poisoned catalysts produced preparations 2.5 and 3 times, respectively, as active as the control catalyst (pre-used once under normal conditions, see above). This peculiar phenomenon suggests that the internal structure of the carrier protein was altered on removal of the metal ions by EDTA to favor the catalytic activity.

Effect of Acetylation.—As previously reported[4], an acetylated silk-palladium catalyst prepared from pre-acetylated silk fibroin and palladous chloride in the usual manner is more active than the unacetylated silk-palladium catalyst in hydrogenating azlactone. Quite unexpectedly, however, it was found in the present investigation that acetylation with acetic anhydride of the pre-formed silk-palladium catalyst greatly depressed the activity. It may be inferred that acetylation of the carrier protein of silk-palladium catalyst blocks the

openings of the fibroin micelles in which the active palladium atoms reside and thus makes it difficult for the substrate to reach the active centers.

In short, there are two types of inhibition; the first, including ethyl mercaptan, inhibits the catalyst by directly combining with palladium atoms and the second, such as ferric ion, cupric ion, and acetylation, by modifying the structure of carrier fibroin. The latter type of inhibition is unique to the silk-palladium catalyst and has special bearings to the structural interpretation of the catalytic activity.

Experimental

I. Preparation of the Catalyst. — The catalyst was prepared by the method of previous papers.

II. Selectivity of the Catalyst.—*1) Reduction of C=C Double Bonds.—a) Reduction of fumaric acid.*—Seventy milliliters of aqueous solution containing 12 g. of fumaric acid adjusted to pH 7.0 with 20% sodium hydroxide and 100 mg. of the catalyst were shaken in 90 kg./cm² of hydrogen at 53°C. The reduction was complete after 28 min. The reaction mixture was filtered and the filtrate evaporated to 60 ml. which was then acidified with 20 ml. of concentrated hydrochloric acid. After standing overnight, 9.5 g. succinic acid (m.p. 184°C) was obtained. It was confirmed that the product is devoid of unsaturated bond by infrared spectroscopy.

b) Reduction of mesityl oxide.—Forty-one grams of mesityl oxide was reduced, using 350 mg. of the catalyst with 90 kg./cm² hydrogen at 30°C. The reduction was complete after an hour and 1 mol. of hydrogen was absorbed per 1 mol. of mesityl oxide. 36 g. of methyl isobutyl ketone (b. p. 113~115°/760 mm Hg) was obtained from the reaction mixture. Infrared spectroscopy showed a disappearance of unsaturated bond.

c) Preparation of Raney-nickel catalyst.—0.5 g. of Raney-nickel-alloy was added to 6 ml. of 25% sodium hydroxide solution in small portions during 5 min. and then heated for an hour on a water bath. The catalyst sludge was washed three times with each 30 ml. of water and washed with methanol decantation.

d) Reduction of crotonaldehyde. — Twenty-one grams of crotonaldehyde dissolved in 30 ml. of ether was reduced at 20°C with 85 kg./cm² hydrogen using 100 mg. of the catalyst. The reduction was continued for 102 min. The reaction mixture was distilled in a 30 cm. dephlegmator. Fifteen grams of butyraldehyde (b. p. 75°C) was obtained and this sample contained no C=C double bonds as examined by infrared spectroscopy.

Analysis of the dinitrophenylhydrazone of this compound showed the following results.

Anal. Found: N, 22.6. Calcd. for $C_{10}H_{12}O_4N_4$: N, 22.2%.

The aldehyde group of crotonaldehyde was never reduced even at higher temperature (130°C).

e) Reduction of acrolein.—When 13 g. acrolein in 20 ml. ether was reduced at 20°C with 85 kg./cm² hydrogen using 350 mg. of the catalyst, hydrogen was readily absorbed and the absorption ceased in 36 min.

On adding 2, 4-dinitrophenylhydrazone to 1/100 portion of the reaction mixture, there was obtained 550 mg. propionaldehyde-2, 4-dinitrophenylhydrazone with m.p. 126°C, indicating a yield of 100% in the reduction.

Anal. Found: N, 23.7. Calcd. for $C_9H_{10}O_4N_4$: N, 23.5%.

By distillation of the residue through 15 cm. Widmer column 2 g. of the distillate of b.p. 49°C was obtained. It was proved infrared spectroscopically that this material has no C=C double bond.

This catalyst did not catalyze the reduction of the aldehyde group of this product even when the reduction was carried out at 120°C.

f) Reduction of acrylonitrile. — Fifty grams of acrylonitrile was reduced at 6°C, 100 atm. with 100 mg. of this catalyst and spontaneously the reaction temperature rose to 24°C. The reaction was concluded for 71 min. By fractional distillation, 41 g. of ethyl cyanide (b. p. 96~98°C) was obtained. The product was identified by infrared spectroscopically and gas-chromatographically.

g) Reduction of phenol.—Phenol was not hydrogenated in methanol at 170°C with 80 atm./cm² hydrogen by 350 mg. of this catalyst.

2) Reduction of Carbonyl Compounds.—a) Reduction of acetone and methyl ethyl ketone.—Thirty grams of acetone (or methyl ethyl ketone) was not reduced with 90 kg./cm² hydrogen at 120°C in the presence of 350 mg. of the catalyst. (Activity of recovered catalyst was tested by the method of previous report[1]). It was clear that the activity of this catalyst was maintained through this condition).

b) Reduction of benzaldehyde.—Eleven grams of benzaldehyde dissolved in 40 ml. of methanol was reduced with 90 kg./cm² hydrogen at 27°C using 350 mg. of catalyst. The reduction was continued for 426 min. absorbing of hydrogen per mol. of the aldehyde. 4.3 g. of toluene was obtained from the reaction product, and identified by infrared spectrum.

3) Reduction of Cyanides.—Reduction of benzyl cyanide.—Using 350 mg. of the catalyst 6.0 g. of benzylcyanide was reduced with 100 kg./cm² hydrogen at 75°C in a mixture of 15 g. acetic anhydride and 30 g. of acetic acid. The reduction was completed in 276 min. Six grams of acetylphenethylamine (b. p. 145°C/5 mm Hg) was obtained by fractional distillation reaction mixture under reduced pressure.

Anal. Found: N, 8.35. Calcd. for $C_{10}H_{13}ON$: N, 8.58%.

4) Reductive Aminations of Carbonyl Compounds.—a) Reductive amination of benzaldehyde.—Using 350 mg. of the catalyst 10.6 g. of benzaldehyde was reduced in the presence of 6.7 ml. of aqueous ammonia and 25 ml. of methanol with 94 kg./cm² hydrogen at 80°C. The reduction was completed in 220 min. By fractional distillation, 6.4 g. of

dibenzylamine (b. p. 156°C/11mmHg) was obtained. The results of analysis were as follows:

Dibenzylamine. *Anal.* Found: N, 6.74. Calcd. for $C_{14}H_{15}N$: N, 7.07%.

Dibenzylamine hydrochloride. *Anal.* Found: N, 5.76. Calcd. for $C_{14}H_{16}NCl$: N, 6.00%.

b) Reductive amination of α-ketoglutaric acid.—Five grams of α-ketoglutaric acid, 14 ml. of 28% ammonia and 36 ml. of methanol were placed in the autoclave. Hydrogenation was carried out at 60°C, 90 kg./cm² for 1 hr. After the reaction mixture was filtered, the filtrate was evaporated in vacuo, dissolved in 5 ml. of water and adjusted to pH 3.2 with hydrochloric acid, then 2.5 g. of glutamic acid was obtained. This product was identified paper-chromatographically and was optically inactive.

5) Reductions of Nitro Compounds.—a) Reduction of nitrobenzene.—This was hydrogenated in the same way as previously reported[1].

b) Reduction of 5-methyl-3-dimethylaminohexyl-(2) p-nitrobenzoate hydrochloride (MDAN).—12.4 g. of MDAN in 40 ml. methanol was reduced at 60°C, 80 atm. for 90 min. with the 350 mg. catalyst. The reaction mixture was concentrated to a volume of 20 ml. and kept at 0°C overnight. There was obtained 8 g. of 5-methyl-3-dimethylaminohexyl-(2) *p*-aminobenzoate hydrochloride (MDAA) with m. p. of 217°C. The mixed melting point with an authentic sample showed no depression.

c) Reduction of ω-nitrostyrene.—When 15 g. of ω-nitrostyrene was hydrogenated at 20°C, 97 atm. in the presence of 350 mg. of the catalyst, the absorption of hydrogen came to an end in 23 min. Filtration, concentration and vacuum-distillation gave 6 g. of the product boiling at 96~108°C/2 mmHg, which crystallized on standing. 4.8 g. of the crystal (m. p. 102°C) obtained. The material was identified as phenylaldoxime.

Anal. Found: N, 10.0. Calcd. for C_8H_9ON: N, 10.4%.

d) Reduction of diethylnitromalonate. — Using 350 mg. of the catalyst 6.9 g. of diethylnitromalonate was reduced in a mixture of 16 ml. of acetic anhydride and 27 ml. of acetic acid with 100 kg./cm² hydrogen at 100°C for 90 min. Two grams of diethyl acetoaminomalonate (m. p. 89°C) was obtained from the reaction mixture after evaporation, addition of 5 ml. of ethyl acetate and overnight standing.

Anal. Found: C, 50.11; H, 7.13; N, 6.52. Calcd. for $C_9H_{15}O_5N$: C, 49.76; H, 6.96; N, 6.45%.

6) Reduction of Azobenzene.—a) Reduction to hydrazobenzene.—5.2 g. of azobenzene was reduced in 50 ml. of ethanol with 94 kg./cm² hydrogen at 20°C. The reduction was concluded for 185 min. absorbing 1 mol. of H_2 per mol. of azobenzene. The reaction mixture was evaporated and the residue was dissolved in 10 ml. of hot methanol. After cooling, 3.0 g. of hydrazobenzene (m. p. 288°C) was obtained as orange crystals. The crystals identified with infrared spectroscopy.

b) Reduction to aniline.—Ten grams of azobenzene was reduced with the 350 mg. catalyst under the same conditions as above except that the reaction temperature was elevated to 70°C. The reduction was concluded for 125 min. Thirteen grams of acetanilide (m. p. 112°C) was obtained from the reaction mixture by the following method. The filtered reaction mixture was evaporated in the presence of 10 g. of acetic anhydride and the residue dissolved in 10 ml. of water and cooled.

III. Durability of Catalyst.—*1) Preparation of the Carbon-Palladium Catalyst.*—The catalyst was prepared by the method of R. Mozingo[5] and stored in wetness.

2) General Procedures.—Hydrogenation experiments were carried out using an autoclave as already reported[1] and the reaction velocity of the hydrogenation of diethyl maleate was calculated according to the equation:

Reaction velocity = 100/reaction time (in min.)

where the reaction time is the time required from 30 to 70% of the hydrogen uptake proceeds.

IV. Poisoning of Catalyst. — *1) General Procedures.* — Hydrogenation velocity of diethyl maleate was measured by the III—*2* method and the reaction velocity of the hydrogenation of nitrobenzene was calculated by the method of the previous paper[1].

2) Inhibition of Mercaptan.—a) Preparation of inhibited catalyst. — Two hundred and fifty milligrams of the silk-palladium or carbon-palladium containing 20 mg. of palladium (In the following experiments, this amount of catalyst was used as a unit.) was mixed with 50 ml. of 0.02% ethyl mercaptan methanol solution, stirred for 15 min. at 20°C, filtered and washed with water and methanol.

b) Poisoning test by substrate containing mercaptan.—b-1) Reduction of nitrobenzene.—The reaction velocity was measured in relation to the reduction system containing 1 mg. of ethylmercaptan, 4 g. of nitrobenzene, 50 ml. of methanol and 1 unit of each catalyst.

b-2) Reduction of dietyl maleate (DEM).—The reaction velocity was measured in relation to 50 g. of DEM containing 4 mg. of ethyl mercaptan.

3) Inhibition by Metal Ions (Metal Ion Effect to the Reduction System of DEM).—Forty milligrams of $Fe(NO_3)_3 \cdot 12H_2O$ or 25 mg. of $Cu(NO_3)_2 \cdot 3H_2O$ was added to the reaction system containing 50 g. of DEM and 1 unit of each catalyst, and the reaction velocity was measured.

4) Reactivation by Ethylenediamine Tetraacetic Acid (EDTA). — Recovered catalysts of experiment (IV-*3*) were boiled with 0.5% EDTA for 5 min., filtered and washed with water and methanol. (Filtered solution was apparently colored with Cu^{2+} or Fe^{3+} EDTA complex.) Using this catalyst, 50 g. of DEM was reduced with hydrogen at 150°C.

5) Effect of Acetylation.—Acetylation of silk-palladium.—Three hundred and fifty milligrams of silk-palladium catalyst was boiled with 50 ml. of an acetic acid anhydride for 2 hr. filtered and washed with water and methanol.

Summary

1. The silk-palladium catalyst was found to catalyze the hydrogenation of

aliphatic C=C double bonds, aldehyde, nitriles and nitro groups directly attached to aromatic rings, azobenzene, and some oximino groups, but relatively weakly that of aliphatic nitro groups. The catalyst was completely inactive to aromatic C=C double bonds, aliphatic carbonyl (with the exception of α-ketoglutarate which was reduced to glutamate in the presence of ammonia), nitriles, and several oximes.

2. The catalyst, when used once, considerably increased its catalytic activity and this elevated activity persisted after several repeated uses.

3. Ethyl mercaptan was poisonous to the catalyst probably due to its combination with the active palladium atoms. Ferric and cupric ions inhibited the catalyst by forming chelate linkages with the carrier protein. The inhibition by these metal ions could be restored by ethylenediamine tetraacetic acid. Acetylation of the catalyst with acetic anhydride markedly reduced the activity, although the treatment of silk fibroin with acetic anhydride prior to the preparation of the catalyst gave a strongly active catalyst.

The author wishes to express his hearty gratitude to Professor Shiro Akabori for encouragement and valuable discussion. He is also indebted to Mr. Yasuo Fujii for cooperation in some phases of this work, to Dr. S. Sakurai and to the Ajinomoto Co., Inc. for the gift of diethyl maleate, and to Dr. R. Sato for his advice on preparing this manuscript.

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