BIOORGANIC CHEMISTRY 22, 387–394 (1994)

Catalytic Properties of Carbonyl Reductase from Rabbit Kidney for Acetohexamide and Its Analogs

YORISHIGE IMAMURA, TOSHIYUKI HIGUCHI, MASAKI OTAGIRI, SHINJI NAGUMO,* AND HIROYUKI AKITA*

*Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1, Oe-honmachi, Kumamoto 862, Japan; and *School of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274, Japan*

Received May 31, 1994

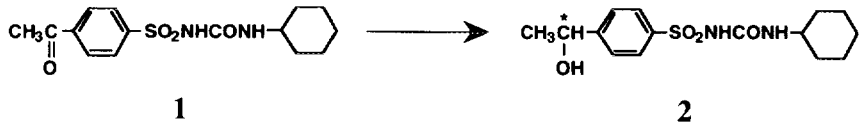
Analogs submitted by ethyl, *n*-propyl, *n*-butyl, and isopropyl groups instead of methyl group adjacent to a ketone group of acetohexamide were synthesized and the structural requirements of carbonyl reductase from rabbit kidney for these analogs were kinetically examined. The hydrophobicities in straight-chain alkyl groups of acetohexamide analogs were found to play an important role in the catalytic activity and substrate-binding capacity of the enzyme. We propose the possibility that a hydrophobic pocket is located in the substrate-binding domain of the enzyme.

INTRODUCTION

Drugs with a ketone group within their structures have been shown to be reduced to the corresponding alcohol metabolites through enzymatic system in the living body (*1*, *2*). The produced alcohol metabolites, in many cases, are pharmacologically active and contribute to their therapeutic effect (*3–5*). For example, an oral antidiabetic drug, acetohexamide [4-acetyl-*N*-(cyclohexylcarbamoyl)benzenesulfonamide], is stereoselectively reduced to (−)-hydroxyhexamide when administered to humans and rabbits, as shown in Fig. 1, and (−)-hydroxyhexamide has a hypoglycemic action (*6*, *7*). For this reason, it is important to examine the nature of enzymes responsible for the metabolic reduction of drugs with a ketone group. The metabolic reduction of drugs with a ketone group is mainly catalyzed by carbonyl reductase (EC1.1.1.184) (*2*, *8–11*), which is a member of aldo–keto reductase family similar to aldehyde reductase (EC 1.1.1.2) and aldose reductase (EC1.1.1.21) (*12*). So far, a variety of carbonyl reductases have been purified from the liver, kidney, lung, and brain of mammalian species (*2*, *8*, *13–16*). However, information on catalytic properties of purified carbonyl reductases for drugs with a ketone group has been limited.

Recently, we have purified a new carbonyl reductase from rabbit kidney by using acetohexamide as a substrate (*10*). The purified enzyme can be regarded as a drug-metabolizing enzyme because it reduces many drugs with a ketone group like befunolol, metyrapone, haloperidol, loxoprofen, and daunorubicin. The purpose of the present study is to synthesize analogs substituted by ethyl, *n*-propyl,

0045-2068/94 $6.00



1 2

FIG. 1. Enzymatic reduction of acetohexamide to (−)-hydroxyhexamide.

n-butyl, and isopropyl groups instead of the methyl group adjacent to a ketone group of acetohexamide and to elucidate the structural requirement of carbonyl reductase from rabbit kidney for acetohexamide and its analogs.

RESULTS

Synthesis of Acetohexamide Analogs

Acetohexamide analogs [4-alkanoyl-*N*-(cyclohexylcarbamoyl)benzenesulfonamides] were synthesized as shown in Fig. 2. Commercially available *n*-propylbenzene (**3**) was treated with chlorosulfonic acid to give 4-*n*-propylbenzenesulfonyl chloride (**4**), which was used for the next reaction without further purification. Treatment of **4** with aqueous NH_3 afforded 4-*n*-propylbenzenesulfonamide (**5**). The para-substitution pattern of **5** was confirmed by the fact that two doublets possessing 8.3 Hz coupling constant appeared at δ 7.30 and δ 7.83, respectively. Oxidation of **5** with chromium trioxide in acetic acid gave 4-*n*-propanoylbenzenesulfonamide (**6**). The existence of the benzyl carbonyl group was confirmed by means of infrared spectrum (1675 cm^{-1}). The obtained **6** was treated with cyclohexylisocyanate in the presence of potassium carbonate (K_2CO_3) to provide 4-*n*-

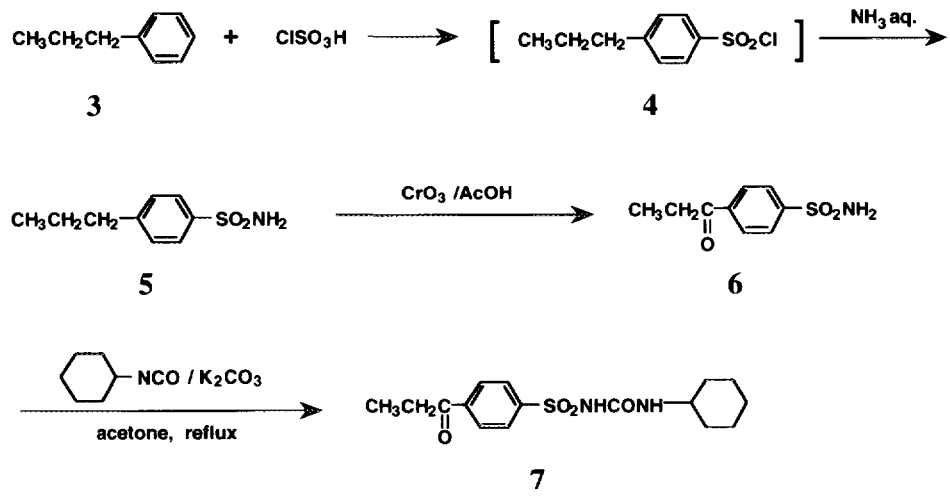
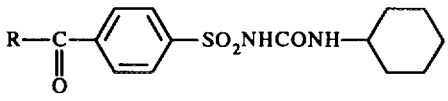


FIG. 2. Synthetic route of acetohexamide analogs.

TABLE I

Substrate Specificity of Carbonyl Reductase from Rabbit Kidney for Acetohexamide and Its Analogs



R	K_m (mM)	V_{max} (units/mg)
Methyl	0.50	1.24
Ethyl	0.36	5.41
n-Propyl	0.23	6.28
n-Butyl	0.12	11.9
Isopropyl	0.34	4.14

propanoyl-*N*-(cyclohexylcarbamoyl)benzenesulfonamide (**7**). The structure of **7** was confirmed by elemental analysis and physical methods (ir and NMR). 4-*n*-Butanoyl-, 4-*n*-pentanoyl-, and 4-isobutanoyl-*N*-(cyclohexycarbamoyl)benzenesulfonamide were synthesized by the same procedure as mentioned above from *n*-butyl-, *n*-pentyl-, and isobutylbenzene, respectively.

Reduction of Acetohexamide and Its Analogs Catalyzed by Carbonyl Reductase from Rabbit Kidney

Table 1 shows the kinetic parameters for the reduction of acetohexamide and its analogs catalyzed by carbonyl reductase from rabbit kidney; K_m and V_{max} are apparent Michaelis constant and maximal velocity, respectively. Judging from these results, it is likely that the hydrophobicities in straight-chain alkyl groups of acetohexamide analogs contribute to catalytic activity of the enzyme. In order to further elucidate the structural requirement of the enzyme for substrate, the common logarithm of k_{cat}/K_m values of the enzyme for acetohexamide and its analogs was plotted against the common logarithm of their partition coefficients (Fig. 3). k_{cat} is referred to as catalytic constant and the k_{cat}/K_m value determines the binding capacity of substrate to enzyme. As expected, a significant correlation was observed between k_{cat}/K_m values of the enzyme for acetohexamide and its analogs with a straight-chain alkyl group and their partition coefficients. On the other hand, k_{cat}/K_m value of the enzyme for acetohexamide analog with isopropyl group was smaller than that with the *n*-propyl group; therefore, in the case of acetohexamide analog with the iospropyl group, the plot was somewhat distant from the regression line of the plots for acetohexamide and its analogs with a straight-chain alkyl group.

DISCUSSION

Our previous papers have demonstrated that arginine and lysine residues located in coenzyme-binding domain and histidine residue located in substrate-binding

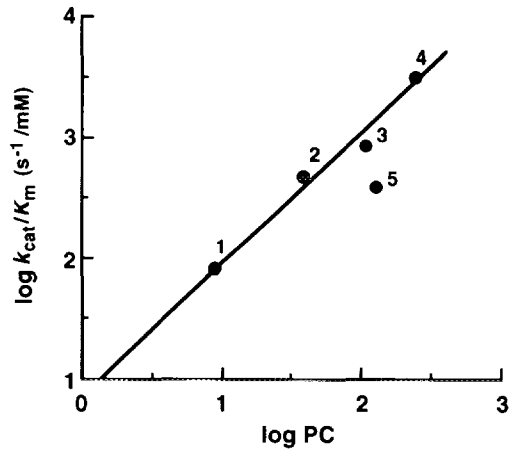


FIG. 3. Relationship between the k_{cat}/K_m values of carbonyl reductase from rabbit kidney for acetohexamide and its analogs and their partition coefficients (PC). *R*: 1, methyl; 2, ethyl; 3, *n*-propyl; 3, *n*-butyl; 5, isopropyl.

domain of carbonyl reductase from rabbit kidney are essential for its catalytic activity (*17*, *18*). In the present study, we provide evidence that the hydrophobicities in straight-chain alkyl groups of acetohexamide analogs have an important role in the catalytic activity and substrate-binding capacity of the enzyme. Interestingly, the k_{cat}/K_m value of the enzyme for acetohexamide analog with the isopropyl group was smaller than that with the *n*-propyl group. This may be because the branched-chain alkyl group causes a steric hinderance in the binding of the analog to the enzyme. Detailed studies should be made to elucidate the structural requirement of the enzyme. However, because of poor solubility of acetohexamide analogs in the enzyme solution, it was difficult to measure the catalytic activity of the enzyme for the analogs with a straight-chain alkly group more than four carbon atoms such as the *n*-pentyl and *n*-hexyl groups.

Recently, we have demonstrated that k_{cat}/K_m values of carbonyl reductase from rabbit kidney for 4-acetylpyridine analogs substituted by straight-chain alkyl groups instead of the methyl group increase with an increasing number of carbon atoms up to the *n*-pentyl group (five carbon atoms), whereas k_{cat}/K_m values of the enzyme for 4-acetylpyridine analogs with *n*-hexyl, *n*-heptyl, and *n*-octyl groups decrease slightly with an increasing number of carbon atoms (*10*). These findings indicate that a hydrophobic pocket, which is fitted most effectively to the *n*-pentyl group, is located in the substrate-binding domain of the enzyme. It is reasonable to assume that the hydrophobic pocket plays an important role in the binding of acetohexamide and its analogs to the enzyme.

Acetohexamide has been reported to be stereoselectively reduced to (−)-hydroxyhexamide when administered orally to humans and rabbits (*6*, *7*). Furthermore, (−)-hydroxyhexamide with almost 100% enantiomeric purity was isolated from the reaction mixture of acetohexamide with the cytosolic fraction of rabbit liver (*19*). Although the absolute configuration of (−)-hydroxyhexamide is not yet

determined, it is predictable that the reduction of acetohexamide catalyzed by carbonyl reductase from rabbit kidney gives (−)-hydroxyhexamide of S-configuration, based on the Baumann–Prelog rule (*20*). (−)-Hydroxyhexamide exhibits stronger hypoglycemic action than acetohexamide itself and contributes largely to the overall hypoglycemic action after oral administration of acetohexamide (*6*). For the development of new oral antidiabetic drugs, we are currently investigating the stereoselective reduction of acetohexamide analogs catalyzed by carbonyl reductase from rabbit kidney and the hypoglycemic action of the alcohol metabolites produced.

EXPERIMENTAL

Synthetic Chemistry

General methods. All melting points were measured on a Yanaco MP-S3 micro-melting point apparatus and were uncorrected. Infrared spectra were measured on a JASCO A-3 spectrophotometer. NMR spectra were measured on a JEOL EX-4000 instrument and $CDCl_3$ was used as a solvent unless otherwise stated, with tetramethylsilane as an internal reference. High-resolution mass spectra (HRMS) were obtained with a JEOL JMS-D 300 spectrometer. All evaporations were performed under reduced pressure. For column chromatography, silicagel (Kieselgel 60) was employed.

4-Alkylbenzenesulfonamides. Chlorosulfonic acid (97%, 28.6 ml, 5 equivalents) was added to a stirred and cooled (0°C) solution of **3** (10 g, 0.083 mol) by means of a dropping funnel, and the mixture was heated at 40°C for 30 min. The reaction mixture was poured into the ice water (150 ml) with stirring and extracted with ether. The ether layer was washed with saturated brine and dried over anhydrous $MgSO_4$. Evaporation of the organic solvent gave a crude **4**, which was used for the next reaction without further purification. The crude **4** was added to an aqueous ammonia solution (70 ml) with stirring and the mixture was heated at 40°C for 30 min. The reaction mixture was diluted with water and extracted with ether. Ether layer was dried over $MgSO_4$ and evaporated to give an oily product, which was chromatographed on silicagel (300 g) to afford a crude **5** from the *n*-hexane–AcOEt (2 : 1, v/v) eluate. Recrystallization of a crude crystal form *n*-hexane–Et_2O gave colorless needles **5** (68% yield from **3**). mp 98–101°C; ^{1}H-NMR δ 0.95 (t, J = 7.3 Hz, 3H, CH_3), 1.61–1.70 (m, 2H, CH_2), 2.65 (t, J = 7.3 Hz, 2H, benzylic-H), 5.13 (br. s, 2H, NH_2), 7.30, 7.83 (each d, J = 8.3 Hz, 2H, aromatic-H); ir (nujol) cm^{-1} 3350, 3250; *Anal.* Calcd for $C_9H_{13}O_2NS$: C, 54.27; H, 6.53; N, 7.04. Found: C, 54.18; H, 6.58; N, 7.21. Other 4-alkylbenzenesulfonamides were obtained by the same procedure as the preparation of **5** from the corresponding alkylbenzenes. 4-*n*-Butylbenzenesulfonamide: Recrystallization from *n*-hexane–Et_2O to give colorless needles, 71% yield from *n*-butylbenzene. mp 95–97°C; ^{1}H-NMR δ 0.93 (t, J = 7.3 Hz, 3H, CH_3), 1.31–1.40 (m, 2H, CH_2), 1.57–1.65 (m, 2H, CH_2), 2.68 (t, J = 7.6 Hz, 2H, benzylic-H), 5.01 (br. s, 2H, NH_2), 7.31, 7.83 (each d, J = 8.3 Hz, 2H, aromatic-H); ir (nujol) cm^{-1} 3350, 3260; *Anal.* Calcd for $C_{10}H_{15}O_2NS$: C,

56.31; H, 7.09; N, 6.57. Found: C, 56.25; H, 7.06; N, 6.48. 4-*n*-Pentylbenzenesulfonamide: Recrystallization from *n*-hexane–AcOEt to give colorless needles, 70% yield from *n*-pentylbenzene. mp 89°C; ^{1}H-NMR δ 0.90 (t, J = 6.8 Hz, 3H, CH_3), 2.67 (t, J = 7.8 Hz, 2H, benzylic-H), 5.00 (br. s, 2H, NH_2), 7.31, 7.83 (each d, J = 8.3 Hz, 2H, aromatic-H); ir (nujol) cm^{-1} 3350, 3250; *Anal.* Calcd for $C_{11}H_{17}O_2NS$: C, 58.12; H, 7.54; N, 6.16. Found: C, 58.03; H, 7.40; N, 6.07. 4-Isobutylbenzenesulfonamide: Recrystallization from *n*-hexane–AcOEt to give colorless plates, 55% yield from isobutylbenzene. mp 96–98°C; ^{1}H-NMR δ 0.91 (d, J = 6.3 Hz, 6H, isopropyl Me), 1.82–1.96 (m, 1H, methine), 2.54 (d, J = 7.3 Hz, 2H, benzylic-H), 7.28, 7.83 (each d, J = 8.3 Hz, 2H, aromatic-H); ir (nujol) cm^{-1} 3340, 3250; *Anal.* Calcd for $C_{10}H_{15}O_2NS$: C, 56.31; H, 7.09; N, 6.57. Found: C, 56.22; H, 7.07; N, 6.59.

4-Alkanoylbenzenesulfonamides. A suspension of chromium trioxide (CrO_3, 22.42 g, 5 equivalents) in acetic acid (50 ml) was added to a stirred and cooled (0°C) solution of **5** (8.165 g, 0.041 mol) in acetic acid (20 ml) and the mixture was brought to ambient temperature and stirred for 5 h. After isopropyl alcohol (10 ml) was added to the reaction mixture, the whole was condensed under the reduced pressure to give a crude residue. It was diluted with water and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to give a crystal, which was recrystallized from *n*-hexane–EtOH to afford colorless needles, 4-*n*-propanoylbenzenesulfonamide **6** (52% yield from **5**). mp 133–135°C; ^{1}H-NMR δ 1.19 (t, J = 7.1 Hz, 3H, CH_3), 3.09 (q, J = 7.1 Hz, 2H, -CH_2CO-), 4.86 (br. s, 2H, NH_2), 8.00, 8.12 (each d, J = 8.5 Hz, 2H, aromatic-H); ir (nujol) cm^{-1} 3300, 3200, 1675; *Anal.* Calcd for $C_9H_{11}O_3NS$: C, 50.69; H, 5.20; N, 6.57. Found: C, 50.49; H, 5.15; N, 6.55. Other 4-alkanoylbenzenesulfonamides were obtained by the same procedure as the preparation of **6** from the corresponding 4-alkybenzenesulfonamides. 4-*n*-Butanoylbenzenesulfonamide: Recrystallization from *n*-hexane–EtOH to afford colorless needles, 52% yield. mp 112–114°C; ^{1}H-NMR δ 1.02 (t, J = 7.3 Hz, 3H, CH_3), 2.98 (t, J = 7.1 Hz, 2H, -CH_2CO-), 5.23 (br. s, 2H, NH_2), 8.01, 8.06 (each d, J = 8.5 Hz, 2H, aromatic-H); ir (nujol) cm^{-1} 3370, 3270, 1675; *Anal.* Calcd for $C_{10}H_{13}O_3HS$: C, 52.85; H, 5.77; N, 6.16. Found: C, 52.85; H, 5.74; N, 6.17. 4-*n*-Pentanoylbenzenesulfonamide: Recrystallization from *n*-hexane–MeOH to afford colorless plates, 61% yield. mp 114–115°C; ^{1}H-NMR δ 0.96 (t, J = 7.3 Hz, 3H, CH_3), 2.99 (t, J = 7.3 Hz, 2H, -CH_2CO-), 5.15 (br. s, 2H, NH_2), 8.01, 8.06 (each d, J = 8.3 Hz, 2H, aromatic-H); ir (nujol) cm^{-1} 3350, 3240, 1675; *Anal.* Calcd for $C_{11}H_{15}O_3NS$: C, 54.75; H, 6.27; N, 5.81. Found: C, 54.71; H, 6.07; N, 5.87. 4-Isobutanoylbenzenesulfonamide: Purification by column chromatography on silicagel from the *n*-hexane–AcOEt (2 : 1) eluate to provide a colorless solid, 38% yield. mp 106–109°C; ^{1}H-NMR (CD_3OD) δ 1.19 (d, J = 6.8 Hz, 6H, isopropyl Me), 3.31 (br. s, 2H, NH_2), 3.61–3.71 (m, 1H, methine), 8.01, 8.11 (each d, J = 8.8 Hz, 2H, aromatic-H); ir (nujol) cm^{-1} 1670 (weak); *Anal.* HRMS Calcd for $C_{10}H_{13}O_3NS$: 227.0616. Found: 227.0623.

4-Alkanoyl-N-(cyclohexylcarbamoyl)benzenesulfonamides. A mixture of **6** (264 mg, 1.24 mmol) and anhydrous K_2CO_3(0.52 g, 3 equivalents) in acetone (15 ml) was refluxed for 2 h with stirring and then cyclohexylisocyanate (0.29 g, 1.8 equivalents) was added to the above reaction mixture. The whole was refluxed

for 20 h with stirring. The reaction mixture was evaporated to give a solid, which was treated with water. After filtration, the filtrate was acidified with 6 N HCl and extracted with AcOEt. The organic layer was dried over $MgSO_4$ to give a crude crystal, which was recrystallized from *n*-hexane–EtOH to afford colorless needles, 4-*n*-propanoyl-*N*-(cyclohexylcarbamoyl)benzenesulfonamide **7** (72% yield from **6**). mp 178–181°C; ^{1}H-NMR (CD_3OD) δ 1.19 (t, J = 7.1 Hz, 3H, CH_3), 3.10 (q, J = 7.1 Hz, 2H, -CH_2CO-), 3.37–3.45 (m, 1H, methine), 4.87 (br. s, 2H, 2 × NH), 8.07, 8.15 (each d, J = 8.5 Hz, 2H, aromatic-H); ir (nujol) cm^{-1} 3360, 3080, 1690, 1660; *Anal.* Calcd for $C_{16}H_{22}O_4N_2S$: C, 56.80; H, 6.51; N, 8.28. Found: C, 56.65; H, 6.57; N, 8.23. Other 4-alkanoyl-*N*-(cyclohexycarbamoyl)benzenesulfonamides were obtained by the same procedure as used for the preparation of **7** from the corresponding 4-alkanoylbenzenesulfonamides. 4-*n*-Butanoyl-*N*-(cyclohexylcarbamoyl)benzenesulfonamide: Recrystallization from $CHCl_3$ to give colorless needles, 73% yield. mp 184–185°C; ^{1}H-NMR δ 1.02 (t, J = 7.4 Hz, 3H, CH_3), 2.98 (t, J = 7.2 Hz, 2H, -CH_2CO-), 3.61–3.63 (m, 1H, methine), 6.46 (d, J = 7.9 Hz, 1H, NH), 7.98, 8.07 (each d, J = 8.8 Hz, 2H, aromatic-H); ir (nujol) cm^{-1} 3350, 3070, 1690, 1655; *Anal.* Calcd for $C_{17}H_{24}O_4N_2S$: C, 57.93; H, 6.86; N, 7.95. Found: C, 57.78; H, 6.89; N, 7.87. 4-*n*-Pentanoyl-*N*-(cyclohexylcarbamoyl)benzenesulfonamide: Recrystallization from *n*-hexane–EtOH to give a colorless prisms, 57% yield. mp 173–176°C; ^{1}H-NMR δ 0.96 (t, J = 7.3 Hz, 3H, CH_3), 3.00 (t, J = 7.6 Hz, 2H, -CH_2CO-), 3.57–3.65 (m, 1H, methine), 6.45 (d, J = 7.8 Hz, 1H, NH), 7.98, 8.07 (each d, J = 8.0 Hz, 2H, aromatic-H); ir (nujol) cm^{-1} 3350, 3070, 1690, 1650; *Anal.* Calcd for $C_{18}H_{26}H_4N_2S$: C, 58.99; H, 7.15; N, 7.64. Found: C, 58.83; H, 7.18; N, 7.64. 4-Isobutanoyl-*N*-(cyclohexylcarbamoyl)benzenesulfonamide: Recrystallization from EtOH to give colorless needles, 47% yield. mp 179–181°C; ^{1}H-NMR δ 1.24 (d, J = 6.8 Hz, 6H, isopropyl Me), 3.50–3.57 (m, 1H, methine), 3.61–3.63 (m, 1H, methine), 6.46 (d, J = 7.9 Hz, 1H, NH), 7.99, 8.07 (each d, J = 8.5 Hz, 2H, aromatic-H); ir (nujol) cm^{-1} 3340, 3070, 1685 (sh), 1650; *Anal.* Calcd for $C_{17}H_{24}O_4N_2S$: C, 57.93; H, 6.86; N, 7.95. Found: C, 57.64; H, 6.92; N, 7.87.

Partition Coefficients

The partition coefficients of acetohexamide and its analogs were determined by the method of Hansch *et al.* (*21*).

Enzyme Reaction

Materials. Carbonyl reductase was purified from cytosolic fraction of rabbit kidney as described previously (*10*). Acetohexamide was supplied by Shionogi Co. (Osaka, Japan). NADPH was purchased from Oriental Yeast Co. (Tokyo, Japan). Other chemicals were of analytical grade.

Assay of enzyme activity. The activity of carbonyl reductase from rabbit kidney was assayed spectrophotometrically (Shimadzu UV-240) by monitoring NADPH oxidation at 340 nm (*10*). The reaction mixture consisted of 0.1 M Na, K phosphate buffer (pH 6.0), 0.25 mM NADPH, 1.0 mM substrate (acetohexamide and its analogs), and enzyme in a total volume of 1.0 ml. The reaction was initiated by

the addition of enzyme. One unit of enzyme activity was defined as the oxidation of 1 μmol of NADPH/min at 30°C. Protein concentration was determined by the method of Bradford (*22*).

Kinematic analysis. K_m and V_{max} values of carbonyl reductase from rabbit kidney for acetohexamide and its analogs were calculated by using a computing program for least-squares linear regression of Lineweaver–Burk plots. k_{cat} value was determined by dividing V_{max} value by enzyme concentration. The enzyme concentration was estimated according to a molecular weight of 28,000 (*10*).

REFERENCES

1. AHMED, N. K., FELSTED, R. L., AND BACHUR, N. R. (1979) *J. Pharmacol. Exp. Ther.* **209,** 12–19.
2. FELSTED, R. L., AND BACHUR, N. R. (1980) *Drug Metab. Rev.* **11,** 1–60.
3. FIELD, J. B., OHTA, M., BOYLE, C., AND REMER, A. (1967) *N. Engl. J. Med.* **277,** 889–894.
4. TANAKA, Y., NISHIKAWA, Y., MATSUDA, K., YAMAZAKI, M., AND HAYASHI, R. (1984) *Chem. Pharm. Bull.* **32,** 1040–1048.
5. SUCKOW, R. F., SMITH, T. M., PERUMAL, A. S., AND COOPER, T. B. (1986) *Drug Metab. Dispos.* **14,** 692–697.
6. MCMAHON, R. E., MARSHALL, F. J., AND CULP, H. M. (1965) *J. Pharmacol. Exp. Ther.* **149,** 272–279.
7. IMAMURA, Y., KOJIMA, Y., AND ICHIBAGASE, H. (1985) *Chem. Pharm. Bull.* **33,** 1281–1284.
8. WERMUTH, B. (1981) *J. Biol. Chem.* **256,** 1206–1213.
9. IMAMURA, Y., NOZAKI, Y., AND OTAGIRI, M. (1989) *Chem. Pharm. Bull.* **37,** 3338–3342.
10. IMAMURA, Y., HIGUCHI, T., NOZAKI, Y., SUGINO, E., HIBINO, S., AND OTAGIRI, M. (1993) *Arch. Biochem. Biophys.* **300,** 570–576.
11. JACOBY, W. B., AND ZIEGLER, D. M. (1990) *J. Biol. Chem.* **265,** 20715–20718.
12. FLYNN, T. G., AND GREEN, N. C. (1993) *in* Enzymology and Molecular Biology of Carbonyl Metabolism 4 (Weiner, H., Grabb, D. W., and Flynn, T. G., Eds.), pp. 351–257, Plenum, New York.
13. SAWADA, H., HARA, A., NAKAYAMA, T., AND KATO, F. (1980) *J. Biochem.* (Tokyo) **87,** 1153–1165.
14. TERADA, T., NIWASE, N., KOYAMA, I., IMAMURA, M., SHINAGAWA, K., TOYA, H., AND MIZOGUCHI, T. (1993) *Int. J. Biochem.* **25,** 1233–1239.
15. SCHIEBER, A., FRANK, R. W., AND GHISLA, S. (1992) *Eur. J. Biochem.* **206,** 491–502.
16. ORITANI, H., DEYASHIKI, Y., NAKAYAMA, T., HARA, A., SAWADA, H., MATSUURA, K., BUNAI, Y., AND OHYA, I. (1992) *Arch. Biochem. Biophys.* **25,** 1233–1239.
17. HIGUCHI, T., IMAMURA, Y., AND OTAGIRI, M. (1994) *Biochim. Biophys. Acta* **1199,** 81–86.
18. IMAMURA, Y., HIGUCHI, T., AND OTAGIRI, M. (1993) *Biochem. Mol. Biol. Int.* **31,** 1105–1110.
19. IMAMURA, Y., KOJIMA, Y., HIGUCHI, T., AKITA, H., OISHI, T., AND OTAGIRI, M. (1989) *J. Phamacobio-Dyn.* **12,** 731–735.
20. BAUMANN, P., AND PRELOG, V. (1958) *Helv. Chem. Acta* **41,** 2362–2379.
21. HANSCH, C., KIEHS, K., AND LAWRENCE, G. L. (1965) *J. Am. Chem. Soc.* **87,** 5770–5773.
22. BRADFORD, M. M. (1976) *Anal. Biochem.* **72,** 248–254.