HACETTEPE BULLETIN OF
NATURAL SCIENCES AND ENGINEERING

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SUBSCRIPTION RATES

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CONTENTS (Cont.)

Glucose-6-Phosphate Dehydrogenase from Rat Liver (II)

The Evidence for a Specific Function of Lysine Residue. Substrate Specificity and Isoelectric Point of the Enzyme.

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Introduction

Glucose-6-phosphate dehydrogenase is widely distributed in nature throughout both the animal and plant kingdoms. The enzyme first discovered in 1931 by Warburg and Chrisitian, and named as the "Zwischenferment". The discovery of this enzyme caused the coenzyme, nicotinamide adenin dinucleotide phosphate to be known.[10]

This enzyme is the first regulatory enzyme in pentose phosphate pathway, which is known as a second way in glucose utilization. The fatty acids, cholesterol, reduced glutation and steroid hormones are required NADPH+H+, for thier synthesis, which is generated by the activity of this enzyme.[10, 13] If the enzyme deficient, these four biosynthesis can not take place in the cells. In this case erythrocytes are not able to survive and may be hemolysed.

It is rather important to know the mechanism of the catalytic activity of this enzyme, and which amino acids are involved in the active center of the enzyme. Probable amino acids which link with coenzyme NADP+ were discussed in the previous paper.[6]

The aim of the presenet paper, is to discuss the amino acid to which substrate glucose-6-phosphate binds in the active center and also to discuss some physical features of the enzyme.

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Pyridoxal 5'-phosphate has been utilized to detecet the ε -amino group of lysine at the active site of various enzymes, including glucose-6-P dehydrogenase from *Leuconostoc mesenteroides*,[12] and *Candida utilis*.[5] Glutamate dehydrogenase,[17] and tryptophanase[9] from *Escherichia coli* and ribonuclease from bovine panceras[11] have been used for further studies of pyridoxal 5'-phosphate (PL 5'-P) binding to the ε -amino group of the lysine residue. These enzymes were first treated with PL 5'-P, then reduced with sodium borohydrid (NaBH₄) and these derivatives were digested with trypsin and chymotrypsin. From these mixtures small peptides, containing 5'-phosphopyridoxyl residue were isolated and the amino acid sequences were determined. PL 5'-P were found to be bounded to the ε -amino group of the lysine residue in all theree experiments.[9, 11, 17]

The isoelectric point and substrate specificity of glucose-6-P dehydrogenase are also discussed in this paper.

Materials and Methods

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP⁺ oxidoreductase, EC. 1.1.1.49) from rat liver was used in all experiments in purified form. Fairly pure enzyme perparation was a gift by Dr Holten.

One unit of glucose-6-phosphate dehydrogenase activity is the amount required to form 1 micromole of NADPH + H⁺ in one minute in the assay employed.[2] The initial reaction velocity was expresssed as micromoles of NADPH + H⁺ formed per minute. Glucose-6-phosphate dehydrogenase activity was measured at 340 mμ wavelength by the initial rate of reduction of NADP⁺ at 30° C.

All solutions were prepared and used frehly and all assays were done in duplictate. In kinetic studies a Gilford model 240 spectrophometer was used and for routine assays, a Beckman model DB-G. The other details and experimental conditions were described in the previous paper.[6]

Electrofocussing Experiment

The experiment was designed according to the manual book of LKB-La-boratories. A 110 ml capacity electrofocussing column was used and glycerol was applied as a fillling material instead of sucrose. Glycerol was prepared in twenty four different concentrations, between 2.5 to 60 % and the amount was 4.6 ml of each. Ampholyne was mixed with glycerol as a carrier substance (Ampholyne, LKB-Producter AB, Stockholm) The best working pH range, was 4-6. Sulfuric acid was first added as a

anodal buffer and then glycerol-ampholyne mixture was applied to the column very carefully in decreasing amounts starting by 60 %. 2.5 % mixture was added finally and the triethanolamine was applied as a cathodal buffer top of the column. 74 units of dialysed pur enzyme were applied to the middle of the column. Two electrodes were connected with a power supply and the experiment was continued for fifty hours with a 900 volts electric current. The column content was removed in 1 ml quantities from the column by a fraction collector. Each sample was tested for pH and enzyme activity. The column tube was enclosed in a glass water jacket through which water at 4° C. was circulated. This was necessary for preventing overheating by high voltage.

Chemicals

Glucose-6-phosphate, NADP$^+$, and 2-deoxy-D-glucose-6-phospate were obtained from Boehringer Mannheim Corporation New York. Galactose-6-phosphate and pyridoxal 5'-phosphate from Sigma Chemical Company St. Louis, Missoury, ampholyne LKB-Laboratory Stockholm and the other chemicals used were analytical reagent grade from Mallincrodt Chemical Company St. Louis Missouri, U.S.A.

' Results

Inhibition of Glucose-6-Phosphate Dehydrogenase by Pyridoxal 5'-Phosphate

Pyridoxal 5'-phosphate was used by several investigators to determine the ε-amino froup of the lysine residue in the active center of different enzymes. Pyridoxal 5'-phosphate (PL 5'-P) was found to inhibit glucose-6-phosphate dehydrogenase (G-6-PDH) from rat liver. 2.7 x 10-^{2} units/ml of enzyme were incubated for 15 minutes at 0° C in the dark with different concentrations of PL 5'-P, and the enzyme activity was measure at 30° C by the addition of glucose-6-phosphate and NADP$^+$ at the same time. The enzyme was incubated with PL 5'-P in dark, in order to prevent the decomposition of PL 5'-P by light. The concentration dependence of pyridoxal 5'-phosphate inhibition in bicine buffer at pH 7.9 was illustrated in Figure 1. 3.5 x 10-^{4} M. PL 5'-P produces 50 % inhibition under the experimental conditions.

Six different concentrations of glucose-6-P, and two different concentrations of PL 5'-P were incubated for 15 minutes at 0° C in the dark and 1 x 10-^{3} M. NADP$^+$ was added last and enzyme activity was measured. The inhibition of PL 5'-P was found to be competitive with glucose-6-P. The result of this experiment was applied to Line-Weaver-
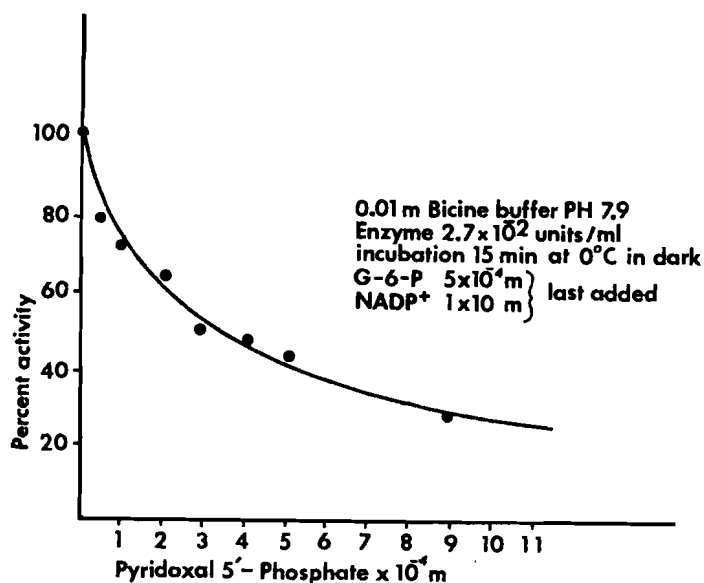
Figure 1

The concentration dependent inhibition effect of pyridoxal 5'-phosphate on glucose-6 -phosphate dehydrogenase. The enzyme was incubated for 15 min. at 0° C in the dark in 0.01 M. bicine buffer at pH 7.9 and at the indicated concentration of PL 5'-P 0.01 ml quantities were removed and the enzyme activity was measured in the routine assay conditions.

Burk plot as shown in Figure 2 The results suggest that PL 5'-P binds to the glucose-6-P binding site on the Enzyme surface. In this experiment the Km value of glucose-6-P was found as $Km = 5.3 \times 10^{-5}$ M. and the inhibiton constant for PL 5'-P was found as $Ki = 5.6 \times 10^{-5}$ M.

In a second experiment 2.7×10^{-2} units/ml of enzyme were incubated in five different concentrations of NADP$^+$ and two different concentrations of PL 5'-P for 15 minutes in the dark at 0° C in bicine buffer at pH 7.9. 2×10^{-3} M. glucose-6-P was added finally and enzyme activity was measured at 30° C. PL 5'-P showed non-competitive inhibition with respect to NADP$^+$. This result indicates that the PL 5'-P binding site is different from the NADP$^+$ binding site on enzyme surface. The Km value of NADP$^+$ was found as $Km = 7.27 \times 10^{-6}$ M. and the inhibition constant of PL 5'-P was found as Ki... 5.6×10^{-5} M. The result is summarized as shown in Figure 3.

The studies with PL 5'-P, strongly suggested that the ε -amino group of the lysine residue was one of the binding sites for glucose-6-P.
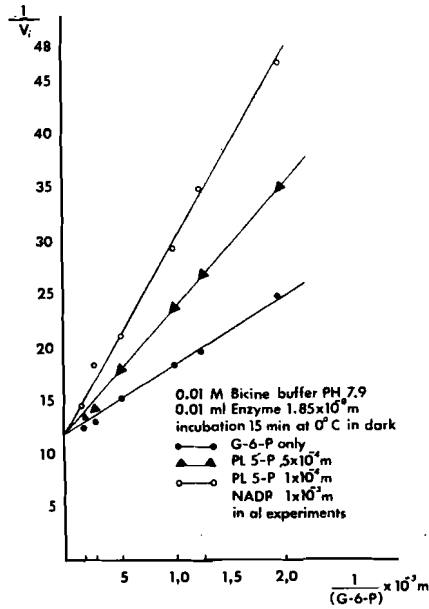
Figure 2

A double riciprocal plot showing competitive inhibition by pyridoxal 5'-phosphate with glucose-6-P in glucose-6-P dehydrogenase. The enzyme was incubated for 15 min. at 0° C. in the dark, in 0.01 M. bicine buffer at pH 7.9 Glucose-6-P and pyridoxal 5'-P concentrations were as indicated. 1 x 10-3 M. NADP+ was added last and enzyme activity was measured.

Figure 3

A double reciprocal plot showing non-compatitive inhibition by pyridoxal 5'-phosphate with to NADP+. The enzyme was incubated for 15 min. at 0° C. in the dark, in 0.01 M. bicine buffer at pH 7.9. Pyridoxal 5'-P and NADP+ concentrations were as indicated. 1 x 10-3 M. glucose-6-P was added last and enzyme activity was mesured.

Substrate Specificity of Glucose-6-Phosphate Dehydrogenase

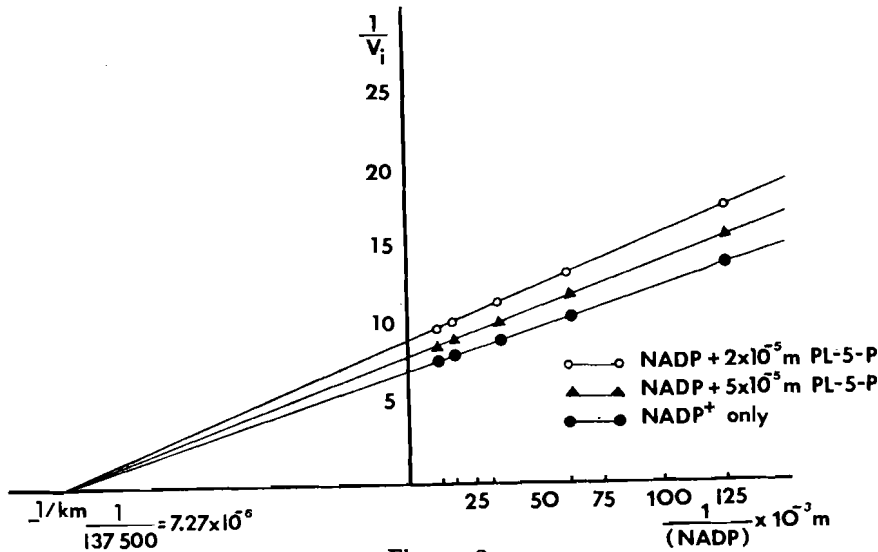Glucose-6-phosphate dehydrogenase from rat liver uses glucose-6-P as a natural subtrate. The enzyme was found to utilize 2-deoxy-D-glucose-6-P and galactose-6-P in addition to glucose-6-P. The relative rates of oxidation of these two compounds were rather low as compaired with that of glucose-6-P. The result is summarized in Table I.

TABLE I

The Relative Oxidation Rates by Glucose-6-Phosphate Dehydrogenase for Glucose-6-Phosphate, 2-Deoxy-D-Glucose-6-Phosphate and Galactose-6- Phosphate.

Substrate	Concentration	Per cent Activity
Glucose-6-P	5×10^{-3} M.	100
2-deoxy-D-glucose-6-P	5×10^{-3} M.	7.5
Galactose-6-P	5×10^{-3} M.	8

Seven different concentrations of glucose-6-P, two different concentrations of 2-deoxy-D-glucose-6-phosphate and 3.6×10^{-2} units/ml of enzyme were mixed and 1×10^{-3} M. NADP$^+$ was added immediately and the enzyme activity was measured. The experiment result is presented in a Line-Weaver-Burk plot in fig. 4. The kinetic behaviour of 2-deoxy-D-glucose-6-P was competitive against to glucose-6-P. The Km value was found as $Km = 6.7 \times 10^{-5}$ M. and the inhibition constant Ki value was found as $Ki = 5 \times 10^{-4}$ M.

Isoelectric Focussing Experiment

The isoelectric focussing experiment was carried out as described in the material and methods section. Ampholyne is a substance which acts as an acid in a strongly acidic medium and as an alkali in a strongly basic medium. During a period of 50 hours under 900 volts a pH gradient is established in the column. The pH is 1 at the bottom of the column and 10 at the top. The enzyme in the column will move up to a definite zone where pH value is equal to its electric charge. In the zone where the pH is equal to the isoelectric point of the enzyme no further migration occurs. At the end of experimental period 1 ml mixture was removed from the column by a fraction collector. pH value and enzyme activity was determined for each tube and result is represented in fig. 5. The maximal activity was found as 2.8 units/ml of enzyme in the tube number 44.

The pH curve intersects the maximal activity peak point at a pH value of 5.3 which is called the isoelectric point (pI) of the enzyme. Two small peaks appeared on both sides of the optimal peak the pH value of which could not be determined because they were too close to the optimal point.
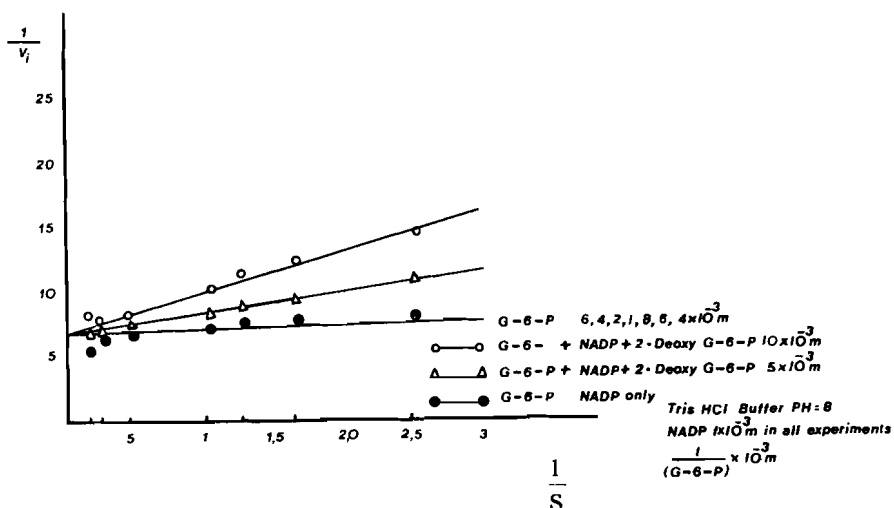
Figure 4

A double reciprocal plot showing competitive inhibition of glucose-6-P dehydrogenase by 2-deoxy-D-glucose-6-P with glucose-6-P. 3.6 units/ml of enzyme were mixed with two different concentrations of 2-deoxy-D-glucos-6-P and six different concentrations of glucose-6-P. Finally 1×10^{-3} M. $NADP^+$ was added immediately and the enzyme activity was measured. $\dfrac{1}{v}$ is plotted against to $\dfrac{1}{S}$.
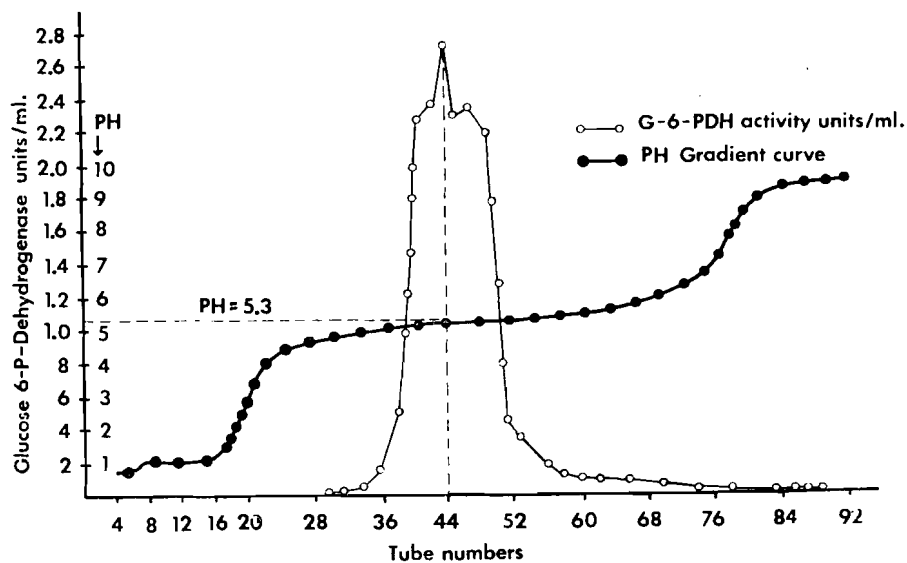
Figure 5

pH curve and the enzyme activity curve was drown after the isoelectric focusing experiment as explained in the text. The isoelectric point of glucose-6-phosphate dehydrogenase from rat liver was found 5.3 as shown in the graph.

Discussion

Pyridoxal 5'-phosphate was used to label the active lysine residues in several enzymes.[5, 9, 11, 12, 17] Pyridoxal 5'-P showed competitive inhibition with glucose-6-P and non-competitive inhibition with NADP+ as shown in fig. 3 and 4. Glucose-6-phosphate dehydrogenase isolated from *Candida utilis* by Domischke and Domagk was traeted with pyridoxal 5'-phosphate (PL 5'-P) and also showed competitive inhibition with glucose-6-P[5]. The same result was also obtained by Olive, Geroch and Levy with glucose-6-P dehydrogenase isolated from *Leuconostoc mesenteroides* by using PL 5'-P in a kinetic method.[12] Olive, Geroch and Levy suggested that the ionization of the phosphate group of glucose-6-P which has a pK' of 6.1 and should exist in the anionic form in order to bind to the protonated ε -amino group of the lysine residue. The second probablitiy is that the aldehyde group of the PL 5'-P forms a Schiff's base with the ε -amino group of a specific lysine residue of the enzyme protein.

To determine the pyridoxyllysine complex in different enzymes some detailed studies were done by several authors. Glutamate decarboxylase isolated from *Escherichia coli* by Strausbach and Fischer,[17] tryptophanase, by Kagamiyama, Morino and Snell[9] and ribonuclease from bovine pancreas by Means and Fenney[11] were incubated with PL 5'-P and then reduced with sodium borohydride (NaBH$_4$). These complexes were digested with trypsin and chymotrypsin and small peptides containing N$^\varepsilon$-pyridoxyllysine residue were isolated. The amino acid sequence of the small peptides were determined by these authors. PL 5'-P was found to be covalently bound to an ε-amino group of the lysyl residue. The amino acid sequences are indicated as follows.

Glutamate decarboxylase Ser-Ile-Ser-Ala-Gly-His-Lys-Phe
                                                        |
                                                   PL 5'-P

Tryptophanase .... Ser-Ala-Lys-Lys-Asp-Ala
                                                        |
                                                   PL 5'-P

In conclusion pyridoxal 5'-P and glucose-6-P were bound to the same site on glucose-6-P dehydrogenase and showed competitive inhibition. The evidence strongly suggests that the ε -amino group of the lysine residue is one of the probable binding sites for glucose-6-P in the structure of glucose-6-P dehydrogenase form rat liver.

Glucose-6-P dehydrogenase was found to utilize 2-deoxy-D-glucose-6-P and galactose-6-P as a substrate. The oxidation rates were found as low as 7.5 and 8 % as compared with glucose-6-P.

The enzyme isolated from human erythrocyte by Yoshida was found to utilize these two compounds as a substrate.[18] Scott and Tatum observed that the enzyme isolated from *Neurospora crassa* also utilized these two substances. Therefore, in this respect there are similarities between the human erythrocyte, rat liver and *Neurospora carssa* Enzymes. From these experiment it is concluded that both substrates can not play an important role in physiological conditions.

Recently isoelectric focussing techniques have been developed for separation of isoenzymes and proteins. These techniques are also avaliable for determination of the isoelectric point of proteins. In our experiment the isoelectric point (pI) of glucose-6-P dehydrogenase was found to be 5.3 as showen in Figure 5, but the two points at both sides of this value remained undetermined. For the enzyme isolated from *Neurospora crassa*, three bands of protein in the pH range of 4.8 to 5.2 were consistently observed by Scott.[15] He estimated a difference in isoelectric point of less than 0.2 pH unit. The resolving power of isoelectric focussing is demonstrated by an experiment of Albers and Scanul. The authors used a narrow pH gradient for human serum lipoproteins and they separated fraction B from C with a pI difference of only 0.09 pH units. Probably using a narrow pH gradient in our experiment it is possible to separate the close points from the main peak.

Acknowledgements

Summary

Glucose-6-phosphate dehydrogenase from rat liver was used in purified form in all experiments.

The binding site of glucose-6-P to the active center of the enzyme was tested with pyridoxal 5'-phosphate by using a kinetic method Pyridoxal 5'-phosphate showed a competitive inhibition with glucose-6-P in the experimental conditions. This result indicates that glucose-6-P and Phyridoxal 5'-phosphate (PL 5'-P) binds to the same site on the enzyme surface. The ε -amino group of the lysine residue was found to be one of the probable binding sites of the substrate glucose-6-P.

Glucose-6-P dehydrogenase from rat liver was found to utilize 2-deoxy-D-glucose-6-P and galactose-6-P in addition to glucose-6-P. The relative oxidation rates of two compounds were very low (7.5 and 8 %) as compared with its natural substrate and ware thus thought not to be important in physiological conditions. 2-deoxy-D-glucose-6-P was run as a second substrate and showed a competitive inhibition with glucose-6-P.

In order to determine the isoelectric point of glucose-6-P dehydrogenase from rat liver an isoelectric focussing experiment was done in a glycerol-ampholyne mixture at a high voltage for 50 hours. The isoelectric point of the enzyme was found 5.3 in our experimental conditions.

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The Isolation of L-Asparaginase and L-Serine Dehydratase from Bacteria*

Nazif Kolankaya** / Atilla Atalay** / Sevda Gökdeniz**

Introduction

Kidd (1953) first observed that certain transplanted lymphomas of mice and rats were strongly suppressed by treatment with guinea pig serum, and Broome (1961) provided evidence that L-asparaginase in the serum is the antitumoral factor. The finding by Mashburn and Wriston (1964) that L-asparaginase derived from Escherichia coli has antitumor activity similar to that of guinea pig serum opened up the possibility of large scale production of the enzyme for ultimate clinical trial. Thus, a large amount of purified enzyme has been supplied and employed in the clinical tests for acute leukemia and other malignant neoplasms in man by Hill, Roberts, Loeb, Khan, Maclellan, Hill (1967), Hill, Loeb, Maclellan, Khan, Roberts, Schields, Hill (1969) and Oetgen, Old, Boyse, Campbell, Philips, Clarkson, Tallal, Leeeper, Schwartz, Kim (1967). Khan and Hill (1969) showed that the administration of such an enzyme protein for a long duration produces the corresponding antibody in the living bodies, and the antibody causes and anaphylactic schok or neutralization of the drug effect. Therefore, the discovery of a new L-asparaginase immunologically different from that of E.coli has been greatly desired. Thus, in addition to E.coli some reports have been published on the production of tumor inhibitory L-asparaginase from Aspergillus terreus by DeAngeli, Pochiari, Tonolo, Zurita, Ciaranfi, Perin (1970), from Erwinia aroideae by Peterson, Ciegler(1969), from E.carotovora by Wade, Elsworth, Herbert, Keppie, Sargeant (1969), from Mycobacterium tuberculosis by Jayaram, Ramakrishnan, Vaidyanathan (1968), from Serratia marcescens by Heinemann, Howard, Palocz (1970), and

from Proteus vulgaris by Tosa, Sano, Yamato, Nakamura, Ando, Chibata (1971),. At the other hand, in recent years Regan, Vodopick, Takeda, Lee, Faulcon (1969) showed that leukocytes from chronic granulocytic leukemia apparently require serine for growth. It has been suggested that L-serine dehydratase might be useful in leukemia therapy.

In the present paper twenty nine strains of bacteria and five strains of fungi were tested for L-asparaginase and L-serine dehydratase activity, and partially purified L-asparaginase from P.vulgaris was measured for the effect of synthesis of DNA, RNA and protein on acute leukemia cells.

Materials and Methods

Bacterial and Fungal Strains: The bacteria and fungi marked with QMB were obtained from Quatermaster Research and Development Center U.S.Army, Natick, Mass. Culture Collection. E.coli N 300, E. coli B,E.coli B_1 and E.coli W were obtained from Michrobiology department, Harvard University. E.Coli $K_{12}687$ and E.coli $K_{12}AB$ 735 were obtained from Microbiology department, California University. E.coli C 600/5 was obtained from Microbiology Department, Sussex University. P.vulgaris A-232 and other strains of bacteria without code number were obtained from Institute of Hıfzısıhha, Ankara,

Media and Culture Method: To screen for L-asparaginase producing bacteria, a medium developed by Bilimoria (1969), containing 0.1 % pepton (Difco), 0.6 % beef extract(Difco), 0.33% KH_2PO_4 and 0.1 % L-asparagine was used. Aspergillus cultures were grown on a medium of Deangeli, Pochiari, Tanola, Zurita, Ciaranfi and Perin (1970), containing 5 % pepton(Difco), 1 % beef extract(Difco) and 5 % mannitol. Media used for bacteria were adjusted to pH 7.0 and for fungi to pH 4.5 befor sterilization at 121°C of presure for 20 min. The media were distributed in 50 ml amounts to 250 ml shaking flasks, sterilized, and inoculated with a loopful of bacteria. Culture was carried out for 24 hours at 37°C on a reciprocating shaker (New Brunsvick Scientific Co) at 175 rev/min. Five days old slant cultures of Aspergillus were suspended with 10 ml of distilled water and 1 ml of this suspention was used for inoculation. The strains of Aspergillus were grown at 30°C for 48 hours on a reciprocating shaker at 175 rev/min. Production was collected by centrifugation and the cell paste was then frozen and kept at-20°C.

L-Asparaginase Assay: L-Asparaginase activities were measured by the procedure described by Peterson and Ciegler (1969) with some modifications Frozen cells were suspended with distilled water. A 0.1 ml sample of cell suspention, 0.9 ml of 0.1 M sodium borate buffer (pH 8.5),

and 1 ml of 0.04 M L-asparagine solution were combined and incubated for 10 min at 37 C. The reaction was stoped by the addition of 0.5 ml of 15 % (W/V) trichloracetic acid. After centrifugation, a 0.5 ml portion of supernatant fluid was diluted to 4 ml with distilled water and treated with 1.0 ml of Nessler's reagent. The colour reaction was allowed to proceed for 15 min. The yellow colour was read at 450 nm in a Bausch-Lomb spectrophotometer. The OD was then compared to a standard curve prepared from solution of ammonium chloride as the ammonia source. One international unit (IU) of L-asparaginase is that amount of enzyme which liberates 1 micromole of ammonia in 1 min at 37 C. The remainder of the cell suspention was dried and the dry weight determined.

L-Serin Dehydratase Assay: L-Serine dehydratase activities were measured by the method of Nelson, Peterson, Ciegler (1973). The only difference of this method from that of L-asparaginase assay is the buffer used in the assay as 0.01 M.

Partial Purification of L-asparaginase: All of the steps described below were carried out at 4 C. Frozen cells were thawed and suspended with 100 ml of 0.05 M phosphate buffer (pH 7.0) per 10 g cell paste, cooled in ice and sonicated in a Artek Model 150 Sonicator for 15 min. Cell free extract was collected by centrifugation (30 min at 30000xg) and treated with 0.05 volumes of 1.0 M $MnCl_2$ to partially remove nucleic acid. After the $MnCl_2$ was added, the suspention was stirred for 1 hr, allowed to stand for 15 min, and then centrifuged to remove precipitate. Solid $(NH_4)_2SO_4$ was added to the supernatant fluid to a concentration of 25 % saturation. After stirring for 30 min, the precipitate was collected by centrifugation at 17.000 rpm for 20 min. The $(NH_4)_2SO_4$ concentration was raised to 40 % and then 80 % saturation. The precipitates of each obtained was centrifuged as before. The $(NH_4)_2SO_4$ precipitated fractions were then dialyzed against 0.05 M phosphate (pH 7.0) and the dializate was kept at −20°C.

Anti Leukemic Test: The anti leukemic action of L-asparaginase was tested against granulocytic leukemia cells. Leukemic blood was obtained from the Children Hospital, Hacettepe University. A 0.25 ml of heparinized blood was incubated in 5 ml of Dulbecco's modified Eagle medium with 10 per cent dialyzed (against 30 volumes of normal saline, with three changes at 4°C for 48 hours) calf serum at 37°C. A 1 IU of L-asparaginase was added to the assay tube. Deoxyribonuclei acid (DNA) and Ribonucleic acid (RNA) synthesis was measured by incorporation of tritiated thymidine (1 uc/ml) and tritiated uridine (1 uc/ml) into a fraction insoluble in trichloracetic acid by using filter disc method of

Bollum (1968). Protein synthesis was measured by the procedure described by Mans and Novelli (1961), using tritiated methionine (1 uc/ml).
Protein Determination: Protein was determined by the method of Lowry, Rosebrough, Farr and Randall (1951).

Results

Twenty nine strains of bacteria and five strains of fungi were screened for L-asparaginase and L-serine dehydratase production. The results of these are summarized in Table I. Proteus vulgaris A-232 was found to contain high levels of these enzymes. Of the other bacteria tested E. coli W, Serratia marcescens QMB 466 were potent sources of L-serin dehydratase. Clinically isolated strain of E.coli was found to synthesize remarkable quantities of L-asparaginase. Proteus vulgaris was selected for further studies because of high productivity on the production of both enzymes.

During the $(NH_4)_2SO_4$ purification steps the highest L-asparaginase activity was found at 80 % saturation and after sonication L-serin dehydratase activity was lost. In addition, when relevant substrates were present in the growth medium, both of the enzymes induced (Table II). Partially purified L-asparaginase which was obtained from P.vulgaris A-232 inhibited DNA, RNA and protein synthesis of acute leukemia cells by 10 %, 3 %, 9 % respectively (Table III).

Conclusion

It has been shown that P.vulgaris A-232 produced both of the enzymes in high levels, this may give an advantage to produce the enzymes from one organism. Nelson, Peterson and Ciegler(1973) reported high yield of L-serine dehydratase from P.vulgaris NRRl B-123, but didn't measure L-asparaginase activity. In addition, an other strain of P.vulgaris was found to have high L-asparaginase activity. by Tosa, Sano, Yamato, Nakamura, Ando and Chibata (1968).

When the appropriate substrates added to the growth medium, the production of enzymes are increased. This suggested that the enzymes are inducible or substrates act as a nitrogen source.

Loosing the activity of L-serin dehydratase by sonication, might be from degradation of molecule during this procedure.

The inhibition of DNA, RNA and protein synthesis of acute leukemia cells by L-asparaginase from P.vulgaris A-232, shows that it has an anti tumoral activity, and possibly willi increase this property with further purification.

TABLE I

Production of Asparaginase and Serine Dehydratase
by Bacteria and Fungi

Organism	L-asparaginase i.u./mg dry weight	L-serine dehydratase i.u./mg dry weight	mg dry weight /ml medium
E.coli B	0.462	0.035	0.70
E.coli W	0.179	0.271	0.46
E.coli N 300	0.086	0.000	0.60
E.coli C 600/5	0.106	0.094	0.76
E.coli B1	0.083	.041	0.60
E.coli K12687	0.123	0.000	0.60
E.coli K12AB 735	0.104	0.000	0.76
E.coli*	1.030	0.083	0.60
Serratia marcescens QMB 466	0.040	0.126	0.34
Bacillus suptilis QMB 1611	0.087	0.000	0.040
Bacillus cereus QMB 1565	0.035	0.000	0.85
Bacillus megaterium QMB 1605	0.000	0.100	0.60
Bacillus circulans QMB 1569	0.000	0.000	−
Bacillus alvei QMB 1593	0.000	0.000	−
Bacillus badius QMB 1595	0.000	0.000	−
Bacillus brevis QMB 1570	0.000	0.000	−
Bacillus licheniformis QMB 1603	0.000	0.000	−
Bacillus sphericus QMB 1609	0.000	0.000	−
Alcaligenes fecalis QMB 1483	0.000	0.000	−
Cellulomonas uda QMB 1534	0.000	0.000	−
Cellvibrio vulgaris QMB 2	0.000	0.000	−
Pseudomonas ovalis	0.000	0.035	0.70
Pseudomonas aeroginosa	0.063	0.037	0.90
Proteus vulgaris A-232	0.648	0.170	0.96
Proteus vulgaris	0.403	0.045	0.75
Proteus vulgaris	0.475	0.015	0.80
Proteus mirabilis	0.079	0.015	0.76
Micrococcus flavus	0.000	0.000	−
Aerobacter aerogenes	0.468	0.000	0.36
Aspergillus foetidus QM 328	0.000	0.000	−
Aspergillus carbonarius QM 331	0.000	0.000	−
Aspergillus niger QM 1273	0.000	0.000	−
Aspergillus phoenicus QM 329	0.000	0.000	−
Aspergillus nidulans QM 1985	0.000	0.000	−

* Clinically isolated strain.

TABLE II

Effect of the Substrates on the Production of Enzymes by P.vulgaris

Experiment*	L-Asparaginase IU/ml medium	L-Serine dehydratase IU/ml medium
1. Control	0.540	0.180
2. + L-asparagine	0.800	0.150
3. + L-serine	0.590	0.210
4. + L-serine and L-asparagine	0.700	0.250

* In all experiments shown in the table, same media (0.1 % pepton, 0.6 % beef extract, 0.33 % KH2PO4) was used, and amino acids were added to this medium in 0.1 % concentrations except control.

TABLE III

Effect of L-asparaginase on the Synthesis of DNA,RNA and Protein on acute leukemia Cells.

	Incorporation (%)		
	H3-Thymidine	H3-Uridine	H3-Methionin
Comlete medium	100	100	100
— L-asparagine	80	77	76
+ L-asparaginase	90.2	97	90.8

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Summary

Twenty nine strains of bacteria and five strains of fungi were screened for L-asparaginase and L-serine dehydratase activity. Of these, one strain of P.vulgaris was found to produce high levels of both enzymes. E.coli W and Serratia marcescens QMB 466 were shown to produce L-serin dehydratase in remarkable quantities. Partially purified L-asparaginase which was extracted from P. vulgaris A-232 by sonication, was found to inhibit DNA, RNA and protein synthesis of acute leukemia cells, by 10,3,9, percent recpectively. In addition when relevant substrates were present in the growth medium, both of the enzymes could be induced.

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# Total Syntheses of Some 7-Methyl Substituted Estrogens

Enis Oskay

Introduction

Within the last ten years many of the steroidal hormones have been totally synthesized.[1-13] A French firm, Roussel, has even started to manufacture most of the steroidal hormones, used in chemical therapy, by total synthesis.[1,2] So far nothing has been reported about the total syntheses of 7-methyl substituted steroids. While studying the effect of the 7-methyl group on a certain biological activity[14-16] several 7-methyl substituted steroids have been prepared by partial synthesis[15,16] with rather poor yields. For this purpose first of all a double bond must be introduced to the steroid molecule at 5-C. The resulting unsaturated molecule should then be oxidized to a 7-keto-Δ^5 steroid. Finally the unsaturated ketone, thus obtained, when reacted with methyl magnesium iodide leads to a 7-oxo methylene steroid which could be hydrogenated with palladium oxide in glacial acetic acid-acetic anhydride mixture. For the oxidation of the Δ^5 steroids to the 7-keto-Δ^5 steroids t-butyl chromate or alternatively chromic oxide in pyridine is used. The first method suffers from a low yield as well as the extreme care necessary in working with large amounts of t-butyl chromate. The second method results in an even lower yield. One other method for the preparation of 7-keto-Δ^5 steroids involves a three step sequence reported by Lenhard and Bernstein.[16] This method involves allylic bromination (C-7) of Δ^5 with N-bromo succinimide followed by reaction of the bromo compound with neutral alumina to produce the 7-OH steroid which can then be oxidized with chromium trioxide in pyridine to 7-keto-Δ^5 steroid.

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In the work of Torgov[5, 6] for the total synthesis of d,1-8, 14-bis-dehydroestrone methyl ether (IV) (see Scheme I) triton B catalyzed condensation of crude 1,2,3,4-tetrahydro-6-methoxy-1-vinyl-1-naphthol (II) with 2-methylcyclopentane-1,3-dione afforded 2-(6-methoxy-1,2,3,4-tetrahydronaphthylidene ethyl)-2-methylcyclopentane-1, 3-dione (III) which on cyclization with p-toluenesulphonic acid in refluxing benzene, afforded d,1-8, 14-bisdehydroestrone methyl ether (IV).

SCHEME I
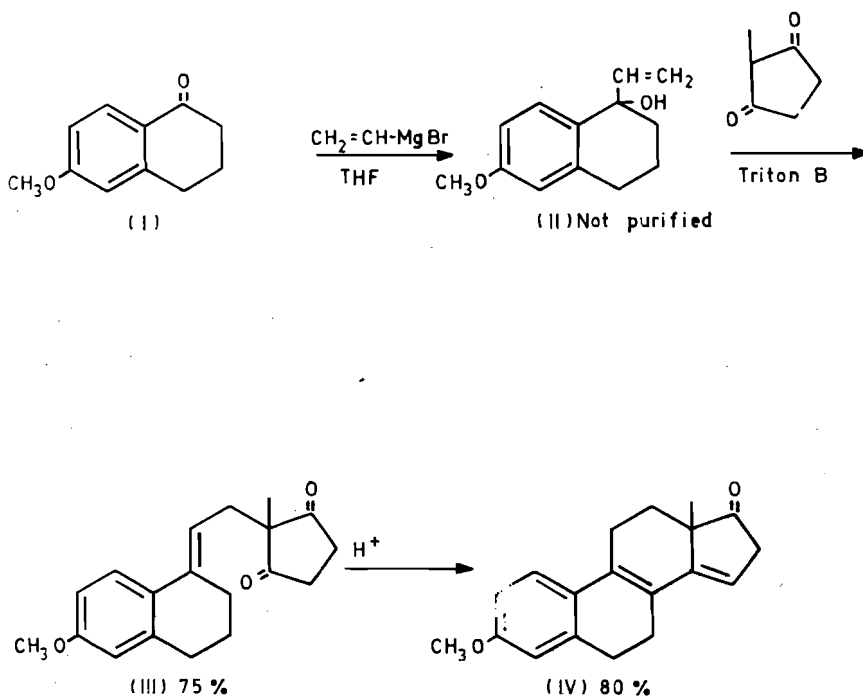
Method

In the present work (see Scheme II) subsititution of the tetralone (I) by a CH_3 at C-3 could, according to the Scheme I, yield methyl substituted derivatives of II, III and IV (XIII, XIV, XV) and XX, XXI which could be converted to some other additional 7-methyl substituted estrogens. Since 3-methyl-6-methoxy tetralone (XII) is not a known compound 7-methyl estrogens described in this paper were totally synthesized from o-methoxybenzyl bromide (see Scheme II).

SCHEME II
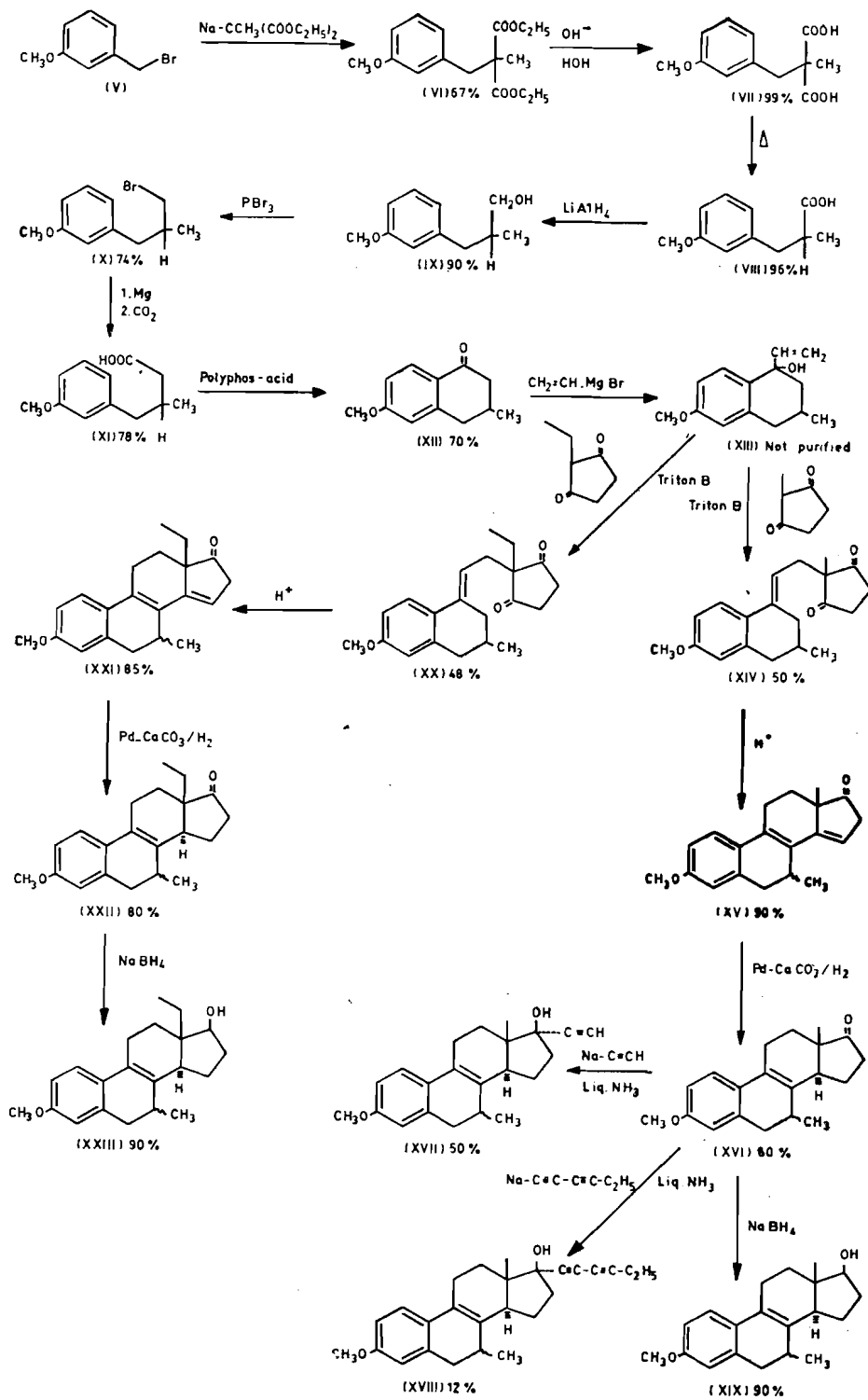
Results

Several 7-methyl substituted estrogenic steroids have been totally synthesized from o-methoxybenzyl bromide.

Experimental

Methyl-o-methoxybenzyl-diethylmalonate (VI). To a freshly prepared solution of sodium ethoxide, prepared from sodium (20 gr., 0.87 atom gram) in super dry ethanol (500 cc.), methyl-diethyl-malonate (151 gr., 0.8 mole gram) was added with stirring. This was followed by the addition of o-methoxybenzyl bromide (174 gr., 0.86 mole gram). The reaction mixture was refluxed (2 hours). Most of the solvent was distilled off and the residue was poured onto crushed ice. The mixture was extracted with ether (3X). The extracts were washed (water) and dried (MgSO$_4$). The solvent was removed and the residue distilled under reduced pressure to give methyl-o-methoxybenzyl-diethylmalonate (VI, 170 gr. 67 %), b.p 138-142°/0.3 mm.; $n_D^{28°}$ 1.4902; (Found: C, 65.20 %; H, 7.60 %. C$_{16}$H$_{22}$O$_5$ requires: C, 65.30 %; H, 7.54 %).

Methyl-o-methoxybenzyl-malonic acid (VII). To a refluxed solution of potassium hydroxide (280 gr., 5 mole gram) in water (290 cc.) methyl-o-methoxybenzyl-diethylmalonate (VI, 295 gr., 1 mole gram) was added (30 minutes). The mixture was refluxed for an additional 2 hours period, cooled and acidified with conc. hydrochloric acid. The precipitate was collected, washed (water) and dried (MgSO$_4$). Yield: 232 gr. (almost quantitative); m.p 146-147°; (Found: C, 60.45 %; H, 5.90 %. C$_{12}$H$_{14}$O$_5$ requires: C, 60.50 %; H, 5.93 %).

α-Methyl-α-o-methoxybenzylacetic acid (VIII). Methyl-o-methoxybenzyl-malonic acid (VII, 77.6 gr., 0.4 mole gram) was heated at 200° for 2 hours and finally distilled to yield α-methyl-α-o-methoxybenzylacetic acid (VIII, 61 gr., 96 %), b.p 140-144°/1 mm.; $n_D^{23°}$ 1.5218; (Found: C, 67.90 %; H, 7.25 %; C$_{11}$H$_{14}$O$_3$ requires: C, 68.03 %; H, 7.27 %).

2-Methyl-3-o-methoxyphenylpropan-1-ol (IX). A solution of α-methyl-α-o-methoxybenzylacetic acid (VIII, 255 gr., 1.30 mole gram) in sodium dried ether (250 cc.) was added (60 minutis) to a susppension of lithium aluminium hydride (70 gr., 1.84 mole gram) in dry ether (500 cc.). The reaction mixture was refluxed for 1 hour and then cooled (0°). Excess of the hydride was carefully decomposed with water (150 cc.). This was followed by the addition of 20 % sulphuric acid (500 cc.). The mixture was extracted with ether. Ethereal layers were washed (water) and dried (MgSO$_4$). Evaporation of the solvent

and distillation of the residue yielded 2-methyl-3-o-methoxyphenylpropan-1-ol (IX, 188 gr., 90 %), b.p $116°/1$ mm.; $n_D^{24°}$ 1.5225; (Found: C, 73.35 %; H, 8.90 %; $C_{11}H_{16}O_2$ requires: C, 73.39 %; H, 8.95 %).

1-Bromo-2-methyl-3-o-methoxyphenylpropane (X). Phosporous tribromide (180 gr., 0.66 mole gram) was added (45 minutes) to a solution of 2-methyl-3-o-methoxyphenylpropan-1-ol (IX, 180 gr., 1 mole gram) in sodium dried benzene (300 cc.) at 0°. The reaction mixture was then stirred (30 minutes) at room temperature and finally refluxed (4 hours). The mixture was cooled and poured onto crushed ice. Benzene layer was separated, washed first with aqueous bicarbonate solution, then with water and dried ($MgSO_4$). The solvent was removed and the residue distilled to yield 1-bromo-2-methyl-3-o-methoxyphenylpropane (X, 180 gr., 74 %), b.p $104°/0.4$ mm.; $n_D^{24°}$1.5405; (Found: C, 54.20 %; H, 6.35 %; $C_{11}H_{15}OBr$ requires: C, 54.34 %; H, 6.25 %).

β-Methyl-γ-o-methoxyphenylbutyric acid (XI). A Grignard reagent was prepared from magnesium (12.2 gr., 0.5 atom gram) and 1-bromo-2-methyl-3-o-methoxyphenylpropane (X, 122 gr., 0.5 mole gram) using a total 260 cc. of dry tetrahydro furane. This reagent was poured onto an excess of solid carbon dioxide and left to stand overnight. The mixture was then diluted with water (300 cc.), acidified (conc. hydrochloric acid) and extracted with ether (3X). Ethereal layers were washed with 30 % aqueous sodium hydroxide solution and the aqueous layers were acidified (conc. hydrochloric acid). Acidic layers were extracted with ether (3X). Ethereal layers were dried ($MgSO_4$) and the solvent removed. The residue was distilled to yield β-methyl-γ-o-methoxyphenylbutyric acid (XI, 81 gr., 78 %), b.p 152°/0.5 mm.; $n_D^{24°}$ 1.5198; (Found: C, 69,15 %; H, 7.80 %; $C_{12}H_{16}O_3$ requires: C, 69.22 %; H, 7.75 %).

3-Methyl-6-methoxy-α-tetralone (XII). A. mixture of polyposphoric acid (125 cc.) and β-methyl-γ-o-methoxyphenylbutyric acid (XI, 42 gr., 0.2 mole gram) was heated (100°) with vigorous shaking (45 minutes). The mixture was poured onto crushed ice, scratched and warmed until the red coloured gum turned to a colourless precipitate. The precipitate was collected, washed (water) and crystallised (petrol ether 40-60°) to yield 3-methyl-6-methoxy-α-tetralone (XII, 26 gr., 70 %), m.p 71-72°; (Found: C, 75.70 %; H, 7.50 %; $C_{12}H_{14}O_2$ requires: C, 75.76 %; H, 7.43 %).

2-(3-Methyl-6-methoxy-1, 2, 3-4-tetrahydronaphthylidene-ethyl)-2-methylcyclopentane-1,3-dione (XIV) and 2-(3-methyl-6-methoxy-1,2,3, 4-tetrahydronaphthylideneethyl)-2-ethylcyclopentane-

1,3-dione (XX). A Grignard reagent was prepared from magnesium (24.4 gr., 1 mole gram) and vinyl bromide (108 gr., 1 mole gram) using a total amount of 400 cc. of dry tetrahydro furane as solvent. This reagent was reacted with 57 gr. (0.3 mole gram) of 3-methyl-6-methoxy-α-tetralone dissolved in 100 cc. of tetrahydro furane and 400 cc. of ether. The crude vinyl carbinol thus obtained was dissolved in 500 cc. of xylene and 110 cc. of tertiary butyl alcohol, and 33 gr (0.3 mole gram) of 2-methylcyclopentane -1,3-dione or 37.8 gr. (0.3 mole gram) 2-ethylcyclopentane-1,3-dione was added. After stirring (15 minutes) at room temperature 10 cc. of 40 % solution of triton B in methanol was added and the mixture stirred and heated at the reflux temperature for 2 hours. After cooling ether (1000 cc.) was added and the mixture stirred with cooling for 4-5 minutes. The mixture was filtered and the filtrate was washed (3X) with 5 % potassium hydroxide solution and with water (3X). The filtrate was then dried ($MgSO_4$) and concentrated in vacuo. The residues were crystallised (petrol ether 40-60°) to yield: a- 2-(3-methyl-6-methoxy-1,2,3,4-tetrahydronaphthylideneethyl)-2-methylcyclopentane-1,3-dione (XIV, 48 gr., 50 %); (Found: C, 76.85 %; H, 7.70 %; $C_{20}H_{24}O_3$ requires: C, 76.90 %; H, 7.75 %), b- 2-(3-methyl-6-methoxy-1, 2, 3, 4-tetrahydronaphthylideneethyl)-2-ethylcyclopentane-1, 3-dione (XX, 55 gr., 48 %), m.p 81°; (Found: C, 77.60 %; H, 8.00 %; $C_{21}H_{26}O_3$ requires: C, 77.27 %; H, 8.03 %).

d,1-8,14-Bisdehydro-7ξ-methyloestronemethylether (XV) and d,1-8,14-bisdehydro-7ξ, 18-dimethyloestronemethylether (XXI). A solution of XIV (20 gr., 0,07 mole gram) or of XX (50 gr., 0.16 mole gram) in methanol (500 cc.) containing 2N HCl (10 cc.) was heated (100°) for 2 hours. The reaction mixtures were diltuted with water (2000 cc.) and extracted (ether, 3X). The ethereal layers were washed (water) and dried ($MgSO_4$). Organic solvents were removed and the residues crystallised to yield: a- d,1-8,14-bisdehydro-7ξ -methyloestrone-methylether (XV, 17 gr., 90 %), m.p 151-152°; (Found: C, 81.50 %; H, 7.60 %; $C_{20}H_{22}O_2$ requires: C, 81.60 %; H, 7.54 %), b- d,1-8,14-bisdehydro -7ξ, 18-dimethyloestronemethylether (XXI, 40 gr., 85%), m.p 105°; (Found: C, 81.65 %; H, 7.90 %; $C_{21}H_{24}O_2$ requires: C, 81.78 %; H, 7.85 %).

d,1-8-Dehydro-7ξ-methyloestronemethylether (XVI) and d,1-8-dehydro-7 ξ-18-dimethyloestronemethylether (XXII). Hydrogenations of XV (15 gr., 0.05 mole gram) and of XXI (15 gr., 0.05 mole gram) in analar benzene (150 cc.) in the presence of prereduced pallladium on calcium carbonate catalyst (5 %, 5 gr.) yielded by spontaneous crystallisation (methanol) from the filtrated, concentrated

reaction products: a- d,l -8-dehydro-7ξ- methyloestronemethylether (XVI, 12 gr., 80 %), m.p 95-97°; (Found: C, 81.00 %; H, 8.20 %; $C_{20}H_{24}O_2$ requires: C, 81.05 %; H, 8.17 %), b- d,l -8-dehydro-7ξ, 18-dimethyloestronemethylether (XXII, 12 gr., 80 %), m.p 100°; (Found: C, 81.20 %; H, 10.30 %; $C_{21}H_{26}O_2$ requires: C, 81.26 %; H, 10.30 %),.

d, 1-3-Methoxy-7 ξ-methylestra-1,3,5 (10), 8-tetraen-17β-o 1 (XIX) and d,1-3-methoxy-7 ξ-18-dimethylestra-1,3,5 (10), 8-tetraen-17β-o 1 (XXIII). Reduction of compounds XVI (2.9 gr., 0.01 mole gram) and XXII (3.1 gr., 0.01 mole gram) by sodium borohydride (1.5 gr., 0.04 mole gram) in methanol yielded: a- XIX (2.6 gr., 90 %), m.p 131.5°; (Found: C, 45 %; H, 8.80 %; $C_{20}H_{26}O_2$ requires: C, 80.50 %; H,8. 78 %), b- XXIII (2.7 gr., 90 %), m.p 129°; (Found: C, 80.50 %; H, 9.00 %; $C_{21}H_{28}O_2$ requires: C, 80.73 %; H, 9.04 %).

d,1-17α-Ethynyl-3-methoxy-7ξ-methylestra-1,3,5 (10), 8-tetraen-17β-ol (XVII) and d,1-17α-(1,3-hexadiynyl)-3-methoxy-7ξ, 18-dimethylestra-1,3,5 (10), 8-tetraen-17 β-o 1 (XVIII). Compounds XVII and XVIII were prepared from XVI and appropriate sodium acetylides in liquid ammonia. Crystallisation (methanol) of the crude reaction products yielded: a- XVII (a gum, 50 %); (Found: C, 81.85, %; H, 8.20 %; $C_{22}H_{26}O_2$ requires: C, 81.95 %; H, 8.13 %) b- XVIII (12 %), m.p 120-122°; (Found: C, 83,15 %; H, 8.20 %; $C_{26}H_{30}O_2$ requires: C, 83.40 %; H, 8.09 %).

Summary

Introduction of a 7-CH_3 group to the steroid skeleton is a tedious method involving several steps and suffering from low yields. Although within the last ten years many of the steroidal compounds have been totally synthesized[1-13] so for nothing has been reported about the total syntheses of 7-methyl substituted steroids. In the present work total syntheses of some 7-methyl steroids, starting with o-methoxybenzyl bromide, are described.

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A Comparison of Hydrocracking and Hydrogenolysis Reactions

Ömer Kuleli*

Introduction

Catalytic cracking of petroleum to obtain lower boiling hydrocarbon fractions is today the most important catalytic process. Reforming of the middle and heavy fractions of cracking products is a close second to this process. Hydrocracking is a special form of catalytic cracking. It is performed in the presence of hydrogen and has proved to be very flexible to meet the fluctuating market demands for gasoline. By applying different operating conditions it is possible to convert 75 % of petroleum to gasoline or 60 % to middle distillates (light fuel oil, diesel, jet fuel) in the same reactor. Light gasoline, a major product of hydrocracking, has a high octane number due to its high isoparaffin content.

The hydrocracking is carried out at a hydrogen pressure of about 35-200 Atm and at 300-450 °C on bifunctional catalysts, such as Pt/ Zeolite. Low space velocities (LHSV) of about one is used.

New restrictions on the lead-content of gasoline force the refiners to find alternative ways to produce gasolines of high octane numbers. The two strong alternatives are:
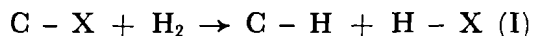
1. Hydrocracking,

2. Alkylation.

Since the erection of the first hydrocracking plant in 1959 the world hydrocracking capacity has been increased rapidly (1968: 25 Miot, 1970: 50 Miot) (1). The trend to no-lead gasoline indicates that many new hydrocracking plants will soon be operating all over the world.

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General

A general equation for the heteregeneous catalytic cracking reaction in the presence of hydrogen is

$$C - X + H_2 \rightarrow C - H + H - X \quad (I)$$

where the atom X is C, N, S, O or a halogen. Hydrocracking requires a dual function (bifunctional) catalyst, having

a) Acid centers for cracking and isomerisation, and

b) Metal or metal oxides for hydrogenation/dehydrogenation.

A side reaction of hydrocracking is hydrogenolysis and it is represented also by the equation (I). It requires monofunctional catalysts. These are usually hydrogen activating metals, such as Ni, Co, Ru, Pt, Ir, Rh, etc. Hydrogenolysis has drawn special attention in the search of alternative ways for the production of SNG (Substitute Natural Gas). Indeed Hydromethanisation (2) and the SNG from naphtha – of British Gas Council – are industrial applications of hydrogenolysis. The addition of methanators to the existing Lurgi Pressure Gasifiers seems to be the most promising solution to satisfy the growing demand for SNG (3) The new and yet not commercially operated methanators require a thorough study of hydrogenolysis reactions.

This study intends to explain the differences between these two related reactions and to which extent hydrogenolysis plays a role in commercial hydrocracking processes.

Experimental

The experiments have been carried out in a flow system, made of stainless steel. High hydrogen pressures (up to 50 Atm) and low hydrocarbon space velocities (0,3-0,5 h^{-1}) have been used. Hydrogen was dried and purified in a Deoxo unit. After being saturated with the hydrocarbon the mixture was preheated to reaction temperature before entering the reactor. Reaction temperature was controlled within $\pm$ 1 °C. About one or two mililiters of catalyst were used for the conversion.

The reaction products were analysed by gas chromatography. Details of temperature programmed GC-analysis were explained elsewhere (4). For the complete analysis of mixtures, two capillary columns (PPG and Squalane) and a packed column (Reoplex/Al$_2$O$_3$) were used.

Bifunctional hydrocracking catalyst (catalyst B), 0,5 % Pt/Ca/Y-Zeolite, was a commercial one and obtained from The Union Carbide Int. Co. Monofunctional 5 % Pt/Al$_2$O$_3$ catalyst (catalyst M); used in

this work, was prepeared as explained below: 0,440 g of PtO_2. xH_2O was mixed with 6,688 g Al_2O_3 in water. This slurry was stirred continuously, water being slowly evaporated. More water was added and this procedure was repeated 5-6 times till PtO_2. xH_2O was uniformly adsorbed on Al_2O_3. This way of preparation ensures an acid-free catalyst which is of vital importance in hydrogenolysis reactions. A reliable hydrogenolysis catalyst must not have acidic properties, otherwise it may initiate hydrocracking reactions of ionic nature.

Results and Discussions

Hydrocracking and hydrogenolysis of methylcyclopentane (MCP) and cyclohexane (CH) were carried out under the same reaction conditions. The aim was to find out the fraction of hydrogenolytic reactions taking part on bifunctional catalysts. A short comparison of the results is given in Table 1.

The bifunctional catalyst, Pt/Ca/Y-Zeolite (B), as compared to monofunctional Pt/Al_2O_3 (M) was much more active. The combination of hydrogenation/dehydrogenation component (Pt) and the acid component (Zeolite) gives excellent rates of conversions. Hydrocarbon reactions on such bifunctional catalysts can be explained through carbenium-ion mechanism.
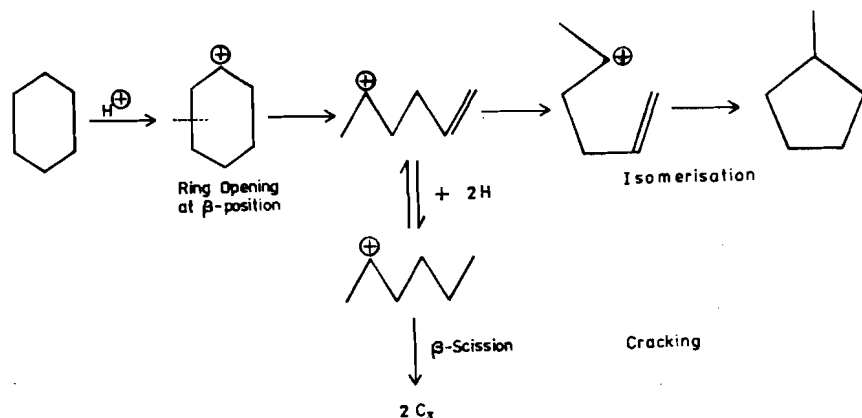
The conversions on M are about three magnitudes of order smaller than those of B. This large difference indicates that the hydrogenolytic reactions are not significant during commercial hydrocracking operations

The careful study of the reaction products reveal the fact that these two reactions proceed quite differently from each other.

TABLE I

Comparison Between Monofunctional and Bifunctional Catalysis of Naphthene Conversion

Catalyst M: 5 % Pt/Al_2O_3 Monofunctional
Catalyst B: 0,5 % $Pt/Ca/Y$–Zeolite   Bifunctional
T: 275°, H_2:HC(*): 20 Mol/Mol, P: 40 Atm, LHSV: 0,3 h^{-1}

Feed	Methylcyclopentane		Cyclohexane	
Catalyst	B	M	B	M
Conversion (Wt %)	27,12	0,07	74,03	0,02
Analysis of products (Wt %)				
Ring opening	25,4	30,0	6,2	—
Isomerization	66,3	57,9	92,3	68,4
Paraffins < C_6	3,9	12,1	0,6	31,6
Naphthenes < C_6	0,2	—	400 ppm	—
Aufbau (> C_6) (**)	4,2	—	0,9	—
Products of ring opening				
n–Hexane	38,3	3,7	41,2	100
2 Me Pentane	32,9	60,4	31,8	—
3 Me Pentane	21,8	35,9	21,7	—
2,2 Di Me Butane	4,2	—	3,1	—
2,3 Di Me Butane	2,8	—	2,2	—
Paraffins < C_6				
C_1	1,3	30,6	2,2	100
C_2	0,4	—	2,2	—
C_3	6,3	—	4,8	—
C_4	52,0	—	48,7	—
C_5	40,0	69,3	42,1	—

(*) H_2: HC: Ratio of hydrogen to hydrocarbon
(**) Aufbau products are hydrocarbons which contain more carbon atoms than the feed hydrocarbons.

1. The main reaction in both cases is isomerization, isomerization of CH being faster than that of MCP.
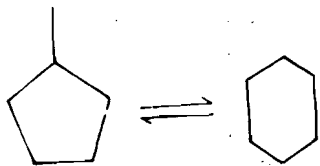
Thermodynamic equilibrium is reached with catalyst B.

2. Ring opening of MCP is faster than that of CH. The hydrogenolytic ring opening of a C_5-ring is favoured over C_6-ring due to its orbital geometry. The low concentrations of n- Hexane among ring opening products indicate that the rupture of secondary-tertiary C-C bonds is very difficult. The ration of b and c type ruptures corresponds to statistical probability; the 2 Me Pentane: 3 Me Pentane ratio for eight different experiments under varying conditions is 2, 1 : 1. A probable scheme of ring opening during hydrocracking is illustrated below:
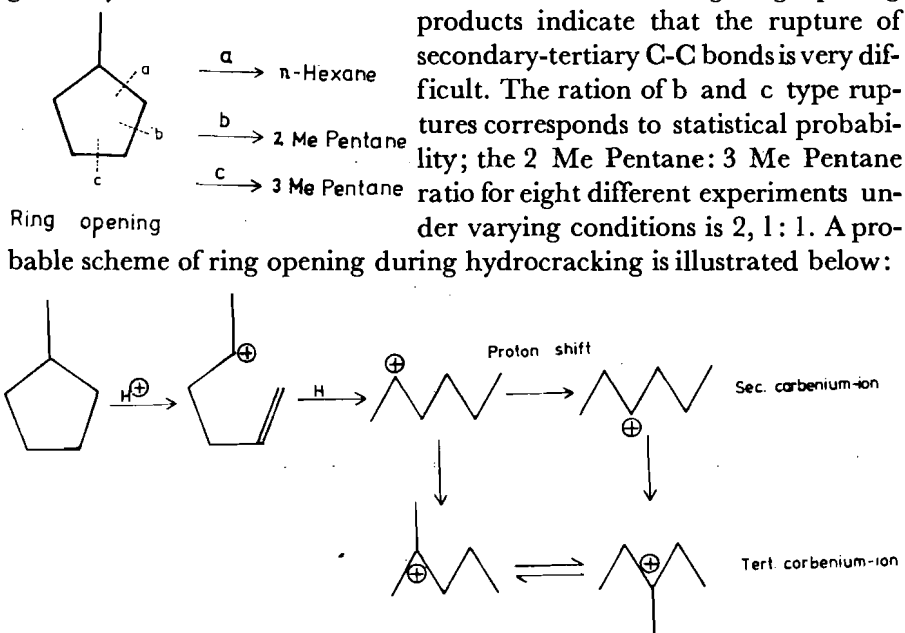
This ionic rearrangement is closely coupled with thermodynamic equilibrium. A set of experiments, performed to show the effect of increasing retention time on the composition of ring opening products, have indeed shown that n-Hexane quantity was increased with the time at the cost of others. At long retention times the amount of n-Hexane exceeded even the thermodynamically predicted concentrations.

3. Cracking on B seems to be a disproportionation reaction. The amount of paraffins smaller than C_6 were almost equal to the Aufbau products larger than C_6. The fact that the Aufbau products were all made of C_7 and C_8-alkylcyclohexanes indicates that two moles of the original hydrocarbon combine to form an intermediate which quickly disproportionates to a paraffin and a naphthene. Cracking on M favours the production of methane, a typical hydrogenolysis reaction. One is encouraged to think that cracking to paraffins smaller than C_6 may proceed without the preliminary ring opening. The only detectable crack-product of cyclohexane hydrogenolysis was methane which was formed by a successive demethanation of an activated (i. e. chemisorbed on an active Platin atom) cyclohexane molecule.

Conclusion

The role of hydrogenolysis reactions taking part on the hydrogenation/dehydrogenation component of a bifunctional hydrocracking catalyst is of no practical importance. The existence of such reactions can at best be suggested by the presence of small amounts of methane and ethane. As is well known a carbenium ion mechanism operating through β-scissions, produces no hydrocarbon smaller than C_3. The reaction velocities of hydrocracking on such powerful catalysts are several magnitudes of order larger than those of hydrogenolysis.

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