SYNTHESIS AND INVESTIGATION OF THE PHYSICAL PROPERTIES OF SOME RELATED SULFONYLUREAS

A

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of the Ohio State University

By

WALTER MOROZOWICH, B.S., M.S.

**********

The Ohio State University
1959

Approved by:

Frank W. Bepe
Adviser
Department of Pharmacy
ACKNOWLEDGEMENTS

I sincerely wish to acknowledge my adviser, Dr. Frank W. Bope, for suggesting the problem and for the generous guidance offered throughout the development of this problem. I remain indebted to many others, faculty members and fellow graduate students, for their helpful suggestions and assistance. I would like to thank the Upjohn Company and the American Foundation for Pharmaceutical Education for offering financial support throughout my stay at Ohio State.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION AND STATEMENT OF PROBLEM</td>
<td>1</td>
</tr>
<tr>
<td>DISCUSSION OF LITERATURE</td>
<td>3</td>
</tr>
<tr>
<td>Hypoglycemics and Their Mechanism of Action</td>
<td>3</td>
</tr>
<tr>
<td>Insulin</td>
<td>3</td>
</tr>
<tr>
<td>Chelating Agents and Diabetes</td>
<td>7</td>
</tr>
<tr>
<td>Guanides and Biguanides</td>
<td>9</td>
</tr>
<tr>
<td>The Sulfonamides</td>
<td>12</td>
</tr>
<tr>
<td>The Mechanism of Action of the Sulfonylureas</td>
<td>17</td>
</tr>
<tr>
<td>EXPERIMENTAL</td>
<td>20</td>
</tr>
<tr>
<td><strong>Synthesis of Sulfonylurea Derivatives</strong></td>
<td></td>
</tr>
<tr>
<td>1. Synthesis of N-p-Tosyl-N-Butylurea</td>
<td>20</td>
</tr>
<tr>
<td>2. Synthesis of N-p-Tosyl-N-Butyl-N-Methylurea</td>
<td>22</td>
</tr>
<tr>
<td>4. Synthesis of N-p-Tosyl-N-Butyl-C-Methylisourea</td>
<td>24</td>
</tr>
<tr>
<td>5. Synthesis of p-Tosyl-N-Butylacetamide</td>
<td>27</td>
</tr>
<tr>
<td>6. Synthesis of N-p-Tosyl-N-Butylthiourea</td>
<td>28</td>
</tr>
<tr>
<td>7. Synthesis of N-p-Nitrophenylsulfonyl-N-Butylurea</td>
<td>29</td>
</tr>
<tr>
<td>8. Synthesis of N-p-Tolyl-N-Butylurea</td>
<td>30</td>
</tr>
<tr>
<td>9. Synthesis of 1-p-Tosyl-5-Butylbiuret</td>
<td>31</td>
</tr>
<tr>
<td>10. Synthesis of N-p-Tosyl-Valeramide</td>
<td>33</td>
</tr>
<tr>
<td>11. Synthesis of N-p-Phenylethylsulfonyl-N-Butylurea</td>
<td>33</td>
</tr>
<tr>
<td><strong>Synthesis of Keto-Sulfonamides</strong></td>
<td>38</td>
</tr>
<tr>
<td>1. Synthesis of p-Tosylacetamide</td>
<td>38</td>
</tr>
<tr>
<td>2. Synthesis of p-Tosyl-p-Toluamide</td>
<td>38</td>
</tr>
<tr>
<td>3. Synthesis of Methanesulfonyl-p-Toluamide</td>
<td>38</td>
</tr>
<tr>
<td>4. Synthesis of Di-Tosylamide</td>
<td>39</td>
</tr>
<tr>
<td><strong>Enolization Study</strong></td>
<td>40</td>
</tr>
<tr>
<td>Discussion</td>
<td>43</td>
</tr>
<tr>
<td><strong>Infrared Spectra Study</strong></td>
<td>49</td>
</tr>
<tr>
<td>Discussion</td>
<td>55</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Ultraviolet Spectra Study</td>
<td>61</td>
</tr>
<tr>
<td>Discussion</td>
<td>64</td>
</tr>
<tr>
<td>Distribution Study</td>
<td>72</td>
</tr>
<tr>
<td>Discussion</td>
<td>74</td>
</tr>
<tr>
<td>Chelation Study</td>
<td>75</td>
</tr>
<tr>
<td>Discussion</td>
<td>80</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>82</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>85</td>
</tr>
<tr>
<td>AUTOBIOGRAPHY</td>
<td></td>
</tr>
<tr>
<td>Table</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>1 - pKa Values of Sulfonyleuraeas</td>
<td>41</td>
</tr>
<tr>
<td>2 - pKa Values of Keto-Sulfonamides</td>
<td>42</td>
</tr>
<tr>
<td>3 - Infrared Spectra of Sulfonyleuraeas</td>
<td>54</td>
</tr>
<tr>
<td>4 - UV Spectra of Sulfonyleuraeas</td>
<td>62</td>
</tr>
<tr>
<td>5 - UV Spectra of Miscellaneous Compounds</td>
<td>63</td>
</tr>
<tr>
<td>6 - Distribution Coefficients</td>
<td>73</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - The Insulin Molecule</td>
<td>5</td>
</tr>
<tr>
<td>2 - The Insulin Molecule</td>
<td>5</td>
</tr>
<tr>
<td>3 - Insulin &amp; The Scheme of Glucose Metabolism</td>
<td>6</td>
</tr>
<tr>
<td>4 - Triphasic Alloxan Response</td>
<td>9</td>
</tr>
<tr>
<td>5 - Mechanism of Biguanide Hypoglycemia</td>
<td>12</td>
</tr>
<tr>
<td>6 - Correlation of Valves and pKa Values</td>
<td>47</td>
</tr>
<tr>
<td>7 - Infrared Spectra</td>
<td>51</td>
</tr>
<tr>
<td>8 - Infrared Spectra</td>
<td>52</td>
</tr>
<tr>
<td>9 - Infrared Spectra</td>
<td>53</td>
</tr>
<tr>
<td>10 - Freezing Point Depressions in Benzene</td>
<td>56</td>
</tr>
<tr>
<td>11 - Effect of Dilution on N Hand Amide</td>
<td>56</td>
</tr>
<tr>
<td>12 - Effect of Dilution on Extinction Coefficients</td>
<td>57</td>
</tr>
<tr>
<td>13 - Sulfonamide Tautomerism</td>
<td>64</td>
</tr>
<tr>
<td>14 - Ultraviolet Spectra</td>
<td>66</td>
</tr>
<tr>
<td>15 - Ultraviolet Spectra</td>
<td>66</td>
</tr>
<tr>
<td>16 - Ultraviolet Spectra</td>
<td>66</td>
</tr>
<tr>
<td>17 - Affect of pH on Spectra</td>
<td>68</td>
</tr>
<tr>
<td>18 - Affect of pH on Spectra</td>
<td>68</td>
</tr>
<tr>
<td>19 - Correlation of Electronic and Spectral Effects</td>
<td>70</td>
</tr>
<tr>
<td>20 - Effect of Metals on Titration Curve</td>
<td>79</td>
</tr>
<tr>
<td>21 - Graphical Determination of Ks</td>
<td>80</td>
</tr>
</tbody>
</table>
INTRODUCTION AND STATEMENT OF PROBLEM

The only effective treatment for the control of the condition known as diabetes mellitus prior to 1955 was the administration of the natural pancreatic hormone insulin, which is deficient in diabetics. The main disadvantages of insulin therapy are the constant threat of hypoglycemic shock from excessive insulin and also the manner of administration, namely parental.

The interest in the field of sulfonamides as oral insulin substitutes began with Janbon's fortuitous observation of the symptoms of hypoglycemia in patients that were being treated for typhoid fever with a new sulfonamide (1). Loubatieres, a close friend of Janbon, studied the strange side-effect of the compound, 2254-R. P., in animals (2). In 1942, he made the amazing postulation that this compound acted pancreatotropically, that is, it stimulated the pancreas to secrete more insulin. It appears now that the hypoglycemic sulfonamides and the sulfonylureas are truly beta-cytotropic by stimulating the beta (β) cells of the pancreas to secrete more insulin. Since the β-cells of the sulfonylurea treated individual no longer store insulin in appreciable amounts in the pancreas, it is quite possible that these compounds effect the release of stored insulin from the mitochondrial-zinc-insulin complex as it normally exists in the pancreas (2,3).

Powerful chelating agents such as alloxan have been found to localize in the β-cells and, after producing an initial hypoglycemic
state, produce a diabetic state with simultaneous destruction of the \( \beta \)-cells (4, 5, 6). This experimental diabetic state can be "cured" through sulfonylurea treatment.

Although the gross mechanism of action of the sulfonylureas is known, the precise site of action within the \( \beta \)-cells is yet to be discovered. This can possibly be done from a greater knowledge of the structure and function of the \( \beta \)-cells and from a better understanding of the physicochemical properties of the sulfonylureas.

This research was undertaken to synthesize a series of closely related sulfonylureas with possible hypoglycemic action from which various physical parameters could be studied concerning the nature of the sulfonylurea moiety. Factors affecting ionization, the electrophilic nature of the substituents on the phenyl ring, electronic conjugation through the sulfonyl function and possibility of enolization were studied because of their importance in the chelate study. It is quite possible that these compounds may exhibit their hypoglycemic action via chelation. Lastly, factors affecting the partitioning of the molecule between organic and aqueous liquids were studied to show the effect of structural modifications of the molecule on their distribution coefficients.
DISCUSSION OF LITERATURE

Hypoglycemics and Their Mechanism of Action

Insulin

Research characterizing the etiology of the diabetic syndrome began in 1869 when Langerhans (7) first showed that diabetes could be produced by extirpation of the pancreas. Minkowski (8), in 1898, reported that diabetes arises from a malfunctioning of the pancreas resulting in an inability of the tissues to oxidize glucose although fructose utilization was unimpaired.

An effective extract of the pancreas was not obtained until 1922, when Banting and Best (9) succeeded in preparing a pancreatic extract which would completely inhibit the hyperglycemia of the depancreat-inized dog.

Through acetone precipitation of a pancreatic extract, a hyperglycemic compound was obtained (10) which was later known as HGF, hyperglycemic-glycogenolytic-factor or glucagon. The separation of the contaminants from insulin was accomplished by Abel in 1926 (11).

It is now known that there are three types of cells in the pancreas: alpha (α) cells which secrete glucagon, beta (β) cells which secrete insulin, and D-cells, whose function is unknown.

In 1953, the amino acid composition of insulin was reinvestigated by means of a new technique (12), countercurrent distribution. Sanger reported extensively on the degradation of insulin with the invaluable terminal amino group scavenger, 2, 4 dinitrofluorobenzene (13, 14). This study, together with the countercurrent distribution study, gave a rather complete picture of the structure of the insulin molecule.

Insulin is a polypeptide with a molecular weight of 12,900 and is composed of 16 different amino acids which are arranged in four chains held together through 6 disulfide linkages. The molecule is composed of two "A" chains each having a molecular weight of 2,750, and two "B" chains each with a molecular weight of 3,700. Two histidine residues are found in each B chain giving a total of four histidine residues.
per molecule. Two zinc ions have been shown to be coordinated or chelated with the histidine groupings (15). This is not too surprising since histidine is one of the strongest naturally occurring chelating agents (16).

Figure 1 is a schematic representation of the four chains of the insulin molecule held together by the disulfide or S bonds. The A chains begin with A (alanine) and end with P (phenylalanine). The B chains begin with A and end with G (glycine). Histidine is represented by the letter H. Figure 2 shows that zinc insulin is actually a dimer in which two insulin molecules are joined through two zinc ions. This zinc-insulin complex is unstable in the presence of acids resulting in the solubilization of free insulin. Most true chelates are decomposed by the presence of acids.
The Insulin Molecule

Figure 1

Figure 2

\[ 2H^+ + Zn_2 - \text{Insulin}_2 \rightleftharpoons 2Zn^{++} + 2 \text{Insulin} - H \]
The mechanism of action of insulin has been studied quite extensively in the past 30 years, as a result, five major theories have been proposed to account for the mechanism of action of insulin.

**Insulin and the Scheme of Glucose Metabolism**

- Glucose
- ATP
- Hexokinase
- Glucose-6-PO₄
- Fructose-1-PO₄
- Fructose-6-PO₄
- Fructose-1,6-PO₄
- Triose-PO₄
- Oxaloacetic acid
- Pyruvic Acid
- Succinic Acid
- Krebs Cycle
- Citric Acid
- A-Ketoglutaric Acid
- Fats
- Protein

*Proposed sites of action of insulin.

Figure 3
From Figure 3, it can be seen that insulin may act on (a) the cytostructural interfaces or the membranes of the cells in merely a physical manner to permit the entry of glucose into the cell (17), (b) catalysis of the hexokinase reaction, (c) the Krebs Cycle, (d) the production of ATP (adenosine triphosphate), and (e) the formation of glycogen from glucose-1-phosphate. Currently, the main action of insulin is believed to be a facilitation of glucose permeation through the cell membranes.

Chelating Agents and Diabetes

The production of diabetes with chelating agents was first observed in 1942 by Okamoto (4) who reported that dithizone or diphenylthiocarbazide (I) was a powerful diabetogenic agent.

\[
\begin{align*}
\text{I} & \quad \text{II} \\
\text{III}
\end{align*}
\]

Diabetogenic action was shown only by those derivatives which could chelate. The O-methyl derivative of 8-hydroxyquinoline, for example, cannot chelate and as a result the compound is inactive as a diabetogenic agent.

The function of the small amounts of zinc present in the pancreas was shown by Maske (19) to be intimately concerned with the storage of insulin within the \(-\)cells of the pancreas. Using pancreatic tissue homogenates and employing ultracentrifugation, he obtained an insulin-zinc-mitochondria complex as one of the fractions. This insulin could not be removed from the complex by washing. This is not too surprising since complexes of the type insulin-zinc-protein
have been known for quite some time with other basic groups, such as protamine or amino acids serving as the protein portion (20). The slow release of insulin from these complexes is the basis of the delayed or prolonged-release insulin preparations currently available.

The effect of naturally occurring zinc binding metabolites of glucose on the insulin-zinc-protein complex has been studied (21) in concentrations of $5 \times 10^{-4}$ to $5 \times 10^{-3}$ molar aqueous solutions. The study showed that oxaloacetic acid, glutathione, cysteine, histidine, and organic phosphates can accomplish the release and subsequent solubilization of insulin from the insoluble form of either zinc-insulin or protamine-zinc-insulin. Glucose has a unique ability to greatly stimulate the metabolism of the islet cells as compared with other tissue cells and the resulting metabolites have been shown to possess a greater affinity for zinc than does insulin.

Using this line of reasoning, Maske(3) theorized that the normal release of insulin from the $\beta$ cells of the pancreas was accomplished by a competitive interaction of the glucose metabolites with the insulin-zinc-mitochondria complex. Thus, when the blood glucose rises, the metabolic rate of the $\beta$-cells greatly increases and the metabolites displace the insulin from the complex, permitting the synthesis of a new molecule of insulin on the mitochondrial template.

That insulin is stored in the pancreas can be shown through histochemical staining (19). The administration of sulfonylureas produces almost a complete degranulation of the $\beta$-cells which has been correlated with a greatly decreased insulin storage within the pancreas. This is similar to the initial hypoglycemic syndrome produced by alloxan as illustrated in Figure 4.
Hypoglycemia is believed to result from a displacement of the insulin from the $\beta$-cells, but because of the ensuing necrosis of the $\beta$-cells, diabetes is eventually produced.

Administration of alloxan to animals pretreated with sulfonylureas prevents the initial hyperglycemic phase A and the secondary phase B, however the diabetic phase C still occurs. This substantiates the theory that degranulation of the $\beta$-cells by the sulfonylureas is a result of a decreased storage of insulin.

Guanides and Biguanides

Guanidine was first recognized as a hypoglycemic agent in 1919 (22) but severe neurotoxicity limited its usefulness (23). N-aryl derivatives were found to be inactive, whereas N-alkyl derivatives possessing a terminal amino group had some activity.

An extensive study of the biguanides (24) led to the discovery of two active compounds, namely synthalin A (IV) and synthalin B (V).
Although they were quite active, they possessed the undesirable side effects of gastro-intestinal irritation, nausea and vomiting.

Replacing the guanidino groups with isothiourea groups (VI) gave only one-half to one-third of the activity of the corresponding biguanide (25).

\[
\begin{align*}
\text{NH}_2 - \text{C} - \text{NH} - (\text{CH}_2)_n - \text{NH} - \text{C} - \text{NH}_2 & \quad n = 10 \\
\text{NH}_2 - \text{C} - \text{S} - (\text{CH}_2)_n - \text{S} - \text{C} - \text{NH}_2 & \quad n = 12
\end{align*}
\]

VI

Prompted by the advent of the sulfonylureas, a series of condensed biguanides completely unrelated to the sulfonylureas were investigated by Ungar (26). The most active of these compounds were phenethyldiguanide (PEDG or DBI)(VII), amylbiguanide (ABI) (VIII), and isoamylbiguanide (IX).

\[
\begin{align*}
\text{Et} - \text{NH} - \text{C} - \text{NH} - \text{C} - \text{NH}_2 & \quad \text{VII} \\
\text{Et-NH-C (NH)} - \text{NH} - \text{C (NH)-NH}_2 & \quad \text{VIII} \\
\text{Et-NH-C (NH)} - \text{NH} - \text{C (NH)-NH}_2 & \quad \text{IX}
\end{align*}
\]

These compounds are similar to the synthalains but are not as potent and do not share their severe histotoxicity. No histologic changes are perceptible after prolonged treatment with the condensed biguanides. However, in extremely high doses, degranulation of the \( \alpha \)-cells occurs (27). Unlike the sulfonylureas, the biguanides are active in the absence of the pancreas or in alloxanized animals. By using animals in which all of the internal organs were removed,
it was shown that DBI increases the rate of peripheral glucose utilization (28). However, unlike insulin, increased glycogen deposition does not occur. The effect on the liver is an inhibition of the production of glucose from glycogen. Although the peripheral uptake of glucose is increased, an increased production of carbon dioxide does not occur. An accumulation of lactic acid does occur which indicates an increased anaerobic glycolysis.

According to a few reports in the literature, the main site of action of the guanides and the biguanide appears to be on certain enzyme systems associated with the Krebs Cycle and the generation of high energy phosphates such as ATP within the mitochondria. Tissue respiration can be completely arrested with moderate doses of biguanides. The reported cytochrome oxidase inhibition caused by these compounds may be the mechanism of this respiratory depression (29).

Since Fe+++ is associated with the activity of cytochrome (30) and since biguanides are powerful chelating agents (31), it would seem likely that the cytochrome oxidase was inactivated through complex formation.

The increased glucose uptake can be explained by the Pasteur Effect, in which anaerobiosis stimulates the uptake of glucose in tissues by overcoming the aerobic inhibitory transfer mechanism of glucose, thus permitting a greater entry of glucose (32). The additional glucose cannot be effectively used, therefore the major portion of the glucose is converted into lactic acid. This is probably the same mechanism that occurs when a diabetic individual exercises—hypoglycemia is produced through an increased anaerobic entry of glucose into the tissues.

Since depressed tissue respiration results in diminished energy production, the resulting decreased gluconeogenesis by the liver should not be unexpected since the formation of insulin requires energy from ATP (33).
Mechanism of Biguanide Hypoglycemia

Increased **Tissue** Anaerobic Glycolysis

Increased **Tissue** Glucose Uptake

**Anoxia** → **HYPOGLYCEMIA** → Decreased **Tissue** Hepatic Glycolysis

**Figure 5**

It should be pointed out that the biguanides are far from being ideal hypoglycemic agents. They control the major symptom of diabetes, which is hyperglycemia, but they do so by essentially suffocating the tissues.

The Sulfonamides

The recognition by Janbon (1), in 1942, of the symptoms of hypoglycemia from the report of Vonkennel (34) led to the important discovery of orally active pancreatotropic agents. The first sulfonamide known to have hypoglycemic action was isopropylsulfathiaiazole (X).

\[
\text{Structure-activity-relationships showed that the sulfanilyl moiety (35) and an alkyl chain were necessary for activity (36). Chen found that the 3-cyclohexyl derivative of X had greater activity than any of the straight chain alkyl derivatives (37. Hypoglycemic action in the sulfonylurea series was first observed by Dantzenberg (38) in 1938 with the compound isopropylsulfanilylurea (X1).}
\]

\[
\text{X1}
\]
Franke and Fuchs, while studying chemotherapeutic agents, came across two sulfonylureas which had remarkable hypoglycemic action (39). They were known as BZ-55 (Tolbutamide, N-p-tosyl-N-butylurea, Orinase) (XII) and D-860 (Carbutamide, N-p-aminophenylsulfonyl-N-butylurea) (XIII).

\[
{\text{XII}}^{\text{XIII}}
\]

Carbutamide was tried clinically by the Lilly Company but was later recalled because of chronic side reactions. Tolbutamide was extensively studied by a number of workers (40, 41, 42) and is currently on the market under the trade name of Orinase (Upjohn).

Little work was published in the area of sulfonylureas until the latter part of 1958. The Boehringer Company of Germany reported (43) on a series of compounds with the general formulae:

\[
{\text{XIV}}^{\text{XV}}^{\text{XVI}}
\]

These compounds were synthesized by the interaction of (a) a sulfonyl-isocyanate with an amine (b) an alkyl isocyanate with a sulfonamide (c) a sulfonyl halide with an isourea followed by hydrolysis of the isourea.
into a urea, or (d) by the addition of water to a sulfonylcarbodiimide. From a study of the pharmacology of the compounds, they concluded that hypoglycemic action demands the presence of an \(-\text{SO}_2^-\text{NH}-\text{C}^\equiv\text{O}\) group. Carbamates (XV) and thiocarbamates were less active than the analogous urea compounds. The nitrogen most distant from the \text{SO}_2 group may be disubstituted and still retain activity. Alkyl groups on XIV were believed necessary to endow a certain degree of lipotropic character to the molecule, although no evidence was given to support this. Aromatic or heterocyclic groups in place of R in XIV gave better activity but increased the toxicity. Lastly, the phenyl group must be joined to the sulfone function as in XIV with an alkyl group for R, and these groups cannot be interchanged.

At the same time, the Farbwerke Hoechst (44) group also reported their findings with the sulfonylureas. The structural modifications studied were similar to those of the Boehringer Company, but their choice was a little more exotic. Compounds having the general formulae:

\[
\begin{align*}
\text{XIV} & : Z - \text{SO}_2^-\text{NH}-\text{C}^\equiv\text{O}^-\text{NH}^-\text{R}^- \quad \text{R}^- = \text{alkyl}, \text{alkylether}, \text{or} \text{cycloalkyl} \\
& \quad Z = \text{OH}, \text{NH}_2, \text{CH}_3, \text{Cl}, \text{Br}, \text{or combinations thereof.} \\
\text{XV} & : R^- = \text{alkyl}, \text{biphenyl} \\
& \quad R^- = \text{alkyl} \\
\text{XVI} & : R^- = \text{alkyl}, \text{alkylether}, \text{or} \text{cycloalkyl} \\
& \quad Z = \text{OH}, \text{NH}_2, \text{CH}_3, \text{Cl}, \text{Br}, \text{or combinations thereof.} \\
& \quad R^- = \text{alkyl}, \text{biphenyl} \\
& \quad R^- = \text{alkyl} \\
& \quad R^- = \text{alkyl} \\
& \quad R^- = \text{alkyl} \\
\end{align*}
\]

were without action. In XIV it was shown that R should contain from 2-7 carbons in order to be active. Replacing R with 2-pyridyl, \(\text{\text{C}_\equiv\text{O}}\), gave good activity but, strangely enough, the 4-pyridyl compound,
was completely inactive. Methyl or halogens as Z, placed in the meta or ortho position did not produce any active compounds. Compounds with strongly electronegative groups, such as HOOC-, ROOC-, R-NH-C=O or NO₂ - on the phenyl ring, as in XX, inactivated the compound.

\[ \text{XX} \]

Compounds XXI, XXII, and XXIII, again shown as general formulae where R and \( R \) were substituted phenyl, alkyl or cycloalkyl group were without activity. The synthesis of these compounds was not reported.

\[ \text{XXI} \]

\[ \text{XXII} \]

\[ \text{XXIII} \]

The Lilly Laboratories reported a series of sulfonyleureas in which the substituents of the phenyl ring as well as the alkyl chain were varied. Their compounds were prepared by reacting an appropriate sulfonamide with an alkylchlorocarbonate to give a urethane. The urethane was not isolated but upon addition of the desired amine, the resulting salt was isolated. The salt was pyrolyzed.
to give the sulfonylurea. The synthesis is illustrated by the following equation:

\[
R - \text{SO}_2 - \text{NH}_2 + \text{O} \overset{\text{Cl}}{\longrightarrow} R - \text{SO}_2 \text{NH} - \text{O}^+ \overset{\text{NH}_2}{\longrightarrow} R - \text{SO}_2 \text{NH} - \text{O}^+ \text{NHR}
\]

An alternate procedure was given using an alkyl isocyanate and a sulfonamide. The activity of these compounds was not reported.

The Pfizer Company (46), during a symposium on chloropropamide or N-p-chlorophenylsulfonyl-N-propylurea, disclosed that the order of activity of a series of phenyl substituted sulfonylureas decreased in the order p-chloro, p-bromo, p-methyl, and p-fluoro. Placing the methyl group meta, decreased the activity by one-third as compared with the corresponding para-derivative. From the slight degree of activity reported for compounds XXIV through through XXVII, it can be seen that the basic pharmacophore is SO2-NH-C-Z, where Z can be any electronegative element, such as N, O, or, as mentioned previously, S.

\[
\text{XXIV} \quad \text{XXVII}
\]

In general, para-substituted aryl compounds had maximum activity and duration of action. Unsubstituted, ortho-substituted and disubstituted aryl compounds were less active.

Unsubstituted sulfonylureas have been prepared from nitrourea and a sulfonamide in 80 percent yield (47).

\[
\text{RSO}_2 \text{NH}_2 + \text{NO}_2 \text{N (Na) CONH}_2 \longrightarrow \text{RSO}_2 \text{N (Na) CONH}_2 + \text{N}_2 \text{O}
\]
Sulfonylureas in low yield can be prepared from prolonged heating of an alkylurea and a sulfonamide (48).

\[ \text{RSO}_2 \text{N} \overset{\text{NaOH}}{\longrightarrow} \text{R} \overset{\text{NH}_2}{\longrightarrow} \text{CONHR} \text{ (Na)} \overset{\text{CONHR}}{\longrightarrow} \text{NH}_3 \]

Carbamyl chlorides readily condense with sulfonamides at elevated temperatures (47), (49), (50) to give sulfonylureas.

\[ \text{RSO}_2 \text{NH}_2 + \text{R}_2 \text{NCOCl} \overset{\text{HCl}}{\longrightarrow} \text{RSO}_2 \text{N} \overset{\text{CONHR}}{\longrightarrow} \text{R}_2 \text{NHCONHR}_2 + \text{HCl} \]

Dialkylcarbamyl halides must be used in this reaction.

Sulfonylisocyanates were first prepared by Billeter (51), 1904, from silver cyanate and the sulfonyl halide. Because of the extreme sensitivity of the compound to moisture and the close boiling point of the parent compound, no other attempts at isolation have been reported. Using vacuum fractionation, we have been able to prepare pure sulfonylisocyanates.

Sulfonyloylanamides, prepared from sulfonylhalides and cyanamide, can be made to react with alcohols to give isoureas or with amines to give guanides (52), (53):

\[ \text{RSO}_2 \text{NHCN} \overset{\text{ROH}}{\longrightarrow} \text{RSO}_2 \text{NHC(OH)NH}_2 \]

\[ \text{RSO}_2 \text{NHC}(\text{NH})\text{NH}_2 \]

These isoureas can be hydrolyzed to give ureas (54). Isoureas can be made by the reaction of sulfonyl chlorides with alkylisoureas (55).

Mechanism of Action of the Sulfonylureas

In 1942, Loubatieres (2) showed that sulfonylisopropylthiadiazole (2254-RP) was inactive in the absence of the pancreas. At that time he postulated that this compound acts in a pancreatotropic manner. In 1955, he presented evidence that these compounds have the same
action as BZ-55, which is N-p-tosyl-N'-butylurea, a sulfonurea derivative.

With 2254-HP, it was possible to cure alloxan diabetes (56); it was postulated that new \( \beta \)-cells were formed from the acinar tissue of the pancreas by these compounds.

Since the \( \beta \)-cells of the pancreas secrete HGF, hyperglycemic factor, it was believed that the sulfonamides and the sulfonureas exerted their action through inhibition of the \( \alpha \)-cells (40, 41). This was later discounted by Volk (57) and Ferner (58), since the compounds are inactive in fully alloxanized animals.

Mirsky (59) suggested that the mechanism for the blood sugar lowering effect of the sulfonureas resides in an inhibition of the hepatic insulinase system, but using \( \text{I}^{131} \)-insulin, Vaughan (60) and Volk (61) could not show evidence of an insulin sparing effect. There was no decrease in the rate of destruction of \( \text{I}^{131} \)-insulin in the presence of the sulfonureas.

The sulfonureas have effects on the liver which could account for their hypoglycemic effect. Glucose-6-phosphatase, which exists in the liver, was shown to be inhibited by the sulfonureas (62). The inhibition of the conversion of fructose to glucose (63) suggests another explanation of the inhibition of gluconeogenesis in the liver. But since the compounds are inactive in the absence of the pancreas, the liver theory cannot be accepted as the ultimate mode of action of these compounds.

At present, most of the evidence is in favor of a \( \beta \)-cytotropic mechanism. This was substantiated by the degranulation of the \( \beta \)-cells after sulfonurea treatment (64, 65). \( \beta \)-cell degranulation correlates with the insulin content of the pancreas (66); the greater the degranulation the lower the insulin content. Unequivocal evidence of an increased endogenous insulin output in response to the sulfonureas was difficult to show until the experiments of Ungar were published (3). He injected a small amount of insulin into the portal vein which leads from the pancreas to the
liver. This resulted in hypoglycemia, but an increased utilization of glucose could not be shown. This is exactly the case with the sulfonylureas; hypoglycemia is produced, but an increased utilization of glucose could not be shown. This inability of the sulfonylureas to enhance the uptake of insulin from the bloodstream, up to this time, was very confusing since it is a known fact that a parenteral injection of insulin causes an increased uptake of glucose by the tissues. However, it should be pointed out that a parenteral injection of insulin produces an unphysiologically high level of insulin in the bloodstream, thus an abnormal glucose uptake is evidenced. Therefore, it appears that in response to sulfonylurea treatment, an increased amount of insulin is secreted into the portal vein. This insulin is bound by the liver and exerts its metabolic action on the liver i.e., gluconeogenesis is decreased. This theory was supported by Weinhouse et al., using isotopically labeled glucose (67). To further substantiate this theory, insulin was administered subcutaneously resulting in slow absorption and in low blood levels. Enhanced glucose uptake by the tissues could not be demonstrated, although hypoglycemia was produced. This, of course, was the result of a suppression of the rate of the glucose released by the liver.

Thus we can now say almost dogmatically that the sulfonylureas are essentially β-cell stimulators. The exact mechanism of this increased release of insulin is yet to be elucidated. From clinical reports, the sulfonylureas have been shown to cure diabetes (56), although in most cases remission occurs. Quite possibly by recognizing and treating the early stages of diabetes, prophylaxis may be achieved.

A great advance in the field of medicine has been made, however elucidation of the specific mechanism of insulin release initiated by sulfonylureas will enable a more rational synthesis of the most active and the least toxic hypoglycemic agent. Physical studies of the sulfonylurea molecule will most certainly give a better insight to this mechanism.
Experimental

Synthesis of Sulfonylurea Derivatives

1. Preparation of N-p-Tosyl-N-Butylurea

The interaction of p-tosyl chloride with inorganic cyanates was first studied as a possible route to sulfonylureas. It was not possible to prepare sulfonyl isocyanates from p-tosyl chloride with sodium or potassium cyanate in anhydrous DMF, benzene, or acetone at reflux temperature. Neither would direct fusion provide a sulfonyl isocyanate. With silver cyanate, the reaction could be accomplished at temperatures above 175°, without a solvent or with nitrobenzene as the solvent. In order to separate the sulfonyl isocyanate from unreacted sulfonyl chloride, vacuum fractionation had to be employed. The sulfonyl isocyanates are very reactive compounds and must be handled in a moisture free atmosphere. Exposure to air for just a few minutes is sufficient to cause formation of a solid film of p-tosyl amide on the surface of p-tosyl isocyanate. p-Tosyl isocyanate was characterized by the formation of p-tosyl amide upon addition to water and by the presence of an intense peak at 2270 cm.\(^{-1}\) in the infrared spectra characteristic of isocyanates. A convenient test for these isocyanates is the liberation of CO\(_2\) in water.

In preparing sulfonyl isocyanates, the quality of the silver cyanate is most imperative. Aged silver cyanate or silver cyanate dried above 40° decreased the yield of sulfonyl isocyanate considerably. The silver cyanate was prepared by slowly adding equimolar 20% aqueous solutions of silver nitrate into a stirred solution of potassium cyanate. The reverse addition resulted in silver cyanate of inferior quality. The silver cyanate was filtered, resuspended in acetone, filtered, washed with acetone, and lastly, washed with ether. The compound was dried in a vacuum at 35° for one hour and
used immediately.

To prepare p-tosyl isocyanate, a solution of 30.0 g. (0.158 mole) of p-tosyl chloride in 150 ml. of nitrobenzene was treated with 26.4 g. (0.176 mole) of silver cyanate in a 250 ml. flask fitted with a stirrer, a thermometer, and a condenser (fitted with a drying tube). The mixture was warmed cautiously to 175°, when the exothermic reaction began, the flask was immersed in a 50° water bath. After a few minutes, the reaction subsided and the contents were kept at 190° for 2 hours. The resulting pale yellow colloidal mixture was centrifuged and the supernatant liquid was decanted. An additional 50 ml. of nitrobenzene were added; the solid was reuspended and then centrifuged. This process was repeated with 3 fifty ml. portions of nitrobenzene. The combined extracts were distilled through a 15 inch column packed with helices. The yield of pure p-tosyl isocyanate was 18.4 g. or 56 percent of the theoretical yield. The boiling point was 90-92°/0.5 mm.

The preparation of p-tosyl isothiocyanate by a similar reaction of potassium thiocyanate or silver thiocyanate with p-tosyl chloride was unsuccessful.

Heat was not necessary for the reaction of p-tosyl isocyanate with butylamine. A solution of 9.90 g. (0.05 mole) of p-tosyl isocyanate in 50 ml. of anhydrous ether was added dropwise to 4.05 g (0.05 mole) of butylamine in 50 ml. of ether in a nitrogen atmosphere. The solution was stirred for two hours and then extracted with four 50 ml. portions of saturated NaHCO₃ solution. The alkaline extracts were additied with HCl to pH 2 and the resulting precipitate was filtered and recrystallized from 50 ml. of 50 percent aqueous acetone. Two recrystallizations gave a constant melting point of 128.0-128.5° by the capillary method using an Anschütz thermometer. Based on the first isolation, the yield was 12.2 g. or 91 percent. This compound was also obtained in 80 percent yield from equimolar amounts of butyl isocyanate and p-tosyl amide in benzene after refluxing for three hours. An equimolar amount of triethylamine was
added as a catalyst (68).

The m.p. of the compound N-p-tosyl-N'-butylurea was 128.0-128.5°, which agrees with that value subsequently reported in the literature (69), 128-129°. An infrared spectrograph showed an NH group at 3300 cm\(^{-1}\), an amide group at 1655 cm\(^{-1}\) and an SO\(_2\) group at 1333 and at 1160 cm\(^{-1}\) (70). Titration of the compound gave a molecular weight of 274 as compared to a calculated value of 270.3.

2. Preparation of N-p-Tosyl-N'Butyl-N'-Methylurea

A solution of 5.91 g. (0.03 mole) of p-tosyl isocyanate in 20 ml. of anhydrous thiophene free benzene was dropped into 3.05 g. (0.035 mole) of butylmethyamine in 20 ml. of benzene. The solution was refluxed for 30 minutes with drying tube fitted to the condenser. The benzene solution was then extracted with four 50 ml. portions of 5 percent KOH. The combined extracts were cooled in an ice bath to precipitate the potassium derivative of the compound. The yield of the potassium compound dried at 60° and 2 mm. was 6.95 g. or 81 percent. After two recrystallizations from 5 percent KOH, the m.p. was 208-210°. This compound was very soluble in water.

The infrared spectrograph gave no indication of -NH- or amide functions. However, -SO\(_2\)- was present at 1320 and 1135 cm\(^{-1}\). An intense absorption at 1590 cm\(^{-1}\) was tentatively assigned to the -C=N- grouping. This would indicate that the compound had enolized. Later, it was shown that N-p-tosyl-O-methyl-N'-butylisourea, which is locked in the enol form, also had a peak in the same region, 1610 cm\(^{-1}\), which is unequivocally due to the -C=N- moiety.

The potassium derivative of N-p-tosyl-N'-butyl-N'-methyurea was dissolved in water, then acidified and the resulting oil extracted with ether. The ether was removed and the oil was dissolved in 20 ml. of 5 percent NaOH solution and cooled to 5°, which resulted in a gel. Upon further dilution to 200 ml. gel formation continued to occur.
The potassium derivative was analyzed for carbon and hydrogen as the tri-hydrate. This was proven by dehydrating 3 molecules of water at 160°. The analyses were done by the Galbraith Microanalytical Laboratories, Knoxville, Tennessee.

Analytical Data

Calculated for $C_{13}H_{20}N_{2}SK_{3}H_{2}O$

$C$, 41.23; Found: 41.42

$H$, 6.51; Found: 5.71

3. The Synthesis of N-p-Tosyl-N-Methyl-N'-Butylurea

A solution of 1.36 g. (0.05 mole of N-p-tosyl-N'-butylurea in 20 ml. of 2 percent aqueous NaOH was kept at 30° for 24 hours. The solution was extracted with ether, however nothing was recovered upon evaporation of the ether. The extracted solution was then acidified with 10 percent HCl to pH 2 resulting in precipitation. The precipitate was recrystallized from 20 ml. of 50 percent aqueous ethanol. The compound proved to be the starting material, N-p-tosyl-N'-butylurea. The same quantities were used in another reaction with absolute ethanol as the solvent and sodium ethoxide as the base. The starting compound was again isolated. Reactions at 40° and 50° were tried but the results were unsuccessful.

The use of diazomethane as the methylating agent was then tried. Ten and one-half grams of nitrosomethylurea were prepared by standard methods (71). The nitrosomethylurea was treated with KOH and ether and distilled to give 200 ml. of diazomethane solution which was standardized by titration of a sample of benzoic acid.

Ninety ml. of the ethereal diazomethane solution containing 0.0375 mole of diazomethane were added slowly to a mixture of 10.00 g. (0.0365 mole) of N-p-tosyl-N'-butylurea in 100 ml. of absolute ether. Solution of the compound slowly occurred upon addition of the diazomethane solution. After complete addition, the ether solution was
decanted from the remaining few crystals of the starting compound. The ethereal solution was extracted with 5 percent aqueous NaOH. Upon acidification of the basic extract, nothing precipitated which indicated that complete reaction of the diazomethane with the sulfonylurea was achieved. One hundred ml. of petroleum ether were added to the extracted ether solution to induce crystallization. Two such recrystallizations gave 9.69 g or 93 percent of N-p-tosyl-N-methyl-N-butylurea as white glossy plates melting at 57-58°.

Analytical Data

Calculated for $C_{13}H_{20}N_{2}O_{3}S$

$C$, 54.90; Found: 54.98
$H$, 7.08; Found: 7.05

4. Synthesis of $N$-$p$-Tosyl-$N$-Butyl-$O$-Methylisourea

This compound was prepared for its later use in proving enolization since it is locked in the enol form. A solution of 17.0 g. (0.02 mole) of butylurea was dissolved in 18.5 g. (0.02 mole of dimethylsulfate and warmed to 70°. At that temperature, an exothermic reaction ensued and the temperature rose to 114°. The reaction mixture was cooled to 90° and kept at this temperature for 15 minutes. The mixture was then cooled to room temperature and 100 ml. of 40 percent KOH were required to liberate butylisomethylurea from its bisulfate salt. The butylisomethylurea which separated was extracted with 50 ml. of ether. The ether extract was dried with anhydrous sodium sulfate and the ether was removed. The resulting crude butylisomethylurea was distilled at 80-85° and 4 mm. Redistillation gave 10.9 g. or 41 percent of pure butylisomethylurea boiling at 63-65° and 1 mm. Curd (72) prepared the same compound from butylcyanamide and methanol. He reported a boiling point of 90° at 10 mm., but did not offer any proof of structure.

Analysis of the infrared spectra of the butylisomethylurea, prepared by the dimethyl sulfate method, showed characteristic
Imine peaks (73) at 3300, 1660 and 1560 cm\(^{-1}\). This supports formula XXVIII rather than XXIX as the structure of butylisomethylurea. The latter would have a doublet in the 3300 cm\(^{-1}\) region and the intense 1560 cm\(^{-1}\) peak would be missing.

\[
\text{OCH}_3 \\
\text{C}_4\text{H}_9 - \text{NH} - \text{C} = \text{NH} \\
\text{XXVIII}
\]

\[
\text{OCH}_3 \\
\text{C}_4\text{H}_9 - \text{N} = \text{C} - \text{NH}_2 \\
\text{XXIX}
\]

That the structure of alkylisoureas varies was first pointed out by Werner in 1914 (74). He prepared \(N\)-ethyl-\(O\)-methylisourea and \(N, N\)-dipropyl-\(O\)-methylisourea. From a study of their degradation products he concluded XXX and XXXI, respectively, as the structures.

\[
\text{OCH}_3 \\
\text{C}_2\text{H}_5 - \text{N} = \text{C} - \text{NH}_2 \\
\text{XXX}
\]

\[
\text{OCH}_3 \\
\text{C}_3\text{H}_7 - \text{N} = \text{C} - \text{NH} \\
\text{XXXI}
\]

Essentially, the method of Cox and Sprague (75) was used for the reaction of \(p\)-tosyl chloride with \(N\)-butyl-\(O\)-methylisourea. A solution of 7.63 g. (0.049 mole) of \(N\)-butyl-\(O\)-methylisourea in 80 percent aqueous acetonitrile was stirred with 8.58 g. (0.045 mole) of \(p\)-tosyl chloride and 14.0 g. of \(K_2\text{CO}_3\) at room temperature. After 10 minutes, solution occurred and a temperature rise was noted. The solution was stirred for 3 hours at room temperature with crystallization slowly occurring. The mixture was concentrated to about 50 ml. and then 200 ml. of \(H_2O\) was added. The resulting precipitate was filtered, washed with \(H_2O\), and dried in a vacuum at 50\(^\circ\). The yield was 12.61 g. or 98 percent. An ether-petroleum ether mixture was found to be an excellent recrystallizing medium. The crude product was dissolved in 50 ml. of ether and 100 ml. of petroleum ether were
added. Lustrous white flakes grew slowly. Within 5 min. crystallization was complete. After a second recrystallization, the product was dried at 60° and 2 mm. The melting point was 80.2° - 80.5°.

\[
\text{XXXII}
\]

\[
\text{XXXIII}
\]

It was necessary to verify the structure of the final product, XXXIII, since reaction with the other nitrogen of N-butyl-O-methylisourea would give a different structure, XXXIV.

\[
\text{XXXIV}
\]

It would be difficult to distinguish between these two possible formulae from their infrared spectra. Hydrolysis should be a means of differentiation since XXXIII should afford N-p-tosyl-N'-butylurea. Acidic hydrolysis proved more effective than basic hydrolysis probably because of less cleavage of the urea portion. A solution of 0.400 g. of the postulated N-p-tosyl-O-methyl-N'-butylisourea in 20 ml. of acetone was mixed with 20 ml.
of 10 percent aqueous HCl. The solution was refluxed for 30 min. The acetone was removed under vacuum with precipitation occurring. The mixture was made basic with 5 percent NaOH solution and the precipitate dissolved. The solution was filtered and acidified; the resulting precipitate was recrystallized from 50 percent aqueous ethanol. The infrared spectra showed the compound to be N-p-tosyl-N-butylurea and a mixed melting point with the authentic sample showed no depression. Thus structure XXXIII was proven correct.

Analytical Data

Calculated for \( \text{C}_{13}\text{H}_{20}\text{N}_2\text{O}_3\text{S} \)

C, 54.90; Found: 54.77
H, 7.08; Found: 6.66

5. Preparation of p-Tosyl-N-Butylacetamide

Unsubstituted sulfonylacetamides were first prepared in 1938 by heating a sulfinate with \( \alpha \)-chloroacetamide in a sealed tube (76). However, using substituted \( \alpha \)-haloacetamides, it was found that the sealed tube technique was unnecessary. Potassium p-tosyl sulfinate was prepared by reducing p-tosyl chloride with zinc according to the method of Vogel (77). \( \alpha \)-Chloro-N-butylacetamide was prepared in 88 percent yield from one equivalent of \( \alpha \)-chloroacetyl chloride and two equivalents of butylamine.

A solution of 11.5 g. (0.06 mole) of \( \alpha \)-chloro-N-butylamine in 100 ml. of absolute ethanol was heated to 50° for 8 hours. Higher temperatures decreased the yield considerably and made purification of the final product difficult. A finely divided precipitate of KCl formed during heating. The solution was filtered and concentrated to 25 ml. The addition of 100 ml. of water resulted in precipitation of the product. The yield of crude p-tosyl-N-butylacetamide was 5.03 g. or 84 percent. The product was recrystallized twice 50 ml. of 80 percent aqueous ethanol. The white
needle-like crystals were dried overnight at 80° under vacuum. The melting point was 106.0-106.6°.

Analytical Data

Calculated for C_{13}H_{19}N\textsubscript{3}O\textsubscript{3}S

C, 57.98; Found: 58.09

H, 7.10; Found: 6.99

6. Synthesis of N-p-Tosyl-N-Butylthiourea

After unsuccessful attempts to prepare p-tosylisothiocyanate, the synthesis of N-p-tosyl-N-butylthiourea was attempted with equimolar quantities of butylisothiocyanate, p-tosylamide and triethylamine in dioxane. The reaction was followed by removing an aliquot of the solution, adding a known amount of butylamine and allowing the solution to stand for 30 minutes. The butylamine reacted with the butylisothiocyanate, and the unreacted butylamine was determined by titration with standard HCl. The results showed that the isothiocyanate did not react with the amide in dioxane, acetone, or toluene at reflux temperatures. However, the reaction did proceed in an aqueous medium. A solution containing 4.27 g. (0.025 mole) of p-tosylamide, 1.25 g. (0.031 mole) of NaOH, and 2.37 g. (0.025 mole) of butylisothiocyanate in 70 ml. of 30 percent aqueous acetone was stirred at 70-80° for 4 hours. Higher temperatures materially decreased the yield, and a strong sulfide odor was noticed. The solution was cooled and acidified to pH 2 with HCl. The resulting oily liquid solidified after being cooled in the refrigerator overnight. The solid material was filtered and dissolved in 40 ml. of 5 percent NaHCO\textsubscript{3} solution. The solution had to be kept above 30° to prevent crystallization of the sodium derivative. The solution was acidified, and an oil, which slowly solidified, was obtained. The solid was recrystallized twice from 50 ml. of 50 percent aqueous ethanol. The oil phase did not occur
in this solvent mixture. The yield of N-p-tosyl-N-butyliolurea was 3.52 g or 51 percent. The melting point was 96-97°.

**Analytical Data**

Calculated for C₁₂H₁₈N₂O₂S₂

C, 50.32; Found: 50.13  
H, 6.33; Found: 6.32

7. Synthesis of N-p-Nitrophenylsulfonfyl-N'-Butylurea.

A solution of 4.06 g. (0.02 mole) of p-nitrophenylsulfonamide, 1.98 g. (0.02 mole) of butylisocyanate, 2.0 g. of triethylamine, in a mixture of 5 ml. of DMF and 25 ml. of benzene, was refluxed for three hours. The benzene was removed under vacuum and the oily residue was dissolved in 60 ml. of 5 percent NaHCO₃ solution. The resulting orange colored solution was decolorized with Darco giving a pale yellow solution which was acidified to pH 2 with HCl. At first a white colloidal precipitate separated which congealed into a large semi-solid mass and solidified after cooling to 0°. The solid material was then dissolved in 30 ml. of ethanol and 10 ml. of water were added, after which crystals slowly grew. The solution was cooled to 0° before filtering, and the compound was recrystallized twice in this manner giving pale yellow plates which melted at 166.0-166.5°. The yield was 4.54 g. or 75 percent.

**Analytical Data**

Calculated for C₁₁H₁₅N₂O₅S  

C, 43.84; Found: 43.83  
H, 5.02; Found: 5.10
8. Synthesis of N-p-Tolyl-N'-Butylurea

A solution of 6.40 g. (0.04 mole) of p-tolyl chloride and 4.64 g. (0.04 mole) of butylurea in 50 ml. of toluene was refluxed until the liberation of HCl fumes ceased; six hours of reflux were required. The solution was concentrated to 20 ml. under vacuum and then cooled in the refrigerator to induce crystallization. The compound was recrystallized from 30 ml. of 95 percent ethanol. The yield was 6.34 g. or 64 percent, after being dried at 70° and 2 mm. The compound was not soluble in 10 percent NaOH, yet most acylureas are soluble in base (93). It was possible that the product was N,N-p-tolyl-N'butylurea (XXXV) rather than N-p-tolyl-N'-butylurea (XXXVI). This possibility was negated by showing that the same compound was obtained by reaction of toluylisocyanate with butylurea as follows:

Toluylisocyanate was prepared by refluxing 19.0 g. (0.12 mole) of p-tolyl chloride with 25.0 g. (0.17 mole) of freshly prepared silver cyanate in 100 ml. of toluene for 6 hours. Care was taken to exclude moisture. The mixture was carefully filtered through a Buchner filter into a Claissen distilling flask. The solvent was removed under vacuum, and p-toluylisocyanate was distilled, with the aid of a stream of nitrogen, at 110-114° and 9 mm. The yield was 8.3 g. or 42 percent. A sample of the isocyanate, when added to water, reacted immediately to yield p-tolylamide which melted at 157-158°, as compared with literature values of 155° and 165° (78). This confirms the formation of p-toluylisocyanate.

A solution of 1.61 g. (0.01 mole) of p-toluylisocyanate in 20 ml. of toluene was added slowly to a solution of 0.74 g. (0.01 mole) of butylamine in 20 ml. of toluene. The resulting solution was refluxed for one hour. The solvent was removed under vacuum and the solid residue was recrystallized from 20 ml. of dioxane. The yield of the dried compound was 1.2 g. or 62 percent. The melting point was 132-133°. There was no depression of the
melting point when mixed with a sample of the compound obtained from reaction of p-toluyl chloride with butylurea. Infrared spectra of both compounds were similar. The structure of XXXVI was thus confirmed.

Analytical Data

Calculated for $\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_2$

$C$, 66.65; Found: 66.59
$H$, 7.73; Found: 7.59

9. Synthesis of 1-p-Tosyl-5-Butylbiuret

A solution of 2.96 g. (0.15 mole) of p-tosylisocyanate, in 20 ml. of ether, was dropped into a solution of 1.74 g. (0.15 mole) of butylurea in 20 ml. of ether and 3 ml. of DMP. A nitrogen atmosphere was provided. A mildly exothermic reaction took place. After stirring the solution for 2 hours, 50 ml. of 5% NaHCO$_3$ solution were added. An amorphous powdery precipitate formed upon agitation. The precipitate was filtered and resuspended in a
mixture of 50 ml. of water and 50 ml. of ether. The aqueous phase was maintained at pH 2. Upon agitation, the sodium derivative slowly dissolved. The ether layer was removed and concentrated to 10 ml., followed by the addition of 20 ml. of petroleum ether. The amorphous precipitate was collected and recrystallization was attempted from other common organic solvents as well as solvent mixtures, without success. The material was finally purified by dissolving the precipitate in 20 ml. of chloroform, decolorizing with Darco, then adding 80 ml. of petroleum ether, which caused an amorphous precipitate to form. This was dried at 80° and 2 mm. The melting point was 97-98° and the yield was 3.99 g. or 85 percent.

At this time, the insolubility of the sodium compound even in hot water could not be rationalized. There was the possibility that the highly reactive p-tosylisocyanate did not selectively attack the unsubstituted NH₂ group of butylurea. This possibility was ruled out after the same compound possessing the same properties was synthesized by another route as follows: p-Tosylurea, prepared from p-tosylisocyanate and anhydrous ammonia, was refluxed with butylisocyanate in toluene, to produce the same compound, 1-p-tosyl-5-butyliuret, as evidenced from a mixed melting point and infrared spectra. This confirmed formula XXXVIII as the correct structure rather than XXXVII.

This compound was later reported (43) with a melting point of 110°.
10. Synthesis of N-p-Tosylvaleramide

N-p-tosylvaleramide was synthesized by refluxing 8.50 g. (0.05 mole) of p-tosylamide with 6.00 g. (0.05 mole) of valeryl chloride in 100 ml. of toluene. After 12 hours the liberation of gaseous HCl ceased. The solvent was removed under vacuum and the solid residue was recrystallized from 80 ml. of 60% aqueous dioxane. After two such recrystallizations, the compound was dried at 70° and 2mm. The yield was 10.5 g. or 81 percent. This method of removing HCl was more suitable than the use of organic bases. Triethylamine caused precipitation immediately when added to a solution of p-tosylamide and valeryl chloride in toluene or acetone. Apparently, quaternization occurred which prevented reaction since unreacted p-tosylamide was isolated after refluxing the mixture for 6 hours.

Analytical Data

Calculated for C_{12}H_{17}NO_{3}S
C, 56.41; Found: 56.63
H, 6.70; Found: 6.62

11. Synthesis of N-\(\beta\)-Phenylethylsulfonyl-N'-Butylurea

Sodium \(\beta\)-phenylethylsulfonate was prepared according to the method of Johnson et. al., (79). A mixture of 20.0 g. (0.108 mole) of \(\beta\)-phenylethyl bromide and 30.0 g. (0.24 mole) of sodium sulfite in 100 ml. of water was refluxed with vigorous stirring; the oily bromide disappeared after 5 hours of refluxing, the solution was cooled in the refrigerator overnight. The crystalline sodium \(\beta\)-phenylsulfonate was collected and dried. The yield was 21.0 g. or 93 percent.

Conversion of sodium \(\beta\)-phenylsulfonate into \(\beta\)-phenylethylsulfonyl chloride was more suitable with POCl_{3} than with PCl_{5} or SOCl_{2}. The latter two caused the sulfonyl chloride to turn dark on
standing even after repeated distillation.

A mixture of 10.0 g. of sodium $\beta$-phenylethylsulfonate and 15.0 g. of POCl$_3$ was refluxed in 200 ml. of benzene for 6 hours. The benzene was removed with an aspirator using gentle heat. After removing all corrosive fumes, the residue was distilled to give 7.4 g. of $\beta$-phenylethylsulfonyl chloride, which boiled at 124-126$^\circ$ and 3 mm.

Five grams of $\beta$-phenylethylsulfonyl chloride were added to 20 ml. of 28% aqueous ammonia solution. The mixture was stirred and refluxed for one hour. Solution gradually occurred. The solution was cooled to give almost a quantitative yield of $\beta$-phenylethylsulfonamide. The melting point was 124-125$^\circ$ which corresponded to the literature value of 124$^\circ$ (79).

A solution of 2.03 g. (0.01 mole) of $\beta$-phenylethylsulfonamide, 1.09 g. (0.01 mole) of butylisocyanate and 1.2 g. of triethylamine in 50 ml. of anhydrous DMF was heated to 90$^\circ$ for three hours. The solvent was removed under vacuum at 40$^\circ$, and the residue was dissolved in 20 ml. of 5 percent aqueous NaOH solution. This solution was acidified to pH 2 with 5 percent HCl and the resulting precipitate was collected and dried. It was recrystallized twice from 30 ml. of 1:1 chloroform-carbon tetrachloride to give a white crystalline compound which, after drying at 70$^\circ$ and 2 mm., melted at 168-169$^\circ$. The yield was 1.52 g. or 54 percent.

Analytical Data

Calculated for C$_{13}$H$_{20}$N$_2$O$_3$S

C, 54.90; Found: 54.96
H, 7.09; Found: 7.27
This compound, a phosphonylurea, should be an interesting compound from the standpoint of biological activity. No. phosphonylureas are reported in the available literature. From a study of phosphorus chemistry (80, 81), a route to the preparation of the phosphonylurea moiety was proposed through reaction of a phosphonylhaloiso cyanate with an amine. Phosphonylhaloiso cyanates have not been previously reported, although phosphonyldi-isocyanates have been reported.

Phenylphosphonylchloroiso cyanate was prepared by refluxing 58.5 g. (0.30 mole) of phenylphosphonyl dichloride with 45.0 g. (0.30 mole) of freshly prepared silver cyanate in 80 ml. of nitrobenzene for one hour. The nitrobenzene was removed under vacuum and the remaining oil was fractionated through a 15 inch column packed with helices. Fraction 1 was obtained at 103-109°/1.5 mm. and amounted to 4.5 g. Fraction 2 boiled at 110-116°/1.5 mm. and amounted to 29.3 g. Fraction 3 distilled at 116-126°/1.5 mm. and weighed 3.2 g. Fraction 2 was refractionated to give 17.4 g. at 90-92°/0.5-1.0 mm.

Fraction 1 was identified as phenylphosphonyl dichloride as evidenced by a negative nitrogen test with the sodium fusion method and by the boiling point. Pure fraction 2 contained nitrogen and halogen, and possessed an intense peak at 2270 cm⁻¹ in the infrared spectra. This fraction was thus identified as phenylphosphonylchloroiso cyanate. Fraction 3 possessed nitrogen but no halogen; two intense peaks near 2270 cm⁻¹ in the infrared spectra, indicative of diisocyanates, showed this compound to be phenylphosphonyl-diisocyanate.

It was desired to take advantage of the greater rates of reactivity of isocyanates than phosphonochloridates (82) by reacting phenylphosphonylchloroiso cyanate with butylamine to give
N-phenylphosphonylchloro-N-buty lurea, and then to methy late this compound with methanol to give N-[phenylphosphonyl- (O-methyl)-] N-buty lurea. However, attempts to react butylamine with the phosphonylchloroisocyanate resulted in elimination of HCl.

The reaction of phenylphosphonylchloroisocyanate with butylamine was carried out with 0.025 molar quantities or 5.0 g. of the former and 1.78 g. of the latter. The phosphonylchloroisocyanate, in 20 ml. of anhydrous ether, was added dropwise, in a nitrogen atmosphere, into the butylamine in 20 ml. of ether. An immediate precipitate formed which began to slowly dissolve. This precipitate, N-phenylphosphonyl-P-chloro-N-buty lurea, could not be isolated in a stable form. The elimination of HCl was apparent by its odor.

Methanalysis of this compound was attempted similar to the method employed by Audrieth (82) in the preparation of phenylphosphonyl-O-methylchloridate. This was done by adding a solution containing an excess of methanol, 5 ml., and one equivalent of pyridine, 2.0 g., directly to the reaction mixture obtained from the interaction of the amine with the phosphonylisocyanate. The mixture was refluxed for 12 hours. The precipitated pyridine hydrochloride was removed and the ether solution was extracted with 30 ml. of 5 percent aqueous NaOH solution. The aqueous extract was acidified carefully to pH 2 with 5 percent HCl. No precipitate was obtained, however, an acidic compound was indicated because an oily liquid was extracted with ether from the basic solution but not from the acidic solution. The oily liquid was soluble in water, acetone, ether, benzene, chloroform, and carbon tetrachloride. The compound was cooled to -5° for 48 hours and by that time, about one-half of the material had crystallized. Petroleum ether (40 ml.) was added (the compound was not soluble in this solvent) and the mixture filtered. The yield of the product was 2.1 g. The infrared spectra showed bonded OH, possibly overlapped with NH. Amide absorption was missing. The melting point of this compound was 108-110°.
It was thought that this compound was possibly O-methyl-phosphonamidate; Audrieth (82) reported a melting point of 111° for this compound. Using his method, stepwise replacement of the
\quad \text{g} \text{ens on phenylphosphonyl dichloride, first by methanolysis in}
\quad \text{the presence of pyridine, then by liquid ammonia, afforded authentic}
\quad \text{O-methyl-phosphonamidate. Infrared spectra of the two compounds were}
\quad \text{the same.}

Apparently the compound XXXIX was formed but during methanolysis, decomposition occurred. The reactions are summarized below.

\[
\begin{align*}
\text{XXXIX + CH}_3\text{OH} & \rightarrow \text{XXXIX + CH}_3\text{OH} & \text{XXXIX + CH}_3\text{OH} \\
\end{align*}
\]
Synthesis of Keto-Sulfonamides

The keto-sulfonamides in this section were prepared for their later use in proving enolization. The keto-sulfonamides are less complex than the sulfonylureas but they possess the same basic structure of $\text{-SO}_2\text{-NH-C}_0\text{-}$. For the study of enolization, it was desirable to have keto-sulfonamides with various groups attached to the basic moiety and compounds in which the attachment to the basic moiety is reversed.

1. Synthesis of p-Tosylacetamide

This compound was synthesized according to the method of Vogel (83) in 84 percent yield from p-tosylamide and acetic anhydride. The melting point was 138-139°, and the recorded melting point was 139° (83).

2. Synthesis of p-Tosyl-p-Toluamide

This was prepared in 87 percent yield by refluxing equimolar quantities of p-tosylamide with p-toluyl chloride in toluene. The melting point was 139-140°; the recorded melting point was 138-139° (84).

3. Synthesis of Methanesulfonyl-p-Toluamide

The method used for the preparation of p-tosyl-p-toluamide was not suitable for the preparation of this compound. Using pyridine as the solvent, reaction was achieved. Two grams (0.02 mole) of methanesulfonylamide and 3.09 g. (0.09 mole) of toluyl chloride were heated in 20 ml. of pyridine for 20 minutes. The reaction mixture was cooled and made acidic with 10 percent HCl. An oil separated and solidified within a few minutes. The solid was
dissolved in 20 ml. of hot ethanol, 20 ml. of water were added, and the solution allowed to cool slowly. The resulting product was re-crystallized twice in this manner to give 4.26 g. or 91 percent yield of long white crystalline needles.

Analytical Data

Calculated for C_{9}H_{11}N\ O_{3}S
C, 50.69; Found: 50.65
H, 5.20; Found: 5.33

4. Synthesis of Di-tosylamide

A mixture of 9.53 g. (0.05 mole) of p-tosyl chloride, 8.55 g. (0.05 mole) of p-tosylamide, 4.0 g. (0.10 mole) of sodium hydroxide, and 100 ml. of 50 percent aqueous acetone was stirred at 70° for 30 minutes. The reaction was mildly exothermic. After the acetone was removed under vacuum, the solution was acidified to pH 1 with HCl. The crystals that formed were removed and dissolved in NaHCO_{3} solution. The solution was acidified and the precipitate recrystallized twice from 50 percent aqueous ethanol. After drying at 100° for 3 hours, the yield was 6.2 g. or 75 percent. The compound melted at 172-173°; the recorded melting point is 174° (85).
Enolization Study

A literature search pertaining to enolization (86, 87, 88, 89, 90) disclosed that little information is available concerning enolization of urea derivatives and nothing has been reported with respect to enolization of sulfonyleureas. In order to study this phenomenon, three studies were made: (a) a pKₐ study of a series of model keto-sulfonamides and a series of sulfonyleureas, (b) an ultraviolet spectral study in the presence of acid and base, and (c) a study of the infrared spectra, especially the bonded N-H region and the keto region.

The pKₐ values of the sulfonyleureas and keto-sulfonamides were determined by titration of approximately 40 mg. of each of the compounds in 20 ml. of 50 percent aqueous ethanol. Double distilled, demineralized water and absolute ethanol were used as the solvent mixture. The ionic strength of the solvent mixture was maintained at 0.10 with potassium nitrate. The compounds to be titrated were dissolved in the solvent mixture and titrated in a 50 ml. jacketed flask at 25°. The solution was magnetically stirred and a nitrogen atmosphere was provided. Approximately 4 ml. of 0.04659 N sodium hydroxide were added through a 5 ml. microburet graduated to 0.01 ml. The pH readings were obtained on a Beckman Model G pH Meter after each 0.1-0.2 ml. additions. Near the endpoint, 0.02 ml. increments were used. The endpoint was found by calculating the greatest change of pH per 0.01 ml. increment. The pKₐ' was taken as the pH at one-half titration. The results are tabulated in tables I and 2.
<table>
<thead>
<tr>
<th>Compound</th>
<th>pKa</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{CH}_3\text{SO}<em>2\text{NH}-\text{C-NH-C}\text{NH}</em>{4}\text{H}_9)</td>
<td>6.67</td>
</tr>
<tr>
<td>(\text{C}_2\text{H}_5\text{SO}<em>2\text{NH}-\text{C-NH-C}\text{NH}</em>{4}\text{H}_9)</td>
<td>6.49</td>
</tr>
<tr>
<td>(\text{CH}_3\text{SO}<em>2\text{NH}-\text{C-NH-C}\text{NH}</em>{4}\text{H}_9)</td>
<td>6.41</td>
</tr>
<tr>
<td>(\text{CH}_3\text{SO}<em>2\text{NH}-\text{C-NH}</em>{4}\text{H}_9)</td>
<td>6.15</td>
</tr>
<tr>
<td>(\text{CH}_3\text{SO}<em>2\text{NH}-\text{C-NH-C}\text{NH}</em>{3}\text{H}_7)</td>
<td>6.03</td>
</tr>
<tr>
<td>(\text{ClSO}<em>2\text{NH}-\text{C-NH-C}\text{NH}</em>{4}\text{H}_9)</td>
<td>5.78</td>
</tr>
<tr>
<td>(\text{CH}_3\text{SO}<em>2\text{NH}-\text{C-NH-C}\text{NH}</em>{4}\text{H}_9)</td>
<td>5.18</td>
</tr>
<tr>
<td>(\text{NO}_2\text{SO}<em>2\text{NH}-\text{C-NH-C}\text{NH}</em>{4}\text{H}_9)</td>
<td>5.04</td>
</tr>
<tr>
<td>(\text{CH}_3\text{SO}<em>2\text{NH}-\text{C-NH-C}\text{NH}</em>{4}\text{H}_9)</td>
<td>3.93</td>
</tr>
<tr>
<td>(\text{CH}_3\text{SO}_2\text{CH}<em>2\text{-C-NH-C}\text{NH}</em>{4}\text{H}_9)</td>
<td>&gt;14</td>
</tr>
<tr>
<td>(\text{CH}<em>3\text{C-NH-C-NH-C}\text{NH}</em>{4}\text{H}_9)</td>
<td>&gt;14</td>
</tr>
<tr>
<td>(\text{CH}_3\text{SO}<em>2\text{N-C-NH-C}\text{NH}</em>{4}\text{H}_9)</td>
<td>a.</td>
</tr>
<tr>
<td>(\text{CH}_3\text{SO}<em>2\text{N=C-NH-C}\text{NH}</em>{4}\text{H}_9)</td>
<td>a.</td>
</tr>
</tbody>
</table>

a. Compound was not acidic.
Table 2

*pK<sub>a</sub> Values of Keto-Sulfonamides

<table>
<thead>
<tr>
<th>Compound</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-SO&lt;sub&gt;2&lt;/sub&gt;-NH-C-(\bigcirc)-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4.88</td>
</tr>
<tr>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-(\bigcirc)-SO&lt;sub&gt;2&lt;/sub&gt;-NH-C-(\bigcirc)-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>5.00</td>
</tr>
<tr>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-(\bigcirc)-SO&lt;sub&gt;2&lt;/sub&gt;-NH-C-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>6.00</td>
</tr>
<tr>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-(\bigcirc)-SO&lt;sub&gt;2&lt;/sub&gt;-NH-SO&lt;sub&gt;2&lt;/sub&gt;-(\bigcirc)-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>2.76</td>
</tr>
</tbody>
</table>

* Represents an average of duplicate determinations agreeing within 0.03.
Discussion

Enolization of saccharin has been postulated to account for the production of the O-ethyl derivative with silver oxide and ethyl iodide. By varying the conditions, the N-ethyl derivative could be obtained; this indicated enolization of saccharin (92).

\[
\text{O} \quad \text{C} \quad \text{NH} \\
\text{SO}_2
\]

\[
\text{OH} \\
\text{N} \\
\text{SO}_2
\]

The sulfonylureas as well as the ketosulfonamides are quite similar to the compound saccharin.

The ionization constants of the keto-sulfonamides show that XLI is a stronger acid than XL.

\[
\text{CH}_3 - \text{SO}_2 - \text{NH-C-CH}_3 \quad \text{CH}_3 - \text{SO}_2 - \text{NH-C-CH}_3
\]

XLI

The order of the acidities can be explained by resonance stabilization of XL, which can be illustrated as follows:

\[
\text{CH}_3 - \text{SO}_2 - \text{NH-C-CH}_3
\]

This will tend to decrease the electron withdrawing power of the sulfone function and as a result the acidity will decrease as compared to XLI. This is supported by the fact that diphenylsulfone has higher SO\textsubscript{2} frequencies than phenylmethylsulfone. The higher frequency is interpreted as indicating more double bond character or greater electron density of the sulfur to oxygen bond.
This same effect is shown with less ambiguity by compounds XLII and XLIII, in which the perturbing group on the parent amide is kept constant.

\[
\text{CH}_3\text{-SO}_2\text{-N=O-CH}_3 \quad \text{pKa} = 6.00
\]

\[
\text{XLII}
\]

\[
\text{CH}_3\text{-SO}_2\text{-N=O-CH}_3 \quad \text{pKa} = 5.00
\]

\[
\text{XLIII}
\]

It is impossible to locate the position of the negative charge of the anion since it probably exists somewhere between the two limiting forms:

\[
\text{CH}_3\text{-SO}_2\text{-N=O-CH}_3^{(-)} \quad \text{and} \quad \text{CH}_3\text{-SO}_2\text{-N=O-CH}_3^{(+)}.
\]
The classical concept of structure assumed that the electrons were held in a rigid arrangement giving rise to two different anions differentiated only by the location of an electron pair. However, this is not entirely correct. The independent existence of two anions in equilibrium is not possible since the electron arrangement is not a stable one, but is subject to constant changes. The more correct structure of the anion could be shown as a resonance stabilized form of:

\[ (-) \begin{array}{c} \text{R-SO}_2\text{-N}^{\equiv} \text{C-R} \end{array} \]

It will be shown later in the infrared study that chelation can act as the driving force to lock the sulfonylureas in the enol form since a C-N frequency is noticed in the spectra of the alkali metal derivatives.
From figure 6, it can be seen that the substituents on the phenyl ring directly affect ionization since a linear relationship is obtained with pKa and \( \sigma \) values. Hammett's \( \sigma \) values are a measure of the electron donating or attracting power of the substituent (91). Apparently, there is conjugation through the sulfone function connecting the ionizing site. Infrared spectral studies on substituted phenylsulfonyl derivatives have shown that conjugation of the type

\[
\begin{array}{c}
\begin{array}{c}
\text{Z-}\text{S-R}
\end{array}
\end{array}
\text{arrow}
\begin{array}{c}
\begin{array}{c}
\text{(+)}\text{Z=}\text{S-R}
\end{array}
\end{array}
\]

exists by virtue of the linear relationship of \( \sigma \) values with the sulfone frequency (91).

The extremely large rho value obtained for the sulfonylureas indicates conjugation with the sulfone function. Rho values for non-conjugated systems such as substituted \( \beta \)-phenylpropionic acids (93) or substituted trans-2-phenylcyclopropanecarboxylic acids (94) are relatively small, ca., 0.2. However, in trans-cinnamic acids, where conjugation can occur, the rho value is 0.466 (93). The rho value of 1.46 for sulfonylureas, indicates strong conjugation through the sulfone function.
Correlation of \( \sigma \)-values and pKa Values

A. \( \text{CH}_3 - \text{SO}_2 - \text{NH-C-NH} \)

B. \( \text{CH}_3 - \text{SO}_2 - \text{NH-C-NH-C}_4\text{H}_9 \)

C. \( \text{Cl} - \text{SO}_2 - \text{NH-C-NH-C}_3\text{H}_7 \)

D. \( \text{NO}_2 - \text{SO}_2 - \text{NH-C-NH-C}_4\text{H}_9 \)

\( \rho = 1.46 \)

Figure 6
An interesting comparison is seen in table 1 from the pK_a value of N-p-tosyl-N-butylurea, 6.41, and N-p-tosyl-N-butylthiourea, 5.18. If ionization of the sulfonylureas occurred directly at the nitrogen, the amide and thioamide groups should exert merely an inductive effect on the ionizing site. From a consideration of the electronegativities of the sulfur atom and the oxygen atom (95), we would expect the amide to be a stronger acid than the thioamide. This is contrary to the results observed. Other workers (96) have noted the same contradiction. The increased acidity of thiourea as compared with urea (97) has been claimed to be the result of greater ease of enolization. Therefore the greater acidity of N-p-tosyl-N-butylurea as compared with the thio derivative offers support in favor of enolization of the sulfonylureas.

Hydrogen bonding of a hydroxyl group with a sulfone group has been studied by the infrared spectral shifts of the hydroxyl frequency (98, 99, 100). Sulfoxides were shown to be capable of forming stronger hydrogen bonds than sulfones. To account for this, Price (101) argues that the additional oxygen of the sulfone increases the dipole of the group thus holding the oxygens closer to the sulfur. The result is a decreased basicity of the oxygens. The two oxygens are considered as a unit in hydrogen bonding, rather than bonding selectively to one oxygen.

The postulation of enolization in the sulfonylureas was first advanced in 1943 by Kumler and Daniels (102) in an attempt to explain the inactivity of sulfanilylurea as a chemotherapeutic agent. However, they gave no evidence to support their postulate.

Correlating all of this information, the enol form of a sulfonylurea in the extreme mesomeric state could be written

\[
\begin{align*}
&\begin{align*}
\text{(-)} & O \\
\text{Z} & \text{S} - \text{N} = \text{C} - \text{NH} - \text{R} \\
\downarrow & O
\end{align*}
\end{align*}
\]

\[
\begin{align*}
\text{OH}
\end{align*}
\]
with a strongly electron donating group (negative sigma value) as Z, and

\[
\begin{align*}
(-) Z &= \text{S-N} \equiv \text{NH-R} \\
(+) &= \text{O} \\
\end{align*}
\]

with a strongly electron attracting group (positive sigma value) for Z. Fisher-Hirschfelder Models of the enolized sulfonylurea were constructed and they showed the possibility of a six membered ring with hydrogen bonding to the sulfone group. This structure is favored by the tetrahedral nature of the sulfur atom, the approximately 90° angle of the sp nitrogen, the planar 120° sp² carbon and the approximately 90° angle of the hydroxyl.

A potential metallic chelating site is apparent here.

**Infrared Spectra Study**

Preliminary gross inspection of the infrared spectra of sulfonylureas showed that some compounds exhibited a tremendous difference in the -NH- region with a simultaneous splitting of the amide peak. This could be interpreted as: (a) hydrogen bonding or (b) partial enolization (103). To elucidate this problem, a quantitative study of the infrared spectra was carried out.

The infrared spectra were recorded on a Perkin-Elmer Model 21 Spectrophotometer using sodium chloride optics and a nujol mull of
the compounds. The \(-\text{NH}\), the amide and the asymmetric \(-\text{SO}_2-\) frequencies are recorded in table 3. The calibration of the spectrographs was accomplished with the partial spectrum of water vapor recorded directly on the graph. The spectrographs are recorded in figures 7, 8, and 9.

If polymerization type hydrogen bonding occurred, molecular weights would easily confirm this. Molecular weight determinations were obtained by the Beckman freezing point method (104) using benzene as the solvent. The benzene was purified by passing anhydrous thiophene free benzene through alumina. To obtain the proper freezing point, 5.5\(^\circ\)C, the benzene had to be recrystallized; the liquid that did not freeze at 4.5-5.0\(^\circ\)C was decanted.

The solubilities of the sulfonylureas were such that 0.03 molar concentrations could not be exceeded. Three determinations of the freezing point were made at each concentration using fresh 2.0 ml. samples of the stock solution each time. The depression of the freezing point of benzene ranged from 0.058 to 0.018\(^\circ\)C. The variation of the readings, with the average, was less than 0.004\(^\circ\)C. The freezing point depression was calculated by subtracting the freezing point of the sulfonylurea solution from the freezing point of the purified benzene sample.
Infrared Spectra

α-Tosyl-N-Butylacetamide

N-p-Tosyl-N'-Butylurea

N-p-Tosyl-N-Methyl-N'-Butylurea

N-(4-Methyl-3-Aminophenylsulfonyl)-N'-Butylurea

Figure 8
Infrared Spectra

Figure 9

N-(\textbeta\text{-Phenylethylsulfonyl})\text{-Butylurea}

N-(\textit{p}-\text{Nitrophenylsulfonyl})\text{-Butylurea}

N-(\textit{p}-\text{Tosyl})\text{-Butylthiourea}

N-(\textit{p}-\text{Chlorophenylsulfonyl})\text{-Propylurea}

N-\text{Valeryl}-\textit{p}-\text{Tosylamide}
Table 3
Infrared Spectra of Sulfonyleureas

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\text{NH}$</th>
<th>Amide</th>
<th>Sulfone</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CH}_3\text{-}\text{SO}_2\text{-NH-}\text{C-NH-C}_4\text{H}_9$</td>
<td>3300</td>
<td>1690</td>
<td>1655</td>
</tr>
<tr>
<td>$\text{Cl}\text{-}\text{SO}_2\text{-NH-}\text{C-NH-C}_3\text{H}_7$</td>
<td>3315</td>
<td>1700</td>
<td>1658</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-}\text{SO}_2\text{-NH-}\text{C-NH-C}_4\text{H}_9$</td>
<td>3320</td>
<td>___</td>
<td>1541*</td>
</tr>
<tr>
<td>$\text{NO}_2\text{-}\text{SO}_2\text{-NH-}\text{C-NH-C}_4\text{H}_9$</td>
<td>3300</td>
<td>1690</td>
<td>1650</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-}\text{SO}_2\text{-N=C-NH-C}_4\text{H}_9$</td>
<td>3380</td>
<td>___</td>
<td>1689</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-}\text{SO}_2\text{-N=C-NH-C}_4\text{H}_9$</td>
<td>3450</td>
<td>1610**</td>
<td>___</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-}\text{SO}_2\text{-NH-}\text{C-NH-C}_4\text{H}_9$</td>
<td>3280</td>
<td>1698</td>
<td>1640</td>
</tr>
<tr>
<td>$\text{CH}_{3}\text{-}\text{SO}_2\text{-NH-}\text{C-NH-C}_4\text{H}_9$</td>
<td>3290</td>
<td>___</td>
<td>1717</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-}\text{SO}_2\text{-CH}_2\text{-NH-C}_4\text{H}_9$</td>
<td>3320</td>
<td>___</td>
<td>1662</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-}\text{SO}_2\text{-NH-}\text{C-NH-C}_4\text{H}_9$</td>
<td>3410</td>
<td>___</td>
<td>1690*</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-}\text{SO}_2\text{-NH-}\text{C-NH-C}_4\text{H}_9$</td>
<td>Bonded</td>
<td>___</td>
<td>1670</td>
</tr>
</tbody>
</table>

* Thioamide frequency.
** =C=N= frequency.
*** Supplied by the Upjohn Co.
Supercooling was noted, but the temperature rose quickly and remained very constant for approximately three minutes. This temperature was recorded as the freezing point.

The change in the theoretical freezing point, obtained from the theorem that one mole of sulfonylurea would give a depression of 5.12° (104), was divided by the observed change in freezing point and the results are presented in figure 10.

The results indicate that dimerization occurred in benzene. The nature of this dimer was elucidated by studying the effect of dilution on the infrared spectra. The solvent, chloroform, was purified by passing analytical reagent chloroform through alumina to remove the preservative, ethanol. Since cells of different lengths were not available, various concentrations of the sulfonylurea were used in a 0.1065 mm. sodium chloride cell. The dependence of the N-H frequency and the amide frequency on dilution is shown in figure 11. Molar extinction coefficients were calculated from the infrared graphs at the various dilutions and the results are plotted in figure 12.

Discussion

Trans association of amides, as shown in XLIV, gives rise to bands at 3500-3400, 3300±20, and 3080±20 (105).

Cis aggregation, XLV provides bands at 3500-3400, 3180±20, 3080±20 cm⁻¹. Cis aggregation would support dimerization.

\[
\begin{align*}
&\text{XLIV} \\
&\text{XLV}
\end{align*}
\]
Freezing Point Depressions in Benzene

![Graph showing freezing point depressions in benzene with molarity on the x-axis and ΔF.P. on the y-axis.]

**Figure 10**

Effect of Dilution on $\nu$ NH and $\nu$ Amide

![Graph showing the effect of dilution on NH and Amide frequencies with molarity on the x-axis and $\nu$ NH and $\nu$ Amide on the y-axis.]

**Figure 11**
Effect of Dilution on Extinction Coefficients

Molar Extinction Coefficients

0.1 M.
0.5 M.
1.0 M.

\[ \frac{\epsilon}{c} \text{ of } N-p\text{-Tosyl-N-Butylurea in CHCl}_3 \]

Figure 12
With 1.0 molar N-p-tosyl-N'-butylurea in CHCl₃, absorption was noted at 3385, 3200, and 3060 cm⁻¹. Figure 10 shows that the compound exists in dimeric aggregates in benzene, therefore, a cis dimer is indicated. This is not too surprising since benzoic acid, as well as many other organic acids, are dimerized in organic solvents (106).

Association in the -NH region and splitting of the amide peak of the sulfonylureas was shown only by those compounds possessing the SO₂-NH-C=O moiety, which, incidentally is the basic pharmacophore endowing hypoglycemic activity(43,44). The -SO₂- frequencies of N-p-tosyl-N'-butylurea are at 1333 and 1160 cm⁻¹, which indicates bonding since the compound N-p-tosyl-N-methyl-N'-butylurea has SO₂ frequencies at 1382 and 1165 cm⁻¹; the latter compound is incapable of SO₂ bonding if cis aggregation occurs. Figure 9 shows a decrease in the -NH and the amide frequencies with increasing concentration. This is a result of increasing aggregation in solution(107). The keto doublet apparently arises from incomplete dimerization in CHCl₃, since the frequency is shifted to the lower values. The shift of a bonded group to lower frequencies as compared with the non-bonded group is a common effect(108). The nature of the dimer can be shown as XLVI.

![Chemical Structure](image-url)
The infrared spectra of potassium N-p-tosyl-N'-butyl-N'-methyl-
urea, fortuitously, provided additional evidence of enolization and
gave indication of chelation. From figure 7 a band is noted at
1590 cm\(^{-1}\) arising from the C=O group. This is verified by N-p-tosyl-
O-methyl-N'-butylisourea which is locked in the enol form and also
has this band at 1610 cm\(^{-1}\). That the compound is a chelate is shown
by a shift of the asymmetric and symmetric frequencies of the -SO\(_2\)-
function, 1333 and 1160 cm\(^{-1}\), to lower frequencies, 1320 and 1130
\(^{cm^{-1}}\); therefore bonding with the -SO\(_2\)- group occurs. It might be
argued that the -SO\(_2\)- band shifts to a lower frequency because of a
greater electron density as in the carboxylate salts. This has been
discounted by the work of Momose et al. (91, 92). By infrared
spectra, they showed that the -SO\(_2\)- function of sulfonamides is
more electron rich than that of sulfones, and, as a result, has a
higher -SO\(_2\)- frequency than the sulfones. The sodium derivative
of 1-p-tosyl-5-butyliuret also showed enolization since a C=O peak
was noticed at 1645 cm\(^{-1}\) with -SO\(_2\)- peaks at 1370 and 1126 cm\(^{-1}\).
Therefore, the structure of potassium N-p-tosyl-N'-butyl-O-methyl-isourea can be shown as XLIII, a chelate.

\[
\text{XLIII}
\]

Sodium 1-p-tosyl-5-butylbiuret, as mentioned before, is unexpectedly insoluble in water. The infrared spectrum shows the presence of the $-\text{SO}_2^-$ group at 1138 and 1375 cm$^{-1}$ and the presence of the amide at 1685 cm$^{-1}$. The peak at 1645 cm$^{-1}$ is undoubtedly due to the C=N group, indicating that the enol form is present. The infrared spectra of the free acid shows the $-\text{SO}_2^-$ at 1370 and 1126 cm$^{-1}$, with the amide at 1690 and 1720 cm$^{-1}$. The C=N frequency was absent. These frequency shifts and the appearance of the C=N group in the salts are indicative of enolization. Additional stability of the chelate is probably provided by the other keto group as depicted in XLIV, which could account for the aqueous insolubility. Fischer-Hirshfelder Models show this to be possible.

\[
\text{XLIV}
\]
Ultraviolet Spectra Study

The ultraviolet spectra of the sulfonylureas was studied to show the effect of phenyl-substitution on the electronic distribution within the molecule in the excited state; the possibility of obtaining further evidence supporting enolization was also foreseen. Biologically, the nature of the electronic distribution in excited states is important for its effect in determining the affinity of a molecule for a receptor site and for its effect in determining the degree and rate of absorption of the molecule (109). The ability of biologically active molecules to assume canonical mesomorphic states has been shown by Kumler and Daniels (110), using bacteriostatic amino compounds, and by Bell and Roblin (111), using the sulfonamides, to be extremely important in explaining biological activity of these compounds.

The ultraviolet spectra of the sulfonylureas and the keto-sulfonamides were obtained at two different concentrations in both 0.05 N HCl and 0.05 N NaOH using double distilled, demineralized water. Most of the sulfonylureas exhibited a weak secondary peak which showed up in the more concentrated solution. The solutions were adjusted to give an extinction of 0.3 to 0.4, the most sensitive region of the instrument. A Beckman DU Spectrophotometer equipped with quartz cells was used with applied cell corrections. Readings were recorded every 3 to 5 μm with 1 μm differentials recorded near the maxima. Molar extinction coefficients were calculated and a synopsis of the results is given in tables 4 and 5.
<table>
<thead>
<tr>
<th>Compound</th>
<th>0.05 M HCl</th>
<th>0.05 N NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_{max}$</td>
<td>$\epsilon \times 10^{-3}$</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-SO}_2\text{-NH-C\text{-NH-C}_4\text{H}_9}$</td>
<td>230</td>
<td>14.13</td>
</tr>
<tr>
<td></td>
<td>263</td>
<td>1.19</td>
</tr>
<tr>
<td>$\text{Cl}\text{-SO}_2\text{-NH-C\text{-NH-C}_4\text{H}_9}$</td>
<td>234</td>
<td>18.21</td>
</tr>
<tr>
<td></td>
<td>258</td>
<td>0.82</td>
</tr>
<tr>
<td>$\text{NO}_2\text{-SO}_2\text{-NH-C\text{-NH-C}_4\text{H}_9}$</td>
<td>259</td>
<td>10.85</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-SO}_2\text{-NH-C\text{-NH-C}_4\text{H}_9}$</td>
<td>228</td>
<td>12.92</td>
</tr>
<tr>
<td>$\text{NH}_2\text{-SO}_2\text{-NH-C\text{-NH-C}_4\text{H}_9}$</td>
<td>264</td>
<td>0.38</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-SO}_2\text{-NH-C\text{-NH-C}_4\text{H}_9}$</td>
<td>230</td>
<td>14.30</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-SO}_2\text{-N-C\text{-NH-C}_4\text{H}_9}$</td>
<td>233</td>
<td>15.31</td>
</tr>
<tr>
<td></td>
<td>263</td>
<td>0.81</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-SO}_2\text{-NH-C\text{-C}_4\text{H}_9}$</td>
<td>231</td>
<td>13.47</td>
</tr>
<tr>
<td></td>
<td>263</td>
<td>0.82</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-SO}_2\text{-N-C\text{-C}_4\text{H}_9}$</td>
<td>230</td>
<td>14.69</td>
</tr>
<tr>
<td></td>
<td>263</td>
<td>0.72</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-SO}_2\text{-CH}_2\text{-C\text{-NH-C}_4\text{H}_9}$</td>
<td>227</td>
<td>4.80</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-SO}_2\text{-NH-C\text{-NH-C}_4\text{H}_9}$</td>
<td>230</td>
<td>13.10</td>
</tr>
<tr>
<td></td>
<td>264</td>
<td>0.74</td>
</tr>
<tr>
<td>$\text{C}_2\text{H}_5\text{-SO}_2\text{-NH-C\text{-NH-C}_4\text{H}_9}$</td>
<td>258</td>
<td>0.21</td>
</tr>
<tr>
<td>$\text{CH}_3\text{-SO}_2\text{-NH-C\text{-NH-C}_4\text{H}_9}$</td>
<td>259</td>
<td>13.41</td>
</tr>
</tbody>
</table>

* In absolute methanol

** In 0.05 N Sodium Methoxide
### Table 5
UV Spectra of Miscellaneous Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{\text{max.}}$ (mu)</th>
<th>$\epsilon \times 10^3$</th>
<th>$\lambda_{\text{max.}}$ (mu)</th>
<th>$\epsilon \times 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$-$\text{C}$-$\text{SO}_2$-$\text{NH}$-$\text{C}$-$CH$_3$</td>
<td>230</td>
<td>13.78</td>
<td>228</td>
<td>13.17</td>
</tr>
<tr>
<td>CH$_3$-$\text{C}$-$\text{NH}$-$\text{SO}_2$-$\text{CH}$</td>
<td>247</td>
<td>14.07</td>
<td>247</td>
<td>16.29</td>
</tr>
<tr>
<td>CH$_3$-$\text{C}$-$\text{SO}_2$-$\text{NH}$-$\text{SO}_2$-$\text{C}$-$CH$_3$</td>
<td>227</td>
<td>23.65</td>
<td>227</td>
<td>23.00</td>
</tr>
<tr>
<td>CH$_3$-$\text{C}$-$\text{NH}$-$\text{C}$-$NH$-$C$_4$H$_9$</td>
<td>244</td>
<td>7.23</td>
<td>245</td>
<td>6.65</td>
</tr>
<tr>
<td>NO$_2$-$\text{C}$-$\text{SO}_2$-$\text{NH}_2$</td>
<td>261</td>
<td>12.32</td>
<td>276</td>
<td>7.51</td>
</tr>
<tr>
<td>CH$_3$-$\text{C}$-$\text{SO}_2$-$\text{NH}_2$</td>
<td>226</td>
<td>13.23</td>
<td>226</td>
<td>9.86</td>
</tr>
<tr>
<td>CH$_3$-$\text{C}$-$\text{NH}$-$\text{SO}_2$-$\text{C}$-$CH$_3$</td>
<td>246</td>
<td>22.69</td>
<td>250</td>
<td>22.88</td>
</tr>
</tbody>
</table>

* In 0.05 N sodium ethoxide.

** In absolute ethanol.
Discussion

Keto-enol tautomerism quite often shows a shift of the UV spectrum of a compound with a change in pH. Base generally favors enol formation (112). Table 4 shows that the sulfonyleureas in general show both a decrease in the extinction coefficients and a decrease in the position of the $\lambda_{\text{max.}}$ in going from acid to base. There are three exceptions: the nitro derivative,3, the acetamide,9, and the phenylethyl derivative,11. It will be shown later that the nitro group has a greater electron affinity than the sulfonyleurea group and as a result the nitro group will achieve a negative charge on the oxygen. It may be argued that the increased chromophoric conjugation gives a shift of the $\lambda_{\text{max.}}$ to the longer wavelengths (113). The constancy of the $\lambda_{\text{max.}}$ of the acetamide,9, indicates direct carbon ionization. It has been shown that sulfonacetamides that cannot enolize, do not shift the position of the $\lambda_{\text{max.}}$, whereas the sulfonacetamides capable of enolization, do shift the position of the $\lambda_{\text{max.}}$ (114). And lastly, the phenylethyl derivative,11, should also be considered as an exception since the sulfone group is not directly attached to the phenyl ring. The weak band noted at 259 mu is characteristic of the benzene ring, therefore, due to insulation of the benzene ring from the ionizing site, a spectral shift should not be expected upon ionization. Another generalization of the sulfonyleureas in table 4, is the decrease in extinction coefficients in going from acid to base.

There is only one report in the literature concerning tautomerism of sulfonamides, that of sulfapyridine and sulfathiazole (115).

Sulfonamide Tautomerism

\[
\text{Sulfonamide Tautomerism}
\]

\[
\text{NH}_2 - \overset{\text{\textbullet}}{\text{\textcircled{\textbullet}}} - \text{SO}_2 - \text{NH} - \overset{\text{\textbullet}}{\text{\textcircled{\textbullet}}} \quad \longleftrightarrow \quad \text{NH}_2 - \overset{\text{\textbullet}}{\text{\textcircled{\textbullet}}} - \text{SO}_2 - \text{N} = \overset{\text{\textbullet}}{\text{\textcircled{\textbullet}}}
\]

Figure 13
These tautomeric systems show a shift of the $\lambda_{\text{max}}$ to lower wavelengths in going from acid to base. The direction of this shift is generally the same with the sulfonyleureas in Table 4. $N^4$-acetyl-sulfapyridine was shown to be 60 percent enolized in water from a comparison of the UV spectra of the $N^1$-methyl derivative, XLIV, with the UV spectrum of the $N$-methyl-pyridyl derivative, XLV.

![Chemical structures](image)

Sulfathiazole, also studied by the same technique, was shown to be 90 percent enolized in water. Using the analogous sulfonyleurea system, the UV spectrum of the parent compound, $N$-$p$-tosyl-$N$-butylurea, shown in Figure 14, practically superimposes the UV spectrum of its $O$-methyl derivative which is locked in the enol form. In base, however, Figure 8 shows that the parent compound becomes dissimilar to both the $O$-methyl derivative and the $N$-methyl derivative. In the case of sulfapyridine and sulfathiazole, base was deemed necessary to produce enolization of the compounds as evidenced from a similarity of the UV spectra of the $N$-methylpyridyl derivative, XLV, and the spectrum of the parent compound in the presence of base. However, from the UV spectra shown in Figures 14 and 15, it appears that the sulfonyleureas are enolized in acid. This possibility was further studied by observing the position of the bands of the sulfonyleureas in solvents of decreasing polarity.

As a rule, solvents of decreasing polarity favor enol formation, and, as a result, a progressive shift of the $\lambda_{\text{max}}$ is noted (116). The maxima of $N$-$p$-tosyl-$N$-butylurea in water, absolute ethanol, and cyclohexane gave $\lambda_{\text{max}}$ values of 230, 229, and 228 nm respectively. This shift of the maxima to lower wavelengths with decreasing solvent
Ultraviolet Spectra

Figure 14

I. CH₃-SO₂-N=O-NH-C₄H₉

II. CH₃-SO₂-N-C-NH-C₄H₉

III. CH₃-SO₂-NH₂-NH-C₄H₉

$\varepsilon \times 10^{2}$

Figure 15

In 0.05N HCl

$\varepsilon \times 10^{3}$

Figure 16

In 0.05N NaOH
polarity appeared to support the theory of enolization; however, it was later shown that this is merely a solvent effect (116) since the peak of N-p-tosyl-N-methyl-N'-butylurea also shifts by 3 mu from 233 in water to 230 mu in cyclohexane. Enolization of this compound is not possible; therefore, the results of this study are inconclusive.

The spectrum of the sulfonylthiourea shown in figure 17 shows an interesting shift of the maximum from 259 mu in acid to 227 mu in base. Thiourea and its alkyl derivatives show an intense peak at 255 to 265 mu which is due to the great chromophoric power of the C=S group since the corresponding urea derivatives are practically transparent in the UV region (117). Using this reasoning, the peak at 259 mu appears to be due to the thione form which disappears in base apparently because of enolization. The resulting peak at 227 mu is reminiscent of the p-tosyl group although the intensity is much greater.

Figure 18 shows the effect of acid and base on the spectra of N-(p-methyl-m-aminobenzenesulfonyl)-N'-cyclohexylurea. In base a peak is noted at 293 mu which reverts to 227 mu in acid. Apparently the p electrons of the amino group are removed from conjugation through salt formation with the acid, since the spectra reverts to that of the simple tosyl derivative, N-p-tosyl-N'-butylurea.

Figure 19, a plot of A<sup>-</sup> versus A<sup>+</sup> values, is an attempt to delineate the substituents on the phenyl ring of the sulfonylureas into those affording positive mesomeric structures and those affording negative mesomeric structures by means of their effect on electronic spectra. The <sup><sigma>-</sup> value of the SO<sub>2</sub>NHCONHR group was obtained from the pKa, 3.54, at 37.5° in water of N-p-carboxyphenyl-N'-butylurea (118). This value was estimated to be 3.64 at 25° from a consideration of the effect of temperature on the ionization constant of acetic acid (119). By using Hammett's definition of sigma as the log <var>K</var> <var>o</var> / <var>K</var> <var>i</var>, where <var>K</var> <var>o</var> is the ionization constant of benzoic acid and <var>K</var> <var>i</var> is the ionization constant of the substituted benzoic acid (N-p-carboxyphenyl-N'-butylurea), a value of -3.58 + 4.20 or + 0.62 was obtained as the <sup><sigma>-</sup> value of the substituent, -SO<sub>2</sub>NHCONHR. This
Affect of pH on Spectra

\[
\text{In 0.05 N NaOH}
\]

\[
\text{In 0.05 N HCl}
\]

Figure 17

\[
\text{In 0.05 N HCl}
\]

\[
\text{In 0.05 N NaOH}
\]

Figure 18
value was subtracted from the $\sigma^-$ values of the various substituents, to give the $\Delta \sigma^-$ value. Doub and Vandenbelt (120), from a careful analysis of the effects of benzene substitution on UV spectra, have shown that benzene may be considered to have a peak at 203.5 mu when calculating $\Delta \lambda$ values. Thus the observed maxima of the various sulfonyleurases minus the hypothetical 203.5 mu value gave the values. The $\Delta \lambda$ values may be considered as the UV manifestation of the net resultant of the electron affinity of the p-phenyl substituent, whether it be electron donating or withdrawing, and the electron withdrawing affinity of the -SO$_2$NHCONHR moiety.

Thus, the groups in the second quadrant of figure 19 have a greater affinity for electrons than the -SO$_2$NHCONHR group and this would necessitate a negative charge on the phenyl substituent in the mesomeric state. It is interesting to note that the compounds in this second quadrant are inactive as hypoglycemics (44). The compounds in the fourth quadrant, all active hypoglycemic agents, would have a positive charge on the phenyl-substituent in the excited state. The chloro derivative should be considered an anomaly since it possesses an electron attracting effect through induction and an electron donating ability through resonance. It should be noted that the $\sigma^-$ values are obtained from the ground state whereas the UV spectra is a function of the excited state, which may confuse the picture with the halogens. It would be interesting to test this plot on a larger series of sulfonyleurases.

The properties of the sulfone function have been shown to support a configuration of:

```
  O     O
- S -
```

this has been postulated from studies of dipole moments (121), ultraviolet spectra (122, 123, 124, 110), and from infrared spectra (91, 92, 125, 126). This would permit the postulation of one structure, XLVI, for electron donating substituents and another, XLVII, for electron attracting substituents in the excited state.
Correlation of Electronic and Spectral Effects

\[ \Delta \lambda \]  
\[ \mu \text{ Calc.} \]

* Obtained from the value of the p-substituent minus the value of SO_2NHCONHR.

** Obtained from the max. observed minus the hypothetical max., 203.5 \text{ mu}, of benzene (121).

*** Max. obtained from Baxter, J.N. Comptes Rend., 210, 60 (1959).

Figure 19
It should be pointed out that the structural formula of the sulfone function written with one S to O single bond and One S to O double bond is a manner of depicting the electronic distribution in support of the properties of the sulfone function. In actuality, the sulfone function should be considered as a dipole with the oxygens acting as a net negative site and the sulfur as the positive site (101).
In order to reach the beta cells of the pancreas, the sulfonylureas must be transported in both the ionized and the unionized states. The ionized species are necessary for transport through the various cell membranes (127, 128). In general, the membranes of mammalian cells are composed of lipoprotein films about 5 nm thick (129). At body pH, the sulfonylureas would essentially be completely ionized, thus the limiting factor would be their intrinsic lipid solubilities. This may explain why a small change in the sulfonylurea structure gives a profound change in the biological activity of the compound.

Partition coefficients of the sulfonylureas were determined from the system chloroform (0.75 ml.), cyclohexane (9.25 ml.) and water (10 ml.). The water was adjusted to pH 1.5 with hydrochloric acid before equilibration. The cyclohexane was purified by washing with sulfuric acid and water, then distilled (130).

Ten ml. of the acidic stock solution of N-p-tosyl-N-butylurea were mixed with 10 ml. of the cyclohexane-chloroform mixture in a 30 ml. vial stoppered with a plastic cap. The mixture was shaken and allowed to stand at 25° in a water bath for 6 hours. A sample of the lower aqueous layer was removed and read directly on the spectrophotometer at 230 nm. The extinction was 0.170; the reading before equilibration was 0.458. Therefore, the amount in the organic layer was represented by an extinction of 0.458 - 0.170 or 0.288. The partition coefficient, defined as the concentration in the organic phase divided by the concentration in the aqueous phase, gave a value of 0.288/0.170 or 1.6. The same procedure was carried out with the sulfonylureas listed in table 6 using the respective max. of the compounds in acid.
Table 6

<table>
<thead>
<tr>
<th>Compound</th>
<th>Distribution Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. $\text{CH}_3\text{-SO}_2\text{-NH-C-NH-C}_4\text{H}_9$</td>
<td>0</td>
</tr>
<tr>
<td>2. $\text{NO}_2\text{-SO}_2\text{-NH-C-NH-C}_4\text{H}_9$</td>
<td>0.11</td>
</tr>
<tr>
<td>3. $\text{CH}_3\text{-SO}_2\text{-NH-C-NH-C}_4\text{H}_9$</td>
<td>0.23</td>
</tr>
<tr>
<td>4. $\text{Cl}\text{-SO}_2\text{-NH-C-NH-C}_3\text{H}_7$</td>
<td>0.40</td>
</tr>
<tr>
<td>5. $\text{CH}_3\text{-SO}_2\text{-NH-C-N-C}_4\text{H}_9$</td>
<td>1.10</td>
</tr>
<tr>
<td>6. $\text{CH}_3\text{-SO}_2\text{-NH-C-C}_4\text{H}_9$</td>
<td>1.50</td>
</tr>
<tr>
<td>7. $\text{CH}_3\text{-SO}_2\text{-NH-C-NH-C}_4\text{H}_9$</td>
<td>1.61</td>
</tr>
<tr>
<td>8. $\text{CH}_3\text{-SO}_2\text{NH-C-NH-C}_4\text{H}_9$</td>
<td>46</td>
</tr>
<tr>
<td>9. $\text{CH}_3\text{-SO}_2\text{N=CH-NH-C}_4\text{H}_9$</td>
<td>66</td>
</tr>
<tr>
<td>10. $\text{CH}_3\text{-SO}_2\text{NH-C-NH-C}_4\text{H}_9$</td>
<td>130</td>
</tr>
</tbody>
</table>
Discussion

From the definition of the partition coefficient, greater aqueous solubility will result in a lower partition coefficient. Table 6 shows that compound 1 has a distribution coefficient of 0. This arises from the fact that at pH 1.5, the amino group on the phenyl ring becomes a hydrochloride through salt formation. This results in a complete localization of the compound in the aqueous layer.

The difference in distribution coefficients between compound 2 and compound 3 shows the greater hydrophilic character of the nitro group as compared with the methyl group. The chloro derivative, 4, should not be directly compared with compounds 2 and 3 since it contains a propyl chain rather than a butyl chain. Compounds 6 and 7 are somewhat surprising in that there is little difference between their portion coefficients. One would expect the inclusion of an NH group, as in 7, to endow a greater water affinity, although this was not noted.

The large partition coefficients of compounds 8 and 9 dramatize the significance of the $N^1$ hydrogen in favoring aqueous solubility. The position of the methyl group, whether it be on the $N^1$ nitrogen or the oxygen is not critical.

The thio compound, 10, gives an unexpectedly high lipid solubility. This could be explained by the decreased tendency of thio compounds to exhibit hydrogen bonding (131), therefore a decreased affinity of water molecules for the compound.

The extremely high partition coefficients of the compounds 8, 9, and 10 may be the reason for their lack of hypoglycemic activity. Thus, in addition to other factors, the partition coefficients appear to influence biological activity.
As mentioned previously, the diabetogenic action of alloxan, which first produces a hypoglycemic state, is directly correlated with the ability to chelate. Other diabetogenic compounds, when modified chemically to prevent chelation, have also been shown to be inactive in the production of diabetes. Since chelation appears to be important with some diabetogenics, it may quite possibly be important in the hypoglycemic action of the sulfonylureas. Since the sulfonylureas have been shown, earlier in this work, to enolize and to chelate with monovalent metals, this study was undertaken to learn the stability of the sulfonylurea chelates with divalent metals, especially zinc.

The equation for the reaction of the sulfonylureas with metal ions may be written as:

\[ R-S=O + M^{+} \stackrel{k}{\rightarrow} R-S-O \cdot M^{+} \]

\[ R-NH \]

\[ L_1 \]

\[ R-S=O \cdot M^{+} + L_1 \stackrel{k_2}{\rightarrow} R-S-O \cdot M \cdot O-C \]

\[ R-NH \]

\[ L_{III} \]

\[ R-S-O \cdot M \cdot O-C \rightarrow R-S-O \cdot M \cdot O-C \]

\[ R-NH \]

\[ L_{IV} \]
If Ke is allowed to represent the chelating agent or the sulfonylurea, the sum of both of the above reactions is:

$$M^{++} + 2Ke \rightarrow MKe_2$$

and, following Bjerrum's notation (132), the stability constant, $K_s$, will be expressed by the following equation:

$$\frac{MKe_2}{M^{++} \times Ke^2} = K_s = k_1 \times k_2$$

The mathematical treatment developed by Bjerrum for the calculation of formation constants of this type involves the quantity $\bar{n}$, the average number of Ke bound to one $M^{++}$. For a 2:1 complex, it has been shown that at $\bar{n} = 0.5$, $1/Ke - k_1$, at $\bar{n} = 1.5$, $1/Ke - k_2$, and at $\bar{n} = 1.0$, $1/Ke^2 = K_0$. In order to determine $\bar{n}$, the method of Calvin et al. (133, 134) was used in which $\bar{n}$ is obtained from the horizontal displacement of the titration curve of the sulfonylurea compared with the titration curve of the sulfonylurea-plus-metal. This displacement, in ml., is a measure of the amount of $H^+$ liberated through chelation. Each $M^{++}$ ion will displace a total of two $H^+$ ions. Therefore, the horizontal displacement in ml., when converted to moles and divided by the total number of moles of metal present, will give $\bar{n}$. The validity of this can be shown mathematically:

$$M^{++} + HKe \rightarrow MKe^+ + H^+$$

$$MKe^+ + HKe \rightarrow MKe_2 + H^+$$

The total amount of Ke chelated is the sum of the $MKe^+$ and $MKe_2$. Therefore, the change in $pH$ upon addition of metal can be used in determining $\bar{n}$ as shown from the equation:

$$\bar{n} = \frac{MKe_2 + MKe^+}{M^{++}} = \frac{Ke \text{ (chelated)}}{M^{++}} = \frac{H^+}{M^{++}}$$
Typical curves in the presence and in the absence of metal ion are shown in figure 20. For example, at pH 6 using copper ion, the displacement amounts to 0.05 ml. and the value of $\bar{n}$ can be calculated by the equation:

$$\bar{n} = \frac{\text{ml.} \times 10^{-3} \times \text{Normality of Base}}{\text{Conc of Metal}}$$

$$\bar{n} = \frac{0.05 \times 10^{-3} \times 1.378 \times 10^{-2}}{1.60 \times 10^{-6}} = 0.42$$

The values for log $K_e$ can be found from the Henderson-Hasselbalch equation, as demonstrated at pH 6.00:

$$\text{pH} = \text{pKa} + \log \frac{\text{Salt}}{\text{Acid}}$$

$$\log K_e = \text{pKa} - \text{pH} - \log K_e$$

$$\log K_e = 5.75 - 6.00 - (-4.80)$$

$$\log K_e = 4.55$$

The values of $\bar{n}$ and log $K_e$ for Cu$^{++}$, Zn$^{++}$, and Co$^{++}$ with N-p-tosyl-N-butyrulea are given in table 6. The value of pKa was determined at one-half titration of the sulfonylurea in the absence of metal. Finally, by plotting the values of log $K_e$ against the $\bar{n}$ values, as shown in figure 21, the value of $K_s$ was read at $\bar{n} = 1$. For Cu$^{++}$, Zn$^{++}$, and Co$^{++}$, the respective $K_s$ values were found to be 3.8, 3.5 and 3.4.

The titrations of the sulfonylurea were carried out at 25° with 100 ml. (1.6 x $10^{-5}$ mole) of the stock solution using 0.01388 N tetramethyl ammonium hydroxide as the titrant. This base has been shown to be preferable to sodium hydroxide since the latter chelates and may distort the results (135). The stock solution was prepared by dissolving 43.78 mg. of the sulfonylurea in 1000 ml. of double distilled, demineralized water. Solution was achieved by warming and agitating the solution for 3 hours. The titration was carried out in a 250 ml. jacketed flask with holes provided in the cover.
for the Beckman G electrodes, a buret, a thermometer and a nitrogen inlet. The solution was stirred magnetically, but had to be turned off before pH reading could be obtained. This was due to the low ionic strength, and it was undesirable to increase the ionic strength since self ionization of the sulfonylureas would be increased. A 1 ml. microburet graduated to 0.002 ml. was used.

Analytical reagent metals were used in preparing the stock solutions of the metals. Ten ml. of the metal stock solutions, containing $1.8 \times 10^{-6}$ moles of the respective metals, were added to 100 ml. of the sulfonylurea solution to obtain the chelate curve.

An attempt was made to isolate chelates by addition of 273 mg. (0.001 mole) of N-p-tosyl-N′-butylurea, 10 ml. of 0.046 N NaOH, and 0.001 mole of the metal chlorides. Cupric chloride gave an immediate pale green precipitate of the copper chelate. Cobaltous chloride would cause precipitation of its chelate only when excess base was added. The zinc chelate could not be isolated; excess base caused solubilization. Infrared spectra of the cobalt or copper precipitates dried at $90^\circ$ for one hour showed little difference as compared with the infrared spectra of the starting compound, N-p-tosyl-N-butyurea. However, infrared spectra of the air dried metal derivatives showed a C=N peak indicating that enolization had occurred. The spectra were ill defined since the compounds contained water of hydration.

Attempts to methylate N-p-tosyl-N-butyurea with equimolar amounts of dimethyl sulfate and two equivalents of aqueous base, either at room temperature or at reflux temperature, failed.
Effect of Metals on Titration Curve

Figure 20
### Table 6

Log Ke and \( \bar{n} \) Values of Chelates

<table>
<thead>
<tr>
<th>pH</th>
<th>log Ke</th>
<th>Cu</th>
<th>Zn</th>
<th>Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.00</td>
<td>4.55</td>
<td>0.42</td>
<td>0.43</td>
<td>0.44</td>
</tr>
<tr>
<td>6.50</td>
<td>4.05</td>
<td>1.09</td>
<td>0.78</td>
<td>0.69</td>
</tr>
<tr>
<td>7.00</td>
<td>3.55</td>
<td>1.33</td>
<td>1.10</td>
<td>0.91</td>
</tr>
<tr>
<td>7.5</td>
<td>3.05</td>
<td>1.95</td>
<td>1.31</td>
<td>1.22</td>
</tr>
<tr>
<td>8.00</td>
<td>2.55</td>
<td>1.58</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td>8.50</td>
<td>2.05</td>
<td>2.10</td>
<td></td>
<td>1.75</td>
</tr>
</tbody>
</table>

---

**Graphical Determination of \( K_s \)**

![Graphical Determination of \( K_s \)](image)

**Figure 21**
Discussion

Since extremely dilute solutions were used, the results are valid only in this system. Chelate stability constants have been previously determined from solutions containing as little as $5 \times 10^{-5}$ moles of chelating agent (136) which is comparable to the value of $1.6 \times 10^{-5}$ moles used in this work. Dilute solutions had to be used since the solubility of the sulfonylureas are limited in water. Aqueous ethanol or dioxane were not used because they raise the $pK_a$ values of the sulfonylureas and result in the precipitation of the metal hydroxides due to the higher pH of the solution. From figure 20 it can be seen that the stability of the metal chelates is in the order $Cu^+ > Zn^+ > Co.++$. This is the same order observed with the chelating agent ethylenediamine (137). The $K_g$ values of $Cu^{++}$, $Zn^{++}$ and $Co^{++}$ are estimated at 3.8, 3.5 and 3.4 respectively from figure 21. The smaller the value of $K_g$, the weaker the chelate. The sulfonylureas form relatively weak chelates comparable to insulin, which has a $K_g$ of 4.4 to 6.1 (164). Values for $K_g$ as high as 15 are not uncommon.

The inability to methylate the sulfonylureas in aqueous base with dimethylsulfate indicates that the ionic form is possibly resonance stabilized. The UV study supports this since the spectra of the sulfonylureas in base are dissimilar to the corresponding O-methylisourea derivatives which are held in the enol form. The infrared spectra show clearly that the solid sodium or potassium chelates of the sulfonylureas are held in the enol form. It appears that chelation acts as a driving force in the formation of the enol structure.
SUMMARY AND CONCLUSIONS

1. Eleven sulfonylureas were synthesized and one phosphonylurea was attempted by the following methods:

(a) The reaction of an appropriate amino compound with p-tosyl isocyanate gave the sulfonylureas N-p-tosyl-N'-butylurea, N-p-tosyl-N'-butyl-N'-methylurea, and 1-p-tosyl-5-butyliurea.

(b) The reaction of butyl isocyanate with a sulfonamide afforded the compounds N-p-tosyl-N'-butylurea, N-p-nitrophenyl-N'-butylurea, and N-\(\beta\)-phenylethyl-N'-butylurea. \(\beta\)-phenylethylsulfonamide was prepared from \(\beta\)-phenylethyl bromide and sodium sulfite, which gave \(\beta\)-phenylethyl sulfonate; this, reacted with \(\text{POCl}_3\) gave \(\beta\)-phenylethylsulfonyl chloride, and this, in turn, with ammonia gave the sulfonamide.

(c) N-p-tosyl-N' butyl-0-methylisourea was prepared from p-tosyl chloride and O-methylbutylisourea. This compound is water insoluble.

(d) N-p-tosyl-N-methyl-N'-butylurea was prepared by the reaction of diazomethane with N-p-tosyl-N'-butylurea. This compound is also insoluble in water.

(e) N-p-tosyl-N'-butylthiourea was prepared from butyl isothiocyanate and p-tosyl amide in aqueous base. Anhydrous organic solvents were unsuitable.

(f) N-p-toluyl-N'-butylurea was prepared from p-toluyl chloride with butylurea and from toluyl isocyanate and butylamine.

(g) Phenylphosphonylchloroisocyanate was prepared from phenylphosphonyl dichloride and silver cyanate by refluxing equimolar amounts in nitrobenzene. Attempts to prepare N-phenylphosphonyl-0-methyl-N'-butylurea by reaction of phenylphosphonylchloroisocyanate with butylamine and then
methanol failed since HCl was liberated from the compound and resulted in cleavage of the molecule to O-methylphenyl-phosphonylamidate.

2. The ability of the sulfonyleureas to enolize was shown from the analogous keto-sulfonamides which were synthesized for this study. Since there is a difference in the pKa values of the two compounds N-p-tosyl-acetamide and N-methanesulfonyl-p-toluamide, enolization is indicated. Direct N ionization should not give rise to a difference in the pKa values of the two compounds.

3. The pKa values of the sulfonyleureas were shown to depend heavily on the electrophilic nature of the substituents on the phenyl ring. Conjugation through the sulfone function is indicated.

4. The infrared spectra of the sulfonyleureas showed considerable perturbation in the -NH- and amide regions. This was shown not to be enolization, but dimerization. Freezing point depressions supported this contention. The infrared spectra of the monovalent metal derivatives of the sulfonyleureas clearly showed that the compounds were in the enol form since a C=N peak appeared and the amide peak disappeared. Since the SO₂ band shifted to the lower frequencies, bonding with the SO₂ was likely.

5. Further evidence supporting enolization was provided by the UV study. In general, the sulfonyleureas exhibited hypsochromy in base. The spectra of the compound N-p-tosyl-N-butylurea, in acid, resembled the compound N-p-tosyl-N-butyl-O-methylurea and thus supported enolization. The compound N-p-tosyl-N-butyl-thiourea also shows enolization since the thione peak disappears, indicating that ionization proceeds via the sulfur atom. The \( \Delta \sigma^\text{max} \) values were shown to be a function of the \( \Delta \sigma^- \) values. The difference between the \( \sigma^- \) values of the phenyl substituent and the sulfonyleureas moiety gives the \( \Delta \sigma^- \) values.

6. The effect of blocking the acidic hydrogen or replacing the urea moiety by a thiourea group, in the sulfonyleureas, was shown to greatly increase the partition coefficients. The partition
coefficients appear to be important in determining biological activity.

7. The stability constants of N-p-tosyl-N-buty lurea with Cu++, Co++, and Zn++ have been found to range between 3 and 4 in dilute aqueous solutions. The chelate stability constant of zinc insulin is between 4 and 6. Therefore, it appears quite possible that the sulfonyleureas may exert their action through chelation. Since insulin is stored in the pancreas as an insulin-zinc-mitochondria complex and since other chelating agents can displace the insulin from the complex, the sulfonyleureas may also act in this manner.
BIBLIOGRAPHY


42. Scope, 2, 2 (1957).


51. Billeter, O. C. Ber., 37, 690 (1904).


60. Vaughan, M., Science, 123, 885 (1956).
65. Scope, 2, 7 (1957).


108. Ibid., p. 162.


117. Ibid., p. 70.


137. Williams, R.J.F., Biological Revs., 28, 381 (1953).
I, Walter Morozowich, was born in Irwin, Pennsylvania, in the year 1933. I received my secondary school education in Irwin. I obtained a Bachelor of Science degree in Pharmacy from Duquesne University, in 1955, and a master of Science degree in Pharmacy here at Ohio State University, in 1958. I held an Upjohn Fellowship from 1955 to 1958 and an American Foundation for Pharmaceutical Education Fellowship the year 1958-1959.