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The Benzodiazepine Story[1]

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Before starting with my lecture proper, I would like to express my deep felt thanks to the Medicinal Chemistry Division of the American Chemical Society for choosing me to receive this prestigious award. It is indeed an honor, gives me great satisfaction, and makes me very happy.

In this lecture, I would like to tell you about the chain of events that started with the synthesis of a new chemical entity and culminated in the discovery of a new class of biologically active agents. Specifically, I shall discuss the development of the group of centrally acting 1,4-benzodiazepines that began with the discovery of a pharmacologically active compound, which received the generic name chlordiazepoxide and is the active ingredient of Librium.

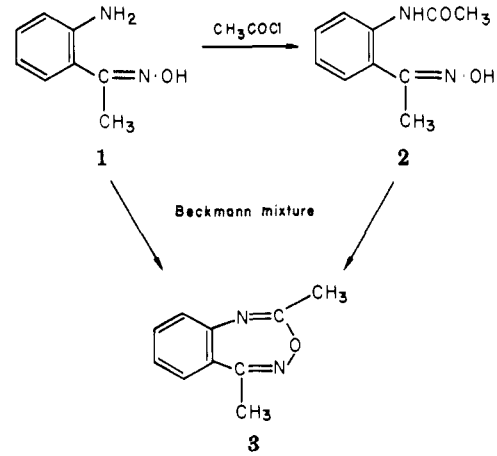
The story starts in the mid 1950s when the tranquilizers, a new class of therapeutic agents, were shown to have considerable clinical value, and Roche decided to embark on a program concerned with the synthesis of products of this type. The pharmacological tests for the screening of sedatives and tranquilizers were well in hand, and we chemists were asked to produce a new compound which would be superior to the then existing tranquilizers.

A chemist faced with a problem of this kind has various approaches at his disposal. He can start in a rather sophisticated manner with a biochemical working hypothesis, with other intelligent speculations, or select a more prosaic approach.

Our knowledge of the processes occurring in the brain was rather limited, and we could not think of an intelligent working hypothesis. Therefore, we decided to take the low road and to attack this problem in a purely empirical manner. Since our main interest was chemical synthesis, we planned to select an approach which would be chemically most attractive, challenging, and satisfying. This left us essentially with two alternatives: to modify existing drugs or to search for a new class of tranquilizers. Molecular modification, sometimes disparagingly called molecular manipulation, of products known to have the desired properties has proven to be very successful in the past. The modification or simplification of the molecular structure of naturally occurring alkaloids, hormones, and antibiotics has given excellent results. The molecular modification of many synthetic drugs, e.g., of the first sulfa drug Prontosil, the first MAO inhibitor, the first synthetic diuretic, and many other synthetic biologically active products has also led to vastly improved medicines. This approach did not appear to be very promising, since the then known tranquilizers were intensively studied by several groups of investigators, e.g., meprobamate (Miltown) at Wallace Laboratories, reserpine by the Ciba research group, and chlorpromazine by the SKF research team.

We therefore considered it more attractive to pursue the second approach and be guided mainly by our interest in synthetic chemical bench work. The class of compounds we were seeking would be expected to fulfill the following criteria: (1) be relatively unexplored, (2) be readily accessible, (3) give the possibility of a multitude of variations and transformations, (4) offer some challenging chemical problems, and (5) "look" as if it could lead to biologically active products. Our search led us to the benzheptoxdiazines, compounds I had worked with during my postdoctoral assistantship years at the University of Cracow.[2] These studies in the early 1930s were concerned with a search for new dyestuffs and dyestuff intermediates and were terminated after we found that the benzheptoxdiazines did not lend themselves to the anticipated uses. Compounds of this type now looked rather attractive to us and seemed to be well suited for a fairly broad synthetic program. The starting materials were readily accessible, and their transformation into benzheptoxdiazines seemed to be a reaction of general applicability.

The first compounds of this type were prepared in 1891 by Auwers and von Meyenburg[3] by treatment of amino- (1) or acetaminoacetophenone oximes (2) with a Beckmann



mixture. The heptoxdiazine structure was "definitely established" in 1924.[4]

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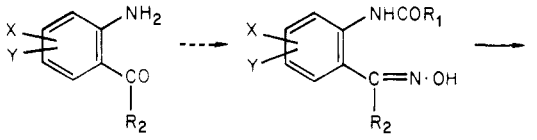
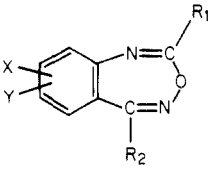
I knew from my past experience that these compounds were readily formed, crystallized very well, and could be easily isolated and purified. A literature search revealed that since our work in Cracow[2] very little had been published about the chemistry of benzheptoxdiazines and that no studies concerned with their biological properties had been carried out. These compounds therefore seemed to be ideally suited for our purposes. We planned to synthesize a number of the relatively readily accessible amino ketones **4** bearing various substituents in the



4 **5**



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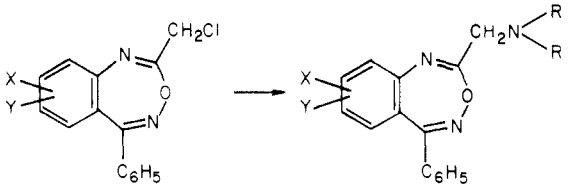
benzene ring and acylate their oximes to products of type **5**. By combining a variety of amino ketones and acids, a large number of new compounds of type **6** would be expected to become available in the shortest time and with a minimum of difficulties.

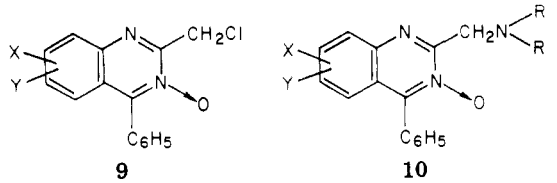
Further transformations of **6** offered the promise of additional interesting possibilities. One of our first objectives was the synthesis of new compounds, e.g., **7**, which, by treatment with amines, could be converted into products possessing basic side chains as in **8**. The reaction



7 **8**

products, we hoped, might have interesting properties, since it is known that basic groups frequently impart biological activity.

In the midst of our work, we began to have serious doubts about the structure of the heptoxdiazines of type **7** and **8**. In particular, the results of hydrogenation experiments were quite revealing. The oxygen was removed with great ease and the products, formed in good yield, were quinazolines. Additional chemical studies showed unequivocally that the so-called heptoxdiazines did not possess the postulated structure but were in fact quinazoline 3-oxides[5] as shown in **9** and **10**. The interesting

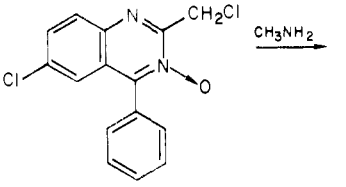


9 **10**

novel structure of these compounds and their facile formation and transformations gave us additional incentive to continue our work. We synthesized a number of quinazoline 3-oxides of type **9**, treated them with secondary amines, and obtained the expected substitution products of type **10**. The reaction occurred readily with the formation of nicely crystallized products, but unfortunately the pharmacological properties were rather disappointing. Neither removal of the *N*-oxide oxygen nor hydrogenation at the 3,4 position yielded anything of interest.[5,6]

At that time (this was the second half of 1955) we had to stop our work in the quinazoline field since other problems seemed to be of greater importance. We became involved with other synthetic projects and the isolation, purification, and degradation of various antibiotics. This intensive work, of little practical value, finally led, in April 1957, to an almost hopeless situation. The laboratory benches were covered with dishes, flasks, and beakers—all containing various samples and mother liquors. The working area had shrunk almost to zero, and a major spring cleaning was in order.

During this cleanup operation, my co-worker, Earl Reeder, drew my attention to a few hundred milligrams of two products, a nicely crystalline base and its hydrochloride. Both the base, which had been prepared by treating the quinazoline *N*-oxide **11** with methylamine, and



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its hydrochloride had been made sometime in 1955. The products were not submitted for pharmacological testing at that time because of our involvement with other problems. Since the compounds were pure and had the expected composition, we submitted the water-soluble salt for pharmacological evaluation in 1957. We again expected to receive negative pharmacological results and thought that our work with quinazoline *N*-oxides would be finished and lead to the publication of some chemically interesting material. Little did we know that this was the start of a program which would keep us busy for many years.

The product was submitted for testing in May 1957 and within a few days we received an enthusiastic telephone call from our pharmacologist, Dr. Lowell Randall. He informed us that this compound possessed unusually interesting properties in the six tests which were generally used for the preliminary screening of tranquilizers and sedatives. Table I shows the comparison of its pharmacological properties with those of the then most used tranquilizers and the hypnotic, phenobarbital.[7] Mice were used in all these tests with the exception of the third one which was carried out with unanesthesized cats. The inclined screen test indicates muscle relaxation and sedation and the foot shock possibly a taming effect. The test with the unanesthesized cat shows muscle relaxation and is quite characteristic for this class of compounds as is the pentylenetetrazole test which indicates sedative and anticonvulsant properties. The two electroshock tests are a measure of their potency as anticonvulsants.[8]

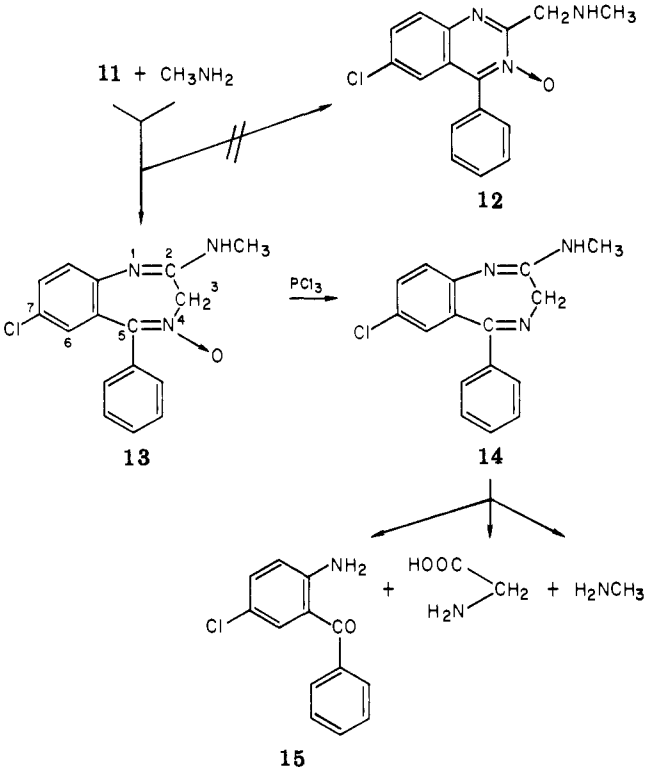
Table I shows that the new compound was much more effective than meprobamate in each of our six preliminary tests. Compared with chlorpromazine, it was weaker in the first two tests, of equal strength as a muscle relaxant in the cat, and had a more pronounced anticonvulsant activity in the mouse. As can be seen in the last line our new compound was superior to phenobarbital in the first four tests but inferior in the two electroshock tests. The absence of direct hypnotic properties below the toxic dose was another interesting feature which differentiated it very characteristically from phenobarbital. It is also worth noting that unlike chlorpromazine and reserpine it had no

Table I. Pharmacological Properties[a] of "New Compound", Meprobamate, Chlorpromazine, and Phenobarbital

compd	inclined screen	foot shock	cat	anticonvulsant tests: pentylene-tetrazole	anticonvulsant tests: electroshock max	anticonvulsant tests: electroshock min
new compound	100	40	2	18	92	150
meprobamate	250	250	100	150	200	167
chlorpromazine	17	20	2.5	42	150	600
phenobarbital	120	80	10	75	18	90

[a] Dose (mg/kg) of orally administered drug required to achieve the desired effect.

Scheme I

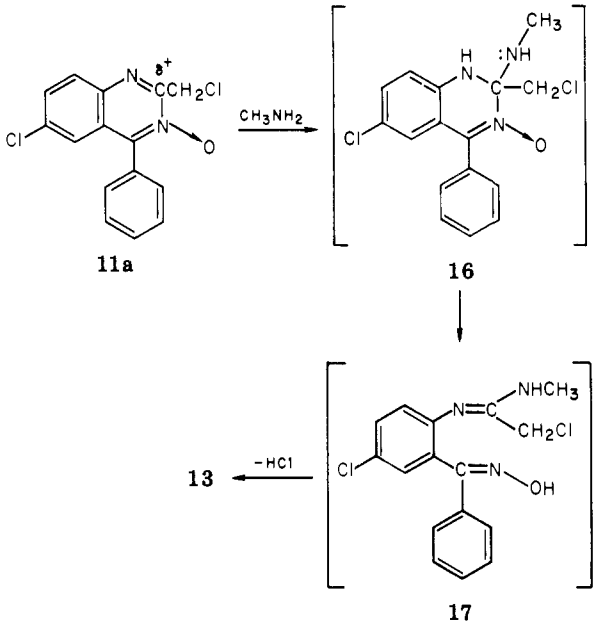


effect whatsoever on the autonomic nervous system. The product had a pronounced taming effect on monkeys. The low toxicity (620 mg/kg in mice) was particularly encouraging. It looked like an ideal compound.

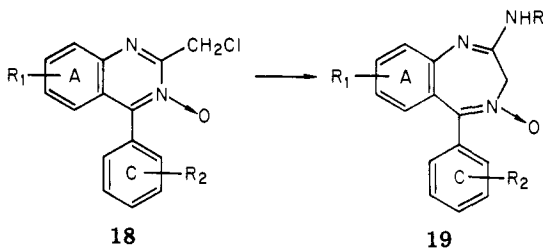
While the compound underwent a whole gamut of sophisticated pharmacological tests by Dr. Randall and his staff,[7] we studied the chemistry of this unusual product. From the very beginning we had reservations about its structure since the UV and IR spectra were completely different from those of the starting material 11 or those of other related quinazoline 3-oxides. Since at that time NMR or mass spectra were not yet used for the structure determination of complicated heterocyclic molecules, we resorted to classical methods. The analytical data showed that the reaction product had the expected molecular weight and elementary composition; the methylamino group and the *N*-oxide function were also present. The degradative studies,[9] which allowed us to establish definitely that the compound had structure **13** rather than **12**, are outlined in Scheme I.

First we removed the *N*-oxide oxygen and then we hydrolyzed compound **14** with acid. This gave the aminobenzophenone used as starting material in quantitative yield. The acid-soluble residue gave, after benzoylation by the Schotten–Baumann procedure, benzoylmethylamine and hippuric acid in greater than 60% yield. The degradation products, glycine and methylamine, could result only from the hydrolysis of the benzodiazepinone **13** via **14** and not from the quinazoline **12** or other conceivable isomers.

This unusual transformation of a quinazoline 3-oxide into a benzodiazepine 4-oxide prompted us to investigate the reaction in depth. The study of various reaction conditions, solvents, and the behavior of a number of analogues[6] led us to conclude that the methylamine attacks the quinazoline *N*-oxide at the 2 position due to its residual positive charge (**11a**) rather than replacing the reactive



chlorine atom as might be expected.[10] This causes the formation of an intermediate of type **16** which most probably is ultimately transformed via **17** into the 1,4-benzodiazepine derivative **13**. The interesting and promising pharmacological properties of this compound led us to synthesize a number of related products (**19**)



obtained by causing quinazoline 3-oxides **18** to react with ammonia and a variety of primary amines.[11]

The synthesis of these analogues and homologues enabled us to file a patent application in May 1958 claiming 2-amino-1,4-benzodiazepine 4-oxides bearing various substituents in the benzo and phenyl rings. Because of the novelty of these products, the patent[12] was granted within a year (July 1959) and without difficulty.

The pharmacological evaluation of all the analogues and homologues on hand showed that none were significantly

Table II. Pharmacological Activity[a] of Chlordiazepoxide and Its Transformation Products

			anticonvulsant tests			
				pentylene tetrazole	electroshock	
compd	inclined screen	foot shock	cat		max	min
13	100	40	2	18	92	150
21	100	20	2	15	150	150
22	75	40	1	6	52	400
23	75	20	1	6	25	61

[a] Dose (mg/kg) of orally administered drug required to achieve the desired effect.

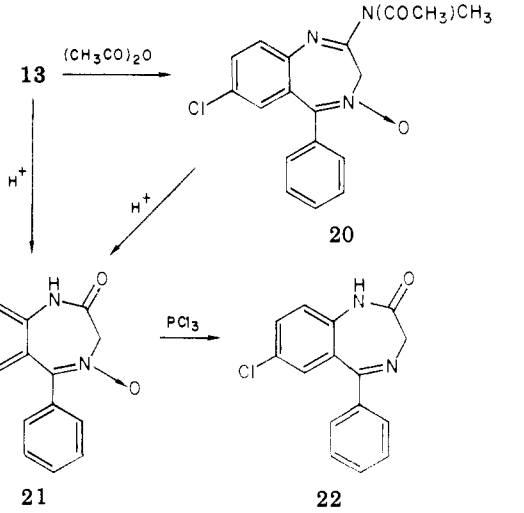
superior to the first product, the methylamino derivative **13**. It was therefore decided to intensify the study of this compound and prepare it for clinical evaluation and possible introduction. Its pharmacological and psychotropic properties in animals were thoroughly explored, the toxicological studies were expanded, and an intensive clinical investigation was started and conducted under the energetic direction of Dr. L. Hines.

It soon became apparent that this compound possessed very valuable tranquilizing (anxiolytic) properties and the interest of the clinical investigators became so great that within a short time thousands of patients had been treated with this drug. These extended successful studies enabled us to file an NDA very quickly and to introduce the compound [7-chloro-2-(methylamino)-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide] in 1960 under the trademark Librium. The generic name which was later generally accepted was chlordiazepoxide. The time elapsed between the first pharmacological testing and introduction was only $2^1/_2$ years.

This record time was made possible by minimizing red tape and by the enthusiasm and frictionless cooperation of all the people involved in the chemical and pharmaceutical production and pharmacological, toxicological, and clinical testing of the new drug. The favorable clinical results and the then existing positive attitude of the FDA were obviously of prime importance.

While this product was being prepared for introduction, it became desirable to find a form which would lend itself to the preparation of a pharmaceutically acceptable elixir or syrup for pediatric and geriatric use, because chlordiazepoxide hydrochloride, the water-soluble clinically used salt, was extremely bitter. This was not at all surprising since it is well known to most medicinal chemists that every useful drug is either bitter, hygroscopic, or unstable. Since this compound was rather valuable, it possessed all three of these properties.

During these studies, we found that a suspension of the quite insoluble, finely pulverized base itself was unsuitable. Moreover, the pharmacologically equipotent acetyl derivative **20** also proved to be too bitter despite its low solubility. Not unexpectedly, aqueous solutions or suspensions of **13** and **20** were relatively unstable. The ensuing study led to the very interesting finding that the substituent at the 2 position was the cause of the instability and was readily removed by acid hydrolysis.[13] To our pleasant surprise, the decomposition product **21** showed the same pharmacological activity as **13** (Table II).[14] A further transformation of this product was the removal of the *N*-oxide function to form **22**. This change also did not affect the pharmacological properties; quite the contrary, the activity even seemed to be slightly enhanced. Thus, it turned out that some of the unique features which seemed so characteristic for chlordiazepoxide were not at all needed for its pharmacological activity. The *N*-oxide

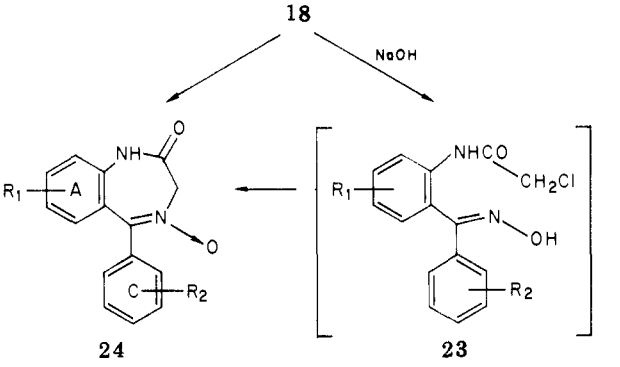


function and particularly the basic substituent, which was the cornerstone of our initial working hypothesis, proved to be only unnecessary adornments. The only features which were common to these biologically active compounds were the 1,4-benzodiazepine ring system bearing a chlorine at the 7 position and a phenyl group at the 5 position.

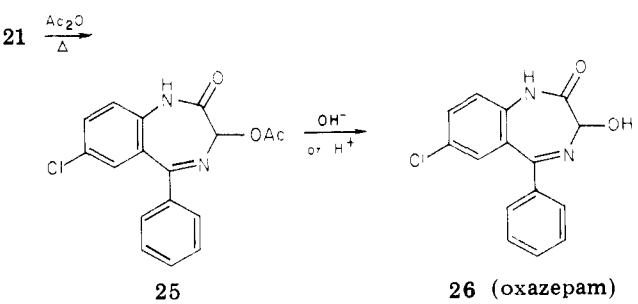
Based on this knowledge, we started a broad program of molecular modification, aiming at the discovery of products which would be superior to chlordiazepoxide.

In order to facilitate our work, we first sought simpler methods which would make compounds of type **21** and **22** more readily accessible.

We found that *N*-oxides of type **21** could be prepared easily by alkaline treatment of 2-(chloromethyl)quinazoline *N*-oxides **18**.



The results of the pharmacological study of these *N*-oxides (**24**) were not very interesting, since different substituents had only minimal effect on the biological spectra and potencies of these compounds. Chemically, however, they undergo an interesting reaction on treatment with acetic acid or anhydride. This so-called Polonovsky rearrangement results in the formation of the 3-acetoxy derivative **25** which on mild hydrolysis yields the bio-



logically active 3-hydroxy derivative **26**. This rearrangement of benzodiazepine 4-oxides was studied by

Table III. Comparison of the Pharmacological Activity[a] of Chlordiazepoxide with That of Diazepam

compd	inclined screen	foot shock	cat	anticonvulsant tests: pentylene-tetrazole	anticonvulsant tests: electroshock max	anticonvulsant tests: electroshock min
chlordiazepoxide	100	40	2	18	92	150
diazepam	30	10	0.2	1.4	6.4	64

[a] Dose (mg/kg) of orally administered drug required to achieve the desired effect.

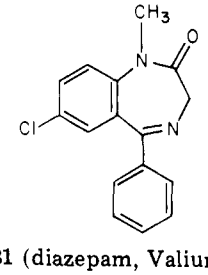
research teams at Wyeth[15] and also at Roche.[6]

The Wyeth investigators were rather successful. They discovered the biological activity first, obtained a patent, and were able to introduce oxazepam in the United States in 1965 under the trade name Serax and under various other names in other countries.

We concentrated our studies on simple benzodiazepinones without the *N*-oxide function. The search for alternative syntheses of these relatively simple compounds resulted in a number of routes leading to the desired products in good yields. Two methods which were used most extensively are shown in Scheme II.[16]

In both cases, *o*-amino ketones **27** were used as starting materials. Treatment of the appropriately substituted aminobenzophenone with a haloacetyl halide yielded a compound of type **28**, which, on treatment with ammonia, gave the benzodiazepinone **30** via an amino derivative **29**. The other method involved treatment of an aminobenzophenone with an amino acid ester hydrochloride in pyridine leading directly from **27** to **30**. The first, multistep method generally gave better overall yields, up to 70–80% of very pure products. The second method facilitated the synthesis of benzodiazepinones bearing substituents at position 3, since many α-amino acids bearing a variety of substituents at the α carbon are commercially available. With these two methods, we prepared first a number of benzodiazepinones bearing numerous substituents at different positions in ring A. A subsequent modification was the introduction of a substituent in position 1 which was readily achieved by treatment with base and an alkylating agent.

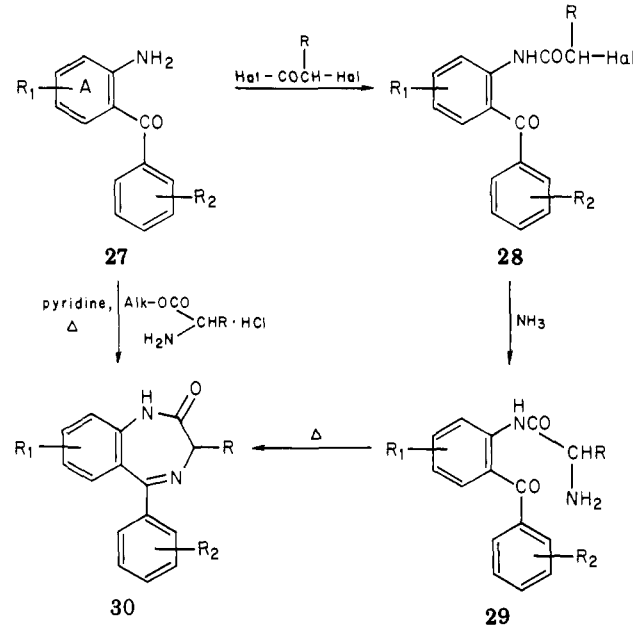
Near the end of 1959, just before the introduction of Librium, we were very much aware of its clinical value and started to look for a superior product. All the compounds which were then on hand had similar activity spectra, but one, the 1-methyl derivative **31** (7-chloro-1,3-dihydro-1-



31 (diazepam, Valium)

methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one), was significantly more potent than chlordiazepoxide. In the hope that this higher potency would be connected with other advantages in its clinical utility, we started an intensive study of this substance. The compound was 3–10 times as potent as chlordiazepoxide, as Table III indicates. Further studies showed that the toxicity was extremely low. Its psychotropic and other pharmacological properties were studied in depth with very favorable results. It was given the generic name diazepam and, after the appropriate toxicological and extended clinical studies, was introduced near the end of 1963 under the trademark of Valium. In this case, the time elapsed between the first

Scheme II



pharmacological testing and introduction was 4 years.

The valuable clinical properties of this new compound led to an expansion of our synthetic program. The chemical staff was enlarged considerably and the Pharmacological Department grew proportionally.

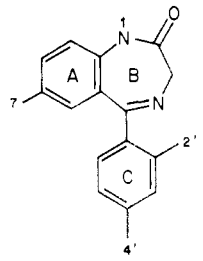
As soon as the Librium patent[12] had appeared, other research centers also started the investigation of benzodiazepine derivatives. This intensive activity led to a number of valuable alternative routes for the synthesis of benzodiazepinones.

Within a few years our intensive efforts resulted in the synthesis and pharmacological evaluation of well over 3000 1,4-benzo- and heterodiazepinones. This involved the preparation, identification, and pharmacological investigation of about 4000 intermediates and byproducts.

The large number of benzodiazepinones at our disposal enabled us to study thoroughly the structure–activity relationships in this series. It became apparent at the very beginning of our studies that the substitution pattern played an important role, of paramount importance being the substituent at the 7 position (ring A). Substituents in rings C and also B had additional effects. Our most significant findings are summarized in Chart I[17] (see also ref 18).

These "rules" proved to be valuable guidelines in the course of our further studies, which led to benzodiazepinones with over 80 different substituents at the 7 position and with hundreds of substituents at position 1. Of particular interest was the 1-*tert*-butyl homologue[19] of diazepam, which is almost completely inactive. Whereas other alkyl groups are readily removed by liver microsomes, the *tert*-butyl group is not attacked, as was shown by our Metabolism Group under the direction of Dr. M. Schwartz.[20] Based on these findings we synthesized a compound which combined all the features known to

Chart I. Effects of Substituents on the Biological Activity of Benzodiazepinones[a]



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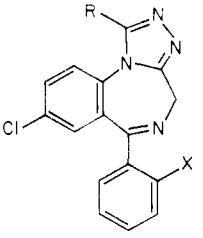
[a] Ring A: (position 7) generally, increased by electron-withdrawing groups, e.g., halogens, NO_2, and CF_3, and decreased by electron-releasing groups such as CH_3 and OCH_3; decreased by any substituents in any positions other than 7. Ring B: increased by a methyl group at position 1; decreased by larger substituents; *tert*-butyl derivative is completely inactive. Ring C: increased by halogens at the 2′ position (e.g., Cl and F); very strongly decreased by a substituent at the 4′ position.

impart high activity: the CH_3 group at 1, the nitro group at 7, and a fluorine at 2′.[14,17] It proved to be, as expected, one of the pharmacologically most potent benzodiazepinones, illustrating the additive or potentiating properties of pharmacophoric groups in the benzodiazepine series. It was introduced in Switzerland in 1975 as a potent hypnotic, acting in 1-mg doses.

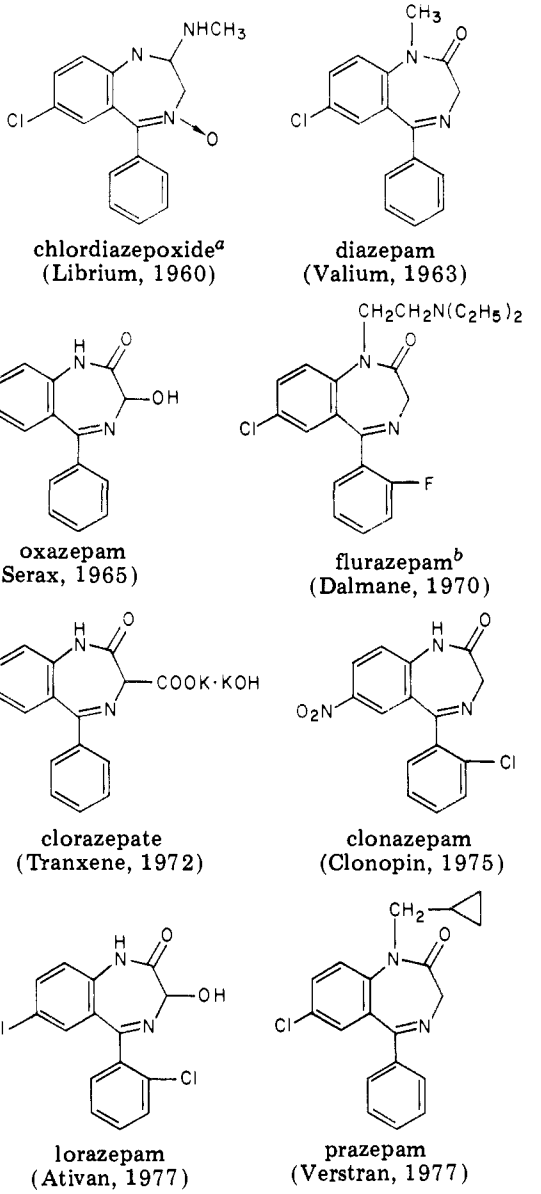
These studies showed that our six preliminary tests gave a good indication of the potency of these compounds. However, differences in the pharmacological spectrum were not very significant since, to date, they unfortunately have not led to compounds which show effects going much beyond those of other 1,4-benzodiazepines. In every case, muscle relaxation and sedation, anxiolytic, anticonvulsant, and hypnotic properties are present to a varying degree. Only the preponderance of one or the other activity seems to vary. It should be noted that this discussion has been limited to benzodiazepinones having a phenyl group at the 5 position, because such compounds were generally biologically most active and also most readily accessible. However, many benzodiazepinones bearing other substituents at the 5 position were synthesized and studied pharmacologically by many research teams, including ours. Few were of interest and some of them were rather difficult to prepare. Only two benzodiazepinones bearing a substituent other than phenyl at the 5 position are currently on the market. One is an α-pyridyl derivative, bromazepam,[21] marketed by Roche; the other is a cyclohexenyl derivative, tetrazepam,[22] which was introduced by Clin-Byla.

Research in the benzodiazepine series has been very active and continues in many industrial centers, leading to various modifications of the basic structure. Some of the most interesting novel developments are derivatives with additional rings joining the diazepine nucleus at the 1 and 2 positions. At the present time, the most interesting are the "triazolobenzodiazepines" 33, which were synthesized and investigated by Takeda[23] and Upjohn[24] research teams. These compounds are generally more potent than the corresponding 1-methylbenzodiazepinones. Two of them are commercially available outside the U.S.



33, R = H, CH_3; X = H, Cl

Chart II



[a] Marketed mainly as the hydrochloride. [b] Marketed as the hydrochloride.

Evidence of the intensive research in the benzodiazepine field still in progress is indicated by the continuous flow of new patents and scientific publications. In the last five years, over 1600 original patents have appeared, and over 12000 papers concerned with the chemistry, pharmacology, and clinical aspects have been published. One of the main objectives is the discovery of products having a narrower spectrum of biological activities. It is to be expected that this search will continue for years to come and might ultimately lead to superior products with more specific properties: anxiolytics, muscle relaxants, or anticonvulsants causing less sedation, compounds with pronounced antidepressant properties, or even drugs acting in psychoses. The general acceptance of this class of compounds is illustrated by the widespread use of the eight benzodiazepine derivatives which are now marketed in the U.S. They are shown in Chart II together with their generic trade names and introduction dates. Six of them are

anxiolytics, flurazepam is a hypnotic, and clonazepam is an antiepileptic. Fourteen additional 1,4-benzodiazepine derivatives are marketed outside the U.S., many of them under several trade names.

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[1-Penicillamine,2-leucine]oxytocin. Synthesis and Pharmacological and Conformational Studies of a Potent Peptide Hormone Inhibitor[1]

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[1-Penicillamine,2-leucine]oxytocin was synthesized by the solid-phase method of peptide synthesis and purified by partition chromatography on Sephadex G-25, followed by gel filtration. The peptide was found to be a very potent competitive inhibitor of oxytocin in the oxytocic assay with a pA_2 of 7.14 and an inhibitor of oxytocin in the milk-ejecting assay. The compound showed no agonist activity in either of these assays, and its inhibitory activity at the uterus was of prolonged duration. The ^{13}C nuclear magnetic resonance spectral properties and the ^{13}C T_1 (spin–lattice) relaxation times of [Pen^1,Leu^2]oxytocin were determined, and the results were compared with previous studies of [Pen^1]oxytocin, a related competitive inhibitor, and oxytocin, the native hormone agonist. These studies indicated that the hormone inhibitors [Pen^1,Leu^2]oxytocin and [Pen^1]oxytocin have similar conformational and dynamic properties which are different than those of the agonist, oxytocin.

Peptide hormone competitive inhibitors (antagonists) constitute a potentially useful class of organic compounds in clinical applications and for studying peptide–receptor interactions and the mechanisms of peptide hormone action. These applications derive from their ability to interact with the receptor in a manner similar to the hormone and their inability to transduce a biological message to effect a change in the target cells metabolism or other properties. Thus a peptide hormone competitive inhibitor can provide information of the hormone–receptor interaction independent of the transduction event and important clues to structural and dynamic features related to both binding and transduction.

Recently we have shown that [1-penicillamine]oxytocin ([Pen^1]oxytocin, S-C$(CH_3)_2$CH(NH_2)CO-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2), a competitive inhibitor of oxytocin,[3,4] has considerably restricted dynamic properties relative to those of oxytocin.[5,6] These studies suggested that certain specific differences in the conformational, dynamic, and structural properties of the hormone and antagonist were related to differences in biological activity.[6]

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