SYNTHETIC STUDIES ON SULFUR CONTAINING BIOACTIVE COMPOUNDS

SYNTHETIC STUDIES ON SULFUR CONTAINING BIOACTIVE COMPOUNDS

MITSUO NUMATA

1982

GENERAL INTRODUCTION

ROLE OF SULFUR IN ACTIVITY: A DISCUSSION FROM THE VIEWPOINT OF ISOSTERISM

I) General Introduction

Role of Sulfur in Activity: A Discussion from the Viewpoint of Isosterism

I-1) Preface

More than 20 years have passed since I joined Dr. Yurugi's group of the Chemistry Laboratories of Takeda Chemical Industries Company Ltd. in 1958. At that time, Dr. Yurugi was devoting himself to the synthesis of lipoic acid, a sulfur containing essential co-factor for the oxidative decarboxylation of pyruvic acid, and he gave me a chance to take part in the synthetic project.

I have been so fortunate to have been able to devote with sulfur containing bio-active compounds such as lipoic acid to begin with, nereistoxin, thiamine propyl disulfide and its metabolites, thiamine tetrahydrofurfuryl disulfide and its metabolites, penicillins and cephalosporins ever since. My assignment has been to develop practical procedures to synthesize not only these useful compounds but also in many cases new derivatives with more potent activities. This dissertion thesis contains these synthetic procedures I have investigated and other new findings obtained during the course of my investigation.

In addition to a blessed encounter with Dr. Yurugi who led me to step in organic sulfur chemistry, I am also fortunate to be acquainted with prof. S. Oae, who is the one of the greatest pioneers in the field of organic sulfur chemistry. His continuing works with ever-green interest in organic sulfur chemistry opened my eyes to the depth

of the organic sulfur chemistry. Even his mere presence have given me a full confidence in sticking around the syntheses of many sulfur containing bio-active compounds.

During my pursuit on synthetic works on useful sulfur-containing medicals, I could not get away myself from embracing a fundamental inquiry, "What role does sulfur have in the biological activities of these sulfur-containing compounds?".

In organic chemistry, sulfur containing compounds are known to exhibit characteristic chemical reactivities such as lability to oxidation, formation of active ionic and/or multi-valent intermediates. Many studies have shown that the sulfur atom in the molecules is responsible for these characteristic reactivities.[rev.1)]

It is highly probable that such chemical properties reflect on biological properties of sulfur containing bio-active compounds and accordingly the sulfur atom in the molecules plays some important roles in the biological activities.

Modern organic sulfur chemistry have allowed us to examine the probability using isosterism approach and to get answers to the above inquiry from the experimental results.

As an introduction to the dissertion, the role of sulfur in biological activities of a select group of bioactive sulfur containing compounds will be discussed. In order to keep the discussion within reasonable dimensions, a somewhat arbitrary selection of compounds has had to be made. The selection is based on the amount of information

available.

I-2) Definition of Isosterism.[rev.2, ref.1)]

The isomeric compounds of a sulfur containing compound which bear an oxygen, methylene or ethylene instead of the sulfur of the mother compound are called oxygen, methylene and ethynylene isosteres, respectively. Since sulfur and these substituents have similarities in dimensions as shown in Table 1 but varied parameters in electronegativity, hydrophobicity, hydrogen bonding capacity or flexibility as shown in Tables 2 to 5, it is likely that the original sulfur compound and its isosteres have similar molecular sizes and shapes but diversified physical and chemical properties. When these isosteres are compared , in many cases if not all, one can co-relate the biological property of a particular molecule to a specific physical or chemical parameter of the sulfur atom.

Such replacement of atom to clarify the role(s) of an element, group or molecule in biological activity is called isosterism.

I-3) Role of Sulfur

The sulfur atom in a sulfur containing bio-active compound may have one or more of the following roles.

(a) Receptor interactions. If the sulfur atom is responsible for an interaction with a receptor or an enzyme, the size, shape, electronic properties and hydrogen bonding capacity of the sulfur containing molecule will

Table 1

Comparison of Thioether, Ether and Methylene Linkages in Dimensions.

CH_3-X-CH_3

	X=	S	O	CH_2
Van der Waals Radius of X (Å)		1.8	1.4	2.0
C-X (Å)		1.82	1.42	1.54
C-X-C (°)		105	111	113.7

*L. Pauling "Nature of Chemical Bond" p260

Table 2

Electronegativity values

S	O	C
2.5	3.5	2.5

*C.A. Coulson "Valence" p140 (1961), Oxford Univ. Press

Table 3

Comparison of Hydrophobicity

Material	log P (octane-H_2O)
$CH_3-(CH_2)_3-CH_3$	2.50
$C_2H_5-S-C_2H_5$	1.95
$C_2H_5-O-C_2H_5$	0.77

* C. Hansch & S.M. Anderson, J. Org. Chem., 32, 2583 (1967)

Table 4

Comparison of Thermodynamic Data for Hydrogen Bonds

		$-\Delta H$ (Kcal/mol)
OH---O	CH_3-COOH	7.0
	H_2O	5.0
	CH_3-OH	4.0
OH---S	C_6H_5-OH+$(C_2H_5)_2S$	3.4

*Z. Simon "Quantum Biochemistry & Specific Interactions" p86 (1976), Abacus Press

Table 5

Comparison of Potential Tortion Barriers (Kcal/mol)

	Kcal/mol
CH_3-CH_3	2.75
CH_3-O-CH_3	2.72
CH_3-S-CH_3	2.13

*E.L. Eliel "Stereochemistry of Carbon Compounds" p134 (1962), McGraw-Hill Book Company, Inc.

be the important parameters.

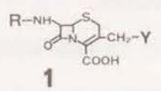
(b) Pharmacokinetics. The sulfur may be necessary for the absorption, transport and excretion of the compound. In this case lipophilicity and hydrogen bonding capacity are likely to be important.

(c) Metabolism or Chemical Reactivity. The sulfur atom may be involved in blocking or aiding the biochemical transformation. In this case the isomeric displacement may cause a shorter half life or a complete loss of biological activity or unexpected side effects such as antagonist activity.

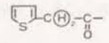
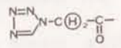
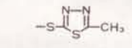
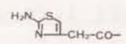
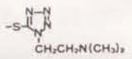
(d) Structural. If the sulfur atom has a structural role in holding other functionalities in a particular geometry, the isosteric replacement may not cause any large change in biological activity, since the sulfur atom may have little contact with the external medium.

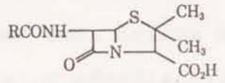
I-4) Cephalosporins Structural Role of Sulfur

Cephalosporin C (1a) was isolated from a fermentation broth of a mold (Cephalosporium acremonium) by Newton and Abraham in 1953.[2] It showed a weaker antibiotic activity against Gram-positive bacteria than that of penicillin G (2) but showed a considerable activity against Esherichia coli, a Gram-negative bacterium, against which the latter is inactive. In 1961, when structure 1a was determined by both chemical[3] and X ray techniques[4] and when its structural relationship to penicillin was realized, a world wide interest in this molecule was generated.

Cephalosporins 

1

	Name	R	Y
1 a	Cephalosporin C	HOOC-CH(NH$_2$)-(CH$_2$)$_3$-CO-	-OCOCH$_3$
1 b	Cephalothin		-OCOCH$_3$
1 c	Cefazolin		
1 d	Cefotiam		

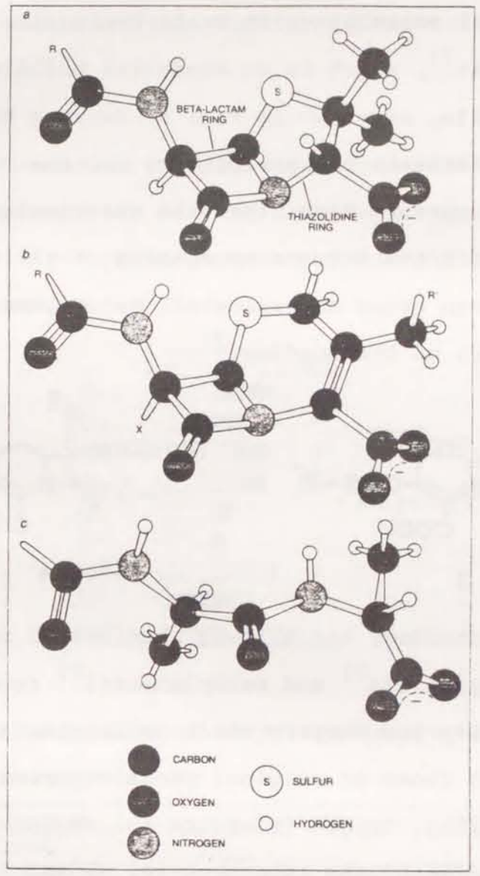


2; Penicillins

Continuing research programs have developed many clinically effective cephalosporins with broad anti-bacterial spectrum covering both Gram-positive and -negative bacteria and they are now widely used for treatments of diseases infected with bacteria. Among them, are cephalothin (1b)[5], cefazolin (1c)[5] and cefotiam (1d)[6], which were developed by E. Lilly in 1962, Fujisawa in 1970 and Takeda company in 1981, respectively. As apparent from the structures, they were yielded from peripheral modifications, i.e. side chain modifications, of cephalosporin C.

Both penicillins and cephalosporins inhibit the growth of bacteria by interfereing the cell wall biosynthesis[7] of bacteria. Since the cell wall is an essential feature of bacterial cells which has no

Fig. 1 Structural Analogy between Penicillins (a), Cephalosporins (b) and N-Acetyl-D-ala-D-ala (c)



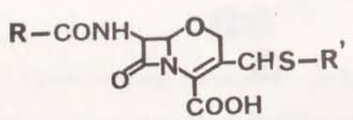
Penicillins

Cephalosporins

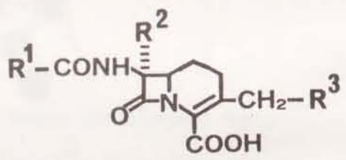
N-Acetyl-D-ala-D-ala

counterpart in animal cells, when they are administered to infected animals, they inhibit the growth of bacteria without inhibiting growth of host cells.

The basis for the inhibition by the antibiotics is their structural resemblence to D-ala-D-ala side chain of peptidoglycan[7], which is an essential building block of the cell walls, as shown in Fig. 1. Enzymes building the cell walls mistake the antibiotics for the required substrate and approach them. Then the antibiotics combine irreversibly with the enzymes by opening of the ß-lactam ring. The hydroxy group of serine of the enzymes may well be the site of the bonding.[7]



3



4

Modern technology has allowed chemists to prepare a number of oxygen (3)[8] and methylene (4)[9] isosteres of cephalosporins and compare their antibacterial activities with those of original cephalosporins. Generally speaking, oxygen isosteres (3) showed superior activities and methylene isosteres (4) showed inferior activities to those of corresponding mother cephalosporins as illustrated in Fig. 2 and Table 6.

The maintenance of antibacterial activity during the isosteric replacements is compatible with and support the mechanism of antibacterial activity of cephalosporins described above. Since the replacement

does not change the structure of cephalosporins largely due to the similar dimensions of sulfur, oxygen and methylene as shown in Table 1, it is reasonable that the mimicking ability to D-ala-D-ala part of peptidoglycan, i.e. antibacterial activity is maintained among the isosteres.

Fig. 2 S-O Isomerism in Gram-negative activity of Cephalosporins bearing different side chains. Open bars: cephalosporins (X=S). Filled bars: oxygen isosteres (X=O).

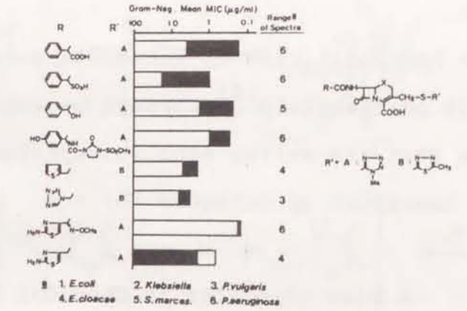


Table 6

Antibacterial Activities of Methylene Isosteres (4) of Cephalosporins

R¹	R²	R³	Staph. aureus 209P JC-1	Strep. pyogenes C-203	E. coli NIHJ JC-2	Kreb. pneumoniae SRL-1	Proteus mirabilis PR-4	Proteus vulgaris CN-329
PhCH(NH₂)–	–H	–CH₂STet	30	6.3	0.8	1.6	1.6	6.3	12.5
		–OCH₃	45	50	25	50	50	100	>100
		–H	56	25	3.1	25	12.5	25	100
PhCH(HNCONHEt)–	–H	–CH₂STet	32	12.5	0.8	0.8	0.8	12.5	12.5
		–OCH₃	46	25	25	6.3	1.6	6.3	12.5
		–H	57	12.5	1.6	3.1	0.8	6.3	12.5
H₂N–	–H	–CH₂STet	35	12.5	0.4	0.05	0.05	0.1	0.2
		–CH₃	39	>100	3.1	6.3	6.3	6.3	12.5
		–H	59	25	0.05	0.05	0.01	0.02	0.05
PhCH₂–	–H	–CH₃	37	6.3	3.1	>100	100	>100	>100
HO–	–OCH₃	–CH₂STet	58	>100	>50	6.3	3.1	6.3	6.3
	–OCH₃	–CH₂OCONH₂	65	>100	>50	25	25	50	50
Cefazolin				0.1	0.1	1.6	1.6	3.1	100.0

Structure: H R¹ / R²CON / O / N / R² / CO₂H

Minimum inhibitory concentrations (μg/ml)*)

a) Minimum inhibitory concentrations were determined by the agar dilution method.

Accordingly, the sulfur atom of cephalosporins has a role of category (d), described in the preceding section, i.e. structural role.

It is worth to discuss the difference of activity among the isosteres. An empirical rule[10] tells that the increase in hydrophilicity of antibiotic improves the Gram-negative activity. As indicated by the data of Table 3, the replacement of sulfur atom with oxygen is likely to increase the hydrophilicity. The oxygen isosteres with superior Gram-negative activities depicted in Fig. 2 might be regarded as examples which follow the empirical rule.

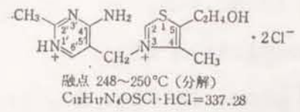
Since the hydrophilicity of antibiotics is known to influence their permiability[10] through bacterial membrane, it is possible that the sulfur atom in cephalosporins has a role in transport of category (b) too.

I-5) Thiamine　A Role of Sulfur in Chemical Reactivity

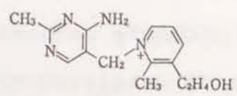
Thiamine was first discovered and extracted from rice rod by Suzuki in 1912[11]. He showed that it relieved chiken which were fed with white rice for a long period and suffered from lethal nerveous inflammation (Polyneuritis gallinarum). Its structure was determined by Williams in 1935[12].

Thiamine is an essential factor for the transformation of sugars into energy in mammalian bodies. More precisely, it exists in organs as an pyrophosphate ester and catalyzes decarboxylation of α-ketoacids, oxidative decarboxylation of α-ketoacids, formation of ketols and mutation of

α-ketols[13].



5; thiamine



6; pyrithiamine

Eq. 5-1

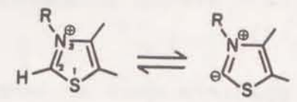
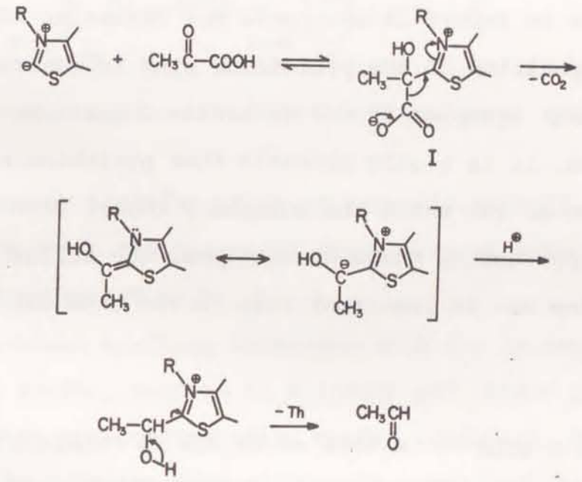


Fig. 3　A Mechanism for the thiamine-catalyzed decarboxylation of pyruvic acid



The catalytically functional part of the molecule is the sulfur containing five membered thiazolium ring. The hydrogen atom at the 2-position is rapidly exchanged in aqueous media. It is now well established that the catalytically active site of the molecule is at the 2-carbon. The presence of an adjacent positive charge

at N-3 and the adjacent sulfur atom enhances the stability of the ylid structure given in Eq.5-1.

The probable mechanism of decarboxylation of pyruvic acid catalyzed by thiamine is shown in Fig. 3.

An ethylenyl isostere (6) named pyrithiamine was synthesized and shown to be a strong antanonist of thiamine by Tracy in 1940[14].

Occupancy theory[15] predicts that an isomer, which can occupy the receptor site as similarly as the original active compound but is not as reactive as the latter , exhibits an antagonist activity.

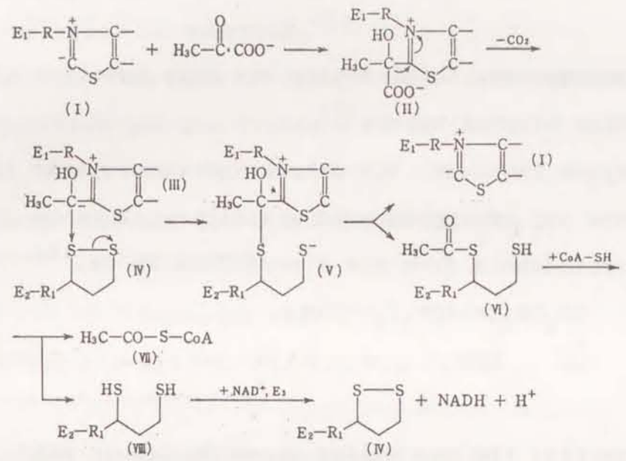
Since no report is available for carbanion formation at the 2-position of the pyridinium ring in contrast to the abundant examples of C-2 carbanion formations of thiazolium, it is highly probable that pyrithiamine (6) is a compound for which the occupancy theory stands.

As apparent by these discussions, the sulfur atom in thiamine has an important role in the chemical reactivity.

I-6) Lipoic acid A Role of Sulfur in Chemical Reactivity

Lipoic acid (7) was isolated from livers of pigs as a co-factor for oxidative transformation of pyruvic acid by Reed in 1951[16]. It accepts a high energetic acetaldehyde from hyroxyethyl thiamine (III in Eq. 6-1) and transfer an acetyl group to co-enzyme A as formulated in Eq. 6-1. Thus lipoic acid contributes to energy transfer system in living organisms.

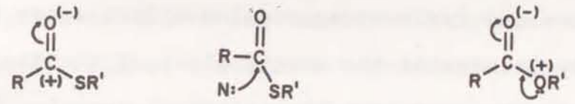
Eq. 6-1



This energy transfer relys on specific natures of thiol esters which are different from those of alcohol esters in a number of ways.

(1) Chemical bonding, consisted with the 3s and 3p orbitals of sulfur, results in a longer and weaker bond than that with the corresponding oxygen compounds. Hence, there will be a lesser interelectronic repulsion, and a more facile ester bond cleavage in nucleophilic displacement reactions at the carbonyl carbon atom with thiol esters than with alcohol esters.

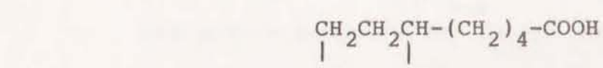
Eq. 6-2



(2) The much smaller tendency of sulfur towards "π-bond" formation as compared with oxygen, results in a far greater localization of charge into the carbonyl group.

In consequence, thiol esters are more reactive than corresponding alcohol esters[17].

An oxygen isostere, 6,8-dihydroxyoctanoic acid (8), did not show any of lipoic acid activities. This is quite understandable from the discussions above.[18]



8

Apparently, the two sulfur atoms in lipoic acid have the key role in its chemical reactivity.

I-7) Peptide Hormones A Structural Role of Sulfur

A) Neurohypophyseal Hormones[19]

From posterior lobe of pituitary (Neurohypophysis) in human are excreted five peptide hormones, oxytocin (9), arginine-vasopressin (10), lysine-vassopressin (11) and vasotocin (12). Oxytocin plays an essential role in lactation. It produces milk ejection by contraction of myoepithelial cells. Oxytocin contracts smooth muscle of the uterus, but it role in parturition is unclear. Oxytocin is widely used for the induction of labor in the parturition and placenta expulsion periods[20].

Arginine- and lysine-vasopressins effect water reabsorption by increasing the permiability of the distal kidney tubles. The important physiological function is

the homeostatic control of extracellular fluid volume. In case of insufficient vasopressin lebels, water reabsorption declines and a large amount of low concentration of urine is excreted.[20]

Vasotocin has both of vasopressin- and oxytocin-like activities but its function is unclear.

1 2 3 4 5 6 7 8 9

Cys-Tyr-Ile-Glu-Asu-Cys-Pro-Leu-Gly-NH_2

Oxytocin (9)

Cys-Tyr-Phe-Glu-Asu-Cys-Pro-Arg-Gly-NH_2

Arginine-vasopressin (10)

Cys-Tyr-Phe-Glu-Asu-Cys-Pro-Lys-Gly-NH_2

Lysine-vasopressin (11)

Cys-Tyr-Ile-Glu-Asu-Cys-Pro-Arg-Gly-NH_2

Vasotocin (12)

All these hormones have a common structural pattern of nine amino acid residues with a 1,6-disulfide bridge.

More than 300 synthetic analogs of neurohypophyseal hormones have been prepared and tested. The results have showed the essentiality of the 20 membered ring of the hormones for the bio-activity. Enlargement or contraction of the ring is accompanied by loss of activity. Peptides of non-cyclic structure are inactive. 1-Deamino analogs of oxytocin, argine- and lysine-vasopressins showed neurohypophyseal hormone activities, thus the terminal

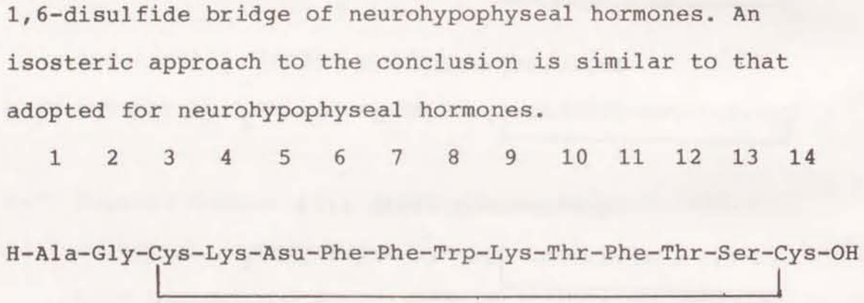
amino group was shown to be not essential for the activity.

1-Deamino-dicarba analogs in which the terminal amino group and the disulfide bridge are replaced by hydrogen and ethylene, respectively, exhibited high neurohypophyseal hormone activity[22].

On the basis of these results, it is concluded that the role of the disulfide is to maintain the essential architechture of the hormones.

B) Somatostatin

The 3,14-disulfide bridge of somatostatin (13) has been concluded to bear a role similar to the role of the 1,6-disulfide bridge of neurohypophyseal hormones. An isosteric approach to the conclusion is similar to that adopted for neurohypophyseal hormones.



Somatostatin (13)

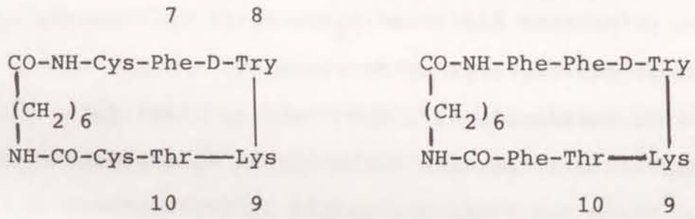
In 1972, Guillenin and collaborators[23] isolated from hypo-thalamus a peptide that inhibits the release of growth hormone from pituitary gland. Structural studies showed the hormone, designated somatostatin (13), as a tetradecapeptide with a 3,14-disulfide bridge forming a 38-member heterodectic ring. The structure was confirmed by the synthesis[24].

It soon became apparent that somatostatin inhibits not only the secretion of several pituitary hormones but also the secretion of insulin, glucagon and sveral

gastrointestinal hormones. These finding suggests a physiological role for somatostatin in regulating the secretion of these hormones.

More than 150 analogs of somatostatin have been synthesized so far. Structure-activity analysis has afforded following information indicating the role of sulfur in hormone activity.

The linear amino terminal (tail) part is not essential for the activity. Thus Des-(Ala^1-Gly^2)-somatostatin exhibits 30-100% of the potency of the native hormone[25]. An analog having the structure (14) which retains only four of the amino acids of the natural hormone (sequence 7-10) and has reduced and constrained rings with two bridges, the first one made of a cystine and the second made of a 7-aminoheptanoic acid, exhibits 2.55 times more potent growth hormone inhibition than that of somatostatin (Table 7)[26]. Replacement of the cystine bridge of the above analog with two phenylalanines affords an analog (15) with reduced but still strong inhibitory activities (Table 7)[26].



somatostatin analog (14) (15)

The high potency of analogs (14) and (15) provides evidence for the essentiality of 7-10 amino acids in a

constrained state and unessentiality of the disulfide bridge.

Table 7

Inhibition of Hormone Release (relative to somatostatin)

Material	Glucagon	Insulin	Growth-hormone
somatostatin	1	1	1
(14)	2.66	3.50	2.55
(15)	0.86	0.88	0.65

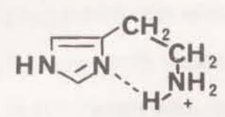
On the basis of these observations, it can be stated that the role of sulfur atoms in somatostatin is to keep the hormone in a specific rigid form favorable to receptor-hormone interaction.

I-8) Thiaburiamide, an Antihistamine, and Related Compounds

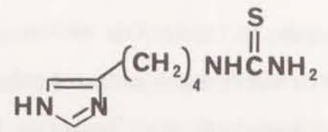
A Role of Sulfur in Receptor Interactions

A major role of histamine (16) is a transmittance of a stimulus from a neuron end to another one[27]. When histamine stimulates histamine H_2-receptor of stomack, secretion of gastric acid takes place.

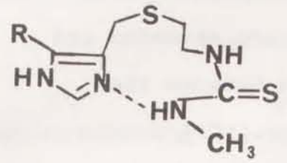
In 1972 Burimamide (17) was found by Black and co-workers[28] to antagonize histamine at H_2-receptors in vitro. Since the finding strongly indicated the potential use in therapy of peptic ulcers, which are caused by too much secretion of gastric acid, it was soon tested in animals. But it was not so much effective in vivo as was expected from the in vitro result.



histamine (16)

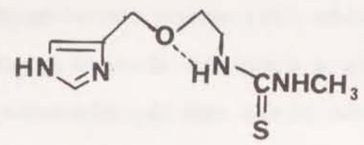


burimamide (17)



R=H; thiaburimamide (18)

$R=CH_3$; metiaburimamide (19)



oxaburimamide (20)

In search for more in vivo effective compounds, many derivatives were synthesized and tested. Among them, thiaburimamide (18) and metiaburimamide (19)[29] are not only 4-5 and 8-9 times, respectively, more active H_2-receptor antagonists than is burimamide (17) in vitro, but also active in vivo. Particularly, metiaburimamide (19) has sufficient oral activity in animals and man to be considered as an inhibitor of evoked gastric acid secretion in therapy. Whereas, oxaburimamide (20)[30] is inactive at all.

Reason for the order of antagonist activities, (19) > (18) > (17) > (20) was sought by X-ray crystallography of these compounds[31]. And it was deduced that the activity orders might be related to the order of conformational analogy of these compounds to histamine (16).

The feature of thiaburimamide (18) and metiaburimamide (19) which distinguishes them from that

of burimamide (17) is the folding of the side chains with the formation of a ten-membered ring, and the intramolecular hydrogen-bonding between the thiourea N-H and the imidazole-ring basic nitrogen atom. This is in contrast with crystalline burimamide (17) and oxa-burimamide (20) where the side chains are extended and there is a complete absence of contact between the imidazole rings and the thiourea groups. CPK models suggest that if the thiaburimamide conformation was adopted by burimamide it would be hindered by repulsions between hydrogens in the side chain. This repulsion is reduced by replacement of the second methylene by a thioether linkage because the C-S are longer than the C-C bonds (Table 1)[31].

These findings suggest that a cyclic intramolecularly hydrogen bonded conformation, which is adopted by histamine (16), thiaburimamide (18) and metiaburimamide (19) in common, may be a favorable conformation for receptor-compounds bindings. These findings also suggest that a role of the thioether sulfur in the side chains of the drugs is to increase the conformational flexibility and to aid drugs in taking the favorable conformation.

I-9) Angiotensin Converting Enzyme Inhibitors[32]

A Role of Sulfur in Chemical Reactivity

In animal blood exists an extremely small quantity of (10 mug/dl) angiotensin II (22), an octapeptide, which has the most potent vassopressor (blood pressure rising) activity hitherto known[33]. It has quite a short

half-life (15 sec) of existence. In a normal state, angiotensin II (22) is constantly generated from inactive angiotensin I (21), which is also present in blood, on enzymatic hydrolysis with a proteolytic enzyme designated angiotensin converting enzyme[34].

Angiotensinogen

↓ ← Renin

H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-OH

Angiotensin I (21)

↓ ← Angiotensin Converting Enzyme

H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH

Angiotensin II (22)

Angiotensin converting enzyme is present in a large amount in lung tissue and vuscular beds. This enzyme has a molecular weight of 140,000-300,000 dalton, in which 26% its weight of polysaccharide and a zink cation (Zn^{++}) are involved[35]. So the enzyme is called a glyco- and metalo-enzyme. Its structure has remained to be solved.

In animal blood, there are a number of kinins that antagonize angiotensin II. For example, bradykinin, a nona-peptide which is generated from kininogen by the hydrolytic action of trypsin has a strong vaso-depressor activity[36].

Angiotensins and kinins in combination constitute a blood pressure regulation system and are called vaso-active tissue hormones.

In 1960, Ferreira[37] discovered that a venom of Brazilian snake (Bothrops jararacca) depress blood

pressure strongly. Later, Bakhle[38] found that the depression is caused by inhibition of angiotensin converting enzyme by the venom. Hence was obtained quite a productive hint that an inhibitor of angiotensin converting enzyme is a potential antihypertensive drug. But the venom was inadequate for the drug, because it was too unstable to be administered orally.

In search for an analog which is avoid of the deficit, many analogs were prepared and tested. Among them, SQ-13297 and SQ-14225 were found by Ondetti[39] to inhibit the angiotensin converting enzyme strongly in vitro. In fact, the latter depressed blood pressure eminently in vivo tests using human hypertensive patients[40].

Table 8

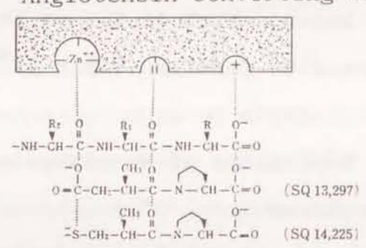
Inhibition of Angiotensin Converting Enzyme

Material	IC_{50} (uM)
$HOOC-CH_2-\underset{\displaystyle CH_3}{\underset{\blacktriangledown}{CH}}-CO-Pro$ (SQ-13297)	18
$HS-CH_2-\underset{\displaystyle CH_3}{\underset{\blacktriangledown}{CH}}-CO-Pro$ (SQ-14225)	0.023
jararacca venom	0.06

The inhibitory activity of SQ-13297 and SQ-14225 has been explained by the postulation that -COOH and -SH of the drugs deprive angiotensin converting enzyme of Zn^{++} ion, which is essential for the enzyme activity,

by ion pair formation as illustrated in Fig. 4. Taking into account that the co-ordination strength between funcional groups and Zn^{++} ion is in the order -SH > -COOH > C=O[41], the postulation goes well with the order of inhibitory activity SQ-14225 > SQ-13297.

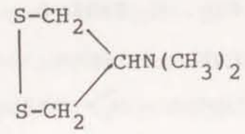
Fig. 4 A Postulated Binding between Angiotensin Converting Enzyme and Its Inhibitors



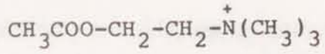
SQ-14225 is now in clinical trials as a potential antihypertensive agent. It may be deduced from the discussions above that the role of sulfur in SQ-14225 is to capture Zn^{++} ion.

I-10) Nereistoxin A Role of Sulfur in Chemical Reactivity

Nereistoxin (23) was isolated from corpses of a marine annelid (Lubriconereis heteropoda) by Nitta in 1934[42]. He showed it neurotoxic to insects and mammals. The structure was proposed to be 4-dimethylamino-1,2-dithiolane (23) through degradation studies by Hashimoto and Okaichi[43] and confirmed by synthesis[44] by chemists including this author at Takeda Chemical Industries Company.



Nereistoxin (23)

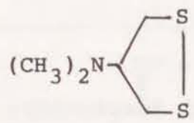
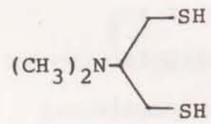
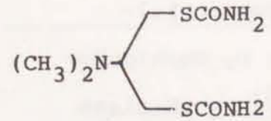
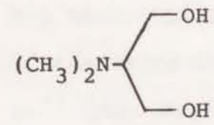
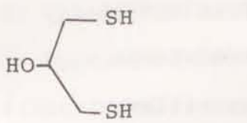
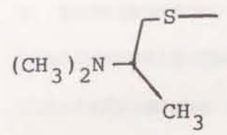


Acetylcholine (24)

As was reported by Nitta, nereistoxin (23) is lethally neurotoxic to insects. For example, toxicity of nereistoxin is 21.8 times as potent as that of BHC against rice stem borer, which is one of the most harmful insects for rice farming (Table 9)[45].

Table 9

Insecticidal Activities of Nereistoxin and Its Derivatives

		Azuki Bean Weevils Dry film contact μg/dish	Rice Stem Borer Topical application μg/g
	(Nereistoxin)	7.9	1.7
		5.9	20
	(Padan)	8	5
		>1000	>50
		>1000	>50
		>1000	>50
BHC		1	37

The potent insecticidal activity of nereistoxin indicated its potentiality to be an insecticide, but unfortunately it was too unstable to be used in fields. Among many analogs successively synthesized, Padan was found to posses not only potent insecticidal activity (Table 9) but also enough stability to be used in fields.

Sakai[46] showed that nereistoxin (23) intervenes acetylcholine (24) mediated transmittance of neuron-excitement and eventually puts insects in lethal coma. He also showed that such thiols as cystein, cysteamine and penicillamine are detoxicants for toxic symptoms caused by nereistoxin.

Inspection of Table 9 apparently indicates essentiality of dimethylamino and two thiols (or a disulfide) groups for the insecticidal activity.

Upon comparison of the structures, it is tempting to propose a mechanism of insecticidal activity of nereistoxin as follows. The disulfide and the dimethyl-amino groups of nereistoxin are isosteric, in a broad sense, to the acetoxy and the trimethylammonio groups

of acetylcholine, respectively; accordingly nereistoxin may be an isostere of acetylcholine. Thus, nereistoxin can react with acetylcholine-receptors in competition with acetylcholine and block transfer of nerve impulses.

Data available are too meager for further discussion, however, the author may be not much wrong to state that the disulfide of nereistoxin is a reaction center in toxin-receptor binding.

I-11) Conclusion

The results of these discussions are very encouraging. It has been shown that isosterism between sulfur and other elements is not only an effective step to understand the role of sulfur in the activity of sulfur containing bio-active compounds but also an effective means to develop more useful analogs. It has also been shown that sulfur atom does play various roles in the activities of sulfur containg bio-active compounds.

References

Reviews

1) Organic Chemistry of Sulfur (Ed. S. Oae) Plenum Press, New York and London, 1977
2) Isosterism and Molecular Modification in Drug Design, C.W. Thornber, Chem. Soc. Rev. 8(4), 563-580 (1979)
3) Drug Design Vol II (Ed. E.J. Ariens), Academic Press, New York and London
4) The Structure and Function of Enzymes, S.A. Bernhard, W.A. Benjamin, Inc., New York and Amsterdam, 1968

5) Vitamines Vol I and Vol II, S. Shimazono and S. Yurugi, Kyoritsu Zensho, Japan, 1980

Literatures

1) A. Korolkovas, Essentials of Molecular Pharmacology, Background for Drug Design, p54-59 (1970), Wiley Inter-Science
2) G.C.F. Newton and E.P. Abraham, Nature (London), 175, 548 (1955)
3) E.P. Abraham and G.C.F. Newton, Biochem. J., 79, 548 (1955)
4) D.C. Hodgkin and E.N. Maslen, Biochem. J., 79, 393 (1961)
5) Structure-Activity Relationships among the Semisynthetic Antibiotics (Ed. D. Perlman) Academic Press, New York, San Francisco and London, 1977
6) M. Numata, I. Minamida, M. Yamaoka, M. Shiraishi, T. Miyawaki, H. Akimoto, K. Naito and M. Kida, J. Antibiotics, 31, 1262 (1978)
7) a) D.J. Tipper, J.L. Strominger, Proc. Nat. Acad. Sci., 54, 1133 (1965), b) B. Lee, J. Mol. Biol., 61, 463 (1971), c) D.B. Boyd, Chem. Education, 53, 483 (1976)
8) K. Murakami, M. Takatsuka, K. Motokawa and T. Yoshida, J. Med. Chem., 24, 88 (1981)
9) S. Uyeo and H. Ona, Chem. Pharm. Bull., 28, 1563 (1980)
10) T. Sawai, K. Matsuda, A. Tamura and S. Yamagishi, J. Antibiotics, 32, 59 (1979)
11) U. Suzuki, T. Shimamura and S. Odake, Biochem. Z., 43, 89 (1912)
12) R.R. Williams, R.E. Waterman, J.C. Keresztesy, E.R.

Buchman, J. Am. Chem. Soc., 57, 536 (1935)

13) a) R. Breslow, Chem. Ind., R1956, 28 and 1957, 893, b) R. Breslow, J. Am. Chem. Soc., 80, 3719 (1958)

14) a) H. Tracy and R.C. Elderfield, Science, 92, 180 (1940), b) H. Tracy and R.C. Elderfield, J. Org. Chem., 6, 54 (1941), c) D.W. Wooley and A.G.C. White, J. Biol. Chem., 149, 285 (1943)

15) A. Korolkovas, Essentials of Molecular Pharmacology, p266-272 (1970), Wiley Interscience

16) L.J. Reed, B.G. Debusk, I.C. Gunsulus and C.S. Hornburger, Science, 114, 93 (1951), b) L.J. Reed, B.G. Debusk, I.G. Gunsulus and G.H.F. Schnakenberg, J. Am. Chem. Soc., 73, 5920 (1951)

17) a) K. Lloyd and T. Young, Chem. Comm., 1968, 1400, b) S. Masamune, Y. Hayase, W. Schilling, W.K. Chan and G.S. Bates, J. Am. Chem. Soc., 99, 6756 (1977)

18) S. Yurugi, M. Numata and T. Fushimi, Yakugaku Zasshi, 80, 1321 (1960), The 6,8-dihydroxyoctanoic acid prepared in this paper was sent to L.J. Reed who checked this sample and found no lipoic acid activity.

19) a) R. Acker, in Polypeptide Hormones; Molecular and Cellular Aspects (Ed. R. Porter and D.W. Fitsimmons) Ciba Foundation Symmposium, No. 41, Excerpta Medica, New York, p31-59 (1976), b) W.H. Sawyer, Fed. Proc., 36, 1842 (1977), c) R. Acher, J. Chauvet and M.T. Chauvet, Nature New Biol., 244, 124 (1973)

20) a) B.M. Altura and B.T. Altura, Fed. Proc., 36, 1853 (1977), b) B.M. Altura, Fed. Proc., 36, 1840

(1977)

21) B.M. Ferrier, D. Jarvis and V. du Vignaud, J. Biol. chem., 240, 4264 (1965)

22) a) T. Yamanaka, S. Hase, S. Sakakibara, I.L. Schwartz, B.M. Dubois and R. Walter, Mol. Pharmacol., 6, 474 (1970), b) S. Hase, S. Sakakibara, M. Wahrenburg, M. Kirchenberger, I.L. Schwartz and R. Walter, J. Am. Chem. Soc., 94, 3590 (1972)

23) a) W. Vale, P. Brazeau, G. Grant, A. Nussey, R. Burugus, J. Rivier, N. Ling and R. Guillemin, C. R. Acad. Sci. Paris, Ser. D, 275, 2913 (1972), b) R. Burugus, N. Ling, M. Butcher and R. Guillemin, Proc. Nat. Acad. Sci., US, 70, 688 (1973)

24) a) W. Vale, P. Brazeau, C. Rivier, M. Brown, B. Boss, J. Rivier, R. Burugus, N. Ling and R. Guillemin, Rec. Progr. Horm. Res., 31, 365 (1975), b) R. Guillemin and J.E. Gerich, Ann. Rev. Med., 27, 379 (1976), c) P. Brazeau, W. Vale, R. Burugus, N. Ling, M. Butcher, J. Rivier and R. Guillemin, Science, 179, 77 (1973)

25) J. Rivier, P. Brazeau, W. Vale and R. Guillemin, J. Med. Chem., 18, 123 (1975)

26) D.F. Veber, F.W. Holly, R.F. Nutt, S.J. Bergstrand, S.F. Brady, R. Hirschmann, M.S. Glitzer and R. Saperstein, Nature, 280, 512 (1979)

27) A.S.F. Ash and H.O. Schild, Br. J. Pharmacol. Chemother., 27, 427 (1966)

28) J.W. Black, W.A.M. Duncan, G.J. Durant, C.R. Ganellin and M.E. Parsons, Nature, 236, 385 (1972)

29) J.W. Black, C.W. Durant, J.C. Emmet and C.R. Ganellin, Nature, 248, 65 (1974)

30) G.J. Durant, in Proceedings of the Sixth International Symposium on Medicinal Chemistry, (Ed. M.A. Simikins), Costwold Oxford, p189 (1979)

31) a) K. Prout, S.R. Critchley, C.R. Ganellin and R.C. Mitchell, J. Chem. Soc. Perkin II, 1977, 68, b) R. Ganellin, J. Med. Chem., 24, 913 (1981)

32) D.W. Cushman and M.A. Ondetti, Progress in Medicinal Chemistry, 17, p42-104 (1980)

33) a) L.T. Skeggs, F.E. Dorer, J.R. Kahn, K.E. Lentz and M. Levine, Am. J. Med., 60, 737 (1976), b) M.J. Peach, Physiological Review, 57, 313 (1977)

34) a) J.H. Laragh, Progr. Cardiovasc. Dis., 21, 159 (1978), b) E.G. Erdoes, Circ. Res., 36, 247 (1975), c) E.G. Erdoes, Am. J. Med., 60, 749 (1976), d) E.G. Erdoes, Fed. Proc., 36, 1760 (1977)

35) a) M. Das and R.L. Soffer, J. Biol. Chem., 250, 6762 (1975), b) M. Das, J.L. Hatley and R.L. Soffer , J. Biol. Chem., 252, 1316 (1977)

36) J.C. McGiff, Chairman, Symposium on Kinin, Renal Function and Blood Pressure Regulation, Fed. Proc., 35, p172-206 (1976)

37) a) S.H. Ferreira and M. Rocha e Silva, Cienc. Cult., 15, 276 (1963), b) S.H. Ferreira, Br. J. Pharmacol., 24, 163 (1965), c) S.H. Ferreira in ; Hypertensive Peptides (Ed. E.G. Erdoes, N. Back and F. Sicuteri) , Springer Verlag, New York, p356-367 (1966)

38) Y.S. Bakhle, Nature, 220, 919 (1968)

39) a) M.A. Ondetti, B. Rubin and D.W. Cushman, Science, 196, 441 (1977), b) B. Rubin, R.J. Raffan, D.G. Kotler, E.H. O'Keefe, D.A. DeMaio and M.E. Goldberg , J. Pharmacol. Exp. Therap., 204, 271 (1978), c) B. Rubin, M.J. Antonaccio and Z.P. Horovitz, Progr. Cardiovasc. Dis., 21, 183 (1978)

40) a) L. Farve, J.W. Hollifield and D.N. McKinstry, J. Tenn. Med. Assoc., 71, 201 (1978), b) H. Garvas, H.R. Brunner, G.A. Trini, G.R. Kershaw, C.P. Tifft , S. Cuttelod, I. Garvas, R.A. Vukovich and D.N. McKinstry, N. Engl. J. Med., 298, 991 (1978)

41) H. Freiser, Symposium on Chelationg Agents, Recent Research on Some Sulfur Containing Chelationg Agents , Japan Analyst, 21, 1300 (1972)

42) S. Nitta, Yakugaku Zasshi, 54, 648 (1934)

43) a) T. Okaichi and Y. Hashimoto, Ann. N. Y. Acad. Sci., 90, 667 (1960), b) T. Okaichi and Y. Hashimoto, Agr. Biol. Chem., 26, 224 (1962)

44) a) H. Hagiwara, M. Numata, K. Konishi and Y. Oka, Chem. Pharm. Bull., 13, 253 (1965), b) M. Numata and H. Hagiwara, Chem. Pharm. Bull., 16, 311 (1968)

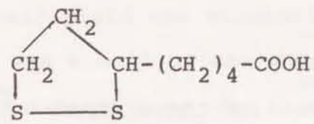
45) K. Konishi, Dissertion "Synthesis of Nereistoxin and Related Compounds" p82 (1968)

46) M. Sakai, Botyu-Kagaku, 32, 21 (1967)

CHAPTER 1

SYNTHESIS OF LIPOIC ACID

As was described in the introduction, lipoic acid (1) is an essential co-factor for oxidative decarboxylation of pyruvic acid in living organisms. Although no deficiency symptom of lipoic acid has been recorded in animals, it is well recognized that when animals suffer malfunctions of the liver or the coronary organs lipoic acid is present in the liver in anomalously low quantity and a supply of lipoic acid to the animals by injection is an effective curative means. Thus, lipoic acid is widely used for clinical treatments of such diseases as liver cirrhosis, diabetes mellitus, liver failure, corronary insufficiency and toxemia of pregnancy[1].



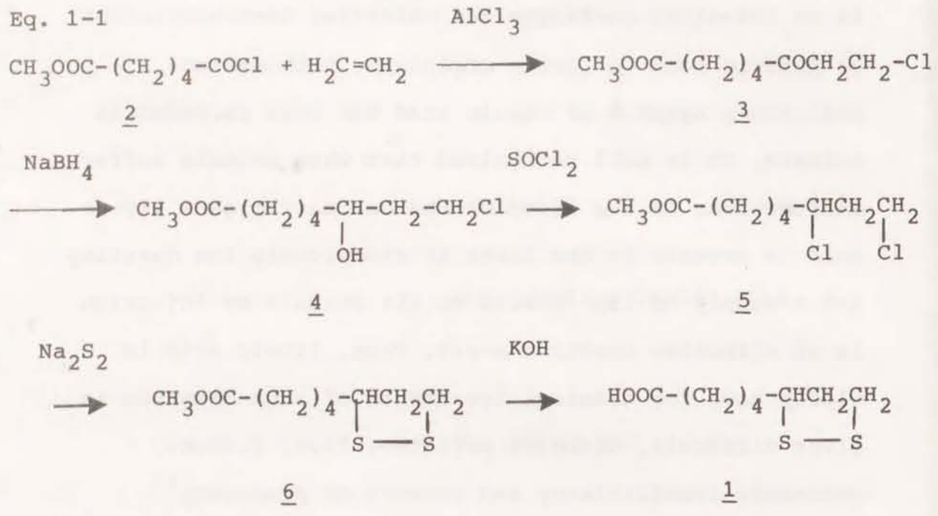
Lipoic acid (1)

This study was undertaken by Dr. Yurugi in 1956 for the purpose of developing a new industrially feasible synthesis of lipoic acid. The author joined his synthetic project in 1958.

Among many routes to lipoic acid which had been reported by that time, the route formulated in Eq. 1-1 which was developed by Reed (1955)[2] and later innovated by Acker (1957)[3] seemed the most industrially feasible one. But this route still have some disadvantages for industrialization, one is that all of the intermediates are oils which require distillation for their isolation, and another one is that sodium borohydride is rather

expensive.

Eq. 1-1



The object of this study was to find a new route to lipoic acid which is avoid of these disadvantages.

Section 1: Synthesis of Alkyl 8-alkoxy- and 8-acyloxy-6-oxooctanoate

Our basic strategy on the first step of the synthesis of lipoic acid was to prepare an octanoic acid bearing two functional groups at 6- and 8-positions, both of which are convertible into thiols.

Precedingly, Yurugi et al.[4] synthesized methyl 8-ethoxy-6-oxooctanoate (12b) starting from an enamine, 1-piperidino-1-cyclopentene (7), via three steps as formulated in Eq. 1-2 wherein R was C_2H_5. Acylation of enamine (7) with 2-ethoxypropionyl chloride (8b) followed by the hydrolysis of the resultant reaction mixture with HCl afforded 1,3-diketone (10b) which on

hydrolysis with NaOH and subsequent esterification with CH_3OH-H_2SO_4 resulted in 12b in a good yield.

As an initial challenge to the synthesis of lipoic acid, the author intended to improve the reaction yield either by variation of R or by insertion of a step affording a crystalline intermediate which would make purification more convenient.

In an analogous manner to preceding procedure, methyl 8-alkoxy-6-oxooctanoate (12a,c,d) were prepared and the results are summarized in Table 1-1, 1-2 and 1-3. As apparent by the data, the over-all yield was improved as R varied to higher alkyl groups. Thus, the highest over-all yield was attained when R was C_4H_9.

Eq. 1-2

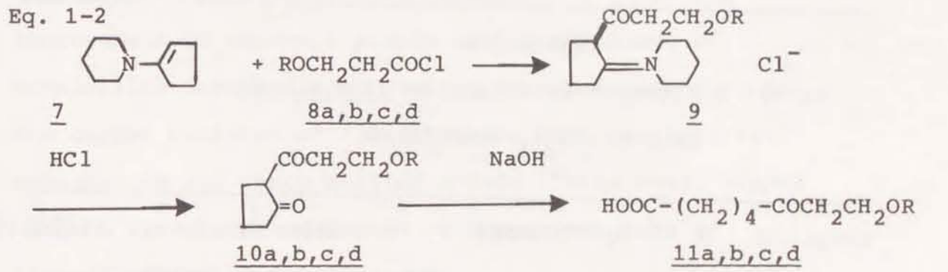


Table 1-1

1,3-Diketones (10a,b,c,d)

compound	R	bp °C(mmHg)	UVmax(nm) in EtOH	log	Yield(%)
10a	CH_3	87-97(0.1)	287	5.12	37

10b	C_2H_5	85-95(0.4)	287	5.69	55
10c	C_3H_7	100-108(0.12)	289	5.56	68
10d	C_4H_9	111-120(0.2)	287	5.51	78

Table 1-2

8-Alkoxy-6-oxooctanoic acids (11a,b,c,d)

compound	R	mp °C	appearance	Yield(%)
11a	CH_3	oil	oil	74
11b	C_2H_5	47-50	leaflets	74
11c	C_3H_5	33-36	leaflets	74
11d	C_4H_5	36-38	needles	85

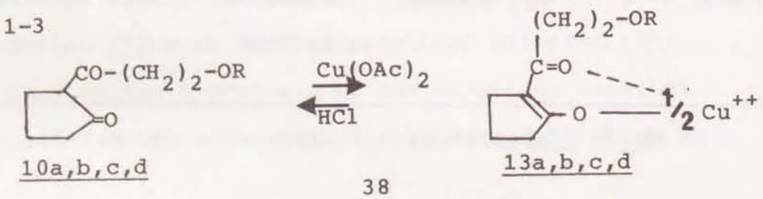
Table 1-3

Methyl 8-Alkoxy-6-oxooctanoate (12a,b,c,d)

$CH_3OOC-(CH_2)_4-COCH_2CH_2OR$

compound	R	bp °C(mmHg)	IR(cm^{-1}) CO	IR(cm^{-1}) ester	IR(cm^{-1}) C-O-C	Yield (%)
12a	CH_3	106-108(0.15)	1739	1250-1143	1115	96
12b	C_2H_5	114-120(0.6)	1739	1250-1143	1115	98
12c	C_3H_7	115-123(0.2)	1724	1250-1143	1111	98
12d	C_4H_9	120-124(0.2)	1724	1250-1143	1111	98

Eq. 1-3



10a,b,c,d 13a,b,c,d

a; R=CH_3, b; R=C_2H_5

c; R=C_3H_7, d; R=C_4H_9

When etherial solution of 1,3-diketones (10a,b,c,d) were shaken with an aqueous solution of $Cu(OAc)_2$, copper chelates of 1,3-diketones (13a,b,c,d) were formed as precipitates, which were recrystallized from appropriate solvents to afford colorful crystals (Table 1-4). Treatment of the chelates with 20% HCl regenerated 1,3-diketones, which were collected by extraction with ether. But the over-all yields of chelate formation and re-generation of 1,3-diketone were about 30%, which were quite poor as compared to isolation yields of 1,3-diketones using distillation (Table 1-1). Thus, the improvement of over-all yields taking advantage of crystalline intermediates was not attained. However, as the copper chelates of 1,3-diketones have characteristic appearances and sharp melting points (Table 1-4), copper chelate formations conferred us convenient ways of identification of 1,3-diketones.

Table 1-4

Copper Chelates of 1,3-Diketones (13a,b,c,d)

compound	R	mp °C	recryst. solvent	appearance	IR(cm^{-1})
13a	CH_3	174-175	Me_2CO	green prisms	1600, 1471, 1422
13b	C_2H_5	170-171	Me_2CO	white green feathers	1597, 1506, 1464

13c	C_3H_7	157-158	Me_2CO	"	1587, 1504, 1460
13d	C_4H_9	135-136	Et_2O	"	1587, 1497, 1456

Since acyloxy group was expected to be more convenient for the following transformations than alkoxy group, in the next place, the author intended to prepare methyl 8-acyloxy-6-oxooctanoate (15) which bears acyloxy group instead of alkoxy of compounds 12.

Preparation of the object compounds by application of reactions shown in Eq. 1-2 with R being acyls was unsuccessful. Whereas, reaction of methyl 6-oxo-7-octenoate (14) with such acids as formic acid, acetic acid, propionic acid and butyric acid in the presence of chataritic amount of sulfuric acid or Lewis acid under heating afforded methyl 8-acyloxy-6-oxooctanoate (15a,b,c,d) in good yields. Among the catalysts investigated, sulfuric acid gave methyl 8-acetoxy-6-oxooctanoate (15b) in the highest yield as summarized in Table 1-5.

Eq. 1-4

$$CH_2=CH\text{-}CO\text{-}(CH_2)_4\text{-}COOCH_3 + R\text{-}COOH \xrightarrow{\text{catalyst}} RCOOCH_2CH_2CO\text{-}(CH_2)_4\text{-}COOCH_3$$

14 → 15a,b,c,d

a; R=H, b; R=CH_3, c; R=C_2H_5, d; R=C_3H_7

Table 1-5

Methyl 8-acetoxy-6-oxooctanoate (15b)

catalyst	temp.	time(hr)	Yield (%)

$Hg(AcO)_2$	reflux	0.5	28.7
$Hg(SO_4)_2$	reflux	0.5	33.4
H_2SO_4	reflux	0.5	30.4
H_2SO_4	water-bath ca. 70°C	2.0	61.0
BF_3-AcOH	reflux	0.5	34.8
BF_3-AcOH	water-bath	2.0	50.0
BF_3-Et_2O	water-bath	2.0	43.5

Table 1-6

Methyl 8-acyloxy-6-oxooctanoate (15a,b,c,d)

compound	R	bp °C(mmHg)	IR(cm^{-1})	Yield (%)
15a	H	132-138(0.12)	1724, 1250-1143	49
15b	CH_3	125-135(0.1)	1739, 1266-1190	61
15c	C_2H5	128-133(0.15)	1739, 1266-1143	38.5
15d	C_3H_7	135-145(0.15)	1724, 1250-1143	34

Among methyl 6,8-bisfunctionalized octanoate (12a-d, 15a-d) thus far prepared, methyl 8-acetoxy-6-oxooctanoate (15b) with the top priority and methyl 8-ethoxy-6-oxooctanoate (12b) with the second priority were selected as basic compounds for the following transformations. The top priority on 15b is based on such good availability that the precursor, methyl 6-oxo-7-octenoate (14)

is easily prepared by dehydrochlorination of methyl 8-chloro-6-oxooctanoate (3)[5], which in turn is prepared according to the reactions shown in Eq. 1-1, and its novelty as a compound. On the contrary, 12a was precedingly prepared by Starker[6] by Michael addition of ethanol into methyl 6-oxo-7-octenoate (14).

Section 2 Reduction of methyl 8-ethoxy- and 8-acetoxy-6-oxooctanoate

Our strategy on the second step is to transform both functions at 6- and 8-positions of octanoic acids (12b and 15b) obtained in the preceding section into thiols. In this section, the reduction of 6-oxo- group into hydroxy group is described.

Many researchers performed the reduction of alkyl 6-oxooctanoate into alkyl 6-hydroxyoctanoate using sodium borohydride or lithium aluminium hydride as summarized in Table 1-7.

Table 1-7

Reduction of Alkyl 6-oxooctanoates

$R^1\text{-}CH_2CH_2CO\text{-}(CH_2)_4\text{-}COOR \longrightarrow R^1\text{-}CH_2CH_2\underset{\displaystyle OH}{\underset{|}{CH}}\text{-}(CH_2)_4\text{-}COOR$

reagent	researcher	R^1
$NaBH_4$	Bullock (1952)[5]	CH_3COS-
	Soper (1954)[7]	$C_6H_5CH_2$-S-
	Starker (1955)[6]	CH_3O-
$LiAlH_4$	Bullock (1957)[8]	Alkyl-S-

The author searched for the reduction which proceeds under industrially more feasible conditions. Catalytic reduction of methyl 8-ethoxy-6-oxooctanoate (12b) with Raney nickel as catalyst and methanol as solvent in a high pressure atmosphere of hydrogen (81 Kg/cm^2) at room temperature afforded methyl 8-ethoxy-6-hydroxyoctanoate (16) in 86.5% yield. Catalytic reduction of methyl 8-acetoxy-6-oxooctanoate (15b) in similar conditions as above except heating at 70-100°C for 3 hours gave rise to methyl 8-acetoxy-6-hydroxy-octanoate (17) in 81.5% yield.

Hydrolysis of methyl 8-acetoxy-6-hydroxyoctanoate (17) on treatment with sodium hydroxide in methanol afforded 6,8-dihydroxyoctanoic acid (18) of mp 77-78°C in 84% yield. This compound corresponds an oxygen isostere of dihydro lipoic acid and has been registered in a patent as a compound of mp 68-69°C[9].

Eq. 1-5

$RO\text{-}CH_2CH_2CO\text{-}(CH_2)_4\text{-}COOCH_3 \xrightarrow[\text{Raney Ni}]{H_2} ROCH_2CH_2\underset{\displaystyle OH}{\underset{|}{CH}}\text{-}(CH_2)_4\text{-}COOCH_3$

12b; R=C_2H_5- 16; R=C_2H_5-

15b; R=CH_3CO- 17; R=CH_3CO-

NaOH ↙

$HO\text{-}CH_2CH_2\underset{\displaystyle OH}{\underset{|}{CH}}\text{-}(CH_2)_4\text{-}COOH$

18

Section 3 New synthesis of 6,8-dihalogenooctanoic acids and its esters

In line with the strategy described in the section 2, transformation of methyl 8-ethoxy-6-hydroxyoctanoate (16) and methyl 8-acetoxy-6-hydroxyoctanoate (17) into 6,8-dihalogenooctanoic acids (20 and 22) was investigated.

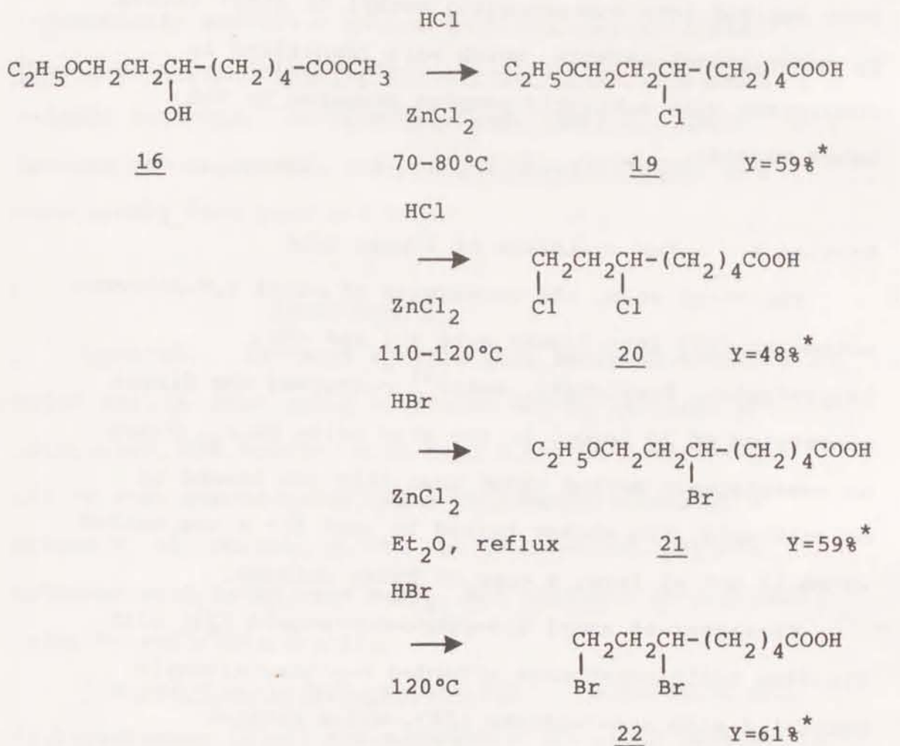
Previously, Reed[2] synthesized 6,8-dihalogeno-octanoic acids by stepwise incorporation of halogens into 8- and then 6-positions of the octanoic acid skelton as exemplified by Eq. 1-1. To gain an advantage over this method, the author aimed to develop a simultaneous incorporation of halogens into both of the 6- and 8-positions.

Treatment of methyl 8 ethoxy-6-hydroxyoctanoate (16) with gaseous HCl in the presence of $ZnCl_2$ at 70-80°C afforded 6-chloro-8-ethoxyoctanoic acid (19) in 59% yield. Repetition of the above reaction at higher temperatures, 110-120°C, resulted in 6,8-dichloro-octanoic acid (20) in 48% yield.

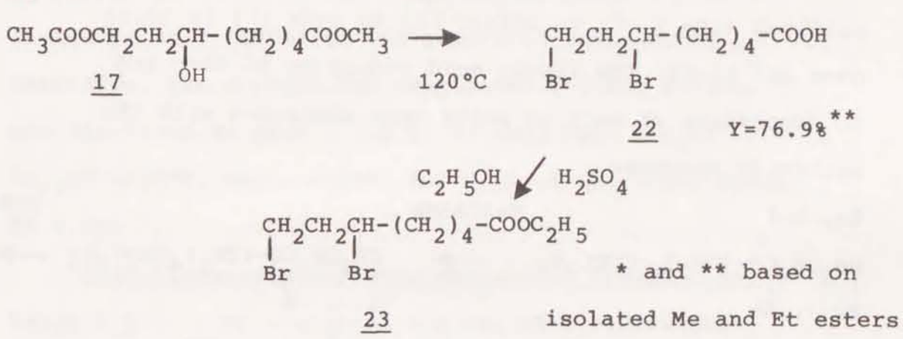
Introduction of gaseous HBr into a refluxing etherial solution of methyl 8-ethoxy-6-hydroxyoctanoate (16) and $ZnCl_2$ gave 6-bromo-8-ethoxyoctanoic acid (21) in 59% yield. Whereas, introduction of HBr gas into 16 under heating at 110-120°C afforded 6,8-dibromooctanoic acid (22) in 61% yield.

Thus, it became apparent that the rather drastic conditions are necessary for simultaneous halogenation of the 6- and 8-positions.

Eq. 1-6



Eq. 1-7



* and ** based on isolated Me and Et esters

Application of the above reaction condition, which afforded 6,8-dibromooctanoic acid (22), to methyl 8-acetoxy-6-hydroxyoctanoate (17) also afforded 22 in 76.9% yield.

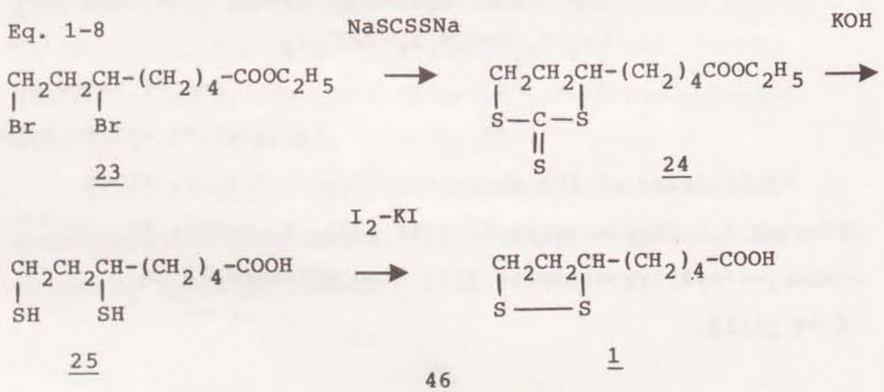
All of these halogenated octanoic acids (19-22) were derived into corresponding methyl or ethyl esters by conventional methods, which were identified in comparison with authentic samples prepared by the known methods.

Section 4 New synthesis of lipoic acid

The final step, the conversion of ethyl 6,8-dibromo-octanoate (23) into lipoic acid (1) was then investigated. Previously, Acker[3] performed the direct conversion of 23 into 1 in one step using Na_2S_2. Since no advantageous method other than this one seemed to be available, the author turned to seek for a new method which is not al least a copy of known methods.

Treatment of ethyl 6,8-dibromooctanoate (23) with disodium trithiocarbonate afforded 4-ethoxycarbonyl-butyl-1,3-dithiane-2-thione (24), which without isolation was successively hydrolyzed with KOH and oxidized with I_2-KI to afford lipoic acid (1) in 39.8% over all yield. The lipoic acid showed mp 56-58°C and no depression of melting point upon admixture with the authentic specimen.

Eq. 1-8



23 24 25 1

In summary, an access to lipoic acid starting from industrially available methyl 5-chloroformylvalerate (2), i.e. (2)-(3)-(14)-(15b)-(17)-(22)-(23)-(24)-(25)-lipoic acid (1), has been attained. Therein, advantageous new processes, i.e. (14)-(15b)-(17)-(22), are successfuly incorporated.

Experimental

General. Infrared spectra were measured in KBr disc, nujol mull or neat using a Hitachi EPI-S_2 infrared spectrophotometer. NMR spectra were determined on a Varian HA-100 or T-60 spectrometer using tetramethylsilane as a standard. All melting points are uncorrected. Organic extracts were dried over $MgSO_4$. All solvents were evaporated by rotor-evaporation.

1-Piperidino-1-cyclopentene (7) A solution of cyclopentanone (30 g) and piperidine (85 g) in benzene (200 ml) was refluxed for 5 hr, during which the water azeotropically distilled was seperated using water seperator. The solvent was evaporated and the residue was distilled to give 7 (50 g) as colorless oil of bp_{17} 107-109°C. Anal. Calcd. for $C_{10}H_{17}N$: N; 9.26. Found. N; 9.02.

2-(3-Alkoxypropionyl)cyclopentanone (10a,b,c,d)

Table 1-1 To a stirred mixture of 1-piperidino-1-cyclopentene (7 ; 0.33 mol) and triethylamine (40 g, 0.4 mol) in $CHCl_3$ (300 ml) was added a solution of appropriate 3-alkoxypropionyl chloride (8a,b,c,d; 0.33 mol) in $CHCl_3$ (150 ml) dropwise. After 1 hr stirring, the

mixture was allowed to stand overnight. The mixture was heated to reflux with 30% HCl (300 ml) for 5 hr. After cooling, the organic layer was seperated, washed with water, dried. The solution was concentrated and the residue was distilled to afford the titled compounds.

Anal. Calcd. for $C_9H_{14}O_3$ (10a): C; 63.51, H; 8.29.
Found. C; 64.06, H; 8.35.
for $C_{10}H_{16}O_3$ (10b): C; 65.19, H; 8.75.
Found. C; 65.22, H; 8.92.
for $C_{11}H_{18}O_3$ (10c): C; 66.64, H; 9.15.
Found. C; 66.65, H; 9.25.
for $C_{12}H_{20}O_3$ (10d): C; 67.89, H; 9.50.
Found. C; 67.67, H; 9.58.

8-Alkoxy-6-oxooctanoic acid (11a,b,c,d) Table 1-2

A mixture of an appropriate 2-(3-alkoxypropionyl) cyclopentanone (10a,b,c,d; 0.05 mol), NaOH (1.6 g, 0.04 mol) and water (32 ml) was heated to reflux for 30 min. The solution was adjusted to pH 6.5 with 5% NaOH and extracted with Et_2O. The aqueous solution was adjusted to pH 1 with 30% HCl and saturated with NaCl and extracted with Et_2O. The organic layer was seperated, washed with brine and dried. The solvent was evaporated to leave the titled acid.

Anal. Calcd. for $C_{10}H_{18}O_4$ (11b): C; 59.39, H; 8.97.
Found. C; 59.10, H; 8.94.
for $C_{11}H_{20}O_4$ (11c): C; 61.09, H; 9.32.
Found. C; 61.48, H; 9.34.
for $C_{12}H_{22}O_4$ (11d): C; 62.58, H; 9.63.
Found. C; 62.47, H; 9.51.

Methyl 8-alkoxy-6-oxooctanoate (12a,b,c,d) Table 1-3

An appropriate 8-alkoxy-6-oxooctanoic acid (11a,b,c,d; 5 g) was heated to reflux with MeOH (5 ml), benzene (80 ml), $MgSO_4$ (18 g) and H_2SO_4 (4 ml) under stirring. The mixture was shaken with water (100 ml) and then with 10% $NaHCO_3$. The organic layer was concentrated and distilled to afford the titled compound.

Anal. Calcd. for $C_{10}H_{18}O_4$ (12a): C; 59.39, H; 8.97.
Found. C; 59.54, H; 8.86.
for $C_{11}H_{20}O_4$ (12b): C; 61.09, H; 9.32.
Found. C; 61.31, H; 9.35.
for $C_{12}H_{22}O_4$ (12c): C; 62.58, H; 9.63.
Found. C; 62.94, H; 9.61.
for $C_{13}H_{24}O_4$ (12d): C; 63.90, H; 9.90.
Found. C; 64.16, H; 9.86.

Copper chelates of 2-(3-alkoxypropionyl)cyclopentanone (13a,b,c,d) Table 1-4

A solution of an appropriate 2-(3-alkoxypropionyl) cyclopentanone (10a,b,c,d; 2 g) in Et_2O (40 ml) was shaken with a solution of $Cu(AcO)_2 \cdot H_2O$ (4 g) in water (80 ml). The precipitates formed were collected with suction and washed with water and dried and recrystallized from an appropriate solvent listed in Table 1-4 to afford the titled compound.

Anal. Calcd. for $C_{18}H_{26}O_6Cu$ (13a): C; 53.79, H; 6.52.
Found. C; 53.81, H; 6.43.
for $C_{20}H_{30}O_6Cu$ (13b): C; 55.86, H; 7.03.
Found. C; 55.79, H; 7.05.
for $C_{22}H_{34}O_6Cu$ (13c): C; 57.69, H; 7.48.

Found. C; 57.50, H; 7.51.

for $C_{24}H_{38}O_6Cu$ (13d): C; 59.30, H; 7.88.

Found. C; 59.49, H; 7.85.

Methyl 8-formyloxy-6-oxooctanoate (15a) To a heated (80°C) and stirred solution of methyl 6-oxo-7-octenoate (14; 8.5 g) was added HCOOH (11.5 g) dropwise. After 2 hr heating and stirring, HCOOH was distilled and the residue was distilled under reduced pressure to afford 15a as an oil of $bp_{0.12}$ 132-138°C, Yield 5.3 g (49%).

Anal. Calcd. for $C_{10}H_{16}O5$: C; 55.54, H; 7.46.

Found. C; 55.12, H; 7.57.

Methyl 8-acetoxy-6-oxooctanoate (15b) Table 1-5 and 1-6 A mixture of methyl 6-oxo-7-octenoate (14; 17 g), AcOH (30 g) and an appropriate catalyst (200 mg or 3 drops) as listed in Table 1-5 was treated in the condition as indicated in the Table. The solution was mixed with water (150 ml) and saturated with NaCl and then extracted with AcOEt. The extract was washed with sat. NaCl and dried. The solvent was removed and the residue was distilled under reduced pressure to afford 15b as a colorless oil.

Anal. Calcd. for $C_{11}H_{18}O_5$: C; 57.38, H; 7.88.

Found. C; 57.27, H; 7.95.

Methyl 8-acyloxy-6-oxooctanoate (15c,d) Table 1-6

A mixture of methyl 6-oxo-7-octenoate (14; 0.1 mol), propionic acid or butyric acid (0.5 mol) and H_2SO_4 (3 drops) was heated on a water bath (80°C) for 3 hr. The solution was mixed with water (15 ml) and saturated with NaCl and extracted with AcOEt. The extract was washed with

10% $NaHCO_3$ and brine successively and dried. The solvent was removed and the residue was distilled under reduced pressure to afford 15c,d.

Anal. Calcd. for $C_{12}H_{20}O_5$ (15c): C; 59.00, H; 8.25.

Found. C; 58.65, H; 8.18.

for $C_{13}H_{22}O_5$ (15d): C; 60.44, H; 8.59.

Found. C; 60.10, H; 8.52.

Methyl 8-ethoxy-6-hydroxyoctanoate (16)

A solution of methyl 8-ethoxy-6-oxooctanoate (12b; 9.5 g) in MeOH (40 ml) was shaken with Raney Ni (5 g) in an autoclave under hydrogen atmosphere (81 kg/cm^2) at 25°C for 3 hr. The mixture was filtered and the filtrate was concentrated. The residual oil was distilled under reduced pressure to afford 16 as a colorless oil of $bp_{0.2}$ 110-117°C. Yield 8.3 g (86.5%).

Anal. Calcd. for $C_{11}H_{22}O4$: C; 60.52, H; 10.16.

Found. C; 60.26, H; 10.16

Methyl 8-acetoxy-6-hydroxyoctanoate (17)

A solution of methyl 8-acetoxy-6-oxooctanoate (15b; 23 g) in MeOH (120 ml) was shaken with Raney Ni (20 g) in an autoclave under hydrogen atmosphere (74 kg/cm^2) at 70-100°C for 3 hr. The mixture was filtered and the filtrate was concentrated. The residue was distilled under reduced pressure to afford 17 as a colorless oil of $bp_{0.2}$ 128-130°C. Yield 18.8 g (81.5%).

Anal. Calcd. for $C_{11}H_{20}O_5$: C; 56.88, H; 8.68.

Found. C; 56.68, H; 8.65.

6,8-Dihydroxyoctanoic acid (18)

A mixture of methyl 8-acetoxy-6-hydroxyoctanoate

(17; 11.6 g), MeOH (25 ml), NaOH (5 g) and water (25 ml) was heated to reflux for 2 hr. The solvent was removed in vacuo and the residue was made acidic with 10% HCl and saturated with NaCl and extracted with AcOEt. The extract was dried and concentrated in vacuo to leave crystalline solids 7.2 g (84%). Recrystallization of the solids from AcOEt to afford an analytical sample of mp 77-78°C, as colorless needles.

Anal. Calcd. for $C_{11}H_{20}O_5$: C; 54.53, H; 9.15.
Found. C; 54.75, H; 8.87.

Methyl 6-chloro-8-ethoxyoctanoate (methyl ester of 19)

(a) To a heated mixture of methyl 8-ethoxy-6-hydroxy-octanoate (16; 22 g) and $ZnCl_2$ (2.5 g) was introduced anhydrous HCl gas at 70-80°C for 3 hr. During which, $ZnCl_2$ (8.5 g) was added in three to four portions. The mixture was diluted with MeOH (70 ml) under ice-cooling and the solution was saturated with HCl. After overnight storage, the mixture was poured into ice-water and extracted with benzene. The extract was washed with water and dried. The solvent was removed and the residue was distilled under reduced pressure to afford the titled compound as colorless oil of $bp_{0.1-0.2}$ 92-104°C. Yield 14 g (59%). The sample was identical with the sample prepared method b) in IR spectra.

(b) A solution of methyl 8-ethoxy-6-hydroxyoctanoate (16; 11 g), $SOCl_2$ (7 g) and pyridine (3 drops) in benzene (20 ml) was stirred on a water-bath at 70-80°C for 30 min. The solution was pored into ice-water (50 ml). The organic layer was seperated, washed with 5% $NaHCO_3$

and water and then dried. After removal of the solvent, the residue was distilled under reduce pressure to afford the titled compound. Yield 8.2 g (69%).

Anal. Calcd. for $C_{11}H_{21}O_2Cl$: C; 55.80, H; 8.94.
Found. C; 56.70, H; 9.42.

IR (liquid); 1724 (strong, carboxyl C=O), 1250-1143 (strong, ester), 1111 (strong, C-O-C) cm^{-1}.

Methyl 6,8-dichlorooctanoate (methyl ester of 20)

A mixture of methyl 8-ethoxy-6-hydroxyoctanoate (16; 11 g) and $ZnCl_2$ (1 g) was heated at 110-120°C and anhydrous HCl was introduced thereto for 2 hr. During the reaction, $ZnCl_2$ (4 g) was added to the reaction mixture in three portions. After cooling, MeOH (50 ml) was added and the mixture was saturated with HCl and allowed to stand overnight. The mixture was poured into water and extracted with benzene. The extract was washed with water and dried. The solvent was removed and the residue was distilled to afford the titled compound as colorless oil of $bp_{0.1}$ 94-115°C. The sample was identical with the authentic sample prepared according to the procedure described by Reed[2] on IR spectral comparison.

IR (liquid); 1739 (strong, carboxyl C=O), 1266-1143 (strong, ester) cm^{-1}.

Methyl 6-bromo-8-ethoxyoctanoate (21)

A solution of methyl 8-ethoxy-6-hydroxyoctanoate (16; 11 g) and $ZnCl_2$ (2.2 g) in Et_2O (40 ml) was heated to reflux and anhydrous HBr was introduced thereto for 3 hr. The solution was poured into ice-water (200 ml).

The organic layer was seperated, washed with water and dried. After removal of the solvent, the residue was distilled under reduced pressure to afford the titled compound as a colorless oil of $bp_{0.4}$ 100-118°C. Yield 8.3 g (59%).

Anal. Calcd. for $C_{11}H_{21}O_3Br$: C; 46.98, H; 7.53.
Found. C; 46.88, H; 7.45.

IR (liquid); 1739 (strong, carboxyl C=O), 1250-1143 (strong, ester), 1111 (strong, C-O-C) cm^{-1}.

Ethyl 6,8-dibromooctanoate (23)

(a) To methyl 8-ethoxy-6-hydroxyoctanoate (16; 11 g) heated at 110-120°C was introduced anhydrous HBr for 3 hr. The oily residue was heated to reflux with EtOH (40 ml), benzene (80 ml) and H_2SO_4 (4 ml) for 8 hr, during which water azeotropically distilled was seperated using water seperator. The mixture was shaken with cold water (200 ml) and benzene (150 ml). The organic layer was washed with 10% $NaHCO_3$ and water and then dried. After removal of the solvent, the residue was distilled under reduced pressure to give the titled compound as an oil of $bp_{0.3}$ 123-140°C. Yield 10.1 g (61%). The sample was identical with the authentic sample prepared according to the procedure described by Reed[2] in IR spectra.

(b) Methyl 8-acetoxy-6-hydroxyoctanoate (17; 11.6g) was treated with HBr and esterified as above to afford the titled compound as an oil of $bp_{0.1}$ 119-132°C. Yield 12.7 g (76.9%). The sample was identical with the authentic sample[2] in IR spectra.

d,l-Lipoic acid

A sodium ethylate solution made from Na (2.3 g) and EtOH (50 ml) was saturated with H_2S. After dropwise addition of CS_2 (5 g) thereto, the solution was heated to reflux for 30 min. After cooling, a solution of ethyl 6,8-dibromooctanoate (23; 16.5 g) in EtOH (50 ml) was added dropwise under stirring. After 2 hr stirring, the mixture was allowed to stand overnight. The precipitated NaBr (10 g; calcd. 10.3 g) was removed with suction, and the filtrate was evaporated to dryness. The residual oil was mixed with KOH (9.3 g), water (93 ml) and EtOH (93 ml) and the mixed solution was heated to reflux under N_2 atmosphere for 2 hr. After removal of EtOH in vacuo, the solution was shaken with Et_2O. The aqueous layer was made pH 3 with HCl and the oil deposited was extracted with $CHCl_3$ (100 ml). To the extract was added I_2-KI solution[10] was added dropwise during 4 hr period to give a permanet brown color. The organic layer was seperated, washed with 1% $Na_2S_2O_3$ and dried. After removal of the solvent, the residue was distilled under reduced pressure to afford lipoic acid (1) as a yellow oil of $bp_{0.2-0.5}$ 160-175°C. Yield 4.1 g (39.8%). After standing in a refregerator, the oil was solidified to show mp57-58°C which did not show depression of melting point on admixture with the authentic sample prepared according to the procedure of Reed[2]. A sample was recrystallized from nBu_2O for analysis, mp61-62°C. It showed the characteristic UV spectrum of lipoic acid, $\lambda_{max.}$ 332nm, ε 157 which is in good accord with the recorded values[2].

Referendes

1) a) F. Rausch, Klin. Wochschr., 34, 737 (1956), b) H. Redetyki, H. Bloedorm and H.W. Bansi, Klin. Wochshr., 34, 845 (1956)

2) L.J. Reed and Ching-I Niu, J. Am. Chem. Soc., 77, 416 (1955)

3) D.S. Acker and W.J. Wayne, J. Am. Chem. Soc., 79, 6483 (1957)

4) S. Yurugi, T. Fushimi and M. Numata, Yakugaku Zasshi , 80, 1165 (1960)

5) M.W. Bullock and J.A. Brockmann et al., J. Am. Chem. Soc., 74, 3455 (1952) and 76, 1828 (1954)

6) L.N. Starker and D.B. Cosulish et al., J. Am. Chem. Soc., 77, 2534 (1955)

7) Q.F. Soper and W.E. Buting et al., J. Am. Chem. Soc., 76, 4109 (1954)

8) M.W. Bullock and J.J. Hand et al., J. Am. Chem. Soc., 79, 1975 (1957)

9) E. Merck: Deutch Reiches Patent 1,046,016 (1958)

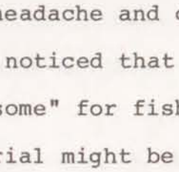
10) R.L. Schriner and R.C. Fuson, "Identification of Organic Compounds," 2nd Ed., John Wiley Sons, Inc., New York, N. Y., p53 (1940)

CHAPTER 2

SYNTHESIS OF NEREISTOXIN AND RELATED COMPOUNDS

In 1922, a doctor, S. Nitta examined a fisherman who came to see him for headache and disorder in respiratory function. The doctor noticed that the man handled a lot of marine-annelid "isome" for fishing baits and suspected that come toxic material might be contained in the corpses of the annelids and it might be the cause of the trouble. Soon, he started to investigate the toxin and eventually in 1934[1] he succeeded in its isolation from chloroform extract of the annelids and named it "Nereistoxin" after the annelid's scientific term "Lubriconereis heteropoda". He proposed a molecular formula, $C_7H_{11}NO_4S_2$, for the oxalate salt of the toxin and showed it neurotoxic to various animals and insects.

In 1961, after 27 years since the first isolation of Nereistoxin, Y. Hashimoto and T. Okaichi[2], who were interested in Nitta's report, reinvestigated the toxin and corrected the molecular formula of its oxalate to $C_7H_{13}NO_4S_2$. Based on degradation studies, they identified Nereistoxin as 4-dimethylamino-1,2-dithiolane (1).



1

Dr. S. Tatsuoka, the former Director of Central Research Division in Takeda Chemical Industries Company, Ltd., happened to attend the meeting where the result was presented by Hashimoto and was interested in its strong toxity to insects, since it suggested the

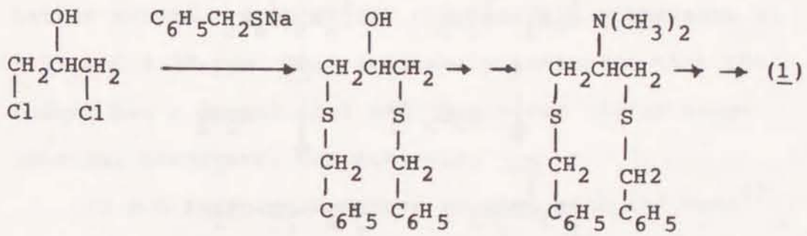
potential utility of the toxin as an insectiside and also it has a unique structure. He then offered Hashimoto co-laboration on its synthetic work.

At first, K. Konishi and his group members who belonged to Agricultural Chemicals Division of this company took charge of the synthesis. But a preliminary exploration soon showed that the synthesis was not so easy as was optimistically expected from the simple structure of the toxin. Then Dr. S. Yurugi and his groups, including the author, who had experienced in the synthesis of lipoic acid having the same 1,2-dithiolane nucleus as Nereistoxin, were asked to join the synthetic project.

In the following two sections, the syntheses of Nereistoxin which we undertook are described.

Section 1: Synthesis of Nereistoxin Starting from 1,3-Bis(benzylthio)-2-propanol

Eq. 2-1 First Strategy



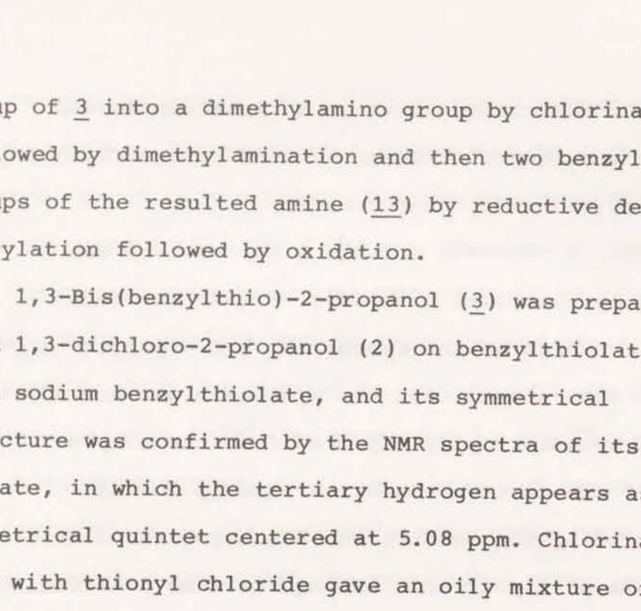
2 3 13 (1)

Since Nereistoxin (1) has a symmetrical structure, a readily available symmetrical alcohol 1,3-bis(benzylthio)-2-propanol (3)[3] was our first choice as the starting material. We intended to convert the hydroxy

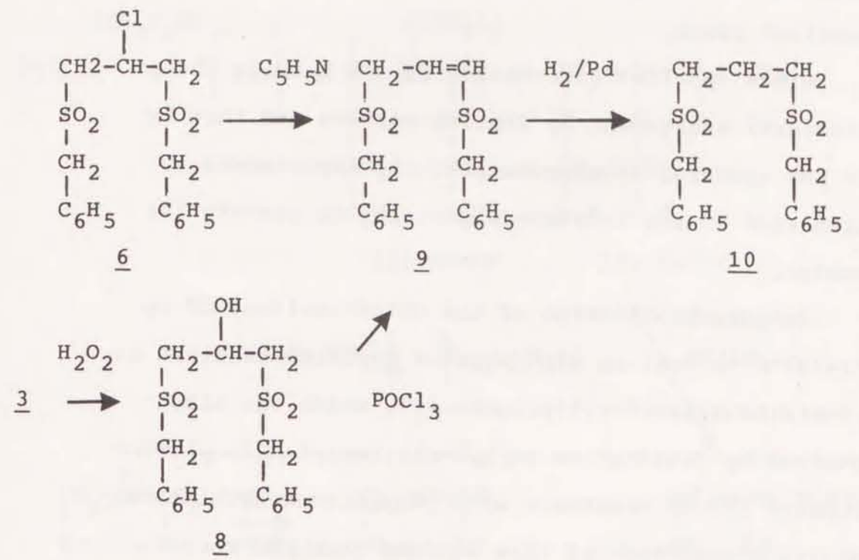
group of 3 into a dimethylamino group by chlorination followed by dimethylamination and then two benzylthio groups of the resulted amine (13) by reductive debenzylation followed by oxidation.

1,3-Bis(benzylthio)-2-propanol (3) was prepared from 1,3-dichloro-2-propanol (2) on benzylthiolation with sodium benzylthiolate, and its symmetrical structure was confirmed by the NMR spectra of its acetate, in which the tertiary hydrogen appears as a symmetrical quintet centered at 5.08 ppm. Chlorination of 3 with thionyl chloride gave an oily mixture of symmetrical (4) and asymmetrical (5) chlorides. Since seperation trials by column chromatography were not successful, their structures were identified by driving them to crystalline and seperable derivatives as follows.

Eq. 2-2



Eq. 2-3



Oxidation of the chloride mixture (4+5) with H_2O_2 afforded solids, from which symmetrical chloro-sulfone (6) of mp 201-202°C, insoluble in chloroform, and asymmetrical chloro-sulfone (7) of mp 135-137°C, soluble in chloroform, were isolated. The former showed a singlet for the benzylic methylenes at 4.30 ppm, whereas the latter showed a doublet for the benzylic methylenes at 4.27 and 4.38 ppm. This apparently indicates that the former has a symmetrical and the latter has an asymmetrical structure, respectively.

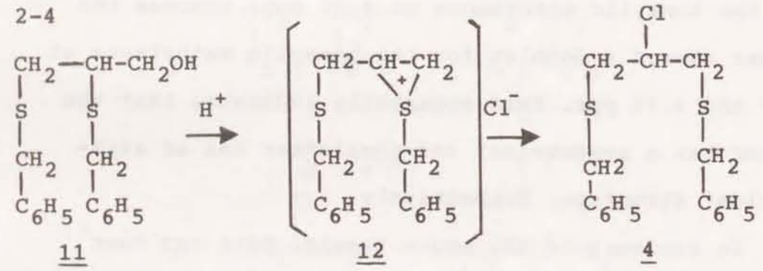
In contrast to the above result, Fitt and Owen[4] ascribed the asymmetrical structure to the chloro-sulfone of mp 204-205°C, which they obtained from 2,3-bis(benzylthio)-1-propanol (11) on treatment with HCl followed by the oxidation with H_2O_2. We repeated the experiment and got the chloro-sulfone of mp 201-202°C

which is identical to the former chloro-sulfone described above.

Since apparent discrepancy exists between the structural assignment by English workers and that by our NMR spectral interpretation, the experiments as formulated in Eq. 1-3 were undertaken to clarify the problem.

Dehydrochlorination of the chloro-sulfone of mp 201-202°C on heating with aqueous pyridine resulted in 1,3-bis(benzylsulfonyl)propene (9), which was also obtained by dehydration of 1,3-bis(benzylsulfonyl)-2-propanol (8) on treatment with phosphorousoxychloride. Catalytic reduction of this sulfone gave the known 1,3-bis(benzylsulfonyl)propane (10). The results definitely supported the assignment based on the NMR spectra interpretation. The reaction which proceeded in Fitt and Owen's experiment could be formulated as below.

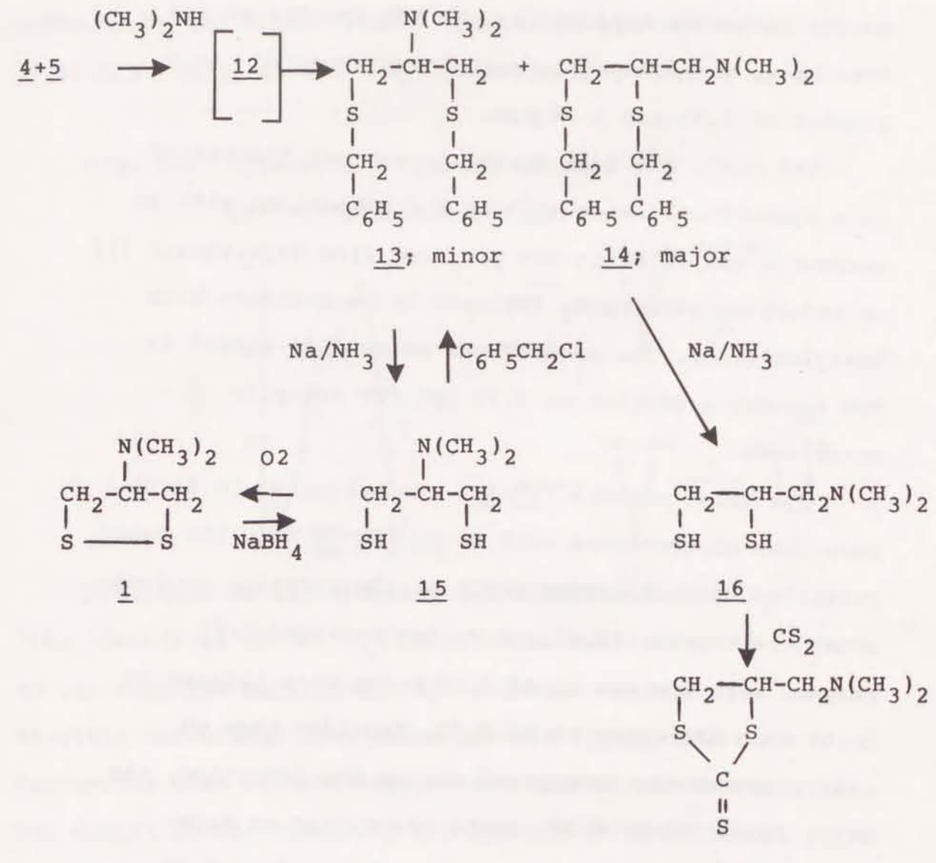
Eq. 2-4



11 12 4

The migration of benzylthio group accompanied by chlorination of 3 to 5 and of 11 to 4 was proposed to have proceeded through an episulfonium intermediate as formulated in 12.

Eq. 2-5



Reaction of the chloride mixture (4+5) with dimethylamine in an autoclave at 160°C for 16 hr afforded an oily mixture of a symmetrical amine (13) and an asymmetrical amine (14), whose thin layer chromatography (TLC) showed two spots visualized by Kraut-Dragendorff reagent with the solow moving spot being far more intense in color than the fast movint spot. The repeated recrystallizations of a hydrogen oxalate salt of the mixture gave a salt of the slow moving isomer in pure state. The asymmetrical

structure (14) for the thus obtained major and slow moving isomer is apparent by the NMR spectra of its free base, whose benzylic methylenes appeared as a doublet at 3.53 and 3.58 ppm.

The minor and fast moving isomer was identified as a symmetrical amine (13) by TLC comparison with an authentic sample which was prepared from Nereistoxin (1) on reduction with $NaBH_4$ followed by benzylation with benzylchloride. The symmetrical amine (13) showed in NMR spectra a singlet at 3.70 ppm for benzylic methylenes.

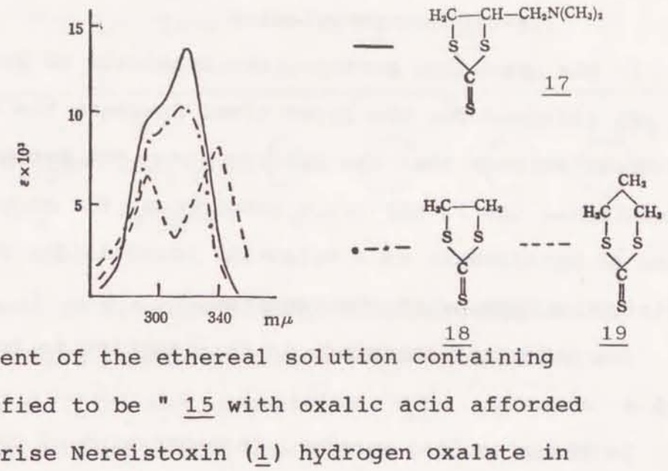
The amine mixture (13+14) was subjected to Birch reduction on treatment with Na in liquid NH_3. The paper partition chormatography (PPC) of the reaction product showed two spots visualized by Kraut-Dragendorff reagent with the one at Rf 0.28 being more intense in color than the other at Rf 0.57. Provided that no rearrangement was intervened during the reduction, the major isomer of Rf 0.28 can be identified as asymmetrical dithiol (14) and the minor one of Rf 0.57 as symmetrical dithiol (15).

When the aqueous solution of the reaction product was shaken with ether, the dithiols were partitioned in two phases with 14 in aqueous phase and 15 in ethereal phase.

The asymmetrical structure of 14 was confirmed by derivatization to 4-dimethylaminomethyl-1,3-dithiolane-2-thione (17), which was obtained on treatment of 14 with CS_2. That the nucleus in 17 is 1,3-dithiolane-

2-thione (18) rather than 1,3-dithiane-2-thione (19) is apparent by the closer resemblance of UV spectrum of 17 to that of 18 rather than 19 as shown in Fig. 2-1.

Fig. 2-1 Ultraviolet Spectra of Some Cyclic Trithiocarbonates



Treatment of the ethereal solution containing "the identified to be " 15 with oxalic acid afforded to our surprise Nereistoxin (1) hydrogen oxalate in 6% yield calculated from amine mixture (13+14). On comparison with natural Nereistoxin hydrogen oxalate, the sample showed identical IR, NMR and UV spectra and no depression of melting point on admixture test. The spontaneous formation of Nereistoxin from 15 without artificial oxidation could be ascribable to the occurrence of air oxidation during the experimental procedures.

Thus the study has completed the synthesis and the structural identification of Nereistoxin, successfully, in spite of the low over-all yield.

It is conceivable that the cause for the low yields resides in the dimethylamination step where the

episulfonium intermediate 12 was formed at the first stage and then dimethylamine attacked 12 preferentially at the less hindered terminal methylene to afford the undesired asymmetrical amine (14) as the major product.

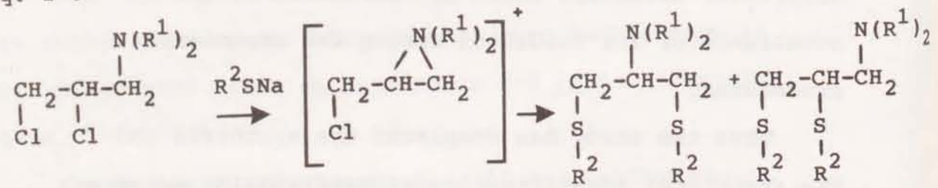
Section 2: Synthesis of Nereistoxin from N,N-Dimethyl-2,3-dichloropropylamine

In the preceding section, the synthesis of Nereistoxin (1) was attained for the first time. However, the over all yield was so poor that the process could not become feasible for the larger scale production. For an evaluation of Nereistoxin as a potential insecticide, a more efficient access to it was required.

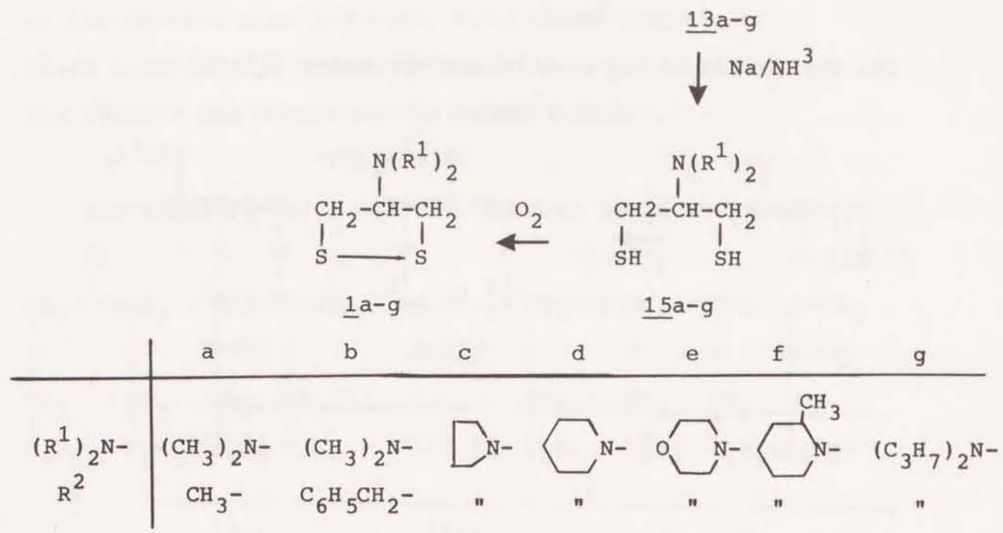
The object of the study in this section is to fine such an access.

In the preceding section, incorporation of two benzylthio groups firstly and dimethylamino group secondly secondly was found to be mechanistically unfavorable for the preparation of the desired symmetrical amine (13). The first motive the author had was "What will happen if the preceding procedure is reversed?".

Eq. 2-6



20a-g 21a-g 13a-g major 14a-g minor



1a-g 15a-g

	a	b	c	d	e	f	g
$(R^1)_2N-$ R^2	$(CH_3)_2N-$ CH_3-	$(CH_3)_2N-$ $C_6H_5CH_2-$	N- "	N- "	O N- "	CH_3 N- "	$(C_3H_7)_2N-$ "

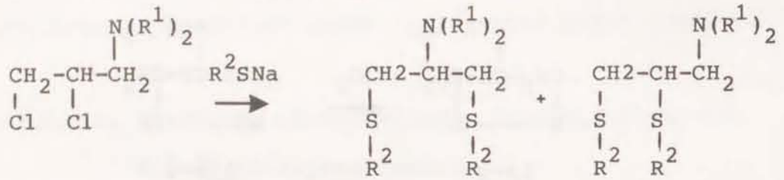
Treatment of N,N-dimethyl 2,3-dichloropropylamine (20a) with sodium benzylmercaptide afforded a mixture of symmetrical (13b) and asymmetrical (14b) amines in 77:23, calculated on the NMR signals. It is reasonably deduced that the thiolation reaction proceeded via a formation of an epi-ammonium intermediate (21a) and the incoming thiols attacked the less hindered terminal methylenes preferentially to afford the symmetrical amine (13b) as the major product.

The Birch reduction of the amine mixture (13b+14b) followed by the air oxidation afforded Nereistoxin (1a) in a 32% over-all yield.

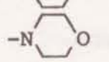
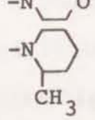
Since the starting material 20a is easily prepared from allylbromide via two steps, i.e. dimethylamination with dimethylamine followed by chlorination, an advanced and economically feasible access to Nereistoxin (1a) was attained in this way.

Table 1

The Thiolation of 2,3-Dichloropropylamines (20a-g) with R^2SNa



20a-g 13a-g 14a-g

	$-N(R^1)_2$	R^2	13 (%)	14 (%)
a	$-N(CH_3)_2$	CH_3-	63[a]	30[a]
b	"	$C_6H_5CH_2-$	77[b]	23[b]
c		"	62[d]	-
d		"	77[c]	-
e		"	59[c]	-
f		"	71[d]	-
g	$-N(C_3H_7)_2$	"	68[d]	-

a) calculated on Gas chromatogram

b) calculated on NMR signals

c) calculated on isolated products

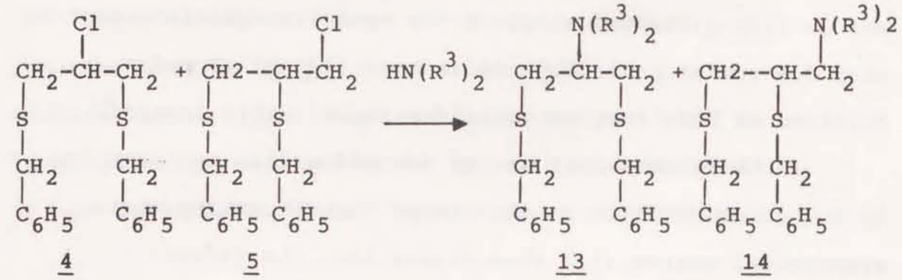
d) calculated on derived sulfone

It should be noted that the thiolation in this section and the amination in the preceding section provide the complementary synthesis of symmetrical (13) and asymmetrical (14) amines, respectively. On variation

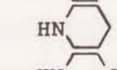
of the substituents involved, i.e. R^1, R^2 and R^3, in these reactions, a number of amines were prepared and the results are summarized in Tables 1 and 2.

Table 2

The Amination of a Chloride Mixture (4+5) with Amines



4 5 13 14

	$HN(R^3)_2$	13 (%)	14 (%)
b	$HN(CH_3)_2$	minor[a]	major[a]
c		-	59[b]
d		-	91[b]
e		-	78[b]
h	$HN(CH_2CH_2OH)_2$	-	65[b]

a) result in the preceding section

b) calculated on the isolated hydrogen oxalates of 14

As seen in the Tables, the bulkier the substituents, the more the more the complementary nature of the reactions becomes apparent. For example, when $(R^1)2N-$ in the thiolation (Table 1) and $(R^3)_2N-$ in the amination (Table 2) are both piperidino and R^2 is benzyl, the thiolation afforded a symmetrical amine (13d) exclusively

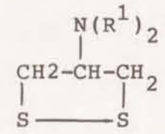
in 77% yield while the amination afforded a corresponding asymmetrical amine (14d) in 91% yield. Both amines were obtained as crystalline products without any difficulties.

The structures of the amines included in the tables were identified by the NMR spectra. The symmetrical amines (13b-g) showed singlets for benzylic methylene protons near 3.8 ppm while asymmetric isomers (14b-h) showed doublets or more complex peaks for them at this region.[6)]

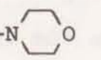
Another convenient way of identification was provided by the empirical rule on thin layer chromatography where symmetrical amines (13) flow faster than the corresponding asymmetrical amines (14), as one example was described in the preceding section.

Table 3

Nereistoxin and its Analogs



1a-f

1	a	c	d	e	f
$-N(R1)_2$	$-N(CH_3)_2$				
mp (°C)	172-174 (oxal.)	192-193 (oxal.)	180-184 (oxal.)	186-189 (oxal.)	87-89 (pic.)
UV_{max}(nm) in H_2O	320	324	321	320	-
e	125	116	134	113	-

On Birch reduction followed by the oxidation with the air, the symmetrical amines (13c-f) were converted into Nereistoxin (1a) and its analogs (1c-f) which showed the UV maximum near 320 nm characteristic of a 1,2-ditholane ring as listed in Table 3.

Thus, taking advantage of the rearrangement reaction from an asymmetrical amine (20b) to a symmetrical amine (13b), Nereistoxin became available in large quantity. This paved the way to the development of new insecticide "Padan" by the other researchers.

Experimental

General — Paper partition chromatography, Toyo Roshi No. 50 BuOH-AcOH-H_2O (4:1:5), asending method. For others see Chapter 1.

1,3-(Bisbenzylthio)-2-propanol (3) — A solution of 1,3-dichloro-2-propanol (2; 64.5 g) in EtOH (200 ml.) was added to a solution of benzylmercaptan (124 g) and sodium (23 g) in EtOH (400 ml) under ice cooling. The mixture was allowed to stand overnight and filtered to remove NaCl. The filtrate was evaporated, the residual oil was dissolved in benzene. The mixture was washed with water and 10% NaOH, dried, evaporated to dryness. The yellow residual oil (132.5 g) was used substantially as the titled compound for the following reaction. The oil was solidified after standing for three months. The solid was recrystallized from ligroin-cyclohexane to give white leaflets (82.8 g), mp 41-45°C.

Anal. Calcd. for $C_{17}H_{20}OS_2$: C; 67.07, H; 6.62, S; 21.05.
Found. C; 67.05, H; 6.52, S; 21.02.

3 was acetylated by the conventional method using acetic anhydride and pyridine to give pale yellowish oil. NMR of acetate of 3 (CDCl3, ppm) ; 2.06 (s, 3H, CH_3CO), 2.64 (d, 4H, 2xC-CH_2S-benzyl), 3.70 (s, 4H, benzylic-CH_2), 3.08 (q, 1H, AcO-CH=).

2-Chloro-1,3-bis(benzylthio)propane (4) and 1-chloro-2,3-bis(benzylthio)propane (5) To a stirred solution of 3 (28.3 g) in benzene (100 ml), $SOCl_2$ (12 g) was added dropwise. The mixture was refluxed for 30 min, and condensed under reduced pressure. The residue was dissolved in benzene and the mixture was washed with water, 10% $NaHCO_3$ and dried. The solvent was removed to give yellowish oil (28 g), which was shown to be a mixture of 4 and 5 by the results of following experiments.

2-Chloro-1,3-bis(benzylsulfonyl)propane (6) and 1-chloro-2,3-bis(benzylsulfonyl)propane (7) To a suspension of above obtained oil (8.2 g) in HCOOH (100 ml), 30% H_2O_2 (14 g) was added under excellent stirring. Afer a while exothermic reaction set in, the temperature rised to about 80°C and the mixture became homogeneous. After the reaction subsided, stirring was continued for 3 hr, and the solvent was removed in vacuo. The residue was poured into ice-water and the precipitated solid was collected by filtration. The solid was suspended in $CHCl_3$ (400 ml) overnight, the insoluble material (1.7 g) was collected by filtration and was recrystallized from

dioxane to give while needles, mp 201-202°C, of 6. The filtrate was evaporated to dryness, the residual solid (4.6 g) was recrystallized from EtOH to give white powders, mp 135-137°C, of 7.
NMR of 6 (d_6-DMF, ppm); 4.30 (s, 4H, 2xbenzylic-CH_2-).
NMR of 7 ($CDCl_3$, ppm); 4.27 and 4.38 (each s, each 2H, 2xbenzylic-CH_2-).
Anal. Calcd. for $C_{17}H_{19}O_4ClS_2$: C; 52.77, H; 4.95, O; 16.54.
Found (6, mp201-202°C): C; 52.50, H; 5.00, O; 16.28.
Found (7, mp135-137°C): C; 52.77, H; 5.07, O; 16.69.

1,3-Bis(benzylsulfonyl)propene (9) a) A mixture of 6 (600 mg), pyridine (20 ml) and water (5 ml) was heated to reflux for 6 hr and evaporated to dryness. To the residual solid, water was added and the solid was collected with suction. The solid was recrystallized from EtOH to afford white leaflets (32o mg), mp177-179°C.
IR (Nujol mull): 1642 and 964 (C=C), 1311 and 1130 (SO_2).
Anal. Calcd. for $C_{17}H_{18}O_4S_2$: C; 58.26, H; 5.18, O; 18.26.
Found. C; 58.58, H; 5.13, O; 18.01.

b) A mixture of 1,3-bis(benzylsulfonyl)-2-propanol (8; 500 mg), $POCl_3$ (1 g) and pyridine (20 ml) was heated at 80°C for an hour. The mixture was poured into ice-water, the precipitated solid was collected by suction and recrystallized from EtOH to afford white leaflets, mp177-179°C. This was identical with the sample obtained above by comparison of IR spectra.
Anal. Found: C; 58.13, H; 5.34.

1,3-Bis(benzylsulfonyl)propane (10) In the pressure bottle of an apparatus for catalytic reduction

were placed 9 (0.6 g), 5% Pd-C (5 g) and dioxane (100 ml) and an initial hydrogen pressure of 3 atm was applied. After 7 hr, the reduction was completed and the contents were transferred to a flask, boiled and filtered. The filtrate was evaporated to dryness and the residual solid was recrystallized from EtOH to give colorless needles, mp207-209°C. Anal. Calcd. for $C_{17}H_{20}O_4S_2$: C; 57.93, H; 5.72. Found: C; 57.94, H; 5.87.

2-Dimethylamino-1,3-bis(benzylthio)propane (13) and N,N-dimethyl-2,3-bis(benzylthio)propylamine (14)

The chloride mixture (4+5) (15 g) was heated with 30% dimethylamine-benzene solution (45 g) in an autoclave at 160°C for 16 hr. The mixture was evaporated in vacuo, the residual oil was taken into Et_2O. The solution was washed with water, dried and condensed to give reddish brown oily mixed amine (13.7 g). On thin layer chromatogram, the oil gave two spots, which were brought out by Karut-Dragendorff reagent after elution with CH_3CN first and then cyclohexane-benzene-CH_3CN on Merck silicagel G plate with the fast moving spot of 13 being weak and the slow moving spot of 14 being more intense.

Hydrogen oxalate of mixed amine (13+14) To a solution of the mixed amine above obtained (4.5 g) in Et_2O (100 ml), was added a saturated solution of oxalic acid in Et_2O (40 ml). The precipitated solid (5.9 g) was collected by filtration and recrystallized from dioxane-Et_2O. There was obtained white leaflets, mp 136-142°C of hydrogen oxalate salt of the mixed

amine.

Anal. Calcd. for $C_{21}H_{27}O_4NS_2$: C; 59.82, H; 6.46, N; 3.32.
Found: C; 59.61, H; 6.58, N; 3.35.

The sample gave two spots on TLC chromatogram performed by the same conditions described in the above experiment.

N,N-Dimethyl-2,3-bis(benzylthio)propylamine (14)

The hydrogen oxalate salt of mixed amine (13+14) above obtained was suspended in boiling $CHCl_3$ (100 ml), the isoluble material was removed by filtration. The filtrate was evaporated to dryness and the solid (1.9 g) was recrystallized from MeOH-Et_2O to give white leaflets, mp145-147°C. The sample showed a single spot corresponding slow moving isomer in the above experiment, which was brought out with Kraut-Dragendorff reagent after elution with MeOH-CH_3OH on Merck silicagel G.

Anal. Calcd. for $C_{21}H_{27}O_4NS_2$: C; 59.82, H; 6.46; N; 3.32.
Found: C; 59.54, H; 6.48; N; 3.25.

The NMR spectrum of the free base was shown in Fig. 2-b.

2-Dimethylamino-1,3-bis(benzylthio)propane (13) hydrogen oxalate from Nereistoxin (1) To a stirred solution of Nereistoxin (1; 740 mg) in MeOH (20 ml) and EtOH (10 ml), $NaBH_4$ (1 g) was added under ice cooling. After 30 min stirring, benzylchloride (8 g) was added dropwise, and the mixture was refluxed for 1 hr. The solvent was removed, the residual oil was taken into Et_2O. To the solution, a saturated ethereal solution of oxalic acid was added until no further precipitation was observed. After standing for 3 hr, the solvent was decanted off. To the residue was added 10% K_2CO_3 (20 ml)

was added and the mixture was shaken with Et_2O. To the organic layer seperated, was added a saturated ethereal solution of oxalic acid and the mixture was allowed to stand over-night. The solid formed was collected with suction and recrystallized from EtOH-Et_2O to give white powders, mp90-93°C (dec.). The sample showed a single spot corresponding to the fast moving isomer of the mixed amine on TLC performed by the same condition described above.

NMR (CCl_4, ppm); 2.13 (s, 6H, $N(CH_3)_2$), 2.26-2.7 (m, 5H, CH_2-CH-CH_2), 3.60 (s, 4H, 2xbenzylic-CH2-).

3-Dimethylamino-1,2-propanedithiol (16) Sodium (1.9 g) and a solution of mixed amine (13+14; 4.6 g) in EtOH (40 ml) were added simultaneously to a stirred mixture of liquid NH_3 (80 ml) and EtOH (40 ml) under dry ice-acetone cooling. After stirring for 30 min, NH_3 was evaporated off at room temperature, EtOH was removed in vacuo and the residue was poured into H_2O. The resulted solution gave two spots on PPC with a spot at Rf 0.28 corresponding 16 being strongly colored and a spot at Rf 0.57 corresponding Nereistoxin (1) being faintly colored with Kraut-Dragendorff reagent. Since 16 was too unstable to be isolated, the solution was used for the following reaction without further purification.

Nereistoxin (1) hydrogen oxalate The above obtained aqueous layer was extracted with Et_2O. To the ethereal extract was added a saturated ether solution of oxalic acid, the precipitated solid (360 mg) was collected and

recrystallized from 95% EtOH. There was thus obtained faintly yellowish needles, mp 173-174°C (dec.).

NMR (D_2O, ppm); 3.00 (s, 6H, $N(CH_3)_2$), 3.53 (d, 4H, CH2-CH-CH_2), 5.60 (q, 1H, -CH=). UV_{max} in H_2O; 320 nm (e 150). The compound did not show depression of melting point on admixture with naturally obtained Nereistoxin (1) hydrogen oxalate. Anal. Calcd. for $C_7H_{13}O_4NS_2$: C; 35.13, H; 5.46, N; 5.85. Found: C; 35.16, H; 5.72, N; 6.08.

4-Dimethylaminomethyl-1,3-dithiolane-2-thione (17)

To a stirred alkaline solution of 16 prepared as described above from 2.6 g of mixed amine (13+14), CS_2 (30 g) was added in one portion. After 30 min, the mixture was extracted with Et_2O and the extract was dried. To the ethereal solution was added a saturated ether solution of oxalic acid. The yellow solid was collected and recrystallized from 50% EtOH to afford the titled compound as yellow leaflets (0.5 g) of mp 187-189°C (dec.). UV_{max} in H_2O: 318 nm (e 14,000). Anal. Calcd. for $C_8H_{13}O_4NS_3$: C; 33.93, H; 4.63; N, 4.94. Found: C; 33.95, H; 4.95, N; 4.94.

1,3-Dithiolane-2-thione (18) A solution of sodium trithiocarbonate in EtOH was prepared by the general method; a solution of Na (2.3 g) in EtOH (50 ml) was saturated with H_2S, CS_2 (5 g) was added to the solution and the mixture was refluxed for 30 min. To the solution was added, a solution of ethylenedibromide (9.5 g) in EtOH (50 ml), and the mixture was allowed to stand overnight. The mixture was taken into water (500 ml)

and extracted with Et_2O. The solvent was removed to afford yellow oil of 18. UV_{max} in EtOH: 318 nm (e 10,600).

1,3-Dithiane-2-thione (19) To a stirred solution of sodium trithiocarbonate prepared as above, was added a solution of trimethylenedibromide (10 g) in EtOH (50 ml). The same procedure as above was repeated to afford yellow solid, which was recrystallized from EtOH to give yellow needles of mp 80°C of 19. UV_{max} in EtOH: 292 nm (e 6,990) and 338 nm (e 8,550). Anal. Calcd. for $C_4H_6S_3$: C; 31.96, H; 4.03, S; 64.00. Found: C; 32.27, H; 4.19, S; 63.55.

N,N-Dialkyl-2,3-dichloropropylamines (20a-g)
These were prepared according to the procedures of Cromwell and Hassner[5] with some modifications.

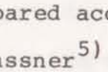
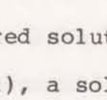
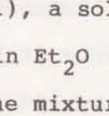
To a stirred solution of allylbromide (60 g, 0.5 mol) in Et_2O (200 ml), a solution of appropriate secondary amine (1 mol) in Et_2O (100 ml) was added dropwise under ice-cooling. The mixture was allowed to stand overnight in a refregerator keeping away the moisture. The precipitate was filtered and the filtrate was treated with dry HCl until no more precipitation occurred. The precipitated allylamine hydrochloride was collected by filtration. The solid material was dissolved in $CHCl_3$ (300 ml), to which Cl_2 was introduced until the calculated weight increase was attained. The mixture was evaporated to dryness to afford oily N,N-dialkyl-2,3-dichloropropyl amine (20) hydrochloride which generally solidified after standing. TLC of the product showed that the compound was homogeneous. For the identification of the new

compounds, they were derived into hydrogen oxalate or picrate salts as listed in Table 4.

Table 4

$CH_2(Cl)-CH(Cl)-CH_2-N(R^1)_2$: Acid

20c-g

20	$-N(R^1)_2$	Acid	mp (°C)	Formula	Analysis Calcd. (Found) C	H	N
c		oxalic	130-131	$C_9H_{15}O_4NCl_2$	39.72 (39.85	5.55 5.59)	- -
e		oxalic	143-145	$C_9H_{15}O_5NCl_2$	37.52 (37.66	5.25 5.36	4.86 4.80)
f		picric	121-123	$C_{15}H_{20}O_7N_4Cl_2$	41.01 (41.25	4.59 4.92	12.75 12.73)
g	$-N(C_3H_7)_2$	oxalic	108-110	$C_{11}H_{21}O_4NCl_2$	43.71 (45.06	7.01 7.44	- -)

2-Dimethylamino-1,3-bis(benzylthio)propane (13b) and N,N-dimethyl-2,3-bis(benzylthio)propylamine (14b) by Eq.2-6
A mixture of N,N-dimethy-2,3-dichloropropylamine (20a) hydrochloride[5] (19.2 g), MeOH (50 ml) and EtOH (150 ml) was added to a solution of KOH (5.6 g) in EtOH (200 ml) under stirring and the KCl deposited was filtered off. To the filtrate was added a solution of sodium benzylmercaptide in EtOH, which was prepared from Na (5.8 g), benzyl mercaptan (31 g) and EtOH (50 ml), and the

mixture was heated to reflux for 2 hr. The deposited NaCl was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in Et_2O, washed with water and dried and evaporated to afford a brown oil (26.4 g). The oil showed two spots on TLC with the faster isomer corresponding 13b being strong in color and the slower isomer corresponding 14b being faint in color with Kraut-Dragendorff reagent. The NMR spectrum was shown in Fig. 2a. The oil was crystallized as hydrogen oxalate salt, which was recrystallized from MeOH to give white powder of mp 90-93°C. Anal. Calcd. for $C_{21}H_{27}O_4NS_2$: C; 59.82, H; 6.46, N; 3.32. Found: C; 59.60, H; 6.47, N; 3.35.

Nereistoxin (1a) hydrogen oxalate by Eq. 2-6

The amine mixture (13b+14b; 8g) above obtained was dissolved in a solution of liquid NH_3 (240 ml) in ether (160 ml) and the reaction vessel was cooled over dry-ice acetone bath. To the stirred mixture was added Li (2.4 g), whereupon the mixture tuned into dark blue. After 20 min, EtOH (24 ml) was added under vigorous stirring and NH_3 was evaporated off in a hood. To the residue H_2O (150 ml) was added and the mixture was extracted with Et_2O. The extract was washed with brine and dried. To the solution was added a saturated ether solution of oxalic acid until no more solid was formed. The yellow solid (2.3 g) was collected with suction and recrystallized from 95% EtOH to afford yellow leaflets of the titled compound, mp 172-174°C, which was identical with a specimen prepared in the preceding section (Y=32%).

2-Dimethylamino-1,3-bis(methylthio)propane (13a)

To the stirred suspension of Nereistoxin (1a) hydrogen oxalate (1g) in 50% MeOH, was added $NaBH_4$ (0.5 g) in MeOH (5 ml) under ice cooling. After 5 min, excess $NaBH_4$ was decompose by drops of CH_3COOH. Keeping the solution alkaline with simultaneous addition of 10% NaOH, Me_2SO_4 (11.6 g) was added dropwise under vigorous stirring during 1 hr. The mixture was extracted with Et_2O and the extract was treated with a ethereal solution of oxalic acid. The solid deposited (200 mg) was collected to afford the titled compound of mp 132°C. The sample was used as the standard for the next experiment. Anal. Calcd. for $C_9H_{10}O_4NS_2$: C; 40.13, H; 7.11. Found: C; 39.64, H; 7.21.

2-Dimethylamino-1,3-bis(methylthio)propane (13a) hydrogen oxalate and N,N-dimethyl-2,3-bis(methylthio) propylamine (14a) by Eq. 2-6

To a solution of KOH (2.1 g) in EtOH (100 ml) was added N,N-dimethyl-2,3-dichloropropylamine (20a) hydrochloride (7g) and KCl deposited was filtered off. To the filtrate was added sodium methylmercaptide in EtOH solution, which was prepared from Na (3.3 g) and methylmercaptan (6.9 g) and EtOH (50 ml), and the mixture was heated to reflux for 2 hr. After standing overnight, NaCl deposited was filtered off and the filtrate was acidified with ethanolic HCl and the solvent was removed in vacuo. The oily residue was dissolved in H_2O (150 ml), and the solution was made alkaline with K_2CO_3 and extracted with Et_2O. To the

solution was added a saturated ether solution of oxalic acid until no more solid was formed. The solid (6.7 g) was collected and recrystallized from MeOH to give white powder of mp 122-123°C. The powder showed 2 spots on TLC with the first moving isomer identical with 13a above obtained being intense in color and the slow moving isomer corresponding 14a being faint in color. The exact coposition of isomers was calculated on Gas Chromatogram of the free base , which is listed in Table 1.

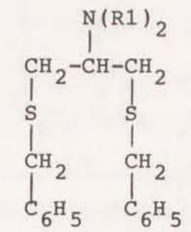
TLC conditions: absorbant; Merck 100 mesh silicagel+ Mallinkrodt 100 mesh silica gel (9:1), developer: MeOH. 13a showed a spot at Rf 0.35 and 14a at Rf 0.18, both of which were visuallized by Kraut-Dragendorff reagent. Anal. Calcd. for $C_9H_{19}O_4NS_2$: C; 40.13, H; 7.11, N; 5.20. Found: C; 40.23, H; 7.39, N; 5.02.

2-N,N-Dialkylamino-1,3-bis(benzylthio)propane (13c-d) by Eq. 2-6

A mixture of N,N-dialkyl-2,3-dichloropropylamine (20c-d) hydrochloride (1 mol), MeOH (50 ml) and EtOH (150 ml) was added to a stirred solution of KOH (5.6 g) in EtOH (200 ml) and KCl was filtered off. To the filtrate was added a solution of sodium benzylmercaptide in EtOH which was made from Na (5.8 g), benzylmercaptan (31 g) and EtOH (50 ml) and the mixture was heated to reflux for 2 hr. The NaCl formed was filtered and the filtrate was evaporated to dryness. The residue was dissolved in Et_2O and the solution was washed and dried. The solvent was removed in vacuo to afford brown oil which solidified

after standing. In cases of oily amines, they were converted into the corresponding sulfones for the identification and the calculation of the yields.

Table 5



13c,e

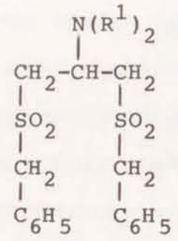
13	$-N(R^1)_2$	mp (°C)	Formula	Anal. Calcd. (Found) C	H	N
c		52-54	$C_{22}H_{29}NS_2$	71.11	7.87	3.77
				(70.95	7.64	3.43)
e		81-84	$C_{21}H_{27}ONS_2$	67.52	7.29	3.75
				(67.48	7.17	3.58)

2-N,N-Dialkylamino-1,3-bis(benzylsulfonyl)propane for identification and calculation of the yields

To the suspension of the above obtained amines (13c,f,g) (0.03 mol) in HCOOH (100 ml), 30% H_2O_2 (14 g) was added under vigorous stirring. After a while an exothermic reaction set in, the temperature rised to about 80°C and the mixture became homogeneous. After the reaction subsided, the stirring was continued for 3 hr. After removal of the solvent in vacuo, the residue was poured

into ice-water and the mixture was made alkaline with 10% K_2CO_3. The precipitated solid was collected and recrystallized from EtOH to give the sulfones.

Table 6



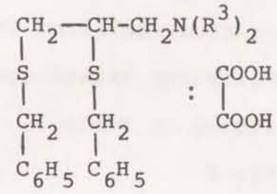
$-N(R^1)_2$	mp (°C)	Formula	Analysis Calcd. (Found) C	H
	151-153	$C_{21}H_{27}O_4NS_2$	59.83	6.46
			(59.94	6.31)
	188-189	$C_{23}H_{31}H4^{NS}{}_2$	61.44	6.95
			(61.40	6.94)
$-N(C_3H_7)_2$	146-148	$C_{23}H_{33}O_4NS_2$	61.16	7.36
			(61.41	7.25)

N,N-Dialkyl-2,3-bis(benzylthio)propylamine (14c,d,e,h) (Table 2)

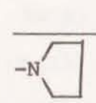
The chloride mixture (4+5) (15 g) was heated with secondary amine (20 g) and benzene (40 ml) at 160°C for 16 hr in an autoclave. The excess amine and solvent were removed in vacuo and the residue was dissolved in benzene and the solution was washed and dried. The solvent was removed to afford a brown oil of 13c,d,e,h. The amines were crystallized as the hydrogen oxalate salts and the yields listed in Table 2 were calculated

on the isolated oxalate salts.

Table 7



14

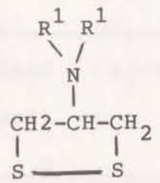
14	$-N(R^3)_2$	mp(°C)	Formula	Analysis Calcd (Found) C	H	N
c		135-137	$C_{23}H_{29}O_4NS_2$	61.72	6.53	3.13
				(61.76	6.43	2.93)
d		129-132	$C_{24}H_{31}O_4NS_2$	62.44	6.77	3.04
				(62.47	6.95	3.04)
e		105-109	$C_{23}H_{29}O_5NS_2$	59.60	6.31	3.02
				(59.67	6.36	2.80)
h	$-N(CH_2CH_2OH)_2$	125-128	$C_{23}H_{31}O_6NS_2$	57.36	6.49	2.91
				(57.09	6.71	2.85)

4-N,N-Dialkylamino-1,2-dithiolane (1c-f) (Table 3)

The amine (13c-f) (8 g) was dissolved in liquid NH_3 (240 ml) and ether (160 ml) and the vessel was cooled over dry ice acetone bath. To the stirred solution Li (2.4 g) was added, whereupon the mixture turned into dark blue. After 20 min, EtOH (24 ml) was added, whereby the color vanished and NH_3 was evaporated off in a hood. The residue was dissolved in H_2O (150 ml) and the mixture was extracted with Et_2O. The air was introduced into the aqueous layer under ice-cooling for 40 min. The aqueous solution was extracted with Et_2O. To the

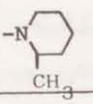
combined ethereal extracts, was added a saturated solution of oxalic acid in Et_2O until no more solid was formed. The solid was recrystallized from 95% EtOH to afford yellow leaflets of the titled compounds. Physical constants are listed in Table 3.

Table 8



:Acid

1c-f

1	$-N(R^1)_2$	Acid	Formula	Analysis Calcd. (Found) C	H
c		oxalic	$C_9H_{15}O_4NS_2$	40.74 (41.21	5.70 5.79)
d		oxalic	$C_{10}H_{17}O_4NS2$	42.99 (43.23	6.13 6.24)
e		oxalic	$C_9H_{15}O_5NS_2$	38.42 (38.20	5.37 5.55)
f		oxalic	$C_{15}H_{20}O_7N_4S_2$	41.66 (41.82	4.64 5.28)

References

1) S. Nitta, Yakugaku Zasshi, 54, 648 (1934)

2) T. Okaichi and Y. Hashimoto, Agr. Biol. Chem., 26, 224 (1962)

3) N.S. Johary and L.N. Owen, J. Chem. Soc., 1955, 1302

4) P.S. Fitt and L.N. Owen, J. Chem. Soc., 1957, 2251

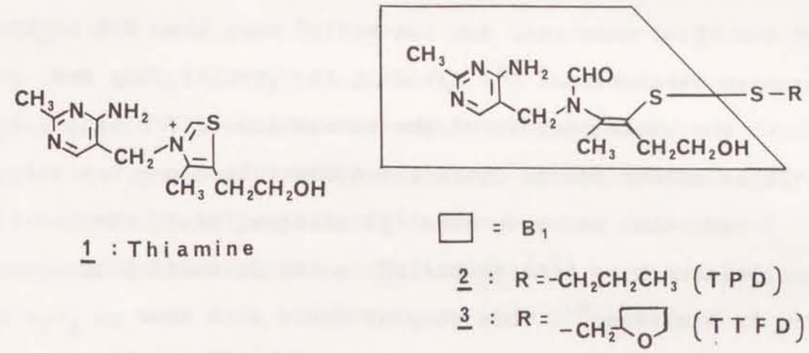
5) N.H. Cromwell and A. Hassner, J. Am. Chem. Soc., 77, 1568 (1955)

6) Y. Asahi, M. Numata and E. Mizuta, Chem. Pharm. Bull., 21, 112 (1973)

CHAPTER 3

STUDIES ON THE METABOLITES OF THIAMINE PROPYL DISULFIDE (TPD) AND THIAMINE TETRAHYDROFURFURYL DISULFIDE (TTFD)

Thiamine propyl disulfide (2; TPD)[1)] and thiamine tetrahydrofurfuryl disulfide (3; TTFD)[1e)] are mixed disulfide consisting of thiol type thiamine (circled and abbreviated as B_1) and alkanethiols. On oral administration to mammals, they are absorbed through intestine into the body quite effectively, where they are cleaved into thiamine (1) and alkanethiols by the action of thiol-peptides such as glutathione.



1 : Thiamine

2 : R = $-CH_2CH_2CH_3$ (TPD)

3 : R = $-CH_2$-(O) (TTFD)

Since thiamine (1), an essential co-factor for various biochemical transformations in the body, is absorbed through intestine into the body only in a limited amount[2)], TPD and TTFD are good transport form of thiamine and thus could be defined as "pro-drugs" of thiamine. They were first synthesized by T. Matsukawa and his co-workers[1)] in these laboratories and on account of these merits they have been on sale from Takeda chemical industries company since 1954 and 1961, respectively.

The alkanethiols, i.e. propanethiol for TPD and 2-tetrahydrofuranemethanethiol for TTFD, confer TPD and TTFD lipophilicity suitable for penetration through

intestinal wall, but the role terminates when thiamine is successfully transported into the body. Thus alkane-thiol moieties are defined as facilitating disposable moieties by E.J. Ariens[3]. Nevertheless, their fates or metabolism after absorption is an important problem in view of the safety of the drugs as a whole.

To clarify the problem, Z. Suzuoki and his co-workers including the author scrutinized urine of the rats which were dosed with radio active TPD and TTFD, S^{35} labelled at the thiol moieties, and identified more than 90% of the urinary metabolites[4)5)]. Based on the results they set forth the whole picture of the metabolism of TPD and TTFD as summarized in Chart 1 and Chart 2.

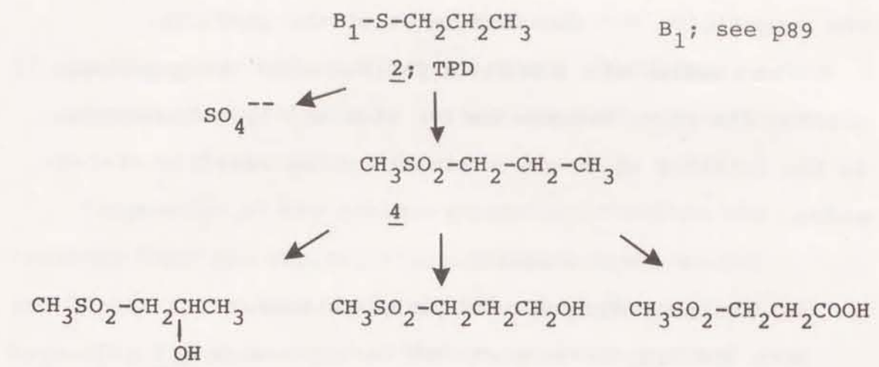
Isolation and structural identification of the metabolites have been described in the dissertion of Dr. K. Nishikawa[6]. This chapter deals with the synthesis of authentic samples of metabolites, which was carried out by the responsibility of the author, and with the chemical phenomena encountered during the synthesis.

Section 1 Mechanism for the Oxidation-Acetylation of Hydroxysulfides Encountered in the Synthesis of Metabolites of Thiamine Propyl Disulfide (TPD)

The metabolism of TPD is summarized in Chart 1. The metabolites of its propane thiol part except for inorganic sulfate are the products of methylation followed by the oxidation.

The synthesis of these metabolites was straightforward.

Chart 1. Metabolism of TPD in Rats



Among them methyl propyl sulfone (4) and 3-methylsulfonyl-propionic acid (7) were known compounds. The other two unregistered compounds, 2-hydroxypropyl methyl sulfone (5) and 3-methylsulfonylpropanol (6) were prepared from the corresponding known sulfides (5s and 6s)[7,8)] by oxidation with H_2O_2 in AcOH.

A sole problem encountered in the synthesis was formation of an acetylated by-product. An equal amount of acetate of 6 (6a) was formed along with the production of 6. While no acetylation was observed on the production of 5.

A similar reaction to obtain an acetylated sulfone from a hydroxy containing sulfide has been found and designated as the oxidation-acetylation by Clingman and Richtmeyer[9], but no explanation has been given to the mechanism of the reaction. Hence, our interest was shifted to obtaining an explanation for the mechanism.

With the aim of obtaining some clue to the mechanism, we attempted to oxidize several hydroxysulfides (5s to 11s)

under the same condition as described above and determined the composition and the structures of the products.

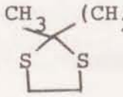
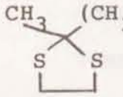
The results are listed in Table 1 with the possible minimum distances between sulfur atom and hydroxy oxygen in the sulfides which are estimated using Dreiding stereo-model.

Table 1

Oxidation of Hydroxysulfides to Sulfones with 30% H_2O_2 in AcOH at 70°C

$-S-C_n-OH \longrightarrow -SO_2-C_n-OH + -SO_2-C_n-OAc$

5s-11s 5-11 5a-11a

Starting Sulfide	Hydroxy Sulfone (%)	Acetylated Sulfone (%)	Reaction time hr.	Minimum distance between S and O in Å
$CH_3S(CH_2)_2OH$ 8s	100[b]	0 [b]	0.5	2.60
$CH_3SCH_2\underset{\underset{OH}{|}}{C}HCH_3$ 5s	100[b]	0 [b]	0.5	2.60
$CH_3S(CH_2)_3OH$ 6s	50[c]	50[c]	0.5	1.60
$CH_3S(CH_2)_4OH$ 9s	59[c]	41[c]	0.5	0.20
 (CH2)2OH 10s	64[c]	36[c]	1.5	1.68
 $(CH_2)_3OH$ 11s	30[d]	70[d]	1.0	0.20
	0 [b,e]	100[b,e]		

a) 0.01 mol of sulfide was treated with 0.026 mol 30% H_2O_2 in 10 ml of AcOH at 70°C until the sulfide was disappeared

b) based on TLC evidence

c) calculated on IR intensity of carbonyl

d) calculated on isolated materials

e) 4 times of volumes of AcOH was applied

Inspection of the table apparently indicates the tendency that the shorter the distance between sulfur and oxygen the higher the yield of acetylation. Typically 11s whose hydroxy oxygen could be located near the sulfur atom more closely than hyroxygroups of other sulfides, afforded the highest yield (70%) of acetylated sulfone (11a). The results strongly suggested that in the oxidation of the sulfur there was an neighbouring group participation towords the hydroxygroup.

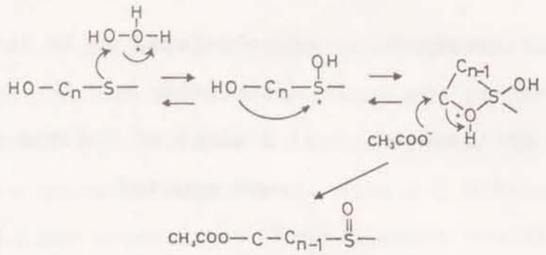
When 11s was oxidized with 30% H_2O_2 in a 4 times volume of acetic acid, the yield of acetylated sulfone (11a) was increased to a quantitative one. Thus, it was apparent that the neighbouring group participation as well as competetion between hydroxy anion and acetate anion were responsible for the product composition.

Taking into account that the mechanism of the oxidation of sulfides to sulfoxides and sulfones is generally accepted to be ionic[10], the present oxidation-acetylation would be most reasonably accounted for by the mechanism as depicted in Fig. 1.

Fig. 1

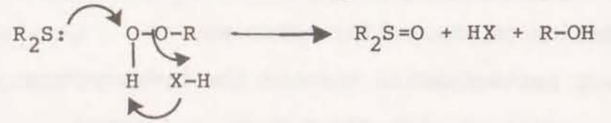
Mechanism for the Oxidation-Acetylation of Hydroxysulfides

$CH_3COOH + HOOH \rightleftharpoons CH_3COO^- + H-\overset{H}{\underset{|}{O}}-\overset{+}{O}-H$



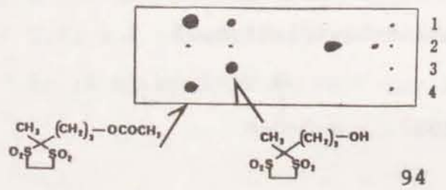
In this regard, it should be mentioned that Barnard et al.[10] preferred a concerted mechanism as formulated below to a protonation mechanism for the oxidation of sulfides. A number of reports which appeared thereafter adopted the concerted mechanism rather than the protonation mechanism.

Concerted Mechanism by Barnard et al.[10]



However our experiment demonstrated that the protonation mechanism appears more plausible for the oxidation-acetylation. As a supporting evidence to this mechanism, a marked retardation of the oxidation rate was observed by addition of sodim acetate to the reaction medium as is apparently visualized by TLC in Fig. 2. The retardation can not be explained by the concerted mechanism.

Fig. 2 Thin Layer Chromatogram of the Oxidation of a Hydroxysulfide (11s)



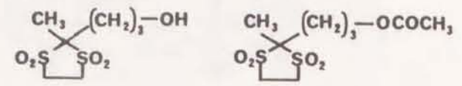
11a 11

1: 11s treated with 30% H_2O_2 at 70°C for 1 hr.

2: 11s treated with 30% H_2O_2-AcOH-AcONa at 70°C for 1 hr.

3: Authentic 11

4: Authentic 11a



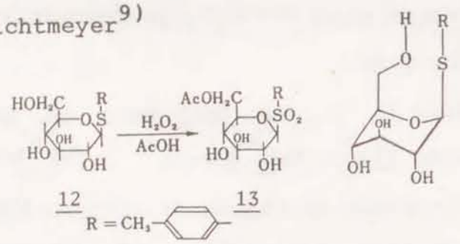
11 11a

These results would be reasonably explicable on the fact that the present oxidation was carried out in a large excess of an acid, in which a protonation mechanism could possibly overweigh a concerted mechanism.

The possibility of intervention of peracids in the reaction would be ruled out from the fact that the rate of formation of a peracid from the parent acid and H_2O_2 under the present condition is much slow[11].

Clingman and Richtmeyer[9] carried out the oxidation of thioglucoside (12) with H_2O_2 in acetic acid and found that 6-O-acetyl sulfone (13) was obtained as a sole product. Although they recognized that the acetylation could have occurred in the oxidation, they made no further investigation. Since the Dreiding stereo model of 12 clearly shows that the hydroxy at C-6 can take a closer position than the other hydroxy groups in the molecule (the minimum distance between S and O is estimated 0.80 Å), it would be pertinent to assume that the reaction would have proceeded by essentially the same mechanism as in the present oxidation-acetylation.

Eq. 3-1 Oxidation-Acetylation Found by Clingman and Richtmeyer[9)]

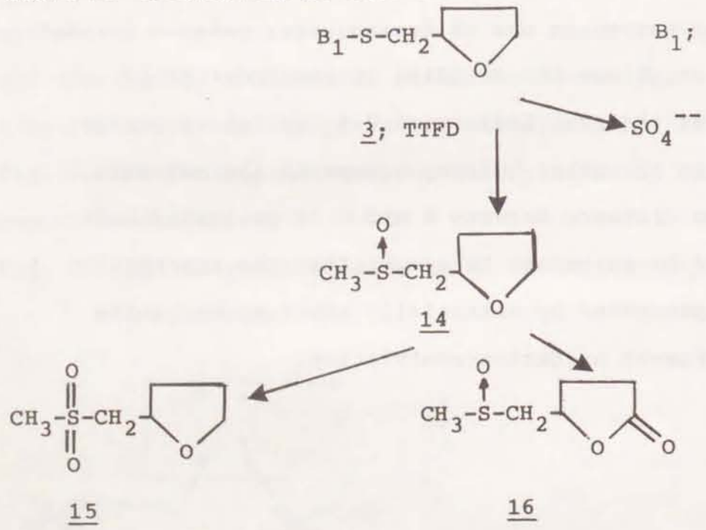


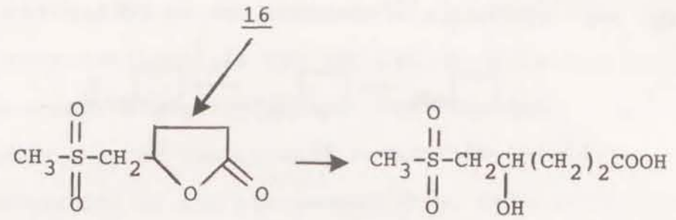
12 R = CH_3- 13

Section 2 Synthesis of Metabolites of Thiamine Tetrahydrofurfuryl Disulfide (TTFD) and Stereochemistry of (-)-Methyl Tetrahydrofurfuryl Sulfoxide Isolated from Rat's Urine

The metabolism of Thiamine tetrahydrofurfuryl disulfide (3; TTFD) is summarized as Chart 2. Metabolites of disposable moiety or tetrahydrofuranemethanethiol moiety were products of methylation followed by oxidation at the sulfur and at the 5 position in the tetrahydrofurane ring and inorganic sulfate.

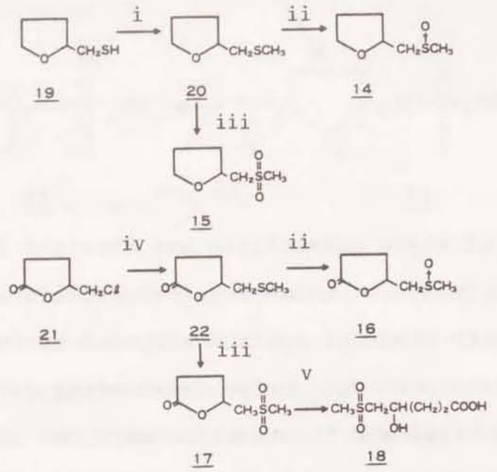
Chart 2. Metabolism of TTFD in Rats





Synthesis of these metabolites was straight forward too (Eq. 3-2). Methylation of known 2-tetrahydrofuranemethanethiol (19)[12)] with dimethyl sulfate afforded methyl sulfide (20), which on oxidation with H_2O_2 under ice-cooling gave methyl sulfoxide (14) and on oxidation with the same oxidant under heating in acetic acid gave methyl sulfone (15). Treatment of known 6-chloromethyldihydro-2(3H)-furanone (21)[13)] with sodium methanethiol afforded 5-methyl-thiomethyldihydro-2(3H)-furanone (22), which on oxidation with H_2O_2 under ice-cooling and under heating afforded corresponding methyl sulfoxide (16) and methyl sulfone (17), respectively. Hydrolysis of the latter 17 with sodium hydroxide followed by acidification of the reaction mixture with HCl and extraction of the mixture with AcOEt and recrystallization of the solid extract from $CHCl_3$ afforded 4-hydroxy-5-methylsulfonylvaleric acid (18). All of these synthetic metabolites showed IR and NMR spectra and TLC flow-rates completely identical with those of corresponding metabolites. The results confirmed the structural identification of the metabolites of TTFD performed by Suzuoki et al.[5)]

Eq. 3-2 Synthesis of metabolites of TTFD



i; $(CH_3)_2SO_4$-KOH, ii; 30% H_2O_2/AcOH at ca. 0°C,
iii; 30% H_2O_2/AcOH at 70°C , iv; CH_3SNa/ CH_3OH,
v; 0.5N-NaOH at 70°C

Only one discrepancy existed between the urinary metabolites of the rats and the corresponding synthetic samples was that an urinary metabolite methyl sulfoxide (14) was levo rotatory[α]$_D$ -60.2° (c=0.77, $CHCl_3$) while corresponding synthetic material was optically inactive.

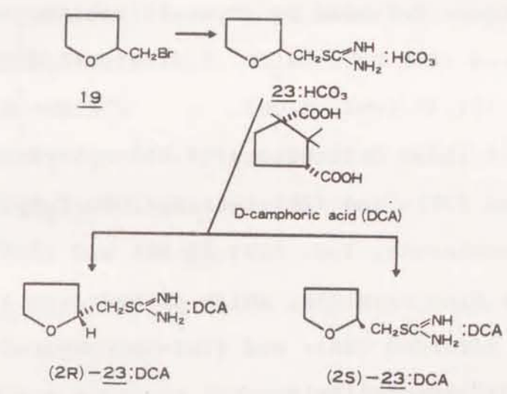
Since of the two asymmetric centers of 14, C_2 and sulfur, C_2 was originally racemic in TTFD, it was assumed that the sulfur asymmetric center which was formed during the metabolism could be chiral as a result of stereospecific biochemical oxidation at the sulfur. In facts, other metabolites 15, 17 and 18 which have no asymmetry at the sulfur were optically inactive. The optical activity of 16 was not checked due to lack of material quantity.

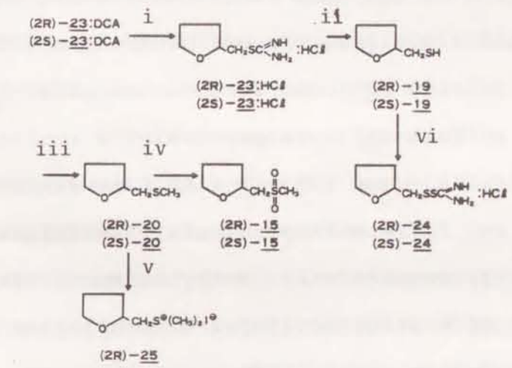
At that time a number of examples of asymmetric

oxidation of sulfides by micro-organisms[14], plants[15] and a worm[16] were available in the literature while that by mammals was not. Hence my interest was directed to the stereochemistry of the urinary metabolite (-)-14.

For clarification of the stereochemistry, the author intended to prepare diastereoisomers of 14 and to investigate the chirality of them.

Eq. 3-3 Chiral Synthesis of Metabolites and Related Compounds





i; c-HCl, ii; $NaHCO_3$ at 90-100°C, iii; $(CH_3)_2SO_4$-KOH,
iv; 30% H_2O_2/AcOH at 70°C, v; CH_3I, vi; 30%H_2O_2-thiourea-c-HCl

Treatment of dl-tetrahydrofurfuryl bromide (19) with thiourea and then with sodium hydrogencarbonate afforded dl-tetrahydrofurfurylthiuronium hydrogencarbonate (23:HCO_3) as white solids. On admixture of the solid with D-camphoric acid (DCA) in water, (2R)-tetrahydrofurfurylthiuronium D-camphorate ((2R)-23:DCA) was precipitated from the solution which was on repeated crystallizations from 50% EtOH to give the sample with constant rotation $[\alpha]_D$ -12.6° (c=1, MeOH). Condensation of the mother liquor followed by crystallizations of the solid resulted from MeOH-CH_3CN (1:4) afforded (2S)-23:DCA with $[\alpha]_D$ +37.3° (c=1, MeOH).

Treatment of these diastereomeric D-camphorates with HCl afforded (2R)- and (2S)-tetrahydrofurfuryl-thiuronium hydrochloride, i.e. (2R)-23:HCl and (2S)-23:HCl, respectively, as fine crystals, which on hydrolysis with aq. $NaHCO_3$ afforded (2R)- and (2S)-tetrahydrofuranemethanethiol (19), respectively.

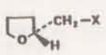
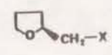
By application of the same reactions formulated in Eq. 3-2, (2R)- and (2S)-19 were transformed into (2R)- and (2S)-methyl sulfide (20) and further into (2R)- and (2S)-methyl sulfone (15), respectively.

Treatment of (2R)- and (2S)-19 with thiourea and H_2O_2 gave (2R)- and (2S)-tetrahydrofurfurylthiothiuronium hydrochloride (24), respectively. Methylation of (2R)-20 with methyl iodide afforded (2R)-2-dimethylthionium-methyl-tetrahydrofurane iodide (25).

The optical rotations of these compounds are listed in Table 2.

Table 2

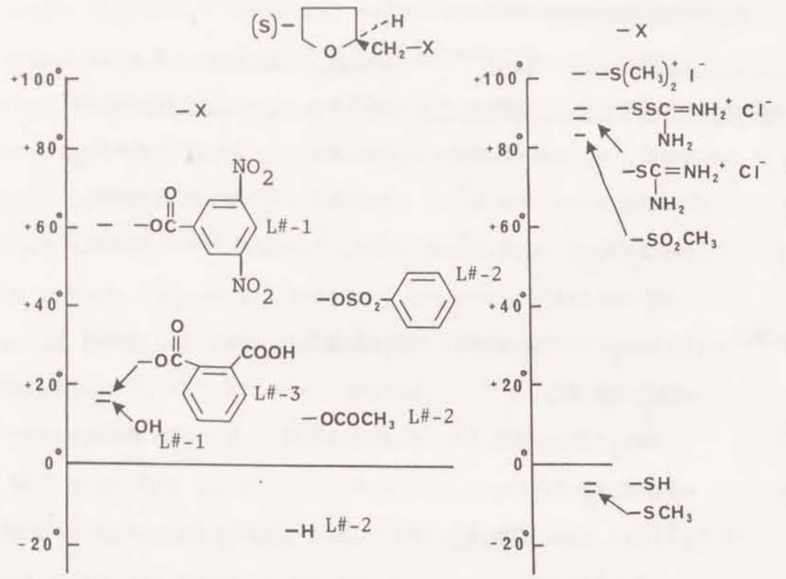
Optical Rotations of (2R)- and (2S)-2'-Substituted 2-methyltetrahydrofurane Derivatives at 589 nm

compound	X	 (2R)	 (2S)	c	solvent
19	-SH	+4.1°	-4.4°	1	$CHCl_3$
20	$-SCH_3$	+5.1°	-5.1°	1	MeOH
15	$-SO_2CH_3$	-50.5°	+50.3°	1	MeOH
23:HCl	$-SC(=NH:HCl)NH_2$	-39.5°	+40.9°	1	MeOH
24	$-SSC(=NH:HCl)NH_2$	-38.4°	+39.1°	1	MeOH
25	$-S(CH_3)_2\ I^-$	-32.4°			

Since the absolute figures of the rotations of each enatiomeric pairs were within the experimental errors of the measurement, it was thought that the optical resolution of 23 using D-camphoric acid and the following transformations afforded the enantiomerically pure compounds which were satisfactory enough for the following investigation on the stereochemistry.

The assignment of their C_2-configurations was performed by comparison of rotations with those of some authentic compounds using Freudenberg's "rule of shift"[17] for the optical rotations of carbon compounds.

Fig. 3 Molecular Rotations of (2S)-2'-Substituted 2-methyltetrahydrofuranes at 589 nm



L#; cited from literatures, 1) F.C. Hartman, R. Barker, J. Org. Chem., 29, 873 (1964), 2) only sign was reported, D. Gagnaire, A. Butt, Bull. Soc. Chim. Fr., 1961, 312, 3)M.P. Balfe et al., J.Chem. Soc., 1941, 312

In Fig. 3 molecular rotations of (2S)-2'-substituted-2-methyltetrahydrofuranes prepared in this report are compared with those of authentic closely related compounds haveng 2S configuration. As is apparent by the Fig., when electron whithdrawing group is introduced into the 2'-position of (2S)-2-methyltetrahydrofurane, a more positive molecular rotation was exhibited by the compound.

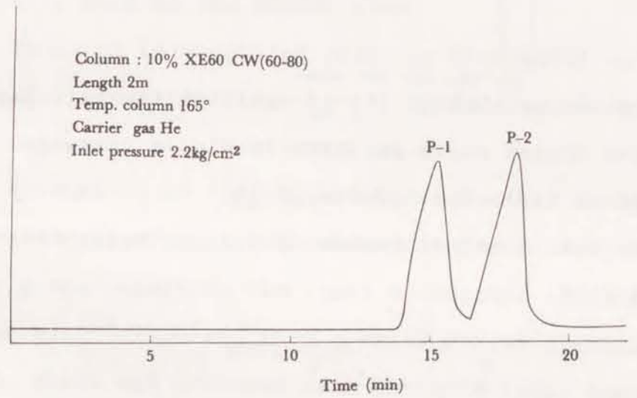
Freudenberg's "rule of shift"[17] states that a similar change in the asymmetric center causes a similar change in the optical rotatory powers of different but similarly

constituted dissymmetric compounds.

Since both series of compounds included in Fig. 3 show shifts of molecular rotations to the same direction as the 2'-substituent increases electronegativity, both series of compounds seem to have the same configuration i.e. (2S).

Oxidation of (2R)-20 by the same procedure as described for dl-20 to dl-14 and gas chromatographic seperation of the diastereomeric mixtures afforded (2R, S-S)-14 of $[\alpha]_D$ +11.4° (c=1, $CHCl_3$) and (2R, S-R)-14 of $[\alpha]_D$ -120.2° (c=1, $CHCl_3$), each of which showed a single peak corresponding P-1 and P-2, respectively, depicted in Fig. 4.

Fig. 4 Seperation of Diastereomers of Methyl Tetrahydrofurfuryl Sulfoxide (14) by Gas Chromatography

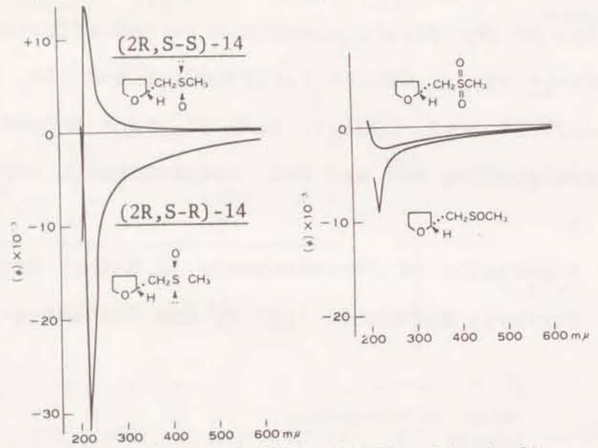


They exhibited Opically Rotatory Dispersion Curves (ORD) with opposite signs of Cotton effects as shown in Fig. 5.

The configurations of the sulfoxide group in these compounds were assigned by application of Mislow's empirical rule[18] which states that (R)- and (S)-sulfoxides

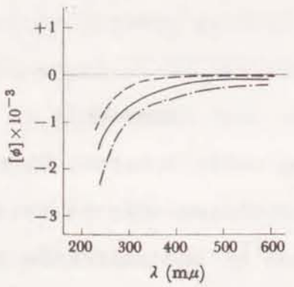
having the formula CH_3-SO-CH_2-R exhibit negative and positive Cotton curves, respectively. He also showed that the contribution of sulfur asymmetry to optical rotation is so strong that it overweighs the contribution of carbon asymmetry, if there is, and desides the sign of Cotton effect of the whole molecule.

Fig.5 Optical Rotatory Dispersion Curves of (2R, S-S)- and (2R, S-R)-Methyl tetrahydrofurfuryl sulfoxides (14)



Since the metabolite (-)-14 exhibits the tail part of negative Cotton curve as shown in Fig. 6, it was identified as (2dl, S-R) isomer of 14.

Fig. 6 Optical Rotatory Curves of Urinary Metabolite (-)-14



——: metabolite ------ : first moving isomer

•-•-•- : slow moving isomer

17)

When van't Hoff's "rule of superposition" that states asymmetric centers in a molecule make independent contributions to the total molecular rotation is applied on the rotations of (2R, S-S)- 14 and (2R, S-R)-14, Eq. 3-3 could be formulated: where (C_2) and (S) are contributions of C_2 and sulfur asymmetry, respectively.

Eq. 3-3

+ 11.4° (rotation of (2R, S-S)-14 at D line)=-(C_2)+(S)

-120.2° (rotation of (2R, S-R)-14 at D line)=-(C_2)-(S)

(S)=11.4°+120.2°/2

Thus, the contribution of sulfur asymmetry to the rotation of an enantiomer of 14 under D-line is calculated to be 65.8°. And it follows that the urinary metabolite (-)-14 of rats with $[\alpha]_D$ -60.2° is calculated to be 91.5% optically pure at the sulfur atom.

Thus, it is concluded that the biological oxidation of sulfur atom affording (-)-14 in rats was highly stereospecific with R-orientation.

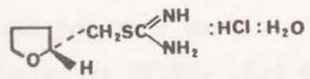
Suzuoki et al.[19] showed that metabolic methylation and oxidation of the disposable moieties of TPD and TTFD takes place mainly in the liber microsomes in rats. Oae et al.[20] reported that a multi enzyme system, P-450, which was obtained from rabbit's liber has an oxidizing ability of various sulfides into sulfoxides with varied stereospecificities. They also showed that the enzyme system conform complexes with the substrate sulfides with so strong force that only a minor part

of oxygen was incorporated from solvent water into the sulfoxides.

Taking into these results into consideration, it is highly probable that the stereospecificity of the metabolite (-)-14 was incorporated from the oxidizing enzyme located in the rat's liber.

X-Ray Crystallographic Analysis Results

X-ray crystallographic analysis of (2R)-(-)-tetrahydrofurfurylthiuronium chloride monohydrate (23: HCl: H_2O) was performed by M. Takamoto and the results are summarized in Fig. 7-9 and Table 3. All of the results confirm the configurational assignment of C_2-asymmetry described above.



23 : HCl : H_2O

Fig. 7 Stereoscopic View of (2R)-(-)-Tetrahydrofurfurylthiuronium chloride monohydrate (23: HCl: H_2O)

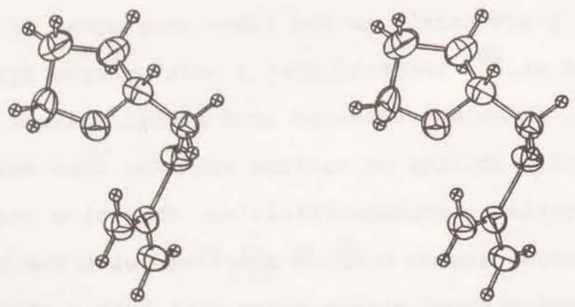
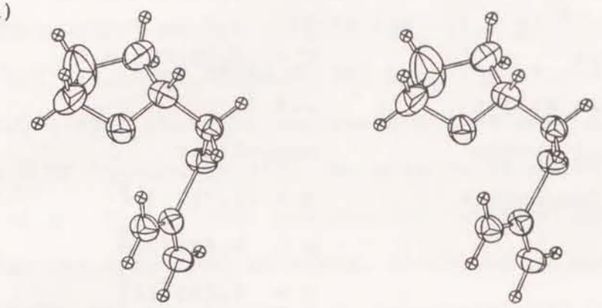


Fig. 8 Stereoscopic views of (a); the other conformer and (b); the molecular arrangement in the unit cell as seen normal to the a-b plane

(a)



(b)

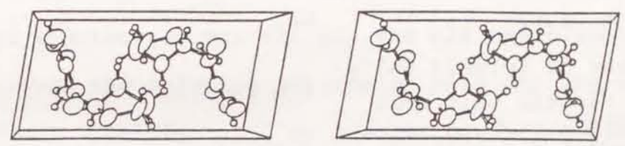
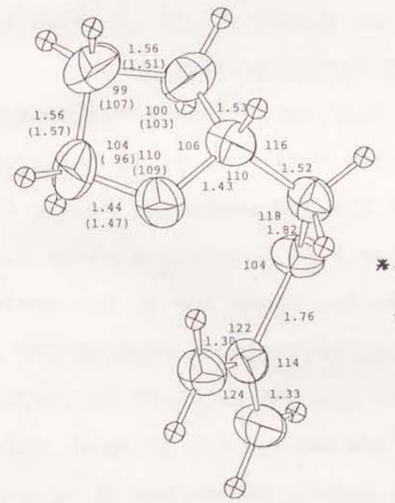


Fig. 9 Bond distances(Å) and Angles(°) for nonhydrogen atoms



*values in parenthes are those of the other conformer

Table 3

Crystal Data

Formula	$C_6H_{12}N_2OS.HCl.H_2O$
Formula Weight	214.7
Crystal System	monoclinic
Cell Dimensions	a = 10.792(3)Å
	b = 9.431(2)Å
	c = 5.343(1)Å
	β = 99.90(4)°
Cell Volume	535.8(2)Å^3
Space Group	$P2_1$
Number of Formula Units in the Unit Cell	Z = 2
Calculated Density	1.331g/cm^3

Experimental

2-Hydroxypropyl methyl sulfone (5) To a solution of 2-hydroxypropyl methyl sulfide (5s; 21.2 g)[7] in AcOH (100 ml) was added dropwise 30% H_2O_2 (50 g) under stirring at such a rate that the temperature does not exceed 70°C. After 2 hr heating at 70°C the mixture revealed a single spot of 5 . The solvent was removed in vacuo and the residue was dissolved in water. The solution made alkaline with K_2CO_3 and the excess H_2O_2 was dicomposed with Na_2SO_3. After condensation of the solution, the residue was extracted with acetone and the extract was evaporated to afford a colorless oil (26 g). The oil was dissolved in $CHCl_3$, and the solution was cooled with dry ice-acetone, whereupon 5 precipitated as a white powdery solid, 18.2 g (66%), mp 47-51°C. IR and NMR spectra were identical with those of metabolites[4]. Anal. Calcd. for $C_4H_{10}O_3S$: C; 34.77, H; 7.30, O; 34.73. Found. C; 34.49, H; 7.16, O; 34.25.

3-Methylsulfonylpropanol (6) and its acetate (6a) 3-Methylpropanol[8] (6s; 21.2 g) was oxidized with 30% H_2O_2 (50 g) in AcOH (100 ml) in the same way as above to give a colorless oil (22.8 g). TLC of the oil revealed two spots corresponding 6 and its acetate. The oil was dissolved in hot AcOEt and the solution was cooled slowly , whereupon 6 precipitated as colorless rods, 10.5 g (38%), mp 32-37°C. NMR and IR spectra were identical with those of the metabolite[4]. Silica gel column chromatography of the mother liquor developed by bezene-acetone

afforded the acetate of 6 (6a) as an oil.

Anal. Calcd for $C_4H_{10}O_3S$: C; 34.49, H; 7.16, S, 23.10. Found: C; 34.93, H; 7.05, S; 23.10.

IR spectra of 6a ($CHCl_3$); 1730 (CO) and 1135 (SO_2) cm^{-1}. NMR spectra of 6a ($CDCl_3$, ppm): 3.07 (s, 3H, $COCH_3$), 2.94 (s, 3H, SO_2CH_3), 4.21 (t, 2H, OCH_2).

Oxidations of Hydroxysulfides (5s-11s) to Sulfones (Table 1) To a stirred solution of a hydroxysulfide (0.01 mol eq. to a sulfur) in AcOH (10 ml) was added 30% H_2O_2 (3.25 g, 0.026 mol) at a rate not to exceed 70°C and a mixture was kept 70°C until the TLC demonstrated no existence of the sulfide and sulfoxide; the time required is listed in the table. The solution was then evaporated to remove excess H_2O_2. Benzene was added to the residue, and the moisture was removed azeotropically and the mixture was evaported to dryness. The residue was dissolved in $CHCl_3$ and the solution was dried. The solvent was removed to afford a mixture of hydroxysulfones (5-11) and the acetates of the hydroxysulfones (5a-11a). The product's composition was determined using acetyl carbonyl band (near 1730 cm^{-1}). The results are listed in Table 1. All the compounds included in the table gave satisfactory analytical data.

dl-Methyl tetrahydrofurfuryl sulfide (20) To a solution of 2-tetrahydrofuranemethanethiol (19; 7.2g)[11] in 20% NaOH (36 ml), was added Me_2SO_4 (7.8 g) under vigorous stirring. The mixture was extracted with Et_2O,

and the extract was washed with water and dried. After evaporation of the solvent, the residue was distilled to give dl-20 as colorless oil, 5.8 g (73%), of bp_{25} 80-82°C. IR (neat): 1058 (ether) cm^{-1}. NMR ($CDCl_3$, ppm): 2.64 (d, 2H, $-CH_2-S-$), 2.18 (s, 3H, SCH_3). Anal. Calcd. for $C_6H_{12}OS$: C; 54.50, H; 9.15, S; 24.25. Found: C; 54.56, H; 8.92, S; 23.56.

dl-Methyl tetrahydrofurfuryl sulfoxide (14) To an ice-cooled solution of 30% H_2O_2 (5.6 g) in AcOH (30 ml), was added dl-20 (6.6 g) at a rate that the temperature not to exceed 40°C. After the exothermic reaction subsided, the solution was left at room temperature for 2 hr and the excess H_2O_2 was decomposed with aq. Na_2SO_3 and the solution was evaporated to dryness. The residue was taken into 10% $NaHCO_3$ and the mixture was extracted with $CHCl_3$. The extract was washed with brine and dried. After removal of the solvent, the residue was distilled to give dl-14 as colorless oil, 5.9 g (80%), of $bp_{0.5}$ 115-120°C. NMR ($CDCl_3$, ppm): 2.66 and 2.69 (each s, 3H, $SOCH_3$). Anal. Calcd. for $C_6H_{12}O_2S$: C; 48.62, H; 8.16, S; 21.63. Found: C; 48.87, H; 8.23, S; 21.01.

dl-Methyl tetrahydrofurfuryl sulfone (15) To a stirred solution of 30% H_2O_2 (14.1 ml) in AcOH (30 ml) was added dl-20 (3.3 g) at a rate that the temperature did not exceed 70°C. After heating at 70°C for 2 hr, the excess H_2O_2 was decomposed with aq. Na_2SO_3 and the mixture was evaporated to dryness. The residue was taken into 10% $NaHCO_3$ and the mixture was extracted with

$CHCl_3$. The extract was washed with water and dried. After removal of the solvent, the residual solid was recrystallized from CCl_4 to afford dl-15, 3.4 g (83%), as leaflets of mp 37-42°C. NMR and IR spectra were identical with those of the corresponding metabolite[5]. NMR ($CDCl_3$, ppm): 3.07 (s, 3H, SO_2CH_3). Anal. Calcd for $C_6H_{12}O_3S$: C; 43.90, H; 7.37. Found: C; 43.96, H, 7.53.

dl-5-Methylthiomethyldihydro-2(3H)-furanone (22)

5-Chloromethyldihydro-2(3H)-furanone[13] (21; 11.9 g) was heated to reflux with 1N-CH_3SNa/CH_3OH (20 ml) for 1.5 hr. After cooling, the solution was made acidic with cooled c-HCl and concentrated in vacuo. The residue was extracted with Et_2O and the extract was washed and dried. After removal of the solvent, the residue was distilled to give a colorless oil of dl-22, 5.3 g (78%), of bp_{12} 140-145°C. IR ($CHCl_3$): 1760 (CO), 1170 (ether) cm^{-1}. NMR ($CDCl_3$, ppm): 2.21 (s, 3H, SCH_3). Anal. Calcd. for $C_6H_{10}O_2S$: C; 49.28, H; 6.90, O; 21.89. Found: C; 49.28, H; 6.84, O; 22.30.

dl-5-Methylsulfinylmethyldihydro-2(3H)-furanone (16)

dl-22 was oxidized with 30% H_2O_2 by the same procedure as described for the preparation of dl-14 to afford dl-16 as an oil which showed in NMR a doublet for CH_3SO- at 2.67 and 2.70 ppm. When the oil was treated with AcOEt, a diastereomeric isomer which gives a singlet for CH_3SO- at 2.67 ppm was obtained as needles of mp 120-123°C. IR of dl-16 ($CHCl_3$): 1780 (CO) and 1170 (ether) cm^{-1}. Anal. Calcd. for $C_6H_{10}O_3S$: C; 44.42, H; 6.22, S; 19.76.

Found: (diastereoisomer of mp 120-123°C): C; 44.32, H; 6.24, S; 18.99.

The metabolite[5] identified as 16 was shown to be identical with the crystalline diastereomer by NMR spectral comparison.

dl-5-Methylsulfonylmethyldihydro-2(3H)-furanone (17)

dl-22 (13.7 g) was oxidized with 30% H_2O_2 by the same procedure as was described for the preparation of dl-15 to afford dl-17, 14.4 g. The material was recrystallized from $CHCl_3$-AcOEt to afford dl-17 as needles of mp 90-92°C, 12.1 g (72%). The compound was identical with the metabolite[5] on IR and NMR spectral comparison. NMR ($CDCl_3$, ppm): 3.03 (s, 3H, SO_2CH_3). Anal. Calcd. for $C_6H_{10}O_4S$: C; 40.43, H; 5.66, S; 17.99. Found: C; 40.57, H; 5.55, S; 17.32.

dl-4-Hydroxy-5-methylsulfonylvaleric acid (18)

dl-17 (1.78 g) was warmed with 0.5N-NaOH (10 ml) at 70°C for 2 hr. After cooling, the solution was made pH 3.0-3.5 with 1N-HCl under ice-cooling. The solution was saturated with NaCl and extracted with AcOEt. The extract was concentrated to afford solid, which was recrystallized from $CHCl_3$ to result prisms of mp 103-105 of dl-18, 1.05 g (54%). The compound was identical with the metabolite[5] on comparison with NMR and IR spectra and electrophoretic flow rates. Anal. Calcd. for $C_6H_{12}O_5S$: C; 36.72, H; 6.17, O; 40.76. Found: C; 36.80, H; 5.90, O; 40.74.

dl-Tetrahydrofurfurylthiuronium hydrogen carbonate

(23:H_2CO_3) A solution of dl-tetrahydrofurfuryl-bromide (19; 330 g) and thiourea (167 g) in EtOH (1 l) was heated to reflux for 8 hr and then evaporated to dryness in vacuo. To the residue a solution of $NaHCO_3$ (168 g) in H_2O (1.7 l) was added rapidly under stirring. The solids deposited were collected with suction and washed with ether to result dl-23·H_2CO_3 as white solid (300 g, 68%). The material was decomposed gradually on standing, therefore it was used immediately for the following experiment.

(2R)-(-)-Tetrahydrofurfurylthiuronium D-camphorate (23:DCA) and (2S)-(+)-Tetrahydrofurfuryl D-camphorate (23:DCA)

dl-23·H_2CO_3 (300 g) and D-camphoric acid (DCA; 272 g) were mixed mechanically in H_2O (2.5 l), whereby caused the instant evolution of CO_2, and the mixture was allowed to stand at room temperature overnight. The solid deposited was collected with suction and recrystallized twise from 33% EtOH to give (2R)-(-)-23: DCA (124.5 g, 51%) as colorless crystals of mp 178°C, $[\alpha]_D$ -12.6° (c=1, MeOH). Condensation of the mother liquor to a qarter volume afforded solid, which was recrystallized three times from 2 volumes of MeOH-MeCN (1:4) to result (2S)-(+)-23: DCA (98 g, 40%) as colorless crystals of mp 152°, $[\alpha]_D$ +37.3° (c=1, MeOH). Anal. Calcd. for $C_{16}H_{28}N_2O_5$: C; 53.31, H; 7.83, N; 7.77. Found: (2R)-(-)-23: DCA -- C; 53.02, H; 8.06, N; 7.83.

(2S)-(+)-23: DCA -- C; 52.90, H; 7.73, N; 7.83.

(2R)-(-)-Tetrahydrofurfurylthiuronium chloride (23:HCl)

(2R)-(-)-23:DCA ($[\alpha]_D$ -12.6° (c=1, MeOH)) (114.4 g),

c-HCl (26.5 ml), H_2O (200 ml) and Et_2O (200 ml) were mixed in a seperator vessel. The aqueous layer was taken, washed with Et_2O (100 ml) and concentrated to dryness in vacuo to afford colorless solid. Twice recrystallizations of the solid from 10 volumes of H_2O-MeCN (1:30) afforded (2R)-(-)-23:HCl·H_2O (62.5 g, 92%) as colorless rhombic crystals of mp 65-67°C, $[\alpha]_D$ -40.5° (c=1, MeOH). Anal. Calcd. for $C_{16}H_{12}N_2OS$:HCl·H_2O: C; 33.56, H; 7.04, N; 13.05. Found: C; 33.56, H; 7.08, N; 12.78.

(2S)-(+)-Tetrahydrofurfurylthiuronium chloride (23:HCl)

Treatment of (2S)-(+)-23:DCA ($[\alpha]_D$ -37.3° (c=1, MeOH)) (36 g) in the same manner as above afforded (2S)-(+)-23:HCl·H_2O (19.5 g, 91%) as colorless rhombic crystals of mp 65-67°, $[\alpha]_D$ +40.9° (c=1, MeOH). Anal. Found: C; 33.49, H; 7.08, N; 13.09.

(2R)-(+)-2-Tetrahydrofuranemethanethiol (19)

To an ice-cooled solution of (2R)-(-)-23:HCl·H_2O ($[\alpha]_D$ -39.5° (c=1, MeOH)) (12.6 g) in H_2O (20 ml), $NaHCO_3$ (5 g) was added under stirring. After 30 min, the mixture was heated at 90-100°C for 1 hr. The oil liberated was extracted with Et_2O (2x20 ml). The combined extract was washed, dried and evaporated to dryness to afford (2R)-(+)-19 (5.8 g, 83%) as colorless oil of $[\alpha]_D$ +4.1° (c=1, $CHCl_3$). TLC and GC analyses showed that the material is homogenous and identical with dl-19 prepared above on the flow rates.

(2S)-(-)-2-Tetrahydrofuranemethanethiol (19)

(2S)-23:HCl·H_2O ($[\alpha]_D$ +39.2° (c=1, MeOH))(12.8 g) was

treated as above to result (2S)-(-)-19 (5.6 g, 79%) as colorless oil of $[\alpha]_D$ -4.4° (c=1, $CHCl_3$). TLC and GC analyses of the oil assured the homogeneity of the compound and also identity with the racemate dl-19 on the flow rates.

(2R)-(+)-Methyl tetrahydrofurfuryl sulfide (20)

To an ice-cooled and stirred solution of (2R)-(+)-19 ($[\alpha]_D$ +4.1° (c=1, $CHCl_3$)) (5.8 g) and KOH (9.4 g) in H_2O (20 ml), Me_2SO_4 (6.3 g) was added in one portion and the mixture caused the immediate formation of an oil. The oil was extracted with Et_2O (2x20 ml) and the extract was washed and dried. Removal of the solvent in vacuo afforded (2R)-(+)-20 (6.0 g, 91%) as colorless oil of $[\alpha]_D$ +5.1° (c=1, MeOH). The compound was identical with the racemate dl-20 in TLC and GC flowing rates.

(2S)-(-)-Methyl tetrahydrofurfuryl sulfide (20)

(2S)-(-)-19 ($[\alpha]_D$ -4.4° (c=1, $CHCl_3$)) (5.6 g) was treated as above to result (2S)-(-)-20 (5.8 g, 91%) as colorless oil of $[\alpha]_D$ -5.1° (c=1, MeOH)). The material was identical with the racemate dl-20 on TLC and GC flowing rates.

(2R)-(-)-Methyl tetrahydrofurfuryl sulfone (15)

(2R)-(+)-20 ($[\alpha]_D$ +5.1° (c=1, MeOH)) (6.0 g) was oxidized with 30% H_2O_2 by the similar manner as was described for the preparation of dl-15 above to afford solid 6.9 g, which was recrystallized from CCl_4 twice to afford (2R)-(-)-15 as colorless leaflets of mp 65-67°C, $[\alpha]_D$ -50.5° (c=1, MeOH).

Anal. Calcd. for $C_6H_{12}O_3S$: C; 43.88, H; 7.38. Found: C; 44.00, H; 7.38.

(2S)-(+)-Methyl tetrahydrofurfuryl sulfone (15)

From (2S)-(-)-20 ($[\alpha]_D$ -5.1° (c=1, MeOH)) (5.1 g) by the same treatment as above, (2S)-(+)-15 was obtained, 6.1 g (80%), as leaflets of mp 65-67°C, $[\alpha]_D$ +50.3° (c=1, MeOH). Anal. Found: C; 43.90, H; 7.56.

(2R)-(-)-Tetrahydrofurfurylthiothiuronium chloride (24) To a mixture of thiourea (4.8 g), c-HCl (7.5 ml) and EtOH (100 ml) was added a solution of (2R)-(+)-19 ($[\alpha]_D$ +4.1° (c=1, $CHCl_3$)) (5.9 g) in 50% EtOH (20 ml) and then 30% H_2O_2 (6.0 g) under cooling at 0-10°C and vigorous stirring. The solid was removed and the mother liquor was concentrated to dryness. The residue was treated with acetone (150 ml) under dry-ice-acetone cooling. The crystalline solid was collected with suction and recrystallized from EtOH-Et_2O to afford (2R)-(-)-24 as crystals of mp 117-118°C, $[\alpha]_D$ -38.4° (c=1, MeOH), 7.55 g (67%). Anal. Calcd. for $C_6H_{12}N_2OS_2$:HCl : C; 31.50, H; 5.72, N; 12.24. Found: C; 31.40, H; 5.76, N; 12.49.

(2R, S-S)-(+)- and (2R, S-R)-Methyl tetrahydrofurfuryl sulfoxides (14) (2R)-(+)-20 ($[\alpha]_D$ +5.1° (c=1, MeOH)) (7.92 g) was oxidized with 30% H_2O_2 in the same manner adopted for the preparation of dl-14 to result an oil, 6.8 g, of $[\alpha]_D$ -53.2° (c=1, $CHCl_3$). The oil was subjected to preparative gas chromatography seperation (column: 20% diethyleneglycol succinate on 60-80 mesh chromosorb W; temperature 173°; carrier He) to give

(2R, S-S)-(+)-14 as a faster eluent of $[\alpha]_D$ +11.4° (c=1, $CHCl_3$), +18.2° (c=1, MeOH) and (2R, S-R)-(-)-14 as a slower eluent of $[\alpha]_D$ -120.2° (c=1, $CHCl_3$), -155.6° (c=1, MeOH). The materials obtained here showed Optical Rotatory Dispersions as depicted in Fig. 5.

References

1) a) T. Matsukawa and S. Yurugi, Proc. Jap. Acad., 28, 146 (1952), b) T. Matsukawa and S. Yurugi, Yakugaku Zasshi, 72, 1602 and 1616 (1952), c) T. Matsukawa and T. Kawasaki, Yakugaku Zasshi, 73, 216 (1953), d) T. Matsukawa, T. Iwatsu and T. Kawasaki, Yakugaku Zasshi, 73, 497 (1953), e) S. Yurugi and T. Fushimi, Yakugaku Zasshi, 78, 602 (1958)

2) a) M. Fujiwara, H. Nanjo, T. Arai and Z. Suzuoki, J. Biochem., 41, 273 (1954), b) Z. Suzuoki and T. Suzuoki , 40, 11 (1953), c) M. Fujiwara, S. Sasakawa, Y. Itokawa and K. Ikeda, J. Vitaminology, 10, 55 (1964), d) K. Takenouchi and K. Aso, J. Vitaminology, 9, 106 (1963)

3) E.J. Ariens in "Drug Design Vol II" (Ed. E.J. Ariens) p38, Academic Press, New York and London (1971)

4) a) Z. Suzuoki, K. Nishikawa and M. Numata, J. Biochem., 58, 279 (1965), b) K. Nishikawa, Z. Suzuoki and M. Numata, J. Pharmacol. Exp. Therap., 157, 589 (1967)

5) Z. Suzuoki, K. Kishiko, S. Kikuchi, K. Nishikawa and M. Numata, J. Pharmacol. Exp. Therap., 158, 353 (1967)

6) K. Nishikawa, Dissertion Thesis "Metabolism of Disulfide Type Thiamine Derivatives"

7) F.G. Bordwell and H.M. Anderson, J. Am. Chem. Soc., 75, 4962 (1953)

8) R.M. McCurdy and J.H. Prager, U.S. Patent 2925406 (1960)

9) A.L. Clingman and N.K. Richtmeyer, J. Org. Chem., 29, 1782 (1964)

10) D. Barnard, L. Bateman and J.I. Cuneen, "Organic Sulfur Compound Vol 1" (Ed. N. Kharasch), Pergamon Press Inc., New York, p229 (1961)

11) F.P. Greenspan, J. Am. Chem. Soc., 68, 907 (1946)

12) V.C. Barry and J.E. McCormick, Proc. Roy. Irish. Acad., 58B, No. 17, 345 (1958)

13) H. Leucks and O. Splattstoesser, Chem. Ber., 40, 304 (1907)

14) a) R.M. Dodson, N. Newman and H.M. Tsuchiya, J. Org. Chem., 27, 2707 (1962), b) B.J. Auret, D.R. Boyd, H.B. Henbest and S. Ross, J. Chem. Soc., (C), 1968, 2371, d) G.S. Fonken and R.A. Johnson, "Chemical Oxidations with Microorganisms" Marcel Dekker, Inc., New York, p227-232 (1972)

15) a) W. Klyne, J. Day and A. Kjaer, Acta Chem. Scand., 14, 215 (1960), b) K.K. Cheung, A. Kjaer and G.A. Sim, Chem. Commun., 100 (1965)

16) F. Lucas and L. Lebenbook, Biochem. J., 100, 473 (1966)

17) See E.L. Eliel "Stereochemistry of Carbon Compounds" McGraw-Hill Book Company, Inc., p110 (1962)

18) a) K. Mislow, M.M. Green, P. Lauer, J.T. Mellis, T. Simmons and L. Tenary, Jr., J. Am. Chem. Soc.,

87, 1958 (1965), b) M.A. Axelrod, P. Bickart, M.L. Goldstein, M.K. Green, A. Kjaer and K. Mislow, Tetrahedron Letters, 1968, 3249

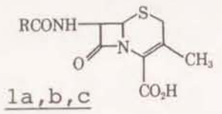
19) T. Fujita, Z. Suzuoki, S. Kozuka and S. Oae, J. Biochem., 74, 723 (1973)

20) a) T. Takahashi, Y.H. Khim, D. Fukushima, K. Fujimori and S. Oae, Heterocycles, 10, 229 (1978), b) Y. Watanabe, T. Numata, T. Iyanagi and S. Oae, Bull. Chem. Soc. Jpn., 54, 1163 (1981), c) S. Oae, T. Takata and K. Fujimori, Bull. Chem. Soc. Jpn., (in print)

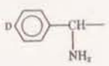
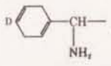
CHAPTER 4

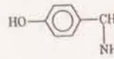
A NOVEL SYNTHESIS OF DESACETOXY-CEPHALOSPORIN FROM PENICILLIN

In 1955, a new antibiotic was isolated by Newton and Abraham[1] from a mold "Cephalosporium acremonium". This antibiotic, Cephalosporin C, had a relatively weak antimicrobial spectrum which, however, displayed remarkable effectiveness against penicillin resistent microorganisms. Chemical modifications together with improvements of fermentation of cephalosporin C have led to the development of many semi-synthetic cephalosporins which are clinically used in large amounts. Among them, cephalexin (1a), cephradine (1b) and cefadroxil (1c) with desacetoxycephalosporin nucleus are characterized for oral activity.



1a,b,c

1a; R=  , cephalexin 1b; R=  , cephradine

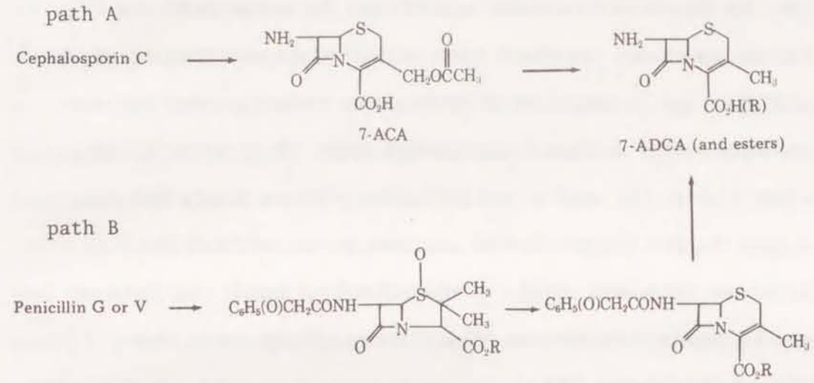
1c; R=  ,cefadroxil

Orally Active Cephalosporins

Compared to parenteral cephalosporins such as cephalothin and cephaloridine which are required to be administered to patients by way of injection with the specific handlings, these are easily administered orally by patients themselves. Therefore, in spite of their relatively weaker antimicrobial spectra than those of parenteral cephalosporins, the desacetoxycephalosporins (1) are conveniently used for the treatment of various infections.

The desacetoxy-cephalosporin nucleus has been

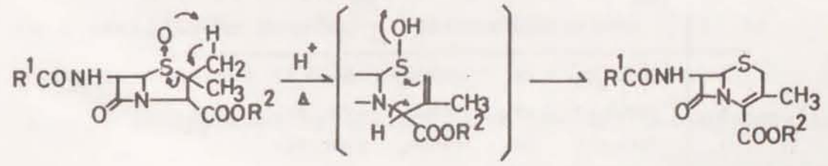
synthesized through two paths, the one by catatlytic reduction[2] of 7-aminocephalosporanic acid (2; 7-ACA) which is derived[3] from cephalosporin C (path A), the other by acid catalyzed thermal ring expansion[4] of penicillin sulfoxide (4) (path B).



The use of a large amount of noble metal catalyst, however, makes the path A to the antibiotic economically unrealistic. Whereas, the abundant availability of penicillin and the efficient overall transformation yield make the path B industrially feasible.

The author was motivated in this study to find another path from penicillin to desacetoxycephalosporin which could compete path B from the viewpoint of patentability and productivity.

Eq. 4-1 Rearrangement Mechanism of Penicillin Sulfoxide into Desacetoxycephalosporin

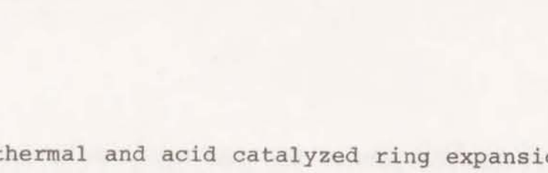


Morin et al (1963)

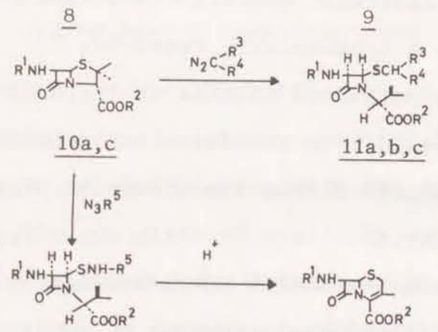
The thermal and acid catalyzed ring expansion of penicillin sulfoxides (4) to desacetoxycephalosporins (5), initially discovered by Morin et al.[4], has been extensively investigated and the intermediacy of a sulfenic acid (7) by a six electron sigmatropic rearrangement as depicted in Eq. 4-1 has been confirmed by many methods.[5]

Since previous studies have shown that sulfonium ylides[6] and sulfilimines[7] bearing ß-hydrogens undergo similar rearrangements, the author considered that if a penicillin sulfonium ylide (8) and a sulfilimine (9) as depicted in Fig. 1 are formed they should rearrange to afford 2-azetidinones (11) and (12), respectively, antd the latter upon some proper treatments might ring close to a desacetoxycephalosporin (5).

Fig. 1



Eq. 4-2



	R^1	R^2	R^3	R^4	R^5
a	$PhOCH_2CO-$	CH_3	$-COOCH_3$	$-COOCH_3$	-
b	$PhOCH_2CO-$	CH_3	$-COOCH_3$	$p-NO_2-Ph-$	-
c	$PhCH_2CO-$	CH_3	$-COOCH_3$	$p-NO_2-Ph-$	-
d	$PhOCH_2CO-$	CH_3	-	-	$-COOC_2H_5$

With these expectations in mind, we carried out following reactions.

Reaction of penicillin V methyl ester (10a) with 6 eq. of dimethyl diazomalonate in the presence of 2 eq. of dimethyl diazomalonate in the presence of 2 eq. $CuSO_4 \cdot H_2O$ in diethyl carbonate at 110°C for 30 min followed by chromatographic seperation of the reaction mixture on silica gel impregnated with 1% oxalic acid using n-hexane-benzene as an eluent afforded 2-azetidinone (11a) as a homogeneous gum in 46% yield.

In a similar manner penicillin V methyl ester (10a) and penicillin G methyl ester (10c) were treated with methyl p-nitrophenyldiazoacetate[8] in the presence of $CuSO_4 \cdot H_2O$ to afford 2-azetidinones (11b) and (11c), respectively, as foams in 70% yields.

The results were in good accordance with the expectation and explicable in terms of an initial attack of the carbenes generated from diazo-compounds onto the sulfur atom of the penicillins (10) to afford the sulfonium ylides (8) and subsequent spontaneous ring opening of 8 into 2-azetidinones (11) through a six electron sigmatropic rearrangement.

In another run, penicillin V methyl ester (10a) was heated with 6 eq. ethyl azidoformate[9] in diethyl carbonate at 90-100°C until 2 eq. nitrogen had been evolved to result in 2-azetidinone bearing a sulfenamido group (12d) as a homogeneous gum in 12% yield.

It is apparent by analogy with the aforegoing results

that a nitrene[9] was initially formed from ethyl azidoformate which attacked the sulfur atom of the penicillin (10a) to form the sulfilimine (9d) and 9d was spontaneously transformed into 2-azetidinone (12d).

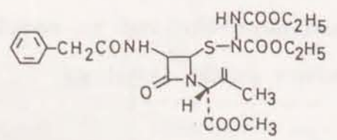
All of the 2-azetidinones thus prepared were new compounds and their structures were supported by their NMR, IR and Mass spectral data and elemental analyses.

The cyclization of 12d to desacetoxycephalosporin (5d) was attained when 12d was heated with diethylamine hydrochloride in dimethylacetamide at 130°C for 2 hr. Desacetoxycephalosporin (5d) was isolated by preparative TLC in 4% yield and identified by comparison with an authentic specimen.[4b]

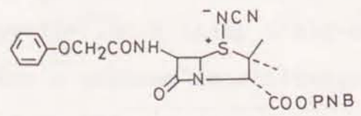
Thus a novel path from penicillin (10) to desacetoxycephalosporin (5) via 2-azetidinone-3-sulfenamide (12) was realized, although the over all yield was rather poor as compared to a nearly quatitative yield of path B.

Comparison with other similar methods

After completion of this study, the conversion of penicillin to desacetoxycephalosporin via 2-azetidinone-3-sulfenamide, on the same basic idea as ours, was tried by at least two other groups. The intermediates isolated by them were sulfenamide (13)[10] and sulfilimine (14).[11]



13



14 PNB= p-nitrobenzyl

On treatment with 30% KOH or aluminum oxide, 13 was converted into corresponding desacetoxycephalosporin in good yields (80-85%). Whereas, attempted conversion of 14 to desacetoxycephalosporin under acidic or basic conditions was unsuccessful.

Since 13 was made from penicillin sulfoxide by two steps, our path is still a sole example which does not deal with penicillin sulfoxide.

Experimental

(3R,4R)-4-Bism(methoxycarbonyl)methylthio-1-(1-methoxycarbonyl-2-methyl-2-propenyl)-3-phenoxyacetamido-2-azetidinone (11a) A mixture of penicillin V methylate (10a; 2.4 g), methyl diazomalonate (6.8 ml) and $CuSO_4 \cdot H_2O$ (2.0 g) was gradually heated to 90°C and kept at the temperature for 1 hr under stirring. 230 ml in total of nitrogen was evolved during the reaction. The temperature was rised to 104°C and kept for 30 min. After cooling, the mixture was dissolved in AcOEt and the insoluble solid was removed with suction. The filtrate was concentrated and the residue was subjected to silica gel (impregnated with 1% oxalic acid; 200 g) column chromatography and developed with n-hexane-AcOEt (1:1) to afford the titled compound, 1.46 g (46%).
IR ($CHCl_3$): 1786 (β-lactam C=O), 1748 (ester) cm^{-1}.
NMR ($CDCl_3$; ppm): 1.89 (s, 3H, $=C-CH_3$), 3.67 (s, 6H, $2xOCH_3$) 3.72 (s, 3H, OCH_3), 4.14 (s, 1H, SCH), 4.49 (s, 2H, $PhO-CH_2$) 4.82 (s, 1H, N-CH-C=C), 4.96 and 5.06 (each s, each 1H,

$=CH_2$), 5.38 (dd, 1H, J=5 and 8 Hz, C_3-H), 5.56 (d, 1H, J= 5 Hz, C_4-H), 6.8-7.4 (m, 6H, amide and aromatic protons).

Anal. Calcd. for $C_{22}H_{24}N_2O_9S$: C; 53.44, H; 5.30, N; 5.67. Found: C; 53.32, H; 5.43, N; 5.35.

$[\alpha]_D$ -77.9° (c=0.69, dioxane).

(3R,4R)-1-(1-Methoxycarbonyl-1-methyl-2-propenyl)-4-(methoxycarbonyl-p-nitrophenyl)methylthio-3-phenoxyacetamido-2-azetidinone (11b) A mixture of penicillin V methylate (10a; 0.364 g), methyl p-nitrophenyldiazoacetate (1.3 g) $CuSO_4 \cdot H_2O$ (0.3 g) and ethyl carbonate (10 ml) was heated at 110-113°C for 15 min, during which 150 ml of nitrogen was evolved. After filtration, the filtrate was concentrated and the residue was subjected to silica gel (impregnated with 1% oxalic acid: 100 g) column chromatography and developed with n-hexane-AcOEt (1:1) to afford the titled compound as yellow crystals of mp ca. 50°C, 0.393 g (70%).

IR (dioxane): 1786 (ß-lactam C=O), 1748 (ester).

NMR ($CDCl_3$; ppm): 1.93 (s, 3H, $=C-CH_3$), 3.69 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 4.34 and 4.54 (ABq, 2H, J=16 Hz, OCH_2CO), 4.73 (s, 1H, SCH), 4.87 (s, 1H, N-CH-C=C), 4.99 and 5.15 (each s, each 1H, $=CH_2$), near 5.3 (m, 1H, C_3-H), 5.40 (unresolved d, 1H, C_4-H), 6.8-7.4 (m, 5H, aromatic), 7.52 and 8.09 (ABq, 4H, J=8 Hz, aromatic).

$[\alpha]_D$ -62.9° (c=1, dioxane).

(3R,4R)-1-(1-Methoxycarbonyl-2-methyl-2-propenyl)-4-(1-methoxycarbonyl-1-p-nitrophenyl)methylthio-3-phenylacetamido-2-azetidinone (11c) A mixture of penicillin

G methylate (10c; 0.348 g), methyl p-nitrophenyldiazoacetate (1.3 g), $CuSO_4 \cdot H_2O$ (0.03 g) and ethyl carbonate (10 ml) was heated at 113°C for 20 min, during which 150 ml of nitrogen was evolved. After filtration, the filtrate was concentrated and the residue was subjected to silica gel (impregnated with 1% oxalic acid; 150 g) column chromatography and developed with n-hexane-AcOEt (1:1) to afford the titled compound as yellow solid of mp near 47°C, 0.371 g (70%).

IR (dioxane): 1786 (ß-lactam C=O), 1754 (ester).

NMR ($CDCl_3$): 1.89 (s, 3H, $=C-CH_3$), 3.54 (s, 2H, $Ph-CH_2$), 3.70 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.74 (s, 1H, SCH), 4.81 (s, 1H, N-CH-C=C), 4.99 and 5.13 (each s, each 1H, $=CH_2$), 5.0-5.3 (m, 2H, C_3- H and C_4-H), 6.45 (unresolved d, 1H, CONH), 7.1-7.4 (m, aromatic), 7.47 and 8.13 (ABq, J=8.5 Hz, aromatic).

$[\alpha]_D$ -37.2° (c=1, dioxane).

(3R,4R)-4-Ethoxycarbamoylthio-1-(1-methoxycarbonyl-2-methyl-2-propenyl)-3-phenoxyacetamido-2-azetidinone (12d)

A mixture of penicillin V methylate (10a; 3.6g), ethyl azidoformate (5.0 ml) and ehyl carbonate (25 ml) was heated at 130°C for 30 min, during which ca. 500 ml of nitrogen was evolved. Solvent was evaporated in vacuo, and the residue was subjected to silica gel (1 Kg) column chromatography and developed with benzene-AcOEt (1:1) to afford the titled compound, 1.107 g (12%) as a slightly yellowish oil.

IR ($CHCl_3$): 3390 (NH), 1770 (ß-lactam C=O), 1733 (ester), 1686 (amide I), 1538 (amide II) cm^{-1}.

NMR ($CDCl_3$, ppm): 1.22 (t, 3H, J=8 Hz, CH_2-CH_3), 1.94

(s, 3H, =C-CH_3), 3.76 (s, 3H, $COOCH_3$), 4.10 (q, 2H, J=8 Hz, $\underline{CH_2}$-Me), 4.56 (s, 2H, OCH_2), 4.83 (s, 1H, N-CH-C=C), 5.01 (d, 1H, J=5 Hz, C_4-H), 5.06 and 5.14 (each s, each 1H, $=CH_2$), 5.49 (q, 1H, J=5 and 8 Hz, C_3-H), 5.52 (s, 1H, SNH), 6.8-7.4 (m, 5H, aromatic), 7.84 (d, J=8 Hz, CONH).
Mass spectrum m/e 451 (M^+=$C_{20}H_{25}N_3O_7S$), 392 (M^+-$COOCH_3$), 363 (M^+-$NHCOOC_2H_5$), 331 (M^+-$SNHCOOC_2H_5$).
$[\alpha]_D$ -38.8° (c=1, dioxane).

Methyl 7β-phenoxyacetamido-desacetoxycephalosporanate (5d) A mixture of 12d (1.107 g), $NH(C_2H_5)_2$:HCl (0.160 g) and dimethylacetamide (100 ml) was heated at 120-130°C for 2.5 hr. The solvent was evaporated in vacuo, and the residue was taken into water and extracted with Et_2O. The extract was washed with 5% $NaHCO_3$ and water and dried. The extract was concentrated and the residue was subjected to silica gel column chromatography and developed with benzene-AcOEt (1:1) to afford the titled compound as solid of mp 129-136°C, 0.852 g. The material was identical with the authentic compound[4b] in comparison with IR and NMR spectra.

References

1) C.G.F. Newton and E.P. Abraham, Nature, 175, 548 (1955)
2) C.W. Ryan, R.L. Simon and E.M. Van Heynigen, J. Med. Chem., 12, 310 (1969)
3) J.E. Dolfini, H.E. Applegate, G. Bach, J. Bernstein, J. Schwartz and F.L. Weinstein, J. Med. Chem., 14, 117 (1971)

4) a) R.B. Morin, B.G. Jackson, E.H. Flynn and R.W. Roeske, J. Am. Chem. Soc., 84, 3400 (1962), b) R.B. Morin, B.G. Jackson, R.A. Mueller, E.R. Lavagnino, W.B. Scanlon and S.L. Andrews, J. Am. Chem. Soc., 85, 1896 (1963), c) ibid. idem., 91, 1401 (1969)
5) a) I. Ager, D.H.R. Barton, G. Lucente and P.G. Sammes, J. Chem. Soc. Chem. Commun., 1972, 601 b) R.D.G. Cooper , L.D. Hatfield and D.O. Spry, Accounts of Chem. Res., 6, 32 (1973).
6) W. Ando, T. Yagihara, S. Tozune, I. Imai, J. Suzuki, T. Toyama, S. Nakaido and T. Migita, J. Org. Chem., 37, 1721 (1972)
7) S. Oae, K. Tsujihara and N. Furukawa, Tetrahedron Letters, 2663 (1970)
8) E. Ciganek, J. Org. Chem., 35, 862 (1970)
9) W. Ando, N. Ogino and T. Migita, Bull. Chem. Soc. Jpn., 44, 2278 (1971)
10) G. Franceschi, M. Foglio, P. Masi, A. Suarato, G. Palamidessi, L. Bernardi, F. Arcamone and G. Cainelli, J. Am. Chem. Soc., 99, 248 (1977)
11) J.E.G. Kemp, D. Ellis and M.D. Closier, Tetrahedron Letters, 3781 (1979)

List of Publications

Chapter 1

1) Studies on Lipoic Acid II. Synthesis of Alkyl 8-Alkoxy- and 8-Acyloxy-6-oxooctanoate (1): S. Yurugi, M. Numata and T. Fushimi, Yakugaku Zasshi, 80, 1170-1175 (1960)

2) Studies on Lipoic Acid III. Synthesis of Alkyl 8-Alkoxy- and 8-Acyloxy-6-oxooctanoate (2): S. Yurugi, M. Numata and T. Fushimi, Yakugaku Zasshi, 80, 1317-1321 (1960)

3) Studies on Lipoic Acid IV. Reduction of Methyl 8-Alkoxy- and 8-Acetoxy-6-oxooctanoate: S. Yurugi, M. Numata and T. Fushimi, Yakugaku Zasshi, 80, 1321-1326 (1960)

4) Studies on Lipoic Acid V. New Synthesis of 6,8-Dihalo-octanoic Acids and its Esters: S. Yurugi, M. Numata and T. Fushimi, 80, 1326-1332 (1960)

5) Studies on Lipoic Acid VIII. New Syntheses of α-Lipoic Acid (3): S. Yurugi, M. Numata and T. Fushimi, Yakugaku Zasshi, 81, 299-301 (1961)

Chapter 2

6) Synthesis of Nereistoxin and Related Compounds I. : H. Hagiwara, M. Numata, K. Konishi and Y. Oka, Chem. Pharm. Bull., 13, 253-260 (1965)

7) Synthesis of Nereistoxin and Related Compounds II.: M. Numata and H. Hagiwara, Chem. Pharm. Bull., 16, 311-319 (1968)

Chapter 3

8) The Fate and Urinary Metabolites of Thiamine Propyl Disulfide in Rats: Z. Suzuoki, K. Nishikawa and M. Numata, J. Biochem., (Tokyo), 58, 279-284 (1965)

9) Further Studies on the Urinary Metabolites of Thiamine Propyl Disulfide in Rats: K. Nishikawa, Z. Suzuoki and M. Numata, J. Pharm. Exp. Therap., 157, 589-598 (1967)

10) Urinary Metabolites of Thiamine Tetrahydrofurfuryl Disulfide in Rats: Z. Suzuoki, K. Murakami, S. Kikuchi, K. Nishikawa and M. Numata, J. Pharm. Exp. Therap., 158, 353-364 (1967)

11) A Novel Oxidation-Acetylation of Hydoxysulfides: M. Numata, M. Yamaoka and K. Masuda, Chem. Pharm. Bull., 18, 221-228 (1970)

12) Chemical Studies on the Metabolites of Thiamine Tetrahydrofurfuryl Disulfide (TTFD). Stereo-chemistry of a Levorotatory Urinary Metabolete, (-)-Methyl Tetrahydrofurfuryl Sulfoxide in Rats: M. Numata, M. Yamaoka and H. Hirano, J. Takeda Res. Laboratories, 31, 1-10 (1972)

Chapter 4

13) Novel Transformations of Penicillins into 2-Azetidinones with Diazo- and Azido-Compounds and a Novel Synthesis of Desacetoxycephalosporin : M. Numata, Y. Imashiro, I. Minamida and M. Yamaoka, Tetrahedron Letters, 5097-5100 (1972)

Other Publications with Involvement of the Author

Lipoic Acid

14) Studies on Lipoic Acid I. New Synthesis of 8-Alkoxy-6-oxooctanoic Acid and its Esters: S. Yurugi, T. Fushimi and M. Numata, Yakugaku Zasshi, 80, 1165 (1960)

15) Studied on Lipoic Acid III. Synthesis of Alkyl 8-Alkoxy- and 8-Acyloxy-6-oxooctanoate (2): S. Yurugi , T. Fushimi and M. Numata, Yakugaku Zasshi, 80, 1317 (1960)

16) Studies on Lipoic Acid VI. New Synthesis of α-Lipoic Acid (1): S. Yurugi, T. Fushimi and M. Numata, Yakugaku Zasshi, 80, 1686 (1960)

17) Studies on Lipoic Acid VII. New Synthesis of α-Lipoic Acid (2): S. Yurugi, H. Yonemoto, T. Fushimi and M. Numata, Yakugaku Zasshi, 80, 1692 (1960)

Nereistoxin and Related 1,2-Dithiolanes

18) Hindered Internal Rotation of N,N-Dimethylpropylamines: Y. Asahi, M. Numata and E. Mizuta, Chem. Pharm. Bull., 21, 112 (1973)

19) Brugierol and Isobrugierol, Trans- and Cis-4-Hydroxy-1,2-Dithiolane-1-Oxide, from Brugiera Conjugata: A. Kato and M. Numata, Tetrahedron Letters, 203 (1972)

Disulfide Type Thiamines and Related Compounds

20) Synthesis of Bis(thiamine) Alkylene Disulfides and Related Compounds: H. Hagiwara, N. Shimahara and M. Numata, J. Takeda Res. Laboratories, 26, 1 (1967)

21) S-Alkylisothioureas III. A Facile Synthesis of Unsymmetrical Disulfides: O. Aki, M. Numata and K. Shirakawa, J. Takeda Res. Laboratories, 32, 1 (1973)

Cephalosporins

22) Syntesis of New Cephalosporins with Potent Antimicrobial Activities: M. Numata, I. Minamida, S. Tsushima, T. Nishimura, M. Yamaoka and N. Matsumoto, Chem. Pharm. Bull., 25, 3117 (1977)

23) New Cephalosporins with 7-Acyl Groups Derived from ß-Ketoacids I. 7-(ß-Ketoacylamino)cephalosporins: M. Numata, M. Yamaoka, I. Minamida, M. Kuritani and

Y. Imashiro, J. Antibiot., 31, 1245 (1978)

24) New Cephalosporins with 7-Acyl Groups Derived from ß-Ketoacids II. Further Modifications of 7-(3-Oxobutyrylamino)cephalosporins: M. Numata, I. Minamida, M. Yamaoka, M. Shiraishi and T. Miyawaki, J. Antibiot., 31, 1252 (1978)

25) A New Cephalosporins, SCE-963: 7-[2-(2-Aminothiazol-4-yl)-acetamido]-3-[[[1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl]-thio]methyl]ceph-3-em-4-carboxylic Acid, Chemistry and Structure-Activity Relationships: M. Numata, I. Minamida, M. Yamaoka, M. Shiraishi, T. Miyawaki, H. Akimoto, K. Naito and M. Kida, J. Antibiot., 31, 1262 (1978)

26) A New Route to Semisynthetic Cephalosporins from Desacetylcephalosporin C I. Synthesis of 3-Heterocyclicthiomethyl-Cephalosporins: S. Tsushima, M. Sendai, M. Shiraishi, M. Kato, N. Matsumoto, K. Naito and M. Numata, Chem. Pharm. Bull., 27, 696 (1979)

27) An Approach to Broad-Spectrum Cephalosporins: K. Morita, H. Nomura, M. Numata, M. Ochiai and M. Yoneda, Phil. Trans. R. Soc. Lond., B289, 181 (1980)

Acknowledgement

I am grateful to late Dr. S. Yurugi for having led me to the organic sulfur chemistry 24 years ago and to Prof. S. Oae for generous guidance in the field of sulfur chemistry which has covered about 20 years ever since I first became acquainted with him.

I acknowledge Drs. E. Ohmura and K. Morita, president and vice president of Central Research Division of Takeda Chemical Industries Ltd., for extending me their leadership to be one of the specialists in the synthesis of sulfur containing bioactive compounds in this division.

This dissertion was compiled under the guidance of Drs. M. Nishikawa and M. Fujino, without this I would have lost a chance to make a period on my earlier works.

This dissertion reports essence of my earlier works on the synthesis of sulfur containing bioactive compounds, which were performed with the help of co-operative works and critical discussions of many research colleagues, Drs. and Messers. M. Yamaoka, K. Nishikawa, I. Minamida, Y. Imashiro, E. Mizuta, O. Aki, Y. Nakagawa, late H. Hagiwara, Y. Oka and H. Asakawa. I am honored to have been associated with them.

Our Analytical Research Department is gratefully acknowledged.