# THE RESOLUTION AND STUDY OF THE PHYSICAL PROPERTIES OF COMPOUNDS BELONGING TO THE EPHEDRINE SERIES

Charles I. Jarowski

Thesis submitted to the Faculty of the Graduate School
of the University of Maryland in partial fulfillment
of the requirements for the degree
of Doctor of Philosophy
1943





ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

ACKNOWLEDGMENT

The author wishes to express his sincere thanks for the consistent assistance proferred by Dr. Walter H. Hartung, under whose direction this study was carried out.

TABLE OF CONTENTS

# LIST OF TABLES

# SECTION I.

## INTRODUCTION

In the year 1808 Malus observed that when ordinary light is allowed to pass through a single plane of tourmaline crystal, this light is changed. The difference may be seen by looking through a single tourmaline plate, then placing another plate on it. On rotating the first plate, it is obvious that there are certain positions in which the two transparent plates acting together form an opaque body and light does not pass through. However when the crystal is rotated at right angles to this position, the plates act as a single plate. The light waves emerging from such a tourmaline crystal vibrate in a single plane and are said to be polarized. Such plane-polarized light will pass through a second crystal so long as the polarizing tendencies of the second crystal are parallel to those of the first; if they are at right angles to each other, the second crystal allows none of the polarized light to pass through.

Biot continued the investigations of Malus and seven years later reported that if a transparent layer of quartz is placed between the two tourmaline plates, then the maximum transmission of light is obtained not when the tourmaline plates are parallel to one another but after one of them has been turned through an angle the magnitude of which depends upon the thickness of the quartz. In other words, light is polarized when it passes through a layer

of tourmaline, but the plane of polarization is rotated when the light passes through a layer of quartz. Some of Biot's quartz crystals turned the plane of polarization to the right (were dextrorotatory), and others were levorotatory (rotated to the left). Biot discovered also that solutions of many organic compounds have the same effect. By passing plane-polarized light through tubes that contained solutions of sugars, salts, and organic acids, he found that some of them were optically active, that is they turned the plane of polarization either to the right or the left, but most of them were optically inactive. He discovered that the rotation produced by a solution of a given optically active compound is proportional to its concentration and proportional also to the length of the column of solution through which the light traverses.

In 1848 Pasteur, who was familiar with Biot's work, and who knew that quartz crystallizes in hemihedral forms reached the conclusion that optical activity might have a definite relationship to crystal form. Six years earlier, Mitscherlich had investigated the sodium ammonium double salts of racemic (inactive) tartaric acid, and of dextrorotatory tartaric acid. Both of these salts have the same chemical composition, the same crystalline form, and the same specific gravity. On examining some large crystals of the dextro salt, he discovered hemihedral facets.

Pasteur theorized that the inactive salt should possess no hemihedral facets. The result of his

investigations was at first disappointing, for he found that inactive tartaric acid yielded hemihedral facets also. His very careful examination of the salt crystals from the inactive acid, however, revealed that they were not all exactly alike. The angles between the faces were identical in all of them, but the crystals were not identical and some of them were mirror images of others. He could pick out right-hand and left-hand crystals, and with a magnifying glass and a pair of tweezers he isolated a supply of both kinds. He dissolved the two types separately and observed that one solution was dextrorotatory and the other was levorotatory. A mixture of equal quantities of the two solutions was optically inactive. Pasteur in explaining his results assumed that in the tartaric acid molecule the atoms are arranged in a right-handed or left-handed spiral (1).

In 1867 Kekule (2) postulated the tetrahedral structure of the carbon atom, though it is doubtful if he really intended to convey the modern stereochemical idea. Two years later, Paterno (3) proposed to explain certain cases of isomerism by means of tetrahedral models but his views attracted little attention. It appears that Wislecenus (4) was the first to bring the matter into prominence. In 1873 he published his important researches on the lactic acids, in which he proved that the structures of the isomeric lactic acids were identical. He showed that there were no less than three lactic acids all having the same chemical structure yet certain different physical

properties:

| | M.P. | B.P. | d.20°/4° | $(\alpha)_D$ |
|---|---|---|---|---|
| dextro lactic acid | 26° | 120°/12mm. | 1.248 | +2.24 |
| levo lactic acid | 26° | 120°/12mm. | 1.248 | -2.24 |
| racemic acid | 16° | 120°/12mm. | 1.248 | 0 |

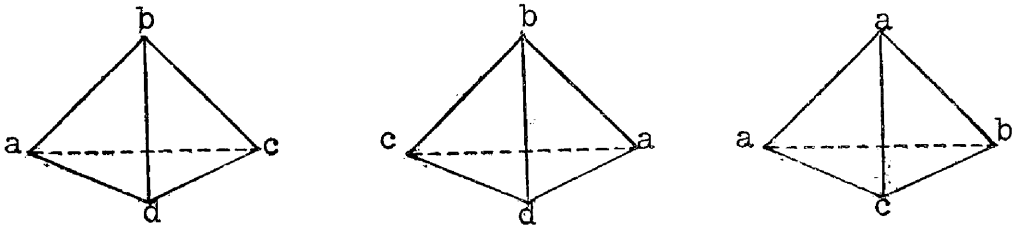
In his paper he stated:"The facts force us to explain the difference between isomeric molecules of the same structure by a different arrangement of atoms in space."

The year 1874 marks the opening of a new era. Van't Hoff (5) and Le Bel (6) simultaneously and independently published papers in which the modern theory of stereochemistry was outlined; and by them the isomers of optically active compounds and geometrical isomers were accounted for. They maintained that the carbon atom is a tetrahedron, and on the basis of solid geometry they were able to explain the isomerism of optically active compounds and geometrical isomers. Applied to the carbon atom the geometrical properties of a tetrahedron permit the following conclusions:

(1) If all four groups attached to the four apices are different, there are two possible arrangements in space.

(2) These two spatial arrangements bear the same relationship to each other as does an object to its mirror image.

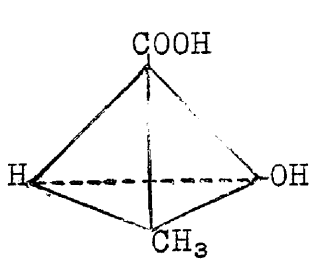
(3) If any two or more of the groups are identical, the above property of the tetrahedron vanishes and there is no plane or center of asymmetry.



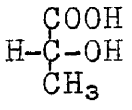

I II III

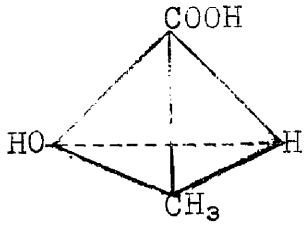
I is the mirror image of II and the two tetrahedra are not mutually superposable; there are no planes or centers of symmetry. III, with two groups similar has a plane of symmetry; a second tetrahedron with the same groups would always be superposable.

The only difference between the theories advanced by the two men is that Le Bel gave his tetrahedron some flexibility whereas van't Hoff maintained a rigid structure. They both pointed out the fact that the compounds then known to exhibit optical activity each had at least one carbon atom attached to four different atoms or groups. Such a carbon atom is called asymmetric. Since it is usual to regard the asymmetric carbon as lying in the center of the tetrahedron the structure of the lactic acids are represented as follows:




dextro lactic acid




levo lactic acid

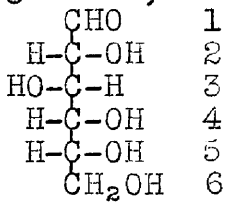
Here it is obvious that with the lactic acids another type of structural isomerism is present, namely,

stereoisomerism (from the Greek, stereos, meaning solid). Such compounds possessing similar atomic linkages but different spatial positions are called stereoisomers or enantiomorphs. Stereochemistry, therefore, is concerned with the chemical and physical phenomena which are believed to be associated with the relative positions in space taken up by atoms within a molecule. This arrangement of the atoms is termed the configuration; and just as the constitution of an organic compound is represented by means of its structural formula, so may its configuration be represented by means of its space formula as indicated for the lactic acids.

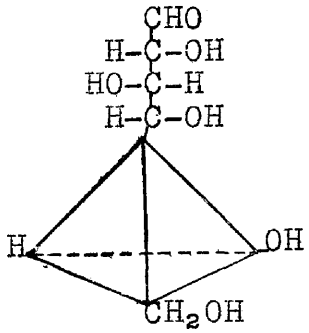
Until such time when it becomes actually possible to see the arrangement of the atoms in a molecule of lactic acid, it will be impossible to establish the absolute configuration of the two optically active isomers; hence, for the present, the scientist must remain content with relative configuration arbitrarily adopting one representation for the dextro and the other for the levo-rotatory isomer. The reference standard arbitrarily adopted is glyceric aldehyde,

$$CH_2OH\text{-}CHOH\text{-}CHO$$

This arbitrary choice goes back originally to Fischer's work on the sugars (7) and is based on the configuration of carbon atom number 5 of glucose,

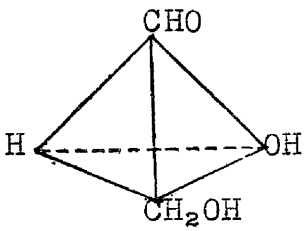


to which he assigned the configuration:

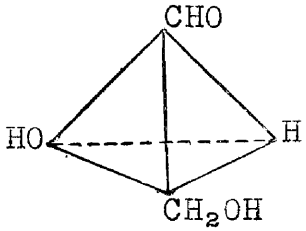



Fischer also established that the asymmetric carbon atom in dextrorotatory glyceric aldehyde has the same relative configuration.

Thus the space models for the enantiomorphs of glyceric aldehyde are:




I




II

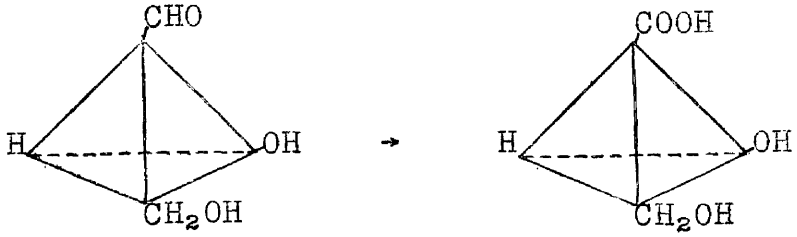
At first sight, these two groupings may appear to be identical; examination will show, however, that they are not superposable but are related to each other as a right hand glove is to a left hand glove. Since absolute configuration cannot be established, that shown in I is arbitrarily selected to represent the dextrorotatory isomeride while II is the levorotatory enantiomorph. An observer stationed at CHO will find that in I the order H-OH-$CH_2OH$ is clockwise while in II the same order is counterclockwise.

Thus with the isomeric glyceric aldehydes as a starting

point, it now becomes possible to establish the configurational relationships of many optically active compounds. Those compounds whose configurations are derived from dextro-glyceric aldehyde are members of the d-series and, similarly all derived from the levo aldehyde belong to the l-series. In order that the optical rotation may also be indicated the (+) and (-) characters, in parentheses, are used; the (+) sign designates clockwise or dextro rotation, the (-), levo or counterclockwise rotation.

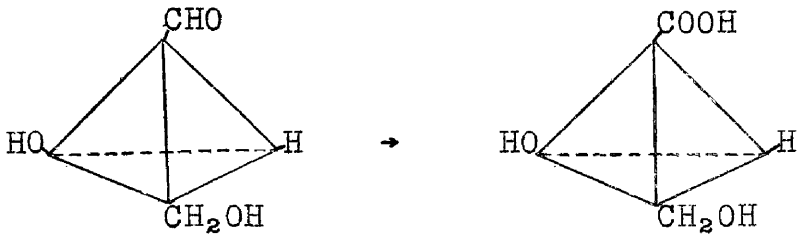
The most reliable method for the determination of the configuration consists in converting the compound studied into a compound of known configuration without altering the asymmetric relationship of the optically active groups or in preparing the compound from another whose configuration has been established.

For example, the aldehyde group may be oxidized to the carboxyl; the space models for the aldehydes and acids are indicated:




I II

d(+) glyceric aldehyde d(-) glyceric acid



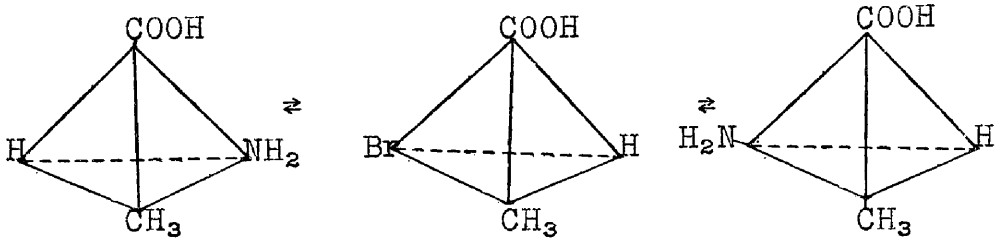

III IV

1(-) glyceric aldehyde 1(+) glyceric acid

The actual specific rotation of the acid from d-glyceric aldehyde in aqueous solution, however, is counterclockwise 2°, that is -2°; yet because of its configurational relationship to the standard it is a member of the d-series. By the same token IV represents l-glyceric acid even though its rotation is to the right. This may at first appear contradictory to have the levo-rotating compound in the d-series and the dextroisomeride in the l-series. But the apparent conflict will resolve itself if one keeps in mind that the prefixes d- and l- signify relative configuration in terms of the glyceric aldehydes.

Similarly, (+) tartaric acid and (+) malic acid were assigned to the d-series by interconversion, after a series of reactions, into d(-) glyceric acid.

The determination of relative configuration is further complicated by the possibility of a shift in the groups from one series to another. Such a phenomenon is called Walden Inversion, named after the man who first observed it. A good example is the conversion of d-alanine into l-alanine by the action of bromine.




d-alanine 1-bromopropionic acid l-alanine

Therefore, in the determination of relative configuration, the possibilities of Walden Inversion must always be taken into account and conclusions modified accordingly.

Naturally occurring mandelic acid has a levo rotation and is a member of the d-series. Its configuration was determined with the utilization of certain rules which have been found to be of considerable assistance. The two utilized are stated briefly:

Rule 1. The rotatory effect of similarly constructed molecules of identical series varies in like manner on change in temperature, dilution, addition of neutral salts, etc.

Rule 2. If the rotation of an acid on conversion into its amide or phenylhydrazide is displaced toward the right, the acid belongs to the d-series. Conversely, if the displacement is toward the left, the acid belongs to the l-series (8,9).

By a comparison of (-) mandelic acid with alpha-hydroxy acids of the d-series such as d(+)tartaric acid, d(-) lactic acid, and d(+) malic acid, Clough found that temperature changes, dilution, and addition of neutral

salts affected the rotatory power similarly.

Freudenberg and coworkers (10) determined the specific rotations of (-) mandelic acid, d(-) lactic acid, d(-) glyceric acid, d(+) tartaric acid and their amides, and by application of Rule 2 they reached a similar conclusion. The experimental data was as follows:

| | $(\alpha)_D$ | $(\alpha)_D$ amide |
|---|---|---|
| d(-) lactic acid | | |
| d(-) glyceric acid | | |
| d(+) tartaric acid | | |
| (-) mandelic acid | -4° | +22° |
| d(-) glyceric acid | -2.1° | +60° |
| d(+) tartaric acid | +6° | +64° |
| (-) mandelic acid | -153° | -95.5° |

In the case of mandelic acid, or other compounds containing one asymmetric carbon atom, there are three forms possible; levo, dextro and racemic. As with the lactic acids the chemical and physical properties of the two enantiomorphic forms are identical in every respect except rotation; they vary only in direction but not in magnitude. In their reactions with symmetrical compounds the resulting products are identical in every respect except in their effect on plane-polarized light; and here again they produce rotations of equal magnitude but of different sign. For example the reaction between the isomers of mandelic acid and ethyl alcohol would produce enantiomorphic esters. With other asymmetrical molecules the two forms of mandelic acid react to yield compounds which are no longer similar. For example the reaction between a racemic base and the optical antipodes of an acid may be represented as follows:

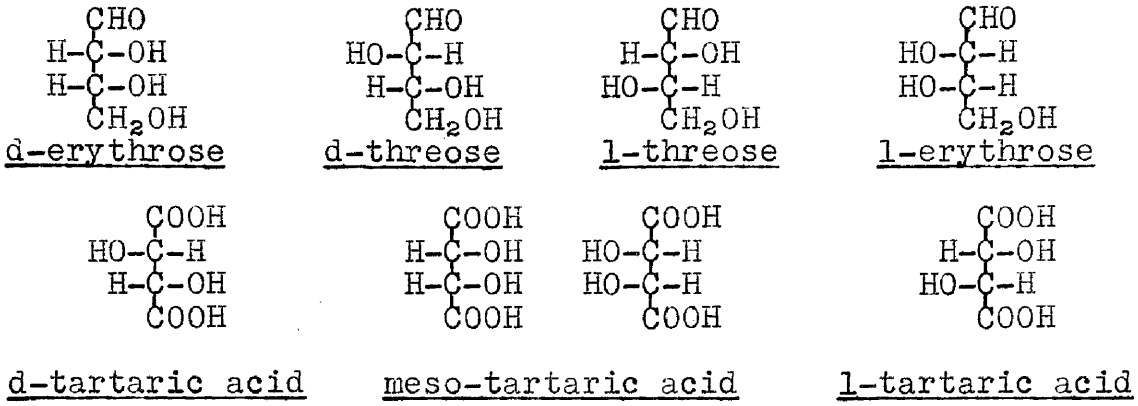
$$2\ \text{dl-B} + 2(-)\ \text{A} \rightarrow \underset{\text{I}}{(+)\ \text{B.(-)A}} + \underset{\text{II}}{(-)\ \text{B.(-)A}}$$

$$2\ \text{dl-B} + 2(+)\ \text{A} \rightarrow \underset{\text{III}}{(+)\ \text{B.(+)A}} + \underset{\text{IV}}{(-)\ \text{B.(+)A}}$$

In the above salts I and IV are true mirror images as II and III. As is true of mirror images, their melting points, solubilities aswell as all other physical and chemical properties are equal. They only differ in their effect on polarized light; the sign of rotation is opposite, however the magnitude is the same. Compounds I and II or III and IV are spatial isomers likewise but they are called diastereoisomers in order to distinguish them from the true mirror images. Since the rotational effect produced by the acid portion is opposite to that of the base in one instance and similar in the other, the two compounds will possess different optical activities both as to magnitude and sometimes direction. They also differ in many other respects; they have different melting points, solubilities, densities and may vary in their rates of reaction.

Examples of diastereoisomerism are encountered everywhere, the best known and most intensively studied being, perhaps, the simple sugars. Examples of such isomerism are given in the case of the four aldotetroses and tartaric acid.

Instead of using space diagrams conventional projection formulas will be employed:




In the aldotetroses d-erythrose and d-threose are diastereoisomers; d-erythrose and l-erythrose are enantiomorphs. In the case of d-erythrose the magnitude of rotation will be greater than that of d-threose for both of its asymmetric carbons are exerting their rotational effects on polarized light in the same direction; d-threose on the other hand has its asymmetric carbons in conflict, i.e. one is exerting its effect in a dextrorotatory direction while its neighbor is influencing polarized light in an opposite direction. The overall effect upon plane-polarized light is a summation of the individual rotations of each of the asymmetric carbon atoms. In the case of meso-tartaric acid, where the rotational effect of the two asymmetric carbon atoms is equal, but of opposite direction, the summation is zero; such a compound is said to be inactive by internal compensation and is called a meso compound.

Even though they are composed of identical structural elements, diastereoisomers are different chemical compounds. There is evidence showing the differences in physical and chemical properties of such isomers. In regard to rates of

reaction it has been shown that one of the components of a racemic mixture may react with an optically active compound at a faster rate than its isomer.

Thus (+) and (-) camphorcarbonic acid were decarboxylated at equal rates when heated with aniline. With (-) nicotine the (+) acid decomposed at a rate about 13 per cent faster than the (-) acid; and if nicotine were used with the racemic acid the (+) component reacted about 8 per cent more rapidly (11). During the esterification of racemic mandelic acid with (-) menthol the dextrorotatory component of the acid reacts more rapidly (12).

Since enzymes are optically active, organic biocatalysts, it is to be expected that the rate and extent of reaction with stereoisomers should differ. Pasteur (13) observed that (+) tartaric acid is more rapidly fermented than its optical antipode. For sugars, as Fischer (14) so ably showed, enzymes exhibit a decided preference for given configurations, leaving diastereoisomers little or not affected. Alpha and $\beta$-glycosides may be qualitatively differentiated by means of enzymes; e.g., $\alpha$-methyl-d-glucoside is hydrolyzed by yeast and $\beta$-methyl-d-glucoside is hydrolyzed by emulsin (15).

Enzymes are active not only in degradation but also in synthetic reactions. If in such syntheses an asymmetric center is formed one configuration is frequently formed at the expense and even to the exclusion of its isomer. Thus it has been found that o-methylcyclohexanone is reduced by hops to a dextrorotatory alcohol (16). Benzoylformic acid is reduced in the presence of milk, hops or beef liver to

(-) mandelic acid. Benzaldehyde and hydrogen cyanide in the presence of emulsin form optically active mandelonitrile (17).

From the above observations it appears that neither the enzyme nor the substrate alone determines the resultant of their reactions; an enzyme may select one isomer of one pair, the other of another, while on the other hand one enzyme may destroy the dextrorotatory component, another the levorotatory antipode of a racemic mixture. In some cases each isomer is destroyed at the same rate; for example, racemic lactic acid subjected to certain ferments is slowly destroyed without developing any optical activity whatever (18), that is the (+) and (-) isomers are acted on equally.

It is interesting to note that the same variation occurs in the case of the combination of asymmetric substances of known constitution. Thus a levorotatory alkaloid may be separated from its mirror image by the insolubility of its salt with an optically active acid, while with another acid the solubility may be reversed and with a third the difference may be too small to afford any separation.

Since enzyme action is responsible for many of the changes occurring in substances as they pass through the living cells, differences in rate and extent of change are to be expected with enantiomorphic substances.

Thus on subcutaneous injection of racemic malic acid into rabbits, (+) malic acid appears in the urine (19).

This indicates that the (-) malic acid is more readily metabolized by the body.

When optically active antipodes of camphor are fed to dogs (20) or rabbits (21) more or the levo isomer than of the dextro escapes as camphorol glucuronate. There is less differentiation between dextro and levo borneol (22) though here also more of the levo appears in the urine as glucuronate.

Similar differences are seen in the relative amounts of some of the isomers of the amino acids which undergo similar destruction in the tissues. The natural amino acids, i.e. of the l-series, are more quickly oxidized. For example, Wohlgemuth (23) found that when racemic forms of tyrosine, leucine, aspartic or glutamic acids are ingested by rabbits, the naturally occurring components are excreted in smaller proportions than the foreign isomer.

In view of the differences in physical and chemical properties of diastereoisomers, a difference in pharmacological response is not unexpected. If, for example, two optical isomers are administered to a living organism, then by reaction with the tissue ( built up of optically active components ) two diastereoisomers are formed. The physiological response may then be convoked by either physical or chemical forces or by a combination of the two working simultaneously.

The earliest evidence that living cells are able to

differentiate between the isomers was offered by Piutti, in 1886 (24). He pointed out that (+) asparagine has a sweet taste, while the natural levo isomer is insipid. Pasteur ascribed this difference to the presence of an optically active substance in the nervous mechanism of taste and pointed out the analogy with the enzymes which he had demonstrated thirty years previously.

The first definite example of difference in the pharmacological effect between two types of stereoisomers was offered by levo and racemic hyoscyamine ( atropine ), which were investigated by Cushny (25). Qualitatively, both isomers were found similar, however, the levo was almost twice as powerful a mydriatic as atropine. From quantitative measurements the inference was drawn that the dextro isomer is practically devoid of action on the myo-neural junctions of the autonomic nervous system. Later experiments in which the dextro and levo isomers were compared confirmed this. Cushny reported the relative of 1:20 for the dextro and levo isomers of hyoscyamine. Laidlaw (26) found them to vary from 1:25 on the cardiac vagus to 1:100 by the direct application to the iris.

Numerous pharmacologically active compounds have been resolved and quantitative studies made with the enantio-morphs. Different workers utilized different animals and studied the effect on various organs. The following table shows a small number of the compounds studied and their relative potencies. This may be considered evidence for quantitative differences in pharmacological response of

diastereoisomers.

| NAME OF COMPOUND | BASIS FOR COMPARISON | REL.STRENGTH | REF. |
|---|---|---|---|
| 1.Hyoscine | Parasympathetic endings | (+):(-)=1:18 | (27) |
| 2.Homatropine | Salivary nerve endings | dl :(+)=5:8 | (28) |
| 3.Nicotine | Toxicity | dl :(-)=1:2 | (29) |
| 4.Epinephrine | Sympathetic endings | (+):(-)=1:15 | (30) |
| 5.Corbasil | Pressor action | (+):(-)=1:30 | (31) |
| 6.Cocaine | Anesthetic<br>Toxicity | (-) slightly more<br>(+):(-)=1:10 | (32) |
| 7.Pseudococaine | Anesthetic | dl :(+)=1:2 | (33) |
| 8.β-Eucaine | Local anesthetic action<br>Toxicity | (+):(-)=1:1<br>(+):(-)=1:2 | (34) |
| 9.Coniine | Toxicity | dl :(+)=1:1 | (35) |
| 10.Synephrine | Pressor action | (+):(-)=1:60 | (36) |

In the pharmacological investigations of enantiomorphic compounds it has been demonstrated that the tissues are able to differentiate between two antipodes, while in other instances no distinction is made between them. There are of course many tissues which fail to give any response to either isomer.

Cushny, who made an excellant review on the subject of the pharmacology of stereoisomers (37), regarded the difference between the physiological activities of two optical isomerides as analogous to the different behaviours of the antimeric forms towards optically active acids. He was of the opinion that an explanation of the influence of molecular dissymmetry on physiological activity would be found along the lines of this analogy, possibly, for example, by the more active

isomeride forming a compound with an optically active receptor in the tissues which possessed different physical properties from that formed with its enantiomorph.

Cushny maintained that optical isomers differ in their pharmacological action proper just as they differ in their reaction with enzymes and that they exhibit the same variability. For example, among the hyoscyamines and hyoscines, the levorotatory forms act 15 times as strongly as the dextro in certain organs in which they are said to exert a "specific action", while in other positions such as in striated muscle or in the nerve ends supplying it, the isomers are equally and only feebly active. A parallel may be drawn with (-) and (+) lactates which differ in their reaction with penicillium, while they are equally responsive to some bacteria; and a further parallel exists between the behaviour of these isomers in the tissues and that originally observed by Pasteur between the tartrates and the cinchona alkaloids.

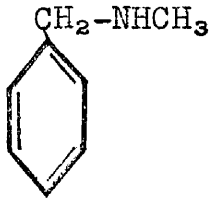
Quoting Cushny,

> There can, I think, be no question that in all these three conditions the differences observed between the action of two isomers arise from the same fundamental cause that, for example, levo-adrenaline contracts the vessels of the conjunctiva more strongly than the dextro adrenaline from the same principle that makes d-tartrate more readily oxidized by penicillium and less easily precipitated by cinchonine than the levo tartrate, that is because in the penicillium's tissues, as in the test tube, the isomers form compounds with some optically active substance and these compounds are no longer mirror images (diasteromers) and are no longer identical in their physical characteristics.

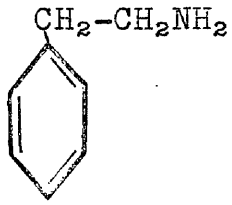
King (38) attributed the differences in the action of optical isomers on the assumption that less of one than of the other reaches the site of action, owing to different amounts being intercepted on the way; he based his view upon the observations of Pasteur that optically active dyes are differentially adsorbed by wool, so that an active isomer may be removed from solution by wool soaked in it. The two isomeric hyoscyamines must be present in the blood stream in equal amounts, for they have equal effects on the terminations on the motor nerves to the muscle, and to explain the difference in their action on the salivary glands by King's view it would be necessary to assume that in their passage from the blood into the receptor on the myoneural junction about 15 times as much of the dextro isomer is detained by adsorption as of the levo; in the brain of the frog on the other hand more of the levo would be arrested in the interval between the plasma and the nerve cell.

Easson and Stedman (39) have elaborated an alternative view according to which there appears to be no reason for differentiating between molecular dissymmetry and structure in regard to the manner in which they influence physiological activity. They consider that the difference in physiological activity of optical isomers may frequently be ascribed to circumstances which are identical with those which cause different symmetrical molecules to exhibit different physiological activities. For example, consider the case of the following

symmetrical, structural isomers:




I

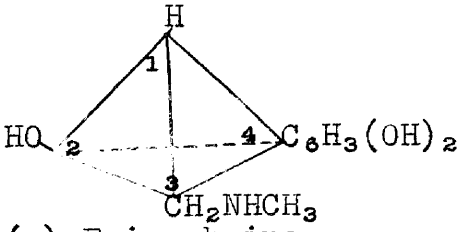



II

The pressor action of II is very much greater than I. According to the proponents of this theory the molecular arrangement of the compound is the factor responsible for difference in physiological response of structural isomers. Their contention is that molecular dissymmetry and the optical activity which accompanies it are merely accidental adjuncts of different molecular arrangements.

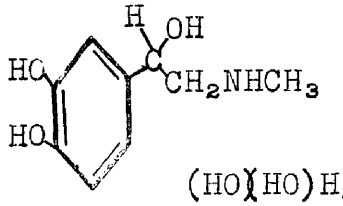
The theoretical basis for their suggestion starts from the postulate that a drug is attached to its specific receptor in the tissues in such a manner that a considerable proportion of the drug molecule is involved. If an asymmetric carbon atom is involved three of the groups present may be concerned in the process.

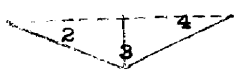
As an example, the epinephrines are represented by models III and IV:



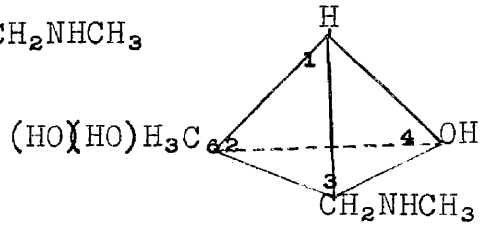

(-) Epinephrine

III




V




(+) Epinephrine

IV

Figure V represents diagrammatically the surface of the specific receptor in the tissues. For the drug molecule to produce a maximum physiological effect it must be attached to the receptor in such a manner that the groups 2,3,4 in the drug coincide with 2,3,4 in the receptor. Such coincidence can only occur with the more active levo isomer. If the hydrogen at position 1 were replaced by hydroxyl the resulting symmetrical compound should still have the same activity as (-) epinephrine since the base of the tetrahedron remains unchanged. The acquisition of such a compound was not feasible. Hence another test was applied to prove the validity of their theory using (+) epinephrine. The hydroxyl at position 4 in (+) epinephrine cannot be concerned with the attachment of the drug to its receptor. This attachment is brought about by the face, 1-3-4 , and the smaller physiological activity of the isomeride is attributed to the less perfect combination which results. By substituting a hydrogen atom for the hydroxyl in (+) epinephrine a symmetrical compound is obtained which, they theorized, should possess the same affinity for the receptor. The fact that this symmetrical compound, 3,4, dihydroxy-$\beta$-phenylethylmethylamine (epinine), does possess the same physiological activity bears out their hypothesis very well.

The frontiers of physics and chemistry can no longer be drawn with the precision that was formerly supposed to

be possible. There is a tendency to attribute the pharmacological effects of substances to physical changes produced. Quoting Beutner (40):

> Every pharmacological action is ultimately due to a physical change which the drug brings about in the living tissue.
>
> Change of chemical constitution, the basis of many theories, primarily affects the physical properties of the substances in question. The real problem, would be, therefore to study the nature of these physical properties and the corresponding changes in living tissue by these substances leading to drug action.

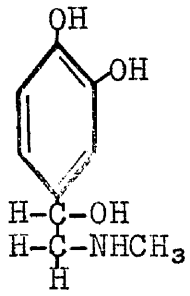
# SECTION II

## THE EPHEDRINE SERIES OF COMPOUNDS

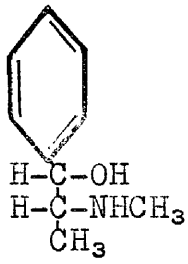
### 1.Spatial Configuration

There have been numerous investigations upon structurally related compounds with the aim of correlating chemical structure with physiological activity. The isolation of epinephrine from the adrenal gland and ephedrine from various species of ephedra, and the recognition of their powerful pharmacological actions stimulated synthetic chemical research. This research has resulted in the production of a number of organic medicinal agents, which, because they have the property of causing results very similar to those caused by the stimulation of the sympathetic nervous system, have been called "sympathomimetic" drugs. Several of these pressors (since they cause a rise in blood pressure) have been resolved into stereoisomeric forms which have been compared pharmacologically. In some instances the dextro form showed the greater activity whereas with others the levo excelled. As with the enzymes, the physiological differentiation does not depend upon the effect on polarized light.

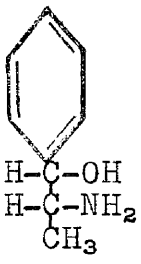
The chemical relationship of a few pressors will be apparent from an inspection of their structural formulas:



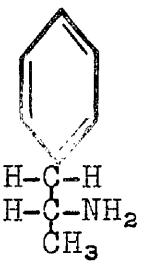
Epinephrine



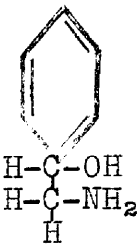
Ephedrine



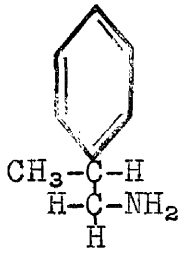
Propadrine



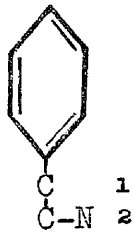
Benzedrine



Phenylethanolamine



β-phenylpropylamine (Isobenzedrine)



In the subsequent discussion of these compounds the method of numbering shown above will be adhered to.

The determination of the relative configuration of the asymmetric carbon atoms in the ephedrine series of bases has been carried out rather completely.

The two asymmetric carbons in ephedrine make possible the existance of four optically active compounds and two racemic mixtures. The following conditions prevail:

| | C No.1 | C No.2 | |
|---|---|---|---|
| a) | + | - | |
| b) | - | + | Racemic mixture (dl-Ephedrine) |
| c) | + | + | |
| d) | - | - | Racemic mixture (dl-Pseudoephedrine) |

Isomers c and d have rotations of greater magnitude than their diastereoisomers a and b, since both asymmetric carbon atoms have similar rotational directions. They represent pseudoephedrine, differentiating them from the diastereoisomeric ephedrines.

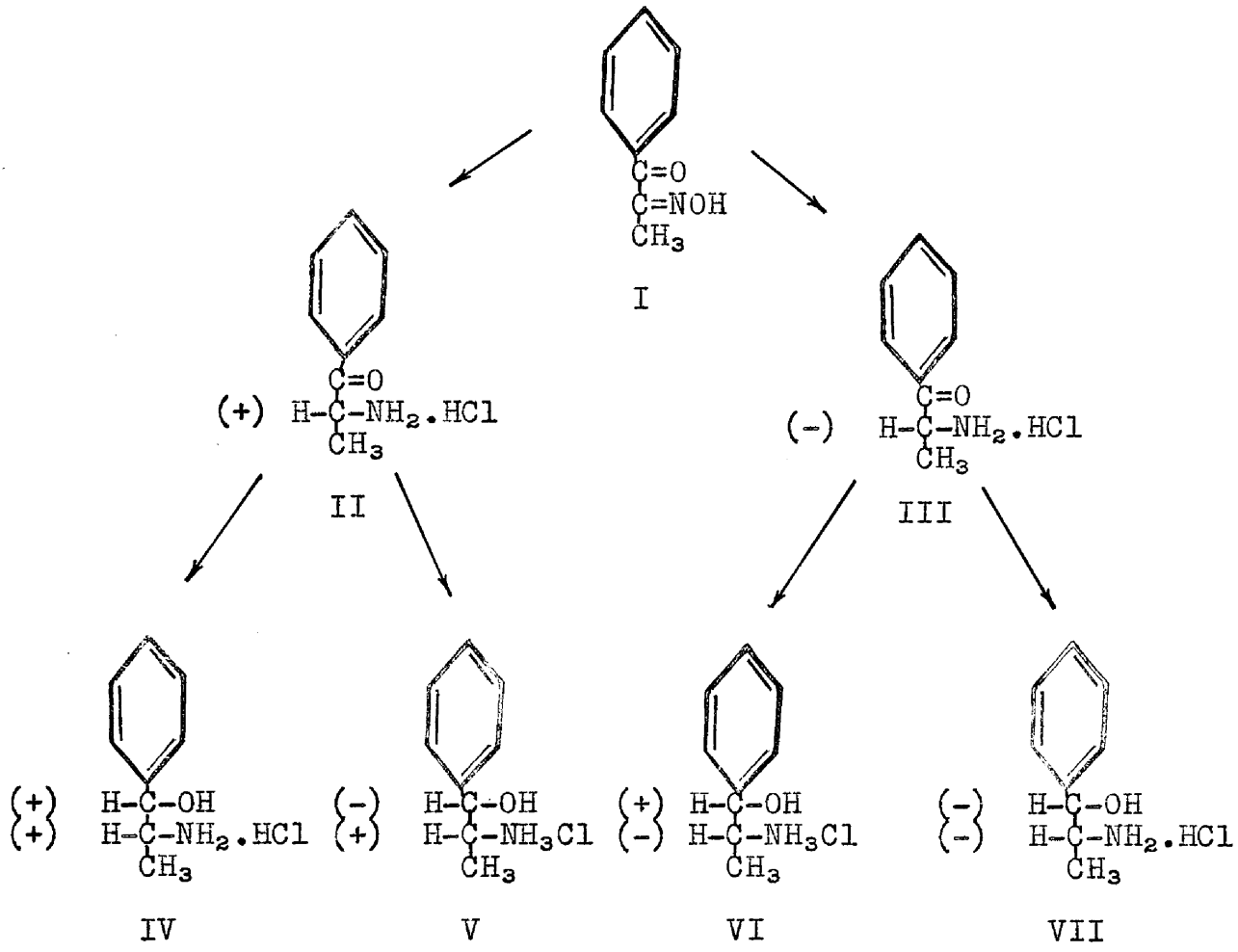
> (-) Ephedrine (b) is the most abundant,and from the Chinese species of ephedra, Ma Huang (41), was the first of the bases to be isolated. It could be converted into a dextrorotatory isomer. However, this (+) isomer, as was shown later, is represented by (c); i.e., it is not the true enantiomorph and when first encountered was called pseudo-(meaning false) ephedrine.

All the possible isomers are known and have been prepared synthetically (42,43,44,45,46,47).

Kanao (48) methylated propadrine in order to procure ephedrine. This method is of interest for in the preparation of propadrine an excellant example of partial asymmetric synthesis (production of a new single configuration in a molecule that is already optically active) is illustrated.

During the catalytic hydrogenation of isonitroso-propiophenone (49) the oximino group is first converted into an amino group; this creates an asymmetric center. Further reduction then converts the carbonyl into a

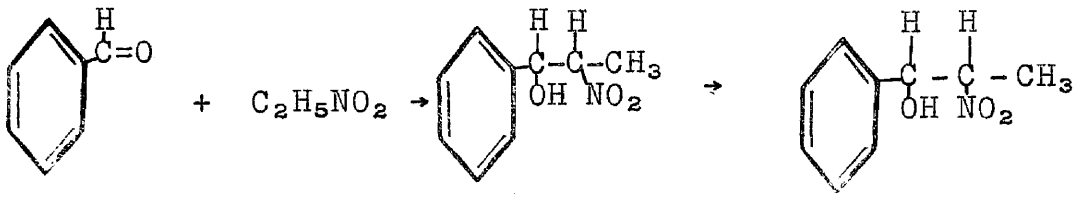
carbinol group. Schematically the possible reduction products are represented as follows:




During the formation of the aminoketone an asymmetric carbon atom is generated, but the (+) and (-) arrangements appear in equal amounts, and the racemic mixture may be represented as II-III. The reduction of the carbonyl group in II might be expected to give the diastereoisomers IV and V, whereas III should then form VI and VII. IV and VII are enantiomorphs and together would form racemic pseudo-propadrine; V and VI represent propadrine. Thus, in the

synthesis represented two racemic mixtures might notmally be expected. However, only one is obtained, namely that represented by V-VI. Hence, the configuration assumed by the ketonic carbon atom when it is converted into an asymmetric carbon atom is influenced by the spatial arrangement of the amino-bearing carbon atom.

On the other hand, if propadrine is prepared from benzaldehyde and nitorethane, through phenylnitropropanol,



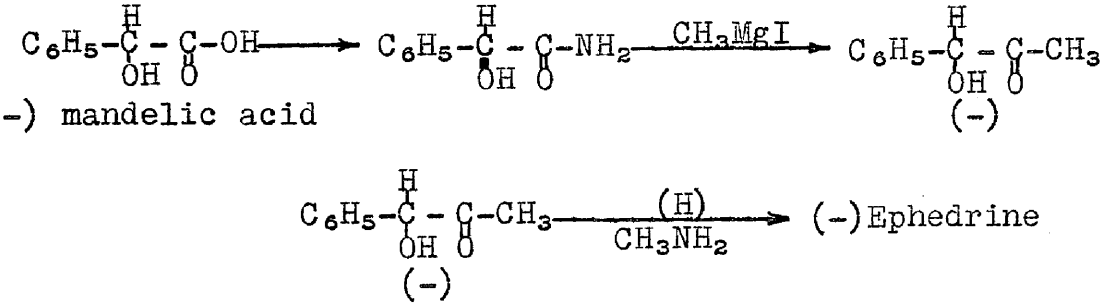
a mixture of equal amounts of the two racemic forms is obtained. In the condensation of nitroethane with benzaldehyde to form the nitroalcohol the two asymmetric carbon atoms are generated simultaneously and all possible configurations are actually obtained. The reduction of the nitro group does not affect the configuration once established.

Kanao utilized the latter method and separated the two racemic mixtures by fractional crystallization of the hydrochlorides from absolute alcohol. Subsequent methylation of dl-propadrine yielded dl-ephedrine; dl-pseudopropadrin was methylated to dl-pseudoephedrine.

The steric relationship between pseudoephedrine and ephedrine was first noticed by Nagai (50). He converted (-) ephedrine into (+) pseudoephedrine by heating with 25% hydrochloric acid in a sealed tube. Subsequent workers (51,52) showed that this transformation was the

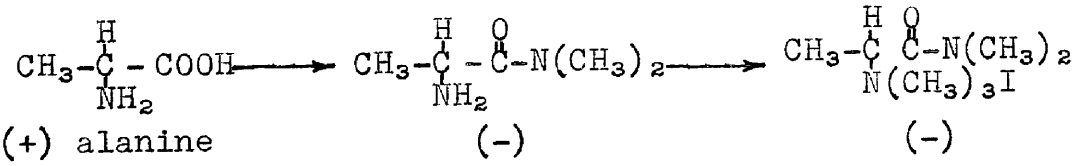
result of an inversion on carbon atom 1. The center of asymmetry 2 in (-) ephedrine and (+) pseudoephedrine is the same as is shown by the fact that on replacement of the alcoholic hydroxyl group both yield the same (+) desoxyephedrine (53).

The relative configuration about the hydroxyl-bearing carbon, number 1, was established by the synthesis of (-) ephedrine from d(-) mandelic acid and is thus placed in the d-series. The reactions are (54),



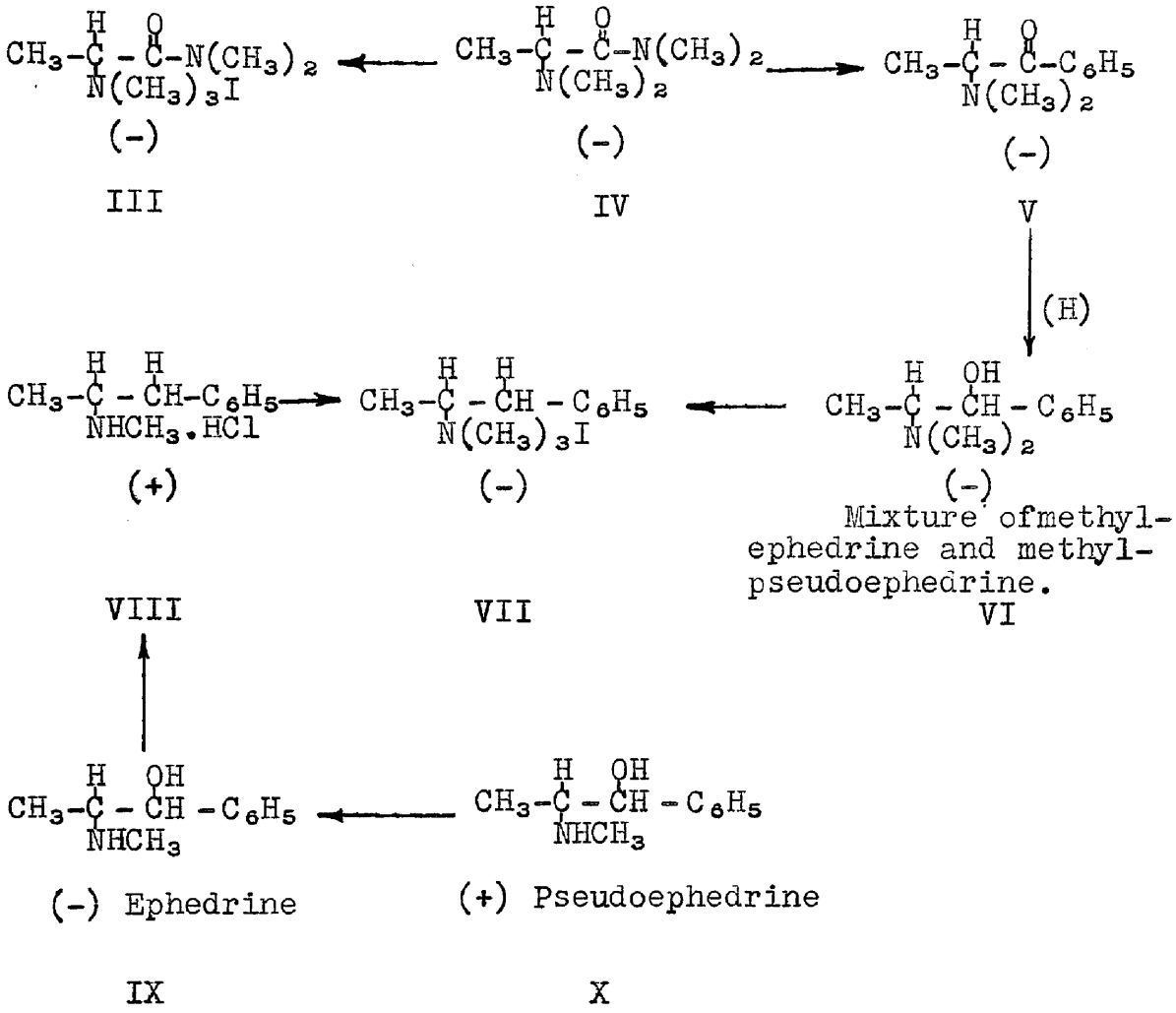
d(-) mandelic acid

Leithe (55) assigned the relative configuration of the methylamino-bearing carbon atom to the d-series. His conclusions are based on a steric comparison of benzedrine (of which the dextro-isomer has the same relative configuration as dextro-desoxyephedrine) with the optically active forms of α-phenethylamine. Freudenberg and Nikolai (56), synthesized desoxyephedrine according to the following series of reactions:

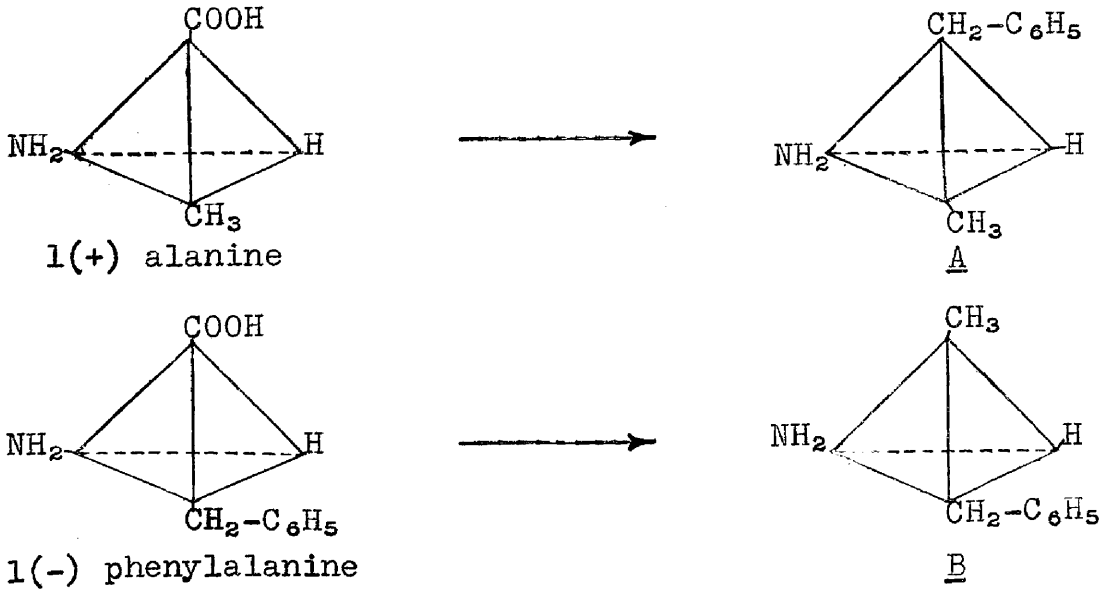


l(+) alanine (-) (-)

I II III




placed the relative configuration of carbon 2 in the l-series. However, there is some question about the correctness of this designation as may be seen from the following:



By starting with l(+) alanine the authors obviously obtained the benzedrine whose relative configuration is represented by space formula A. Had they started with l(-) phenylalanine and reduced the carboxyl to a methyl they would have obtained the isomer represented by space formula B. A and B are mirror images.

> An observer standing at position H, figure A, would find the order $CH_3$-$NH_2$-$C_6H_5CH_2$ to be counterclockwise, whereas in figure B the same order is clockwise.

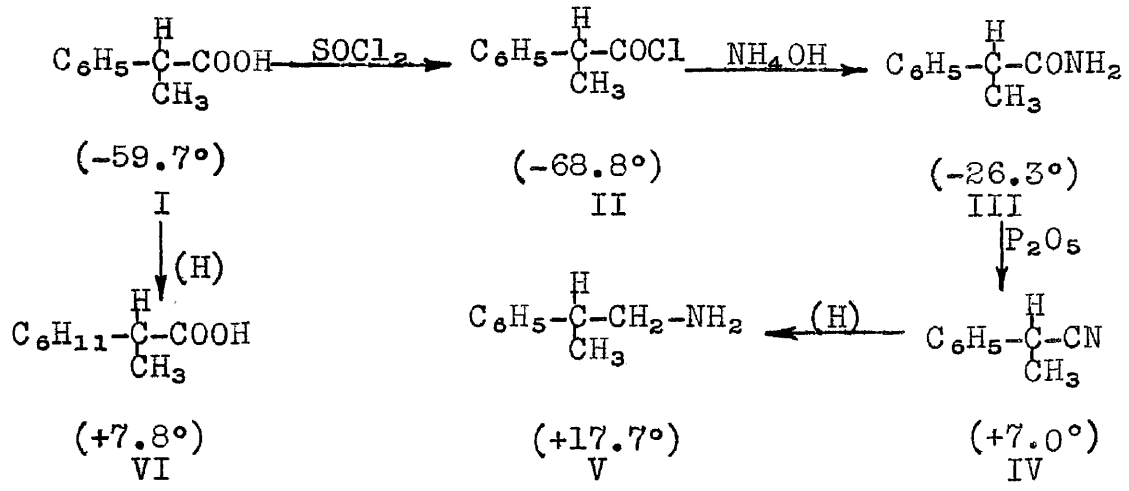
In other words A has the relative configuration of l-alanine or d-phenylalanine, depending on which amino acid is taken as the reference standard.

The relative spatial arrangements of the asymmetric centers in the propadrine isomers are ostensibly analogous to those of ephedrine. This is indicated by the fact that (+) propadrine results from the reduction of the oxime of $C_6H_5$-CHOH-$COCH_3$, which is procured from l(+) mandelamide and $CH_3MgI$. As mentioned before, d(-) mandelamide ultimately yielded (-) ephedrine upon similar treatment and reduction in the presence of methylamine. Conversely if l(+) mandelamide had been employed in the latter reaction (+) ephedrine would have resulted.

In the case of benzedrine it follows that if Freudenberg's work is conclusive the asymmetric carbon in the dextro form belongs to the l-series (or d-series, related to phenylalanine).

(+) Isobenzedrine is a member of the d-series. This is indicated by the work of Levene. In this case the asymmetric carbon atom corresponds to the hydroxyl-bearing

carbon in ephedrine and propadrine. The reactions involved are as follows:




The shift in rotation to the right upon conversion to the amide places the levorotatory acid into the d-series (see Rule 2, p.10). The dextrorotation (VI) upon replacement of the phenyl with a cyclohexyl group likewise points to a d-series configuration (57,58).

No reference has been found to the relative configuration of the asymmetric center in phenylethanolamine.

## 2. Pharmacology

Analogous to the steric relationship of the groups about the asymmetric centers in the compounds mentioned is the rather marked qualitative similarity in their effect on blood pressure upon administration. Pharmacodynamic studies have likewise shown qualitative and quantitative differences between these sympathomimetic drugs as well as between stereoisomers.

Thus ephedrine resembles epinephrine in that it

produces a marked rise in blood pressure, but the effect is less intense and more prolonged (59). While ephedrine is active upon oral or systemic administration, epinephrine is inactive orally. Animals become tolerant upon repeated injections of ephedrine; the intensity effect of epinephrine is closely proportional to the quantity injected (60).

Qualitatively the optical isomers of ephedrine possess the same physiological actions: the mydriatic action, the inhibition of the isolated rabbit's intestine, the contraction of the isolated guinea pig's uterus (virgin), the astringent property on the human nasal mucous membranes and the pressor action.

Quantitatively the pharmacological activities of the optical isomers of ephedrine, according to Chen, Wu and Henriksen (61), are shown in the following table:

TABLE I.

Quantitative Pharmacology of the Ephedrine Isomers

| Isomer | Mydriasis | Ratio of Pressor Action in Animals | Pressor Action in man per os | M.L.D. (rabbit) mgm./kgm. |
|---|---|---|---|---|
| (-) | +++++ | 35.15 | + | 60 |
| dl | +++++ | 26.40 | + | 60 |
| (+) | +++ | 11.90 | - | 80 |
| (+) ps. | ++++ | 6.80 | + | 75 |
| dl ps. | +++ | 4.00 | + | 70 |
| (-) ps. | + | 1.00 | - | 80 |

For epinephrine the ratio of pressor activities for the three isomers has been found to be (-):dl:(+)=1:1/2:1/20. The (-) isomer is also 6-20 times more toxic than the

(+) isomer (62).

Propadrine acts similarly to ephedrine. Its action on swollen mucous membranes is more prolonged than ephedrine upon local application. It is less toxic than ephedrine, with less stimulation of the anxiety complex.

According to Hirose (63-cited by Kanao), the order of activity of the optical isomers of propadrine is as follows:

(+) Pseudopropadrine $>$ dl-propadrine = (-) propadrine

(+) Propadrine $<$ (-) propadrine

(+) Pseudopropadrine $>$ (-) pseudopropadrine $<$ (-) propadrine

Chen, Wu, and Henriksen (61) reported that (+) pseudopropadrine has a weaker action than (-) or dl-ephedrine, but a stronger action than (+) pseudoephedrine.

These comparisons appear inconsistent and not in harmony with the knowledge of the N-methylpropadrines (ephedrines). Perhaps a more careful investigation of the pharmacodynamic properties of these diastereoisomers should be made.

Benzedrine is likewise active orally. It has a greater stimulating effect of the central nervous system than either propadrine or ephedrine; its toxicity however is greater. Comparison of the pressor effects in normal persons of the dextro, racemic and levo-isomers of benzedrine was reported by Alles (64). Following oral administration of the compounds their pressor responses were quite closely similar. It was found that the central effects of the (+) isomer are

approximately 1 1/2 to 2 times that of the racemic isomer, and 3 to 4 times that of the levo-isomer (65). According to Tainter (66) there is no apparent difference in toxicity between the dextro and racemic forms; the levo-isomer is 1/10 as toxic.

Isobenzedrine qualitatively compares to benzedrine in its pressor action. It is inactive upon oral administration and is less stimulating to the central nervous system than benzedrine but its toxicity is much lower (67,68).

Phenylethanolamine has a blood pressure effect in rabbits that is initially greater than and finally comparable with that of ephedrine, and a lower toxicity in guinea pigs by subcutaneous injection. It is inactive orally but its action on the congested mucous membrane is in every respect comparable to that of ephedrine. Compared to benzedrine its central effect is negligible (69).

# SECTION III

## Purpose of The Work

In view of the chemical and physical differences between diastereoisomers and the quantitative, physiological variations demonstrable upon the administration of antimers, a correlation of physicochemical properties of spatial isomers with pharmacological activity is to be expected. In this connection an interesting observation was made in the solubilities of the salts of the isomeric ephedrines. With d(-) mandelic acid, for example the solubility of the salts increases in the following order:

(-) Ephedrine (-) mandelate
(+) Ephedrine (-) mandelate
(+) Pseudoephedrine (-) mandelate
(-) Pseudoephedrine (-) mandelate

The physiological activities of the ephedrines, when measured by the rise produced in blood pressure, increase in the same order. Whether there is a mathematical parallelism between the two phenomena has not yet been established.

The object of the present work is to make a careful study of some physical and chemical properties of the ephedrine series of compounds in the endeavour to ascertain whether there is some definite relationship in the diastereoisomeric, physical and chemical differences between the more active compound and its antipode.

# SECTION IV

# EXPERIMENTAL

## 1.General

The experimental work consists of the synthesis of racemic mandelic acid, its resolution into enantiomorphs by the use of (-) ephedrine, and the utilization of the (+) and (-) mandelic acids in the resolution of isobenzedrine, benzedrine, propadrine, pseudopropadrine and phenylethanolamine in order to study the physical properties of the diastereoisomeric salts.

The bases were procured through the courtesy of the following:

Benzedrine-----Dr.F.P.Nabenhauer, Smith, Kline and French Laboratories, Philadelphia,Pa.

β-Phenylpropylamine-----Dr.M.L. Moore, Sharp and Dohme,Inc. Glenolden, Pennsylvania.

(-) Ephedrine-----Abbott Laboratories, North Chicago, Ill. Sharp and Dohme.

Propadrine (Racemic Mixture of the 4 bases)-----from the Commercial Solvents Corp.,Indiana.

Phenylethanolamine-----Mr.Robert Simonoff, University of Maryland.

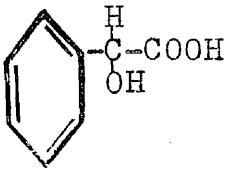
All temperatures recorded in this investigation are Centigrade degrees and were determined with Anschutz "stem-immersion" thermometers. In all cases the rate of heating was so adjusted that the bath temperature never rose more rapidly than 1° per minute.

The source of monochromatic light was a Zeiss sodium vapor lamp for the major portion of the rotations. The

rotatory values for the pseudopropadrine bases and (-) phenylethanolamine were determined with sodium light procured by heating sodium chloride to incandescence in a direct flame.

In all instances 95% alcohol was the solvent employed for the polarimetric measurements. The concentration values are in grams per 100 cc.

2.The Preparation of Racemic Mandelic Acid [70]



M.W.=152.06

Quantities used:

NaCN----------150 gms.
$C_6H_5CHOHCOOH$------318 gms.
$NaHSO_3$----------1500 gms.
Cracked ice-------900 gms.
HOH----------2500 gms.
HCl (38%)----------425 cc.

Reactions:

$$C_6H_5CHO + NaHSO_3 \rightarrow C_6H_5CHOHOSO_2Na$$

$$C_6H_5CHOHOSO_2Na + NaCN \rightarrow C_6H_5CHOHCN + Na_2SO_3$$

$$C_6H_5CHOHCN + HCl + 2HOH \rightarrow C_6H_5CHOHCOOH + NH_4Cl$$

Procedure:

Into a 4-liter, wide-mouthed glass jar, fitted with a mechanical stirrer, is placed a solution of 150 gms. (3 moles) of NaCN,

This reaction and the subsequent hydrolysis should be carried out in a good hood for HCN is liberated.

The NaCN used was the technical cyan-egg 92-95%.

which was dissolved in 500 cc. of water and 318 gms. (3 moles) of U.S.P. benzaldehyde. The stirrer is started and 850 cc. of a saturated solution of $NaHSO_3$ is

This solution is best prepared by stirring 1500 gms. of technical $NaHSO_3$, 97-100%, with 2 liters of water and filtering to remove the excess salt. The specific gravity of the solution varies between 1.37-1.39.

added to the mixture, slowly at first and then in a thin stream. The time of addition is 10-15 minutes. During the addition of the first half of this solution 900 gms. of cracked ice is added to the reaction mixture, a handful at a time. The layer of mandelonitrile which appears during the addition of the sulfite solution is separated from the water in a separatory funnel. The water is extracted once with about 1500 cc. of benzene, the benzene is evaporated and the residual nitrile is added to the main portion.

The crude nitrile (about 300 cc.) is placed at once

The mandelonitrile should be mixed with HCl as soon as it is separated from the water for it undergoes rapid rearrangement to the isonitrile (71).

in a 25 cm. evaporating dish, and 425 cc. of C.P. concentrated HCl (Sp.Gr. 1.19) is added. The hydrolysis is allowed to proceed in the cold for about 12 hours,

The hydrolysis may be carried out with heat but the compound procured is highly colored.

after which the mixture is filtered. The filtrate is heated on a steam bath to remove the water and excess HCl. After heating for 5-6 hours it is best to cool the

mixture and filter the ammonium chloride-mandelic acid

> It is advisable to cool and stir in order to break up any lumps forming and thus the product can be more easily filtered.

mixture. This residue is added to the solid material obtained before. The product is deeply colored and must be dried in the air and light for at least 24 hours. The total yield of the crude mandelic acid-ammonium chloride mixture was 582 gms.(moist). The mixture is ground in a mortar, transferred to a 2 liter flask and washed twice with 750 cc. portions of cold benzene.

> If the crude product is not first washed with cold benzene the final product is usually colored. Very little mandelic acid is lost by this washing.

The insoluble portion is transferred to a suction funnel and sucked dry.

The solid mixture is transferred to a 2 liter flask and shaken ten minutes with 750 cc. of ether. The ether solution is decanted through a suction filter and the solid thrown onto the filter and sucked dry. The solid is then returned to the flask and shaken with 400 cc. of ether. The mixture is filtered with suction and the solid washed twice with 250 cc. portions of ether. Each portion is allowed to drain through the filter several times while the solid is kept porous with a spatula. The combined ether filtrate is then filtered through an ordinary glass funnel.

> An occasional water layer should be removed in a separatory funnel.

The ether solution is placed in a 3 liter round-bottom

flask and 750 cc. of toluene (benzene or xylene may be used instead) is added. The mixture is distilled on a steam bath through an efficient fractionating column. Heat until the temperature rises to 95°. The residual toluene solution is cooled and the crystals of mandelic acid which separate are filtered with suction.

Melting point found to be 118° and the yield amounted to 75% of the theoretical.

### 3.Resolution of dl-Mandelic Acid

The following methods are recorded in the literature:

A.The resolution of mandelic acid employing (+) cinchonine is described by Gatterman and Wieland (72). The melting point of the (+) cinchonine (+) mandelate reported by Manske and Johnson (73) is 177°. The pure (+) acid obtained melted at 133-134°.

B.Manske and Johnson (73) obtained pure (-) mandelic acid by the resolution of the dl-form with (-) ephedrine. Their optically pure (-) ephedrine (-) mandelate melted at 170° and the pure (-) mandelic acid at 133.5°. The (+) acid was procured by repeated crystallizations of the racemic acid with (+) ephedrine; its melting point was 132.5°.

C.Roger (74) accomplished the resolution of dl-mandelic acid with (-) ephedrine alone. He reported a specific rotation of the (-) enantiomorph of -189.9° (in acetone); the (+) acid had a specific rotation of +189.1°.

D. Resolution is also accomplished by the use of a mold, which when grown on a solution of the ammonium salt of the acid destroys the levo-isomer (75).

E.By partially esterifying mandelic acid with (-) menthol the non-esterified acid is then levorotatory as the dextro-acid is somewhat more readily esterified (76).

The method employed in this investigation was essentially that described by Roger with slight modifications.

Procedure:Twenty grams of (-) ephedrine (0.12 mol.) and 20 gms. of dl-mandelic acid (0.13 mol.) were each dissolved separately in 50 cc. of 95% alcohol. The two solutions were mixed and heated on a steam bath for one hour. Upon cooling there was a copious deposition of crystals. The mixture was filtered with suction and the mother liquor was reserved. The crystals were washed with two 10 cc. portions of alcohol and these washings were collected for the second crystallization. The weight of crystals amounted to 16.8 gms., $(\alpha)_D^{27} = -43.68$ (c=1.4365).

The mother liquor was gently heated on the steam bath for 30 minutes and then 20 gms. each of (-) ephedrine and dl-mandelic acid were added simultaneously with stirring. Heating is continued for another 30 minutes and the clear solution was allowed to cool gradually. It is filtered with suction and set aside. The mother liquor is now saturated with the more soluble diastereoisomer (-) ephedrine (+) mandelate. The residue on the filter was washed with alcohol and the washings reserved for the next

crystallization. Yield of crystals equalled 23 gms.

The collected washings were heated on a steam bath and the two batches of crystals (39.8 gms.) were added. Sufficient alcohol was added to effect a solution in the hot. Upon cooling 35 gms. of crystals were procured, $(\alpha)_D^{25} = -63.3°$ (c=1.4050).

Recrystallization yielded 28 gms. of optically pure (-) ephedrine (-) mandelate, softened at 168°, melted at 170°. The following rotatory values were observed at different concentrations:

$(\alpha)_D^{26.5} = -70.6°$ (c=2.4056)
$(\alpha)_D^{25} = -78°$ (c=1.8680)
$(\alpha)_D^{25} = -82°$ (c=0.9719)
$(\alpha)_D^{25} = -85°$ (c=0.9316)

To 150 cc. of water was added sufficient concentrated HCl calculated to react with the weight of complex added, according to the following equation:

$$C_6H_5\text{-}CHOH\text{-}\underset{\underset{NHCH_3.C_6H_5CHOH\text{-}COOH}{|}}{CH}\text{-}CH_3 + HCl \rightarrow C_6H_5CHOH\text{-}\underset{\underset{NHCH_3.HCl}{|}}{CH}\text{-}CH_3 +$$

(-) ephedrine (-) mandelate
M.W.=317.19

$$C_6H_5CHOH\text{-}COOH$$

The crystals were added with stirring and extracted with ether. Most of the ether was recovered by gentle distillation over a hot water bath and the concentrated ethereal solution was transferred to a crystallizing dish and allowed to cool and dry. The crystals were dissolved in hot acetone, filtered and the acetone allowed to evaporate spontaneously. The yield of (-) mandelic acid was practically quantitative, m.p. 133-134°, $(\alpha)_D^{25} = -178.4°$

(c=0.6948).

The addition of excess alkali and extraction yielded (-) ephedrine for future resolutions.

The mother liquor containing the more soluble diastereoisomer was evaporated down to about one half of its volume on a steam bath in a current of air. The solution is cooled and diluted with three times its volume of ether with efficient stirring. Any crystals separating consisted of impure (-) ephedrine (-) mandelate which should be removed by filtration. The filtrate was denuded of ether and alcohol by distillation on a steam bath under reduced pressure. Upon cooling a viscous, uncrystallizable, brown residue remained which consisted of (-) ephedrine (+) mandelate.

Acidification and extraction with ether as described for the recovery of (-) mandelic acid yielded (+) mandelic acid, m.p. 132-133°, $(\alpha)_D^{25}$ = +173° (c=0.6907). If the crystals were highly colored they were purified by dissolving them in a minimum of hot acetone and adding an excess of benzene or toluene to the acetone solution.

The acid solution was made basic and the (-) ephedrine recovered by extraction with ether.

As described above the (-) ephedrine (+) mandelate was not procured in crystalline form. A crystalline product was obtained by dissolving (-) ephedrine and (+) mandelic acid in ether separately and then mixing the two solutions with rapid and prolonged stirring; m.p. 78-91° (repeated crystallizations produced no change in the fusion interval), $(\alpha)_D^{25.5}$ +21.29° (c=2.8180).

## 4.Resolution of dl-Benzedrine

### A.Utilizing (-) mandelic acid

Experiments with various solvents were carried out in order to ascertain the best solvent to employ for resolution. (-) Mandelic acid (0.5 gm.) and racemic benzedrine (0.4 gm.) were simultaneously dissolved in 10 cc. of petroleum ether, xylene, benzene, toluene, ether, diisopropyl ether, methyl alcohol, 95% ethyl alcohol, absolute alcohol, and isopropyl, normal butyl and isoamyl alcohols. The first six were abandoned for the desired benzedrine mandelates were practically insoluble in them. In general, the higher the molecular weight of the alcohol employed the less soluble was the complex. Of the group, absolute alcohol proved to be most efficacious and hence was employed in the following procedure.

Procedure 1:Twenty grams of dl-benzedrine and 25 gms. of (-) mandelic acid (0.15 mol. in both cases) were each dissolved separately in 20 cc. of absolute alcohol. The two solutions were mixed and heated on a steam bath for 30 minutes. The clear solution is allowed to cool gradually. The weight of crystalline deposit amounts to 38 gms. After five recrystallizations from 50 cc. portions of absolute alcohol 21.6 gms. of optically pure (+) benzedrine (-) mandelate was procured, m.p. 162-163°, $(\alpha)_D^{25} = -50.0°$ (c=2.2368).

The addition of (+) benzedrine (-) mandelate to NaOH solution (20%), extraction with ether, drying of the ethereal solution over anhydrous $Na_2CO_3$ and removal of the

ether by suction-pump yielded (+) benzedrine, $(\alpha)_D^{24.5} = +3.8°$ (c=3.4168).

> Application of heat in the removal of ether showed that exposure of the free base to a temperature of 90° for 30 minutes caused racemization.

For the acquisition of the more soluble diastereoisomer the mother liquors from the first three crystallizations were combined and concentrated to one-half the original volume on a steam bath in a current of air. The residue was cooled and then three times as much ether was added to the alcoholic solution remaining. The crystals were filtered with suction and redissolved in a minimum of hot absolute alcohol. Again ether was added after cooling and the process was repeated for a third time to yield (-) benzedrine (-) mandelate, m.p. 166°, $(\alpha)_D^{26} = -68.6°$ (c=1.7054). The free base, (-) benzedrine had a specific rotation of -3.8°, at 28°, (c=2.5633).

Procedure 2:The solubility of (-) benzedrine (-) mandelate was found to be 2.46 gms. per 100 gms. of absolute alcohol. Eleven grams of dl-benzedrine (0.08 mol.) and 12.4 gms. of (-) mandelic acid (0.08 mol.) were simultaneously dissolved in a quantity of absolute alcohol (475 gms.) calculated to dissove merely the 16.33 gms. of (-) benzedrine (-) mandelate theoretically formed. The solution was gently heated on a steam bath for 30 minutes and after cooling 12.2 gms. of crystalline deposit was procured. The crystals were dissolved in 50 cc. of hot absolute alcohol. Upon cooling 11 gms. of optically pure (+) benzedrine (-) mandelate

was obtained.

The mother liquor from the first crystallization was evaporated on a steam bath until the volume decreased by about 100 cc. The residue was cooled and the crystals of impure (+) benzedrine (-) mandelate were removed by filtration. Further concentration as directed under procedure 1 (p.46) and addition of ether, etc., ultimately yielded (-) benzedrine (-) mandelate.

B.Utilizing (+) Mandelic acid

Procedure:The method followed was similar to procedure 2 (p.46) except that the dextro-acid was employed. The less soluble diastereoisomer procured was (-) benzedrine (+) mandelate, m.p. 163°, $(\alpha)_D^{28}$ = +49.8° (c=1.6045). The rotation of the free base was -3.8° at 27.5° (c=2.6407).

All attempts to procure the more soluble diastereoisomer in a state of optical purity proved unsuccessful. After eleven recrystallizations from ether-alcohol solution the crystals melted at 163.5° and $(\alpha)_D^{25}$ = +53.6° (c=1.8449).

C.Preparation of dl-Benzedrine.dl-Mandelate

Procedure:Racemic benzedrine (0.01 mol.) and racemic mandelic acid (0.01 mol.) were each dissolved separately in 20 cc. of ether. Upon admixture of the two solutions there was a quantitative precipitation of the amine salt, m.p. 156.5°.

D.Density determination

The diastereoisomers (-) benzedrine (-) mandelate and (-) benzedrine (+) mandelate showed a significant difference in density. The method employed simply consisted in the determination of the volume of toluene displaced by a given weight of compound. The values found:

(-) Benzedrine (-) mandelate = 1.27

(-) Benzedrine (+) mandelate = 1.19

5.Resolution of dl-Isobenzedrine

A.Utilizing (-) mandelic acid

Procedure: The method followed was similar to procedure 1 (p.45) with the exception that secondary butyl alcohol was the solvent employed for the recrystallization of the less soluble diastereoisomer. This alteration was made for the mandelate of isobenzedrine was recrystallized with difficulty from absolute alcohol on account of its great solubility.

The physical properties of the less soluble (-) isobenzedrine (-) mandelate were: m.p. 127-127.5°, $(\alpha)_D^{25.6} = -57.8°$, (c=2.0735). The free base, (-) isobenzedrine: $(\alpha)_D^{29.8} = -18.82°$ (c=2.5492).

The more soluble complex procured by three recrystallizations from ether-alcohol melted at 118.5-119°, $(\alpha)_D^{29.8} = -47.5°$ (c=1.2841). The free base, (+) isobenzedrine: $(\alpha)_D^{29.8} = +18.8°$ (c=2.4651).

B.Utilizing (+) mandelic acid

Procedure: Following the procedure 1 (p.45) the less soluble diastereoisomer (+) isobenzedrine (+) mandelate was procured in optically pure form, m.p. 127-127.5°, $(\alpha)_D^{29.8}$ = + 58.7° (c=1.5818).

C.Preparation of dl-isobenzedrine.dl-mandelate

Procedure: The method followed is described under part C (p.47). The compound had a melting point of 119.5-120.5°.

6.Resolution of dl-Propadrine and dl-Pseudopropadrine

A.Separation of racemic propadrine from racemic pseudopropadrine

Procedure: The crude mixture of the 4 bases (m.p.62°) was purified by recrystallization from absolute alcohol, m.p. 64.5°. The bases were dissolved in benzene and dry HCl gas was bubbled into the cooled solution for at least 4 hours.

The HCl was generated by dropping concentrated $H_2SO_4$ upon concentrated HCl and passing the moist vapors through a $CaCl_2$ tube.

The yield of the hydrochlorides was quantitative.

If the crude product was dissolved in benzene and the hydrochlorides precipitated, the impurity which was formed in a side reaction in the condensation of benzaldehyde and nitroethane, was separated by recrystallization from water. The impurity, an amine hydrochloride, being less soluble than the propadrine salts is separated by filtration. The mother liquor is made basic and the free bases recovered, upon reprecipitation from benzene solution as the hydrochlorides are utilized for the subsequent resolutions.

The impurity was analyzed by Mr.Bernard Sussman. He reported: N-5.67% Cl-16.75%.

The hydrochloride of dl-propadrine is less soluble than dl-pseudopropadrine hydrochloride in absolute alcohol. Separation was effected by repeated recrystallizations from absolute alcohol. Racemic propadrine hydrochloride melted at 194°; reported 192° (77) and 194° (78). The free base recrystallized from benzene had a fusion point of 104-105°.

Pure dl-pseudopropadrine hydrochloride was procured by concentrating the first mother liquor down to the point of incipient crystallization, cooling and then adding a volume of ether three times as great as that of alcohol. The process was repeated at least twice to yield crystals melting at 170.5-171.5°; reported 169° (78).

Another method just as expeditious consisted in evaporating the mother liquor from the initial crystallization to dryness on a water bath and dissolving the residue in secondary butyl alcohol. Recrystallization of the crystals procured in this manner from a fresh portion of secondary butyl alcohol yielded pure dl-pseudopropadrine hydrochloride.

The free dl-pseudopropadrine base had a melting point of 71.5° after recrystallization from toluene.

B.Resolution of dl-propadrine utilizing (-) mandelic acid

Procedure: Following the method described under the resolution of benzedrine, procedure 1 (p.45) and employing absolute alcohol the less soluble diastereoisomer procured

was (-) propadrine (-) mandelate, m.p. 171.5-172°, $(\alpha)_D^{29} = -70.6°$ (c=2.3506). The free base procured by the addition of excess alkali was washed free of alkali with several portions of distilled water; the m.p. 102°, $(\alpha)_D^{30} = -19.9°$ (c=0.2641). Kanao (78) reported a melting point of 50° upon recrystallization of the free base from water and a specific rotation of -14.56° at a temperature of 20° in absolute alcohol.

(+) Propadrine (-) mandelate isolated by utilizing ether-alcohol for each recrystallization melted at 164.5-165°, $(\alpha)_D^{29.5} = -42.8°$ (c=1.2390). The free base, m.p. 102°, $(\alpha)_D^{32} = +20.8°$ (c=0.2400).

C.Resolution of dl-propadrine utilizing (+) mandelic acid

Procedure: The method followed is described under the resolution of benzedrine, procedure 1 (p.45). The less soluble form, (+) propadrine (+) mandelate, has a melting point of 171.5-172°, $(\alpha)_D^{29.5} = +70.7°$ (c=2.3460).

D.Resolution of dl-pseudopropadrine utilizing (-) mandelic acid

Procedure: The method followed is described under the resolution of benzedrine, procedure 1 (p.45).

The less soluble diastereoisomer (+) pseudopropadrine (-) mandelate melted at 170°, $(\alpha)_D^{32.4} = -45.3°$ (c=1.1905). The free base, (+) pseudopropadrine has a m.p. of 77.5-78°, and $(\alpha)_D^{20} = +33.14$ (absolute alcohol) according to Kanao (78). In this investigation the hydrochloride of the base was utilized for the determination of the specific rotation. (+) Pseudopropadrine hydrochloride melted at 179°, $(\alpha)_D^{29} = +41°$

(c=0.1584, in distilled water).

(-)ψ-Propadrine (-) mandelate is procured by concentration of the first mother liquor and addition of excess ether. The crystals thus procured were recrystallized from secondary butyl alcohol several times to yield optically pure (-) pseudopropadrine (-) mandelate, m.p. 163.5°, $(\alpha)_D^{32.4} = -41.3°$ (c=0.7999). (-) Pseudopropadrine hydrochloride melted at 178°, $(\alpha)_D^{29} = -38.7°$ (c=0.2100, in distilled water).

E.Preparation of dl-propadrine.dl-mandelate

Procedure: The method followed is similar to that described under part C (p.47). The compound dl-propadrine. dl-mandelate has a melting point of 161-162°.

F.Preparation of dl-pseudopropadrine.dl-mandelate

Procedure: The method followed is similar to that described under part C (p.47). The compound dl-pseudopropadrine.dl-mandelate melted at 162.5-163°. Mixture of the two racemic mandelates (E and F) melted at 156-158°.

7.Resolution of dl-Phenylethanolamine

A.Utilizing (-) mandelic acid

Procedure: Three grams of racemic phenylethanolamine (0.02 mol.) and 3.3 gms. of (-) mandelic acid (0.02 mol.) were dissolved simultaneously in 20 cc. of commercial absolute alcohol. Lengthy cooling and scratching of the sides of the beaker caused no formation of crystals. The addition of a large excess of ether caused an almost immediate precipitation. The crystals were dissolved in secondary butyl alcohol and allowed to cool gradually. The melting point of the

(-) phenylethanolamine (-) mandelate procured was 144-145°, $(\alpha)_D^{31} = -58.3°$ (c=0.8916).

The free base had a specific rotation of -20.9° at a temperature of 30° (c=0.0956).

B. Preparation of dl-phenylethanolamine.dl-mandelate

Procedure: The method followed is described under part C (p.47). The compound dl-phenylethanolamine-dl-mandelate melted at 129.5-130°.

TABLE II

Physical Data of Antipodes Studied

| Name of compound | M.P. | $(a)_D$ | T | Concn. | Remarks |
|---|---|---|---|---|---|
| (-) Ephedrine | 34-40 | -3.47 | 27 | 4.3130 | Most active isomer of ephedrine |
| (-) Propadrine | 102 | -19.9 | 30 | 0.2641 | More active pressor than (+) |
| (+) Propadrine | 102 | +20.8 | 32 | 0.2400 | |
| (+) Pseudopropadrine hydrochloride | 179 | +41 | 29 | 0.1584 | More active as a pressor than (-) |
| (-) Pseudopropadrine hydrochloride | 178 | -38.7 | 32 | 0.2100 | |
| (+) Benzedrine | | +3.8 | 25 | 3.4168 | More active stimulant of C.N.S. |
| (-) Benzedrine | | -3.8 | 28 | 2.5633 | |
| (-) Isobenzedrine | | -18.82 | 30 | 2.5492 | |
| (+) Isobenzedrine | | +18.8 | 30 | 2.4651 | |
| (-) Phenylethanolamine | | -20.9 | 30 | 0.0956 | |

## 8.Determination of Solubility

General Procedure: Ten cubic centimeters of distilled water and normal saline solution were placed in 25 cc. test tubes. The diastereoisomer was added in small portions until the solution was saturated and an excess of amine salt remained on the bottom of the test tube. The test tubes were stoppered and suspended in a constant temperature bath for six hours. Every half hour the tubes were vigorously shaken up and down for two minutes.

At the end of six hours 3 cc. samples were collected in accurately weighed weighing bottles. For procuring solutions free of undissolved matter, either filtration through a pledget of raw cotton inserted in the neck of a small funnel, or utilization of a 5 cc. pippette whose tip was wrapped in raw cotton were the methods employed.

The weights of the solutions were determined and the major portion of the distilled water was removed by heating the solutions in the weighing bottles over a steam bath for 5-6 hours. The residues were dried to constant weight at 100°. All values are expressed in grams per 100 grams of distilled water.

In the case of normal saline solutions the weight of sodium chloride was subtracted from the total weight of the residue and the solubility values still represent grams of diastereoisomer soluble in 100 grams of distilled water. The weight of sodium chloride was determined by multiplying the weight of distilled water by the percentage of sodium

chloride in normal saline for 37° or 25° as actually determined rather than by ashing each residue. The accuracy of this method was substantiated in the case of (+) benzedrine (-) mandelate (37°) and (-) benzedrine (+) mandelate (37°) as shown by the following values:

| | Calculated wt. of NaCl | Found (Ash) |
|---|---|---|
| (+) benzedrine (-) mandelate | 0.0371 | 0.0374 |
| (-) benzedrine (+) mandelate | 0.0462 | 0.0464 |

TABLE III.

The Physical Properties and the Solubility of Diastereoisomers Studied

| Name of compound | M.P. | $(a)_D$ | T | Concn. | Solubility 37° Distilled water | Solubility 37° Normal saline | Difference | Solubility 25° Distilled water | Solubility 25° Normal saline | Difference |
|---|---|---|---|---|---|---|---|---|---|---|
| (-)Ephedrine(-)mandelate | 170 | -70.6 | 26.5 | 2.4056 | 6.27 | 6.99 | 0.72 | 6.00 | 5.95 | -0.05 |
| (-)Ephedrine(+)mandelate | 78-91 | +21.29 | 25.5 | 2.8180 | 80.61 | 92.34 | 11.73 | 77.48 | 88.68 | 11.20 |
| (-)Propadrine(-)mandelate | 171.5-172 | -70.6 | 29 | 2.3506 | 7.39 | 7.36 | -0.03 | 6.08 | 6.30 | 0.22 |
| (+)Propadrine(-)mandelate | 164.5-165 | -42.8 | 29.5 | 1.2390 | 10.69 | 10.19 | -0.50 | 7.70 | 7.26 | -0.44 |
| (+)Propadrine(+)mandelate | 171.5-172 | +70.7 | 29.5 | 2.3460 | 8.36 | 7.34 | -1.02 | 5.58 | 5.95 | 0.37 |
| dl-Propadrine.dl-mandelate | 161-162 | | | | 8.08 | 8.14 | 0.06 | 5.35 | 6.00 | 0.65 |
| (+)Pseudopropadrine(-)mandelate | 170 | -45.3 | 32.4 | 1.1905 | 8.43 | 9.56 | 1.13 | 6.22 | 6.23 | 0.01 |
| (-)Pseudopropadrine(-)mandelate | 163.5 | -41.3 | 32.4 | 0.7999 | 18.74 | 19.01 | 0.27 | 11.95 | 15.02 | 3.07 |
| dl-Pseudopropadrine.dl-mandelate | 162.5-163 | | | | 11.41 | 11.06 | -0.35 | 8.67 | 8.66 | -0.01 |
| (+)Benzedrine(-)mandelate | 162-163 | -50.0 | 25 | 2.2368 | 4.63 | 4.77 | 0.14 | 3.93 | 4.18 | 0.25 |
| (-)Benzedrine(-)mandelate | 166 | -68.6 | 26 | 1.7054 | 7.25 | 7.56 | 0.31 | 5.98 | 6.24 | 0.26 |
| (-)Benzedrine(+)mandelate | 163 | +49.8 | 28 | 1.6045 | 4.80 | 4.85 | 0.05 | 3.94 | 4.21 | 0.27 |
| dl-Benzedrine.dl-mandelate | 156.5 | | | | 6.98 | 7.42 | 0.44 | 5.73 | 6.19 | 0.46 |
| (-)Isobenzedrine(-)mandelate | 127-127.5 | -57.8 | 25.6 | 2.0735 | 25.30 | 28.08 | 2.78 | 14.59 | 15.59 | 1.00 |
| (+)Isobenzedrine(-)mandelate | 118.5-119 | -47.5 | 29.8 | 1.2841 | 63.46 | 61.15 | -2.31 | 51.15 | 48.88 | -2.27 |
| (+)Isobenzedrine(+)mandelate | 127.0-127.5 | +58.7 | 29.8 | 1.5818 | 27.23 | 31.95 | 4.72 | 14.39 | 15.54 | 1.15 |
| dl-Isobenzedrine.dl-mandelate | 119.5-120.5 | | | | 20.13 | 18.36 | -1.77 | 11.50 | 11.31 | -0.19 |
| dl-Phenylethanolamine.dl-mandelate | 129.5-130 | | | | 18.56 | 19.79 | 1.23 | 11.63 | 11.78 | 0.15 |
| (-)Phenylethanolamine(-)mandelate | 144-145 | -58.3 | 31 | 0.8916 | | | | | | |

# SECTION V.

## DISCUSSION OF RESULTS

Pharmacodynamic information about the isomers of isobenzedrine and phenylethanolamine is not available. However, from analogies with other compounds, as described below, the prediction is ventured that the levorotatory isomer will prove to be the more active of each pair.

Of each isomeric pair the base forming the less soluble salt with d(-) mandelic acid is the more active physiologically. For example, (-) propadrine (-) mandelate is 78.8% as soluble (25°) as (+) propadrine (-) mandelate (68.8% at 37°). (+) Pseudopropadrine (-) mandelate is 52% as soluble (25°) as (-) pseudopropadrine (-) mandelate (44% at 37°). For (+) benzedrine (-) mandelate and (-) benzedrine (-) mandelate the values are 42% (25°) and 64% (37°). Of the enantiomorphous pairs (-) propadrine, (+) pseudopropadrine, and (+) benzedrine are the more active.

In benzedrine, where the amino-bearing carbon atom is asymmetric, the more active form is dextrorotatory. In epinephrine, where the hydroxyl-bearing carbon atom is asymmetric, the levo-rotating isomer is more active. In ephedrine and propadrine, which have two asymmetric centers, one corresponding to the hydroxyl-bearing carbon of epinephrine and the other to the amino-bearing carbon of benzedrine, the most active of the four optical isomers is that configuration in which the carbinol carbon atom

is levorotatory and the carbinamine carbon atom is dextrorotatory.

If in (-) ephedrine, according to the data of Chen, Wu and Henriksen (Table I.,page 33), the configuration of the carbon atom 1 is reversed, i.e. in (+) pseudoephedrine, the pressor activity is considerably decreased but the mydriatic potency remains unchanged. If instead the configuration of carbon 2 is changed, that is (-) pseudoephedrine, both pressor and mydriatic activities are very markedly decreased.

If in (-) pseudoephedrine the configuration about carbon number 1 is reversed, that is (+) ephedrine, the mydriatic and pressor action are both increased but not as much as if the configuration of carbon 2 is changed.

From these results one is lead to believe that the optimum configuration for activity is found in those isomers in which carbon number 1, if asymmetric, is dextrorotatory (d-series according to Freudenberg) and in which carbon number 2, if asymmetric, is dextrorotatory (d-series if referred to phenylalanine, l-series if referred to alanine, see page 29).

Other correlations between solubility and physiological effects do not appear in the data thus far obtained. It is not unlikely, however, that as more information about the physical and physiological properties becomes available that further correlations may be found.

The experimentally determined solubilities of (-) benzedrine (+) mandelate and (+) benzedrine (-) mandel-

ate are in good agreement; this is to be expected since the two salts are enantiomorphs. The values determined for (-) propadrine (-) mandelate and (+) propadrine (+) mandelate, another pair of enantiomorphous salts, are not in such good agreement. These should be carefully checked before concluding that the differences are significant.

The difference in densities between the diastereoisomeric salts (-) benzedrine (-) mandelate and (-) benzedrine (+) mandelate is large and is suggestive that similar differences may be expected in cases of other diastereoisomers. Whether these density values may be correlated with physiological properties can be determined only after more such information becomes available.

This study reveals a striking parallelism of properties between isobenzedrine and phenylethanolamine, as may be seen from table IV.

TABLE IV.

Comparison of the Physical Properties of Isobenzedrine and Phenylethanolamine

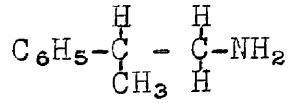
| Name of compound | M.P. | $(a)_D$ |
|---|---|---|
| (-)Isobenzedrine(-)mandelate | 127-127.5 | -57.8 |
| (-)Ethadrin(-)mandelate | 144-145 | -58.3 |
| dl-Isobenzedrine.dl-mandelate | 119.5-120.5 | |
| dl-Ethadrin.dl-mandelate | 129.5-130 | |
| (-)isobenzedrine | | -18.82 |
| (-)ethadrin | | -20.7 |

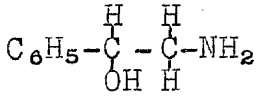
SOLUBILITY

| | 37° | | 25° | |
|---|---|---|---|---|
| | Water | Saline | Water | Saline |
| dl-isobenzedrine.dl-mandelate | 20.13 | 18.36 | 11.50 | 11.31 |
| dl-ethadrin.dl-mandelate | 18.56 | 19.79 | 11.63 | 11.78 |

---

This suggests a tantalizing type of isosterism,

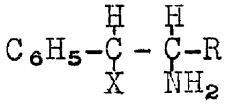




in which a hydroxyl group of a physiologically active compound may be replaced by a methyl and only relatively minor changes in physical properties are produced.

This isosteric analogy apparently holds for physiological properties. When given parenterally both are effective pressors; on the other hand oral administration shows no activity. Furthermore both compounds exert only a slight stimulant action on the central nervous system.

These unusual correlations suggest the desirability of comparing the physical and physiological properties of an active molecule of the type



in which X represents the first element in groups IV, V, VI, and VII of the periodic table joined by a single bond to the remainder of the molecule, the other valences being satisfied with hydrogen atoms, thus

| $-CH_3$ | $-NH_2$ | $-OH$ | $-F$ |
|---|---|---|---|
| (15) | (16) | (17) | (19) |

The molecular weights of the four isosters would be nearly the same and there should be little difference in electronic structure and molecular size.

# SECTION VI

## SUMMARY

1.A literature survey is presented which deals with the relationship of asymmetry and physiological activity.

2.The spatial configuration and the pharmacological activity of the pressor amines of the ephedrine series is discussed.

3.Propadrine, pseudopropadrine, benzedrine, β-phenylpropylamine, and phenylethanolamine were resolved into their enantiomorphous forms. For this purpose mandelic acid was prepared and resolved into (+) and (-) forms with (-) ephedrine. A study of the physical properties of the free bases as well as the diastereoisomeric mandelates was carried out.

4.The correlation of solubility with physiological activity is discussed on the basis of a quantitative determination of the solubility of the mandelates in normal saline and distilled water at 25° and 37°.

5.The isosteric, physical and physiological analogy between phenylethanolamine and isobenzedrine is pointed out.

## SECTION VII

## BIBLIOGRAPHY

(1) Pasteur, L.
"Recherches sur la dissymmetrie moleculaire des produits organiques naturels", Paris (1861).

(2) Kekule, F.A.
Zeitschr. f. Chemie, N.F. 3, 217, (1867).

(3) Paterno, E.
Giorm. di Scienze Naturali ed Econ. V.Palermo (1869). Through: Stewart, A.W., "Stereochemistry" 2 nd. edition, Longmans, Green & Co.,New york. (1919).

(4) Wislecenus, J.
Ann. 167, 343, (1873).

(5) Van't Hoff, J.H.
"Voorstell tot uitbreidung der struktuur formules in de ruimte Utrecht" (1874). Through Stewart.

(6) Le Bel, J. A.
Bull. soc. chim. ii, 22, 377 (1874).

(7) Fischer, E.
Ber. 24, 1836, 2683, (1891).
Ber. 27, 3208, (1894).

(8) Clough, G.W.
J. Chem. Soc. 113, 526-554, (1918).

(9) Hudson, C. S.
J. Am. Chem. Soc. 32, 338, (1910).
J. Am. Chem. Soc. 40, 813, (1918).

(10)Freudenberg, K., Brauns, F., and Siegel, H.
Ber. 56, 193, (1923).

(11)Bredig, G., and Fajans, K.
Ber. 41, 752, (1908).

(12)Marckwald, W., and McKenzie, A.
J. Chem. Soc. 964, (1899).

(13)Pasteur, L.
Compt. rend. 51, 298, (1860).

(14)Fischer, E.
Ber. 27, 2035, (1894).

(15)Fischer, E.
Z. physiol. Chem. 26, 60, (1898).

(16)Akamatsu, S.
Biochem. Z. 142, 188, (1923).

(17)Rosenthaler, L.
Biochem. Z. 14, 238, (1909).

Krieble, V. K.
J. Am. Chem. Soc. 35, 1643, (1913).

(18)Ehrlich, F.
Biochem. Z. 63, 379, (1914).

(19)Tomita, M.
Biochem. Z. 123, 231, (1921).

(20)Mayer, A.
Biochem. Z. 9, 439, (1908).

(21)Hamalainen
Skand. Arch. f. Phys. 23, 86, (1910).

(22) Magnus-Levy, A.
Biochem. Z. 2, 329, (1907).

(23)Wohlgemuth, J.
Ber. 38, 2064, (1905).

(24)Piutti
Compt. rend. 103, 134, (1886).

(25)Cushny, A.R.
Journ. Phys. 30, 176, (1904).

(26)Laidlaw, P. P.
J. Chem. Soc. 95 II, 1966, (1909).

(27)Cushny, A. R.
Journ. Pharm. and Exper. Ther. 17, 41, (1921).

(28)Cushny, A. R.
Journ. Pharm.and Exper. Ther. 15, 105, (1920).

(29)Mayor, A.
Through Pictet, A., Ber. 37, 1234, (1904).

(30)Cushny, A. R.
Journ. Phys. 37, 130, (1908).
Journ. Phys. 38, 259, (1909).

(31)Tiffeneau, M.
Cong. de Physiol., Paris, (1920).

(32)Gottlieb, R.
Arch. exp. Path. Pharmakol. 97, 113, (1923).

(33)Poulsson, E.
Arch. exp. Path. Pharmakol. 27, 309, (1890).

(34) King, H.
Trans. Chem. Soc. 125, 41,(1924).(or J. Chem.Soc.).

(35) Ladenburg, A., and Falck
Ann. 247, 83, (1888).

(36) Tainter, M.L., and Seidenfeld, M.H.
J. Pharmacol. 40, 23, (1930).

(37) Cushny, A. R.
"Biological Relations of Optically Isomeric Substances", Williams and Wilkins, Baltimore, (1926).

(38) King, H.
Through Cushny, page 62. See (37).

(39) Easson, L.H., and Stedman, E.
Biochem. J. 27, 1257-66, (1933).

(40) Beutner, R.
J. Pharmacol. 31, 305-318, (1927).

(41) Nagai, N.
Pharm. Ztg. xxxii, 700, (1887).

(42) Nagai, N.
J. Pharm. Soc. Japan cccxxix, 739, (1909).

(43) Eberhard, A.
Arch. Pharm. ccliii, 62, (1915).

(44) Eberhard, A.
Arch. Pharm. cclviii, 97, (1920).

(45) Fourneau, E., and Kanao, S.
Bull. soc. chim. 4 th. ser. xxxv, 614, (1924).

(46) Spath, E., and Gohring, R.
Monatsch. Chem. xli, 319, (1920).

(47) Spath, E., and Koller, G.
Ber. 58, 1268, (1925).

(48) Kanao, S.
J. Pharm. Soc. Japan 560, (Vol.48, No.10) October, (1928).

(49) Jenkins, G. L., and Hartung, W. H.
"The Chemistry of Organic Medicinal Products", John S. Swift Co., Inc. (1941) p.435.

(50) Nagai, N.
J. Pharm. Soc. Japan 121, 200, (1892).

(51)Gadamer, J.
Arch. Pharm. 246, 566-74, (1908).

(52)Schmidt, E.
Ar. 251, 320, (1913).

(53)Leithe, W.
Ber. 64B, 2827-32, (1931).

(54)Freudenberg, K., Schoffel, E., and Braun, E.,
J. Am. Chem. Soc. 54, 234-236, (1932).

(55)Leithe, W.
Ber. 65B, 660-666, (1932).

(56)Freudenberg, K., and Nikolai, F.
Ann. 510, 223, (1934).

(57)Levene, P. A., Mikeska, L. A., Passoth, K.
J. Biol. Chem. 88, 27-59, (1930).

(58)Reihlen, H., Knopfle, L., and Sapper, W.
Ann. 534, 247-75, (1938).

(59)Nagel, A.
Arch. exp. Path. Pharmakol. cx, 129, (1925).

(60)Chen, K.K., and Schmidt, C.F.
Journ. Pharm. and Exp. Ther. xxiv, 339, (1924).

(61)Chen, K. K., Wu, Chang-Keng, and Henriksen, E.
Journ. Pharm. and Exp. Ther. 36, 363-400, (1929).

(62)Tainter, M.L.
J. Pharmacol. 40, 43, (1930).

Launoy, L., and Menguy, B.
Compt. rend. Soc. Biol. 87, 1066, (1922).

(63)Kanao, S.
Journ. Pharm. Soc. Japan xlviii, 947, 1070, (1928).

(64)Alles, G. A.
Univ. Calif. Publ. Pharm. 1, 129, (1939).

(65)Alles, G. A., and Prinzmetal, M.
American Journal of the Medical Sciences No.5
200, 665-673, (1940).

(66)Tainter, M. L., Schulte, J. W., Reif, E. C., Bacher, Jr.
J. A., and Lawrence, W. S.
J. Pharmacol. 71, 62-74, (1941).

(67)Hartung, W. H., and Munch, J. C.
J. Am. Chem. Soc. 53, 1875, (1931).

(68) Hauschild, F.
Arch. exp. Path. Pharmakol. 195, 647-680, (1940).

(69) Chen, K. K., and Schmidt, C. F.
Medicine 9, 88, (1930).

(70) Corson, B. B., Dodge, Ruth A., Harris, S. A., Yeaw, J. S.,
Organic Synthesis VI, 58-62, John Wiley & Sons,
Inc.

(71) Wood, C. E., and Lilly, H. S.
J. Chem. Soc. 127, 95, (1925).

(72) Gatterman, L., and Wieland, H.
"Laboratory Methods of Organic Chemistry", The
Macmillan Co., (1932) page 219.

(73) Manske, R. H. F., and Johnson, T. B.
J. Am. Chem. Soc. 51, 1906-1909, (1929).

(74) Roger, R.
J. Chem. Soc. 1544, (1935).

(75) Sudborough, J. J., and Bernthsen, A.
"Text Book of Organic Chemistry", D. Van Nostrand
Co., Inc., (1931) page 492.

(76) Marckwald, W., and McKenzie, A.
Ber. 32, 2130, (1899).
Ber. 34, 469, (1901).

(77) Hey, D. H.
J. Chem. Soc. 1234, (1930).

(78) Kanao, S.
Journ. Pharm. Soc. Japan No.560, Vol.48, No.10
October (1928).