I. STUDIES ON THE MESCAL ALKALOIDS AND RELATED COMPOUNDS

II. PREPARATION OF SEVERAL PHOSPHO-ORGANIC COMPOUNDS

Thesis by

Paul R. Saunders

In Partial Fulfilment

of the Requirements for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1943

Acknowledgment

TABLE OF CONTENTS

PART I

STUDIES ON THE MESCAL ALKALOIDS AND RELATED COMPOUNDS

INTRODUCTION

Pellote (peyote, peyotl, mescal buttons) is the drug used by the Indians of northern Mexico and the southwestern United States for the hallucinatory effects produced by it when eaten. The actions of the drug are probably entirely due to the alkaloids present, eleven of which have been isolated to date.

Pellote is obtained from the cactus which is now most commonly classified as Lophophora williamsii (Fam. Cactaceae). It has also been classified as Anhalonium Lewinii, which was formerly most generally accepted, Echinocactus williamsii, and others. The tops of the plant, which is a small spineless cactus covered with wooly hairs, are cut off and eaten either fresh or dried, and are commonly known as "mescal buttons". It is found chiefly in northern Mexico and near the Rio Grande River in Texas, and though its use has spread somewhat, it is still confined to a comparatively few tribes of this general area. The term mescal button should not be confused with the fermented drink made in Mexico known as mescal, which is a distilled beverage prepared from various species of Agave.

The use of pellote in religious ceremonies by the Indians of the Rio Grande valley has been known for many years, and an interesting account of the actual ceremony is given by James Mooney (1). Quoting briefly from his description: "The ceremony occupies from twelve to fourteen hours, beginning at about 9 or 10 o'clock and lasting

sometimes until nearly noon the next day. The exercises
open with a prayer by the leader, who then hands each man
four mescals, which he takes and eats in rapid succession.
In eating, the dry mescal is first chewed in the mouth, then
rolled into a large pellet between the hands, and swallowed.
After this first round the leader takes the rattle, while
his assistant takes the drum, and together they sing the
first song four times, with full voices, at the same time
beating the drum and shaking the rattle with all the strength
of their arms. The drum and rattle are then handed to the
next couple, and so the song goes on round and round the
circle - until perhaps 9 o'clock the next morning, when the
instruments are passed out of the tepee, the sacred foods
are eaten, and the ceremony is at an end." There appear to
be no undesirable after-effects, and the drug is often used
by the Indians for therapeutic purposes, as in the treat-
ment of fevers and headaches.

There are many descriptions of the effects produced
by pellote in man, among which might be mentioned those of
Mitchell (2), Havelock Ellis (3), Prentiss and Morgan (4),
and Fernberger (5). The general picture of the effects of
the crude drug is well-known, but the actions of the various
alkaloids present have not been studied at all thoroughly,
and a great deal of work remains to be done on this subject.

The outstanding action of pellote and the one probably
chiefly responsible for its use by the Indians is the pro-
duction of color hallucinations. The first phase of the

intoxication is marked by a general feeling of euphoria, and often stimulation is observed, leading to the loss of a sense of fatigue. When the second stage is reached, the individual begins to have the characteristic color visions, and in some cases other sensory effects. Descriptions of the visions indicate a great individual variation in response to the drug, but in general they are characterized by an incessant flow of hallucinations of great variety of form and color, sometimes being composed of geometric figures or familiar objects, usually in constant motion, and sometimes various grotesque figures are observed, all intensely colored. The visions are most often obtained with the eyes shut or in the dark, but are sometimes seen in the light. During this period the subject is completely conscious and aware of his surroundings. In addition, there are sometimes hallucinations of hearing and an increase in auditory acuity. The general sensibility may be affected in such a manner that the subject has the illusion of being without weight, or of the doubling of the ego. There may be kinesthetic disturbances leading to difficulty in ascertaining the location of the arms or legs. Accompanying these sensations are various physical changes, such as mydriasis, drop in pulse rate, and reddening of the face.

The subjective sensations experienced are illustrated by the following quotation from Mitchell (2). After taking six buttons he "soon became aware of a violet haze, and also had the subjective sensation of an increased competence in

dealing with problems, which was not born out in practice". On lying in a darkened room, he saw "delicate floating films of color .....zig-zag lines of very bright colors, in rapid motion .....I began to see for the first time definite objects associated with colors. A white spear of grey stone grew up to huge height, and became a tall Gothic tower of elaborate design .....covered with clusters of what seemed to be huge precious stones. These were green, purple, red, and orange. All the colors I have ever beheld are dull as compared with these .....the vast pendant masses of emerald green, ruby red, and orange began to drip a slow rain of color". A series of visions followed, until the effects of the drug had disappeared in a few more hours. In some descriptions it is stated that sensory and auditory stimuli often have an effect on the intensity and nature of the visions produced.

Extensive studies on the "mescal psychosis" are described by Beringer (6), Rouhier (7), and Klüver (8). The three main constituents of the psychosis seem to be (1) sensory abnormalities, (2) a change in conscious attitude and condition, and (3) emotional abnormalities.

Actions of mescal alkaloids on man

Mescaline. The subjective sensations of mescal intoxication in man are fairly closely duplicated by the action of the chief alkaloid, mescaline, at doses of about 200 mgm. or larger. Inasmuch as it is customary to take about five buttons to obtain the typical hallucinations,

and as about 200 mgm. of mescaline is present in this number of the buttons, it is probable that mescaline is responsible for the major portion of the action.

Mescaline has been studied quite intensively, particularly in regard to the generation of various psychotic states. Its use in psychiatry (Stockings (9)) is based on its ability to reproduce in a normal subject phenomena closely related to those of certain psychopathological states (especially schizophrenia), and also upon the fact that these changes occur in a state of clear consciousness, enabling the subject to describe the effects to an observer.

Some of the sensations experienced by schizophrenics are known from their drawings and the descriptions given by them after the administration of sodium amytal, which often produces a temporary lucid period in a patient who is ordinarily uncommunicative. The hallucinations resulting from mescaline (visual, auditory, and somatic) are very similar to those described by these patients. The dissociation of the personality which is the most characteristic feature of schizophrenia is often experienced by persons who have taken mescaline. Other points of similarity include the catatonia produced, disturbances of mood associated with the feeling of unreality, difficulties in carrying out a logical train of thought, and other manifestations.

The mechanism of action of mescaline is unknown. From the work of Quastel and Wheatley (10) on brain respiration, it is known that mescaline, as well as a number of related

amines (tyramine, phenethylamine, and others) and typical narcotics, has the property of inhibiting the oxidation of glucose, lactate, and pyruvate.

Richter (11) found that 58% of the mescaline fed to man could be recovered in the urine, and that the remainder was unaccounted for. It is unlikely that amine oxidase is responsible for any appreciable destruction of mescaline, for Blaschko, Richter, and Schlossmann (12) found that it was attacked only very slightly in the presence of this enzyme. Slotta and Müller (13) found that 50% of the mescaline fed to guinea pigs and dogs could be recovered from the urine as 3,4,5-trimethoxy-phenylacetic acid, but none of this compound could be isolated in human experiments.

Mention is made in the literature of some visual disturbances with some of the other alkaloids, although their actions have not been investigated in this respect at all thoroughly. Heffter (14) reported the following effects after taking several of the other alkaloids by mouth:

Anhalonine (100 mgm. dose) - slight narcosis. It is reported that lassitude is experienced, together with a sensation of gliding.

Lophophorine (20 mgm. dose) - headache, reddening of the face.

Anhalonidine (100-250 mgm. dose) - slight narcosis.

Pellotine (80-240 mgm. dose) - narcosis, fatigue, aversion to mental and physical labor. Pellotine has been studied more thoroughly than any of the other alkaloids with the

exception of mescaline, particularly by Robles and Robleda (15). They reported that it causes mental confusion, visual hallucinations without the production of color, auditory and kinesthetic hallucinations, and disturbances of concepts of space and time. In doses of 15-30 mgm. it was formerly used clinically as a narcotic (Hutchings (16)).

There are no data available on the actions of the other known alkaloids of mescal on man.

Pharmacological actions of mescal alkaloids

The pharmacological effects of the various alkaloids on animals have been studied very little, with the exception of mescaline and pellotine, and even in the case of the latter compounds it is not possible at the present time to draw many conclusions from the data which have been obtained.

Mescaline. Grace (17) made a careful study on several animals of the pharmacological actions of mescaline, and some of his findings are reported below:

(1) Lethality. The lethal dose in frogs by injection into the dorsal lymph sac is 750 mgm. per kilogram body weight, and in mice by intraperitoneal injection is 500-600 mgm. per kilogram. The symptoms observed in frogs were progressive motor paralysis, arrest of respiration, and abolition of reflexes. Death was due to respiratory failure, and the main action seemed to be depression of the central nervous system. These results agree with those of Heffter on frogs, but are opposed to those of Dixon (18), who states that mescaline possesses a strychnine-like convulsant action.

(2) <u>Action on voluntary muscle</u>. Mescaline at a concentration of 1:500 paralyzed the isolated frog gastrocnemius to electrical stimulation.

(3) <u>Circulation and respiration</u>.

(a) Ten mgm. per kilogram of mescaline given intravenously to a cat anesthetized with ether-chloroform resulted in a blood pressure fall from 110 to 50 mm. Hg, which was accompanied by slowing of the respiration. The pulse rate was unchanged, although the strength of the beat was lessened. This action on the blood pressure and the respiration appears to be of a central rather than a peripheral nature, for if the vagi are sectioned, a slight rise in blood pressure is observed, and the respiration is unchanged.

(b) The action of mescaline on the decerebrate cat is similar to the action on an anesthetized animal.

(c) Ten mgm. per kilogram of mescaline given intravenously to a decapitate cat caused a rise in blood pressure of 60 mm. Hg. This pressor action is not affected when the ganglia are paralyzed by nicotine.

(4) <u>Action on the isolated heart</u>. In low concentration (1:50,000), mescaline caused a slight decrease in the amplitude of the beat, but no change in the rate. In high concentration (1:1000), there was a pronounced decrease in the amplitude, accompanied by a decrease in rate, and the heart stopped in diastole. This action seems to be directly on the cardiac muscle, for atropine was without effect. The action on the perfused rabbit heart was of the same nature

at similar concentrations.

(5) Action on involuntary muscle.

(a) The isolated intestine of the rabbit and the cat were not affected by concentrations from 1:100,000 to 1:2500. However, the intestine of the intact animal was strongly stimulated by the intravenous injection of 11 mgm. per kilogram, and the contraction was no longer obtained after an injection of atropine.

(b) The isolated uterus was not affected, while the uterus in situ was strongly stimulated.

Raymond-Hamet (19) studied the actions of mescaline on dogs under chloralose anesthesia. Artificial respiration was given to these animals, and both vagi were cut. He found that with doses of 2-8 mgm. per kilogram a slight fall or rise in blood pressure occurred, but that with doses of 20 mgm. per kilogram a fall was always observed, accompanied by slowing of the heart. This action is opposed to the observations of Grace, which were obtained with different animals and anesthetics. In addition, Raymond-Hamet showed that small doses (2-4 mgm. per kilogram) inhibited the intestine in situ, and that larger doses first stimulated, and then depressed the tonus, similarly to nicotine. However, mescaline still produced this effect after a large dose of sparteine, which abolishes the nicotine ganglionic stimulation, and therefore Raymond-Hamet concluded that mescaline acts directly on the muscle. However, Grace found that mescaline did not produce stimulation of the intestine

after the injection of atropine, so it appears that some other mechanism must be involved. The fall in blood pressure, slowing of the heart, and stimulation of the intestine indicate that mescaline does not have appreciable sympathomimetic activity.

Clerc, Paris, and Janot (20) worked with dogs anesthetized with chloralosane, and also observed a blood pressure fall, which was less pronounced after the injection of atropine or section of the vagi. They noted an increase in both the rate and amplitude of respiratory movements, which is contradictory to the results obtained by Grace on cats under chloroform-ether anesthesia.

It is difficult at present to suggest any mechanism that will satisfactorily explain the various actions of mescaline observed by these authors, although the central nervous system depression is probably related to the inhibition of brain respiration found by Quastel and Wheatley (10). With regard to the unique action of mescaline in producing color hallucinations, it seems likely that the most fruitful method of approach to the problem would be through objective observations on man, rather than attempting to study the problem by the analysis of subjective sensations or by animal experimentation.

Pellotine. Heffter (14) observed that in the frog pellotine first produced narcosis and diminished reflex activity. In about one hour the reflex activity increased, and convulsive breathing and tetanus occurred, which were

sometimes still present twelve hours later. The toxicity
for a number of animals was determined by Robles and Rob-
leda (15), and the lethal dose in mgm. of pellotine hydro-
chloride per kilogram of body weight was found to be as
follows: frog (500), rat (500), guinea pig (300), cat (150),
and pigeon (100). The pigeon, which is very sensitive to
the action of pellotine, showed incoordination, loss of
equilibrium, rapid breathing, mydriasis, and finally con-
vulsions preceding death.

Clerc, Janot, and Paris (21) found that in the rabbit
anesthetized with chloral, a dose of 5 mgm. per kilogram of
pellotine hydrochloride lowered the blood pressure by as
much as 80 mm. Hg, and at the same time slowed the heart,
and that this action was abolished by sections of the vagi.
They also noted a hyperglycemic action in rabbits, with a
rise in blood sugar of as much as 50%.

Other alkaloids. Very little work has been done on
the pharmacology of the other alkaloids of mescal buttons.
Heffter (14) performed some preliminary experiments, as did
Dixon (18), but the latter's work is of doubtful value, be-
cause several of the compounds used by him were obviously
impure. Mogilewa (22) investigated the actions on the per-
fused intact frog heart.

(1) Anhalonine: Heffter showed that a dose of 10
mgm. of anhalonine hydrochloride in the frog caused central
paralysis, which disappeared after about one hour, when a
condition of increased reflex activity began to appear.

This latter effect often persisted for 24 hours, and sometimes for several days. The perfused frog heart is slowed.

(2) <u>Anhalonidine</u>. The actions of anhalonidine seem to be similar to those of anhalonine, and in addition it is reported to cause paralysis of motor nerve endings.

(3) <u>Lophophorine</u>. This alkaloid does not show the paralyzing action of anhalonine and anhalonidine, but causes an immediate increase in reflex excitability in frogs in doses of 0.25-1.0 mgm., which persists for many hours. The frog heart is also slowed by lophophorine.

(4) <u>Anhalamine</u>. Anhalamine produces in the frog a preliminary narcosis followed by increased reflex excitability, similar to that observed with several of the other alkaloids.

No mention is made in the literature of pharmacological work done on any of the other alkaloids of mescal buttons, which have been isolated more recently.

Chemistry of mescal alkaloids

At the present time eleven alkaloids have been isolated from mescal buttons, and the structures of all of them are known (see table on page 15). This table indicates the close structural relationships amongst these compounds, and it is possible that some of the missing members of these series may later be found to occur in small amounts in the buttons. The theory (Robinson (23), Hahn and Rumpf (24), and Schöpf and Bayerle (25)) that isoquinolines are formed in plants through ring-closure of the corresponding phenethylamines would suggest the presence, in small amounts at least, of 3-methoxy-4,5-methylenedioxyphenethylamine and 3,4-dimethoxy-5-hydroxyphenethylamine.

The references to the isolation of the alkaloids are as follows, in the order of their discovery, from 1888 to 1939: anhalonine (Lewin (26)), pellotine (Heffter (27)), mescaline, anhalonidine, and lophophorine (Heffter (28)), anhalamine (Kauder (29)), anhalinine (Späth and Becke (30)), anhalidine (Späth and Becke (31)), N-methylmescaline (Späth and Bruck (32)), N-acetylmescaline (Späth and Bruck (33)), and O-methyl-d-anhalonidine (Späth and Bruck (34)). Anhalonine, lophophorine, and O-methyl-d-anhalonidine occur in the optically active forms, while pellotine and anhalonidine, which also possess an asymmetric carbon atom, are isolated in the racemic form.
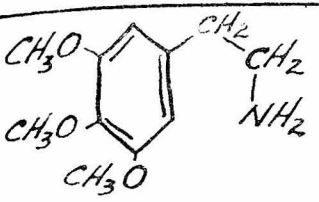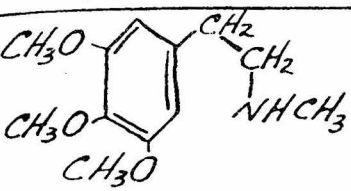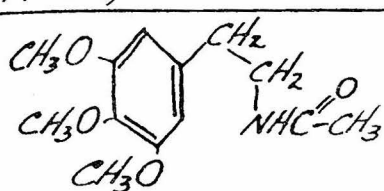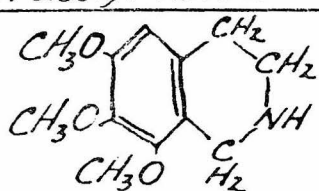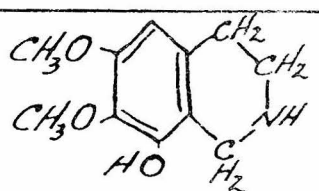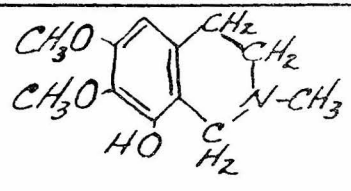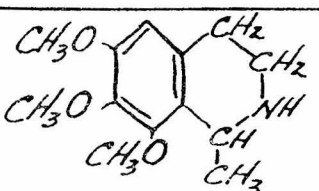
The amounts of the alkaloids found in the dried buttons vary widely, according to the different investigators.

Kauder (29) reported: mescaline (0.9%), anhalonine (0.25%), lophophorine (0.25%), pellotine (0.2%), anhalonidine (0.2%), and anhalamine (small amount). When Späth first worked on the isolation of the alkaloids (35), he obtained: total non-phenolic bases (0.2%), including mescaline (0.1%), lophophorine (0.0015%), and anhalinine (0.0075%); total phenolic bases (0.4%), including anhalamine (0.1%), and anhalidine (0.001%). The yields of the other alkaloids are not given. In his later work (32) he obtained what are described as much fresher buttons, and was able to increase the yields ten times over those of the older material. The only figures he gives are for mescaline (1.25%), anhalonine (0.35%), and N-methylmescaline (0.025%). The yields obtained in the present work from mescal buttons which were several years 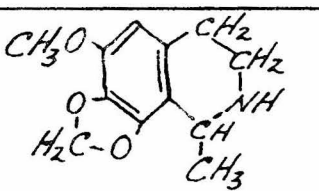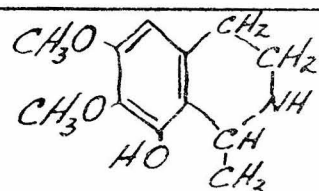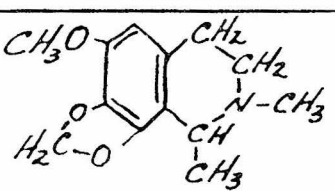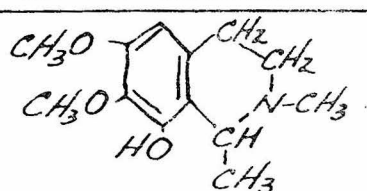old were: mescaline (0.85%), anhalonine (0.12%), lophophorine (0.07%), pellotine (0.15%), and anhalonidine (0.02%). Rouhier (7) also states that the proportion and amounts of the alkaloids vary both seasonally and environmentally, and this is of course partially responsible for the great variation in the physiological responses to the crude drug.

Anhaline, which was isolated from the closely related cactus Anhalonium fissuratum by Heffter (27), has not been found in mescal buttons. It was shown by Späth (47) to be identical with hordenine, isolated from barley malt germs, and possessing the formula 4-hydroxyphenethyldimethylamine.

The present work was undertaken with the view to

MESCAL ALKALOIDS

TRIMETHOXY COMPOUNDS	METHOXY-METHYLENE-DIOXY COMPOUNDS	DIMETHOXY-HYDROXY COMPOUNDS
mescaline		
N-methyl mescaline		
N-acetyl mescaline		
anhalinine		anhalamine
		anhalidine
o-methyl d-anhalonidine	l-anhalonine	anhalonidine
	l-lophophorine	pellotine

preparing a number of the mescal alkaloids and related substances in order to be able to make a study of the physiological properties of the compounds. The complete series of the trimethoxy-substituted compounds was prepared, including N-dimethylmescaline. Homomyristicylamine (3-methoxy-4,5-methylenedioxyphenethylamine) has been synthesized by a new method, and mescaline, anhalonine, lophophorine, pellotine, and anhalonidine have been isolated from the mescal buttons in sufficient quantities for preliminary study. The action of amine oxidase[1] on a number of these compounds was investigated.

N-dimethylmescaline has not been found in Lophophora. Luduena (36) states that the cactus alkaloid trichocereine from Trichocereus terscheki has been identified as N-dimethylmescaline, but no chemical data on this substance could be found. Guggenheim (37) refers to a publication by Reti (38) in which it is stated that Trichocereus terscheki contains on a dry weight basis 0.05% mescaline and 0.2% trichocereine. Unfortunately this paper could not be obtained at the present time. This compound has been synthesized in the present work, and the two N-methyl-tetrahydroisoquinolines of the 6,7,8-trimethoxy series have been prepared.

Homomyristicylamine has been prepared by Salway (39), Decker and Becker (40), Späth and Gangl (41), Späth and Kesztler (42), and Hahn and Schales (43). Their methods are reported to give moderate yields, but were not considered

---

[1] This work was made possible through the kindness of Dr. G.A. Alles.

suitable for the convenient preparation of large quantities
of the compound, and therefore the homoamine synthesis of
Julian and Sturgis (44), which was known to give good re-
sults when veratraldehyde or piperonal were used as inter-
mediates, was applied using 3-methoxy-4,5-methylenedioxy-
benzaldehyde. This synthesis proceded by the following
reactions:
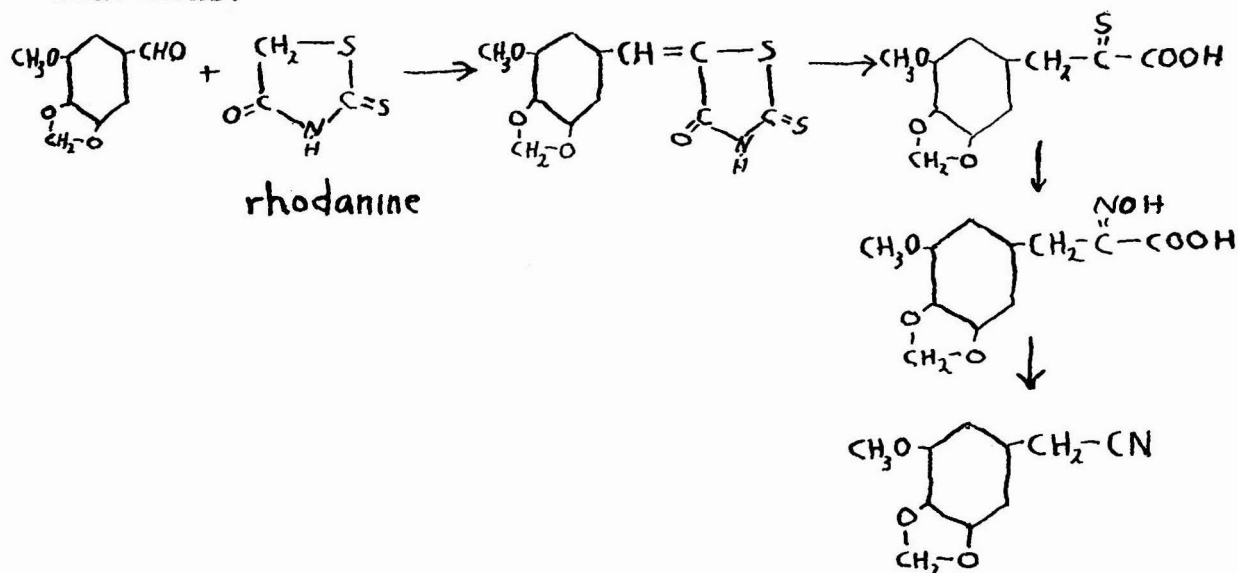
The nitrile prepared in this manner was reduced at high
hydrogen pressure, in the presence of methanolic ammonia in
order to decrease formation of secondary amine.

Späth and Gangl (41), and Späth and Kesztler (42)
have reported the synthesis of dl-anhalonine by ring closure
of N-acetylhomomyristicylamine with P_2O_5, followed by re-
duction of the 3,4-dihydroisoquinoline. They obtained a
dihydroisoquinoline which was apparently entirely the de-
sired 6-methoxy-7,8-methylenedioxy compound. However, in
this work a mixture of this substance and the isomeric 6,
7-methylenedioxy-8-methoxy compound was obtained, which
could not be separated, so the hydrogenation was conducted

on the mixture. By fractional crystallization of the hydro-
chloride of the reaction product it was possible to obtain
pure dl-anhalonine.

The preparation of 3,4-dimethoxy-5-hydroxyphenethyl-
amine requires 3,4-dimethoxy-5-hydroxybenzaldehyde as an
intermediate, and an attempt was made to develop a method
of preparing this substance in the large quantities neces-
sary for subsequent syntheses. Späth and Rader (45) attempt-
ed its preparation by the Sn-HCl reduction of 5-nitrover-
atraldehyde, and subsequent diazotization of the amino
aldehyde and decomposition of the diazonium salt to the
phenolic compound. However, the only substance which they
could isolate from the reaction mixture was 3-methoxy-4,
5-dihydroxybenzaldehyde. They succeeded in preparing the
desired compound by the rather laborious method of separating
the various products resulting from the methylation of gal-
lic acid, and in this way accumulated a sufficient quantity
of 3,4-dimethoxy-5-hydroxybenzoic acid to prepare the cor-
responding aldehyde through the Rosenmund reduction of the
acid chloride. The only other preparation of the aldehyde
is that given by Mauthner (46), who used a similar method.
It was thought that the compound might be prepared in large
amounts conveniently by the following series of reactions,
where the aldehyde group is blocked during the hydrogenation
by forming the dimethylacetal:
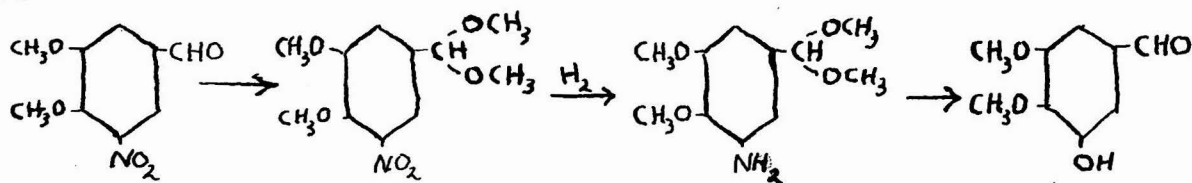
The reactions apparently proceeded satisfactorily up to the decomposition of the diazonium salt formed in the presence of either HCl or H_2SO_4, but none of the desired compound could be isolated from the reaction mixture after the decomposition of the diazonium salt. However, when HCl was used in the diazotisation and $CuSO_4$ solution in the decomposition, 3,4-dimethoxy-5-chloro-benzaldehyde could be isolated in good yield.

solution with 50% aqueous KOH to separate the non-phenolic bases from the phenolic bases. When this was tried it was found to be impossible to isolate appreciable quantities of the phenolic compounds. Titration experiments were carried out to investigate this point, and it was found that about 50% of the basic material was removed from the ether by one shaking with 50% KOH solution. When the KOH fraction was neutralized with HCl, followed by the addition of 2% K_2CO_3, and extracted with ether, only about 10% of this phenolic fraction could be recovered. In a preliminary experiment using 25% KOH solution, 85% of the phenolic fraction could be recovered. When this method was applied to the large batch, only 50% of the phenolic bases could be recovered by ether extraction, and extraction with several other solvents did not increase the yield of alkaloids obtained from this fraction. 0.34 equivalents of base were present in the ether (Fraction A) after shaking with 25% KOH solution, representing 52% of the original basic fraction. 0.14 equivalents of base could be recovered from the 25% KOH solution by ether extraction after neutralizing with HCl and making alkaline with K_2CO_3. This represents 45% of the basic material which had been removed by the alkali treatment, and it was worked up separately as will be described later (Fraction B).

It was thought possible that the KOH solution had resulted in the reaction of the phenolic compounds with some other substance present in the extract, giving a

product which was insoluble in ether. Therefore, the residue from the ether extraction of the 25% KOH solution was neutralized and made 2N with HCl, and refluxed 4 hours. K_2CO_3 was then added until the solution was alkaline, and another ether extraction was carried out. In this way 0.046 equivalents of base were obtained (Fraction C), which represents 16% of the total phenolic base fraction.

Isolation of non-phenolic bases from fraction A

The ether solution of fraction A was concentrated to about 1 liter and the basic material was extracted by repeated shakings with 2% acetic acid. The acetic acid solution was made alkaline with K_2CO_3 and extracted with ether. 0.29 equivalents of base were recovered, representing 85% of the original non-phenolic fraction.

Isolation of mescaline sulfate. The ether was removed at 20 mm. Hg, and an oily brown residue was obtained, which was dissolved in 250 cc. of 95% EtOH, and a 10% solution of H_2SO_4 in EtOH was added to a slight acidity to litmus. A heavy precipitate was obtained, which was dried over $CaCl_2$, and weighed 70 gm. It was recrystallized from 220 cc. of boiling water, the crystals filtered at 5^0 C., and washed with 95% EtOH. The mother liquor from the recrystallization and the wash solution were added to the original. The crystals weighed 41.0 gm., and possessed m.p. 186-9^0 C. (All melting points reported were taken on a Fisher hot-stage apparatus, and are corrected.) Späth (47) gives m.p. 183-6^{0}C. for mescaline sulfate. The picrate was prepared from

equivalent amounts of mescaline sulfate and picric acid in water, and melted at 217-8°C. Späth gives m.p. 216-8°C. The hydrochloride was prepared from an aqueous solution of the sulfate by decomposition with excess NaOH, followed by benzene extraction. The benzene was removed at 20 mm. Hg, the base neutralized with methanolic HCl, and precipitated with absolute ether. The white hydrochloride melted at 183-4°C. Hahn and Wassmuth (48) give m.p. 182°C. for mescaline hydrochloride. It was recrystallized from absolute ether-MeOH (2:1), and dried at room temperature over P_2O_5.

	C	H
Calculated for $C_{11}H_{18}O_3NCl$	53.33	7.32
Found	53.48	7.35

Isolation of anhalonine. Anhalonine was isolated from the filtrate from the mescaline sulfate precipitation in the following manner: the alcohol was removed in vacuo, 2% K_2CO_3 added, and the solution extracted with ether. 0.063 equivalents of base were obtained. The ether was removed in vacuo, and an oil weighing 15 gm. was obtained. After the addition of 50 cc. of 2N HCl, a crystalline precipitate was immediately formed, which was filtered at 5°C., washed with 5% HCl and dried. Five and one-half grams of this crude salt was obtained, and it was purified by liberating the free base, extracting with ether, and distilling. The base distilled at 144-6°C./0.07 mm. Hg, and the distillate crystallized at once, with m.p. 84-5.5°C. Späth and Kesztler (42) give m.p. 84-5.5°C. The quaternary iodide

was prepared according to Späth and Gangl (41), recrystallized from water containing a little NaI and then from MeOH. It melted at 222-4°C., then solidified, and remelted at 238-41°C., as described by Späth and Gangl. Anhalonine hydrochloride was prepared from the free base in absolute ether by passing in anhydrous HCl gas. It melted at 256-9°C. (d.), and by recrystallization from MeOH needles were obtained, which were dried at room temperature over P_2O_5.

	$\underline{C}$	$\underline{H}$
Calculated for $C_{12}H_{16}O_3NCl$	55.92	6.26
Found	55.87	6.37

Optical rotation of anhalonine:

$$c = 2.6 \ (\text{MeOH, 1 dm. tube})$$
$$[\alpha]_D^{25°C} = \frac{100(-1.68)}{2.6} = -64.6°$$

Späth and Kesztler (42): c = 2.62 (MeOH, 1dm. tube).

$$[\alpha]_D^{25°C} = -64.1°$$

Isolation of lophophorine hydrochloride. The filtrate from the anhalonine hydrochloride precipitation was made alkaline with K_2CO_3 and extracted with ether (0.013 equivalents of base). The ether was removed in vacuo, and the residue taken up dilute HCl. This solution was decolorized with 100 mgm. of Merck animal charcoal and concentrated in a desiccator, until white crystals formed, which were filtered and washed with 95% EtOH. The filtrate was concentrated further to a syrup, which was dis-

solved in hot 95% EtOH, and on cooling this solution 1.4 gm. of white crystals (needles) were deposited. These two crystal fractions were combined, and weighed 3.2 gm. The picrate was prepared from equivalent amounts of the hydrochloride and picric acid in hot absolute EtOH, and possessed m.p. 161-2.5°C. Späth and Kesztler (42) give 162-3°C. as the melting point of lophophorine picrate. The hydrochloride was decomposed with NaOH solution, the base extracted with ether and distilled after the removal of the ether in vacuo. A colorless oil was obtained at 120-5°C./0.05 mm. Hg.

The quaternary iodide was prepared in the same manner as anhalonine methiodide. After recrystallization from MeOH, it melted partially at 225°C., and completely at 238-40°C. Späth and Gangl (41) give partial melting at 223°C., and complete melting at 239-41°C.

The hydrochloride was prepared from the base with methanolic HCl, and precipitated with ether. It was recrystallized from methyl ethyl ketone for analysis, and dried at room temperature over P_2O_5.

	C	H	Methoxyl
Calculated for $C_{13}H_{18}O_3NCl$	57.45	6.68	11.42
Found	57.96	6.67	11.11

Optical rotation of lophophorine hydrochloride:

c = 4.2 (water, 1 dm. tube)

$$[\alpha]_D^{25°C} = \frac{100\ (-0.62)}{4.2} = -14.8°$$

Späth and Kesztler (42): c = 4.425 (water, 1 dm. tube)

$$[\alpha]_D^{25°C} = -16.3°$$

No further crystalline material could be obtained from the non-phenolic base fraction.

Isolation of pellotine hydrochloride from fraction B.

After removal of the ether from fraction B, a black oily residue was obtained. Excess aqueous HCl was then added, and the solution was kept at 0°C. for 3 days. A small amount of non-basic material precipitated and was filtered off. The filtrate was made alkaline with K_2CO_3, a resinous precipitate filtered, and the filtrate extracted with ether (0.046 equivalents base). The ether was removed in vacuo, the residue dissolved in 150 cc. of dilute HCl, and after decolorization with Merck animal charcoal the solution was evaporated to dryness in a desiccator over KOH. The residue was treated with absolute EtOH, and a white insoluble residue was obtained which weighed 7.3 gm. and was shown to be pellotine hydrochloride.

The picrate was prepared from equivalent amounts of the hydrochloride and picric acid in water. An oil separated, which partially crystallized at 0°C. After recrystallization of this material from water, it possessed m.p. 167-8°C. Späth and Becke (35) give the melting point of pellotine picrate as 165-7°C.

The free base was prepared by extraction with ether of an aqueous solution of the hydrochloride which was made

alkaline with NH_4OH, and after removal of the ether the base was distilled. The fraction boiling at 135-40°C./ 0.06 mm. Hg was collected, and this fraction crystallized immediately and showed m.p. 111-2°C. Späth and Becke (49) give the melting point of pellotine as 110-2°C.

The methiodide was prepared with methyl iodide in MeOH, and the reaction mixture allowed to stand at room temperature for two days. The MeOH and methyl iodide were removed <u>in vacuo</u> and the residue, after recrystallization from MeOH, possessed a melting point of 197-98°C. The melting point given by Kauder (29) for pellotine methiodide is 198°C.

Pellotine hydrochloride was prepared from the base dissolved in ether by passing HCl gas through the solution. After filtration, it was recrystallized from MeOH-ether (1:3) for analysis, and dried at room temperature over P_2O_5.

	<u>C</u>	<u>H</u>
Calculated for $C_{13}H_{20}O_3NCl$	57.01	7.36
Found	57.17	7.30

No further crystalline substances could be isolated from this fraction.

Isolation of Anhalonidine from fraction C

The ether was removed from fraction C (0.046 equivalents) <u>in vacuo</u>, and the black oily residue was dissolved in 50 cc. of hot 2N HCl. On cooling, 8.7 gm. of non-crystalline material separated, which was dissolved in 25 cc.

of 0.5N HCl. This solution was decolorized with Merck ani-
mal charcoal, concentrated, and cooled. In this way 3.7 gm.
of impure hydrochloride was obtained, which was dissolved
in water and the base extracted with ether after making the
solution alkaline with K_2CO_3. The ether was removed _in vacuo_,
and the residue subjected to slow sublimation at a bath temp-
erature of 120-60°C./0.05 mm. Hg. A fraction was obtained
melting at 145-55°C., which was extracted twice with boiling
absolute ether, and the residue, which weighed 220 mgm. then
melted at 159-61°C. According to Späth and Becke (35), anhal-
onidine melts at 157-61°C.

The picrate was prepared from equivalent amounts of
the hydrochloride and picric acid in water, and after re-
crystallization from water possessed m.p. 200-2°C. Späth
(50) gives m.p. 201-8°C.

Anhalonidine hydrochloride was prepared from the base
with methanolic HCl, precipitated with ether, and recrys-
tallized for analysis from MeOH-ether (1:2).

	C	H
Calculated for $C_{12}H_{18}O_3NCl$	55.49	6.98
Found	55.69	7.14

Only a small amount of basic material was present in
the filtrate from the anhalonidine hydrochloride precipi-
tation, and no other pure substances could be isolated from
this fraction.

II - SYNTHETIC WORK

N-methylmescaline (Späth and Bruck (32))

To 4.6 gm. of freshly distilled mescaline was added 2.22 gm. of benzaldehyde, and the solution warmed on the water bath for a few minutes. The water formed was removed at 50°C. in vacuo, 3.2 gm. of methyl iodide added, and the solution allowed to stand for 19 hours at room temperature and then heated at 60-70°C. for 9 hours. The viscous glassy product was dissolved in 40 cc. of MeOH, 5 cc. of water added, and the solution refluxed for 15 minutes. Fifty cubic centimeters of water was then added, the MeOH and benzaldehyde were boiled off, the solution made acid with acetic acid and extracted with ether. The acid solution was then made alkaline with NaOH solution, extracted with ether, and the producted distilled after removal of the ether. N-methylmescaline distilled at 130-40°C./1.0 mm. Hg, and the colorless oil obtained weighed 2.9 gm. The hydrochloride was prepared in absolute ether with HCl gas, and melted at 187-8°C. After recrystallization from acetone-MeOH (9:1), it melted at 201-201.5°C. Späth and Bruck (32) give m.p. 201-2°C.

The picrate was prepared from the hydrochloride in water, and possessed m.p. 174-6°C.

N-dimethylmescaline

An unsuccessful attempt was made to prepare the compound from N-methylmescaline, using an equivalent amount of

methyl iodide, but the quaternary salt was formed chiefly, leaving considerable unreacted N-methylmescaline. The following method of preparation was found to give satisfactory results: 3.0 gm. of N-methylmescaline was dissolved in 5 cc. of absolute EtOH, and 1.1 cc. of 40% formaldehyde and 40 mgm. of Adams catalyst ($PtO_2 \cdot H_2O$) were added. This mixture was reduced with hydrogen in a Paar bomb at room temperature and 42 pounds pressure, for 2 hours. The catalyst was filtered off, the MeOH removed _in vacuo_, and water added. The solution was made alkaline and extracted with ether, and distilled after removal of the ether. One and one-half grams of N-dimethylmescaline was obtained at 128-31°C./0.6 mm. Hg. The hydrochloride was prepared with HCl gas in ether, recrystallized from acetone-MeOH (9:1), and had m.p. 208-9°C. A mixed melting point determination with 2-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride, which melts at 216-8°C., gave a melting point of 175-85°C. A sample was dried at room temperature over P_2O_5 for analysis.

	C	H
Calculated for $C_{13}H_{22}O_3NCl$	56.60	8.04
Found	56.71	8.17

The picrate was prepared from the base in 95% EtOH by adding an equivalent amount of picric acid in water, and after recrystallization from 95% EtOH, it melted at 171-2°C. It was dried at 80°C. over P_2O_5 for 24 hours.

	C	H
Calculated for $C_{19}H_{24}O_{10}N_4$	48.72	5.16
Found	48.77	5.13

6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline (Anhalinine) (Späth (51))

To 13.5 gm. of freshly distilled mescaline was added 15 cc. of water and 7.0 cc. of 40% formaldehyde with shaking. The solution was heated for 1 hour on the water bath, and then the separated aqueous layer was removed, and the residue washed with 20 cc. of water. The white solid product was dissolved in 95 cc. of hot 2N HCl, and allowed to crystallize. After recrystallization from 6N HCl, 7.0 gm. of the hydrochloride was obtained, which melted in an evacuated tube at 247-9°C. A sample was dried at 80°C. over P_2O_5 for 24 hours for analysis.

	$\underline{C}$	$\underline{H}$
Calculated for $C_{12}H_{18}O_3NCl$	55.49	6.98
Found	55.65	6.81

The picrate was prepared according to Späth, and melted at 183-4°C.

2-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline

To 3.6 gm. of freshly distilled 6,7,8-trimethoxy-1,2, 3,4-tetrahydroisoquinoline in a tube were added 2.2 cc. of 40% formaldehyde and 1.24 cc. of 98% formic acid. There was an immediate evolution of gas, and the solution was heated on the water bath for one-half hour, until gas evolution had ceased. The tube was then sealed and heated at 100°C. for 12 hours, after which the reaction product was made alkaline with NaOH solution, and extracted with ether. The residue after the removal of the ether distilled at 123-6°C./0.06 mm. Hg. The hydrochloride was prepared in ether with HCl gas, and recrystallized from a solution of

ethyl acetate-absolute EtOH (1:1). This material, which melted at 216-8°C., was dried at room temperature over P_2O_5.

	C	H
Calculated for $C_{13}H_{20}O_3NCl$	57.02	7.36
Found	57.18	7.57

The picrate was prepared from the hydrochloride and an equivalent amount of picric acid in EtOH-water (1:1), and recrystallized from MeOH-water (1:1). It possessed a melting point of 136-7.5°C., and was dried for analysis at 80°C. over P_2O_5.

	C	H
Calculated for $C_{19}H_{22}O_{10}N_4$	48.93	4.76
Found	49.66	4.72

1-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline (Späth (51), and Späth and Bruck (34)).

To 17.6 gm. of mescaline was added 10.0 cc. of freshly distilled acetic anhydride, and this solution heated at 150-60°C. for 1.5 hours. The volatile material was removed at 150°C. at 20 mm. Hg, and a portion of the residue distilled at 185-90°C./0.05 mm. Hg. The distillate crystallized immediately, and after recrystallization from ether containing 10% MeOH the product melted at 90-1°C.

The remainder of the N-acetylmescaline (18.0 gm.) was dissolved in 160 cc. of anhydrous toluene, and 70 gm. of P_2O_5 added in small portions to the gently refluxing solution. The reaction mixture was cooled, and after the addition of water was heated on the water bath for 10 minutes,

and then allowed to stand overnight. The toluene layer was washed twice with 50 cc. of 3N HCl, and the washings added to the aqueous solution. The latter was made alkaline with KOH solution, extracted with ether, the ether removed in vacuo, and the residue distilled. The fraction boiling at 135-40°C./0.02 mm. Hg was collected, and a light yellow oil was obtained, which weighed 14.0 gm. The picrate was prepared in benzene solution, and melted at 181-2°C. Späth (51) also gives 181-2°C.

Reduction of the 3,4-dihydro-isoquinoline with granulated zinc in HCl solution containing a little $CuSO_4$ (Späth and Bruck (34)) gave a low yield of the corresponding tetrahydroisoquinoline, and therefore a catalytic method was adopted, which gave a quantitative yield. Fourteen grams of the dihydroisoquinoline was dissolved in 75 cc. of MeOH, 42 mgm. of Adams catalyst added, and the hydrogenation conducted at room temperature and 1 atmosphere pressure. The theoretical amount of hydrogen was taken up in 1 hour and 20 minutes. The catalyst was filtered off, the MeOH removed at 20 mm. Hg, and the hydrochloride prepared from the residue with methanolic HCl and precipitated with absolute ether. After recrystallization from methyl ethyl ketone, it melted at 153-8°C., and was dried for analysis at room temperature over P_2O_5.

	C	H
Calculated for $C_{13}H_{20}O_3NCl$	57.02	7.36
Found	56.93	7.14

The picrate was prepared from the free base in 95%
EtOH, and precipitated through the addition of an equal
volume of water. After recrystallization from EtOH-water
(1:1), the melting point was 172-4°C. Späth (51) gives the
melting point as 172-3°C.

1,2-dimethyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline

Four and one-half grams of freshly distilled 1-methyl-
6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline was mixed
with 1.45 cc. of 98% formic acid and 2.6 cc. of 40% form-
aldehyde in a tube. The solution was heated on the water
bath for 1 hour until gas evolution had ceased, and the
tube was then sealed and heated for 13 hours at 100°C. The
reaction product was made alkaline with NaOH solution, ex-
tracted with ether, and after removal of the ether *in vacuo*,
the product distilled. Four and one-half grams of a color-
less oil was obtained at 108-10°C./0.12 mm. Hg. In order
to remove any unreacted secondary amine, the base was dis-
solved in 5 cc. of absolute ether and 10 cc. of freshly dis-
tilled acetic anhydride was added. After standing for one
hour, the solution was treated with NaOH solution and ex-
tracted with ether. After removal of the ether, the hydro-
chloride was prepared from the residue with methanolic HCl
and precipitated with 4 volumes of absolute ether at 0°C.,
while scratching with a glass rod. In this way the hydro-
chloride could be prepared in white crystalline form. It
is quite hygroscopic, absorbing 1 mol of water from the at-
mosphere in about 10 minutes. The salt was recrystallized

by dissolving in the minimum amount of hot MeOH and adding absolute ether until a turbidity was produced. The crystals obtained on cooling were exposed to the atmosphere until a constant weight was obtained, and the compound then possessed m.p. 125-8°C. The analysis was run on the monohydrate, dried in air.

	$\underline{C}$	$\underline{H}$
Calculated for $C_{14}H_{22}O_3NCl \cdot H_2O$	54.98	7.91
Found	55.22	7.88

The picrate was prepared from equivalent amounts of the base and picric acid in EtOH, and precipitated with an equal volume of water. An oil separated, which crystallized in a few hours at 0°C. This material was filtered, and after recrystallization from EtOH-water (1:1), with scratching to avoid oil formation, it melted at 122-3.5°C, and was dried at 80°C. over P_2O_5 for analysis.

	$\underline{C}$	$\underline{H}$
Calculated for $C_{20}H_{24}O_{10}N_4$	50.00	5.04
Found	50.57	5.27

Preparation of homomyristicylamine (3-methoxy-4,5-methylenedioxyphenethylamine)

Myristicin

Myristicin was obtained by the fractionation (8 inch column packed with helices) of 3.15 Kgm. of oil nutmeg (heavy) obtained from the Dodge and Olcott Co. 1.6 Kgm.

of myristicin was collected at 154-7°C./18mm. Hg.

Isomyristicin

A solution of 540 gm. of myristicin in 1400 cc. of 95% EtOH containing 250 gm. of KOH was refluxed for 24 hours, then neutralized with HCl, and the EtOH distilled off at 20 mm. Hg. The residue was poured into 1 liter of water, the separated oil washed twice with 500 cc. of water, and fractionated. Isomyristicin was collected at 170-3°C./ 15 mm. Hg, and after standing a few hours crystallized in needles with m.p. 41°C. Power and Salway (52) give m.p. 44°C.

Myristicinaldehyde (Salway (53))

Thirty grams of isomyristicin was stirred vigorously with 150 cc. of water at 60°C., and a solution of 60 gm. of KMnO$_4$ in 1.5 liters of water was then added dropwise over a period of 1.5 hours, while maintaining the temperature at 60-4°C. The precipitated MnO$_2$ was filtered off after cooling, washed with 500 cc. of ice-water, and dried at 70°C. Several such batches were run, and the combined MnO$_2$ was then extracted for 12 hours with chloroform in a Soxlet extractor. The chloroform was evaporated off on the water bath, and the solid residue washed with cold ether. The yield was usually about 45% of the theoretical, and the aldehyde melted at 132°C. Späth and Gangl (41) give m.p. 132°C.

Preparation of rhodanine (Julian and Sturgis (44))

Anhydrous NH_3 was passed into a solution of 250 gm. of CS_2, 200 cc. of absolute EtOH, and 200 cc. of absolute ether for 3.5 hours, at 0-5°C. The ammonium dithiocarbamate was filtered off, and washed with cold EtOH and ether. A solution of sodium chloroacetate was prepared from 84 gm. of NaOH in 140 cc. of water and 205 gm. of chloroacetic acid in 175 cc. of water. Na_2CO_3 was added until the solution was neutral to litmus, and then the ammoniumdithiocarbamate was added to this solution with good cooling and stirring. The resulting solution was added to 275 cc. of concentrated HCl at 85°C., and the precipitated material recrystallized from 200 cc. of glacial acetic acid. It weighed 175 gm., and melted at 163-6°C.

Myristicinalrhodanine

Fifty grams of myristicinaldehyde and 37 gm. of rhodanine were dissolved in 95 cc. of glacial acetic acid, and 69 gm. of anhydrous sodium acetate dissolved in 95 cc. of glacial acetic acid added. An orange precipitate formed at once, and the mixture was refluxed for 15 minutes. It was then poured into 1.5 liters of water, filtered, and washed with water, EtOH, and finally ether. The yield was 75 gm. of material with m.p. 252-5°C. It was recrystallized for analysis from acetone-pyridine (3:1), and dried at 110°C. over P_2O_5.

	$\underline{C}$	$\underline{H}$
Calculated for $C_{12}H_9O_4S_2N$	48.80	3.07
Found	49.00	3.14

α-thioketo-β-(3-methoxy-4,5-methylenedioxyphenyl)-propionic acid

Seventy-six grams of myristicinalrhodanine and 350 cc. of 15% NaOH were heated on the water bath for one hour with stirring, until complete solution occurred, and then the contents of the flask were heated to 100°C. for 10 minutes. After cooling, the product was precipitated by the rapid addition of 350 cc. of 10% HCl. The yellow product was washed with water and after drying in air weighed 58 gm. This thioketo acid could not be crystallized, and the next step was carried out without further purification of this intermediate.

α-oximino-β-(3-methoxy-4,5-methylenedioxyphenyl)-pyruvic acid

A solution of hydroxylamine was prepared by adding a solution of sodium ethylate prepared from 16.5 gm. of sodium and 475 cc. of absolute EtOH to a warm solution of 49.5 gm. of hydroxylamine hydrochloride in 44 cc. of water, followed by filtration of the precipitated NaCl. To this solution was added 58 gm. of the above thioketo acid, and the solution was heated on the water bath for 40 minutes, and the alcohol then removed in vacuo. The oily residue was dissolved in 120 cc. of 5% NaOH, the solution filtered,

and then precipitated slowly with 120 cc. of 10% HCl, scratching as the precipitation proceeded to avoid oil formation. In this way 48.2 gm. of a yellow-brown solid was obtained, which was dried for 48 hours over KOH and $CaCl_2$. The compound could be obtained in crystalline form by dissolving in toluene at 85-90°C. and allowing to cool slowly. Microscopic needles which were washed with petroleum ether were obtained, with m.p. 145-6°C. The compound was dried at room temperature over P_2O_5 and paraffin.

	$\underline{C}$	$\underline{H}$
Calculated for $C_{11}H_{11}O_6N$	52.17	4.38
Found	52.57	4.85

3-methoxy-4,5-methylenedioxyphenyl-acetonitrile

Forty-eight grams of the crude oximino acid was dropped into 150 cc. of acetic anhydride at a temperature of 60-80°C., at a rate which gave moderate foaming. At the end of the reaction the acetic anhydride was removed in vacuo, the residue taken up in ether and water, and the ether solution washed three times with one-half volume of 10% Na_2CO_3 solution. The ether was removed in vacuo, and the residue distilled. The nitrile was collected at 170-5°C./1.5 mm. Hg, and weighed 22 gm. It solidified quickly, and after recrystallization from MeOH melted at 88-9°C. Hahn and Schales (43) give m.p. 90°C. for this compound, prepared by another method.

Homomyristicylamine

Twenty-three grams (0.12 mol) of the above nitrile was reduced in an American Instrument Co. high pressure hydrogenation bomb. Two cubic centimeters of Raney Ni catalyst was used, at 600 pounds pressure and 125°C., in the presence of 72 cc. of methanolic NH_3 (9-11N). The hydrogenation was run for 4 hours, and after cooling, the catalyst was filtered off, and the MeOH and water were removed _in vacuo_. The residue was distilled, and 16.0 gm. of homomyristicylamine was collected at 120-4°C./0.2 mm. Hg. This colorless oil was dissolved in 30 cc. of 10% HCl, and evaporated to dryness in a desiccator over KOH. After recrystallization from 95% EtOH-ethyl acetate (1:1), the hydrochloride was dried at 56°C. over P_2O_5, and melted at 164-5°C. Salway (39) gives the melting point of homomyristicylamine hydrochloride as 165°C.

	C	H
Calculated for $C_{10}H_{14}O_3NCl$	51.84	6.09
Found	51.93	6.32

The picrate was prepared from the hydrochloride in water, and melted at 193-4°C.

dl-anhalonine (Späth and Gangl (41), and Späth and Kesztler (42))

To a solution of 6.7 gm. of homomyristicylamine in 14 cc. of anhydrous benzene was added dropwise 5.5 cc. of freshly distilled acetic anhydride. Reaction occurred immediately, and the volatile material was then removed in

a desiccator containing H_2SO_4, KOH, and paraffin. The impure N-acetylhomomyristicylamine melted at 122-3°C., and after recrystallization from ligroin (90-100°C.) melted at 133-4°C. Späth and Gangl give m.p. 130°C.

	$\underline{C}$	$\underline{H}$
Calculated for $C_{12}H_{15}O_4N$	60.75	6.37
Found	61.23	6.27

Eight grams of the acetyl compound was dissolved in 110 cc. of anhydrous toluene, and 32 gm. P_2O_5 was added in about 2 gm. portions to the refluxing solution, over a period of 30 minutes. After all the P_2O_5 was added, the reaction mixture was cooled, water added, and the solution extracted with ether. After making the solution alkaline with NaOH the base was extracted with ether. The ether was removed *in vacuo*, the residue fractionally distilled, and the product boiling at 146-9°C./0.075 mm. Hg was collected. The distillate solidified immediately, and melted at 55-67°C. Späth and Gangl (41) give m.p. 60-2°C.

A solution of 5.2 gm. of the above 3,4-dihydroisoquinoline in 50 cc. of MeOH was **hydrogenated** at room temperature and 1 atmosphere pressure, in the presence of 23 mgm. of Adams catalyst. The theoretical amount of hydrogen was taken up in 45 minutes. The catalyst was removed by filtration, the MeOH removed *in vacuo*, and the material distilling at 144-6°C./0.08 mm. Hg collected.

The quaternary methiodide prepared according to Späth and Gangl melted at 205-225°C. The hydrochloride

of the base was prepared with methanolic HCl, and precipitated with ether. This salt, which weighed 4.1 gm., was recrystallized from 1N HCl, and the first crystal fraction then recrystallized from MeOH, giving a product which melted at 241-5°C. The quaternary iodide of this compound melted at 245-8°C. Späth and Gangl give m.p. 242-3°C. 1-Anhalonine methiodide prepared from 1-anhalonine isolated from the mescal buttons melted at 222-4°C., then recrystallized and remelted at 238-41°C. Späth and Gangl showed that racemization occurred at a temperature of about 230°C., and that the higher temperature represents the melting point of dl-anhalonine methiodide. A sample of 1-anhalonine methiodide was heated at 235°C. for three minutes, and the product recrystallized from MeOH-water (1:1). It then melted at 240-5°C., and a mixture of this material and the methiodide prepared from the synthetic product melted at 242-6°C. The isomeric quaternary methiodide of 1,2-dimethyl-6,7-methylenedioxy-8-methoxy-1,2,3,4-tetrahydroisoquinoline (prepared by the action of methyl magnesium iodide on cotarnine iodide) melts at 228-9°C. (Freund (54)). The dl-anhalonine hydrochloride was recrystallized from MeOH for analysis, and dried at room temperature over P_2O_5.

	C	H
Calculated for $C_{12}H_{16}O_3NCl$	55.92	6.26
Found	56.37	6.30

Attempted preparation of 3,4-dimethoxy-5-hydroxy-benzaldehyde

5-nitro-vanillin (Bentley (55))

To a solution of 455 gm. of vanillin (Monsanto U.S.P.) in 1800 cc. of glacial acetic acid at 10°C. was added drop-wise a solution of 135 cc. of fuming HNO_3 (sp. gr. 1.5) in 500 cc. of glacial acetic acid. The temperature was allowed to rise slowly to 30°C. at the end of the reaction, and after cooling to 15°C. the yellow-green product was fil-tered, and washed free of acetic acid with MeOH. The dried product weighed 414 gm., and melted at 174-5°C.

Potassium salt of 5-nitro-vanillin

One hundred and forty grams of 5-nitro-vanillin was suspended in 3.5 liters of water, and 49 gm. of KOH in 150 cc. of water was added. A clear, dark red solution was ob-tained, to which was added 50% KOH solution, until no fur-ther precipitation of reddish-orange crystals was obtained. On acidifying the filtrate with HCl, a small amount of 5-nitro-vanillin remaining in solution was precipitated. The potassium salt, after washing with 95% EtOH and drying at 130°C. for 4 hours, weighed 140 gm.

5-nitro-veratraldehyde (Späth and Roder (45))

Späth and Roder state that the potassium salt of 5-nitro-vanillin should be heated with dimethyl sulfate at 170-5°C. Only a very low yield was obtained under these conditions, so a lower temperature was used in this pre-paration. Two hundred grams of the potassium salt was

suspended in 625 cc. of freshly distilled dimethyl sulfate, and heated with stirring. At approximately 145°C. the color changed abruptly to yellow, and the reaction mixture was cooled immediately to room temperature. The dimethyl sulfate was distilled off in vacuo, the residue dissolved in ether, and the ether solution washed with 5% NaOH to remove unreacted 5-nitro-vanillin. The ether was removed by distillation, the residue recrystallized from EtOH-water (2:1), and dried over $CaCl_2$. The yield of 5-nitro-veratraldehyde was 144 gm., m.p. 88-90°C. The oxime was prepared and recrystallized from EtOH, and melted at 150-1°C. Brady and Manjunath (56) give m.p. 151°C. for this derivative.

5-nitro-veratraldehyde-dimethylacetal

To a solution of 118 gm. of 5-nitro-veratraldehyde in 250 cc. of MeOH was added concentrated HCl to give a concentration of 0.5%. This solution was allowed to stand at room temperature for 1 week, and the HCl was then neutralized with a solution of sodium methylate in MeOH, and the MeOH was distilled off in vacuo. On fractional distillation, 113 gm. of the dimethylacetal was collected at 138-44°C./0.2 mm. Hg. Crystals formed on standing, with m.p. 31-6°C., which after recrystallization from petroleum ether (60-70°C.) melted at 34-6°C. A sample was dried over P_2O_5 and paraffin for analysis.

	C	H
Calculated for $C_{11}H_{15}O_6N$	51.36	5.88
Found	51.93	6.27

Reduction of 5-nitro-veratraldehyde-dimethylacetal

Fifty grams of 5-nitro-veratraldehyde-dimethylacetal was dissolved in 100 cc. of MeOH, and 10 cc. of Raney Ni catalyst in EtOH added. The hydrogenation was conducted in the high pressure hydrogenation bomb at an initial hydrogen pressure of 1000 pounds, at $23^{\circ}C$. The reduction was stopped when the theoretical amount of hydrogen had been absorbed, and the catalyst was filtered off. No attempt was made to isolate the amino-aldehyde.

Diazotization of amino-aldehyde and decomposition of diazonium salt

The MeOH was distilled off _in vacuo_ from a sample (one-tenth of the total) of the above solution, and the residue dissolved in 200 cc. of ice-water containing 5 cc. of concentrated H_2SO_4. The calculated amount of $NaNO_2$ dissolved in a little water was added slowly, until the diazotization was completed, as indicated by the starch-iodide reaction. The excess nitrous acid was decomposed with urea, and the solution heated on the water bath in a stream of CO_2 for 1 hour. A dark solution resulted, and after cooling, was extracted with ether, and the ether removed _in vacuo_. No crystalline compounds could be isolated from the residue, and all further attempts to prepare 3,4-dimethoxy-5-hydroxybenzaldehyde by diazotization with other acids and decomposition under milder conditions were unsuccessful.

However, another substance could be prepared in reasonable yield by decomposition of the diazonium compound

formed in the presence of HCl, by means of $CuSO_4$ solution.
A quantity of the amino-aldehyde solution corresponding to
5.0 gm. of 5-nitro-veratraldehyde-dimethylacetal before re-
duction, was evaporated _in vacuo_. The residue was taken up
in 50 cc. of 4N HCl, and diazotized with the calculated
amount of $NaNO_2$ at 0-5°C. This solution was then added
dropwise to a solution of 75 gm. of $CuSO_4 \cdot 5H_2O$ in 100 cc.
of water, at a temperature of 85-90°C. A smooth evolution
of nitrogen occurred, and at the end of the reaction the
solution was extracted with benzene. The benzene was dis-
tilled off, and the residue distilled _in vacuo_. A color-
less oil weighing 2.3 gm. was obtained at 110-2°C./0.75 mm.
Hg, and this product immediately crystallized. After re-
crystallization from ligroin, it melted at 57°C. It was
dried at room temperature over P_2O_5 and paraffin for the
analysis, which showed the compound to be 3,4-dimethoxy-
5-chlorobenzaldehyde.

	C	H	Cl
Calculated for $C_9H_9O_3Cl$	53.88	4.52	17.68
Found	53.94	4.66	18.27

This substance has been prepared by another method
by Hann (57), and its melting point is given as 57°C.

III - ACTION OF AMINE OXIDASE ON MESCAL ALKALOIDS

AND RELATED COMPOUNDS

A partially purified amine oxidase preparation was made as described by Alles and Heegaard (58): a liver (70-90 gm.) from a freshly killed rabbit was ground with ice and water in a Waring blendor, the volume made up to 500 cc. and the pH adjusted to 8,0. Fiber was screened out, the mixture passed twice through a Sharples super-centrifuge, and dialyzed in Visking tubing for 20 hours in 10 liters of water at $5^{o}C$. The pH of the dialyzed solution was adjusted to 6.0 with acetic acid, and the precipitate centrifuged off. This precipitate was re-suspended in one liter of distilled water, the pH adjusted to 6.0, the solids centrifuged off, and the centrifugate discarded. The solids were then re-suspended in water with the addition of concentrated buffer solution and 1:1000 phenylmercuric acetate solution, and the pH adjusted. The final solution had a volume of 250 cc. and contained 0.1M sodium phosphate buffer of pH 7.0 and 1:10,000 phenylmercuric acetate.

The oxidation system consisted of 2.0 cc. of purified amine oxidase in 0.1M phosphate buffer at pH 7.0 and 0.3 cc. water, to which was added 0.2 cc. 0.1M amine-hydrochloride solution. The oxygen uptake at $30^{o}C$. was measured in the usual way with the Warburg apparatus. A control was run simultaneously without amine, as well as a com-

parison standard containing 0.2 cc. of 0.1M phenethylamine. The rate recorded was the maximum rate, because with many substrates the rate of oxygen uptake decreases with time, possibly due to inhibition by reaction products (Hare (59)), and this rate was expressed in terms of per cent relative to the rate of oxidation of phenethylamine. The following compounds were tested in this manner:

Mescaline, N-methylmescaline, N-dimethylmescaline, homo-myristicylamine, 6,7,8- trimethoxy-1,2,3,4-tetrahydroisoquinoline, 2-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline, 1-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline, 1,2-dimethyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline, dl-anhalonine, l-anhalonine, lophophorine, anhalonidine, and pellotine.

Mescaline had been previously studied in regard to the action of amine oxidase and the finding of Blaschko, Richter, and Schlossmann (12) and Pugh and Quastel (60) that it is not appreciably oxidized in the presence of amine oxidase was confirmed in the present work. Only one of the compounds listed above gave an uptake of oxygen, namely homo-myristicylamine. It was oxidized at a rate corresponding to 72% of the rate of oxidation of phenethylamine.

It is quite generally true that compounds not attacked by amine oxidase (ephedrine, benzedrine, mescaline) are active when given by mouth, while substances which are oxidized are not effective when administered orally (Beyer and Lee (61)). It therefore seems likely that homomyristicylamine

would not be expected to show strong activity when given orally, and in contrast to the other compounds tested that amine oxidase is at least partially responsible for its destruction in the body.

SUMMARY

1. The alkaloids of mescal buttons have been investigated, and five of the known alkaloids have been isolated and identified.

2. Six compounds of the 3,4,5-trimethoxyphenethylamine and the 6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline types have been synthesized from mescaline.

3. Homomyristicylamine has been prepared by a new method, and used to synthesize dl-anhalonine.

4. An unsuccessful attempt was made to develop a method for the preparation of large quantities of 3,4-dimethoxy-5-hydroxy-benzaldehyde for use as an intermediate in the synthesis of 3,4-dimethoxy-5-hydroxyphenethylamine.

5. The action of amine oxidase on a number of these compounds was studied.

REFERENCES

1. Mooney, Therap. Gazette, 20, 7 (1896)

2. Mitchell, Brit. Med. J., 2, 1625 (1896)

3. Ellis, Lancet, 1, 1540 (1897)

4. Prentiss and Morgan, Therap. Gazette, 20, 4 (1896)

5. Fernberger, Am. J. Psychol., 36, 267 (1923)

6. Beringer, Der Meskalinrausch, Berlin (1927)

7. Rouhier, Le Peyotl, Paris (1927)

8. Klüver, Mescal, London (1928)

9. Stockings, J. Mental Sci., 86, 29 (1940)

10. Quastel and Wheatley, Biochem. J., 27, 1609 (1933)

11. Richter, Biochem. J. 32, 1763 (1938)

12. Blaschko, Richter, and Schlossmann, Biochem. J., 31, 2187 (1937)

13. Slotta and Müller, Z. physiol. Chem., 238, 14 (1936)

14. Heffter, Arch exp. Path. Pharmakol., 40, 385 (1898)

15. Robles and Robleda, Anales inst. biol. Mex., 2, 15 (1931)

16. Hutchings, State Hosp. Bulletin, St. Lawrence, No. 1 (1897)

17. Grace, J. Pharmacol., 50, 359 (1934)

18. Dixon, J. Physiol., 25, 69 (1899)

19. Raymond-Hamet, Arch, exp. Path. Pharmakol., 169, 97 (1933)

20. Clerc, Paris, and Janot, Compt rend. soc. biol., 121, 1300 (1936)

21. Clerc, Janot, and Paris, Compt rend. soc. biol., 119, 828 (1935)

22. Mogilewa, Arch. exp. Path. Pharmakol., 49, 137 (1903)

23. Robinson, J. Chem. Soc., 876 (1917)

24. Hahn and Rumpf, Ber., 71, 2141 (1938)

25. Schöpf and Bayerle, Ann., 513, 190 (1934)

26. Lewin, Arch. exp. Path. Pharmakol., 24, 401 (1888)

27. Heffter, Ber., 27, 2975 (1894)

28. Heffter, Ber., 29, 216 (1896)

29. Kauder, Arch. Pharm., 237, 190 (1899)

30. Späth and Becke, Ber., 68, 501 (1935)

31. Späth and Becke, Ber., 68, 944 (1935)

32. Späth and Bruck, Ber., 70, 2446 (1937)

33. Späth and Bruck, Ber., 71, 1275 (1938)

34. Späth and Bruck, Ber., 72, 334 (1939)

35. Späth and Becke, Monatsh., 66, 327 (1935)

36. Ludueña, Compt. rend. soc. biol., 121, 368 (1936)

37. Guggenheim, Die biogenen Amine, Basel (1940)

38. Reti, X Congr. Internat. chim., Rome (1938)

39. Salway, J. Chem. Soc., 1208 (1910)

40. Decker and Becker, Ann., 395, 328 (1913)

41. Späth and Gangl, Monatsh., 44, 103 (1923)

42. Späth and Kesztler, Ber., 68, 1663 (1935)

43. Hahn and Schales, Ber., 67, 1486 (1934)

44. Julian and Sturgis, J. Am. Chem. Soc., 57, 1126 (1935)

45. Späth and Roder, Monatsh., 43, 93 (1922)

46. Mauthner, Ann., 449, 102 (1926)

47. Späth, Monatsh. 40, 129 (1919)

48. Hahn and Wassmuth, Ber., 67, 696 (1934)

49. Späth and Becke, Ber., 67, 266 (1934)

50. Sp–äth, Monatsh., <u>43</u>, 477 (1923)

51. Sp–äth, Monatsh., <u>42</u>, 97 (1921)

52. Power and Salway, J. Chem. Soc., 2054 (1907)

53. Salway, J. Chem. Soc., 1204 (1909)

54. Freund, Ber. <u>36</u>, 4257 (1904)

55. Bentley, Amer. Chem. J., <u>24</u>, 171 (1900)

56. Brady and Manjunath, J. Chem. Soc., 1060 (1924)

57. Hann, J. Wash. Acad. Sci., <u>24</u>, 124 (1934)

58. Alles and Heegaard, J. Biol. Chem., <u>147</u>, 491 (1943)

59. Hare, Biochem. J., <u>22</u>, 968 (1928)

60. Pugh and Quastel, Biochem. J., <u>31</u>, 2306 (1937)

61. Beyer and Lee, J. Pharmacol. <u>74</u>, 155 (1942)

PART II

PREPARATION OF SEVERAL PHOSPHO-ORGANIC COMPOUNDS

INTRODUCTION

At the present time it is recognized that phospho-organic compounds involved in cellular metabolism may be classified roughly into two types. In the first group can be placed those compounds in which the phosphate is linked to an alcoholic hydroxyl group, and which are hydrolyzed with a free energy change of approximately -3000 calories. In the second group are included creatine phosphate, arginine phosphate, phospho-enolpyruvic acid, phosphoglyceryl phosphate, adenosine tri-phosphate, and acetyl phosphate, which are hydrolyzed with a free energy change of approximately -10,000 calories. (1)

Acetyl phosphate was shown by Lipmann (2) to be a product of the oxidation of pyruvic acid by Bacterium Delbrueckii:

$$CH_3\text{-}CO\text{-}COOH + H_3PO_4 + O_2 \rightarrow CH_3\overset{O}{\overset{\|}{C}}\text{-}O\text{-}PO_3H_2 + CO_2 + H_2O_2$$
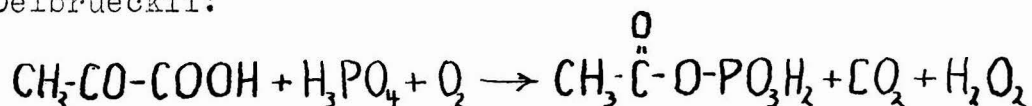
The properties of carboxyl phosphate suggest that compounds containing this structure may be utilized in various biological syntheses, as for example in the formation of the ester group of fats and of the peptide bond. In this connection it was considered to be of interest to prepare benzoyl phosphate, the anhydride of benzoic acid and phosphoric acid.

A method was developed for the preparation of this substance, although it has not been found possible to obtain it pure. In addition, acetyl phosphate has been prepared according to the method of Lynen (3), and phospho-enolpyruvic acid has been prepared and partially purified by the method of Kiessling (4).

EXPERIMENTAL WORK

PREPARATION OF ACETYL PHOSPHATE (3)

Dibenzyl phosphoric acid

Two hundred and twenty grams of benzyl alcohol (Merck reagent) was dissolved in 200 cc. of absolute ether, and 90 gm. of P_2O_5 was added to this solution in portions of about 2 gm., with moderate cooling to keep the temperature at 25-30°C. At the end of the reaction, 100 cc. of water was added, and the solution heated on the water bath, while 200 gm. of $BaCO_3$ and then 1 liter of saturated $Ba(OH)_2$ solution were added to bring the solution to alkalinity to phenolphthalein. The hot solution was filtered and the precipitate boiled twice with 500 cc. of water, filtered, and the three filtrates combined, concentrated at 100°C. to 500 cc., cooled, and the first crystal fraction collected. Further concentration yielded more product of the same purity. The total weight of Ba-salt was 26.6 gm., with m.p. 255-61°C. (d.). Twenty-five grams of the Ba-salt was dissolved in 750 cc. of hot water, and the calculated amount of $AgNO_3$ dissolved in a little water was added. The Ag-salt crystallized at once in needles, and after filtering and drying it weighed 24.6 gm., and melted at 215-16.5°C. Lynen gives m.p. 216°C. The compound was dried at room temperature over P_2O_5 for analysis.

	C	H
Calculated for $C_{14}H_{14}O_4PAg$	43.66	3.66
Found	44.15	3.83

Palladium-charcoal catalyst

A solution of 0.3 gm. of palladium chloride in 30 cc. of 0.1N HCl was added dropwise to a shaking suspension of 6.0 gm. of acid-washed Norite A in 300 cc. of distilled water, while hydrogen was passed through in a continuous stream. The catalyst was centrifuged off after shaking for 20 minutes, washed twice with absolute EtOH, centrifuged, and dried in vacuo over H_2SO_4. It was then dried over P_2O_5 at 110°C. for 4 hours in vacuo. This preparation was quite active, as shown by the rapid hydrogenation of cinnamic acid in 80% MeOH.

Ag-salt of acetyl phosphate

The reaction was carried out in a wide-mouth bottle fitted with a dropping funnel and a Pyrex filter stick, and moisture was carefully excluded during the reaction and subsequent filtration. One and one-half grams of the Ag-salt of dibenzyl phosphoric acid and 5 cc. of absolute ether were introduced into the bottle, and then 0.7 gm. of freshly distilled acetyl chloride dissolved in 2 cc. of absolute ether was added dropwise, at room temperature, over a period of several minutes. The mixture was allowed to stand for one hour, then 20 cc. of absolute ether was added, and the bottle was shaken vigorously. The ether solution was then filtered, and the ether and excess acetyl chloride removed by distillation in vacuo at 30°C. The oily residue was dissolved in 30 cc. of absolute ether, and 250 mgm. of palladium-charcoal catalyst added. The acetyl-dibenzyl-phosphate

was then hydrogenated at 25°C. and one atmosphere pressure,
with the hydrogenation flask protected from moisture by a
U-tube containing Drierite. The uptake of hydrogen stopped
after the theoretical amount of hydrogen had been absorbed
in 25 minutes. The catalyst was filtered off at 0°C., the
ether solution shaken twice with 5 cc. of ice-water, and the
aqueous solution quickly neutralized with saturated Ba(OH)$_2$
solution to phenolphthalein. These operations were con-
ducted in the cold, because of the great instability of
acetyl phosphate in acid solution. The insoluble barium
phosphate was centrifuged off, and 4 cc. of 25% AgNO$_3$ solu-
tion added to the supernatant, followed by an equal volume
of acid-free EtOH. The Ag-salt crystallized at once in
needles, and was centrifuged, washed with cold EtOH and
ether in the centrifuge, and dried. The weight was 488 mgm.

According to Lynen the Ag-salt of acetyl phosphate
hydrolyzes quantitatively as follows:

$$3\,Ag_2(CH_3CO)PO_4 + 5\,OH^- \longrightarrow 2\,Ag_3PO_4 + HPO_4^= + 3\,CH_3COO^- + 2\,H_2O$$
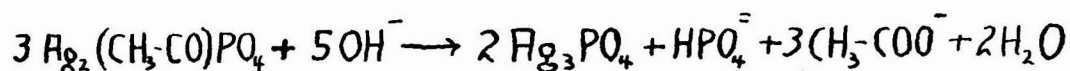
A titration conducted after hydrolysis of the Ag-salt in
boiling water for 10 minutes indicated a purity of 77%.
The substance was further purified as described below. The
Ag-salt was stirred up with slightly less than the calcu-
lated amount of KCl solution at 0°C. and pH 7.0, the pre-
cipitated AgCl was centrifuged off, and the Ag-salt re-
precipitated by the dropwise addition of 25% AgNO$_3$ solution.
The crystals were washed with EtOH and ether, and dried over
H$_2$SO$_4$. An acetyl determination according to Pregl gave:

		Acetyl
Calculated for $C_2H_3O_5PAg_2$		12.15
Found		11.90

PREPARATION OF BENZOYL PHOSPHATE

When the Ag-salt of dibenzyl phosphate was reacted with freshly distilled benzoyl chloride in the same manner as with acetyl chloride, that is by using two times the calculated amount of the acid chloride, only two-thirds of the calculated amount of hydrogen was taken up, and the metal salts isolated subsequently were obtained in low yield and were of a purity of about 25%, as determined by titration of acid formed on hydrolysis, and by benzoyl determination. Further investigation led to the isolation of one of the products formed from benzoyl chloride and Ag-dibenzyl-phosphate when the reaction was conducted in this manner. Removal of the ether *in vacuo* after the reaction gave an oil, which could be crystallized by vigorous scratching at 0°C. This product was washed free of benzoyl chloride with ligroin (85-100°C.), and dried. Two grams were obtained from 3.0 gm. of Ag-dibenzyl-phosphate, with m.p. 61-75°C. By repeated recrystallizations through dissolving in absolute ether and adding four volumes of ligroin, a product of constant melting point of 113-6°C. was obtained. Repeated recrystallization from anhydrous isopropyl ether gave a product of the same melting point. Several fractions were dried at room temperature over P_2O_5 and paraffin for analysis. This intermediate should be

benzoyl-dibenzyl-phosphate:
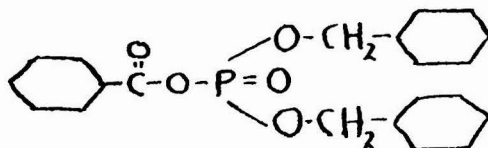
	C	H	P
Calculated for $C_{21}H_{19}O_5P$	65.96	5.01	8.12
Fraction of m.p. 61-75°C.	66.64	4.99	8.12
Fraction of m.p. 82-90°C.	65.36	4.58	
Fraction of m.p. 113-6°C.	64.08	4.61	5.88

The above analyses indicate clearly that the material of constant m.p. 113-6°C is not the desired intermediate.

The method finally adopted for the preparation of benzoyl phosphate is described below. Three grams of Ag-dibenzyl-phosphate was treated dropwise at 25°C. with 0.98 cc. (mol/mol) benzoyl chloride over a period of about 5 minutes, in the presence of 10 cc. of absolute ether. The mixture was shaken during this addition, then allowed to stand at 25°C. for three hours, 40 cc. of absolute ether added, and the AgCl filtered off as previously described. There was no detectable odor of benzoyl chloride in the filtrate. This ether solution was then hydrogenated after the addition of 500 mgm. of palladium-charcoal catalyst. The uptake of hydrogen stopped after the theoretical amount had been absorbed in 25 minutes. All subsequent procedures were conducted at 0-5°C., in order to reduce hydrolysis. The catalyst was filtered off, and the ether solution then shaken twice with 10 cc. of water, and the aqueous solution

neutralized at once to phenolphthalein with 60 cc. of
0.2N NaOH. Ten cubic centimeters of 10% $CaCl_2$ solution
was added, and rosettes of crystals formed immediately,
which were centrifuged off, washed with acid-free EtOH and
ether in the centrifuge, and dried. In this way was ob-
tained 1.25-1.50 gm. of the Ca-salt of benzoyl phosphate of
a purity of about 75% as determined by titration of a sample
hydrolyzed in boiling water for 10 minutes. A benzoyl de-
termination according to Pregl on the material combined
from several preparations gave the following: 19.8 mgm.
used 5.05 cc. of 0.0126N NaOH. Calculated for 19.8 mgm.:
6.55 cc. of 0.0126N NaOH. A purity of 77% is indicated by
this determination.

The Ag-salt is very insoluble, while the Ba-salt is
somewhat more soluble than the Ca-salt. The preparation of
the pure Ag-salt through first precipitating the inorganic
phosphate with $Ba(OH)_2$ solution was not possible because of
the limited solubility of the Ba-salt of benzoyl phosphate.
The Ca-salt was used because it was thought likely that it
could more easily be separated from inorganic impurities.
However, repeated attempts to obtain the pure Ca-salt by
dissolving the impure material in water at $0^{o}C$. and pH 7.0
(the solubility under these conditions is 80 mgm. per 100
cc. of water), and fractionally precipitating with 10% $CaCl_2$
solution and EtOH, were unsuccessful.

PREPARATION OF PHOSPHO-ENOLPYRUVIC ACID (4)

A solution of 18 cc. of freshly distilled phosphorus oxychloride in 30 cc. of redistilled quinoline was added dropwise to a solution of 30 gm. of pyruvic acid (b.p. 74-5°C./22 mm. Hg) in 30 cc. of quinoline. There was a strong evolution of heat, and the temperature was maintained at 60-70°C. by cooling. The reaction product was cooled to 5°C., and dissolved by slowly adding 25% NaOH solution, avoiding an excess of alkali. Two more preparations were made as described, the solutions combined, and the quinoline separated off. The pH was adjusted to 8.9, two volumes of 95% EtOH added, and a small amount of precipitate filtered. The filtrate was precipitated with 200 cc. of 25% Ba(OAc)$_2$ solution, and the precipitate filtered and washed with 2:1 EtOH-water.

This precipitate was suspended in 100 cc. of water, 35 cc. of 25% H$_2$SO$_4$ solution added, and the mixture was stirred for 1 hour. Then the suspension was treated with magnesia mixture in ammoniacal solution, and allowed to stand for 4 hours, after which time it was centrifuged. To the supernatant was added 75 cc. of 25% Ba(OAc)$_2$ solution and an equal volume of 95% EtOH at pH 7.0, and the precipitate which formed was filtered off and stirred with 200 cc. of water to which sufficient HCl was added to give a pH of 4.0. After centrifuging, the pH of the supernatant was adjusted to 7.5, and on the addition of an equal volume of 95% EtOH, a precipitate was obtained which was dried over

$CaCl_2$, and weighed 6.5 gm.

The phospho-enolpyruvic acid was determined according to the method of Meyerhof and Kiessling (5). This method makes use of the fact that phospho-enolpyruvic acid is split quantitatively by I_2/alkali with the liberation of inorganic phosphate, which can be determined colorimetrically. The difference between the alkali-hydrolyzable phosphate, and the I_2/alkali liberated phosphate is a measure of the phospho-enolpyruvic acid present. By this method the above product was shown to contain 35% of the Ba-salt of phospho-enolpyruvic acid.

SUMMARY

1.   The silver salt of acetyl phosphate has been prepared and obtained pure.

2.   A method has been developed for the preparation of the calcium salt of benzoyl phosphate with a purity of approximately 75%.

3.   The barium salt of phospho-enolpyruvic acid has been prepared and obtained with a purity of 35%.

REFERENCES

(1941)
1. Lipmann, Advances in Enzymology, Vol. I, 99

2. Lipmann, Enzymologia, $\underline{4}$, 65 (1937)

3. Lynen, Ber., $\underline{73}$, 367 (1940)

4. Kiessling, Ber., $\underline{69}$, 2331 (1936)

(1935)
5. Meyerhof and Kiessling, Biochem. Z., $\underline{281}$, 249