THE SYNTHESIS OF STILBENE COMPOUNDS HAVING POTENTIAL CARCINOGENIC AND HORMONAL ACTIVITY

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PREFACE

Synthetic compounds having hormonal activity are of great value in elucidating the mechanism of hormonal activity as well as having potential usefulness as substitutes for the natural material. Stilbestrol and stilbestrol derivatives have been found to possess estrogenic potency for numerous experimental animals and man. The usefulness of many of these compounds is limited, however, by their toxicity resulting in nausea and vomiting in many patients.

When given in large doses, stilbestrol and some of its derivatives cause the development of mammary cancer in certain strains of susceptible mice. There is wide variation in the carcinogenic potency in these substances, and the development of compounds of even greater carcinogenic potency would provide a useful tool in cancer research.

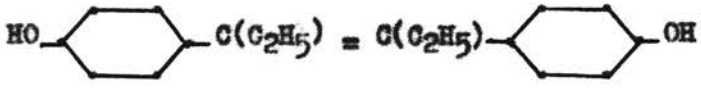
This research was, therefore, undertaken with the purpose of synthesizing compounds possessing potential carcinogenic and hormonal activity.

TABLE OF CONTENTS

HISTORICAL BACKGROUND

The isolation from natural sources of a compound with biological activity and the elucidation of its structure will always stimulate scientists to either duplicate synthetically the original biological compound or find a simpler compound having the same biological activity. This was especially true in the case of estrone, the female sex hormone isolated from human pregnancy urine by Doisy and his group (4).

In the course of their research on potential carcinogens, the group of Cook, Dodds and Hewitt became interested in finding compounds which would have biological activity similar to that of estrone. From 1933 to 1937 these men and their associates synthesized numerous compounds which, although having biological activity similar to estrone, did not have the potency of the natural product. In many instances these synthetic compounds were extremely toxic. In 1938, as a result of much work, Dodds, et al. (2), reported for the first time the synthesis of 4,4'-dihydroxy-α,β-diethylstilbene (stilbestrol) from desoxyanisoin. This synthesis marked the beginning of a new field of research in which the prime purpose was to synthesize compounds related structurally to stilbestrol,



and possibly having greater biological activities. Much work has been done along this line and an excellent review of the field has been written by Solmssen (5).

In his review Solmssen compares the activities of compounds in which the hydroxyl groups have been shifted from the para positions, compounds in which three carbons have been located between the two phenyl groups, and compounds in which aromatic, aliphatic or heterocyclic substitution has been made on the carbons located between the two phenyl groups. None of these modifications has given compounds superior in activity to stilbestrol.

INTRODUCTION

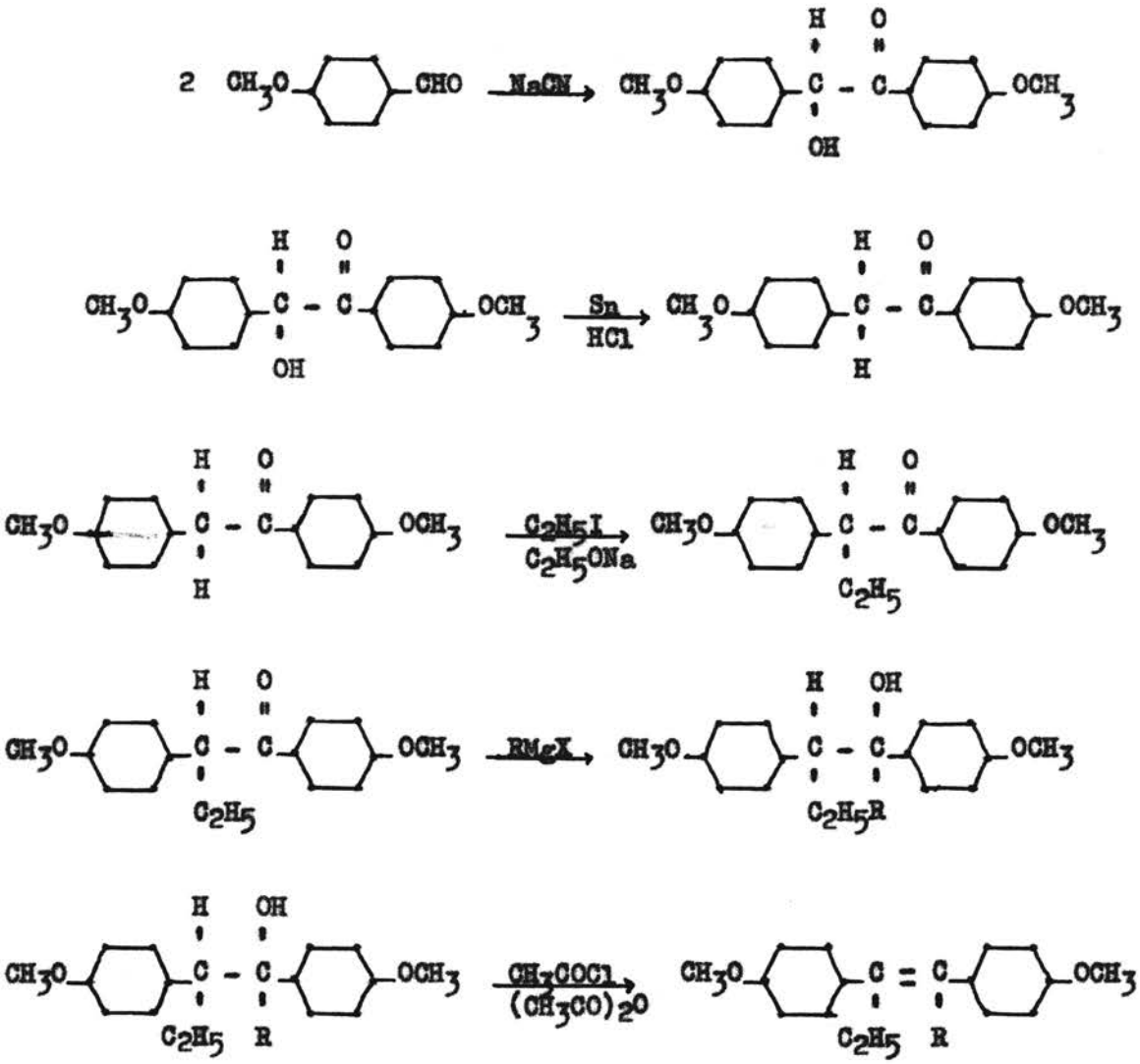
In order to keep the number of variable factors at a minimum it was decided at the beginning of this work to retain in the compounds synthesized one ethyl group on the ethylene portion of the stilbene molecule. The ethyl group was retained because those compounds of the stilbene type having the highest biological activity have one ethyl group or one methyl group on the ethylene portion of the molecule. In order to prolong the activity of these compounds, methoxy instead of hydroxy groups were placed in the para positions of the phenyl groups of the stilbene molecule.

The general scheme of the synthesis is outlined in Table I. A benzoin type condensation converted anisaldehyde to anisoin which upon reduction with tin and hydrochloric acid gave desoxyanisoin. The desoxyanisoin was ethylated with ethyl iodide and sodium ethoxide according to the method suggested by Dodds and co-workers (3). By means of a Grignard reaction the α-ethyldesoxyanisoin was arylated on the β-carbon to give a tertiary alcohol which was then dehydrated to the substituted stilbene.

Unsuccessful attempts were made to prepare the α-naphthyl, the benzyl, the β-styryl, the 3-pyridyl, and the cyclohexyl derivatives.

Although the phenyl derivative had been prepared by Dodds and co-workers (1), it was decided to repeat his experiment. It is interesting to note that the melting point of the compound prepared in this work is 110 - 111° C. (uncorr.), whereas that of the compound prepared by Dodds is of 80 - 81° C. The 1,2-dianisyl-1-phenylbutanol-1 used in the preparation of the dehydrated product has the same melting point as that prepared by

TABLE I



R = phenyl, p-phenoxyphenyl, p-tolyl, p-isobutylphenyl

Dodds. Carbon and hydrogen analyses confirmed the empirical formula of 4,4'-dimethoxy-α-ethyl-β-phenylstilbene. This suggests that the difference in the method of dehydration may result in formation of isomers.

The biological testing of the compounds prepared was beyond the scope of this research. However, these compounds will be tested and the results submitted for publication at a later date.

EXPERIMENTAL

Anisoin

In a 12-liter, round-bottom flask equipped with a reflux condenser were placed 2 kilograms (14.7 moles) of anisaldehyde, 3.5 liters of methanol and 500 grams of sodium cyanide dissolved in 1.6 liters of water. After the mixture was refluxed for two hours, an additional 50 grams of sodium cyanide dissolved in 500 ml. of water was added and the mixture was refluxed for an additional two hours. The reaction mixture was chilled in an ice bath and 900 ml. of water was added with continuous stirring. Stirring was continued until crystallization occurred. The crystals were separated in a basket centrifuge, washed with methanol and dried. Yield, 699 grams (34.9% of the theoretical). Melting point: obtained, 109 - 110° C.; reported in the literature, 110 - 111° C.

Desoxyanisoin

Into a 12-liter, round-bottom flask equipped with a reflux condenser were placed 1450 grams (5.33 moles) of anisoin, 5.5 liters of isopropyl alcohol, 860 grams of mossy tin, and 2.3 liters of concentrated hydrochloric acid. After the mixture was refluxed for thirteen hours, the hot solution was decanted from the residual tin. The crystals which formed when the solution cooled were separated by filtration from the chilled solution, washed with methanol and dried. Yield, 1211 grams (88% of the theoretical). Melting point: obtained, 110 - 111° C.; reported in the literature, 111 - 112° C.

α-Ethyldesoxyanisoin

Two and one-tenth liters of absolute ethanol was placed in a 12-liter, round-bottom flask fitted with an efficient reflux condenser. To the alcohol was added 113 grams (4.93 moles) of sodium. The rate of reaction was controlled by means of an ice bath. When all the sodium had reacted, 1244 grams (4.57 moles) of desoxyanisoin and 763 grams (4.89 moles) of ethyl iodide were added rapidly. While this mixture was being refluxed for fifteen minutes, a second charge of sodium ethoxide was prepared by adding 41.3 grams (1.79 moles) of sodium to 700 ml. of absolute ethanol. To this solution was then added 284 grams (1.82 moles) of ethyl iodide. The second charge was then added to the main reaction mixture which was then refluxed for four and one-half hours. At the end of this time the alcohol was distilled and the residue taken up in ten liters of ethyl ether. The unreacted desoxyanisoin and the sodium iodide formed in the reaction were removed by filtration. The ether solution was washed with water, dilute aqueous sodium thiosulfate solution and then with water again. The ether solution was concentrated to three liters and placed in an icebox overnight. The following morning any desoxyanisoin that had settled out was removed by filtration. The ether was distilled and the residue transferred to a 2-liter Claisen flask. The residue was distilled at reduced pressure and that fraction distilling at 220 - 245° C. at a pressure of 2 mm. was collected. The product was dissolved in two liters of anhydrous ether and placed in an icebox overnight. The desoxyanisoin which crystallized was removed and the product was distilled a second time. The fraction boiling at 220 - 230° C. at a pressure of 1 - 2 mm. was saved. Yield, 1013 grams (77% of the theoretical).

1,2-Dianisyl-1-phenylbutanol-1

Into a 500-ml. three-neck, round-bottom flask equipped with a reflux condenser, mercury-sealed stirrer and a dropping funnel were placed 3.43 grams (0.141 moles) of magnesium turnings and 50 ml. of anhydrous ethyl ether. With the dropping funnel 22.17 grams (0.141 moles) of phenyl bromide dissolved in 90 ml. of ether was added with stirring. After the reaction subsided, a solution of 25 grams (0.089 moles) of α-ethyldesoxyanisoin in 50 ml. of ether was added to the Grignard reagent. After the addition was complete, the reaction mixture was kept at reflux for an additional twenty-four hours.

The reaction mixture was decomposed by pouring on cracked ice and making the solution acid to Congo red with hydrochloric acid (1:1). The ether layer was separated and the aqueous layer was re-extracted with ether. The ether extracts were combined, washed with water, dried with anhydrous sodium carbonate, and distilled to dryness on a water bath. The residue was recrystallized from Skellysolv "B". Yield, 25.8 grams (80.8% of the theoretical). Melting point, obtained, 104 - 105° C.; reported in literature, 107 - 108° C.

4,4'-Dimethoxy-α-ethyl-β-phenylstilbene

Ten grams (0.027 moles) of 1,2-dianisyl-1-phenylbutanol-1 was dissolved in 45 ml. of glacial acetic acid. The reaction flask was placed in an ice bath and dry hydrogen chloride gas passed through the reaction mixture for six hours. The crystals formed were removed by filtration and recrystallized from ethanol. Yield, 5.9 grams (62% of the theoretical). Melting point, 110 - 111° C. The melting point of the 4,4'-dimethoxy-α-ethyl-β-phenylstilbene obtained by Dodds is 80 - 81° C. Analysis: C, 83.45%; H, 6.82%; $C_{24}H_{24}O_2$ requires C, 83.66%; H, 7.02%.

4,4'-Dimethoxy-α-ethyl-β-(p-tolyl)stilbene

The Grignard reagent was prepared by adding to a mixture of 3.8 grams (0.156 moles) of magnesium turnings and 25 ml. of anhydrous ether, 27.1 grams (0.156 moles) of p-tolyl bromide dissolved in 90 ml. of anhydrous ether. A solution of 15 grams (0.052 moles) of α-ethyldesoxyanisoin dissolved in 30 ml. of anhydrous ether was added to the prepared reagent. After the reaction subsided, the ether was distilled and 100 ml. of anhydrous benzene was added. The reaction mixture was refluxed and stirred for six hours and then decomposed by pouring it on ice and making the solution acid to Congo red with hydrochloric acid (1:1). The benzene layer was separated and the aqueous layer re-extracted with benzene. The benzene fractions were combined, dried with anhydrous sodium carbonate, and then distilled to dryness. To the residue (26.1 grams) was added 29 ml. of acetyl chloride and 58 ml. of acetic anhydride. The mixture was refluxed for eight hours. The reaction mixture was poured on ice, and the aqueous solution extracted with ether. The ether extracts were washed with water, and then dilute sodium bicarbonate solution and dried with anhydrous sodium carbonate. The ether was distilled and the residue crystallized from methanol to which a small amount of ether had been added to eliminate turbidity. The product was recrystallized from methanol. Yield, 7.1 grams (27% of the theoretical). Melting point, 94.5 - 95.5° C. Analysis: C, 83.36%; H, 7.2%; $C_{25}H_{26}O_2$ requires C, 83.7%; H, 7.3%.

4,4'-Dimethoxy-α-ethyl-β-(p-phenoxyphenyl)stilbene

The Grignard reagent was prepared by adding to a mixture of 3.2 grams (0.132 moles) of magnesium turnings and 50 ml. of anhydrous ether, a solution of 25.6 grams (0.103 moles) of p-bromodiphenyl ether in 90 ml. of anhydrous ethyl ether. Since reaction rate was very slow, a reflux

and stirring period of twenty hours was required for formation of the Grignard reagent.

A solution of fifteen grams (0.052 moles) of ∝-ethyldesoxyanisoin in 49 ml. of anhydrous ether was added. After the reaction had subsided, the ether was distilled and 100 ml. of anhydrous benzene was added to the reaction mixture. Refluxing and stirring was continued for an additional six hours.

The reaction mixture was decomposed by pouring on ice and making the solution acid to Congo red with hydrochloric acid (1:1). The benzene layer was removed and the aqueous fraction was re-extracted with benzene. The benzene fractions were combined, washed with water, dried with anhydrous sodium carbonate and distilled to dryness. The residue (31 grams) was refluxed for eight hours with 70 ml. of acetic anhydride and 35 ml. of acetyl chloride. The reaction mixture was poured on ice and the aqueous solution extracted with ether. The ether extract was washed with water and then with dilute aqueous sodium bicarbonate solution, dried with anhydrous sodium carbonate and distilled to dryness. The residue was crystallized from ethanol and the resulting crystals recrystallized from isopropyl alcohol. Yield, 14.2 grams (52.2% of the theoretical). Melting point, 129 - 130° C. Analysis: C, 82.66%; H, 6.59%; $C_{30}H_{28}O_3$ requires C, 82.52%; H, 6.47%.

4,4'-Dimethoxy-∝-ethyl-β-(p-isobutylphenyl)stilbene

A Grignard reagent was prepared by adding to a mixture of 10.2 grams (0.41 moles) of magnesium turnings and 50 ml. of anhydrous ether, a solution of 39.8 grams (0.187 moles) of p-bromoisobutylbenzene in 90 ml. of anhydrous ether. A solution of 30 grams (0.105 moles) of ∝-ethyldesoxyanisoin in 60 ml. of anhydrous ether was then added to this reagent.

After the addition was complete, the ether was distilled and 125 ml. of anhydrous benzene added to the reaction mixture. Refluxing and stirring was continued for six hours.

The reaction mixture was decomposed by pouring it on ice and acidifying to Congo red with hydrochloric acid (1:1). The aqueous layer was re-extracted with benzene. The benzene extracts were combined, washed with water, dried with anhydrous sodium carbonate and distilled to dryness.

The residue (63.5 grams) was dehydrated by refluxing for eight hours with 84 ml. of acetyl chloride and 169 ml. of acetic anhydride. The reaction product was poured into ice and extracted with ether. The ether extract was washed with water and dilute aqueous sodium bicarbonate solution, dried over anhydrous sodium carbonate, and distilled to dryness.

The residue was crystallized from ethanol. The resulting crystals were recrystallized from isopropyl alcohol. Yield, 14.5 grams (31% of the theoretical). Melting point, 95 - 96° C. Analysis: C, 83.99%; H, 7.95%; $C_{28}H_{32}O_2$ requires C, 83.94%; H, 8.05%.

Attempted Synthesis of 1,2-Dianisyl-1-(β-styryl)butanol-1

Into a 500-ml. round-bottom flask equipped with dropping funnel, a mercury-sealed stirrer, and a condenser were placed 3.8 grams (0.156 moles) of magnesium turnings and 25 ml. of anhydrous ether. A solution of 28.9 grams (0.158 moles) of β-bromostyrene in 90 ml. of anhydrous ether was then added. A solution of 15 grams (0.052 moles) of α-ethyldesoxyanisoin dissolved in 30 ml. of anhydrous ether was added to the resulting Grignard reagent. The reaction mixture was refluxed for forty-three hours and then decomposed in the usual manner. This reaction yielded 1.2 grams of a yellow crystalline material which softened and flowed rather than melted at 145.5 - 146° C. Analysis: C, 90.15%; H, 6.17%; $C_{26}H_{28}O_3$ requires

C, 80.37%; H, 7.27.

The analysis indicates that the compound obtained is not 1,2-dianisyl-1-(-styryl)butanol-1.

Attempted Synthesis of 1,2-Dianisyl-1-(3'-pyridyl)butanol-1

To a mixture of 2.5 grams (0.102 moles) of magnesium and 25 ml. of anhydrous ether was added a solution of 16.8 grams (0.106 moles) of 3-bromopyridine in 90 ml. of anhydrous ether. The Grignard reagent did not form even after eighteen hours of constant stirring and refluxing.

Attempted Synthesis of 1,2-Dianisyl-1-benzylbutanol-1

Into a 500-ml. round-bottom flask equipped with a mercury-sealed stirrer, a reflux condenser and a dropping funnel was introduced 7.8 grams (0.321 moles) of magnesium turnings and 30 ml. of anhydrous ether. To this was added a solution of 39.9 grams (0.315 moles) of benzyl bromide in 100 ml. of anhydrous ether. A vigorous reaction occurred. A solution of 30 grams (0.105 moles) of α-ethyldesoxyanisoin in 60 ml. of anhydrous ether was then added. The ether was removed by distillation and 125 ml. of benzene added. The reactants were refluxed for six hours and then decomposed in the usual manner. No crystalline products were isolated from the oils.

Attempted Synthesis of 1,2-Dianisyl-1-(α-naphthyl)butanol-1

Into a 500-ml. round-bottom flask equipped with a mercury-sealed stirrer, a condenser, and a dropping funnel were placed 3.4 grams (0.14 moles) of magnesium and 25 ml. of anhydrous ether. To these was added a solution of 29.2 grams (0.14 moles) of α-bromonaphthalene in 90 ml. of anhydrous ether. To the Grignard reagent was added a solution of 25 grams (0.088 moles) of α-ethyldesoxyanisoin in 60 ml. of anhydrous ether. The reaction mixture refluxed with stirring for forty-eight hours.

The reaction product was decomposed in the usual manner. The only crystalline product obtained proved to be naphthalene. All attempts to obtain other crystalline products from the reaction product failed.

Attempted Preparation of 1,2-Dianisyl-1-cyclohexylbutanol-1

A Grignard reagent was prepared from 3.8 grams (0.156 moles) of magnesium suspended in 25 ml. of anhydrous ether, to which a solution of 25.8 grams (0.158 moles) of cyclohexyl bromide in 90 ml. of anhydrous ether was added. A solution of 15 grams (0.052 moles) of α-ethyldesoxyanisoin in 30 ml. of anhydrous ether was added to the Grignard reagent. The reaction was refluxed for forty-eight hours. The reaction mixture was decomposed in the usual manner and gave 8.7 grams of a white crystalline solid. The melting point was 138.5 - 139.5° C. Analysis: C, 80.83%; H, 8.35%; $C_{24}H_{32}O_3$ requires C, 78.2%; H, 8.75%. Apparently the compound isolated is not the expected carbinol. The nature of this compound is unknown.

DISCUSSION

One explanation for the failure of repeated attempts to prepare the cyclohexyl, β-styryl, benzyl, and α-naphthyl derivatives might be that in each case the halide combined with itself to form a biaryl compound. This is indicated by the fact that when the α-ethyldesoxyanisoin was added to the Grignard reagents no appreciable heat of reaction was noted. In general it was found, with the exception of the phenyl bromide, that the formation of the Grignard reagents leading to those compounds synthesized, was slow and laborious. The p-phenoxyphenyl Grignard reagent in particular required twenty hours of heating to complete its formation.

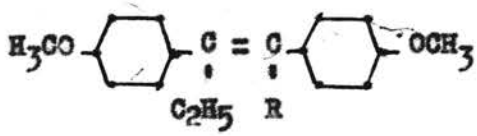
It seems, in view of some of the negative results acquired, that a new approach is needed in order to successfully synthesize compounds of this type.

It might be advantageous to use amalgamated magnesium in order to slow down the formation of the Grignard reagent and thus possibly prevent side reactions. In addition organo-lithium compounds might be considered instead of organo-magnesium compounds. The Wurtz reaction although good for preparing symmetrically substituted products would not be very useful for making the unsymmetrical compounds now sought. The use of the Diels-Alder type reaction will however be investigated as it seems to offer most hope at the present time.

SUMMARY

4,4'-Dimethoxy-α-ethyl-β-phenylstilbene, 4,4'-dimethoxy-α-ethyl-β-(p-tolyl)stilbene, 4,4'-dimethoxy-α-ethyl-β-(p-phenoxyphenyl)stilbene, and 4,4'-dimethoxy-α-ethyl-β-(p-isobutylphenyl)stilbene have been prepared. The physical constants for these compounds are shown in Table II.

TABLE II



R	Melting Point	Analysis			
		Calculated		Found	
	(° C.)	Carbon (%)	Hydrogen (%)	Carbon (%)	Hydrogen (%)
phenyl	110 - 111	83.66	7.02	83.45	6.82
p-tolyl	94.5 - 95.5	83.7	7.3	83.36	7.2
p-phenoxyphenyl	129 - 130	82.52	6.47	82.66	6.59
p-isobutylphenyl	95 - 96	83.94	8.05	83.99	7.95

The phenyl derivative prepared in this work is probably a geometric isomer of the same compound prepared by Dodds.

Attempts were made to prepare the cyclohexyl, β-styryl, benzyl, α-naphthyl and 3-pyridyl derivatives.

These compounds will be tested for both carcinogenic and hormonal activity in rats and mice. The results of such tests will be published at a later date.

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AUTOBIOGRAPHY

Milton J. Allen was born in New York City, July 3, 1918. In 1935, he entered Oklahoma A. & M. College and remained there until 1937 when he accepted a summer appointment with the Research Division of the New York Department of Health. Upon the termination of this appointment, he became Research Chemist in the Department of Endocrinology, Post-Graduate Medical School, Columbia University.

In 1939, he left Columbia University to become Director of the Hormone Division of Hospital Liquids, Inc. In 1941, he founded The Allen Laboratories for the manufacture of fine chemicals and pharmaceuticals. This was sold in 1943, and Mr. Allen became a consultant to such companies as Vitamins, Inc., Lee Foundation for Nutritional Research, American Biosynthetics Corporation, Lincoln Laboratories and Quaker Oats Company.

From 1941 to 1943, he was a consulting chemist to the Department of Physiology, Northwestern University. In 1945, he accepted an appointment as senior Biochemist in the Illinois Department of Public Health. He resigned this position to return to the University of Chicago, where he was awarded the Bachelor of Science degree in 1946.

Mr. Allen entered Oklahoma A. & M. College for graduate work September, 1946. He served until January, 1947, as a Graduate Fellow and from that time to completion of the work for the Master of Science degree, he was a Research Fellow in the Agricultural Experiment Station.

Mr. Allen was awarded a National Institute of Health Research Fellowship and a University Scholarship at Johns Hopkins University,

effective September 1947, for research in cancer chemistry.

He is a member of the American Association for the Advancement of Science and a Fellow of the British Chemical Society.

Typist: Anna Lee Phillips