AN ABSTRACT OF THE THESIS OF

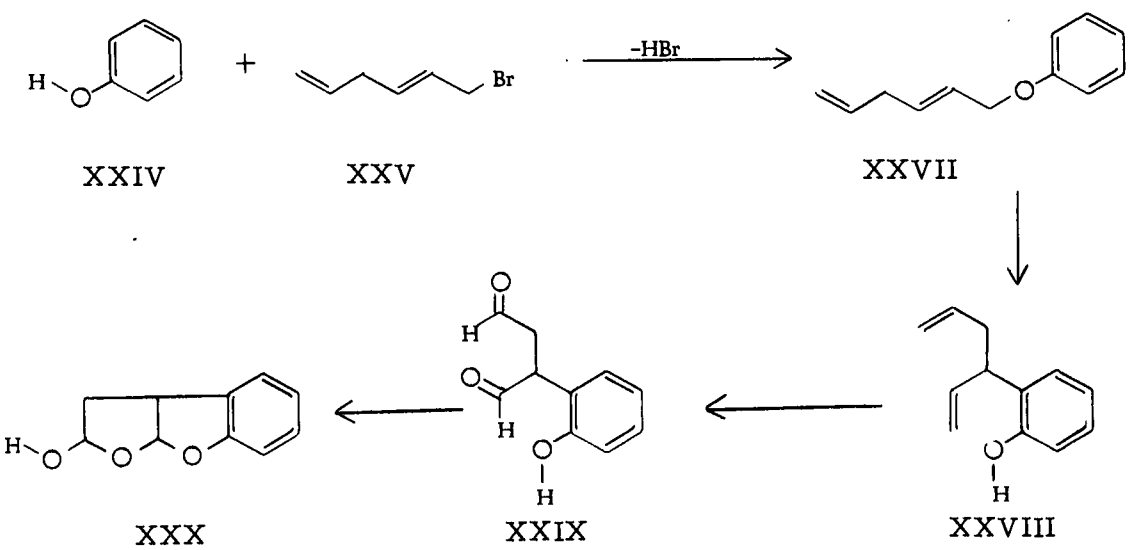
DAVID JAMES JONES for the MASTER OF SCIENCE
(Name) (Degree)

in Food Science presented on July 13, 1972
(Major) (Date)

Title: SYNTHESIS OF THE 2, 3, 3a, 8a-TETRAHYDRO-2-HYDROXYFURO[2, 3-b] BENZOFURAN RING SYSTEM, AN AFLATOXIN MOIETY

Abstract approved: _____
Dr. Norman E. Pawlowski

A new method for the preparation of the 2, 3, 3a, 8a-tetrahydro-2-hydroxyfuro[2, 3-b] benzofuran, XXX, ring system has been developed. The synthesis envolved a substitution reaction of phenol, XXIV, with 1-bromo-2, 5-hexadiene, XXV, to give 2, 5-hexadien-1-yl phenyl ether, XXVII. The ether was rearranged via an ortho-Claisen rearrangement to 3-(o-hydroxyphenyl)-1, 5-hexadiene, XXVIII. The double bonds of the phenolic diene were cleaved to dialdehyde XXIX which cyclized to the desired product, XXX. The structure of XXX was verified by infrared, ultraviolet, nuclear magnetic resonance, and mass spectral data, as well as by a preparation from an adaptation of a published route.



XXIV XXV XXVII

XXVIII XXIX XXX

Synthesis of the 2, 3, 3a, 8a-Tetrahydro-2-hydroxy-furo[2, 3-b]benzofuran Ring System, An Aflatoxin Moiety

by

David James Jones

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1973

APPROVED:

Assistant Professor of Food Science and Technology
in charge of major

Head of Department of Food Science and Technology

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Date thesis is presented July 13, 1972

Typed by Susie Kozlik for David James Jones

ACKNOWLEDGEMENTS

The author wishes to express most sincere gratitude to Dr. Norman E. Pawlowski for his guidance, encouragement, and patience. Thanks to Professors R. O. Sinnhuber and D. J. Lee for their inspiration. Thanks also to fellow graduate student James K. Yoss for his timely advice and encouragement. Many thanks are extended to all the other staff, faculty, and graduate students in the Department of Food Science and Technology for their recommendations, enthusiasm, and cooperation.

The most profound thanks go to my parents Mr. and Mrs. Gale H. Jones for their encouragement and understanding during my graduate study.

This study was supported by the National Institute of Health Grant ES-0256 from the Division of Environmental Health Science and training grant FD-00010 from the Food and Drug Administration.

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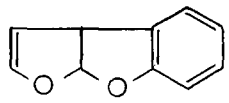
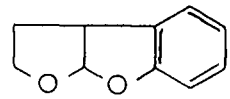
INTRODUCTION

In 1960 the outbreak of "turkey X disease" in England and a nationwide trout hepatoma epizootic in the United States led to the discovery of the aflatoxins. The aflatoxins are metabolites produced by certain species of Aspergillus, a common mold in stored agricultural products (20). These toxins belong to a unique class of potentially dangerous mycotoxins containing the 3a, 8a-dihydrofuro[2, 3-b]benzofuran, I, or the 2, 3, 3a, 8a-tetrahydrofuro[2, 3-b]benzofuran, II, ring system. The furo[2, 3-b] benzofuran mold metabolites have been



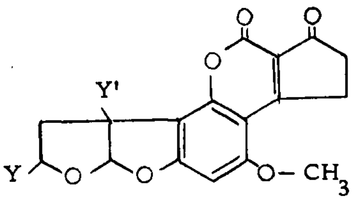
I



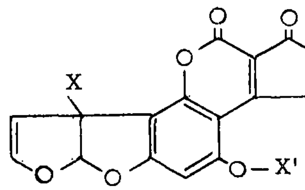
II

divided into three groups (37): 1) substituted coumarins, the aflatoxin derivatives; 2) substituted xanthones, the sterigmatocystin derivatives; and 3) substituted anthraquinones, the versicolorin derivatives. Some naturally-occurring structures of the groups are shown in Figures 1, 2, and 3, respectively.

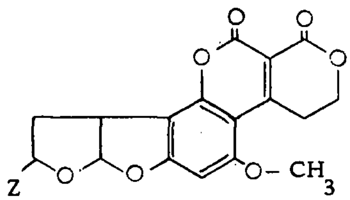
Figure 1. Substituted coumarins, the aflatoxins.



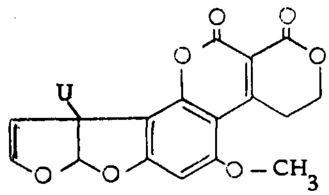
Name	Y	Y'	Ref.
B_2	H	H	3
B_{2a}	OH	H	17
M_2	H	OH	33



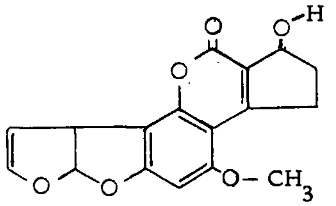
Name	X	X'	Ref.
B_1	H	CH_3	3
M_1	OH	CH_3	26
P_1	H	H	13



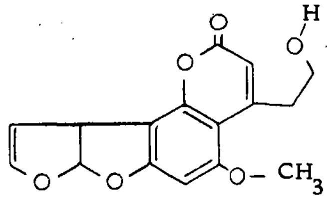
Name	Z	Ref.
G_2	H	3
G_{2a}	OH	16



Name	U	Ref.
G_1	H	3
GM_1	OH	31

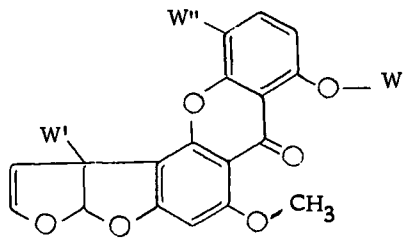


Name	Ref.
aflatoxicol (R_o)	14, 36



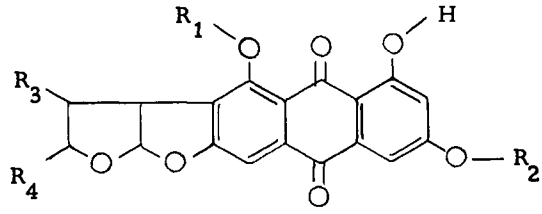
Name	Ref.
Parasiticaol (B_3)	22

Figure 2. Substituted xanthones, the sterigmatocystin derivatives.



Name	W	W'	W''	Ref.
Sterigmatocystin	H	H	H	9
O-methylsterigmatocystin	CH_3	H	H	11
Aspertoxin	CH_3	OH	H	32, 43
5-Methoxysterigmatocystin	H	H	OCH_3	24

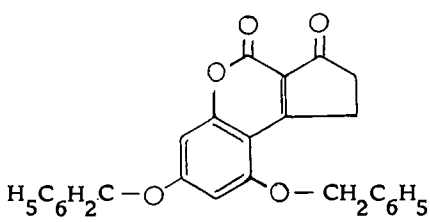
Figure 3. Substituted anthraquinones, the versicolorin derivatives.



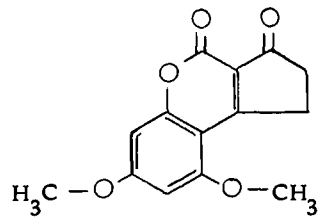
Name	R_1	R_2	R_3	R_4	Ref.
Versicolorin A	H	H	H	H	21
Versicolorin B	H	H	H, H	H, H	21
Versicolorin C		racemate of B			21
Aversin	CH_3	CH_3	H, H	H, H	10

Aflatoxin Structure and Biological Activity

Frequently a close relation has been found between biological activity and a similar chemical structure. The aflatoxins have two similar moeties in common, a furofuran and a coumarin portion. Dickens (15) attributed most of the biological activity of the aflatoxins to the α, β -unsaturated lactone function, the coumarin portion. Parks and Wang (32) prepared 5, 7-dibenzoxycyclopentenone[2, 3-c] coumarin, III, and found it produced no histological effects when orally administered to ducks or intraperitoneally to rats. Wogan, Edwards, and Newberne (44) showed that 5, 7-dimethoxycyclopentenone[2, 3-c]coumarin, IV, was neither toxic nor carcinogenic to rats and ducklings at more than 200 times the dose level of an effective response to aflatoxin B_1. Ayres et al. (5) also found coumarin IV not to be carcinogenic to rainbow trout. This data indicates that the furofuran ring system is important to the adverse biological effects of the aflatoxins. Other biological data with the aflatoxins seems to substantiate this conclusion.



III



IV

Acute tocicity and carcinogenicity studies provide useful data for associating biological activity with the furofuran function. Comparable data for acute toxicity with the aflatoxins and day-old ducklings is given in Table 1. The LD_{50} values in this table permit the following general statements concerning furofuran structure and toxicity. Saturation of the vinyl ether double bond reduces the toxicity by two to four times, i.e. B_1 vs B_2, G_1 vs G_2, and M_1 vs M_2. Addition of water across this double bond detoxifies the compound by greater than one hundred fold, i.e. B_1 vs B_{2a} and G_1 vs G_{2a}. Hydroxylation of aflatoxin B_1 and B_2 to M_1 and M_2, respectively, affects acute toxicity to ducklings very little.

In many cases, the carcinogenic properties of the natural furo[2,3-b]benzofuran compounds correspond to their acute toxicities, although the two biological effects are quite possibly unrelated. Table 2 shows the comparative oral hepatocarcinogenicity in rainbow trout for some of the aflatoxins. Again it can be seen that the integrity of the vinyl ether double bond appears to have a significant effect on the biological activity.

Chemistry of the Furo[2,3-b]benzofuran Ring System

The reactivity of the furofuran portion in aflatoxins B_1 and B_{2a} appear to be consistent with that of acetals and hemiacetals in general.

Table 1. Acute oral toxicity of aflatoxins to day-old ducklings.

Toxin	Approx. Wt. of duckling in g	Vehicle[a]	Duration in days	LD_{50} in mg/kg[b]	Ref.
Aflatoxin B_1	38	DMF	6	0.36	12[c]
Aflatoxin B_2	38	DMF	6	1.70	12[c]
Aflatoxin G_1	38	DMF	6	0.78	12[c]
Aflatoxin G_2	38	DMF	6	3.45	12[c]
Aflatoxin B_1	50	DMSO	14	0.73	45[d]
Aflatoxin B_2	50	DMSO	14	1.76	45[d]
Aflatoxin G_1	50	DMSO	14	1.18	45[d]
Aflatoxin G_2	50	DMSO	14	2.83	45[d]
Aflatoxin B_1	51	Prop Gly	2	0.56	4[d]
Aflatoxin G_1	51	Prop Gly	2	1.80	4[d]
THDOA B_1[e]	51	Prop Gly	2	No deaths[f]	4[d]
Aflatoxin B_1	45	WG Oil	7	0.24	33[d]
Aflatoxin M_1	45	WG Oil	7	0.33	33[d]
Aflatoxin M_2	45	WG Oil	7	1.24	33[d]
Aflatoxin B_{2a}	--	--	--	No deaths[f]	17[c]
Aflatoxin G_{2a}	--	--	--	No deaths[f]	17[c]

[a]DMF is dimethylformamide. DMSO is dimethyl sulfoxide. Prop Gly is propylene glycol. WG Oil is wheat germ oil.

[b]Milligrams toxin per kilogram body weight lethal to 50% of the animals.

[c]Khaki Campbell ducklings.

[d]White Pekin ducklings.

[e]THDOA B_1 is tetrahydrodesoxoaflatoxin B_1.

[f]No deaths occurred when the following maximum doses were administered: THDOA B_1 @ 1.00 mg/kg; Aflatoxin B_{2a} @ 24.0 mg/kg; and Aflatoxin G_{2a} @ 36.0 mg/kg.

Table 2. Oral hepatocarcinogenicity of aflatoxins in rainbow trout.

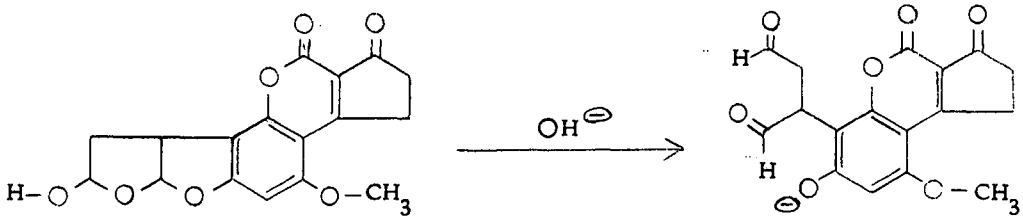
Toxin	Dose in ppb[a]	12 Months Nos.	12 Months %	16 Months Nos.	16 Months %	Ref.
Control	0	0/20	0	0/40	0	5
Aflatoxin B_1	4	10/40	25	14/40	35	5
Aflatoxin B_1	8	40/57	70	32/40	80	5
Aflatoxin B_1	20	62/80	78	--	--	5
Aflatoxin B_2	20	1/20	5	0/40	0	5
Aflatoxin G_1	20	1/20	5	7/40	17	5
Aflatoxin G_2	20	0/20	0	0/40	0	5
THDOA B_1[b]	20	1/80	1	--	--	5
5,7-DMCPC[c]	20	0/80	0	--	--	5
Control	0	1/100	1	--	--	40
Aflatoxin B_1	4	25/106	24	--	--	40
Aflatoxin M_1	8	8/106	8	--	--	40
Aflatoxin M_1	16	30/103	29	--	--	40
Aflatoxin M_1	32	33/106	31	--	--	40
Aflatoxin M_1	64	31/110	28	--	--	40

[a] Toxin dose was in parts per billion of purified daily diet.

[b] THDOA B_1 is tetrahydrodesoxoaflatoxin B_1.

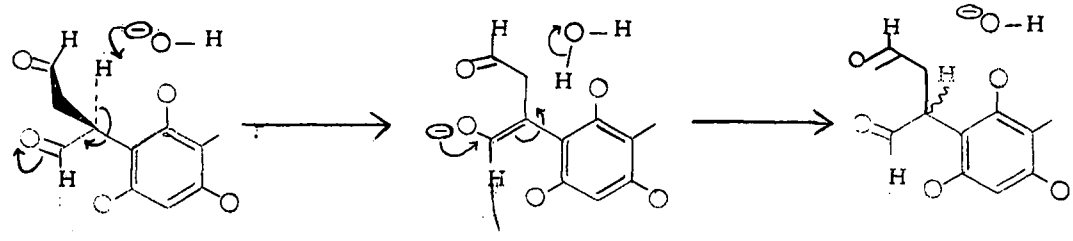
[c] 5,7-DMCPC is 5,7-dimethylcyclopentenone[2,3-c]coumarin.

Dilute acidic conditions catalyze the formation and hydrolysis of both acetals and hemiacetals. Dilute basic conditions catalyze the formation and hydrolysis of only hemiacetals. In the ultraviolet spectrum of aflatoxin B_{2a}, Buchi et al. (8) observed a bathochromic shift characteristic for phenols after the addition of base. The furofuran rings were believed to be opened by base to the phenoxide ion, V. Aflatoxin B_1 showed no bathochromic shift with the same basic treatment indicating



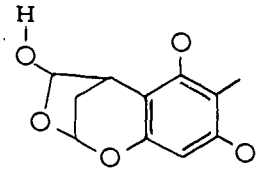
aflatoxin B_{2a} V

that both the lactone and furofuran portions are unaffected. In this same study, it was also noted that the optical rotation of aflatoxin B_{2a} approached zero within minutes after addition of base (mechanism illustrated below). Acidification followed by extraction led to recovery of the racemic hemiacetal, aflatoxin B_{2a}.

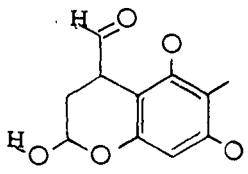


(-) (±)

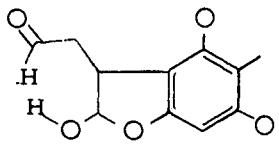
The fact that the furofuran function is reformed from phenoxide ion V indicates its stability over other isomeric structures. These may include partial structures VI, VII, and VIII. (VI has never been



VI



VII



VIII

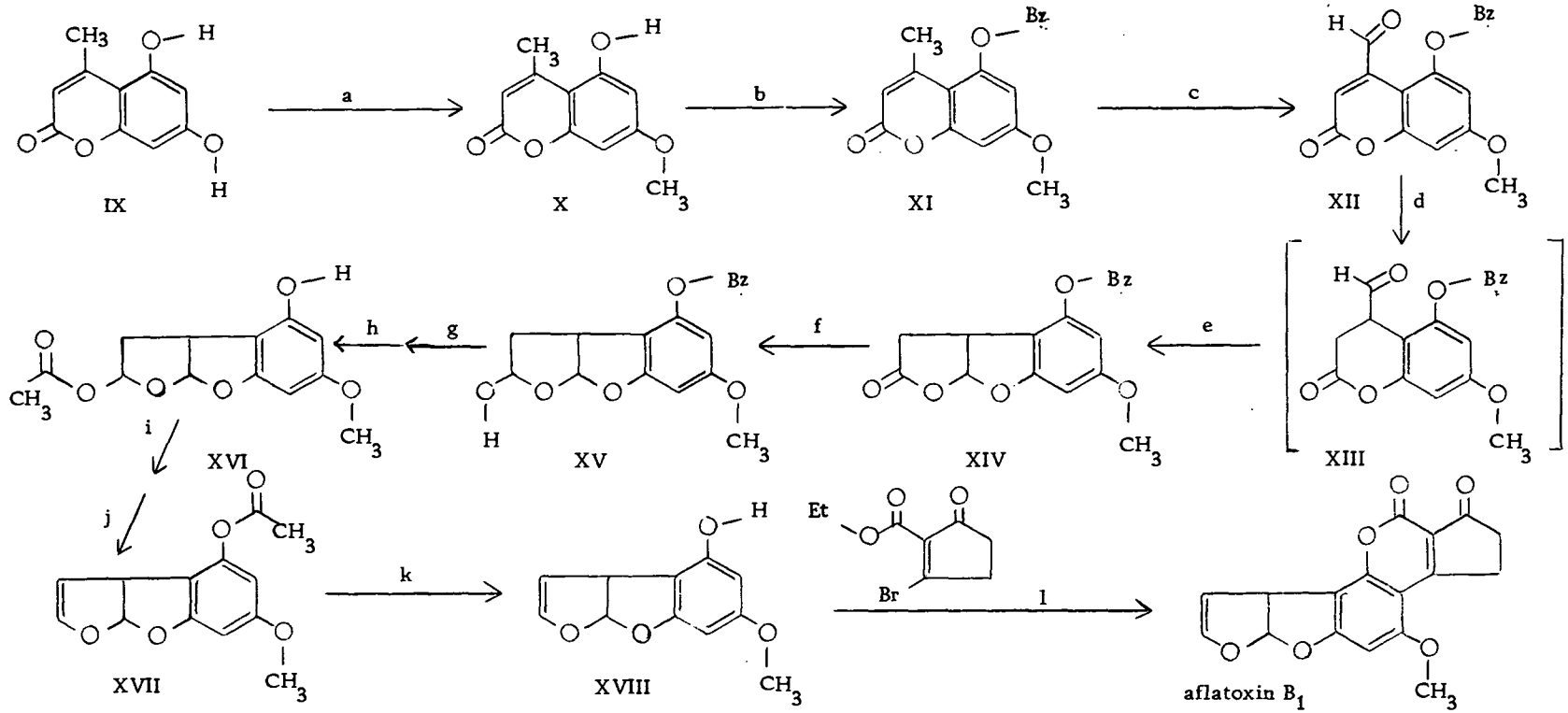
postulated in relation to the chemical behavior of the furofuran hemiacetals). If the furofuran structure were preferred, all three partial structures would be expected to rearrange to the same hemiacetal analogous to that of aflatoxin B_{2a}. In one instance, a similar rearrangement has been observed. Buchi (8) found that lactone XIII formed a good yield of lactone XIV (Figure 4, e) in what was termed a β-acyl lactone rearrangement.

Synthesis of the Furo[2,3-b]benzofuran Ring Systems

Buchi et al. (7) announced the total synthesis of racemic[1] aflatoxin B_1 in 1966 and described it in greater detail late in 1967 (8). In 1971 Buchi and Weinreb (6) reported an improved synthesis for aflatoxin B_1 which is shown in Figure 4. The improved method

[1] All synthetic 3a,8a-dihydro- and 2,3,3a,8a-tetrahydrofuro[2,3-b]benzofurans are racemic.

Figure 4. Improved synthesis of aflatoxin B_1 (6, 8).



a. $(CH_3)_2SO_4$, K_2CO_3

b. $BrCH_2C_6H_5$, NaI, $NaCO_3$

c. SeO_2

d. CH_3CO_2H, Zn

e. Existing conditions

f. $HAl(C_4H_9)_2$

g. $CH_3CO_2COCH_3$, CH_3CO_2Na

h. Pd-C, H_2

i. $CH_3CO_2COCH_3$, CH_3CO_2Na

j. 400° C

k. K_2CO_3, H_2O

l. $ZnCO_3$, $NaHCO_3$

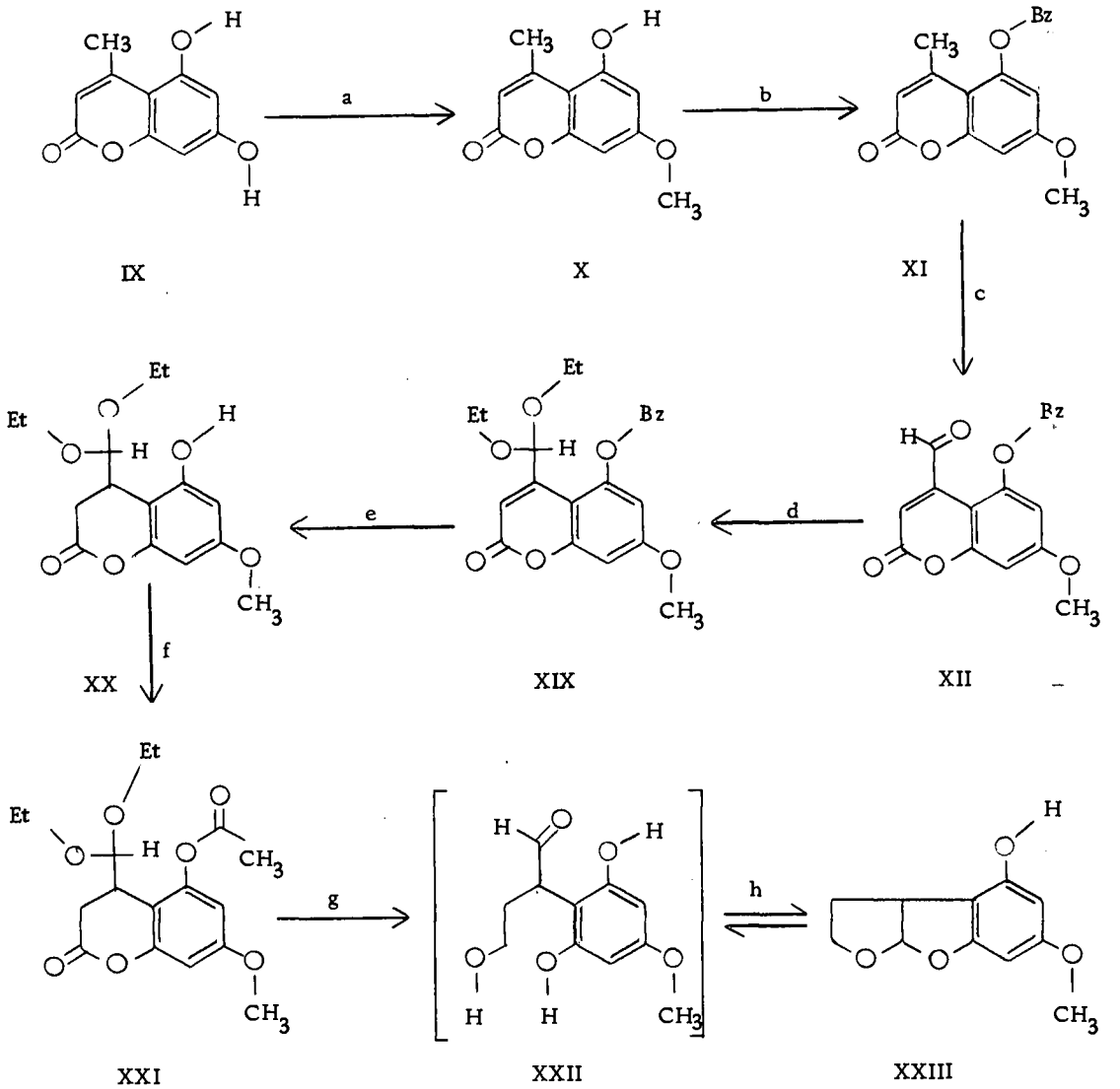
involved 12 steps and gave an overall yield of 3.1%. The furofuran synthetic steps to lactone X were retained from the old method (8).

Knight, Roberts, and Roffey (28) have constructed 2,3,3a,8a-tetrahydro-4-hydroxy-6-methoxyfuro[2,3-b]benzofuran, XXIII (Figure 5), in a manner similar to that of Buchi (8). The primary difference was in the method of reducing the 3,4-double bond of 4-formylcoumarin, XII. Knight further reduced the 2-carbon (Figure 5, f) from an ester to an alcohol function with lithium aluminum hydride. The saturated final product, XXIII, was later used to make aflatoxin B_2 (35) and dihydro-O-methylsterigmatocystin (34).

Synthetic Furo[2,3-b]benzofuran Ring Systems as a Model for Biological Experimentation

The data presented above showed that small changes in the furo-[2,3-b]benzofuran ring system significantly alters the biological response. These responses, no doubt, reflected the different chemical behavior of the various modifications in the furofuran ring system. In order that more light may be shed on the biological effects of this function, we undertook the preparation of a model by a new synthetic route.

Figure 5. Synthesis of 2, 3, 3a, 8a-tetrahydro-4-hydroxy-6-methoxy-furo[2, 3-b] benzofuran (28).



RESULTS AND DISCUSSION

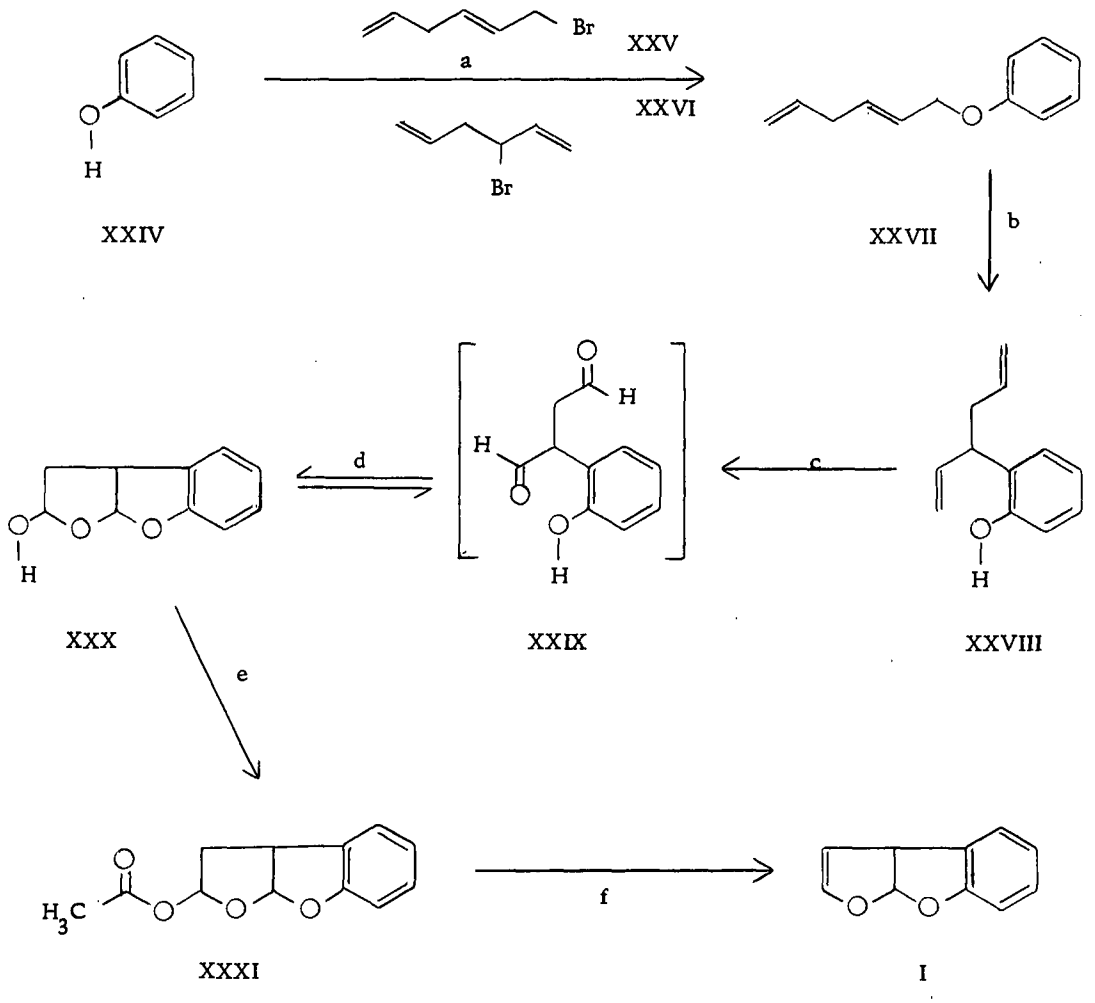
Interest in the synthesis of the furofuran ring system was aroused by the significant role it seems to play in the biological activity of compounds such as the aflatoxins. In order to prepare a model for biological studies, 2,3,3a,8a-tetrahydro-2-hydroxyfuro[2,3-b]benzofuran, XXX (Figure 6), was synthesized by a new route. The structure of XXX was confirmed by an alternate synthetic route using an adaptation of Buchi's route to compound XV (Figure 4).

A New Synthesis for 2,3,3a,8a-Tetrahydro-2-hydroxyfuro[2,3-b]benzofuran

The new synthesis was based on the preparation of o-hydroxyphenylsuccinaldehyde, XXIX, which has structural similarity to phenoxide ion V observed by Buchi (8). Intermediate XXIX would be expected to form hemiacetal XXX (Figure 6, d) under mildly acidic conditions as was theorized by Buchi for V.

The initial step of the synthesis (Figure 6, a) required a substitution reaction of phenol, XXIV, with 1-bromo-2,5-hexadiene, XXV, to yield 2,5-hexadien-1-yl phenyl ether, XXVII. Fortunately, 3-bromo-1,5-hexadiene, XXVI, an isomer formed during the preparation of bromide XXV, also reacted with phenol to yield ether XXVII. The mechanism of this reaction is no doubt an allylic rearrangement known as an Sn2' reaction. The yield of ether XXVII was 89.5% with

Figure 6. Synthesis of 3a, 8a-dihydrofuro[2, 3-b]benzofuran.



a. K_2CO_3

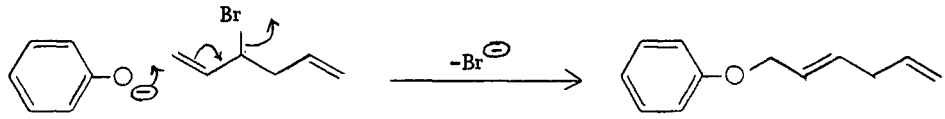
b. BCl_3

c. OsO_4, $NaIO_4$

d. Existing Conditions

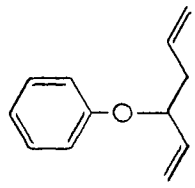
e. $CH_3CO_2COCH_3$, CH_3CO_2Na

f. Pyrolysis



XXVI XXVII

less than 1.0% of the undesired ether XXXII formed. Substantial amounts of ether XXXII[2] could be produced when the bromide reagent was composed of higher amounts of isomer XXVI while heating to 75 - 100° C and excluding the use of a solvent.



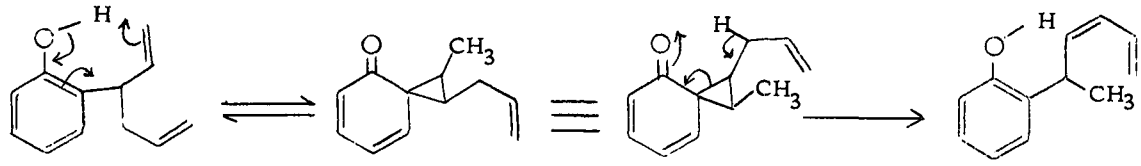
XXXII

In the second step (Figure 6, b) ether XXVII was converted to 3-(o-hydroxyphenyl)-2,5-hexadiene, XXVIII, by an ortho-Claisen rearrangement. Traditionally, this rearrangement is accomplished by heating to approximately 200° C (41). When ether XXVII was heated neat or in N,N-dimethylaniline (bp 193-195° C), a number of unidentified products and polymers were produced. Two side reactions may have taken place: a Cope rearrangement (23) of diene XXVIII could form 1-(o-hydroxyphenyl)-1,5-hexadiene, XXXIII; and an abnormal

[2] Spectral data (ir, nmr, and mass spectrum) of a preparative gas-liquid chromatographic fraction are consistent with the structure of XXXII.

XXVIII XXXIII

Claisen rearrangement (30) of XXVIII could form 5-(o-hydroxyphenyl)-1,3-hexadiene, XXXIV. Both side reactions generate thermodynamically more favorable conjugated products.



XXVIII XXXIV

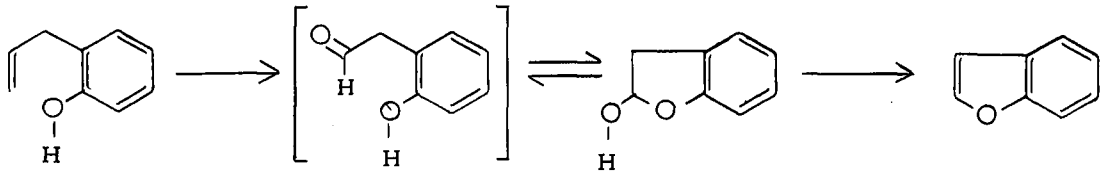
The desired Claisen rearranged product, XXVIII, was successfully produced without heating using boron tricloride as a catalyst (18). The yield was found to depend upon the solvent. Fahrni, Habich, and Schmid (18) found that the catalytic rearrangement of allyl phenyl ether in chlorobenzene solution gave better yields of 2-allylphenol than the ether did neat or in nitrobenzene. Ether XXVII only gave 20 to 25% yields of the desired diene, XXVIII (Figure 6, b), in chlorobenzene solution during catalytic rearrangement. A more volatile solvent than chlorobenzene (bp 132° C) was sought so carbon tetrachloride (bp 77° C), chloroform (bp 61° C), and methylene chloride (bp 40° C) were used. Carbon tetrachloride and methylene chloride gave yields

similar to that of chlorobenzene. Chloroform (ethanol free), as a solvent, gave a 49% yield of the phenolic diene, XXVIII. Varying the temperature between +10 and -40° C had little or no effect on the yield.

The third step in the synthetic scheme (Figure 6, c) required an oxidative cleavage of both double bonds of substituted diene XXVIII to dialdehyde XXIX. As mentioned earlier, XXIX would be expected to form the furofuran ring system spontaneously under acidic conditions.

The primary concern in this step was to have oxidizing reagents which were specific in order not to oxidize the phenolic function. Thus it was not surprising that potassium permanganate and hydrogen peroxide-formic acid proved to be unsatisfactory oxidizing agents for XXVIII.

At this point in the synthesis, 2-allylphenol, XXXV, was used as a readily accessible[3] model for the oxidation reaction. Aldehyde XXXVI would form hemiacetal XXXVII analogous to that in Figure 6, d. When compound XXXVII was made, it was not purified as such but was identified by its mass spectrum and conversion to benzofuran,[4] XXXVIII, with orthophosphoric acid (2).

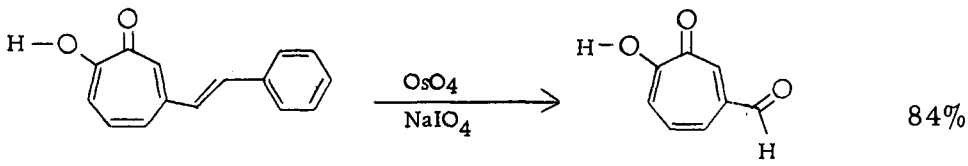


XXXV XXXVI XXXVII XXXVIII

[3] Aldrich Chemical Co.

[4] The synthetic benzofuran was identical (tlc and glc behavior) to the benzofuran from Aldrich Chemical Co.

A single step reaction using osmium tetroxide and sodium periodate was found to be the oxidation method of choice. The procedure employed was analogous to that used by Tarbell, Williams, and Sehm (42) on 4-styryltropolone, XXXIX. If the oxidizing reagents would not attack the tropolone function significantly, it would seem that the phenol function should be equally safe from attack. 2-Allyl-



84%

XXXIX XL

phenol, XXXV, gave hemiacetal XXXVII as a major product when exposed to the conditions described for 4-styryltropolone; however, much of the starting phenol, XXXV, remained unreacted. Increasing the amount of sodium periodate or allowing the reaction to stir longer did not seem to consume more 2-allylphenol. Increasing the osmium tetroxide 40 fold (alkene to OsO_4 ratio = 10:1) did alleviate the problem.

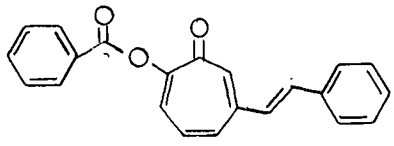
Hemiacetal XXX was produced (Figure 6, c and d) in 28% yield by the osmium tetroxide-sodium periodate method described above for 2-allylphenol. The greatest problems with this reaction envolved increasing the yield, finding reaction conditions to consume all the starting material, and finding the best means of product isolation. Not all of diene XXVIII was oxidized even when a molar ratio for XXVIII to

osmium tetroxide was five to one. Table 3 shows some representative molar ratios of alkene to osmium tetroxide under similar oxidation conditions. The ratio of alkene to catalyst can be quite high illustrating that the osmium tetroxide is very efficient. Inspite of many variations, the efficiency of the catalyst in the presence of diene XXVIII could not be increased.

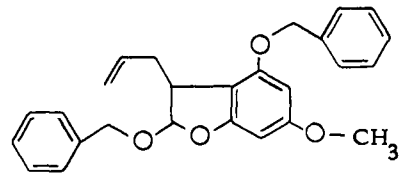
Table 3. Molar ratio of alkene to osmium tetroxide.

Compound	Molar Ratio alkene to OsO_4	Yield	Ref.
4-Styryltropolone, XXXIX	400 : 1	84%	42
4-Styryltropolone benzoate, XLI	25 : 1	50%	42
Compound XLII	10 : 1	63%	6
Phenol XXVIII	5 : 1[a]	28%	--

[a]One mole of XXVIII is two equivalents of alkene.



XLI



XLII

Rylander (39) has indicated that pH can be important to the catalytic properties of osmium tetroxide. Two one-hundredths molar phosphate-citrate buffer solutions (pH 3, 4, 5, and 6) were used in place of water in the reaction media. Simultaneous thin-layer chromatographic analysis of the buffer reaction mixtures showed that there

was no difference in the four solutions. Warming the reaction mixture, excluding atmospheric oxygen, filtering off the sodium iodate formed, using less water and dioxane, stirring overnight, or adding more sodium periodate did not consume all of the starting material. The unreacted starting material became quite evident when an extraction procedure was used to recover the product.

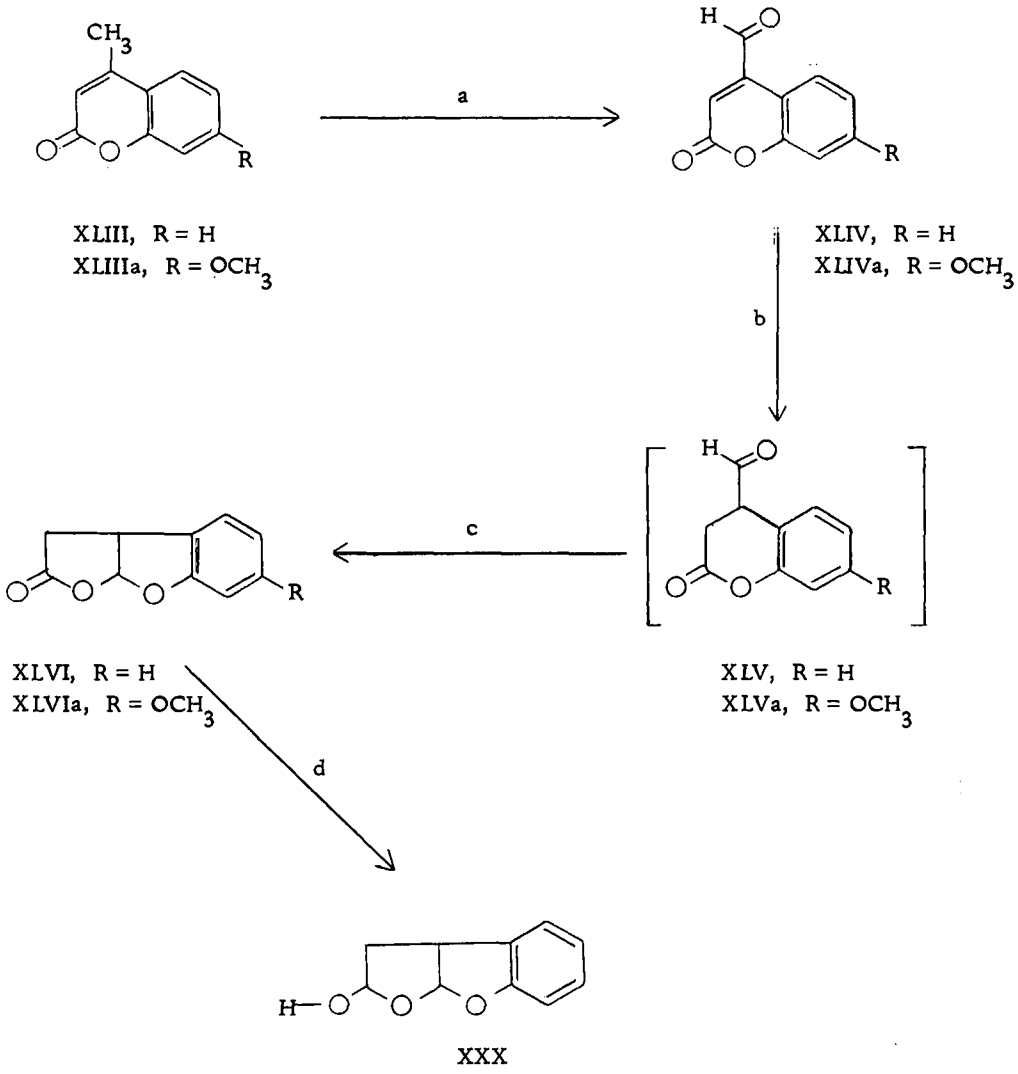
Dilution of the reaction media with water and extraction with ether or chloroform gave an organic layer which turned black in less than five minutes. When the organic layer was dried and concentrated, an oily black residue remained. The black color could not be removed with column chromatography. This color is characteristic of osmium tetroxide reacting with an olefin (39). The best work up procedure was to filter the reaction mixture and remove the volatiles in vacuo. This eliminated the formation of the black osmium complex.

Hemiacetal XXX prepared above was compared to that made by analogous methods of Buchi (8). This provides additional proof for the structure of XXX.

Synthesis of 2,3,3a,8a-Tetrahydro-2-hydroxyfuro-[2,3-b]benzofuran by the Methods of Buchi

Hemiacetal XXX was made by analogous methods of Buchi et al. (8) and Buchi and Weinreb (6) in their preparation of hemiacetal XV (Figure 4). This is schematically shown in Figure 7. In the first

Figure 7. Synthesis of 2, 3, 3a, 8a-tetrahydrofuro[2, 3-b]benzofurans by the method of Buchi (6, 8).



a. SeO_2

b. Zn, CH_3CO_2H

c. Existing conditions

d. $(C_4H_9)_2$ AlH, HCl

step (Figure 7, a) bromobenzene was used in place of xylene as the solvent for the selenium dioxide oxidation of the methyl group in 4-methylcoumarin, XLIII. When xylene was used as the solvent, the oxygen of the selenium dioxide was converted to water quantitatively, but 25% of the 4-methylcoumarin remained unchanged. This seemed to indicate that some benzaldehydes were produced from the solvent. When bromobenzene was used, the oxidation was nearly quantitative to produce aldehyde XLIV.

Buchi (8) reported that aldehyde XII produced lactone XIV (Figure 4, d and e) in 80% yield with a zinc-acetic acid reduction. Intermediate XIII could be isolated; however, they chose reaction conditions which rearranged XIII to XIV in situ. Aldehyde XLIV could be converted to lactone XLVI in only 19% yield using the analogous procedure of Buchi. Variation of the reaction conditions failed to increase the yield. For the reason of low yield, lactone XLVIa was prepared but the yield during the zinc-acetic acid reduction was nearly identical to that of XLVI. Possibly a hydrogenation similar to that used by Knight, Roberts, and Roffey (28) for compound XIX (Figure 5) may have given higher yields.

Hemiacetal XXX was prepared from lactone XLVI (Figure 7, d) by an analogous method of Buchi and Weinreb (6) in Figure 4, f. The product was identical to that produced from diene XXVIII.

Comparison of the Two Methods of Preparation

Each of the preparations just described for compound XXX has three basic steps. The overall yield of XXX from the new synthetic method was 14% compared to 7% from the adapted methods of Buchi.

While the yields of the two different routes were not as high as desired, the apparent major product, XXX, of both methods of preparation had the furo[2,3-b]benzofuran structure. This supports other observations that the furofuran ring system is preferred over partial structures VI, VII, and VIII.

Both methods could be employed to produce derivatives with substituents on the benzene ring. Thus it is possible that the procedures of the new synthesis could be adapted for preparation of vinyl ether XVIII in route to aflatoxin B_1 (Figure 4). However, the use of an unsymmetrical phenol would generate a mixture of two isomers after the ortho-Claisen rearrangement, assuming both ortho positions of the phenol were unsubstituted in the starting material. The method of Buchi is limited to the accessibility of the desired unsymmetrical coumarin.

SUMMARY AND CONCLUSION

A new method for the preparation of 2,3,3a,8a-tetrahydro-2-hydroxyfuro[2,3-b]benzofuran, XXX, ring system has been developed. The new method has comparable efficiency in our laboratory with the adapted methods of Buchi. Both methods of preparation gave the same desired product which lends strong proof as to the structure. All spectral data were consistent with compound XXX.

EXPERIMENTAL

The reported melting points (mp) and boiling points (bp) are uncorrected values. The infrared spectra (ir) were recorded on a Beckman IR-5 and IR-18 spectrophotometers. Ultraviolet spectra (uv) were obtained on a Beckman DK-1 recording spectrophotometer. The symbol λ_{max} (EtOH-NaOH) refers to a spectrum obtained when one drop of 0.1 N sodium hydroxide was added to the 3 ml sample cell. Nuclear magnetic resonance spectra (nmr) were recorded on a Varian HA-100 spectrometer. Chemical shifts are given in parts per million downfield from the internal standard, tetramethylsilane. The number of hydrogens at a chemical shift is followed by the letter H. The abbreviations s, d, t, q, and m indicate singlet, doublet, triplet, quartet, and multiplet, respectively. Coupling constants (J) are given in cycles per second (Hz). Mass spectra (ms) were obtained on a Varian MAT CH-7 mass spectrometer. The ms data are given in mass-to-charge ratio followed by the percent relative intensity (ionizing voltage 70 eV). All gas-liquid chromatographic analyses (glc) were taken from an Aerograph A-700 gas chromatograph with a thermoconductivity detector. The column was an 1/8" X 5', 5% SE-30 on 100-120 mesh Chromosorb G. The injector port was kept at 220° C; the detector was held at 325° C; and the column temperature was varied according to the compound's mobility. Helium was used as the carrier gas with

a flow rate of 30 cc/min. Thin-layer chromatography (tlc) was employed routinely for monitoring reactions. A 0.25 mm thickness of Silica Gel GF-254 (Brinkmann Instruments, Westbury, N. Y.) served as the adsorbent. The developed plates were visualized with an ultraviolet lamp (Black-Ray UNSL-22, Ultraviolet Products Inc., San Gabriel, Calif.) and/or after being exposed to iodine vapor.

1-Bromo-2,5-hexadiene, XXV

1-Bromo-2,5-hexadiene was prepared according to Hwa and Sims (27) in the preparation of 1,3,5-hexatriene from allyl magnesium bromide and acrolein. The crude bromohexadiene weighed 145.1 g (0.90 mol, 83%). The colorless liquid was dried with anhydrous calcium chloride and distilled at 35 mm Hg yielding two fractions. The first fraction (69.1 g, 0.43 mol) was taken at 39-54° C and contained about 50% of each isomer according to glc analysis. The second fraction (59.8 g, 0.37 mol) was taken at 54-65° C and contained 81% of the higher boiling isomer, 1-bromo-2,5-hexadiene. The following properties were obtained from preparative glc fractions:

3-bromo-1,5-hexadiene, XXV

bp 134° C.

ir (neat) 3050, 1645, 1430, 985, 924, 740, 690 cm^{-1}.

nmr (CCl_4) δ 5.80 (2 H, m), 5.08 (4 H, m), 4.36 (1 H, q, J = 7 Hz), 2.61 (2 H, t, J = 6.5 Hz).

ms 81 (100), 38 (48), 79 (45), 40 (45), 55 (41), 27 (31), 80 (28), 77 (21), 53 (17), 160, 162 (molecular ions not detected).

1-bromo-2,5-hexadiene, XXVI

bp 148° C.

ir (neat) 3050, 1665, 1640, 1210, 995, 967, 920 cm^{-1}.

nmr (CCl_4) δ 5.65 (3 H, m), 4.95 (2 H, m), 3.80 (2 H, d, J = 5.5 Hz), 2.77 (2 H, t, J = 5 Hz).

ms 76 (100), 40 (52), 38 (44), 55 (43), 74 (39), 27 (29), 75 (11), 72 (15), 53 (13), 65 (10), 160, 162 (molecular ions not detected).

2,5-Hexadien-1-yl Phenyl Ether, XXVII

Ether XXVII was prepared according to an analogous substitution reaction of Allen and Gates (1) in the preparation of o-eugenol from guaiacol (o-methoxyphenol) and allyl bromide. Glyme (1,2-dimethoxyethane) was used in place of acetone as the solvent (Buchi and Weinreb, 6). A stirred mixture of 4.7 g (50 mmol) of phenol (Matheson Coleman and Bell Co.), 8.85 g (55 mmol) of 81% 1-bromo-2,5-hexadine, 7.0 g (51 mmol) of anhydrous potassium carbonate, and 20 ml of dry glyme (distilled from calcium hydride) was stirred at

at room temperature for 48 hours. Glc analysis of the mixture indicated a product to phenol ratio of 97:3. The reaction mixture was diluted with water and extracted with diethyl ether. The ether layer was washed with 5% sodium hydroxide, washed with water, dried with anhydrous sodium sulfate, and concentrated under vacuum. The product distilled at 51-53° C (0.03 mm Hg) affording 7.78 g (44.7 mmol, 89.5%) 2,5-hexadien-1-yl phenyl ether which by glc analysis was found to be 99% pure.

ir (neat) 3015, 1645, 1603, 1495, 1248, 975, 920, 756, 693 cm^{-1}.

uv λ_{max} (EtOH) 277, 270, 219 mμ (ε 1520, 1870, 9790).

nmr (CCl_4) δ 6.97 (5 H, m), 5.67 (3 H, m), 5.05 (1 H, m), 4.91 (1 H, s), 4.34 (2 H, d, J = 3 Hz), 2.73 (2 H, t, J = 5 Hz).

ms 80 (100), 79 (76), 41 (44), 94 (43), 81 (43), 39 (39), 65 (23), 77 (22), 27 (18), 50 (13), 66 (11), 133 (6), 174 (molecular ion, 2).

3-(o-Hydroxyphenyl)-1,5-hexadiene, XXVIII

Diene XXVIII was prepared by a boron trichloride-catalyzed ortho-Claisen rearrangement similar to that described by Fahrni, Habich, and Schmid (18) for allyl phenyl ethers. A mixture of 7.0 g (40 mmol) 2,5-hexadien-1-yl phenyl ether and 210 g of ethanol free chloroform under nitrogen was cooled in an ice bath while boron trichloride (Matheson, Coleman and Bell Co.) was slowly bubbled into

the solution. After 20 minutes addition of the catalyst, a tlc analysis (benzene, eluting solvent) indicated a complete disappearance of the reacting ether with the formation of two products. The lower spot (R_f 0.3) was found to be phenol. The upper spot (R_f 0.6) was the desired product. After methanolysis, the solution was warmed to room temperature, washed with water, dried with anhydrous sodium sulfate, and concentrated under vacuum. The product distilled between 65 and 68° C (0.01 mm Hg) to give 3.43 g (19.5 mmol, 49%) of 3-(o-hydroxyphenyl)-1,5-hexadiene.

ir (neat) 3450, 3050, 1645, 1570, 1508, 1491, 1457, 999, 917, 753 cm^{-1}.

uv λ_{max} (EtOH) 274 mu (ϵ 2570).

uv λ_{max} (EtOH - NaOH) 294, 241 mμ (ϵ 3450, 7180).

nmr (CCl_4) δ 6.83 (4 H, m), 5.82 (2H, m), 5.29 (1 H, s), 4.98 (4 H, m), 3.65 (1 H, q, J = 7 Hz), 2.47 (2 H, t, J = 7 Hz).

ms 133 (100), 105 (34), 131 (18), 77 (18), 120 (12), 79 (11), 134 (10), 39 (9), 103 (8), 49 (8), 174 (molecular ion, 2).

2,3,3a,8a-Tetrahydro-2-hydroxyfuro[2,3-b]benzofuran, XXX, from Compound XXVII

Hemiacetal XXX was prepared by an osmium tetroxide-catalyzed periodate oxidation similar to that used by Tanbell, Williams, and Sehm (42) on 4-styryltropolones. To a stirred solution of 0.87 g

(5 mmol) of diene XXVIII, 250 mg (1 mmol) osmium tetroxide in 19 ml dioxane (purified according to Wiberg, 44), and 5 ml of water was added 2.3 g (11 mmol) of sodium metaperiodate during a 30 minute period. After two hours of additional stirring the mixture was filtered and the filter cake was washed with acetone. The combined filtrate and washings were taken to dryness in vacuo. The oily residue was chromatographed on silica gel (5 g of Silicar CC-7, 200-325 mesh, Mallincknodt Chemical Works) with 5% absolute ethanol in benzene to give 0.25 g (1.4 mmol, 28%) of 2,3,3a,8a-tetrahydro-2-hydroxyfuro-[2,3-b)benzofuran.

mp 123-125° C.

ir (KBr pellet) 3470, 2940, 1601, 1489, 1257, 1222, 1190, 752, 744 cm^{-1}.

uv λ_{max} (EtOH) 278, 273, 115(sh) mμ (ε 2290, 2280, 6230).

uv λ_{max} (EtOH-NaOH) 287, 228 mμ (ε 2920, 5820).

nmr ($CDCl_3$) δ 7.16 (4 H, m), 6.45 (1 H, d, J = 6 Hz), 5.70 (1 H, m), 4.15 (1 H, m), 2.50 (2 H, m; 1 H, -OH).

ms 131 (100), 77 (51), 91 (40), 160 (31), 51 (31), 39 (31), 147 (30), 103 (29), 123 (28), 89 (27), 178 (molecular ion, 7).

4-Methylcoumarin, XLIII

4-Methylcoumarin was prepared according to the method of Woodruff (46) via the Pechmann reaction which condensed phenol and

ethyl acetoacetate in the presence of aluminum chloride. The yield at one-half scale after two recystallization was 51.5 g (0.32 mol, 31%) of white crystals having a melting point of 80° C (literature value 83-84). A third recrystallization and/or sublimation (at 0.03 mm Hg) did not elevate the melting point.

ir ($CHCl_3$) 1727, 1633, 1458, 1395, 1375, 1195, 862 cm^{-1}.

uv λ_{max} (EtOH) 310, 270 mμ (ε 6580, 11500).

nmr ($CDCl_3$) δ 7.46 (4 H, m), 6.24 (1 H, s), 2.44 (3 H, s).

4-Formylcoumarin, XLIV

4-Formylcoumarin was prepared by a method analogous to that of Buchi et al. (8) except that bromobenzene was used in place of xylene as the solvent. A mixture of 8.0 g (50 mmol) of 4-methylcoumarin and 8.3 g (75 mmol) of resublimed selenium dioxide (J. T. Baker Chemical Co.) in 540 ml of freshly distilled bromobenzene were heated to reflux for 18 hours under a slow stream of nitrogen. The filtered solvent was completely removed by vacuum and the residue was recrystallized with benzene to yield 7.1 g of bronze-colored crystals. The concentrated mother liquor was recrystallized from chloroform to give 1.1 g of similar colored crystals, yielding a total of 8.2 g (47 mmol, 94%). Tlc analysis (4% acetone in chloroform) of both crystalline crops showed them pure and free of 4-methylcoumarin.

mp 154-155° C.

ir ($CHCl_3$) 1732, 1718, 1609, 1062 cm^{-1}.

uv λ_{max} (EtOH) 310, 273 mμ (ε 5740, 10000).

nmr ($CDCl_3$) δ 8.58 (1 H, d, J = 7 Hz), 7.45 (4 H, m), 6.85 (1 H, s).

2,3,3a,8a-Tetrahydro-2-oxofuro[2,3-b]benzofuran, XLVI

Lactone XLVI was prepared by a method analogous to that of Buchi et al. (8). Zinc dust (270 mg, 4.1 mmol) was slowly added to a vigorously stirred solution of 174 mg (1.0 mmol) of 4-formylcoumarin in 10 ml of glacial acetic acid at 100° C. After two hours at 110-120° C, the mixture was cooled, diluted with an equal volume of chloroform, filtered, and concentrated under vacuum. The residual solid was taken up in chloroform, washed with water, dried with anhydrous sodium sulfate, and concentrated. The crude product was recrystallized from ethanol-water and sublimed (0.03 mm Hg) to give 33 mg (0.19 mmol, 19%) of 2,3,3a,8a-tetrahydro-2-oxofuro[2,3-b]benzofuran.

mp 126-128° C.

ir ($CHCl_3$), 1795, 1607, 1487, 1472, 1172, 1151, 1068, 1026, 1009, 980, 904 cm^{-1}.

uv λ_{max} (EtOH) 281, 275 mμ (ε 2530, 2880).

nmr ($CDCl_3$) δ 7.07 (4 H, m), 6.47 (1 H, d, J = 6 Hz), 4.18 (1 H, m), 3.08 (1 H, pair of d, J = 18 Hz, J = 9 Hz), 2.71 (1 H, pair of d, J = 18 Hz, J = 2 Hz).

2,3,3a,8a-Tetrahydro-2-hydroxyfuro[2,3-b]benzofuran, XXX, from Compound XLVI

Hemiacetal XXX was prepared by an analogous method of Buchi and Weinreb (6). A solution of 30 mg (0.17 mmol) of 2,3,3a,8a-tetrahydro-2-oxofuro[2,3-b]benzofuran in 5 ml of dry toluene was cooled to -25°C. Diisobutylaluminum hydride (24 mg, 0.17 mmol) in 1 ml of dry toluene was slowly added to the cold solution. The solution was stirred for one hour at -25° C, 10% hydrochloric acid was added, and the mixture was stirred at room temperature for ten minutes. The organic layer was separated, dried with anhydrous sodium sulfate, and concentrated in vacuo. The residue was sublimed (0.03 mm Hg) and chromatographed on 5 g of silica gel (Silicar CC-7, 200-325 mesh; 5% absolute ethanol in benzene, eluting solvent) to give 12 mg (0.07 mmol, 41%) of 2,3,3a,8a-tetrahydro-2-hydroxyfuro[2,3-b]benzofuran. The spectral data was identical to that of hemiacetal XX prepared from diene XVIII. A mixture of the two compounds prepared by separate methods was inseparable using tlc analysis.

7-Methoxy-4-methylcoumarin, XLIIIa

Coumarin XLIIIa was prepared by an adapted methylation of Buchi and Weinreb (6). A stirred mixture of 1.75 g (10 mmol), of 7-hydroxy-4-methylcoumarin (Aldrich Chemical Co.), 1.54 g (11 mmol) of anhydrous potassium carbonate, and 20 ml dry glyme was

brought to reflux. Dimethyl sufate (1.39 g, 11 mmol) was added over a period of one hour and heating was continued for an additional 24 hours. Analysis (tlc, 8% acetone in chloroform) showed a quantitative conversion. The warm mixture was filtered and the solid was washed with chloroform. The combined solvents were removed in vacuo. The solid residue was recrystallized from ethanol to give 1.48 g of white crystals. The mother liquor was concentrated to dryness and the residue was chromatographed on 5 g of silica gel (Silicar CC-7, 200-325 mesh) with 5% acetone in chloroform to give 0.33 g of similar white crystals. The total yield of 7-methoxy-4-methylcoumarin was 1.81 g (9.5 mmol, 95%).

mp 158-159° C.

ir ($CHCl_3$) 1720, 1618, 1395, 1372, 1299, 1289, 1153, 1140, 1078 cm^{-1}.

uv λ_{max} (EtOH) 319, 219 mμ (ϵ 15500, 18900).

nmr ($CDCl_3$) δ 8.48 (1 H, d, J = 7 Hz), 7.30 (1 H, m), 6.92 (2 H, m), 6.71 (1 H, s), 3.93 (3 H, s).

4-Formyl-7-methoxycoumarin, XLIVa

Coumarin XLIVa was prepared by the procedure of 4-formylcoumarin. After six hours of refluxing, tlc analysis (5% acetone in chloroform) showed a quantitative yield. The warm filtered solvent was removed in vacuo and the residue was recrystallized in benzene to

yield 1.52 g (7.45 mmol, 94%) of brown crystalline 4-fromyl-7-methoxycoumarin.

mp 197-198°C.

ir ($CHCl_3$) 1733, 1718, 1610, 1155, 1064 cm^{-1}.

uv λ_{max} (EtOH) 323, 217 mμ (ε 11800, 13600).

nmr ($CDCl_3$) δ 8.48 (1 H, d, J = 7 Hz), 7.30 (1 H, m), 6.92 (2 H, m), 6.71 (1 H, s), 3.93 (3 H, s).

2,3,3a,8a-Tetrahydro-6-methoxy-2-oxofuro-[2,3-b]benzofuran, XLVIa

Lactone XLVIa was prepared by the procedure of lactone XLVI. The crude product was sublimed (at 0.03 mm Hg) to give 210 mg (1.03 mmol, 21) of pure 2,3,9,10-tetrahydro-6-methoxy-2-oxofuro-[2,3-b]benzofuran.

mp 129-130° C.

ir ($CHCl_3$) 1795, 1633, 1601, 1504, 1343, 1289, 1160, 1145, 1005, 975 cm^{-1}.

uv λ_{max} (EtOH) 281, 220 mμ (ε 3130, 4990).

nmr ($CDCl_3$) δ 7.11 (1 H, m), 6.57 (2 H, m), 6.47 (1 H, d, J = 6 Hz), 4.16 (1 H, m), 3.82 (3 H, s), 3.08 (1 H, pair of d, J = 18 Hz, J = 9 Hz), 2.71 (1 H, pair of d, J = 18, J = 2 Hz).

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