Methyl 1-Methoxy-5-ethyl-7-azaisoquinoline-3-carboxylate (21). A solution of 117 mg of **20** in 16 ml of 6% acetic acid was left standing at room temperature for 18 hr. The resultant precipitate was filtered, the filtrate neutralized with sodium bicarbonate, and a second precipitate also filtered. Sublimation of the combined solids, 72 mg, yielded colorless crystals of **21**, mp 130–132°; spectra: [infrared (Nujol)] C=O 5.80(s), C=C 6.16(w), 6.26(w), and 6.41(m) μ; [ultraviolet (methanol)] λ_{max} 211, 221, 250, 321, and 334 mμ (log ϵ 4.41, 4.38, 3.94, 3.90, and 3.90); $\lambda_{shoulder}$ 287 mμ (log ϵ 3.74); (pmr) three-proton triplet 1.38 (J = 7.5 cps) (C–Me), two-proton quartet 3.02 (J = 7.5 cps) (methylene), three-proton singlets 4.02 and 4.23 (methoxyls), and one-proton singlets 8.12, 8.56, and 9.40 ppm (aromatic hydrogens).

Anal. Calcd for $C_{13}H_{14}O_3N_2$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.39; H, 5.82; N, 11.35.

After treatment of a solution of 70 mg of **21** in 2 ml of methanol with excess of dry hydrogen chloride the solvent was removed and the residue heated at 160° for 30 min. The infrared spectrum of the product contained absorption bands characteristic of the isocarbostyril nucleus. A solution of the solid in 5 ml of methanol saturated with hydrogen chloride was kept at room temperature for 2 days. The solvent was removed, the residue dissolved in 2 ml of water, and the solution neutralized. Filtration of the resultant precipitate yielded 27 mg of the isocarbostyril **14**, mp 200–202°; the infrared spectrum was identical with that of the above sample.

The Total Synthesis of Racemic Aflatoxin B_1[1]

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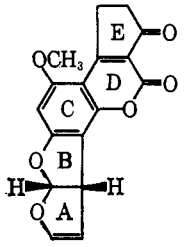
Contribution from the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts. *Received August 2, 1967*

Abstract: Aflatoxin B_1 has been prepared in the form of its racemate from phloroglucinol by a 12-step sequence.

Aflatoxin B_1 (**1**) belongs to a group of acutely toxic and highly carcinogenic mold metabolites produced by *Aspergillus flavus*.[4] After having completed structural studies on these toxins,[5] we turned to contemplation of their synthesis.



1

Taking notice of the lability which the vinyl ether grouping imparts to the molecule, it was decided to introduce this functionality at the very end of the synthesis. As a precursor for such a group we chose a lactone function. The Pechmann reaction seemed admirably suited for the construction of the coumarin ring and we[5] as well as others[6,7] have demonstrated with a model compound that the cyclopentenone ring could be closed by dehydration of the corresponding carboxylic acid. For the elaboration of the phenol **19** containing rings A, B, and C, we chose a 4-methylcoumarin already having the required number of carbon atoms. This plan for the construction of the tricyclic intermediate incidentally is the result of some speculative thinking on the biogenesis of the aflatoxins. The initial phase of the synthesis was thus concerned with the preparation of 5-benzyloxy-7-methoxy-4-methylcoumarin (**7**).

Acetylation of phloroacetophenone (**2**) with 2 equiv of hot acetic anhydride produced comparable amounts of 2,4-diacetoxy-6-hydroxyacetophenone (**3**) and 2,6-diacetoxy-4-hydroxyacetophenone (**4**). Crystallization from chloroform gave the phenol **4** and the chelate **3** could be isolated from the mother liquor. The nuclear magnetic resonance spectrum of **4** revealed a symmetrical arrangement of substituents while the spectrum of the unsymmetrical isomer **3** exhibited three distinct methyl signals and an AB quartet for the nonequivalent aromatic protons. Methylation of the phenol **4** with diazomethane followed by acid hydrolysis afforded phloroacetophenone 4-methyl ether (**5**) identical with a sample prepared in poor yield by direct methylation of **2**.[8] Alkylation with 1 equiv of benzyl bromide led to the ether **6** which was transformed to the coumarin **7** with the aid of a Wittig reaction. This synthetic sequence (Chart I) leads to a coumarin of defined structure but the over-all yield is low and we subsequently developed two more satisfactory procedures.

Addition of 1 equiv of benzyl bromide to a suspension of potassium carbonate in acetone–tetrahydrofuran containing the readily accessible 5,7-dihydroxy-4-methylcoumarin (**8**) afforded the 5,7-dibenzyloxy ether **9** and what turned out to be the desired 5-benzyloxy-7-hydroxy-4-methylcoumarin (**10**). The latter was isolated in 20% yield from the insoluble part of the reaction mixture. Methylation gave the coumarin **7** identical with that prepared by the structurally unambiguous

(1) Announced previously in a communication to the editor by G. Büchi, D. M. Foulkes, M. Kurono, and G. F. Mitchell, *J. Am. Chem. Soc.*, **88**, 4534 (1966).

(2) National Science Foundation Graduate Fellow, 1964–1966.

(3) National Institutes of Health Postdoctoral Fellow, 1966–1967.

(4) For a summary, *cf.* G. N. Wogan, *Bacteriol. Rev.*, **30**, 460 (1966).

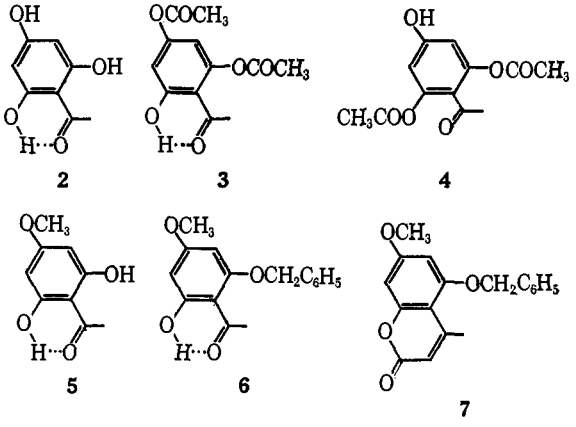
(5) T. Asao, G. Büchi, M. M. Abdel-Kader, S. B. Chang, E. L. Wick, and G. N. Wogan, *J. Am. Chem. Soc.*, **85**, 1706 (1963); **87**, 882 (1965); S. Brechbühler, G. Büchi, and G. Milne, *J. Org. Chem.*, **32**, 2641 (1967).

(6) J. G. Underwood and J. S. E. Holker, *Chem. Ind.* (London), 1865 (1964).

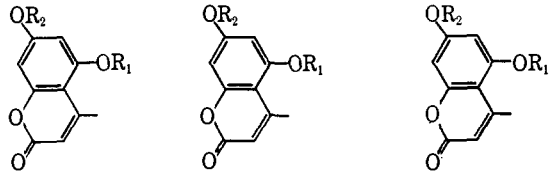
(7) R. S. Bhute, V. Sankaran, and G. S. Sidhu, *Indian J. Chem.*, **4**, 96 (1966).

(8) A. Sonn and W. Bülow, *Ber.*, **58**, 1691(1925).

Chart I



route already described. The isomeric phenol **11** was undoubtedly also formed in the benzylation of the dihydroxycoumarin **8** and to be certain that we could differentiate it and its methyl ether **12** from the desired isomers, efforts were made to prepare these compounds in pure form. Catalytic debenzylation of the dibenzyloxycoumarin **9** furnished isolable quantities of 5-hydroxy-7-benzyloxy-4-methylcoumarin (**11**). Methylation transformed it to the methyl ether **12** which was distinct by the usual spectral and physical properties from the isomer **7**.



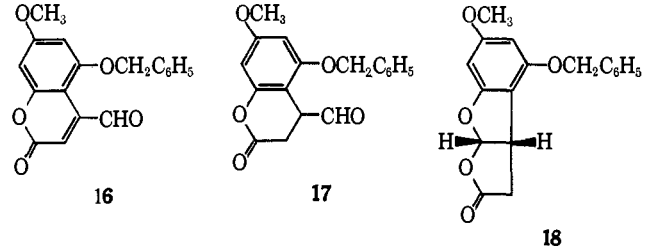
8, $R_1 = R_2 = H$ **10**, $R_1 = CH_2C_6H_5$; $R_2 = H$ **13**, $R_1 = H$; $R_2 = CH_3$
9, $R_1 = R_2 = CH_2C_6H_5$ **11**, $R_1 = H$; $R_2 = CH_2C_6H_5$ **14**, $R_1 = R_2 = CH_3$
12, $R_1 = CH_3$; $R_2 = CH_2C_6H_5$ **15**, $R_1 = CH_3$; $R_2 = H$

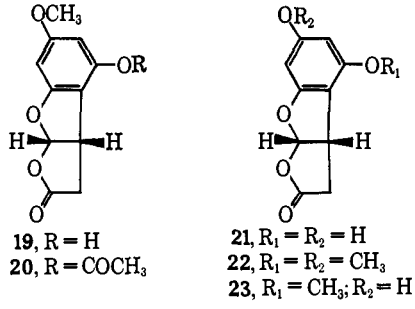
While measuring the ultraviolet absorption properties of the two phenols **10** and **11**, we observed that their spectra differed significantly in basic ethanol but not in the neutral medium. More interestingly, the spectrum of 5,7-dihydroxy-4-methylcoumarin (**8**) in basic ethanol closely resembled the base spectrum of 5-benzyloxy-7-hydroxy-4-methylcoumarin (**10**), indicating preferential anion formation at the 7-hydroxy function. Clearly, an opportunity exists for selective substitution of the 7-hydroxy group in **8** and indeed what appeared to be a selective methylation had already been described in the literature.[9,10] We have investigated this reaction in some detail and found that methylation of an aqueous solution of **8** containing 1 equiv of *sodium hydroxide* with dimethyl sulfate afforded 34% of 7-methoxy-5-hydroxy-4-methylcoumarin (**13**), 12% of the dimethyl ether **14**, and only very minor amounts of the isomeric monomethyl ether **15**. Benzylation of **13** produced the benzyl ether **7**[10] in essentially quantitative yield.

The question as to why the benzylation of the dihydroxycoumarin **8** gave mainly the 5-benzyloxy derivative **10** remains unanswered unless the insolubility of this particular isomer in the solvent combination used simply prevented it from being alkylated further.

The intermediate **7** now had to be transformed into the aldehyde **16** and when the former was oxidized with selenium dioxide[11] the yellow aldehyde **16**, synthesized independently by Roberts,[10] was produced smoothly. It was expected that reduction of **16** with zinc in acetic acid would lead to the corresponding 3,4-dihydrocoumarin **17** which we hoped to rearrange to the lactone **18** in a separate operation. In fact, this combination of reagents brought about not only the anticipated reduction but also isomerization to the lactone **18**. There is precedent for this isomerization which has been called "β-acyl lactone rearrangement."[12,13] Catalytic debenzylation of **18** over a palladium catalyst was an easy matter and yielded the tricyclic phenol **19** characterized further by its acetate **20** (Chart II).

Chart II





19, R = H
20, R = $COCH_3$

21, $R_1 = R_2 = H$
22, $R_1 = R_2 = CH_3$
23, $R_1 = CH_3$; $R_2 = H$

Before proceeding along the main synthetic pathway, we made efforts to prepare the crucial intermediate **19** by selective monomethylation of the seemingly less hindered hydroxyl group in the readily accessible dihydroxylactone **21** (see Experimental Section). Unfortunately such an attempt afforded the dimethoxylactone **22**, the undesired monomethyl ether **23**, and only a minor portion of the useful isomer **19**.

Returning to the synthetic sequence leading to aflatoxin B_1 (**1**), we now had to add the coumarin ring. In analogy to the earlier model studies,[5,6] a mixture of the phenol **19** and ethyl methyl-3-oxoadipate[14] was exposed to 86% sulfuric acid. Only 5% of the anticipated product **24** was isolable from the reaction mixture. Two additional compounds converted into the methyl esters by brief exposure to ethereal diazomethane were isolated in 2 and 10% yield, respectively. The former was identified as the substituted isofurocoumarin **25** by comparing its ultraviolet

(9) P. L. Sawhney and T. R. Seshadri, *Proc. Indian Acad. Sci.*, **37A**, 592 (1953).

(10) While the present investigation was in progress, a paper by J. A. Knight, J. C. Roberts, and P. Roffey [*J. Chem. Soc., Sect. C*, 1308 (1966)] appeared, confirming the earlier report.

(11) Method of A. Schiavello and E. Cingolani, *Gazz. Chim. Ital.*, **81**, 717 (1951).

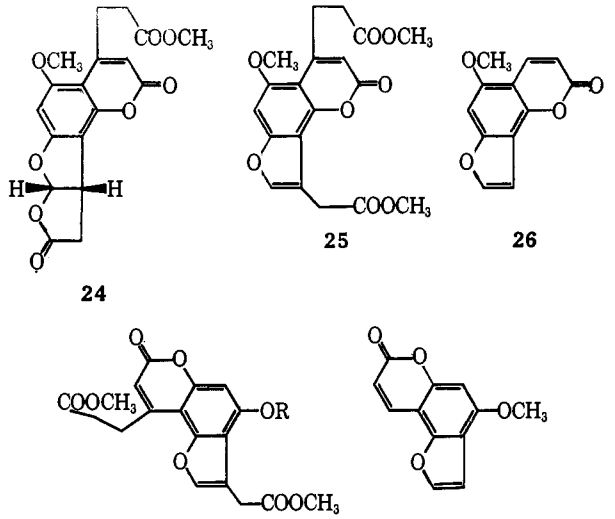
(12) C. L. Lange, H. Wamhoff, and F. Korte, *Chem. Ber.*, **100**, 2312 (1967).

(13) A. Lawson, *J. Chem. Soc.*, 144 (1957).

(14) D. K. Banerjee and K. M. Sivanandaiah, *J. Org. Chem.*, **26**, 1634 (1961). In later preparations we used the more convenient method of E. C. Taylor and A. McKillop, *Tetrahedron*, **23**, 897 (1967).

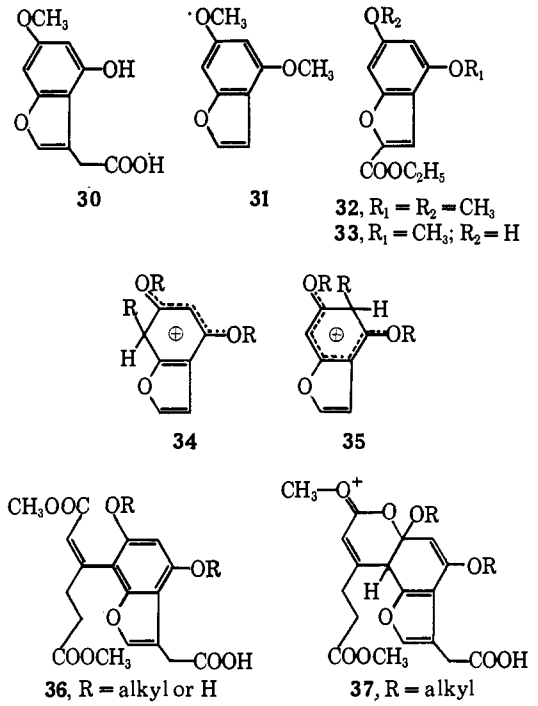
spectrum, λ_{max} 223, 230 (s), 247 (s), 253, 269, and 309 mμ, with that of isobergaptene (**26**), λ_{max} 255, 270, and 310 mμ.[15] The third product brought forth by the Pechmann reaction was the phenol **27**. For structural identification it was converted to the methyl ether **28** (Chart III) whose ultraviolet absorption properties, λ_{max} 215, 253, 258 (s), 308, and 340 mμ, placed it into the allobergaptene series. (Allobergaptene (**29**) has λ_{max} 219, 252, 308, and 338 mμ.[16])

Chart III



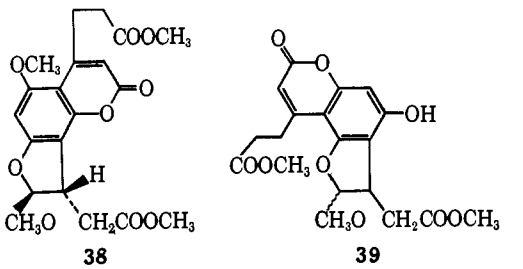
The formation of the allofurocoumarin **27** was neither desired nor anticipated and we made extensive efforts to find a tricyclic precursor leading to larger proportions of either **24** or **25**. Early experiments along these lines produced exactly opposite results. For example, condensation of the lactones **18**, **20**, **21**, and **22** with ethyl methyl-3-oxoadipate in sulfuric acid produced mostly substances containing the allobergaptene skeleton (See Experimental Section). After having demonstrated that the conditions used in these condensations were not severe enough to convert isobergaptenes to allobergaptenes, we began to suspect that the products observed were not derived from the dihydrobenzofurans (*e.g.*, **19**) directly but rather from intermediary benzofurans (*e.g.*, **30**). Electrophilic substitution of such intermediates at C_7 would then lead to a cinnamic ester **36** and then to the allobergaptenes actually observed. This hypothesis conforms with the experience of Robertson and his collaborators who found that electrophilic agents attack the three benzofurans **31**, **32**, and **33**[17] at the C_7 position. This might be construed as a reflection of the relative stabilities of the two intermediates **34** and **35** (Chart IV). The positive charge in the former can be delocalized over five atoms without disturbing the furan ring while charge delocalization in the latter disrupts the aromatic ring. There is also precedent for ether cleavage[18,19]

Chart IV



in Pechmann condensations and we believe these to occur by phenyl–oxygen cleavage *via* intermediates of type **37**.

After these unsuccessful attempts to prepare useful quantities of the tetracyclic intermediate **24**, we reasoned that the coumarin synthesis might take the desired course provided the formation of the intermediate benzofuran **30** could be prevented from occurring. We were pleased to find that the acetal **38** was formed when the phenol **19** and the β-keto ester were allowed to react in methanol solution containing hydrogen chloride. Variation of experimental conditions eventually leads to a procedure giving pure acetal in reproducible yields approaching 60%. The structure of the acetal **38** is defined by the proton spectrum and conversion into the isobergaptene **25** accomplished by brief exposure to hot polyphosphoric acid. The substituents on the hydrofuran ring are *trans* oriented because the vicinal coupling of the acetal proton was observed to be 1.7 cps while the analogous coupling in the *cis*-fused lactones and in the aflatoxins themselves is of the order of 6–7 cps.



Before closing the discussion of this phase in the synthesis, we cannot restrain ourselves from mentioning that condensation of the dihydroxylactone **21** with ethyl methyl 3-oxoadipate in methanolic hydrogen chloride solution produced mainly a new acetal **39**, the corre-

(15) F. Wessely and J. Kotlan, *Monatsh.*, **86**, 430 (1955); G. Rodighiero and C. Antonello, *Chem. Abstr.*, **50**, 12037 (1956).

(16) G. Caporale, *Ann. Chim.* (Rome), **50**, 1135 (1960); *Chem. Abstr.*, **55**, 21106 (1961); R. T. Foster, W. N. Howell, and A. Robertson, *J. Chem. Soc.*, 930 (1939).

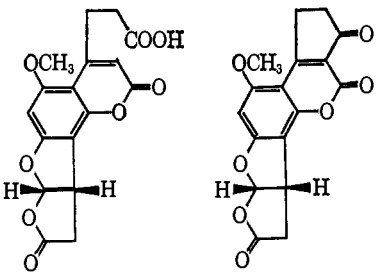
(17) R. T. Foster and A. Robertson, *ibid.*, 921 (1939); R. T. Foster, W. N. Howell, and A. Robertson, *ibid.*, 930 (1939); J. R. Clarke, G. Glaser, and A. Robertson, *ibid.*, 2260 (1948).

(18) D. Chakravarti and B. Majumdar, *J. Indian Chem. Soc.*, **15**, 136 (1938).

(19) A. Robertson, R. B. Waters, and E. T. Jones, *J. Chem. Soc.*, 1681 (1932).

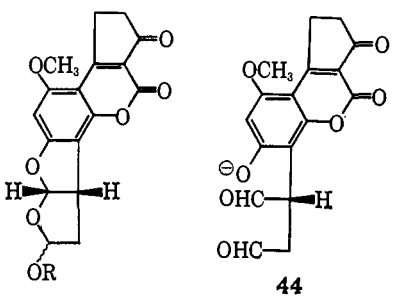
sponding *cis* isomer, and the previously encountered allobergaptene **27**. Evidence presented in the Experimental Section proved that the acetal **39** also belongs to the allobergaptene series. This finding clearly demonstrates how seemingly minor changes in the substrate structure have a pronouced effect on the course of the Pechmann coumarin synthesis. We do not pretend to have predicted this situation.

In any event, the acetal **38** was readily hydrolyzed to the tetracyclic lactone carboxylic acid **40**. Various attempts to dehydrate the latter by the agency of polyphosphoric acid[5,6] led to no useful results. By adding stannic chloride to a suspension of the acid **40** in trifluoroacetic anhydride, we obtained the symmetrical anhydride but not a trace of the ketone. The acid, however, did combine readily with oxalyl chloride and when the resulting crude acid chloride was treated with aluminum chloride in methylene chloride solution the pentacyclic ketone **41** was formed.



40 **41**

The γ-lactone function in **41** was anticipated to be an exceptionally good hydride acceptor (infrared absorption at 1790 cm^{-1}!) and when the substance was reduced with disiamylborane[20] the desired hemiacetal **42** was formed in poor yield. Subsequent work by Milne[21] led to an improved procedure. Chromatographic separation of the reaction products led to the racemic hemiacetal **42** and unaltered starting material. At this point we were able to establish a connection with "natural" material



42, R = H
43, R = $COCH_3$

44

because the optically active hemiacetal **42**[22] could be prepared by trifluoroacetic acid catalyzed hydration of natural aflatoxin B_1 (**1**). The infrared and ultraviolet spectra in solution of racemic and optically active hemiacetal were identical and the two substances could not be separated by thin layer chromatography.

In contrast to aflatoxin B_1 (**1**) the ultraviolet spectra of the pentacyclic lactone **41** and the hemiacetal **42** display a pronounced bathochromic shift in basic medium which is reversible upon acidification. Clearly, the spectra observed in base are those of the phenoxide anions (*e.g.*, **44**). The anions retain a single asymmetric center located next to an aldehyde function and consequently it should be possible to racemize the optically active compounds in basic solution. As anticipated, the optical rotation of a basic solution of the "natural" hemiacetal approached zero within minutes. Acidification followed by extraction led to racemic hemiacetal **42** identical in every detail with synthetic material. To reach aflatoxin B_1 the synthetic hemiacetal **42** had to be dehydrated. We have not yet been able to realize this change in a single operation. When the corresponding acetate **43**[23] was heated for 10 min at 240° under reduced pressure (0.05 mm), it was converted into racemic aflatoxin B_1 (**1**) which was identical with a sample of natural origin.

The biological properties of some of the compounds prepared will be discussed in forthcoming papers by Professor G. N. Wogan, Department of Food Science and Nutrition, M.I.T.

Experimental Section

Elemental analyses were performed by Midwest Microlabs, Inc., Indianapolis, Ind., and Dr. S. M. Nagy at the Massachusetts Institute of Technology. Melting points (mp) were determined on a hot-stage microscope and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 237 instrument; only selected high-intensity bands are listed. Ultraviolet spectra were obtained on a Cary Model 14 recording spectrophotometer. The symbol λ_{max} EtOH–NaOH refers to the spectrum obtained when one drop of 0.1 *N* sodium hydroxide was added to the sample cell (3 ml). In all cases, the original spectrum was restored by addition of hydrochloric acid to the basic solution. Nuclear magnetic resonance (nmr) spectra were recorded on a Varian A-60 spectrometer. Chemical shifts are given in parts per million downfield from tetramethylsilane as internal standard; coupling constants (*J*) are given in cycles per second. The abbreviations s, d, t, q, and m indicate singlet, doublet, triplet, quartet, and multiplet, respectively. The nmr data are given by listing the chemical shift, number of protons, multiplicity, and coupling constants. When appropriate and significant, the chemical shifts for AB or ABX systems were determined by correction of the observed chemical shift difference with the formula[24] $(\delta_A - \delta_B)_{obsd}^2 = (\delta_A - \delta_B)^2 + J_{AB}^2$. Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter. Thin layer chromatography (tlc) was used routinely for monitoring reactions and chromatographic separations. Plates coated with Merck silica gel G were generally developed with 3–5% methanol in chloroform. Inspection of tlc plates was conveniently performed with an ultraviolet lamp (Blak-Ray UVL-22, Ultraviolet Products Inc., San Gabriel, Calif.) since the coumarins exhibited a blue fluorescence. Merck silicic acid or silica gel was used for column chromatography. Chloroform extracts were dried with anhydrous sodium sulfate and ethyl acetate extracts were dried with anhydrous magnesium sulfate.

2,6-Diacetoxy-4-hydroxyacetophenone (4). A suspension of phloroacetophenone (5.045 g, 30 mmoles) in acetic anhydride (6.18 g, 60 mmoles) was heated at 110–165° for 2 hr and solvent then removed under reduced pressure. The residual solid was dissolved in 40 ml of chloroform and kept at 0° for 2 days and the resulting precipitate was recrystallized from methanol–water to yield 3.046 g (12 mmoles, 40%) of 2,6-diacetoxy-4-hydroxyacetophenone (**4**) as colorless

(20) H. C. Brown and D. B. Bigley, *J. Am. Chem. Soc.*, **83**, 486 (1961).

(21) We are much indebted to Mr. George Milne, M.I.T., for his contribution to this phase of the synthesis.

(22) The hydration of aflatoxin B_1 was first investigated by P. J. Andrellos and G. R. Reid, *J. Assoc. Offic. Agr. Chemists*, 47, 801 (1964), but the hemiacetal was not isolated. Optically active hemiacetal **42**, however, has been isolated from crude mixtures of toxins produced by *Aspergillus flavus* by J. G. Heathcote and M. F. Dutton, *Biochem. J.*, in press, and by A. Ciegler, U. S. Department of Agriculture, private communication.

(23) The optically active acetate was described by K. J. van der Merwe, L. Fourie, and de B. Scott, *Chem. Ind.* (London), 1660 (1963).

(24) L. M. Jackmann, "Applications of Nuclear Magnetic Resonance Spectroscopy," Pergamon Press, Inc., New York, N. Y., 1959, p 89.

needles: mp 154–155°; nmr (CD_3SOCD_3), 2.30 (6 H, s), 2.42 (3 H, s), 6.74 (2 H, s), 10.9 (1 H, broad s).

An nmr spectrum ($CDCl_3$) of the isomeric phloroacetophenone diacetate **3** exhibited three distinct methyl singlets, an AB quartet (J = 2) for the nonequivalent aromatic protons, and a sharp singlet at 13.64 ppm for the chelated phenolic proton. The melting point of this unsymmetrical diacetate was 86–87°.

Phloroacetophenone 4-Methyl Ether (5). To an ethereal diazomethane solution (140 mmoles) at room temperature was added a solution of 2,6-diacetoxy-4-hydroxyacetophenone (**4**) (30.2 g, 120 mmoles) in 100 ml of dioxane. After standing overnight, the reaction mixture was concentrated and the residual pale yellow oil was heated at reflux for 8 hr with methanol (200 ml) and 1% hydrochloric acid (100 ml). The cooled reaction mixture was neutralized with sodium bicarbonate and concentrated to 100 ml to afford needles (14.75 g). The filtrate was extracted with ether to furnish another 3.474 g, a total of 18.227 g (83%) of phloroacetophenone 4-methyl ether. Recrystallization from water gave colorless needles: mp 139–139.5° (lit.[8] mp 136–137°); nmr (CD_3SOCD_3), 2.69 (3 H, s), 3.89 (3 H, s), 6.15 (2 H, s), 12.6 (2 H, broad s).

2-Benzyloxy-6-hydroxy-4-methoxyacetophenone (6). To a stirred mixture of 145.6 g (1.054 moles) of finely powdered anhydrous potassium carbonate and 19.24 g (0.105 mole) of phloroacetophenone 4-methyl ether (**5**) in acetone was added 18.85 g (0.105 mole) of benzyl bromide in acetone (200 ml) during 30 min at room temperature under nitrogen. After 14 hr the mixture was filtered and the precipitate was dissolved in water and extracted with chloroform. The extract and initial acetone filtrate were combined, concentrated, and redissolved in chloroform (200 ml). This was washed, dried, and concentrated, and the crude product was recrystallized from ethanol to give 23.57 g (82%) of 2-benzyloxy-6-hydroxy-4-methoxyacetophenone (**6**) as colorless needles: mp 110–111.5°; nmr ($CDCl_3$), 2.58 (3 H, s), 3.85 (3 H, s), 5.17 (2 H, s), 6.15 (1 H, d, J = 2), and 6.22 (1 H, d, J = 2, AB), 7.57 (5 H, s), 14.70 (1 H, s).

5-Benzyloxy-7-methoxy-4-methylcoumarin (7) *via* the Wittig Reaction. An intimate mixture of the mixed ether **6** (272 mg, 1.0 mmole) and carbethoxymethylenetriphenylphosphorane (964 mg, 2.2 mmoles) was heated at 170° for 19 hr. Chromatography of the crude product on silicic acid with chloroform as eluent gave a white solid which was recrystallized from ethanol to afford 212 mg (0.72 mmole, 72%) of 5-benzyloxy-7-methoxy-4-methylcoumarin (**7**), mp 142–143° (lit.[10] 138–141°).

5,7-Dibenzyloxy-4-methylcoumarin (9) and 5-Benzyloxy-7-hydroxy-4-methylcoumarin (10). A 12-l. flask equipped with mechanical stirrer and addition funnel was charged with 250 g (1.30 moles) of 5,7-dihydroxy-4-methylcoumarin, which was dissolved in acetone (6 l.) and tetrahydrofuran (1.2 l.) under a nitrogen atmosphere. Finely powdered anhydrous potassium carbonate (540 g, 3.9 moles) was added, followed by 244 g (1.43 moles) of benzyl bromide added dropwise during 5 hr. Additional potassium carbonate (135 g) was introduced 37 hr later. After 64 hr the reaction mixture was filtered and the precipitate was washed with acetone. The solid cake was added to ice and acidified with 820 g of concentrated hydrochloric acid and the resulting precipitate was collected. To remove the dibenzyloxycoumarin from this mixture, it was suspended in 500 ml of chloroform for 1 hr and filtered. The chloroform-insoluble portion (45 g) was recrystallized from methanol to afford 30.0 g of 5-benzyloxy-7-hydroxy-4-methylcoumarin (**10**).

The initial acetone filtrate was concentrated under reduced pressure, the residue was partially dissolved in chloroform (500 ml) and filtered, and this filtrate was concentrated to leave 255 g of a yellow solid. Two recrystallizations from ethanol afforded 120 g (0.323 mole, 25%) of 5,7-dibenzyloxy-4-methylcoumarin (**9**).

A sample of **9** was recrystallized from ethanol for analysis: mp 132–135°; ν_{max} ($CHCl_3$) 1720, 1615, 1610, 1450 cm^{-1}; λ_{max} (EtOH) 208, 245, 255, 318 mμ (ε 73,000, 8480, 7700, 13,300); nmr ($CDCl_3$), 2.34 (3 H, d, J = 1), 5.00 (4 H, s), 5.85 (1 H, q, J = 1), 6.42 (2 H, s), 7.39 (10 H, s).

Anal. Calcd for $C_{24}H_{20}O_4$: C, 77.40; H, 5.41. Found: C, 77.59; H, 5.32.

A sample of 5-benzyloxy-7-hydroxy-4-methylcoumarin (**10**) was recrystallized from chloroform–ether, then from ethanol, for analysis: mp 221–223°; ν_{max} (KBr) 3250, 1690, 1610, 1560 cm^{-1}; λ_{max} (EtOH) 206, 247, 256, 324 mμ (ε 63,900, 7140, 7140, 14,580); λ_{max} (EtOH–NaOH) 235, 270, 372 mμ (ε 16,000, 9070, 19,900); nmr (CD_3SOCD_3), 2.40 (3 H, s), 5.13 (2 H, s), 5.85 (1 H, s), 6.34 (1 H, d, J = 2), 6.46 (1 H, d, J = 2), 7.45 (5 H, s), 10.50 (1 H, broad s).

Anal. Calcd for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00. Found: C, 72.28; H, 5.17.

5-Hydroxy-7-benzyloxy-4-methylcoumarin (11). A mixture of 5,7-dibenzyloxy-4-methylcoumarin (**9**) (14.6 g, 39 mmoles) and palladium-on-carbon catalyst (1.5 g of a 10% preparation) in 400 ml of ethyl acetate was allowed to consume 1 equiv of hydrogen (960 ml, 4.5 hr). After filtration to remove catalyst, the concentrated filtrate was suspended in chloroform (250 ml) and extracted with an equal volume of 1% sodium hydroxide. The basic extract was acidified and the precipitate (3.4 g) was recrystallized from ethanol to give a mixture of the two monobenzyloxycoumarins. The mother liquor from a second recrystallization was sufficiently enriched to allow crystallization of 5-hydroxy-7-benzyloxy-4-methylcoumarin (**11**) (0.6 g, 2.1 mmoles, 5%). Further recrystallization from methanol afforded a sample for analysis: mp 209–213°; ν_{max} (KBr) 3200, 1680, 1625 cm^{-1}; nmr (CD_3SOCD_3), 2.54 (3 H, d, J = 1), 5.16 (2 H, s), 5.92 (1 H, q, J = 1), 6.48 (2 H, s), 7.45 (5 H, s), 10.66 (1 H, broad s); λ_{max} (EtOH) 208, 249, 257, 319 mμ (ε 55,000, 7600, 8530, 16,000); λ_{max} (EtOH–NaOH) 273, 319, 384 mμ (ε 15,300, 10,000, 9780). This spectrum in basic solution was markedly different from that obtained for the isomeric coumarin **10**. The tlc R_f of **11** in 4% methanol in chloroform was 0.43, while the R_f of **10** was 0.35.

Anal. Calcd for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00. Found: C, 72.59; H, 5.18.

5-Benzyloxy-7-methoxy-4-methylcoumarin (7). To 15.9 g (56 mmoles) of 5-benzyloxy-7-hydroxy-4-methylcoumarin (**10**) dissolved in acetone (600 ml) and tetrahydrofuran (50 ml) under a nitrogen atmosphere was added finely powdered anhydrous potassium carbonate (23.5 g, 0.17 mole) and methyl iodide (24.1 g, 0.17 mole). After 9 hr another 6.9 g of potassium carbonate was added and after 21 hr the mixture was filtered. The filtrate was concentrated to leave a white solid which was taken up in chloroform, washed with water, dried, and freed of solvent. The crude product (17.8 g) was recrystallized from ethanol to give 11.8 g (40 mmoles, 71%) of 5-benzyloxy-7-methoxy-4-methylcoumarin (**7**) as white needles, mp 141–142°. A second crop was collected to give a total of 13.1 g (44 mmoles, 79%). The analytical sample was recrystallized from ethanol: mp 141–142° (lit.[10] mp 138–141°); ν_{max} ($CHCl_3$) 1725, 1615, 1495, 1450, 1390 cm^{-1}; λ_{max} (EtOH) 208, 245, 255, 320 mμ (ε 52,000, 7550, 7200, 14,900); nmr ($CDCl_3$), 2.39 (3 H, d, J = 1), 3.74 (3 H, s), 5.02 (2 H, s), 5.83 (1, H, q, J = 1), 6.32 (2 H, s), 7.36 (5 H, s).

Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.96; H, 5.35.

5-Methoxy-7-benzyloxy-4-methylcoumarin (12). A solution of 5-hydroxy-7-benzyloxy-4-methylcoumarin (**11**) (332 mg, 1.18 mmoles) in tetrahydrofuran was treated with excess ethereal diazomethane at room temperature overnight. Solvent was then removed and the residue recrystallized twice from methanol to afford 173 mg (0.58 mmole, 50%) of 5-methoxy-7-benzyloxy-4-methylcoumarin (**12**): mp 116–118°; ν_{max} ($CHCl_3$) 1720, 1610, 1465, 1420, 1385, 1355 cm^{-1}; λ_{max} (EtOH) 209, 246, 255, 320 mμ (ε 54,000, 7740, 7460, 16,750); nmr ($CDCl_3$), 2.46 (3 H, d, J = 1.2), 3.79 (3 H, s), 5.05 (2 H, s), 5.87 (1 H, q, J = 1.2), 6.32 (1 H, d, J = 2.5), 6.44 (1 H, d, J = 2.5), 7.36 (5 H, s).

Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.86; H, 5.37.

5-Hydroxy-7-methoxy-4-methylcoumarin (13). To a cool solution (10°) of sodium hydroxide (40.0 g, 1.0 mole) in water (450 ml) was added 150 g (0.782 mole) of 5,7-dihydroxy-4-methylcoumarin. While the reaction mixture was maintained at 0–5°, dimethyl sulfate (98.5 g, 0.782 mole) was added over 30 min. The solution was stirred at 0–5° for 2 hr and then allowed to come to room temperature. After 4 hr the solution was brought to pH 7 with concentrated hydrochloric acid and the solid was filtered and washed with water. The yellow solid was suspended in 1 l. of 5% sodium hydroxide and refiltered. This process was repeated three or four times or until the residual solid (mostly 5,7-dimethoxy-4-methylcoumarin) became nearly colorless. 5,7-Dimethoxy-4-methylcoumarin was isolated after recrystallization from methanol: mp 169–171°; yield, 21.6 g (12%).

Acidification followed by filtration of the alkaline filtrate gave crude 7-methoxy-5-hydroxy-4-methylcoumarin as a tan, spongy solid. This was recrystallized while still wet from ethanol to give 54.2 g (34%) of 7-methoxy-5-hydroxy-4-methylcoumarin, mp 255–256° (lit.[10] mp 252–254°).

5-Benzyloxy-7-methoxy-4-methylcoumarin (7) was prepared by benzylation of the phenol **13** as described in ref 10.

5-Benzyloxy-7-methoxy-4-formylcoumarin (16). A mixture of 5-benzyloxy-7-methoxy-4-methylcoumarin (**7**) (19.22 g, 0.065 mole) and resublimed selenium dioxide (10.1 g, 0.091 mole, Alfa

Inorganics) in xylene (700 ml) was heated at reflux for 5 hr with a Dean–Stark trap for removal of water and filtered while hot. Dark yellow crystals were collected from the cooled filtrate and a second crop was obtained by recrystallizing the concentrated mother liquor from benzene. The two crops were combined, dissolved in hot methylene chloride, and filtered to remove residual selenium metal. The concentrated filtrate was recrystallized from benzene to give 16.8 g of the yellow aldehyde. A second crop (2.0 g) was produced by recrystallization from chloroform–ether, making a total of 18.8 g (0.0606 mole, 93%) of 5-benzyloxy-7-methoxy-4-formylcoumarin (**16**). An analytical sample was recrystallized from ethyl acetate: mp 189–190.5° (lit.[10] mp 189–191°); ν_{max} ($CHCl_3$) 1730, 1620 cm^{-1}; nmr ($CDCl_3$), 3.84 (3, H, s), 5.13 (2 H, s), 6.23 (1 H, s), 6.45 (2 H, broad s), 7.36 (5 H, s), 10.38 (1 H, s); λ_{max} (MeCN) 244 (s), 341 mμ (ε 8500, 9520). To obtain an ultraviolet spectrum in ethanol, heating was required to dissolve the aldehyde and the spectrum resembled that of a simple coumarin: λ_{max} (EtOH) 208, 247, 256, 324 mμ (ε 54,900, 8100, 7120, 13,750) (hemiacetal formation!).

Anal. Calcd for $C_{18}H_{14}O_5$: C, 69.66; H, 4.55. Found: C, 69.76; H, 4.56.

5,7-Dibenzyloxy-4-formylcoumarin was prepared by the procedure described for **16**. Chromatography of mother liquors on silicic acid with chloroform eluent could also be utilized to free the product of selenium metal. A sample of 5,7-dibenzyloxy-4-formylcoumarin was recrystallized from benzene, then from ethyl acetate–methylene chloride, for analysis: mp 198–199°; ν_{max} ($CHCl_3$) 1730, 1620, 1600, 1350 cm^{-1}; λ_{max} (MeCN) 243 (s), 340 mμ (ε 10,050, 10,000); nmr ($CDCl_3$–CD_3SOCD_3), 5.16 (2 H, s), 5.20 (2 H, s), 6.16 (1 H, s), 6.64 (2 H, s), 7.39 (10 H, s), 10.37 (1 H, s).

Anal. Calcd for $C_{24}H_{18}O_5$: C, 74.60; H, 4.70. Found: C, 74.34; H, 4.72.

2,3,3a,8a-Tetrahydro-2-oxo-4-benzyloxy-6-methoxyfuro[2,3-*b*]benzofuran (18). To a solution of 5-benzyloxy-7-methoxy-4-formylcoumarin (**16**) (9.7 g, 31.2 mmoles) in glacial acetic acid (150 ml) at 100° was added cautiously 8.2 g (125 g-atoms) of zinc dust with vigorous mechanical stirring. After 1.5 hr at 115–120° the mixture was cooled slightly, diluted with an equal volume of chloroform, filtered, and concentrated. The residual solid was taken up in chloroform, washed with water, dried, and concentrated. The crude product (10.6 g) was recrystallized from chloroform–diisopropyl ether to give, in two crops, 7.86 g (25.2 mmoles, 80%) of benzyloxymethoxylactone **18** as white needles. An analytical sample recrystallized from methanol had mp 166–167°; ν_{max} ($CHCl_3$) 1795, 1635, 1605, 1510, 1445 cm^{-1}; λ_{max} (EtOH) 208.5, 259 (s), 264.5, 268, 276.5 mμ (s) (ε 53,300, 700, 820, 858, 625); nmr ($CDCl_3$), 2.90 (2 H, d, J = 6), 3.72 (3 H, s), 4.13 (1 H, q, J = 6), 5.03 (2 H, s), 6.15 (2 H, s), 6.44 (1 H, d, J = 6), 7.35 (5 H, s).

Anal. Calcd for $C_{18}H_{16}O_5$: C, 69.21; H, 5.17. Found: C, 69.15; H, 5.10.

2,3,3a,8a-Tetrahydro-2-oxo-4,6-dibenzyloxyfuro[2,3-*b*]benzofuran. Using the method described for the preparation of **18**, 5,7-dibenzyloxy-4-formylcoumarin was reduced with zinc in acetic acid to furnish the dibenzyloxylactone as white needles. An analytical sample recrystallized from ethanol for analysis had mp 162–162.5°; ν_{max} ($CHCl_3$) 1795, 1635, 1610, 1505 cm^{-1}, λ_{max} (EtOH) 209, 258, 264, 268, 277 mμ (s) (ε 63,800, 1012, 1072, 1057, 700); nmr ($CDCl_3$), 2.90 (2 H, d, J = 6), 4.12 (1 H, q, J = 6), 4.97 (2 H, s), 5.01 (2 H, s), 6.23 (2 H, s), 6.43 (1 H, d, J = 6), 7.35 (10 H, s).

Anal. Calcd for $C_{24}H_{20}O_5$: C, 74.21; H, 5.19. Found: C, 74.37; H, 5.15.

2,3,3a,8a-Tetrahydro-2-oxo-4-hydroxy-6-methoxyfuro[2,3-*b*]benzofuran (19). Palladium-on-carbon catalyst (190 mg of a 10% preparation) was prereduced in ethanol (65 ml) and 851 mg (2.72 mmoles) of benzyloxymethoxylactone **18** in a glass boat dropped into the stirred suspension. After an uptake of 1 equiv of hydrogen (2 hr) the mixture was filtered and concentrated. The crude product (602 mg, 2.71 mmoles, 100%) was recrystallized from ethanol–water for analysis: mp 166–167.5°; ν_{max} (KBr) 3490, 1785, 1645, 1625, 1510, 1440 cm^{-1}; λ_{max} (EtOH) 225 (s), 268 mμ (ε 8800, 576); λ_{max} (EtOH–NaOH) 215, 239 (s), 270 (s), 275 mμ (ε 35,800, 10,500, 968, 1005); nmr (CD_3SOCD_3),[24] 2.69 (1 H, q, J = 2.5, J_{gem} = 18), 3.10 (1 H, q, J = 8.5, J_{gem} = 18), 3.68 (3 H, s), 4.17 (1 H, octet, J = 6, J = 8.5, J = 2.5), 6.08 (2 H, s), 6.60 (1 H, d, J = 6), 9.9 (1 H, broad s).

Anal. Calcd for $C_{11}H_{10}O_5$: C, 59.46; H, 4.53. Found: C, 59.39; H, 4.51.

2,3,3a,8a-Tetrahydro-2-oxo-4-acetoxy-6-methoxyfuro[2,3-*b*]benzofuran (20). Hydroxymethoxylactone **19** (2.80 g, 12.6 mmoles) was heated on a steam bath for 30 min with acetic anhydride (20 ml), then kept at room temperature for 6r hr. Solvent was removed under reduced pressure and the crude product was recrystallized from chloroform–diisopropyl ether to give 2.711 g (10.3 mmoles, 82%) of acetoxymethoxylactone **20**: mp 126–127.5° (from CH_3OH); λ_{max} (EtOH) 225, 278 mμ (ε 8930, 2940); nmr ($CDCl_3$), 2.26 (3 H, s), 2.76 (1 H, d, J = 4), 2.82 (1 H, d, J = 7.5), 3.72 (3 H, s), 4.10 (1 H, octet, J = 6, J = 4, J = 7.5), 6.28 (1 H, d, J = 2), 6.37 (1 H, d, J = 2), 6.46 (1 H, d, J = 6); ν_{max} ($CHCl_3$) 1795, 1765, 1640, 1605, 1505, 1445, 1375 cm^{-1}.

Anal. Calcd for $C_{13}H_{12}O_6$: C, 59.09; H, 4.57. Found: C, 59.09; H, 4.42.

2,3,3a,8a-Tetrahydro-2-oxo-4,6-dihydroxyfuro[2,3-*b*]benzofuran (21). In the same manner as described for the hydrogenolysis of benzyloxymethoxylactone **18**, dibenzyloxylactone was converted into dihydroxylactone **21** in quantitative yield. The starting lactone was insoluble in ethanol, but gradually dissolved as the hydrogenolysis proceeded. A sample was recrystallized from ethanol–water (or ethyl acetate–chloroform) to furnish **21** for analysis: mp 200–204°; ν_{max} (KBr) 3400, 3250, 1745, 1645, 1630 cm^{-1}; λ_{max} (EtOH) 206, 226 (s), 269.5, 273 (s), 277 mμ (s) (ε 41,800, 8900, 644, 585, 474); λ_{max} (EtOH–NaOH) 213, 238 (s), 269.5, 273, 277 mμ (ε 36,000, 10,500, 1000, 1005, 980); nmr (CD_3SOCD_3),[24] 2.69 (1 H, q, J = 2, J_{gem} = 17.5), 3.08 (1 H, q, J = 8.5, J_{gem} = 17.5), 4.10 (1 H, octet, J = 8.5, J = 2, J = 6), 5.88 (1 H, d, J = 2), 6.00 (1 H, d, J = 2), 6.55 (1 H, d, J = 6), 9.42 (1 H, s), 9.73 (1 H, s).

Anal. Calcd for $C_{10}H_8O_5$: C, 57.70; H, 3.87. Found: C, 57.94; H, 3.99.

Partial Methylation of Dihydroxylactone 21. To a solution of dihydroxylactone **21** (1.40 g, 6.74 mmoles) in acetone (50 ml) at 0° was added anhydrous potassium carbonate (4.82 g, 35 mmoles) and 0.995 g (7.0 mmoles) of methyl iodide in acetone (7 ml). The mixture was stirred at room temperature for 14 hr and then filtered. The concentrated filtrate was taken up in ethyl acetate, washed with dilute hydrochloric acid and water, dried, and concentrated to give an orange oil (1.76 g). This was chromatographed on silicic acid (35 g). Dimethoxylactone **22** was eluted with chloroform (245 mg, 1.04 mmoles, 15%); increasing the polarity with 0.5–2% methanol brought through methoxyhydroxylactone **23** (617 mg, 2.78 mmoles, 41%). Starting material (309 mg, 22%) was recovered by further elution with 5–10% methanol–chloroform.

Examination of fractions by tlc using ethyl acetate–chloroform (1:1) indicated the presence of hydroxymethoxylactone **19** in later fractions eluted with 2% methanol–chloroform. However, the amount of isomer **19** was estimated to be 10% or less of the total of the two monomethoxylactones.

A sample of dimethoxylactone **22** recrystallized from ethanol for analysis had mp 154–156°: ν_{max} ($CHCl_3$) 1795, 1635, 1610, 1505 cm^{-1}; λ_{max} (EtOH) 228 (s), 268, 276 mμ (s) (ε 8440, 689, 533); nmr ($CDCl_3$), 2.89 (2 H, d, J = 6), 3.74 (3 H, s), 3.77 (3 H, s), 4.12 (1 H, q, J = 6), 6.10 (2 H, broad s), 6.45 (1 H, d, J = 6).

Anal. Calcd for $C_{12}H_{12}O_5$: C, 61.01; H, 5.12. Found: C, 60.91; H, 4.80.

An analytical sample of methoxyhydroxylactone **23** was recrystallized from isopropyl alcohol–diisopropyl ether: mp 168.5–170°; ν_{max} (KBr) 3330, 1755, 1635, 1610, 1505, 1480, 1430 cm^{-1}; λ_{max} (EtOH) 227 (s), 270, 277 mμ (s) (ε 7970, 735, 618); λ_{max} (EtOH–NaOH) 249, 277 mμ (s) (ε 7800, 2120).

Anal. Calcd for $C_{11}H_{10}O_5$: C, 59.46; H, 4.54. Found: C, 59.42; H, 4.67.

2,3,3a,8a-Tetrahydro-2-oxo-6-acetoxy-4-methoxyfuro[2,3-*b*]benzofuran. Methoxyhydroxylactone **23** (159 mg, 0.72 mmole) was treated with excess acetic anhydride at 25° for 8 hr, then solvent was removed under reduced pressure. The residual oil was crystallized from chloroform–diisopropyl ether to give 163 mg (0.62 mmole, 86%) of the methoxyacetoxylactone. A sample recrystallized from ethanol for analysis had mp 137–138°; ν_{max} ($CHCl_3$) 1795, 1765, 1625, 1500, 1465, 1450, 1430, 1420, 1370 cm^{-1}; λ_{max} (EtOH) 224 (s), 270, 276 mμ (ε 7320, 980, 940); nmr ($CDCl_3$), 2.24 (3 H, s), 2.91 (2 H, d, J = 6), 3.80 (3 H, s), 4.15 (1 H, q, J = 6), 6.25 (1, H, d, J = 2), 6.32 (1 H, d, J = 2), 6.46 (1, H, d, J = 6).

Anal. Calcd for $C_{13}H_{12}O_6$: C, 59.09; H, 4.58. Found: C, 59.33; H, 4.66.

Hydroxymethoxylactone 19 and 3-Oxoadipate in Sulfuric Acid. To 229 mg (1.03 mmoles) of hydroxymethoxylactone **19** at 0° was added ethyl methyl 3-oxoadipate (250 mg, 1.24 mmoles) and 5.0 ml of 86% sulfuric acid. After 64 hr at 0° the brown, fluorescent solution was carefully added to a slight excess of sodium bicarbonate in ice water. Extraction with chloroform gave an oil (98 mg) from which was obtained, by crystallization with methanol, 16 mg (0.045

mmole, 4.3%) of lactone ester **24** identified by its infrared and nmr spectra.

The fluorescent aqueous portion was acidified with hydrochloric acid and extracted continuously with ethyl acetate for 65 hr and the concentrated extract was treated with ethereal diazomethane in tetrahydrofuran for 30 min at 0°. Recrystallization of the residue from alcohol gave 3-carbomethoxymethyl-4,6-dihydroxy-β-(2′-carbomethoxyethyl)-7-benzofuranacrylic acid δ-lactone (**27**) (38 mg, 0.105 mmole, 9.7%), identified by its nmr (no aromatic methyl ether peak), infrared (no lactone band at 1790 cm^{-1}), and ultraviolet spectra, and tlc R_f (0.27 in 3% methanol–chloroform). A sample of **27** recrystallized from ethanol for analysis had mp 222–225°; ν_{max} (KBr) 3425, 1735, 1685, 1625, 1590 cm^{-1}; λ_{max} (EtOH) 223, 246, 255, 262 (s), 309 (s), 344 mμ (ε 28,800, 15,650, 16,550, 12,700, 7750, 12,400); λ_{max} (EtOH–NaOH) 229, 246 (s), 285, 307 (s), 399 mμ (ε 37,800, 17,300, 10,200, 6000, 22,900); nmr (CD_3SOCD_3), 2.74 (2 H, t, J = 7.5), 3.28 (2 H, t, J = 7.5), 3.68 (6 H, s), 3.87 (2 H, s), 6.06 (1 H, s), 6.57 (1 H, s), 7.87 (1 H, s), approximately 11.0 (1 H, very broad).

Anal. Calcd for $C_{18}H_{16}O_8$: C, 60.00; H, 4.47. Found: C, 59.81; H, 4.39.

The remainder of the esterified ethyl acetate extract was chromatographed (silicic acid, chloroform eluent) and one of the fractions obtained was crystallized from alcohol to afford the isofurocoumarin **25** (7 mg, 0.02 mmole, 2%). The infrared, ultraviolet, and nmr spectra differed from those of the allofurocoumarin methyl ether **28**. Complete analytical data are given in the Experimental Section for isofurocoumarin **25**.

3-Carbomethoxymethyl-6-hydroxy-4-methoxy-β-(2′-carbomethoxyethyl)-7-benzofuranacrylic Acid δ-Lactone (28). A solution of **27** (41 mg, 0.11 mmole) in acetone (15 ml) was treated with methyl iodide (142 mg, 1.0 mmole) and anhydrous potassium carbonate (183 mg, 1.3 mmoles) for 44 hr at room temperature. The reaction mixture was filtered and the concentrated filtrate was suspended in chloroform and refiltered to afford 57 mg of a yellow solid having an nmr spectrum consistent with structure **28**. A sample was recrystallized from ethanol: mp 150–151, then 159–161°; ν_{max} ($CHCl_3$) 1730, 1620, 1590, 1440, 1385, 1340 cm^{-1}; λ_{max} (EtOH) 215, 253, 258 (s), 308, 340 mμ (ε 25,600, 19,650, 18,000, 8150, 10,450); nmr ($CDCl_3$), 2.73 (2 H, t, J = 7.5), 3.37 (2 H, t, J = 7.5, d, J = 1), 3.72 (3 H, s), 3.75 (3 H, s), 3.85 (2 H, d, J = 1), 3.94 (3 H, s), 6.08 (1 H, t, J = 1), 6.61 (1 H, s), 7.60 (1 H, t, J = 1).

Anal. Calcd for $C_{19}H_{18}O_8$: C, 60.96; H, 4.85. Found: C, 60.81; H, 4.85.

Dimethoxylactone 22 with 3-Oxoadipate in Sulfuric Acid. A mixture of dimethoxylactone **22** (2.124 g, 9.0 mmoles) and 3-oxoadipate (2.364 g, 11.7 mmoles) in 86% sulfuric acid (36 ml) was stirred at 25° for 234 hr, then added to ice water (700 ml). This was made basic with sodium bicarbonate and extracted with ethyl acetate, but very little neutral material was obtained. The aqueous portion was reacidified and extracted continuously with ethyl acetate for 100 hr and the concentrated extract was heated at reflux with 200 ml of methanol and 1 ml of sulfuric acid for 6 hr. The reaction mixture was added to chloroform, which was then washed with aqueous sodium bicarbonate, dried, and concentrated to leave 3.395 g of crude product. Chromatography on silicic acid (40 g) with chloroform eluent afforded 1.382 g (3.7 mmoles, 41%) of the allofurocoumarin **28**, mp 148–150°. Further elution with methanol–chloroform gave, after recrystallization from methanol, 380 mg (1.05 mmoles, 12%) of the phenol **27**, mp 210–217°.

Benzyloxymethoxylactone 18 and 3-Oxoadipate in Sulfuric Acid. Benzyloxymethoxylactone **18** (312 mg, 1.0 mmole) and 3-oxoadipate (304 mg, 1.5 mmoles) were allowed to react in 86% sulfuric acid (5 ml) for 142 hr at room temperature and the mixture was then added to 150 ml of ice water. Addition of excess sodium bicarbonate and extraction with ethyl acetate gave very little neutral material. Consequently the aqueous portion was reacidified and extracted continuously with ethyl acetate for 168 hr. The concentrated extract was kept at reflux in methanol (100 ml) with sulfuric acid (10 drops) for 8 hr and this mixture was added to cold ethyl acetate, which was washed, dried, and concentrated. The residual solid (281 mg) was chromatographed on silicic acid (10 g) with chloroform as eluent to afford, after recrystallization from methanol, 140 mg (0.39 mmole, 39%) of the phenol **27**, mp 210–217°.

Acetoxymethoxylactone 20 and 3-Oxoadipate in Sulfuric Acid. In the manner described for the transformation of **18**, 3-oxoadipate and acetoxymethoxylactone **20** were treated with 86% sulfuric acid. The continuous ethyl acetate extract was treated with ethereal diazomethane and the product was recrystallized from ethanol to give a 33% yield of the methyl ether **28**.

Dihydroxylactone 21 and 3-Oxoadipate in Sulfuric Acid. To a mixture of 1.31 g (6.29 mmoles) of dihydroxylactone **21** and 1.74 g (8.65 mmoles) of 3-oxoadipate at 0° was added 20.0 ml of 86% sulfuric acid. After stirring for 22 hr at 25° the brown, fluorescent solution was added slowly to 400 ml of ice-cold methanol and this was stirred at 25° overnight and then heated at reflux for 30 min. Sodium bicarbonate (52.7 g, 0.628 mole) was added carefully and the mixture was concentrated and then added to 400 ml of ice water. The aqueous mixture was acidified and extracted with three portions of ethyl acetate. The combined extracts were washed, dried, and concentrated to leave 2.5 g of brown solid which was crystallized from methanol. The greenish crystals (1.068 g) were recrystallized from methanol–chloroform, affording 0.925 g of material, mp 218–225°, having ultraviolet absorption indicative of the phenol **27**. The original acidic aqueous portion was extracted continuously with ethyl acetate for 40 hr, the concentrated extract was combined with previous mother liquors, and the mixture was treated with methanol and sulfuric acid to ensure complete esterification. This was worked up as previously described, affording 1.3 g of brown material which was chromatographed (silicic acid, chloroform to 20% methanol–chloroform). Recrystallization of a fraction eluted with 2% methanol gave 0.314 g of **27** adding to a total yield of 1.238 g (3.44 mmoles, 55%).

Other fractions and mother liquors were combined in tetrahydrofuran and treated with excess ethereal diazomethane overnight. The crude methylated material was chromatographed on silicic acid (chloroform) and 0.327 g (0.875 mmole) of **28** was collected, giving a total yield of 4.315 mmoles (69%) of the allofurocoumarins **27** and **28**.

3-Carbomethoxymethyl-2,3-dihydro-2,6-dimethoxy-4-hydroxy-β-(2′-carbomethoxyethyl)-5-benzofuranacrylic Acid δ-Lactone (38) from Hydroxymethoxylactone (19). To a solution of 6.14 g (27.6 mmoles) of the phenol **19** and 6.16 g (30.4 mmoles) of β-ketoadipate in absolute methanol (300 ml) maintained at −12 to −20° was added dry hydrogen chloride for 1 hr. The reaction was allowed to warm to 3–5° and was stirred at this temperature for 18 hr. Methanol (250 ml) was removed at room temperature under reduced pressure. Ether was added and the crystalline solid filtered to give 4.58 g of acetal **38**. The filtrate was evaporated to dryness, dissolved in chloroform, and washed three times with water. The organic phase was then dried over sodium sulfate and evaporated *in vacuo* to give 8.5 g of a brown oil. The oil was chromatographed on 200 g of silica gel G with chloroform as eluent. *Note:* Ethanol had to be removed from the chloroform before chromatography by rapidly passing reagent grade chloroform through a column of alumina. An additional 700 mg of crystalline acetal plus 1.1 g (6.38 g, 57%) of oily acetal was recovered. Examination of the oily fractions by nmr indicated the presence of a mixture (1.5:1) of *trans*-acetal (**38**) and the less stable *cis*-acetal. The mixture showed nmr absorption, in addition to that reported below, at 5.77 (doublet for *cis*-acetal proton, J = 6), and 3.51 (singlet for *cis*-acetal $-OCH_3$).

A sample was recrystallized from methanol to give pure *trans*-acetal **38**: mp 129–130°; ν_{max} ($CHCl_3$) 1735, 1630, 1610, 1440 cm^{-1}; λ_{max} (EtOH) 255, 261, 327 mμ (ε 8200, 9400, 12,700); nmr ($CDCl_3$),[24] 2.53 (1 H, q, J = 9.5, J_{gem} = 17), 2.98 (1 H, q, J = 4.5, J_{gem} = 17), 2.58 (2 H, t, J = 7), 3.21 (2 H, t, J = 7), approximately 3.7 (1 H, not visible), 3.56 (3 H, s), 3.72 (6 H, s), 3.90 (3 H, s), 5.56 (1 H, d, J = 1.7), 5.96 (1 H, s), 6.40 (1 H, s).

Anal. Calcd for $C_{20}H_{22}O_9$: C, 59.11; H, 5.46. Found: C, 59.37; H, 5.67.

3-Carbomethoxymethyl-4-hydroxy-6-methoxy-β-(2′-carbomethoxyethyl)-5-benzofuranacrylic Acid δ-Lactone (25) (from Acetal 38). Acetal **38** (70 mg, 0.17 mmole) was heated on a steam bath with polyphosphoric acid (3.9 g, Matheson Coleman and Bell) for 20 min, cooled quickly, and 25 ml of water was added. Extraction with ethyl acetate and chromatography of the concentrated extract (49 mg) on silicic acid with chloroform gave the isofurocoumarin **25**. The infrared spectrum was identical with that of material obtained from the sulfuric acid catalyzed condensation of **19** with oxoadipate. An analytical sample was recrystallized from methanol–methylene chloride to furnish pure **25** as white plates: mp 154–155°; ν_{max} ($CHCl_3$) 1735, 1635, 1610, 1480, 1455, 1445 cm^{-1}; λ_{max} (EtOH) 223, 230 (s), 247 (s), 253, 269, 309 mμ (ε 18,900, 17,100, 14,850, 18,600, 14,850, 10,010); nmr ($CDCl_3$), 2.60 (2 H, t, J = 7.5), 3.27 (2 H, t, J = 7.5), 3.73 (3 H, s), 3.79 (3 H, s), 3.95 (5 H, s), 6.09 (1 H, s), 6.87 (1 H, s), 7.54 (1 H, t, J = 1).

Anal. Calcd for $C_{19}H_{18}O_8$: C, 60.96; H, 4.84. Found: C, 60.68; H, 4.91.

3-Carbomethoxymethyl-2,3-dihydro-4,6-dihydroxy-2-methoxy-β-(2′-carbomethoxyethyl)-7-benzofuranacrylic Acid δ-Lactone (39).

Anhydrous hydrogen chloride was bubbled through a solution of dihydroxylactone **21** (735 mg, 3.5 mmoles) and 3-oxoadipate (860 mg, 4.25 mmoles) in 55 ml of methanol for 1.5 hr at −50 to 0°. After 15 hr of stirring at 25°, most of the solvent was removed and the residue was added to ice water (100 ml). Extraction with ethyl acetate followed by chromatography of the extract (1.456 g) on silicic acid gave 811 mg (2.06 mmoles, 59%) of material, eluted with 1–2% methanol–chloroform, which was predominantly the acetal phenol **39**. Examination by nmr of one fraction indicated a mixture (2:1) of *cis*-acetal **39** and of the allofurocoumarin **27**. An earlier fraction was recrystallized from chloroform–ether to afford the *trans*-acetal **39** for analysis: mp 130–132°; ν_{max} ($CHCl_3$) 3250, 1730, 1625, 1580 (weak), 1445, 1370 cm^{-1}; λ_{max} (EtOH) 210, 230 (s), 247 (s), 257, 320 mμ (ε 43,300, 15,900, 6400, 6150, 15,700); λ_{max} (EtOH–NaOH) 221, 238 (s), 271, 375 mμ (ε 31,000, 18,800, 8350, 21,400). The spectrum observed in basic solution is indicative of a 7-hydroxy-5-alkoxycoumarin: nmr ($CDCl_3$), 2.68 (2 H, t, J = 7), 3.24 (2 H, t, J = 7), approximately 2.7 (2 H, m, AB of ABX), approximately 3.6 (1 H, m, X of ABX), 3.55 (3 H, s), 3.71 (3 H, s), 3.73 (3 H, s), 5.52 (1 H, d, J = 1.5), 5.96 (1 H, s), 6.55 (1 H, s), 8.92 (1 H, s).

Anal. Calcd for $C_{19}H_{20}O_9$: C, 58.16; H, 5.14. Found: C, 58.22; H, 5.06.

3-Carbomethoxymethyl-2,3-dihydro-2,4-dimethoxy-6-hydroxy-β-(2′-carbomethoxyethyl)-7-benzofuranacrylic Acid δ-Lactone. Miscellaneous mother liquors and fractions containing the phenol **39** were treated with excess ethereal diazomethane in tetrahydrofuran overnight. Solvent was evaporated and the residue was chromatographed (silicic acid, chloroform) to give fractions which were crystallized from methanol. The material obtained was largely the methyl ether of **39** but it was contaminated with minor amounts of the allofurocoumarin **28**. Recrystallization from methylene chloride–diisopropyl ether gave a sample of the methyl ether for analysis, mp 100–102°. The nmr spectrum indicated a mixture of *trans*- and *cis*-acetals (2:1): nmr ($CDCl_3$), 2.66 (2 H, t, J = 7), 3.24 (2 H, t, J = 7), approximately 3.1 (1 H, m, not visible), approximately 2.8 (1 H, m, not visible), 3.51 (part of 3 H, s, *cis*-acetal $-OCH_3$), 3.56 (part of 3 H, s, *trans*-acetal $-OCH_3$), approximately 3.9 (1 H, m), 3.71 (6 H, s), 3.85 (3 H, s), 5.58 (part of 1 H, d, J = 1.7, *trans*-acetal proton), 5.79 (part of 1 H, d, J = 6.5, *cis*-acetal proton), 6.00 (1 H, s), 6.45 (1 H, s); ν_{max} ($CHCl_3$) 1735, 1625, 1440, 1360 cm^{-1}; λ_{max} (EtOH) 211, 232, 256, 315 mμ (ε 45,600, 17,400, 6400, 14,800).

Anal. Calcd for $C_{20}H_{22}O_9$: C, 59.11; H, 5.45. Found: C, 59.20; H, 5.38.

Allofurocoumarin 28 from the Methyl Ether of 39. The methyl ether of **39** (55 mg, 0.135 mmole) and 1.0 g of polyphosphoric acid were heated in a centrifuge tube at 75° for 25 min while stirring with a glass rod. Methanol (4 ml) was added and the white precipitate which formed was collected and recrystallized from ethanol to give 48 mg (0.128 mmole, 95%) of the allofurocoumarin **28** identified by its infrared and ultraviolet spectra.

2,3,3a,8a-Tetrahydro-2-oxo-4-hydroxy-6-methoxy-β-(2′-carboxyethyl)-5-furo[2,3-*b*]benzofuranacrylic Acid δ-Lactone (40). The acetal **38** (136 mg, 0.34 mmole) was treated with 5 ml of glacial acetic acid, 5 ml of water, and 0.5 ml of concentrated hydrochloric acid at 25° for 24 hr. Solvent was removed under reduced pressure to leave 120 mg (0.35 mmole, 100%) of lactone carboxylic acid **40**, having infrared absorption (Nujol) at 1790, 1725, 1700 cm^{-1}. The material could be recrystallized from acetic acid–water, but efforts to free the product of solvent failed. A sample recrystallized from acetonitrile–methanol for analysis showed mp 252–256° dec; ν_{max} (KBr) 3450 broad, 1795, 1725, 1635, 1610, 1485, 1440, 1355 cm^{-1}; λ_{max} (EtOH) 251, 259, 319 mμ (ε 7800, 8350, 11,100); nmr (CD_3SOCD_3),[24] 2.84 (1 H, q, J = 2.5, J_{gem} = 18), 3.26 (1 H, q, J = 9, J_{gem} = 18), 2.49 (2 H, t, J = 7), 3.09 (2 H, t, J = 7), 3.89 (3 H, s), 4.41 (1 H, octet, J = 6, J = 9, J = 2.5), 6.00 (1 H, s), 6.71 (1 H, s), 6.77 (1 H, d, J = 6), 11.8 (1 H very broad).

Anal. Calcd for $C_{17}H_{14}O_8$: C, 58.96; H, 4.08. Found: C, 59.10; H, 4.20.

2,3,3a,8a-Tetrahydro-2-oxo-4-hydroxy-6-methoxy-β-(2′-carbomethoxyethyl)-5-furo[2,3-*b*]benzofuranacrylic Acid δ-Lactone. Lactone carboxylic acid **40** was converted into its methyl ester by treatment of a suspension in tetrahydrofuran with excess ethereal diazomethane. Recrystallization from ethanol–chloroform gave a sample of lactone ester for analysis: mp 210–213°; λ_{max} (EtOH) 251, 259, 321 mμ (ε 7150, 8090, 10,800); ν_{max} ($CHCl_3$) 1795, 1730, 1625, 1610, 1485, 1470, 1435 cm^{-1}; nmr (CD_3SOCD_3),[24] 2.58 (2 H, t, J = 7), 3.14 (2 H, t, J = 7), 2.83 (1 H, q, J = 2.5, J_{gem} = 17.5), 3.26 (1 H, q, J = 9, J_{gem} = 17.5), 3.64 (3 H, s), 3.88 (3 H, s), 4.42 (1 H, octet, J = 6, J = 9, J = 2.5), 6.03 (1 H, s), 6.73 (1 H, s), 6.78 (1 H, d, J = 6).

Anal. Calcd for $C_{18}H_{16}O_8$: C, 60.00; H, 4.48. Found: C, 60.31; H, 4.75.

2-(2,3,3a,8a-Tetrahydro-2-oxo-4-hydroxy-6-methoxyfuro[2,3-*b*]-benzofuran-5-yl)-5-oxo-1-cyclopentene-1-carboxylic Acid δ-Lactone (41). To a cold (5°) suspension of 2.0 g (5.78 mmoles) of the acid **40** (dried at 80° for 36 hr *in vacuo*) in methylene chloride (10 ml) was added oxalyl chloride (20 ml). (All glass equipment was flame dried immediately before use.) After mixing, the solution was stirred at room temperature for 48 hr. Excess oxalyl chloride was removed under reduced pressure and the residual brown solid dried *in vacuo* for 2 hr. The acid chloride was suspended in methylene chloride (300 ml) and cooled to −5° (ice–salt bath). Aluminum chloride (2.28 g, 17.2 mmoles) was added in three portions (0.760 g each) over 20-min intervals. The solution was stirred (care was taken to use a large magnetic stir bar and as rapid a stir rate as possible) at −5 to +5° for 10 hr. The solvent was removed under reduced pressure and hydrochloric acid (20 ml, 6 *N*) was then added to the yellow solid. After 2 hr, the pink solid was filtered and washed with water. An aqueous slurry of the crude products was transferred to a centrifuge tube, the water was removed, and the product washed with 5% sodium bicarbonate (four times) and water (two times). The pentacyclic ketone **41** was filtered, washed with methanol, and dried: yield, 690 mg (37%); mp >320° dec; ν_{max} (Nujol) 1790, 1760, 1685, 1630, 1605, 1560 cm^{-1}; λ_{max} (MeOH) 216 (s), 240 (s), 264, 362 mμ (ε 16,600, 11,180, 9220, 15,800); λ_{max} (MeOH–NaOH) 247, 289, 401 mμ (ε 10,140, 8650, 33,200); nmr (CF_3COOH), 2.77 (2 H, m) and 3.21 (2 H, m, A_2B_2), 2.9 (2 H, m), 3.61 (3 H, s), 4.07 (1 H, m), 6.22 (1 H, s), 6.33 (1 H, d, J = 6).

The bicarbonate-soluble portion of the reaction product was acidified and extracted, giving 978 mg of a solid material. Crystallization gave unaltered lactone carboxylic acid **40** (900 mg).

2-(2,3,3a,8a-Tetrahydro-2,4-dihydroxy-6-methoxyfuro[2,3-*b*]-benzofuran-5-yl)-5-oxo-1-cyclopentene-1-carboxylic Acid δ-Lactone (42) (Optically Active Aflatoxin B Hemiacetal). Aflatoxin B_1 (**1**) (170 mg, 0.54 mmole) was suspended in 5 ml of water and 3 ml of trifluoroacetic acid was added. After the solid had dissolved (30 min), another 5 ml of water was added and the green fluorescent solution was stirred for an additional 2 hr. Cooling at 0° for 4 hr caused crystallization. The precipitate was collected, washed with water, and dried to give hemiacetal **42** (120 mg), mp 233–235° dec. The filtrate was neutralized with sodium bicarbonate (no excess) and extracted with chloroform to furnish another 60 mg of crude hemiacetal **42** (a total of 180 mg, 0.54 mmole, quantitative conversion).

The material would not crystallize well from any of the numerous solvents tried. Methanol–isopropyl alcohol gave an off-white microcrystalline sample which was submitted for analysis: λ_{max} (EtOH) 217 (s), 240 (s), 265, 363 mμ (ε 17,450, 12,300, 10,100, 20,200); λ_{max} (EtOH–NaOH) 249, 290 402 mμ (ε 12,300, 10,800, 43,200); nmr (CD_3SOCD_3), 2.13 (2 H, m), 2.45 (2 H, m, A_2 of A_2B_2), 3.22 (2 H, m, B_2 of A_2B_2), 3.90 (3 H, s), 4.05 (1 H, m), 5.16 (1 H, m, hemiacetal proton), 6.45 (1 H, d, J = 6), 6.55 (1 H, s) (the hydroxyl proton was not detected); ν_{max} (Nujol) 3460, 1755, 1680, 1630, 1590, 1550 cm^{-1}.

Anal. Calcd for $C_{17}H_{14}O_7$: C, 61.82; H, 4.27. Found: C, 61.42; H, 4.43.

Optically Active Aflatoxin B Lactone (41). A solution of aflatoxin B hemiacetal **42** (59 mg, 0.18 mmole) in 4 ml of glacial acetic acid with one drop of water added was treated with 2.7 ml of a solution of chromium trioxide (120 mg) in 10 ml of acetic acid–water (9:1). After stirring for 18 hr at 25° the solid which formed was collected and washed with water (22 mg after drying). The filtrate was concentrated, diluted with water, and extracted with chloroform to furnish another 12 mg of the lactone **41** (34 mg total, 0.103 mmole, 57%). A sample of the dried initial precipitate was submitted for analysis: mp >350° dec; ν_{max} (Nujol) 1790, 1760, 1685, 1630, 1605, 1560 cm^{-1}. This infrared spectrum (Nujol) was identical with that of synthetic **41**: $[\alpha]^{25}D$ −544° (*c* 0.01452, 10% CH_3OH–$CHCl_3$), $[\alpha]_{546}$ = −688°, $[\alpha]_{578}$ −577.

Anal. Calcd for $C_{17}H_{12}O_7$: C, 62.20; H, 3.68. Found: C, 61.96; H, 3.88.

Racemic Aflatoxin B Hemiacetal 42 (from Reduction of 41). Pentacyclic ketone **41** (126 mg, 0.384 mmole) was partially dissolved in 100 cc of freshly distilled diglyme at 60° with stirring and was treated, under N_2, with 5 equiv of disiamylborane in tetrahydrofuran (6.4 cc). The reaction was monitored by tlc, and after 84 hr water was added. The reaction mixture was neutralized and evaporated to dryness. The residue was extracted with chloroform–acetone (4:1), and the soluble portion was separated by prepara-

tive tlc, using the same solvent. Removal and extraction (again with chloroform–acetone) of the bands yielded 20.6 mg (0.063 mmole, 16.2%) of hemiacetal **42**, mp 182°, and 31.4 mg (0.095 mmole, 25%) of recovered lactone **41**.

2-(2,3,3a,8a-Tetrahydro-2-acetoxy-4-hydroxy-6-methoxyfuro[2,3-*b*]-benzofuran-5-yl)-5-oxo-1-cyclopentene-1-carboxylic Acid δ-Lactone (43) (Racemic Aflatoxin B Hemiacetal Acetate). To synthetic hemiacetal **42** (21 mg, 0.064 mmole) dissolved in acetic acid (2 ml) and acetic anhydride (1.5 ml) were added several small crystals of *p*-toluenesulfonic acid. After 12 hr at ambient temperatures, the excess reagents were removed *in vacuo* and the product was isolated by preparative tlc. The racemic hemiacetal acetate **43** obtained (17 mg, 0.045 mmole, 70%) had mp 245–246° (from chloroform–ether. Its infrared spectrum was identical with that of **43** obtained from natural aflatoxin B_1 (**1**): ν_{max} ($CHCl_3$) 1760, 1750, 1685 (weak), 1625, 1600, 1555, 1485, 1440, 1380, 1310 cm^{-1}.

Optically Active Aflatoxin B Hemiacetal Acetate (43). A solution of aflatoxin B_1 (**1**) (115 mg, 0.37 mmole) in 10 ml of glacial acetic acid and 1 ml of acetic anhydride was stirred at 25° for 168 hr in the presence of 4 mg of toluenesulfonic acid. Sodium bicarbonate (70 mg) was added and the reaction mixture was concentrated. The resulting solid was washed with water, dried, and recrystallized from chloroform–cyclohexane and then ethyl acetate to afford 98 mg (0.26 mmole, 70%) of aflatoxin B hemiacetal acetate **43**: mp 233–235° (lit.[23] mp 227°); nmr ($CDCl_3$), 1.73 (3 H, s), 2.60 (2 H, t, J = 6) and 3.44 (2 H, t, J = 6, A_2B_2), 2.48 (2 H, d, J = 5), 4.00 (3 H, s), 4.22 (1 H, m), 6.41 (1 H, s), 6.47 (1 H, m), 6.56 (1 H, d, J = 6). The ultraviolet spectrum was identical with that of aflatoxin B_1 and the infrared spectrum in $CHCl_3$ was indistinguishable from that of the racemic acetate **43**.

Racemic Aflatoxin B_1 (1). Racemic aflatoxin B hemiacetal acetate **43** (16 mg, 0.043 mmole) was pyrolyzed at 240° for 15 min under reduced pressure (0.01 mm). The brown residue was applied to preparative tlc plate and the band corresponding to **1** was removed. Extraction of the silica gel with chloroform–methanol yielded 5.3 mg (0.017 mmole, 40%) of racemic aflatoxin B_1 (**1**), mp 255–256°, having infrared, ultraviolet, and mass spectra identical with those of the natural material.

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Synthesis of Secretin. II. The Stepwise Approach[1,2]

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Abstract: A heptacosapeptide amide with the amino acid sequence proposed by Jorpes and Mutt for porcine secretin was synthesized. The stepwise strategy was applied, active esters were used in the acylation reactions, and all the protected intermediates were isolated. After removal of the protecting groups and purification, the synthetic peptide showed the characteristic biological activities of natural (porcine) secretin.

Jorpes, Mutt, and their collaborators isolated porcine secretin in pure form[4] and proposed [5–7] sequence I for its amino acid constituents.

His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-
1 2 3 4 5 6 7 8 9 10 11 12 13

Arg-Asp-Ser-Ala-Arg-Leu-Glu(NH_2)-
14 15 16 17 18 19 20

Arg-Leu-Leu-Glu(NH_2)-Gly-Leu-Val-NH_2
21 22 23 24 25 26 27

I[7]

The synthesis of a protected tetradecapeptide corresponding to sequence 14–27 has already been described.[1] The present paper reports the continuation of the stepwise synthesis to the completion of the entire chain of I.

Details of the synthesis are summarized in Chart I and here only its more important features are outlined. Nitrophenyl esters[8] were used in all the chain-lengthening steps with the exception of the last acylation, in which both the *p*-nitrophenyl ester of bisbenzyloxycarbonyl-L-histidine and the azide of benzyloxycarbonyl-L-histidine could be applied equally well. For the protection of this single histidine residue the benzyloxycarbonyl protecting group was used; for the masking of all other α-amino functions, the *t*-butoxycarbonyl group[9] was selected, since it could be removed under mild conditions with trifluoroacetic acid after each chain-lengthening step. In this manner the nitro groups on the arginine moieties, the benzyl ester groups on the side-chain carboxyls of aspartic acid and glutamic acid residues, and the benzyl-ether linkages on the serines were not affected and undesired acylation of the alcoholic hydroxyls in the threonine and serine side chains were avoided. The protected intermediates were isolated as solids, several of them in crystalline form, all in excellent yield.

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(2) The present synthesis was reported in a preliminary form: M. Bodanszky, M. A. Ondetti, S. D. Levine, V. L. Narayanan, M. V. Saltza, J. T. Sheehan, N. J. Williams, and E. F. Sabo, *Chem. Ind.* (London), 1757 (1966).

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