Total Syntheses of Aflatoxins M_1 and G_1 and an Improved Synthesis of Aflatoxin B_1

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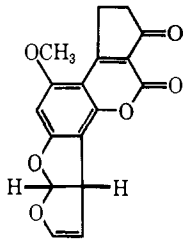
Abstract: Starting with 4,6-dihydroxybenzofuran-3[2*H*]-one (**4**) the tricyclic phenol **29** was prepared in 11 steps. Condensation of **29** with the vinyl bromide **33** in methylene chloride solution in the presence of zinc carbonate produced racemic milk toxin (**3**). This new coumarin synthesis seems to be generally applicable and particularly useful whenever acid-sensitive phenols forbid the practice of the classical Pechmann synthesis. An improved synthesis of aflatoxin B_1 (**1**) resulted when the new method was applied to the phenol **42**. Finally aflatoxin G_1 (**2**) was prepared analogously from the phenol **42** and the bromo lactone **47**.

The highly toxic and exceedingly carcinogenic aflatoxins are mold metabolites produced by a number of *Aspergillus* and *Penicillium* species.[2] Systematic investigations have shown the major toxins to be aflatoxin B_1 (**1**) and aflatoxin G_1 (**2**). Their fate in animals



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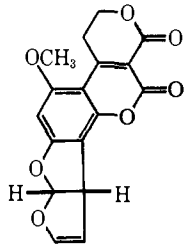
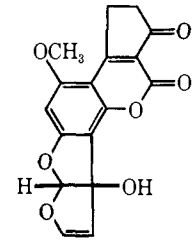


2

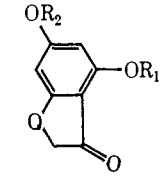
is of interest for several reasons. A matter of practical importance concerns the presence of toxic derivatives in edible animal products when the animals are fed rations containing aflatoxins. Of more academic interest is the role metabolites play in producing the biological effects of the compounds.

Lactating cattle fed sublethal levels of aflatoxins excrete in their milk a toxin which has been named aflatoxin M_1 (milk toxin).[3,4] The same metabolite was subsequently isolated from the urine of sheep[5,6] and the livers of rats dosed with aflatoxin B_1.[7] In addition, small quantities of aflatoxin M_1 have been found in cultures of *Aspergillus flavus*.[6] Structural studies have shown that aflatoxin M_1 is the hydroxyaflatoxin B_1 represented by structure **3**.[6,8] The acute toxicity of M_1 seems established[9] but lack of material has left unanswered the more important question of its carcinogenicity. We have developed a total synthesis of aflatoxin M_1 which makes this metabolite available for further biological studies.[10]



3

The initial phase of the synthesis was concerned with transformation of the dihydroxybenzofuranone (**4**)[11] to the benzyl methyl ether **5**. Methylation of the phenol **4** with 1 equiv of diazomethane is known to give the undesired monomethyl ether **6**,[11] and our efforts to selectively attack one of the two hydroxy groups in **4** with other agents (*e.g.*, acetic anhydride, benzyl bromide) met with failure. A study aimed at removing one of two identical protecting groups was commenced with the readily available dibenzyl ether **7**. With the hope of removing the sterically more accessible benzyl group the dibenzyl ether **7** was hydrogenated over a palladium catalyst until 1 equiv of hydrogen had been absorbed. Contrary to expectation the major product was the undesired phenol **8** whose structure was established by methylation to **9** followed by hydrogenolytic debenzylation to the known phenol **6**.[11]

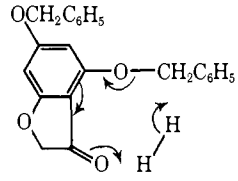


4, $R_1 = R_2 = H$
5, $R_1 = CH_2C_6H_5$; $R_2 = CH_3$
6, $R_1 = CH_3$; $R_2 = H$
7, $R_1 = R_2 = CH_2C_6H_5$
8, $R_1 = H$; $R_2 = CH_2C_6H_5$
9, $R_1 = CH_3$; $R_2 = CH_2C_6H_5$
10, $R_1 = R_2 = CH_3$
11, $R_1 = H$; $R_2 = CH_3$

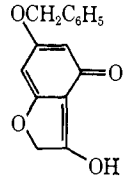
The preferential debenzylation observed can be rationalized in at least two different ways. If the substrate **7**

(1) National Institutes of Health Postdoctoral Fellow, 1967–1968.
(2) For a review see: L. A. Goldblatt, Ed., "Aflatoxin," Academic Press, New York, N. Y., 1969.
(3) R. Allcroft and R. B. A. Carnaghan, *Vet. Rec.*, **75**, 259 (1963).
(4) H. DeIongh, R. O. Vles, and J. G. van Pelt, *Nature (London)*, **202**, 466 (1964).
(5) R. Allcroft, J. Nabney, and P. E. Best, *ibid.*, **209**, 154 (1966).
(6) C. W. Holzapfel, P. S. Steyn, and I. F. H. Purchase, *Tetrahedron Lett.*, 2799 (1967); J. G. Heathcote and M. F. Dutton, *Tetrahedron*, **25**, 1497 (1969).
(7) W. H. Butler and J. I. Clifford, *Nature (London)*, **206**, 1045 (1965).
(8) M. S. Masri, R. E. Lundin, J. R. Page, and V. C. Garcia, *ibid.*, **215**, 753 (1967).
(9) I. F. H. Purchase, *Food Cosmet. Toxicol.*, **5**, 339 (1967).
(10) Announced previously in a communication to the editor: G. Büchi and S. M. Weinreb, *J. Amer. Chem. Soc.*, **91**, 5408 (1969).
(11) T. A. Geissman and E. Hinreiner, *ibid.*, **73**, 782 (1951).

is adsorbed on the catalyst surface through the carbonyl oxygen atom, the cleavage of one benzyl group becomes facilitated by a proximity effect. Perhaps equally reasonable is a scheme involving 1,6 addition of hydrogen (arrows in A) followed by tautomerization of the intermediate enol B.



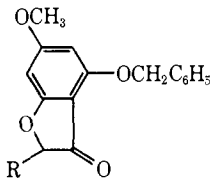
A



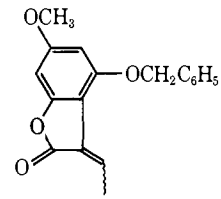
B

We next turned our attention to the dimethyl ether **10** which has been prepared from the bisphenol **4** and dimethyl sulfate in aqueous sodium hydroxide.[12] In our hands the yield was low due to formation of a large proportion of a C-methylated product. Use of dimethyl sulfate and potassium carbonate in acetone again gave the C-methylated product in addition to the desired ether and substances resulting from the self-condensation of acetone. The base-stable dimethoxyethane (glyme) is reported[13] to promote O-alkylation of enolates and indeed the dimethyl ether **10** could be prepared in 78% yield by treating a refluxing solution of the phenol **4** in glyme with dimethyl sulfate in the presence of suspended potassium carbonate. No C-alkylated product was detected and we have generally found glyme to be far superior to acetone for the O-alkylation of phenols.

Partial cleavage of the dimethyl ether **10** with 2 equiv of anhydrous aluminum chloride afforded the phenol **11** identical with material prepared by a much less efficient but structurally unambiguous route.[14] Phenol **11** was now alkylated with benzyl bromide and the resulting ether **5** converted to the bromo ketone **12** by treatment with phenyltrimethylammonium perbromide.[15] Exposure of the bromide **12** to benzyl alcohol in the presence of calcium carbonate produced the colorless benzyloxy ketone **13** (65%) and the bright yellow crystalline benzylidene lactone **14** in 1% yield.

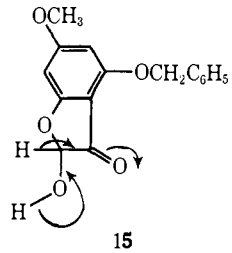


12, R = Br
13, R = $OCH_2C_6H_5$



14

To rationalize the formation of the latter substance it is assumed that the hydroxy ketone **15**, formed by combination of the bromide **12** and water (from the reaction of calcium carbonate with hydrogen bromide), undergoes an intramolecular hydride transfer to the hydroxy lactone **16** which is followed by alkylation with benzyl bromide (from the reaction of excess benzyl alcohol with hydrogen bromide) and terminated by dehydration of the resulting carbinol **17**.



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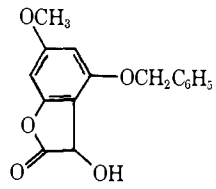

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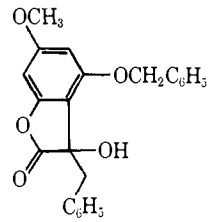

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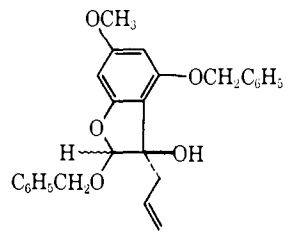
Condensation of the benzyloxy ketone **13** with excess allylmagnesium bromide afforded a mixture of epimeric allyl alcohols **18** which on oxidation with osmium tetroxide–sodium periodate in aqueous dioxane[16] buffered with sodium bicarbonate gave a 7:1 mixture of chromatographically separable hydroxy aldehydes **19** and **21** in 72% yield based on benzyloxy ketone **13**. The major epimer tentatively was assigned structure **19** on the assumption that the Grignard reagent attacks from the side opposite to that of the space-demanding benzyloxy group.[17]



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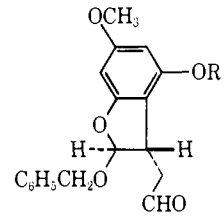
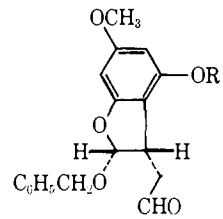


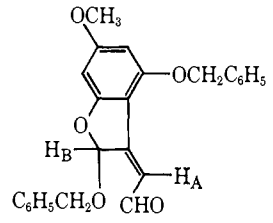
19, R = $CH_2C_6H_5$
20, R = $COCH_3$



21, R = $CH_2C_6H_5$
22, R = $COCH_3$

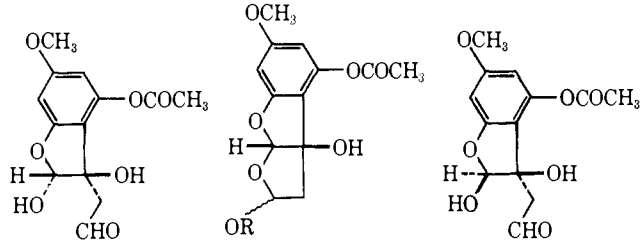
In the presence of only trace amounts of acids both β-hydroxy aldehydes were transformed to the unsaturated aldehyde **23**. The coupling constant between H_A and H_B in this compound was found to be 2 Hz, indicating the transoid arrangement of protons already shown in structure **23**.[18]

(12) T. P. C. Mulholland and G. Ward, *J. Chem. Soc.*, 1642 (1953).
(13) For a discussion of the factors controlling alkylation of ambident anions see W. J. LeNoble, *Synthesis*, 1, 1 (1970).
(14) L. A. Duncanson, J. F. Grove, J. MacMillan, and T. P. C. Mulholland, *J. Chem. Soc.*, 3555 (1957).
(15) A. Marquet and J. Jacques, *Tetrahedron Lett.*, **9**, 24 (1959); prepared as described by W. S. Johnson, J. D. Bass, and K. L. Williamson, *Tetrahedron*, **19**, 861 (1963).
(16) R. Pappo, D. S. Allen, Jr., R. U. Lemieux, and W. S. Johnson, *J. Org. Chem.*, **21**, 478 (1956).
(17) See D. J. Cram and D. R. Wilson (*J. Amer. Chem. Soc.*, **85**, 1245 (1963)) for a discussion of the stereochemistry of addition of organometallics to α-alkoxy ketones.
(18) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed, Pergamon Press, Oxford, 1969, pp 316–328.



23

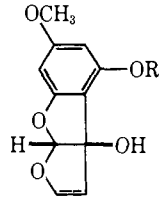
We planned to next remove both benzyl protecting groups in the hydroxy aldehydes **19** and **21** in a single hydrogenolysis, but, in fact, the products thus formed were exceedingly unstable and decomposed before they could be transformed to more stable intermediates. This difficulty was overcome when it was found that hydrogenation of either epimer **19** or **21** over a palladium/carbon catalyst in benzene solution in the presence of acetic anhydride and sodium acetate gave the much more stable monoacetates **20** and **22**, respectively. Further hydrogenation of acetate **22** over the same catalyst, but in ethyl acetate solution, followed by acetylation of the crude product with acetic anhydride in pyridine at −30°, afforded the crystalline tricylic diacetate **26**. An intermediate in this conversion is undoubtedly the bicyclic hemiacetal **24** which cyclizes to the tricyclic hemiacetal **25**.



24 **25**, R = H; **26**, R = $COCH_3$ **27**

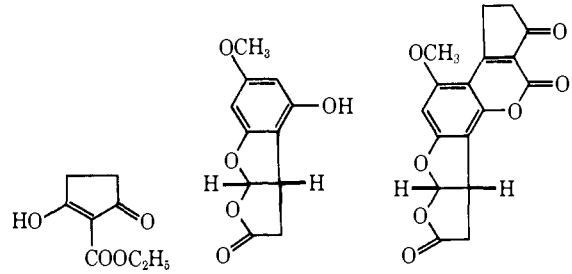
The tricyclic diacetate **26** was also available in essentially the same yield from the epimeric monoacetate **20** by the same sequence of reactions, and in preparative runs it was found to be advantageous to use the mixture of epimeric hydroxy aldehydes **19** and **21** for further transformations. This situation can be explained if the bicyclic hemiacetal **27**, initially formed in the hydrogenolysis of the benzyl ether **20**, isomerizes to the tricylic hemiacetal **25** with the more stable cis- rather than trans-fused hydrofuran rings *via* the corresponding monocyclic dialdehyde and the epimeric bicyclic hemiacetal **24**.

The acetate **26** was transformed further to the vinyl ether **28** by short contact time pyrolysis and thence to the phenol **29** by saponification with aqueous sodium bicarbonate.



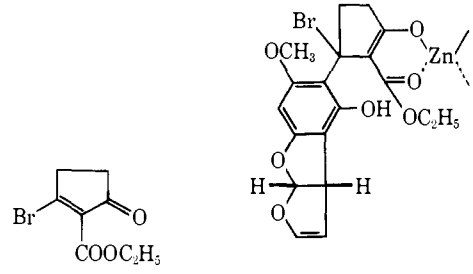
28, R = $COCH_3$
29, R = H

It was our intention to combine the tricyclic phenol **29** and 2-carbethoxycyclopentane-1,3-dione (**30**)[19] in a von Pechmann coumarin synthesis and we were much encouraged when the phenol **31**[20] condensed with the dione **30** in the presence of phosphorus pentoxide in benzene solution to give the pentacyclic lactone **32**.



30 **31** **32**

Phenol **29**, however, was found to be exceptionally sensitive to acidic reagents and polymerized rapidly under the conditions used for the synthesis of the model compound **32**. After all efforts to effect the crucial condensation in the presence or absence of acidic catalysts failed, it was decided to replace the β-tricarbonyl compound **30** with a more electrophilic analog. Our hope that the bromide **33**, readily available from the enol **30** and oxalyl bromide, would be highly reactive and condense with the phenol **29** under mild conditions proved correct. Both zinc carbonate and magnesium carbonate in methylene chloride solution catalyzed the coumarin synthesis to produce racemic aflatoxin M_1 identical, except for optical rotation, with natural material. Sodium and potassium carbonate, on the other hand, proved ineffective, suggesting that creation of a chelate (*e.g.*, **34**) facilitates the formation of the new carbon–carbon bond. Elimination of zinc bromide followed by cyclization of the hydroxy ester would complete the overall process.



33 **34**

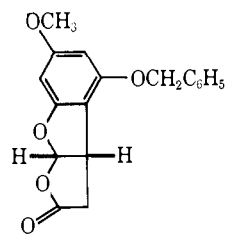
Since the low-yield steps in the previously reported aflatoxin B_1 synthesis[20] were associated with the construction of the coumarin ring, we decided to try our new procedure in what turned out to be an improved synthesis of this toxin.

Reduction of the lactone **35**[20] with diisobutylaluminum hydride[21] afforded the hemiacetal **36**, which on acetylation with acetic anhydride in the presence of sodium acetate was transformed to a 4:1 mixture of epimeric acetates. The acetyl methyl signal in the nuclear magnetic resonance spectrum of the major epimer occurs at δ 2.02 while it is shifted upfield to δ 1.66 in the minor epimer by long-range shielding from the benzene ring. Hydrogenolysis of a mixture of acetates **37** and **38** yielded a mixture of phenols **39**, which without further

(19) G. Büchi and E. C. Roberts, *J. Org. Chem.*, 33, 460 (1968).

(20) G. Büchi, D. M. Foulkes, M. Kurono, G. F. Mitchell, and R. S. Schneider, *J. Amer. Chem. Soc.*, 89, 6745 (1967).

(21) J. Schmidlin and A. Wettstein, *Helv. Chim. Acta*, 46, 2799 (1963).



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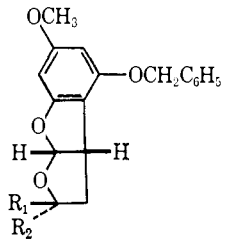
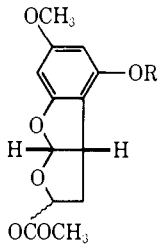
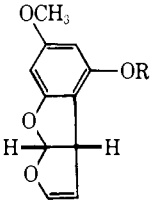


36, $R_1 = OH$; $R_2 = H$
37, $R_1 = CH_3COO$; $R_2 = H$
38, $R_1 = H$; $R_2 = CH_3COO$



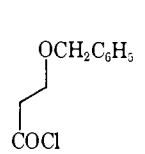
39, R = H
40, R = $COCH_3$



41, R = $COCH_3$
42, R = H

purification was acetylated to the diacetates **40**. These were pyrolyzed and the resulting crystalline vinyl ether (68% overall yield from **37**, **38**) was hydrolyzed with aqueous potassium carbonate to the key intermediate **42** in essentially quantitative yield. Condensation with the bromide **33** in methylene chloride solution, using freshly precipitated zinc carbonate with sodium bicarbonate added as hydrobromic acid scavenger, gave racemic aflatoxin B_1 in 36% yield.

To test the new coumarin synthesis further we turned our attention to aflatoxin G_1 (**2**), the only member of this group of natural products which had not been reached by synthesis. 3-Benzyloxypropionyl chloride (**43**),[22] when condensed with diethyl ethoxymagnesiomalonate,[23] gave the acylmalonate **44** which according to nmr analysis is approximately 50% enolic in carbon tetrachloride solution. The alcohol **45**, prepared by hydrogenolysis of the corresponding benzyl ether **44**, when heated in toluene afforded the lactone **46**. This cyclic acylmalonate is completely enolic as judged by the presence of a one-proton singlet at δ 14.30 in its nmr spectrum. Its infrared spectrum with absorptions at 1736, 1726, 1640, and 1600 cm^{-1} and ultraviolet spectra with maxima at 248 mμ (ϵ 11,300) in ethanol and 258 mμ (ϵ 18,100) in sodium hydroxide are also in full accord with formula **46**. The enol **46** when subjected to the action of oxalyl bromide was smoothly transformed to the



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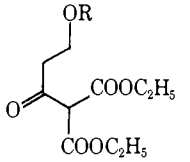
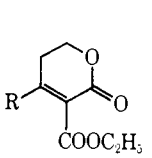


44, R = $CH_2C_6H_5$
45, R = H



46, R = OH
47, R = Br
48, R = I

bromide **47**. The latter turned out to be much less reactive than the bromide **30** and gave only low yields of aflatoxin G_1 (**2**) on condensation with the tricyclic phenol **42** in the presence of zinc carbonate. Addition of finely powdered anhydrous lithium iodide, however, improved the yield significantly, presumably due to formation of the more reactive vinyl iodide **48**. Identity of racemic aflatoxin G_1 (**2**) with natural material was established by comparison of infrared, ultraviolet, and mass spectra as well as by thin-layer chromatographic behavior.

(22) J. H. Sperna Weiland, *Recl. Trav. Chim. Pays-Bas*, 83, 81 (1964).
(23) Prepared according to J. A. Price and D. S. Tarbell, "Organic Syntheses," Collect. Vol. IV, Wiley, New York, N. Y., 1963, p 285.

Experimental Section

General. Microanalyses were performed by the M.I.T. Microchemical Lab and by Midwest Microlab, Inc., Indianapolis, Ind. Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Ultraviolet (uv) spectra were recorded on a Cary 14 instrument. Infrared (ir) spectra were recorded on a Perkin-Elmer Model 237 grating spectrophotometer. Nuclear magnetic resonance (nmr) spectra were measured on Varian Associates T-60, A-60, HA-60, and HA-100 instruments, and are given in parts per million (δ) downfield from an internal tetramethylsilane standard. The abbreviations s, d, t, q, and m refer to singlet, doublet, triplet, quartet, and multiplet, respectively. Mass spectra were determined on a Hitachi RMU6D instrument. Merck silica gel PF_{254} used for both thin-layer and column chromatography and Merck silica gel 0.50–0.20 mm used for column chromatography were obtained from Brinkmann Instruments. Florisil (100–200 mesh), obtained from Fisher Scientific, was also used for column chromatography.

4,6-Dibenzyloxybenzofuran-3[2*H*]-one (7). A mixture of 830 mg (5.0 mmol) of 4,6-dihydroxybenzofuran-3[2*H*]-one (**4**), 1.5 g (11 mmol) of anhydrous potassium carbonate, 1.8 g (10.5 mmol) of benzyl bromide in 25 ml of dimethoxyethane, and 5 ml of dimethylformamide was stirred at room temperature for 3 hr and then warmed on the steam bath overnight. The mixture was evaporated *in vacuo*, diluted with water, and extracted with chloroform. The extract was dried ($MgSO_4$), filtered through a short column of Florisil in chloroform, and evaporated to a light yellow oil. This material was chromatographed on 50 g of 0.05–0.20-mm silica gel in methylene chloride, affording 710 mg (41%) of white crystals. An analytical sample was recrystallized from benzene–hexane: mp 105–106°; ir ($CHCl_3$) 1695, 1615, 1600, 1240 cm^{-1}; uv max (C_2H_5OH) 286, 310 mμ (ϵ 22,900, 6360); nmr ($CDCl_3$) δ 7.16 (10 H, s), 5.95 (2 H, AB, J = 2 Hz), 5.04 (2 H, s), 4.86 (2 H, s), 4.40 (2 H, s).

Anal. Calcd for $C_{22}H_{18}O_4$: C, 76.29; H, 5.24. Found: C, 76.32; H, 5.16.

4-Hydroxy-6-benzyloxybenzofuran-3[2*H*]-one (8). A solution of 692 mg (2.0 mmol) of 4,6-dibenzyloxybenzofuran-3[2*H*]-one (**7**) in 20 ml of ethyl acetate was hydrogenated at atmospheric pressure with 100 mg of 10% palladium/carbon. Hydrogen (1 equiv) was absorbed in 0.75 hr and the reaction was stopped. The mixture was filtered through Celite and the solvent was evaporated, affording partially crystalline material which was recrystallized from ether in two crops, 310 mg (62%). An analytical sample was recrystallized from ethyl acetate–hexane: mp 127–128°; ir ($CHCl_3$) 3680, 3610, 3475, 1675, 1640, 1610, 1220 cm^{-1}; uv max (C_2H_5OH) 281, 315 mμ (ϵ 23,300, 4580); uv max (NaOH) 287, 352 mμ (ϵ 21,100, 5240); nmr ($CDCl_3$) δ 7.32 (5 H, s), 6.05 (2 H, AB, J = 2 Hz), 5.05 (2 H, s), 4.55 (2 H, s); the –OH proton was not detected.

Anal. Calcd for $C_{15}H_{12}O_4$: C, 70.31; H, 4.72. Found: C, 70.61; H, 4.57.

4-Methoxy-6-benzyloxybenzofuran-3[2*H*]-one (9). A well-stirred mixture of 275 mg (1.1 mmol) of 4-hydroxy-6-benzyloxybenzofuran-3[2*H*]-one (**8**), 0.5 g of anhydrous potassium carbonate, and 0.3 ml of dimethyl sulfate in 15 ml of dry acetone was heated at reflux for 0.5 hr. After diluting with water, the mixture was extracted with chloroform, dried ($MgSO_4$), and evaporated *in vacuo* to a colorless oil which crystallized on addition of petroleum ether. One recrystallization from benzene–petroleum ether gave 210 mg (73%) of white needles. A sample recrystallized from benzene–hexane for analysis had mp 133–135°; ir ($CHCl_3$) 1692, 1620, 1598 cm^{-1}; uv max (C_2H_5OH) 282, 310 mμ (ϵ 23,900, 5940); nmr ($CDCl_3$) δ 7.38 (5 H, s), 6.12 (2 H, AB, J = 2 Hz), 5.08 (2 H, s), 4.54 (2 H, s), 3.84 (3 H, s).

Anal. Calcd for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22. Found: C, 70.72; H, 5.33.

4-Methoxy-6-hydroxybenzofuran-3[2*H*]-one (6). To a solution of 13 mg (0.048 mmol) of 4-methoxy-6-benzyloxybenzofuran-3-[2*H*]-one (**9**) in 10 ml of ethyl acetate was added 10 mg of 10% pal-

ladium/carbon. The mixture was hydrogenated at atmospheric pressure for 20 min, filtered through Celite, and evaporated to yield 6 mg (66%) of white needles, mp 279–281° (lit.[11] mp 290–292°). This material was identical with an authentic sample prepared by the method of Geissman.[11]

4,6-Dimethoxybenzofuran-3[2*H*]-one (10). A mixture of 83 g (0.5 mol) of 4,6-dihydroxybenzofuran-3[2*H*]-one (**4**) and 150 g (1.08 mol) of anhydrous potassium carbonate was suspended in 1.2 l. of dimethoxyethane and brought to reflux. Dimethyl sulfate (134 g, 1.05 mol) was added over a period of 0.5 hr and heating was continued for an additional 2.5 hr. Most of the solvent was evaporated *in vacuo* and 1 l. of water was added. The resulting solid precipitate was filtered, washed well with water, and pressed dry. This material was dissolved in chloroform and filtered through a column of Florisil (100 g) in chloroform. The total eluent was evaporated *in vacuo* and crystallized from benzene–hexane, yielding 76.2 g (79%) of crystals: mp 138–140° (lit.[12] mp 138–139°); ir ($CHCl_3$) 1690, 1615, 1595, 1240 cm^{-1}; uv max (C_2H_5OH) 222, 282, 310 (sh) mμ (ε 15,800, 21,200, 5570); nmr ($CDCl_3$) δ 6.09 (2 H, AB, J = 2 Hz), 4.60 (2 H, s), 3.92 (3 H, s), 3.88 (3 H, s).

4-Hydroxy-6-methoxybenzofuran-3[2*H*]-one (11). To a stirred suspension of 27 g (0.20 mol) of anhydrous aluminum chloride in 200 ml of methylene chloride was added 19.4 g (0.10 mol) of 4,6-dimethoxybenzofuran-3[2*H*]-one (**10**), and the resulting dark solution was heated at reflux for 1.25 hr. The solvent was evaporated *in vacuo* and 200 ml of 10% hydrochloric acid was added carefully. After bringing to reflux, the mixture was cooled to room temperature and filtered, giving 11.5 g (64%) of brown crystals, mp 125–135°. A sample of this material was purified by passing through a column of silica gel in 20% ether–methylene chloride followed by recrystallization from hexane: mp 140–142° (lit.[14] mp 144°); ir ($CHCl_3$) 3460, 1678, 1640, 1610, 1470 cm^{-1}; nmr ($CDCl_3$) δ 7.80 (1 H, broad s), 6.10 (2 H, AB, J = 2 Hz), 4.61 (2 H, s), 3.81 (3 H, s).

4-Benzyloxy-6-methoxybenzofuran-3[2*H*]-one (5). A mixture of 11.5 g (0.064 mol) of crude 4-hydroxy-6-methoxybenzofuran-3[2*H*]-one (**11**), 14 g (0.10 mol) of anhydrous potassium carbonate, 15 g (0.088 mol) of benzyl bromide, 60 ml of dimethoxyethane, and 25 ml of dimethylformamide was heated at reflux and stirred vigorously for 1.25 hr. The solvent was evaporated and chloroform was added. The organic layer was separated and the aqueous phase was extracted several times with chloroform. The organic extracts were combined, dried ($MgSO_4$), and filtered through a column of approximately 75 g of Florisil in chloroform. Evaporation of the eluate *in vacuo* gave a solid which was recrystallized from benzene–hexane to afford 12.8 g (74%) of needles, mp 158–166°. An analytical sample was recrystallized from benzene: mp 167–168°; ir ($CHCl_3$) 1695, 1615, 1245 cm^{-1}; uv max (CH_3CN) 226, 278, 308 mμ (ε 21,400, 19,100 5900); nmr ($CDCl_3$) δ 7.4 (5 H, m), 6.10 (2 H, AB, J = 2 Hz), 5.23 (2 H, s), 4.60 (2 H, s), 3.80 (3 H, s).

Anal. Calcd for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22. Found: C, 71.45; H, 5.36.

2-Bromo-4-benzyloxy-6-methoxybenzofuran-3[2*H*]-one (12). A solution of 13.5 g (0.050 mol) of 4-benzyloxy-6-methoxybenzofuran-3[2*H*]-one (**5**) in 300 ml of warm, dry tetrahydrofuran was treated in portions over 15 min with 19.5 g (0.052 mol) of phenyltrimethylammonium perbromide.[15] The mixture was evaporated *in vacuo* and chloroform and water were added. The organic layer was separated, dried ($MgSO_4$), and evaporated *in vacuo*. A single crystallization from benzene–hexane afforded 15.4 g (88%) of pale yellow crystals. An analytical sample was recrystallized from benzene–hexane, mp 170–172°; ir ($CHCl_3$) 1715, 1625, 1592, 1225 cm^{-1}; uv max (CH_3CN) 235, 298 mμ (ε 10,800, 16,300); nmr ($CDCl_3$) δ 7.35 (5 H, m), 6.40 (1 H, s), 6.10 (2 H, AB, J = 1 Hz), 5.20 (2 H, s), 3.80 (3 H, s).

Anal. Calcd for $C_{16}H_{13}O_4Br$: C, 55.04; H, 3.75. Found: C, 54.83; H, 3.70.

2,4-Dibenzyloxy-6-methoxybenzofuran-3[2*H*]-one (13). A mixture of 13.0 g (0.037 mol) of 2-bromo-4-benzyloxy-6-methoxybenzofuran-3[2*H*]-one (**12**), 40 ml of benzyl alcohol, and 15 g of calcium carbonate was heated on the steam bath for 1.5 hr. The yellow mixture was taken up in chloroform, filtered, and evaporated *in vacuo*. The residue was again taken up in chloroform, filtered, and passed through a column of 40 g of Florisil in chloroform. The total eluate was evaporated *in vacuo* and recrystallized three times from ether, affording 9.0 g (65%) of white crystals. An analytical sample was recrystallized from carbon tetrachloride–ether: mp 45–47°; ir ($CHCl_3$) 1710, 1620, 1595, 1225 cm^{-1}; uv max (CH_3CN) 225, 288, 315 (sh) mμ (ε 17,400, 19,000, 5100); nmr ($CDCl_3$) δ 7.35 (10 H, m), 6.05 (2 H, AB, J = 1.5 Hz), 5.35 (1 H, s), 5.18 (2 H, s), 4.80 (2 H, poorly resolved AB, J = 11 Hz), 3.75 (3 H, s).

Anal. Calcd for $C_{23}H_{20}O_5$: C, 73.39; H, 5.36. Found: C, 73.58; H, 5.44.

3-Benzylidene-4-benzyloxy-6-methoxybenzofuran-2[3*H*]-one (14). The combined recrystallization mother liquors from conversion of 73.0 g of the bromo ketone **12** to the benzyloxy ketone **13** were evaporated to an oil (35 g) and chromatographed on 700 g of silica gel PF_{254}–silica gel 0.05–0.20 mm (2:1) in chloroform. The early fractions contained a yellow crystalline product which was recrystallized from ethyl acetate–petroleum ether: mp 138–140°; 1 g; ir ($CHCl_3$) 1775, 1620, 1585 cm^{-1}; uv max (C_2H_5OH) 252, 363 mμ (ε 11,800, 17,100); nmr ($CDCl_3$) δ 7.72 (1 H, s), 6.6–7.3 (10 H, m), 6.25 (2 H, AB, J = 2 Hz), 4.82 (2 H, s), 3.75 (3 H, s).

Anal. Calcd for $C_{23}H_{18}O_4$: C, 77.08; H, 5.06. Found: C, 77.03; H, 5.13.

***trans*- and *cis*-2,4-Dibenzyloxy-3-hydroxy-6-methoxy-2,3-dihydrobenzofuran-3-acetaldehyde (19 and 21).** A solution of 0.750 g (0.002 mol) of 2,4-dibenzyloxy-6-methoxybenzofuran-3[2*H*]-one (**13**) in 10 ml of dry tetrahydrofuran was cooled in ice and treated with excess allylmagnesium bromide in ether.[24] The resulting solution was stirred for 10 min, poured onto saturated sodium bicarbonate solution, and extracted with chloroform. The organic extract was dried ($MgSO_4$) and evaporated *in vacuo* to a colorless oil. This oil was dissolved in 15 ml of dioxane and 7 ml of water, and 50 mg of osmium tetroxide was added, followed by 1 ml of saturated sodium bicarbonate solution. Sodium metaperiodate (1 g) was added in portions over 0.5 hr. Stirring was continued for 0.5 hr, and the mixture was diluted with saturated sodium bicarbonate solution and extracted with chloroform. The extract was washed with sodium sulfite solution and then with sodium bicarbonate solution, dried ($MgSO_4$), and evaporated *in vacuo*. The residual oil was chromatographed on 50 g of silica gel PF_{254} in 2% ether–methylene chloride. The first component from the column consisted of 530 mg (63%) of a colorless oil. A middle fraction was submitted for analysis: ir ($CHCl_3$) 3540, 1710, 1625, 1605, 1148 cm^{-1}; uv max (C_2H_5OH) 268, 357 mμ (ε 975, 357); nmr ($CDCl_3$) δ 9.76 (1 H, t, X of ABX, J = 3 Hz), 7.35 (10 H, s), 6.06 (2 H, AB, J = 2 Hz), 5.40 (1 H, s), 5.15 (2 H, AB, J = 18 Hz), 4.88 (2 H, AB, J = 11 Hz), 3.70 (3 H, s), 3.49 (1 H, s, –OH), 2.95 (2 H, AB of ABX, J = 3, 15 Hz).

Anal. Calcd for $C_{25}H_{24}O_6$: C, 71.42; H, 5.75. Found: C, 71.31; H, 5.78.

The second component was recrystallized from benzene–petroleum ether: 75 mg (9%) of white needles; mp 93–94°; ir ($CHCl_3$) 3520, 1710, 1622, 1600, 1205 (s), 1150 (s) cm^{-1}; uv max (C_2H_5OH) 264 mμ (ε 1050); nmr ($CDCl_3$) δ 9.93 (1 H, s), 7.41 (5 H, s), 7.34 (5 H, s), 6.08 (2 H, s), 5.56 (1 H, s), 4.99 (2 H, s), 4.70 (2 H, AB, J = 12 Hz), 3.61 (3 H, s), 3.20 (2 H, AB, J = 19 Hz), 3.10 (1 H, s, –OH).

Anal. Calcd for $C_{25}H_{24}O_6$: C, 71.42; H, 5.75. Found: C, 71.63; H, 5.80.

Unless extreme care was taken throughout this procedure to exclude traces of acid from all glassware, a third component, moving faster than the other two, was found in varying quantities. It proved to be the unsaturated aldehyde **23**: mp 127–129° after recrystallization from benzene–hexane; ir ($CHCl_3$) 2740, 1660 1610 (s), 1350 1160 (s) cm^{-1}; uv max (C_2H_5OH) 253, 358 mμ (ε 9800, 26,500); nmr ($CDCl_3$) δ 9.78 (1 H, d, J = 8 Hz), 7.32 (10 H, s), 6.66 (1 H, d of d, J = 2, 8 Hz), 6.53 (1 H, d, J = 2 Hz), 6.10 (2 H, AB, J = 2 Hz), 5.12 (2 H, s), 3.85 (2 H, AB, J = 11 Hz), 3.76 (3 H, s).

Anal. Calcd for $C_{25}H_{22}O_5$: C, 74.61; H, 5.51. Found: C, 74.70; H, 5.56.

***trans*- and *cis*-2-Benzyloxy-3-hydroxy-4-acetoxy-6-methoxy-2,3-dihydrobenzofuran-3-acetaldehyde (20 and 22).** A solution of 700 mg (1.7 mmol) of major aldehyde **19** in 10 ml of acetic anhydride and 7 ml of benzene was treated with 1.5 g of sodium acetate and 150 mg of 10% palladium/carbon and the mixture was hydrogenated at atmospheric pressure. After 1.5 hr, 250 ml (11 mmol) of hydrogen was absorbed. The mixture was filtered through Celite, washed with water, dried ($MgSO_4$), and evaporated *in vacuo* to 500 mg of colorless oil. This material appeared homogeneous by tlc but could not be crystallized, chromatographed, or distilled: ir ($CHCl_3$) 3540, 2720, 1755, 1720, 1625, 1600 cm^{-1}; nmr ($CDCl_3$) δ 9.85 (1 H, X of ABX, J = 2.5 Hz), 7.43 (5 H, s), 6.40 (2 H, AB, J = 2 Hz), 5.52 (1 H, s), 4.97 (2 H, AB J = 12 Hz), 3.87 (3 H, s),

(24) D. A. Shirley, "Preparation of Organic Intermediates," Wiley, New York, N. Y., 1951, p 5.

3.44 (1 H, broad s, -OH), 2.98 (2 H, AB of ABX, J = 2.5, 15 Hz), 2.41 (3 H, s).

A solution of 95 mg (0.26 mmol) of the crystalline minor aldehyde **21** was dissolved in 5 ml of benzene and 5 ml of acetic anhydride and 100 mg of 10% palladium/carbon was added along with 0.5 g of sodium acetate. The mixture was hydrogenated at atmospheric pressure for 2 hr, filtered through Celite, washed with water, dried ($MgSO_4$), and evaporated *in vacuo* to give 70 mg of colorless oil. This material was also homogeneous by tlc but could not be purified further: ir ($CHCl_3$) 3490, 2720, 1755, 1720, 1630, 1595 cm^{-1}; nmr ($CDCl_3$) δ 9.64 (1 H, t, J = 1.5 Hz), 7.08 (5 H, s), 6.32 (2 H, AB, J = 2 Hz), 5.63 (1 H, s), 4.80 (2 H, AB, J = 11 Hz), 3.75 (3 H, s), 3.68 (1 H, s, -OH), 3.04 (2 H, d, J = 1.5 Hz), 2.30 (3 H, s).

2,4-Diacetoxy-3a-hydroxy-6-methoxy-2,3,3a,8a-tetrahydrofuro[2,3-*b*]benzofuran (26). A mixture of *trans*- and *cis*-dibenzyloxyaldehydes **19** and **21** (4 g, 9.5 mmol) (chromatographed on silica gel PF_{254} in 5% ether–methylene chloride) was dissolved in 50 ml of acetic anhydride and 25 ml of benzene. Sodium acetate (6.5 g) was added along with 1 g of 10% palladium/carbon, and the mixture was hydrogenated for about 1.5 hr at atmospheric pressure until hydrogen uptake was complete. After filtering through Celite, the solution was washed with water, dried ($MgSO_4$), and evaporated *in vacuo*.

The residual oil was taken up in 50 ml of ethyl acetate, 0.7 g of 10% palladium/carbon was added, and the mixture was hydrogenated for 1.5 hr at atmospheric pressure until hydrogen uptake had stopped. The mixture was filtered through Celite, added to a solution of 40 ml of dry pyridine and 15 ml of acetic anhydride at −70°, and stored overnight at −30°. The solution was washed twice with 10% hydrochloric acid, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residual oil on 50 g of 0.05–0.20 mm silica gel in 10% ether–methylene chloride afforded 830 mg (27%) of white crystals. An analytical sample was recrystallized from ethyl acetate–petroleum ether: mp 131–132°; ir ($CHCl_3$) 3550, 1765 (s), 1645, 1615, 1240 (s) cm^{-1}; (Nujol) 3490, 1765, 1715, 1635, 1250, 1200 cm^{-1}; uv max (CH_3CN) 225, 278 mμ (ϵ 6950, 2450); nmr ($CDCl_3$) δ 6.32 (2 H, AB, J = 2 Hz), 6.26 (1 H, X of ABX, J = 5 Hz), 6.02 (1 H, s), 3.78 (3 H, s), 3.10 (1 H, s, -OH), 2.65 (2 H, AB of ABX, J = 5, 13 Hz), 2.33 (3 H, s), 2.11 (3 H, s); mass spectrum (80 eV) *m/e* (rel intensity) 324 (28), 282 (11), 265 (9), 236 (10), 222 (57), 193 (87), 179 (43), 167 (100).

Anal. Calcd for $C_{15}H_{16}O_8$: C, 55.56; H, 4.97. Found: C, 55.49; H, 5.07.

3a-Hydroxy-4-acetoxy-6-methoxy-3a,8a-dihydrofuro[2,3-*b*]benzofuran (28). Over a period of 1.25 hr a solution of 100 mg (0.31 mmol) of the tricyclic diacetate **26** in 30 ml of toluene was passed through a column of glass helices (1 × 15 cm, washed with dilute ammonia and then with water) heated to 450°. A slow stream of nitrogen was passed through the system during the reaction. The condensed pyrolysate was evaporated *in vacuo* and the residue was filtered through a pipette of silica gel (1 g) in 20% ether–methylene chloride. The total eluent was evaporated and recrystallized from ether–petroleum ether, affording 60 mg (73%) of crystals. An analytical sample was recrystallized from benzene–hexane: mp 121–123°; ir ($CHCl_3$) 3540, 1755, 1620 cm^{-1}; uv max (CH_3CN) 238, 282 mμ (ϵ 8000, 4000); nmr ($CDCl_3$) δ 6.49 (1 H, A of AX, J = 3 Hz), 6.30 (2 H, AB, J = 2 Hz), 6.23 (1 H, s), 5.39 (1 H, X of AX, J = 3 Hz), 3.72 (3 H, s), 3.07 (1 H, s, -OH), 2.29 (3 H, s); mass spectrum (80 eV) *m/e* (rel intensity) 264 (52), 222 (45), 204 (24), 193 (100), 176 (49).

Anal. Calcd for $C_{13}H_{12}O_6$: C, 59.09; H, 4.58. Found: C, 59.22; H, 4.43.

3a,4-Dihydroxy-6-methoxy-3a,8a-dihydrofuro[2,3-*b*]benzofuran (29). A solution of 30 mg (0.14 mmol) of vinyl ether **28** in 2 ml of methanol and 1 ml of water was treated with 1 ml of saturated sodium bicarbonate solution and stirred at room temperature for 0.75 hr under nitrogen. The solution was acidified with 10% hydrochloric acid and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and evaporated *in vacuo* to an oil. Filtration through a pipet of silica gel (1 g) in 40% ether–methylene chloride gave 24 mg (94%) of an oil which appeared homogeneous on tlc. The material crystallized on cooling but melted below room temperature: ir ($CHCl_3$) 3550, 3300, 1620, 1145 cm^{-1}; nmr ($CDCl_3$) δ 6.53 (1 H, d, J = 3 Hz), 6.30 (1 H, s), 6.04 (2 H, AB, J = 2 Hz), 5.55 (1 H, d, J = 3 Hz), 3.70 (3 H, s).

2-Carbethoxy-3-bromocyclopentenone (33). An ice-cold solution of 55 mg (0.34 mmol) of 2-carbethoxycyclopentane-1,3-dione (**30**)[19] in 10 ml of benzene and 2 ml of methylene chloride was treated with 0.2 ml of redistilled oxalyl bromide. After stirring for 2 hr, the solution was evaporated at 0° *in vacuo*. The residue was filtered through a pipet of Florisil (1 g) in 10% ether–methylene chloride, affording 48 mg (65%) of yellow crystals. An analytical sample was recrystallized from ether–hexane, giving colorless needles, mp 49–50°. The compound decomposed slowly at room temperature: ir ($CHCl_3$) 1745, 1725, 1615 cm^{-1}; uv max (dioxane) 237 mμ (ϵ 14,200); nmr ($CDCl_3$) δ 4.38 (2 H, q, J = 7 Hz), 2.9 (4 H, A_2B_2), 1.38 (3 H, t, J = 7 Hz).

Anal. Calcd for $C_8H_9O_3Br$: C, 41.22; H, 3.89. Found: C, 40.97; H, 4.05.

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 (32). A solution of 22 mg (0.10 mmol) of the tricyclic phenol **31** and 25 mg (0.15 mmol) of 2-carbethoxycyclopentane-1,3-dione (**30**) in 10 ml of benzene was treated with 0.5 g of phosphorus pentoxide. The mixture was stirred at room temperature for 1.5 hr and cooled at 0° for an additional 1.5 hr, and ethyl acetate and 10% hydrochloric acid were added. The organic layer was separated, washed with sodium bicarbonate solution, dried ($MgSO_4$), and evaporated *in vacuo*, affording 10 mg (30%) of pentacyclic lactone **32** which was identical with an authentic sample[20] in uv, ir, and tlc.

Racemic Aflatoxin M_1 (3). Sodium bicarbonate (1.5 g) and zinc carbonate (1.0 g)[25] were ground together and suspended in 20 ml of methylene chloride. The vinyl bromide **33** (40 mg, 0.17 mmol) and the tricyclic phenol **29** (21 mg, 0.095 mmol) were added and the mixture was stirred at room temperature under nitrogen for 20 hr. The reaction mixture was transferred to a Soxhlet thimble and extracted continuously for 24 hr with 2% methanol–chloroform. The extract was washed with sodium bicarbonate solution and dried (Na_2SO_4).

The solid left in the thimble was dissolved in 10% hydrochloric acid and extracted with chloroform. The extract was washed with sodium bicarbonate solution, dried (Na_2SO_4), and combined with the chloroform solution from the Sohlet extraction. Evaporation of the combined extract gave a solid residue which was chromatographed on 1 g of Florisil in 5% methanol–chloroform, affording 10 mg (32%) of racemic aflatoxin M_1 having ir ($CHCl_3$), mass spectrum, and quantitative uv identical with those of natural aflatoxin M_1, as well as identical thin-layer chromatographic behavior.[26] A sample recrystallized from methanol had mp 274–276°.

2-Hydroxy-4-benzyloxy-6-methoxy-2,3,3a,8a-tetrahydrofuro[2,3-*b*]benzofuran (36). A solution of lactone **35**[20] (624 mg, 2.0 mmol) in 50 ml of dry toluene was cooled to −22° and 2.6 ml (2 mmol) of diisobutylaluminum hydride in toluene was added slowly. The solution was stirred for 1 hr at this temperature, 10% hydrochloric acid was added, and the mixture was stirred at room temperature for 10 min. The organic layer was separated, dried ($MgSO_4$), and evaporated *in vacuo* affording a white solid. This material was chromatographed on 45 g of silica gel 0.05–0.20 mm (10% ether–methylene chloride) and gave 460 mg (74%) of white crystals. An analytical sample recrystallized from ethyl acetate–hexane had mp 143–144°; ir ($CHCl_3$) 3610, 3450, 1715 (very weak), 1625, 1500, 1435 cm^{-1}; nmr ($CDCl_3$) δ 7.35 (5 H, s), 6.31 (1 H, d, J = 6 Hz), 6.08 (2 H, s), 5.6 (1 H, m), 5.05 (2 H, s), 4.0 (1 H, m), 3.70 (3 H, s), 2.5 (2 H, m; 1 H, -OH); uv max (CH_3OH) 268 mμ (ϵ 935); uv max (NaOH) 268 mμ (ϵ 1140).

Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.80; H, 6.00.

2-Acetoxy-4-benzyloxy-6-methoxy-2,3,3a,8a-tetrahydrofuro[2,3-*b*]-benzofurans (37 and 38). To a solution of 150 mg (0.475 mmol) of hemiacetal **36** in 20 ml of benzene was added 1.0 g of anhydrous sodium acetate and 1.5 ml of acetic anhydride. The mixture was stirred overnight at room temperature, heated at reflux for 1 hr, and filtered. The filtrate was washed with water, dried ($MgSO_4$), and evaporated *in vacuo* to give 170 mg (100%) of a colorless oil. Analysis of this material by nmr showed a 4:1 mixture of epimeric acetates.

Recrystallization from ether–hexane afforded the pure major isomer **37**: mp 103–104°; ir ($CHCl_3$) 1745, 1630, 1500, 1440 cm^{-1}; nmr ($CDCl_3$) δ 7.32 (5 H, s), 6.35 (2 H, m), 6.18 (2 H, s), 5.02 (2 H, s), 4.10 (1 H, m), 3.70 (3 H, s), 2.45 (2 H, m), 2.02 (3 H, s); uv max (C_2H_5OH) 268 mμ (ϵ 942).

Anal. Calcd for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66. Found: C, 67.28; H, 5.24.

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(25) Zinc carbonate was precipitated from 0.1 *M* zinc sulfate solution by addition of 0.1 *M* sodium carbonate solution and was dried *in vacuo*.

(26) We are much indebted to Drs. A. C. Keyl and A. C. Waiss, Western Regional Research Service, U. S. Department of Agriculture, Albany, Calif., for a sample of natural aflatoxin M_1.

Preparative tlc (5% ether–methylene chloride) of the recrystallization mother liquors afforded the minor isomer **38** which was recrystallized from hexane: mp 93–94°; ir ($CHCl_3$) 1745, 1625, 1610, 1500, 1435 cm^{-1}; nmr ($CDCl_3$) δ 7.35 (5 H, s), 6.35 (2 H, m), 6.10 (2 H, s), 5.05 (2 H, s), 4.02 (1 H, m), 3.75 (3 H, s), 2.50 (2 H, m), 1.66 (3 H, s); uv max (C_2H_5OH) 268 mμ (ϵ 1000).

Anal. Calcd for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66. Found: C, 67.61; H, 5.90.

4-Acetoxy-6-methoxy-3a,8a-dihydrofuro[2,3-*b*]benzofuran (41). A solution of 170 mg (0.475 mmol) of a mixture of epimeric acetates **37** and **38** in 20 ml of ethyl acetate was treated with 50 mg of 10% palladium/carbon and hydrogenated at atmospheric pressure for 2.5 hr. The mixture was filtered through Celite and evaporated *in vacuo* to afford a colorless oil. This material was immediately dissolved in 20 ml of benzene and 0.8 g of anhydrous sodium acetate and 1.5 ml of acetic anhydride were added. The mixture was stirred at room temperature for 20 hr, filtered, and evaporated *in vacuo*, affording 155 mg of an oil.

A toluene solution (35 ml) of this material was passed through a column of glass helices (1 × 15 cm) (washed with ammonia and then with water) heated at 400° under a slow stream of nitrogen. The condensate was evaporated and the residue was filtered through a pipet of silica gel 0.05–0.20 mm (1 g) in methylene chloride. Evaporation of the solvent gave an oil (80 mg, 68%) which crystallized. An analytical sample was recrystallized from hexane: mp 88–89°; ir ($CHCl_3$) 1755, 1635, 1620, 1605, 1495 cm^{-1}; nmr ($CDCl_3$) δ 6.66 (1 H, d, J = 7 Hz), 6.42 (1 H, t, J = 2.5 Hz), 6.37 (1 H, d, A of AB, J = 2 Hz), 6.21 (1 H, d, B of AB, J = 2 Hz), 5.16 (1 H, t, J = 2.5 Hz), 4.48 (1 H, t of d, J = 2.5, 7 Hz), 3.74 (3 H, s), 2.30 (3 H, s); uv max (C_2H_5OH) 278, 225 (sh) mμ (ϵ 3300, 2900).

Anal. Calcd for $C_{13}H_{12}O_5$: C, 62.90; H, 4.87. Found: C, 62.81; H, 4.90.

4-Hydroxy-6-methoxy-3a,8a-dihydrofuro[2,3-*b*]benzofuran (42). A methanol solution (1.5 ml) containing 30 mg (0.125 mmol) of vinyl ether **41** was treated with 100 mg of potassium carbonate in 1 ml of water. The solution was allowed to stand for 0.5 hr at room temperature under nitrogen, was acidified with 10% hydrochloric acid, and was extracted with ethyl acetate. The extract was washed with water, dried ($MgSO_4$), and evaporated, giving 25 mg (96%) of an oil which crystallized on cooling. A sample which was recrystallized for analysis from ether–petroleum ether had mp 128–130°; ir ($CHCl_3$) 3580, 3325, 1635, 1515, 1450 cm^{-1}; nmr ($CDCl_3$) δ 6.62 (1 H, d, J = 7 Hz), 6.38 (1 H, t, J = 2.5 Hz), 5.88 (2 H, AB, J = 2 Hz), 5.70 (1 H, broad, –OH), 5.32 (1 H, t, J = 2.5 Hz), 4.50 (1 H, t of d, J = 2.5, 7 Hz), 3.68 (3 H, s); uv max (C_2H_5OH) 230, 270 mμ (ϵ 8100, 650); uv max (NaOH) 230, 272 mμ (ϵ 8500, 870).

Anal. Calcd for $C_{11}H_{10}O_4$: C, 64.08; H, 4.89. Found: C, 63.94; H, 5.18.

Racemic Aflatoxin B_1 (1). Zinc carbonate[25] (1.0 g) and sodium bicarbonate (1.5 g) were ground together and suspended in 20 ml of methylene chloride. The vinyl bromide **33** (40 mg, 0.17 mmol) and the tricyclic phenol **42** (18 mg, 0.087 mmol) were added and the mixture was heated at reflux under nitrogen for 3 hr, then stirred at room temperature overnight. The reaction mixture was transferred to a Soxhlet apparatus and was extracted for 3 hr with ethyl acetate. The extract was evaporated *in vacuo* to afford an oil which was slurried with cold ether. The residue was chromatographed on silica gel 0.05–0.20 mm (1 g) in chloroform, to give 10 mg (36%) of racemic aflatoxin B_1 having tlc, ir ($CHCl_3$), uv, and mass spectrum identical with those of the natural material.

Diethyl 3-Benzyloxypropionylmalonate (44). Diethyl malonate (4.8 g, 0.03 mol) in 3.2 ml of absolute ethanol and 0.1 ml of carbon tetrachloride was added to magnesium turnings (0.74 g, 0.03 g-atom).[23] A vigorous reaction ensued and the mixture was cooled briefly with an ice bath. Ether (15 ml) was added and the mixture was heated at reflux for 3 hr until the magnesium had dissolved. The solution was evaporated *in vacuo*, benzene (10 ml) was added, and the solvent was again removed *in vacuo*. The residual oil was dissolved in 30 ml of ether and added dropwise over 0.25 hr to an ice-cold stirred solution of 5.94 g (0.03 mol) of 3-benzyloxypropionyl chloride (**43**)[22] in 20 ml of ether. The reaction mixture was stirred at room temperature for an additional 2 hr and poured onto 125 ml of ice-cold 10% hydrochloric acid. The organic layer was separated, washed with water, dried ($MgSO_4$), and evaporated *in vacuo* to afford 9.4 g (97%) of pale yellow oil. Analysis by nmr and tlc showed this material to be very pure. It decomposed, however, upon distillation or silica gel chromatography: ir (film) 1760, 1735, 1650, 1610, 1250 cm^{-1}; nmr ($CDCl_3$) δ 13.6 ($^1/_2$ H, s), 7.26 (5 H, s), 4.54 ($^1/_2$ H, s), 4.48 (2 H, s), 4.16 (4 H, q, J = 7 Hz), 3.70 (2 H, t, J = 6 Hz), 2.8 (2 H, m), 1.22 (6 H, t, J = 7 Hz); uv max (C_2H_5OH) 255 mμ (ϵ 6000); uv max (NaOH) 270 mμ (ϵ 12,400).

3-Carbethoxy-4-hydroxy-5,6-dihydro-2-pyrone (46). Diethyl 3-benzyloxypropionylmalonate (**44**) (20.5 g, 0.086 mol) was dissolved in 125 ml of ethyl acetate, 1.5 g of 10% palladium/carbon was added, and the mixture was hydrogenated at atmospheric pressure for 2 hr until hydrogen was no longer absorbed. The mixture was filtered through Celite and evaporated *in vacuo*. The residue was dissolved in 100 ml of toluene and refluxed for 2.5 hr. The solvent was removed *in vacuo* and the residue was recrystallized from ether, affording 7.5 g (64%) of white crystals, mp 74–77°. An analytical sample recrystallized from ether had mp 74–76°; ir ($CHCl_3$) 1736, 1726, 1640, 1600 cm^{-1}; nmr ($CDCl_3$) δ 14.30 (1 H, broad), 4.42 (2 H, q, J = 7 Hz), 4.38 (2 H, t, J = 6 Hz), 2.78 (2 H, t, J = 6 Hz), 1.40 (3 H, t, J = 7 Hz); uv max (C_2H_5OH) 248 mμ (ϵ 11,300); uv max (NaOH) 258 mμ (ϵ 18,100).

Anal. Calcd for $C_8H_{10}O_5$: C, 51.61; H, 5.41. Found: C, 51.71; H, 5.24.

3-Carbethoxy-4-bromo-5,6-dihydro-2-pyrone (47). Oxalyl bromide (0.6 ml) was added to a solution of 572 mg (3.1 mmol) of enol **46** in 10 ml of benzene and the solution was allowed to stand at room temperature overnight. Evaporation of the solution *in vacuo* gave a yellow oil which was passed through a column of 25 g of silica gel 0.05–0.20 mm in 6% ether–methylene chloride, affording 730 mg (96%) of a colorless liquid which crystallized on cooling, mp 43–45°. An analytical sample recrystallized from ether–petroleum ether had mp 44–45°; ir ($CHCl_3$) 1740, 1635, 1400, 1310, 1100 cm^{-1}; nmr ($CDCl_3$) δ 4.40 (2 H, q, J = 7 Hz), 4.28 (2 H, t, J = 6 Hz), 3.00 (2 H, q, J = 6 Hz), 1.35 (3 H, t, J = 7 Hz); uv max (CH_3CN) 231, 265 (sh) mμ (ϵ 8150, 2100).

Anal. Calcd for $C_8H_9O_4Br$: C, 38.58; H, 3.64. Found: C, 38.47; H, 3.72.

Racemic Aflatoxin G_1 (2). A finely ground mixture of zinc carbonate[25] (1.5 g) and anhydrous lithium iodide (1.0 g) was added to a solution of the tricyclic phenol **42** (19 mg, 0.092 mmol) and the vinyl bromide **47** (50 mg, 0.20 mmol) in 20 ml of methylene chloride. The reaction mixture was stirred at room temperature for 3 hr, heated at reflux for 7 hr, and stirred at room temperature overnight. Ethyl acetate and 10% hydrochloric acid were added, the organic layer was separated, and the aqueous phase was extracted thoroughly with ethyl acetate. The combined organic extract was washed with sodium bicarbonate solution, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was slurried with cold ether and was chromatographed on 1 g of silica gel 0.05–0.20 mm in chloroform to give 4.2 mg (14%) of racemic aflatoxin G_1 (**2**) having ir ($CHCl_3$), uv, tlc, and mass spectrum identical with that of material of natural origin.

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