The Absolute Configuration of the Aflatoxins

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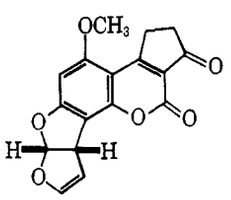
Structural investigations of the mold metabolites aflatoxin B_1 and G_1 culminated in the proposals **1** and **3** (without stereochemical designations).[2] X-Ray crystallographic investigations revealed *cis*-fused hydrofuran rings in aflatoxin G_1 (**3**)[3] and B_2 (**2**),[4] while the present paper reports the absolute configuration of the toxins.



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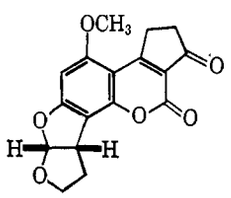

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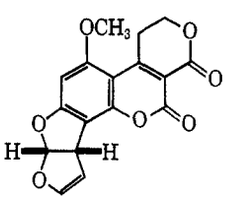

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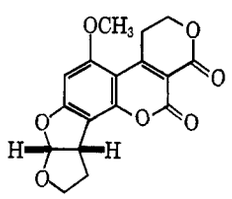

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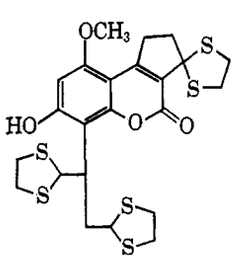
Aflatoxin B_1 (**1**) was transformed to the trithioketal **5** by the action of ethanedithiol in the presence of anhydrous zinc chloride. Desulfurization by Raney Nickel W_2 in ethanol solution yielded the phenol **6** further characterized by the optically active acetate **7**. Exhaustive ozonolysis of the acetate **7** followed by oxidation with hydrogen peroxide gave glutaric acid and 2-methylbutanoic acid (**8**) identified by comparison of gas chromatographic retention times of it and the corresponding methyl ester with those of authentic samples. The 2-methylbutanoic amide (**9**), prepared from the acid (**8**), had $[\alpha]^{25}D$ +14.2°.



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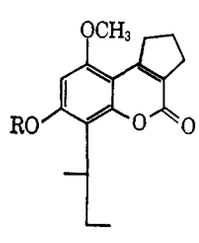
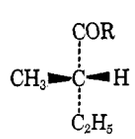


6, R = H
7, R = CH_3CO



8, R = OH
9, R = NH_2

Kuhn–Roth oxidation provided a much more efficient method of degradation. Oxidation of the phenol **6** with 2 *N* chromic acid followed by steam distillation of the volatile acids gave acetic acid (5%), propionic acid (5%), and (+)-(*S*)-2-methylbutanoic acid (**8**) (90%), $[\alpha]^{25}D$ +19.2° (lit.[5] $[\alpha]^{25}D$ +19.3). The corresponding (+)-(*S*)-2-methylbutanoic amide (**9**), $[\alpha]^{25}D$ +22.4° (lit.[5] $[\alpha]^{25}D$ +22.5°), was identical with the derivative prepared from authentic (+)-(*S*)-2-methylbutanoic acid (**8**).[5] The absolute configuration of dextrorotary 2-methylbutanoic acid has previously been established by relating it to L-(+)-isoleucine[6] and to L-(+)-2-methylpentanoic acid[7–9] which in turn were correlated with L-(−)-glyceraldehyde. Consequently the absolute configuration of aflatoxin B_1 is that implied in structure **1**. The absolute configuration of aflatoxin B_2 (**2**) follows because it can be prepared by reduction of B_1 (**1**).[10–13] Catalytic reduction of aflatoxin G_1 gave aflatoxin G_2[11,12] and since the circular dichroism curves[14] of B_1 and G_1 are essentially superimposable the two toxins again have the absolute configurations implied in structures **3** and **4**.

Experimental Section

Elemental analyses were performed by Midwest Microlabs, Inc., Indianapolis, Ind., and Dr. S. M. Nagy and associates at the Massachusetts Institute of Technology. Melting points 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. Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter using a 1-dm tube. Thin layer chromatography was used routinely for monitoring reactions and chromatographic separations.

Thioketal 5.—A mixture of aflatoxin B_1 (375 mg, 1.2 mmoles), anhydrous sodium sulfate (807 mg), freshly melted zinc chloride (711 mg), and freshly distilled ethanedithiol (7 ml) was stirred at 25° for 2.5 hr. The excess ethanedithiol was then removed *in vacuo* and the residue was extracted with chloroform–methanol (9:1). The residue from the chloroform extract was chromatographed on a 6:5 mixture of silica gel G and Hyflo Super-Cel (15 g). Thioketal **5** (390 mg, 60%) was eluted with 1% methanol–chloroform: mp 169–170 dec; $[\alpha]^{26}D$ +278° (*c* 1.02, $CHCl_3$); ultraviolet maxima (95% ethanol) at 257, 267, and 341 mμ (ε 10,900, 12,300, 16,600); and infrared absorption ($CHCl_3$) at 1725, 1604, and 1570 cm^{-1}.

Anal. Calcd for $C_{23}H_{26}O_4S_6$: C, 49.43; H, 4.69. Found: C, 49.50; H, 4.65.

Phenol 6.—A solution of thioketal **5** (219 mg, 0.394 mmole) in 95% ethanol (15 ml) was treated with Raney Nickel W_2 for 100 hr at room temperature. The reaction mixture was filtered and the catalyst was extracted with five 100-ml portions of ethanol. Evaporation of the filtrates and chromatography of the residue on silica gel G–Hyflo Super-Cel (6:5) (14 g) yielded, upon elution with chloroform and recrystallization from methanol–chloroform,

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(14) We are much indebted to Professor Guy Ourisson, Strasbourg, France, for these measurements.

pure phenol **6**: prisms; mp 277–278; ultraviolet maxima (95% ethanol) at 256, 264, and 333 mμ (ε 16,700, 18,200, 26,400); and infrared absorption (Nujol) at 3160, 1668, 1601, 1570, 1500, and 1460 cm^{-1}.

Anal. Calcd for $C_{17}H_{20}O_4$: C, 70.83; H, 6.99. Found: C, 70.71; H, 6.93.

Acetate 7.—A mixture of phenol **6** (60 mg, 0.21 mmole), pyridine (15 ml), and acetic anhydride (25 ml) was stirred for 8 hr at room temperature. Excess reagent was removed *in vacuo* and the residue chromatographed on a 6:5 mixture of silica gel G and Hyflo Super-Cel (0.25% methanol–chloroform). The yellowish crystals (58 mg, 84%) were sublimed at 100° (0.005 mm) to yield the acetate **7**. Recrystallization from chloroform–hexane gave colorless needles: mp 144–145°; $[\alpha]^{25}D$ +3.1° (*c* 2.77, chloroform); ultraviolet maxima (95% ethanol) at 250 and 315 mμ (ε 17,760, 29,000); and infrared absorption ($CHCl_3$) at 1760, 1710, 1630, and 1600 cm^{-1}.

Anal. Calcd for $C_{19}H_{22}O_5$: C, 69.07; H, 6.71. Found: C, 68.86; H, 6.66.

Ozonolysis of Acetate 7.—The acetate **7** (720 mg, 2.3 mmoles) was ozonolyzed in 100 ml of methylene chloride at 15° for 12 hr. Volatile components from the reaction mixture were collected by passing the effluent gases through a Dry Ice–acetone trap. Additional methylene chloride was added periodically to the reaction vessel to keep the volume of the reaction mixture at *ca.* 100 ml. The liquid in the trap and the reaction mixture were then combined and treated with 10% sodium hydroxide (30 ml). Methylene chloride was removed *in vacuo* and the reaction mixture was further treated with ethanol (45 ml) and hydrogen peroxide (7 ml of 30%) for 0.5 hr at room temperature followed by 0.5 hr at reflux. The reaction mixture was extracted with chloroform and then neutralized with dilute hydrochloric acid.

Steam distillation of the reaction mixture yielded (+)-(*S*)-2-methylbutanoic acid (**8**) (40 mg, 17%) identified by the vpc retention time of the acid and its methyl ester and by conversion to (+)-(*S*)-2-methylbutanoic amide (**9**). The acid was found to be dextrorotary, but the exact rotation could not be determined. Continuous chloroform extraction of the aqueous phase yielded, after chromatography of the extract, glutaric acid (21 mg, 16%), mp 92–93° characterized by comparison with an authentic sample.

(+)-(*S*)-2-methylbutanoic Amide (9).—To (+)-(*S*)-2-methylbutanoic acid (43 mg, 0.42 mmole), obtained from the ozonolysis of acetate **7**, was added 1 equiv of oxalyl chloride. The mixture was heated to 45° for 2 hr. A small amount of ether was added and gaseous ammonia was passed through the solution for 1 hr. The ether mixture was filtered and evaporated to dryness. Recrystallization from benzene–ether–ethanol (5:5:1) yielded (+)-(*S*)-2-methylbutanoic amide (**9**) as colorless plates: mp 109–110°; $[\alpha]^{25}D$ +14.2° (*c* 0.244, chloroform); $M^{25}D$ +14.3 (lit.[5] mp 109.9–110.3°; $[\alpha]^{25}D$ +22.5; $M^{25}D$ +22.7).

Kuhn–Roth Oxidation of Phenol 6.—Kuhn–Roth oxidations were carried out according to the procedure of Wiesenberger.[15] The phenol **6** (52 mg, 0.18 mmole) was treated with 4 *N* chromic acid (4 ml) and water (4 ml). Steam distillation was immediately begun with addition of 2 ml of water for each 2 ml of distillate. The distillate was titrated against phenolphthalein with 0.05 *N* NaOH (0.17 mequiv of acid found). This distillate was reduced to a volume of 3 ml and acidified (pH < 1) with concentrated sulfuric acid. Extraction with five 10-ml portions of ether and vpc separation of the products (4 ft × 0.25 in., 4% phosphoric acid, 20% diethylene glycol succinate on Chrom W (60–80 mesh) at 110°) yielded acetic acid (5%), propionic acid (5%), and (+)-(*S*)-2-methylbutanoic acid (**8**) (11 mg, 90%), $[\alpha]^{25}D$ +19.2° (lit.[5] $[\alpha]^{25}D$ +19.3°). The (+)-(*S*)-2-methylbutanoic acid (**8**) was characterized by conversion into (+)-(*S*)-2-methylbutanoic amide (**9**), $[\alpha]^{25}D$ +22.4° (*c* 0.32, chloroform) (lit.[5] $[\alpha]^{25}D$ +22.5°).

Registry No.—**1**, 1162-65-8; **2**, 1389-06-6; **3**, 1165-39-5; **4**, 7241-98-7; **5**, 13133-70-5; **6**, 13133-71-6; **7**, 13133-72-7; **8**, 1730-91-2; **9**, 13133-74-9.

Acknowledgment.—We are indebted to the National Cancer Institute for financial support.

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Stereochemistry of the Alkaloid Gitingensine

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The steroidal alkaloid gitingensine was isolated from the leaves of *Kibatalia gitingensis* Woods[1] in low yields and structure **1a** was proposed for this compound, without assignment of configuration. Moreover, it was shown[1] that N-methylgitingensine (**1b**), although isomeric with, was different from paravallarine (**2**).[2] This difference seemed to be due to the configuration at C-3 and/or at C-20 in gitingensine (**1a**).

It has been shown recently[3] that primary and secondary amines react readily with dimedone to form condensation compounds. These substances possess a vinylogous amide group exhibiting strong ultraviolet absorption in the 280-mμ region. When in an asymmetric surrounding, this chromophore is optically active, as in the case of similar derivatives in the amino acid series.[4] Moreover, the sign of the Cotton effect reflects the configuration of the asymmetric center adjacent to the chromophore.

In order to assign the configuration to the primary amine in gitingensine (**1a**), its dimedone condensation derivative (**3**) was prepared and submitted to optical rotatory dispersion (RD) and circular dichroism (CD) examination.[5] As shown in Figure 1, the Cotton effect[5] associated with the vinylogous amide group of compound **3** at *ca.* 280 mμ is strongly positive (a = +214, $[\theta]_{288}$ +13,400).

As reference compounds, the adduct of 3β-amino-5α-pregnane (**4a**) and its 3α isomer (**4b**) were then prepared. Although their specific rotations at the sodium D line are very similar (see Experimental Section), compound **4a** of absolute configuration (3*S*), and presenting a stereochemistry at C-3 opposite that of **4b** (3*R*), exhibits a negative Cotton effect (a = −70) and (**4b**, a = +62) a positive one. This shows that inversion of the configuration of the 3-amino grouping gives rise to opposite Cotton effects for the dimedone condensation compounds. Since gitingensine (**1a**) and its dimedone derivative (**3**) present a double bond at C-5, the condensation compound of 3β-amino-20β-hydroxypregn-5-ene (**5**) was prepared for comparison purposes in order to make sure that the Δ^5 double bond does not invert the sign of the Cotton effect associated with the (3*S*) configuration. The sign of the rotatory dispersion curve of the condensation product (**5**, a = −183) is the same as in **4a**, but the molecular

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