Table III. Estimated[a] Quantities of the Major Compounds in Whole Essence Oil from Valencia Oranges

compound	% area under curve: overripe ess. oil	control ess. oil
α-pinene	0.34	0.46
myrcene	1.98	2.76
limonene	92.6	93.7
linalool	0.71	0.74
trans-2,8-*p*-menthadien-1-ol	0.14	0.04
cis-2,8-*p*-menthadien-1-ol	0.36	0.16
decanal	0.48	0.32
trans-carveol	0.25	0.06
carvone	0.12	0.13
perillaldehyde	0.11	0.04
dodecanal	0.08	0.04
β-elemene	0.09	0.07
β-caryophyllene	0.05	0.01
valencene	1.49	0.70
epi-α-selinene	0.08	0.04

[a] Estimates are based on gas chromatographic data.

ously believed to be the best source for obtaining valencene.

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Stereochemistry of Aflatoxicol B

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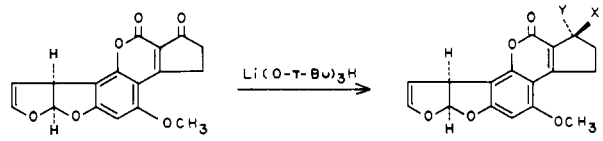
The relative stereochemistry of aflatoxicol B, one of the two diastereoisomers produced upon reduction of aflatoxin B_1 with lithium tris(*tert*-butoxy)aluminum hydride, has been determined by single-crystal X-ray diffraction analysis and corresponds to structure 2. Aflatoxicol B crystallized in the orthorhombic space group $P2_12_12_1$ with a = 16.463 (2), b = 19.178 (2), and c = 4.342 (1) Å. The structure was solved by direct methods and refined by full-matrix, least-squares methods to R = 0.04.

Aflatoxicols A and B (Cole et al., 1972; Detroy and Hesseltine, 1970) (aflatoxin R_0) are biological metabolites of aflatoxin B_1 and have been shown to produce many of the toxic effects found in aflatoxin B_1. Although less toxic than aflatoxin B_1, the aflatoxicols are carcinogens to trout (Schoenhard et al., 1974) and are active in the duckling bioassay (Detroy and Hesseltine, 1970) and in a microsomal mediated bacterial assay. Of the two diastereoisomers, aflatoxicol A (silica gel G-HR, chloroform–acetone 93:7 v/v, R_f 0.30) has been shown to be more toxic than aflatoxicol B (R_f 0.26).

Although the gross chemical structures of the aflatoxicols have been known for some time, the stereochemical detail of their structures has not been reported. Because the two diastereoisomers differ in toxicity, it was of interest to determine the stereochemistry of these isomers by single-crystal X-ray diffraction analysis.

RESULTS AND DISCUSSION

The reduction of aflatoxin B_1 (1) with lithium tris(*tert*-butoxy)aluminum hydride produce the two diastereoisomers 2 and 3 (Pawlowski et al., 1977). Separation by TLC



1

2: X=H, Y=OH
3: X=OH, Y=H

and recrystallization from chloroform–hexane produced suitable crystals of aflatoxicol B (R_f 0.26) for X-ray structure determination. The crystals were determined to be orthorhombic and belong to space group $P2_12_12_1$. Intensity measurements of diffraction maxima provided 813 unique nonzero reflections used in the structural analysis. The structure was determined by direct methods and refined by full-matrix least-squares procedures to an R = 0.040 (see Supplementary Material Available paragraph). Carbon and oxygen atoms were refined anisotropically and hydrogen atoms isotropically.

Tables I and II contain bond lengths and angles calculated from the final positional coordinates and Figure 1 shows a stick diagram and numbering scheme for the structure. The numbering scheme follows that in the previously reported structure of aflatoxin B_1 (van Soest and Peerdeman, 1970a,b,c). Bond lengths and angles are

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Table I. Distances between Bonded Atoms in Aflatoxicol B

atoms	distance, Å (esd)[a]	atoms	distance, Å (esd)[a]
C_1-C_2	1.421 (10)	C_{13}-O_5	1.454 (9)
C_1-O_1	1.404 (8)	C_{13}-O_6	1.425 (9)
C_1-O_2	1.222 (8)	C_{14}-C_{15}	1.497 (10)
C_2-C_3	1.513 (9)	C_{15}-C_{16}	1.336 (10)
C_2-C_6	1.356 (8)	C_{16}-O_6	1.388 (9)
C_3-C_4	1.535 (10)	C_{17}-O_4	1.432 (9)
C_3-O_3	1.440 (9)	C_3-HC_3	1.21 (8)
C_4-C_5	1.543 (10)	C_4-H_1C_4	1.09 (8)
C_5-C_6	1.507 (10)	C_4-H_2C_4	1.09 (7)
C_6-C_7	1.448 (8)	C_5-H_1C_5	1.03 (7)
C_7-C_8	1.418 (8)	C_5-H_2C_5	1.07 (8)
C_7-C_{12}	1.403 (9)	C_9-HC_9	1.07 (6)
C_8-C_9	1.392 (9)	C_{13}-HC_{13}	1.08 (8)
C_8-O_4	1.369 (8)	C_{14}-HC_{14}	1.22 (10)
C_9-C_{10}	1.382 (10)	C_{15}-HC_{15}	1.11 (8)
C_{10}-C_{11}	1.377 (8)	C_{16}-HC_{16}	1.21 (8)
C_{10}-O_5	1.378 (8)	C_{17}-H_1C_{17}	0.98 (9)
C_{11}-C_{12}	1.383 (8)	C_{17}-H_2C_{17}	1.08 (8)
C_{11}-C_{14}	1.507 (10)	C_{17}-H_3C_{17}	1.15 (7)
C_{12}-O_1	1.385 (7)	O_3-HO_3	1.15 (11)
C_{13}-C_{14}	1.550 (9)		

[a] The estimated standard deviations given in parentheses do not contain cell constant errors and have not been corrected for thermal motion.

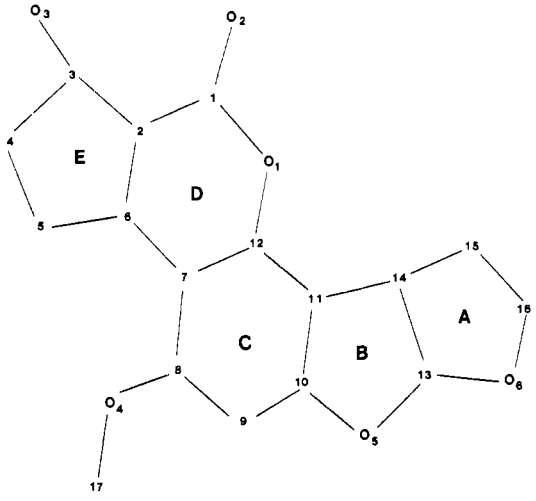

Figure 1. A "stick" representation of the structure of aflatoxicol B showing the numbering scheme used in the analysis.

in excellent agreement with those determined for aflatoxin B_1 and B_2 and with commonly accepted values.

Of primary importance in the study was the determination of the stereochemistry at the new asymmetric center at C_3. Inspection of the ORTEP stereodiagram (Johnson, 1971) in Figure 2 clearly shows that the orientation of the OH group on C_3 is trans to the A–B ring junction. Since the absolute stereochemistry of aflatoxin B_1 has been established, the configuration of the new center in aflatoxicol B is found to be R. Thus, the total structure of aflatoxicol B corresponds to structure 2.

Table II. Angles between Bonded Atoms in Aflatoxicol B

atoms	angles, deg (esd)	atoms	angles, deg (esd)
C_2-C_1-O_1	116.5 (5)	C_9-C_{10}-C_{11}	125.2 (6)
C_2-C_1-O_2	128.7 (6)	C_9-C_{10}-O_5	122.5 (5)
O_1-C_1-O_2	114.7 (6)	C_{11}-C_{10}-O_5	112.3 (6)
C_1-C_2-C_3	123.9 (5)	C_{10}-C_{11}-C_{12}	117.0 (6)
C_1-C_2-C_6	123.2 (6)	C_{10}-C_{11}-C_{14}	110.3 (5)
C_3-C_2-C_6	112.9 (6)	C_{12}-C_{11}-C_{14}	132.7 (5)
C_2-C_3-C_4	102.4 (5)	C_7-C_{12}-C_{11}	122.3 (5)
C_2-C_3-O_3	112.2 (7)	C_7-C_{12}-O_1	122.4 (5)
C_4-C_3-O_3	108.8 (6)	C_{11}-C_{12}-O_1	115.3 (5)
C_3-C_4-C_5	107.6 (6)	C_{14}-C_{13}-O_5	107.8 (6)
C_4-C_5-C_6	103.6 (6)	C_{14}-C_{13}-O_6	106.8 (6)
C_2-C_6-C_5	110.9 (5)	O_5-C_{13}-O_6	107.7 (6)
C_2-C_6-C_7	120.3 (6)	C_{11}-C_{14}-C_{13}	101.1 (5)
C_5-C_6-C_7	128.8 (5)	C_{11}-C_{14}-C_{15}	114.2 (7)
C_6-C_7-C_8	126.5 (6)	C_{13}-C_{14}-C_{15}	102.2 (5)
C_6-C_7-C_{12}	116.4 (5)	C_{14}-C_{15}-C_{16}	109.2 (6)
C_8-C_7-C_{12}	117.1 (5)	C_{15}-C_{16}-O_6	113.6 (7)
C_7-C_8-C_9	122.4 (6)	C_1-O_1-C_{12}	121.2 (5)
C_7-C_8-O_4	114.8 (5)	C_8-O_4-C_{17}	117.8 (5)
C_9-C_8-O_4	122.9 (5)	C_{10}-O_5-C_{13}	108.3 (5)
C_8-C_9-C_{10}	116.0 (5)	C_{13}-O_6-C_{16}	108.1 (5)
C_2-C_3-HC_3	107 (3)	O_5-C_{13}-HC_{13}	104 (4)
C_4-C_3-HC_3	115 (4)	O_6-C_{13}-HC_{13}	110 (4)
O_3-C_3-HC_3	111 (3)	C_{11}-C_{14}-HC_{14}	120 (4)
C_3-C_4-H_1C_4	114 (4)	C_{13}-C_{14}-HC_{14}	109 (4)
C_3-C_4-H_2C_4	109 (3)	C_{15}-C_{14}-HC_{14}	108 (4)
C_5-C_4-H_1C_4	110 (4)	C_{14}-C_{15}-HC_{15}	129 (4)
C_5-C_4-H_2C_4	106 (3)	C_{16}-C_{15}-HC_{15}	121 (4)
H_1C_4-C_4-H_2C_4	110 (5)	C_{15}-C_{16}-HC_{16}	127 (3)
C_4-C_5-H_1C_5	115 (4)	O_6-C_{16}-HC_{16}	119 (3)
C_4-C_5-H_2C_5	110 (4)		
C_6-C_5-H_1C_5	115 (4)	O_4-C_{17}-H_1C_{17}	109 (4)
C_6-C_5-H_2C_5	111 (4)	O_4-C_{17}-H_2C_{17}	110 (3)
H_1C_5-C_5-H_2C_5	102 (6)	H_1C_{17}-C_{17}-H_2C_{17}	102 (6)
C_8-C_9-HC_9	122 (4)	H_1C_{17}-C_{17}-H_3C_{17}	120 (6)
C_{10}-C_9-HC_9	122 (4)	H_2C_{17}-C_{17}-H_3C_{17}	105 (6)
C_{14}-C_{13}-HC_{13}	120 (4)	C_3-O_3-HO_3	83 (6)

An ORTEP stereodiagram of the unit cell contents is shown in Figure 3. An interesting feature of the molecular packing is the hydrogen bonding between pairs of molecules related by the crystallographic screw symmetry along the z axis. The intermolecular O_2–O_3 distance is 2.930 Å, which is indicative of H bonding between the hydroxyl group and the carbonyl of the lactone. Of the remaining six intermolecular contacts less than 3.5 Å, only the O_6–O_6 contact of 2.971 Å related by the screw axis along z is noteworthy.

EXPERIMENTAL SECTION

Reduction of Aflatoxin B_1. Aflatoxicol A and B were prepared by the lithium tris(*tert*-butoxy)aluminum hydride reduction of aflatoxin B_1 using the previously reported procedure (Pawlowski et al., 1977). The diastereomers were separated on a 500-μm Analtech alumina GF TLC plate using a benzene–acetone–ethyl acetate

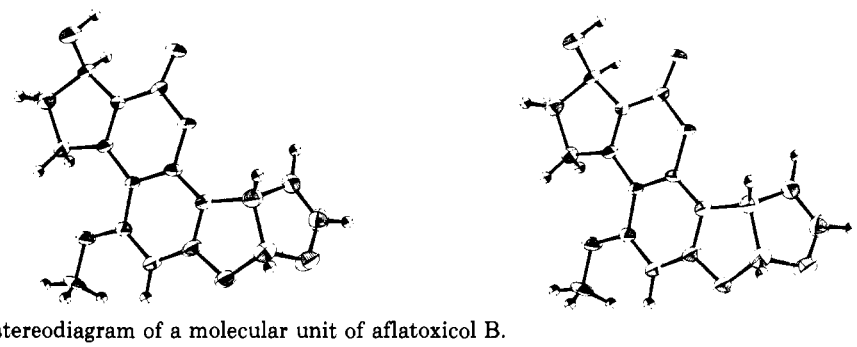

Figure 2. An ORTEP stereodiagram of a molecular unit of aflatoxicol B.

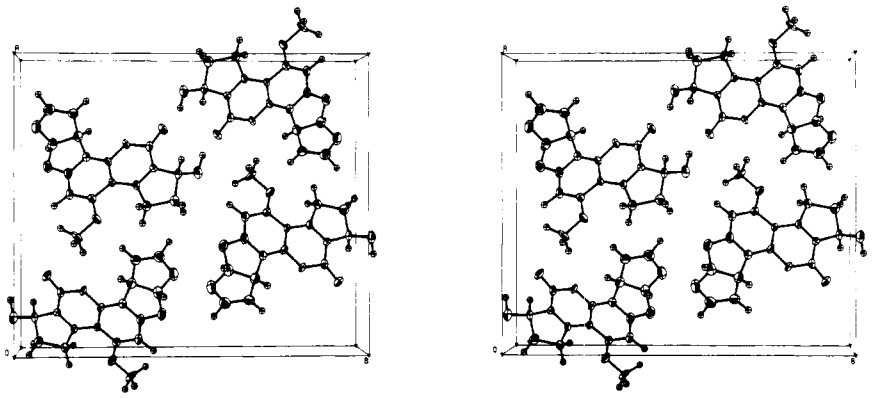

Figure 3. An ORTEP stereodiagram of the unit cell contents. The view is down the *c* axis and the *b* axis is horizontal.

Table III. Summary of Crystallographic Data for Aflatoxicol B

molecular formula	$C_{17}H_{14}O_6$
molecular weight	314.3 g/mol
linear absorption coefficient	9.07 cm^{-1} (Cu K_α)
density (calcd)	1.45 g/cm^3
space group	$P2_12_12_1$
cell dimensions	a = 16.463 (2), b = 19.178 (2)
	c = 4.342 (1) Å
	Z = 4
	V = 1371 $Å^3$
crystal size	0.3 × 0.1 × 0.08 mm
number of reflections	1695 (measured)
	813 (obsd)
final R	0.040
R_w	0.039

(100:12:24) solvent mixture to develop the plate. The TLC plate was first developed for 10 min and dried, developed an additional 15 min and dried, and finally developed an additional 20 min. After scraping the isomers from the plate, the aflatoxicols were eluted from the alumina using chloroform. Recrystallization of the aflatoxicols from chloroform–hexane resulted in crystals of aflatoxicol that were suitable for a single-crystal X-ray structure determination.

X-ray Structure Determination. Although the recrystallization of aflatoxicol B produced clumps of needles, a single crystal could be easily separated from the mass. An Enraf-Nonius CAD-4 diffractometer with graphite monochromator and CuK radiation was used in all diffraction measurements. The unit cell dimensions were determined from 15 accurately centered reflections ranging from 8.4 to 27.1°θ. The space group was determined as $P2_12_12_1$ from systematic absences. A summary of pertinent crystallographic information is given in Table III.

Diffraction maxima were collected using ω–2θ scan technique to a maximum θ = 75°. Of the 1695 reflections measured, 813 were considered unique, nonzero ($I > 3\sigma_I$) maxima and used in the diffraction analysis. Control reflections monitored after each set of 100 reflections indicated no significant decomposition of the crystal during data collection. The data were corrected for Lorentz–polarization effects and placed on an absolute scale by the method of Wilson (Stout and Jensen, 1968). Initial phases were determined using MULTAN (Main et al., 1974) with E's ≥ 1.35. The phase set with a significantly higher figure of merit (1.62) produced an E-map which provided starting positions for the 22 non-hydrogen atoms in the structure. Full-matrix, least-squares refinement of these starting positions, first isotropically then anisotropically, gave an R of 0.07. Hydrogen positions were then located from difference maps and refined isotropically giving a final R of 0.04 (R_w 0.039).

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Supplementary Material Available: Tables of calculated and observed structure factor comparisons and final positional and thermal parameters (8 pages). Ordering information is given on any current masthead page.

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