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Determination of Absolute Configuration of Epishamixanthone, a Metabolite of *Aspergillus rugulosus*, by Anomalous Scattering of Light Atoms

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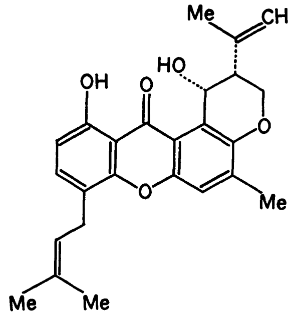
The absolute configuration of epishamixanthone has been determined from the Bijvoet differences by anomalous scattering of light atoms for Cu $K\alpha$ radiation. To verify the determination, the absolute configuration of sterigmatocystin which was already determined has been examined by the same procedure. The reliability of the Bijvoet differences calculated from the structure has been estimated by means of the probability method. The errors in the measured values of the Bijvoet differences have been analysed by the normal probability plot. The accuracy of the measurement may be affected considerably by the habit of crystal. The present analysis has established that the configurations of two asymmetric carbon atoms in epishamixanthone are both *R*.

Many natural products are isolated as optically active substances, and the determinations of their structures, including the absolute configurations, are of interest in connection with the study of stereospecificity in biological systems. Although the X-ray method for the determination of the absolute configuration seems to be more direct and more reliable than the spectroscopic[1)] and chemical methods,[2)] until recently the application of the X-ray method was confined to a crystal containing heavy atoms. The developments of the isolation technique of minor products and the direct method of the X-ray structure analysis have enhanced the need for a reliable method for the determination of the absolute configuration without further chemical modification. Recently, some authors described the determinations of the absolute configurations by using anomalous scattering of light atoms.[3,4)] In the present investigation, the absolute configuration of epishamixanthone **1**, a metabolite of *Aspergillus rugulosus*, has been determined by the method described by Engel.[4)] In addition, the reliability of this method has been examined by using sterigmatocystin **2**, whose absolute configuration had already been known from its bromo derivative.[5)]



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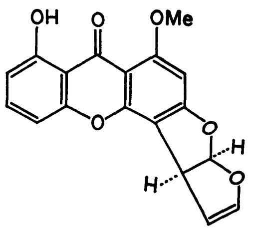

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Structure Determination

The isolation technique, spectral data, and relative structure of epishamixanthone have been published previously.[6)]

Crystallographic Measurement of Epishamixanthone. The crystals of epishamixanthone were grown as greenish-yellow needles elongated along the b axis. The cell constants were obtained by least-squares fits of the θ values for 49 reflections measured by a four-circle diffractometer. Two sets of intensity data were collected to analyze the errors in the calculated Bijvoet differences: the first data-set was collected by the stationary-crystal stationary-counter method and the second set was by the θ-2θ scan method. The experimental conditions for each set are summarized in Table 1(a) together with the crystal data. The intensities were corrected only for the Lorentz and polarization factors.

Structure Determination of Epishamixanthone. The initial structure was determined by using the first data-set. The orientation and location of the xanthone skeleton were determined by application of vector-search method.[7)] The location of the remaining non-hydrogen atoms were successively determined by Fourier syntheses. The coordinates and temperature factors were initially refined by the block-diagonal least-squares method by using the carbon scattering factor. The atomic species were determined by inspection of the temperature factors as well as chemical information. The positions of hydrogen atoms were assigned from a difference Fourier synthesis. Including the hydrogen atoms, the refinement reduced the R value to 0.081 for 1452 reflections, where the following weighting scheme was adopted: $w=0.63$ for $F_o=0$, $w=1.0$ for $0<F_o\leqq10$, and $w=1.0/(1.0+0.71(F_o-10))$ for $10<F_o$.

Further refinement was made using the second data-set and the block-diagonal least-squares method. The four strongest reflections, 001, 210, -201, and -311, were rejected from the subsequent calculations. The methyl hydrogen atoms attached to the C(25) atom could not be located; this is probably due to a large thermal motion of the group. Some of the hydrogen atoms obtained from the first set were found to be mislocated. A weighting scheme, $w=0.25$ for $F_o=0$, $w=1.0$ for $0<F_o\leqq8$, and $w=1.0/(1.0+0.5(F_o-8))$ for $8<F_o$, was used in the final calculation. The final R value was 0.058 for 1719 reflections ($R=0.055$ for non-zero reflections). The final atomic parameters refined by the second set are given in Tables 2(a) and 3(a).

Structure Refinement of Sterigmatocystin. The intensity measurement and structure analysis of sterigmatocystin have been described.[8)] In order to estimate the effect of error in the atomic parameters on B_{th}^*,

TABLE 1. CRYSTAL DATA AND EXPERIMENTAL CONDITIONS

(a) Epishamixanthone
  (i) Crystal data
    $C_{25}H_{26}O_5$, $M=406.5$
    monoclinic, space group $P2_1$
    $a=15.038(6)$, $b=6.251(4)$, $c=11.595(5)$ Å
    $\beta=105.14(4)°$, $V=1052.1$ Å^3
    $D_o=1.28$ g/cm^3
    $D_x=1.28$ g/cm^3 (for $Z=2$)
    μ(for Cu $K\alpha$) $=7.3$ cm^{-1}
    $N_o/N_c=0.20$
  (ii) First intensity data-set
    Ni-filtered Cu $K\alpha$ radiation
    Toshiba four-circle diffractometer
    stationary-crystal stationary-counter
      peak counting time: 30 s
      background: function of 2θ
    $\sin\theta/\lambda<0.53$
    independent reflections: 1452
  (iii) Second intensity data-set
    Ni-filtered Cu $K\alpha$ radiation
    Rigaku four-circle diffractometer
    θ-2θ scan mode
      scan range: $\Delta\theta=1.2°+0.15°\tan\theta$
      scan speed: $2°/\min(\theta)$
      background counting time: 8 s
    $\sin\theta/\lambda<0.562$
    independent reflections: 1723
(b) Sterigmatocystin
  (i) Crystal data
    $C_{18}H_{12}O_6$, $M=324.3$
    monoclinic, space group $P2_1$
    $a=16.056(4)$, $b=4.324(2)$, $c=11.218(3)$ Å
    $\beta=113.13(5)°$, $V=716.2$ Å^3
    $N_o/N_c=0.33$
  (ii) Second intensity data-set
    Ni-filtered Cu $K\alpha$ radiation
    Rigaku four-circle diffractometer
    θ-2θ scan mode
      scan range: $\Delta\theta=1.0°+0.15°\tan\theta$
      scan speed: $2°/\min(\theta)$
      background counting time: 8 s
    $\sin\theta/\lambda<0.562$
    independent reflections: 1227

intensities were remeasured in different conditions. (The intensity data collected in the condition described in the previous paper is noted as the first set.) The cell constants were remeasured before data collection. The experimental condition for the second set is summarized in Table 1(b) together with refined cell constants. The intensities were corrected only for the Lorentz and polarization factors.

The structure was refined by the block-diagonal least-squares method with the second set. The five strongest reflections, -111, -201, -211, 201, and -312, were omitted from the calculations. The weighting scheme used in the final cycle of the refinement was: $w=0.2$ for $F_o=0$, $w=1.0$ for $0<F_o\leq 14$, and $w=1.0/(1.0+0.6(F_o-14))$ for $14<F_o$. The final R value was 0.039 for 1222 reflections ($R=0.036$ for non-zero reflections). Since the final atomic parameters obtained from the second set seem to be more accurate than those from the first set, they are documented in Tables 2(b) and 3(b). A list of the observed and calculated structure factors is deposited at the office of the Chemical Society of Japan (Document No. 7802).

Determination of Absolute Configuration

Measurement of Bijvoet Differences. The sets of Bijvoet and reference reflections with large $BA_{\rm th}$* and with relatively large intensity were chosen by hand. Unless otherwise stated, the calculations of the structure factors were executed with $\Delta f_o''=0.02$ and $\Delta f_c''=\Delta f_H''=0.0$. The Bijvoet differences of both compounds were measured under the same experimental condition. A crystal was mounted with its b axis parallel to the ϕ axis of a Rigaku off-line four-circle diffractometer. The θ-2θ scan technique was adopted using Ni-filtered Cu $K\alpha$ radiation with a scan speed of 0.5°/min (θ) and a scan width of 0.8° (θ). Backgrounds were counted for 26 s at both sides of the scan range. In this experimental condition, the intensities of the Bijvoet pair ($h_1\ k_1\ l_1$ and $h_1\ \bar{k}_1\ l_1$) and its reference pair ($h_2\ k_2\ l_2$ and $h_2\ \bar{k}_2\ l_2$) were measured alternately 10 times. To diagnose the error of measurement, the intensities of equivalent reflections ($\bar{h}_1\ k_1\ \bar{l}_1$, $\bar{h}_1\ \bar{k}_1\ \bar{l}_1$, $\bar{h}_2\ k_2\ \bar{l}_2$, and $\bar{h}_2\ \bar{k}_2\ \bar{l}_2$) were also measured in the same way. The results of the measurements for each compound are summarized in Table 4. The standard deviation of mean value calculated from 10 individual BA's, $\sigma(BA_e)$, is not significantly large compared with that expected from counting statistics, $\sigma(BA_c)$. The signs of DELA for sterigmatocystin are minus through all reflections, while for epishamixanthone a few exceptions are found.

Effect of Error in Atomic Parameters on $B_{\rm th}$. The magnitude of $B_{\rm th}$ for the enantiomer-sensitive reflection in a light atom structure is fairly small. It is inferred, however, that this fact does not mean that very accurate atomic parameters are necessary to evaluate the $B_{\rm th}$. The effect of the error in atomic parameters has been quantitatively estimated as listed in Table 5, which shows that each of the $\sigma(B_{\rm th})$ is small enough to hold the sign of the $B_{\rm th}$ invariable. Further, the estimation of the $\sigma(B_{\rm th})$ has been assessed by means of a half-normal probability plot[9] (Fig. 1). These plots show that the $\sigma(B_{\rm th})$'s have been correctly estimated. It should be noted that $B_{\rm th}$ is insensitive to overall thermal parameters; even though the overall thermal parameters obtained from the second data-set for sterigmatocystin are significantly smaller than those from the first set, the slope for sterigmatocystin is approximately 1.0. For the enantiomer-sensitive reflections, all of the signs of the $B_{\rm th}$ calculated at the

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* The notations are same as those in Engel's paper.[4) DEL$=B_{\rm ex}/B_{\rm th}$, $B=(Q_{\rm H1}-1)/(1/2)(Q_{\rm H1}+1)$, DELA$=BA_{\rm ex}/BA_{\rm th}$, $BA=(Q_{\rm H1}/Q_{\rm H2}-1)/(1/2)(Q_{\rm H1}/Q_{\rm H2}+1)$, $Q_{{\rm H}i}=F_{{\rm H}i}/F_{\bar{\rm H}i}$; H1: Bijvoet reflection; H2: reference reflection.

TABLE 2. FINAL ATOMIC PARAMETERS[a] ($\times 10^4$) OF NON-HYDROGEN ATOMS, WITH ESTIMATED STANDARD DEVIATIONS IN PARENTHESES

(a) Epishamixanthone

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C (1)	3477 (3)	4671 (8)	4593 (4)	48 (2)	242 (12)	121 (4)	6 (9)	42 (5)	−7 (13)
C (2)	3239 (3)	3298 (7)	5403 (4)	44 (2)	226 (13)	106 (4)	16 (8)	44 (4)	6 (11)
C (3)	2601 (2)	1615 (7)	4948 (3)	41 (2)	241 (12)	102 (4)	34 (8)	38 (4)	18 (11)
C (4)	2297 (3)	46 (7)	5689 (4)	44 (2)	249 (13)	105 (4)	37 (9)	37 (4)	39 (12)
C (5)	1657 (3)	−1562 (7)	5081 (4)	40 (2)	216 (12)	122 (4)	−10 (8)	41 (4)	9 (12)
C (6)	1340 (3)	−3233 (8)	5721 (4)	48 (2)	273 (13)	125 (4)	19 (9)	51 (5)	50 (13)
C (7)	744 (3)	−4772 (9)	5105 (5)	52 (2)	288 (15)	161 (5)	−39 (10)	69 (5)	−5 (15)
C (8)	453 (3)	−4718 (10)	3878 (5)	59 (2)	358 (18)	154 (5)	−96 (11)	87 (6)	−93 (16)
C (9)	731 (3)	−3118 (10)	3204 (4)	53 (2)	447 (20)	132 (5)	−76 (12)	58 (5)	−76 (17)
C (10)	1340 (3)	−1589 (8)	3837 (4)	47 (2)	316 (16)	122 (4)	−29 (10)	58 (5)	−41 (14)
C (11)	2232 (3)	1491 (8)	3723 (4)	44 (2)	321 (15)	113 (4)	−23 (10)	39 (4)	8 (14)
C (12)	2452 (3)	2951 (9)	2923 (4)	49 (2)	330 (15)	113 (4)	−23 (10)	36 (5)	29 (14)
C (13)	3065 (3)	4546 (8)	3345 (4)	50 (2)	311 (15)	123 (4)	18 (10)	61 (5)	57 (14)
C (14)	3333 (3)	6137 (10)	2521 (4)	75 (3)	352 (17)	138 (5)	−24 (13)	59 (6)	130 (17)
C (15)	4700 (3)	6109 (10)	6092 (4)	55 (2)	367 (18)	133 (5)	−76 (12)	32 (5)	19 (16)
C (16)	4137 (3)	5779 (8)	6978 (4)	54 (2)	253 (13)	113 (4)	−2 (9)	30 (5)	−5 (13)
C (17)	4671 (3)	6024 (9)	8272 (4)	78 (3)	281 (15)	123 (5)	−23 (12)	20 (6)	−23 (15)
C (18)	4119 (4)	6099 (18)	9146 (5)	81 (3)	911 (44)	129 (5)	−33 (22)	59 (7)	−133 (29)
C (19)	5592 (4)	6129 (13)	8621 (5)	67 (3)	570 (27)	147 (5)	−46 (16)	−8 (6)	−85 (23)
C (20)	3652 (3)	3638 (8)	6733 (4)	44 (2)	217 (11)	117 (4)	19 (8)	28 (4)	17 (12)
C (21)	441 (4)	−3061 (14)	1855 (5)	84 (3)	659 (31)	140 (6)	−212 (19)	46 (7)	−118 (23)
C (22)	−302 (4)	−4582 (14)	1254 (6)	87 (3)	560 (29)	165 (6)	−120 (18)	56 (8)	−124 (23)
C (23)	−1186 (3)	−4135 (11)	857 (4)	71 (3)	490 (21)	95 (4)	−77 (14)	28 (5)	−27 (17)
C (24)	−1820 (5)	−5913 (16)	271 (5)	121 (5)	685 (35)	140 (6)	−163 (25)	62 (9)	−95 (26)
C (25)	−1669 (8)	−2153 (17)	907 (7)	236 (10)	574 (34)	190 (9)	239 (34)	112 (15)	−69 (31)
O (1)	4300 (2)	1997 (6)	7228 (3)	61 (2)	271 (9)	142 (3)	29 (7)	8 (4)	52 (10)
O (2)	2575 (2)	51 (6)	6806 (3)	71 (2)	321 (10)	102 (3)	−51 (8)	26 (3)	53 (9)
O (3)	1610 (2)	−3305 (6)	6922 (3)	74 (2)	335 (11)	129 (3)	−35 (8)	37 (4)	106 (11)
O (4)	1614 (2)	−70 (6)	3170 (3)	56 (1)	369 (11)	108 (3)	−94 (7)	34 (3)	−20 (10)
O (5)	4101 (2)	6280 (6)	4912 (3)	69 (2)	305 (10)	128 (3)	−76 (8)	42 (4)	53 (10)

(b) Sterigmatocystin

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C (1)	4391 (2)	886 (8)	2594 (2)	32 (1)	487 (20)	67 (2)	13 (9)	20 (3)	−9 (13)
C (2)	3993 (2)	1958 (8)	1334 (2)	33 (1)	414 (18)	69 (2)	14 (8)	39 (3)	−20 (12)
C (3)	3091 (2)	1098 (7)	496 (2)	30 (1)	389 (17)	63 (2)	29 (8)	26 (3)	−27 (12)
C (4)	2631 (2)	2145 (8)	−838 (2)	36 (1)	461 (18)	66 (2)	64 (9)	38 (3)	−11 (12)
C (5)	1722 (2)	938 (8)	−1546 (2)	33 (1)	402 (18)	65 (2)	44 (8)	28 (3)	−48 (12)
C (6)	1218 (2)	1685 (8)	−2862 (2)	41 (1)	481 (20)	65 (2)	70 (10)	26 (3)	−34 (13)
C (7)	355 (2)	562 (9)	−3500 (3)	49 (2)	528 (21)	69 (3)	51 (11)	−9 (3)	−41 (14)
C (8)	−22 (2)	−1343 (9)	−2861 (3)	40 (1)	533 (23)	100 (3)	31 (11)	−7 (3)	−46 (16)
C (9)	446 (2)	−2168 (9)	−1569 (3)	36 (1)	517 (22)	93 (3)	−5 (10)	9 (3)	−21 (15)
C (10)	1311 (2)	−1002 (8)	−947 (2)	34 (1)	425 (18)	70 (3)	34 (9)	14 (3)	−36 (13)
C (11)	2628 (2)	−904 (7)	1012 (2)	30 (1)	386 (17)	61 (2)	7 (8)	28 (3)	−48 (12)
C (12)	3013 (2)	−1987 (7)	2256 (2)	34 (1)	359 (17)	62 (2)	24 (8)	22 (3)	−13 (11)
C (13)	3881 (2)	−1072 (8)	3013 (2)	37 (1)	404 (18)	53 (2)	46 (9)	18 (3)	4 (12)
C (14)	3496 (2)	−4173 (8)	4351 (3)	53 (2)	478 (20)	64 (2)	21 (10)	30 (3)	24 (13)
C (15)	2378 (2)	−1750 (10)	4732 (3)	74 (2)	657 (27)	128 (4)	−12 (14)	127 (5)	−20 (19)
C (16)	1996 (2)	−2285 (9)	3486 (3)	55 (2)	523 (22)	98 (3)	19 (11)	74 (4)	67 (15)
C (17)	2655 (2)	−3943 (7)	3061 (2)	44 (1)	375 (18)	69 (3)	−15 (9)	37 (3)	25 (12)
C (18)	5371 (2)	4504 (10)	1569 (3)	32 (1)	753 (27)	110 (3)	−50 (11)	35 (3)	51 (18)
O (1)	4422 (1)	3884 (6)	821 (2)	32 (1)	589 (15)	82 (2)	−31 (7)	32 (4)	28 (10)
O (2)	2968 (1)	3968 (6)	−1387 (2)	42 (1)	723 (18)	83 (2)	−24 (8)	39 (2)	135 (11)
O (3)	1579 (1)	3531 (7)	−3504 (2)	52 (1)	770 (18)	72 (2)	37 (8)	33 (2)	97 (11)
O (4)	1756 (1)	−1901 (5)	326 (2)	32 (1)	505 (13)	67 (2)	−29 (6)	10 (2)	39 (9)
O (5)	4193 (1)	−2260 (6)	4239 (2)	44 (1)	534 (14)	61 (2)	−23 (7)	5 (2)	48 (9)
O (6)	3248 (2)	−2931 (8)	5335 (2)	72 (1)	915 (21)	72 (2)	−9 (10)	61 (3)	−68 (12)

a) Anisotropic temperature factors are in the form: $\exp[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+\beta_{12}hk+\beta_{13}hl+\beta_{23}kl)]$.

Table 3. FINAL ATOMIC COORDINATES ($\times 10^3$) AND ISOTROPIC TEMPERATURE FACTORS ($\times 10$) OF HYDROGEN ATOMS, WITH ESTIMATED STANDARD DEVIATIONS IN PARENTHESES

(a) Epishamixanthone

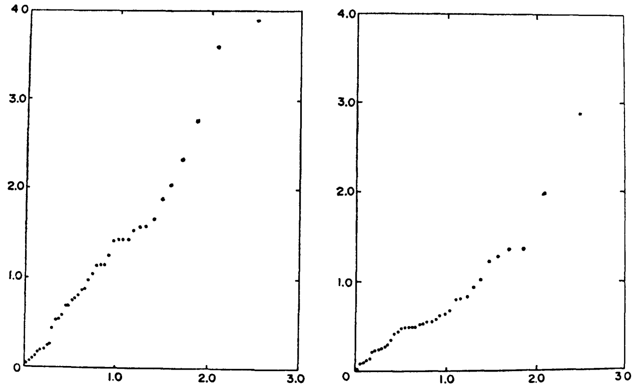
	x	y	z	B/Å^2
H(C7)	56(4)	−568(11)	560(5)	64(15)
H(C8)	9(3)	−568(11)	343(4)	55(14)
H(C12)	209(3)	284(10)	200(4)	44(12)
H(C14)	309(4)	574(14)	154(5)	81(17)
H(C14)	316(4)	753(13)	274(5)	69(16)
H(C14)	399(3)	586(11)	261(4)	48(12)
H(C15)	507(4)	719(13)	615(5)	67(16)
H(C15)	511(3)	487(10)	617(4)	40(12)
H(C16)	362(3)	674(10)	674(4)	45(12)
H(C18)	359(5)	484(14)	890(6)	94(20)
H(C18)	371(5)	748(17)	912(6)	105(22)
H(C18)	434(4)	679(12)	999(5)	66(15)
H(C19)	585(4)	627(12)	942(5)	63(14)
H(C19)	603(4)	618(11)	789(5)	62(14)
H(C20)	308(3)	355(9)	718(4)	30(10)
H(O1)	380(5)	107(14)	723(6)	92(18)
H(O3)	193(4)	−206(13)	719(6)	80(18)
H(C21)	100(4)	−288(13)	157(5)	69(16)
H(C21)	9(5)	−165(16)	165(6)	97(21)
H(C22)	−5(4)	−639(16)	117(6)	93(20)
H(C24)	−230(4)	−537(12)	77(5)	73(17)
H(C24)	−165(4)	−786(15)	22(5)	84(18)
H(C24)	−210(4)	−570(14)	−69(6)	89(19)

(b) Sterigmatocystin

	x	y	z	B/Å^2
H(C1)	502(2)	146(10)	319(3)	31(8)
H(O3)	217(3)	366(13)	−303(4)	57(11)
H(C7)	1(2)	118(10)	−446(3)	37(9)
H(C8)	−65(2)	−225(11)	−333(3)	36(8)
H(C9)	15(2)	−346(12)	−117(3)	49(10)
H(C14)	379(2)	−628(10)	467(3)	34(8)
H(C15)	212(2)	−78(12)	529(4)	52(10)
H(C16)	140(2)	−146(12)	285(4)	53(10)
H(C17)	244(2)	−614(10)	266(3)	34(8)
H(C18)	570(2)	265(12)	175(3)	49(10)
H(C18)	548(3)	540(15)	257(4)	70(12)
H(C18)	556(2)	594(13)	114(4)	55(11)

early stage of the refinement were equal to those at the final stage. Therefore, it is not necessary to refine the structure extremely for application of the method described by Engel,[4] if the structure is correctly solved. The other reflections with smaller Bijvoet differences may have a larger value of $\sigma(B_{\mathrm{th}})/|B_{\mathrm{th}}|$. Average $\sigma(\Delta|F_c|)$ value was discussed by Parthasarathy.[10]

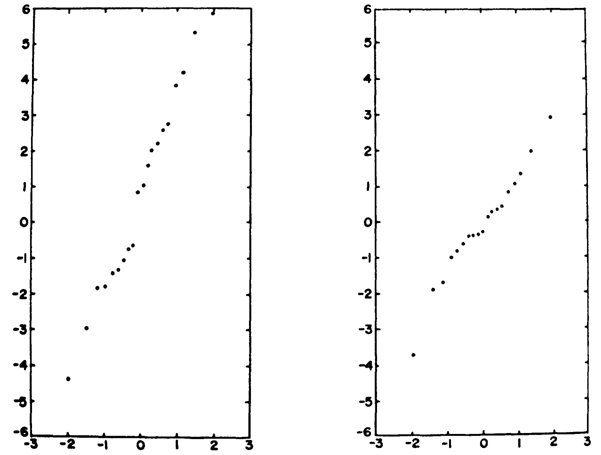
Two kinds of B_{th} were calculated: $B_{\mathrm{th}}(1)$ with $\Delta f_o''=0.032$, $\Delta f_c''=0.009$, and $\Delta f_H''=0.0$,[11] and $B_{\mathrm{th}}(2)$ with $\Delta f_o''=0.018$ and $\Delta f_c''=\Delta f_H''=0.0$. In the $B_{\mathrm{th}}(2)$, the $\Delta f_o''$ corresponds to the effective anomalous scattering factor of oxygen atom defined by Engel,[4] and the contribution of hydrogen atoms is neglected. For the reflections in Table 5, the values given by $\sum|B_{\mathrm{th}}(1)-B_{\mathrm{th}}(2)|/\sum(1/2)(|B_{\mathrm{th}}(1)|+|B_{\mathrm{th}}(2)|)$ are 0.105 and 0.051 for epishamixanthone and sterigmatocystin respectively.



(a) Epishamixanthone (b) Sterigmatocystin

Fig. 1. Half-normal probability plot: the vertical axis is statistic δp_i; the horizontal axis is the expected δp_i.
$$\delta p_i=||B_{\mathrm{th}}(1)_i|-|B_{\mathrm{th}}(2)_i||/\{\sigma^2(B_{\mathrm{th}}(1))_i+\sigma^2(B_{\mathrm{th}}(2))_i\}^{1/2},$$
where $B_{\mathrm{th}}(1)_i$, $B_{\mathrm{th}}(2)_i$, $\sigma(B_{\mathrm{th}}(1))_i$, and $\sigma(B_{\mathrm{th}}(2))_i$ are calculated from two sets of atomic parameters and their estimated standard deviations obtained from independent structure refinement.

Error in Intensity Measurement. Various kinds of experimental errors in the detection of the small intensity difference of Bijvoet pair have been eliminated appreciably by the use of the reference reflection; for epishamixanthone mean DEL and DELA are −1.04 ±0.22 and −1.14±0.15 respectively, and for sterigmatocystin the corresponding values are −1.26±0.12 and −0.98±0.07. However, the standard deviation of mean DELA for each compound calculated from individual DELA's is still larger than the corresponding value expected from $\sigma(BA_c)$'s or $\sigma(BA_e)$'s. This fact is visualized in the normal probability plot[9] (Fig. 2),



(a) Epishamixanthone (b) Sterigmatocystin

Fig. 2. Normal probability plot: the vertical axis is statistic δm_i; the horizontal axis is the expected δm_i.
$$\delta m_i=\{(\mathrm{DELA}_{hkl})_i-(\mathrm{DELA}_{\bar{h}k\bar{l}})_i\}/\{\sigma^2(\mathrm{DELA}_{hkl})_i+\sigma^2(\mathrm{DELA}_{\bar{h}k\bar{l}})_i\}^{1/2},$$
where the $\sigma(\mathrm{DELA}_{hkl})_i$ and $\sigma(\mathrm{DELA}_{\bar{h}k\bar{l}})_i$ are calculated from 10 individual DELA's. The plot is independent of BA_{th}.

TABLE 4. RESULTS OF THE BIJVOET MEASUREMENTS

BA_{ex} is the mean values of 10 individual BA's. $\sigma(BA_c)$ and $\sigma(BA_e)$ are standard deviations of the mean expected from counting statistics and that calculated from 10 individual BA's respectively. BA_{th}, BA_{ex}, $\sigma(BA_c)$, and $\sigma(BA_e)$ are given in %.

(a) Epishamixanthone

h_1	k_1	l_1	h_2	k_2	l_2	BA_{th}	BA_{ex}	$\sigma(BA_c)$	$\sigma(BA_e)$	DELA
4	2	0	4	2	1	0.99	−1.38	0.23	0.20	−1.40
							−1.09	0.24	0.34	−1.11
−7	2	1	−8	2	2	0.55	0.02	0.15	0.17	0.04
							−0.57	0.15	0.15	−1.04
6	1	1	7	1	1	−1.09	0.31	0.20	0.25	−0.28
							0.81	0.20	0.20	−0.74
2	1	3	1	1	3	1.03	−0.19	0.21	0.20	−0.19
							−1.66	0.21	0.19	−1.61
−3	2	1	−4	2	1	1.10	−1.88	0.32	0.41	−1.71
							−0.87	0.32	0.39	−0.79
1	2	1	0	2	1	1.79	−2.13	0.47	0.83	−1.19
							−7.81	0.47	0.51	−4.37
1	1	5	2	1	5	−0.72	−0.22	0.31	0.48	0.31
							1.17	0.31	0.44	−1.64
−5	1	2	−6	1	1	−1.01	0.51	0.49	0.54	−0.51
							2.68	0.48	0.53	−2.65
−2	3	2	−1	3	2	1.06	−1.61	0.36	0.41	−1.51
							−0.80	0.36	0.40	−0.75
−5	2	4	−5	2	5	1.65	−2.29	0.35	0.53	−1.39
							−1.39	0.35	0.44	−0.84
3	1	4	3	1	5	−0.72	−0.20	0.30	0.62	0.28
							0.63	0.30	0.47	−0.87
0	2	4	0	2	3	1.17	−2.53	0.27	0.45	−2.15
							−0.11	0.27	0.33	−0.09
6	1	4	5	1	4	−1.37	−0.75	0.49	0.51	0.54
							2.26	0.50	0.60	−1.65
−4	2	6	−4	2	5	1.56	−0.97	0.49	0.29	−0.62
							−2.99	0.48	0.68	−1.92
2	3	0	1	3	0	0.72	−1.31	0.34	0.25	−1.83
							−0.15	0.34	0.59	−0.21
3	1	2	2	1	2	1.45	−1.78	0.53	0.62	−1.23
							−0.94	0.53	0.52	−0.65
−4	2	2	−3	2	2	−0.75	2.36	0.31	0.37	−3.13
							1.02	0.29	0.25	−1.36
−8	1	3	−6	1	3	0.97	−0.22	0.49	0.77	−0.23
							−2.58	0.49	0.86	−2.67
−2	1	2	−2	1	3	−0.97	0.82	0.13	0.21	−0.84
							1.04	0.13	0.14	−1.07
8	2	3	7	2	4	−1.18	1.74	0.61	0.76	−1.47
							1.01	0.62	0.76	−0.85

(b) Sterigmatocystin

h_1	k_1	l_1	h_2	k_2	l_2	BA_{th}	BA_{ex}	$\sigma(BA_c)$	$\sigma(BA_e)$	DELA
2	1	1	1	1	1	−1.25	1.52	0.11	0.23	−1.21
							1.68	0.11	0.11	−1.35
2	2	2	3	2	2	2.23	−2.35	0.38	0.31	−1.05
							−2.17	0.38	0.45	−0.97
−7	1	2	−6	1	3	−1.77	1.03	0.22	0.32	−0.58
							1.19	0.22	0.23	−0.68
−5	1	5	−4	1	5	−1.44	1.16	0.33	0.31	−0.80
							1.39	0.33	0.52	−0.97
6	1	1	5	1	1	−1.27	0.25	0.16	0.12	−0.19
							0.73	0.16	0.23	−0.58
−11	1	3	−11	1	2	0.81	−0.96	0.29	0.45	−1.19
							−1.23	0.29	0.42	−1.51
−3	2	1	−3	2	0	−1.66	1.85	0.34	0.25	−1.12
							0.97	0.34	0.38	−0.59

Table 4. (Continued)

(b) Sterigmatocystin										
h_1	k_1	l_1	h_2	k_2	l_2	BA_{th}	BA_{ex}	$\sigma(BA_c)$	$\sigma(BA_e)$	DELA
4	2	2	5	2	2	−0.54	0.25	0.18	0.18	−0.46
							1.17	0.18	0.17	−2.17
8	2	0	7	2	0	−1.96	3.99	0.56	0.61	−2.04
							2.87	0.56	0.57	−1.46
−4	1	7	−3	1	7	1.00	−0.98	0.21	0.42	−0.98
							−0.58	0.21	0.26	−0.58
−9	1	2	−10	1	2	−1.65	1.55	0.40	0.32	−0.94
							1.46	0.40	0.68	−0.88
5	1	6	6	1	6	−1.09	1.24	0.24	0.20	−1.14
							0.46	0.24	0.18	−0.43
3	2	4	2	2	4	−1.06	1.90	0.51	0.76	−1.79
							1.55	0.50	0.43	−1.46
−7	2	5	−7	2	4	−1.63	0.97	0.65	0.66	−0.60
							0.72	0.67	0.61	−0.44
7	1	0	6	1	0	1.73	−1.29	0.48	0.49	−0.75
							−2.02	0.46	0.52	−1.17
0	1	5	−1	1	6	−1.77	1.42	0.43	0.43	−0.80
							2.52	0.43	0.48	−1.42
4	1	5	5	1	5	−1.27	1.06	0.35	0.43	−0.84
							0.53	0.35	0.26	−0.42
−1	2	6	−1	2	5	−1.73	1.72	0.33	0.40	−1.00
							1.95	0.33	0.51	−1.13
8	1	4	9	1	4	−0.74	0.52	0.28	0.40	−0.70
							0.90	0.28	0.22	−1.21

Table 5. Effect of error in atomic parameters on B_{th}(%)[a]

(a) Epishamixanthone				(b) Sterigmatocystin			
h	k	l	B_{th}	h	k	l	B_{th}
8	2	3	−0.99(3)	2	1	1	−1.14(2)
−2	1	2	−0.77(2)	2	2	2	1.54(6)
−8	1	3	1.08(5)	−7	1	2	−1.25(4)
−4	2	2	−0.72(3)	−5	1	5	−0.92(4)
3	1	2	1.50(10)	6	1	1	−1.37(2)
2	3	0	0.63(5)	−11	1	3	0.89(2)
−4	2	6	1.24(5)	−3	2	1	−1.48(5)
6	1	4	−1.38(5)	4	2	2	−0.80(1)
0	2	4	0.71(2)	8	2	0	−1.86(7)
3	1	4	−0.80(3)	−4	1	7	1.13(2)
−5	2	4	1.24(6)	−9	1	2	−0.92(3)
−2	3	2	0.95(4)	5	1	6	−0.67(1)
−5	1	2	−0.59(11)	3	2	4	−1.11(4)
1	1	5	−0.56(1)	−7	2	5	−1.63(7)
1	2	1	1.07(18)	7	1	0	1.22(5)
−3	2	1	1.18(11)	0	1	5	−1.47(6)
2	1	3	0.60(2)	4	1	5	−0.94(2)
6	1	1	−0.90(2)	−1	2	6	−1.06(2)
−7	2	1	0.31(1)	8	1	4	−0.78(1)
4	2	0	0.72(3)				

[a] $\sigma(B_{th})$ is given in parentheses with respect to the least significant digit. $\sigma(B_{th})$ has been calculated by the formula: $\sigma^2(B_{th})=\sum_i\{\partial B_{th}/\partial P_i\}^2(\sigma P_i)^2$, where P_i and σP_i are the i-th parameter and estimated standard deviation, respectively, obtained from a block-diagonal least-squares calculation. The parameters and estimated standard deviations in the most refined stage (Tables 2 and 3) have been used in this calculation.

although the number of samples is small. The slopes, especially for epishamixanthone, are larger than 1.0. This indicates the existence of some errors due to geomery of the instrument and/or habit of crystal which were not fully eliminated by the method. This is partly explained by the fact that the width of the peak-profile of epishamixanthone changes more drastically, as the direction of the X-ray beam changes, than that of sterigmatocystin.

The errors exist to a great extent in experimental measurements of Bijvoet difference. Since the method described by Engel is insensitive to experimental errors such as the instability and geometrical error of an instrument, the method may generally give a more significant enantiomer-indication than the R method. Various sources of error were discussed by Engel.[4)]

The absolute configuration of epishamixanthone is correctly represented by the parameters in Tables 2(a) and 3(a), if the left-handed coordinate system is adopted. The absolute configuration of sterigmatocystin determined by the present analysis agrees with that determined by its bromo derivative.[5)] For each of the present compounds, the probability that the selected absolute configuration is wrong is estimated to be far less than 0.01%, assuming a t-distribution of DELA values.

Crystal and Molecular Structure

The crystal and molecular structure of epishamixanthone is shown in Fig. 3, together with the numbering scheme of atoms used in this paper. The figure is drawn with correct absolute configuration. The bond

TABLE 6. BOND LENGTHS AND ANGLES FOR EPISHAMIXANTHONE

(a) Bond length (Å)			
C (1)-C (2)	1.386(7)	C (10)-O (4)	1.355(6)
C (1)-C (13)	1.420(7)	C (11)-C (12)	1.402(7)
C (1)-O (5)	1.358(6)	C (11)-O (4)	1.384(6)
C (2)-C (3)	1.429(6)	C (12)-C (13)	1.359(7)
C (2)-C (20)	1.520(7)	C (13)-C (14)	1.505(8)
C (3)-C (4)	1.454(6)	C (15)-C (16)	1.508(8)
C (3)-C (11)	1.385(7)	C (15)-O (5)	1.432(7)
C (4)-C (5)	1.442(6)	C (16)-C (17)	1.513(8)
C (4)-O (2)	1.253(6)	C (16)-C (20)	1.515(7)
C (5)-C (6)	1.433(7)	C (17)-C (18)	1.469(13)
C (5)-C (10)	1.396(7)	C (17)-C (19)	1.339(10)
C (6)-C (7)	1.380(7)	C (20)-O (1)	1.428(6)
C (6)-O (3)	1.346(6)	C (21)-C (22)	1.494(12)
C (7)-C (8)	1.375(8)	C (22)-C (23)	1.317(11)
C (8)-C (9)	1.399(9)	C (23)-C (24)	1.506(12)
C (9)-C (10)	1.393(8)	C (23)-C (25)	1.445(14)
C (9)-C (21)	1.510(11)		
(b) Bond angles (°)			
C (2)-C (1)-C (13)	122.3(5)	C (9)-C (10)-O (4)	115.8(5)
C (2)-C (1)-O (5)	123.8(4)	C (3)-C (11)-C (12)	122.8(5)
C (13)-C (1)-O (5)	113.9(4)	C (3)-C (11)-O (4)	123.8(5)
C (1)-C (2)-C (3)	118.2(4)	C (12)-C (11)-O (4)	113.5(5)
C (1)-C (2)-C (20)	119.4(4)	C (11)-C (12)-C (13)	119.6(5)
C (3)-C (2)-C (20)	122.4(4)	C (1)-C (13)-C (12)	118.8(5)
C (2)-C (3)-C (4)	124.2(4)	C (1)-C (13)-C (14)	119.5(5)
C (2)-C (3)-C (11)	118.1(4)	C (12)-C (13)-C (14)	121.7(5)
C (4)-C (3)-C (11)	117.7(4)	C (16)-C (15)-O (5)	109.6(5)
C (3)-C (4)-C (5)	117.0(4)	C (15)-C (16)-C (17)	114.5(5)
C (3)-C (4)-O (2)	122.6(4)	C (15)-C (16)-C (20)	108.8(4)
C (5)-C (4)-O (2)	120.4(4)	C (17)-C (16)-C (20)	112.4(4)
C (4)-C (5)-C (6)	121.7(4)	C (16)-C (17)-C (18)	115.9(6)
C (4)-C (5)-C (10)	121.2(4)	C (16)-C (17)-C (19)	123.0(6)
C (6)-C (5)-C (10)	117.1(4)	C (18)-C (17)-C (19)	121.1(7)
C (5)-C (6)-C (7)	120.0(5)	C (2)-C (20)-C (16)	111.3(4)
C (5)-C (6)-O (3)	120.6(4)	C (2)-C (20)-O (1)	111.2(4)
C (7)-C (6)-O (3)	119.5(5)	C (16)-C (20)-O (1)	108.4(4)
C (6)-C (7)-C (8)	120.4(5)	C (9)-C (21)-C (22)	116.5(7)
C (7)-C (8)-C (9)	122.4(6)	C (21)-C (22)-C (23)	126.3(8)
C (8)-C (9)-C (10)	116.6(6)	C (22)-C (23)-C (24)	117.6(7)
C (8)-C (9)-C (21)	123.2(6)	C (22)-C (23)-C (25)	129.8(8)
C (10)-C (9)-C (21)	120.1(6)	C (24)-C (23)-C (25)	112.7(8)
C (5)-C (10)-C (9)	123.6(5)	C (10)-O (4)-C (11)	119.8(4)
C (5)-C (10)-O (4)	120.6(4)	C (1)-O (5)-C (15)	115.0(4)

lengths and angles for non-hydrogen atoms are shown in Table 6. The configurations of the C(16) and C(20) atoms are both *R*. The absolute configuration of shamixanthone has been assigned by Chexal *et al.* by application of the Horeau asymmetric synthesis.12) Thus the configuration of C(20) in epishamixanthone is the same as that of the corresponding atom in shamixanthone, while that of C(16) is opposite. From the biosynthetic study,13,14) it was shown that both the carbon atoms are incorporated from the methyl carbon of acetate, and was suggested that the dihydropyran ring is formed by cyclization between 3-methyl-2-butenyloxy and aldehyde groups in the precursor. Chexal *et al.*14) discussed the mechanism of the formation of substituted dihydropyran rings. Although epishamixanthone is one of epimers of shamixanthone, it is unlikely, from the absolute configurations of these metabolites, that the metabolites arise through ene-reaction with transition states differing to each other only in the conformation of aldehyde group in the precursor.

The equations of the least-squares planes and the deviations of atoms from each plane are given in Table 7. The ring composed of C(1), C(2), C(20), C(16), C(15), and O(5) forms a half-chair conformation. The torsion angles of C(15)–C(16)–C(17)–C(19) and

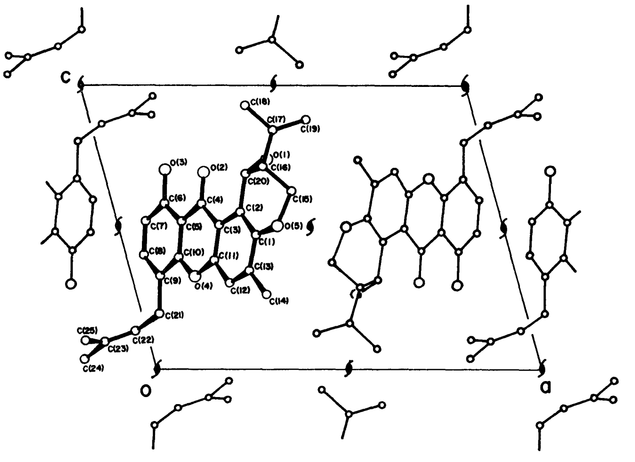


Fig. 3. The crystal and molecular structure of epishamixanthone viewed along the b-axis.

TABLE 7. DEVIATIONS (Å) OF ATOMS FROM LEAST-SQUARES PLANES

In the equations of the plane, X, Y, and Z are $ax+cz\cos\beta$, by, and $cz\sin\beta$ respectively.

Plane: C(1)—C(13), O(2), O(4)

$0.809X-0.584Y-0.058Z=1.036$

[C(1) −0.066, C(2) −0.027, C(3) −0.006, C(4) 0.021, C(5) 0.024, C(6) −0.002, C(7) −0.030, C(8) −0.036, C(9) 0.001, C(10) 0.014, C(11) 0.019, C(12) 0.036, C(13) 0.003, O(2) 0.030, O(4) 0.029, C(21) −0.044 C(14) 0.003, O(5) −0.138, C(20) 0.008, O(1) −1.227, O(3) 0.015]

Plane: C(1), C(2), C(20), O(5)

$0.784X-0.620Y-0.037Z=1.024$

[C(1) 0.015, C(2) −0.011, C(20) 0.005, O(5) −0.008, C(15) −0.452, C(16) 0.331]

Plane: C(16)—C(19)

$0.030X-0.995Y+0.091Z=-2.766$

[C(16) 0.002, C(17) −0.007, C(18) 0.002, C(19) 0.002]

Plane: C(21)—C(25)

$0.380X+0.262Y-0.887Z=-2.308$

[C(21) 0.002, C(22) −0.005, C(23) 0.004, C(24) 0.001, C(25) −0.002]

C(10)-C(9)-C(21)-C(22) are −11.8 and −172.5° respectively. There are two intramolecular hydrogen bonds, O(1)-H(O1)···O(2) and O(3)-H(O3)···O(2). The distances of O(2)···H(O1) and O(2)···H(O3) are 1.89 and 1.76 Å respectively. The angles of O(1)-H(O1)···O(2), O(3)-H(O3)···O(2), H(O1)···O(2)-C(4), and H(O3)···O(2)-C(4) are 157, 144, 108, and 102° respectively. The interplanar distance of xanthone skeletons stacking along the b axis is about 3.65 Å. The intermolecular distances less than 3.8 Å are listed in Table 8. There are neither intermolecular hydrogen bonds nor abnormally short contacts: the crystal is composed of molecules held by van der Waals forces.

The atomic scattering factors were taken from International Tables for X-ray Crystallography.[15] Most of calculations were performed on a TOSBAC-3400 computer at the Tottori University Computing Center by the use of programs written by the authors. The block-diagonal least-squares refinement and the vector-search calculation were executed on a NEAC-2200 N700 computer at the Computation Center of Osaka University using programs written by T. Ashida and I. Tanaka respectively.

TABLE 8. INTERMOLECULAR CONTACTS LESS THAN 3.8 Å EXCLUDING HYDROGEN ATOMS

The superscripts refer to the following equivalent positions: I x, $1+y$, z; II $-x$, $1/2+y$, $1-z$; III $1-x$, $1/2+y$, $1-z$.

$\mathrm{C}(1)\cdots\mathrm{C}(5^{\mathrm{I}})$	3.763	$\mathrm{C}(20)\cdots\mathrm{O}(3^{\mathrm{I}})$	3.671
$\mathrm{C}(2)\cdots\mathrm{C}(6^{\mathrm{I}})$	3.680	$\mathrm{C}(5)\cdots\mathrm{C}(7^{\mathrm{II}})$	3.733
$\mathrm{C}(3)\cdots\mathrm{C}(7^{\mathrm{I}})$	3.633	$\mathrm{C}(6)\cdots\mathrm{C}(7^{\mathrm{II}})$	3.722
$\mathrm{C}(11)\cdots\mathrm{C}(8^{\mathrm{I}})$	3.613	$\mathrm{C}(6)\cdots\mathrm{C}(8^{\mathrm{II}})$	3.602
$\mathrm{C}(12)\cdots\mathrm{C}(8^{\mathrm{I}})$	3.758	$\mathrm{C}(25)\cdots\mathrm{O}(3^{\mathrm{II}})$	3.467
$\mathrm{C}(12)\cdots\mathrm{C}(9^{\mathrm{I}})$	3.644	$\mathrm{O}(2)\cdots\mathrm{C}(25^{\mathrm{II}})$	3.720
$\mathrm{C}(13)\cdots\mathrm{C}(9^{\mathrm{I}})$	3.765	$\mathrm{O}(3)\cdots\mathrm{C}(8^{\mathrm{II}})$	3.744
$\mathrm{C}(13)\cdots\mathrm{C}(10^{\mathrm{I}})$	3.696	$\mathrm{O}(3)\cdots\mathrm{C}(24^{\mathrm{II}})$	3.521
$\mathrm{C}(14)\cdots\mathrm{O}(4^{\mathrm{I}})$	3.726	$\mathrm{C}(14)\cdots\mathrm{O}(1^{\mathrm{III}})$	3.537
$\mathrm{C}(16)\cdots\mathrm{O}(2^{\mathrm{I}})$	3.527	$\mathrm{C}(15)\cdots\mathrm{C}(1^{\mathrm{III}})$	3.773

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