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STEREOCHEMISTRY OF CERCOSPORIN+

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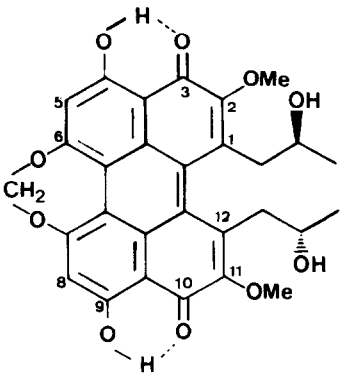
Abstract - The absolute configuration of the asymmetric carbons and the axial chirality of the natural mold metabolite cercosporin (from Cercospora sp.) have been established on the basis of X-ray analysis and chemical reactions. The results confirm the inherent dissymmetry of the perylenequinone ring, the twisting of which gives rise to the diastereoisomer isocercosporin. The energy barrier for the conversion of cercosporin into isocercosporin has been evaluated.

Cercosporin (1a) is a polyketide-derived perylenequinone produced by many species of the deuteromycete Cercospora[1] the pathogenic fungus which is the causal organism of diseases of many plants. Recent interest has arisen in the photodynamic antibacterial activity[2] and in the mechanism of the phytotoxic activity[3] of cercosporin.

Cercosporin was isolated and its chemistry studied by Kuyama and Tamura[4], but its complete structure (1a) was established by ourselves in 1971[5]. A detailed study, encompassing many results on the structural chemistry and partially elucidating the stereochemistry of cercosporin, was published later by Yamazaki[6], who also investigated the biosynthesis of (1a)[7]. Similar partial results were obtained by other groups[8]. It is the purpose of this paper to further clarify the interesting stereochemical features of cercosporin.



1a,b

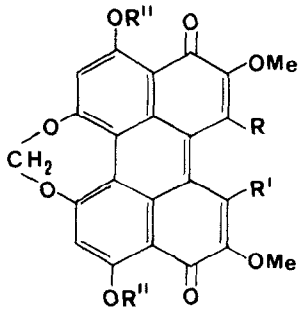
By heating in various solvents, cercosporin isomerizes into an almost equimolar equilibrium mixture of cercosporin and isocercosporin (1b)[5], and the same happens for (1b). Both 1a and 1b possess two asymmetric centers in the side chains.

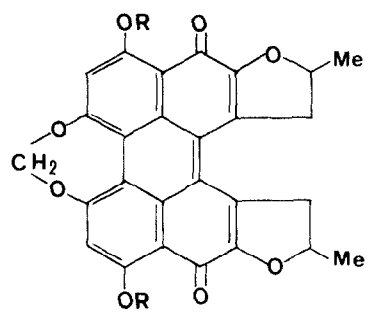
+ Part 12 of the series "Secondary mold metabolites". Part 11: G. Assante et al., J. Agr. Food Chem. 29, 785 (1981).

As isocercosporin has the same structure as cercosporin, and as the configuration of the side chain centers cannot be modified by the thermal isomerization process, isocercosporin must be a diastereoisomer of (1a), the difference between the two consisting in the opposite twisting of the perylenequinone ring. This is also shown by comparison of CD spectra of (1a) and (1b)[6]. As this twisting introduces an inherent dissymmetry, the sheer existence of the two diastereoisomers requires that in both 1a and 1b the two chiral carbon atoms of the chains must have the same absolute configuration[6,9]. The twisting of the ring can be due to the presence of the methylenedioxy bridge, or to Van der Waals repulsion between the bulky side chains, or to both factors together.

As a matter of fact, the non-planarity of the ring, although inferred from optical data, in particular the high value of the optical rotation and CD spectra, has not been demonstrated unambiguously. Yamazaki[6] was able to degrade the side chains via a retroaldol reaction to the compound (2) that is optically inactive. However, since the reaction required heating at reflux in aqueous solutions the compound could have racemized at high temperature during the reaction. On the other hand, cyclization of cercosporin and isocercosporin with concentrated sulfuric acid at room temperature to the corresponding noranhydroderivatives (8) gave two identical compounds, with superimposable CD spectra. We suppose that in this case the relief of steric hindrance due to the folding of the side chains into the dihydrofuran rings, lowers the inversion barrier enough to give a planar ring. The optical activity of (8) measured on the acetates (9) is due to the chiral carbon atoms, adjacent to the strong perylenequinone chromophore. This is consistent with the low intensity of the CD spectrum, in comparison with that of (1a) and (1b). Therefore it seems that the methylenedioxy ring alone is not enough to prevent racemization at room temperature[10].

We proceeded then to the destruction of the asymmetric centers in mild conditions in order to maintain the side chains. Oxidation of (1a) with CrO_3-pyridine in methylene chloride at room temperature gave a product (3a) where, however, only one of the secondary alcohol groups was transformed into a ketone, and with poor yields. Then both cercosporin and isocercosporin were converted with CH_3I and silver oxide into





2 R = R' = CH_3 R'' = H

3a R = $CH_2CHOHCH_3$ R' = CH_2COCH_3 R'' = H

4a,b R = R' = $CH_2CHOHCH_3$ R'' = CH_3

5a,b R = R' = CH_2COCH_3 R'' = CH_3

6a R = $CH_2CHOHCH_3$ R' = CH_2COCH_3 R'' = CH_3

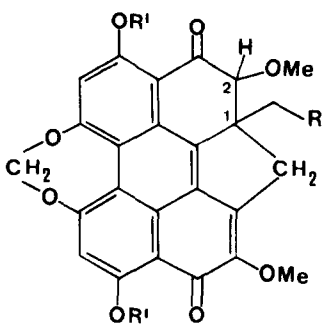
7a R = $CH_2CH(OCOCH_3)CH_3$ R' = $CH_2CH(OCOPh)CH_3$ R'' = H

8 R = H

9 R = $COCH_3$

the dimethylethers (4a,b), which maintain the optical properties (CD) of the parent compounds. Oxidation of these ethers with CrO_3-pyridine afforded the expected ketones (5a,b) which show identical IR, NMR spectra and TLC behaviour, but have opposite CD spectra (fig. 2). They are therefore enantiomers. This result confirms the inherent non-planarity of the ring in (1a) and (1b) and the importance of the side chains for the high inversion barrier. The similarity of the CD spectra of (5) with those of all the other derivatives of cercosporin and respectively isocercosporin allows the easy correlation of the relative configurations of the twisted rings.

A product (6a) of partial oxidation with only one side chain with a carbonyl group was also obtained. Moreover, during attempts to cleave the side chains of (1a) with basic reagents two new yellow compounds were isolated. The spectral data for these compounds are consistent only with the cyclic structures (10) and (12). The formation of (10) and (12) can be easily explained by a retroaldol reaction on one or both side chains, followed by the nucleophilic attack of the methylene anion onto the position β to the quinone carbonyl.



10 R = $CHOHCH_3$ R' = H
11 R = $CH(OR')CH_3$ R' = $COCH_3$
12 R = H R' = H
13 R = H R' = $COCH_3$

The principal reason for the existence of a barrier to the inversion of the ring in the conversion of cercosporin into isocercosporin is the steric hindrance to the movement of the side chains one respect to the other. We hoped that the magnitude of the barrier could be estimated by dynamic NMR experiments, but even heating at 180°C a mixture of 1a and 1b did not induce any coalescence of the signals of the isomers in the NMR spectrum. We had therefore to revert to more traditional methods, such as the measure of the rate constants of mutarotation at different temperatures[11]. The method has the disadvantage, in our case, of the high absorption of cercosporin even at long wavelengths, and of the inaccuracy of measures of almost equimolar mixtures, due to the low total optical rotation of such mixtures. Search for a suitable solvent led to the choice of the unusual acetylacetone, as e.g. ether or CH_2Cl_2 are too low-boiling, toluene has scarce solubilizing power, and pyridine induces the decomposition of the solutes. The rate of mutarotation was measured at 76°, 85°, 96° and 100.2°C. (Table 1). A set of good rate constant k_m was obtained. If the interconversion of the two diastereoisomers is given by eq. (1), k_1 can be obtained by combining the two equations (2) and (3), where K is derived from $[\alpha]_D$ values of the equilibrium mixture at each temperature.

$$\text{Cerc} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Isocerc} \qquad (1)$$

$$k_m = k_1 + k_{-1} \qquad (2)$$

$$K = k_1/k_{-1} \qquad (3)$$

From the absolute rate constant equation[12], the values of $\Delta H^{\ddagger}$ = 20.0 kcal/mole and $\Delta S^{\ddagger}$ = 20.8 e.u. could be obtained (r = 0.975). The unusually high value of the entropy change, which can be attributed to the change of conformation of the side

TABLE 1

Mutarotation of 1a to the equilibrium mixture in acetylacetone

Temp.°C	c (g/l)	no.points	$k_m \times 10^5$ sec^{-1}	r
76	0.003	10	3.277	0.996
85	0.003	10	9.62	0.997
96	0.003	10	21.56	0.999
100	0.003	10	25.78	0.997

chains in the interconversion process[2], can be invoked to explain the long half-time for the isomerization, with an energy barrier not too different from that of other, faster interconverting systems[11].

As it was said above, the two asymmetric carbons of the side chains must have the same absolute configuration. In order to establish this configuration, the dimethylether (4a) of cercosporin was reacted with D,L-phenyl-butyric anhydride according to Horeau[13]. As the recovered 2-phenyl-butyric acid was dextrorotatory, and on the assumption that the aryl-CH_2 group(where aryl is the whole ring)is larger than a methyl[14], the absolute configuration of both carbons must be R. The following step was the determination of the absolute configuration of the helical ring. In this case, the method of choice could possibly be only X-ray analysis. Crystallization of cercosporin or isocercosporin is hampered by the fact that they isomerize by heating even at boiling point of ether. Fortunately, however, during the screening of many Cercospora species for new metabolites[1], we found in C.setariae a natural ester (7a) the monoacetate monobenzoate of the side chain hydroxyls, which crystallized in form suitable for X-ray analysis just by evaporation of solvent at room temperature. Mild hydrolysis of this ester gave pure cercosporin, thus confirming the identity of configuration with the parent compound[1].

The X-ray analysis, whose details are given below, could give the helicity of the ring, by correlation with the known configuration of the asymmetric carbon atoms of the side chain. As, however, the Horeau method which has been employed to establish the configuration is not exempt from ambiguities, being based on an empirical scale of bulkiness of the substituents, oxygen anomalous dispersion data were collected. This analysis confirmed the R configuration for both carbons of the side chain, and indicated a definite twisting of the rings (fig. 1). Assuming the axis C_6-C_{17} as a chirality axis, the axial chirality of cercosporin must be R (or M)[15].

These results enable us to obtain also the axial chirality of some other natural perylenequinones, by correlation of the CD spectra with those of cercosporin and isocercosporin. This will be the subject of a forthcoming paper.

Inspection of the X-ray data also shows that cercosporin exists in the solid state as one of the two hydroxyquinone tautomers. A study of the tautomerism of cercosporin and related perylenequinones in solution is also in progress.

X-RAY ANALYSIS OF (7a)

Preliminary cell parameters obtained photographically were refined by least-squares from the setting angles of 12 reflections accurately measured on the diffractometer: a = 12.327(3), b = 15.552(3), c = 17.074(3) A; V = 3273.3 A^3; M_r =680.7; Z = 4; D_c = 1.38 cm^{-3}; u(CuK) = 8.23 cm^{-1}; space group $P2_12_12_1$. Diffracted intensities were measure at room temperature with a Siemens AED single crystal diffractometer using CuK radiation (λ = 1.5418 A) with

Table 2. Fractional atomic coordinates ($\times 10^4$)

	x/a	y/b	z/c		x/a	y/b	z/c
O1	1940(2)	2068(1)	-92(1)	C30	9(3)	-1888(2)	-1783(2)
O2	94(2)	1858(1)	-143(1)	C31	-798(3)	-1501(2)	-1245(2)
O3	-2629(2)	959(2)	-1793(2)	C32	-1880(3)	-1514(3)	-1449(2)
O4	-2221(2)	104(2)	-2997(1)	C33	-2634(3)	-1143(3)	-949(3)
O5	-792(2)	-381(1)	-4135(1)	C34	-2308(4)	-779(3)	-250(3)
O6	4418(2)	-1478(2)	-2673(1)	C35	-1223(4)	-758(3)	-50(2)
O7	5365(2)	-22(2)	-2010(2)	C36	-464(3)	-1120(2)	-545(2)
O8	5251(2)	1261(2)	-1090(2)	C37	-1380(5)	-1174(3)	-4181(3)
O9	2336(2)	1152(2)	-3869(1)	C38	5451(3)	-1365(4)	-3029(3)
O10	3817(2)	381(2)	-4141(2)	H1	-2800(36)	681(31)	-2186(27)
O11	1020(2)	-1813(1)	-1550(1)	H2	5302(39)	710(32)	-1247(30)
O12	-254(2)	-2252(2)	-2393(2)	H3	-1772(24)	1657(20)	-644(18)
C1	-112(2)	1402(2)	-815(2)	H4	3872(30)	2057(23)	-294(22)
C2	-1205(2)	1385(2)	-987(2)	H5	1214(25)	1073(22)	291(18)
C3	-1569(2)	960(2)	-1646(2)	H6	967(25)	2029(21)	728(20)
C4	-820(2)	552(2)	-2148(2)	H7	1119(32)	-649(26)	-4331(23)
C5	295(2)	566(2)	-1964(1)	H8	2312(26)	-492(23)	-3778(20)
C6	703(2)	989(2)	-1279(2)	H9	127(38)	732(30)	-5004(30)
C7	-1226(2)	142(2)	-2840(2)	H10	301(33)	1271(26)	-4197(27)
C8	-426(2)	-169(2)	-3393(2)	H11	988(36)	1668(28)	-4982(28)
C9	643(2)	-145(2)	-3252(2)	H12	4845(51)	1503(42)	-3101(38)
C10	1029(2)	95(2)	-2463(2)	H13	3443(43)	2082(38)	-2856(35)
C11	2069(2)	-92(2)	-2198(2)	H14	4064(42)	2017(33)	-3725(31)
C12	2722(2)	-822(2)	-2478(2)	H15	1410(27)	-1614(22)	-2989(20)
C13	3818(2)	-789(2)	-2421(2)	H16	2695(25)	-2016(20)	-3062(18)
C14	4362(2)	-82(2)	-2023(2)	H17	3499(40)	-2712(31)	-1755(26)
C15	3682(2)	504(2)	-1579(2)	H18	2421(36)	-2845(35)	-1027(32)
C16	2530(2)	469(2)	-1627(2)	H19	3130(36)	-1936(30)	-1220(26)
C17	1853(2)	1015(2)	-1166(1)	H20	-2078(34)	-1852(30)	-2079(27)
C18	2435(2)	1559(2)	-638(2)	H21	-3221(41)	-1155(34)	-1058(31)
C19	3544(2)	1640(2)	-641(2)	H22	-2979(35)	-542(30)	139(26)
C20	4180(2)	1134(2)	-1110(2)	H23	-1015(39)	-592(32)	501(28)
C21	1044(3)	1698(3)	273(2)	H24	408(30)	-1180(24)	-408(19)
C22	1433(2)	-252(2)	-3931(2)	H25	-2118(44)	-1235(38)	-3757(33)
C23	1696(3)	595(3)	-4359(2)	H26	-868(47)	-1683(38)	-3941(37)
C24	727(4)	1129(3)	-4622(2)	H27	-1645(47)	-1346(38)	-4614(33)
C25	3402(4)	1002(3)	-3827(3)	H28	5655(43)	-2019(39)	-3123(35)
C26	3989(6)	1664(4)	-3357(5)	H29	6082(41)	-1040(34)	-2729(31)
C27	2173(2)	-1656(2)	-2714(2)	H30	5559(54)	-799(49)	-3290(41)
C28	1859(3)	-2231(2)	-2019(2)	H31	1422(23)	-2767(20)	-2312(16)
C29	2763(4)	-2459(4)	-1463(3)	H32	2227(24)	404(20)	-4814(18)

nickel monochromator. 3476 independent reflections ($\pm h,k,\ell$) were measured and 2531 were used in the structure analysis having considered as unobserved the reflections whose intensities were $< 2\sigma(I)$. The structure was solved by direct methods with MULTAN[16] and refined by block-diagonal least-squares to a final $R = 7.7\%$. In order to have better structure analysis results, a new set of diffraction data was collected (h,k,ℓ and $-h,-k,-\ell$) on a Syntex $P2_1$ diffractometer. A total of 4536 independent reflections was collected and 296 of these, having $I < 2\sigma(I)$, were considered unobserved and excluded by the refinement. The coordinates resulted in the preceeding refinement were used with the new data. The R and R_w values obtained in the refinement of the enantiomer were $R = 0.0442$ and $R_w = 0.0428$. According to the Hamilton[17] test this is a significant difference.

List of the structure factors calculations and of thermal parameters of atoms is available from the authors (G.D.A., G.B., P.S.) on request.

Geometry and configuration. The atomic coordinates, bond lenghts, valency angles are given in Tables 2-4. The atom numbering is shown in Fig. 1 which reports a projection of the structure on the plane perpendicular to the C16-C17 bond line and rotated by -15°around x and by 30°around y. The six condensed rings are arranged in an helix fashion which is right-handed from the benzoate to acetate chain. In the perylene moiety the delocalisation is limited and an alternation of short and long bonds is observed. Other bond distances are as expected while the strains due to the helix conformation determine some variations in the angles of the rings with respect to the normal values. All the central rings, but the C1-C6, are

Table 3. Bond distances (Å)

O1-C18	1.367(4)	C1-C6	1.432(4)	C15-C16	1.424(4)
O1-C21	1.393(4)	C2-C3	1.380(5)	C15-C20	1.406(4)
O2-C1	1.373(4)	C3-C4	1.411(4)	C16-C17	1.427(4)
O2-C21	1.392(4)	C4-C5	1.410(4)	C17-C18	1.429(4)
O3-C3	1.331(4)	C4-C7	1.433(5)	C18-C19	1.373(4)
O4-C7	1.257(4)	C5-C6	1.433(4)	C19-C20	1.369(4)
O5-C8	1.385(4)	C5-C10	1.443(4)	C22-C23	1.541(5)
O5-C37	1.433(5)	C6-C17	1.431(4)	C23-C24	1.523(6)
O6-C13	1.371(4)	C7-C8	1.448(4)	C25-C26	1.493(9)
O6-C38	1.422(5)	C8-C9	1.340(4)	C27-C28	1.536(5)
O7-C14	1.240(4)	C9-C10	1.477(5)	C28-C29	1.506(6)
O8-C20	1.335(4)	C9-C22	1.523(4)	C30-C31	1.482(5)
O9-C23	1.440(5)	C10-C11	1.390(4)	C31-C32	1.379(5)
O9-C25	1.337(6)	C11-C12	1.472(4)	C31-C36	1.396(5)
O10-C25	1.217(6)	C11-C16	1.426(5)	C32-C33	1.388(6)
O11-C28	1.461(4)	C12-C13	1.336(4)	C33-C34	1.381(7)
O11-C30	1.313(4)	C12-C27	1.518(4)	C34-C35	1.381(7)
O12-C30	1.229(5)	C13-C14	1.456(4)	C35-C36	1.381(6)
C1-C2	1.379(4)	C14-C15	1.452(4)		

far from a planar geometry and the deformations are certainly due to the strains of the helix conformation. The seven membered ring exhibits a "boat" conformation with C1, C6,C18,O1 in a plane and the remaining atoms in the same part of the plane (see Table 6). The internal sp^2 angles are greater than 120°, while that at C21 is near to a normal sp^3 angle. The distorsion of this ring at C21 is clearly indicated by the two torsion angles O2-C21-O1-C18 = 88.5(3)° and C1-O2-C21-O1 = 76.3(4)°. The two asymmetric carbons C23 and C28, as predicted above, show both the *R* configuration. The substituents to the chiral carbons are *trans* with respect to the perylene mean plane. The torsion angles C12-C27-C28-O11 = 66.0(3)°, C9-C22-C23-O9 = 69.5(4)° show that the benzoic and acetic groups orient themselves almost parallel and *endo* to the mean propeller plane. The molecular packing does not show significant intermolecular contacts

EXPERIMENTAL

M.ps are uncorrected. UV spectra were measured in 95% EtOH. NMR spectra were

Table 4. Bond angles (°)

C18-O1-C21	114.8(3)	C8-C9-C10	119.2(3)	O1-C18-C19	113.2(3)
C1-O2-C21	119.3(2)	C10-C9-C22	121.1(2)	C18-C19-C20	121.3(3)
C8-O5-C37	114.8(3)	C5-C10-C9	117.7(2)	O8-C20-C19	117.8(3)
C13-O6-C38	121.4(3)	C9-C10-C11	122.8(3)	O8-C20-C15	123.3(3)
C23-O9-C25	117.7(3)	C5-C10-C11	119.5(3)	C15-C20-C19	118.9(3)
C28-O11-C30	117.8(2)	C10-C11-C16	117.5(3)	O1-C21-O2	111.4(3)
O2-C1-C6	124.4(3)	C10-C11-C12	124.1(3)	C9-C22-C23	113.7(3)
O2-C1-C2	111.6(3)	C12-C11-C16	118.4(2)	O9-C23-C22	110.7(3)
C2-C1-C6	124.0(3)	C11-C12-C27	120.1(2)	C22-C23-C24	116.2(3)
C1-C2-C3	120.0(3)	C11-C12-C13	119.5(3)	O9-C23-C24	105.9(3)
O3-C3-C2	118.3(3)	C13-C12-C27	119.7(3)	O9-C25-O10	121.9(4)
C2-C3-C4	119.9(3)	O6-C13-C12	119.0(3)	O10-C25- C26	125.5(5)
O3-C3-C4	121.9(3)	C12-C13-C14	121.4(3)	O9-C25-C26	112.7(4)
C3-C4-C7	118.2(2)	O6-C13-C14	119.2(2)	C12-C27-C28	113.9(3)
C3-C4-C5	119.7(3)	O7-C14-C13	121.6(3)	O11-C28-C27	110.1(3)
C5-C4-C7	122.1(2)	O7-C14-C15	121.3(3)	C27-C28-C29	116.0(3)
C4-C5-C10	118.2(2)	C15-C14-C13	116.9(2)	O11-C28-C29	106.5(3)
C4-C5-C6	122.1(3)	C14-C15-C20	119.7(3)	O11-C30-O12	123.2(3)
C6-C5-C10	119.7(2)	C14-C15-C16	121.5(3)	O12-C30-C31	122.4(3)
C1-C6-C5	114.3(2)	C16-C15-C20	119.7(3)	O11-C30-C31	114.4(3)
C5-C6-C17	118.1(3)	C11-C16-C15	117.4(3)	C30-C31-C36	120.3(3)
C1-C6-C17	127.4(3)	C15-C16-C17	121.9(3)	C30-C31-C32	119.1(3)
O4-C7-C4	122.5(3)	C11-C16-C17	120.5(2)	C32-C31-C36	120.5(3)
C4-C7-C8	116.6(2)	C6-C17-C16	119.2(2)	C31-C32-C33	119.1(4)
O4-C7-C8	120.6(3)	C16-C17-C18	114.0(2)	C32-C33-C34	120.5(4)
O5-C8-C7	117.0(2)	C6-C17-C18	126.8(3)	C33-C34-C35	120.4(4)
C7-C8-C9	122.9(3)	O1-C18-C17	123.3(2)	C34-C35-C36	119.7(4)
O5-C8-C9	119.4(3)	C17-C18-C19	123.5(3)	C31-C36-C35	119.8(4)
C8-C9-C22	119.3(3)				

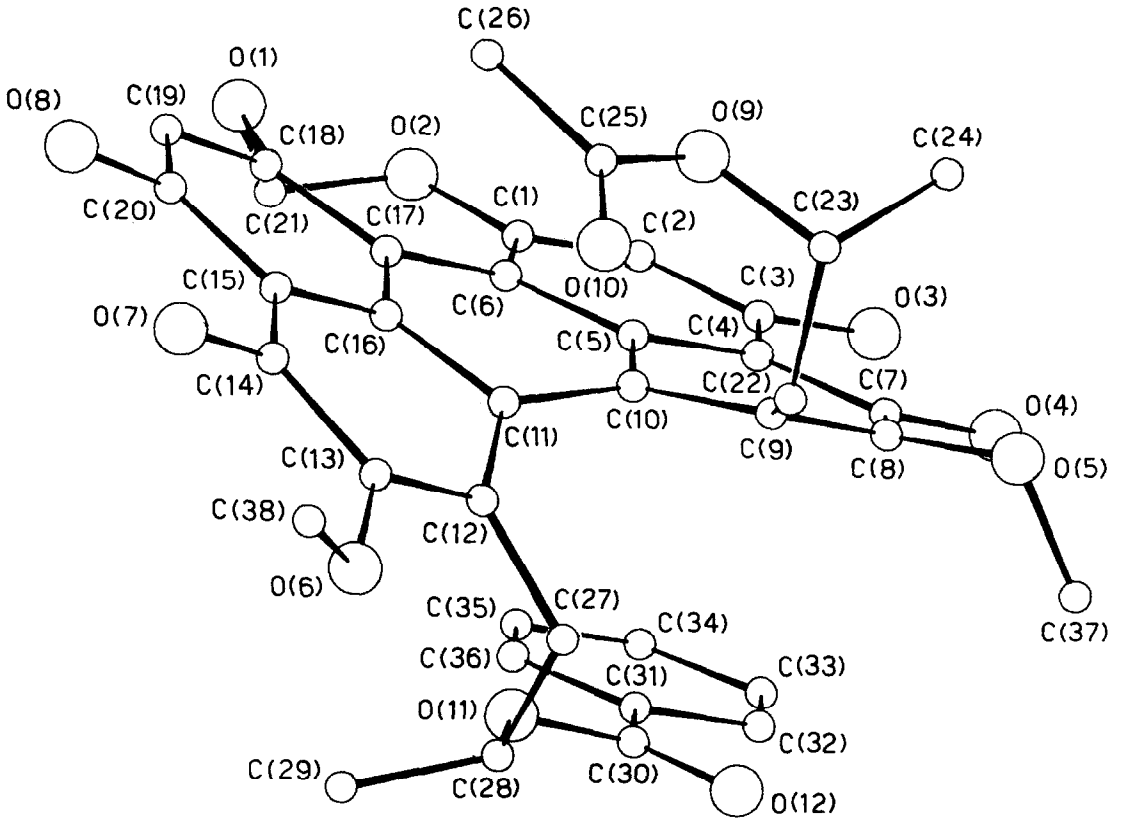


Fig. 1. Projection of the molecule (7a)

recorded with a Varian XL-100-15 spectrometer; the chemical shifts are given in ppm (δ) relative to internal Me_4Si. Mass spectra were measured with a Hitachi RMU6D instrument, at 70 eV. Column chromatography and tlc were performed with silica gel. Where not otherwise indicated, the purity of the products was checked by tlc, NMR and MS and deemed sufficient for the purpose of structural elucidation.

Isolation and purification of cercosporin (1a).

A strain of Cercospora Kikuchii 128.27 obtained from Centraal Bureau voor Schimmelcultures, Baarn, grown on potato-agar in Roux flasks was extracted twice with EtOAc after 2 weeks growth at room temp. The extracts were dried on Na_2SO_4 and evaporated in vacuo at 30°. Pure cercosporin (1a)[1] (30 mg for flask) was obtained after dissolution in $CHCl_3$ of the extracts, filtration and precipitation with hexane.

Isocercosporin (1b).

0.5 g of cercosporin, dissolved in 100 ml of toluene were refluxed for 30 min; evaporation of the solvent and PLC (Merck plates) with C_6H_6-Et_2O-formic acid (50:50:1% v/v) as eluent gave 200 mg of pure (1b) and about 200 mg of unchanged (1a).

Oxidation with CrO_3-Pyridine of (1a).

200 mg of CrO_3 were added to 320 mg of dry pyridine in 5 ml of dry CH_2Cl_2 and stirred at room temp. for 15 min; 100 mg of (1a) dissolved in CH_2Cl_2 were added. After 30 min the organic phase, washed with water, was purified by PLC using C_6H_6-Et_2O-formic acid (50:50:1% v/v). The elution of the upper band gave 20 mg of the compound (3a) red powder, m.p. 135-138°, λ_{max}^{nujol} cm^{-1} 1710 (aliphatic CO). Mass 532 (M^+), 514 (M^+-18). ^{1}H-NMR ($CDCl_3$); δ 0.52 (d, J=6, 2 Me), 2.07 (s, COMe), 2.5-2.8 and 3.2-3.8 (m, CH (OH)-Me and CH_2-CO), 4.05 and 4.19 (s, 2 OMe), 5.68 (s, OCH_2O), 6.92 and 7.01 (s, 2 arom. H), 14.74 and 14.84 (2 chel. OH).

Methylation of cercosporin.

To 15 ml of a dry acetone solution containing 300 mg of (1a), 1.2 g of

Table 5. Selected torsion angles (°)

C23-O9-C25-C26	175.1(4)	C12-C27-C28-O11	66.0(3)
C23-O9-C25-O10	-5.4(6)	C27-C28-O11-C30	-82.9(3)
C22-C23-O9-C25	-80.8(4)	C29-C28-O11-C30	150.7(3)
C24-C23-O9-C25	152.5(4)	C28-O11-C30-O12	-3.9(5)
C9-C22-C23-C24	-51.3(4)	C28-O11-C30-C31	175.7(3)
C9-C22-C23-O9	69.5(4)	O11-C30-C31-C36	-1.8(5)
C8-C9-C22-C23	86.3(4)	O11-C30-C31-C32	177.5(3)
C10-C9-C22-C23	-85.7(4)	O12-C30-C31-C32	-2.9(5)
C37-O5-C8-C7	-74.8(4)	O12-C30-C31-C36	177.8(3)
C37-O5-C8-C9	114.3(4)	C1-C6-C17-C18	-10.0(5)
O4-C7-C8-O5	9.6(4)	C6-C17-C18-O1	8.2(5)
C38-O6-C13-C12	145.3(4)	C17-C18-O1-C21	-38.8(4)
O6-C13-C12-C27	-8.2(4)	C18-O1-C21-O2	-88.5(3)
O6-C13-C12-C11	-179.0(3)	O1-C21-O2-C1	-76.3(4)
C13-C12-C27-C28	91.2(4)	C21-O2-C1-C6	-22.5(4)
C12-C27-C28-C29	-54.8(4)	O2-C1-C6-C17	-4.2(5)
C11-C12-C27-C28	-79.6(4)		

Table 6. Equations of least-squares planes with atomic deviations (Å) and angles between planes (°)

1. C1-C6 $0.1042\,X + 0.8425\,Y - 0.5285\,Z = 2.5498$
C1 0.008, C2 0.001, C3 -0.008, C4 0.006, C5 0.002, C6 -0.010, C17 0.070, O2 0.026, C10 -0.071, C7 0.041, O3 -0.013

2. C4,C5,C7-C10 $0.1063\,X + 0.8920\,Y - 0.4393\,Z = 2.2394$
C4 0.030, C5 0.058, C10 -0.125, C9 0.083, C8 0.016, C7 -0.073, C22 0.548, O5 0.230, O4 -0.138, C3 0.122, C6 0.184, C11 -0.447

3. C5,C6,C17,C16,C11,C10 $0.1813\,X + 0.7716\,Y - 0.6097\,Z = 2.7851$
C5 0.005, C6 -0.110, C17 0.061, C16 0.037, C11 -0.145, C10 0.123, C12 -0.584, C15 0.286, C18 0.294, C1 -0.279, C4 -0.070, C9 0.570

4. C11-C16 $0.0642\,X + 0.5868\,Y - 0.8072\,Z = 2.9607$
C11 0.148, C12 -0.080, C13 -0.042, C14 0.098, C15 -0.033, C16 -0.090, O6 -0.276, O7 0.214, C10 0.602, C17 -0.281, C20 -0.065

5. C15-C20 $-0.0160\,X + 0.6879\,Y - 0.7256\,Z = 2.4664$
C15 -0.044, C16 0.001, C17 0.027, C18 -0.056, C19 0.012, C20 0.039, O1 -0.178, O8 0.129, C11 0.117, C14 -0.134, C6 0.162

6. C31-C36 $0.1066\,X + 0.8777\,Y - 0.4672\,Z = -1.1576$
C31 -0.003, C32 0.000, C33 0.008, C34 -0.010, C35 0.002, C36 0.003, C30 0.004, O11 0.053, O12 -0.041

7. O9,C25,O10,C26 $0.1437\,X + 0.5402\,Y - 0.8292\,Z = 6.8593$
O9 0.000, C25 0.003, O10 -0.001, C26 -0.003

8. C1,C6,C18,O1 $0.0014\,X + 0.7682\,Y - 0.6402\,Z = 2.5727$
C1 -0.007, C6 0.008, C18 -0.009, O1 0.002, O2 -0.197, C17 -0.082, C21 -0.841

1-2 = 5.9	1-3 = 7.6	2-3 = 12.7
3-4 = 17.0	3-5 = 14.0	4-5 = 8.8

Ag_2O and 3 ml of MeI were added. The solution was kept stirring for 10 h at room temp. and in the dark. The reaction mixture was then filtered and washed with acetone, the filtrate evaporated and chromatographed by PLC using $CHCl_3$-MeOH (90:10). Two main compounds were eluted, dimethylcercosporin (4a) and a trimethyl-derivative.

Dimethylcercosporin (4a).

90 mg, orange-red crystals (from acetone), m.p. 100-105°, UV λ max (nm): 222, 261sh, 270, 330 and 460 (ε 45,000, 32,800, 34,600, 5200, 27,800); CD (in EtOH, c $1.42.10^{-2}$ g/100 ml): 233, 249, 294,365, 402, 480 nm (Δε : +12.25, +11.5, -11.8, -5.5, -4.3, +7.9); λ_{max}^{nujol} cm^{-1} 1620 (conj. CO). Mass 564 (M^+ +2) ^{1}H-NMR

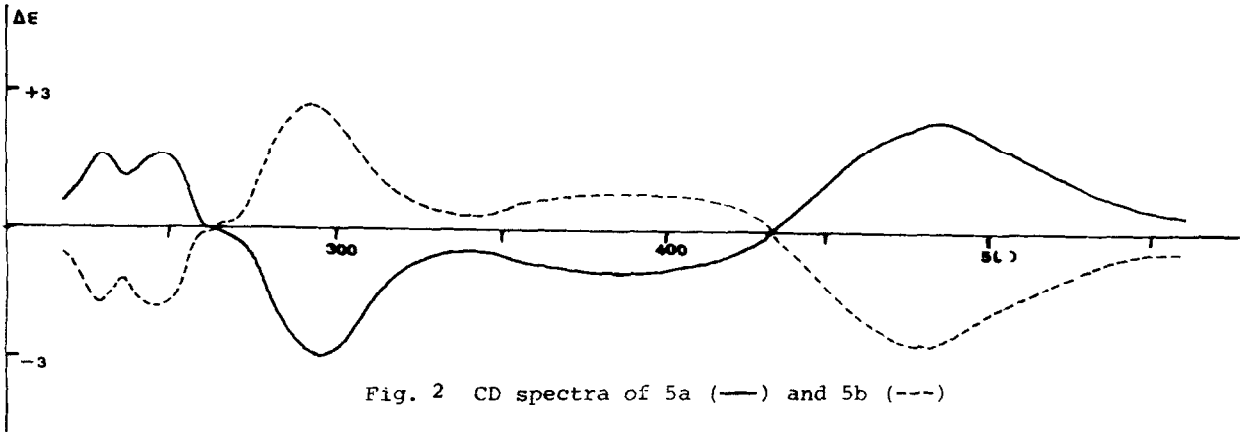


Fig. 2 CD spectra of 5a (——) and 5b (---)

(acetone-d_6); δ 0.53 (d, J = 6, 2Me), 2.6-2.8 and 3.3-3.6 (m, 2-CH_2), 3.8-3.95 (m, 2-CH-OH), 4.00 (s, 4OMe), 5.78 (s, OCH_2O), 7.14 (s, H-5 and H-8).

Trimethylcercosporin.

150 mg, red solid, m.p. 115°, UV λ max (nm): 223, 273, 332 and 475 (ε 39,000, 36,000, 6,200, 17,500). Mass 578 (M^+ +2), 545, 460, 445.

Trimethylcercosporin monoacetate.

To 2 ml of pyridine, 100 mg of the trimethylderivative and 4 ml of acetic anhydride were added. The solution was allowed to stand overnight. It was then poured into ice-water, the orange-yellow precipitate was collected and chromatographed by PLC using $CHCl_3$-MeOH (15:1), to give 80 mg of a glassy solid-^{1}H-NMR (acetone-d_6); δ 0.43 (Me-CH-OMe), 0.64 (Me-CH-OAc), 1.58 (OAc), 2.9-3.5 (CH_2-CH-OAc, CH_2CH-OMe, J=7.0 and 5.5), 3.05 (CHOMe), 4.05 (4 OMe), 4.64 (CH-OAc), 5.83 (OCH_2O), 7.22 (H-5 and H-8).

Dimethylisocercosporin (4b).

Isocercosporin (350 mg) in 20 ml of dry acetone was methylated with Ag_2O (1 g) and MeI (4 ml) for 20 h at room temp. Filtration, evaporation and PLC with CH_2Cl_2-MeOH (15:1) gave the dimethylisocercosporin (4b) and the trimethylderivative. (4b). 130 mg, red crystals, m.p. 165° and 255-256°, UV λ max (nm): 261sh, 269, 330 and 465 (ε 32,700, 33,800, 5,100, 26,400); CD (in EtOH, c $1.36.10^{-2}$ g/100 ml): 229, 249, 270, 294, 360, 400, 480 nm (Δε : -12.15, -10.3, +3.9, +12.36, +4.9, +3.3, -8.86). Mass 564 (M^+ +2), 562, 531, 518, 499, 460, 445, 429; ^{1}H-NMR (acetone-d_6); δ 0.92 (d, J=6, 2Me), 2.7-3.0 and 3.2-3.4 (m, -2CH_2), 3.5-3.7 (m, 2CH-OH), 4.01-4.02 (4OMe), 5.83 (s, OCH_2O), 7.16 (s, H-5 and H-8).

Trimethylisocercosporin.

160 mg as a glassy solid, m.p. 135-140°; Mass 578 (M^+ +2), 576, 545, 459, 340.

Oxidation with CrO_3-Pyridine of (4a).

100 mg of (4a) were oxidized with the CrO_3-Py complex as described for (1a) at room temp.; after 4 hours the organic phase was chromatographed by PLC using CH_2Cl_2-MeOH (15:1). The elution of the upper band gave 20 mg of (5a) as a red powder, m.p. 210°, ν_{max}^{nujol} cm^{-1} 1720 (aliphatic CO); UV λ max (nm) 266, 332, 465 and 530sh (ε 13,850, 2000, 9400, 2600); CD (EtOH, c $1.48.10^{-2}$ g/100 ml): 232, 249, 290, 482 nm (Δε :+3.01, +3.01, -3.58, +3.20). Mass 560 (M^+ +2), 542, 527, 500, 485, 471, 457. ^{1}H-NMR (pyridine-d_5) δ 2.07 (s, 2Me-CO), 3.93 (s, 2OMe), 4.15 (s, 2OMe), 4.00 and 4.60 (2 arylCH_2CO, AB, J=16), 5.82 (OCH_2O), 7.12 (s, H-5 and H-8). 50 mg of (6) were also obtained from the same reaction, m.p. 125°, ν_{max}^{KBr} cm^{-1} 1720(aliphatic CO); UV λ max (nm 270, 332 and 460 (ε 27,750, 5800, 14,500). Mass 562 (M^+ +2), 542 (M^+ -18), 525, 509. ^{1}H-NMR ($CDCl_3$); δ 0.62 (d, J=6, Me), 2.06 (s, COMe), 2.4-2.8 and 3.4-3.8 (m, CH_2-CHOH-Me and CH_2-CO), 3.98, 4.08, 4.10 and 4.12 (s, OMe), 5.70 (OCH_2O), 7.06 and 7.09 (s, 2 arom. H).

Oxidation with CrO_3-Pyridine of (4b).

100 mg of (4b) were oxidised with CrO_3-py as for (4a) at room temp. and (5b) was separated by PLC in CH_2Cl_2-MeOH (15:1) as eluent; m.p. 198-200°, CD (EtOH, c $1.35.10^{-2}$): 230, 250, 290, 482 (Δε -2.90, -2.85, +3.40, -3.29); Mass 560 (M^+ +2), 558 (M^+), 542, 527, 499. ^{1}H-NMR ($CDCl_3$): δ 2.06 (s, 2Me-CO), 3.5-4.2 (AB, J=16, 2 arylCH_2), 4.00 (s, 2OMe), 4.10 (s, 2OMe), 5.70 (OCH_2O), 7.08 (s, H-5 and H-8).

Noranhydrocercosporin (8).

(1a) (100 mg) was dissolved in 5 ml

of concentrated sulphuric acid and, after 10 min, the mixture was poured in ice. The precipitate was collected, dried, and chromatographed on acidic silica gel using $CHCl_3$-MeOH (15:1) as eluent. (8) (70 mg) has m.p. 300°, UV λ max (nm) 274, 330sh, 525, 550sh (ε 21,950, 2700, 11,700; 9800); Mass 470 (M^+). The same product was obtained from (1b).

Compounds (8) from (1a) or (1b) were acetylated with pyridine and acetic anhydride in the usual manner. Both derivatives appear identical as shown by comparison of their NMR and CD spectra. m.p. 223-225°. CD (dioxane c $1.73.10^{-2}$): 249, 280.5, 306, 334, 430, 508 nm ($\Delta\varepsilon$: +8.1, +10.0, -2.6, +2.9, -1.33, +2.0).

Alkali degradation of (1a) with sodium methoxide and ethyleneglycol.

Cercosporin (300 mg) and sodium methoxide (300 mg) were heated in 5 ml of ethyleneglycol for 2 h at 100° with stream of N_2. The reaction mixture was poured into water and, after acidification with diluted HCl, extracted with chloroform. The organic layer was chromatographed by PLC (Merck plates) using benzene-ether-formic acid (50:50:1% v/v), to give two main yellow compounds (10) and (12).

(10) 100 mg, m.p. 230°, $[\alpha]_D^{20} = -153°$ (c 0.1; pyridine), UV λ max (nm) 263, 290sh, 334, 413 (ε 30,300, 12,000, 5500, 20,600); Mass 490 (M^+) (100), 431 (66), 401 (50), 399 (50).

Compound (10) was acetylated in the usual manner; PLC in hexane-EtOAc (50:50) gave (11). The triacetate of (10) has m.p. 230-235°; Mass 616 (M^+), 574 (M^+ -42), 532 (-42), 490 (-42), 431, 401. H^1-NMR ($CDCl_3$) δ 1.17 (d, J=6, Me-CHOAc), 1.40 and 2.20 (dd, J_{AB}=13, CH_2-CHOAc), 2.26, 2.40 and 2.50 (3OAc), 3.42 and 3.48 (J_{AB}=17, CH_2), 3.6 (CHOAc, 3.70 (OMe-2), 4.08 (OMe), 4.47 (H-2), 5.63 and 5.79 (J_{AB}=7, OCH_2O), 7.11 and 7.15 (H-5 and H-8).

(12) 30 mg, m.p. 270-275° (dec), UV λ max (nm) 262, 408 (ε 17,000, 12,500); Mass, 446 (M^+), 431, 417, 401, 385, 371.

The acetylation of (12) gave, after PLC in hexane-EtOAc (50:50) the diacetate (13) as a yellow solid, m.p. 155-160°; Mass, 530 (M^+), 488 (M^+-42), 446 (-42). ^{1}H-NMR ($CDCl_3$) δ 1.33 (s, Me), 2.28 and 2.30 (2OAc), 3.54 (s, CH_2), 3.76 (OMe-2), 4.13 (OMe-11), 4.29 (H-2), 5.56 and 5.92 (AB, J=7, OCH_2O), 7.11 and 7.15 (H-5, H-8).

Reaction of (4a) with 2-phenylbutyric anhydride.

65 mg of 2-phenylbutyric anhydride were added to 50 mg of (4a) in 1 ml of dry pyridine. The solution was kept for 20 h at room temp. (+)-2-phenylbutyric acid $[\alpha]_D^{20} = +2.8°$ (c = 0.5, pyridine) was obtained by working up the reaction mixture according to lit. 13.

Mutarotation of cercosporin.

Optical rotations were measured in acetylacetone at 589 nm using a 1 dm polarimetric tube. Mutarotation experiments were run in tubes immersed in a circulating oil bath. The first reading was normally taken 5 minutes after dissolution. The rate constants of mutarotation were obtained using the first order integrated rate law expression for the process of unimolecular mutarotation. The results are collected in table 1.

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