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John M. Jacyno, John S. Harwood, Horace G. Cutler, and Mee-Kyoung Lee

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ISOCLADOSPORIN, A BIOLOGICALLY ACTIVE ISOMER OF CLADOSPORIN FROM *CLADOSPORIUM CLADOSPORIOIDES*

JOHN M. JACYNO,*

College of Pharmacy, Ohio Northern University, Ada, Ohio 45810

JOHN S. HARWOOD,

NMR Facility, Department of Chemistry, University of Georgia, Athens, Georgia 30602

HORACE G. CUTLER,

USDA, ARS, Russell Research Center, PO Box 5677, Athens, Georgia 30613

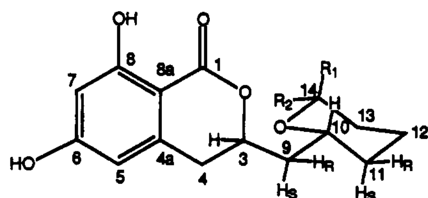
and MEE-KYOUNG LEE

Korea Ginseng and Tobacco Research Institute, Daejon, Korea 305-345

ABSTRACT.—Extraction of the fungus *Cladosporium cladosporioides* yielded the known isocoumarin, cladosporin [**1**], and a new compound. This metabolite, which inhibited the growth of etiolated wheat coleoptiles slightly more than did cladosporin, was characterized as a diastereoisomer of cladosporin at C-14 and was named isocladosporin [**2**].

Cladosporium cladosporioides (Fres.) de Vries (Dematiaceae) is a common phylloplane fungus and a contaminant of many types of seeds (1). Some strains of this species appear to be weakly phytopathogenic (2). Biologically active metabolites which have been isolated from *C. cladosporioides* include cladosporin [**1**] (1), cladospolides A and B (3), and the calphostins (4). Cladosporin inhibits the growth of etiolated wheat coleoptiles (5), cladospolides affect the growth of lettuce seedlings (3), and the calphostins are potent inhibitors of protein kinase C from animal tissue (6).

Continuing our examination of *C. cladosporioides*, we have isolated a new compound which proved to be a diastereoisomer of cladosporin and which we have named isocladosporin [**2**]. This report describes the isolation, characterization, and preliminary observations on the effects on plant growth of the new metabolite.



1 R_1=Me, R_2=H
2 R_1=H, R_2=Me

$J_{3\text{-}9R}$=9.3 (8.7) $J_{3\text{-}9S}$=2.3 (4.1)
$J_{10\text{-}9R}$=2.3 (2.7) $J_{10\text{-}9S}$=10.2 (10.4)
$J_{10\text{-}11R}$=ca. 1 (2.6) $J_{10\text{-}11S}$=9.6 (7.7)
$J_{9R\text{-}9S}$=14.7 (14.5)

FIGURE 1. Approximate solution state conformation of isocladosporin [**2**]. Relevant ^{1}H-^{1}H coupling constants are given in Hz. Values for cladosporin diacetate (11) are in parentheses.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were obtained using the following instruments: ^{1}H/^{13}C nmr, Bruker AMX 400; eims, Hewlett-Packard 5985B; uv, Beckman Model 35; ir, Analect FX-6160 FTIR. Mp's were measured on a microscope hot-stage apparatus and are uncorrected. Tlc was performed on Analtech Si gel GF #02521 Uniplates.

CULTURE AND ISOLATION.—*C. cladosporioides* (NRRL 5507) was grown at 25° for 5 days in 12 constantly shaken 2.8-liter Fernbach flasks, each containing 500 ml of a yeast (2%)/sucrose (15%) medium. The culture was then homogenized and filtered, and the filtrate was extracted with EtOAc. After evaporation of solvent, the residue was chromatographed on Si gel, eluting sequentially with C_6H_6, *t*-butyl Me ether, and EtOAc. Crude cladosporin [**1**] was obtained from the *t*-butyl Me ether and EtOAc fractions and, after further chromatography, recrystallized from Me_2CO. The mother liquor from the recrystallization was evaporated to dryness, and the residue was subjected to

gradient chromatography on Si gel, using a C_6H_6 (500 ml) to C_6H_6 (1 liter)/EtOAc (1 liter) gradient and collecting 20-ml fractions. A component with R_f 0.84 [hexane-EtOAc (1:1)] was present in fractions 40–46, and another, with R_f 0.75, corresponding to cladosporin, in fractions 47–64. Fractions 40–46 were combined and rechromatographed on C_{18} reversed-phase silica, eluting first with MeCN-H_2O (1:1), then with MeCN-H_2O (3:1). The desired component was present in fractions from the latter solvent system. On standing for several days at room temperature, a product (20 mg) **2** crystallized from the concentrated fractions as clusters of colorless needles.

Isocladosporin [**2**].—Mp 164–166°; uv (95% EtOH) λ max 218, 231 (shoulder), 269, 306; ir (KBr) ν cm^{-1} 3185 (br), 2932, 1671, 1629, 1606, 1464, 1385, 1334, 1260, 1239, 1159, 1114, 1098, 1045; eims *m/z* (rel. int.) 292 (7), 179 (15), 151 (23), 99 (100), 81 (44), 69 (10), 55 (8); ^1H and ^{13}C nmr see Table 1.

Cladosporin [**1**].—Ir (KBr) ν cm^{-1} 3234 (br), 2935, 1640 (shoulder), 1621, 1590, 1467, 1424, 1382, 1350, 1304, 1275, 1233, 1185, 1162, 1118, 1041, 694; ^1H and ^{13}C nmr see Table 1.

BIOASSAY.—The effect of isocladosporin [**2**] on the growth of etiolated wheat coleoptiles was determined as described previously for cladosporin [1] (5).

RESULTS AND DISCUSSION

The ms and uv spectra of **2** were almost identical to those of cladosporin [**1**]; the ir spectra were also quite similar, although clearly not identical. These observations strongly indicated that **2** was a stereoisomer of **1**. Two stereoisomers of cladosporin have previously been reported (7): one, mp 155–156°, was isolated as a minor metabolite from a strain of *Aspergillus flavus*; the other, mp 136–138°, was obtained as a side product from the reaction of 8-*O*-methylcladosporin with BCl_3. Our compound **2**, mp 164–166°, differs significantly in its ir and uv spectra from those reported for the higher melting isomer, although the four ir absorptions given for the lower melting isomer are very close in frequency to those appearing in the spectrum of **2**. Further comparison is precluded by the absence of any other spectroscopic data for the lower melting isomer.

The structure and approximate solution-state conformation of isocladosporin [**2**] were determined using 2D NOESY (8) and ROESY (9,10) nmr experiments, together with comparisons to the data published previously for cladosporin [**1**] (5) and its diacetate (5,11). The ^1H- and ^{13}C-nmr chemical shift assignments used in the structure and conformation determinations were obtained using 1D spectra and 2D COSY (12), TOCSY (13), and ^1H, ^{13}C chemical shift correlated (HETCORR) (12) spectra. Relevant ^1H-^1H coupling constants were obtained using 1D homonuclear decoupling experiments. In addition, we also obtained various spectra of an authentic sample of cladosporin [**1**] to compare with the isocladosporin [**2**] spectra and with the published assignments for **1** (5). The ^1H and ^{13}C chemical shift assignments for cladosporin and isocladosporin are listed in Table 1.

Comparisons of the ^1H and ^{13}C chemical shift assignments of **1** and **2** show that, in most cases, the chemical shifts of comparable nuclei are similar. The principal differences are observed in the chemical shifts of the Me-substituted oxane ring (see Table 1, C-10–C-15 and H-10–H-15). This suggested the possibility that there could be conformational differences between these regions of the cladosporin and isocladosporin molecules. This possibility was borne out by inspection of the NOESY and ROESY spectra of **2**, which showed a strong nOe response between H-10 and H-14 on the oxane ring. This response, which is absent in the ROESY spectrum of **1**, indicates that the H-10 and H-14 protons are both axial, and therefore spatially close enough to show an nOe. In turn, this requires that the oxane Me substitutent in **2** be equatorial, as opposed to its axial location in **1**. The TOCSY spectra of **1** and **2** are also consistent with the differing orientation of this Me group. The TOCSY spectrum of **2** showed a significant coupling between the H-10 and H-14 protons. This

TABLE 1. ^1H and ^{13}C Chemical Shifts of Cladosporin [1] and Isocladosporin [2].

Position	Compound			
	1		**2**	
	δ ^{13}C	δ ^1H	δ ^{13}C	δ ^1H
1	169.68		170.10	
3	76.31	4.70	75.94	4.82
4	33.66[a]	2.83	33.64	2.82
4a	141.85		141.94	
5	106.48	6.16[b]	106.67	6.17
6	162.34[c]		162.84[c]	
7	101.96	6.29[b]	101.92	6.30
8	164.34[c]		164.30[c]	
8a	102.04		101.69	
9	39.47[a]	1.96[e]	41.79	1.89[e]
		1.84[d]		1.75[d]
10	66.35	4.10	73.16	3.70
11	30.86	1.65[d]	31.86	1.56[d]
		1.35[e]		1.22[e]
12	18.19	ca 1.6	23.47	1.81
		ca 1.7		1.56
13	30.95	1.65	33.17	1.59
		1.35		1.21
14	67.83	3.99	74.02	3.47
15	18.92	1.23	22.02	1.15

[a,b]These assignments are reversed from those in Springer *et al.* (5).
[c]These assignments may be interchanged.
[d]*Pro-R* hydrogen.
[e]*Pro-S* hydrogen.

response was absent in the TOCSY spectrum of **1** obtained under the same conditions, suggesting also that the H-10 and H-14 protons in **2** are diaxial, whereas they are not in **1**.

Apart from the orientation of the oxane Me group, the stereochemistry and solution-state conformation of isocladosporin [**2**] are essentially the same as those reported for cladosporin diacetate (11). In order to compare the solution-state conformation of **2** to that of cladosporin diacetate, ^1H-^1H coupling constants were obtained for the H-3, H-9, H-10, and H-11 spin system. These coupling constants determine the approximate geometry of the C-3–C-9–C-10 linkage and are shown in Figure 1.

As can be seen from Figure 1, most of the coupling constants obtained for **2** are very similar to those obtained for cladosporin diacetate (11). The only significant differences are observed for the H-10–H-11 couplings, and these could be the result of small changes induced in the geometry of the oxane ring as a result of the Me group orientation (equatorial vs. axial) and not the result of changes in overall solution state conformation. Therefore, we infer that the geometry of the C-3–C-9–C-10 linkage is approximately the same in **2** as in cladosporin diacetate. This would suggest that the solution-state conformation of **2** closely resembles that reported for cladosporin diacetate (11), which, in turn, is essentially the same conformation as that reported in the X-ray crystal structure of cladosporin [**1**] (5).

However, we must point out that

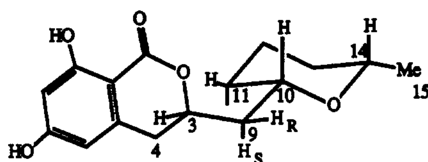


FIGURE 2. Possible alternative conformation of isocladosporin [**2**]. For clarity, one of the H-11 protons has been omitted.

there is an alternative conformation possible for the oxane portion of isocladosporin [**2**] that we have not been able to rule out on the basis of the available nmr data. [We thank a referee for pointing out the possibility of an alternative conformer.] This conformation is shown in Figure 2.

We had hoped to obtain nOe data that would strongly favor one of the two possible structures. Thus, with reference to Figure 1, we found strong nOe responses in the NOESY and ROESY spectra between the H-4 and H-9 protons. From models of this structure, we observed that the distance between the H-4 and H-9 protons should be comparable to the distance between the H-9 and H-11 protons, and we therefore hoped to see a fairly strong nOe interaction between the latter set of protons in support of this conformation. Unfortunately, we were only able to find weak evidence, in the form of a low-intensity cross-peak between H-9_S and H-11_R, of such an nOe interaction in a NOESY spectrum obtained with a mixing time of 700 msec; NOESY spectra obtained with mixing times of 300 and 500 msec and the ROESY spectrum (300 msec mixing time), did not show significant cross peaks between the H-9 and H-11 protons. Similarly, after building molecular models, we had hoped that the conformation shown in Figure 2 would exhibit an nOe response between the H-3 and H-11 protons. In this case, the same NOESY and ROESY spectra noted above failed to provide any evidence in confirmation of this particular structure. Finally, although the chemical shifts of many of the oxane ring protons and carbons are different, we cannot conclude a priori that any of those shift differences proves the existence of either possible structure. Thus, in the absence of any evidence to the contrary, we conclude, on the basis of the available nOe data, that the solution-state conformation of isocladosporin [**2**] is that depicted in Figure 1.

When tested in the etiolated wheat coleoptile bioassay, isocladosporin [**2**] was slightly more potent than cladosporin [**1**], producing 100% and 50% inhibition of growth, compared to 81% and 50% for **1**, at 10^{-3} and 10^{-4} M, respectively. No inhibition was observed from either compound at concentrations of 10^{-5} or 10^{-6} M.

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