

STRUCTURE AND STEREOCHEMISTRY OF PHASEOLINIC ACID: A NEW ACID FROM *MACROPHOMINA PHASEOLINA*

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Macrophomina phaseolina (Tassi) Goid (Phomaceae) is a widely distributed fungus reported to have phytotoxic activity (1,2). The fungus produces a nonspecific exotoxin that inhibits the seed germination of *Phaseolus mungo* L. (3). The structure of the toxin, phaseolinone, isolated from the culture filtrate of the fungus has been reported (4). This paper reports the isolation of a new acid designated phaseolinic acid [**1**] from the culture filtrate and determination of its structure and stereochemistry on spectral grounds and by single crystal X-ray crystallography. The acid **1**, unlike phaseolinone [**2**], does not display any phytotoxic activity.

Phaseolinic acid [**1**] was found to have the molecular formula $C_{11}H_{18}O_4$ on the basis of its IR. It exhibits IR absorption bands at 3150, 1760, and 1740 cm^{-1} attributable, respectively, to hydrogen bonded hydroxyl, five-membered lactone, and acid carbonyl. The 1H -NMR spectrum displays signals that are in conformity with the depicted structure. The MS shows fragment ion peaks which are in accordance with the structure.

In order to determine the structure

unambiguously, a single crystal X-ray analysis of **1** was carried out. Suitable crystals of **1** for X-ray analysis were obtained from EtOAc. An ORTEP (5) drawing of the structure (less hydrogen atoms) is shown in Figure 1. The fractional atomic co-ordinates are listed in Table 1.

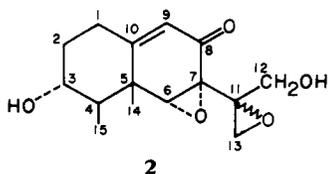
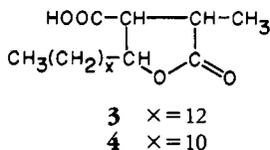
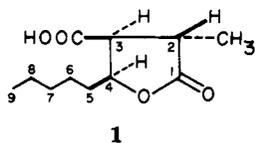
The CD curve of phaseolinic acid shows a positive maximum ($\Delta\epsilon = +1.76$, $c = 7.47$ mmol/liter in dioxane) due to the lactone chromophore. According to the Klyne lactone sector rule (6) or the Sneath lactone rule (7), the absolute configuration of phaseolinic acid is considered to be 2*R*, 3*R*, and 4*R* as shown in Figure 1.

It may be mentioned that phaseolinic acid [**1**] is a homologue of nephromopsic acid [**3**] (8-10) and nephrosteranic acid [**4**] (11,12) which have been isolated from lichens. However, it appears that absolute conformations of both the acids **3** and **4** are yet to be settled.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—

The IR spectrum was recorded in a nujol mull on a Perkin-Elmer model 177 instrument, and the



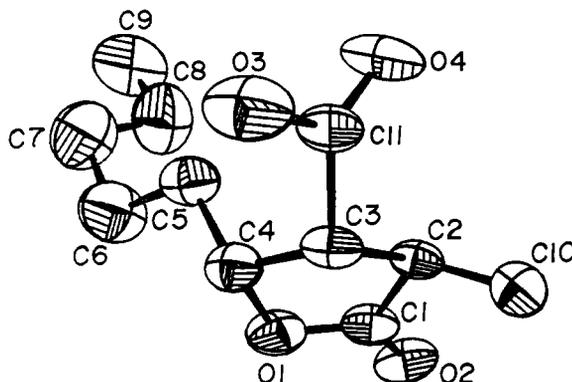


FIGURE 1. An ORTEP drawing of phaseolinic acid [1].

^1H -nmr spectrum was obtained on a JEOL FX-100 Fourier-transform spectrometer operating at 99.6 MHz in CDCl_3 . The low resolution mass spectrum was taken on a Hitachi model RMU-6L mass spectrometer operating at 70 eV, and the high resolution mass spectrum was recorded on a JEOL JMS-DX 300 instrument. Tlc analysis was carried out on silica gel plates (Merck 60 HF₂₅₄, solvent C_6H_6 -MeOH, 90:10), and the compound was visualized under uv light. The crystallographic analysis was carried out on a Rigaku AFC-5 FOS four circle diffractometer using graphite monochromated $\text{Cu-K}\alpha$ radiation. Cd analysis was done in a JASCO J-20A automatic recording spectropolarimeter.

FUNGAL MATERIAL.—The fungus *M. phaseolina* was identified by Dr. A.K. Banerjee, Burdwan University, Burdwan, West Bengal, India. A voucher specimen is preserved in The Department of Microbiology of the Indian Institute of Chemical Biology.

ISOLATION OF PHASEOLINIC ACID [1].—The

fungus *M. phaseolina* was grown in rice medium (50g/250 ml H_2O) for 10 days at 30° . The unused rice and mycelial mat were separated by filtration under suction, and the residue was washed with H_2O (200 ml). The filtrate was treated with activated charcoal, washed with distilled H_2O , dried, and eluted with CHCl_3 . CHCl_3 was distilled off, the residue was dissolved in a minimum volume of MeOH, and the solution was poured into excess H_2O and centrifuged (10,000 rpm). The H_2O layer contained phaseolinone [2]. The H_2O insoluble part was dried, dissolved in Et_2O , and to this solution was added an equal volume of *n*-hexane when precipitates appeared which were separated by centrifugation. The clear solution thus obtained was concentrated and left overnight when a crystalline solid was obtained. This crystalline matter on chromatographic purification on a silica gel column followed by crystallization from EtOAc yielded needles of phaseolinic acid [1], 50 mg from 100 g infected rice. Mp 139 – 140° ; $[\alpha]_D -150^\circ$ (c , 0.2 CHCl_3); $\text{ir } \nu_{\text{max}}$ 3100–3200 (hydrogen bonded OH), 1760 (five mem-

TABLE 1. Fractional Atomic Coordinates ($\times 10^4$)

Atom	x	y	z
C1	1611 (6)	0 (19)	9812 (14)
C2	1117 (5)	1785 (16)	8472 (12)
C3	1587 (5)	2106 (15)	6441 (12)
C4	2398 (5)	1636 (19)	7330 (14)
C5	2867 (6)	3707 (20)	8271 (16)
C6	3617 (6)	3001 (27)	9297 (21)
C7	4076 (6)	5035 (34)	10413 (20)
C8	3762 (7)	5837 (33)	12577 (22)
C9	4278 (9)	7750 (32)	13837 (23)
C10	329 (6)	1119 (22)	8030 (15)
C11	1498 (5)	4624 (18)	5440 (13)
O1	2316 (3)	-128 (13)	9148 (10)
O2	1422 (4)	-1168 (13)	11361 (10)
O3	1211 (4)	6288 (13)	6249 (10)
O4	1768 (4)	4687 (13)	3387 (9)

bered lactone), 1740 (acid carbonyl), 1260, 1190, 1128, 1020, 990, 935, 870, 700 cm^{-1} ; ^1H nmr (CDCl_3 , 99.6 MHz, δ) 0.76-1.76 (14 H, m, methyl and methylene protons), 2.88-3.32 (2H, m, 2-H and 3-H), and 4.68 (1H, m, 4-H); ms m/z (30 eV) (%) 214 (3.5) (M^+), 196 (13.1) ($\text{M}^+ - \text{H}_2\text{O}$), 168 (21.9) ($\text{M}^+ - \text{H}_2\text{O} - \text{CO}$), 155 (17.5) ($\text{M}^+ - \text{CO}_2 - \text{CH}_3$), 143 (100) ($\text{M}^+ - \text{C}_5\text{H}_{11}$), 115 (65.7), 97 (61.3), 87 (62), 69 (54), 55 (40.8). Accurate mass m/z 214.1198, calcd for $\text{C}_{11}\text{H}_{18}\text{O}_4$, 214.120.

CRYSTALLOGRAPHIC MEASUREMENTS.—Colorless crystals of phaseolinic acid were obtained from ETOAc. A specimen with dimensions ca $0.15 \times 0.45 \times 0.90$ mm was selected for the X-ray measurements. The 993 unique intensities, which included both observed (=972) and unobserved (=21) reflections, were collected by the 2θ - ω scan method within $2\theta < 120^\circ$ on a Rigaku AFC-5 FOS four circle diffractometer using graphite monochromated $\text{Cu-K}\alpha$ ($\lambda = 1.5418 \text{ \AA}$) radiation. Crystal data¹: $\text{C}_{11}\text{H}_{18}\text{O}_4$, $a = 17.8400$ (41), $b = 5.6135$ (6), $c = 5.9977$ (7) \AA , $\beta = 94.699$ (14), $V = 598.62$ (17) \AA^3 , space group monoclinic, $P2_1$ ($Z = 2$). All the non-hydrogen atomic positions were revealed by the direct method (MULTAN) (13). The positions of hydrogen atoms except for that of the carboxylic acid proton were generated computationally on the basis of stereochemical and geometrical considerations. The block-diagonal least-squares refinements for the total reflections with anisotropic thermal factors for non-hydrogen atoms and isotropic thermal factors for hydrogen atoms converged to the final R value of 0.089 (UNICS III) (14).

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¹Atomic coordinates for the structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Rd., Cambridge, CB2 IEW, UK.