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STRUCTURE OF PHASEOLINONE, A NOVEL PHYTOTOXIN FROM MACROPHOMINA PHASEOLINA

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Abstract: Structure of phaseolinone, a phytotoxin isolated from the fungus Macrophomina phaseolina (Tassi) Goid, has been established as 1.

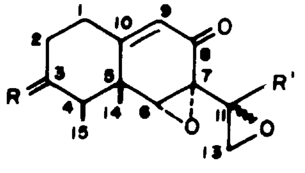
Macrophomina phaseolina (Tassi) Goid is a widely distributed fungus which causes inhibition of seed germination, wilting and root rot of many plants including soya bean, ground nut, beans, tobacco etc.[1] The fungus is known to produce a nonspecific exotoxin, which inhibits the seed germination of black gram (Phaseolus mungo L.)[2]. Herein we present evidences that the toxin designated as phaseolinone, isolated[3] from the culture filtrate of the fungus has the eremophilane structure 1.

Phaseolinone (1), $C_{15}H_{20}O_5$; m.p. 126-128°C; $[\alpha]_D$ +94.5° (c 0.8, EtOH); ν_{max} (nujol) 3600-3360 (OH), 1668, 1628 (C=C-C=O) cm^{-1}; λ_{max} (EtOH) 245 nm (ϵ 10,000); CD(MeOH): $\Delta\epsilon_{332}$ +3.3, $\Delta\epsilon_{247}$ +5.65, $\Delta\epsilon_{219}$ -3.3, formed an amorphous diacetate (2), $[\alpha]_D$ +120.7° (c 0.7, EtOH); ν_{max} (nujol) 1740, 1670, 1630 cm^{-1}; λ_{max} (EtOH) 245 nm (ϵ 8,000); m/z 322(M^+-42). The IR, UV, PMR (Table I) and CMR spectra[4] of 1 and 2 indicated the presence of one secondary methyl, one tertiary methyl, one primary alcohol, one secondary alcohol, one 1,1-disubstituted epoxide, one trisubstituted epoxide, one β-substituted six membered enone, two methylenes and three tetrasubstituted sp^3 carbons in 1.

Evidence for the epoxypropanol side chain was obtained as follows. Treatment of 2 with Ac_2O/NaOAc (100°C, 6 hr) yielded the triacetate (3, M^+,m/z 424), while benzoylation of 1 with PhCOCl/pyridine yielded the benzoate (4), ν_{max} (nujol) 1720, 1665 and 1600 cm^{-1}; m/z 386, 384 (M^+-2 x Ph-COOH). Treatment of 1 with 4N sulphuric acid in acetone yielded the acetonide (5), ν_{max} (nujol) 3520, 3420, 1640 cm^{-1}, m/z 323 (M^+-15) for which the alternative 1,3-acetonide structure could be ruled out from the deshielding of the -CH_2OH protons on acetylation to 6. Treatment of 1 with 0.1N aqueous sulphuric acid (60°C, 12 hr) yielded the tetrol (7), ν_{max} (nujol) 3530-3000, 1640 cm^{-1}; λ_{max} (MeOH) 242nm (ϵ 10,000). The naphthofuran (8) was obtained as a minor product, the yield of which increased to about 50% on longer reaction time (48 hr). The same compound could also be obtained from 7 under similar conditions. Periodate oxidation of 7 followed by acetylation yielded the amorphous acetate (9), the IR spectrum of which showed absorptions for OAc (1710 cm^{-1}), COOH (3620-3360, 1660 cm^{-1}) and enone groups (1660 and 1600 cm^{-1}) and the PMR spectrum lacked the signals for C-12 and C-13 protons. The UV (λ_{max} 243 nm) and CD ($\Delta_{\epsilon 248}$ +7.3, $\Delta_{\epsilon 328}$ +1.7) spectra were also very similar to those of 1. On oxidation with pyridinium dichromate 1 yielded the ketone (10), ν_{max} ($CHCl_3$) 3560–3420, 1710, 1660 cm^{-1}; m/z 278 (M^+) and the crystalline keto aldehyde (11), m.p. 202°C, ν_{max} (nujol) 1720, 1710 and 1670 cm^{-1}; m/z 276 (M^+), the PMR spectrum of which showed deshielding of one of the C-13 protons by about 0.5 ppm.

The part structure CH_3-CH-CH(OH)-CH_2-CH_2-C=CH-C=O was derived by homonuclear proton spin decoupling experiments on 2 and 4 which permitted all the chemical shifts and coupling constants of these protons to be determined (Table I). A rational combination of the two part structures and the rest of the molecule i.e. one tertiary methyl, one quarternary carbon and one trisubstituted epoxide led to the structure 1 for phaseolinone. The equatorial orientation of the C_3-OH and C_4-Me groups could be deduced from the coupling constants of the C-3 and C-4 protons. The *cis* relationship of the C-5 and the C-7 substituents was inferred from the observation that modifications of the C-11, C-12 and C-13 functionalities considerably affected the chemical shift of the C-14 protons.

Supporting evidence for the structure of phaseolinone was obtained by its acid catalysed dehydration, (*vide supra*) to the naphthofuran 8, $C_{15}H_{14}O_2$,

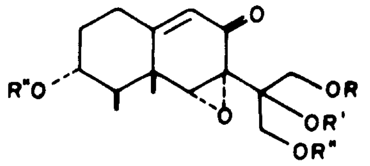


1 R = $<^{\cdot OH}_{H}$, R' = $\overset{12}{C}H_2OH$

2 R = $<^{\cdot OAc}_{H}$, R' = CH_2OAc

10 R = O, R' = CH_2OH

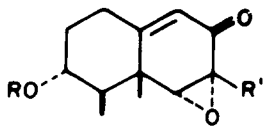
11 R = O, R' = CHO



3 R" = R = Ac, R' = H

5 R" = H, R + R' = $\times^{CH_3}_{CH_3}$

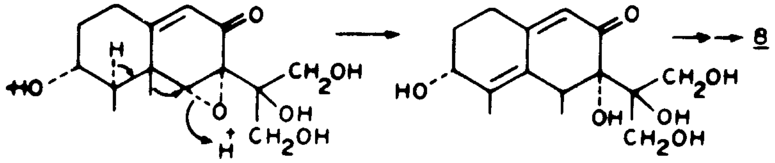
6 R" = Ac, R + R' = $\times^{CH_3}_{CH_3}$



9 R = Ac, R' = COOH

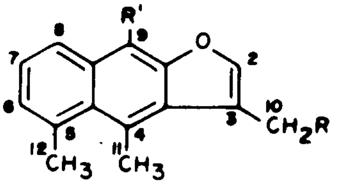
4 R = COPh, R' = $-\overset{Cl}{C}(CH_2OCOPh)_2$

15 R = H, R' = $-\underset{\underset{CH_2}{\|}}{C}-CH_2OH$



7

14



8 R = OH, R' = H

12 R = H, R' = OAc

13 R = OAc, R' = H

Table I. PMR Chemical shifts (∂ units) of phaseolinone and its derivatives (100 MHz, $CDCl_3$)*

Compound	14-H	15-H	13-H	6-H	9-H	12-H	3-H	Others
1	1.13	1.22d (6.6)	3.11d(4.6) 2.62d(4.6)	3.62	5.75d (1.5)	4.14d(12.2) 3.84d(12.2)	3.60m (4.3,11,11.4)	-
$2^{\dagger}$	1.18	1.13d (6.8)	2.99d(4.9) 2.65d(4.9)	3.56	5.76d (1.5)	4.66d(12.2) 4.40d(12.2)	4.84m (4.3,11,11.5)	2.08,2.07(OAc)
3	1.26	1.16d (6.6)	4.44d(12) 4.30d(12)	3.82	5.74d (1.5)	4.76d(12) 4.52d(12)	4.88m (4.3,11,11.5)	2.01,2.04,2.08(OAc); 3.38(OH)
$4^{\dagger}$	1.36	1.22d (7)	4.22d(12) 3.90d(12)	4.02	5.84d (1.5)	5.06d(12) 4.85d(12)	5.18m (4.3,11,11.4)	7.56m,8.12m(Ar)
5	1.20	1.26d (7)	3.71s	3.71	5.72d (1.5)	4.16s	3.62m (4.3,11,11.4)	1.43,1.33(Me_2)
6	1.27	1.15d (7)	4.39d(12) 4.22d(12)	3.74	5.75d (1.5)	4.28d(8.3) 4.16d(8.3)	4.90m (4.3,11,11.3)	2.01,2.07(OAc); 1.42,1.31(Me_2)
7	1.20	1.28d (7)	3.86d(12) 3.73d(12)	3.87	5.76d (1.5)	4.06d(12) 3.90d(12)	3.65m (4.3,11,11.3)	-
9^{**}	1.24	1.06d (7)	-	3.50	5.70brs.	-	4.83m (4.3,11,11.5)	2.04(OAc)
10	1.12	1.22d (7)	3.16d(4.2) 2.65d(4.2)	3.60	5.94d (1.5)	4.20d(12.2) 3.91d(12.2)	-	-
11	1.27	1.24d (6.6)	3.18d(4.5) 3.10d(4.5)	3.50	6.09brs.	9.36	-	-

* Figures in parentheses indicate J values in Hz. ** In DMSO - D_6

† For $-CH_2CH_2-$

∂	H-1ax	H-1eq	H-2ax	H-2eq	H-4	J	1ax,1eq	1ax,2ax	1ax,2eq	2ax,2eq
2	2.56	2.34	1.42	2.16	2.03		14.0	14.0	5.0	13.0
4	2.56	2.41	1.58	2.30	2.25		14.0	14.0	4.2	13.5

m.p. 128-32°C, ν_{max} (nujol) 3460-3300 (OH) cm^{-1}; λ_{max} (MeOH) 242 (ϵ57,700), 248 (ϵ62,250), 304 (ϵ5,540), 316 (ϵ7,250), 327 (ϵ6,100) and 342 (ϵ5,540) nm. The structure of 8 follows from the UV spectrum which is in excellent agreement with that reported[5] for the naphthofuran (12) and from the PMR spectrum which showed signals for one Ar-CH_2OH (δ 4.94, CH_2; δ 1.70, OH; $J_{CH,OH}$ 5.5 Hz; δ 5.35, CH_2; δ 2.10, $COCH_3$ in acetate 13), two Ar-CH_3 (δ 2.96 and 3.14) and five aromatic protons (7.25 *m*, H-7 and H-8; 7.64*s*, H-2; 7.70*s*, H-9; 7.70*m*, H-6). The PMR spectrum recorded in presence of $Eu(fod)_3$ not only simplified the aromatic signals but the relative LIS values ($\Delta\delta$ H-2, 0.88; H-6, 0.14; H-7, 0.11; H-8, 0.10; H-9, 0.23; H-11, 0.72; H-12, 0.09; H-10, 2.06; OH, 9.78 ppm) indicated the proximity of H-2 and 4-Me group to the CH_2OH group. The formation of 12 is presumably triggered by protonation of the 6,7-epoxide group with migration of the methyl group and elemination of C-4 proton followed by cyclisation and dehydration of the intermediate (14). This provides additional support for the relative stereochemistry at C-4, C-5 and C-6 in 1.

Thus, phaseolinone is the 11,12-epoxide of phomenone (15) isolated earlier from *Phoma exigua*[6]. Comparison of the CD and PMR data of the two compounds indicates that they have not only same stereochemistries at C-5, C-6 and C-7 but also the same absolute stereochemistry. The stereochemistry of phaseolinone at C-11, however, remains to be settled.

References and Notes

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2. K.A.I. Siddiqui, A.K. Gupta, A.K. Paul and A.K. Banerjee, *Experientia*, 35, 1222 (1979).
3. Isolation was guided by bio-assay against seed germination of *P. mungo*. The pure toxin causes complete inhibition of seed germination of *P. mungo* at a concentration of 25 µg/ml. It also possesses strong mutagenic property against *E. Coli* and *Salmonella* spp. (K.A.I. Siddiqui, unpublished results).
4. ^{13}C Chemical shifts (δ values) in $CDCl_3$ of 1 and 2 (in parenthesis) : C-1, 30.91 (30.47); C-2, 35.02 (31.17); C-3, 70.72 (72.74); C-4, 44.45 (41.45); C-5, 41.16 (41.15); C-6, 63.75 (63.58); C-7 62.80 (61.99); C-8, 193.60 (192.27); C-9, 120.60 (120.88); C-10, 164.90 (162.56); C-11, 56.81 (54.89); C-12, 61.70 (63.23); C-13, 48.74 (48.84); C-14, 18.80 (18.55); C-15, 11.32 (11.23).
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