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# SOLANAPYRONES A, B AND C, PHYTOTOXIC METABOLITES FROM THE FUNGUS ALTERNARIA SOLANI

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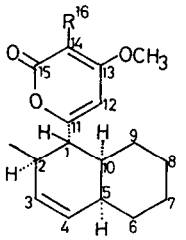
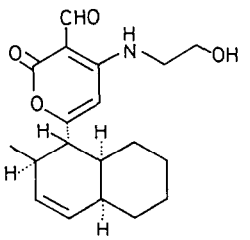
Abstract: On the basis of spectroscopic data and chemical reaction, structures 1, 2 and 3 are proposed for solanapyrones A, B and C, phytotoxins from the fungus Alternaria solani, parasite of potato.

Alternaria solani, causal organism of early blight disease of tomato and potato produces several metabolites whose structures have been clarified.[1] Recently it has been reported that A. solani secretes two host specific toxins which induce necrotic symptoms typically associated with the disease.[2] In this communication we would like to report the structure determination of three new phytotoxins, solanapyrones A(1), B(2) and C(3) isolated from culture filtrate of the fungus. Solanapyrone A(1) induced a necrotic lesion on the leaf of potato at 100μg/100μl(MeOH). The fungus was grown by surface culture on a potato glucose



1 R=CHO
2 R=$CH_2OH$



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medium at 25°C for 25 days. The chloroform extracts of the culture filtrate were subjected to silica gel column chromatography eluting with a mixture of benzene and ethyl acetate to give solanapyrones A(1), B(2) and C(3). The physicochemical, UV and IR data of these phytotoxins are shown in table 1.

Solanapyrone A(1) has a molecular formula $C_{18}H_{22}O_4$ from the high resolution mass(HR-MS) spectrum. The IR and UV spectra indicated a α-pyrone ring and an aldehyde(Table 1). The $^{13}C$ NMR spectrum showed the presence of two methyls, four methylenes, eight methines and four quaternary carbons.[3] The $^1H$ NMR spectrum exhibited signals at $\delta$ 4.10 due to a methoxyl and at $\delta$ 10.15 to an aldehyde. Therefore remaining two oxygens were characterized as a pyrone ring. Since only one signal due to aromatic proton was observed at $\delta$ 6.22 as a singlet, trisubstituted pyrone ring was deduced. The presence of pyrone moiety was also supported by the EI-MS, in which intence peak was observed at $^m/z$ 158, $C_7H_5O_4^+$. The substitution pattern of

pyrone ring was deduced by comparison of the chemical shifts with those of known pyrone derivatives in $^1H$ and $^{13}C$ NMR spectra[4]. Validity of the pyrone moiety was also confirmed by

Table 1. Physiochemical and spectral data of solanapyrones.

| | Solanapyrone A(1) | Solanapyrone B(2) | Solanapyrone C(3) |
|---|---|---|---|
| HR-MS found | 302.1505 ($M^+$) | 304.1668 ($M^+$) | 331.1785 ($M^+$) |
| calcd. | 302.1517 ($C_{18}H_{22}O_4$) | 304.1673 ($C_{18}H_{24}O_4$) | 331.1784 ($C_{19}H_{25}NO_4$) |
| $[\alpha]_D$ | -67.3°(c 2.26, $CHCl_3$) | -59.0°(c 0.4, $CHCl_3$) | -5.0(c 0.88, $CHCl_3$) |
| UV $\lambda_{max}^{EtOH}$ nm(ε) | 232(9200), 327(9400) | 303(8500) | 238(19900), 282(6900) 320(7300) |
| IR $\nu_{max}^{neat}$ $cm^{-1}$ | 1740, 1700, 1680, 1600 | 3400, 1680, 1630, 1550 | 3400, 1700, 1660, 1600, 1570 |

the fact that treatment of 1 with KOH-MeOH-$H_2O$ yielded a keto ester, 4[5], EI-MS m/z 292($M^+$), IR $\nu_{max}^{neat}$ $cm^{-1}$ 1750, 1600, 1440, $^1H$ NMR $\delta_{TMS}^{CDCl_3}$ 0.88(3H, d, J=6.8Hz, CH-$CH_3$), 3.33(2H, s, $COCH_2$), 3.68(3H, s, $OCH_3$), 5.34(1H, m, =H), 5.53(1H, m, =CH), 5.58(1H, s, =CH). From the number of unsaturation of 1, the remaining moiety was deduced to be bicyclic hydrocarbon including a disubstituted cis double bond from the $^1H$ NMR spectrum( $\delta$ 5.44, 5.67, each 1H, J=9.8Hz). Extensive decoupling experiment in the $^1H$ NMR spectrum(Fig. 1) proved the structure and stereochemistry of the dehydrodecalyl moiety. The large coupling constants (J=11.7, 9.8Hz) of 1-H means that the proton locates axially to the two vicinal protons, 2-H and 10-H. Further the moderate coupling constant(J=4.0Hz) of 10-H with 5-H indicates that the ring juncture must be cis. The structure 1 for solanapyrone A is compatible with the biogenetic consideration in which 1 is produced through polyketide pathway.

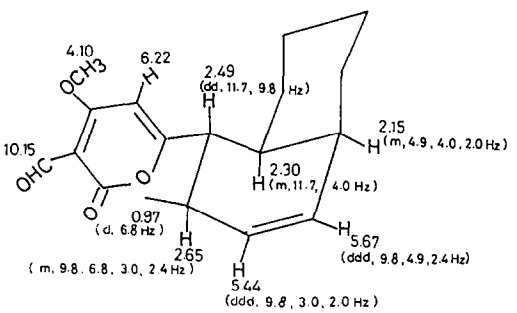
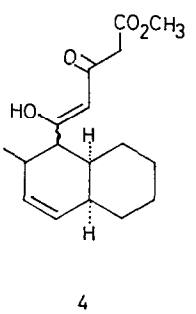



Fig. 1

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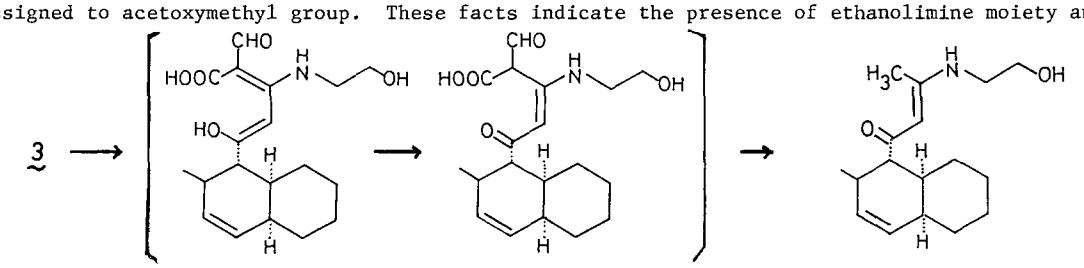
Solanapyrone B(2) has a molecular formula $C_{18}H_{24}O_4$ from the HR-MS. The IR spectrum (Table 1) showed the presence of hydroxyl group but no aldehyde group. The $^{13}C$ NMR spectrum revealed the presence of two methyls, five methylenes, seven methines and four quaternary carbons[6]. The facts indicate that the aldehyde group in 1 would be replaced to the hydroxymethyl group in 2. In fact the $^1H$ NMR spectrum (Table 2) of 2 exhibited a signal at $\delta$ 4.56 ascribed to the hydroxymethyl group. Further, treatment of 2 with acetic anhydride in pyridine gave an acetate, $C_{20}H_{26}O_5$ EI-MS m/z 346($M^+$), IR $\nu_{max}^{neat}$ $cm^{-1}$ 1720, 1640, 1555, $^1H$ NMR $\delta_{TMS}^{CDCl_3}$ 2.07(3H, s), 4.98(2H, s). The structure 2 was confirmed by the corelation with 1 as follows. Oxidation of 2 with pyridinium chlorochromate yielded an aldehyde, whose spectroscopic data are identical with 1.

Table 2. $^1$H NMR Spectra of solanapyrones B(2) (400MHz in $CDCl_3$) and C(3) (500MHz in $CDCl_3$)

| Solanapyrone B(2) | | | Solanapyrone C(3) | | |
|---|---|---|---|---|---|
| chemical shift ($\delta$ ppm) | number of proton | coupling constants ( Hz ) | chemical shift ($\delta$ ppm) | number of proton | coupling constants ( Hz ) |
| 0.93 | 3 | d J=6.8 | 0.93 | 3 | d J=6.7 |
| 1.18 | 3 | m | 1.08~1.28 | 3 | m |
| 1.42 | 3 | m | 1.47 | 3 | m |
| 1.71 | 2 | m | 1.71 | 2 | m |
| 2.13 | 1 | m | 2.14 | 1 | m |
| 2.29 | 1 | m | 2.28 | 1 | m |
| 2.41 | 1 | dd J=11.7, 9.8 | 2.36 | 1 | dd J=11.6, 12.2 |
| 2.59 | 1 | m | 2.59 | 1 | m |
| 3.93 | 3 | s | 3.53 | 2 | q J=5.5 |
| 4.56 | 2 | s | 3.89 | 2 | dd J=5.5, 4.9 |
| 5.44 | 1 | ddd J=9.8, 3.0 2.0 | 5.43 | 1 | ddd J=9.8, 3.0, 2.0 |
| 5.66 | 1 | ddd J=9.8, 4.9 2.4 | 5.65 | 1 | ddd J=9.8, 4.9, 2.4 |
| 6.09 | 1 | s | 6.00 | 1 | s |
| | | | 9.96 | 1 | s |
| | | | 10.79 | 1 | s |

Solanapyrone C(3) has a molecular formula $C_{19}H_{25}NO_4$ from the HR-MS. The UV and IR spectra of 3 (Table 1) are very similar with solanapyrone A(1). The $^{13}$C NMR spectrum revealed the presence of one methyl, six methylenes, eight methines and four quaternary carbons.[7] The $^1$H NMR spectrum of 3 (Table 2) was also closely resemble with that of 1. However two signals due to two methylene groups were observed at $\delta$ 3.53 and 3.89, but no signals due to methoxyl group. Acetylation of 3 with acetic anhydride in pyridine gave a monoacetate, $[\alpha]_D$ -25.4° (c=1.4, $CHCl_3$), $C_{21}H_{27}NO_5$ from the HR-MS $^m$/z 373.1872(calcd. 373.1887), UV $\lambda_{max}^{EtOH}$ nm($\varepsilon$) 237 (22800), 282(7500), 321(9100), IR $\nu_{max}^{neat}$ $cm^{-1}$ 3200, 1740, 1700, 1640, 1610, 1570. The $^1$H NMR spectrum of the acetate showed the signals at $\delta$ 3.63 and 4.27, in which later signal should be assigned to acetoxymethyl group. These facts indicate the presence of ethanolimine moiety and




3a 3b 4

lead to structure 3 for solanapyrone C. Further evidence for structure 3 was obtained by the hydrolysis product of 3. Thus, treatment of 3 with $K_2CO_3$ in $H_2O$-EtOH at 80°c for 4 hr gave a product 5, white solid, $C_{17}H_{27}NO_2$ from the HR-MS $^m$/z227.2051(calcd. 277.2041), UV $\lambda_{max}^{EtOH}$ nm($\varepsilon$)

309(4200), IR $\nu_{max}^{KBr}$ $cm^{-1}$ 3360, 1700, 1590, 1540, $^1H$ NMR $\delta_{TMS}^{CDCl_3}$ 1.99(3H, s), 5.06(1H, s). The formulation of 5 from 3 would be rationalized by the scheme through 3a and 3b. The structural similarity of the solanapyrones with betaenones[8] and compactin[9] anticipates promising biological activity not only phytotoxicity but also enzyme inhibiting activity.

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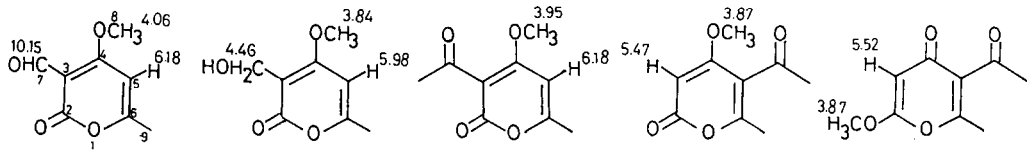
3. $^{13}C$ NMR (25, 1MHz) $\delta_{TMS}^{CDCl_3}$ 20.25($CH_3$), 20.98($CH_2$), 25.85($CH_2$), 28.39($CH_2$), 29.71($CH_2$), 35.17(CH), 36.08(CH), 36.81(CH), 47.90(CH), 57.80($CH_3$), 95.93(CH), 101.77(C), 130.00(CH), 131.49(CH), 162.06(C), 173.84(C), 176.37(C), 186.39(CH).
4. Following compounds at hand were used for comparison. Cf. A. Ichihara, K. Murakami and S. Sakamura, Tetrahedron Lett, 22, 4005(1981).

$^1H$ NMR

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6, $^{13}C$ NMR(25.1MHz) $\delta_{TMS}^{CDCl_3}$ 21.08(q, 9-C), 57.59(q, 8-C), 94.87(d, 5-C), 101.16(s, 3-C), 161.21(s, 2-C), 170.61(s, 6-C), 174.49(s, 4-C), 185.82(d, 7-C).

5. The enolized carbonyl is tentative.
6. $^{13}C$ NMR(25.1MHz) $\delta_{TMS}^{CDCl_3}$ 20.21($CH_3$), 20.92($CH_2$), 25.96($CH_2$), 28.30($CH_2$), 29.71($CH_2$), 35.04(CH), 35.74(CH), 36.08(CH), 46.76(CH), 54.50($CH_2$), 56.43($CH_3$), 95.98(CH), 103.53(C), 130.08(CH), 131.13(CH), 165.06(C), 166.05(C), 168.10(C).
7. $^{13}C$ NMR(50.1MHz) $\delta_{TMS}^{CDCl_3}$ 20.29($CH_3$), 21.05($CH_2$), 26.02($CH_2$), 28.38($CH_3$), 29.84($CH_2$), 34.69(CH), 35.54(CH), 36.82(CH), 45.05($CH_2$), 47.42(CH), 60.88($CH_2$), 94.87(C), 96.01(CH), 130.35(CH), 131.45(CH), 160.54(C), 164.21(C), 172.27(C), 191.20(CH).
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