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STRUCTURES OF ALTILOXINS A AND B, PHYTOTOXINS FROM PHOMA ASPARAGI SACC

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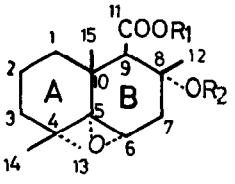
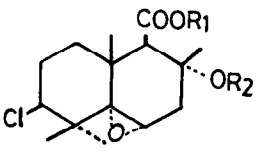
Abstract: By spectroscopic data, the structures of two new phytotoxins, altiloxins A and B from Phoma asparagi Sacc., the incitant of stem blight disease on asparagus are elucidated.

Two phytotoxins, altiloxins A(1) and B(2) were isolated from culture filtrate of Phoma asparagi Sacc., causal fungus of stem blight disease on asparagus.[1] Altiloxins A(1) and B(2) showed weak inhibitory activity to the root elongation of lettuce seedlings, but these inhibited the root growth of the host plant 48.2% and 48.5% respectively at 10 ppm.

Herein we present evidences that the toxins have the drimane-type structures 1 and 2.





1 R_1,R_2=H
3 R_1=CH_3,R_2=H
5 R_1=CH_3,R_2=Ac

2 R_1,R_2=H
4 R_1=CH_3,R_2=H
6 R_1=CH_3,R_2=Ac

The fungus was grown by surface culture on a potato glucose medium at 26°C for 18 days. The culture filtrate was extracted with ethyl acetate and the extracts were chromatographed four times on silica gel columns. Each fraction was checked by bioassay using the lettuce seedlings, and from active fractions altiloxins A(1) and B(2) were isolated.

Altiloxin A(1), mp 136~137°C, $[\alpha]_D^{20}$-16.9°(c 0.70, $CHCl_3$), has a molecular formula $C_{15}H_{24}O_4$ from the high resolution MS m/z 268.1601 (M^+, calcd. 268.1672), IR ν_{max}^{KBr} cm^{-1} 3350 (OH), 1710(C=O), 1380(gem. $C-CH_3$). The ^1H NMR (400 MHz in $CDCl_3$) of 1 indicated the presence of four tertiary methyls.[2]

Altiloxin B(2), mp 145~148°C, $[\alpha]_D^{20}$-10.9° (c 0.88, $CHCl_3$), has a molecular formula $C_{15}H_{23}O_4Cl$ from the high resolution MS m/z 286.1165 (M^+-H_2O, calcd. 286.1150). The IR spectrum exhibited the presence of a hydroxyl and carboxyl groups (3100~3450cm^{-1}, 1750cm^{-1}), and a gem. dimethyl(1380cm^{-1}). The ^1H NMR spectrum showed the presence of four tertiary methyls.[3]

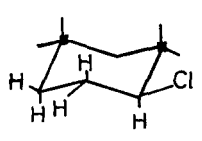
Treatment of altiloxins A(1) and B(2) with diazomethane gave corresponding methyl esters, 3, mp 120~121°C, $[\alpha]_D^{20}$-24.73°(c 1.65, $CHCl_3$), molecular formula $C_{16}H_{26}O_4$ from elemental analysis and high resolution MS m/z 282.1825 (M^+, calcd. 282.1820), IR ν_{max}^{KBr} cm^{-1} 3500(OH), 1750, 1720(C=O), 1380(gem. $C-CH_3$), and 4, mp 138~139°C, $[\alpha]_D^{20}$-24.13°(c 1.73, $CHCl_3$),

molecular formula $C_{16}H_{25}O_4Cl$ from elemental analysis and high resolution MS m/z 318.1386(M^+, calcd. 318.1363), IR ν_{max}^{KBr} cm^{-1} 3500(OH), 1740, 1720(C=O). ^1H NMR spectra of 3 and 4 are shown in Table 1. Acetylation and subsequent methylation[4] of 1 and 2 afforded monoacetates 5[5] IR ν_{max}^{KBr} 1730(C=O), and 6[6], IR ν_{max}^{KBr} 1740, 1720(C=O), which have no longer absorption due to hydroxyl group. Since 1 and 2 contain a carboxylic acid and a hydroxyl group, remaining one oxygen atom must be included in an epoxide or an ether linkage.

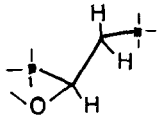
Table 1. ^1H NMR Spectra of Methyl Esters of Altiloxins A and B (400 MHz in $CDCl_3$)

Altiloxin A methyl ester(3)				Altiloxin B methyl ester(4)			
chemical shift(δ)		multiplicity	assignment	chemical shift(δ)		multiplicity	assignment
ppm				ppm			
0.76	(3H)	s	13-CH_3	0.94	(3H)	s	13-CH_3
1.14	(3H)	s	14-CH_3	1.20	(3H)	s	12-CH_3
1.18	(3H)	s	12-CH_3	1.22	(3H)	s	14-CH_3
1.30	(1H)	ddd J=13.2,13.2, 3.9Hz	1-Hax	1.45	(3H)	s	15-CH_3
1.43	(3H)	s	15-CH_3	1.46	(1H)	ddd J=14.6,13.7, 3.9Hz	1-Hax
1.43 ~1.52	(2H)	m	3-H	1.79	(1H)	ddd J=14.6,3.4, 3.4Hz	1-Heq
1.53	(1H)	m	2-Heq	2.02	(1H)	dddd J=13.7,3.9, 3.9,3.4Hz	2-Heq
1.66	(1H)	ddd J=13.2,3.9, 3.4Hz	1-Heq	2.14	(1H)	dddd J=13.7,13.7, 12.5,3.4Hz	2-Hax
1.73	(1H)	ddddd J=13.2,13.2, 13.2,3.9, 3.9Hz	2-Hax	2.15	(1H)	d J=16.1Hz	7-Hax'
2.15	(1H)	d J=16.1Hz	7-Hax'	2.39	(1H)	dd J=16.1,3.9Hz	7-Heq'
2.35	(1H)	dd J=16.1,3.9Hz	7-Heq'	3.08	(1H)	s	9-H
3.09	(1H)	s	9-H	3.18	(1H)	d J=3.9Hz	6-H
3.15	(1H)	d J=3.9Hz	6-H	3.68	(3H)	s	OCH_3
3.66	(3H)	s	OCH_3	3.97	(1H)	dd J=12.5,3.9Hz	3-H

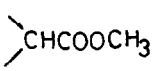
Similarity of the spectral data of 1 (and 3) with those of 2 (and 4) indicates that altiloxins A(1) and B(2) have closely related structures. Actually, ^{13}C NMR spectra of 3[7] and 4[8] prove this view. From the molecular formula, these phytotoxins have a tricyclic structure and only difference between 1 and 2 is the fact that one of the proton of 1 is replaced by a chlorine atom in 2. Since the signals of altiloxin B methyl ester (4) in ^1H NMR spectrum are clearly separated compared with those of 3, extensive decoupling experiments were carried out with 4 and the partial structures (a), (b), (c) and (d) were deduced.



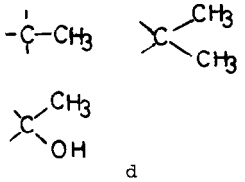
a



b



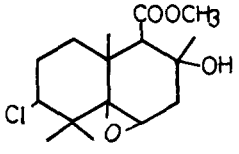
c



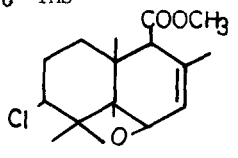
d

The large coupling constant (J=12.5Hz) of 3-Hax with vicinal 2-Hax indicates that the chlorine atom is equatorially oriented in chair cyclohexane. The partial structure (c) was deduced by the fact that the signal at δ 3.08(1H, s) ascribable to α methine (9-H) to the ester group was observed. The presence of tertiary hydroxyl group was confirmed by ^{1}H NMR spectrum of the acetate 5 which has no α-methine nor methylene protons to the acetoxyl group. The hydroxyl group was placed at neighboring with the partial structure c, since an intramolecular hydrogen bond between the ester carbonyl and the hydroxyl group was observed at 3589cm^{-1} under high dilute conditions in the IR spectrum. On the basis of these data together with biogenetic considerations of sesquiterpenoids, a planar structure 4a which has a drimane skeleton was elucidated for altiloxin B methyl ester.

The stereochemistry of 4 was confirmed as follows. The carbomethoxy group of 4 is equatorially oriented, since alkaline treatment only recovered the starting material. In addition, the fact that the signals due to 15-CH_3 in a series of compounds (1-4) in the ^{1}H NMR spectra appear in rather lower field (δ 1.35~1.45) indicates β-configuration of the ester group. Treatment of 4 with thionyl chloride-pyridine gave a mixture of 7, ^{1}H NMR $\delta^{CDCl_3}_{TMS}$ 1.71(3H, t, J=1.5Hz, =C-CH_3), 5.66(1H, m, =CH), and 8, $\delta^{CDCl_3}_{TMS}$ 4.69, 4.88(each 1H, m, $=CH_H$) in a ratio



4a



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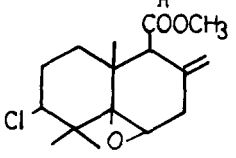
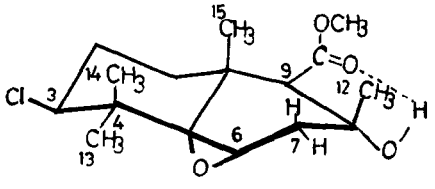


8

of 2 : 1. These findings mean that the hydroxyl group is located in trans to the ester group and trans elimination toward 9-H is inhibited. The regio-and stereochemical features of 4 were confirmed by additional ^{1}H NMR data. Thus, Nuclear Overhauser Effect was observed for, besides 14-CH_3, both the methine proton signals due to 3-H and 6-H when the 13-CH_3 signal was saturated in a difference NOE experiment on 4 in $CDCl_3$. Irradiation of 12-CH_3 increased the intensities of the signals due to 15-CH_3, OCH_3 and 7-Hax. Also, irradiation of 15-CH_3 increased the intensities of the signals due to 14-CH_3, 12-CH_3, 7-Hax and 1-Heq. These observations surely support the relative configuration of altiloxin B methyl ester as depicted in 4b, in which the epoxide has α configuration[9] and O-axial conformation in a twisted boat form, and 7-Hax is located closely with 15-CH_3.[10] Therfore the structure 2 is suggested for altiloxin B itself. Structure (1) of altiloxin A was also elucidated by the same way on the basis of spectral analyses. Very recently, correlations of altiloxin A with altiloxin B and then with known drimane-8,11-diol have been completed and the results will be reported elsewhere.[11]



4b

Acknowledgement: We wish to thank Dr. F. Kodama, Hokkaido Central Agricultural Experiment Station, Naganuma, for supplying the fungus Phoma asparagi Sacc. ADL-8.

References and Footnotes

1. Phytotoxins produced by Phoma sp are betaenones from Phoma betae Fr., A. Ichihara, H. Oikawa, K. Hayashi, S. Sakamura, A. Furusaki, T. Matsumoto, J. Am. Chem. Soc., 105, 2907 (1983), A. Ichihara, H. Oikawa, M. Hashimoto, S, Sakamura, T. Haraguchi, H. Nagano, Agric. Biol. Chem., 47, 2965 (1983), and others are cited in W. B. Turner, D. C. Aldridge, "Fungal Metabolites Ⅱ", Academic Press, New York, 1983.

2. 1, ^{1}H NMR (400 MHz) $\delta^{CDCl_3}_{TMS}$ 0.76(3H, s, 13-CH_3), 1.14(3H, s, 14-CH_3), 1.29(3H, s, 12-CH_3), 1.31(1H, ddd, J=13.2, 13.2, 3.9Hz, 1-Hax), 1.40(3H, s, 15-CH_3), 1.40～1.52(2H, m, 3-H), 1.54(1H, m, 2-Heq), 1.75(1H, ddddd, J=13.2, 13.2, 13.2, 3.9, 3.9Hz, 2-Hax), 1.93(1H, brd, J=13.2, 1-Heq), 2.19(1H, d, J=16.1Hz, 7-Hax'), 2.40(1H, dd, J=16.1, 3.9Hz, 7-Heq'), 3.15 (1H, s, 9-H), 3.16(1H, d, J=3.9Hz, 6-H).

3. 2, ^{1}H NMR (400 MHz) $\delta^{CDCl_3}_{TMS}$ 0.95(3H, s, 13-CH_3), 1.21(3H, s, 14-CH_3), 1.30(3H, s, 12-CH_3), 1.45(3H, s, 15-CH_3), 1.48(1H, ddd, J=14.7, 13.7, 3.9Hz, 1-Hax), 2.00～2.20(3H, m, 1-Heq, 2-Heq, 2-Hax), 2.18(1H, d, J=16.1Hz, 7-Hax'), 2.43(1H, dd, J=16.1, 3.9Hz, 7-Heq'), 3.14 (1H, s, 9-H), 3.20(1H, d, J=3.9Hz, 6-H), 3.97(1H, dd, J=12.2, 3.9Hz, 3-H).

4. Treatment of the methyl esters 3 and 4 with acetic anhydride in pyridine gave no acetates 5 and 6, and recovered the starting materials.

5. 5, ^{1}H NMR (400 MHz) $\delta^{CDCl_3}_{TMS}$ 0.76(3H, s, 13-CH_3), 1.14(3H, s, 14-CH_3), 1.39(3H, s, 15-CH_3), 1.26～1.53(5H, m, 1-Hax, 2-Hax, 2-Heq, 3-Hax, 3-Heq), 1.65(3H, s, 12-CH_3), 1.73(1H, ddddd, J=13.7,13.7,13.7, 3.9, 3.9Hz, 2-Hax), 1.96(3H, s, $COCH_3$), 2.20(1H, dd, J=16.6, 1.5Hz, 7-Hax) 2.81(1H, dd, J=16.6, 3.4Hz, 7-Heq'), 3.10(1H, dd, J=3.4, 1.5Hz, 6-H), 3.33(1H, s, 9-H), 3.63(3H, s, OCH_3).

6. 6, ^{1}H NMR (400 MHz) $\delta^{CDCl_3}_{TMS}$ 0.94(3H, s, 13-CH_3), 1.21(3H, s, 14-CH_3), 1.42(3H, s, 15-CH_3), 1.40～1.45(1H, m, 1-Hax), 1.59～1.67(1H, m, 1-Heq), 1.65(3H, s, 12-CH_3), 1.96(3H, s, $COCH_3$), 2.01～2.07(1H, m, 2-Heq), 2.12(1H, dddd, J=13.9, 13.9, 13.9, 3.4Hz, 2-Hax), 2.24 (1H, dd, J=16.8, 1.5Hz, 7-Hax'), 2.83(1H, dd, J=16.8, 3.4Hz, 7-Heq'), 3.15(1H, dd, J=3.4, 1.5Hz, 6-H), 3.28(1H, s, 9-H), 3.64(3H, s, OCH_3), 3.96(1H, dd, J=12.2, 4.9Hz, 3-H).

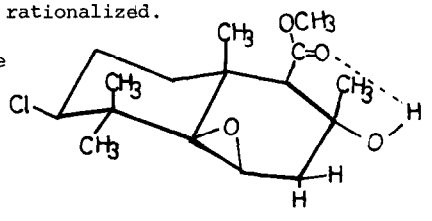
7. 3, ^{13}C NMR(OFR, 25.1 MHz) $\delta^{CDCl_3}_{TMS}$ 17.93(q, 13-CH_2), 18.11(q, 14-CH_3), 26.66(q, 12-CH_3), 26.78(q, 15-CH_3), 29.00(t, 1-CH_2), 34.34(s, 4-C), 36.45(t, 2-CH_2), 37.09(t, 3-CH_2), 38.38 (t, 7-CH_2), 39.08(s, 10-C), 50.98(q, 16-CH_3), 53.32(d, 9-CH), 61.64(d, 6-CH), 66.58(s, 5-C), 69.90(s, 8-C), 172.61(s, 11-C=O).

8. 4, ^{13}C NMR (OFR, 25.1 MHz) $\delta^{CDCl_3}_{TMS}$ 17.93(q, 13-CH_3), 21.62(q, 14-CH_3), 22.50(q, 12-CH_3), 28.83(t, 1-CH_2),, 29.00(q, 15-CH_3), 35.57(t, 2-CH_2), 36.74(s, 4-C), 38.67(t, 7-CH_2), 41.02 (s, 10-C), 51.04(q, 16-CH_3), 53.44(d, 9-CH), 60.64(d, 6-CH), 66.91(s, 5-C), 68.20(d, 3-CH), 69.20(s, 8-C), 172.03(s, 11-C=O).

9. Rather high field shift of the signal due to 13-CH_3 in 1, 2, 3 and 4 would be rationalized by the shielding effect of the oxirane ring. Cf. D. Lavie, Y. Kashman, E. Glotter, Tetrahedron, 22, 1103(1966).

10. If the epoxide in 4 has β configuration, 15-CH_3 and 7β-H are very far apart each other as illustrated in 4c and the NOEs would not be rationalized.

11. A. Ichihara, Y. Kawakami, S. Sakamura, to be published.



4c

(Received in Japan 24 March 1984)