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Structure and Synthesis of Alboatrin, a Novel Phytotoxic Metabolite from Verticillium albo-atrum

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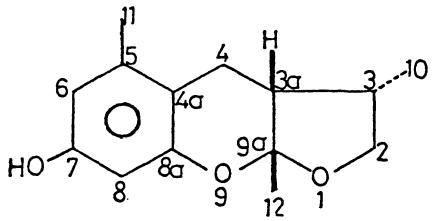
Structure of Alboatrin, a novel phytotoxin isolated from Verticillium albo-atrum has been elucidated on the spectroscopic data and has been confirmed by the synthesis. A biogenesis for the unique phytotoxin is suggested.

Alboatrin (1) is a phytotoxic metabolite which was isolated from the culture filtrate of Verticillium albo-atrum Reinke & Berthord, causal fungus of vascular-wilt disease on alfalfa. Alboatrin inhibited the root growth of the host plant (Maris Kabul) 49.0% at 50 ppm. In this communication, we wish to describe the isolation, structure determination and synthesis of this phytotoxin.



1

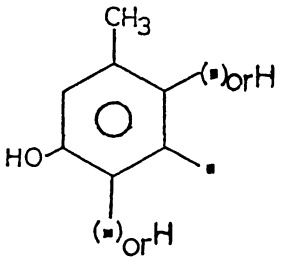
The fungus was grown in potato-sucrose (2%) medium at 22 °C for 40 days in the dark. The culture filtrates were concentrated in vacuo and the residue was extracted with ethyl acetate. The extracts were purified through chromatography, checking with bioassay using growth test of alfalfa seedlings to give three phytotoxic compounds, orcinol, orcinol monomethyl ether and alboatrin (1). Among these toxins, 1 exhibited the highest phytotoxic activity.

Alboatrin (1), mp 146-149 °C, $[\alpha]_D^{22.5}$ + 8.8 (c 0.5, $CHCl_3$), has a molecular formula $C_{14}H_{18}O_3$ from the high resolution MS m/z 234.1248 (M^+, calcd, 234.1265). The UV spectrum showed a benzenoid absorption at λ_{EtOH}^{max} nm(ε) 282(2095). The IR spectrum exhibited the presence of a hydroxyl (3350 cm^{-1}) and a phenyl (1600 cm^{-1}) groups. Acetylation of 1 with acetic anhydride in pyridine yielded a mono-acetate,[1] $C_{16}H_{20}O_4$, whose IR spectrum showed a signal at 1760 cm^{-1} due to a

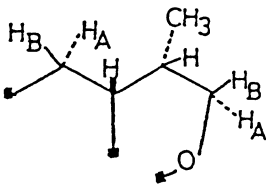
phenolic acetate and no more signals ascribable to hydroxyl groups. Therefore among three oxygens in 1, two other oxygens would form ether linkages. The INEPT experiments in the ^{13}C NMR spectrum[2] of 1 showed presence of 3 methyl signals, 2 methylene signals, 4 methine signals and 5 quaternary carbon signals. Among these 14 carbon signals, two of the methine signals (δ101.76, 109.76) are assigned to aromatic carbons. Extensive decoupling experiments of 1 in the ^1H NMR spectrum (Table 1) revealed the presence of the partial structures, (a), (b),and (c). The aromatic moiety (a) was deduced by the fact that the signals at δ6.21 and 6.27 due to two aromatic protons (6-H, 8-H) showed a meta coupling (J=2.4 Hz). The carbon linkage from 2-CH_2 to 4-CH_2 was also confirmed by the coupling constants to lead the partial structure (b). The singlet signal at δ1.51 was assigned to the methyl group (12-CH_3) attached to acetal carbon.

Table 1. ^1H NMR spectrum of alboatrin (1)

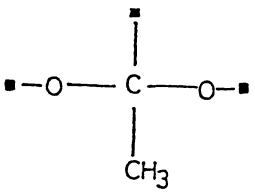
Chemical shift(δ)	Number of proton	Multiplicity(Hz)	Assignment
1.05	3H	d J=6.7	10-H
1.51	3H	s	12-H
1.93	1H	ddd J=10.8, 5.5, 1.8	3a-H
2.10	1H	m	3-H
2.18	3H	s	11-H
2.66	1H	dd J=17.0, 1.8	4-H_A
2.71	1H	dd J=17.0, 5.5	4-H_B
3.51	1H	dd J=8.5, 8.5	2-H_A
4.17	1H	dd J=8.5, 8.5	2-H_B
4.65	1H	brs	7-OH
6.21	1H	d J=2.4	6-H
6.27	1H	d J=2.4	8-H



a



b

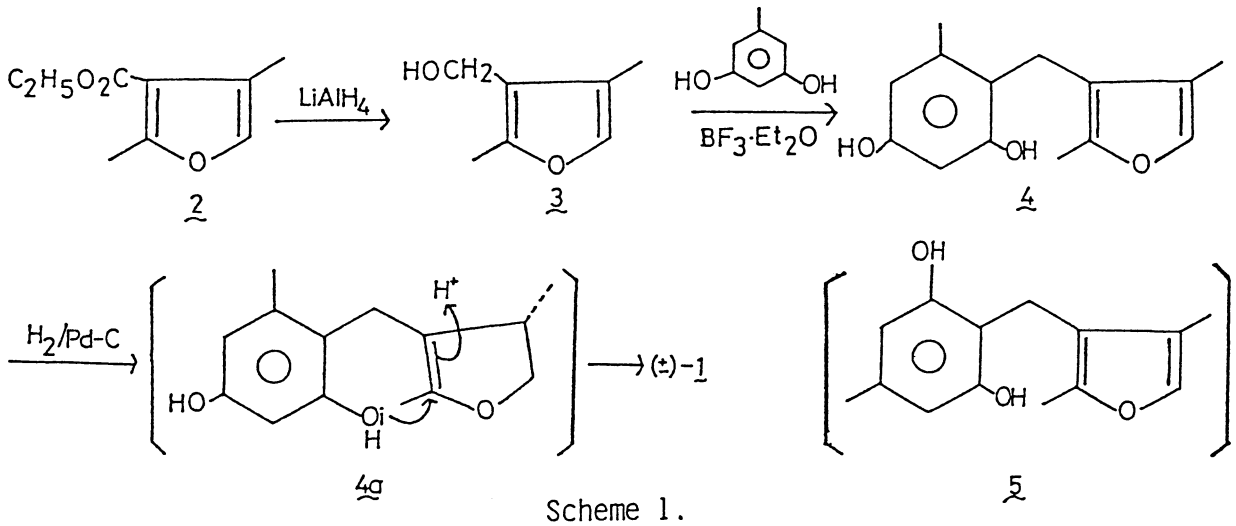


c

From the molecular formula, alboatrin has four possible tricyclic structures[3] containing an aromatic moiety (a). The regio- and stereochemical structure of 1 were confirmed by additional ^1H NMR data. Thus, nuclear Overhauser effect was observed for the signals due to 2-H_A, 3-H, 3a-H, and 4-H_A when the 10-CH_3 signal was saturated in a difference NOE experiment on 1 in $CDCl_3$. Also, irradiation of 2-H_A increased the intensities of the signals due to 2-H_B and 10-CH_3. Irradiation of 12-CH_3 increased the intensities of the signals due to 4-H_A, and 3a-H. These

observations excluded three other structures,[3] and elucidate the regiochemistry as well as relative configuration of alboatrin as depicted in 1.

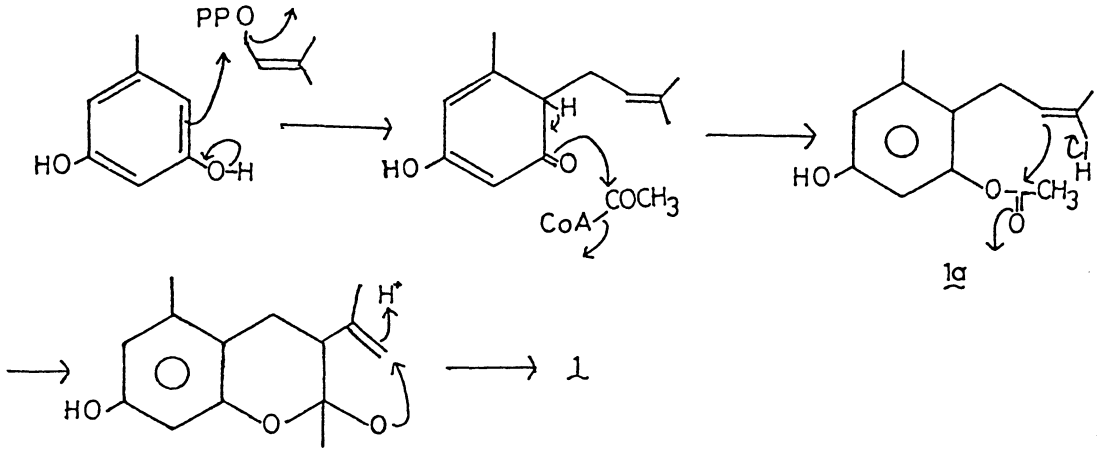
In order to confirm the structure and to develope an effective synthetic method, synthesis of 1 has been undertaken as follows (Scheme 1). Reduction of ethyl 2, 4-dimethyl-3-furoate (2), which was prepared from known procedure,[4] with $LiAlH_4$ in ether yielded an alcohol 3(85%). The Friedel-Crafts reaction[5] of orcinol with the alcohol 3 in the presence of boron trifloride etherate (0.5%) in chloroform afforded an alkylated product[6] 4 (10%) and the regioisomer[7] 5 (6%). Catalytic hydrogenation of 4 on 5% Pd-C in acetic acid at room temperature produced directly (±)-alboatrin (64%) as a single product and no other stereoisomer has been obtained. This stereoselectivity would be rationalized by presuming a dihydrofuran intermadiate 4a, in which protonation from less hindered side to the 10-CH_3 group and simultaneous anti-addition of the phenolic 9-OH to the 9a-C would be occurred to give (±)-1. Optical resolution of (±)-alboatrin (1) has been carried out by using chiral column[8] to give optical active (±)-1, $[\alpha]_D^{22.5}$ + 8.0°(c 0.15, $CHCl_3$) and (-)-1, $[\alpha]_D^{22.5}$ - 6.0°(c 0.16, $CHCl_3$). Both enantiomers, (+)-1 and (-)-1, inhibited the growth of the roots and hypocotyls of alfalfa seedlings (Maris Kabul) at 10^{-3}M — 10^{-4}M.



Scheme 1.

Though alboatrin (1) has an unique structure, the biogenesis would be rationalized by the polyketide pathway involving prenylation as follows (Scheme 2). Prenylation of orcinol with prenyl pyrophosphate and subsequent acetylation would yield prenyl acetate 1a, which is converted through double cyclization (1b) to alboatrin (1).

very recently other phytotoxic metabolites from the same fungus isolated from potato have been obtained and detail of these results will be reported elsewhere.[9]



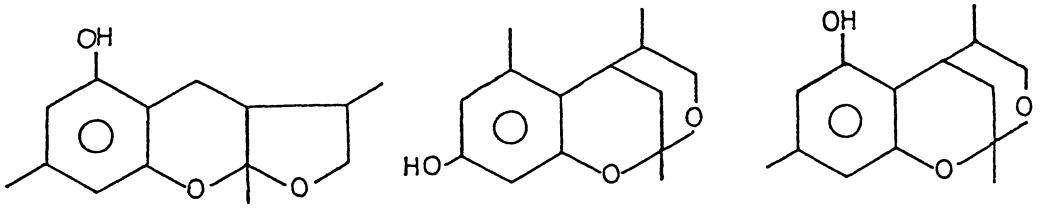
Scheme 2.

References

1) Alboatrin monoacetate, $C_{16}H_{20}O_4$ from the high resolution MS m/z 276.1368 (M^+, calcd 276.1361), $[\alpha]_D^{22.5}$ + 12°(c 0.1, $CHCl_3$); ^1H NMR spectrum (100 MHz) $\delta_{TMS}^{CDCl_3}$ppm 1.05 (3H, d, J=6.1 Hz), 1.51 (3H, s), 1.90 (1H, m), 2.1 (1H, m), 2.23 (3H, s), 2.26 (3H, s), 2.72 (2H, m), 3.52 (1H, dd, J=8.3, 8.3 Hz), 4.18 (1H, dd, 8.3, 8.3 Hz), 6.41 (1H, d, J=2.2 Hz), 6.49 (1H, d, J=2.2 Hz).

2) (+)-1, ^{13}C NMR spectrum (57.25 MHz): $\delta_{TMS}^{CDCl_3}$ppm 15.97 (CH_3), 19.33 (CH_3), 21.58 (CH_2), 22.95 (CH_3), 35.45 (CH), 48.32 (CH), 73.96 (CH_2), 101.76 (CH), 107.22 (C), 109.58 (C), 109.76 (CH), 138.14 (C), 153.85 (C), 154.84 (C).

3) Three other structures are depicted as follows.



4) J. W. Batty, D. D. Howes, and C. J. M. Stirling, J. Chem. Soc., Perkin Trans. 1, 1973, 65; D. D. Howes and C. J. M. Stirling, Org. Synth., 53, 1 (1973).

5) R. K. Razdan, H. C. Dalzell, and G. R. Handrick, J. Am. Chem. Soc., 96, 5860 (1974).

6) 4, $C_{14}H_{16}O_3$ from the high resolution MS m/z 232.1091 (M^+, calcd, 232.1100); $IR\nu_{max}^{KBr}cm^{-1}$: 3400, 1600; ^1H NMR (90 MHz) $\delta_{TMS}^{CDCl_3}$ppm 1.79 (3H, d, J=1.2 Hz), 2.07 (3H, s), 2.24 (3H, s), 3.62 (2H, s), 4.71 (1H, br s), 5.09 (1H, br s), 6.18 (1H, d, J=2.2 Hz), 6.27 (1H, d, J=2.2 Hz), 7.09 (1H, br s).

7) 5, $C_{14}H_{16}O_3$ from the high resolution MS m/z 232.1090 (M^+, calcd, 232.1100); $IR\nu_{max}^{KBr}cm^{-1}$: 3350, 1620, 1580; ^1H NMR (90 MHz) $\delta_{TMS}^{CDCl_3}$ppm 1.78 (3H, d, J=2 Hz), 2.15 (6H, s), 3.68 (2H, s), 5.05 (2H, br s), 6.15 (2H, s), 6.98 (1H, br s).

8) Optical resolution of (±)-alboatrin (1) was carried out using CHIRAL-OC column (1×25 cm, DAICEL CHEMICAL INDUSTRY, LTD) eluting with hexane-i-PrOH (9:1 v/v).

9) H. Oikawa, T. Yokota, M. Nonaka, A. Ichihara, S. Sakamura, A. Tazimi, and R. Sato, to be puplished.

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