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PHYTUBERIN: A NOVEL ANTIFUNGAL TERPENOID FROM POTATO

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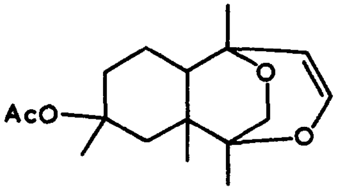
A recent publication on stress metabolites produced by potato tubers[1] includes a proposed structure (I) for phytuberin, an antifungal compound first investigated by Varns[2] in studies on the resistance of potato tuber slices to infection by the late blight fungus Phytophthora infestans. We have isolated phytuberin from potato tubers inoculated with the bacterium Erwinia carotovora var atroseptica[3] and our spectral and chemical observations leading to a revised structure (IIa) are given below.

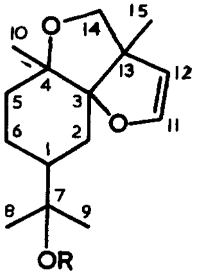
Phytuberin, $C_{17}H_{26}O_4$ ($M^{+\cdot}$294) is a neutral oil which gave no reactions for hydroxyl, ketone or aldehyde groups and had no ultraviolet absorption above 210 nm. The 25.2 MHz cmr spectrum in $CDCl_3$ (TMS as internal standard), obtained with proton noise decoupling, showed 16 singlet signals at δ16.5, 21.6, 22.2, 23.1, 23.4, 29.0, 34.3, 43.7, 45.0, 73.6, 83.8, 93.0, 94.4, 104.4, 146.2 and 170.0. The multiplicities of these signals determined under single-frequency off-resonance decoupled conditions indicated together with chemical shift considerations[4], that the 17 carbon atoms of phytuberin were present in the following skeletal units: one $-\underline{C}(=O)-O-$, one $-O-\underline{C}H=$, one $-\underline{C}H=$, three $\geq\underline{C}-O-$, one $-\underline{C}H_2-O-$, one $\geq\underline{C}-$, one $\geq\underline{C}-H$, three $-\underline{C}H_2-$ and five $-\underline{C}H_3$.

The 100 MHz pmr spectrum of phytuberin in $CDCl_3$ (TMS as internal standard) revealed 5 singlet methyl signals at δ1.04, 1.44, 1.47, 1.56 and 1.99, two olefinic protons 4.67 (1H, d J 2.8Hz, $-\underline{CH}=$) and 6.44 (1H, d J 2.8Hz, $-O-\underline{CH}=$), an AB system due to a $-\underline{CH}_2-O-$ moeity 3.26 (1H, d J 8.5Hz), and 3.39 (1H, d J 8.5Hz), and seven remaining protons as a complex multiplet centred at δ1.6.

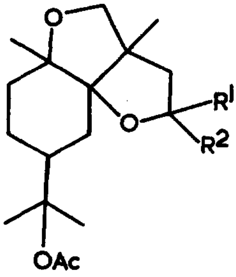
The methyl group at δ1.99 was assigned to an acetate group (infrared ν_{max} 1728 and 1260 cm^{-1}) which was inert to alkaline hydrolysis (NaOH/MeOH) but was removed by reduction ($LiAlH_4$) to give the tertiary alcohol desacetylphytuberin (IIb) $C_{15}H_{24}O_3$ ($M^{+\cdot}$252). The 100 MHz pmr spectrum of (IIb) in $CDCl_3$ showed only 3 distinct methyl signals at δ0.99. (3H,s, bridgehead methyl), 1.18 (6H,s) and 1.53 (3H,s). Removal of the acetate group from phytuberin had



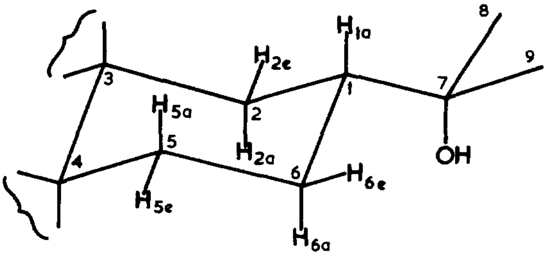
(I)



(IIa, R = Ac)
(IIb, R = H)



(IIIa, $R^1 = R^2 = H$)
(IIIb, $R^1 = OH, R^2 = H$ & $R^1 = H, R^2 = OH$)
(IIIc, $R^1, R^2 = O$)



(IV)

resulted in an average upfield shift of δ0.28[5] for the two methyl groups of the acetyloxyisopropyl sidechain.

Dihydrophytuberin (IIIa) $C_{17}H_{28}O_4$ ($M^{+\cdot}$296), mp 62°C (from n-pentane) was obtained by catalytic hydrogenation of phytuberin in the presence of Pt catalyst in glacial acetic acid; the two olefinic protons in phytuberin are present as part of a vinyl ether function (infrared ν_{max} 735, 1088, 1620 and 3080 cm^{-1}) and the observed δ values and the coupling constant J 2.8 Hz between these protons indicated that this moiety was part of a dihydrofuran ring[6]. Acid catalysed hydration (H^+/H_2O, dioxan) of the double bond in phytuberin gave an epimeric mixture of lactols (IIIb) which on mild oxidation (CrO_3/pyridine) provided the γ-lactone (IIIc), $C_{17}H_{26}O_5$ ($M^{+\cdot}$310) ν_{max} 1776 cm^{-1}. The 100MHz pmr spectrum of (IIIc) in C_6D_6 exhibited two methylene AB quartets associated with the methylene group adjacent to the

TABLE 1. P.m.r. data on desacetylphytuberin (IIb) before and after the addition of the europium shift reagent Eu(fod)$_3$d$_{27}$

Signal identification	$\delta^{0.00}$	$\delta^{1.00}$	$\Delta\delta=\delta^{1.00}-\delta^{0.00}$
CH$_3$ at C-15	0.99	3.30	2.3
CH$_3$'s at C-8,C-9	1.18	$\left\{\begin{matrix}9.10\\9.04\end{matrix}\right\}$	7.9
CH$_3$ at C-10	1.53	6.17	4.6
H at C-14	3.23	8.52	5.3
H' at C-14	3.37	8.82	5.4
H at C-12	4.64	7.36	2.7
H at C-11	6.41	8.01	1.6
A	1.6	10.92	9.3
B	1.6	8.92	7.3
C	1.6	8.20	6.6
D	1.6	7.83	6.2
E	1.6	7.10	5.5
F	1.6	6.75	5.2
G	1.6	4.09	2.5

$\delta^{0.00}$ is chemical shift relative to TMS in the absence of Eu(fod)$_3$

$\delta^{1.00}$ is the chemical shift in the presence of a 1:1 mole ratio of Eu(fod)$_3$ to (IIb)

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carbonyl function (δ 2.19 and 2.54 J 18.5 Hz) and a further methylene group adjacent to oxygen (δ3.25 and 3.44 J 9 Hz).

Since phytuberin possesses only one carbon-carbon double bond and only one carbon--oxygen double bond (present in an acetate group) it must possess a tricyclic structure and the remaining oxygen atom can only be present in a saturated ether ring system. The observed δ values and coupling constants for the protons of the -CH$_2$-O- moiety both in phytuberin and the lactone (IIIc) indicate the presence of a saturated 5-membered ether ring in phytuberin.

Evidence for a 1,3,3',4,4'-substituted cyclohexane ring in a rigid chair conformation in phytuberin was obtained from pmr data on desacetylphytuberin (IIb) in the presence of the deuteriated lanthanide shift reagents Eu(fod)$_3$d$_{27}$ and Pr(fod)$_3$d$_{27}$[7]. In the 100 MHz pmr spectrum of (IIb) determined in CDCl$_3$ in the absence of a shift reagent the protons H_{1a}, H_{2a}, H_{2e}, H_{5a}, H_{5e}, H_{6a} and H_{6e} of the cyclohexane ring (IV) overlap and form a broad multiplet centred around δ1.6. Addition of 0.4-1.0 moles of Eu(fod)$_3$ per mole of (IIb) caused downfield shifts,which were approximately linear with respect to concentration of Eu(fod)$_3$, of all the signals in the spectrum and resulted in separation of the signals due to the individual protons of the cyclohexane ring. Table 1 lists the δ values for all the protons in the spectrum of (IIb) before and after the addition of 1 mole of shift reagent per mole of substrate. In the Table the observed signals due to the protons of the cyclohexane ring are labelled A,B,C,D,E,F, and G in order of decreasing europium-induced shift $\Delta\delta$. A comparison of the line shapes of the observed signals A to G with the predicted first order line shapes for the protons H_{1a}, H_{2a}, H_{2e}, H_{5a}, H_{5e}, H_{6a} and H_{6e}, assuming the following parameters $J_{geminal} = J_{aa'} = 13$ Hz, and $J_{ae'} = J_{ea'} = J_{ee'} = 4$ Hz, showed an exact 1:1 correlation as follows $A = H_{1a}$, $B = H_{6e}$, $C = H_{2a}$, $D = H_{2e}$, $E = H_{6a}$, $F = H_{5a}$ and $G = H_{5e}$. Double irradiation pmr experiments carried out on the europium shifted spectrum verified the above deductions and these will be discussed in a later publication together with further details of the lanthanide shift experiments.

One further structural feature confirmed by the data in Table 1 was the presence of the hydroxyisopropyl side chain in (IIb); the two methyl groups at C-8 and C-9 each shifting by an average $\Delta\delta$ of 7.9 ppm.

On the basis of biogenetic considerations and the summarised data presented above we propose the novel structure (IIa) for phytuberin. An X-ray crystallographic study of dihydrophytuberin (IIIa) is in progress to provide complete stereochemical detail.

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