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TOXICOLOGICAL RESEARCH ON SUBSTANCES FROM FUSARIUM NIVALE III. THE STRUCTURE OF NIVALENOL AND ITS MONOACETATE

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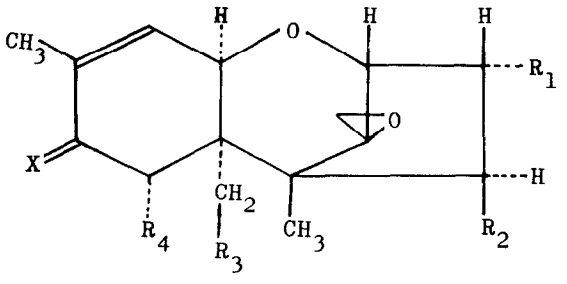
Numerous observations(1) of intoxication in animals fed scabby grains prompted us to isolate toxic principles from the grains infected with Fusaria. Scirpene type sesquiterpenoids, nivalenol (I): mp 222-223° and fusarenon: mp 78-80° have been obtained by Tatsuno et al(2) and Morooka et al.(3) from rice grains polluted by Fusarium nivale Fn-2, a strain originally isolated from actually damaged wheat. In addition, nivalenol monoacetate (II) possessing a marked cytotoxic activity has recently been isolated from a culture broth of the fungus by Y. Ueno. S. G. Yates et al.(4) reported that scirpene type sesquiterpenoid (III) was obtained from Fusarium nivale which isolated from tall fescue, as well as a butenolide. Diacetoxyscirpenol (IV) and 4,15-diacetoxy-3α,7α-dihydroxyscirp-9-en-8-one (V), have been isolated from the plant parasitic fungus Fusarium equiseti and from culture filtrates of F. diversisporum, F. sambucinum and F. tricinctum by P. W. Brian et al.(5), H. P. Sigg et al.(6) and J. R. Bamburg et al.(7). The structure and absolute configuration of these sesquiterpenoids have already been established as in III(7), IV(6,8) and V(9).

The structure (I), 3α,4β,7α,15-tetrahydroxyscirp-9-en-8-one, is now proposed for nivalenol by the interconnection with diacetoxyscirpenol and the following chemical and spectroscopic properties. Nivalenol (I): $C_{15}H_{20}O_7$, M^+ ion m/e 312, λ_{max}^{MeOH} 218mμ (ε, 6300), ν^{KBr} 3500-3200 (OH), 1680, 1610 cm^{-1} (αβ-unsaturated ketone), $\delta^{d\text{-DMSO}+D_2O}$ 0.95 (3H, s, CH_3-C-), 1.17 (3H, d, J=1 cps, CH_3-C=CH-), 2.87

and 2.97 (each 1H, d, J=5 cps, CH_2-C-), 6.58 (1H, dd, J=5 cps, 1 cps, HC=C-). Nivalenol showed the positive epoxide test(10) with sodium thiosulfate and its NMR spectrum indicated the presence of an ethylene oxide ring (typical AB system at 2.87 and 2.97). The NMR spectrum of nivalenol did not show any signal due to an aldehyde proton, but nivalenol was oxidised with Tollen's reagent under mild conditions, thereby suggesting the presence of an α-ketol function in the molecule. Hydrogenation of nivalenol on Pd-C gave the dihydro derivative (mp 242-245 , ν^{KBr} 1700 cm^{-1}).



I R_1=R_2=R_3=R_4=OH, X=O

II R_1=R_3=R_4=OH, R_2=OAc, X=O

III R_1=OH, R_2=R_3=OAc, R_4=H, X=H, $O_2CCH_2CH(CH_3)_2$

IV R_1=OH, R_2=R_3=OAc, R_4=H, X=H_2

V R_1=R_4=OH, R_2=R_3=OAc, X=O

VI R_1=R_2=R_3=R_4=OAc, X=O

VII R_1=R_2=R_3=OAc, R_4=H, X=O

Acetylation of nivalenol with acetic anhydride-pyridine afforded tetraacetylnivalenol (VI) : $C_{23}H_{28}O_{11}$, mp 168-170°, M^+ ion m/e 480, λ_{max}^{MeOH} 227 mμ (ε, 7900), ν^{KBr} 1740, 1690 cm^{-1}, δ^{CDCl_3} 0.85 (3H, s), 1.88 (3H, d, J=1.5 cps) , 2.70 and 3.13 (each 1H, d, J=4 cps), 4.03 (1H, d, J=5 cps), 4.28 and 4.67 (each 1H, d, J=12 cps), 4.70 (1H, d, J=4 cps), 5.25 (1H, dd, J=5 cps, 3 cps), 5.87 (1H, d, J=3 cps), 6.09 (1H, s), 6.62 (1H, dd, J=6 cps, 1.5 cps).

The above chemical and spectral properties of nivalenol indicated the presence of four hydroxyl groups, a tertiary methyl group , one methyl group at a double bond, an ethylene oxide ring and αβ-unsaturated carbonyl group : the remaining oxygen atom was attributed to an ether linkage. The NMR spectrum of tetraacetylnivalenol, in conjunction with the NMR data (Fig. 1), disclosed the arrangement of the hydrogens on C^2-C^3-C^4 and C^{16}-C^9-C^{10}-C^{11}.

The close relationship between nivalenol and scirpene type sesquiterpenoids was evident by the above chemical and spectroscopic properties of nivalenol and its tetraacetate. The chemical conversion of diacetoxyscirpenol (IV) to 3α,4β,15-triacetoxyscirp-9-en-8-one (VII) has already been achieved by H. P. Sigg et al(6).

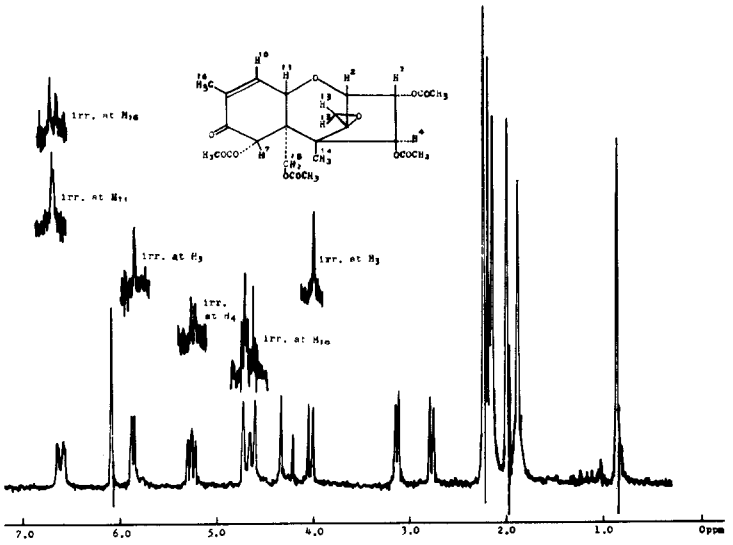


Fig. I. NMR and NMDR spectra of tetraacetylnivalenol (VI)

Reduction of tetraacetylnivalenol with zinc-acetic acid produced the dehydroxy derivative which was completely identical with 3α,4β,15-triacetoxyscirp-9-en-8-one (VII) in TLC, mp and IR spectrum.

Thus, nivalenol (I) is 3α,4β,7,15-tetrahydroxyscirp-9-en-8-one. The configuration of the C-7 hydroxyl group can be shown as in I based on the following observation. The methyl group which is spatially close to a hydroxyl group is known to produce a marked upward shift by acetylation of the hydroxyl group(11). This relationship was employed to establish the stereochemistry of the C-7 hydroxyl group of V as α-configuration by B. K. Tidd(9). We also applied this relationship for the assignment of configuration of the C-7 hydroxyl group in nivalenol. Acetylation of nivalenol caused an upward shift by 20 cps of the C-14 methyl signal. Thus, the C-7 hydroxyl group is in α-configuration and nivalenol is 3α,4β,7α,15-tetrahydroxyscirp-9-en-8-one.

The NMR spectrum of tetraacetylnivalenol (VI) showed chemical shifts and coupling constants similar to those attributed to the hydrogens of 3α,4β,7α,15-tetraacetoxyscirp-9-en-8-one, which was presumably derived from V by B. K. Tidd(9). Thus, it may be deduced that tetraacetylnivalenol (VI) is 3α,4β,7α,15-tetraacetoxyscirp-9-en-8-one.

Nivalenol-monoacetate (II) : $C_{17}H_{22}O_8$, mp 91-92°, M^+ ion m/e 354, ν^{KBr} 3400 (OH), 1725 (acetate), 1685 cm^{-1} (αβ-unsaturated ketone). II was hydrolysed to nivalenol with methanolic ammonium hydroxide, while acetylation of II with acetic anhydride-pyridine gave tetraacetylnivalenol (VI). The NMR spectrum of II showed chemical shifts and coupling constants similar to those attributed to the hydrogens at position 2,3,7,10,11,13,14 and 15 in nivalenol and the C-4 hydrogen resonates at 1.2 ppm down field compared with that of nivalenol.

Thus, II is concluded to be nivalenol-4-O-acetate.

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