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GLUTINOSONE, A NEW ANTIFUNGAL SESQUITERPENE FROM *NICOTIANA GLUTINOSA* INFECTED WITH TOBACCO MOSAIC VIRUS

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Key Word Index—*Nicotiana glutinosa*; Solanaceae; tobacco mosaic virus; sesquiterpene; glutinosone; phytoalexin.

Abstract—Glutinosone is an antifungal substance induced in leaves of *Nicotiana glutinosa* following infection with tobacco mosaic virus. From chemical, spectroscopic and biogenetic evidence it has been assigned structure (**1**).

INTRODUCTION

The concept of "chemical defence" by plants against fungal attack has received much attention in recent years and a wide range of natural antifungal compounds is now known [1]. In general these compounds are absent or occur at very low levels in healthy plants but their concentrations increase considerably on infection. In much of the earlier research inoculation with fungi was employed but recently it has been shown that virus treatment can also induce the formation of fungitoxic compounds [2,3].

In this paper we report the structural elucidation of "glutinosone", a new antifungal sesquiterpene isolated from leaves of *Nicotiana glutinosa* bearing small necrotic lesions which had arisen from infection with tobacco mosaic virus. The compound could not be detected in healthy uninoculated leaves.

RESULTS AND DISCUSSION

Glutinosone was isolated as a colourless mobile oil and has the molecular formula $C_{14}H_{20}O_2$ as determined by MS. Spots of the compound on thin layer plates containing fluorescent indicator quenched strongly under UV light and gave an orange colour when sprayed with 2,4-dinitrophenylhydrazine reagent. Glutinosone has a single UV maximum at 237 nm (ϵ 15400) while a prominent IR absorption peak occurs at 1685 cm^{-1}. These data suggest the presence of an unstrained α,β-unsaturated ketone and an olefinic singlet at δ 5·88 in the PMR spectrum further implies that this is trisubstituted.

Glutinosone contains an isopropenyl group as revealed by IR absorption at 1630 and 895 cm^{-1} and resonances at δ 1·68 (3H, vinylic methyl) and 4·69 (2H, H_2C=). An hydroxyl group is responsible for IR absorption at 3520 cm^{-1} and a broad PMR peak at δ 3·73 which disappears on addition of D_2O. The presence in the PMR spectrum of a one proton doublet (J 12 Hz) at δ 3·77 which undergoes a marked downfield shift to δ 5·10 on acetylation further suggests that the alcohol is secondary. The remaining functional group in glutinosone is a secondary methyl which appears as a distorted doublet (δ 1·20) in $CDCl_3$ but as a well resolved doublet (δ 1·30, J 6 Hz) in deuteriopyridine.

The necessity of assembling these functional groups into a structure having a molecular formula $C_{14}H_{20}O_2$ requires that glutinosone be bicyclic. Further information is obtained from a diol which was obtained by sodium borohydride reduction of glutinosone. Here the olefinic singlet appears at δ 5·30 displaying a considerable upfield shift as a result of the removal of the deshielding effect of the carbonyl group. The alcoholic methine proton resonating as a doublet at δ 3·77 in glutinosone also underwent an upfield shift, this time appearing as a triplet at δ 3·18. This suggests that

the secondary alcohol is located α to the carbonyl in glutinosone, the observed 1:2:1 triplet in the diol being readily explained if the alcoholic methine is now coupled equally with a methine proton on each of the adjacent carbons with J 9 Hz. Hence glutinosone is an α-ketol.

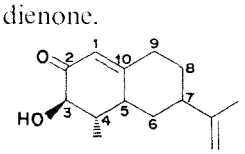
That the α,β-unsaturated ketone was part of a 6-membered ring was shown by reacting glutinosone with concentrated hydrochloric acid in glacial acetic acid. The major product obtained was a phenol $C_{14}H_{18}O$ (M^+ 202) which gave an orange-red colour with diazotized *p*-nitroaniline and had a low intensity aromatic UV absorption maximum at 280 nm (ϵ 2100). In the PMR spectrum, the phenol exhibited signals from two aromatic protons (multiplet δ 6·47), a single hydroxyl group (δ 4·50, disappearing with D_2O), a sharp three proton singlet at δ 2·18 and a six proton singlet at δ 1·65. This last resonance, together with the absence of olefinic absorption near 4·7 indicates that the isopropenyl group had isomerized to an isopropylidene function while the methyl singlet at 2·18 is in the expected position for an aromatic methyl and is evidently derived from the secondary methyl of glutinosone. This locates the secondary methyl in glutinosone as being α- to the secondary alcohol, the methine protons of these two groups in glutinosone being coupled with J 12 Hz.

The production of a benzene ring implies that one of the rings in glutinosone is 6-membered and bearing in mind the necessity of accommodating a trisubstituted double bond, the most logical structure is that of a decalin with the double bond sited at one ring junction (as in **1**). The data already provided are sufficient to locate all the functional groups in ring *A* and the formation of the phenol is now readily explained in terms of a dehydration and subsequent tautomerism of the resulting dienone.

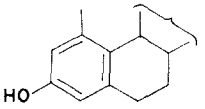
The position of the isopropenyl group in ring *B* remains to be determined but some information on this is provided by a further consideration of the phenol discussed above. This has the low intensity absorption (ϵ 2100) associated with an isolated phenolic chromophore, a suitable model compound being 1-methyloestrone (**2**) which has λ_{max} 282 (ϵ 1820) [4]. The location of the isopropylidene group at C_6 or C_9 would give rise to a styryl chromophore with a considerably enhanced extinction coefficient. As this is not observed in the phenol, the original isopropenyl group in glutinosone must be sited at C_7 or C_8.

A decision in favour of C_7 can be made on biogenetic grounds when it is recognized that glutinosone is very probably a sesquiterpene of the eudesmane class. A similar sesquiterpene which has also lost a methyl group at C_{10} is rishitin (**3**) an induced antifungal compound in potato [5,6], and tomato [7]. Hence glutinosone is tentatively formulated as (**1**) and the phenol obtained by acid treatment is (**4**).

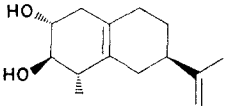
The complete stereochemistry of glutinosone is not known at present. However, the observed coupling constant of 12 Hz for the methine protons at C_3 and C_4 is only consistent with these protons being *trans*-diaxial hence the methyl and hydroxyl groups are *trans*-diequatorial. In the diol (**5**) derived by borohydride reduction, the NMR data can be readily explained if ring *A* exists in the half-chair conformation with all the substituents orientated equatorially. In this conformation the dihedral angle between the olefinic proton at C_1 and the methine proton at C_2 is approximately 90° (Dreiding model). This accounts for the lack of vicinal coupling [8] between these two protons, the methine proton at C_2 appearing at δ 4·05 as a slightly broadened doublet with J 9 Hz.



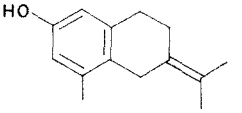
Glutinosone (**1**)



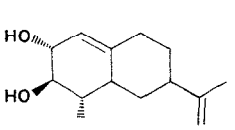
(**2**)



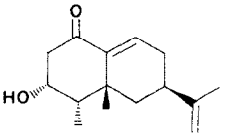
Rishitin (**3**)



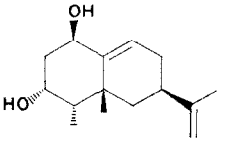
(**4**)



(**5**)



Capsenone (**6**)



Capsidiol (**7**)

The structural similarity of glutinosone (**1**) and rishitin (**3**) has already been mentioned and it is also of interest to note that related antifungal sesquiterpenes capsenone (**6**) and capsidiol (**7**) have been isolated [9,10] from sweet pepper inoculated with fungi. As tobacco, potato, tomato and sweet pepper all belong to the same family, the Solanaceae, this is clearly of phytochemical interest.

Previous work [2] with *Phaseolus vulgaris* has indicated that compounds produced following virus infection may function as phytoalexins [1]. Information is not yet available to indicate whether glutinosone is also produced in fungal-infected tissue of *N. glutinosa*. Nevertheless, its fungitoxicity and chemical similarity to the phytoalexins discussed above strongly suggest that glutinosone may function as a phytoalexin. Its antifungal spectrum and details of its formation and localization will be published elsewhere.

EXPERIMENTAL

Unless otherwise stated, all PMR data are for $CDCl_3$ soln while IR and UV measurements were in $CHCl_3$ and EtOH respectively.

Isolation of glutinosone. Young plants (6–8 weeks old) of *N. glutinosa* with 4–5 fully expanded leaves were inoculated with TMV in aq suspension. Carborundum was used to aid virus adsorption. When brown lesions had been produced (5–7 days), the leaves (2 kg fr. wt) were harvested, deep frozen at −20° for several days and then extracted with C_6H_6 (3 ×). The solvent was removed from the crude extract which was then introduced onto a column of silicic acid (200 g) and eluted with hexane acetone (20:1). Glutinosone was readily detected in the eluate by its quenching of UV light on Merck F_{254} silica plates, orange 2,4-DNP coloration and yellow–green colour with vanillin-phosphoric acid reagent. Fractions containing glutinosone were then bulked, evaporated and rechromatographed on TLC using hexone–acetone (3:1). Glutinosone (87 mg) was thus isolated as a clear oil. It was homogeneous on TLC, having an R_f of 0·32 with hexane–acetone (3:1) and R_f of 0·48 with $CHCl_3$–EtOH (97:3). Characteristic retention times on GLC were 3·15 min (3% OV-17, 194°, 50 ml N_2/min) and 6·4 min (2% OV-225, 160°, 50 ml N_2/min). In the MS peaks were at *m/e* 220 (22%), 192 (15), 191 (8), 162 (50), 147 (20), 134 (20), 121 (78) and 94 (100).

Acetylation of glutinosone. Glutinosone (20 mg) was refluxed with Ac_2O (0·5 ml) and pyridine (0·5 ml) for 1 hr and the acetate (12 mg) was isolated after purification on TLC (C_6H_6–$CHCl_3$, 1:1).

Sodium borohydride reduction of glutinosone. Glutinosone (20 mg) was reacted with $NaBH_4$ (15 mg) in EtOH (3 ml) for 2 hr. The reaction mixture was chromatographed on TLC (hexane–acetone, 3:1) and the major product located by spraying the plate edge with vanillin-phosphoric acid when a purple colour was obtained on heating. On recovery from the silica, the diol crystallized from hexane–EtOAc as needles (13 mg), m.p. 129–132°.

Acid treatment of glutinosone. Glutinosone (25 mg) was dissolved in HOAc (1 ml) and 6 drops conc HCl were added. The mixture was shaken at room temp. for 1 hr, diluted with H_2O and ether extracted. TLC (hexane–EtOAc 9:1) of the extract afforded a major phenolic product (7 mg) which quenched weakly but which gave an intense orange colour with diazotized *p*-nitroaniline.

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