Studies in Terpenoid Biosynthesis. Part 31. Some Aspects of the Chemistry and Biosynthesis of the Steroidal Antibiotic, Demethoxyviridin†

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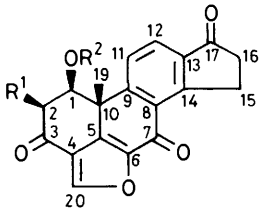
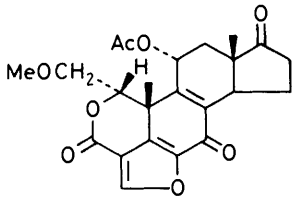
The stereochemistry of some reactions of demethoxyviridin including the reduction of ring A and the preparation of deuteriated dehydroxydemethoxyviridin, is described. The detection of the latter in the fungus, *Nodulisporium hinnuleum* and its conversion into demethoxyviridin, is reported.

The fungal metabolite, demethoxyviridin (**1**),[1]† is one member of a small but interesting group of steroidal antifungal antibiotics. Unlike other naturally occurring steroids, they contain a furan ring attached to C-4 and C-6 and some possess an aromatic ring C. They differ from one another in the oxidation pattern of ring A. The structure of viridin (**2**)[2]† was established by a combination of oxidative studies and spectroscopic measurements and it was confirmed by an *X*-ray analysis.[3] The structure, but not the absolute configuration of demethoxyviridiol was also established by *X*-ray analysis.[1] The structure of wortmannin (**3**) was determined by hydrolysis and interrelationship with a common degradation product of viridin.[4] We have examined a number of aspects of the biosynthesis of these compounds[5,6] including the enrichment and labelling patterns of demethoxyviridin (**1**) biosynthesized by *Nodulisporium hinnuleum* from [1-^{13}C]-, [1,2-$^{13}C_2$]-acetate, [2-^{13}C]- and [5-^{13}C]-mevalonate and from [2]-, [4]-, and [5]-^{2}H and ^{3}H-mevalonates. In this paper we describe the preparation of stereospecifically labelled dehydroxydemethoxyviridin and its role in the biosynthesis of demethoxyviridin (**1**). No chemical studies on demethoxyviridin have been reported. Since we also required some compounds to facilitate the assignment of the carbon-13 n.m.r. spectra in this series,[5] we have examined some features of the chemistry of demethoxyviridin. Like viridin[2] the chemistry of demethoxyviridin is dominated by its alkali instability and hence it is handled, wherever possible, in mildly acidic solution.

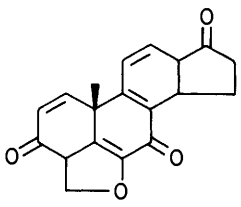


(**1**) $R^1 = R^2 = H$
(**2**) $R^1 = OMe$, $R^2 = H$
(**4**) $R^1 = H$, $R^2 = Ac$
(**5**) $R^1 = H$, $R^2 = Ms$

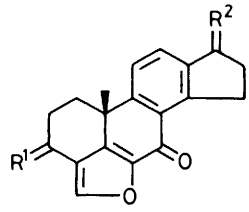


(**3**)

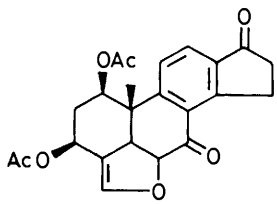
Demethoxyviridin (**1**) readily gave a mono-acetate (**4**) on treatment with acetic anhydride in pyridine but unlike viridin it did not easily form the Δ^2-enol acetate. In the acetate an aromatic ^{1}H n.m.r. signal, which was consequently assigned to 11-H, moves upfield to become co-incident with the 12-H signal. This permits these signals to be distinguished, a feature which is of interest in determining the stereochemistry of the biosynthetic aromatization of ring C. On treatment with methanesulphonyl chloride, demethoxyviridin gave a monomethanesulphonate (**5**) which underwent a facile elimination to afford the Δ^1-$\alpha\beta$-unsaturated ketone (**6**) (δ 6.38 and 7.64, *J* 10 Hz). In several instances this elimination occurred without the isolation of the intermediate methanesulphonate.



(**6**)



(**7**) $R^1 = R^2 = O$
(**8**) $R^1 = \alpha$-H, β-OAc, $R^2 = O$
(**9**) $R^1 = \alpha$-H, β-OAc, R^2 = H, OAc



(**10**)

Catalytic hydrogenation of the unsaturated ketone (**6**) over palladium on charcoal gave the corresponding saturated ketone (**7**). This selective hydrogenation was only successful on rigorously purified material particularly when the unsaturated ketone (**6**) had been obtained directly from demethoxyviridin. Since the biosynthesis of the viridin series of compounds involves the oxidative modification of ring A, the reduction was carried out using deuterium gas to afford material in which the stereochemistry of labelling could be defined. The deuterium gas was prepared by the action of [$^2H_2$]water on sodium in tetrahydrofuran and was used immediately. The catalyst was pre-saturated with deuterium. Although 98.3% [$^2H_2$]water was used, the isotope distribution in the product, determined mass spectrometrically, on the molecular ion revealed the presence of 21.1% $^2H_0$, 48.7% $^2H_1$, 29.7% $^2H_2$, and 0.6% $^2H_3$ species. The substantial quantities of mono- and un-deuteriated species may arise from adverse isotope effects in the preparation of the deuterium gas, the catalytic reduction and possible subsequent exchange at C-2 during the work-up. The stereochemistry of the reduction followed from a careful examination of the high-field ^{1}H n.m.r. spectra. Signals at δ 2.24, 2.74, 2.81, and 3.00 were assigned to the 1α-H, 1β-H, 2α-H, and 2β-H protons respectively on the basis of the magnitude of the coupling

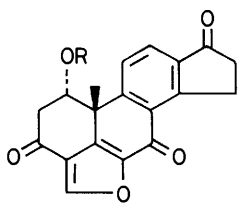
† Viridin is 1β-hydroxy-2β-methoxy-18-norandrosta-5,8,11,13-tetraeno[6,5,4-*bc*]furan-3,7,17-trione.

Table. Coupling constants (determined at 360 MHz) in (**7**)

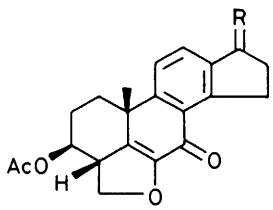
Proton	Signal	Coupling constants (Hz)
1-α	2.24	13.5 ($J_{1\alpha.1\beta}$), 13.5 ($J_{1\alpha.2\beta}$), 5 ($J_{1\alpha.2\alpha}$)
1-β	2.74	Obscured by the 16-H signals
2-α	2.81	18 ($J_{2\alpha.2\beta}$), 5 ($J_{1\alpha.2\alpha}$), 2 ($J_{1\beta.2\alpha}$)
2-β	3.00	18 ($J_{2\alpha.2\beta}$), 13.5 ($J_{1\alpha.2\beta}$), 5.3 ($J_{1\alpha.2\beta}$)

constants (see Table 1). The decrease in the relative integrals in the ^{1}H n.m.r. spectrum of the material from the catalytic deuteriation showed 0.6 ^{2}H at 1α-H, 0.5 ^{2}H at 2α-H, and 0.1 ^{2}H at 2β-H. Because of the overlap of signals it was not possible to calculate reliably the (small) amount of deuterium at 1β-H. The ^{2}H n.m.r. spectrum (determined at 55.3 MHz) contained signals at δ 2.24, 2.80, and 2.99 with relative integrals 1:0.7:0.09. This corresponds to predominant attack from the α-face of ring A directed by the β-oriented C-10 angular methyl group. However, the extent of the deuteriation was disappointing and it was not improved by the addition of deuterioacetic acid to the medium. On occasions a variable amount of a more polar product was obtained from the hydrogenation. The formation of this polar material could be prevented by careful monitoring (t.l.c.) of the hydrogenation. Acetylation of the polar product and further purification gave the acetate (**8**), (*vide infra*).

The αβ-unsaturated ketone (**6**) underwent conjugate axial addition of methanol and water under acidic conditions to afford the methyl ether (**11**) and the 1α-epimer of demethoxyviridin (**12**). The stereochemistry of these adducts followed from



(**11**) R = Me
(**12**) R = H



(**13**) R = O
(**14**) R = H,OAc

the coupling constants of the equatorial 1β-H signal which appeared as a triplet (δ 4.51, *J* 3.3 Hz) in the ether and as a broad triplet (δ 5.03) in the alcohol. The 1α-H signal in the normal series appears as a double doublet (*J* 5 and 10 Hz). As might be expected the chemical shift of 11-H reflects the stereochemistry of a hydroxy group at C-1. A C-1β (equatorial) hydroxy group produces a significant deshielding (Δδ 0.90 p.p.m.) compared to the desoxy compound whilst the 1α-C epimer produces a smaller deshielding of 0.3 p.p.m.

The lability to alkali shown by demethoxyviridin and some of its derivatives may be, in part, due to facile conjugate addition to the furan ring at C-20 under the influence of the carbonyl groups at C-3 and C-7. Consequently the selective reduction and esterification of the C-3, C-7, and C-17 carbonyl groups was examined. Reduction of the ketone (**7**) with sodium (triethoxy)-borohydride followed by acetylation of the resultant mixture gave a 3β-monoacetate (**8**) as the major product and the 3β,17-diacetates (**9**) as the minor products. The structure and stereochemistry of the 3β-monoacetate (**8**) followed from its ^{13}C and ^{1}H n.m.r. spectra. Whilst the ^{13}C n.m.r. spectra lacked the signal at δ 191 p.p.m. assigned to the C-3 carbonyl, the ^{1}H n.m.r. spectrum contained a triplet resonance (δ 5.78, *J* 6 Hz) assigned to the 3α-hydrogen atom. As expected the C-10 methyl group has directed hydride attack to the α-face of the molecule. This compound was also obtained by acetylation of the more polar components of the catalytic hydrogenation of the αβ-unsaturated ketone (**6**). The minor product was a mixture of the 3β,17β- and 3β,17α-diacetates.* The ^{1}H n.m.r. spectrum contained resonances at δ 5.86 (1 H, t, *J* 8 Hz) assigned to the 3-H and at δ 6.24 (1 H, m) assigned to the 17-H. There were three singlets at δ 2.03 (3 H), 2.10 and 2.13 (together 3 H) which were assigned to the 3β- and 17-acetoxy groups. The formation of epimers at C-17 is not surprising since there is no major directing effect at this centre. Similar reduction of demethoxyviridin followed by acetylation gave the diacetate (**10**) which was identical with the diacetate of demethoxyviridiol obtained from *Nodulisporium hinnuleum*.

On occasions, particularly when ethanol–tetrahydrofuran (1:1) was used as a solvent, products arising from the partial reduction of the furan ring in the ketone (**7**) were also obtained. The mixture was acetylated and the products were purified as their acetates to afford the mono- and di-acetates (**13**) and (**14**). The stereochemistry of the acetate (**13**) was assigned from an analysis of the ^{1}H n.m.r. spectrum and, in particular, the coupling constants between the C-3, C-4, and C-21 proton resonances. The C-3 proton resonance (δ 4.78) is readily distinguishable. Spin decoupling studies involving irradiation at this position led to the identification of the C-4 proton resonances (δ 3.58). Irradiation at this position revealed the C-20 proton resonances and the coupling pattern as shown in the Figure. The magnitude of the coupling constants suggests

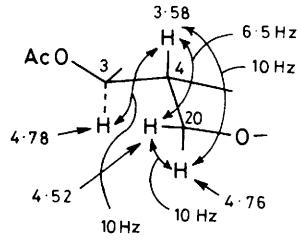


Figure.

that there is a *trans* diaxial relationship between the C-3 and C-4 protons leading to a 3α-H:4β-H-stereochemistry. The diacetate (**14**) showed a similar relationship. When the reduction was repeated with sodium borodeuteride in [^{2}H]methanol, the deuterium label, apart from appearing at C-3 and C-4, was, as shown by the ^{1}H n.m.r. spectrum, evenly distributed between the C-20 hydrogen atoms. Hence this labelled material could not be used to examine the stereochemistry of biosynthetic dehydrogenation reactions involving C-20.

Nevertheless, the dehydroxydemethoxyviridin (**7**) was used to examine the last stage in the biosynthesis of demethoxyviridin by *Nodulisporium hinnuleum*. When the fungus was grown on the laboratory scale, it was not possible to isolate any metabolites which might be intermediates in the biosynthesis of demethoxyviridin despite variations in the media and period of incubation. Hence we used a trapping experiment with a fermentation fed with [1-^{14}C]acetate which established the formation of dehydrodemethoxyviridin (**7**) by the fungus. [^{14}C]Demethoxyviridin (**1**) was readily prepared by incubating [1-^{14}C]acetate of [2-^{14}C]mevalonate with *Nodulisporium hinnuleum*.[5] Dehydration *via* the 1-methanesulphonate and catalytic reduction of the 1-ene, afforded [^{14}C]dehydroxy-demethoxyviridin (**7**). Incubation with *N. hinnuleum* gave

* In Table 1 of ref. 5 the ^{13}C n.m.r. spectrum of this compound is erroneously quoted; the data given in the Table is that of the diacetate (**12**).

[^{14}C]demethoxyviridin (**1**) (3.9% incorporation). [1α,2α-$^{2}H_2$]Dehydroxydemethoxyviridin (**7**) was also fed to *N. hinnuleum*. The resultant demethoxyviridin (**1**) was converted into its more soluble 1-acetate (**4**) with acetic anhydride in pyridine. The ^{2}H n.m.r. spectrum (determined at 55.3 MHz in chloroform) showed a signal at δ 5.44 corresponding to the 1α-proton bearing a label; *i.e.* hydroxylation had proceeded as anticipated, with an overall retention of configuration. However the absence of a signal corresponding to the 2-H implied that deuterium had exchanged from this position either during the biosynthesis or during the preparation of the derivative.

Experimental

^{1}H N.m.r. spectra were determined at 220 (PCMU, R34) and 360 MHz (Brucker WH360).

Derivatives of Demethoxyviridin.—*Demethoxyviridin 1β-acetate* (**4**). This, prepared with acetic anhydride in pyridine, crystallized from acetone as needles, m.p. 220—225 °C (decomp.) $[\alpha]_D$ −67° (Found: C, 69.2; H, 4.5. $C_{21}H_{16}O_6$ requires C, 69.2; H, 4.4%), $\nu_{max.}$ 1 740, 1 700, 1 660, 1 575, and 1 525 cm^{-1}; $\lambda_{max.}$ 301 (ε 12 000) and 238 nm (32 600); δ 1.76 (3 H, s, 19-H), 2.29 (3 H, s, OAc), 2.69 (2 H, m, 16-H_2), 3.02 (2 H, m, 2-H_2), 3.7 (2 H, m, 15-H_2), 5.44 (1 H, dd, *J* 5 and 10 Hz, 1-H), 7.93 (2 H, s, 11- and 12-H), and 8.24 (1 H, s, 20-H).

The *methanesulphonate* (**5**), prepared with methanesulphonyl chloride in pyridine at 0 °C, crystallized from acetone as prisms, m.p. 150—155 °C (decomp.), $[\alpha]_D$ +3.7° (Found: C, 59.6; H, 4.4. $C_{20}H_{16}O_7S$ requires C, 59.9; H, 4.0%), $\nu_{max.}$ 1 720br, 1 670, and 1 580 cm^{-1}; δ (90 MHz), 1.7 (3 H, s, 19-H), 2.6 (2 H, m, 16-H), 3.07 (3 H, s, $MeSO_2$), 3.23 (2 H, m, 2-H_2), 3.7 (2 H, m, 15-H_2), 5.16 (1 H, dd, *J* 6 and 10 Hz, 1-H), 7.94 and 8.07 (ABq, *J* 7 Hz, 11-and 12-H), and 8.2 (1 H, s, 20-H). When the reaction was carried out at room temperature the product was *dehydroxydemethoxy-1,2-didehydroviridin* (**6**) which crystallized from acetone as needles, m.p. 228—232 °C (decomp.), $[\alpha]_D$ −104° (*c* 0.4) (Found: C, 70.4; H, 4.9. $C_{19}H_{12}O_4 \cdot H_2O$ requires C, 70.8; H, 4.9%), $\nu_{max.}$ 1 750, 1 725, 1 700, 1 660, 1 580, and 1 520 cm^{-1}; $\lambda_{max.}$ 240 (ε 33 400) and 309 nm (9 500); δ 1.76 (3 H, s, 19-H), 2.73 (2 H, m, 16-H), 3.73 (2 H, m, 15-H), 6.38 and 7.64 (each 1 H, d, *J* 10 Hz, 1- and 2-H), 7.8 and 8.02 (each 1 H, d, *J* 8 Hz, 11- and 12-H), and 8.27 (1 H, s, 20-H).

Dehydroxydemethoxyviridin (**7**).—The unsaturated ketone (**6**) (1 g) in ethyl acetate (150 ml) was stirred with 10% palladium on charcoal (500 mg) under an atmosphere of hydrogen for 1 h at room temperature. The reduction was followed by t.l.c. The catalyst was filtered off and the solvent was evaporated to afford *dehydroxydemethoxyviridin* (**7**) which crystallized from acetone–light petroleum as needles, m.p. 236—246 °C (decomp.), $[\alpha]_D$ −83° (*c* 0.4) (Found: C, 74.7; H, 4.7. $C_{19}H_{14}O_4$ requires C, 74.5; H, 4.6%), $\nu_{max.}$ 1 700, 1 670, 1 630, and 1 580 cm^{-1}; δ 1.65 (3 H, s, 19-H), 2.24 (1 H, ddd, *J* 5, 13.5, 13.5 Hz, 1α-H), 2.69—2.74 (3 H, m, 1β-H and 16-H), 2.81 (1 H, ddd, *J* 2, 5, and 18 Hz, 2α-H), 3.00 (1 H, ddd, *J* 5.3, 13.5, and 18 Hz, 2β-H), 3.69 and 3.80 (ABq, *J* 6 Hz, 15-H), 7.62 and 7.9 (each 1 H, d, *J* 8 Hz, 11- and 12-H), and 8.20 (1 H, s, 20-H). On occasions a variable amount of polar material was formed. Acetylation and purification of this material gave the acetate (**8**) identical (i.r. and n.m.r.) to the material obtained in the sodium borohydride reduction (see below).

Catalytic Deuteriation.—Deuterium gas was generated by the addition of deuterium oxide (containing 1.7% water by n.m.r.) in tetrahydrofuran to sodium pieces in tetrahydrofuran. A solution of dehydrodemethoxyviridin-1-ene (160 mg) in ethyl acetate (25 ml) was stirred with pre-reduced 10% palladium on charcoal (60 mg) until reduction was complete (t.l.c. control) and the reaction mixture had a characteristic blue-green fluorescence. The suspension was filtered and the solvent was evaporated to afford a residue which was recrystallized from acetone–light petroleum to afford [1,2-$^{2}H_2$]dehydroxymethoxyviridin (40 mg), m.p. 247—250 °C (decomp.). The reduction was repeated in the presence of [$^{2}H_4$]acetic acid (10 μl) but there was no significant difference in the ^{1}H or ^{2}H n.m.r. spectra of the product.

Dehydroxydemethoxy-1α-methoxyviridin (**11**).—The unsaturated ketone (**6**) (1 g) in methanol (59 ml) was treated with sulphuric acid (0.1 ml) for 10 h at room temperature. The solution was poured into water and the product was recovered in ethyl acetate. The organic phase was washed with water and dried (Na_2SO_4). The solvent was evaporated to afford *dehydroxydemethoxy-1α-methoxyviridin* (**11**) (800 mg) which crystallized from acetone as needles, m.p. 255—260 °C (decomp.), $[\alpha]_D$ −16° (*c* 0.6) (Found: C, 71.5; H, 5.1. $C_{20}H_{16}O_5$ requires C, 71.4; H, 4.8%), $\nu_{max.}$ 1 710, 1 700, 1 630, and 1 590 cm^{-1}; $\lambda_{max.}$ 239 (ε 33 900) and 301 nm (11 900); δ 1.63 (3 H, s, 19-H), 2.73 (2 H, m, 16-H_2), 3.07 (2 H, m, 2-H), 3.77 (2 H, m, 15-H_2), 3.2 (3 H, s, OMe), 4.5 (1 H, t, *J* 3.3 Hz, 1-H), 7.6 and 8.0 (each 1 H, d, *J* 8 Hz, 11- and 12-H), and 8.23 (1 H, s, 20-H).

1α-Hydroxydemethoxyviridin (**12**).—The unsaturated ketone (**6**) (500 mg) in acetone (100 ml) was treated with dilute acetic acid (30 ml) for 48 h at room temperature. The solution was concentrated and poured into water. The product was recovered in ethyl acetate. *1α-Hydroxydemethoxyviridin* (**12**) (160 mg) crystallized from acetone as needles, m.p. 238—240 °C (decomp.), $[\alpha]_D$ −49° (*c* 0.4) (Found: C, 66.2; H, 4.4. $C_{19}H_{14}O_5 \cdot H_2O$ requires C, 67.0; H, 4.7%), $\nu_{max.}$ 3 100, 1 690, 1 660, 1 620, and 1 590 cm^{-1}; $\lambda_{max.}$ 238 (ε 28 900) and 301 nm (10 400); δ(DMSO), 1.63 (3 H, s, 19-H), 2.75 (4 H, m, 2- and 16-H), 3.75 (2 H, m, 15-H_2), 5.03 (1 H, br t, *J* 3 Hz), 8.00 (2 H, br s, 11- and 12-H), and 8.2 (1 H, s, 20-H).

Sodium Borohydride Reductions.—Dehydroxydemethoxyviridin (**7**) (1 g) in methanol (500 ml) was treated dropwise with sodium borohydride (100 mg) in ethanol (20 ml) over 1 h. The reaction was followed by t.l.c. and stopped when the major part of the starting material had been consumed. Acetic acid (1 ml) was added and the solvent was removed under reduced pressure. The residue was taken up in pyridine (20 ml) and treated with acetic anhydride (10 ml) for 5 h. The solution was poured into dilute hydrochloric acid and the product was recovered in ethyl acetate and separated by preparative layer chromatography on silica in ethyl acetate. The upper band afforded *dehydroxydemethoxyviridin 3β-acetate* (**8**) (550 mg) which crystallized from methanol as needles, m.p. 222—225 °C (decomp.), $[\alpha]_D$ −120° (*c* 0.6) (Found: C, 72.0; H, 5.3. $C_{21}H_{18}O_5$ requires C, 72.0; H, 5.2%), $\nu_{max.}$ 1 710, 1 660, 1 615, and 1 580 cm^{-1}; δ 1.58 (3 H, s, 19-H), 2.09 (3 H, s, OAc), 3.67 (2 H, m, 15-H), 5.78 (1 H, t, *J* 6 Hz, 3-H), 7.49 and 7.82 (each 1 H, d, *J* 8 Hz, 11- and 12-H), and 7.76 (1 H, s, 20-H). The lower band afforded the *diacetates* (**9**) (120 mg) which crystallized from methanol as needles, m.p. 166—170 °C (Found: C, 70.5; H, 5.6. $C_{23}H_{22}O_6$ requires C, 70.0; H, 5.6%), $\nu_{max.}$ 1 720, 1 660, 1 580, and 1 525 cm^{-1}; δ 1.62 (3 H, s, 19-H), 2.03 (3 H, s, OAc), 2.10 and 2.13 (together 3 H, OAc), 3.64 (2 H, m, 15-H_2), 5.87 (1 H, t, *J* 8 Hz, 3-H), 6.24 (1 H, m, 17-H), 7.42 and 7.83 (each 1 H, d, *J* 9 Hz, 11- and 12-H), and 7.77 (1 H, s, 20-H).

(b) Demethoxydehydroxyviridin (1.4 g) in ethanol–tetrahydrofuran (1:1) (140 ml) was treated with sodium borohydride (300 mg) at 0 °C. The solution was allowed to attain room temperature. The solvents were partially evaporated and the solution was poured into water and the products recovered in

ethyl acetate. The solvent was evaporated and the residue was acetylated as above and separated by preparative layer chromatography to afford three fractions. The first was identified as dehydroxydemethoxyviridin 3β-acetate (**8**) (880 mg) by its n.m.r. spectrum. The second fraction (320 mg) was the *dihydro compound* (**14**), m.p. 172—173 °C (Found: C, 68.3; H, 6.1. $C_{23}H_{24}O_6 \cdot 0.5H_2O$ requires C, 68.1; H, 6.2%), $\nu_{max.}$ 1 730, 1 680, and 1 660 cm^{-1}; δ 1.58 (3 H, s, 19-H), 2.15 (6 H, s, OAc), 3.6 (3 H, m, 4-H, 15-H_2), 4.6 (3 H, m, 3- and 20-H), 6.2 (1 H, m, 17-H), 7.5 and 7.7 (each 1 H, d, *J* 9 Hz, 11- and 12-H). The third fraction (130 mg) was the *dihydro compound* (**13**), m.p. 264—265 °C (Found: C, 71.6; H, 5.5. $C_{21}H_{20}O_5$ requires C, 71.6; H, 5.7%), $\nu_{max.}$ 1 730, 1 680, 1 660, and 1 240 cm^{-1}; δ 1.60 (3 H, s, 19-H), 2.10 (3 H, s, OAc), 2.75 (2 H, m, 16-H), 3.58 (1 H, sext., *J* 6.5, 10 and 10 Hz, 4-H), 3.75 (2 H, m, 15-H), 4.52 (1 H, dd, 6.5 and 10 Hz, 20-H), 4.76 (1 H, t, *J* 10 Hz, 20-H), 4.78 (1 H, br t, *J* 10 Hz, 3-H), and 7.62 and 8.0 (each 1 H, d, *J* 9 Hz, 11- and 12-H).

(c) *Demethoxyviridiol diacetate.* Demethoxyviridin (1 g) in methanol (300 ml) was treated with sodium borohydride in ethanol (100 mg in 20 ml) dropwise over 1 h. The reaction was followed by t.l.c. and stopped when only a trace of starting material remained. Acetic acid (1 ml) was added and the solvent was removed under reduced pressure. The residue was taken up in pyridine (20 ml) and treated with acetic anhydride (10 ml) for 5 h. The solution was poured into dilute hydrochloric acid and the product was recovered in ethyl acetate and purified by preparative layer chromatography to afford *demethoxyviridiol diacetate* (**10**) (500 mg) which crystallized from methanol as needles, m.p. 230—235 °C (decomp.), $[\alpha]_D$ −99° (*c* 0.4) (Found: C, 67.7; H, 5.1. $C_{23}H_{20}O_7$ requires C, 67.6; H, 4.9%), $\nu_{max.}$ 1 740, 1 700, 1 665, 1 615, and 1 580 cm^{-1}; $\lambda_{max.}$ 244 (ε 28 500) and 311 nm (13 000); δ 1.80 (3 H, s, 19-H), 2.13 (3 H, s, OAc), 2.30 (3 H, s, OAc), 2.73 (4 H, m, 2- and 16-H_2), 3.47 (2 H, m, 15-H), 5.13 (1 H, dd, *J* 6 and 11 Hz, 1-H), 5.92 (1 H, dd, *J* 7 and 9 Hz, 3-H), 7.9 (1 H, 20-H), and 7.93 (2 H, s, 11- and 12-H).

Reduction with Sodium Borodeuteride.—Dehydroxydemethoxyviridin (430 mg) was treated with sodium borodeuteride (100 mg) in methanol–tetrahydrofuran (1:1) (80 ml) as above. The product was acetylated and purified by chromatography to afford the [3,17-$^2H_2$]diacetate (**9**) (115 mg) and the [3,4,17,20-$^2H_4$]diacetate (**14**) (30 mg). The integral of the ^1H n.m.r. signals at δ 4.5 and 4.76 corresponded to *ca.* 0.5 H each in the latter.

Trapping Experiment.—[1-^{14}C]Sodium acetate (0.08 mC in 4 ml of sterile water) was distributed between 4 Roux bottles of 7-day old *Nodulisporium hinnuleum* cultures. After a further 3 days dehydroxydemethoxyviridin (190 mg) in dimethyl sulphoxide (2 ml) was evenly distributed between the 4 flasks. After a further 24 h the fermentation was harvested. The dried mycelium was extracted with acetone and the extract chromatographed on silica to afford dehydroxydemethoxyviridin (108 mg) which was recrystallized to constant radioactivity (125 d.p.m. mg^{-1}).

Incubation of [1α,2α-^2H]*Dehydroxydemethoxyviridin with* N. hinnuleum.—[1α,2α-^2H]Dehydroxydemethoxyviridin (**7**) (43 mg) was dissolved in the minimum of ethanol and drop of Tween 80 was added. It was distributed evenly between 12 10-day old cultures of *N. hinnuleum.* The fermentation was harvested after a further 11 days. The dry mycelium was extracted with acetone. The extract was evaporated and then triturated with hot petroleum. The residue was taken up in acetone, boiled with charcoal, filtered, and concentrated to afford demethoxyviridin (200 mg) as a pale grey solid. The demethoxyviridin was treated with acetic anhydride in pyridine at room temperature overnight to afford the acetate (167 mg) which crystallized from acetone–light petroleum as a powder, m.p. 218—223 °C identified by its n.m.r. spectrum. The ^2H n.m.r. spectrum (determined in chloroform at 55.3 MHz) showed a signal at δ 5.44 p.p.m.

Incubation of Dehydroxydemethoxyviridin (**7**) *with* Nodulisporium hinnuleum.—Dehydroxydemethoxyviridin (**7**) (40 mg, 1.5, 10^6 d.p.m.) in ethanol (10 ml) was evently distributed between 10 10-day old surface cultures of *N. hinnuleum.* Growth was allowed to continue for a further 10 days and the metabolites were isolated as described previously to afford demethoxyviridin (61 mg) which crystallized from acetone as needles, m.p. 150—153 °C. It was further purified by preparative layer chromatography to afford demethoxyviridin, m.p. 149—150 °C, 940 d.p.m. mg^{-1} (3.9% incorporation).

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