Biosynthesis of Demethoxyviridin

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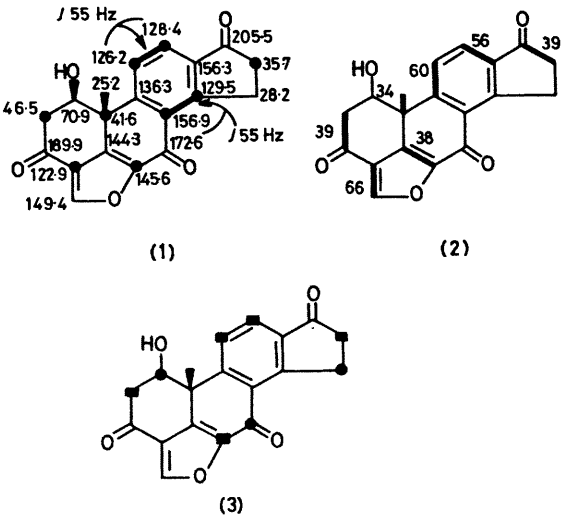
Summary Demethoxyviridin has ^{2}H and ^{13}C enrichment and coupling patterns when derived from [2-$^{2}H_3$]-, [1-^{13}C]-, and [1,2-$^{13}C_2$]-acetate and [2-$^{2}H_2$]-, [5-$^{2}H_2$]-, [2-^{13}C]-, and [5-^{13}C]-mevalonate, consistent with a triterpenoid origin.

DEMETHOXYVIRIDIN (**1**) which is produced by the fungus *Nodulisporium hinnuleum* is one of an interesting group of fungal metabolites with a steroid-like structure yet possessing an aromatic ring c[1] inviting comparison with mammalian steroid biosynthesis. Earlier degradative experiments on viridin (**1**; 2β-OMe) biosynthesized from [2-^{14}C]mevalonate located labels at C-1, C-7, and C-15 consistent with its formation from two farnesyl residues in a steroid-like manner.[2] Some preliminary experiments have also been reported[3] on the biosynthesis of a similar fungal metabolite, wortmannin, which does not have an aromatic ring c. We now report some experiments which define the origin of the carbon skeleton of demethoxyviridin.

The ^{13}C n.m.r. resonances of demethoxyviridin and six of its derivatives were assigned (see **1**). The optimum time for incorporation studies with the fungus was determined. Sodium [1-^{13}C]- and [1,2-$^{13}C_2$]-acetate, and [2-^{13}C]- and [5-^{13}C]-mevalonate were then fed separately to the fungus. The ^{13}C enrichment (ranging from 0·4—1%) and ^{13}C–^{13}C coupling patterns of the resultant samples of demethoxyviridin are shown in (**1**), (**2**), and (**3**).

The coupling patterns, including the induced coupling, $J_{11,12}$ and $J_{8,14}$ which arise from adjacent centres enriched by [1-^{13}C]acetate, are in accordance with a triterpenoid biosynthesis. They show that the aromatic ring has been formed without rearrangement and that the extra carbon atom at C-4 originates from the 3′-position of mevalonate and thus the 4β-methyl group of a protolanosterol/lanosterol precursor. This is of interest since the fungus also produces ergosterol which lacks both methyl groups



Structure (**1**) shows ^{13}C n.m.r. chemical shifts (p.p.m. from Me_4Si; in Me_2SO) and enrichment pattern for sample derived from $CH_3{}^{13}CO_2Na$; structure (**2**) coupling constants (in Hz) for sample derived from $^{13}CH_3{}^{13}CO_2Na$; structure (**3**) enrichment pattern for sample derived from [2-^{13}C]mevalonate (●) and [5-^{13}C]mevalonate (■).

at this centre, and hence the additional atom might have arisen from the C_1 pool. There are also chemical analogies for the formation of the furan ring at this position by the intramolecular condensation of a 6α-ester.[4] In fusidic acid biosynthesis[5] by the fungus *Fusidium coccineum*, it is the other methyl group (4α-methyl) derived from C-2 of mevalonate which is retained. The [2-^{13}C]mevalonate results are in accordance with the earlier carbon-14 work whilst the [5-^{13}C]mevalonate results distinguish five of the isoprene units which go to form demethoxyviridin providing further evidence for excluding a diterpenoid precursor (*cf.* ref. 2).

The ^{1}H n.m.r. signals of demethoxyviridin and its derivatives were assigned from the 220 MHz spectra. [2-$^2H_3$]Acetate, and [2-$^2H_2$]- and [5-$^2H_2$]-mevalonate were then fed to the fungus. The demethoxyviridin was isolated (0·3—2% incorporation) and converted into its more toluble acetate. The ^{2}H n.m.r. spectra of the samples of she acetate were determined at 30·3 MHz† and the relative integrals of the signals were compared. The signals at δ 8·32 (20-H, furan-H), 5·48 (1-H), 3·76 (15-H), and 1·76 (19-H_3) were labelled in the ratio 1·0 : 0·9 : 0·8 : 3·1 from the [2-$^2H_3$]acetate whilst the signals at δ 3·76 (15-H) and 5·48 (1-H) were equally labelled from [2-$^2H_2$]mevalonate. The aromatic signals at δ 8·08 (11- and 12-H) bore approximately 1·5 labels compared to the 2-H and 16-H signals (δ 2·88) which bore a total of 4 labels from the [5-$^2H_2$]-mevalonate. When [2(*R*)-2-^{3}H,2-^{14}C]mevalonate (^{3}H: ^{14}C, 2·76 : 1) was incubated with the fungus, the demethoxyviridin had a ^{3}H : ^{14}C ratio corresponding to the retention of 0·67 atom/mole tritium based on the incorporation of three [2-^{14}C]mevalonoid labels. Furthermore, when [4-(*R*)-4-^{3}H,2-^{14}C]mevalonate was fed to *Nodulisporium hinnuleum*, no tritium was incorporated into the demethoxyviridin (3·3% incorporation, ^{14}C).

If it is assumed that the 1β-acetoxy group replaces a *pro*-2(*R*)-mevalonoid hydrogen atom,[6] the [2-^{2}H]- and [2-^{3}H]-mevalonate results show that a *pro*-2(*S*) mevalonoid hydrogen atom is lost from C-15. Although the 15α- and 15β-hydrogen resonances are too close for a confident distinction to be made (δ 3·74 and 3·86, respectively), only the 15α-resonance appears to be labelled in accordance with inversion at this centre and the loss of a substituent from C-14. The loss of hydrogen from C-11 and C-12 is in accordance with the intervention of squalene and the loss of one hydrogen from the two farnesyl pyrophosphate residues. We have shown that [11-^{14}C]squalene was incorporated into viridiol (0·45%) by *Gliocladium deliquescens*. The [2-$^2H_3$]acetate results show that the 19-methyl group retains all three deuterium labels, thus excluding a cyclopropanoid precursor related to cycloartenol from the biosynthesis. An interesting feature of the [2-$^2H_3$]acetate experiment is that the signals arising *via* the 2-methylene group of mevalonate show a small drop in integral compared to those derived *via* the 3′-methyl group, reflecting the action of prenyl isomerase.

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