Biosynthesis of Flavoglaucin. Stereochemistry of Aromatic Isoprenylation

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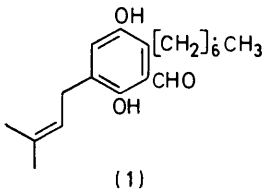
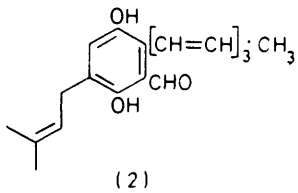
Incorporation studies with [1,2-^{13}C]acetate have established the regular polyketide nature of flavoglaucin modified by isoprenylation. The aromatic isoprenylation occurs without any change in stereochemistry of the olefin in the dimethylallyl moiety.

FLAVOGLAUCIN (1) and the related pigment auroglaucin (2) occur widely in *Aspergillus* spp.[1] The structure of flavoglaucin was established by chemical degradation [2-5] and by synthesis of a reduction product.[6]



(1)



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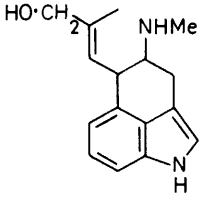

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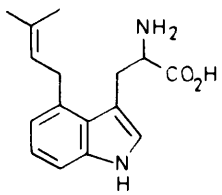
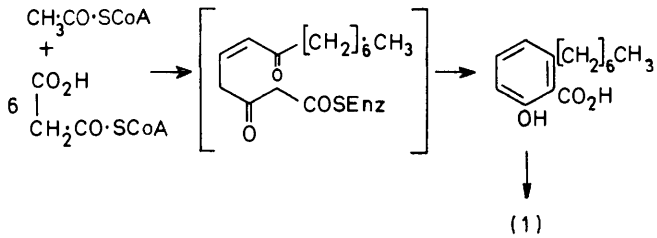


(3)

It has been suggested [7,8] that flavoglaucin is a regular polyketide, modified by aromatic hydroxylation, reduction of the carboxy-group to an aldehyde, and aromatic isoprenylation (Scheme 1). Feeding experiments with [^{14}C]acetate and mevalonate which gave incorporation into auroglaucin support the polyketide hypothesis, and show that the dimethylallyl moiety is derived from mevalonic acid.[9] A number of closely related fungal metabolites exhibit this type of cyclisation of a polyketide chain.[7] [^{14}C]Acetate has been incorporated into palitantin,[10] and [^{14}C]-acetate and -formate have been incorporated into pulvilloric acid.[11]



SCHEME 1

We have used [1,2-^{13}C]acetate to verify this expected cyclisation of the regular heptaketide chain of flavoglaucin. This technique, based on the detection of ^{13}C,^{13}C-coupling in the enriched metabolite, was first used to confirm the structure of and establish the biosynthetic pathway to dihydrolatumicidin.[12] The label-

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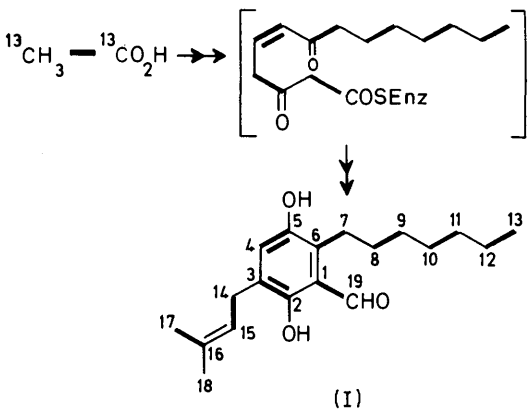
ling pattern obtained on incorporation of [1,2-^{13}C]acetate is shown in Scheme 2. An acetyl-CoA starter unit is



SCHEME 2

thought to condense successively with six malonyl-CoAs on a multienzyme complex.[13]

We used lightly labelled [^{14}C]acetate to determine conditions for obtaining adequate incorporations and checked these with the relatively inexpensive [2-^{13}C]-acetate. The results of these incorporations and the carbon n.m.r. assignments in flavoglaucin are given in the Table. The carbon-13 shift assignments are based both on signal multiplicity (off-resonance and coupled spectra with nuclear Overhauser effect studies) and chemical shift criteria. These criteria are not unambiguous for the methylene carbon resonances, especially in the n-heptyl chain. However, the [2-^{13}C]acetate incorporation experiments assist in establishing the order of these shifts without affecting our arguments concerning the biosynthetic pathway. Thus the methyl carbon atoms C-17 (17.8 p.p.m.) and C-18 (25.8 p.p.m.) in the dimethylallyl group and the n-heptyl methyl carbon atom (14.0 p.p.m.) are distinguished by their characteristic shifts, and most notably the steric γ-shift at C-17.[14] Furthermore, the methylene resonance at 27.0 p.p.m., the methine resonance at 121.2 p.p.m. and the quarternary carbon resonance at 133.7 p.p.m. are assigned to C-14, -15, and -16, respectively, by analogy with the corresponding carbon atoms in 3,7-dimethylocta-1,3,6-triene (26.6, 122.8, and 131.8 p.p.m.)[14] and related monoterpenes.[14] We have used comparisons with published data for 2,5-dihydroxybenzoic acid,[15] exchanging the carboxylic acid function for an aldehyde and substituting methyl groups in the 3- and 6-positions. Minor consideration was given to steric crowding features.[15,16] The assignments for the aromatic ring are thus (calculated values in parentheses): C-1, 117.3 (120.4); C-2, 155.8 (156.1); C-3, 128.5 (127.8); C-4 aromatic methine, 125.7 (125.5); C-5, 145.0 (150.5); and C-6, 128.7 (124.0) p.p.m. The C-3 and C-6 assignments are ambiguous, though the J_{CC} couplings observed in the experiments with doubly labelled material support the present assignment. Our initial attempts to assign resonances for the n-heptyl chain were based on comparison with data for n-hexylbenzene.[17] However, this approach does not offer a clear distinction of the methylene carbon resonances. The [2-^{13}C]acetate incorporation work shows that alternate labelling occurs at the methylene carbon atoms C-7, C-9, and C-11 and the methyl carbon atom C-13 of the n-heptyl chain, thus distinguishing three methylene carbon atoms from the other three carbon atoms. The various chemical shift analogies in long chain alkanes and their derivatives[18] do not provide details for the assignment of the methylene carbon atoms C-9—12 (positions remote from the aryl substituent), though in the present case the order of the J_{CC} values (35 and 29 Hz between C-10 and -11 and C-8 and -9) caused us to revise our initial assignments of the C-8 and C-10 resonances to those given in the Table. The unusually high field shift of the C-7 methylene carbon atom (24.0 p.p.m.) suggests steric crowding by the adjacent aldehyde and hydroxy-groups. The single labelling experiment confirms this assignment.

The incorporation of the [1,2-^{13}C]acetate gave us a simple means of looking at the stereochemistry of the aromatic isoprenylation reaction. The formation and

^{13}C Chemical shifts of flavoglaucin, excess ^{13}C at individual positions in [2-^{13}C]acetate-enriched flavoglaucin, and coupling constants ($^{1}J_{CC}$/Hz) of [1,2-^{13}C]acetate-enriched flavoglaucin

Carbon no.	Signal [a]	Excess ^{13}C(%)	J_{CC}
1	117.3 (s)	50	55
2	155.8 (s)		65
3	128.6 [b] (s)		65
4	125.8 (d)		65
5	145.0 (s)	58	65
6	128.7 (s)		43
7	24.0 (s)	49	43
8	29.1 (t)		29
9	29.6 (t)	59	29
10	32.0 (t)		35
11	31.8 (t)	52	35
12	22.6 (t)		35
13	14.1 (q)	57	35
14	27.0 (t)		43
15	121.2 (d)	70	43
16	133.7 (s)		42
17	17.8 (q)	46	42
18	25.8 (q)	100	
19	195.6 (d)		55

[a] In p.p.m. downfield from Me_4Si. Multiplicities indicated are from proton off-resonance and coupled spectra with nuclear Overhauser enhancement. [b] Owing to overlap in the spectrum of unenriched material no percentage excess ^{13}C could be measured.

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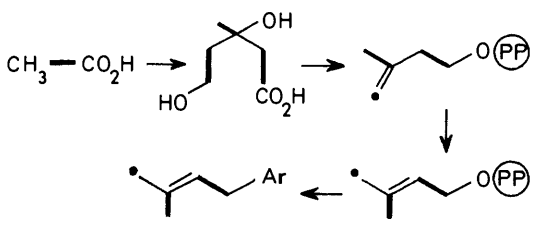
structural variety of such compounds of mixed biosynthetic origin has been summarised.[19] There has been considerable mechanistic speculation about the formation of various isoprenylated indole metabolites, in particular in the ergot alkaloids where the substitution of the isoprene grouping is at the relatively electron-deficient 4-position.[20-23] The precursor of the ergot alkaloids is 4-dimethylallyltryptophan [24] (**3**), and the next well established intermediate is chanoclavine I [25] (**4**). In chanoclavine I the methyl group, which is *cis* to the alkyl group is labelled by [2-^{14}C]mevalonate.[25] [2-^{14}C]Mevalonate gives rise to dimethylallyl pyrophosphate labelled in the *trans*-methyl group and in this case if the aromatic isoprenylation reaction occurs without change in stereochemistry around the double bond, then there must have been an inversion in the cyclisation reaction(s).

The data presented here constitute the first evidence that in simple aromatic isoprenylation there is no change in stereochemistry around the double bond. The ^{13}C n.m.r. data (Table) clearly show that the *cis*-methyl group (C-17) is strongly coupled to the adjacent olefinic carbon atom. The *trans*-methyl group, although enriched, is not coupled to any other carbon atom, as it arises from C-2 of mevalonic acid and C-1 has been lost by decarboxylation (Scheme **3**).



SCHEME 3

EXPERIMENTAL

Carbon-13 n.m.r. spectra were determined at 67.89 MHz in the Fourier transform mode with a Bruker HFX-270 spectrometer. Proton noise decoupling at 270 MHz was provided by a Bruker BSV-3BX decoupler unit. On average a 15 μs pulse, corresponding to a tilt angle of 60°, was employed. For a spectral width of 15 kHz at least 16 K data points were accumulated and an acquisition time of 0.54 s per pulse was employed for 4—8 K pulses. Coupled spectra with nuclear Overhauser enhancements were determined under similar conditions; a 5 s repetition rate was used with data collection occurring 0.1 s after the decoupler power was terminated. Sample concentrations of 0.3M in deuteriochloroform were employed. The solvent provided the signal for the deuterium lock channel. Tetramethylsilane (2%) was added to each solution as an internal reference.

Feeding and Isolation.—Aspergillus amstelodami was grown as surface cultures on a medium (200 ml per 1 l flask) consisting of sucrose (270 g), $NaNO_3$ (2.0 g), KH_2PO_4 (1.0 g), $MgSO_4,7H_2O$ (0.5 g), KCl (0.5 g), $FeSO_4,7H_2O$ (0.01 g), $ZnSO_4,7H_2O$ (0.01 g), and $CuSO_4,5H_2O$ (0.005 g) (all weights per litre). After 5 days the surface of the culture began to turn yellow as pigment production started. The precursors [labelled NaOAc (25 mg) in sterile water (1 ml) (per flask)] were added aseptically at this stage and growth was continued for a further 5 days. The mycelial mats were washed with water and extracted successively with boiling acetone (2 × 200 ml per 4 flasks) and dichloromethane (3 × 300 ml). The combined extracts were evaporated to a small bulk, water (300 ml) was added, and the suspension was extracted with dichloromethane (3 × 300 ml). The extracts were dried ($MgSO_4$) and evaporated, and the residue was extracted with boiling light petroleum (b.p. 40—60°; 2 × 200 ml); the extracts were filtered hot and evaporated. The residue was dissolved in the minimum of dichloromethane and applied to preparative t.l.c. plates (8 plates 20 × 20 cm × 0.75 mm thick of Merck silica gel GF_{254}) and the plates were developed in hexane–ethyl acetate (85 : 15 v/v). The yellow zone of flavoglaucin (R_F 0.5—0.7) was scraped off and eluted with dichloromethane; the eluate was evaporated and the residue crystallized from hexane to afford flavoglaucin (*ca.* 90 mg per 4 flasks) as golden yellow plates, m.p. 102—103° (lit.,[2] 103°).

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