Biosynthesis of Hirsutic Acid C using ^{13}C Nuclear Magnetic Resonance Spectroscopy

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Summary The ^{13}C labelling patterns in hirsutic acid C and complicatic acid, isolated from cultures of *Stereum complicatum* (Fr.) Fr. supplemented with [1-^{13}C]acetate and [2-^{13}C]acetate have been elucidated by ^{13}C n.m.r. spectroscopy.

THE application of ^{13}C n.m.r. spectroscopy to terpenoid biosynthesis has so far found limited use.[1] We report on its use in studies on the biosynthesis of hirsutic acid C (I) and complicatic acid (II)† fungal sesquiterpenes, recently isolated by us from fermentations of *Stereum complicatum* (Fr.) Fr.[2,3]

Several theories have been advanced for the enzymic construction of the hirsutane skeleton from farnesyl pyrophosphate,[4,5] but at the outset of this work experimental evidence supporting any of the possibilities was unavailable.

Initially, an unambiguous assignment was made of the natural abundance ^{13}C n.m.r. spectra of (I) and (II); the chemical shifts (from the proton decoupled spectra) are reported in the Table.

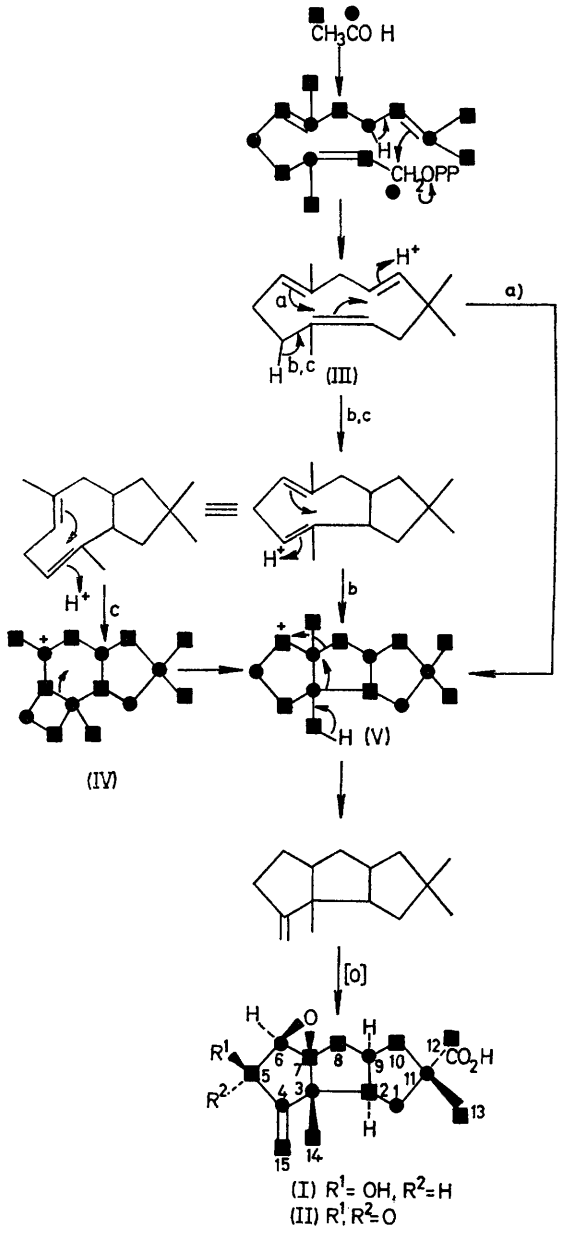
TABLE

^{13}C N.m.r. data for (I) and (II)[a]

Carbon	(I)[b]	(II)[b]
1	36·6	36·9
2	48·5	49·6
3	48·5	46·4
4	158·3	152·8
5	74·0	197·8
6	63·7	60·9
7	75·4	76·5
8	30·0	29·8
9	39·2	39·3
10	46·3	46·1
11	53·2	53·3
12	183·7	183·8
13	24·2	24·2
14	17·0	17·4
15	111·8	120·5

[a] P.p.m. to low field of Me_4Si. [b] Spectra obtained for solutions in $CDCl_3$.

Resonances corresponding to C-4, C-7, C-12, and C-15 are assigned from their chemical shift and "off-resonance" multiplicities. Comparison between the spectra of (I) and (II) enables assignment of the lines corresponding to C-5 and C-6, the quaternary centres C-3 and C-11, and the methyl groups C-13 and C-14. Of the two remaining tertiary carbon signals (C-2 and C-9), C-2 was assigned to the low field resonance because of the greater degree of substitution at C-3 than at C-8.



SCHEME

† Complicatic acid is an antimicrobially active metabolite shown by us to be formed from (I) by the fungus.[3]

Addition of successive amounts of $Eu(fod)_3$ (30 mg) to (II) (250 mg in 2 ml $CDCl_3$) causes lanthanide induced shifts (L.I.S.) which are approximately linear with respect to concentration of $Eu(fod)_3$. The behaviour of the lines assigned above clearly shows that major complexation occurs at the carboxylic acid function, and on this basis the secondary carbons C-1 and C-10 may be distinguished from C-8 by their considerably greater L.I.S. The appearance of C-1 to higher field of C-10 is expected because of the steric interaction between protons on C-1 and C-14 (clearly seen from models).

A previous report[7] suggests that lanthanide complexes may not be used in conjunction with carboxylic acids in organic solvents since decomposition occurs, although lanthanide ions have been used in aqueous solution.[8] We experienced no difficulty in this respect either with ^{13}C or ^{1}H n.m.r. studies.

Sodium [1-^{13}C]acetate (76·7 atom %, total 650 mg) and sodium [2-^{13}C]acetate (90 atom %, total 650 mg) were fed, in parallel, to 5 × 100 ml shaken cultures of *Stereum complicatum*,[2] additions being made to each flask after 127, 167, and 185 h. The fermentations were harvested after 302 h and the extracted mixtures of (I) and (II) treated with $NaBH_4$ in EtOH, converting (II) into (I). ^{13}C Enriched hirsutic acid C (232 mg and 257 mg, respectively) was obtained after purification.

The proton decoupled ^{13}C n.m.r. spectrum of ^{13}C enriched (I) derived from [1-^{13}C]acetate showed approx. 100% peak enhancement‡ of carbons C-1, C-4, C-6, C-9, and C-11. Signals due to carbons C-5, C-7, C-8, C-10, C-12, C-13, C-14, and C-15 were similarly enriched from [2-^{13}C]acetate. The signal at 48·5 (C-2 + C-3) in both labelled samples of (I) showed ^{13}C enrichment. A greater enhancement was seen in the sample derived from [2-^{13}C]acetate suggesting that the more intense tertiary C-2 signal had been labelled only. This was confirmed by oxidation of both labelled samples ($MnO_2/CHCl_3$) to (II) when labelling of C-2 from [2-^{13}C] acetate became obvious. Additions of $Eu(fod)_3$ to both enriched samples of (II) separated the overlapping signals of C-3 and C-10 and clearly showed that C-3 was enriched from [1-^{13}C]acetate.

These results are consistent with a mechanism involving the prior formation of a humulene-type precursor (III) from farnesyl pyrophosphate and proceeding through the carbonium ion intermediate (V) (Scheme). Several routes for the formation of (V) from (III) satisfy the labelling pattern in (I). Pathway (a) involves the least number of steps and is an abridged form of route (b) previously suggested by Scott.[5] Route (c) invokes the intermediacy of the protoilludane precursor (IV), recently shown to be involved in the biosynthesis of the illudins[10] and probably marasmic acid.[11] However, our results require modification of the suggested theory,[4] in which (I) would be derived from protoilludane.

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‡ The resonances were deliberately broadened by multiplication of the F.I.D. by an exponentially decreasing function to minimise intensity errors associated with digitization.[9]

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