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ACETATE-[2-^{13}C,2-$^{2}H_3$] AS A PRECURSOR IN POLYKETIDE BIOSYNTHESIS: DETECTION OF DEUTERIUM INCORPORATION WITH ^{13}C-NMR[1)]

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The fate of acetate hydrogen in polyketide biosynthesis is an interesting subject which has not been studied in detail. ^{2}H and ^{3}H-NMR spectrometries have been applied to tracing acetate hydrogen in the biosynthesis of griseofulvin[2)] and penicillic acid.[3)] Recently we reported a new method utilizing acetate-[2-^{13}C, 2-$^{2}H_3$] as a precursor in polyketide biosynthesis to clarify the mode of incorporation of acetate hydrogen.[4)] Since ^{2}H is directly bonded to ^{13}C in deuterated acetate, the incorporation of ^{2}H into target compound can be detected by measuring ^{13}C-NMR. ^{13}C bearing ^{2}H gives a signal showing ^{13}C-^{2}H coupling and a shift from the corresponding ^{13}C-^{1}H signal. In addition to highly resolved ^{13}C signals, the characteristic coupling and shift facillitate to locate ^{2}H-labellings. This communication deals with the results of our incorporation experiments carried out to clarify the scope and limitation of this approach.

SCYTALONE(1)

In our previous communication we reported that scytalone(1) labelled by acetate-[2-^{13}C, 2-$^{2}H_3$][5)] showed the ^{13}C-^{2}H signal of C-4, a triplet($J_{^{13}C-^{2}H}$ = 20 Hz) centred 0.3 ppm[6)] high field from the corresponding ^{13}C-^{1}H signal in proton noise decoupled ^{13}C-NMR. Although the incorporation of ^{2}H into C-5 was deduced from the lower intensity of ^{13}C-^{1}H signal, the ^{13}C-^{2}H signal was not observed due to its low sensitivity. This problem has now overcome by measuring ^{13}C-NMR under deuterium noise decoupled condition(Fig 1). The ^{13}C-^{2}H signal of C-4 appears as a doublet ($J_{^{13}C-^{1}H}$=127 Hz) centred 38.4 ppm and that of C-5 as a singlet at 108.7 ppm, 0.2 ppm higher than the ^{13}C-^{1}H signal. As expected from previous result signals corresponding to ^{13}C-^{2}H species of C-2 and C-7 were not observed in the spectrum. The loss of ^{2}H from potential sites of labelling is the subject of further study.

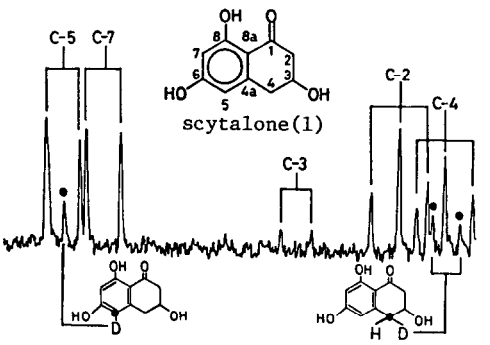

Fig 1 Deuterium decoupled ^{13}C-NMR of scytalone(1) labelled by acetate-[2-^{13}C, 2-$^{2}H_3$]

(+)RUGULOSIN(2)

(+)Rugulosin(2) is a toxic anthraquinonoid produced by *Penicillium brunneum* and other fungi Extensive studies on the chemistry and biosynthesis of anthraquinonoids have clarified that modified bis-anthraquinones such as 2 are derived from partially hydrogenated anthraquinone, which is derivable from anthraquinone or anthrone, *via* dimerization step.[7,8] The proton noise decoupled ^{13}C-NMR spectra of 2 enriched with acetate-[2-^{13}C] and [2-^{13}C, 2-$^{2}H_3$] are given in Fig 2. The details of ^{13}C-NMR assignment will be reported in separate paper.[9] A pair of spectra is a typical example showing the effect of ^{2}H-labelling resulting in lowering ^{13}C-^{1}H signal intensities. The signals due to C-1,3,8 and 11 are markedly lowered compared with non deuterated signals. The ^{13}C-^{2}H signals can be detected directly by measuring deuterium noise decoupled ^{13}C-NMR (Fig 3). The incorporation of ^{2}H into C-1 and 3 indicates the retention of acetate hydrogen throughout the course of biosynthesis. It is in good accord with the suggested mechanism of dimerization, the Diels-Alder type cycloaddition of two molecules of monomeric intermediate.[8] Another interesting observation is the absence of ^{2}H at C-6. Decarboxylation of carboxyl or its equivalent functional group originally bonded to C-6 may occur after aromatization. The methyl groups show ^{13}C-$^{2}H_3$(singlet) and ^{13}C-$^{2}H_2$(doublet; $J_{^{13}C-^{1}H}$= 128 Hz) signals, which indicate the presence of reversible reaction between acetylCoA and malonylCoA.

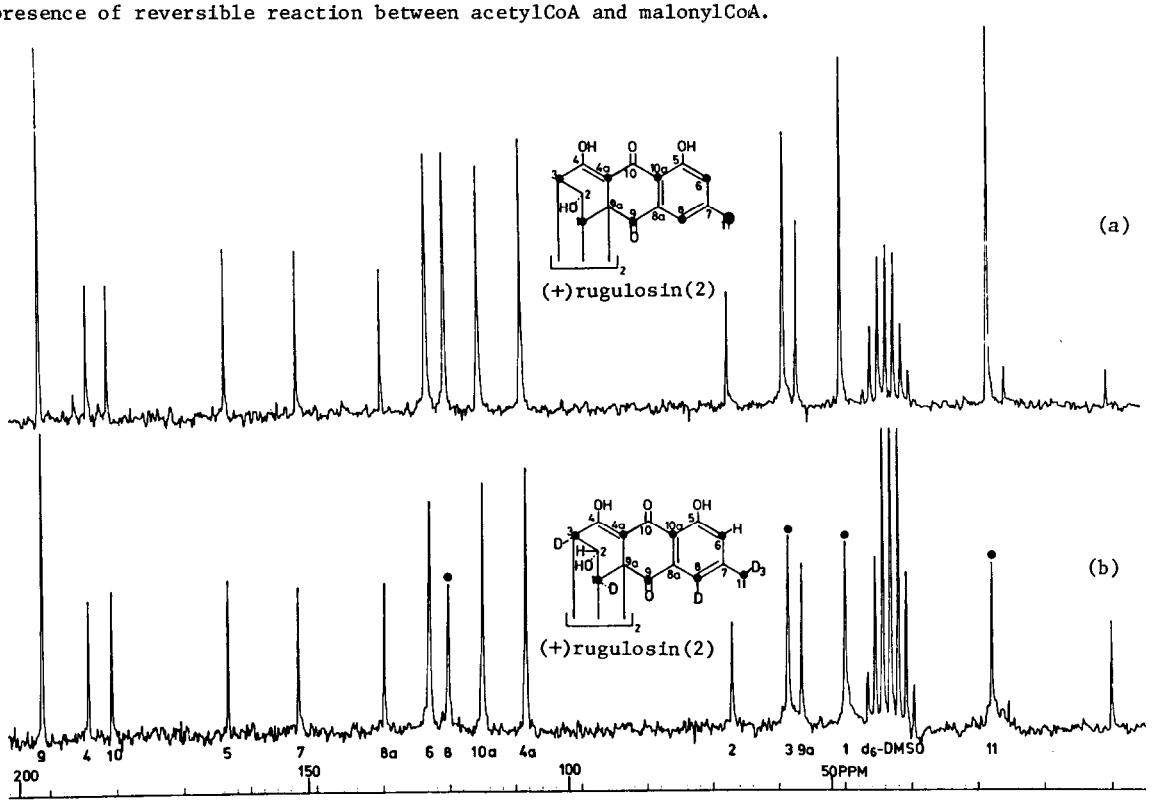

Fig 2 Proton noise decoupled ^{13}C-NMR spectra of (+)rugulosin(2) enriched with (a)acetate-[2-^{13}C]; (b)acetate-[2-^{13}C,2-$^{2}H_3$].

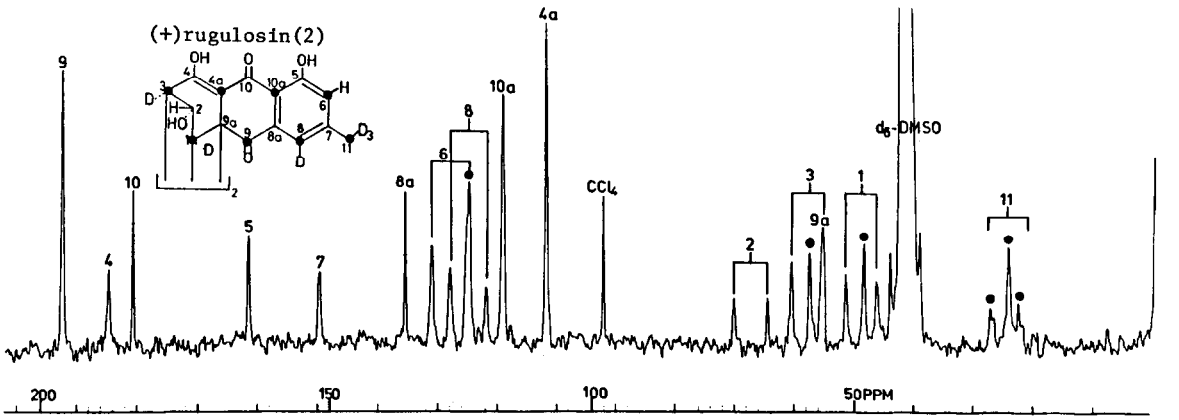

Fig 3 Deuterium noise decoupled ^{13}C-NMR spectrum of (+)rugulosin(2) enriched with acetate-[2-^{13}C,2-$^{2}H_3$].

2-HEXYL-5-PROPYLRESORCINOL(3)

2-Hexyl-5-propylresorcinol(3) is an antibiotic produced by unidentified *Pseudomonas* strain and is claimed to be active against Gram positive and negative bacteria, yeast and fungi.[10] The production of this metabolite is so efficient that we chose 3 as a model for fatty acid biosynthesis to clarify the mode of incorporation of acetate-[2-^{13}C, 2-$^{2}H_3$] into saturated alkyl chains. The ^{13}C-NMR of 3 was assigned from the literature values of reference compounds such as simple alkanes and alkylbenzenes, and also by detailed analysis of the ^{13}C-NMR data of 3 enriched with acetate-[1-^{13}C], [2-^{13}C] and [1,2-$^{13}C_2$]. The assignments shown in the Table are further supported by T_1 values. As T_1 value is comparable to the segment movement of carbon atom, a methylene group more remote from aromatic ring possesses larger T_1 value. The ^{13}C-NMR of 3 labelled by acetate-[2-^{13}C,2-$^{2}H_3$] shows three triplets ($J_{^{13}C-^{2}H}$ = 19Hz) arising from ^{13}C-^{2}H species of C-2', 4' and 1"(Fig 4). The triplets are centred 0.4 ppm high field from the corresponding ^{13}C-^{1}H signals. The methyl groups, however, give complicated signals as the sum of variously deuterated methyl signals. One of the hydrogen bearing aromatic carbons is the potential site of labelling, but only a very weak ^{13}C-^{2}H signal was observed. Since aromatic protons of 3 are easily exchanged on treatment with $^{2}H_2O$, the loss is not a biological phenomenon. There are no reasons to doubt the chiral nature of the methylenes labelled by deuterium. Stereochemical course of the reduction step of double bond in the biosynthesis would be clarified by determining the chirality of the labelled methylene.

The ratios of ^{2}H retention versus enriched ^{13}C were 30-60% in methylene, methin and

Carbon	Chemical shifts ppm (multiplicity)	^{13}C-^{13}C coupling[c] (Hz)	T_1 (sec)
C-1,C-3	154.4(s)[a]	67, 70	-
C-2	112.9(s)[b]	70	-
C-4,C-6	108.3(d)[b]	67	-
C-5	141.9(s)[a]	44	-
C-1'	23.1(t)[a]	34	1.1
C-2'	29.3(t)[b]	32	1.3
C-3'	29.5(t)[a]	32	1.6
C-4'	31.8(t)[b]	34	2.1
C-5'	22.7(t)[a]	35	2.5
C-6'	14.0(q)[b]	34	3.2
C-1"	37.7(t)[b]	43	1.9
C-2"	24.1(t)[a]	35	2.6
C-3"	13.8(q)[b]	35	2.8

a; Enriched with acetate-[1-^{13}C] b; Enriched with acetate-[2-^{13}C] c; Coupling in 3 enriched with acetate-[1,2-$^{13}C_2$].

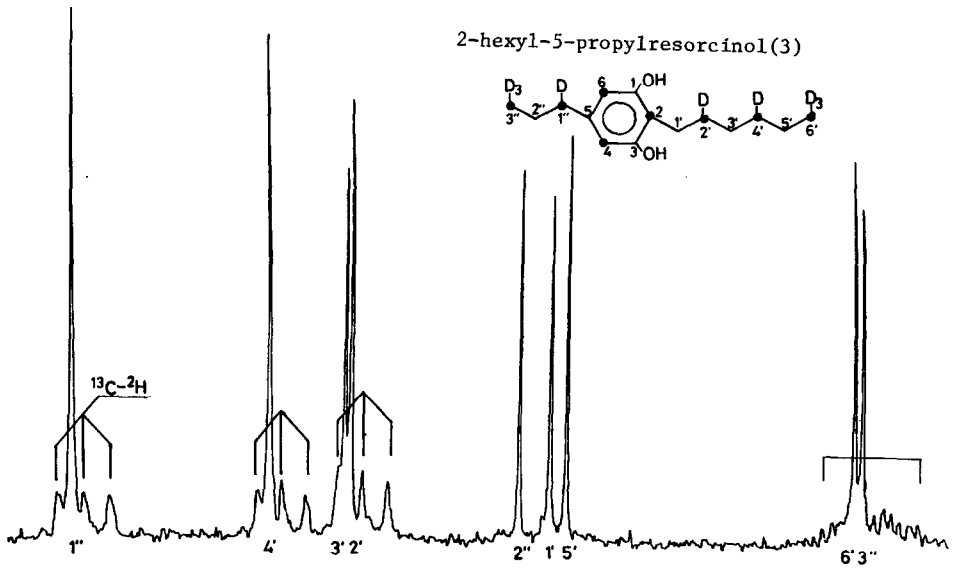

Fig 4 Proton noise decoupled ^{13}C-NMR spectrum of 2-hexyl-5-propylresorcinol(3) enriched with acetate-[2-^{13}C,2-$^{2}H_3$].

aromatic carbons. In the methyl groups, about 90% of enriched ^{13}C retains more than one ^{2}H. If we take into account the fact that methylene hydrogen atoms of malonylCoA were readily exchanged with the protons of environmental water in the experiment of fatty acid biosynthesis using an enzyme preparation,[11] the ratios of ^{2}H retention in our experiments are rather high. The turnover of malonylCoA in intact biological systems should be very rapid.

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References and Notes

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5) We prepared labelled acetate from malonic acid-[2-^{13}C] by exchanging the protons with $^{2}H_2O$ followed by decarboxylation.
6) In our previous communication (ref.4) this value was mistakenly reported as 1.3 ppm.
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