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Biosynthesis of 2-Hexyl-5-propylresorcinol: Biosynthetic Incorporation of Deuterium from [2-^{13}C, 2-$^{2}H_3$]-Acetate[1)]

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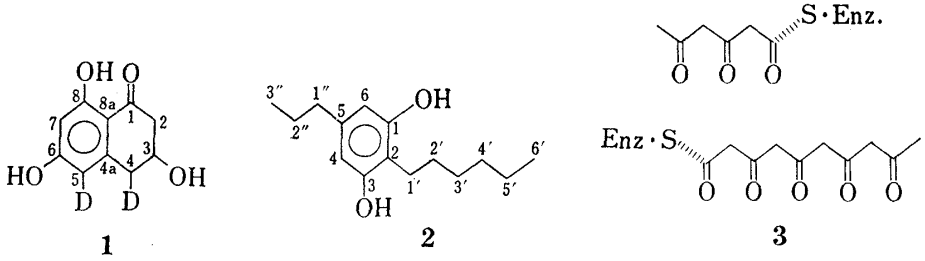
Biosynthetic incorporation of ^{2}H from [2-^{13}C,2-$^{2}H_3$]-acetate into 2-hexyl-5-propylresorcinol was investigated in *Pseudomonas*. The ^{13}C nuclear magnetic resonance spectrum of labelled 2-hexyl-5-propylresorcinol showed ^{13}C-^{2}H signals of expected methylene and methyl carbons in the side chains, but no ^{2}H was found on the aromatic carbons. The main species of methyl groups labelled with ^{2}H were ^{13}C-$^{2}H_3$ and ^{13}C-$^{2}H_2{}^{1}H$. The incorporation experiments unambiguously demonstrate that 2-hexyl-5-propylresorcinol is biosynthesized from two polyketide chains.

Keywords——biosynthesis; 2-hexyl-5-propylresorcinol; ^{2}H, ^{13}C; [2-^{13}C,2-$^{2}H_3$]-acetate; *Pseudomonas*; antibiotic; isotopic shift

Introduction

In previous papers, we reported a new method to trace the fate of acetate hydrogen in polyketide biosynthesis by using [2-^{13}C, 2-$^{2}H_3$]-acetate. Incorporation of ^{2}H into C-4 and -5 of scytalone (**1**) from [2-^{13}C, 2-$^{2}H_3$]-acetate was demonstrated by ^{13}C nuclear magnetic resonance (NMR) measured under ^{2}H-decoupled as well as ^{1}H-decoupled conditions. The absence of ^{2}H at the other potential sites of labelling, C-2 and -7, indicated that ^{2}H originally bonded to those carbons was lost in the course of biosynthesis.[1)] As a continuation of our previous work, we further investigated the usefulness of multiple-labelled precursors in biosynthetic studies. 2-Hexyl-5-propylresorcinol (**2**) is an antibitic produced by an unidentified strain of *Pseudomonas sp.* (strain No. 9004) and claimed to be active against Gram-positive and -negative bacteria, yeast and fungi.[2)] This compound consists of two distinct parts, alkyl side chains and an aromatic ring. The former can be regarded as groups with biosynthetic origin similar to fatty acid and the latter as a typical polyketide. Incorporation studies with multiple-labelled precursors would clarify the scope and limitations of the use of the multiple-labelled precursor in polyketide biosynthesis and also provide information concerning the formation of the dialkylresocinol structure, which is thought to be derived from two polyketide chains (**3**).



1 **2** **3**

Fig. 1

Results and Discussion

The assignment of the ^{13}C-NMR spectrum of 2-hexyl-5-propylresorcinol (**2**) was very difficult, because the chemical shift values of methylenes and methyls are very similar. For instance, six methylene groups except for C-1″, gave signals between 22.7 and 31.8 ppm. In contrast, the assignment of aromatic carbons were easily made with the aid of calculated values and multiplicity in the off resonance spectrum. The ^{13}C-NMR data of dialkylresorcinol (**2**) enriched with [1-^{13}C], [2-^{13}C] and [1,2-$^{13}C_2$]-acetate were extensively used for the assignment. The culture of *Pseudomonas* in a modified Henneberg's medium gave 200—250 mg of **2** per flask (150 ml). The specific incorporation ratios of labelled acetate into **2** were 21—60% (Table I), and the enrichment per carbon was calculated to be 3—7%.

Table I. Incorporation of Labelled Acetate into 2-Hexyl-5-propylresorcinol (**2**)

[1-^{14}C]-Acetate		[^{13}C]-Acetate	mg	2-Hexyl-5-propylresorcinol (**2**)			
Specific act. (dpm/mM)	Total act. (dpm)			Isolated (mg)	Specific act. (dpm/mM)	Specific inc. (%)	Total inc. (%)
1.25×10^6	1.53×10^6	[1-^{13}C]	100.6	231.7	3.04×10^5	24.4	19.5
1.24×10^6	1.52×10^6	[2-^{13}C]	101.0	256.3	2.64×10^5	21.3	18.8
1.27×10^6	1.56×10^6	[1,2-$^{13}C_2$]	100.7	231.8	2.96×10^5	23.4	18.7
1.95×10^6	3.02×10^6	[2-^{13}C,2-$^{3}H_3$]	131.7	209.3	1.17×10^6	59.9	34.3

The origin of carbon atoms was easily defined from the signal enhancement observed in the ^{13}C-NMR spectra of **2** enriched with [1-^{13}C] and [2-^{13}C]-acetate. However, the values of ^{13}C-^{13}C coupling constant in **2** labelled with [1,2-$^{13}C_2$]-acetate are very similar among the alkyl carbons except for that of C-1″, which couples with an aromatic carbon (C-5) and has a larger coupling constant than the other alkyl carbons.[3] Therefore, the values of coupling constant cannot be used for signal assignment except for C-1″. The signals arising from the propyl group were assigned by decoupling experiments. In ^{1}H-NMR, C-2″ protons appears at relatively lower field, δ 1.6, for they are phenethyl protons. This assignment was confirmed by a single frequency decoupling experiment in the ^{13}C-NMR. When ^{1}H-signals around δ 1.6 were irradiated, a methyl proton signal at lower field showed an enhanced signal height, indicating that C-3″ methyl protons resonate at lower field that C-6′ methyl protons. Upon irradiation at slightly higher field than C-6′ protons, *ca.* δ 0.8, the signal observed at 14.0 ppm in ^{13}C-NMR showed considerable enhancement. When the irradiation point was shifted downfield, the signal at 13.8 ppm became a singlet. These results unambiguously demonstrated that the signals at 13.8 ppm arise from C-3″, and that at 14.0 ppm from C-6′. The chemical shift values of the propyl carbons are very similar to those reported for propylbenzene.[4]

Since the ^{13}C-NMR data of hexylbenzene were available as a reference, signals due to C-3′, -4′ and -5′ were assigned according to the chemical shift values of corresponding carbons in hexylbenzene.[4] It has been reported that the introduction of a hydroxyl group *ortho* to alkyl groups in alkylbenzene causes considerable upfield shifts in the signals of alkyl carbons.[5] The chemical shift values of carbon signals assignable to C-1′ and -2′ are in good accord with this observation. The complete assignment of the ^{13}C-NMR of 2- hexyl-5-propylresorcinol (**2**) is shown in Table II. This assignment is supported by T_1 values of alkyl carbons, which were measured by the progressive saturation method. Carbon atoms more remote from the benzene ring show a longer T_1 time. The ^{1}H-decoupled ^{13}C-NMR spectrum of **2** labelled by [2-^{13}C, 2-$^{2}H_3$]-acetate showed ^{13}C-^{2}H signals due to C-2′, -4′ and -1″ as triplets. Each ^{13}C-^{2}H signals were observed at *ca.* 0.4 ppm higher than the corresponding ^{13}C-^{1}H signals. The retention of ^{2}H, which was calculated from the decrease of signal intensities caused by

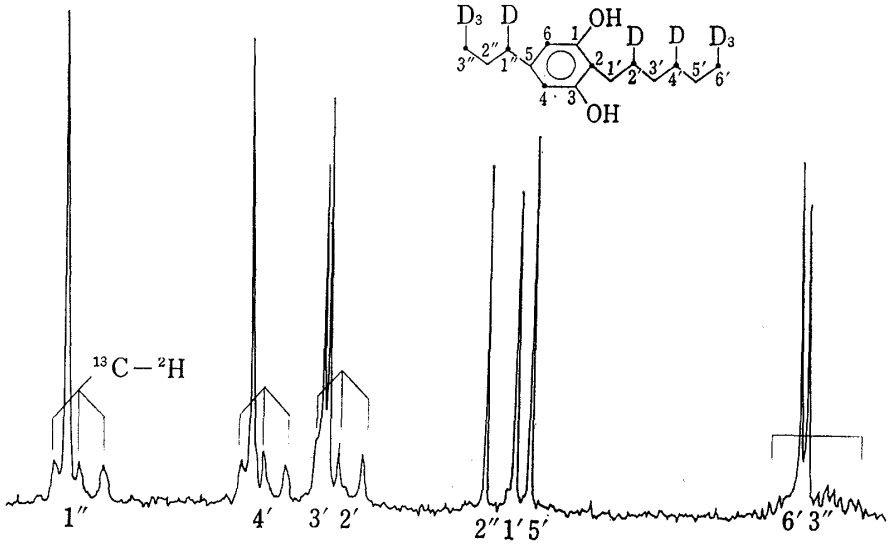


Fig. 2. ^{1}H-Decoupled ^{13}C-NMR Spectrum of 2-Hexyl-5-propylresorcinol (**2**) labelled by [2-^{13}C,2-$^{2}H_3$]-Acetate

TABLE II. ^{13}C-Chemical Shifts and Coupling Constants of 2-Hexyl-5-propylresorcinol (**2**)

Carbon	Chemical shifts ppm (Multiplicity)	^{13}C-^{13}C coupling[c]	T_1 (s)
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	2.1
C-3″	13.8(q)[b]	35	2.8

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

^{2}H labelling, were 48—57% at the methylene carbons and 90% at the methyl carbons. ^{2}H-Decoupled ^{13}C-NMR revealed that the signals due to two methyl groups mainly consist of $^{2}H_3{}^{13}C$ and $^{1}H^{2}H_2{}^{13}C$ in an approximate ratio of 1:1 (Fig. 3). In contrast, C-4 and -6, the other potential sites of ^{2}H labelling did not show siganls corresponding to ^{13}C-^{2}H, indicating that ^{2}H bonded to ^{13}C was lost almost completely during the course of biosynthesis.

The steps involved in the biosynthesis of fatty acid have been extensively studied by using chirally labelled acetate and malonate[6] and also with enzyme systems.[7] On the other hand, McInnes *et al.* demonstrated the incorporation of [2-^{13}C, 2-$^{2}H_3$]-acetate into palmitic acid in a prokaryotic alga, *Anacystis nidulans*.[7] Retention of ^{2}H at the methyl group was more than 90%, whereas retention in methylenes varied according to position in a regular manner. This was accounted for by the difference of residence time at the cysteine residue of β-keto acyl ACP synthetase.[7] As can be seen in Fig. 2, no appreciable regularity was observed in the retention of ^{2}H (48—57%) in the three methylenes. This may reflect differences in

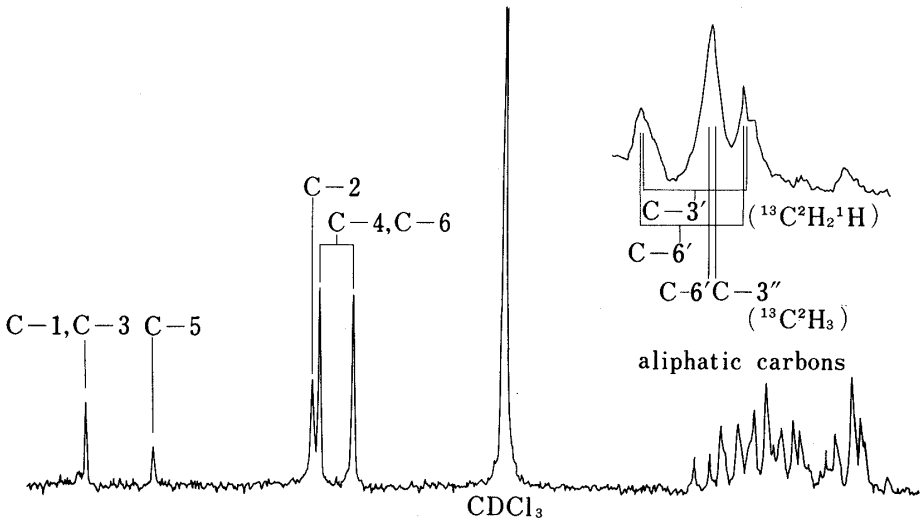


Fig. 3. ^{2}H-Decoupled ^{13}C-NMR Spectrum of 2-Hexyl-5-propylresorcinol(**2**) labelled by [2-^{13}C,2-$^2H_3$]-Acetate

biosynthetic mechanisms between polyketide and fatty acid. The loss of ^{2}H from the aromatic ring is presumably caused by an exchange with environmental water at the resorcinol stage, since aromatic hydrogens of resorcinol were easily exchanged with ^2H_2O.[8] The results so far obtained demonstrate that 2- hexyl-5-propylresorcinol (**2**) is formed from two polyketide chains. [2-^{13}C, 2-$^2H_3$]-Acetate is thus proved to be an effective precursor for use in biosynthetic studies of polyketides in a reduced state.

Experimental

Proton noise decoupled ^{13}C-NMR spectra were measured with a JEOL FX-100 and deuterium noise decoupled ^{13}C-NMR spectrum was obtained with a Varian XL-100. ^{13}C-Labelled acetates were purchased from Merck Sharp and Dohme Canada Ltd., and ^{14}C-labelled acetate from the Radioisotope Association.

Incorporation of Labelled Compounds——Stock culture of *Pseudomonas* No. 9004 was grown on slant of the following composition; polypeptone (1%), meat extract (1%), NaCl (0.2%), agar (1.5%), pH 7.0. Liquid medium was a modified Henneberg's medium; sucrose 30 g, polypeptone 3 g, $NaNO_3$ 0.04 g, K_2HPO_4 0.02 g, $MgSO_4 \cdot 7H_2O$, KCl 0.01 g, $FeSO_4 \cdot 7H_2O$ trace, $CaCO_3$ 8 g in 1 l H_2O, pH 7.0. *Pseudomonas* No. 9004 was incubated in 500 ml Erlenmeyer flask containing 150 ml of liquid medium and grown on a shaker (200 rpm) in the dark at 28°C for 48 h. Two ml aliquots were inoculated in the same medium for production culture. On the 2nd day of production culture, labelled compounds dissolved in sterilized water were added to the flasks and the culture was continued for a further 3 d. The culture was harvested on the 5th day and centrifuged to remove bacteria. The supernatant was adjusted to pH 2 with 2 N HCl and extracted with $CHCl_3$. After removal of $CHCl_3$ *in vacuo*, the extract was chromatographed on silica gel by using benzene as a solvent. Yields and incorporation ratios are shown in Table I. A fraction eluted slightly later than 2-hexyl-5-propylresorcinol (**2**) gave a compound which is very similar to **2**. This compound was identified as 2-butyl-5-propylresorcinol. mp 79—81°C (hexane). MS Calcd for $C_{13}H_{20}O_2$: 208.1463. Found: 208.1446. IR ν_{max}^{KBr} cm^{-1}: 3420, 3380, 2920, 1635, 1585, 1521. UV λ_{max}^{EtOH} (10 g ε): 230 sh (1.96), 273 (1.93). ^{13}C-NMR ($CDCl_3$) ppm: 13.8 (q, C-4′), 14.0 (q, C-3″), 22.9 (t, C-3′ and C-1′), 24.1 (t, C-2″), 31.5 (t, C-2′), 37.7 (t, C-1″), 108.2 (d, C-4 and C-6), 112.7 (s, C-2), 142.0 (s, C-5), 154.4 (s, C-1 and C-3).

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