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# $Eu(fod)_3$-SHIFTED $^2H$-NMR AS A PROBE OF CHIRALITY DUE TO DEUTERIUM SUBSTITUTION: STEREOSPECIFIC DEUTERIUM INCORPORATION INTO 2-n-HEXYL-5-n-PROPYLRESORCINOL, A POLYKETIDE PRODUCED BY PSEUDOMONUS SP. B-9004.[1]

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Summary: $Eu(fod)_3$-shifted $^2H$-NMR of partially deuterated 1S-n-hexylphenylcarbinol (2) and 1S-n-propylphenylcarbinol (3) derivatized from biosynthetically deuterated 2-n-hexyl-5-n-propylresorcinol (1) revealed a stereospecificity of deuterium incorporation into the side chains of 1.

Enzymatic method was succesfully applied in our laboratory to demonstrate stereospecific incorporation of deuterium from [2-$^2H_2$]malonyl-CoA into fatty acid which was prepared in cell free system of fatty acid synthetase[2]. In the present article, we employed a physico-chemical method[3] for a determination of stereospecificity of deuterium incorporation[4] from [2-$^2H_3$]acetate into polyketide, 2-n-hexyl-5-n-propylresorcinol (1), by intact bacteria, Pseudomonus sp. B-9004, and found a higher stereospecificity than in the case of fatty acid[2].

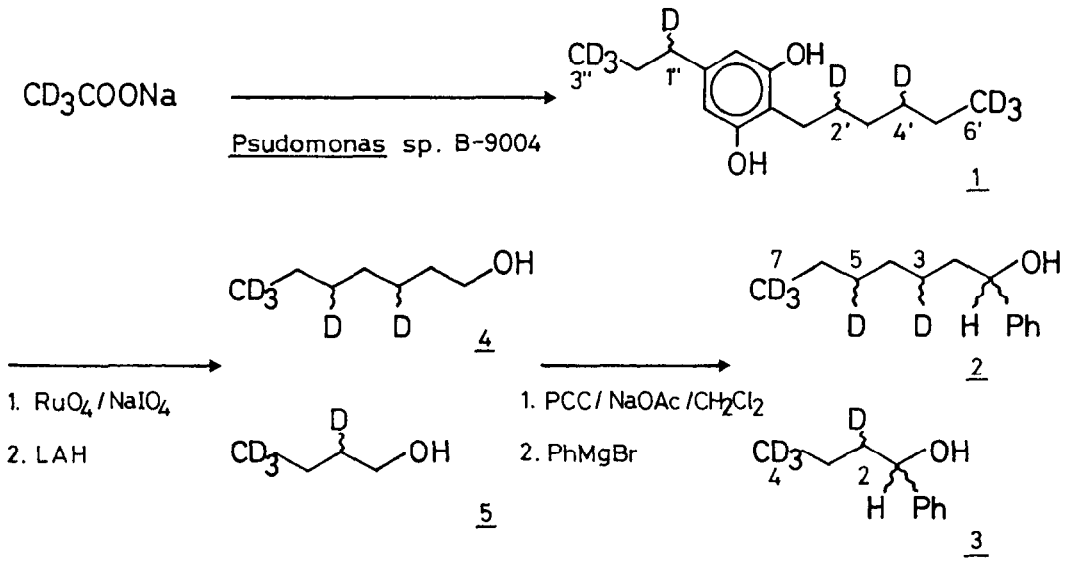
Pseudomonus sp. B-9004 which was grown on a slant[5,6] for 24 hours at 27°C was inoculated into a liquid medium[7]. The inoculated medium was shaken[8] for 48 hours, then 2 ml aliquots of the content were seeded in the same liquid medium[7] and these flasks were shaken[8] for another 48 hours, at that time 100 mg of $CD_3COONa$ in water was added into each flask. After additional 72 hours shaking[8] the bacteria were collected by a centrifuge and were extracted with acetone then with chloform[9] to give 3 gram of crude 2-n-hexyl-5-n-propylresorcinol (1) from mycelia of 10 litter fermentation. $^2H$-NMR measurement of the compound showed deuterium incorporation into side chains[4].

The crude material was oxidized by $RuO_2.2H_2O$-$NaIO_4$ in $CH_2Cl_2$-$H_2O$ at room temperature as was indicated in SCHEME 1. Aromatic ring of the resorcinol derivative 1 (3 gram) was oxidized to give crude mixture (2 gram) contained heptanoic acid and butyric acid[10]. These acids originated from the side chains of 1 were added to excess LAH/Ether to reduce to corresponding

alcohols, n-heptanol (4) and n-butanol (5). These alcohols were oxidized to aldehydes by PCC/NaOAc oxidation. After completion of the reaction, the reaction mixture was directly loaded on a silica gel column to remove inorganics. After the solvent was carefully removed using a Vigreux column, resulted aldehydes mixture was added into excess amount of PhMgBr in ether. Alkylphenylcarbinols formed were separated by silica gel column chromatography to give n-hexylphenylcarbinol (2) (450 mg) and n-propylphenylcarbinol (3) (200 mg). It should be noted that no loss of deuterium on C-2 in the course of the derivatization of 5 into 3 was checked by GC-MS in a model experiment on [2,3-$^2H_2$]pentadecanol. Alkylphenylcarbinols 2 and 3 obtained above were derivatized into their l-(-)-menthoxycarbonyl esters, and the four l-(-)-menthoxycarbonyl esters of 2 and 3 were separated by preparative GLC[11,12]. LAH reduction of the separated esters gave corresponding optically active alkylphenylcarbinols 1S-2, 1R-2, 1S-3 and 1R-3. Optical purity and absolute configuration of C-1 of these alcohols were checked by GLC of their R-(-)-menthoxycarbonyl esters[12] and by $^1$H-NMR measurements of their R-(+)-MTPA esters[13]. Both of these methods gave the coincidental results[14].

Because of the presence of assymmetry on C-1, methylene protons of these alcohols 2 and 3 became diastereotopic, and the diastereotopic non-equivalence was enhanced by the addition of Eu(fod)$_3$ to give well separated signals for four protons on C-2 and C-3. And the assignments of these four protons were made already by measuring $^1$H-NMR spectra of stereoselectively deuterated compounds at various concentrations in the presence of Eu(fod)$_3$ in our previous work[3]. Considering chemical shift paralellism between $^1$H- and $^2$H-NMR, Eu(fod)$_3$-shifted $^2$H-NMR measurements of the alkylphenylcarbinols resolved concerning C-1 were expected to reveal deuterated position and stereochemistry of the deuteration of the alkyl chain.

SCHEME 1:



$Eu(fod)_3$-shifted $^1H$- and $^2H$-NMR data of 1S-2 and 1S-3 were shown in TABLE 1 and 2 in which deuterium content for each deuterated position was calculated by comparing integration of $^2H$-NMR signals of the deuterated alcohols with that of deutero-chloroform which exist in natural abundunce, 0.015 %, in "cold" chloroform which was used as the solvent for the $^2H$-NMR measurements(60 MHz, 5000 scans). From these data , it became clear that alkyl groups of the alkylphenylcarbinols 2 and 3 which were derived from the side chains of 2-n-hexyl-5-n-propylresorcinol (1) contained deuterium in $H_{3S}$, $H_5$ and $H_7$ of 2, and $H_{2S}$ and $H_4$ of 3 in the percentage indicated in FIGURE 1.

Consequently, deuterium incorporation from [2-$^2H_3$]acetate into 2-n-hexyl-5-n-propylresorcinol (1) by the bacteria, Pseudomonus sp. B-9004, was summarized as follows.

1) Deuterium were found on C-2', C-4', C-6', C-1" and C-3".

2) Absolute configuration of C-2' and C-1" were predominantly S.

3) Deuterium content were calculated as in FIGURE 2.

TABLE 1: $Eu(fod)_3$-shifted $^1H$- and $^2H$-NMR: 5.0 mg(26 μmol) of 1S-2 in the presence of 27.0 mg(26 μmol) of $Eu(fod)_3$ in 0.50 ml of $CDCl_3$ or $CHCl_3$ at room temperature.

| | $H_{2R}$ | $H_{2S}$ | $H_{3R}$ | $H_{3S}$ | $H_4$ | $H_5$ | $H_6$ | $H_7$ |
|---|---|---|---|---|---|---|---|---|
| $^1H$-NMR (400 MHz) | 9.55 ppm | 8.50 | 5.47 | 5.88 | 3.00 | 2.16 | 1.76 | 1.10 |
| $^2H$-NMR (60 MHz) | - | - | 5.5<br>0.2%(0.16)* | 5.9<br>3.2%(0.77) | - | 2.2<br>1.4%(0.76) | - | 1.1<br>5.8%(4.78) |

* Deuterium content (relative integration to $CDCl_3$ signal) for $H_{3R}$ might be inaccurate because of low S/N value.

TABLE 2: $Eu(fod)_3$-shifted $^1H$- and $^2H$-NMR: 3.0 mg(20 μmol) of 1S-3 in the presence of 10.4 mg(10 μmol) of $Eu(fod)_3$ in 0.50 ml of $CDCl_3$ or $CHCl_3$ at room temperature.

| | $H_{2R}$ | $H_{2S}$ | $H_{3R}$ | $H_{3S}$ | $H_4$ |
|---|---|---|---|---|---|
| $^1H$-NMR (400 MHz) | 5.60 | 4.97 | 3.38 | 3.62 | 1.70 |
| $^2H$-NMR (60 MHz) | - | 5.1<br>2.7%(0.57)* | - | - | 1.7<br>5.7%(3.58) |

* Deuterium content (relative integration to $CDCl_3$ signal)

FIGURE 1: Distribution and content(%) of deuterium in 1S-2 and 1S-3.

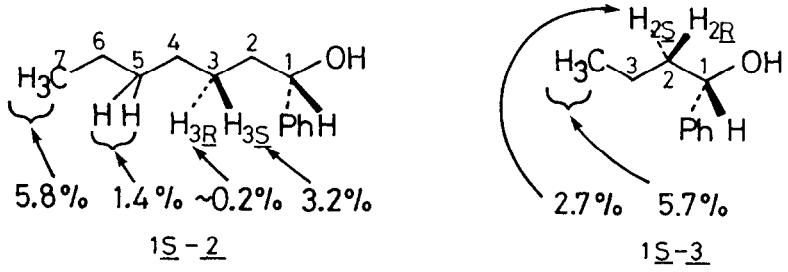
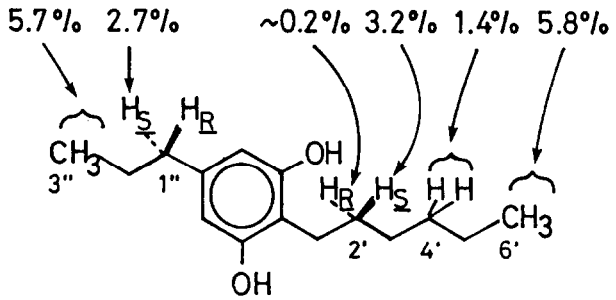


FIGURE 2: Incorporation of deuterium in 1.

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3) J. Furukawa, S. Iwasaki and S. Okuda, Tetr. Lett., the previous report in this volume.
4) U. Sankawa, H. Shimada, and K. Yamasaki, Chem. Pharm. Bull., 29 3061 (1981).
5) The medium contained 1 g meat extract, 1 g polypepton, 0.2 g NaCl and 1.5 g agar in 100 ml $H_2O$. This was adjusted to pH 7.0 before sterilization.
6) Pseudomonus sp. B-9004 was grown on the slant for 24 hours at 27°C and stocked at room temperature. Inoculation on to a new slant every one month was recomended to keep the strain.
7) The solution contained 30 g sucrose, 3 g polypepton, 0.04 g $NaNO_3$, 0.02 g $K_2HPO_4$, 0.5 g $MgSO_4.7H_2O$, 0.01 g KCl, 5 mg $FeSO_4$ and 8 g $CaCO_3$ per litter. The medium was adjusted to pH 7.0 before sterilization.
8) The bacteria were cultured in 500 ml Erlenmyer flasks each contained 150 ml medium on a rotary shaker at 27°C.
9) Only small amount of 2-n-hexyl-5-n-propylresorcinol was detected in the broth.
10) The formation of these acids were followed by GLC: SP-1000, 1.5%, 1.0 m, 180°C.
11) 15% EGS, 1.5 m x 4 mmID was used for the preparative purpose.
12) J. W. Westley and B. Halpern, J. Org. Chem., 33, 3978 (1968).
13) F. Yasuhara and S. Yamaguchi, Tetr. Lett., 21, 2827 (1980).
14) Data for 1S-2, 1R-2, 1S-3 and 1R-3 were listed in the table below.

| | Chemical shift(δ, $CDCl_3$) of R-(+)-MTPA ester | | Retention time(min) of R-(-)-methoxycarbonyl deriv., 5% EGS, 1.5 m, 160°C |
|---|---|---|---|
| | $-OCH_3$ | $-H_1$ | |
| 1S-2 | 3.53 | 5.87 | 16.8 |
| 1R-2 | 3.45 | 5.95 | 18.7 |
| 1S-3 | 3.54 | 5.89 | 8.2 |
| 1R-3 | 3.44 | 5.97 | 9.1 |

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