

BIOSYNTHESIS OF SCYTALONE

Ushio Sankawa\*, Hisao Shimada, Toshitsugu Sato and Takeshi Kinoshita

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-Ku, Tokyo 113, Japan

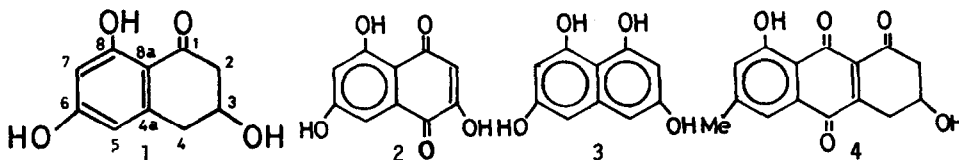
Kazuo Yamasaki

Institute of Pharmaceutical Sciences, School of Medicine, Hiroshima University, 1-2-3 Kasumi,

Hiroshima 737, Japan

(Received in Japan 11 December 1976; received in UK for publication 6 January 1977)

Scytalone(1), a simple derivative of tetralone, was first isolated from a fungus belonging to *Scytalidium*.<sup>1)</sup> Later, *Phialophora lagerbergii* was found to produce scytalone(1) in a good yield along with flaviolin(2) and using this organism polyketide nature of scytalone(1) has been demonstrated by the incorporation experiment with acetate-[1-<sup>13</sup>C].<sup>2)</sup> On the other hand, Bycroft and his co-workers observed that NaBH<sub>4</sub> reduction of 1,3,6,8-tetrahydroxynaphthalene(3) yielded scytalone(1).<sup>3)</sup> Chemical feasibility of aromatic reduction seemed to suggest that scytalone(1) might be formed from the aromatic intermediate(3) in the biosynthesis. In our chemical and biosynthetic studies on fungal modified anthraquinones such as rugulosin, a key intermediate in the biosynthesis has been clarified to be a partially hydrogenated anthraquinone (4; dihydroemodin) which can be derived from anthraquinone and anthrone.<sup>4)</sup> The structural and biosynthetic similarities of scytalone(1) to the modified anthraquinones let us to investigate further details of the biosynthesis of scytalone(1). This communication reports the results of the incorporation and <sup>13</sup>C-NMR experiments using single and double labelled acetate-<sup>13</sup>C as well



as acetate doubly labelled with  $^2\text{H}$  and  $^{13}\text{C}$ .

Cultures of *Phialophora lagerbergii* (IMI 96745) were supplemented with either acetate- $[1-^{13}\text{C}]$  or  $[2-^{13}\text{C}]$  to give scytalone showing anticipated enhancements of  $^{13}\text{C}$  resonance intensities as required by the polyketide structure(1). In the  $^{13}\text{C}$ -NMR spectrum of scytalone labelled with acetate- $[1,2-^{13}\text{C}]$ , eight of the ten signals showed multiple splitting patterns not encountered in usual double labelled experiments. In addition to the singlets arising from natural abundancen, the signals show four satellite peaks which can be attributed to the two kinds of  $^{13}\text{C}$ - $^{13}\text{C}$  couplings arising from intact acetate units incorporating into scytalone with two different dispositions. A complete spectral assignment and the values of  $^{13}\text{C}$ - $^{13}\text{C}$  coupling constant are summarized in the Table ; the assignments are consistent with those reported except C-2, C-4, C-6 and C-8.<sup>2)</sup> C-6 and C-8 were easily assigned by comparing their coupling constants with those of the adjacent carbons. Since C-2 and C-4 showed the same coupling constant values, this method could not be applied. The assignments were made by the NMR investigation of deuterated scytalone, which was obtained by treating scytalone with  $\text{KO}^2\text{H}-^2\text{H}_2\text{O}$ . The  $^1\text{H}$ -NMR spectrum of this sample showed that the protons on C-2, C-5 and C-7 were exchanged with  $^2\text{H}$ . In the  $^{13}\text{C}$ -NMR measured under the standard condition of Pulse Fourier Transform(PFT) method, the sensitivity of  $^{13}\text{C}$  carbon labelled with  $^2\text{H}$  is markedly suppressed by a longer relaxation time ( $T_1$ ) and by the absence of Nuclear Overhauser Effect(NOE). The  $^{13}\text{C}$ -NMR of deuterated scytalone showed a signal at  $\delta 38.8$ , whereas a signal observed at  $\delta 47.1$  in non-deuterated sample could not be detected.

| Carbon | multiplicity in off resonance spectrum | ppm <sup>a)</sup> | $^1\text{J}_{\text{C-C}}$<br>Hz | $^2\text{J}_{\text{C-C}}$<br>Hz |
|--------|--|-------------------|---------------------------------|---------------------------------|
| C-1    | s                                      | 202.0             | 40,55                           | -                               |
| C-2    | t                                      | 47.1              | 38,40                           | 9                               |
| C-3    | d                                      | 66.3              | 37                              | -                               |
| C-4    | t                                      | 38.8              | 38,40                           | -                               |
| C-4a   | s                                      | 145.7             | 40,63                           | -                               |
| C-5    | d                                      | 108.9             | 63                              | -                               |
| C-6    | s                                      | 165.4             | 63,67                           | -                               |
| C-7    | d                                      | 101.3             | 67,70                           | -                               |
| C-8    | s                                      | 165.9             | 61,71                           | -                               |
| C-8a   | s                                      | 111.4             | 54,59                           | 9                               |

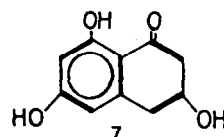
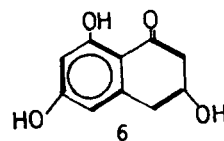
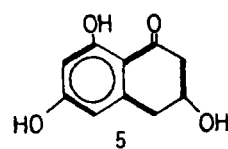


Table. The  $^{13}\text{C}$ -chemical shifts of scytalone and  $^{13}\text{C}$ - $^{13}\text{C}$  coupling constants of acetate- $[1,2-^{13}\text{C}]$  enriched scytalone. a) Relative to TMS

Thus the signals at  $\delta$  38.8 and 47.1 were assigned to C-4 and C-2 respectively. These assignments are also confirmed by a long range coupling ( $J=9$  Hz) between C-2 and 8a.

The  $^{13}\text{C}$ -NMR signals of scytalone labelled by acetate-[1,2- $^{13}\text{C}$ ] are accompanied by additional small satellites. In distinct cases such as C-1 and C-4a, four small satellites are observed in addition to four big satellites arising from  $^{13}\text{C}$ - $^{13}\text{C}$  couplings of intact acetate units. The small satellites are caused by the further  $^{13}\text{C}$ - $^{13}\text{C}$  coupling between the carbons labelled by intact acetate-[1,2- $^{13}\text{C}$ ] molecules and the adjacent carbons labelled by natural abundance  $^{13}\text{C}$  (8 and 9) or by the other acetate-[1,2- $^{13}\text{C}$ ] molecules (10 and 11). The signals due to C-1 and the labelling patterns of the molecules causing additional couplings are schematically shown in Fig.1.

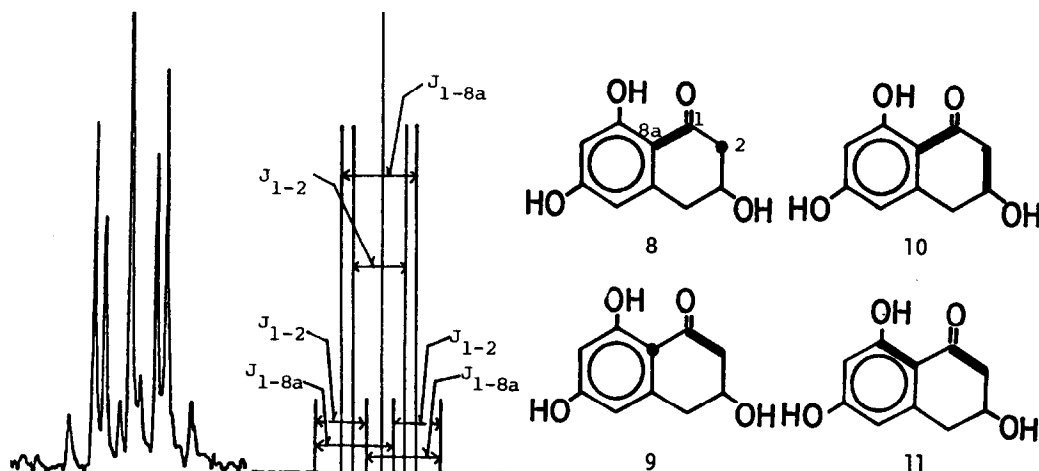
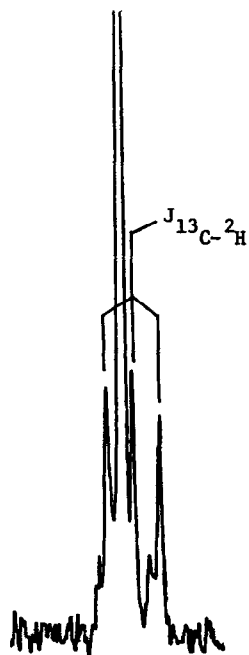


Fig.1. C-1 signal of scytalone labelled by acetate-[1,2- $^{13}\text{C}$ ].

As appears in the Table, the respective carbons show  $^{13}\text{C}$ - $^{13}\text{C}$  couplings with both of the adjacent carbons indicating the presence of two different mode of acetate arrangements as shown in (5) and (6). Since  $^{13}\text{C}$ - $^{13}\text{C}$  coupling between C-4a and C-8a could not be detected, the participation of the other possible arrangement of acetate (7) is ruled out.

Next, we have tested the incorporation of acetate-[2- $^2\text{H}_3$ , 2- $^{13}\text{C}$ ] to obtain information concerning hydrogen. Recently, the application of  $^2\text{H}$  and  $^3\text{H}$ -NMR to the biosynthetic investigation of fungal metabolites have been reported and the labelling patterns of penicillic acid<sup>5)</sup> and griseofulvin<sup>6)</sup> from acetate-[ $^3\text{H}$ ] and [ $^2\text{H}$ ] were unambiguously established. In this study we have tested another possible method,  $^{13}\text{C}$ -NMR, for the detection of  $^2\text{H}$  incorporation by using double labelled acetate-[2- $^2\text{H}_3$ , 2- $^{13}\text{C}$ ]. The incorporation of  $^2\text{H}$  can be detected by  $^{13}\text{C}$ - $^2\text{H}$



coupling or by the decrease of signal intensity. Labelled scytalone obtained from the cultures fed with acetate-[2-<sup>2</sup>H<sub>3</sub>, 2-<sup>13</sup>C] showed enhancements in <sup>13</sup>C-NMR signals due to C-2,4,5,7 and 8a indicating the extensive loss of <sup>2</sup>H in the biosynthetic process. Signal showing <sup>13</sup>C-<sup>2</sup>H coupling was only observed in C-4. In addition to singlet, C-4 showed a triplet (1:1:1, J=20 Hz) centred at δ37.5, 1.3 ppm higher than the singlet signal, as shown in Fig.2. The triplet is attributed to <sup>13</sup>C signal coupled with one <sup>2</sup>H and clearly demonstrates that a part of <sup>13</sup>C present in C-4 carries one <sup>2</sup>H. Judging from signal intensity, C-5 seems to be labelled by <sup>2</sup>H in a significant ratio, but <sup>13</sup>C-<sup>2</sup>H coupling was not observed in this case. Contrary to C-4 and C-5, the signals of C-2 and C-7 did not show any indication of the presence of <sup>2</sup>H and the labelling ratios of <sup>2</sup>H to <sup>13</sup>C should be lower than those of C-4 and C-5. From the decreases of signal intensity the labelling ratios of <sup>2</sup>H to <sup>13</sup>C in C-4 and C-5 were calculated to be 30-40%. Further

Fig.2. The signal due to investigations using <sup>2</sup>H-decoupling method or a paramagnetic relaxation C-4 of scytalone labelled reagent are necessary to establish the exact ratios of <sup>2</sup>H label in these by acetate-[2-<sup>2</sup>H<sub>3</sub>, 2-<sup>13</sup>C]. carbons.

The results, especially the presence of the two different arrangements of acetate units, (5 and 6), provided a strong evidence for the involvement of the symmetrical intermediate such as 3 in the biosynthesis of scytalone. The presence of two different arrangements(5 and 6) can be explained by assuming that the reduction of aromatic ring takes place in one of the rings of 3 in an equal probability.

**Acknowledgements.** The authors thank Mr.K.Matsushita of JEOL Co.Ltd. for the measurement of <sup>13</sup>C-NMR.

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