DEOXYGENATION IN THE BIOSYNTHESIS OF POLYKETIDES: MECHANISM OF BIOMIMETIC REDUCTION OF TETRAHYDROXYNAPHTHALENE

Koji ICHINOSE, Yutaka EBIZUKA and Ushio SANKAWA*

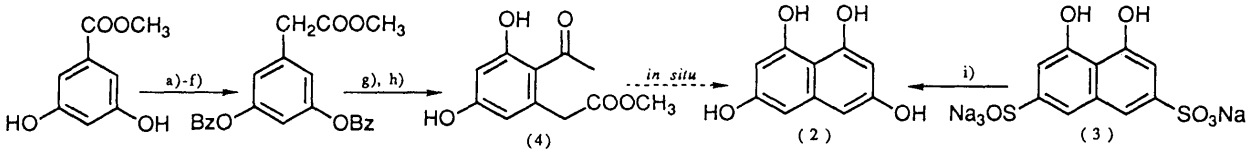
Faculty of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

A biomimetic synthesis of scytalone, a simple derivative of tetralone, was re-investigated using NMR spectroscopy. Scytalone was formed from 1,3,6,8-tetrahydroxy-naphthalene (1,3,6,8-THN) by sodium borohydride reduction only in the presence of sodium methoxide. This suggests that the formation of the keto-tautomer of 1,3,6,8-THN requires an alkaline condition. The investigation of the reactive species using NMR spectroscopy clarified its structure.

KEYWORDS biomimetic; mechanism; keto-tautomer; scytalone; polyketide

Two different mechanisms have been known for deoxygenation of acetate-derived oxygen in the biosynthesis of aromatic polyketides of microbial origin. In the biosynthesis of 6-methylsalicylic acid, an oxygen atom is lost before aromatization.[1] The other mechanism of deoxygenation is post-aromatic reduction followed by dehydration to produce deoxy-type aromatic polyketides. The biosyntheses of 1,8-dihydroxynaphthalene and chrysophanol fall in this category.[2] This paper deals with a biomimetic reaction of the post-aromatic deoxgenation of polyketides.

The compound investigated, scytalone (1), is a simple derivative of tetralone, which occurs in phytopathogenic fungi such as Scytallidium sp.,[3] Phialophora lagerbergii[4] and Verticillium dahliae.[5] In some phytopathogenic fungi it is an important intermediate in the biosynthesis of melanin, a polymer of 1,8-dihydroxynaphthalene, which is essential in forming penetrating hyphae and acts as a chemical factor in subsequent phathogenesis.[6] Recently, non-fungicidal anti-blast chemicals were shown to inhibit the dehydration of scytalone (1), resulting in the accumulation of scytalone (1).[7] In our previous studies of the biosynthesis of scytalone (1), [1,2-$^{13}C_2$]-acetate was incorporated into scytalone (1) with two different labelling patterns in a 1:1 ratio.[8] This strongly indicates that scytalone (1) is biosnthesized via a symmetrical intermediate, 1,3,6,8-tetrahydroxy-naphthalene (1,3,6,8-THN) (2). Wheeler demonstrated later that 1,3,6,8-THN (2) was reduced to scytalone (1) by the cell-free extracts of Verticillium dahliae in the presence of NADPH.[9] Our previous conclusion was partly supported by the observation that scytalone (1) could be formed from 1,3,6,8-THN (2) by sodium borohydride reduction.[10] To obtain further information on the biomimetic reduction of aromatic ring, we reinvestigated the reduction of 1,3,6,8-THN (2) with sodium borohydride.



a) BzBr, K_2CO_3 / Acetone (96 %), b) $LiAlH_4$ / E_2O (98 %), c) CH_3SO_2Cl, NEt_3 / CH_2Cl_2 (84 %), d) NaCN, $BzNEt_3Cl$ / $CHCl_3$-H_2O (69 %) e) $Ba(OH)_2$ / Dioxane-H_2O (92 %), f) CH_2N_2 / Et_2O (q.y.), g) Ac_2O, 60 % $HClO_4$ aq. / AcOH (99 %), h) Pd / C (5 %) / AcOEt-EtOH (97 %), i) NaOH, $Ba(OH)_2$, KOH / N_2, 260° (55 %)

Chart 1. Synthesis of Materials

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When 1,3,6,8-THN (2), which was prepared from chromotropic acid (3) by alkali fusion,[11] was treated with sodium borohydride in methanol, scytalone (1) was not formed. In the previous paper reported by Bycroft et al.,[10] 1,3,6,8-THN was prepared from methyl curvulinate (4),[12] and sodium borohydride reduction was carried out in situ. So this reaction condition was reinvestigated. Methyl curvulinate (4) was synthesized (Chart 1) and treated with sodium methoxide to obtain 1,3,6,8-THN (2). The addition of sodium borohydride to the reaction mixture yielded scytalone (20%) and 6,8-dihydroxy-1-methylisochroman-3-one (5%) (5)[13] along with quinonic compounds such as flaviolin (6) formed by air oxidation.

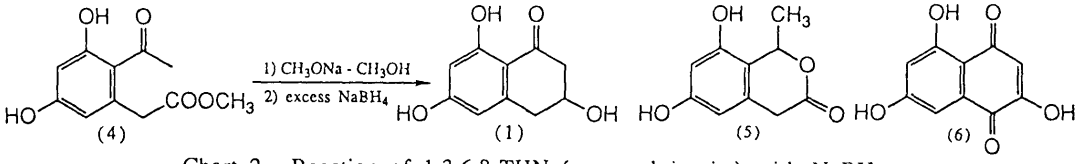

Chart 2. Reaction of 1,3,6,8-THN (prepared *in situ*) with $NaBH_4$

Apparently the sodium borohydride reduction proceeded only under the strong alkaline condition. Next we tried sodium borohydride reduction of 1,3,6,8-THN (2) in methanol in the presence of sodium methoxide. Scytalone (1) was obtained in 25% yield along with the quinonic compounds. The results so far obtained indicate that 1,3,6,8-THN (2) was converted into its keto-tautomer only in the presence of strong alkali and this reactive species was reduced by sodium borohydride. Then we tried to identify the reactive species using NMR spectroscopy.[14]

To measure the ^{13}C-NMR spectra of the reactive species, methyl curvulinate (4) was cyclized in deuteromethanol (CD_3OD) containing sodium deuteromethoxide (CD_3ONa) and a solution of the reaction mixture was directly submitted to NMR measurement. On the other hand, the NMR spectra of 1,3,6,8-THN (2) was measured in a CD_3ONa-CD_3OD solution. The ^{13}C-NMR spectra of these two samples were almost identical. The ^1H-decoupled ^{13}C-NMR spectra showed ten significant signals, indicating that the reactive species is not a symmetrical compound (Fig. 1). Two signals at δ:192.2 and 192.6 were attributed to conjugated carbonyl and enol carbons. Four broad signals at δ:41.0, 102.1, 105.3 and 112.2 were assignable to carbon atoms bearing deuterium, which were formed by protium-deuterium exchange. This was supported by ^1H-NMR spectra in which there was no significant signal. In the ^1H,^2H-decoupled ^{13}C-NMR spectra the four respective signals changed into sharp singlets (Fig. 1).

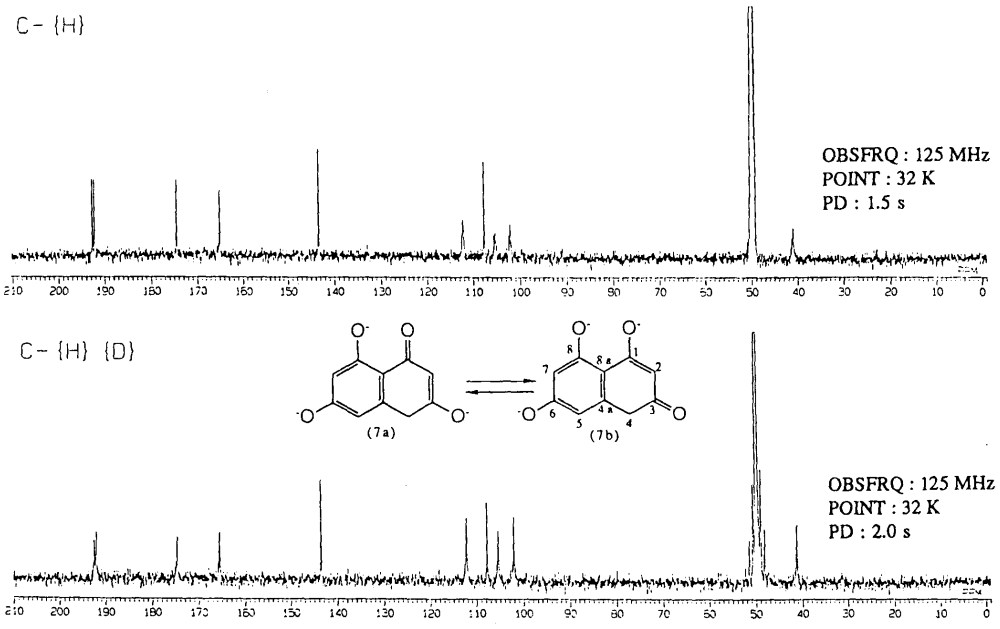

Fig. 1 ^1H-Decoupled and ^1H,^2H-Decoupled ^{13}C-NMR Spectra of 1,3,6,8-THN in CD_3ONa-CD_3OD

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To assign all the carbon signals by ^{13}C-^{1}H correlation spectra, we measured the NMR spectra of 1,3,6,8-THN (2) in CD_3OD-CH_3OH (1:1) containing sodium methoxide. Three singlet ^{1}H signals appeared at δ:5.16, 5.88 and 6.02, which were correlated with the signals at δ:102.1, 105.3 and 112.2 in a C-H COSY spectrum, respectively. An HMBC (^{1}H-detected heteronuclear multiple bond connectivity)[15] spectrum revealed the presence of the ^{1}H-^{13}C long-range chemical shift correlations shown in Table I. A final assignment was made with a doubly labelled 1,3,6,8-THN (2). Methyl curvulinate (4) doubly labelled at the acetyl moiety was synthesized by the acetylation of methyl 3',5'-dibenzyloxyphenylacetate with [1,2-$^{13}C_2$]-acetic acid and trifluoroacetic anhydride.[16] The labelled compound was cyclized in alkali and subjected to NMR measurement. The ^{1}H-decoupled ^{13}C-NMR spectrum gave two pairs of ^{13}C-^{13}C-coupled signals, δ:102.1 and 192.6 (J=63 Hz); δ:105.3 and 165.0 (J=68 Hz).

The results show that the reactive species is 7a or 7b, and 1,3,6,8-THN (2) can be easily converted into its keto-tautomer in strong alkaline condition. The tautomer 7a would be an active species that is reduced by sodium borohydride. This kind of keto-tautomer is probably involved in the biosynthesis of scytalone (1). Studies on theoretical calculations to prove the presence of the keto-tautomer of 1,3,6,8-THN (2) under an alkaline condition are in progress.

Table I. NMR Spectral Data for 1,3,6,8-THN in CD_3ONa-CD_3OD

Position	^{13}C-NMR (ppm)[a]	^{1}H-NMR[b] (ppm)	Carbons correlated in HMBC spectrum[b]
C-1	192.6	----	-----
C-2	102.1	5.16	C-4,8a
C-3	192.2	----	-----
C-4	41.0	c)	-----
C-4a	143.4	----	-----
C-5	112.2	6.02	C-4,6,7,8a
C-6	174.5	----	-----
C-7	105.3	5.88	C-5,6,8,8a
C-8	165.0	----	-----
C-8a	107.7	----	-----

a) Relative to TMS, b) measured in CD_3OD-CH_3OH (1:1) solution of sodium methoxide, c) overlapped with methanol signal.

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