INCORPORATION OF MOLECULAR OXYGEN INTO A MORPHINE ALKALOID, 14-HYDROXYCODEINONE BY *Trametes sanguinea*.

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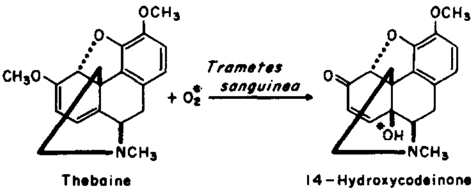
Concerning the mechanism of biological oxidation, it is necessary to determine the origin of oxygen. Regarding the microbial transformation of alkaloid, it was reported that the oxygen at C_6 of 6-hydroxynicotinic acid, oxidation product of nicotinic acid by *Pseudomonas fluorescens* originated from water and not from air (Hunt *et al.*, 1958). Among a series of studies of the mocrobial transformation of alkaloids conducted in our Institute, one of the reactions found was the transformation of thebaine, a morphine alkaloid, to 14-hydroxycodeinone by a wood rotting fungus, *Trametes sanguinea* (Iizuka *et al.*, 1962). This paper describes that the oxygen of the 14-hydroxyl group of the product originates from air and not from water.



Mycelia of *Trametes sanguinea* were prepared as described previously. The filtered mycelia, 2.4 g dry weight, were suspended in 150 ml of medium consisting of koji extract, 0.3 % corn steep liquor and 100 mg of thebaine, in a 1.4 1 gastight suction flask. The atmosphere in the flask was replaced with a mixture of oxygen labelled with oxygen-18 and nitrogen (1:1,V/v), and the suspension was then shaken continuously for 24 hours at 30°C on a reciprocating shaker. During the incubation, thebaine was oxidized almost quantitatively to 14-hydroycodeinone by the organism.

The product was extracted with chloroform, purified by silica gel column chromatography and recrystallized from ethanol. The identity and the purity of the crystals were confirmed by the mixed melting point (269-271°C) and by thin layer chromatography. The content of oxygen isotope was determined by the method of Rittenberg and Ponticorvo (1956). The results shown in Table I clearly indicate that one atom of oxygen in 14-hydroxycodeinone was incorporated from molecular oxygen.

Table I

Incorporation of molecular oxygen into 14-hydroxycodeinone

Exp.	Oxygen-18 atom % excess in			Enrichment %
	O_2	14-hydroxycodeinone		
		theoretical *	found	
I	7.768	1.942	1.905	98.1
			1.985	102.2
II	8.099	2.025	2.137	105.5

* It is calculated as one atom incorporation into only 14-hydroxyl group of 14-hydroxycodeinone.

Although 14-hydroxycodeinone has four oxygen atoms in its molecule, only two of them are necessary to be considered in the oxidation process, the oxygen atoms of the 14-hydroxyl and 6-carbonyl groups. As a general rule, the oxygen atom of the carbonyl group is easily exchangeable with that of water, but the oxygen atom of hydroxyl group is difficult to exchange under these conditions (Cohn and Urey, 1938). Therefore, we examined the possibility of these exchange reaction.

Twenty mg of unlabelled 14-hydroxycodeinone was dissolved in a mixture of 1 ml of ^{18}O-water and 1 ml of citrate buffer (pH 4.0), and the solution was kept at 30°C for 24 hours. Crystals of 14-hydroxycodeinone which appeared by the addition of a few drops of dilute sodium hydroxide solution were separated immediately by filtration and dried *in vacuo*. Data in Table II indicate that an exchange reaction of one atom oxygen actually took place almost to completion under these conditions, and it was assumed that the oxygen atom exchanged was that of a carbonyl group rather than hydroxyl group. Therefore, we postulate that one atom of oxygen incorporated in 14-hydroxycodeinone by *Trametes sanguinea* is present in the 14-hydoxyl group.

Table II

Chemical exchange reaction of oxygen

Exp.	Oxygen-18 atom % excess in				Percentage of oxygen exchanged
	O_2	H_2O	14-hydroxycodeinone		
			expected*	found	
I	6.09	-	0	0.00	0
II	-	5.75	1.44	1.46	101

* It is calculated as one atom exchange of 6-carbonyl oxygen.

However, the possibility still remains that oxygen atoms of both the 14-hydroxyl and 6-carbonyl groups were incorporated biochemically and that the latter was lost by chemical exchange reaction. Attempts to study the microbial transformation process in cell-free extracts have been unsuccessful to date, and it has not been determined whether this reaction is catalized with true oxygenase or mixed function oxygenase (hydroxylase).

We employed whole fungal cells in the experiment, then it is possible to consider another mechanism of incorporation of molecular oxygen. Thebaine can be transformed chemically to 14-hydroxycodeinone by oxidation with 30 % hydrogen peroxide in conc. acetic acid solution (Fel'dman et al., 1945). It might be possible that the incorporation of molecular oxygen was carried out by an indirect mechanism: namely, hydrogen peroxide is first formed by hydrogen peroxide producing oxidase, and then thebaine is oxidized by some type of peroxidase. However, possibility that this type of coupling reaction was actually carried out by the fungus seems not to be the case.

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