Phosphorylation of 3,3'-Dimethyl-4-Hydroxybenzophenone by Soil Microorganism (*Bacillus* sp.)

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3, 3' - Dimethyl - 4 - methoxybenzophenone (methoxyphenone, NK-049) is a new synthetic herbicide¹⁻⁴⁾ having an inhibitory effect to carotenoid biosynthesis.⁵⁾ The transformation of NK-049 into 3,3'-dimethyl-4-hydroxybenzophenone (NK-049-OH) in paddy soils was observed.⁶⁾ Further degradation of NK-049-OH was investigated by isolated microorganism (*Bacillus* sp.) capable of degrading NK-049. The isolated water soluble metabolite of NK-049-OH was its phosphorylation product. Phosphorylation of phenolic hydroxy group by biochemical reaction has been unknown. The present paper deals with the phosphorylation of the hydroxy group of NK-049-OH by the microorganism.

Five mg of ¹⁴C-NK-049-OH (0.1 μ Ci) labeled at carbonyl carbon was incubated with the microorganism in the 150 ml of mineral glucose medium [0.4% of NH₄NO₃, 0.15% of KH₂PO₄, 0.15% of Na₂HPO₄, 0.02% of MgSO₄·7H₂O, 0.0001% of FeSO₄·7H₂O, 0.01% of CaCl₂·2H₂O, 0.05% of yeast extract (Difco) and 0.5% of glucose] (pH 6.8) for three days at 30°C with shaking. Non-metabolized NK-049-OH was removed from the culture medium by extraction with ethyl acetate at pH 6.0-7.0. The remaining aqueous solution was acidified to pH 1.5-2.0 with 2N hydrochloric acid, and extracted with ethyl acetate. The radioactive compound in the ethyl acetate extract was retained at origin by silica-gel thin layer chromatography (*tlc*) using benzene/ethyl acetate (3 : 1). The radioactive compound methylated with diazomethane was moved on *tlc* (*R*_f=0.30) with the same solvent system. After purification by *tlc*, about 3 mg of the methylat-

ed metabolite was obtained in oily state.

The mass spectrum of the methylated metabolite showed the M⁺ ion at *m/e* 334, and the fragment ions at 319 (M⁺-CH₃), 243 (M⁺-CH₃C₆H₄ base peak) and 119 (CH₃C₆H₄-CO⁺). The signals of CMR spectrum in CDCl₃ assigned to two methyl carbons (δ 16.4 and 21.3), two methoxy carbons (δ 54.9 and 55.3), carbonyl carbon of benzophenone (δ 196.0) and benzene carbon substituted with *O,O*-dimethylphosphoryloxy group (δ 152.0). The IR spectrum gave absorption at 1,660 cm⁻¹, 1,600 cm⁻¹, and 1,500 cm⁻¹ due to benzophenone, 1,290 cm⁻¹ (P=O) and 1,050 cm⁻¹ (P-O-alkyl). From these data, the chemical structure of the methylated metabolite was presumed to be *O,O*-dimethyl *O*-[2-methyl-4-(3'-methylbenzoyl)phenyl] phosphate. The methylated metabolite was identical with the synthetic compound based on the spectra data of MS, CMR, and IR.

These facts suggest that the metabolite of NK-049-OH should be *O*-[2-methyl-4-(3'-methylbenzoyl)phenyl] phosphate.

The phosphorylation of NK-049-OH described above was also investigated in the absence of phosphate buffer in the washed cell suspension. That is, the cells grown in bouillon medium and collected by centrifugation were incubated with NK-049-OH in 0.1 M Tris-NaOH buffer (pH 7.0). The phosphorylation metabolite was obtained by the same procedure. This indicated that phosphoric acid moiety was derived from cell contents.

Fig. 1 Chemical structure of methoxyphenone and its related compounds.

要 約

3,3'-ジメチル-4-メトキシベンゾフェノン (メトキシフェノン, NK-049) の水田土壌中の主分解物である 3,3'-ジメチル-4-ヒドロキシベンゾフェノン (NK-049-OH) を, 土壌より分離した NK-049 分解菌 (*Bacillus* sp.) で代謝させ, その水酸基のリン酸化化合物を分離した. このような微生物によるフェノール性水酸基のリン酸化についてはいまだ報告はみられない. NK-049-OH を添加した本菌の培養液から, 代謝物を pH 1.5-2.0 で酢酸エチル抽出し, ジアゾメタンでメチル化後, *tlc* を用いて分離精製した. このメチル化代謝

物の MS, CMR および IR は合成した *O,O*-ジメチル *O*-[2-メチル-4-(3'-メチルベンゾイル) フェニル] フォスフェートと一致した. このことより NK-049-OH の水溶性代謝物の構造は, *O*-[2-メチル-4-(3'-メチルベンゾイル) フェニル] フォスフェートであると推定された.

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