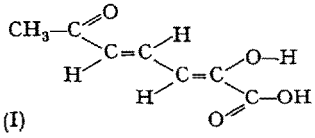
Formation of 2-Hydroxy-6-oxo-2,*trans*-4,*trans*-heptadienoic Acid from 3-Methylcatechol by a *Pseudomonas*[1]

The growth of *Pseudomonas desmolyticum* on different aromatic substrates induces the formation of both pyrocatechase (1,2-oxygenase) and metapyrocatechase (2,3-oxygenase); protocatechuic acid and catechol are oxidized to 3-oxoadipic acid (*ortho*-cleavage), while 3- and 4-methylcatechol are metabolized to compounds which are homologous of hydroxymuconic semialdehyde (*meta*-cleavage)[2]. The ring fission product of 4-methylcatechol was isolated and then identified as 2-hydroxy-5-formyl-2,4-hexadienoic acid[3,4]; otherwise the corresponding oxidation product from 3-methylcatechol had not yet been isolated. However, ICHIHARA et al.[5], DAGLEY et al.[6] and RIBBONS[7] found that different strains of *Pseudomonas* oxidized 3-methylcatechol to a yellow substance with a maximum at 398 nm in alkaline solution. This compound was supposed to be 2-hydroxy-6-oxo-2,4-heptadienoic acid, because it was further degraded to acetaldehyde and 2-oxo-4-hydroxyvalerate[4].

In the present work the extraction and the identification of the *meta*-cleavage product of 3-methylcatechol by *Ps. desmolyticum* are described as 2-hydroxy-6-oxo-2,*trans*-4,*trans*-heptadienoic acid (I).



(I)

Materials and methods. The organism and the conditions of growth have been described[3]. 3-Methylcatechol from Light's was purified by vacuum distillation and sublimation. The spectrophotometric determinations were carried out in the Zeiss P.M.Q. II. The NMR spectra were recorded by a R 10 Perkin-Elmer spectrograph (tetramethylsilane as internal reference). The model 137 Infracord spectrophotometer (Perkin-Elmer Ltd.) was used to obtain IR-absorption spectra. Proteins were determined by the method of WARBURG and CHRISTIAN[8]. 3-Methylcatechol was detected as described by EVANS[9].

Results. In order to isolate a sufficient amount for identification, a large scale incubation was carried out as follows: to a 1000 ml solution containing 500 ml of a buffer suspension of cells (0.30 mg N/ml) and 500 ml of phosphate buffer pH 7, incubated at 30 °C by shaking, aliquots of a M/100 solution of 3-methylcatechol were added, at a rate of 1 µmole/ml of cells suspension. About 10 additions were made at 15 min intervals, when methylcatechol had disappeared. In order to follow the reaction, 1 ml aliquots of the incubation mixture were removed and assayed with Evans reagent for diphenols. After incubation the cells were separated by centrifugation and the supernatant solution, acidified to pH 3 and saturated with ammonium sulphate, was extracted with ether; the ether extract was dried with sodium sulphate and evaporated to dryness at low temperature. The residue was then crystallized from ether. Yellow crystals were obtained, m.p. 122–124 °C, with decomposition. Anal. Calculated for $C_7H_8O_4$: C 53.84; H 5.16. Found C 54.12; H 5.11. The S-benzylisothiouronium salt has m.p. 108–110 °C, with decomposition (from water). Anal. Found: C 55.45; H 4.83; N 8.92. $C_{15}H_{18}N_2O_4S$ requires C 55.80; H 5.58; N 8.69.

The UV-spectrum of the acid in water showed a maximum at 325 nm ($\varepsilon = 25{,}000$); in 0.2*N* NaOH solution the maximum was at 389 nm ($\varepsilon = 41{,}500$). The IR-spectrum (in nujol) of the compound showed bands at 3300, 2300–2500, 1675, 1640 and 1570 cm^{-1}. All these properties are consistent with the presence of a conjugated enol, a carbonyl and a carboxyl group. The NMR-spectrum (in d_5-pyridine) suggests the structure of 2-hydroxy-6-oxo-2,*trans*-4,*trans*-heptadienoic acid. In fact, the following signals were observed: at 11.28 δ a broad singlet of the 2 protons of the carbonyl and the enol groups; a double doublet at 8.26 δ of the proton on 4-C, which is β to the keto group; this signal is shifted in the double doublet ($J_{3,4} = 12$ cps and $J_{4,5} = 17$ cps) because this proton is coupled to the vicinal proton on 3-C and to the *trans*-proton on 5-C. The proton on 3-C absorbs as a doublet ($J_{3,4} = 12$ cps) at 6.86, and the proton on 5-C is responsible for the doublet at 6.43 ($J_{4,5} = 17$ cps). The protons of the methyl group absorb as a singlet at 2.23 δ. The surprising *trans*-configuration of the 4-5 double bond probably arises from acidic treatment during the extraction of the *cis*-precursor, and we could suppose that the system is not very stable because, by addition of deuterated water to pyridine solution, the signals of 3-C and 5-C protons disappeared, and the double doublet of 4-C proton was simplified to a broad singlet.

The structure of (I) was proved by its transformation into 6-methylpicolinic acid in ammonia or ammonium acetate solution[4]. The acid isolated from this reaction had m.p. 122–123 °C, undepressed on admixture with an authentic specimen. Anal. Found C 60.89; H 5.15; N 10.13. $C_7H_7NO_2$ requires C 61.31; H 5.15; N 10.21.

Its UV-spectrum in diluted ammonia solution showed $\lambda_{max} = 272$ nm ($\varepsilon = 4480$), identical with the authentic sample, whose IR-spectrum was wholly superimposable to that of the acid isolated from (I): $\nu_{max} = 3500$, 3350, 1680, 1660 and 1610 cm^{-1} [10].

Riassunto. Ps. desmolyticum ossida la 3-metilpirocatechina ad acido 2-ossi-6-oxo-2,*trans*-4,*trans*-eptadienoico. Vengono descritte le proprietà chimicofisiche che hanno condotto all'identificazione di questo intermedio metabolico.

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