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MICROBIAL CONVERSION OF MURRAYANINE TO MUKOEIC ACID

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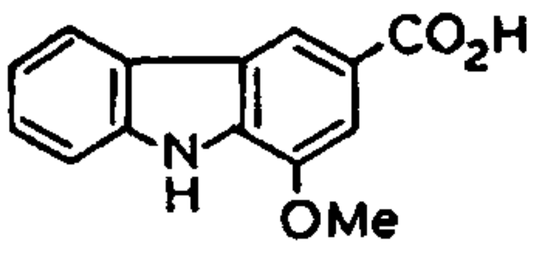
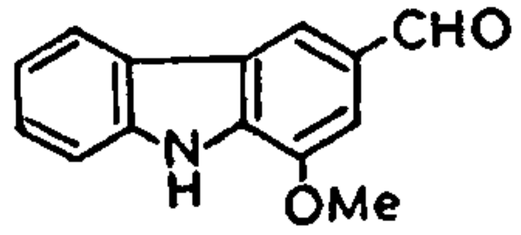
MUKOEIC acid[1], a carbazole alkaloid isolated from *Murraya koenigii*, has been assigned structure (I) based on physical evidences and also based on its formation from murrayanine (II)[2,3]. The biological oxidation of murrayanine to mukoeic acid was suggested based on the occurrence of both of them in the same plant[4]. The present study was undertaken to investigate microbial means of conversion of (II) into (I).

Initially, we searched for an appropriate organism capable of oxidizing an aromatic aldehyde to its corresponding acid, since the natural product II contains an aromatic aldehyde group. As a model system, we investigated the possibility of microbial oxidation of salicylaldehyde (III), a commercially available compound. For this purpose, strains of *Pseudomonas aeruginosa*, *Escherichia coli* B and *Bacillus firmis* were grown in medium (pH 6.8) containing KH_2PO_4 1 g, K_2HPO_4 2 g, $CaCl_2$ 0.02 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, KNO_3 1 g, NaCl 0.2 g, yeast extract 0.3 g, and glucose 10 g per litre. Salicylaldehyde was added to a concentration of 250 μg/ml after 24 h of growth. After incubation for another 48 h the culture filtrates were extracted with ethyl acetate. On removal of solvent, only the *Pseudomonas* culture filtrate yielded a white solid. The solid was crystallized from aqueous alcohol. The crystalline compound (yield 60%), m.p. 159°C, was identified as salicylic acid (IV) from its physical and chemical properties and by direct comparison with a pure sample of salicylic acid.

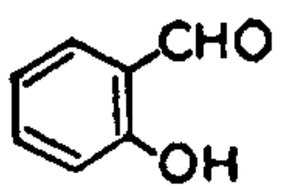
In a similar study *P. aeruginosa* was also found to be quite efficient in converting compound II to I. Maximum conversion could be achieved 24 h after addition of the substrate to a final concentration of 150 μg/ml to the culture. The culture filtrate was extracted with ethyl acetate. On removal of the solvent a semi-solid mass was obtained. It was then chromatographed over silica gel using a series of solvents of increasing polarity, and three compounds were isolated. One was identified as the unconverted compound (II). The second compound (yield 50%), m.p. 241°C, has been assigned molecular formula $C_{14}H_{11}NO_3$ (M^+ 241). The UV spectrum (λ_{max} 236, 270 and 320 nm log ε 4.50, 4.58, 3.92) and IR spectrum (ν_{max} 3431, 1690, 1635, 1615, 1610 cm^{-1}) of the compound were strikingly similar to those of mukoeic acid (I), and identity was finally confirmed by direct comparison with natural mukoeic acid (m.m.p., UV, IR). The other compound, m.p. 228°C, molecular formula $C_{13}H_9NO_3$ (M^+ 227), gave violet coloration with $FeCl_3$, indicating the presence of phenolic hydroxyl group. The UV spectrum (λ_{max} 240, 266, 286, 315 and 323 nm, log ε 4.59, 4.64, 3.8, 3.78, and 3.18) bears a strong similarity to that of 1-hydroxycarbazole with carboxyl substitution at the 3 position[5]. The IR spectrum indicated it was an aromatic substance with an NH (3430 cm^{-1}), one hydroxyl (3200 cm^{-1}) and one carboxylic acid group (1690 cm^{-1}). From all these data the compound has been assigned the structure V.



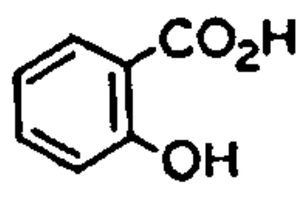
(I)



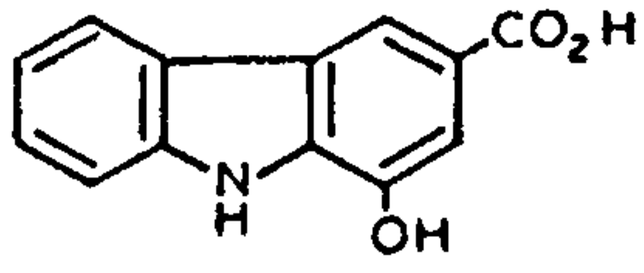
(II)



(III)



(IV)



(V)

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ACETYLENE REDUCTION ACTIVITY OF SOME BLUE-GREEN ALGAE

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MOST aerobic nitrogen-fixing blue-green algae are filamentous forms and N_2 fixation takes place in differentiated cells called heterocysts which serve as N_2-fixing factories[1]. Certain N_2-fixing, heterocystous blue-green algae develop associations with algae, fungi, bryophytes, the water fern *Azolla*, gymnosperms and the angiosperm *Gunnera*[2]. These associations are of interest because the algae supply fixed nitrogen to the host plant and are, in many ways, analogous to rhizobia[3]. Relative to free-living isolates, symbiotically associated blue-green algae have a five-fold or even higher frequency of heterocysts[4,5]. The present paper describes the acetylene reduction activity (ARA) of six blue-green algae in relation to their heterocyst frequency and age. These estimates would serve as a reference for inoculum selection, growth and biochemical studies.

The blue-green algal isolates used are listed in table 1. Axenic cultures of the blue-green algae were grown in BG-11 medium[6] under continuous light at 50 μmol photons $m^{-2} \cdot s^{-1}$ at 25°C. The cultures were harvested after 3, 7 and 30 days, washed with sterile BG-11 medium[7] and suspended in the same medium. ARA was measured by the acetylene reduction assay[7]. The cultures (5 ml each) were placed in 15 ml serum vials with 10% (v/v) acetylene as the gas phase at 25°C and a photon flux of 250 μmol $m^{-2} \cdot s^{-1}$. Gas samples (1 ml) in two replicates were collected after 30 min and analysed in a gas chromatograph (model Tracor 540). The heterocyst frequency was calculated as per cent of total cells by light microscope observations of the filaments. Chlorophyll *a* was estimated by the method of Mackinney[8].

Table 1 shows the data on relative ARA and heterocyst frequency of six blue-green algal strains. Based on their ARA values and heterocyst frequency, the algae can be separated into two main groups: one with high ARA and high heterocyst frequency (*Nostoc anthoceros, Anabaena cycadeae*) and the other with low ARA and low heterocyst frequency (*Anabaena* 7120, *A. variabilis, A. doliolum* and *Nostoc linckia*). The free-living isolates from *Anthoceros* and *Cycas* had high ARA values (4.47 and 4.04 nmol C_2H_4 per μg Chl *a* per hour respectively) compared with the other isolates. This can be explained by the fact that these isolates had average

Table 1 *Acetylene reduction activity and heterocyst frequency of blue-green algae*

Isolate	Source	Acetylene reduction activity* (nmol C_2H_4 per μg Chl *a* per hour) Young culture (3-day)	log-phase culture (7-day)	old culture (30-day)	Heterocyst frequency (%)
Anabaena doliolum	Isolated from paddy field (BHU)	1.47	3.38	0 586	4.95
Anabaena 7120	Gift from B. Bergman (Uppsala, Sweden)	1.42	2.98	0.284	4.55
Anabaena variabilis	Gift from A. K. Kashyap (BHU)	1.29	3.39	0.691	4.65
Nostoc linckia	—"—	1.51	3.60	0.713	4.72
Anabaena cycadeae	Original isolate from corralloid root of *Cycas circinalis* (BHU)	1.71	4.04	1.29	6.00
Nostoc sp.	Original isolate from *Anthoceros* gametophyte (Shillong)	1.88	4.47	1.59	8 90

*Average of six independent experiments.