Formation of *O*-Methyl- and *O*-Pentyl-homoserine from Methanol and *n*-Pentanol in the Presence of L-Homoserine by Bacteria

Sir:

Previous papers^{1,2)} showed that *Corynebacterium* sp. E17 produced *O*-ethylhomoserine, *O*-propylhomoserine, and *O*-butylhomoserine when it was grown in media containing ethanol, *n*-propanol and *n*-butanol, respectively. The bacterial product from ethanol was found to be identical with *O*-ethyl-L-homoserine which was synthesized chemically.³⁾ This compound has also been found to occur in almost all strains of *Brevibacterium*, *Corynebacterium*, *Bacillus*, *Mycobacterium*, *Nocardia* and *Streptomyces* tested.⁴⁾ In experiments using resting cells of *Corynebacterium* sp. E17, *O*-ethylhomoserine, *O*-propylhomoserine and *O*-butylhomoserine were formed from ethanol, *n*-propanol and *n*-butanol respectively in a few hours and their formation was greatly enhanced by addition of L-homoserine.⁴⁾

In this paper, new compounds, *O*-methylhomoserine and *O*-pentylhomoserine are shown to be formed from methanol and *n*-pentanol respectively in the presence of L-homoserine by resting cells of the bacterium.

Corynebacterium sp. E17 was grown in chemically defined medium containing (g/liter water): (NH₄)₂HPO₄, 2; KH₂PO₄, 1; MgSO₄·7H₂O, 0.5; NaCl, FeCl₃·6H₂O and MnCl₂·4H₂O, 0.01 and ethanol as a carbon source, 10, for 40~48 hr at 32°C. The cells were harvested by centrifugation, washed twice with saline, and then resuspended in 0.1 M potassium phosphate buffer at pH 7.5 at a density of about 10 mg of cells (dry wt.)/ml.

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2) Y. Murooka and T. Harada, *Agr. Biol. Chem.*, **31**, 1035 (1967).

3) Y. Murooka, T. Harada and Y. Izumi, *Bull. Chem. Soc. Japan*, **41**, 633 (1968).

4) Y. Murooka and T. Harada, *J. Bact.*, in press.

To 100 ml volumes of the cell suspensions in 500 ml conical flasks, 1 ml of methanol or *n*-pentanol was added in the presence of 100 mg of L-homoserine and 200 mg of urea, and the mixtures were incubated for 20~24 hr at 32°C. After incubation, the reaction mixture was centrifuged at 6000 rpm for 10 min. The supernatant fluid was passed through a column of cation-exchange resin (Dowex 50, H⁺-form) and then a column of anion-exchange resin (Dowex 2, OH⁻-form) to remove inorganic salts. The eluate was evaporated to dryness *in vacuo*. The dried residue was placed on a cellulose column and eluted with *n*-butanol:acetic acid:water (4:1:5). The fractions of eluate were analyzed by paper chromatography. The ninhydrin positive compounds M and P with *R_f* values of 0.70 and 0.88 with the solvent (phenol:ethanol:water (10:7:3 v/v)), respectively, were detected in the appropriate fractions. Both compounds were derived from methanol and *n*-pentanol in the presence of L-homoserine. These fractions which contained the compounds were evaporated to dryness *in vacuo*.

Compounds M and P were isolated by recrystallization of the material in the dry residues from water and ethanol (yield M, 54 mg; P, 150 mg from 1 liter of reaction mixture). These yields were much less than those in the case of *O*-ethylhomoserine, *O*-propylhomoserine and *O*-butylhomoserine. Compound M was very soluble in water, slightly soluble in methanol and hardly soluble in ethanol, acetone or ether, while compound P was soluble in water and methanol, slightly soluble in ethanol, and hardly soluble in acetone or ether.

Compound M, mp 233°C, [α]_D²⁰ -20°, (*c*=2, water), *Anal.* Found: C, 45.07; H, 8.23; N,

FIG. 1. a Infrared Spectra (KBr) of *O*-Ethylhomoserine, *O*-Propylhomoserine and *O*-Butylhomoserine. b Infrared Spectra (KBr) of Amino Acids (M and P) Formed from Methanol and *n*-Pentanol, Respectively.

10.61. Calcd. for $C_5H_{11}NO_3$: C, 45.10; H, 8.33; N, 10.52%. Compound P, mp 268°C, $[\alpha]_D^{30} -6^\circ$, ($c=2$, water), *Anal.* Found: C, 57.36; H, 9.91; N, 7.49. Calcd. for $C_9H_{19}NO_3$: C, 67.11; H, 10.12; N, 7.40%. The infrared spectra of both compounds M and P are shown in Fig. 1 with those of *O*-ethylhomoserine, *O*-propylhomoserine and *O*-butylhomoserine which have already been described.²⁾ All compounds had absorption bands at 2950, 2600, 1585 and 1510 cm^{-1} , which are typical of a zwitter-ionic amino acid.⁵⁾ The strong absorption band at 1120~1125 cm^{-1} , which is typical of a disturbed stretching vibration of a C-O-C configuration, was observed in

the infrared spectra of compounds M and as well as with those of the control compounds. Some differences between these compounds were seen in the region of 840~1000 cm^{-1} .

From comparison of the results of elementary analysis and specific rotation value and infrared spectra with those of *O*-ethylhomoserine, *O*-propylhomoserine and *O*-butylhomoserine, compounds M and P were indicated to be *O*-methylhomoserine and *O*-pentylhomoserine respectively.

Corynebacterium sp. E17 could not produce *O*-methylhomoserine and *O*-pentylhomoserine when grown in culture media containing methanol and *n*-pentanol, respectively. The *O*-alkylhomoserines could also not be formed in reaction mixtures unless L-homoserine was added. This may be because L-homoserine is scarcely formed, if at all, from methanol or *n*-pentanol by the organism. Addition of urea resulted in a gradual increase of pH which might favor the formation of *O*-alkylhomoserine. The formation of *O*-methylhomoserine and *O*-pentylhomoserine by cell suspensions was also observed with *Nocardia corallina* IFO 3338. Both the *O*-alkylhomoserines may be formed by many bacteria since *O*-ethylhomoserine is generally formed in strains of *Brevibacterium*, *Corynebacterium*, *Bacillus*, *Mycobacterium*, *Nocardia* and *Streptomyces*.⁴⁾ However, the physiological role of these *O*-alkylhomoserines is still unknown.

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