O-ALKYL-L-HOMOSERINES: THREE NEW AMINO ACIDS FORMED FROM ALCOHOLS BY CORYNEBACTERIUM ETHANOLAMINOPHILUM SP.NOV.

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In the course of studies (Harada et al., 1966) on the formation of glycine from ethanolamine by a soil bacterium, the bacterium (E17) was found to form a new amino acid, when grown in well aerated medium containing ethanol, n-propanol or n-butanol. The amino acids (E, P and B) formed from ethanol, propanol and butanol, respectively, have now been shown to be O-ethylhomoserine, O-propylhomoserine and O-butylhomoserine. The bacterium strain E17 was named Corynebacterium ethanolaminophilum by Harada and Murooka. No detectable amount of any amino acid was formed with succinate or glucose as a carbon source. The medium used contained 1% alcohol, 0.25% urea, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, and 0.001% NaCl, $MgCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$ in distilled water and was adjusted to pH 7.2. When fermentation was complete, the culture fluid (about 1 l) was centrifuged at 6000 r.p.m. for ten minutes. The supernatant fluid was passed through a column containing cation-

exchange resin (Dowex 50, H^+-form) and then through a column containing anionic-exchange resin (Dowex 2, OH^--form) to remove inorganic salts. The eluate was introduced into a column containing cellulose powder and eluted with butanol:acetic acid: water (4:1:5). The fractions of eluate were analyzed by paper chromatography. Fractions which contained compound E, P or B, were concentrated to dryness. Compounds E, P and B were isolated by recrystallization from the dry residues with water and ethanol (E, 250 mg; P, 300 mg; B, 200 mg). Compound E, Found: C, 49.03; H, 8.37; N, 9.83, Anal. Calcd. for $C_6H_{13}NO_3$: C, 48.97; H, 8.90; N, 9.52. Compound P, Found: C, 51.97; H, 9.14; N, 9.03, Anal. Calcd. for $C_7H_{15}NO_3$: C, 52.16; H, 9.38; N, 8.69. Compound B, Found: C, 54.64; H, 9.74; N, 8.23, Anal. Calcd. for $C_8H_{17}NO_3$: C, 54.84; H, 9.78; N, 7.99.

Compound E; mp 262°C and $[\alpha]_D^{30}$-14 (c 2.5, water).

Compound P; mp 265°C and $[\alpha]_D^{30}$-11 (c 2.0, water).

Compound B; mp 267°C and $[\alpha]_D^{30}$-8 (c 2.0, water).

The infrared spectra of these compounds are shown in Fig. 1. All the infrared spectra showed absorption bands at 2950 and 2600, 1585 and 1510 cm^{-1}, which are typical of a zwitter-ionic amino acid (Bellamy, 1960). The strong absorption band at 1125 cm^{-1} is typical for a disturbed stretching vibration of a C-O-C configuration. The absence of absorption bands near 3300 and 1700 cm^{-1} indicated the absence of a hydroxy radical and lactone ring.

The nmr spectra of these compounds showed absorptions at ca. 1.0, 2.0 and 3.6 p.p.m. due to three, two and five protons, respectively (E); at ca. 0.9, 1.4, 2.0 and 3.6 p.p.m. due to three, four, two and five protons, respectively (P); at ca. 0.9, 1.4, 2.0 and 3.6 p.p.m. due to three, four, two and five protons, respectively (B).

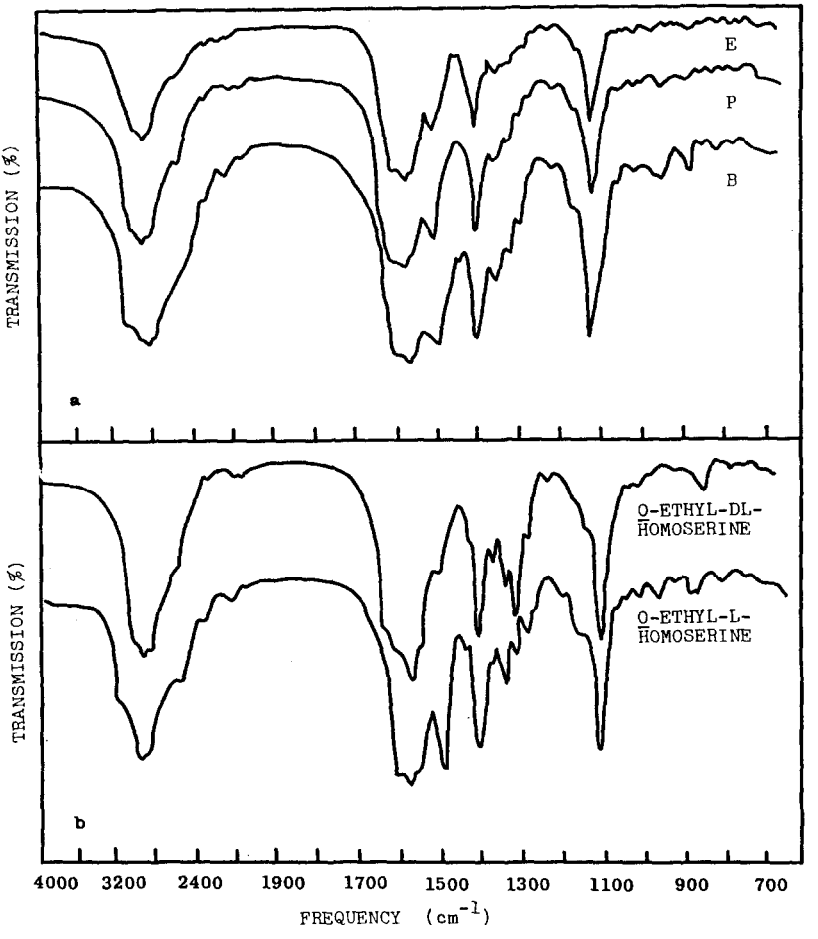
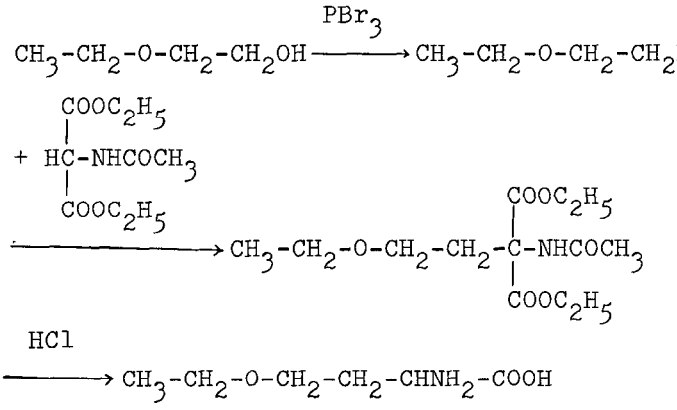


Fig. 1. a, Infrared spectra (KBr) of the amino acids (E, P and B) formed from ethanol, n-propanol, and n-butanol, respectively. b, Infrared spectra (KBr) of O-ethyl-DL-homoserine and O-ethyl-L-homoserine.

The triplet at ca. 1.0 or 0.9 p.p.m. indicates $-CH_3$ group adjacent to a $-CH_2-$ group. The multiple at 1.4 p.p.m. indicates a $-CH_2-$ (P) or $-CH_2-CH_2-$ (B) pattern flanked by carbon atoms which do not have functional groups. The multiple at 3.6 p.p.m. was seen in all the spectra.

From the results of elementary analyses, infrared spectra and nmr spectra, these compounds were considered to be O-ethyl-

homoserine (E), O-propylhomoserine (P) and O-butylhomoserine (B). The mass spectra of these compounds supported these structures, giving the molecular weights of compounds E, P and B as 147, 161 and 175, respectively since the parent molecular ion - COOH (102 (E), 116 (P) and 130 (B)) peaks were observed. To determine the structures of these compounds, the DL-racemate of O-ethyl-homoserine was first synthesized as follows.



O-ethyl-L-homoserine was separated from the DL-racemate by the method of Bergmann and Fraenkel-Conrat (1937). The infrared spectra of O-ethyl-L- and DL-homoserine are shown in Fig. 1.

The spectrum of the bacterial product (E) was accordant with that of the synthetic L-compound while that of the DL-racemate was rather different with that of the L-compound. Thus, it was confirmed that compound E was O-ethyl-L-homoserine. Synthesis of O-propylhomoserine and O-butylhomoserine are in progress. No other ether compounds of amino acids are yet known. The physiological role of these alkyl homoserines is not clear.

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REFERENCES

L. J. Bellamy, The Infra-red Spectra of Complex Molecules, New York, N.Y., Wiley (1960), p234.
M. Bergmann and H. Fraenkel-Conrat, J. Biol. Chem., 119, 707 (1937)
T. Harada and Y. Murooka, J. Ferment. Technol., 44, 192 (1966)