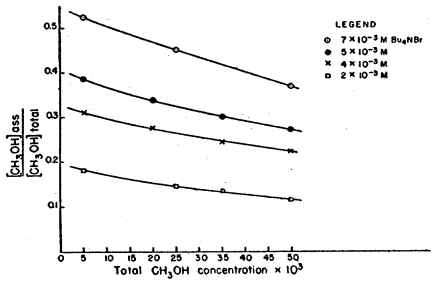

Fig. 2. Effect of various concentrations of tetrabutylammonium bromide on the equilibrium between free and associated methanol.

The effect of changing the anion is striking. Thus, for several tetrabutylammonium salts, the molar-extinction coefficient of associated methanol is as follows: for the picrate, ~ 0.0; for nitrate, 6.5; for bromide, 11.0; and for chloride, 12.0. Evidently the effectiveness of the anion in producing association increases in the same order as the anion charge density. This suggests that association in these cases is not self-association but ion-dipole association—that is, solvation.

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27 July 1959

Occurrence of β-Aminoisobutyric Acid in Mytilus edulis

Abstract. β-Aminoisobutyric acid was isolated from organ extracts of *Mytilus edulis*. Ion-exchange resins and large-scale paper chromatography were used to isolate minute quantities of the compound. β-Aminoisobutyric acid was identified by paper chromatography in several solvents and by conversion to DNP-β-aminoisobutyric acid and subsequent chromatography of the derivative in several solvents.

β-Aminoisobutyric acid was first found in human urine by Crumpler *et al.* (*1*). They observed that some individuals excrete large amounts of this amino acid and that the excretion is an individual characteristic determined genetically. Several papers dealing with genetic factors involved in the excretion of this amino acid (*2*) have appeared since the paper by Crumpler *et al.* was published. Fink *et al.* found that β-aminoisobutyric acid is formed in the rat from thymine (*3*). Awapara and Shullenberger (*4*) observed that leukemic patients excrete large quantities of this acid only when given nitrogen mustard (methyl-bis[β-chloroethyl]amine hydrochloride) or thymine.

During an investigation of nitrogen metabolism in marine invertebrates, we observed that extracts of *Mytilus edulis* contain a compound with all the characteristics of β-aminoisobutyric acid as determined by paper chromatography. It was not an α-carboxyl amino acid, as was shown by treatment with copper carbonate (*5*). Extracts of *M. edulis* contain a large number of amino acids in large quantities. Proper identification by paper chromatography became difficult. The quantity of the unknown was not very large, and to isolate it in sufficient quantities for chemical analysis would be something of a tour de force. We have, however, isolated minute amounts of this compound and shown it to be chromatographically identical with β-aminoisobutyric acid.

For isolation, 100 gm of pooled organs of *Mytilus* were extracted with 80 percent ethanol (*6*). The extract was treated first with Amberlite CG-50 H^+ to remove basic compounds, then with Dowex 50, H^+, to remove all amino acids except taurine and other strongly acidic substances. The amino acids were displaced from the column with 4*N* NH_4OH. The effluent was evaporated under a vacuum. The residue was dissolved in 4 ml of water and decolorized with activated charcoal. To isolate the unknown from all other ninhydrin-reactive compounds, the solution was chromatographed on several sheets of filter paper. Known β-aminoisobutyric acid was chromatographed on the same paper to serve as a guide. The area corresponding to β-aminoisobutyric acid was cut and eluted. The eluate was chromatographed three more times with different solvents until only one ninhydrin-reactive substance was obtained. The R_f values of the unknown and known β-aminoisobutyric acid were identical in several solvents, and when mixed they could not be separated chromatographically (Table 1). A DNP derivative was prepared by the method of Sanger (*7*). Chromatography of the DNP derivative and of DNP-β-aminoisobutyric acid showed that they had identical R_f values in several solvents (Table 2). No separation occurred when they were mixed. From this evidence we concluded that the unknown was β-aminoisobutyric acid.

This compound is present in all parts of the animal. Analyses were carried out chromatographically, and the estimated concentrations are shown in Table 3. This compound appears to be present in other marine invertebrates, but its identity needs to be established with certainty. It is interesting that mammals and invertebrates catabolize thymine by a similar mechanism. β-Alanine also seems to be present in *Mytilus* and in several species of marine invertebrates studied (*8*). If these two amino acids are found in more species, there will be little doubt that thymine and uracil are catabolized to give rise to β-aminoisobutyric acid and β-alanine, respectively (*9*).

Table 1. R_f values of unknown and β-aminoisobutyric acid.

Solvent	R_f
Lutidine, water (62:38)	0.34
Phenol, water (72:28)	0.58
n-Butanol, acetic acid, water (120:30:50)	0.43
n-Butanol, formic acid, water (75:15:10)	0.48

Table 2. R_f values of DNP-β-aminoisobutyric acid and DNP-unknown.

Solvent	R_f
n-Butanol, ethanol, water (40:10:50)	0.71
n-Butanol, water (saturated)	0.53
n-Butanol, 0.1% NH_3 (saturated)	0.40
m-Cresol, 0.3% NH_3 (saturated)	0.74

Table 3. Concentration of β-aminoisobutyric acid in organs of *Mytilus edulis*.

Organ	Concentration (μmole/100 gm of fresh tissue)
Mantle	44
Gill	103
Viscera	62
Foot	93

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9. This work was supported by grants from the Robert A. Welch Foundation, Houston, Tex., and from the National Institutes of Health.

17 July 1959