of the *Trypoxylon* nests showed clearly that this wasp is much more constant in its habits when bringing mud for nest construction. From Fig. 2 it can be seen that whole tubes of a *Trypoxylon* nest will be made of mud from a single source before there is a change to another source of mud. Moreover, *Sceliphron* often thickens the outside of the walls of its nests by plastering on balls of mud from a variety of sources (Fig. 1), whereas *Trypoxylon* does any thickening there may be on the inside. Fired *Trypoxylon* nests also reveal that the prepupal cocoon is composed primarily of grains of sand and clay chewed from the inner wall of the cell. The fired cocoon is white with localized spots of mineral colours derived from the clay it contains.

Some of the fired nests gave distinctive colours which were connected with a specific source, so that an estimate could be made of the flight range of the wasps. In a systematic study, kiln-firing would probably indicate the comparative usages by different species, seasonal changes in nest-building activity, and perhaps information about the rate of nest construction as well.

I thank Mr. Douglas Ferguson, of the Pigeon Forge Pottery, Pigeon Forge, Tennessee, for suggesting that kiln-firing might discriminate between different sources of mud.

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[1] Shinn, A. F., *Health Physics*, **10**, 619 (1964).
[2] Bartram, J., *Phil. Trans. Roy. Soc., London*, **43**, 363 (1746).
[3] Buckman, H., and Brady, N. C., *The Nature and Properties of Soil* (The Macmillan Co., New York, 1960).

Isolation of 2-Aminoethylphosphonic Acid from Phospholipids of the Abalone (*Haliotis midae*)

IN the course of an investigation of the phospholipids of the abalone shellfish (*Haliotis midae*), a ninhydrin-reactive substance was detected which showed properties similar to phosphoryl ethanolamine. For example, no separation between phosphoryl ethanolamine and the unknown compound was obtained by column chromatography on the cation exchange resin 'Amberlite *C.G.* 120' type 2 with an eluting buffer of *p*H 3·25. Hydrolysis with 2 N hydrochloric acid at 120° C for 100 h failed, however, to liberate ethanolamine and phosphoric acid. This substance was identified as 2-aminoethylphosphonic acid.

Horiguchi and Kandatsu[1,2] were the first investigators to describe the isolation of 2-aminoethylphosphonic acid from rumen protozoa and *Tetrahymena pyriformis W*. Recently, this compound has also been isolated from the sea anemone (*Anthopleura elegantissima*) in which it occurs free and esterified with glycerol and sphingosine[3-5]. It appears that the abalone is the highest organism in which the presence of this phosphonic acid has so far been demonstrated.

Lipids of the abalone (shell-free 3,800 g) were extracted with chloroform–methanol (2 : 1 by volume). The water-soluble impurities were removed by two washings according to Folch *et al.*[6]. After taking the material to dryness *in vacuo* it was taken up in petroleum ether (40–60° C) and filtered. The lipids (23·7 g; phosphorus 2·1 per cent), which were obtained after removal of the petroleum ether, were refluxed for 48 h with 600 ml. 6 N hydrochloric acid. The reaction mixture was filtered, the filtrate evaporated to dryness *in vacuo* and the residue taken up in about 20 ml. of water. The phosphonic acid was separated from other compounds by chromatography over a column of 'Zeokarb 225' (20 × 2 cm; 52–100 mesh, hydrogen form, 4·5 per cent divinyl benzene). The eluting agent was 0·1 N hydrochloric acid, 5 ml. fractions were collected and each fraction was tested for the presence of 2-aminoethylphosphonic acid by paper chromatography in 80 per cent *n*-propanol (R_F 0·10). One fractionation was insufficient, due to the presence of large quantities of choline, ethanolamine, serine, etc., and the separation was therefore repeated on an identical column. Fractions containing the compound (tube numbers 8–30 for the first separation and 50–100 for the second) were combined and evaporated to dryness *in vacuo*.

The resulting oil, which could not be induced to crystallize, was taken up in 2 ml. of water and transferred to a small column of 'Dowex 1–*X*8' (4 × 2 cm; acetate form). Elution was carried out with 0·5 N acetic acid; the first 20 ml. contained all the desired material. After evaporation of the acetic acid the material became crystalline. Recrystallization from 50 per cent ethanol gave 58 mg of 2-aminoethyl phosphonic acid, which melted at 284°–286° C and decomposed at 295° C. Horiguchi and Kandatsu[1] give 295°–297° C (decomposition); Kittredge, Roberts and Simonsen[3] give 280°–281° C (decomposition).

Authentic 2-aminoethylphosphonic acid was supplied by Dr. Horiguchi of the University of Tokyo. No depression in melting point was observed when mixing this substance and the isolated material; moreover, the infra-red spectra were identical. Horiguchi and Kandatsu[7] have recently shown dimorphism in 2-aminoethylphosphonic acid, resulting in two infra-red spectra. The substance described in this report corresponded to the metastable 'α-form' of these authors.

It was shown by column chromatography of the lipids on silicic acid according to Hanahan, Dittmer and Warashina[8] that the phosphonic acid was present in the phospholipids of the abalone. The phosphonic acid-containing lipid, which was eluted from the silicic acid column with chloroform–methanol 3 : 2 (by volume), emerged just before phosphatidyl inositol and phosphatidyl choline. This phospholipid fraction was very rich in sphingosine and it seems, therefore, that 2-aminoethylphosphonic acid is part of a sphingolipid, the structure of which is still to be established.

A complete report of the composition of the abalone phospholipids will be published elsewhere.

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[1] Horiguchi, M., and Kandatsu, M., *Nature*, **184**, 901 (1959).
[2] Kandatsu, M., and Horiguchi, M., *Agric. Biol. Chem.*, Tokyo, **26**, 721 (1962).
[3] Kittredge, J. S., Roberts, E., and Simonsen, D. G., *Biochemistry*, **1**, 624 (1962).
[4] Rouser, G., Kritchevsky, G., Heller, D., and Lieber, E., *J. Amer. Oil Chemists' Soc.*, **40**, 425 (1963).
[5] Kittredge, J. S., and Hughes, R. R., *Biochemistry*, **3**, 991 (1964).
[6] Folch, J., Ascoli, I., Lees, M., Meath, J. A., and le Baron, F. N., *J. Biol. Chem.*, **191**, 833 (1951).
[7] Horiguchi, M., and Kandatsu, M., *Agric. Biol. Chem.*, Tokyo, **28**, 408 (1964).
[8] Hanahan, D. J., Dittmer, J. C., and Warashina, E., *J. Biol. Chem.*, **228**, 685 (1957).

MICROBIOLOGY

Inability of Decenylsuccinic Acid to protect *Escherichia coli* against Damage by Freezing

CHEMICAL protection against injury due to damage by freezing has been extensively studied by various workers[1-8]. Recently Kuiper[9] reported that decenylsuccinic acid induced resistance to desiccation, cold and frost in young bean plants. When decenylsuccinic acid penetrates into the lipid layer of the membrane of bean root cells it increases water permeability. This permeability was only slightly temperature dependent[10]. Our laboratory has been interested in the protection against damage by freezing in bacteria[11]. We obtained samples of