Isolation of Asparagine from the Hæmolymph of *Melolontha* Larvæ

DURING a study of the amino-acids of the hæmolymph of insects, a substance was isolated from the blood of *Melolontha vulgaris* which turned out to be asparagine. This finding may be of interest because, to my knowledge, this substance has not been met with in animal fluids or tissues before, while it is a characteristic constituent of many plants.

The blood of some hundred larvæ (15 gm.) was deproteinized with trichloroacetic acid, and the filtrate was freed from the acid by extraction with ether. Hexon bases and some peptide or peptides were precipitated with phosphotungstic acid, and dicarbonic acids were precipitated with barium hydroxide and alcohol. After concentration *in vacuo* and removal of barium with sulphuric acid, mercuric acetate and sodium hydroxide were added by drops until no further precipitate would form.

The precipitate was suspended in water, decomposed with hydrogen sulphide and after removal of mercuric sulphide the solution was concentrated on a steam bath. First tyrosine crystallized out and was removed, and the mother liquor was concentrated further. Irregular crystals of another substance now separated out. They were freed from the glassy mother liquor by digestion with warm 50 per cent alcohol in which the crystals were but little soluble. The substance was repeatedly recrystallized and the resulting sample of an apparently pure substance (10 mgm.) was analysed after drying at 110° C. The results are presented below with the theoretical values for asparagine.

	Found (%)	Theory (%)
Total nitrogen	20·7	21·2
Amino nitrogen (per mol titr.)	11·0	10·6
Amid nitrogen (4 hr. hydrolysis with *n* hydrochloric acid)	9·2	10·6

The crystals resembled in every respect those of asparagine.

The concentration of asparagine in the blood of the larvæ can only be roughly estimated, due to the inevitable losses on recrystallization. 150–250 mgm. per cent should be a probable guess. Blood from larvæ of the related *Oryctis nasicornis* was found to contain 23·7 mgm. per cent amide nitrogen, corresponding to 223 mgm. per cent asparagine, but the material did not suffice for an isolation. So it still remains to be seen if asparagine is a peculiarity in *Melolontha* or a typical product of insect metabolism.

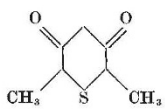
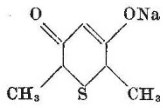
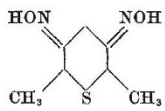
HANS H. USSING.

Laboratory of Zoophysiology,
University of Copenhagen.

Antibacterial Activity of 2 : 6-Dimethyl-Penthiane-3 : 5-Dione

IN a recent speculation Gulland and Farrar[1] have elaborated a hypothesis for the mode of action of *cyclo*telluro-pentane-3 : 5-dione and homologues, in which substrate competition with pyridoxin is postulated as a possible explanation of their high bactericidal activity. The structural resemblance of these tellurium compounds to that of pyridoxin is pointed out, and it is suggested that analogous substances containing an oxygen or a sulphur atom instead of tellurium might possess correspondingly high bacteriostatic or bactericidal activity without the extreme toxicity of the tellurium compounds.

As we were interested in heterocyclic ring compounds containing sulphur[2], we have prepared and tested the sulphur analogue of 2 : 6-dimethyl-cyclotelluro-pentane-3 : 5-dione, which is the most active representative of the tellurium series of homologues. 2 : 6-Dimethyl-penthiane-3 : 5-dione is a white crystalline substance of m.p. 124–125° C., soluble in hot water. It dissolves easily in cold caustic soda solution, but does not give a coloration with alcoholic ferric chloride except on prolonged standing, in which it resembles *cyclo*telluro-pentane-3 : 5-dione[3]. It forms a white crystalline dioxime of m.p. 181–183° C., slightly soluble in hot water. The accompanying table shows the results of the bacteriological examination and gives the minimum concentrations inhibiting the growth of one loopful of inoculum.

	Staph. aureus NCTC 4163 glucose broth (18 hr. at 37°)	*Bact. coli* NCTC 86 Lemco broth (18 hr. at 37°)	*Bact. coli* NCTC 86 Reader's medium (24 hr. at 37°)
	1 : 1,600 (1 : 600,000–1 : 10,000,000)*	< 1 : 1,000 (1 : 9,000,000–1 : 10,000,000)	1 : 6,400
	<1 : 1,000	<1 : 1,000	1 : 3,200
	1 : 1,000–1 : 2,000	<1 : 1,000	1 : 6,400

* The figures given in brackets are the bactericidal concentrations of the analogous tellurium compound reported by Morgan *et al.*[4].

Contrary to expectations, the compounds show little or no activity against the organisms tested. It is interesting to note that enolization, which is a prerequisite in Gulland and Farrar's speculation, in our case decreases or abolishes bacteriostatic activity altogether. The dioxime of 2 : 6-dimethyl-penthiane-3 : 5-dione, however, in contrast to the corresponding tellurium compound, is as active as the parent substance. It would appear, therefore, that other factors, apart from the arrangement $-C.(CH_3).C.(OH).C-$, as suggested by Gulland and Farrar (*loc. cit.*) are fundamentally concerned in determining the bacteriological properties of this class of compounds. Our results certainly do not favour their speculation of substrate competition with pyridoxin.

F. BERGEL.
A. L. MORRISON.
A. R. MOSS.
H. RINDERKNECHT.
J. L. WARD.

Research Department,
Roche Products, Ltd.,
Welwyn Garden City,
Herts.
Feb. 26.

[1] Gulland and Farrar, *Nature*, **154**, 88 (1944).
[2] Avison *et al.*, *Nature*, **154**, 549 (1944).
[3] Morgan *et al.*, *J. Chem. Soc.*, **117**, 1464 (1929).
[4] Morgan *et al.*, *Biochem. J.*, **18**, 196 (1924).