0·1 N sulphuric acid at 62° C, the acid neutralized with barium carbonate and the mixture filtered and concentrated (sample II). Both samples gave a positive Molisch reaction and there was no reducing action with aniline hydrogen phthalate reagent[3].

Samples I and II were further hydrolysed separately for 5 h with an equal volume of 2 N sulphuric acid and neutralized with barium carbonate, filtered and concentrated to 1–2 ml. The hydrolysates were run for 30 h on paper chromatograms (Whatman No. 1 paper descending technique) using the organic layer from a freshly prepared *n*-butanol–acetic acid–water mixture (4 : 1 : 5 v/v/v) (ref. 4). The chromatograms were sprayed with aniline hydrogen phthalate reagent and then dried in an oven at 110° C. The chromatograms showed a pale spot corresponding to authentic xylose (Fig. 1)—a sugar more generally obtained from plants.

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Homarine (*N*-Methyl Picolinic Acid) in Muscles of some Australian Crustacea

During the examination of guanidino compounds in trichloroacetic acid extracts of crustacean muscles, it was observed that treatment of paper chromatograms with alkaline α-naphthol : diacetyl[1] sometimes resulted in the appearances of a yellow spot in addition to the pink spots characteristic of guanidine and its mono- and di-*N*-substituted derivatives. The unknown compound responsible for this yellow spot was present in muscle extracts from the marine crustacea, Moreton Bay lobster (*Thenus orientalis*), king prawn (*Penaeus plebejus*), tiger prawn (*Penaeus esculentus*), and spiny crayfish (*Jasus verreauxi*), but was absent from the corresponding extracts from the fresh-water organisms, Murray River crayfish (*Euastacus elongatus*) and fresh-water crayfish (*Cherax albidus*). This variation in distribution prompted further investigations, and this communication reports evidence for the identification of the compound as homarine[2].

Preliminary experiments with paper chromatograms of crude extracts indicated that the compound strongly absorbed ultra-violet light in the region of 270 mμ and that the formation of yellow colour was due to the alkali and α-naphthol contained in the Barritt reagent[1]. Isolation of the compound was achieved by the following method. 10 kg of freshly excised prawn muscle (from either *P. plebejus*, or *P. esculentus*) was homogenized with 10 l. of methanol in a Waring blender and the mixture allowed to stand, with occasional stirring, for several days at room temperature. The mixture was then filtered, the residue was re-extracted with 10 l. of methanol and the methanolic extracts were combined and concentrated to approximately 500 ml. by vacuum distillation. The copious precipitate which formed during this distillation was removed and discarded. The remaining solution was passed several times through columns of 'Dowex-1' (OH^- form; 500 g) and 'Dowex-50' (H^+ form; 500 g) ion-exchange resins. Progress of the compound was followed by ultra-violet absorption (273 mμ) and paper chromatography of eluate samples. The compound passed unretarded through the anion exchanger, but was retarded on the cation exchanger and appeared in the eluate only after the passage of three bed-volumes of distilled water. This retardation was in contrast to suggestions that homarine is firmly bound to strong cation-exchangers[3,4]. Eluates containing the compound were pooled and concentrated to a viscous oil by vacuum distillation. This oil was dried *in vacuo* over phosphorus pentoxide and recrystallized twice from absolute ethanol to yield 4 g of white hygroscopic crystals. A sample of these crystals was further purified by chromatography on a cellulose column using *n*-butanol, saturated with water, as the mobile phase. Paper-chromatographic examination of the recrystallized product, using five different solvent systems, revealed only one ultra-violet-absorbing spot in each case. No ninhydrin-reacting materials were detected on these chromatograms.

Comparison of the ultra-violet spectrum of the product with published spectra[5] led to tentative identification as homarine. This was confirmed by comparisons of other properties of the isolated compound with those of homarine synthesized by oxidation of α-picoline to α-picolinic acid and methylation of the latter by dimethylsulphate[6,7] (Table 1). The isolated and synthetic compounds also showed identical behaviours on ion-exchange resins and gave the same R_F values after ascending paper chromatography in several solvent systems. Thus, R_F values at 25° C (Whatman No. 1 paper) were 0·30 in *n*-butanol : glacial acetic acid : water (73 : 10 : 17) and 0·75 in *tert.*-butanol : ammonia : water (60 : 30 : 10).

Table 1. Comparisons of Isolated Compound with Homarine

Compound	Ultra-violet spectra			
	λ_{max}, *p*H 7	λ_{min}, *p*H 7	λ_{max}, *p*H 1	ε at λ_{max}, *p*H 7
Isolated compound	274 mμ	241 mμ	271 mμ	6,200
Homarine, refs. 4 and 5	273·5 mμ	240 mμ	270 mμ	6,325

Compound	Elementary analysis (%)*		
	C	H	N
Picrate of isolated compound (m.p. 153°–154° C)	42·7	2·8	15·1
Synthetic homarine picrate (m.p. 155°–156° C)	42·6	2·8	15·3

* Values for synthetic homarine picrate calculated for $C_{13}H_{10}O_9N_4$.

Synthetic homarine, when spotted on filter paper and sprayed with alkaline α-naphthol (equal volumes of 5 N sodium hydroxide and 1 per cent α-naphthol in ethanol), also gave the yellow colour. A number of other related compounds were tested with this reagent and yellow colours were obtained with *N*-methyl pyridinium iodide, pyridine betaine, α-picoline methiodide, and trigonelline (*N*-methyl nicotinic acid). Negative results were obtained with pyridine, pyridine *N*-oxide, nicotinic acid, picolinic acid, pyridoxine hydrochloride, betaine hydrochloride, and choline chloride. From these limited investigations it appears that a pyridinium ring system, containing an alkyl or substituted alkyl radical as the *N*-substituent, is required for this colour reaction.

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