benzoquinone instead of chloranil. This can be explained, considering that: a) picric acid is a well recognized catalyst in dehydrogenation reactions by means of quinones[22]; b) the alcohol causes presumably a weakening of the intramolecular hydrogen bonds holding the bilirubin molecule in a ridge tile-shaped conformation[23] and, as a consequence, a lowering of the activation energy for the conversion of the biladiene molecule into the planar bilatriene skeleton[24]; c) the oxidation potential of chloranil is significantly higher than that of 1,4-benzoquinone[22]. Primary (and secondary) alcohols are not recommended as promoters since a considerable esterification of biliverdin was observed using methanol (or isopropanol) in place of t-butanol.

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Metabolism in Porifera. X. On the intermediary of a formamide moiety in the biosynthesis of isonitrile terpenoids in sponges

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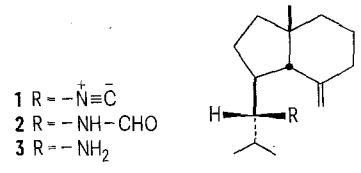
Summary. Feeding the sponge *Axinella cannabina* with labelled axamide-1, the presumptive precursor of axisonitrile-1, resulted in the recovery of nonradioactive isonitrile.

A number of sesquiterpenes and diterpenes carrying isonitrile groups have been recently isolated from sponges[1], often accompanied by the corresponding formamide and isothiocyanate.

The co-occurrence of the isonitrile-formamide pair has been claimed by different authors as strong evidence that a formamide is the biogenetic precursor of the rare isonitrile function[2,3].

We have now investigated the intermediacy of a formamide moiety in the biosynthesis of isonitriles in the sponge *Axinella cannabina,* which contains[4,5] axisonitrile-1 (**1**) as a major isonitrile sesquiterpene, accompanied by trace amounts of the corresponding formamide, axamide-1 (**2**)[3].



1, on reaction with glacial acetic acid, was transformed to **2**, which in turn on alkaline hydrolysis yielded the amine **3**, $C_{15}H_{27}N$ (by accurate mass measurement), $[\alpha]_D^{CHCl}$ 3+65, n_D 1.4919.

The amine **3** (16 mg) was converted into axamide-1 ^{14}C-labelled at the formamide carbon on treatment with ethyl-^{14}C-formate[6] (250 μCi; 1 mCi/mmole) at room temperature for 16 h.

The labelled axamide-1 was purified by preparative TLC and the resulting product (2.5 mg; 620 μCi/mmole) was administered in ethanol (0.2 ml) to the sponge *Axinella cannabina* maintained in well aerated sea water (10 l).

After 5 days, the sponge was collected and the metabolites isolated in the usual way[3,4], after the addition of carrier axamide-1. Up to 15% of the administered radioactivity was recovered in the axamide-1 fraction, while the axisonitrile-1 fraction was found devoid of radioactivity.

A consistent amount of radioactivity (0.1% of administered radioactivity) associated with the free fatty acids fraction, isolated by silica gel column chromatography after conversion into methyl esters (diazomethane), indicates that the administered precursor was taken up and metabolized by the sponge.

The failure by the sponge to transform axamide-1 into axisonitrile-1, under the experimental conditions adopted,

suggests that the formamide cannot act as the precursor of the isonitrile function, although a biosynthesis proceeding at very slow rate could be invoked. Although these findings are not conclusive, it can be suggested that the formamide should be considered as the co-occurring isothiocyanate, a product of further transformation of the isonitrile function.

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Di- and tripeptides containing homoleucine: synthesis and biological assays on insects[1]

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Summary. Some di- and tripeptides containing homoleucine (2-amino-4-methylhexanoic acid) have been synthetized. These compounds have been submitted to biological tests on insects for their hormonal activity in comparison with the known tripeptide pivaloyl-alanyl-p-aminobenzoic acid ethyl ester. Only 2 of them (3 and 4) caused morphological changes on larvae of *Pyrrhocoris apterus*.

In the search for compounds with potential hormonal activity on insects, our attention was attracted by an amino acid[3], 2-amino-4-methylhexanoic acid (homoleucine, **I**, Z=H) whose structure is in some way related to the homoisoprenic unit present in the *Cecropia* juvenile hormone (**V**)[4]. The biosynthetic derivation of this hormone from homomevalonic acid[5], as well as the biogenetic relationships between leucine and mevalonic acid[6], are well documented: therefore it seemed reasonable to us to check whether there could be any interference between this unusual amino acid and the hormonal system of insects. We first studied[7] the stereochemistry of the amino acid by resolving the diastereomeric mixture obtained in the synthesis and assigning the absolute stereochemistry to the natural compound whose definition was rather tentative.

In this paper we now report the synthesis and properties of a number of derivatives of homoleucine, and the results of the experiments performed on some insects. From the work of Slama and coworkers[8], it seemed to us that the best way for administering the amino acid to the insects was after conversion into peptide derivatives of low mol.wt (preliminary experiments showed that the amino acid itself has no action). In particular, Slama found that the most active compounds are tripeptides with an L-alanyl unit as central amino acid and a p-aminobenzoate ester as C-terminal unit. This seems to mimic the structure of juvabione (**VI**)[9].

A strictly related compound, isoleucyl-alanyl-p-aminobenzoic acid ethyl ester, was found to be very active by Zaoral[10] both for its morphogenic effects on insects and for its properties as local anesthetic.

Accordingly, we prepared a few tripeptides with basic structure (**III**). Due to the length of the alkyl chain of homoleucine we thought it logical to test also the dipeptides (**II**) in which the isomerically pure homoleucines are directly linked to the p-aminobenzoate moiety; actually, in these compounds the length of the chain attached to the aromatic ring approaches that of juvabione.

The synthesis of the compounds was done with the dicyclohexylcarbodiimide method[10]. The amino group was protected through the carbobenzyloxy derivative (CBO) and the N-protected amino acid coupled in ethyl acetate with the proper amino counterpart. After filtration of the dicyclohexylurea and evaporation of the solvent, most of the compounds were crystallized directly, except compound 5, which was purified by column chromatography on silicagel. The CBO group was then eliminated by hydrogenolysis in methanol solution using palladium on carbon as catalyst. In table 1 we have reported the physicochemical data for the

Table 1. Physico-chemical data for new compounds*

Compound	Structure	Z	X	Stereochemistry of homoleucine	m.p. or b.p./torr (°C)	$[\alpha]_D$ C=1 in MeOH
1	**I**	CBO		2S, 4S	180/0.4	−11.9°
2	**I**	CBO		2R, 4S	200/1.4	+10.9°
3	**II**	H		2S, 4S	oil	+19.5°
4	**II**	H		2R, 4S	69–71	− 8.9°
5	**II**	CBO		2S, 4S	38–40	−12.6°
6	**II**	CBO		2R, 4S	91–93	+19.7°
7	**III**	H	$COOC_2H_5$	2S, 4S	oil	−46.9°
8	**III**	H	$COOC_2H_5$	2R, 4S	oil	−21.1°
9	**III**	CBO	$COOC_2H_5$	2S, 4S	147–149	−57.3°
10	**III**	CBO	$COOC_2H_5$	2R, 4S	182–184	−29.0°
11	**III**	H	C_2H_5	2S, 4S	109–111	−53.0°
12	**III**	CBO	C_2H_5	2S, 4S	158–159	−56.0°
13	**IV**	CBO	C_2H_5		138–140	−43.3°
14	**IV**	CBO	$COOC_2H_5$		161–163	−49.6°

* Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured with a Perkin Elmer 141 polarimeter. All the compounds gave satisfactory elemental analysis.