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.

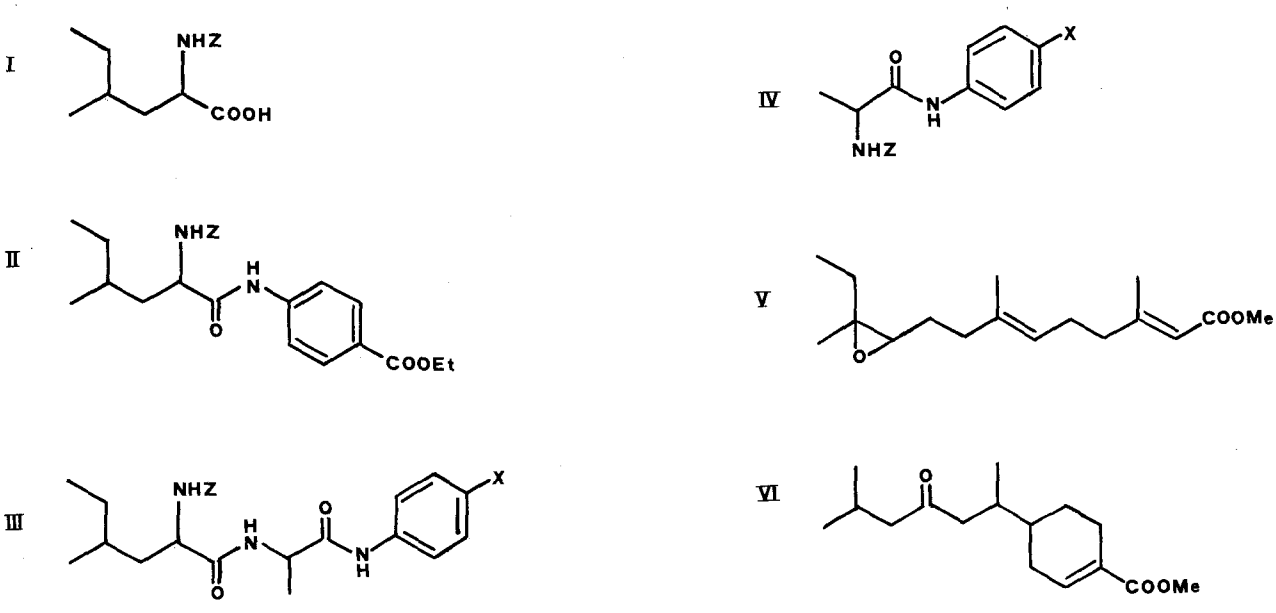
Accordingly, we prepared a few tripeptides with basic structure (**III**). Due to the length of the alkyl chain of homoleucine we thought it logical to test also the dipeptides (**II**) in which the isomerically pure homoleucines are directly linked to the p-aminobenzoate moiety; actually, in these compounds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



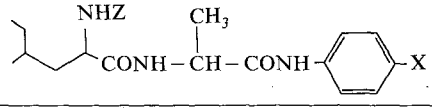

new compounds. In table 2 we have collected the relevant NMR-data of the tripeptides, both free and protected as CBO: the alkyl residue of homoleucine always appears as an unresolved signal, but in all the compounds its integral is consistent with the other signals in the spectrum. To enlarge the spectrum of substances to submit to activity tests, we have also prepared a few peptides where the carboxyl function on the aromatic ring is replaced by an alkyl group or a methylendioxy system; they are obviously prepared by the same route starting from the corresponding anilines. As a reference compound, one of the most active peptides described by Slama[8], namely pivaloyl-alanyl-p-aminobenzoic acid ethyl ester, has been resynthetized.

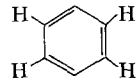
The biological tests were performed on larvae of different species: *Pyrrhocoris apterus, Tenebrio molitor, Pieris brassicae, Spodoptera littoralis, Tribolium confusum, Anagasta kuehniella, Musca domestica, Aedes aegypti.* The compounds were applied topically on the first 4 species, whereas they were fed along with normal food to *T. confusum, A. kuehniella* and *M. domestica* and suspended in water for *A. aegypti.*

Morphological changes were taken as a measure of activity for *P. apterus* and *T. molitor,* whereas for the other species the evaluation of the assay results was simply based on the percentage of individuals undergoing moulting. Most of the compounds did not show any significant activity. Only the 2 dipeptides (3 and 4) were found to inhibit the normal development of *P. apterus* larvae causing formation of superlarvae and adultoids. However, an 80% inhibition occurred when about 20 $\gamma$/insect were applied, that is a large dose if compared with the activity of the standard. The activity was exactly the same for the 2 compounds 3 and 4, thus indicating that the stereochemistry at C-2 of the aminoacid is not relevant.

To verify whether any variation could depend on the configuration at C-4, we synthetized also tripeptides (**III**) and dipeptides (**II**) starting from unresolved mixtures of the 4 isomers of homoleucine (thus including also the 2R,4R and the 2S,4R isomers) but the overall picture in the biological tests did not change. Also inactive were 2 other tripeptides derived from structure **III** by substituting p-ethylaniline with p-isopropylaniline and 3,4-methylen-

Table 2. $^1$H-NMR.-data for tripeptides 7–12 (in $CDCl_3$)




| Compound | Z | C*H*–NHZ | C*H*–$CH_3$ | CH–*C*$H_3$ |  | X |
|---|---|---|---|---|---|---|
| 7 | | 3.5 m (1 H) | 4.75 m (1 H) | 1.45 d (3 H) | 7.62 d (2 H)<br>7.95 d (2 H) | 4.32 q (2 H)<br>1.35 t (3 H) |
| 8 | | 3.45 m (1 H) | 4.80 m (1 H) | 1.50 d (3 H) | 7.61 d (2 H)<br>7.90 d (2 H) | 4.30 q (2 H)<br>1.32 t (3 H) |
| 9 | 7.32 s (5 H)<br>5.12 s (2 H) | 4.0–4.8 m (2 H) | | 1.37 d (3 H) | 7.65 d (2 H)<br>8.02 d (2 H) | 4.35 q (2 H)<br>1.33 t (3 H) |
| 10 | 7.20 s (5 H)<br>5.10 s (2 H) | 4.0–4.8 m (2 H) | | 1.35 d (3 H) | 7.57 d (2 H)<br>7.88 d (2 H) | 4.32 q (2 H)<br>1.32 t (3 H) |
| 11 | | 3.40 m (1 H) | 4.70 m (1 H) | 1.43 d (3 H) | 7.00 d (2 H)<br>7.42 d (2 H) | 2.55 q (2 H)<br>1.15 t (3 H) |
| 12 | 7.25 s (5 H)<br>5.05 s (2 H) | 4.1–4.9 m (2 H) | | 1.37 d (3 H) | 7.02 d (2 H)<br>7.45 d (2 H) | 2.56 q (2 H)<br>1.15 t (3 H) |

dioxyaniline; their physicochemical data are not reported since they were prepared only from diastereomeric mixtures of homoleucine and not from pure isomers.

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## Flavonoids from pollens and stigmas of male and female flowers of four species of the genus *Cucurbita*

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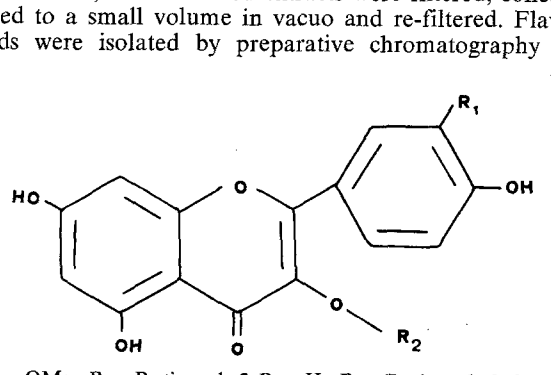
*Summary.* Differences have been found between the flavonoid patterns of pollens and corresponding stigmas of *Cucurbita pepo, C. maxima, C. moschata* and *C. ficifolia*. The major flavonoids have been identified as isorhamnetin-3-O-rutinoside (**1**), kaempferol-3-O-rutinoside (**2**), kaempferol-3-O-robinobioside (**3**) and rutin (**4**). A flavonol glycoside previously isolated from stigmas of *C. pepo* is absent in this material.

There is no evidence that the flavonoids of pollens and stigmas are connected with sex expression of plants. Hartshorne[2] examined flavonoids from both male and female flowers of several plants and could find no relationship between anthocyanidin type and sex. Barber[3] reported, however, that the anthers and stigmas respectively of male and female flowers of *Cucurbita pepo* contain different glycosides of different quercetin methyl ethers.

In view of the great interest in biological function and physiological properties of flavonoid compounds, in the present work the flavonoids of pollens and stigmas of *Cucurbita maxima, C. moschata* and *C. ficifolia* have been studied and the flavonoids of pollen and stigmas of *C. pepo* have been re-examined.

*Material and methods.* For paper chromatography and TLC the solvent mixtures used were: A, 1-butanol-acetic acid-water (4:1:5, upper phase); B, 1-butanol-ethanol-water (4:1:2,2); C, acetic acid-water (5:95); D, acetic acid-conc. HCl-water (30:3:10); E, phenol saturated with water F, 1-butanol-pyridine-water (6:4:3); G, ethyl acetate-butanone-formic acid-water (5:3:1:1); H, 1-butanol-acetic acid-ethyl ether-water (9:6:3:1:1); I, chloroform-ethyl acetate (1:1); L, chloroform-acetic acid (9:1).

Fresh flowers of *Cucurbita pepo, C. maxima, C. moschata* and *C. ficifolia* were collected in Catania. Pollens and homogenized stigmas were extracted 3 times with boiling 95% ethanol; the combined extracts were filtered, concentrated to a small volume in vacuo and re-filtered. Flavonoids were isolated by preparative chromatography on Whatmann 3MM paper in solvent A. Bands were cut off, eluted with 70% ethanol, concentrated and rechromatographed in solvents C and B. When complete separation was not achieved, further purification was obtained by preparative $SiO_2$ TLC in solvent G.




**1** $R_1$ = OMe; $R_2$ = Rutinosyl, **2** $R_1$ = H; $R_2$ = Rutinosyl, **3** $R_1$ = H; $R_2$ = Robinobiosyl, **4** $R_1$ = OH; $R_2$ = Rutinosyl.

Flavonoids were identified by UV-spectral analysis with usual shift reagents[4], total acid hydrolysis with 2 N HCl (1 h at 100 °C), controlled acid hydrolysis with 10% acetic acid (3.5 h under reflux), methylation ($Me_2SO_4$-$K_2CO_3$-$Me_2CO$) followed by acid hydrolysis and $R_f$ data; identifications of **2** and **4** were confirmed by paper co-chromatography with authentic samples (solvents A, B, C, E). Aglycones obtained by total acid hydrolysis of **1**, **2** and **4** were identified respectively as isorhamnetin, kaempferol and quercetin by UV-spectral analysis with shift reagents[4], paper co-chromatography with authentic samples (solvents A, B, D and E) and $SiO_2$ TLC (solvent L); the sugars obtained by total acid hydrolysis of **1**, **2** and **4** were identified as glucose and rhamnose by paper co-chromatography (solvents A and F), $SiO_2$ TLC (solvent H) and GLC of their TMS ethers[5]. Controlled acid hydrolysis of **1**, **2** and **4** gave rutinose, glucose and rhamnose identified as above. Methylation followed by acid hydrolysis of **1** and **4** gave 5,7,3′,4′-tetra-O-methylquercetin, 2,3,4-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose; 5,7,4′-tri-O-methylkaempferol and the above methylated sugars were obtained from **2**. The partially methylated aglycones were identified by UV-spectral analysis[4], MS and paper co-chromatography with authentic samples (solvents A and C); 2,3,4-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose were identified by paper chromatography according to Petek[6] and $SiO_2$ TLC (solvent I)[7]. 3 minor flavonoids (**5**–**7**) were isolated from pollens of *C. maxima* and *C. ficifolia* but were not present in sufficient amount for analysis; UV-spectra, $R_f$ data and colours (dark to yellow in UV + $NH_3$) suggest that they may be flavonol-3-O-monoglycosides.

*Results and discussion.* The major flavonoids (table) have been identified as isorhamnetin-3-O-rutinoside (**1**), kaempferol-3-O-rutinoside (**2**), kaempferol-3-O-robinobioside (**3**) and rutin (**4**); the minor flavonoids (**5**–**7**) may be flavonol-3-O-monoglycosides. Re-examination of pollen and stigmas of *C. pepo* confirms the report of Barber[3] that flavonoids of these materials are different. Barber reported[3],