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Potential juvenile hormone analogues containing piperidine ring: Synthesis and biological assays on insects[1,2]

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Summary. Some compounds containing piperidine rings have been synthesized and submitted to biological tests on insects for their hormonal activity on pupae of *Tenebrio molitor* L. and *Bombyx mori* L. Some of them display a hormone-analogue or toxic activity.

Following the discovery[3] that the abdomen of the adult male of *Hyalophora cecropia* is a rich source of juvenile hormone (JH) and the determination of the structure of its major component[4], many hundreds of synthetic mimics have been prepared[5], most of them containing in their structure a branched carbon chain characteristic of acyclic isoprenoids. In spite of the number of juvenoids synthesized up to now, it is of continuing interest to have new, active substances at our disposal and to characterize the structure-activity relationship better. Going on with our work on the synthesis and biological screening of compounds related to juvenile hormones, like **1** (JH III)[6], we describe in this paper the synthesis of new amides of 4-substituted piperidines with acyclic isoprenoidic acids and the results of their bioassays.

The compounds synthesized may be formally derived from the structures of farnesic or geranic acid in which the 1st isoprenic unity is replaced by a piperidine ring; the amide bond avoids the too-high polarity of an amino group which, as is known[5], could decrease or abolish the biological activity.

In a 1st attempt, the synthesis of compounds **6(a–g)** was carried out by coupling commercially available 4-piperidone hydrate hydrochloride **2** with the suitable carboxylic acid via diphenylphosphinic acid mixed anhydride; this method was described by us[7] as the most convenient for amide bond formation between amines and α,β-unsaturated carboxylic acids. Since 4-piperidone is present in the reaction mixture in a stable hydratated form the yields of the reaction in this case were lower than usual, even using an excess of the reagent. Therefore the synthesis was achieved utilizing 4-piperidone ethylene ketale **3** as the substrate, in chloroform solution: compounds **5(a–g)** were obtained in very high yields (more than 90%) and the hydrolysis to the compounds **6(a–g)** was performed according to conventional methods or, better, by treatment with acidic wet silica gel in methylene chloride solution[8].

Moreover, considering that an α,β-unsaturated alkoxycarbonyl group is present in the structure of many JH mimics. compounds of the general formula **7** were synthesized by a modified Wittig reaction[9] of ketone **6** with trimethylphosphonoacetate.

All the acids utilized for the synthesis of the compounds of general formulas **5**, **6** and **7** were prepared by standard procedures. The mixture of 2E and 2Z geranic acids **4a** and **4b** was obtained by Ag_2O oxidation[10] of commercially available citral in a ratio 2E/2Z 6/4 and used for the subsequent reaction. The 2 isomers, corresponding to **5a** and **5b**, were separated by silica gel chromatography.

Hydrogenation of citral on $Pd/CaCO_3$ (5%)[11] followed by Ag_2O oxidation of the resulting 2,3-dihydrocitral gave 2,3-dihydrogeranic acid **4c** with a 90% overall yield. Commercially available tiglic acid and dimethylacrylic acid were used for the synthesis of the compounds **5f, 6f, 7f** and **5g, 6g, 7g** respectively. The synthesis of the compound **6d** was achieved by m-Cl perbenzoic acid epoxidation of **6c**; compound **6e** was easily obtained by oxymercuriation of **5c** followed by reduction with $NaBH_4$ in methanolic solution[12] and then by hydrolysis of the ketale protecting group.

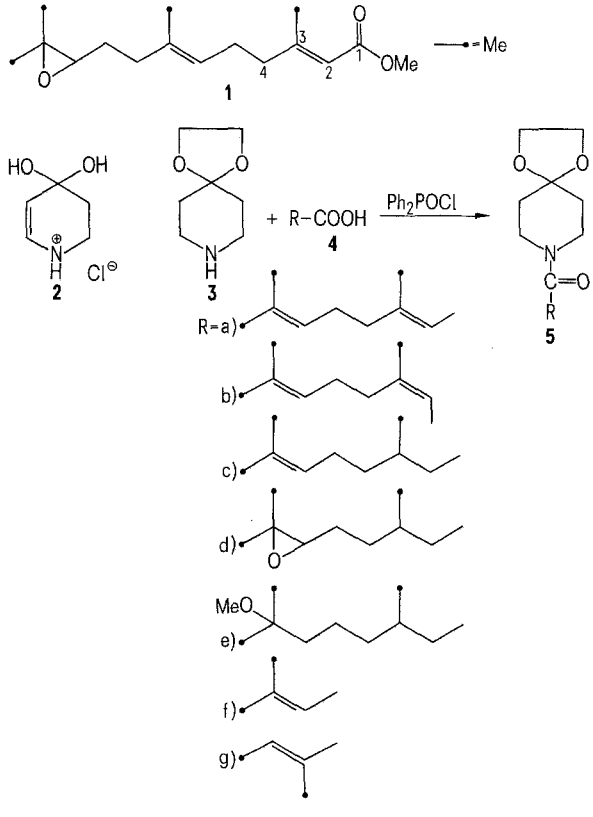
The physicochemical data for the new compounds are reported in the table.

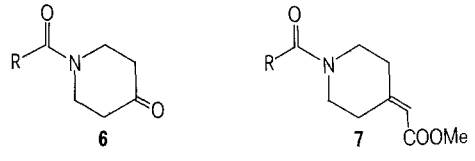
Bioassays. The compounds were assayed on the species and by the methods described below. *Tenebrio molitor L.* (Coleoptera, Tenebrionidae)-administration by contact to

Bombyx mori adults with malformations of wings, legs and abdomen, which emerged from pupae treated with **5g**.

Physico-chemical data for new compounds

Compound	m.p. or b.p./torr (°C)	IR: ν_{max} = cm^{-1}	^{1}H-NMR ($CDCl_3$/TMS) δ (ppm)
5a	180/0.2	1615 (film)	5.80 (m, 1 H, C=CH–CO), 5.11 (m, 1 H, C=CH), 4.00 (s, 4 H, OCH_2CH_2O), 3.62 (m, 4 H, CH_2NCH_2), 1.85 (bs, 3 H, CH_3C=CH), 1.70 (bs, 3 H, CH_3C=CH), 1.60 (bs, 3 H, CH_3C=CH)
5b	180/0.2	1625 (film)	5.80 (m, 1H, C=CHCO), 5.11 (m, 1H, C=CH), 4.00 (s, 4 H, OCH_2CH_2O), 3.65 (m, 4 H, CH_2NCH_2), 1.86 (bs, 3 H, CH_3C=CH), 1.70 (bs, 3 H, CH_3C=CH), 1.62 (bs, 3 H, CH_3C=CH)
5c	200/0.4	1635 (film)	5.12 (m, 1H, C=CH), 4.00 (s, 4 H, OCH_2CH_2O), 3.62 (m, 4 H, CH_2NCH_2), 1.70 (bs, 3 H, CH_3C=CH), 1.60 (bs, 3 H, CH_3C=CH), 0.95 (d, 3 H, J = 6 Hz, CH_3CH)
5d	oil	1620 (film)	4.00 (s, 4 H, OCH_2CH_2O), 3.55 (m, 4 H, CH_2NCH_2), 1.30 and 1.25 (2 s, 3 H each, $(CH_3)_2C$–O–C), 0.97 (d, 3 H, J = 6 Hz, CH_3CH)
5e	220/0.7	1640 (film)	4.00 (s, 4 H, OCH_2CH_2O), 3.62 (m, 4 H, CH_2NCH_2), 3.20 (s, 3 H, OCH_3), 1.12 (s, 6 H, $(CH_3)_2C$–O), 0.95 (d, 3 H, J = 6 Hz, CH_3CH)
5f	50–52 after distillation at 165/0.2	1615 ($CHCl_3$)	5.70 (m, 1H, C=CH), 3.90 (s, 4 H, OCH_2CH_2O), 3.65 (m, 4 H, CH_2NCH_2), 1.70 (bs, 6 H, $(CH_3)_2C$=C)
5g	170/0.2	1610 ($CHCl_3$)	5.50 (m, 1H, C=CH), 3.90 (s, 4 H, OCH_2CH_2O), 3.51 (m, 4 H, CH_2NCH_2), 1.70 (m, 3 H, CH_3CH=C), 1.60 (bs, 3 H, CH=C–CH_3)
6a,b	180/0.2	1715, 1620 (film)	5.90 (m, 1H, C=CH–CO), 5.11 (m, 1H, C=CH), 3.85 (t, 4 H, J = 6.5 Hz, CH_2NCH_2), 2.42 (t, 4 H, J = 6.5 Hz, CH_2COCH_2), 1.91 (bs, 3 H, CH_3C=CH), 1.68 (bs, 3 H, CH_3C=CH), 1.60 (bs, 3 H, CH_3C=CH)
6c	170/0.3	1715, 1640 (film)	5.00 (m, 1H, C=CH), 3.70 (t, 4 H, J = 7 Hz, CH_2NCH_2), 2.30 (t, 4 H, J = 7 Hz, CH_2COCH_2), 1.62 (bs, 3 H, CH_3C=CH), 1.55 (bs, 3 H, CH_3C=CH), 0.90 (d, 3 H, J = 6 Hz, CH_3CH)
6d	200/0.2	1715, 1635 (film)	3.90 (m, 4 H, CH_2NCH_2), 2.45 (t, 4 H, J = 6 Hz, CH_2COCH_2), 1.28 and 1.25 (2 s, 3 H each, $(CH_3)_2C$–O–C), 0.95 (d, 3 H, J = 6 Hz, CH_3CH)
6e	180/0.2	1715, 1635 (film)	3.76 (m, 4 H, CH_2NCH_2), 3.20 (s, 3 H, CH_3O), 2.45 (t, 4 H, J = 6 Hz, CH_2COCH_2), 1.12 (s, 6 H, $(CH_3)_2C$–O), 0.97 (d, 3 H, J = 6 Hz, CH_3CH)
6f	47–48 after distillation at 135/0.5	1715, 1615 (nujol)	5.80 (m, 1H, C=CH), 3.70 (t, 4 H, J = 6 Hz, CH_2NCH_2), 2.45 (t, 4 H, J = 6 Hz, CH_2COCH_2), 1.95 (bs, 3 H, CH_3C=CH), 1.87 (bs, 3 H, CH_3C=CH)
6g	140/0.5	1715, 1620 (film)	5.68 (m, 1H, C=CH), 3.80 (t, 4 H, J = 6 Hz, CH_2NCH_2), 2.41 (t, 4 H, J = 6 Hz, CH_2COCH_2), 1.90–1.60 (m, 6 H, CH_3CH=CCH_3)
7a,b	200/0.3	1710, 1625 (film)	5.80 (m, 2 H, C=CHCON and C=CHCOO), 5.15 (m, 1H, C=CH), 3.70 (s, 3 H, CH_3OCO), 1.90 (bs, 3 H, CH_3C=CH), 1.70 and 1.61 (2 bs, 6 H, $(CH_3)_2C$=C)
7f	34–37	1715, 1630 (film)	5.70 (m, 1H, C=CH), 5.60 (bs, 1H, C=CHCOO), 3.60 (s, 3 H, CH_3OCO), 1.90 (bs, 3 H, CH_3C=CH), 1.80 (bs, 3 H, CH_3C=CH)
7g	51–52	1710, 1625 (film)	5.70 (m, 1H, C=CHCOO), 5.65 (m, 1H, C=CH), 3.68 (s, 3 H, CH_3OCO), 1.90–1.60 (m, 6 H, CH_3CH=CCH_3)





1–20 h pupae of 200 µg of active substance per individual (2 samples of 25 individuals).

Bombyx mori L. (Lepidoptera, Bombicidae)-administration by contact to eggs (samples of (100) of 200 µg of active substance; administration by ingestion to larvae in the 4th instar of 100 µg of active substance per individual on discs of mulberry leaves *(Morus alba)* of 7 cm^2; administration by contact to larvae in the 5th instar of 200 µg per individual; administration by contact to 1–3 h pupae of 400 µg per individual. Assays on larvae and pupae were performed on 2 groups of 10 individuals each. All the substances (in acetone solution) were first tested on *T. molitor;* those showing even a weak activity on this species were subsequently administered to the afore mentioned instars of *B. mori.* For each assay, control samples were grown which evolved normally in all the cases.

The results reported relate only to the assays in which the compounds tested manifested a hormone-analogue or toxic activity.

Group 5 compounds: the formulations **5a** and **5b** had similar effects; the juvenoid action on *T. molitor* was 20% and 10% respectively; hatching inhibition of *B. mori* eggs

was 15% and 25%; both formulations showed a repellent activity on the lepidoptera larvae treated by ingestion, whereas only **5b** caused mortality, in 10% of individuals in the contact tests. Compounds **5c** and **5d** were active only on *T. mori* (30% and 20%). The most active were **5f** and **5g**; both were active on 20% of the individuals treated; hatching inhibition of *B. mori* eggs was 13% and 15% respectively; **5g** caused mortality in 20% of the contact-treated larvae of this species. It was very interesting to observe the activity on pupae where 30% and 40% respectively of treated emerged adults presented malformations (figure). Furthermore, **5g** inhibited emergence in 20% of pupae.

Group 6 compounds: the formulation **6a** had caused slight juvenoid effects on *T. molitor* in 20% of the population treated; subsequently tested on *B. mori* this formulation inhibited hatching in 10% of the eggs and had a repellent effect on the ingestion-treated larvae. Compounds **6f** and **6g** were both active on *T. molitor* in 10% of the cases and on *B. mori* eggs in 13% and 10% of the cases, respectively; furthermore the former induced malformations in adult lepidoptera emerged from 10% of treated pupae whilst the latter caused mortality in 10% of the contact treated larvae.

Group 7 compounds: slight malformations in *T. molitor* were induced by **7a** and **7g** (10%) and **7f** (15%). Tests on *T. molitor* for inhibition of emergence showed that **7g** (14%) and particularly subjected to contact tests malformations in 20% of the adults and emergence inhibition in 20% of the eggs.

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Effects of scavengers of superoxide radicals, hydrogen peroxide, singlet oxygen and hydroxyl radicals on malondialdehyde generation from arachidonic acid by bovine seminal vesicle microsomes

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Summary. Superoxide dismutase, catalase and sodium formate did not inhibit the formation of malondialdehyde (MDA) from arachidonic acid, suggesting that $O_2^{\cdot}$, H_2O_2 and $OH^{\cdot}$ are not involved in the enzymatical oxidation of arachidonic acid. Sodium azide was found to be an inhibitor of MDA production.

Since the observation by Babior et al.[1] that phagocytosing polymorphonuclear leukocytes, effector cells of the acute inflammatory response, release large amounts of superoxide anions ($O_2^{\cdot}$) into the medium in which the activated cells are suspended, evidence has been found for the possible involvement of oxygen species as mediators of inflammation. In 1971 Vane[2] proposed that the anti-inflammatory action of aspirin-like drugs is based on their ability to inhibit prostaglandin (PG) biosynthesis. After that, many authors reported findings supporting the idea that mediation of inflammation is correlated with lipid peroxidation[3,4], in general, and with oxygenation of arachidonic acid[2,5-9] in particular. In addition, anti-oxidants have been demonstrated to inhibit lipoxidase[10] and PG synthetase[10], and to potentiate the anti-inflammatory action of indometacin[11]. The following question has become a question of considerable interest; what oxygen species is (are) implicated in peroxidation of polyunsaturated lipids and in oxygenation of arachidonic acid[5,6,12,13]?

Linolenate was found to be peroxidized by xanthine oxidase acting aerobically upon acetaldehyde[12]. Superoxide dismutase (SOD) as well as catalase inhibited this lipid peroxidation, indicating that both $O_2^{\cdot}$ and hydrogen peroxide (H_2O_2) were essential intermediates. Scavengers of singlet oxygen ($^1O_2$) also inhibited the peroxidation of linolenate, whereas agents known to scavenge hydroxyl radicals ($OH^{\cdot}$) did not. Therefore, $^1O_2$ was proposed to be responsible for linolenate peroxidation.

The oxidation of arachidonic acid has been considered to require H_2O_2 by some authors[5] and $O_2^{\cdot}$ by others[6].

This paper deals with the effect of SOD, catalase, sodium formate and sodium azide on the in vitro production of malondialdehyde by PG synthetase from bovine seminal vesicles, in order to discover which oxygen species is involved in the peroxidation of arachidonic acid.

Materials and methods. Chemicals. Superoxide dismutase (2800 units/mg protein, from bovine blood) and catalase (3600 units/mg protein, from beef liver) were purchased from Sigma Chemical Co., St. Louis, USA. Methylene blue was obtained from E. Merck, Darmstadt, G.F.R. All other chemicals and organic solvents were of analytical grade and purchased from J.T. Baker Chemicals B.V., The Netherlands.

Preparation of prostaglandin synthetase. PG synthetase was prepared from bovine seminal vesicles essentially according to the method of Takeguchi et al.[14]. The precipitated microsomes were suspended (not lyophilized) in 50 mM Tris-HCl buffer (pH = 8.3) and stored at −20 °C in small containers. The yield of microsomal protein from 1414 g of vesicles was 20.8 g, as determined by the method of Lowry et al.[15].

Inhibition of malondialdehyde formation. The convenience