Original Article

Effects of Inhibitors on Juvenile Hormone III Biosynthesis in Corpora Allata of the Cockroach *Periplaneta americana* L., *in Vitro*

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The phenyl glycidyl ether 1-(2,3-epoxypropoxy)-3-prop-2-ynyloxybenzene, 6,7-methylenedioxy-2,2-dimethylchromen, naphth[1,2-d]-1,3-oxathiole and 8-methoxynaphth [1,2-d]-1,3-oxathiole, all mixed function oxidase inhibitors, were better acute inhibitors of juvenile hormone III-biosynthesis than precocene II in the corpora allata of *P. americana* *in vitro*. Some of these compounds apparently have an inhibitory action at biosynthetic step(s) prior to the epoxidation of methyl farnesoate, apart from any action they may have on methyl farnesoate epoxidase.

INTRODUCTION

In the search for chemicals with anti-juvenile hormone activity, groups of compounds that have been examined for their ability to inhibit juvenile hormone (JH) biosynthesis in insects include mevalonate analogues,¹⁻³⁾ 1,3-benzodioxole derivatives,^{4,5)} precocene II⁶⁾ and the fungal metabolite compactin.^{7,8)} As part of a larger study of JH-biosynthesis and its inhibition^{9,10)} we have employed the bioassay of Pratt and Finney,⁹⁾ which utilises corpora allata (CA) of *P. americana* cultured *in vitro*, to examine the inhibition of the terminal steps of methyl (2E, 6E)-10 R-3,7,11-trimethyl-10,11-epoxy-dodecadienoate (JH III) biosynthesis (*i.e.* esterification of farnesoic acid, FA, then conversion of the resulting methyl farnesoate, MF, into its 10,11-epoxide, JH III) by non-terpenoid molecules similar in size to precocene II.

MATERIALS AND METHODS

1. Compounds

6,7-Dimethoxy-2,2-dimethylchromen (**I**; precocene II) (Aldrich Chemical Co.) and *trans*-1,2,

4-trimethoxy-5-propenylbenzene (**V**; α -asarone, Sigma London) were used as received and 2-(4-chlorophenoxy)-2-methylpropionic acid (**VII**; clofibric acid, Sigma London) was converted into its methyl ester¹¹⁾ with diazomethane. Methyl 2-fluorophenoxyacetic acid (**VI**) was the gift of Dr. A. N. Clements (Shell Research Ltd.). 3,4-Dihydroprecocene II (**II**),¹²⁾ mp 55-57°C, 222 (M⁺) (Varian CH5 Mass Spectrometer; direct insertion at 70 eV), NMR (Jeol FX90Q, CDCl₃, TMS); δ 1.31 (s, 6 H; 2,2-dimethyl), 1.76 and 2.69 (t, 6.6 Hz, 2 H; t, 6.6 Hz, 2 H; CH₂CH₂), 3.80 (s, 6 H); 6.37 (s, 1 H) and 6.55 (s, 1H), aromatic H; 6,7-methylenedioxy-2,2-dimethylchromen (**III**),¹³⁾ mp 54.5-55.5°C; 1-(2,3-epoxypropoxy)-3-prop-2-ynyloxybenzene (**VIII**)¹⁴⁾; naphth[1,2-d]-1,3-oxathiole (**IX**) and 8-methoxynaphth[1,2-d]-1,3-oxathiole(**X**),¹⁵⁾ were all prepared as previously described. 2,2-Dimethyl-5-hydroxy-1,3-benzodioxole, prepared from 1,2,4-trihydroxybenzene by the method of Gates and Gillon,¹⁶⁾ was converted via its sodium salt (NaH), heated in toluene with 3-chloro-3-methyl-1-butyne, into the corresponding 1,1-dimethyl-2-propynyl ether. This cyclised *in situ* to give 6,7-iso-

I, $R^6=R^7=CH_3O$

II, 3,4-Dihydro-I

III, $R^6=R^7=-OCH_2O-$ IV, $R^6=R^7=-OC(CH_3)_2O-$ V, $R^1=R^3=R^4=CH_3O$, $R^2=trans-propenyl$ VI, $R^1=OCH_2COOCH_3$, $R^2=F$, $R^3=R^4=H$ VII, $R^1=OC(CH_3)_2COOCH_3$, $R^2=R^4=H$, $R^3=Cl$ VIII, $R^1=OCH_2C\equiv CH$, $R^2=R^3=H$, $R^4=OCH_2CH_2OCH_3$ IX, $R=H$ X, $R=CH_3O$

propylidenedioxy-2,2-dimethylchromen (**IV**), mp 58–60°C, 232 (M^+), NMR: δ 1.39 (s, 6 H; 2,2-dimethyl), 1.65 (s, 6 H; 6,7-isopropylidenedioxy), 5.37 and 6.12 (d, 10 Hz, 1 H; d, 10 Hz, 1 H; 3,4-CH=CH); 6.23 (s, 1 H), 6.31 (s, 1 H), aromatic H, after purification by TLC on silica gel plates (E, Merck HF254; 0.75 mm) developed with hexane and ethyl acetate (9+1 by volume).

2. In Vitro Bioassay

The spontaneous biosynthesis of JH III from [methyl- ^{14}C] methionine¹⁷⁾ in surgically isolated pairs of cockroach corpora allata¹⁸⁾ was compared in the presence and absence of several appropriate inhibitor concentrations using the method of Pratt and Finney,⁹⁾ which incorporates inhibitors into the culture medium without the inclusion of organic solvents. Briefly female corpora allata were bilaterally randomised in groups of four per 0.1 ml of culture medium (TC-199; Gibco-Biocult Ltd), into pairs of control and experimental tubes, duplicated for each inhibitor concentration. Tubes were incubated for 30 min at 30°C, with or without an appropriate concentration of chemical inhibitor, prior to a 3 hr radiolabelling incubation in fresh medium (0.1 ml) containing ^{14}C -methionine (0.3 mM) with or without the inhibitor. In some experiments, 2-[3H] farnesoic acid (2.5×10^{-5} M) was also added to examine any effect of inhibitors on the farnesoic acid *O*-methyl transferase (OMT; EC 2.1.1.-) step. Previously described procedures of solvent extraction, silica TLC separation in the presence of authentic non-radioactive products, and radiometric assay were used to measure the control and inhibited biosynthesis of both JH III and its precursor, methyl farnesoate.¹⁷⁾

Effective inhibitory concentrations (EC_{50} s) were obtained from eye-fitted plots of percent inhibition versus four appropriate inhibitor concentrations in the range 10^{-7} to 10^{-3} M.

RESULTS AND DISCUSSION

The method measures the amounts of epoxide (JH III) and MF present, and the total ester (MF+JH III) biosynthesised under the chosen experimental protocol. Figure 1 compares the inhibitory effects of precocene II (**I**) with those of the other compounds. Previous work⁶⁾ showed that **I** inhibited spontaneous JH-biosynthesis in the CA of adult female *P. americana* only at relatively high concentrations. Furthermore, the inhibition profiles for JH III formation and total ester production were quantitatively indistinguishable and there was no accumulation of MF. A similar result was obtained in the presence of excess exogenous farnesoic acid (FA); the rate of esterification of 3H -farnesoic acid was as sensitive to inhibition as was the subsequent epoxidation to JH III, although the system overall was about 2-fold less sensitive to **I** when FA was present (Fig. 1). Thus **I** at these concentrations *in vitro* appeared to inhibit farnesoic acid *O*-methyl transferase, thereby restricting the supply of MF for the final enzymic epoxidation. Reduction of the 3,4-double bond in **I** gave **II**, with similar inhibitory characteristics, although the double bond is required for anti-juvenile hormone activity *in vivo*.¹⁹⁾

In contrast to **I** and **II**, the 1,3-benzodioxole-analogue of **I** (**III**; Fig. 1), was a good inhibitor of the epoxidase which only affected total ester formation at high concentrations, over a range similar to that shown by **I**, when assayed in the presence of exogenous FA. Dimethyl substitution of the 1,3-dioxole group gave **IV**, and eliminated the epoxidase inhibitory activity, as expected. **IV** then became a precocene-like molecule which again apparently inhibited JH biosynthesis at or before the OMT step (Fig. 1). α -Asarone (**V**) gave 17% inhibition of JH III biosynthesis at 10^{-4} M and methyl 2-fluorophenoxy acetate (**VI**) had no effect at 10^{-3} M, whereas methyl clofibrate (**VII**), with the 2,2-dimethyl-substituted ether moiety found in the precocenes, was a moderately effective inhibitor. **VII** and its analogues were

Fig. 1 Effective concentrations (EC_{50} s) for inhibition of spontaneous (S) or farnesoic acid stimulated (FAS) juvenile hormone III biosynthesis (solid bar) and total ester (open bar; methyl farnesoate plus JH III) biosynthesis *in vitro*. Details in text.

Fig. 2 *In vitro* inhibition of spontaneous JH III biosynthesis (open symbols) and total ester biosynthesis (solid symbols) by oxathiole IX, showing the congruence of inhibitory profiles for the two processes. Each point represents one experiment with four randomised pairs of corpora allata.

previously shown to have toxic and non-specific developmental effects on *Oncopeltus fasciatus*, but no obvious anti-juvenile hormone activity.²⁰⁾

The anti-JH action of the precocenes *in vivo* apparently results from a cytotoxic action which eventually destroys the ability of the CA to synthesise JH.²¹⁾ With the possible exception of fluoromevalonate,²⁾ the ideal of anti-JH activity effected by the straightforward inhibition of specific biosynthetic enzymes by non-juvenoid compounds has not been attained, although it should be possible in principle. In this connection the *o*- and *p*-isomers of the propynyl ether VIII¹⁴⁾ were found to be better inhibitors of JH-biosynthesis *in vitro* than I.⁹⁾ Although these compounds are putative epoxidase inhibitors, their activity profiles evidently resemble that of I (e.g. see VIII in Fig. 1) indicating a preferential action at the level of OMT, or earlier in the biosynthetic pathway. We do not know if, like I, they inhibit OMT in the presence of exogenous farnesoic acid. The oxathioles IX and X are potent mixed function oxidase inhibitors,¹⁵⁾ which, by analogy with III and in contrast to I, should inhibit MF-epoxidase. However, they also were found to inhibit

spontaneous total ester formation as efficiently as they inhibited JH III biosynthesis (Figs. 1 and 2) and apparently act preferentially at a biosynthetic step(s) prior to the epoxidation which remains to be elucidated. These oxathioles may combine the requisite enzyme inhibitory properties with appropriate physical properties for action *in vivo*, since both exhibit precocene-like anti-JH action, with X more effective, on the milkweed bug, *Oncopeltus fasciatus* (Brooks and Mace, unpublished results).

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要 約

ワモンゴキブリ, *Periplaneta americana* L., アラタ体における *in vitro* での幼若ホルモン III 生合成に及ぼす阻害剤の影響

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ワモンゴキブリ, *Periplaneta americana* L. のアラタ体を供試して, *in vitro* での juvenile hormone III 生合成に及ぼす各種化合物の影響を検討した結果, 混合機能オキシダーゼ (mfo) の阻害剤である次の4化合物, phenyl glycidyl ether 1-(2,3-epoxypropoxy)-3-prop-2-ynyloxybenzene, 6,7-methylenedioxy-2,2-dimethylchromen, naphth[1,2-d]-1,3-oxathiole, 8-methoxy-naphth[1,2-d]-1,3-oxathiole は precocene II よりも強く JH III の生合成を阻害した。JH III 生合成過程において, これらの化合物は最終過程に関与する methyl farnesoate epoxidase を阻害するものと期待されたが, 必ずしもこの酵素を阻害するものではなく methyl farnesoate のエポキシ化よりも前の生合成過程を阻害するものもあることが明らかとなった。