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ASPECTS OF THE MODE OF ACTION OF PRECOCENES ON MILKWEED BUGS (Oncopeltus fasciatus) AND LOCUSTS (Locusta migratoria)

G.T. Brooks, A.F. Hamnett, R.C. Jennings, A.P. Ottridge and G.E. Pratt

Agricultural Research Council Unit of Invertebrate Chemistry and Physiology, Sussex University, Brighton BN1 9RQ

Summary 2,2-Dimethyl-7-methoxy-2H-1-benzopyran (precocene 1) and 6,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (precocene 2) isolated from Ageratum houstonianum by Bowers, show interesting differences in the magnitude of their biological effects on milkweed bugs (Oncopeltus fasciatus) and Locusts (Locusta migratoria). The structure-activity relationships of several analogues have been compared in the two species and are discussed in relation to the hypothesis that oxidative metabolism of precocenes in the corpora allata of sensitive insects is a lethal synthesis.

INTRODUCTION

Precocious metamorphosis is induced in the immature stages of certain hemiptera by 2,2-dimethyl-7-methoxy-2H-1-benzopyran (precocene 1; P1) and 6,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (precocene 2; P2) isolated from the plant Ageratum houstonianum (Bowers et al, 1976). When tested on Oncopeltus fasciatus as a jar deposit, P1 is less effective than P2 in inducing precocious metamorphosis. Consequently, P2 has been more closely investigated; in addition to its effect on metamorphosis it also produces sterile, but otherwise normal, adults when applied to appropriate immature stages and sterilises newly-moulted female adults (Bowers et al, 1976; Bowers & Martinez-Pardo, 1977; Unnithan et al, 1977; Unnithan & Nair, 1979).

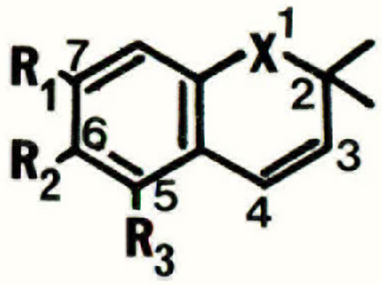
The effects appear to be species specific but recent work shows that both precocenes induce precocious metamorphosis in Locusta (Pener et al, 1978; Němec et al, 1978; Pederson 1978). Large doses of P1 are also effective on Schistocerca gregaria (Chênevert et al, 1978) and P2 has a morphogenetic effect on the pea aphid (Mackauer et al, 1979). P2 causes atrophy of the corpora allata (CA) in at least two species (Bowers & Martinez-Pardo, 1977; Unnithan et al, 1977; Pener et al, 1978; Unnithan & Nair, 1979; Schooneveld, 1979) and juvenile hormone (JH) production is suppressed in what appears to be the first example of chemical allatectomy. This paper reports preliminary studies on the mode of action of precocenes, with emphasis on P1 and its derivatives.

METHODS AND MATERIALS

Chemicals

Precocenes 1 and 2 (p1, P2,1 and 2, Fig.1), the benzodioxole analogue of P2 (MDP; 3, Fig.1), 2,2-dimethyl-7-hydroxy-2H-1-benzopyran (12) and its 2-propynyl- (8), 2-propenyl- (9) and isopropyl- (10) ether analogues were made by the method of Bowers (Bowers et al, 1976; Bowers, 1977a). 2,2-Dimethyl-5-hydroxy-1,3-benzodioxole (Gates

Fig.1. Structures of precocene analogues and derivatives



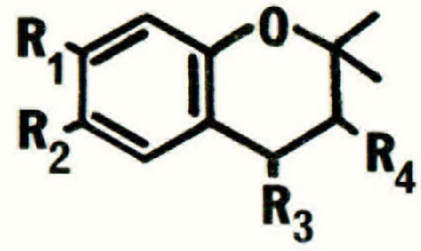
X=O

	R_1	R_2	R_3
1	OMe	H	H (P1)
2	OMe	OMe	H (P2)
3	$-OCH_2O-$		H (MDP)
4	$-OC(Me)_2O-$		H
5	NMe_2	H	H
6	NEt_2	H	H

X=NH

13	OMe	H	H
14	H	H	OMe

	R_1	R_2	R_3
7	H	H	NMe_2
8	$OCH_2C{\equiv}CH$	H	H
9	$OCH_2CH{=}CH_2$	H	H
10	$OCH(Me)_2$	H	H
11	H	OMe	H
12	OH	H	H



	R_1	R_2	R_3	R_4
15	OMe	H	H	H
16	OMe	OMe	H	H
17	$-OCH_2O-$		H	H
18	OMe	H	-O-	
19	OMe	H	OH	Br
20	OMe	OMe	-O-	
21	OMe	H	OH	OH

(cis-, trans-)

Active compounds in the jar deposit test on Oncopeltus were P2, 10, P1, 4 and 8, with relative potencies (++++; 100% precocious metamorphosis at 1µgcm^{-2}), (+++), (++), (++), and (+; inconsistently gave about 5% precocious metamorphosis at 1µgcm^{-2}), respectively.

and Gillon, 1971), 3-dimethylaminophenol and 3-diethylaminophenol were converted via their sodium salts (NaH), heated in toluene with 3-chloro-3-methyl-1-butyne, into the corresponding 1,1-dimethyl-2-propynyl ethers, which cyclised spontaneously to the corresponding 2,2-dimethyl-2H-1-benzopyrans (4), (5) plus (7), and (6), respectively. The isomers (5)(60%) and (7)(40%) were separated by thin layer chromatography (TLC) on silica gel in 60-80°C petroleum ether containing 8% EtOAc.

m-Anisidine, stirred at ambient temperature with 3-chloro-3-methyl-1-butyne (1 equiv.), in ether and triethylamine containing $CuCl_2$ and Cu bronze, gave the corresponding N-1,1-dimethyl-2-propynyl-derivative (Hennion & Hanzel, 1960), which was purified by column chromatography on silica gel. This, on heating at 78°C in dioxan containing one equivalent of $CuCl_2$ (Dillard et al, 1973) gave a mixture (3:2) of the dihydroquinolines (13) and (14) which had m.p. 57-60°C and 117-119°C, respectively, when separated by silica gel TLC with 60-80°C petroleum ether containing 10% EtOAc as solvent.

P1, P2 and MDP (Fig.1) were reduced with polymethylhydrogensiloxane (Aldrich; 1 equiv.) in acidic (HCl) 95% EtOH with 10% Pd/C (Lipowitz & Bowman, 1973), to give the 3,4-dihydro-compounds (15), (16) and (17), respectively. P1 epoxide (18, Fig.1) was prepared from the bromohydrin (19) (Jennings & Ottridge, 1979).

Chemical structures were confirmed by mass spectrometry and NMR.

Insects

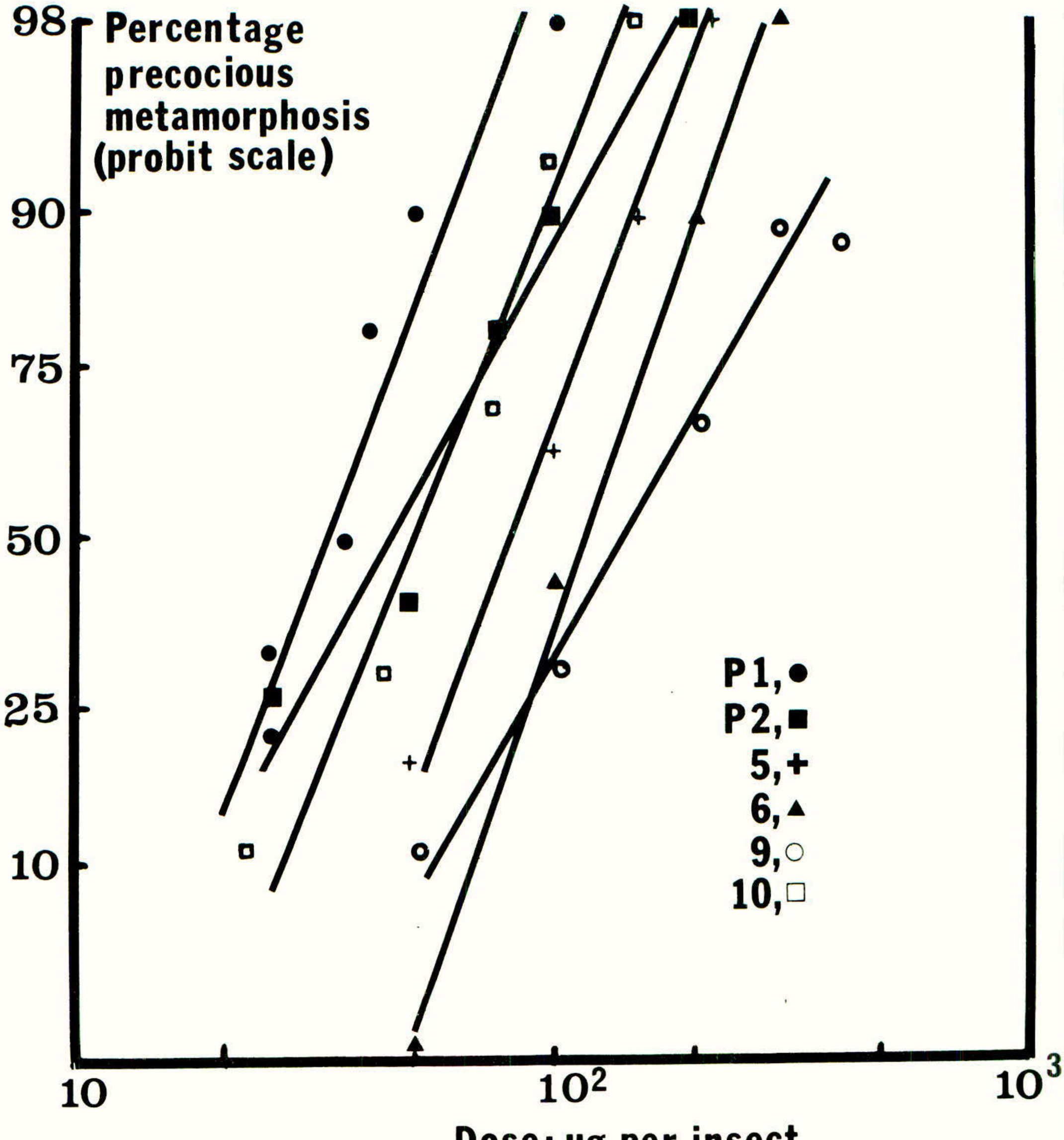
In tests for the induction of precocious metamorphosis, groups of 20 early 2nd instar Oncopeltus were confined (27°C; 16:8 light/dark regime) throughout development in glass jars of surface area 100cm^2 (2 or 3 groups per treatment) containing milkweed seed and water, and surface treated with deposits of the test chemical (10 to 0.125µgcm^{-2}, two-fold serial dilutions). They were compared with groups similarly treated with P1, and P2, or impregnating solvent (ether/acetone;9:1,v/v) only. Other groups were topically treated with the test compounds (5, 2.5 and 1.25µg per insect) in acetone (0.5µl) and similarly confined. Insects that survived exposure to chemicals moulted normally into 3rd or 4th instars, and, if affected, into precocious adults at either the 4th or the 5th instar moult. The total of precocious 4th plus precocious 5th instars as a % of surviving insects was used as a measure of the response.

Early 4th instar Locusta (within 12h of the moult) were topically treated (groups of 20) with acetone (10µl) alone, or containing the test compound (serial doses between 400µg and 25µg, as appropriate) and confined in perforated plastic pots containing wheat shoots and rolled oats in a light/dark (16:8, 40°/25°C)regime . Groups were examined at the next moult for normal 5th instars and precocious adults. The latter are quite distinctive, having the appearance and colour of small normal adults, with unexpanded wings. Dose was plotted on the log scale (Fig.2) against % precocious metamorphosis on the probit scale and the lines fitted by eye to give an estimate of ED50.

RESULTS AND DISCUSSION

The jar test on Oncopeltus (Bowers et al, 1976) is convenient for detecting activity but poses some problems due to the steep slope of the dose-response relationship, the volatility of these compounds during impregnation and the fact that exposure to large amounts of active compounds may result in completely arrested development, so that the insects never moult from the 2nd instar. We found that low activities are not readily detected by this method, so topical application was used as a secondary test, although it appeared to be less effective with P2 on Oncopeltus (W.S. Bowers, personal communication) and on Rhodnius prolixus (G.E. Pratt

Fig.2. Effect of precocene analogues topically applied in acetone to early 4th instar Locusta migratoria; extent of precocious metamorphosis measured at the 5th instar.



unpublished results). Early reports indicated activity in a few closely related compounds and only towards hemiptera (Bowers et al, 1976; Bowers, 1977b). However, P1, although less effective than P2 on Oncopeltus, is somewhat more effective than P2 on Locusta. This, together with an apparent lack of effect on cockroaches and the requirement for very large doses of P1 for effect on the desert locust, Schistocerca gregaria (Chênevert et al, 1978) indicates the need for a close examination of (a) bioassay techniques and (b) other possible reasons for the differing responses to these compounds.

Of the compounds in Fig.1, only P1, P2, (4), (8) and (10) showed activity on Oncopeltus as jar deposits. Activity increased in the order (8)<(9)<P1≃(4)<(10)<P2. Compound (8) was earlier found to be inactive morphogenetically but had sterilant effects (Bowers, 1977b). In the present tests, it caused significant mortality at $2\mu gcm^{-2}$ but a small proportion of the survivors showed precocious metamorphosis. Compound (9) was ineffective as a deposit but 5µg topically applied gave 24% precocious metamorphosis. The isopropyl-ether (10) was more effective than P1 as a deposit (it strongly retarded development at $5\mu gcm^{-2}$) and as effective as P2 by topical application. Generally similar results were found on topical application to Locusta, except that the dimethylamino-(5) and diethylamino-(6) analogues of P1 were also effective, and the approximate order of activity (Fig.2) was (8)<(9)≃(6)<(5)<(10)≃P2<P1. The low agonist activity of compound (8) was confirmed in Locusta and the isopropylenedioxy-analogue (4) of P2 also caused about 5% precocious metamorphosis when topically applied at a dose of 300µg/insect, above which high mortality occurred.

These results suggest that the progressive reduction in electron-donating ability of the 7-substituent in P1 effects a corresponding reduction in activity. Besides being a poorer electron donor than theallyloxy-group (in 9), the propargyloxy-group (in 8) may confer mixed function exidase (MFO) inhibitory properties on the molecule, as found with insecticide synergists that contain this moiety (Wilkinson, 1976). Nevertheless, compound (8) retains weak agonist activity toward both species, in contrast to the benzodioxole (3), which is inactive and antagonises the action of P1 and P2 (Brooks et al, 1979). Slight activity on Locusta and significant activity on Oncopeltus is apparent when the methylene group is substituted, as in compound (4). It is well known that such substitution eliminates synergistic (MFO inhibitory) activity in benzodioxoles, and (4) should behave more like a normal P2 analogue, apart from any steric effects of the methyl groups disposed across the molecular plane. Replacement of the 7-methoxy-group of P2 by 7-isopropoxy- improves activity toward Oncopeltus (Bowers, 1977b). Increased stability toward enzymic O-dealkylation may contribute to this difference, which is also found between (10) and P1 in Oncopeltus.

The destructive effect of P2 on the CA and the metabolic formation of its 3, 4-dihydrodiol (21,R_2=OMe) in some insects (Ohta et al, 1977) suggested (Brooks, 1977) that precocene action might be mediated by attack of an electrophilic moiety such as epoxide (20) on cellular nucleophiles, in analogy with the cytotoxic effects of polycyclic aromatic hydrocarbons in vertebrates. The analogue (5) of P1 was synthesised because the dimethylamino- group is a good electron donor and a prominent feature of dyes that show strong charge separation on oxidation. The apparent lack of activity of (5) and (6) on Oncopeltus is unexplained, whereas the inactivity of the 5-substituted derivatives, (7) and (14), and the 6-substituted compound (11) supports previous findings regarding substituent effects (Bowers, 1977b). However, the dihydroquinoline (13) has no effect on either insect, suggesting that -NH- is an inappropriate substitute for oxygen in the pyran ring.

The 3,4-dihydro-compounds, (15) and (16), are inactive, showing that the double bond is required for activity of P1 and P2. Further, the antagonism of precocene action by MDP (Brooks et al, 1979) supports our view that oxidative biotransformation is necessary for activity. Accordingly, we have examined the metabolism of

{4-^{3}H}-P1 (34mCi/mmole) by adult female Locusta CA in vitro, using previously developed short term culture methods for Orthopteran CA (Pratt et al, 1975) and compared this with the metabolism by mid-gut Malpighian tubules and fat body. Tissue incubates were assayed for radioactivity in aqueous, non-extractable and organic extractable fractions, and the latter were further analysed by reverse phase (C_{18}) TLC followed by silica gel HPLC. The CA metabolised P1 cleanly to a mixture of cis- and trans- 3,4-dihydrodiols (21) plus labelled non-extractables, whereas other tissues produced a range of polar metabolites, with relatively little labelling of non-extractable macromolecules. Considering their small biomass the CA (ca.400 micron diam.), metabolise P1 very rapidly (up to 400 pmole/pair glands/hour). The common involvement of an enzymic oxidation in the formation of both dihydrodiols and labelled non-extractables was shown by the graded inhibition of this metabolism by an MFO inhibitor (ethyl 4-(3,4-methylenedioxyphenoxy)-2E-crotonate;EMC) over the range $5x10^{-6}$M to $2x10^{-4}$M. This inhibition paralleled that by EMC of methyl 10, 11-epoxyfarnesoate (C_{16}JH) biosynthesis from exogenous farnesenic acid in a standard JH-biosynthesis inhibitor assay (Pratt & Finney, 1977) which led to the accumulation of the unepoxidised intermediate methyl farnesoate. Accordingly, the possibility is currently under investigation that the metabolic conversion of P1 to its 3,4-dihydrodiols (21), and the metabolic labelling of non-extractable cellular components by P1 residues may be effected by the same epoxidase which forms part of the physiological JH biosynthetic pathway. We consider it unlikely that simple competition between P1 and methyl farnesoate for the epoxidase will explain the anti-allatal action of precocenes, because of the profound nature of their irreversible cytotoxicity, and because preliminary biochemical observations on CA from another insect revealed pre-emptive inhibition of earlier enzyme(s) in the biosynthetic pathway (Pratt & Bowers, 1977); all of which is consistent with the prime role of a biosynthesised ultimate cytotoxin, presumably the 3,4-epoxide.

We find that synthetic P1-epoxide (18) (Jennings & Ottridge, 1979) is rapidly hydrolysed, following pseudo-first order kinetics over a wide pH range. The rate constant in 0.01M sodium phosphate buffer (pH 7.5) at 25°C is 0.9 sec^{-1}, indicating considerably greater reactivity than the 7,8-dihydrodiol-9,10 epoxides of benzo(a)-pyrene, which are known for their cytotoxic/mutagenic action (Yang et al, 1977). This accords with a putative role of P1-epoxide as an alkylating agent in the CA in vivo, through the initial generation of a carbonium ion at C_4. The limited flexibility of the pyran ring maintains coplanarity of the benzene ring and the 3,4-double bond, thereby maximising electronic interaction between them. Also the electron donating effect of the pyran and 7-methoxyl-oxygens is expected to stabilise the postulated C_4-carbonium ion, which we propose is the active species.

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