Phytochemistry, Vol. 28, No. 3, pp. 781–787, 1989.
Printed in Great Britain.

0031 9422/89 $3.00 + 0.00

INHIBITION OF CELERY CELL GROWTH AND STEROL BIOSYNTHESIS BY THE ENANTIOMERS OF PACLOBUTRAZOL

PENNY A. HAUGHAN, RAYMOND S. BURDEN,* JOHN R. LENTON* and L. JOHN GOAD†

Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.; *Department of Agricultural Sciences, University of Bristol, Institute of Arable Crops Research, Long Ashton Research Station, Long Ashton, Bristol BS18 9AF, U.K.

(*Received* 26 *May* 1988)

Key Word Index—*Apium graveolens*; Umbelliferae; celery cell culture; plant growth regulator; paclobutrazol; enantiomers; sterol biosynthesis; cell growth.

Abstract—The four enantiomeric forms of the triazole plant growth retardant, paclobutrazol, [(2*RS*, 3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)-pentan-3-ol] were tested as inhibitors of cell growth and sterol composition using a celery suspension culture. The (2*R*,3*R*)- and (2*R*,3*S*)-enantiomers were potent inhibitors of cell growth and caused a large accumulation of the 14α-methylsterols obtusifoliol, cycloeucalenol, 14α,24-dimethylcholest-8-en-3β-ol and 14α,24-dimethylcholesta-8,24(28)-dien-3β-ol. The (2*S*,3*S*)- and (2*S*,3*R*)-enantiomers on the other hand were only inhibitory to growth and sterol 14α-demethylation at higher concentrations. It is concluded that the (2*R*)-configuration confers the highest potency both for retarding cell proliferation and for inhibition of the cytochrome P-450 dependent sterol 14α-demethylation reaction in celery cells. A lowering of the stigmasterol:sitosterol ratio was noted, particularly with the (2*R*,3*R*)- and (2*S*,3*S*)-enantiomers, which suggested that the sterol Δ^{22}-desaturase may also be a target for inhibition by triazoles. The relevance of these results to the mode of action of (2*RS*,3*RS*)-paclobutrazol as a plant growth retardant is discussed.

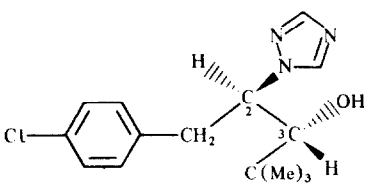
INTRODUCTION

A wide range of synthetic azole compounds are now in use in medicine and agriculture as antifungal compounds and plant growth regulators [1–5]. The fungitoxic azole compounds effectively inhibit the cytochrome P-450 dependent 14α-demethylation reaction in sterol biosynthesis so that there is a decrease in ergosterol content and an increase in 14α-methylsterols (usually lanosterol, 24-methylenelanosterol or obtusifoliol) in the fungal cells [1–3]. The azole compounds exhibiting plant growth retardant properties inhibit the cytochrome P-450 dependent *ent*-kaurene oxidase which is a key enzyme in gibberellin (GA) biosynthesis [5–8]. It is this inhibition of GA production which has been generally believed to represent the main mode of action of the plant growth retardants. However, it is recognised that some plant growth retardants also manifest properties as antifungal compounds or *vice versa* [9–15]. Thus, in addition to blocking GA biosynthesis some of the triazole plant growth regulators are potent inhibitors of sterol biosynthesis at the 14α-demethylation step in fungi [11–15] and plants [15–21]. Moreover, a recent report [21] has described the interaction of triazole compounds with a cytochrome P-450 dependent obtusifoliol 14α-methyl demethylase from maize.

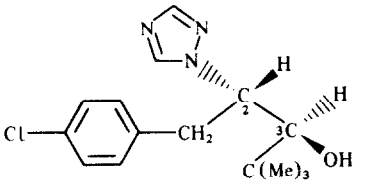
Several of the commercial triazole compounds exist as mixtures of stereoisomers by virtue of either two chiral centres (paclobutrazol, triadimenol) or one chiral centre and an olefinic bond (uniconazol, triapenthenol). Evidence is now being obtained to show that the various biological properties of these compounds may be associated preferentially with particular isomers [5, 15]. With the vinyltriazoles (uniconazol and triapenthenol) it is only the *E*-geometrical isomers which show biological activity and with the chiral centre bearing a hydroxyl in the *R*-configuration the compound is fungitoxic while the *S*-enantiomer is a plant growth retardant [11, 12, 22].

Paclobutrazol is marketed as the enantiomeric mixture (2*RS*, 3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)-pentan-3-ol and the name strictly refers to this diastereoisomer. It has been separated into (2*R*, 3*R*)- and (2*S*, 3*S*)-enantiomers (**1** and **2**, respectively) and these were tested for biological activity [22]. The (2*R*,3*R*)-enantiomer exhibited high fungicidal activity against cereal mildews and rust while the (2*S*,3*S*)-enantiomer showed plant growth retardant activity when tested on apple seedlings [23]. A computer graphics study revealed that the (2*R*,3*R*)-enantiomer structure would superimpose upon that of lanosterol and the (2*S*,3*S*)-enantiomer upon that of *ent*-kaurene thus indicating how paclobutrazol might be envisaged to interact with the protohaem group of the cytochrome P-450 systems involved in sterol and GA biosynthesis, respectively [23]. A subsequent examination [24] of structure–activity relationships was extended to include the resolved (2*R*,3*S*)- and (2*S*,3*R*)-enantiomers (**3** and **4**, respectively) of the (2*RS*,3*SR*)-diastereoisomer of paclobutrazol which had been reported previously [23] to have little biological activity. The paclobutrazol enantiomers were compared with the enantiomeric pairs of triadimenol for their effects on fungal growth, *ent*-kaurene oxidation in a cell free homogenate of *Cucurbita maxima* endosperm, growth of barley seedlings and 14α-methylsterol accumulation in

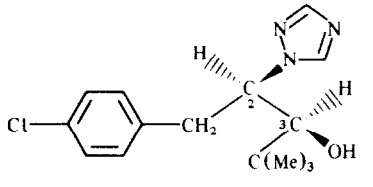
†Author to whom correspondence should be addressed.



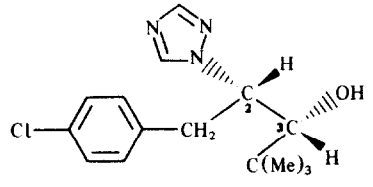
1



2



3



4

the seedlings [24]. In both series of compounds it was found that the *R*-configuration at the alcohol bearing carbon atom conferred fungistatic activity whereas the *S*-configuration at this carbon caused an inhibition of *ent*-kaurene oxidase. However, the relationship between the absolute stereochemistry of the different enantiomers and the inhibition of plant sterol 14α-demethylation activity was less clear. The diastereoisomers and the resolved enantiomers of triadimenol have been tested using wheat and barley seedlings [15–17] and a correlation observed between the extent of growth retardation and the inhibition of sterol synthesis.

We have reported [18–20] that the (2*RS*,3*RS*) mixture of paclobutrazol retards growth of a celery cell suspension culture and inhibits sterol biosynthesis in these cells at the 14α-demethylation step leading to an accumulation of 14α-methylsterols. The paclobutrazol inhibition of celery cell proliferation can be overcome by addition of sterol to the growth medium [18–20]. Furthermore, using paclobutrazol as a probe we have established that there are two sterol requirements; one is for a 'bulk' sterol, possibly for membrane production, while the second is for small amounts of 'trigger' sterol which apparently plays some essential role in the process of cell division and is fulfilled specifically by a 24α-ethylsterol. In this paper we extend our observations [17–20, 24] on the mode of action of paclobutrazol and examine the effects of the resolved enantiomers on cell growth and sterol biosynthesis of a celery cell suspension culture. The results are compared with those obtained in previous studies using whole plants.

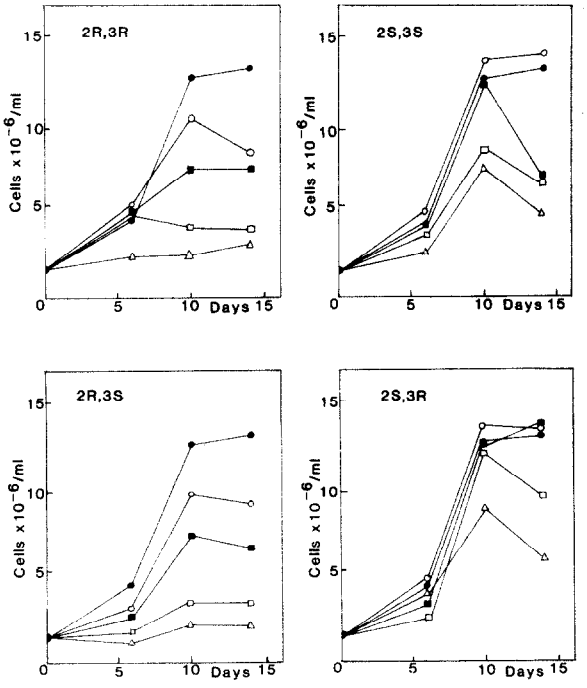


Fig. 1. Effects of increasing concentrations of the four resolved enantiomers of paclobutrazol on growth of celery cell suspension cultures. -●-●- Control; -○-○- 1 μM; -■-■- 3 μM; -□-□- 10 μM; -△-△- 30 μM paclobutrazol.

RESULTS AND DISCUSSION

Celery cell cultures were grown for 15 days in media containing the (2*R*,3*R*)-, (2*R*,3*S*)-, (2*S*,3*R*)- and (2*S*,3*S*)-enantiomers of paclobutrazol at concentrations of 1, 3, 10 and 30 μM. The (2*R*,3*R*)- and (2*R*,3*S*)-enantiomers were equally effective inhibitors of celery cell growth (Figs 1 and 2) and were considerably more potent than the (2*S*,3*S*)- and (2*S*,3*R*)-enantiomers which only showed marked inhibition of growth at concentrations above 10 μM. From these results it can be concluded that it is the enantiomers with the *R*-configuration at C-2 which are the most potent inhibitors of cell proliferation in celery suspension cultures (Fig. 2). The growth inhibition observed at the higher concentrations of the (2*S*,3*S*)- and (2*S*,3*R*)-enantiomers, may have resulted from the presence of 1–3% of the (2*R*,3*R*)- and (2*R*,3*S*)-enantiomers,

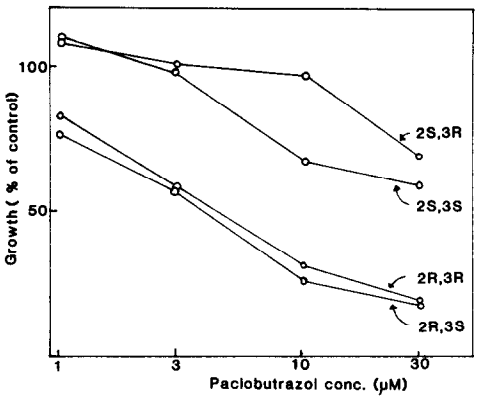


Fig. 2. Growth response of celery cell suspension cultures measured 10 days after exposure to increasing concentrations of the (2*R*,3*R*)-, (2*S*,3*S*)-, (2*R*,3*S*)- and (2*S*,3*R*)-enantiomers of paclobutrazol.

respectively, in these preparations. However, an inhibition of growth at relatively high concentrations of the various paclobutrazol enantiomers could also result from the direct incorporation of the triazoles into cellular membranes. This might result in disruption of membrane function with inhibition of growth rather than their acting through the more specific inhibition of sensitive cytochrome P-450 dependent oxidases. A similar situation has been recognised in fungi when treated with high concentrations of imidazole antifungal drugs [1].

Celery cell cultures grown with the four paclobutrazol enantiomers in the presence of [2-^{14}C]-mevalonic acid incorporated radioactivity into the 4-demethylsterol and 4-methylsterol fractions (Table 1). The latter fraction included both the 4,4-dimethyl- and 4α-methylsterols, some of which possess a 14α-methyl group (cycloartenol, cycloeucalenol, obtusifoliol). There was significantly more radioactivity in the 4-methylsterols obtained from the cells grown in the presence of the (2*R*,3*R*)- and (2*R*,3*S*)-enantiomers. Cells treated with the (2*S*,3*S*)- and (2*S*,3*R*)-enantiomers showed a distribution of radioactivity in the two sterol fractions comparable to the control culture. Analysis of the sterols isolated from these cultures showed the accumulation of considerable amounts of the 14α-methylsterols obtusifoliol and cycloeucalenol, in the cultures treated with the (2*R*,3*R*)- and (2*R*,3*S*)-enantiomers (Table 1). In addition, GC-MS analysis showed that appreciable amounts of 14α,24-dimethylcholest-8-en-3β-ol and 14α,24-dimethylcholesta-8,24(28)-dien-3β-ol also accumulated but they were not resolved from campesterol on the GC column employed to permit accurate quantitation. Much smaller amounts of these 14α-methylsterols were present in the cultures grown in the presence of the (2*S*,3*S*)- and (2*S*,3*R*)-enantiomers while a negligible amount of 14α-methylsterol was present in the control culture. The accumulation of 14α-methylsterols was observed also in the cells harvested at the end of growth experiments employing various concentrations of the paclobutrazol enantiomers (Table 2). The addition of increasing concentrations of the enantiomers to the cultures led to the accumulation of progressively larger amounts of obtusifoliol and to a lesser extent cycloeucalenol. The (2*R*,3*R*)- and (2*R*,3*S*)-enantiomers at low concentrations were significantly more active than the (2*S*,3*S*)- and (2*S*,3*R*)-enantiomers for inducing 14α-methylsterol production. With the two former enantiomers higher amounts of 14α,24-dimethylcholesta-8-en-3β-ol and 14α,24-dimethylcholesta-8,24(28)-dien-3β-ol were formed and they essentially replaced campesterol in the cells. The sterol patterns resulting from exposure of the celery cells to the (2*R*,3*R*)-enantiomer for 6 and 10 days are shown in Table 3. At day 6 appreciable amounts of obtusifoliol and the other 14α-methylsterols were apparent even in the presence of 3 μM (2*R*,3*R*)-paclobutrazol which had not retarded growth at this stage (Fig. 1, Table 3). By day 10 there was a considerable accumulation of 14α-methylsterols and cell proliferation was severely curtailed. These data show that even in the most retarded cells the sterol biosynthetic pathway apparently remains operative up to the 14α-demethylation step which is the reaction inhibited by paclobutrazol.

The effects of increasing concentrations of the four enantiomers on the amounts of the individual 4-demethylsterols were not always consistent (Table 2). However, the higher concentrations of the (2*R*,3*R*)-enantiomer caused a decrease in the proportion of stigmasterol while the other enantiomers apparently had little effect on the amounts of this sterol. Also, with all four enantiomers the content of sitosterol tended to increase. These results

Table 1. Effects of paclobutrazol enantiomers on the incorporation of [2-^{14}C]-mevalonic acid into sterols and the accumulation of obtusifoliol and cycloeucalenol in a celery cell suspension culture*

	Radioactivity (dpm × 10^{-3})		14α-Methylsterols (ng/10^6 cells)	
Enantiomer	4-Demethylsterol	4-Methylsterol	Obtusifoliol	Cycloeucalenol
None	318	37	—	—
(2*R*,3*R*)	240	67	1440	810
(2*S*,3*S*)	252	27	250	60
(2*R*,3*S*)	307	91	1910	930
(2*S*,3*R*)	254	28	190	170

*[2-^{14}C]-Mevalonic acid (2.0 μCi) was added to the growth medium at the start of incubation and cells were harvested after 18 days. Paclobutrazol enantiomers were added to give a final concentration of 3 μM.

Table 2. Sterol composition (ng/10^6 cells) of celery cells harvested after 15 days growth in the presence of paclobutrazol enantiomers

Enantiomer	Concentration (μM)	Chol	Camp	14αMS	Stig	Sito	Obt	Cye	Stig:Sito ratio
None	—	80	2260†	5460	3250	—			1.68
(2*R*,3*R*)	1	80	3600	6310	5990	2210	680		1.05
	3	140	5340	4730	7480	1730	950		0.63
	10	290	6520	4340	6070	1710	1830		0.71
	30	60	6300	2760	4200	9200	870		0.66
(2*S*,3*S*)	1	50	2430	2450	3590	140	270		0.68
	3	130	3780	7000	5730	280	350		1.22
	10	150	4020	5340	5830	530	450		0.92
	30	80	4130	3630	6360	1740	2360		0.57
(2*R*,3*S*)	1	60	2800	4740	3920	930	560		1.21
	3	90	4370	5280	5050	2090	980		1.05
	10	440	8370	5710	2450	9220	1800		2.33
	30	130	11180	6390	6820	13000	3830		0.93
(2*S*,3*R*)	1	50	2910	6580	3990	110	270		1.65
	3	50	2260	4890	3150	130	220		1.55
	10	90	3310	6110	4420	310	320		1.38
	30	140	3630	6340	6950	1780	530		0.91

*Abbreviations: Chol = cholesterol; Camp = campesterol; 14αMS = 14α,24-dimethylcholest-8-en-3β-ol and 14α,24-dimethylcholesta-8,24(28)-dien-3β-ol; Stig = stigmasterol; Sito = sitosterol; Obt = obtusifoliol; Cye = cycloeucalenol.

†Only campesterol was present in control cells. The 14α-methylsterols which cochromatographed with campesterol by GC were present in variable amounts in the cells treated with paclobutrazol enantiomers.

Table 3. Sterol content (ng/10^6 cells) of celery cells exposed to 3 or 30 μM (2*R*,3*R*)-paclobutrazol for 6 and 10 days

Conc. (μM)	Days exposure	Cell population ($\times 10^{-7}$)*	Chol	Camp and 14α-MS	Stig	Sito	Obt	Cye	Stig:Sito ratio
0	6	3.6	310	2850	6120	4700	—	—	1.28
3	6	3.6	580	3860	6240	4270	770	1330	1.46
30	6	1.8	540	4560	6020	6340	5280	1540	0.94
0	10	6.0	440	2830	6080	4580	—	—	1.32
3	10	2.4	310	4680	6330	8160	3820	—	0.78
30	10	1.9	250	7410	5140	5530	10270	1590	0.93

*Cells per 12 ml culture at time of harvest.

Abbreviations as in Table 2.

contrast with our previous observations [20] which revealed a marked decrease in the amounts of both stigmasterol and sitosterol following growth of celery cells in the presence of (2*RS*,3*RS*)-paclobutrazol.

We have noted previously that (2*RS*,3*RS*)-paclobutrazol causes a decrease in the stigmasterol:sitosterol ratio in celery cell cultures [20] and a similar observation has recently been made with maize treated with other triazoles [21]. In the present work with celery cells the different paclobutrazol enantiomers had varying effects on the relative proportions of stigmasterol and sitosterol. In the control cells stigmasterol was the major sterol (Tables 2 and 3). Although the results are not entirely clear cut in all incubations it is apparent that the cells grown in the presence of the (2*R*,3*R*)- and (2*S*,3*S*)-enantiomers had a lowered stigmasterol:sitosterol ratio, particularly at the higher concentrations of the inhibitor (Table 2) and after longer culture periods (Table 2 and 3). The change in the stigmasterol:sitosterol ratio could arise from inhibition of the conversion of sitosterol into stigmasterol. However, there is some doubt [25] concerning the role of sitosterol as the immediate precursor of stigmasterol. Thus, a block in the production of stigmasterol from some other compound may occur. Alternative precursors could be stigmast-7-en-3β-ol or isofucosterol [stigmasta-5,24(28)*E*-dien-3β-ol] both of which can also act as a common precursor to sitosterol and therefore lead to an elevated sitosterol content at the expense of stigmasterol in cells treated with paclobutrazol. Recently it was reported that ergosterol biosynthesis in yeast requires a cytochrome P-450 dependent mono-oxygenase to introduce the side chain Δ^{22}-bond [26]. If the Δ^{22}-desaturase in higher plants which is required for stigmasterol production is also a cytochrome

P-450 dependent enzyme it might be susceptible to inhibition by a triazole such as paclobutrazol. If this proves to be the case then the changes in the stigmasterol : sitosterol ratios recorded in Table 2 indicate that the (2*R*,3*R*)- and (2*S*,3*S*)-enantiomers apparently have the greater ability to inhibit this enzyme. In fungi, there is evidence that the pyrimidine fungicide fenarimol may both inhibit the 14α-demethylation and Δ^{22}-desaturase enzymes involved in ergosterol biosynthesis [27].

The results presented here are in accord with our previous demonstration that the (2*RS*,3*RS*)-mixture of paclobutrazol inhibits sterol biosynthesis in celery cells at the 14α-demethylation step [18–20]. It was observed previously [20] that 10 μM (2*RS*,3*RS*)-paclobutrazol inhibited growth by *ca* 50% compared with the control culture. The present results on the supression of growth observed with the resolved (2*R*,3*R*)- and (2*S*,3*S*)-enantiomers now allows us to conclude that the growth inhibition observed previously with the racemic mixture results mainly, if not exclusively, from the effects of the (2*R*,3*R*)-enantiomer (Fig. 2) and the inhibition of sterol biosynthesis by this enantiomer may be the prime factor in regulating cell proliferation (Fig. 1, Table 3).

The (2*S*,3*S*)-enantiomer of paclobutrazol has been shown to specifically inhibit *ent*-kaurene oxidase which is a key enzyme in GA biosynthesis [8, 24]. However, we have been unable to detect significant amounts of GA by GC-MS in untreated celery cell cultures (unpublished results). Moreover, the inhibition of celery cell growth by paclobutrazol cannot be reversed by addition of GA_3 to the treated cultures (unpublished results). By contrast, growth of paclobutrazol-inhibited cultures can be restored by addition of cholesterol together with a trace of a 24α-ethylsterol, such as stigmasterol, to the paclobutrazol-inhibited culture [18–20]. Thus, the retardation of cell production caused by high concentrations of (2*S*,3*S*)-paclobutrazol (Fig. 2) may be attributed to an inhibition of sterol biosynthesis resulting either from trace amounts of the (2*R*,3*R*)-enantiomer present as an impurity or low inherent activity of the (2*S*,3*S*)-enantiomer on the plant sterol 14α-demethylase system. The experimental evidence suggests that interference with sterol production is a major factor in the regulation of plant cell growth by paclobutrazol in suspension cultures. Köller [16] has related inhibition of shoot growth to the lowered sterol production in whole wheat plants treated with the fungicide triadimenol. Inhibition of growth of etiolated wheat seedlings by the four enantiomers of triadimenol was associated with their relative potencies as inhibitors of sterol 14α-demethylation and the inhibition of growth could not be reversed by application of GA to the seedlings [16]. However, subsequent work has shown that (1*R*,2*S*)-triadimenol is a relatively potent inhibitor of *ent*-kaurene oxidase [24] and some retardation of plant growth might be expected to result from the inhibition of GA production in the seedlings. A requirement for sterol in plant cell growth was also suggested by the reversal of tetcyclasis inhibition of growth of cell suspension cultures by the addition of cholesterol to the medium [28, 29] although in this case it was not clear whether tetcyclasis directly affected sterol biosynthesis in the cells. Indeed, a recent investigation failed to reveal any significant interference in sterol production in maize seedlings exposed to tetcyclasis [21].

In contrast to the results obtained with the different enantiomers of triadimenol on the growth of etiolated wheat seedlings, (2*S*,3*S*)-paclobutrazol, the most effective inhibitor of *ent*-kaurene oxidase [8, 24], was also the most potent inhibitor of shoot growth of light-grown wheat seedlings (Lenton J. R., unpublished observations). However, the high concentrations of the enantiomers of paclobutrazol which inhibit plant sterol biosynthesis also retard shoot growth. As predicted from its potency in the *ent*-kaurene oxidase assay, (2*S*,3*S*)-paclobutrazol was shown to be an effective inhibitor of GA production in intact wheat seedlings. In a GA-insensitive, dwarf wheat line, 1 μM (2*S*,3*S*)-paclobutrazol inhibited GA_1 production without affecting shoot growth whereas the (2*R*,3*R*)-enantiomer was ineffective [30]. This result shows that the primary site of action of the (2*S*,3*S*)-enantiomer at low concentrations is an inhibition of GA biosynthesis and that the reduced GA content is not a consequence of reduced growth caused by some other mechanism. In a near-isogenic, tall, GA-responsive wheat line, 1 μM (2*S*,3*S*)-paclobutrazol caused a 10-fold reduction in GA_1 concentration in the expansion zone of wheat leaves resulting in a 30% reduction in final leaf length [30]. This inhibition of leaf growth was reversed completely by simultaneous addition of GA.

Burden *et al.* [24] showed that the relative activities of the paclobutrazol enantiomers on the accumulation of 14α-methylsterols and decrease of normal sterols in light grown barley seedlings were (2*R*,3*S*)>(2*R*,3*R*) >(2*S*,3*S*)>(2*S*,3*R*). Similar results are reported here using celery suspension cultures. When tested [24] for inhibition of *ent*-kaurene oxidase activity in cell-free preparations from endosperm of *Cucurbita maxima* the effectiveness of the enantiomers was (2*S*,3*S*)> (2*R*, 3*S*)>> (2*R*,3*R*)> (2*S*,3*R*). The four enantiomers produced *ca* equal reductions in shoot height of barley seedlings [24]. However, since relatively high concentrations of the enantiomers were applied to barley seedlings it is possible that an inhibition of both GA and sterol production accounted for the decrease in shoot growth. Indeed some 14α-methylsterol accumulation was seen in barley seedlings treated with each of the four enantiomers [24].

If the results obtained from the present work with celery cell cultures can be extrapolated to intact plants then relatively low concentrations of the (2*R*,3*R*)- and (2*R*,3*S*)-enantiomers of paclobutrazol might be expected to reduce meristematic activity and, as a consequence, retard growth. In light-grown shoots of wheat (see above) and apple [24], which are dependent on GA for growth, the (2*S*,3*S*)-enantiomer is the most potent growth retardant. However, the (2*R*,3*R*)- and (2*R*,3*S*)-enantiomers are far more active than the (2*S*,3*S*)-enantiomer at inhibiting root growth in wheat seedlings (Lenton J. R., unpublished observations). Thus, although it is generally considered that the primary mode of action of paclobutrazol, and other azole growth retardants, is an inhibition of GA biosynthesis, the present results clearly indicate that inhibition of sterol biosynthesis may be an additional site of action. Such an inhibition of sterol production could reduce meristematic activity and also possibly impair the function of cellular membranes, which may in turn adversely affect GA action in elongating cells. The nature of the growth response obtained in retardant-treated plants will depend, therefore, on the inherent capacity of the compound to inhibit GA and sterol biosynthesis, the concentration applied and the relative

dependence of the tissues on GA and sterols for growth.

The precise mechanism of retardation of cell division following inhibition of the sterol biosynthetic pathway at the 14α-demethylation step remains to be established. The suggestion has been made that accumulation of excessive amounts of 14α-methylsterol and depletion of normal sterol may be harmful to membrane structure and function [1–3, 31, 32]. However, evidence is now appearing to show that 'normal' sterols play, in addition to a structural role in membranes, some other essential role in the cell cycle in yeast [2, 33–35], protozoa [36], higher plants [18–20, 37] and animals [38]. Our work with the celery cell suspension culture [18–20, unpublished observations] and that of Tal and Nes [37] using sunflower cultures indicates that a supply of newly synthesized 24α-ethylsterol is essential for cell division to proceed. The interruption in the production of 24α-ethylsterol by paclobutrazol may be a potentially important factor in the plant growth retarding effects of this triazole compound.

EXPERIMENTAL

The (2*R*,3*R*)-, (2*R*,3*S*)-, (2*S*,3*R*) and (2*S*,3*S*)-enantiomers of paclobutrazol were prepared from the (2*RS*,3*RS*)-mixture and the (2*RS*,3*SR*)-diastereoisomers as described previously [24]. The purity of enantiomers was verified by GC [23]: (2*R*,3*R*)-enantiomer 99%; (2*S*,3*S*)-enantiomer 99%; (2*S*,3*R*)-enantiomer 99%; (2*R*,3*S*)-enantiomer 97%.

Cell culture. A celery (*Apium graveolens*) cell suspension culture was kindly provided by Dr Hamish Collin (Department of Botany, University of Liverpool). It was cultured on Murashige and Skoog medium (Flow Laboratories) supplemented with sucrose (30 g/l), 2,4-D (0.5 mg/l) and kinetin(0.6 mg/l) and adjusted to pH 5.5. Media (10 ml) was dispensed into 50 ml conical flasks, autoclaved, and 2 ml of celery cell suspension (6×10^6 cells/ml) added. Cultures were grown for 15 to 21 days at 18° with a 6 hr light–6 hr dark cycle and continuous shaking (50 rpm). Paclobutrazol was added in Me_2CO soln. The final concn of Me_2CO was less than 1% v/v and at this concn it had no significant effect on growth of a control culture. [2-^{14}C]-Mevalonic acid (Amersham sp. act. 51.4mCi/mmol) was sterilized by Millipore filtration prior to addition to the culture medium. Cell growth was determined by cell counting [39]. CrO_3 soln (8%, 0.2 ml) was added to 0.1 ml of cell suspension, heated to 70° for 15 min and then passed 10× through a No. 16 syringe needle before counting on a haemocytometer.

Analysis of sterols. Sterols were extracted from the celery cells and purified by prep. TLC on silica gel ($CHCl_3$–EtOH, 49:1) as described previously [18–20]. 26,27-Dinorcholesta-5,22-dien-3β-ol (100 μg) was added as an int. standard for sterol quantification by GC on a 12 m × 0.22 mm BP-5 fused silica capillary column [SGE (UK) Ltd]. The initial oven temp was 240° held for 2 min and then programmed at 5°/min to 290°. Injection and FID temperatures were 290 and 300°, respectively. The carrier gas (He) flow rate was 1.5 ml/min. The sterols were chromatographed either as the free sterol or the TMS ether derivatives. Sterol identities were confirmed by GC-MS using a VG 70-70H instrument with a 25m × 0.22 mm BP-1 [SGE (UK) Ltd] capillary column [20]. The mass spectra of the sterols agreed with those reported previously [20].

Acknowledgements—We thank Mrs C. S. James for the isolation and purification of the paclobutrazol enantiomers used for this study and the Science and Engineering Research Council for a CASE Studentship awarded to P. A. H. Long Ashton Research Station is financed through the Agricultural and Food Research Council.

REFERENCES

1. Kato, T. (1986) in *Chemistry of Plant Protection* (Haug, G. and Hoffmann, H., eds), pp. 1–24. Springer, Berlin.
2. Weete, J. D. (1987) in *Ecology and Metabolism of Plant Lipids* (Fuller, G. and Nes, W. D., eds), pp. 268–283. American Chemical Society, Washington, D.C.
3. Vanden Bossche, H. (1985) in *Current Topics in Medical Mycology* Vol. 1 (McGinnis, M. R., ed.), pp. 313–351. Springer, New York.
4. Kuck, K. H. and Scheinpflug, H. (1986) in *Chemistry of Plant Protection* Vol. 1 (Haug, G. and Hoffmann, H., eds), pp. 65–96. Springer, Berlin.
5. Lenton, J. R. (1987) *Br. Plant Growth Regul. Group News Bull.* **9**, 1.
6. Rademacher, W., Fritsch, W., Graebe, J. E., Sauter, H. and Jung, J. (1987) *Pestic. Sci.* **21**, 241.
7. Sisler, H. D., Ragsdale, N. N. and Waterfield, W. F. (1984) *Pestic Sci.* **15**, 167.
8. Hedden, P. and Graebe, J. E. (1985) *J. Plant Growth Regul.* **4**, 111.
9. Buchenauer, H. and Röhner, E. (1981) *Pestic. Biochem. Physiol.* **15**, 58.
10. Fletcher, R. A., Hofstra, G. and Goa, J. (1986) *Plant Cell Physiol.* **21**, 367.
11. Izumi, K., Kamiya, Y., Sakurai, A., Oshio, H. and Takahashi, N. (1985) *Plant Cell Physiol.* **26**, 821.
12. Takano, H., Oguri, Y. and Kato, T. (1986) *J. Pestic. Sci.* **11**, 373.
13. Baldwin, B. C. and Wiggins, T. E. (1984) *Pestic. Sci.* **15**, 156.
14. Wiggins, T. E. and Baldwin, B. C. (1984) *Pestic. Sci.* **15**, 206.
15. Köller, W. (1987) *Pestic. Sci.* **18**, 129.
16. Köller, W. (1987) *Physiol. Plant.* **71**, 309.
17. Burden, R. S., Clark, T. and Holloway, P. J. (1987) *Pestic. Biochem. Physiol.* **27**, 289.
18. Haughan, P. A., Lenton, J. R. and Goad, L. J. (1987) in *The Metabolism, Structure and Function of Plant Lipids* (Stumpf, P. K., Mudd, J. B. and Nes, W. D., eds), pp. 91–94. Plenum Press, New York.
19. Haughan, P. A., Lenton, J. R. and Goad, L. J. (1987) *Biochem. Biophys. Res. Commun.* **146**, 510.
20. Haughan, P. A., Lenton, J. R. and Goad, L. J. (1988) *Phytochemistry* **27**, 2491.
21. Taton, M., Ullman, P., Benveniste, P. and Rahier, A. (1988) *Pestic. Biochem. Physiol.* **30**, 178.
22. Lüssen, K. (1987) in *Hormone Action in Plant Development–A Critical Appraisal* (Hoad, G. V., Lenton, J. R., Jackson, M. B. and Atkin, R. K., eds), pp. 133–144. Butterworth London.
23. Sugavanam, B. (1984) *Pestic. Sci.* **15**, 296.
24. Burden, R. S., Carter, G. A., Clark, T., Cooke, D. T., Croker, S. J., Deas, A. H. B., Hedden, P., James, C. S. and Lenton, J. R. (1987) *Pestic. Sci.* **21**, 253.
25. Huang, L.-S. and Grunwald, C. (1986) *Phytochemistry* **25**, 2779.
26. Hata, S., Nishimo, T., Katsuki, H., Aoyama, Y. and Yoshida, Y. (1987) *Agric. Biol. Chem.* **51**, 1349.
27. Sisler, H. D., Ragsdale, N. N. and Waterfield, W. W. (1984) *Pestic. Sci.* **15**, 167.
28. Nitsche, K., Grossmann, K., Sauerbrey, E. and Jung, J. (1985) *J. Plant Physiol.* **118**, 209.
29. Grossmann, K., Weiler, E. W. and Jung, J. (1985) *Planta* **164**, 370.

30. Lenton, J. R., Hedden, P. and Gale, M. D. (1987) in *Hormone Action in Plant Development—A Critical Appraisal* (Hoad, G. V., Lenton, J. R., Jackson, M. B. and Atkin, R. K., eds), pp. 145. Butterworth, London.
31. Bloch, K. (1983) *CRC Crit. Reviews Biochem.* **14**, 47.
32. Vanden Bossche, H., Marichal, P., Gorrens, J., Bellens, D., Verhoeven, H., Coene, M-C., Lauwers, W. and Janssen, P. A. J. (1987) *Pestic. Sci.* **21**, 289.
33. Rodriguez, R. J., Low, C., Bottema, C. D. K. and Parks, L. W. (1985) *Biochim. Biophys. Acta* **837**, 336.
34. Pinto, W. J. and Nes, W. R. (1983) *J. Biol. Chem.* **258**, 4472.
35. Dahl, C., Biemann, H. B. and Dahl, J. (1987) *Proc. Natl Acad. Sci. U.S.A.* **84**, 4012.
36. Whitaker, B. D. and Nelson, D. L. (1987) *Lipids* **22**, 386.
37. Tal, B. and Nes, W. D. (1987) *Plant Physiol. Suppl.* **83**, 161.
38. Popjak, G., Meenan, A. and Nes, W. D. (1987) *Proc. R. Soc. London* **B232**, 273.
39. Dixon, R. A. (1985) *Plant Cell Culture*. IRL Press, Oxford.