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DISTRIBUTION OF PODOLACTONE-TYPE PLANT GROWTH INHIBITORS IN THE CONIFERAE

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Key Word Index—*Podocarpus elatus;* Podocarpaceae; Coniferae; podolactone-type plant growth inhibitors; *nor*diterpene dilactones; harringtonolide; momilactone B; lycoricidinol.

Abstract—Plant growth inhibitors of the podolactone-type have been detected by bioassay in ten further species of *Podocarpus*. The most active extracts in *P. elatus* were from root tips, root cortex and very young leaves. Fifty-seven other conifers were examined for this type of activity. It is present in *Cephalotaxus harringtonia* where it is probably due to the presence of harringtonolide, which, like momilactone B from rice husks, shows podolactone-type inhibition of the growth of etiolated dwarf pea hooks.

INTRODUCTION

Thirty-five *nor-* and *bisnor*-diterpene dilactones have been isolated from seeds, bark, wood, leaves and root tissue of 17 species of the gymnosperm genus *Podocarpus*, and many have strong plant growth regulatory effects [1–4]; some also show cytostatic activity with certain carcinomas [5, 6] and some are insecticidal [7, 8]. Their occurrence, chemistry and biological activity have been reviewed [9, 10] but some earlier structures have been recently corrected [11, 12]. Our interest in the role of the compounds in plants has led us to explore their occurrence in detail in one *Podocarpus* species and to see if they occur more widely in this and other genera.

Attempts to estimate *nor-* and *bisnor*-diterpene dilactones by GC/MS were only partially successful because of their varied fragmentation patterns. Other analytical techniques, such as GC, HPLC and TLC were tried but they lacked specificity and sensitivity. Spectrophotometric techniques could not be used for similar reasons. So the etiolated dwarf pea hook bioassay, which is particularly sensitive to the members of this group of compounds with inhibitory activity [2, 3], remains the only method of detecting them on a small scale. In the assay, the inhibitory activity can be distinguished from that caused by other inhibitors (e.g. abscisic acid) and is referred to as 'podolactone-type' [2]. Using this assay, we have explored the distribution of this type of plant growth regulator in parts of *Podocarpus elatus* R. Br. at various stages of maturity, and have tested other members of the Podocarpaceae as well as other conifers for podolactone-type plant growth inhibitory activity.

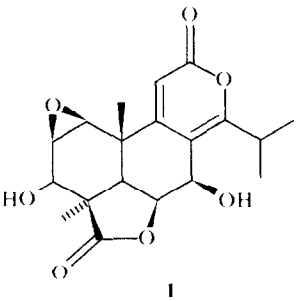
RESULTS AND DISCUSSION

The only *nor*diterpene dilactone identified so far from extracts of *P. elatus* is nagilactone C (**1**), which is one of the most frequently isolated *nor*diterpene dilactones [10] but one of the less active plant growth inhibitors [2–4]. There is evidence from TLC for the presence of other such lactones in leaf extracts of the same plant (M.N.G. and Professor Shô Itô, private communication), which might be expected, as a series of *nor-* and *bisnor*-diterpene dilactones of varying inhibitory activity have been shown to exist in each of several species of *Podocarpus* [10, 13]. Our extraction procedure (Experimental) allows the passage of the known free *nor*diterpene dilactones into the final extract, but any glucosides would remain in the aqueous phase. Other neutral inhibitors could also be present, but representative compounds only affect the growth of etiolated dwarf pea hooks slightly, and do not inhibit their greening (Table 1). Nevertheless, we only ascribe podolactone-type activity to a compound or extract if there is the marked inhibition of growth and obvious prevention of greening characteristic shown by the pure *nor-* and *bisnor*-diterpene dilactones [2].

Extracts of mature seeds, very young leaves and mature leaves of *P. elatus* show increasing inhibition with increasing concentration (Table 2) and extraction of appropriate fresh weights of tissue has allowed us to compare various parts of *P. elatus* (Table 3). The inhibitors are distributed throughout the plant, but the levels of activity vary with tissue and age (Tables 3 and 4). The sources of the most active extracts are young root tips, root cortex and very young leaves (Table 3).

Brown and Sanchez [9] suggested that *nor*diterpene dilactones, if transferred from the leaves to the ground by rain or fog drip, might be the compounds responsible for allelopathic effects in *Podocarpus nagi* (Thunberg) Pilger forests [14]. Inhibitory activity can be detected in leachates of some leaves of *P. elatus* (Table 3), and it correlates well ($r = 0.95$, $P < 0.01$) with the activity of extracts from the same leaves. However, we have not been able to show activity in extracts from soil around a mature tree, or around a seedling, suggesting that an allelopathic role is unlikely.

The activities of extracts from leaves of representative conifers are shown in Table 5. Not all species of *Podocarpus* showed activity at the tissue equivalents tested, and, except for *P. andinus*, the presence or absence



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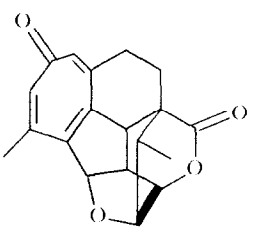

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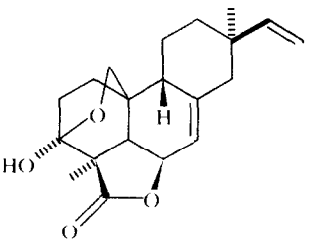

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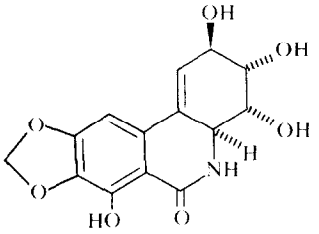

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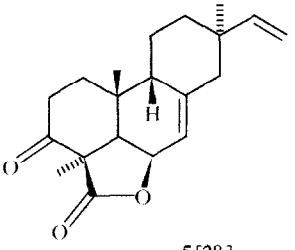

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Table 1. Effect of plant growth inhibitors on plumular hooks from etiolated dwarf pea seedlings. Results expressed as a percentage of control growth, with non-significant values indicated

Inhibitor	Concentration (M)	% Control growth*	Colour after growth period†
Coumarin	10^{-5}	109	+ + +
Cucurbitacin B	10^{-5}	97	+ + +
	10^{-4}	100	+ + +
Duvatrienediol	10^{-6}	79	+ + +
	10^{-5}	92	+ + +
Vernolepin	10^{-6}	81	+ + + (brown ends)
	10^{-5}	80	+ + + (brown ends)
Harringtonolide (2)	10^{-8}	85	+ + +
	10^{-7}	92	+ + +
	10^{-6}	68	+ +
	10^{-5}	29	−
Momilactone B (3)	10^{-7}	99	+ + +
	10^{-6}	94	+ + +
	10^{-5}	36	+
Momilactone A (5)	10^{-7}	103	+ + +
	10^{-6}	99	+ + +
	10^{-5}	84	+ + +

* All values over 85% not significant at $P = 0.05$.

† Colour scores: −, cream or white; +, pale green; + +, slightly paler green than control; + + +, as green as control.

Table 2. Podolactone-type inhibitory effects of concentration ranges of extracts from young and mature leaves and mature seeds of *Podocarpus elatus* on plumular hooks from etiolated dwarf pea seedlings. Results expressed as percentage of control growth

Tissue equivalent (g fr. wt)	Young leaves		Mature leaves		Mature seeds	
	% Growth	Colour*	% Growth	Colour*	% Growth	Colour*
0.001	91	+++				
0.01	75	+++	89	+++	95	+++
0.1	38	+	69	++	54	+
1.0	8	−	32	−	43	−
3.0	−2	−				
8.0			3	−	21	−
LSD:						
$P = 0.05$	16		15		7	

*See Table 1.

Table 3. Comparison of the podolactone-type inhibitory activity of extracts from young, mature or senescent tissues of mature trees and 2- and 4-year seedlings of *Podocarpus elatus* on plumular hooks from etiolated dwarf pea seedlings. Results expressed as a percentage of control growth†

Part of plant	Tissue equivalent fr. wt (g)	% Control growth	Colour of hooks after growth*
Male cones			
Young	0.1	54	+
Mature	0.1	50	+
LSD†		26	
Female receptacles			
Young	0.1	64	++
Mature	0.1	100	+++
LSD		14	
Leaves			
Experiment 1			
Young	0.1	52	+
Mature	0.1	68	++
LSD		9	
Experiment 2			
Young (1978 growth)	0.1	50	+
Mature (1977 growth)	0.1	71	++
Senescent (1976 growth or older)	0.1	58	++
LSD		11	
Leachates from leaves			
Experiment 1			
Young	1.0	46	+
Mature	1.0	76	++
LSD		12	
Experiment 2			
Young (1978 growth)	1.0	84	++
Mature (1977 growth)	1.0	98	+++
Senescent (1976 growth or older)	1.0	85	++
LSD		11	
4-Year seedlings			
Experiment 1			
Leaves: very young	0.1	9	−
mature	0.1	67	++

Table 3. (*Continued*)

Part of plant	Tissue equivalent fr. wt (g)	% Control growth	Colour of hooks after growth*
Stem	0.1	48	+
Root: main	0.1	32	+
nodules	0.1	41	+
	0.01	78	+ +
LSD		19	
Experiment 2			
Young roots and tips	0.1	0	–
Main root: cortex	0.1	0	–
stele	0.1	30	+
Very Young Leaves	0.1	2	–
LSD		v. small	
Experiment 3 (After repotting and 1 month's growth)			
Maturing leaves	0.1	40	–
Stem: cortex	0.1	38	–
stele	0.1	50	+
Root tips	0.1	5	–
Root nodules	0.1	10	–
Sap	0.72	40	+
LSD		22	
2-year seedlings and embryos from mature seed			
Young roots and tips	0.01	52	+
Main root cortex	0.01	76	+ +
Root nodules	0.01	103	+ + +
Embryos (seed stored at 4° for 3 months)	0.01	83	+ + +
LSD		14	

*See Table 1.

†The least significant difference (LSD) for each experiment is shown at $P = 0.05$.

Table 4. Podolactone-type inhibitory effects of extracts from leaves and seed of *Podocarpus elatus* harvested at different times. Results expressed as percentage of control growth

	Leaves		Seeds					
	Tissue		Epimatium			Endosperm and embryo		
Time of harvest	equivalent Experiment	0.1 (i)	0.1 (ii)	0.1	0.5 (i)	0.5 (ii)	0.1	0.5 (i)	0.5 (ii)
1974									
October									
Senescent		68	64						
Young		52							
1975									
February		61							
March				29			62		
April				31			57		
May				29			54		
June		71			37				
July					39		43	37	
August					36			39	
September					42*			46*	
October		83			37*			37*	

Table 4. (*Continued*)

Time of harvest	Tissue equivalent Experiment	Leaves 0.1 (i)	Leaves 0.1 (ii)	Seeds Epimatium 0.1	Seeds Epimatium 0.5 (i)	Seeds Epimatium 0.5 (ii)	Seeds Endosperm and embryo 0.1	Seeds Endosperm and embryo 0.5 (i)	Seeds Endosperm and embryo 0.5 (ii)
1976									
February		87	95						
December									
Early				42					
Late				66					
1977									
February				72			74		
April				75			43		
June			64	82			57		
September				94†			88†		
LSD ($P = 0.05$)		9	15	16	14	9	11	10	11

*Stored open to the air at room temperature.
†After storage in plastic bags at 4° for 3 months.

Table 5. Presence of *nor*- or *bisnor*-diterpene dilactones or podolactone-type plant growth inhibitory activity in gymnosperms. Nomenclature follows Dallimore and Jackson [15]

Plant	Presence	Source or reference	Equiv. leaf wt tested (g)
ORDER CONIFERALES			
Podocarpaceae *Podocarpus*			
Section *Dacrycarpus*			
P. cinctus Pilger	−*	UNSW 4208	0.4 dry
P. dacrydioides A. Richard	−	RBG Melb.	1.0 fr.
P. imbricatus Blume	−	CSIRO	0.4 dry
Section *Nageia*			
P. blumei Endlicher	+	CSIRO	0.4 dry
P. nagi (Thunberg) Makino	+	[10]	
Section *Afrocarpus*			
P. gracilior Pilger	+	[10]	
P. gracillimus Stapf.§	+	'Ripponlea'	0.1 fr.
P. falcatus R. Br.	+	UNSW 6997	0.1 fr.
Section *Sundacarpus*			
P. amarus Blume	−	UNSW 3728	0.4 dry
Section *Stachycarpus*			
P. andinus Peoppigg ex Endlicher	+	RBG Melb.	1.0 fr.
P. ferrugineus D. Don	−	UNSW 9295	0.4 dry
P. spicatus R. Br.	−	UNSW 9296	0.4 dry
Section *Podocarpus*			
P. acutifolius Kirk	+	[7]	
P. archboldii Gray	+	UNSW 4300	0.4 dry
P. dispermus White	+	CSIRO	0.4 dry
P. elatus R.Br.	+	[1]	

Table 5. (*Continued*)

Plant	Presence	Source or reference	Equiv. leaf wt tested (g)
P. hallii Kirk	+	[10]	
P. henkelii Stapf.	+	RBG Melb.	0.1 fr.
P. lambertii Klotzch	+	[10]	
P. lawrencii Hooker (*P. alpinus* R.Br.)	+	Uni. Melb.	0.1 fr.
P. macrophyllus (Thunb.) D. Don var. *nakaii* (Hayata) Li et Keng	+	[10]	
P. milanjianus Rendle	+	[25]	
P. nagi (Thunberg) Pilger	+	[13]	
P. neriifolius D. Don	+	[10]	
P. nivalis Hooker fil.	+	[10]	
P. nubigenus Lindley	+	[10]	
P. philippinensis Foxworthy	+	[26]	
P. pilgeri Foxworthy	+	CSIRO	0.4 dry
P. polystachus R.Br.	+	[27]	
P. purdieanus Hooker	+	[28]	
P. salignus D. Don	+	[10]	
P. sellowii Klotzch	+	[10]	
P. spinulosus (Smith) R.Br	+	Burwah, Q.	0.1 fr.
P. totara D. Don ex Lambert	+	[7]	
Phyllocladus			
P. asplenifolius (Labillardière) Hooker fil.	−*	Uni. Melb.	1.0 fr.
P. trichomanoides D. Don	−	RBG Melb.	1.0 fr.
Microstrobos			
M. fitzgeraldi (F. Mueller) Garden et Johnson	−	Yamina	1.0 fr.
M. niphophilus Garden et Johnson	−*	Yamina	1.0 fr.
Microcachrys			
M. tetragona Hooker fil.	?†	Yamina	1.0 fr.
Dacrydium			
D. balansae Brongniart et Grisebach	−*	UNSW 7567	1.0 fr.
D. bidwillii Hooker fil.	−‡	UNSW 7569	1.0 fr.
D. biforme (Hooker) Pilger	−	UNSW 9292	1.0 dry
D. cupressinum Solander	−	UNSW 7568	1.0 dry
D. franklini Hooker fil.	?‡	UNSW 7570	1.0 fr.
D. kirkii F. Mueller	?†	UNSW 9293	0.4 dry
D. laxifolium Hooker fil.	−	UNSW 9294	1.0 dry
Araucariaceae *Agathis*			
A. robusta (C. Moore) F. M. Bailey	−	RBG Melb.	1.0 fr.
Araucaria			
A. bidwillii Hooker	−	RBG Melb.	1.0 fr.
A. cunninghamii D. Don	−	RBG Melb.	1.0 fr.
A. heterophylla (Salisbury) Franco	−*	RBG Melb.	1.0 fr.
Cephalotaxaceae *Cephalotaxus*			
C. harringtonia (Forbes) K. Koch cv *Prostrata*	+	Yamina	0.2 fr.
var. *drupacea* (Siebold et Zuccarini) Koidzumi	+	RBG Melb.	1.0 fr.
Cupressaceae *Actinostrobus*			
A. pyramidalis Miquel	−	Uni. Melb.	1.0 fr.
Callitris			
C. oblonga A et L.C. Rich.	−	Central Vic.	1.0 fr.

Table 5. (*Continued*)

Plant	Presence	Source or reference	Equiv. leaf wt tested (g)
Chamaecyparis			
C. formosensis Matsumura	–	RBG Melb.	1.0 fr.
C. obtusa (Siebold et Zuccarini) Endlicher	–‡	RBG Melb.	1.0 fr.
C. pisifera (Siebold et Zuccarini) Endlicher	–*	RBG Melb.	1.0 fr.
C. thyoides (L.) Britton, Sterns et Poggenberg	–	RBG Melb.	1.0 fr.
Cupressus			
C. abramsiana C.B. Wolf	–	RBG Melb.	1.0 fr.
C. arizonica Greene	–	RBG Melb.	1.0 fr.
C. macrocarpa Hartweg	–	RBG Melb.	1.0 fr.
C. sempervirens L.	–*	RBG Melb.	1.0 fr.
C. torulosa Don	–*	RBG Melb.	1.0 fr.
Diselma			
D. archeri Hooker fil.	–	Yamina	1.0 fr.
Juniperus			
J. chinensis L.	–‡	RBG Melb.	1.0 fr.
J. sabina L.	–‡	RBG Melb.	1.0 fr.
Libocedrus			
L. plumosa (D. Don) Sargent	?*	RBG Melb.	1.0 fr.
Thuja			
T. standishii (Gordon) Carrière	–‡	RBG Melb.	1.0 fr.
Widdringtonia			
Widdringtonia sp.	–	RBG Melb.	1.0 fr.
Pinaceae *Abies*			
A. cilicica (Antione et Kotschy) Carrière	–*	RBG Melb.	1.0 fr.
Cedrus			
C. deodara (Roxburgh) G. Don in Loudon	–*	RBG Melb.	1.0 fr.
Larix			
L. decidua Miller	–‡	RBG Melb.	1.0 fr.
L. gmelini (Ruprecht) Kuzeneva	–*	RBG Melb.	1.0 fr.
Picea			
P. glauca (Moench) Voss	–	RBG Melb.	1.0 fr.
P. schrenkiana Fischer et Meyer	–	RBG Melb.	1.0 fr.
P. smithiana (Wallich) Biossier	–*	RBG Melb.	1.0 fr.
Pinus			
P. centerta Douglas	–*	RBG Melb.	1.0 fr.
P. helepensis Miller var. *stankewiczii* (Sukaczev) Fomin	–	RBG Melb.	1.0 fr.
P. koraiensis Siebold et Zuccarini	–	RBG Melb.	1.0 fr.
P. patula Schlechtendal et Chamisso	–	RBG Melb.	1.0 fr.
P. radiata D. Don	–	RBG Melb.	1.0 fr.
P. roxburghii Sargent	–	RBG Melb.	1.0 fr.
Tsuga			
T. canadensis (L.) Carrière	–	RBG Melb.	1.0 fr.
Taxodiaceae *Cryptomeria*			
C. japonica (L. fil.) Don cv *Lobbii*	–	RBG Melb.	1.0 fr.

Table 5. (*Continued*)

Plant	Presence	Source or reference	Equiv. leaf wt tested (g)
Cunninghamia			
C. lanceolata (Lambert) Hooker fil.	–	Uni. Melb.	1.0 fr.
Metasequoia			
M. glyptostroboides Hu et Cheng	–	RBG Melb.	1.0 fr.
Sequoia			
S. sempervirens (D. Don) Endlicher	–	RBG Melb.	1.0 fr.
Taxodium			
T. ascendens Brongniart	–	RBG Melb.	1.0 fr.
T. distichum (L.) Richards	–	RBG Melb.	1.0 fr.
ORDER TAXALES			
Taxaceae *Taxus*			
T. cuspidata Siebold et Zuccarini cv *Luteo-Baccata*	–‡	RBG Melb.	1.0 fr.
ORDER GINKOALES			
Gingkoaceae *Gingko*			
G. biloba L.	–	Uni. Melb.	1.0 fr.

*Significant inhibitory activity ($P = 0.05$).
†Strong inhibitory activity (growth < 50% control).
‡Moderate inhibitory activity (growth < 70% control).
§Tentatively identified by C. J. Quinn.
?Slight inhibition of greening or "patchy" coloration.
Sources: UNSW, Herbarium, University of N.S.W.; RBG, Royal Botanic Gardens, Melbourne; CSIRO, collected and determined by J. Wolmesley and V. Moriarty for the CSIRO division of Applied Organic Chemistry, Melbourne; 'Ripponlea', 'Ripponlea' Gardens, Melbourne; Uni. Melb., Systems Garden, University of Melbourne; Yamina, Yamina Rare Plant Nursery, Olinda, Victoria; Burwah, Q., collected by H. T. Clifford, Botany Department, Q'land University, Brisbane.

of activity follows the sections of the genus as described by Dallimore and Jackson [15]. The taxonomy of *Podocarpus* is under discussion [16–18]; podolactone-type inhibitory activity might be a useful marker in this field.

Only two extracts, apart from those from *Podocarpus* species, showed a strong positive response. These were from *Cephalotaxus harringtonia* cv Prostrata and *C. harringtonia* var. *drupacea* Kiodzumi. Seeds of *C. harringtonia* cv Fastigiata are known to contain a strong plant growth inhibitor, harringtonolide (**2**) [19]. Bioassay of this compound showed that it had strong podolactone-type activity (Table 1), so the activity of the two extracts can probably be ascribed to harringtonolide.

Another plant growth inhibitor, momilactone B (**3**), which was isolated from rice husks [20], also shows podolactone-type activity (Table 1), as does lycoricidinol (**4**) [2], isolated from *Lycoris radiata* Herb. [21] and *Narcissus pseudonarcissus* L. cv Golden Harvest [22]. Momilactone A (**5**), which does not have the bridge in ring A, was only slightly inhibitory at the highest concentration tested. The structures of these compounds differ considerably from each other, and from *nor*- and *bisnor*-diterpene dilactones, but inspection of models suggests that overall molecular dimensions and shape may be important in their activity, as well as the modifying effects of functional groups, substituents and polarity as illustrated in the *nor*- and *bisnor*-diterpene dilactone series [3, 4].

Thus, while *nor*- and *bisnor*-diterpene dilactone structures do not appear widespread, being limited so far to the genus *Podocarpus*, podolactone-type inhibitory activity is somewhat more so, being a property of compounds isolated from the gymnosperm genera *Podocarpus* and *Cephalotaxus*, and three monocots. Selection and testing of other plant products may show whether this type of plant growth regulatory activity is more general.

EXPERIMENTAL

Plant material. Cones, leaves and receptacles were harvested from mature male and female specimens of *P. elatus* (*ca* 10 m high) growing in the System Garden, University of Melbourne. The harvesting of leaves continued for more than 2 years; in the second year, mature leaves could be distinguished from younger leaves by their dark colour and woodiness of the stems carrying them. Seedlings were grown first in the glasshouse for 12 months, then in the open. Tissues were harvested and extracted immediately, or held at −15° until required. Seeds collected in August, 1975 lost viability on drying in air; in 1977, mature seeds were stored at 4° in plastic bags, after the method of Noel and Van Staden [23] for maintaining viability in *P. henkelii* Stapf.

Bleeding sap was collected in a plastic tube placed above the cut stem of a seedling decapitated just above ground level. Leaves from other conifers were collected in the System Garden, University of Melbourne, the Royal Botanic Garden, Melbourne, Yamina Rare Plant Nursery, Olinda, Victoria, and 'Ripponlea', Elsternwick, Victoria. Where possible, young leaves were used. Tissue equivalents of 1 g fr. wt or 0.4 g dry wt were tested unless the inhibition was extremely strong, when 0.1 g samples were used. Where possible, a single sample of a tissue equivalent of 5 g was also assayed. Voucher specimens are deposited in the Herbarium, Botany Department, University of Melbourne. Extracts from dry leaves collected and determined by J. Wolmesley and V. Moriarty for CSIRO, and dry leaves from collections in the Herbarium, University of New South Wales were also tested. Nomenclature follows Dallimore and Jackson [15].

Extraction: General procedure. Fresh tissue was ground 3 × with MeOH (*ca* 30 ml/g total) and the resulting slurry was filtered, concd to give a concn of approx. 60% aq. MeOH, and extracted 3 × with *n*-hexane. The aq. phase was concd until all (MeOH) was removed, then extracted with 2 vols of EtOH–$CHCl_3$ (1:1). The lower phase was evapd to dryness, the residue taken up in a min vol (usually *ca* 1 ml/g orig.) of EtOAc–EtOH (2:1), and separated on a small column of neutral alumina (Unilab) (deactivated by standing overnight in the same solvent). The fraction eluted from the column with this solvent was evapd to dryness and the residue dissolved in MeOH.

Leachates. Leaves (5 g) were steeped in distilled water (750 ml) at room temp. for 48 hr. The leachate was filtered, concd to 5 ml, 5 ml $CHCl_3$–EtOH (1:1) added, and the lower phase treated in the usual manner.

Extracts from soil. Extracts were prepared from soil under a mature tree (325.6 g, H_2O content 23% w/w) and from a pot containing a 2½ year seedling (390 g, H_2O content 22% w/w). The extract from soil below the tree was prepared by extracting with MeOH by stirring and decantation and then by blending. After filtration, the MeOH was treated as for extracts from leaves. An extract equivalent to 130 g of damp soil was tested in the bioassay. Soil from the pot was shaken with MeOH twice, for 3 and 18 hr, and treated as before. Extracts equivalent to 5 and 50 g of soil were tested.

Bioassay. The method of ref. [2] was used, except that *Pisum sativum* L. cv Melbourne Market was used in some experiments. Samples of the extracts, equivalent to particular ws of plant material, were pipetted into small petri dishes and the solvent evapd in air before adding 2% sucrose. The replication was three or four-fold and the growing period 44 hr. Increases in the fr. wts of the plumular hooks were measured, the results submitted to analysis of variance, and the least significant difference calculated ($P = 0.05$). For comparisons between experiments, mean values of the increases were recalculated as percentages of control growth so that increasing inhibition is represented by decreasing percentage values. In preliminary tests, the blank solutions did not inhibit the growth of the plumular hooks significantly. The colour of the hooks after the growth period was scored as shown in Table 1.

In the bioassay for inhibitors, compounds such as phenols or carboxylic acids (e.g. indoleacetic acid, gibberellic acid or abscisic acid) cannot contribute to promotion or inhibition of growth, as they are retained on the alumina column, as are zeatin and similar bases. Inhibitors such as coumarin, cucurbitacins, duvatrienediols and sesquiterpenoid lactones (e.g. vernolepin and xanthinin) would be expected to pass through such columns. Xanthoxin would also reach the final extract, but as it is about half as active as abscisic acid in the coleoptile straight-growth test [24], it would inhibit growth of plumular hooks only at 10^{-5} M or above, and should not inhibit greening. Coumarin, cucurbitacin B and vernolepin were tested on pea hooks in the usual manner but harringtonolide, momilactones A and B and duva-1,6,10-triene-3,15-diol were first dissolved in Me_2CO before diluting with H_2O to 2×10^{-5} M in 1% (v/v) Me_2CO, then assayed with the appropriate control.

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