Assay of p-*hydroxybenzaldehyde oxidase.* Reaction mixture consisted of 0·5 ml of enzyme extract; 1 μmol NAD; 350 μmol KH_2PO_4 buffer, pH 8·5 containing 5 mM mercaptoethanol; 2 μmol *p*-hydroxybenzaldehyde. Extraction and assay of *p*-hydroxybenzoic acid was as above. The reaction mixture was incubated at 30° for 2 hr.

Identification of reaction products; *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde and *p*-hydroxybenzyl alcohol were identified by their colour reaction with *p*-nitroaniline and R_f's in 2% formate and C_6H_6–HOAc–H_2O (10:7:3) which were very similar to those of authentic samples. Their UV spectra were also very similar to those of standards. Acetic acid was identified in the reaction mixture by GLC. Six separate reaction mixtures containing a total of 120 mg enzyme extract, 6 μmol NAD, 3·5 mmol KH_2PO_4, pH 8·5, containing 5 mM mercaptoethanol were incubated at 30° for 3 hr. The reaction was terminated with 6N HCl and extracted into 3 vols ether. The ether was reduced to dryness and the sample re-suspended in a minimum of ether. 1 μl was used in GLC in a Tracor 550 Gas Chromatogram using a Chromosorb 101 column (Mesh 60/80,) obtained from Alltech. The conditions were carrier (He) flow rate 60 ml/min at pressure 8 psi. Detector flow rate H_2 50 ml/min; at 12 psi; Air 1·2 CFH at 20 psi. Temperature program 70°–250° @ 15°/min. Flame ionisation detector. Protein was estimated by the method of Lowry [11].

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REFERENCES

1. El-Basyouni, S. Z., Chen, D., Ibrahim, R. K., Neish, A. C. and Towers, G. H. N., (1964) *Phytochemistry* **3,** 485.
2. Grisebach, H. and Vollmer, K. O. (1964) *Z. Naturforsch* **B19,** 781.
3. Zenk, M. H. (1966) in *Biosynthesis of Aromatic Compounds* (edited by Billek, G.), p. 45. Pergamon Press, Oxford.
4. Zenk, M. H. (1965) in *Proc. 2nd Meeting European Biochem. Soc.* Vienna (edited by Billek, G.), p. 45. Pergamon Press, Oxford.
5. Alibert, G. and Ranjeva, R. (1971) *FEBS Letters* **19,** 11.
6. Alibert, G., Ranjeva, R. and Boudet, A. (1972) *Biochim. Biophys. Acta* **279(2),** 282.
7. Kindl, H. and Ruis, H. (1971) *Z. Naturforsch* **B26,** 1379.
8. Toms, A. and Wood, J. M. (1970) *Biochemistry* **9,** 337.
9. Camm, E. L. and Towers, G. H. N. (1973) *Phytochemistry* **12,** 1575.
10. Vance, C. P., Tregunna, E. B., Nambudiri, A. M. D. and Towers, G. H. N. (1974) *Biochim. Biophys. Acta* **343,** 138.
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265.

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INHIBITORY ACTIVITY OF THE PHENOLIC GLUCOSIDE PSILOTIN AND ITS REVERSAL BY GIBBERELLIC ACID AND THIOLS

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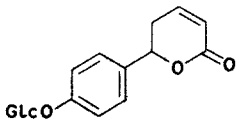
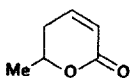
Key Word Index—*Psilotum nudum*; Psilotales; psilotin; unsaturated lactone; seed germination inhibitor; reversed by thio-compounds and gibberellin A_3.

Psilotin, 6-[4′-β-D-glucopyranosyloxyphenyl]-5,6-dihydro-2-oxo-2H-pyran (**1**), was first isolated by McInnes *et al.* [1] from *Psilotum nudum.* Subsequently, Tse *et al.* [2] isolated the compound from *Tmesipteris tannensis* but reported it to be absent from lycopods. McInnes *et al.* [1] called attention to the common structural relationship between psilotin (**1**), parasorbic acid (**2**) and massoilactone, namely the αβ-unsaturated δ-lactone ring, and with these comparisons we suggest inclusion of coumarin. Evidence for growth inhibitory activity in such structures [3] led to the suggestion that psilotin, too, might be biologically active. We can now report that psilotin is in fact an active inhibitor of seed germination and plant growth.



Psilotin (**1**)



Parasorbic acid (**2**)

RESULTS AND DISCUSSION

Seed germination was inhibited in all three species (Table 1). Seeds held for 4–6 days after maximum germination of controls failed to yield additional seedlings, hence were considered dead or dormant. Turnip seed germination and linear growth of seedlings required higher psilotin concentrations than corresponding inhibitions in the other species, and lettuce exhibited the greatest sensitivity. These differences were not reflected in fresh weight data. At a concentration of 10 mmol/liter, psilotin inhibited turnip and lettuce germination completely and limited onion to only 12%.

Table 1. Inhibitory activity of psilotin: ID_{50} values in seed germination and seedling growth by psilotin

Species	Germination	ID_{50} (mM) Seedling Growth (7 days) fresh wt	root length	shoot length
Turnip	5·2 ± 0·4	2·2 ± 0·2	1·6 ± 0·2	2·8 ± 0·2
Onion	1·6 ± 0·2	3·1 ± 0·2	0·8 ± 0·1	1·6 ± 0·2
Lettuce	1·6 ± 0·2	3·4 ± 0·2	0·3 ± 0·1	0·9 ± 0·1

Values were obtained by graphical interpolation of the concentration required for 50% inhibition. Inhibition data for germination was taken when control levels attained this maxima, 24, 48 and 72 hr respectively for turnip, onion and lettuce.

In general, root length was more sensitive to psilotin than other growth parameters in each species, and inhibi-

Table 2. Reversal of the inhibitory effects of psilotin in turnip germination by thio-compounds and GA_3

Antagonist	mM	Psilotin (mM) 0	1	2	5
None	—	86 ± 6	75 ± 6	70 ± 5	45 ± 4
Glutathione	1	81 ± 5	85 ± 7	90 ± 7	78 ± 7
	2	83 ± 6	83 ± 7	86 ± 7	48 ± 5
Thiourea	1	84 ± 7	87 ± 6	92 ± 7	80 ± 7
Thioproline	1	85 ± 7	86 ± 7	90 ± 8	76 ± 6
Hydroxyproline	1	87 ± 6	77 ± 7	71 ± 6	40 ± 5
GA_3(K salt)	0·03	87 ± 7	—	86 ± 7	70 ± 6
	0·075	89 ± 9	—	88 ± 7	74 ± 7
	0·30	89 ± 8	—	91 ± 8	84 ± 7

tion of linear growth differed more among test species than did overall fresh weight. Even the most sensitive assay, root extension in lettuce required a psilotin concentration of 0·3 mM. Levels of this magnitude are not indicative of a particularly potent regulator. However, the reported psilotin contents of 0·13–1·22% (fr. wt) [1,2] corresponds to *ca* 4–37 mmol/kg of tissues hence fall easily within the range of inhibitory concentrations.

The concept of psilotin as part of a regulatory system is reinforced by the demonstration that its inhibitory effects are reversed by glutathione, other thio-compounds and gibberellin A_3 (Table 2). Glutathione, thiourea and thioproline at 1 mM restore nearly full germination to seeds near the ID_{50} level in 5 mM psilotin. Hydroxyproline, lacking the reducing group, is inactive, but gibberellin A_3 is *ca* 10–100-fold more active than the thiols. These responses underscore the coumarin-like character of psilotin [4–6] as well as its potential role in growth regulation.

EXPERIMENTAL

Test materials used in the survey included seeds of turnip, *Brassica rapa* cv Purple Top White Globe; lettuce, *Lactuca sativa* cv Iceberg; and onion, *Allium cepa* cv Yellow Globe. Seed and seedling tests were carried out in petri dishes on moist filter paper at 24° under 500 lx daylight fluorescent light. Germination data are based upon 4–5 replicates each with 75–125 seeds. Growth measurements were based upon triplicates of 35–50 seedlings each. ID_{50} values (concentrations required for 50% inhibition) were obtained by graphical interpolation. Psilotin was provided by Professor G. H. N. Towers, University of British Columbia.

REFERENCES

1. McInnes, A. G., Yoshida, S. and Towers, G. H. N. (1965) *Tetrahedron* **21**, 2939.
2. Tse, A. and Towers, G. H. N. (1967) *Phytochemistry* **6**, 149.
3. Haynes, L. J. and Jones, E. R. H. (1946) *J. Chem. Soc.* 954.
4. Mayer, A. M. and Pioljakoff-Mayer, A. (1961) *Plant Growth Regulation* (R. Klein, ed.), pp. 235–250. Iowa State University Press.
5. Van Overbeek, J. (1966) *Science* **152**, 721.
6. Van, S., Cotterill, J., Degreef, J. and Kint, J. (1972) *Recent Adv. in Phytochemistry* **4**, 165.

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DIARYLPROPANOID FROM *VIROLA MULTINERVIA**

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Key Word Index—*Virola multinervia*; Myristicaceae; 1-(4′-hydroxy-2′-methoxyphenyl)-3-(3″-hydroxy-4″-methoxyphenyl)-propane.

Plant. Virola multinervia Ducke (Myristicaceae), trivial name "ucuúba grande", occurs in the western part of Amazonas State.

Previous work. The root contains *N,N*-dimethyltryptamine and 5-methoxy-*N,N*-dimethyltryptamine [2]. A sample of trunk wood (5·3 kg) was extracted successively with C_6H_6 and EtOH. The C_6H_6-ext. gave sitosterol, stigmasterol, 1-(2′-hydroxy-4′-methoxyphenyl)-3-(3″,4″-methylenedioxyphenyl)-propane (virolane) and 2-hydroxy-1-(2′-hydroxy-4′-methoxyphenyl)-3-(3″,4″-methylenedioxyphenyl)-propane (virolanol) [3].

Present work. The EtOH-ext. (70 g) was extracted successively with boiling light petrol and C_6H_6. The C_6H_6-soln. was evaporated and the residue (20 g) chromatographed on a SiO_2 column. Elution with C_6H_6–$CHCl_3$ 7:3 to 1:1 gave an oil from which a compound, $C_{17}H_{20}O_4$ (150 mg), was isolated by preparative TLC. This was recognized as a dihydroxy-dimethoxy-1,3-diarylpropane by its ^{1}HMR spectrum in which appeared signals due to a CH_2 flanked by two $ArCH_2$ systems, two OMe groups and six aromatic protons. One of the aryls is 3,4-dioxygenated, as evidenced by proton signals between τ 3·15 and 3·35 whose multiplicity can be analysed in the spectrum of the diacetate. The other one is 2,4-dioxygenated as shown by a doublet (τ 3·62, *J* 2 Hz) and a triplet (τ 3·58, *J* 8 & 2 Hz) at the high field, and a doublet (τ 3·00, *J* 8 Hz) at the low field sides of the aromatic region. The signals due to all six aromatic protons were shifted paramagnetically upon formation of the diacetate, reason why one OH, and, consequently, one OMe, must be located on each of the rings. This symmetrical distribution is indicated also by the simplicity of the MS, compatible with the formation of fragments of identical mass from either ring. Intense peaks

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