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.

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DIARYLPROPANOID FROM *VIROLA MULTINERVIA**

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(*Received* 13 *September* 1975)

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

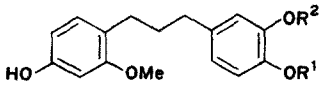
Plant. Virola multinervia Ducke (Myristicaceae), trivial name "ucuúba grande", occurs in the western part of Amazonas State.

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

* Part 6 in the series "The Chemistry of Brazilian Myristicaceae". For Part 5 see Ref. [1]. Sponsored by Ministério do Planejamento (Financiadora de Estudos e Projetos S.A.) through Academia Brasileira de Ciências and by Instituto Nacional de Pesquisas da Amazônia, Manaus.

due to *o*-quinonemethide ions characterize 1,3-diarylpropanes with *o*-hydroxyl [1,3]. In the present case such a peak would appear at *m/e* 136. Its absence locates the methoxyl at the *o*-position. Since thus the OH in the ring under scrutiny must occupy the *p*-position, a positive Gibbs test of the compound locates the OH of the other ring at the *m*-position, as in 1a. This is the isomer of the Gibbs test negative metabolite 1b of *Iryanthera coriacea* Ducke for which very similar ^{1}HMR and MS were reported [3].



(1a) R^1 = Me, R^2 = H
(1b) R^1 = H, R^2 = Me

1-(4′-Hydroxy-2′-methoxyphenyl)-3-(3″-hydroxy-4″-methoxyphenyl)-propane (1a), oil. M found 288·1358, $C_{17}H_{20}O_4$ requires 288·1362, ν_{max}^{film} (cm^{-1}): 3400, 1620, 1600, 1525, 1470, 1240, 1200, 1160, 1035. λ_{max}^{EtOH} (nm): 225 inf., 280 (ε 19500, 7800). $\lambda_{max}^{EtOH+NaOH}$ (nm): 242, 294 (ε 19050, 10950). ^{1}HMR (100 MHz, $CDCl_3$, τ): 3·00 (*d*, *J* 8·0 Hz, H-6′), 3·15–3·35 (*m*, H-2″, 5″, 6″), 3·58 (*dd*, *J* 8·0, 2·0 Hz, H-5′), 3·62 (*d*, *J* 2·0 Hz, H-3′), 6·10 (*s*, OMe), 6·21 (*s*, OMe), 7·25–7·50 (*m*, $2ArCH_2$), 7·9–8·3 (*m*, $CH_2C\underline{H}_2CH_2$). MS (*m/e*): 288 (64%) M, 151 (43), 138 (81), 137 (100), 107 (16). *Diacetate*, oil. ν_{max}^{film} (cm^{-1}): 1760, 1618, 1580, 1500, 1465, 1260, 1200, 1035. ^{1}HMR (100 MHz, $CHCl_3$, τ): 2·88 (*d*, *J* 8·0 Hz, H-6′), 3·05 (*dd*, *J* 8·0, 2·0, H-5′ or 6″), 3·24 (*dd*, *J* 8·0, 2·0 Hz, H-6″ or 5′), 3·12 (*d*, *J* 2·0 Hz, H-2″), 3·15 (*d*, *J* 8·0 Hz, H-5″), 3·40 (*d*, *J* 2·0 Hz, H-3′), 6·19 (*s*, OMe), 6·21 (*s*, OMe), 7·25–7·60 (*m*, $2ArCH_2$), 7·9–8·3 (*m*, $CH_2C\underline{H}_2CH_2$), 7·80 (*s*, 2COMe).

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Phytochemistry, 1976, Vol 15, pp. 568–569. Pergamon Press. Printed in England.

FORMATION OF UBIQUINONE BY TOBACCO PLANT CELLS IN SUSPENSION CULTURE*

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(*Received* 22 *October* 1975)

Key Word Index—*Nicotiana tabacum*; Solanaceae; cell suspension culture; ubiquinone-10.

INTRODUCTION

Since their discovery by Lester [1] and Morton [2], ubiquinones have received much attention from many workers in various fields of research. In the case of cultured plant cells, Threlfall and Goodwin [3] reported the occurrence of ubiquinone-10 in Paul's scarlet rose while Thomas and Stobart examined the time-course of ubiquinone and α-tocopherol formation in *Kalanchoë crenata* callus [4].

According to our investigations [5], tobacco cells in suspension culture appear to contain much more ubiquinone-10 than the parent plants and so might be a suitable source for the large scale production of this compound. In order to obtain basic information on the synthesis of ubiquinone-10 by cultured plant cells, some observations on the variation in the ubiquinone content during the growth of the cultured cells have been made and are detailed in this paper.

RESULTS AND DISCUSSION

The time-course of ubiquinone formation during cell growth was examined using three kinds of cultured cells which have a high growth rate in suspension culture (Fig 1.) The clone BY-2 reached a maximum dry weight 6 days after inoculation, while their ubiquinone content decreased during the early period of the logarithmic phase of growth and then increased reaching the highest level (360 μg per g-dry weight) on the 10th day (Fig. 1). Changes in the ubiquinone content of Xanthi cells were

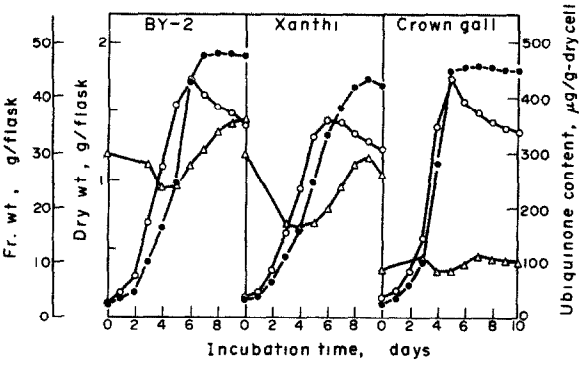

Fig. 1. Cell growth and ubiquinone content in cultured tobacco cells. For the culture media and conditions, see text. Cell growth: ●—● fr. weight; ○—○ dry weight; ubiquinone content: △—△.

*Studies on the Culture Conditions of Higher Plant Cells in Suspension Culture. Part 7.