EXPERIMENTAL

N damascena cells (10 g) were inoculated into 250 ml of Murashige and Skoog medium and grown for 7 days before incubation with 6 mg [2-^{14}C]ABA (35 kBq). Cells (65 g) were harvested after 72 hr by filtration and homogenized in EtOAc–MeOH (5 2). After filtration of the homogenate the extract was evaporated to dryness and dissolved in 500 μl EtOAc–MeOH for chromatography. The culture filtrate, obtained after filtration of cells, was extracted with EtOAc–*n*-PrOH–*n*-BuOH (10 1 2) (×4). The combined extracts were evaporated to dryness. The extracts were subjected to TLC on Si gel HF_{254} (Merck) developed with toluene–EtOAc–MeOH–HOAc (50 30 7 4). The developed plates were scanned for radioactivity and the zones containing the new metabolite (**2**) (R_f 0.43, ABA R_f 0.68) eluted with MeOH. Further purification by CC on Sephadex LH-20 using 1,2-dichloroethane–MeOH (2 1) gave 1.8 mg **2** as a colourless intractable gum. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH, *br*), 1668 (C=C–C=O), 1600 (>C=C<), UV λ_{max}^{MeOH} nm (log ε): 256 (3, 24), EIMS, 80 eV, *m/z* (rel. int.): 280 $[M]^+$ (2), 262 $[M-H_2O]^+$ (17), 244 $[M-2H_2O]^+$ (20), 229 $[M-2H_2O-Me]^+$ (14), 224 **a** (13), 218 $[M-H_2O-CO_2]^+$ (41), 206 (**a** – H_2O) (75), 188 (**a** – $2H_2O$) (92), 161 (83), 122 (48), 111 **b** (78), 97 (100). ^{1}H NMR (200.13 MHz, $CDCl_3$, TMS): δ 1.01 (3H, *s*, Me-6'), 1.10 (3H, *s*, Me-6'), 2.03 (3H, *s*, Me-3), 2.28 and 2.44 (each 1H, *d*, J = 18 Hz, H-5'), 4.24 and 4.46 (each 1H, *d*, J = 18 Hz, CH_2OH-2'), 5.75 (1H, *s*, H-2), 6.18 (1H, *s*, H-3'), 6.14 (1H, *d*, J = 16 Hz, H-5), 7.65 (1H, *d*, J = 16 Hz, H-4).

3: EIMS, 80 eV, *m/z* (rel. int.): 294 $[M]^+$ (1), 276 $[M-H_2O]^+$ (2), 263 $[M-OMe]^+$ (2), 258 $[M-2H_2O]^+$ (2), 243 $[M-2H_2O-Me]^+$ (2), 238 **a** (4), 220 (**a** – H_2O) (6), 206 (**a** – MeOH) (25), 188 (**a** – MeOH – H_2O) (34), 161 (22), 125 **b** (51), 83 (100).

The MS were recorded on a Varian MAT 111 instrument (direct inlet system, electron energy 80 eV, source temp. *ca* 300°, inlet temp. *ca* 120°).

Acknowledgement—We wish to thank Dr. H. Bohm for providing cell suspension cultures.

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Phytochemistry, Vol. 22, No. 5, pp. 1278–1280, 1983
Printed in Great Britain

0031-9422/83/051278-03$03.00/0

ISOLATION AND CHARACTERIZATION OF DIHYDROMALEIMIDE AND ITS GLUCOSIDE AS GROWTH INHIBITORS FROM DWARF PEA

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(*Revised received* 27 *September* 1982)

Key Word Index—*Pisum sativum*, Leguminosae, dwarf pea, growth inhibitor, *R*-dihydromaleimide, *R*-dihydromaleimide-β-D-glucoside.

Abstract—Two new growth inhibitors, *R*-dihydromaleimide and *R*-dihydromaleimide β-D-glucoside, were isolated from 2-week-old pea shoots.

In spite of many studies [1–7], plant dwarfism is not fully understood in terms of growth inhibitors. We report now on the isolation of two new growth inhibitors (**1** and **2**) from 12 kg fr. wt of dwarf pea (cv Progress No. 9) shoots grown under *ca* 1 klx of white fluorescent light at 18–23° for 2 weeks. The bioassay procedures used were the lettuce germination and hypocotyl elongation tests.

Compound **1** (0.8 g), colourless prisms, mp 103–105°, $C_4H_5NO_2$, $[\alpha]_D^{23}$ −126°, was characterized as (−)-dihydromaleimide (**1**) by its spectroscopic properties, and by direct comparison with (±)-dihydromaleimide derived from maleimide by a partial reduction by sodium boro-

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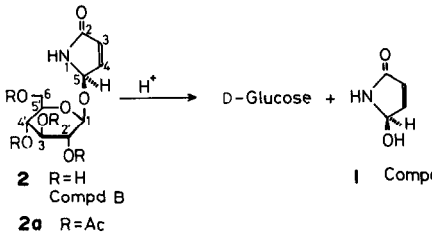

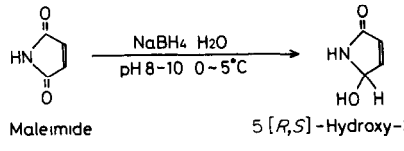

hydride in a pH-controlled aqueous solution.

Compound **2** (7 5 g), colourless prisms, mp 185–188°, $C_{10}H_{15}NO_7$, $[\alpha]_D^{22\,5}$ −73.7°, gave D-glucose ($[\alpha]_D^{23\,5}$ +41.1°) and compound **1** on hydrolysis with dilute hydrochloric acid. This and the ^{13}C NMR spectrum of **2** suggested that it was the β-D-glucoside of **1**. The slow disappearance of a broad signal at δ 6.77 in the ^1H NMR spectrum of the tetra-acetate (**2a**) of **2** after the addition of D_2O indicated that the above signal was not due to the hydroxyl but to the NH proton Thus, **2** was deduced to be the *O*-β-D-glucoside of **1**.

The CD curves of **1** and **2** showed negative Cotton effects at the π–π^* region ($\Delta\varepsilon_{205} = -1\,42$ and $\Delta\varepsilon_{200} = -14.91$ for **1** and **2**, respectively) and the absolute configuration of C-5 was deduced to be *R* according to Uchida and Kuriyama's rule [8].

Liu and Castelfranco [9] isolated from pea plants (cv Radio) a glucoside for which they proposed a plane structure identical with that of **2** and claimed that it might be identical with pisatoside reported by Kocourek *et al.* [10], based merely on the colour reaction to a diphenylamine reagent, mp and IR spectrum. The physicochemical properties and colour reaction of pisatoside and of its aglycone are mostly identical with those of **2** and **1**, respectively, and those of Liu and Castelfranco's glucoside are also similar to those of our compound **2** However, the mp and $[\alpha]_D$ values of the tetra-acetate of pisatoside are considerably different from those of **2** tetra-acetate (**2a**). It may be assumed that pisatoside has the same plane structure as that of **2** but is different in the stereochemistry at C-5. It is not clear, however, with which compound Liu and Castelfranco's glucoside is identical because of the lack of its $[\alpha]_D$ data

Compound **1** is not an artefact produced in the isolation process because a quantitative study showed that the distributions of **1** and **2** in dwarf pea shoots differ from each other [Miyamoto, K., Hashimoto, T. and Hasegawa, K., unpublished results].

The growth inhibitory activities of dihydromaleimide were shown with intact seedlings of dark-grown dwarf pea (cv Progress No 9) ($\geqq 20\,\mu g$/plant; I_{50} 300 μg/plant) and normal pea (cv Alaska) ($\geqq 200\,\mu g$/plant; I_{50} 600 μg/plant) as well as with those of dark grown barley (I_{50}, 3×10^{-3} M) and light-grown lettuce ($\geqq 5 \times 10^{-5}$ M; I_{50} 2.5×10^{-4} M). Inhibitory activities were also found in the indoleacetic acid induced elongation of wheat coleoptile sections ($\geqq 10^{-5}$ M; I_{50} 3×10^{-3} M) as well as in lettuce seed germination ($\geqq 10^{-4}$ M; I_{50}, 5×10^{-4} M). The glucoside was active only when applied to the root systems, suggesting that the aglycone released on hydrolysis may exert the action. The activities of *R*- and *R,S*-dihydromaleimides were not different in lettuce hypocotyls, indicating that both isomers were equally active. Although the effective dosage level of **1** was fairly high, it was comparable to that of plant growth retardants such as CCC (Cycocel), AMO-1618 and maleic hydrazide in the lettuce hypocotyl tests under the same bioassay conditions

EXPERIMENTAL

Isolation of **1** *and* **2** Shoots (12 kg fr wt) of light-grown pea (*Pisum sativum* L. cv Progress No 9) seedlings were extracted with 80% aq Me_2CO and, after removing lipid fractions at pH 7 and 3, the remaining aq fraction was adsorbed on charcoal (250 g) and eluted with 80% aq Me_2CO The eluate was chromatographed on a reverse phase Si gel (silanized Si gel, Merck) column with H_2O followed by 50% aq MeOH The former eluate was chromatographed on a Sephadex LH-20 column, the MeOH eluate of which gave **2** (7 5 g) Purification of the mother liquor by charcoal CC (eluted by Me_2CO) followed by Si gel chromatography (eluted by MeOH) gave **1** (0 8 g)

Compound **1** UV $\lambda_{max}^{CHCl_3}$ nm (ε) 237 (1030), IR ν_{max}^{KBr} cm^{-1} 3300, 1688, 1646, 1588, ^1H NMR (CD_3OD) δ 7 07 (1H, *dd*, *J* = 6, 2 Hz, H-4), 6 08 (1H, *d*, *J* = 6 Hz, H-3), 5 63 (1H, *d*, *J* = 2 Hz, H-5), ^{13}C NMR (CD_3OD) δ 175 0 (C-2), 150 8 (C-4), 128 1 (C-3), 82 2 (C-5), CD (H_2O, *c* 0 103) nm (Δε) 240 (+0 26), 205 (−1 42) (Found C, 48 5; H, 5 1; N, 13 4% $C_4H_5NO_2$ requires C, 48 5, H, 5 1, N, 14 1%, MW 99.)

Compound **2** UV λ_{max}^{MeOH} nm (ε) 198 (9000), 234 (1490), IR ν_{max}^{KBr} cm^{-1} 3300, 1692, 1660, 1594, ^1H NMR (CD_3OD–D_2O) δ 7 67 (1H, *dd*, *J* = 6, 1 8 Hz, H-4), 6 65 (1H, *d*, *J* = 6 Hz, H-3), 6 28 (1H, *br s*, H-5), 4 93–3 78 (7H, *m*, glucose), ^{13}C NMR (D_2O–CD_3OD) δ 176 2 (C-2), 148 7 (C-4), 128 6 (C-3), 101 3 (C-1'), 87 8 (C-5), 77 3 (C-3'), 76 8 (C-5'), 74 0 (C-2'), 70 6 (C-4'), 61 8 (C-6'), CD (H_2O, *c* 0 033) nm (Δε) 242 5 (+2 48), 200 (−14 91) (Found C, 45 8, H, 5 8, N, 5 3% $C_{10}H_{15}NO_7$ requires C, 46.0; H, 5 8, N, 5 4%, MW 261.)

Tetra-acetate of compound **2** (**2a**). Colourless prisms, mp 144–145°, $[\alpha]_D^{23}$ +213 3° ± 7 3° (dioxane, *c* 0 35), UV $\lambda_{max}^{CHCl_3}$ nm (ε). 198 (12 500), 235 (1420), IR ν_{max}^{KBr} cm^{-1}. 3300, 1756, 1742, 1716, 1701, 1601, 1237, ^1H NMR ($CDCl_3$) δ 6 93 (1H, *ddd*, *J* = 6, 2, 1 Hz, H-4), 6.77 (1H, *m*, NH), 6 10 (1H, *ddd*, *J* = 6, 1, 1 Hz, H-3), 5 63 (1H, *m*, H-5), 5 25–4 92 (3H, *m*, glucose), 4 70 (1H, *d*, *J* = 7 Hz, glucose), 4 23 (1H, *s*, glucose), 4 17 (1H, *d*, *J* = 1 Hz, glucose), 3 72 (1H, *m*, glucose), 2 10, 2 05, 2 02, 1 98 (each 3H, *s*, COMe); ^{13}C NMR ($CDCl_3$) δ 172 7 (C-2), 170 8 (acetyl CO), 170 2 (acetyl CO), 169 5 (× 2, acetyl CO), 140 6 (C-4), 128 7 (C-3), 96.9 (C-1'), 85 8 (C-5), 72.8 (C-3'), 72 1 (C-5'), 70 9 (C-2'), 68.3 (C-4'), 61 9 (C-6'), 20.7 (acetyl Me), 20 5 (× 3, acetyl Me) (Found C, 50 4, H, 5 5, N, 3 0% $C_{18}H_{23}NO_{11}$ requires C, 50 4, H, 5 4, N, 3.3%, MW 429.)

Reduction of maleimide An ice-cooled aq soln of $NaBH_4$ (7 00 g) adjusted to pH 8.0 with dilute HCl was slowly added to an ice-cooled aq soln of maleimide (7 24 g), keeping the pH at 8–10 according to ref [11] The reaction was stopped by lowering the pH below 5 0 by addition of dilute HCl

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Phytochemistry, Vol 22, No 5, pp 1280–1281, 1983
Printed in Great Britain

0031–9422/83/051280–02$03 00/0
Pergamon Press Ltd

AN URUSHIOL DERIVATIVE FROM POISON SUMAC

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(*Received* 31 *August* 1982)

Key Word Index—*Toxicodendron vernix*, Anacardiaceae, urushiol derivative, catechols

Abstract—GC analysis of the ethanolic extract of poison sumac (*Toxicodendron vernix*) showed five urushiol components four of which were identical with those from poison ivy (*T radicans*) The new component was determined to be 3-*n*-pentadec-8,11,13-trienylcatechol

INTRODUCTION

In a previous publication, the analysis of the different components of poison ivy (*Toxicodendron radicans*) and poison oak (*T diversilobum*) urushiols were reported [1] The third most widely distributed member of the contact dermatitis causing plants of the family Anacardiaceae is poison sumac (*T vernix*) whose urushiol has not been fully examined and is reported here

RESULTS AND DISCUSSION

GC analysis of poison sumac (*Toxicodendron vernix*) urushiol showed that four of its components were identical with those of poison ivy urushiol and represent 40% of the total The remaining 60% was a single unidentified component GC/MS analysis of the TMSi derivative showed that the new component had the same MW as the triolefinic isomer of poison ivy urushiol and catalytic hydrogenation gave a single product, 3-*n*-pentadecylcatechol (PDC) This indicated that the new component must be a double bond isomer of the 8,11,14-triene component of poison ivy urushiol

The mixture was separated by reversed phase HPLC GC analysis of the separated components showed that the new triene was contaminated with 10% of the 8,11,14-triene The two isomers were only separable by GC and thus the mixture was used for spectral analysis without further purification The presence of a vinyl methyl and the absence of a terminal methylene signal in ^{1}H NMR and ^{13}C NMR of the new triene fixed the position of the terminal double bond at C-13 Osmium tetroxide–potassium periodate oxidation of the dimethyl ethers of the triene mixture gave ω-(2,3-dimethoxyphenyl)-capryl aldehyde Isolation of this aldehyde indicated that the first double bond in the new triene was at C-8 The position of the middle double bond was determined by subjecting the dimethyl ether of the triene mixture and that of pure 8,11,14 triene to osmium tetroxide oxidation, the product of each reaction was then partially oxidized with potassium periodate. GC/MS analysis indicated that two peaks are produced, the first due to ω-(2,3-dimethoxyphenyl)-capryl aldehyde and the second showed ions at m/z 337, 320 and 302 in both cases The presence of identical mass spectral fragments in the partial oxidation reaction products of both trienes indicated that the position of the middle double bond is the same in both compounds This confirms the structure of the new poison sumac component to be 3-*n*-pentadec-8,11,13-trienylcatechol The same triolefinic component has been reported from Japanese Lac urushiol by Sunthankar and Dawson [4], but the assignment of the second and third double bonds was not unequivocal The allerginicity of poison sumac urushiol was found to be similar to that of poison ivy urushiol and the same product could be used for production of tolerance and desensitization to both urushiols [5]