Phytochemistry, Vol. 21, No. 8, pp. 1935–1937, 1982.
Printed in Great Britain.

0031–9422/82/081935–03$03.00/0

PHENOLIC PLANT GROWTH INHIBITORS FROM THE FLOWERS OF *CUCURBITA PEPO*

HIDEJI ITOKAWA, YOSHIAKI OSHIDA, AKIRA IKUTA, HIDEO INATOMI* and TAKASHI ADACHI*

Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji, Tokyo, Japan; *Faculty of Agriculture, Meiji University, 5158 Ikuta, Tamaku, Kawasaki, Kanagawa, Japan

(*Revised received* 2 *December* 1981)

Key Word Index—*Cucurbita pepo*; Cucurbitaceae; male flowers; plant growth inhibitor; benzyl glucoside; 4-methoxybenzyl glucoside; 3, 4-dimethoxybenzyl glucoside.

Abstract—Eleven compounds isolated from the growth inhibiting active fraction of male flowers of *Cucurbita pepo*, were identified as *p*-hydroxybenzaldehyde, anisyl alcohol, *p*-hydroxybenzyl methyl ether, *p*-hydroxybenzyl alcohol, veratryl alcohol, isovanillyl alcohol, *p*-coumaric acid, phloretic acid, benzyl-β-D-glucoside, 4-methoxybenzyl-β-D-glucoside and 3, 4-dimethoxybenzyl-β-D-glucoside. Each compound was assayed for growth inhibiting activity using lettuce seedlings; three showed strong activity, whereas the glucosides were inactive.

INTRODUCTION

Many plant growth regulators are known to occur in seeds of *Cucurbita pepo* [1]. On the basis of these observations, the occurrence of new plant growth regulators was surveyed in flowers of *C. pepo*. In the course of this investigation, we have previously reported two new flavonol glycosides [2]. We now wish to report the isolation and identification of plant growth inhibitors in an active fraction of the male flowers.

RESULT AND DISCUSSION

Fractionation of the methanolic extract of the male flowers was followed by a growth inhibiting bioassay using lettuce seedlings which afforded four fractions. The strong inhibiting activity was found in fraction 2, which was chromatographed on a Si gel column in chloroform–methanol and *n*-hexane–ethyl acetate to give eight substances **1–8** and a mixed fraction.

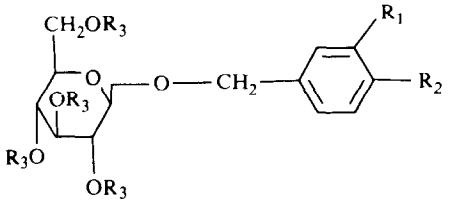
Compounds **1–8** were identified by standard procedures (UV, IR, ^{1}H NMR and mass spectra and by comparison with authentic samples) as *p*-hydroxybenzaldehyde **1**, anisyl alcohol **2**, *p*-hydroxybenzyl methyl ether **3**, *p*-hydroxybenzyl alcohol **4**, veratryl alcohol **5**, isovanillyl alcohol **6**, *p*-coumaric acid **7** and phloretic acid **8**.

After acetylation of the mixed fraction, the three acetylated compounds **9–11** were isolated by HPLC.

These compounds appeared to be benzyl glycoside derivatives from UV and ^{1}H NMR studies and ^{13}C NMR showed the presence of a benzyl group. Six carbons of each sugar moiety suggested the presence of β-glucose. Hydrolysis by β-glucosidase of the deacetylated compounds **12–14** afforded as aglycones benzyl alcohol, anisyl alcohol and veratryl alcohol respectively. Thus these glycosides are benzyl-β-D-glucoside **12**, 3-methoxybenzyl-β-D-glucoside **13** and 3, 4-dimethoxybenzyl-β-D-glucoside **14**. They were further identified by comparison with synthetic samples prepared from tetra-acetylglucosyl bromide and the corresponding benzyl alcohol.

Six compounds (**4–6** and **12–14**) isolated from the male flowers are reported for the first time from natural sources. Few benzyl derivatives have been reported before, especially as their glucosides [3].

The growth inhibition of the isolated compounds were studied in lettuce seedlings. As shown Table 1, *p*-hydroxybenzaldehyde **1**, anisyl alcohol **2** and *p*-coumaric acid **7** showed strong inhibition and phloretic acid was moderately inhibitory. *p*-Coumaric acid [4] and *p*-hydroxybenzaldehyde are known as growth inhibitors, the latter, for example, inhibits the growth of *Chlorella* [5].



9 $R_1 = R_2 = H$, $R_3 = Ac$
10 $R_1 = H$, $R_2 = OMe$, $R_3 = Ac$
11 $R_1 = R_2 = OMe$, $R_3 = Ac$
12 $R_1 = R_2 = R_3 = H$
13 $R_1 = R_3 = H$, $R_2 = OMe$
14 $R_1 = R_2 = OMe$, $R_3 = H$

Although benzoic acid derivatives have been previously reported as being capable of inhibiting growth [4], anisyl alcohol is newly reported as having growth inhibiting activity. It is interesting physiologically that three of the eleven substances present together in male flower parts show growth inhibiting activity. It is also of interest that the benzyl alcohol glucosides do not occur in other parts of this plant except the buds and flowers (Table 2). Juneja *et al.* [6] reported that the major neutral metabolite of benzyl alcohol is benzyl-β-D-glucoside in wheat, sorghum and barley. Also in gibberellin studies, glucosides are generally less active than the free gibberellins [7–9]. In our own case, it is also apparent that benzyl glucoside are inactive, whereas some of the free phenols of the male flowers show significant inhibitory activities.

EXPERIMENTAL

TLC on Si gel ($60F_{254}$, Merck) in (1) *n*-hexane–EtOAc (1:1), (2) $CHCl_3$–MeOH (9:1), (4:1), (3) $CHCl_3$–MeOH–HOAc (6:1:0.1), (4) *n*-BuOH–HOAc–H_2O (5:1:1), (5) pyridine–EtOAc–HOAc–H_2O (5:1:1:3). The MeOH extract obtained from the male flowers of *Cucurbita pepo* was dissolved in H_2O and successively extracted with *n*-hexane, EtOAc and *n*-BuOH (fraction 3). The *n*-hexane and EtOAc extract was evaporated and then separated into H_2O-soluble (fraction 2) and insoluble fractions (fraction 1). Fraction 2 was separated by CC with Si gel, eluting with $CHCl_3$–MeOH and *n*-hexane–EtOAc. Mixed acetates were separated by HPLC (*n*-hexane–EtOAc, 5:4) and obtained as compounds **9–11**.

Benzyl-β-D-glucoside tetra-acetate, **9**. Mp 96–97° colorless needles (*n*-hexane–Et_2O), $[\alpha]_D^{25}$ −50.1° (MeOH; *c* 1.05). UV

Table 1. Effect of compounds **1–8** and **12–14** on the germination and growth of *Lactuca sativa*

Tested compound	Part*	Concentration (mol/l.) 5×10^{-4}	10^{-3}	2×10^{-3}	5×10^{-3}	10^{-2}	0
1	R	−20.9	−24.4	−28.5	−51.2	−100	
	H	−20.1 (95.0)†	−23.7 (95.0)	−30.2 (95.0)	−52.6 (62.5)	−100 (0)	(90.0)
2	R	−9.9	−16.8	−35.3	−72.6	−100	
	H	−10.3 (88.5)	−16.2 (88.1)	−28.1 (89.5)	−66.7 (70.5)	−100 (0)	(90.0)
3	R	+0.9	+2.6	+3.2	+1.2	+1.3	
	H	−0.1 (100)	−0.5 (100)	+0.1 (99.4)	−1.4 (100)	−0.2 (99.4)	(100)
4	R	+4.2	+9.0	+7.5	+2.7	−22.6	
	H	+1.4 (94.4)	+1.1 (96.9)	−0.7 (97.4)	−4.3 (97.4)	−23.9 (95.0)	(96.3)
5	R	−4.6	−11.9	−27.4	−37.5	−51.3	
	H	−2.4 (99.4)	−0.6 (98.8)	−4.0 (95.0)	−11.0 (96.2)	−34.5 (89.4)	(98.8)
6	R	−2.1	−3.9	−9.0	−10.9	−21.7	
	H	−3.4 (93.8)	−3.8 (93.1)	−6.9 (92.5)	−11.3 (91.9)	−24.2 (86.3)	(94.4)
7	R	+4.5	−7.8	−40.8	−79.3	−99.8	
	H	−2.1 (100)	−4.5 (98.8)	−10.4 (97.5)	−56.8 (69.4)	−99.8 (0.6)	(100)
8	R	−0.7	0	−0.3	−26.7	−97.8	
	H	−1.0 (99.4)	−1.0 (99.4)	−1.6 (99.4)	−15.2 (87.5)	−94.3 (6.9)	(100)
12	R	−0.3	−0.8	−0.5	−1.6	−11.1	
	H	0 (98.1)	−0.6 (100)	−1.9 (98.8)	−4.1 (99.4)	−10.3 (98.8)	(98.8)
13	R	−2.5	−11.1	−19.9	−30.0	−36.0	
	H	−5.1 (98.8)	−8.9 (100)	−9.3 (99.4)	−16.1 (96.9)	−23.4 (88.1)	(100)
14	R	−2.0	−4.5	−5.7	−17.6	−23.7	
	H	−0.2 (99.4)	−0.8 (100)	−2.7 (100)	−6.0 (100)	−14.7 (99.4)	(100)

*R, radicle; H, hypocotyl.
†Germination percentage.

Table 2. Quantity of the glycoside mixture from bud stage to flowering stage

	Stage										
	Flower	Bud									
Node	0	1	2	3	4	5	6	7	8	9	10
Average fr. wt/part (g)	6.9	3.4	2.4	1.9	1.5	1.2	0.9	0.7	0.6	0.5	0.5
Glycoside mixture (ppm)	3.1*	2.1	0.8	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

*__12__:__13__:__14__ = 10:10:1.
N.D., not detected.

λ_{max}^{MeOH} nm (ϵ): 218 (1400), 247 (150), 252 (180), 258 (220), 264 (170), 268 (110). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 1750, 1220, 1040, 750. ^{1}H NMR (δ, ppm, CCl_4): 1.94–2.06 (12H, *s*), 3.5–5.0 (9H), 7.21 (5H, *m*). MS *m/z* (%): 438 $[M]^+$ (0.4), 347 (7.8), 331 (15.8), 259 (17.5), 245 (35.3), 216 (12.5), 169 (17.5), 157 (25), 152 (40.6), 139 (67.5), 115 (23.8), 97 (42.5), 91 (100). ^{13}C NMR (δ, ppm, $CDCl_3$): 171.0 (*s*), 169.3 (*s*), 128.6 (*s*), 128.4 (*d*), 128.3 (*d*), 127.7 (*d*), 99.3 (*d*), 72.9 (*d*), 71.8 (*d*), 71.3 (*d*), 70.6 (*t*), 68.5 (*d*), 62.0 (*t*), 20.6 (*q*).

Deacetylate **12**. Colorless oil. UV λ_{max}^{MeOH} nm (ϵ): 217 (1400), 247 (100), 252 (110), 258 (130), 264 (110), 267 (80). IR ν_{max} cm^{-1}: 3260, 1660, 1390, 1380, 1080, 1040. ^{1}H NMR (δ, ppm, CD_3OD): 3.6–5.0 (9H), 7.40 (5H, *m*). MS *m/z* (%): 271 $[M+1]^+$ (1.2), 253 (2.5), 209 (5.6), 163 (30.0), 143 (27.5), 108 (22.5), 91 (100).

4-*Methoxybenzyl-β-*D*-glucoside tetra-acetate* **10**. Colorless oil, $[\alpha]_D^{25} - 43.1°$ (MeOH; *c* 0.96) UV λ_{max}^{MeOH} nm (ϵ): 227 (9600), 273 (1500), 280 (sh, 1300). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1280–1200, 1080–1020, 755. ^{1}H NMR (δ, ppm, CCl_4): 1.94–2.06 (12H, *s*), 3.75 (3H, *s*), 3.5–5.0 (9H) 6.74 (2H, *d*, J = 8 Hz), 7.12 (2H, *d*, J = 8 Hz). MS *m/z* (%): 468 $[M]^+$ (3.8), 348 (3.8), 331 (6.3), 259 (2.5), 245 (5.6), 169 (13.8), 152 (51.3), 139 (33.1), 122 (38.8), 121 (100). ^{13}C NMR (δ, ppm, $CDCl_3$): 170.6 (*s*), 170.2 (*s*), 169.3 (*s*), 159.5 (*s*), 129.6 (*s*), 128.6 (*d*), 113.9 (*d*), 98.9 (*d*), 72.9 (*d*), 71.9 (*d*), 71.4 (*d*), 70.5 (*t*), 68.6 (*d*), 62.1 (*t*), 55.3 (*q*), 20.6 (*q*).

Deacetylate **13**. Mp 139–140.5° colorless needles (*n*-hexane–Et_2O). UV λ_{max}^{MeOH} nm (ϵ): 227 (9900), 273 (1400), 280 (sh, 1200). IR ν_{max}^{KBr} cm^{-1}: 3260, 1610, 1510, 1250, 1070, 1030. ^{1}H NMR (δ, ppm, CD_3OD): 3.80 (3H, *s*), 3.6–4.9 (9H), 6.92 (2H, *d*, J = 8 Hz); 7.38 (2H, *d*, J = 8 Hz). MS *m/z* (%): 300 $[M]^+$ (2.3), 163 (9.4), 122 (100), 121 (88.2).

3,4-*Dimethoxybenzyl-β-*D*-glucoside tetra-acetate*, **11**. Mp 123–124° colorless needles (*n*-hexane–Et_2O), $[\alpha]_D^{25} - 51.2°$ (MeOH, *c* 0.26). UV λ_{max}^{MeOH} nm (ϵ): 211 (8100), 232 (8900), 278 (3100), 282 (sh, 2800). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3010, 1750, 1270–1210, 1040. ^{1}H NMR (δ, ppm, CCl_4): 1.98–2.08 (12H, *s*), 3.79 (6H, *s*), 3.4–5.0 (9H), 6.70 (3H, *m*). MS *m/z* (%): 498 $[M]^+$ (1.4), 331 (5.7), 289 (7.1), 169 (22.9), 166 (17.9), 157 (23.0), 152 (25.7), 151 (100), 115 (43.6), 98 (33.6).

Deacetylate, **14**. Colorless oil. UV λ_{max}^{MeOH} nm (ϵ): 210 (5600), 232 (6100), 278 (2100), 282 (sh, 1900). IR ν_{max} cm^{-1}: 3240, 1660, 1520, 1260, 1080, 1020. ^{1}H NMR (δ, ppm, $CDCl_3$): 3.82 (6H, *s*), 3.6–4.9 (9H); 7.02 (2H, *m*). MS *m/z* (%): 330 $[M]^+$ (9.5), 168 (33.3), 152 (31.7), 151 (100).

Synthesis of **9–11**. The appropriate benzyl alcohol was condensed with tetra-acetylglucosyl bromide in dry Et_2O in the presence of Ag_2O [14]. The product was purified by the HPLC (*n*-hexane–EtOAc, 5:4) and recrystallized from *n*-hexane–Et_2O.

Growth inhibiting expts. Lactuca sativa seeds (40) treated with $HgCl_2$ were placed on one filter paper in a 9 cm Petri dish with 3 ml test soln per dish, and the control plants treated with H_2O alone. pHs were uncorr. The dishes were placed in a growth room at 20° in the dark. After 98 hr, the test and control plants were compared. The results reported represent the average of four expts.

$$\text{Activity of growth inhibition } (-\%) = 100 - \frac{\text{Length after treatment of the samples}}{\text{Length after treatment of the controls}} \times 100.$$

Acknowledgement—We acknowledge the assistance of members of the Central Analytical Laboratory of this University.

REFERENCES

1. Fukui, H., Koshimizu, K., Usuda, S. and Yamazaki, Y. (1977) *Agric. Biol. Chem.* **41**, 175.
2. Itokawa, H., Oshida, Y., Ikuta, A., Inatomi, H. and Ikegami, S. (1981) *Phytochemistry* **20**, 2421.
3. Kosheleva, L. I. and Nikonov, G. K. (1968) *Farmatsiya* **17**, 40.
4. Shibata, S. (1978) *Biological Active Natural Products* pp. 6–33. Ishiyakushupan, Japan.
5. Dedonder, A. and Sumere, C. F. V. (1971) *Z. Pflanzenphysiol.* **65**, 70.
6. Juneja, P. S., Pearcy, S. C. and Gholson, P. K. (1975) *Plant Physiol.* **56**, 385.
7. Yamane, H., Murofushi, N., Osada, H. and Takahashi, N. (1977) *Phytochemistry* **16**, 831.
8. Yamane, H., Yamaguchi, I., Yokota, T., Murofushi, N. and Takahashi, N. (1973) *Phytochemistry* **12**, 255.
9. Yokota, T., Kobayashi, S., Yamane, H. and Takahashi, N. (1978) *Agric. Biol. Chem.* **42**, 1811.