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Combined Effect on Plant Growth of (−)-Epicatechin and Hydroquinone, Compounds from *Aesculus californica* NUTT. (Hippocastanaceae)

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Hydroquinone and (−)-epicatechin were isolated from seeds of *Aesculus californica* NUTT. (Hippocastanaceae), and their effect on the growth of lettuce and rice seedlings was examined. Hydroquinone inhibited germination at high concentrations (above 100 μg/ml) but it stimulated growth at low concentrations for both lettuce and rice seedlings. (−)-Epicatechin showed little activity by itself. However, when applied with hydroquinone, (−)-epicatechin tended to counteract the growth inhibitory activity of hydroquinone.

Keywords——*Aesculus californica*; California buckeye; hydroquinone; (−)-epicatechin; combined effect; plant growth regulator

The California buckeye, *Aesculus californica* NUTT. (Hippocastanaceae), is a small tree that is found in the canyons and dry hillsides of the Pacific Coast Range and in the foothills of Sierra Nevada. The seeds of this tree are quite large and are toxic.[1] Californian Indians used to grind up these seeds with water and use the resulting mixture as a fish poison; they also used the seeds as a foodstuff after they had leached out the toxins. We were interested in the fact that other plants seem not to grow under this tree, suggesting that metabolites of this tree may include an allelopathic compounds. Therefore, as a part of our search for biologically active compounds, we examined the growth-inhibitory properties of components of the seeds of *A. californica*.

In our preliminary screening test for anti-germination activity towards lettuce seeds,[2] a methanol extract of the seeds of *A. californica* showed activity at 5000 ppm. Separation of the methanol extract into fractions soluble in *n*-hexane, ethyl acetate, *n*-butanol, and water, and bioassay at 500 ppm indicated that the active component was in the ethyl acetate fraction. Two major compounds **1** (0.74% of the weight of the methanol extract) and **2** (0.60% of the weight of the methanol extract) in the active ethyl acetate fraction were isolated by silica gel column chromatography. These two compounds **1** (mp 238—240 °C, MW 290) and **2** (mp 165 °C, MW 110) were identified as (−)-epicatechin[3] and hydroquinone,[4] respectively, on the basis of various spectral data (infrared (IR) spectrum, mass spectrum (MS), ultraviolet (UV) spectrum, specific optical rotation ([α]), proton (^{1}H-) and carbon-13 (^{13}C-) nuclear magnetic resonance (NMR) spectra).

The effect of hydroquinone on the growth of lettuce and rice[5] seedlings was examined and the results are listed in Table I. At concentrations larger than 100 μg/ml in the lettuce tests, hydroquinone inhibited hypocotyl and root growth. Concentrations less than 30 μg/ml stimulated the growth of lettuce seeds. When tested on rice seed germination, hydroquinone showed a similar pattern of inhibition above 100 μg/ml and growth stimulation at lower concentrations.

(−)-Epicatechin showed little growth inhibitory activity at concentrations less than

TABLE I. Effect of Hydroquinone on the Growth of Lettuce and Rice Seedlings

Concentration (μg/ml)	Length (mm)			
	Lettuce		Rice	
	Hypocotyl	Root	Shoot	Root
0	2.50±0.19	9.95±1.38	34.45±2.00	101.65±4.65
1	2.85±0.32	10.25±1.51	33.55±1.67	103.05±3.65
3	2.95±0.20	13.25±2.05	33.40±1.94	100.75±5.03
10	2.74±0.22	12.79±1.77	37.10±2.00	118.25±2.81
30	2.30±0.34	8.35±1.63	34.70±1.34	128.50±3.78
100	0	0	32.80±2.26	86.40±8.45
300	0	0	27.25±1.05	8.60±0.69
1000	0	0	12.90±1.25	0

TABLE II. Combined Effect of (−)-Epicatechin and Hydroquinone on the Growth of Lettuce Seedlings

Hydroquinone (μg/ml)	Growth (mm)							
	Hypocotyl				Root			
	(−)-Epicatechin (μg/ml)				(−)-Epicatechin (μg/ml)			
	0	3	30	100	0	3	30	100
0	9.6±0.7	9.6±0.4	10.1±0.5	10.2±0.8	60.4±3.0	60.4±4.3	58.7±2.7	57.3±3.9
10	9.9±0.4	9.9±0.7	9.8±0.4	10.0±1.1	62.0±3.6	62.1±3.0	59.1±2.2	55.4±3.1
30	8.4±0.6	8.6±0.6	9.9±0.7	10.8±0.8	55.4±4.6	56.3±3.8	50.4±4.8	49.6±5.7
50	6.8±0.8	6.0±0.7	9.0±0.7	9.3±0.8	42.1±5.1	39.1±4.9	46.1±4.5	45.7±4.9

TABLE III. Combined Effect of (−)-Epicatechin and Hydroquinone on the Growth of Rice Seedlings

Hydroquinone (μg/ml)	Growth (mm)							
	Hypocotyl				Root			
	(−)-Epicatechin (μg/ml)				(−)-Epicatechin (μg/ml)			
	0	3	30	100	0	3	30	100
0	43.7±5.3	44.4±2.7	44.5±8.4	46.8±6.8	126.9±5.7	129.3±8.3	140.7±6.9	138.6±8.3
30	42.1±6.7	44.4±5.0	44.0±9.6	47.2±3.9	141.6±7.9	138.9±8.3	143.9±7.2	129.3±8.9
100	35.8±5.9	38.7±4.8	37.1±7.0	40.6±6.8	112.2±8.5	120.4±6.5	112.7±7.0	141.2±9.1
300	29.8±2.8	32.0±3.2	37.1±5.5	42.3±4.7	13.9±2.4	15.1±1.5	21.1±2.2	34.5±3.9

300 μg/ml. Since an antagonistic effect was observed in the case of nagilactone E and (−)-epicatechin isolated from *Podocarpus nagi* ZOLL. *et* MORITZI (Podocarpaceae),[6] the combined effect of (−)-epicatechin with hydroquinone on the growth of lettuce and rice seedlings was investigated. The results are listed in Tables II and III, and show that when applied with hydroquinone, (−)-epicatechin tends to counteract the growth-inhibitory activity of hydroquinone. In the case of lettuce, the activity is limited to the hypocotyl growth. In the case of rice, blocking of the activity of hydroquinone by (−)-epicatechin was seen in both root and shoot growth.

It is possible that *A. californica* utilizes hydroquinone to inhibit the growth of surrounding plants in order to compete more effectively for nutrients and water. However, the role of hydroquinone and (−)-epicatechin in relation to the survival of buckeye seeds remains to be elucidated.

Experimental

Melting points were determined using a Sybron Thermolyne Mp-12615, and are uncorrected. Elemental analysis for hydroquinone was performed in Osaka City University. UV spectra were measured using a Hitachi 100-80 instrument and IR spectra were taken on a Hitachi 215. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL PS-200 and JEOL FX-100 spectrometers, respectively, using tetramethylsilane as an internal reference. MS were taken on a Hitachi RMU 6-MG.

Isolation——Seeds of *A. californica* were collected in California, U.S.A., in September 1983. After storage of the ground seeds (500 g) in MeOH for 3 d, the mixture was filtered and the MeOH was then removed to give a residue (24.3 g). This residue was separated into fractions soluble in *n*-hexane (0.23 g), EtOAc (1.0 g), *n*-BuOH (8.6 g), and H_2O (14.5 g). Column chromatography (silica gel, 30 g, $CHCl_3$–MeOH) of 0.98 g of the EtOAc fraction yielded 176 mg of **1** and 143 mg of **2**.

(−)-Epicatechin (1)——Colorless needles of mp 238—240 °C (dec.) (recryst. from $CHCl_3$–EtOAc); $[\alpha]_D^{24}$ −56.4° (c=0.06, MeOH). Electron impact (EI)-MS m/z: 290 (M^+). The IR, UV, and ^{1}H-NMR spectra of **1** were identical with those of an authentic sample.

Hydroquinone (2)——Colorless needles of mp 165 °C (recryst. from $CHCl_3$–Me_2CO). *Anal.* Calcd for $C_6H_6O_2$: C, 65.33; H, 5.46. Found: C, 65.45; H, 5.49. High resolution MS Calcd for $C_6H_6O_2$: 110.0368. Found: 110.0363. The IR, UV, and ^{1}H-NMR spectra of **2** were identical with those of an authentic sample.

Bioassay——In the case of lettuce (*Lactuca sativa*, L., *cv.* Grand Rapids), tests were carried out according to the method of Kamisaka,[2] and in the case of rice (*Oryza sativa*, L., *cv.* Norin 20), according to our method.[5,6] The bioassay was repeated ten times at each concentration. The results are listed in Tables I, II, and III.

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References

1) L. Fowden and A. Smith, *Phytochemistry*, **7**, 809 (1968).
2) S. Kamisaka, *Plant Cell Physiol.*, **14**, 747 (1973).
3) R. S. Thompson, D. Jacques, E. Haslam, and R. J. N. Tanner, *J. Chem. Soc., Perkin Trans. 1*, **1972**, 1387.
4) L. Gattermann and H. Wieland, "Die Praxis des Organishen Chemikers," De Gruyter and Co., Berlin, 1961, p. 266.
5) M. Taniguchi and Y. Satomura, *Plant Cell Physiol.*, **12**, 295 (1971).
6) I. Kubo, T. Matsumoto, F. J. Hanke, M. Taniguchi, and Y. Hayashi, *Experientia*, submitted.