Short Communication

Identification of Jasmonic Acid and Abscisic Acid as Senescence-promoting Substances from *Cleyera ochnacea* DC

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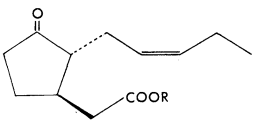
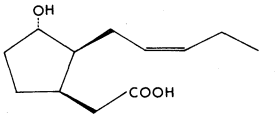
Recently we reported the isolation of (−)-methyl jasmonate (methyl (1*S*,2*R*)-3-oxo-2-(2′-*cis*-pentenyl)-cyclopentane-1-acetate; **1a**) as a senescence-promoting substance from wormwood (*Artemisia absinthium* L.).[1] It has been also found as an odoriferous compound in the essential oils of *Jasminum glandiflorum* L.[2] and *Rosmarinus officinalis* L.[3] The free acid of this ester (jasmonic acid; **1b**) has been isolated for the first time from the culture filtrates of *Lasiodiplodia theobromae* as a plant growth inhibitor,[4] while it has also been isolated as a growth inhibitor from the plant extracts obtained from the leaves and galls of chestnut (*Castanea crenata* Sieb et Zucc.), the immature seeds of *Phaseolus vulgaris* L. and *Dolichos lablab* L.,[5] and the pericarp of *Vicia faba* L.[6] Cucurbic acid (**2**) and its derivatives, which are growth inhibitors structurally related to jasmonic acid, are known to occur in *Cucurbita pepo* L.[7] Strenuous efforts to learn the distribution of jasmonic acid and its related inhibitory compounds in the plant kingdom resulted in the identification of jasmonic acid and abscisic acid in the extract from *Cleyera ochnacea* DC. These results are briefly described in this paper.

Mature leaves (2.0 kg fresh weight) of *C. ochnacea* which were purchased locally were extracted with 80% aqueous acetone three times. The extract was concentrated *in vacuo* to give an aqueous residue. The aqueous solution was extracted with ethyl acetate at pH 2.5. The ethyl acetate fraction was separated into acidic and neutral fractions by the usual acid-base partition procedure. The senescence-promoting activity was observed only in the acidic fraction, using the oat leaf assay previously described.[1] The acidic crude material (11.2 g) was purified in a charcoal column (Wako charcoal for chromatography; 3.0 × 52 cm). Elution was done with acetone–water, increasing the acetone content by 10% steps for every 1.2 liter. Senescence-promoting activity was found in the eluates with 80% acetone in water. About a third of this eluate (2.0 g) was charged onto a silicic acid column (Wako gel C-100, 30 g; 1.0 × 17 cm) impregnated with 0.5 M aqueous formic acid (18.8 ml) and eluted with *n*-hexane–ethyl acetate, increasing the ethyl acetate content by 1 to 10% steps for every 200 ml. The active compounds were eluted with 1% (A-1) and 60% ethyl acetate in *n*-hexane (A-2).

The A-1 fraction was further purified on preparative TLC (0.25 mm thickness) developed with benzene–ethyl acetate–acetic acid (10 : 1 : 1, v/v/v). The active zone of *Rf* 0.43～0.50 was extracted with ethyl acetate and finally purified on the same preparative TLC using a solvent system of *n*-hexane–ethyl acetate–acetic acid (10 : 1 : 1, v/v/v). The active zone of *Rf* 0.34～0.48 was eluted with ethyl acetate, methylated with ethereal diazomethane, then subjected to GC-MS (2.0 mm × 1 m glass column packed with 1% OV-1 on 80-100 mesh Chromosorb W (AW-DMCS), 140°C isothermal, 21 eV). The mass spectrum



1a; R = CH_3
1b; R = H



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of the main peak at 4.6 min revealed fragment ions characteristic of methyl jasmonate at *m/z* 224 (M^+ 29%), 206 (10), 193 (17), 177 (16), 156 (41), 151 (64), 135 (16), 133 (33), 121 (16), 109 (25) and 83 (100). Thus, the major active substance in the A-1 fraction was confirmed to be jasmonic acid (**1b**). The yield of jasmonic acid was estimated by gas chromatography to be about 6 μg per kg fresh weight of *C. ochnacea* leaves. Jasmonic acid at concentrations higher than 5 mg/liter was effective in promoting senescence and at 50 mg/liter it inhibited the senescence-retarding effect induced by 2 mg/liter kinetin to about 50%.

The A-2 fraction was also purified on preparative TLC developed with ethyl acetate–chloroform–*n*-hexane–acetic acid (20 : 8 : 20 : 1, v/v/v/v). The active zone of *Rf* 0.16～0.23 was extracted with ethyl acetate, methylated with ethereal diazomethane, then subjected to GC-MS (2.0 mm × 2 m glass column packed with 1% OV-1 on 80-100 mesh Chromosorb W (AW-DMCS), 210°C isothermal, 21 eV). The fragment ions of the peak at 4.3 min were at *m/z* 278 (M^+ 3%), 260 (6), 246 (6), 205 (12), 190 (100), 162 (38), 134 (32) and 125 (44) which are characteristic of *cis,trans*-ABA methyl ester. Thus, the major active substance in the A-2 fraction was identified as *cis,trans*-ABA. The yield of *cis,trans*-ABA was estimated by gas chromatography to be about 18 μg per kg fresh weight of the tissue. *cis,trans*-ABA at concentrations higher than 0.1 mg/liter was effective in promoting senescence and at 26.4 mg/liter it could completely eliminate the anti-senescence activity of 2 mg/liter kinetin.

The successful identification of jasmonic acid, as well as ABA, as senescence-promoting factors in the mature leaves of *Cleyera ochnacea* DC suggests that these compounds might be concerned with growth and development, especially senescence in this tissue.

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REFERENCES

1) J. Ueda and J. Kato, *Plant Physiol.*, **66**, 246 (1980).
2) E. E. Demole, E. Lederer and D. Mercier, *Helv. Chim. Acta*, **45**, 675 (1962).
3) L. Crabalona, *Comp. Rend. Acad. Sci. Paris, Ser. C*, **264**, 2074 (1967).
4) D. C. Aldridge, S. Galt and D. Giles, *J. Chem. Soc. (C)*, **1971**, 1623.
5) H. Yamane, H. Takagi, H. Abe, T. Yokota and N. Takahashi, *Plant and Cell Physiol.*, **22**, 689 (1981).
6) W. Dathe, H. Rönsh, A. Preiss, W. Schade, G. Sembdner and K. Schreiber, *Planta*, **153**, 530 (1981).
7) H. Fukui, K. Koshimizu, Y. Yamazaki and S. Usuda, *Agric. Biol. Chem.*, **41**, 189 (1977).