Phytochemistry, 1973, Vol. 12, pp. 1149 to 1152. Pergamon Press. Printed in England.

FLAVONOID GLYCOSIDES FROM ILLUMINATED CELL SUSPENSION CULTURES OF *PETROSELINUM HORTENSE*

FRITZ KREUZALER and KLAUS HAHLBROCK

Lehrstuhl für Biochemie der Pflanzen, Biologisches Institut II der Universität, D-7800 Freiburg i. Br., Germany

(*Received* 24 *October* 1972. *Accepted* 17 *November* 1972)

Key Word Index—*Petroselinum hortense*; Leguminosae; cell suspension cultures; flavones; flavonols; malonylated glycosides.

Abstract—Twenty-four different flavonoid glycosides were isolated from illuminated cell suspension cultures of parsley (*Petroselinum hortense*). The chemical structures of fourteen of these compounds were further characterized. The aglycones identified were the flavones apigenin, luteolin and chrysoeriol, and the flavonols quercetin and isorhamnetin. The flavones occurred either as 7-*O*-glucosides or as 7-*O*-apioglucosides, while the flavonols were 3-*O*-monoglucosides or 3,7-*O*-diglucosides. One-half of these glycosides were electrophoretically mobile and substituted with malonate residues.

INTRODUCTION

THE FORMATION of flavonoid glycosides in cell suspension cultures of *Petroselinum hortense* has been shown to be dependent on treatment of the cells with light.[1] Two of the major flavone glycosides occurring in parsley plants, apiin (7-*O*-[β-D-apiofuranosyl(1→2)β-D-glucosyl]-apigenin) and graveobiosid-B (3'-methoxyapiin)[2] were also reported to be formed by illuminated cell suspension cultures of parsley.[1]

Results presented in this communication demonstrate that treatment of parsley cell suspension cultures with light induces the production of a large number of glycosides of several flavones and flavonols.

RESULTS

A total of 24 different flavonoid glycosides was isolated from the cell cultures after continuous illumination for 24 hr. The compounds were extracted with hot 80% ethanol and then separated by PC. Amounts sufficient for the identification of the aglycones and the sugar moieties were obtained from 14 of these glycosides. Table 1 summarizes the results of this investigation and lists the solvent systems used for purification of the individual glycosides.

The aglycones identified were the flavones apigenin, luteolin and chrysoeriol, and the flavonols quercetin and isorhamnetin.

Glucose and apiose were the only sugars detected after hydrolysis of the purified glycosides. The two major flavones apigenin and chrysoeriol both occurred as glucosides and as apioglucosides, while no apiose was found in the flavonol glycoside fractions. The two flavonols were found to be glucosylated either in the 3-position alone (quercetin) or in both the 3- and the 7-positions (quercetin and isorhamnetin).

One-half of the glycosides listed in Table 1 migrated in an electric field. From all but one of these compounds, malonic acid was isolated after acid hydrolysis and identified by PC.

[1] HAHLBROCK, K. and WELLMANN, E. (1970) *Planta* **94**, 236.
[2] GRISEBACH, H. and BILHUBER, W. (1967) *Z. Naturforsch.* **22b**, 746.

Other non-volatile dicarboxylic acids or phosphoric and sulfuric acids could not be detected.

The two malonylated 3,7-diglucosides of isorhamnetin (compounds XIII and XIV in Table 1) could be separated by PC. Glycoside XIV migrated in an electric field almost twice as fast as glycoside XIII. Although malonic acid was not measured quantitatively, an estimation of the intensity of the color reaction used for the identification of malonic acid on the paper chromatogram suggested that the second of the two glycosides bears an additional malonate residue.

TABLE 1. CHARACTERIZATION OF SOME OF THE FLAVONE AND FLAVONOL GLYCOSIDES ISOLATED FROM ILLUMINATED CELL SUSPENSION CULTURES OF PARSLEY

Compound No.	Aglycone	Glucose	Apiose	Position of glycosylation	Electrophoretic mobility	Malonic acid	Molar ratio Aglycone: glucose: apiose	Purification (solvent systems)
I	Apigenin	+	−	7	−	−	1:1·2	a, b, c
II	Apigenin	+	−	7	+	+	1:1·2	a, b, c, d, e
III	Apigenin	+	+	7	−	−	1:1·3:0·8	a, b, d, e
IV	Apigenin	+	+	7	+	+	1:1·2:0·9	a, b, c, d, e
V	Luteolin	+	−	7	−	−	1:1·3	a, b, d, e
VI	Chrysoeriol	+	−	7	−	−	1:0·8	a, b, c
VII	Chrysoeriol	+	−	7	+	+	1:0·9	a, b, c
VIII	Chrysoeriol	+	+	7	−	−	1:1·0:1·0	a, b, c
IX	Chrysoeriol	+	+	7	+	+(?)	1:1·1:1·1	a, b, c, d, e
X	Quercetin	+	−	3	−	−	1:1·3	a, b
XI	Quercetin	+	−	3 + 7	−	−	1:2·2	a, b
XII	Quercetin	+	−	3	+	+	n.d.	a, b, c
XIII	Isorhamnetin	+	−	3 + 7	+	+	1:2·1	a, b, c, d, e
XIV	Isorhamnetin	+	−	3 + 7	++	+	1:1·7	a, b, c, d, e

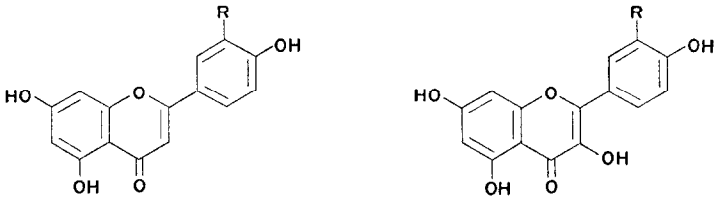
DISCUSSION

In addition to the two flavone glycosides apiin and graveobiosid B which are already known to occur in illuminated cell suspension cultures of parsley,[1] a number of neutral and malonylated glucosides and apioglucosides of five closely related flavones and flavonols were isolated from these cells. No glycoside of kaempferol, the flavonol corresponding to the flavone apigenin, was isolated in amounts sufficient for unequivocal characterization. However, recent studies by Fritsch and Grisebach on the composition and the relative amounts of the aglycones formed by parsley cell cultures after illumination for about 5 days resulted in the isolation of all of the six compounds listed.[3]

Complete separation and purification of the various glycosides could only be achieved by a series of chromatographic steps. Inevitably, this procedure caused a significant loss of material. As already mentioned above, at least 24 flavonoid glycosides, all differing with regard to their chromatographic properties, were formed by the illuminated cells, although only 14 of these compounds were obtained in amounts sufficient for further identification. Nine different types of glycosides could be characterized (four based on flavones and five on flavonols) so it is possible that some of the unidentified compounds were variants involving luteolin, kaempferol, quercetin and isorhamnetin (see Table 1).

[3] FRITSCH, H.-J. and GRISEBACH, H., personal communication.

No attempts have been made to determine the nature of the glycosidic linkage between the two sugars apiose and glucose in the 7-*O*-apioglucosides of apigenin and chrysoeriol from the cell cultures. However, Hulyalkar *et al.* have shown that the sugar moiety of apiin isolated from intact parsley plants is β-D-apiofuranosyl-(1 → 2)-β-D-glucose.[4] Furthermore, Sutter *et al.*[5] and Ortmann *et al.*[6] have purified enzymes from illuminated parsley cell cultures which catalyse the formation of apigenin 7-*O*-β-D-glucoside from UDP-D-glucose and apigenin, and of apiin from UDP-D-apiose and apigenin 7-*O*-β-D-glucoside. It must therefore be assumed that compounds III, IV, VIII and IX (Table 1) are flavone 7-*O*-β-D-apiofuranosyl-(1 → 2)-β-D-glucosides.



Flavones:

Apigenin R = H
Luteolin R = OH
Chrysoeriol R = OMe

Flavonols:

Kaempferol
Quercetin
Isorhamnetin

FLAVONES AND FLAVONOLS OCCURRING AS GLYCOSIDES IN ILLUMINATED CELL SUSPENSION CULTURES FROM PARSLEY.

Recently, a malonyl CoA:flavone glycoside malonyltransferase has also been demonstrated in cell-free extracts of these cell cultures.[7] The enzyme catalyzed the transfer of the malonyl residue from malonyl CoA to apiin, resulting in a product identical with compound IV in Table 1. The malonyl apiin formed enzymatically contained 1 mole of malonate per mole of apiin, most likely linked to the glucose moiety of the glycoside.[7] Although precise data on the position of the malonate residue were not obtained, the isolation of two 6″-*O*-malonyl isoflavone 7-*O*-glucosides from clover leaves[8] suggests that compounds II, IV, VII, IX, XII, XIII and XIV (Table 1) are also malonylated in the 6 position of the glucose moiety. In this case, it would seem quite possible that the flavonol diglucosides bear malonyl residues in both of the two glucose moieties as suggested for compound XIV.

No phosphate or sulfate residues which would account for the high electrophoretic mobility of compound XIV could be detected. These inorganic acids were tested because Harborne and Williams recently reported the isolation of a 7-*O*-bisulfate of luteolin 3′-*O*-glucoside and other charged flavones in a number of palm species.[9]

There has been much controversy in the literature concerning the occurrence or non-occurrence of glycosides of apigenin, luteolin, chrysoeriol, and isorhamnetin in parsley plants (see Refs. 2, 10, 11). This confusion might be due at least in part to variations in the isolation techniques and in the age of the plant material used in different laboratories and

[4] HULYALKAR, F. K., JONES, J. K. N. and PERRY, M. B. (1965) *Can. J. Chem.* **43**, 2085.
[5] SUTTER, A., ORTMANN, R. and GRISEBACH, H. (1972) *Biochim. Biophys. Acta* **258**, 71.
[6] ORTMANN, R., SUTTER, A. and GRISEBACH, H. *Biochim. Biophys. Acta* in press.
[7] HAHLBROCK, K. (1973) *FEBS Letters* in press.
[8] BECK, A. B. and KNOX, J. R. (1971) *Australian J. Chem.* **24**, 1500.
[9] HARBORNE, J. B. and WILLIAMS, C. A. (1971) *Z. Naturforsch.* **26b**, 490.
[10] KARRER, W. (1958) *Konstitution und Vorkommen der organischen Pflanzenstoffe*, Birkhäuser Verlag, Basel.
[11] BILHUBER, W. (1967) dissertation, Freiburg.

should be clarified by the results presented here. Furthermore, the isolation and the partial characterization of malonylated flavone and flavonol glycosides are reported for the first time.

Indirect evidence for the occurrence of malonylated flavonoid glycosides in *Petrosilinum* and *Cicer* sp. has also been obtained by Davenport and Dupont,[12] who reported the isolation from these plants of enzymes capable of liberating malonate from flavone glycoside fractions.

EXPERIMENTAL

Cell suspension cultures of parsley (*Petroselinum hortense*) were grown, illuminated for 24 hr, and harvested as described previously.[13]

80 g (fr. wt) of cells were boiled for 5 min in 400 ml of 80% EtOH. Insoluble material was filtered off from the hot mixture. The solvent was then evaporated, and the residue was dissolved in 20 ml of 50% EtOH.

The following solvent systems were used for chromatography of the glycosides on pre-washed Whatman 3 MM paper: (a) 10% HOAc; (b) 20% EtOH; (c) *tert*-BuOH–HOAc–H_2O (3:1:1); (e) 15% HOAc. Column chromatography on polyamide was carried out with benzene–MeOH–MeCOEt–acetyl acetone (15:10:5:1) (solvent system d). Conditions for paper electrophoresis were as described elsewhere.[7]

Hydrolysis of the glycosides was achieved by incubating a solution of 1 mg in 1 ml of 50% EtOH with 1 ml of CF_3CO_2H for 16 hr at 70°. The mixture was then evaporated to dryness, and the aglycones were extracted with ether and the sugars and malonic acid with H_2O.

Flavonoid glycosides and the aglycones were detected under a UV lamp at 350 nm. The aglycones, sugars and malonic acid were identified by PC and TLC. In addition, characteristic changes in the UV spectra of the flavones and flavonols were measured under various conditions.[14] Positions of glycosylation of the flavonoids were determined by the same method.[14] Glucose[15] and apiose[16] were estimated by spectrophotometric assays, and malonic acid was detected after PC with a spray reagent.[17] Color reactions were used for attempts to identify sulfate[18] or phosphate[19] after hydrolysis of the glycosides.

Acknowledgement—This work was supported by the Deutsche Forschungsgemeinschaft.

[12] DAVENPORT, H. E. and DUPONT, M. S. (1972) *Biochem. J.* **129,** 18 P.
[13] HAHLBROCK, K., EBEL, J., ORTMANN, R., SUTTER, A., WELLMANN, E. and GRISEBACH, H. (1971) *Biochim. Biophys. Acta* **244,** 7.
[14] MABRY, T. J., MARKHAM, K. R. and THOMAS, M. B. (1970) *The Systematic Identification of Flavonoids,* Springer, Berlin.
[15] BERGMEYER, H. U. and BRENT, E. (1962) in *Methoden der enzymatischen Analyse* (BERGMEYER, H. U., ed.), 123, Chemie, Weinheim.
[16] SANDERMANN, JR., H. (1969) *Phytochemistry* **8,** 1571.
[17] PASKOVA, J. and MUNK, U. J. (1960) *J. Chromatog.* **4,** 241.
[18] SCHNEIDER, J. J. and LEWBART, M. L. (1956) *J. Biol. Chen.* **222,** 787.
[19] DAWSON, R. M. C., ELLIOTT, D. C., ELLIOTT, W. H. and JONES, K. M. (1969) *Data for Biochemical Research,* p. 575, Clarendon Press, Oxford.