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SHORT COMMUNICATION

THE PHENOLIC COMPOUNDS OF *OENOTHERA*

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Abstract—24 species of *Oenothera*, representing 8 subgenera, were examined for their phenolics by TLC. Several phenolic acids, hydrolysable tannins, a quercetin-glucoside and galactosides of quercetin, kaempferol and myricetin were identified. In addition delphinidin and cyanidin were found.

INTRODUCTION

ALTHOUGH the Oenotheraceae are known as accumulators of polyphenols, there are relative few reports on the phenolic pattern of the genus *Oenothera*.[1,2] In order to better elucidate the taxonomy of *Oenothera* from the chemical standpoint, it was necessary to have more data on the phenolic constituents of this genus. Therefore a detailed study of these compounds has been made and the first results are reported in this paper.

RESULTS AND DISCUSSION

The pattern of phenolic substances in hydrolyzed leaf extracts of 24 species of *Oenothera* is shown in Table 1. From these results, it is clear that a distinction can be made between the various subgenera firstly on the basis of their phenolic acids and secondly on the basis of their flavonols. The subgenera, Hartmannia, Kneiffia, Lavauxia, Raimannia and Oenothera contain all the phenolic acids listed in Table 1, whereas the representatives of the subgenera Pachylophus and Heterostemon contain no neochlorogenic acid and Megapterium has neither caffeic nor neochlorogenic acid. Besides the identified compounds, other as yet unidentified phenolic acids were noted on the chromatograms. The flavonoid pattern of *Oenothera* is characterized by a relative paucity of the rarer flavonols; only quercetin, kaempferol and myricetin were found. The subgenera Oenothera and Raimannia contain no myricetin. These results are in full agreement with those of Howard and Mabry[3] who have independently examined the genus. In the subgenera Pachylophus and Megapterium, no kaempferol was detected. Common to all species were the anthocyanidins, delphinidin and cyanidin.

Examination of different plant organs of a representative of each of the subgenera Oenothera (*O. erythrosepala*), Megapterium (*O. missouriensis*), and Raimannia (*O. mollissima*) showed that there are quantitative and qualitative differences in the phenolic pattern between the various parts of a plant (Table 2). It was clearly shown that as a rule the leaves contain the highest concentration and the largest number of phenols, but there are few exceptions. For example, in *O. missouriensis* kaempferol was found only in the stamen and the style and digallic acid in all parts of the plant but the leaves. Kaempferol had already

[1] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Vol. 5, p. 224, Birkhauser, Basel (1969).
[2] E. C. BATE-SMITH, *J. Linn. Soc.* (*Bot.*) **58**, 95 (1961).
[3] G. HOWARD and T. J. MABRY, private communication.

TABLE 1. THE PHENOLIC CONSTITUENTS IN HYDROLYSED LEAF-EXTRACTS OF VARIOUS SPECIES OF *Oenothera*

Oenothera Subgenus and species	Ellagic acid	Gallic acid	Digallic acid	Neochlorogenic acid	Caffeic acid	*p*-Coumaric acid	*o*-Coumaric acid	Myricetin	Quercetin	Kaempferol	Delphinidin	Cyanidin
1. Hartmannia Munz												
O. rosea L'Hér. ex Ait.	+	+	+	+	(+)	+	+	(+)	+	+	+	+
O. speciosa Nutt.	+	+	−	+	(+)	+	+	+	+	+	+	+
O. tetraptera Cav.	+	+	+	+	(+)	+	+	(+)	+	+	+	+
2. Kneiffia Munz												
O. tetragona Roth subspec. glauca Munz	+	+	+	+	(+)	+	+	+	+	+	+	+
O. fruticosa L.	+	+	+	+	(+)	+	+	+	+	+	+	+
O. perennis L.	+	+	+	+	+	+	+	+	+	+	+	+
4. Megapterium Munz												
O. missouriensis Sims.	+	+	+	−	−	+	+	+	+	−	+	+
5. Lavauxia Jepson												
O. acaulis Cav.	+	+	(+)	+	+	+	+	+	+	+	+	+
O. flava Garett	+	+	(+)	+	+	+	+	+	+	+	+	+
6. Pachylophus (Spach) Jepson												
O. caespitosa	+	+	+	−	+	+	+	+	+	−	+	+
7. Raimannia Munz												
O. drummondii Hook.	+	+	+	+	+	+	+	−	+	+	+	+
O. berteriana Spach	+	+	+	+	+	+	+	−	+	+	+	+
O. mollissima L.	+	+	+	+	+	+	+	−	+	+	+	+
O. odorata Jaq.	+	+	+	+	+	+	+	−	+	+	+	+
9. Oenothera												
O. dalton Clel.	+	+	+	+	+	+	+	−	+	+	+	+
O. ammophila Focke et Borb.	+	+	+	+	+	+	+	−	+	+	+	+
O. parviflora L.	+	+	+	+	+	+	+	−	+	+	+	+
O. bauri Boedijn.	+	+	+	+	+	+	+	−	+	+	+	+
O. hookeri T. et G.	+	+	+	+	+	+	+	−	+	+	+	+
O. biennis L.	+	+	+	+	+	+	+	−	+	+	+	+
O. jamesii Gigas	+	+	+	+	+	+	+	−	+	+	+	+
O. erythrosepala Borb.	+	+	+	+	+	+	+	−	+	+	+	+
O. strigosa (?)	+	+	+	+	+	+	+	−	+	+	+	+
12. Heterostemon Nutt.												
O. tanacetifolia T. et G.	+	+	(+)	−	+	+	+	+	+	+	+	+

(+) indicates a very low concentration of a particular compound.

TABLE 2. THE DISTRIBUTION OF PHENOLIC COMPOUNDS IN VARIOUS ORGANS OF *O. erythrosepala* BORB.—1, *O. missouriensis* SIMS.—2, AND *O. mollissima* L.—3

Phenolic compounds	Plant organ: Shoot axis	Leaf	Root	Calyx	Corolla	Filament
Ellagic acid	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3
Gallic acid	1 2 3	1 2 3	1 2 3	2	(1) 2	(1) 2 3
m-Digallic acid	1 2 3	1 3	(1) 2 (3)	(2) 3	1 (2) 3	(2) 3
Neochlorogenic acid	1 3	1 3	1 3	1 3	1 3	(1) 3
Caffeic acid	(1) 3	1 3	1 3	1 3	1 3	1 3
p-Coumaric acid	(1) 2 3	1 2 3	2	2 3	1 2 3	1 2 3
o-Coumaric acid	(1) 2 3	1 2 3	1 2 3	2 3	(1) 3	1 2 3
Myricetin	2	2	—	2	2	2
Quercetin	1 (2) 3	1 2 3	—	1 2 3	1 2 3	1 2 3
Kaempferol	1	1 3	(1)	1 3	(1)	1 2 3
Delphinidin	1 2 3	1 2 3	(1)	1	1	1 (2)
Cyanidin	2 3	1 2 3	—	(2) 3	1	1 3

Phenolic compounds	Hypanthium	Style + stigma	Ovary	Fruit wall	Seed
Ellagic acid	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3
Gallic acid	2	(1) 2 3	1 2 3	1 2 3	1 2 3
m-Digallic acid	2 3	(1) (2) 3	(1) 2 3	1 2 3	1 2
Neochlorogenic acid	1 3	1 3	1 3	1 3	3
Caffeic acid	1 3	(1) 3	3	3	1 3
p-Coumaric acid	2 3	1 2 3	1 2 3	1 2 3	2
o-Coumaric acid	2 3	1 2 3	(1) 2 3	(1) 2 3	1 2 3
Myricetin	2	2	2	2	—
Quercetin	1 2 3	1 2 3	1 2 3	1 2 3	3
Kaempferol	1	1 2 (3)	1	1 (3)	(1) 3
Delphinidin	1	1 (2)	1 3	1 3	1 2 3
Cyanidin	3	(2) 3	1 2 3	1 3	1 2 3

Figures in brackets indicate a very low concentration of a particular compound.

earlier been found in pollen grains by Wiermann.[4] Flavonols and anthocyanidins are hardly present in roots and it is also striking that there is a paucity of phenolics in the calyx and the hypanthium. Additionally it should be noted that quinic and sinapic acids were detected in the leaves of the plants examined. Non-hydrolysed plant extracts contained characteristic hydrolysable tannins[5] and a preliminary report on their structure is being prepared.

Besides the tannins and several still unidentified tannin-like substances (probably glycosides of phenol carboxylic acids) there are about 10 flavonol glycosides, based on quercetin, kaempferol and myricetin. These are glycosylated with various sugars including glucose, galactose, rhamnose and possibly arabinose. The preliminary identification of these glycosides in *O. erythrosepala*, *O. mollissima*, and *O. missouriensis* are shown in Table 3.

[4] R. WIERMANN, *Ber. Dtsch. Bot. Ges.* **81**, 3 (1968).
[5] H. D. ZINSMEISTER, H. HOPF, H. KAUER und S. BARTL, *Planta Med.* **18**, 160 (1970).

The myricetin galactoside found in *O. missouriensis* is chromatographically identical with myricetin-3-*O*-β-galactoside which Kagan[6] isolated from *O. lavandulaefolia*. The quercetin galactoside is obviously hyperoside (quercetin-3-*O*-galactoside). Some of the other unidentified glycosides may be identical with those which Kowalewski *et al.*[7] isolated from *O. biennis*. Both the phenolic acids and the flavonol glycosides seem to be valuable

TABLE 3. FLAVONOLGLYCOSIDES IN THE LEAVES OF 3 SPECIES OF *Oenothera*

Glycoside	*O. erythrosepala*	*O. missouriensis*	*O. mollissima*
Quercetin glucoside	+	+	+
Quercetin galactoside	+	+	+
Kaempferol galactoside	+	−	+
Myricetin galactoside	−	+	−

markers for the chemotaxonomy of *Oenothera*. This has already been established for flavonol glycosides by Howard and Mabry[3] who discuss the implication of these compounds in taxonomy. The results reported here confirm their findings and enlarge the survey of the chemical patterns of *Oenothera* by adding the results on phenolic acids as well as including 12 more species.

The subgenera Oenothera and Raimannia seem to be the most advanced since they contain no myricetin the loss of which is believed to be a step in the evolution of flavonoids in higher plants.[8]

EXPERIMENTAL

Plant material. All the plants were cultivated outdoors. As a rule they were extracted immediately after harvesting. In some cases for storage purposes, the material was frozen at −35° to −40°, this treatment did not affect the phenolic pattern of the plants, even after a longer storage period. The classification of the plants was according to Munz.[9]

Extraction procedure. Three g of fresh or frozen plant tissue was homogenized in 100 ml 50% acetone and extracted for 24 hr in the cold. The residue was filtered off and the filtrate was reduced *in vacuo* at 25° to 2–3 ml, and acetone then added to give a final concentration of 50%. Such samples could be stored for long times at 0°.

Hydrolysis. One ml of extract was hydrolysed by 5 ml 2n HCl at 100° for 1 hr. The hydrolysate after cooling was neutralized with KOH and extracted with a small amount of n-AmOH.

TLC. The samples were applied on plates of cellulose microcrystalline (Avicel), using standard techniques of one and two dimensional chromatography. Solvents were secBuOH/HOAc/H_2O (70:5·25); HOAc (6%); HOAc/HCl/H_2O (60:6.20), Toluol/HCO_2Et/HCO_2H (5·4:1), n-BuOH/HOAc/H_2O (6·2.2), n-BuOH/Pyridine/H_2O/HOAc (60:40:30·3).

The compounds were identified by their R_f-value, fluorescence in the UV, with and without NH_3-vapour, Diphenylboric acid-β-amino-ethyl-ester (= Naturstoffreagenz A), KIO_3, basic $AgNO_3$, aniline phthalate, acid hydrolysis and co-chromatography.

[6] J. KAGAN, *Phytochem.* **6,** 317 (1967).
[7] Z. KOWALEWSKI, M. KOWALSKA and L. SKRZYPCZAKOWA, *Diss. Pharmaceuticae et Pharmacologicae* **20,** 573 (1968).
[8] K. KUBITZKI, Laubblattflavonoide als systematisches Merkmal, *Ber. Dtsch. Bot. Ges.* **80,** 757 (1967).
[9] M. MUNZ, *North American Flora*, Vol. *Onagraceae*, New York (1968).