Analysis and Distribution of Flavonoid Glycosides and Rosmarinic Acid in 40 *Mentha* × *piperita* Clones

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A reversed-phase high-performance liquid chromatographic method has been developed for the qualitative and quantitative analysis of a caffeic acid derivative, rosmarinic acid, and the main flavone and flavanone glycosides in leaves of *Mentha* × *piperita*, namely eriocitrin, luteolin 7-*O*-rutinoside, hesperidin, isorhoifolin, diosmin, eriodictyol 7-*O*-glucoside, and narirutin. The last two were identified for the first time in *M.* × *piperita* and *Mentha* genus, respectively. Forty clones, belonging to the three varieties of *M.* × *piperita*, were investigated. The eight phenolic constituents are present in all clones. The flavonoid content of the leaves ranged from 8.6 to 17.8% on dry weight basis. In all instances, eriocitrin, with a concentration range of 6.6–15.0%, is the dominant flavonoid glycoside accompanied by an appreciable amount of luteolin 7-*O*-rutinoside, hesperidin, and rosmarinic acid. The chemotaxonomic and therapeutic relevances of these results are discussed.

INTRODUCTION

Peppermint, *Mentha* × *piperita* L. (Lamiaceae), is widely grown in temperate zones of the world, particularly in the United States and Europe, for its volatile oil, obtained by steam distillation from aerial parts. *Menthae piperitae folium* (*European pharmacopoeia*, 1985) is a well-known herbal remedy, used for its aromatic, stomachic, choleretic, carminative, and stimulant properties. The broad spectrum of bioactivity of the plant has usually been ascribed to the components of its essential oil. Although intensive pharmacological and clinical studies have been carried out for the spasmolytic activity of peppermint oil on gastrointestinal smooth muscle [for a recent review, see Brandt (1988); Hills and Aaronson, 1991), little attention has been paid to spasmolytic and choleretic properties of flavonoids from *M.* × *piperita* leaves (Lallement-Guilbert and Bezanger-Beauquesne, 1970; Pasechnik, 1966; Pasechnik and Gella, 1966). The chemical composition of peppermint oil has been widely investigated [for literature, see Lawrence et al. (1989)] as it is extensively used as a flavoring agent. Comparatively, few studies have been done to characterize the flavonoid glycosides of peppermint extracts, and the following compounds have been isolated: luteolin 7-*O*-glucoside and 7-*O*-rutinoside, apigenin 7-*O*-glucoside and 7-*O*-rutinoside (isorhoifolin) with the 4′-*O*-caffeoyl esters of apigenin glycosides (respectively, piperitoside and menthoside), and the 7-*O*-rutinosides of diosmetin (diosmin), hesperetin (hesperidin), and eriodictyol (eriocitrin) (Braun, 1930; Gella et al., 1966, 1967; Hoffmann and Lunder, 1984; Horhammer, 1961; Kohlmunzer et al., 1975; Lallement-Guilbert and Bezanger-Beauquesne, 1970; Semrau, 1958).

From a morphological standpoint, the sterile natural hybrid from *Mentha aquatica* L. (water mint) and *Mentha* × *spicata* L. (spearmint), *M.* × *piperita*, is subdivided into three distinct varieties (Graham, 1951): var. *vulgaris* Sole or Mitcham mint, which is the most widespread throughout the world; var. *sylvestris* Sole or Hungarian mint; and var. *officinalis* Sole or white mint. They are well characterized by the chemical composition of the essential oil (Gasic et al., 1987; Gilly et al., 1986; Lamaison et al., 1987; Maffei and Sacco, 1987; Pasquier, 1989, 1990). It seems that no phytochemical research had been undertaken on the distribution of flavonoids in different *M.* × *piperita* clones. To obtain a better understanding of the nonvolatile fractions that are present in the clones of the three peppermint varieties and which may also be involved in the pharmacological effects observed, our interest was directed to the phenolic constituents, i.e., caffeic acid derivatives and flavone and flavanone glycosides. This investigation describes the occurrence and distribution of flavonoid glycosides and rosmarinic acid contained in the leaves of 40 clones. The determination is performed using a high-performance liquid chromatographic (HPLC) method. Increased selectivity in UV detection has been obtained by the application of a microprocessor-controlled photodiode array detector (PAD) to acquire spectral data during the chromatographic separation.

MATERIALS AND METHODS

Plant Material. The investigation was started by collecting European and North American clones from the three varieties of *M.* × *piperita*. The clones investigated, classified by variety and group, are given in Table 1 together with a reference number, provenance, and origin. Botanical data of each group have been previously developed (Pasquier, 1989, 1990). They were cultivated under identical field conditions (parcels of 6 m² each) at the Conservatoire National des Plantes Médicinales, Aromatiques et Industrielles (CNPMAI), Milly-la-Forêt, France. The plants were propagated in June 1989 through root cuttings and harvested at the stage of flowering in mid-July 1990. After drying in the shade at room temperature, the leaves were separated from the aerial parts. Voucher specimens are deposited at the CNPMAI.

Chemicals. The reference substances (Figure 1) of standard quality (diosmin, eriocitrin, eriodictyol 7-*O*-glucoside, hesperidin, isorhoifolin, narirutin) and rosmarinic acid were obtained from Extrasynthèse, Genay, France. Luteolin 7-*O*-rutinoside was generously provided by Dr. T.-L. Lunder (Research Centre Nestlé, Vevey, Switzerland). The solvents used were of analytical or HPLC grade and were used without further purification.

Sample Preparation. One gram of powdered leaves was extracted twice with methanol (50 mL) at 45 °C for 15 min each. After filtration, the solution was adjusted to a final volume of 100.0 mL in a volumetric flask (HPLC determination of ros-

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Table 1. *M.* × *piperita* Clones Collected for Investigation

variety (group)	ref no.	clone	provenance[a]	origin
vulgaris (I)	1	Men 139	1	New York
	2	Men 148	1	New Hampshire
	3	Men 141	1	Oregon
	4	Men 146	1	California
	5	Men 144	1	New Jersey
	6	Men 550	1	Oregon
	7	Men 138	1	Leiden (The Netherlands)
	8	Men 143	1	Michigan
	9	Men 140	1	Oregon
	10	Men 142	1	Michigan
	11	Men 560	1	Oregon
vulgaris (IIA)	12	Men 134 (Men 133 tetraploid)	1	Michigan
	13	Men 133 Mitcham	1	England
	14	Men 145 Mitcham	1	Pennsylvania
	15	Ribécourt no. 19	2	Ribécourt (France)
	16	Mitcham–Milly	2	Milly-la-Forêt (France)
	17	AMF	2	Milly-la-Forêt (France)
	18	52	3	Pessione (Italy)
	19	38	3	Digne (France)
	20	59	3	Surrey (England)
	21	90	3	Carmagnola (Italy)
	22	44	3	Turin (Italy)
	23	13	3	Drôme (France)
vulgaris (IIB)	24	Men 200 Murray's Mitcham	1	Michigan
	25	Men 199 Todd's Mitcham	1	Michigan
vulgaris (IIC)	26	Men 147	1	Amsterdam
sylvestris (I)	27	104	3	Giessen (Germany)
	28	49	3	Pessione (Italy)
sylvestris (II)	29	Hongrie no. 5	2	Hungary
	30	39	3	Bulgaria
	31	Auvergne	4	Auvergne (France)
	32	42	3	Savoie (France)
officinalis (I)	33	87	3	Adelaide (Australia)
	34	53	3	Pessione (Italy)
	35	43	3	Turin (Italy)
officinalis (II)	36	AMC	2	Milly-la-Forêt (France)
	37	15	3	Montpellier (France)
	38	Maine-et-Loire	5	Maine-et-Loire (France)
officinalis (III)	39	Priluskaya	6	USSR (?)
officinalis (IV)	40	Men 135	1	Indiana

[a] Provenance of clones: 1, NCGR, Corvallis, OR; 2, CNPMAI 91, Milly-la-Forêt, France; 3, INRA 06, Antibes, France; 4, ARRAPAM 63, Clermont-Ferrand, France; 5, ITEPMAI 49, Chemillé, France; 6, Brno, Czechoslovakia.

marinic acid and flavonoid glycosides except eriocitrin). One milliliter of this solution was diluted to 10 mL with the same solvent (HPLC determination of eriocitrin). Before injection, samples were passed through a 0.45-μm filter. A standard solution containing 0.2 mg/mL of each reference compound in methanol/dimethyl sulfoxide (80:20 v/v) was prepared.

High-Performance Liquid Chromatography (HPLC). Qualitative HPLC analyses were performed using a Waters 600E liquid chromatograph (Waters Associates, Milford, MA), a Waters 990 photodiode array detector in combination with a NEC-APC IV personal computer, and a Waters 990 plotter. UV spectra (220–400 nm) were recorded on-line by the PAD system.

Quantitative HPLC analyses were carried out with a HP 1050 series liquid chromatograph equipped with an automated injection system (Hewlett-Packard, Waldbronn, Germany). For integration, a Chromjet integrator (Spectra Physics, Santa Clara, CA) was used. Quantification was done using external standards and the peak area as a parameter. For all substances a linear relationship between peak area and concentration (0.5–0.05 mg/mL) was observed with a correlation coefficient always better than $r = 0.998$.

Chromatographic Conditions. HPLC analyses were performed on a Nucleosil C_{18} column (5 μm, 250 × 4.6 mm i.d., Macherey Nagel, Düren, Germany) with a linear gradient of acetonitrile/water (adjusted to pH 2.5 with phosphoric acid)/17–23% acetonitrile over 35 min. The flow rate was 1 mL/min; detection was carried out at 280 nm, attenuation 0.05. The system was left to stabilize for 10 min between consecutive injections. The injected volume was 10 μL.

Thin-Layer Chromatography (TLC) of Narirutin. The plates used were precoated sheets (E. Merck, Darmstadt, Germany). The mobile and stationary phases were as follows: A, 5% acetic acid; B, 15% acetic acid; C, 1-pentanol/acetic acid/water (50:25:25 v/v); D, 1-butanol/acetic acid/water (60:20:20 v/v) (cellulose plates); E, ethyl acetate/ethyl methyl ketone/formic acid/water (50:30:10:10 v/v); F, ethyl acetate/pyridine/water/methanol (80:20:10:5 v/v); G, ethyl acetate/acetic acid/formic acid/water (100:11:11:27 v/v) (silica gel 60). Ten microliters of peppermint extracts (1.0 g of powdered leaves was extracted with 10 mL of methanol) and of narirutin (2 mg/mL) was applied on the layer. Narirutin was visualized as a yellow (visible) and a dark green spot (UV–365 nm light) after the layer was sprayed with natural products reagent (Neu, 1957).

RESULTS AND DISCUSSION

Qualitative Analysis of *M.* × *piperita* Clones. A previous study was undertaken to optimize the separation by HPLC. The effects of C_{18} column type, of water/2-propanol or water/acetonitrile ratio, of addition of tetrahydrofuran and of pH on the retention behavior of these phenolic constituents were investigated. The linear gradient described allowed a satisfactory separation of all components, each one yielding a well-resolved peak within a short time (35 min). The chromatogram of a mixture of the reference compounds (1–8) is shown in Figure 2. The detection was carried out at 280 nm, the UV absorption maximum of flavanone glycosides. The UV spectra corresponding to each peak in the chromatogram were determined by using the PAD system. Individual peak

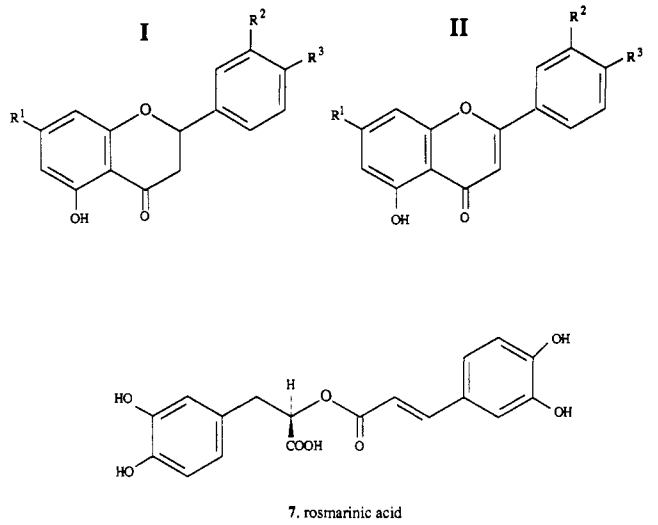

Figure 1. Structural formulas of compounds 1–8.

compound	aglycon	R^1	R^2	R^3
(1) eriocitrin	I	*O*-rutinose	OH	OH
(2) eriodictyol 7-*O*-glucoside	I	*O*-glucose	OH	OH
(3) luteolin 7-*O*-rutinoside	II	*O*-rutinose	OH	OH
(4) narirutin	I	*O*-rutinose	H	OH
(5) hesperidin	I	*O*-rutinose	OH	OCH_3
(6) isorhoifolin	II	*O*-rutinose	H	OH
(8) diosmin	II	*O*-rutinose	OH	OCH_3

purity was tested by examining the UV spectra at different points of the peak.

A typical chromatogram for the analysis of an extract of *M.* × *piperita* leaves is shown in Figure 3. Eriocitrin (1) dominates the chromatogram, with luteolin 7-*O*-rutinoside (3), hesperidin (5), and rosmarinic acid (7) assuming secondary importance. These constituents are accompanied by eriodictyol 7-*O*-glucoside (2), narirutin (4), isorhoifolin (6), and diosmin (8) in low yield. The analysis of *M.* × *piperita* clones showed similar HPLC fingerprint chromatograms. From the flavonoid glycosides investigated, compounds 2 and 4 had hitherto not been identified in *M.* × *piperita*. Narirutin seems to be identified for the first time from the genus *Mentha*, while eriodictyol 7-*O*-glucoside has already been described in *M. aquatica* L. by Burzanska-Hermann et al. (1977). To confirm the presence of narirutin in samples analyzed by HPLC, a detailed thin-layer chromatographic comparison was investigated with the corresponding reference substance. Only the sample (clone Priluskaya) containing a sufficient amount of narirutin (see below) showed a characteristic spot [R_f = 0.55 (A); 0.85 (B); 0.70 (C); 0.60 (D); 0.45 (E); 0.60 (F); 0.50 (G)] when the plates were sprayed with natural products reagent. *M.* × *piperita* clones contain only flavanone (compounds 1, 4, 5) and flavone (compounds 3, 6, 8) rutinosides. These data correspond to the distribution of the isomeric disaccharide pairs of flavanones observed in natural citrus species and citrus hybrids with either rutinosyl [rhamnosyl(α1→6)-glucose] or neohesperidosyl [rhamnosyl(α1→2)glucose] glycosides (Kamiya et al., 1979; Nishiura et al., 1971). They can be easily distinguished by their taste, the rutinosides being tasteless and the neohesperidosides intensely bitter.

The UV spectra obtained by examination of the peaks of reference substances were similar to the spectra from the peaks eluting at the corresponding retention times in peppermint samples (Figure 4). No traces of luteolin 7-*O*-glucoside and apigenin 7-*O*-glucoside could be detected in the extracts. A few secondary peaks were not identified. The UV spectrum of the substance corresponding to peak

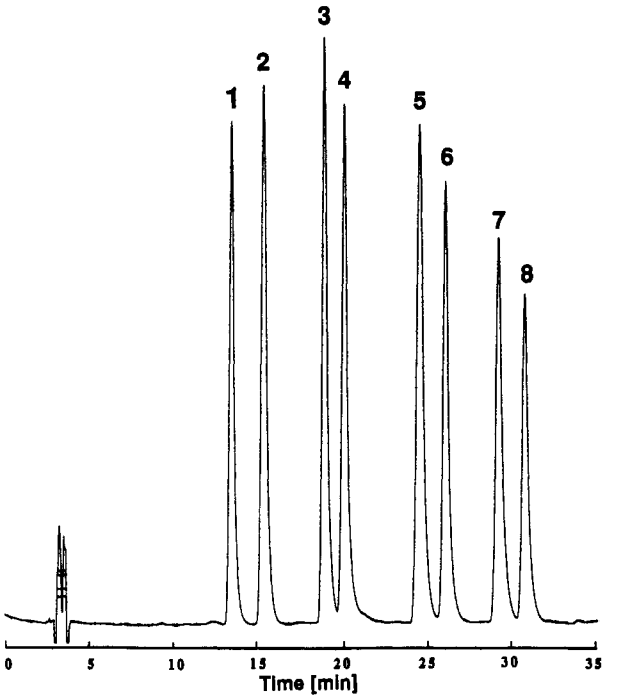

Figure 2. HPLC separation of the mixture of reference substances. Peak identification: (1) eriocitrin; (2) eriodictyol 7-*O*-glucoside; (3) luteolin 7-*O*-rutinoside; (4) narirutin; (5) hesperidin; (6) isorhoifolin; (7) rosmarinic acid; (8) diosmin.

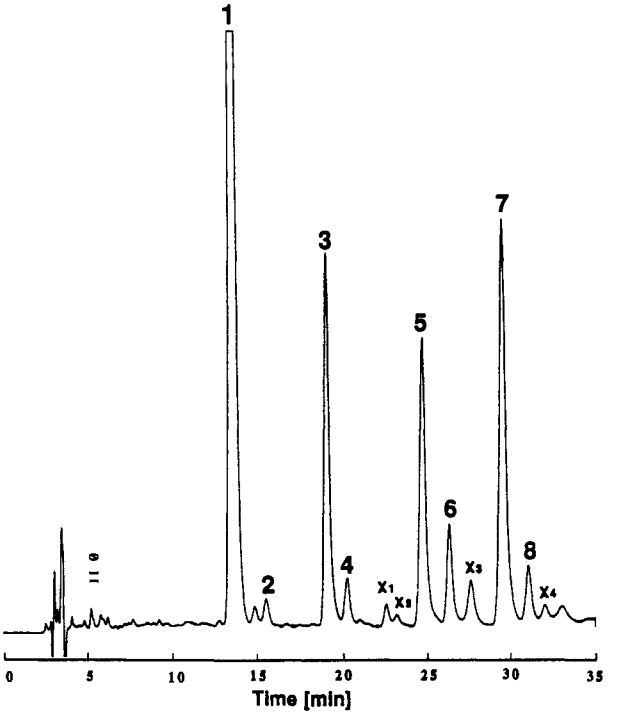

Figure 3. HPLC separation of the flavonoid glycosides and rosmarinic acid of *M.* × *piperita* leaves (clone 36). Peak identification: (1) eriocitrin; (2) eriodictyol 7-*O*-glucoside; (3) luteolin 7-*O*-rutinoside; (4) narirutin; (5) hesperidin; (6) isorhoifolin; (7) rosmarinic acid; (8) diosmin. X_1, X_2, X_3, and X_4 correspond to unknown substances.

X_1 is identical to that of luteolol glycosides, and it may be assumed that this compound corresponds to luteolol 7-*O*-glucuronide, already described in *Mentha longifolia* L. by Bourwieg and Pohl (1973). According to the hypsochromic shift of band I (cinnamoyl ring, λ_{max} = 320 nm, instead of 340 nm), the UV spectrum of the substance corresponding to peak X_4 could be ascribed to caffeic acid derivatives of apigenin glycosides (Gella et al., 1966, 1967). On the other

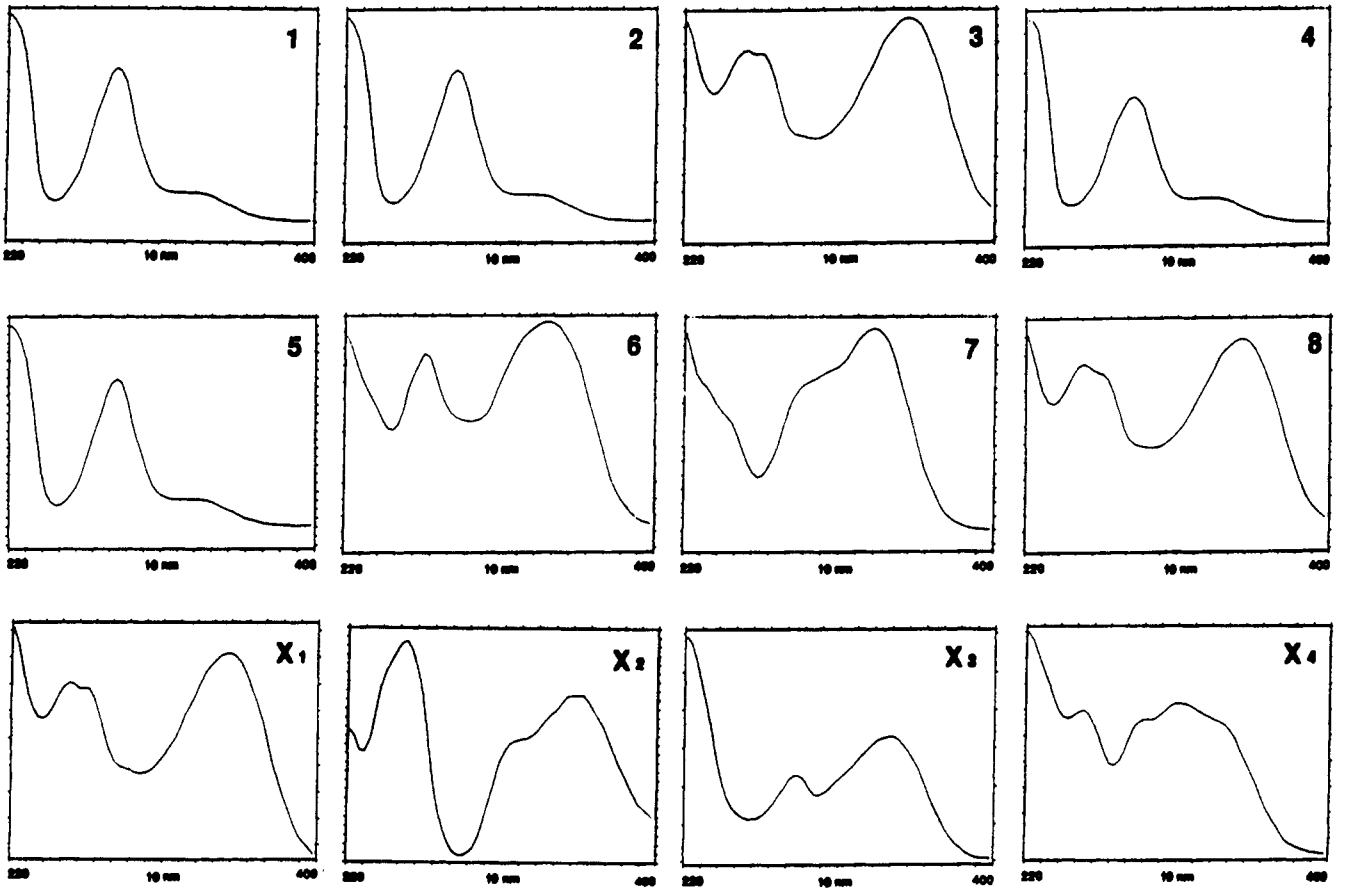

Figure 4. UV spectra of the main flavonoid glycosides and rosmarinic acid of *M.* × *piperita* recorded on-line by the PAD (for peak identities see Figure 3).

hand, the substances corresponding to peaks X_2 and X_3 have UV absorption maxima that are not in accordance with those of constituents described in the literature.

Quantitative Analysis of Phenolic Constituents in *M.* × *piperita* Clones. Quantitative determination was carried out using external standards for calibration. To assess the precision of the analysis, one sample (clone 36) was analyzed repeatedly five times using the sample preparation procedure followed by HPLC. The precision of the entire analytical process for compounds 1–8 in terms of the relative standard deviation was comprised between 0.8 and 1.7%. Table 2 reports the concentrations of flavonoid glycosides and rosmarinic acid found in leaves of *M.* × *piperita* clones. The amplitude of the variation in total glycosides among the peppermint clones ranged from 8.6 to 17.8%. It is noteworthy that the content of eriocitrin, varying from 6.6 to 15.0% (mean 10.8%), was high in all samples. The higher concentrations in eriocitrin and total flavonoid glycosides are present in the Mitcham's clones (*vulgaris* group II). Eriocitrin accounted for about 80% of the total glycosides. It was accompanied by a relatively high yield of luteolin 7-*O*-rutinoside (1.0–2.3%), rosmarinic acid (1.0–3.9%), and, secondarily, hesperidin (0.35–1.3%). Lower contents were found for the other flavonoid glycosides.

As far as the *rubescens* form (*vulgaris* and *sylvestris* varieties) is considered, the amount and composition of phenolic constituents are somewhat characteristic for each group. The *vulgaris* group I was distinguished by a relatively higher content in eriodictyol 7-*O*-glucoside, isorhoifolin, and rosmarinic acid, while eriocitrin was found in a lesser amount in this group and in the *sylvestris* group I. In return, there were larger quantitative differences between the clones of the *officinalis* variety and those of the *rubescens* form. Three glycosides occurred in larger amounts in different *officinalis* groups (eriodictyol 7-*O*-glucoside in groups I and IV; hesperidin and diosmin in groups II and III), while the clone Priluskaya showed a peculiar accumulation of narirutin (1.6%). A similar content in narirutin was found in another Russian clone, Krasnadorskaya, not investigated in the present study. The highest level of luteolin 7-*O*-rutinoside and isorhoifolin was obtained for the *officinalis* group II. Rosmarinic acid was present in low yield in the three *vulgaris* subgroups II in comparison with the *vulgaris* group I and the *officinalis* variety.

Contrary to the chemical composition of the essential oil, the significance and usefulness of flavonoid glycosides as chemotaxonomical character are limited when the clones belonging to the *rubescens* form are compared. In return, the content of some flavonoid compounds in the *officinalis* variety, namely narirutin, hesperidin, and diosmin, may be useful taxonomic markers.

Therapeutic indications of the peppermint preparations are, e.g., in France, symptomatic treatment of digestive disorders and adjuvant treatment for the painful component in spasmodic colitis (Herbal remedies, 1990). Owing to the fact that *M. piperita* clones are characterized by a high content of phenolic constituents, particularly flavonoid glycosides, they are possibly involved in the pharmacological effects observed as the spasmolytic (Capasso et al., 1991), and the anti-inflammatory [for a review, see Alcaraz and Jimenez (1988)] actions of flavonoids may contribute to the healing of some digestive disorders. Moreover, with extraction rates of ca. 75 and 20% of, respectively, the flavonoid compounds and the lipophilic peppermint oil components in infusion (Duband et al., 1992), the question as to whether the essential oil

Table 2. Contents (Percentage, Dry Weight Basis) of Flavonoid Glycosides and Rosmarinic Acid in *M.* × *piperita* Clones

variety (group)	ref no.	compound[a] 1	2	3	4	5	6	7	8	9
vulgaris (I)	1	9.32	0.16	1.45	0.06	0.46	0.27	3.57	0.10	11.82
	2	7.77	0.13	1.16	0.08	0.36	0.19	2.32	0.07	9.76
	3	9.02	0.12	1.05	0.07	0.62	0.36	3.68	0.10	11.34
	4	7.70	0.12	1.23	0.09	0.44	0.25	3.36	0.07	9.90
	5	7.49	0.13	1.29	0.08	0.67	0.25	2.93	0.10	10.01
	6	9.82	0.18	1.45	0.07	0.41	0.31	2.92	0.07	12.31
	7	6.61	0.09	1.14	0.07	0.34	0.26	2.88	0.07	8.58
	8	9.16	0.13	1.34	0.11	0.55	0.26	2.52	0.06	11.61
	9	7.93	0.12	1.33	0.07	0.38	0.23	2.63	0.09	10.15
	10	7.64	0.12	1.30	0.08	0.45	0.29	2.70	0.10	9.98
	11	7.67	0.13	1.25	0.07	0.44	0.30	3.18	0.12	9.98
	av	**8.19**	**0.13**	**1.27**	**0.08**	**0.47**	**0.27**	**2.97**	**0.09**	**10.49**
vulgaris (IIA)	12	14.87	0.09	2.00	0.12	0.55	0.10	2.47	0.06	17.79
	13	12.16	0.04	1.53	0.08	0.41	0.08	2.16	0.05	14.35
	14	10.84	0.04	1.55	0.06	0.42	0.08	1.85	0.04	13.03
	15	11.41	0.06	1.45	0.19	0.34	0.09	1.84	0.03	13.57
	16	11.98	0.05	1.62	0.07	0.64	0.08	1.37	0.08	14.52
	17	10.65	0.03	1.72	0.10	0.56	0.07	1.76	0.08	13.21
	18	13.50	0.05	1.57	0.08	0.41	0.07	1.75	0.03	15.71
	19	12.46	0.05	1.48	0.08	0.58	0.07	1.68	0.05	14.77
	20	11.48	0.03	1.80	0.08	0.60	0.10	1.41	0.05	14.14
	21	13.41	0.03	1.76	0.08	0.57	0.08	1.23	0.05	15.98
	22	11.39	0.05	1.50	0.05	0.52	0.10	1.13	0.08	13.69
	23	13.01	0.03	1.55	0.05	0.46	0.11	1.28	0.07	15.28
	av	**12.26**	**0.05**	**1.63**	**0.09**	**0.50**	**0.09**	**1.66**	**0.06**	**14.67**
vulgaris (IIB)	24	14.30	0.05	1.73	0.10	0.39	0.08	1.69	0.06	16.71
	25	14.98	0.09	1.68	0.15	0.37	0.11	1.60	0.05	17.43
	av	**14.64**	**0.07**	**1.70**	**0.12**	**0.38**	**0.09**	**1.64**	**0.05**	**17.07**
vulgaris (IIC)	26	11.72	0.03	1.24	0.04	0.44	0.05	0.97	0.03	13.55
sylvestris (I)	27	9.79	0.04	1.73	0.13	0.61	0.13	2.35	0.04	12.47
	28	8.88	0.03	1.68	0.04	0.91	0.12	2.26	0.09	11.75
	av	**9.33**	**0.03**	**1.70**	**0.08**	**0.76**	**0.12**	**2.30**	**0.06**	**12.11**
sylvestris (II)	29	11.88	0.04	2.16	0.06	0.76	0.10	1.94	0.12	15.12
	30	10.35	0.04	1.80	0.05	0.74	0.10	1.73	0.11	13.19
	31	11.59	0.04	2.12	0.05	0.90	0.14	2.25	0.13	14.97
	32	10.12	0.04	1.90	0.04	0.68	0.12	2.07	0.11	13.01
	av	**10.98**	**0.04**	**1.99**	**0.05**	**0.77**	**0.11**	**2.00**	**0.12**	**14.07**
officinalis (I)	33	11.61	0.39	0.95	0.16	0.79	0.11	2.60	0.07	14.08
	34	9.38	0.32	1.05	0.14	0.68	0.06	2.55	0.09	11.72
	35	12.38	0.42	1.20	0.12	0.48	0.09	2.77	0.05	14.74
	av	**11.12**	**0.38**	**1.07**	**0.14**	**0.65**	**0.09**	**2.64**	**0.07**	**13.51**
officinalis (II)	36	11.72	0.05	2.20	0.14	1.31	0.50	1.94	0.47	16.39
	37	9.18	0.04	1.85	0.14	1.23	0.26	2.75	0.29	12.99
	38	11.88	0.10	2.32	0.17	1.18	0.31	3.86	0.20	16.16
	av	**10.93**	**0.06**	**2.12**	**0.15**	**1.24**	**0.36**	**2.85**	**0.32**	**15.18**
officinalis (III)	39	11.56	0.18	1.92	1.59	1.28	0.16	1.91	0.23	16.92
officinalis (IV)	40	12.16	0.32	1.06	0.09	0.53	0.08	2.85	0.06	14.30

[a] (1) eriocitrin; (2) eriodictyol 7-*O*-glucoside; (3) luteolin 7-*O*-turinoside; (4) narirutin; (5) hesperidin; (6) isorhoifolin; (7) rosmarinic acid; (8) diosmin; (9) total flavonoid glycosides (1–6, 8).

would contribute to the pharmacological activity can be raised. Furthermore, due to the fact that the flavonoid fingerprints are dominated by flavanone glycosides (compounds 1, 2, 4, 5), well-known as bioflavonoids, the therapeutic effects of the plant drug should be reconsidered. Indeed, it has been known for a long time that the bioflavonoids or citrus flavonoids are suitable sources of vasotonic drugs with a P vitamin activity (Rusznyák and Szent-Györgi, 1936). It will be interesting to consider the use of the postdistillation waste as a potential source of flavonoid glycosides, particularly of eriocitrin, first isolated from lemon peel (Horowitz and Gentili, 1960).

LITERATURE CITED

Alcaraz, M. J.; Jimenez, M. J. Flavonoids as anti-inflammatory agents. *Fitoterapia* **1988**, *59*, 25–38.

Bourwieg, D.; Pohl, R. The Flavonoids of *Mentha longifolia*. *Planta Med.* **1973**, *24*, 304–314.

Brandt, W. Z. Spasmolytic activity of essential oils. *Z. Phytother.* **1988**, *9*, 33–39.

Braun, H. A. The non-volatile constituents of *Mentha piperita*, *Am. J. Pharm.* **1930**, *102*, 202–219.

Burzanska-Hermann, Z.; Rzadkowska-Bodalska, H.; Olechnowicz-Stepien, W. Isolation and identification of flavonoid compounds of *Mentha aquatica* L. Herb. *Rocz. Chem.* **1977**, *51*, 701–709.

Capasso, A.; Pinto, A.; Mascolo, N.; Autore, G.; Capasso, F. Reduction of agonist-induced contractions of guinea-pig isolated ileum by flavonoids. *Phytother. Res.* **1991**, *5*, 85–87.

Duband, F.; Carnat, A. P.; Carnat, A.; Petitjean-Freytet, C.; Clair, G.; Lamaison, J. L. The aromatic and polyphenolic composition of peppermint *Mentha piperita* L. tea. *Ann. Pharm. Fr.* **1992**, *50*, 146–155.

European Pharmacopoeia, 2nd ed.; Maisonneuve: Sainte Ruffine, France, 1985; p 406; *Menthae piperitae folium*.

Gasic, O.; Mimica-Dukic, N.; Adamovic, D.; Borojevic, K. Variability of content and composition of essential oil in different genotypes of peppermint. *Biochem. Syst. Ecol.* **1987**, *15*, 335–340.

Gella, E. V.; Makarova, G. V.; Borisyuk, Y. G.; Litvinenko, V. I. Flavonoids of *Mentha piperita*. *Farm. Zh. (Kiev)* **1966**, *21*, 58–66; *Chem. Abstr.* **1966**, *65*, 13810.

Gella, E. V.; Makarova, G. V.; Borisyuk, Y. G. *Mentha piperita* Flavonoids. *Farm. Zh. (Kiev)* **1967**, *22*, 80–85; *Chem. Abstr.* **1968**, *68*, 49985t.

Gilly, G.; Garnero, J.; Racine, P. Peppermints. Chromatographic analysis of chemical composition. *Parfums, Cosmet., Aromes* **1986**, *71*, 79–86.

Graham, R. A. Mint Notes. IV *Mentha piperita* and the British peppermint. *Watsonia* **1951**, *2*, 30–35.

Herbal remedies. Notice to applicants for marketing authorization. In *Bulletin Officiel 90/22 bis* [English translation of B.O. 90/22 bis]; Direction des journaux officiels: Paris, 1990; 92–11bis, p 25.

Hills, J. M.; Aaronson, P. I. The mechanism of action of peppermint oil on gastrointestinal smooth muscle. *Gastroenterology* **1991**, *101*, 55–65.

Hoffman, B. G.; Lunder, L. T. Flavonoids from *Mentha piperita*. *Planta Med.* **1984**, *51*, 361.

Horhammer, L. Presented at the Ber. XXI Intern. Kongr. Pharm. Wiss., Pisa, Sept 1961.

Horowitz, R. M.; Gentili, B. Flavonoid compounds of Citrus III. Isolation and structure of eriodictyol glycoside. *J. Am. Chem. Soc.* **1960**, *82*, 2803–2806.

Kamiya, S.; Esaki, S.; Konishi, F. Flavonoids in citrus hybrids. *Agric. Biol. Chem.* **1979**, *43*, 1529–1536.

Kohlmunzer, S.; Grzybek, J.; Sodzawiczny, K. Polyphenolic compounds in the post-distillation waste *Herba menthae piperitae*. *Herba Pol.* **1975**, *21*, 130–137.

Lallement-Guilbert, N.; Bezanger-Beauquesne, L. Flavonoids of some medicinal Lamiaceae (rosemary, peppermint, garden sage). *Plant. Med. Phytother.* **1970**, *4*, 92–107.

Lamaison, J. L.; Carnat, A. P.; Carnat, A. Differentiation of peppermint, *Mentha piperita* Mitcham and Hungary types, grown in Auvergne. *Plant. Med. Phytother.* **1987**, *21*, 252–261.

Lawrence, B. M.; Shu, C. K.; Harris, W. R. Peppermint oil differentiation. *Perfum. Flavor.* **1989**, *14* (6), 21–30.

Maffei, M.; Sacco, T. Chemical and morphometrical comparison between two peppermint notomorphs. *Planta Med.* **1987**, *53*, 214–216.

Neu, R. Diarylboric acid chelates with aliphatic oxyalkylamines as reagents for the identification of oxyphenyl-benzo γ-pyrones. *Naturwissenschaften* **1957**, *44*, 181–182.

Nishiura, M.; Kamiya, S.; Esaki, S. Flavonoids in Citrus and related genera. Part 3. Flavonoid pattern and Citrus taxonomy. *Agric. Biol. Chem.* **1971**, *35*, 1691–1706.

Pasechnik, I. K. Study of choleretic properties specific to flavonoids from *Mentha piperita* leaves. *Farmakol. Toksikol.* **1966**, *29*, 735–737.

Pasechnik, I. K.; Gella, E. V. Choleretic preparation from peppermint. *Farm. Zh. (Kiev)* **1966**, *21*, 49–53; *Chem. Abstr.* **1967**, *66*, 36450y.

Pasquier, B. *Mentha piperita* L. Some morphological and chemical data for the classification. In *Compte-rendu technique*; Conservatoire National des Plantes Médicinales Aromatiques et Industrielles: Milly-la-Forêt, 1989; pp 47–64.

Pasquier, B. Study of the morphological and chemical variability in 45 pippermint clones. In *Compte-rendu technique*; Conservatoire National des Plantes Médicinales Aromatiques et Industrielles: Milly-la-Forêt, 1990; pp 43–55.

Rusznyák, S.; Szent-Györgyi, A. Vitamin P: flavonols and vitamins. *Nature* **1936**, *138*, 27.

Semrau, R. On flavones in the Labiate family. Inaug. Dissertation, München, 1958; 71 pp.

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