Phytochemistry, 1971, Vol. 10, pp. 611 to 614. Pergamon Press. Printed in England.

FLAVONOIDS OF *LASTHENIA CONJUGENS* AND *LASTHENIA FREMONTII*

NABIEL A. M. SALEH and BRUCE A. BOHM
Botany Department, University of British Columbia, Vancouver, B.C., Canada
and
ROBERT ORNDUFF
Botany Department, University of California, Berkeley, California, 94720, U.S.A.

(*Received* 20 *June* 1970)

Abstract—The flavonoid complement of *Lasthenia conjugens* and *L. fremontii* (Compositae) as well as hybrids derived from these two species has been determined. The compounds observed were: quercetin-3-*O*-glucoside, quercetin-3-*O*-glucuronide, patuletin-3-*O*-glucoside, patuletin-3-*O*-glucuronide, patuletin-7-potassium bisulfate, patuletin-3, 7-di-*O*-glucoside, patuletin-3-*O*-glucoside-7-potassium bisulfate, and a quercetin-3-*O*-bioside. This latter compound was present in very small concentration in the hybrids only and yielded glucose and glucuronic acid. The hybrids lacked patuletin-7-potassium bisulfate.

INTRODUCTION

AS PART of a chemosystematic study of *Lasthenia* (Compositae) several collections of *L. conjugens* and *L. fremontii* and two hybrids thereof were examined. We now describe the flavonoid complement of these plants including several hitherto unknown patuletin derivatives. Patuletin glycosides have been reported previously: in the Compositae in *Tagetes*,[1-3] *Matricaria*,[4] *Hymenoxys*,[5] *Anthemis* and *Chrysanthemum*;[6] in the Chenopodiaceae, *Spinacia*[7]; in the Leguminosae, *Prosopis*;[8] and in the Eriocaulaceae, *Eriocaulon*.[9] The present work is the first report of patuletin derivatives in *Lasthenia*.

RESULTS AND DISCUSSION

Paper chromatographic analysis of whole plant extracts of *Lasthenia conjugens*, *L. fremontii*, and artificial hybrids between these two species yielded a total of eight flavonoids, seven being the most observed in any single specimen. The aglycones were shown to be quercetin and patuletin by standard methods (u.v., R_fs, fluorescence, comparison with standards). In addition, patuletin was demethylated and the product compared with authentic quercitagetin. Preliminary observation showed five mono- and three diglycosides. These compounds are listed in Table 1. Tables 2 and 3 record the chromatographic and spectral characteristics of these compounds, respectively.

[1] P. S. RAO and T. R. SESHADRI, *Proc. Indian Acad. Sci.* **14A**, 265, 643 (1941).
[2] L. R. ROW and T. R. SESHADRI, *Proc. Indian Acad. Sci.* **23A**, 140 (1946).
[3] N. R. BANNERJEE and T. R. SESHADRI, *Proc. Indian Acad. Sci.* **44A**, 284 (1956).
[4] L. HÖRHAMMER, H. WAGNER and B. SALFNER, *Arzneimittel-Forsch.* **13**, 33 (1963).
[5] M. B. THOMAS and T. J. MABRY, *Phytochem.* **7**, 787 (1968).
[6] J. B. HARBORNE, V. H. HEYWOOD and N. A. M. SALEH, *Phytochem.* **9**, 2011 (1970).
[7] A. ZANE and S. H. WENDER, *J. Org. Chem.* **26**, 4718 (1961).
[8] R. C. SHARMA, A. ZAMAN and A. R. KIDWAI, *Indian J. Chem.* **2**, 83 (1964).
[9] E. C. BATE-SMITH and J. B. HARBORNE, *Phytochem.* **8**, 1035 (1969).

TABLE 1. THE DISTRIBUTION OF FLAVONOIDS IN *Lasthenia conjugens, L. fremontii* AND HYBRIDS

Species		Compound*							
		Q-3-G I	Q-3-Gu I	P-3-G IV	P-3-Gu V	P-7-KS VI	P-3, 7-DG VII	P-3-G 7-KS VIII	Q-3-DGly III
Lasthenia conjugens	6054[2]†	+	+	+	+	+	+	+	?
Lasthenia fremontii	4865	+	+	+	+	+	+	+	?
Lasthenia fremontii	4936	+	+	+	+	+	+	+	?
Hybrid	6054 × 4865	+	+	+	+	−	+	+	?
Hybrid	6054 × 4936	+	+	+	+	−	+	+	+

* Explanation of abbreviations: Q-3-G, quercetin-3-*O*-glucosides; Q-3-Gu, quercetin-3-*O*-glucuronide; P-3-G, patuletin-3-*O*-glucoside; P-3-Gu, patuletin-3-*O*-glucuronide; P-7-KS, patuletin-7-potassium bisulfate; P-3, 7-DG, patuletin-3, 7-*O*-diglucoside; P-3-G-7-KS, patuletin-3-*O*-glucoside-7-potassium bisulfate; and Q-3-DGly, quercetin-3-diglycoside. (Roman numerals refer to structures in text.)

† Plant collection numbers; see Ref. 16.

TABLE 2. CHROMATOGRAPHIC CHARACTERISTICS OF ISOLATED GLYCOSIDES

	Colours		R_f values*			
	u.v.	u.v./NH_3	H_2O	HOAc	BAW	PhOH
Quercetin						
3-glucoside	brown	yellow	12	27	61	31
3-glucuronide			14	27	51	3
3-bioside			18	34	57	4
Patuletin						
3-glucoside	dark brown	yellow	18	34	56	52
3-glucuronide		brown	22	35	44	6
3,7-diglucoside	yellow	yellow	60	61	4	2
	brown					
7-K-bisulfate	yellow	yellow	6	12	22	14
3-glucoside-7-K bisulfate	yellow brown	yellow	60	63	13	20

* See Experimental for solvent key.

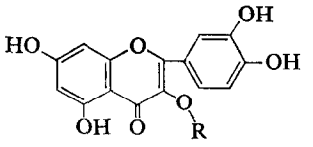
TABLE 3. U.V. SPECTRA OF ISOLATED GLYCOSIDES

Glycosides λ_{max} (nm)	Ethanol	Treatment +$AlCl_3$ Band II	+NaOAc Band I	+H_3BO_3/NaOAc Band II
Quercetin-3-*O*-glucuronide	258, 364	50	13	19
Patuletin-3-*O*-glucoside	261, 360	44	14	30
Patuletin-3-*O*-glucuronide	261, 362	46	12	23
Patuletin-3, 7-*O*-diglucoside	265, 273*, 356	30	0	32
Patuletin-7-K-bisulfate	259, 379	57	0	21
Patuletin-3-*O*-glucoside-7-K-bisulfate	258, 362	43	0	26

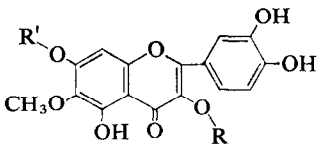
* Inflection.

Two quercetin monoglycosides were encountered. One was shown to be identical to quercitin-3-*O*-glucoside (I) by direct comparison with an authentic sample. The second yielded glucuronic acid on hydrolysis and gave u.v. data indicating that the sugar was attached at position 3 (II).

Three of the monoglycosides yielded patuletin after acid hydrolysis. Patuletin-3-*O*-glucoside (IV) and patuletin-3-*O*-glucuronide (V) were identified by standard methods.[5] The third patuletin derivative gave no sugars on acid hydrolysis and no acyl functions could be detected after alkaline hydrolysis. Emulsin had no effect on the compound. Sodium



I R = glucose
II R = glucuronic acid
III R = glucose + glucuronic acid



IV R = glucose; R′ = H
V R = glucuronic acid; R′ = H
VI R = H; R′ = SO_3^- K^+
VII R = R′ = glucose
VIII R = glucose; R′ = SO_3^- K^+

acetate failed to produce a shift in the A-ring peak of the spectrum indicating that a substituent was located at position 7. $AlCl_3$ did produce a shift in the A-ring peak indicating free hydroxyl group in position 5. Electrophoresis of a sample of the compound showed movement toward the positively charged pole while an authentic sample of patuletin-7-*O*-glucoside remained at the starting line. Re-examination of the acid hydrolyzate showed the presence of sulfate ($BaCl_2$ ppt). Blanks of water and chromatogram eluate were negative. These results are in accord with the existence of patuletin-7-bisulfate (VI). Atomic absorption spectroscopy demonstrated the presence of potassium.

Three diglycosides were observed, two from each *L. conjugens* and *L. fremontii* which yielded patuletin on hydrolysis and a small amount of one from the hybrids which yielded quercetin. One of the diglycosides yielded patuletin-7-*O*-glucoside and glucose on controlled acid hydrolysis (0·05 N). H_2O_2 oxidation[10] yielded glucose so that the initial compound must have been patuletin-3, 7-di-*O*-glucoside (VII). U.v. observations confirmed this conclusion.

The second patuletin derivative in this group gave only the aglycone and glucose on normal acid hydrolysis (2 N) but very mild acid treatment gave patuletin-3-*O*-glucoside. Alkaline hydrolysis failed to reveal the presence of an acyl function. Hydrolysis with emulsin produced glucose and patuletin-7-potassium bisulfate, previously identified. Thus, this compound is patuletin-3-*O*-glucoside-7-potassium bisulfate (VIII). The hydrolytic results are summarized in Table 4.

The diglycoside from the hybrids yielded quercetin, glucose, and glucuronic acid on acid hydrolysis. U.v. data suggested that the compound was substituted only at position 3 (III). Detailed examination of this quercetin-3-*O*-bioside was prevented by the limited amount of compound available from the hybrid plants.

Flavonoid bisulfates are amongst the rarest of naturally occurring flavonoids. Kawaguchi and Kim[11] reported the occurrence of isorhamnetin-3-potassium bisulfate in *Polygonum*

[10] B. V. Chandler and K. A. Harper, *Australian J. Chem.* **14**, 586 (1961).
[11] R. Kawaguchi and K. W. Kim, *J. Pharm. Soc. Japan* **57**, 108 (1937).

TABLE 4. SUMMARY OF HYDROLYSES OF PATULETIN DERIVATIVES

Compound	Treatment				
	Acid Hydrol.	Partial Acid Hydrol.	Alkaline Hydrol.	H_2O_2 Oxidation	Emulsin Hydrol.
Patuletin-7-K bisulfate	Patuletin	————	no change	no sugars	no change
Patuletin-3-*O*-glucoside-7-K bisulfate	Patuletin + glucose	Patuletin-3-*O*-glucoside as intermediate*	no change	glucose	patuletin-7-K-bisulfate
Patuletin-3,7-*O*-diglucoside	Patuletin + glucose	Patuletin-7-*O*-glucoside as intermediate*	no change	glucose	————

* The aglycone plus glucose were observed as well.

hydropiper var. *vulgare*. Hörhammer and Hänsel[12] and Tatsuta[13] also isolated rhamnezin-3-potassium bisulfate from the same species. Isorhamnetin-3 potassium bisulfate has been reported in *Polygonum thunbergii*,[14] *Polygonum perfoliatum*,[15] and in *Oenanthe stolonifera* (Umbelliferae).[15] To the best of our knowledge, the present observation of patuletin bisulfate derivatives marks the first report of such compounds in the Compositae as well as the first such compounds having the bisulfate function at position 7.

EXPERIMENTAL

Plant Material

The plants used were collected by R. Ornduff, the identifying numbers being his. Details of the collection sites and the procedures whereby the hybrids were obtained have been recorded.[16]

Chromatography

Standard methods were followed using Whatman No. 1 and 3MM papers. Solvents used for flavonoid separations were: water; 15% aq. HoAc. *n*-BuOH–HOAc–H_2O (4:1:5) (BAW); and PhOH–H_2O (4:1) (PhOH). Sugars were separated using the PhOH system and benzene–*n*-BuOH–pyridine–H_2O (1:5:3:3).

Hydrolytic Procedures

Total acid hydrolysis was carried out using 2N HCl while partial, or controlled, hydrolysis attempts were made using 0·05 N HCl. Alkaline hydrolysis was carried out with 2 N NaOH. Hydrolysis mixtures were maintained in a boiling water bath. Enzymatic hydrolysis with emulsin was conducted in an acetate buffer (pH 5).

Instrumental Methods

Electrophoresis was carried out on Whatman No. 3MM paper using an acetate–formic acid buffer (pH 2). The voltage was 3 kV and the time of application 30 min.

Acknowledgements—We thank the National Research Council of Canada for support of the analytical portion of this work. Collection and hybridization studies were supported by National Science Foundation grants to R. Ornduff. We also thank Prof. T. Geissman, Chemistry Department, U.C.L.A., for samples of quercetin-3-*O*-glycosides, Dr. I. E. P. Taylor, of this department, for use of the electrophoresis apparatus, and Mr. B. Von Spindler, Soil Sciences Department, for the atomic absorption analysis.

[12] L. HORHAMMER and R. HÄNSEL, *Arch. Pharm.* **286**, 153 (1953).
[13] H. TATSUTA, *Sci. Rep. Tohoku Univ.* 1 **39**, 239 (1956).
[14] H. TATSUTA, *Sci. Rep. Tohoku Univ.* 1 **39**, 236 (1956).
[15] H. TATSUTA and Y. OCHII, *Sci. Rep. Tohoku Univ.* 1 **39**, 243 (1956).
[16] R. ORNDUFF, *Univ. Calif. Pub. Bot.* No. 40, 86–87 (1966).