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SHORT COMMUNICATION

THE FLAVONOID PIGMENTS OF *LIATRIS SPICATA*

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Abstract—The flavonoid pigments of *Liatris spicata* were isolated and identified as the 3-glucoside, 3-rutinoside and 3-glucoside-7-rhamnoside of quercetin.

INTRODUCTION

THE RANGE of distribution of *Liatris spicata* (L.) Willd. (family Compositae) extends east of the Mississippi river from Southern Ontario to the Gulf of Mexico. Several varieties have been described and hybrids are frequent.[1] The flavonoid pigments of these plants and related species were found to be important since they could be used in documenting natural hybridization by comparing two dimensional paper chromatograms of crude plant extracts.[2] In this study, we analyzed a population near Zion, Ill. which was very homogeneous and which did not show any sign of hybridity, either morphologically or chromatographically.[3]

RESULTS

The plants were collected at the end of September 1967, shortly after their flowering peak. After air drying, the stems, leaves and flowers (740g) were ground and extracted continuously with chloroform for 3 days. No flavonoid was detected in this extract. The plant material was then continuously extracted with methanol for 2 days. This solution was taken to dryness under vacuum in presence of nylon powder, and the coated powder was washed successively with chloroform, water and methanol. These last two solutions contained flavonoid pigments.

The methanol wash was taken to dryness and was chromatographed over nylon powder in a short column, 2 cm × 0·5 cm. Water eluted flavonoid I, while solutions containing increasing amounts of methanol eluted flavonoids II and III in that order. The separation between these two compounds on the column was very poor and the process was repeated using a longer column and 25 per cent methanol as eluent. I was further purified by chromatography on cellulose (with water), on silica gel (with 5 per cent MeOH in EtOAc) and finally on nylon. A total of 280 mg of I, 20 mg of II and 60 mg of III were obtained.

Flavonoid I, after recrystallization from methanol, melted at either 220–222° or 245–253°. As no special precaution was taken to dry the methanol solutions, the samples probably differed in their water of crystallization.

The absorption spectrum of I showed maxima at 353 (Band I), 268 and 258 nm (Band II)

[1] L. O. GAISER, *Rhodora* **48**, 178, 216 (1946).
[2] D. A. LEVIN, *Brittonia* **19**, 248 (1967); *Evolution* **22**, in press (1968).
[3] D. A. LEVIN, personal communication.

in MeOH. Addition of sodium acetate shifted Band I to longer wavelength by 55 nm, indicating a free 4′-OH, but left Band II unchanged, thus showing the absence of a free 7-OH.[4] A free 3′-OH was confirmed by observing that boric acid did not cancel the acetate shift of Band I. A free 5-OH, but the absence of a 3-OH, was indicated by the 50 nm shift of Band I in methanolic $AlCl_3$. The presence of a substituent at the 3-position was deduced from the NMR spectrum, which lacked a signal corresponding to a proton at that position. The spectrum displayed doublets with J = 2 c/s at 6·48 and 6·84 corresponding to H-6 and H-8 respectively, doublets with J = 7 c/s at 6·92 and 7·68 corresponding to H-5′ and H-6′ respectively and a singlet at 7·67 assigned to H-2′. The signals for H-2′ and H-6′ as well as those for H-5′ and H-8 were partially overlapping. Integration of the NMR spectrum showed I to be a diglycoside. The sugars' H-1 protons were seen at 5·59 (rhamnose) overlapping the broad glucose signal which appeared to be centred near 5·52. The other sugar protons were seen between 3·1 and 4·0 and at 1·15 (rhamnose methyl).

Hydrolysis of I in 2 *N* HCl for 30 min liberated equimolar amounts of glucose and rhamnose, which were determined gas chromatographically.[5] The aglycone was found to be identical to quercetin chromatographically and spectroscopically and its m.p., 300–310°, was not depressed on admixture with authentic material.

The problem of deciding whether I was a 3-glucoside-7-rhamnoside or a 3-rhamnoside-7-glucoside of quercetin was solved by hydrolyzing it with β-glucosidase. Hydrolysis of 20 mg of I proceeded very slowly at room temperature in aqueous solution. After standing for 1 week, the solution was filtered through a small column of nylon powder. The eluate was concentrated to dryness and was trimethylsilylated in pyridine; gas chromatographic analysis showed that glucose was the only sugar liberated by the enzyme. The flavonoid (12 mg) eluted from the nylon column with methanol had the characteristic orange fluorescence of flavonols in u.v. light and showed a 65 nm spectroscopic shift of Band I in presence of $AlCl_3$. There was no shift of Band II with sodium acetate, which clearly demonstrated that glucose had been hydrolyzed from the 3-position and that the 7-position still carried a substituent. The product of enzymatic hydrolysis, quercetin 7-rhamnoside, melted at 274–276° and behaved chromatographically like other flavonol 7-glycosides.[6] It was hydrolyzed in 2 *N* HCl to quercetin and rhamnose.

Compound II melted at 182–185° after recrystallization from methanol. Compound III melted at 188–190° when recrystallized from dry ethyl acetate and at 220–230° when recrystallized from water. Both flavonoids had the same absorption spectra, with maxima at 354 and 268 (infl.) and 258 nm, and gave the spectral shifts characteristic of quercetin 3-glycosides.[4] On hydrolysis in 2 *N* HCl for 30 min, II yielded quercetin, as well as glucose and rhamnose in equal proportions. II was identified as quercetin 3-rutinoside by direct comparison with authentic rutin. Acid or β-glucosidase hydrolysis of III liberated quercetin and glucose, and since the integration of its NMR spectrum proved it to be a monoglycoside, III is therefore quercetin 3-β-glucoside (isoquercitrin).

Whereas rutin and isoquercitrin have been reported in many plants, the distribution of quercetin 3-glucoside-7-rhamnoside appears to be much more restricted. It was first reported in *Tilia argentea* (family Tiliaceae) without any structural proof.[7] Its melting point was

[4] L. JURD, in *The Chemistry of Flavonoid Compounds*, (edited by T. A. GEISSMAN) p. 122, Pergamon Press, Oxford (1962).
[5] J. KAGAN and T. J. MABRY, *Anal. Chem.* **37**, 288 (1965).
[6] J. B. HARBORNE, in *Comparative Biochemistry of the Flavonoids*, p. 66, Academic Press (1967).
[7] L. HÖRHAMMER, L. STICH and H. WAGNER, *Naturwissenschaften* **46**, 358 (1959).

given as 186–189°. However, this low value does not necessarily indicate that the original structural assignment was in error since in this work we obtained two different melting points for the diglycoside. The same compound has also been recently isolated from *Vincetoxicum officinale* (family Asclepiadaceae).[8]

EXPERIMENTAL

β-Glucosidase was purchased from General Biochemicals, Chagrin Falls, Ohio. The NMR spectra were determined with a Varian A-60A spectrometer in dimethylsulfoxide-d_6 and they are expressed in ppm on the δ-scale using tetramethylsilane as internal standard. The absorption spectra were recorded on a Beckman DB-G spectrometer. The sugar analyses were performed on 3 per cent SE-52 columns in a F & M 402 gas chromatograph. The melting points are uncorrected.

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[8] F. KOZJEK and P. LEBRETON, *Compt. Rend.* **264**, 2409 (1967).
[9] A specimen has been deposited at the herbarium of the University of Illinois, Chicago, Ill.