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SHORT COMMUNICATION

THE FLAVONOID PIGMENTS OF *OPUNTIA LINDHEIMERI*

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Abstract—Quercetin 3-galactoside (hyperin) and the isorhamnetin 3-rutinoside (narcissin), 3-galactoside and 3-rhamnogalactoside were found in the flowers of *Opuntia lindheimeri*. The last two compounds had not been previously described.

INTRODUCTION

Two new flavonoid glycosides have been encountered among the pigments of the flowers of *Opuntia lindheimeri*. The scarcity of information on the composition of the flavonoid pigments in the genus *Opuntia* and more generally in the family Cactaceae contrasts with the current interest placed in the structure of the betacyanin and betaxanthin pigments which are restricted to this family and to related ones of the order Centrospermae.[1] Isorhamnetin was identified by Arcoleo[2] after hydrolysis of an extract from the flowers of *O. ficus-indicus*, while Nair[3] reported the presence of unspecified glucosides of isorhamnetin and quercetin in *O. dillenii*. We now describe the structures of the flavonoid pigments of the flowers of *O. lindheimeri*. The betaxanthin pigments will be the subject of a later communication.

ISOLATION PROCEDURES

A methanol extract of the fresh yellow flowers collected in May, 1964, around Austin, Texas was concentrated to a small volume under vacuum. The residue was partitioned between water and *n*-butanol, all the flavonoids being extracted in the butanol phase. The betaxanthin pigments remained in the aqueous phase. Two-dimensional paper chromatographic analysis of the butanol fraction in the solvents *t*-butanol: acetic acid: water (3:1:1) and 15% acetic acid revealed only two spots originally assigned to one monoglycoside and one diglycoside. The flavonoid solution was concentrated to dryness and the residue was treated with methanol. The monoglycoside fraction precipitated readily, and the diglycoside was isolated from the mother solution by preparative paper chromatography in 15% acetic acid. The diglycoside, compound *A*, crystallized from dilute methanol and was homogeneous when examined by thin-layer chromatography on cellulose, silica gel and polyamide[4] with a variety of solvents. The monoglycoside fraction was a mixture of two components, which were clearly resolved on polyamide thin-layer plates with the solvent system methyl ethyl ketone: methanol: chloroform (1:3:5).[5] Polyamide column chromatography using the same

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[1] Leading references: *a.* M. PIATTELLI and L. MINALE, *Phytochem.* **3**, 307 (1964); *b.* M. PIATTELLI, L. MINALE and G. PROTA, *Tetrahedron* **20**, 2325 (1964); *c.* T. MABRY, In *Comparative Phytochemistry* (Edited by T. SWAIN). Academic Press, London (In press).

[2] A. ARCOLEO, M. RUCCIA and S. CUSMANO, *Ann. Chim.* (*Rome*), **51**, 751 (1961).

[3] A. G. R. NAIR and S. S. SUBRAMANIAN, *J. Sci. Ind. Research* (*India*) **20B**, 507 (1961).

[4] H. RÖSLER, Ph.D. Thesis, Munchen (1960).

[5] K. EGGER, *Angew. Chem.. Int. Ed.* **3**, 593 (1964).

solvent system separated compound *B*, the faster moving component, from compound *C*. Flowers were collected again at the same location in May 1965 and were extracted in 50% methanol. The flavonoids were removed from the extract by passing it through a column of charcoal[6] from which, after washing with water followed by methanol, they were desorbed by a saturated aqueous solution of phenol containing an equimolar amount of ammonium hydroxide. Chromatography of this flavonoid fraction over polyamide with ethyl acetate·methanol (4:1) eluted successively the compounds *B*, a mixture of *A* and *D*, and *C*, the last in very small quantity.

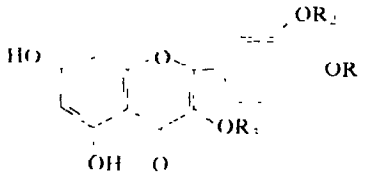
STRUCTURE OF THE FLAVONOIDS

Compounds *A* and *B* had identical absorption spectra, with absorption maxima at 357 mμ (ε 15,000), Band I, and 254 mμ (ε 18,000), Band II, and inflexions at 305 mμ and 266 mμ. The addition of sodium acetate disclosed[7] the presence of hydroxyls at the 4′-position (shift of Band I by 23 mμ) and 7-position (shift of Band II by 20 mμ). The presence of a free hydroxyl at the 5-position was indicated by a positive ferric chloride test and by the shift of 43 mμ of Band I in the presence of $AlCl_3$ and by the dark fluorescence of the glycoside when examined under u.v. light, which excluded the presence of a free 3-hydroxyl. The presence of an additional substituent in the 3′-position was suggested by the pronounced inflexion at 266 mμ.

Upon acid hydrolysis these two glycosides *A* and *B* yielded the same aglycone, m.p. 300–304 (dec.), λ_{max} 430 (infl.), 372, 302 (infl.) and 255 mμ, which now possessed a free hydroxyl in the 3-position since the shift of Band I in presence of $AlCl_3$ was 60 mμ and the compound had a bright yellow fluorescence under u.v. light typical of flavonols. The aglycone was trimethylsilylated and its NMR spectrum was measured in CCl_4.[8] It showed doublets (J = 3 c/s) at 6·10 ppm and 6·44 ppm corresponding to the protons at the positions 6 and 8 respectively, doublets at 6·80 ppm (J = 8 c/s) and 7·67 ppm (J = 2 c/s) for the protons at the positions 5′ and 2′ respectively and one quartet at 7·54 ppm (J_{ortho} = 8 c/s, J_{meta} = 2 c/s) for the proton at the 6′ position. A sharp signal at 3·81 ppm, integrating for 3 protons, was typical of a methoxyl on an aromatic system. This methoxyl must be at the 3′-position, all the others being already accounted for from the u.v. and NMR data.

The presence of a hydroxyl at the 4′- rather than at the 3′-position was also suggested (in addition to the u.v. data) by the failure of the Fremy's salt oxidation of the aglycone. A quinone is normally obtained when the para position is not substituted.[9]

In order to confirm that the aglycone was isorhamnetin (I) and not tamarixetin (II), hesperidin (3′,5,7-trihydroxy-4′-methoxyflavone 7-rutinoside) was converted in about 70



I: $R_1 = R_3 = H$, $R_2 = CH_3$. II: $R_1 = CH_3$, $R_2 = R_3 = H$. III: $R_1 = R_2 = R_3 = H$.

[6] H. ROSLER, T. J. MABRY and J. KAGAN, *Chem. Ber.* **98**, 2193 (1965).
[7] L. JURD. In *The Chemistry of Flavonoid Compounds*. (Edited by T. A. Geissman) p. 122. Pergamon Press, Oxford (1962).
[8] *a*. T. J. MABRY, J. KAGAN and H. ROSLER. *Phytochem.* **4**, 177 and 487 (1965); *b*. T. J. MABRY, J. KAGAN and H. ROSLER. *Nuclear Magnetic Resonance Analysis of Flavonoids*. Publ. 6418. Univ. of Texas Press, Austin (1964).
[9] L. HORHAMMER, H. WAGNER, H. ROSLER, M. KECKEISEN and L. FARKAS. *Tetrahedron* **21**, 969 (1965).

per cent yield into tamarixetin 7-rutinoside by the method of Pacheco *et al.*[10] Tamarixetin was obtained by hydrolysis of this glycoside and the NMR spectrum of its trimethylsilyl ether in CCl_4 was different from that of our aglycone. The only difference in the spectra was for the 6′ proton which was found at lower field (7·76 ppm) in tamarixetin.

Compounds *A* and *B* must therefore be glycosides of isorhamnetin. Compound A, m.p. 177° (dec.), was not attacked by β-glucosidase but was readily hydrolyzed by dilute acids. Glucose and rhamnose in equal proportions were identified gas chromatographically.[11] After trimethylsilylation, the NMR spectrum of *A* confirmed that it was a diglycoside and that rhamnose, with the C-1 proton at 4·25 ppm could not be directly linked to the flavonoid nucleus.[8] The NMR spectrum was typical for a rutinoside[12] linked to the 3-position of flavonols. In order to confirm this position of attachment, the diglycoside was methylated with dimethylsulfate in acetone using the previously reported[6] modification of the standard technique. The methylated product was hydrolyzed in 1% HCl at reflux for 20 min and the crystalline material thus obtained was found identical in all respects with 3-hydroxy-3′,4′,5,7-tetramethoxyflavone, m.p. 192–193 (lit. 192–194,[13]) obtained by methylation followed by acid hydrolysis of rutin (III: R = rutinose). Compound *A*, isorhamnetin-3-β-rutinoside, must therefore be identical with narcissin, m.p. 173–174 (dec.), originally isolated from the flowers of *Narcissus tazetta*[13] and from the pollen of *Lilium auratum*.[14]

Compound *B*, m.p. 219–220°, was hydrolyzed for 20 min at reflux with 1% HCl. Gas chromatographic analysis of the sugar fraction showed the three peaks characteristic of galactose and matching those of an authentic sample. The NMR spectrum of the trimethylsilyl ether of *B* confirmed that it was a monogalactoside and that the sugar, with its C–1 proton at 5·71 ppm ($J = 7$ c/s) had a β-linkage to the flavonoid nucleus.

Compound *B* is therefore isorhamnetin 3-β-D-galactoside and to our knowledge this is the first report of its occurrence in nature.

Compound *C*, m.p. 232–235° (dec.), was also examined by NMR (as the trimethylsilyl ether). It lacked the methoxyl but had the same pattern of nuclear protons as the two isorhamnetin 3-glycosides. That the aglycone was indeed quercetin (III) was confirmed after acid hydrolysis by direct comparison with an authentic sample. The sugar fraction was identified as galactose gas chromatographically. Since the shift of the u.v. absorption maximum in $AlCl_3$ was only 40 mμ, compared to 60 mμ for quercetin, compound *C* must be a quercetin 3-glycoside. It was indeed found to be identical (m.p., NMR, u.v., and i.r. spectra and chromatographic behavior) with hyperin (III, R_3 = galactose).

The mixture of compounds *A* and *D* from the 1965 collection appeared homogenous when examined by thin-layer chromatography and by 2-dimensional paper chromatography and was originally believed to be identical with *A*. It is especially noteworthy that a single spot was observed on polyamide plates with Egger's solvent,[5] indicating the presence of a single type of flavonoid nucleus. However, the NMR spectrum of the trimethylsilyl ether of the mixture disclosed that it contained isorhamnetin 3-rutinoside (Compound *A*) and another rhamnoglycoside. The second component, Compound *D*, present in about 40 per cent of the mixture was identified as isorhamnetin 3-rhamnogalactoside. This assignment is based on the gas chromatographic finding of galactose, glucose and rhamnose after hydrolysis, and on the

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[11] J. Kagan and T. J. Mabry. *Anal. Chem.* **37**, 288 (1965).
[12] H. Rösler, T. J. Mabry, M. Cranmer and J. Kagan. *J. Org. Chem.* In press.
[13] T. Kubota and T. Hase. *J. Inst. Polytech., Osaka City Univ. Ser. C*, **5**, 49 (1956).
[14] M. Kotake and H. Arakawa.. *Naturwissenschaften*, **43**, 327 (1956).

identity in the NMR spectra of the C-1 sugar proton peaks with those of the rhamnogalactoside portion of robinin (kaempferol 7-rhamnoside, 3-rhamnogalactoside). Furthermore, the B-ring protons of the minor component as well as its C-1 galactose proton are, as expected from the proposed formula, at the positions encountered in compound *B*, isorhamnetin 3-galactoside (Table 1). In all the isorhamnetin glycosides the *A*-ring protons were found at

TABLE 1. NMR DATA FOR FLAVONOIDS

Compounds	B-ring protons				Sugar H-1 protons			
	H-2′		H-6	H-5	Glucose	Galactose	Rhamnose	
Isorhamnetin 3-rutinoside and rhamnogalactoside	7·79 $J=2$ c/s	7·51 $J=2$ c/s	7·35[b] $J_m=2$ c/s $J_o=8$ c/s	6·82 $J=8$ c/s	5·95 $J=6$ c/s	5·68 $J=7$ c/s	4·34 $J=1$ c/s	4·25 $J=1$ c/s
Isorhamnetin 3-rutinoside		7·52 $J=2$ c/s	7·34 $J_m=2$ c/s $J_o=8$ c/s	6·83 $J=8$ c/s	5·94 $J=6$ c/s			4·26 $J=1$ c/s
Isorhamnetin 3-galactoside	7·80 $J=2$ c/s		7·46 $J_m=2$ c/s $J_o=8$ c/s	6·81 $J=8$ c/s		5·70 $J=7$ c/s		
3-Rhamnogalactoside portion of Robinin						5·73 $J=7$ c/s	4·35 $J=1$ c/s	

[b] A signal (probably a quartet) centered at about 7·42 ppm could be detected from the integration curve but was obscured by the signal at 7·51 ppm and the low field doublet of the signal at 7·35 ppm

6·14 and 6·46 ppm ($J=2$ ppm). A conspicuous difference of the *B*-ring proton signals in flavonols bearing a 3-galactoside compared to the 3-rutinoside or to the aglycone has already been recorded in the case of hyperin compared to rutin and quercetin.[8]

The sugar moiety of this previously unreported isorhamnetin 3-rhamnogalactoside is almost certainly robinobiose (6-L-rhamnosido-D-galactose) in view of the identity of the NMR signals for their C-1 sugar protons with the corresponding ones in robinin. By analogy with the NMR spectra of rhamnoglucosides, obvious differences would be expected if the rhamnose were linked to a secondary hydroxyl of galactose.[8,12] Upon critical scrutiny of the NMR spectrum of compound *A* from the 1964 collection, it was observed that the rhamnogalactoside was present in very minute amounts. Traces of galactose could also be detected in the gas chromatograph of its sugar portion. Surprisingly, no isorhamnetin 3-glucoside was detected in the flowers. This substance could have been either the intermediate in the stepwise glycosylation of isorhamnetin to isorhamnetin 3-rutinoside or a product of partial hydrolysis of this compound.

EXPERIMENTAL

The u.v. spectra were recorded with a Beckman DU spectrophotometer, the NMR spectra with a Varian A-60 spectrometer using tetramethylsilane as internal standard and carbon tetrachloride as solvent. The melting points are not corrected. The gas chromatographic analysis of the sugars were performed as previously reported.[11]

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