

SHORT COMMUNICATION

MYRICETIN 3- β -GALACTOSIDE, THE MAIN FLAVONOID FROM *OENOTHERA LAVANDULAEFOLIA*, T. AND G.

JACQUES KAGAN

Department of Biological Sciences and Department of Chemistry, University of Illinois
at Chicago Circle, Chicago, Illinois 60680, U.S.A.

(Received 15 July 1966)

Abstract—The main flavonoid from *Oenothera lavandulaefolia*, T. and G., has been isolated and shown to be myricetin 3- β -galactoside.

WE WISH to report the isolation of myricetin 3- β -galactoside in pure form from natural sources. Arbusculoside, a myricetin galactoside, has previously been isolated from *Salix arbuscula*¹ but was not further characterized, while a sharp melting yellow compound (224–225°, dec.) obtained from tea leaves has been claimed to be an equimolar mixture of myricetin 3-glucoside and 3-galactoside.²

EXTRACTION PROCEDURE

An air-dried collection of *O. lavandulaefolia*³ (60 g) was extracted with cold methanol. The extract was taken to dryness in presence of nylon powder, and the coated powder was thoroughly washed, first with CHCl₃, then with water and finally with CH₃OH. All the flavonoids were found in this last solution. The solvent was evaporated, and the residue was chromatographed over silica gel. The main component (I) was eluted with ethyl acetate. It was crystallized from dil. CH₃OH and dark orange crystals, m.p. 198–201°, were obtained (0.380 g).

DISCUSSION

The u.v. spectrum of I in CH₃OH was typical of a flavone,⁴ with maxima at 363 nm (ϵ 16,000), 308 nm (infl., ϵ 8500) and 262 and 256 nm (ϵ 16,000). The presence of 4'- and 7-hydroxyls was indicated by the bathochromic shift of the long (27 nm) and short (18 nm) wave length maxima in presence of sodium acetate. Boric acid did not affect the shift of the 363 nm maximum obtained with acetate, showing the presence of a 3'-hydroxyl. In presence of AlCl₃ the spectrum had maxima at 410, 370 (infl.), 310 and 272 nm. The magnitude of the long wave length shift (44 nm) is typical for a 5-OH and rules out a free 3-OH which would have produced a shift of about 60 nm. That the substance has no free 3-OH group was also indicated by the lack of bright yellow fluorescence when I was examined under u.v. light.

¹ J. RABATE, *J. Pharm. Chim.* **28**, 44 (1938).

² Y. TAKINO, H. IMAGAWA and H. YOSHIDA, *Agr. Biol. Chem. (Tokyo)* **26**, 699 (1962).

³ A specimen (No. 247162) has been deposited at the Herbarium of the University of Texas, Austin, Texas.

⁴ L. JURD, In *The Chemistry of Flavonoid Compounds* (Edited by T. A. GEISSMAN), p. 107 Macmillan, New York (1962).

The NMR spectrum of the trimethylsilyl ether of I in CCl_4 ^{5, 6} disclosed that it was a β -monoglycoside, with the sugar 1-proton appearing as a doublet at 5.26 ppm ($J=7$ c/s). The flavonoid portion of the molecule lacked a 3-hydrogen and consisted in two doublets ($J=2$ c/s) at 6.15 and 6.43 ppm, each integrating for one proton (H-6 and H-8 respectively) and one sharp signal at 7.22 ppm integrating for two protons. This signal indicated that there were only two B-ring protons, in a *para* or *meta* relationship (*ortho* protons give signals with $J=8$ c/s). The first alternative is ruled out by the fact that in all flavones, the 2'- and 5'-proton signals are separated by at least 0.3 ppm, whereas the 2'- and 6'-proton signals often coincide, even when the B-ring is unsymmetrically substituted.⁶

Compound I gave an acetate, m.p. 118°, with acetic anhydride in pyridine. Its NMR spectrum in CDCl_3 showed it to contain nine acetate groups. It had u.v. absorption maxima at 312 (infl.), 298 and 250 nm, which did not shift in presence of AlCl_3 or sodium acetate.

Compound I was hydrolyzed for 20 min at 80° with 1 N HCl, and the sugar was analyzed gas chromatographically.⁷ Three peaks were observed, matching separately and in mixture those produced by a sample of galactose previously equilibrated in pyridine.

The aglycone had u.v. absorption maxima at 378 ($\epsilon 17,000$) and 254 nm ($\epsilon 15,000$), the former shifting to 432 nm in presence of AlCl_3 , thus indicating the presence of a 3-hydroxyl, to which the galactose must therefore have been attached in I. The aglycone was acetylated and the product, m.p. 210–212°, was shown by NMR to contain six acetate groups.

The aglycone must therefore be myricetin, 3,5,7,3',4',5-hexahydroxyflavone. Proof of this assignment was secured by direct comparison with an authentic sample.

The glycoside I is therefore myricetin 3- β -galactoside and *O. lavandulaefolia* represents a rich source for this compound which was found in the flowers as well as in the leaves. Traces of three other flavonoids were detected by paper chromatography, but they were not isolated in sufficient amount for chemical characterization.

Oenothera and related genera in the family Onagraceae are rather poorly defined botanically.⁸ This represents the first report of the isolation of flavonoid constituents in these plants. We are now investigating related species, and we hope that the chemical information will help clarify their botanical relationship.

EXPERIMENTAL

The melting points are not corrected. The NMR spectra were recorded on a Varian A-60 spectrometer and are expressed on the δ -scale in ppm downfield from an internal standard of tetramethylsilane. The u.v. spectra were recorded on a Beckman DB or on a Bausch and Lomb 505 spectrophotometer.

Acknowledgements—We wish to thank Professor M. C. Johnston (Dept. of Botany, University of Texas) for identifying the plants and for his interest in this work. Financial support was received from the Research Board of the University of Illinois.

⁵ T. J. MABRY, J. KAGAN and H. RÖSLER, *Phytochem.* 4, 177, 487 (1965).

⁶ T. J. MABRY, J. KAGAN and H. RÖSLER, *The University of Texas Publ. No.* 6418, Austin, Texas (1964).

⁷ J. KAGAN and T. J. MABRY, *Anal. Chem.* 37, 288 (1965).

⁸ For example, the plant herein studied has recently been called *Calylophus lavandulifolius*,⁹ a change not generally accepted.¹⁰

⁹ P. H. RAVEN, *Brittonia* 16, 276 (1964).

¹⁰ P. A. MUNZ, In *North American Flora* (Ser. II, part 5), p. 1. New York Botanical Garden, New York (1965).