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A RHAMNETIN GLUCURONIDE TRISULPHATE FROM THE LEAVES OF *TAMARIX APHYLLA*

NABIEL A. M. SALEH, HASSAN I. EL-SISSI and MAHMOUD A. M. NAWWAR
National Research Centre, El-Dokki, Cairo, Egypt

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Key Word Index—*Tamarix aphylla*; Tamaricaceae; new sulphated flavonol glycoside; rhamnetin-3′-glucuronide; 3,5,4′-trisulphate.

Plant and source. *Tamarix aphylla*, collected from the Barrage Gardens, Cairo, and identified by Prof. Dr. Vivi Tackholm, Department of Botany, Cairo University. *Previous work.* Leaves [1] and galls [2].

Plant part examined. The leaves of *Tamarix aphylla* were extracted with 70% EtOH, followed by column chromatography (polyamide). Five fractions were collected, and the first fraction eluted with H_2O contained the free sugars, along with a novel flavonoid glycoside (*F*). The glycoside exhibited chromatographic properties of a highly glycosylated flavonoid. Acid hydrolysis released rhamnetin and glucuronic acid; the hydrolysate also gave a white ppt. with $BaCl_2$. The glycoside was unchanged on treatment with β-glucosidase. Controlled acid hydrolysis with 10% HOAc (or 0·05 N HCl) gave rise to four intermediates (F_1, F_2, F_3 and F_4) as well as rhamnetin.

The final intermediate (F_4) was the most stable and gave rhamnetin and glucuronic acid on both acid and enzymic hydrolysis with β-glucuronidase. Methylation of F_4 with dimethylsulphate for 4 hr followed by acid hydrolysis gave quercetin-7,4′-dimethylether (m.m.p., UV and co-chromatography). This indicated (along with the UV data, unstable with NaOEt) that the glucuronic acid moiety is present in position 3′, and (F_4) is rhamnetin-3′-β-glucuronide.

The electrophoretic and paper chromatographic data indicate that a single bisulphate group is present in F_3, two in each of F_1 and F_2, while the parent compound *F* contains three bisulphate groups. The presence of the bisulphate groups is proved by the ppt. given with $BaCl_2$ along with quantitative analysis of sulphur (Found: S, 10·8%. Calc. for $C_{22}H_{17}O_{22}S_3K_3.2H_2O$: S, 10·89%). Potassium was detected by flame spectrophotometry. The

Table 1. Properties of the sulphated products of flavonoid and its partial hydrolysis

	Chromatographic properties			Electrophoresis*	Ultra-violet data†	
	Colour under UV	R_f (× 100) BAW‡	AW§	(distance travelled, cm)	EtOH (nm)	NaOEt
F	Brown	4	87	12·5	270, 320‖, 355‖	Stable
F_1	Brown	12	74	10·1	268, 342, 350‖	Stable
F_2	Yellow	16	65	9·5	248‖, 268, 315‖, 360	Stable
F_3	Yellow	40	47	8·0	265, 325‖, 360	Stable
F_4	Yellow	53	20	1·2	252, 265, 295‖, 325‖, 366	Unstable
Rhamnetin	Yellow	81	0	0	257, 370	Unstable
Quercetin-7,4′-dimethyl ether¶					258, 290‖, 360	Stable

* pH 2, 0·75 M HCO_2H, 50 vol./cm, 30°, 90 min.
† *F*–F_4 gave no shifts with NaOAc or boric acid.
‡ *n*-BuOH–HOAc–H_2O (4:1:5, upper).
§ HOAc–H_2O (1:9).
‖ Inflection.
¶ From F_4 after methylation and acid hydrolysis.

results of electrophoresis, paper chromatography and UV data are outlined in Table 1.

The three bisulphate groups are most probably present on the flavonoid moiety. Thus, on treatment with β-glucuronidase (free of sulphatase), the original material gave rise to a single product (brown on chromatograms under UV). Acidification (0·05 N HCl) of the product gave three intermediates along with rhamnetin. The intermediates disappeared after 10 min heating. The acidity of $AlCl_3$ was apparently high enough to dissociate the sulphate groups, and shifts due to $AlCl_3$ are therefore of no diagnostic value in this particular case.

It is concluded that (*F*) is rhamnetin-3′-glucuronide esterified with potassium bisulphate in positions 3,5 and 4′. Although the presence of a bisulphate group on the glucuronic acid is doubted, yet the possibility should not be excluded. This is the second report of such a highly esterified flavonoid [3], while esterification with one mole of potassium bisulphate has been reported in other moisture-loving plants [4–6]. The presence of such a flavonoid in *Tamarix* is thus not all that surprising, in view of the fact that *Tamarix* species are found in marshy habitats. Furthermore, it has been argued that the formation of flavonoid esters is associated with the adaptability of plants to these environments [7, 8].

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N-N′-DI-O-TOLYLETHYLENDIAMINE FROM *CACHRYS SICULA*

M. Pinar and A. Alemany

Instituto de Química Orgánica General del C.S.I.C. calle Juan de la Cierva, 3-Madrid-6; Spain

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Key Word Index—*Cachrys sicula*; Umbelliferae; *N-N′*-di-o-tolyl-ethylendiamine.

Plant. Cachrys sicula L. or *Hippomarathrum pterochlaenum* Boiss [1]. Endemic species from the Mediterranean region, very common in the S. of Spain. *Previous work.* None. *Present work.* From an acidic fraction of the methanol extract of the whole dried plant, a compound was isolated in 0,01% yield. This compound was shown to be, by UV, IR and NMR, *N-N′*-di-o-tolylethylendiamine. The structure of this diamine, hitherto not isolated from a natural source, was supported by direct comparison (m.p., m.m.p., IR and NMR) with a synthetic sample [2].

EXPERIMENTAL

Plants were collected in Lucena (Córdoba, Spain) June 1973. Legit et determinavit Dr. J. Borja. Voucher specimens (no. 15742) were deposited in the Herbarium Faculty of Pharmacy (Ciudad Universitaria, Madrid).