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A SULPHATED KAEMPFEROL 7,4'-DIMETHYL ETHER AND A QUERCETIN ISOFERULYLGLUCURONIDE FROM THE FLOWERS OF *TAMARIX APHYLLA*

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Key Word Index—*Tamarix aphylla*; Tamaricaceae; sulphated and acylated flavonols; kaempferol 7,4'-dimethyl-ether-3-sulphate; quercetin 3-*O*-isoferulyl-β-glucuronide.

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, 2] and galls [3].

Plant part examined. Fresh flowers of *Tamarix aphylla* were extracted with 70% EtOH, followed by column chromatography (polyamide). Six fractions were collected. The 2 successive fractions eluted with 20 and 30% EtOH contained the 2 flavonol glycosides (*Fa*, mp 204° decomp) and (*Fb*, mp 225° decomp.), respectively.

Acid hydrolysis of *Fa* released kaempferol 7,4'-dimethyl ether [4], (mmp, UV and co-chromatography, Table 1). Kaempferol was obtained on demethylation with HI and no sugar residue was detected but the hydrolysate also gave a white ppt. with $BaCl_2$. Controlled acid hydrolysis of *Fa* with 10% HOAc (or 0·05N HCl) only gave rise to kaempferol 7,4'-dimethyl ether and no other intermediate was detected. The electrophoretic distance travelled in buffer solution of pH 2, 0·75M HCO_2H, 50 vol/cm, 30°, 90 min = 6·5 cm and paper chromatographic data (Table 1) as well as the absence of intermediate on controlled hydrolysis indicate that a single sulphate group is present in *Fa*; (Found: S, 0·70%, Calc. for $C_{17}H_{13}O_9SK.H_2O$: S, 0·69%). Potassium was detected by flame spectrophotometry and by the ppt. obtained with sodium cobaltinitrite. Spectral properties of *Fa* (Table 1) as well as the release of 3-hydroxy-5,7,4'-trimethoxyflavone [5] on methylation of *Fa* followed by acid hydrolysis proved the substitution of SO_3H to be at C-3.

It should be noted, however, that rhamnetin 3-glucuronide-3,5,4'-trisulphate, recently isolated from the leaves of *Tamarix aphylla* [2], was also detected on the present work in the first fraction (eluted with H_2O) of the polyamide column.

Acid and alkaline hydrolysis of *Fb* released quercetin, isoferulic acid and glucuronic acid. Isoferulic acid was separated from the hydrolysate, by paper chromatography, and identified by UV and co-chromatography [3] (Table 1). *Fb* remained unchanged on treatment with β-glucuronidase. Controlled acid hydrolysis gave rise to a single intermediate Fb_1 together with quercetin and isoferulic acid. Fb_1 was separated from the hydrolysate by PC, and gave quercetin and glucuronic acid on both acid and enzymic hydrolysis with β-glucuronidase. Methylation of both *Fb and* Fb_1 with Me_2SO_4 for 4 hr. followed by acid hydrolysis gave quercetin 7,3',4'-trimethyl ether (mmp, UV and co-chromatograph, Table 1). This indicated (along with the mp, UV data and R_f-values of Fb_1, Table 1) the presence of glucuronic acid in position 3, and Fb_1 is thus, quercetin 3-*O*-β-glucuronide.

It is concluded that (*Fb*) is quercetin 3-*O*-β-glucuronide acylated with isoferulic acid in the glucuronic acid moiety (UV properties and R_f-values are outlined in Table 1).

Table 1. Properties of new flavonols and their partial hydrolysis products.

	Colour under UV	Chromatographic properties R_f (×100) BEW	BAW	H_2O	AW	UV spectra λ_{max} (nm) in EtOH	$\Delta\lambda$ (nm) $AlCl_3$ Band II	NaOAc Band I	NaOEt Band II	H_3BO_3 Band II
Fa	brown	63	65	54	60	268, 340	+55	0	+37	—
Kaempferol 7,4'-dimethyl ether	yellow	78	91	00	00	269, 322*, 365	+55	0	+45	—
Kaempferol	yellow	74	85	00	00	268, 368				
Fb	brown	43	63	38	45	268, 365	+43	+6	+50	+13
Fb_1	brown	35	37	73	48	295, 364	+43	+6	+55	+10
Isoferulic acid	mauve	72*	95	39	50	292, 325	—	—	+18	—
Quercetin	yellow	64	65	00	00	255, 374				
Quercetin 7,3',4'-trimethyl ether**	yellow	80	90	00	00	254, 303*, 366				

BEW = *n*-BuOH–EtOH–H_2O (4:1:2·2); BAW = *n*-BuOH–HOAc–H_2O (4:1:5, upper); AW = HOAc–H_2O (15:85); * = Inflection; ** = From *Fb* and Fb_1 after methylation and acid hydrolysis.

It should be noted, however, that H_2O_2 oxidation as well as partial hydrolysis of *Fb* did not release isoferulylglucuronic acid as expected, but only released glucuronic acid. This may be due to the fact that while flavonol glucuronides are stable [6, 7] the linkage between isoferulic acid and glucuronic acid is more labile.

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A FLAVONOL GLYCOSIDE WITH ANTICANCER ACTIVITY FROM *TEPHROSIA CANDIDA**

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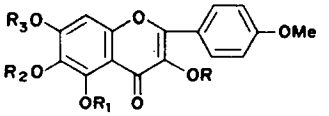
Key Word Index—*Tephrosia candida*; Leguminosae; flavonol glycoside; 6-hydroxykaempferol 4'-methyl ether; anticancer activity.

A programme of screening Indian plants for biological activities led to the observation of activity against human epidermoid carcinoma of the nasopharynx in tissue culture (9KB), in a 50 per cent EtOH extract of the aerial part of *Tephrosia candida* (Roxb) DC [1]. Chemical investigations on *Tephrosia* species have yielded rotenoids and flavonoids and root bark, seeds and leaves of *Tephrosia candida* contain four rotenoids [2,3]. This communication records the isolation and structure elucidation of a flavonol glycoside with anticancer activity.

Solvent fractionation of the EtOH extractive of the air dried plant (aerial portion) located the anticancer activity in the defatted *n*-BuOH soluble fraction. Column chromatography on silica gel yielded the biologically active compound as a yellow microcrystalline substance, mp 192–4°. It gave an olive-green colour with $FeCl_3$ and a magenta colour with Mg-HCl, characteristic of flavonoids and analysed for $C_{28}H_{32}O_{15}$. IR showed OH and α,β unsaturated C=O absorption at 3500–3400 and 1668 cm^{-1} respectively and a broad C–O stretching band in the region 1100–1000 cm^{-1} suggesting its glycosidic nature. Acid hydrolysis gave a new flavonoid aglycone, characterised as 6-hydroxy-kaempferol 4'-methyl ether (II), and L-rhamnose.



(1) R = R_3 = Rha; R_1 = R_2 = H
(2) R = R_1 = R_2 = R_3 = H
(3) R = R_1 = R_2 = R_3 = Me
(4) R = R_1 = R_3 = H; R_2 = Me

The aglycone mp 262–3°, analysed for $C_{16}H_{12}O_7$ (M^+ 316) had ν_{max} 3400 (phenolic hydroxyls) and 1667 cm^{-1} (α,β unsaturated carbonyl). Its UV spectrum in MeOH had maxima at 270 and 365 nm, characteristic of a flavonol having a free 3-OH group. A bathochromic shift with decreased intensity of band I on addition of NaOMe suggested a substituted 4'-hydroxyl while a bathochromic shift of band II in the presence of NaOAc indicated a free 7-OH group in the aglycone-A. Shift of band I (Table 1) with $AlCl_3$–HCl suggested 3,5-dihydroxy substituents in the molecule.

The location of a 4'-methoxyl was evident from the NMR spectrum. A 3 proton $\underline{CH_3}O$ signal appeared at

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Table 1. UV spectral data of *Tephrosia* flavonols

Solvent	λ_{max}. nm (log ϵ) 1	2	4
MeOH	285 (4·18), 325 (4·07)	270 (4·23), 305 *sh* (4·01), 365 (4·20)	265, 360
NaOMe	290 (4·70), 395 (4·16)	275 (4·51), 324 *sh* (4·07, 428 (4·11)	272, 420
$AlCl_3$	290 (4·56), 367 (4·39)	278 (4·37), 302 *sh* (4·13), 364 (4·19), 423 (4·17)	275, 368*sh*, 428
$AlCl_3$/HCl	289 (4·55), 368 (4·36)	277 (4·36), 364 (4·19), 423 (4·06)	275, 360 *sh*, 428
NaOAc	285 (4·25), 325 (4·15)	277 (4·13), 372 (3·98)	284, 370
NaOAc/H_3BO_3	284 (4·22), 326 (4·16)	272 (4·13), 366 (4·11)	285, 370