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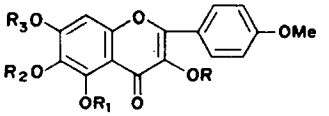
Key Word Index—*Tephrosia candida*; Leguminosae; flavonol glycoside; 6-hydroxykaempferol 4'-methyl ether; anticancer activity.

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

* CDRI Communication No. 1975.

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

Table 2. NMR data of permethyl derivatives of the *Tephrosia* aglycone, nevadensin and eupalitin

Proton	Chemical shifts (ppm) Permethyl nevadensin*	Permethyl eupalitin*	Permethyl *Tephrosia* aglycone
3	6·61(*s*)	—	—
8	—	6·78(*s*)	6·80(*s*)
3′,5′	7·06(*d*)†	7·06(*d*)†	7·08(*d*)†
2′,6′	7·91(*d*)†	8·10(*d*)†	8·15(*d*)†
Methoxyls	3·88–4·12	3·92–4·07	3·9–4·1

* Data from the literature [4,5].
† *J* 9 Hz.

3·8 ppm and the 4 protons of *para* substituted ring B resonated as symmetrical doublets centred at 7·0 and 8·08 ppm (*J* 9 Hz). As an OH had been assigned to position 3, the one proton signal at 6·60 ppm could be due to either H-6 or H-8. No other olefinic proton signal was observed in the spectrum. The aglycone was thus a tetrahydroxymonomethoxyflavone. The presence of four free hydroxyls was confirmed by its methylation with Me_2SO_4, to give a pentamethoxyflavone, $C_{20}H_{20}O_7$ (M^+ 372), mp 152–3°, 5 × $OC\underline{H}_3$ NMR signals between 3·9–4·1 ppm. This methyl ether appeared to be identical with 3,5,6,7,4′-pentamethoxyflavone (permethyleupalitin) [4] rather than with 5,6,7,8,4′-pentamethoxyflavone (permethylnevadensin) [5]. Although reported mps of both compounds are the same, there are differences in their NMR spectra (Table 2).

The gossypetone test, given by dihydroxyflavones, was negative and also supported the absence of C-8 hydroxyl. Further support for the assignment of the 1H singlet at 6·60 ppm in the NMR spectrum of the aglycone to H-8 was obtained by examining the effect of shift reagent $Eu(FOD)_3$ [6]. Addition of this reagent has been found to cause a downfield shift ('S' values) of 1·25–0·08, 1·18–1·56 and 5·70–7·16 of H-3, H-8 and H-6 proton signals respectively. With the permethylated aglycone, the chemical shift ('S' value) for the singlet at 6·80 ppm was found to be 1·0 and corresponded to the H-8 proton. The permethylated aglycone is thus 3,5,6,7,4′-pentamethoxyflavone (**3**). The identification of anisic acid on alkaline degradation of the original aglycone confirmed the location of the methoxyl group at position 4′. This is, therefore, 6-hydroxykaempferol-4′-methyl ether (**2**). The polyhydroxy nature of ring A and the presence of a methoxy at 4′ was also evident from the mass spectrum of **2**. Retro-Diels-Alder type fragmentation was not observed and is in agreement with the presence of 5,6,7-substitution of ring A. The presence of a phenolic methoxyl group was indicated by the loss of Me and $-CH_2$ and a loss of 43 *m/e* from M^+ due to the loss of Ac a characteristic of phenolic ethers [7], to give rise to ion *m/e* 273.

Acid hydrolysis of the original glycoside yielded two moles of rhamnose for every mole of aglycone. Its UV spectrum in MeOH showed maxima at 285 and 325 nm (Table 1). The appearance of band I at a lower wavelength in comparison to that of II indicated that the glycoside has a 3-rhamnosyl substituent. The bathochromic shift of 43 nm in band I in the presence of $AlCl_3$–HCl is characteristic of 3,7-glycosylflavonols [8]. There was also no shift of band II in the presence of NaOAc thus supporting substitution at position-7. Thus rhamnose units are attached to both 3 and 7 positions and the glycoside is the 3,7-dirhamnoside of 6-hydroxykaempferol 4′-methyl ether. This structure was confirmed by methylation with diazomethane followed by acid hydrolysis. The aglycone **4** so obtained, mp 186–9°, analysed for $C_{17}H_{14}O_7$ (M^+ 330). A study of UV shifts with standard reagents (Table 1) showed it was the expected 6,4′-dimethoxy-3,5,7-trihydroxyflavone.

EXPERIMENTAL

Uncorrected capillary mp are reported. UV, IR and 60 Mcs NMR spectra were determined in MeOH, KBr and $CDCl_3$ with TMS as internal standard, unless indicated.

Isolation of glycoside **1**

Air dried, powdered plant material (5 kg) was percolated with 90% EtOH (5 × 15 l) at room temp. and the extract concd. The dark green mass obtained was triturated and extracted successively with C_6H_{14} (4 × 1 l), $CHCl_3$ (5 × 500 ml) and *n*-BuOH (5 × 400 ml). The respective extracts were washed with water thrice and concd. The brown residue (25 g) obtained from the *n*-BuOH fraction showed maximum anticancer activity (9KB). It was chromatographed over Si gel (600 g), using C_6H_6, C_6H_6–$CHCl_3$ (1:1), EtOAc, EtOAc–MeOH (19:1, 9:1, 4:1, 1:1 and 1:3) and pure MeOH. The residue obtained from the EtOAc and EtOAc–MeOH (19:1) fractions (2·4 g) on rechromatography over Si gel gave **1** from EtOAc–MeOH (49:1) eluted fractions, which was crystallized from aqueous MeOH (1·6 g), mp 192–4°. TLC: R_f 0·52 (EtOAc–MeOH–H_2O, 10·5:2:1·5); R_f 0·59 (EtOAc–EtCOMe–HCOOH–H_2O, 5:3:1:1), developed with 1% $FeCl_3$ in EtOH. ν_{max}: 3500–3400, 2950, 1668, 1620, 1500, 1385, 1210, 1100–1000, 910, 845, 820 cm^{-1}. Found: C, 54·94; H, 5·45. $C_{28}H_{32}O_{15}$ requires: C, 55·26; H, 5·26%.

Hydrolysis. **1** (800 mg) in 5 ml MeOH and 7% H_2SO_4 (20 ml) was refluxed at 100° for 4 hr to give a yellow, viscous oily product (A) which on chromatography over Si gel, and elution with C_6H_6–Et_2O (1:1) gave **2** which was crystallized from Et_2O (310 mg) mp 262–3°. TLC: R_f 0·8 (EtOAc–MeOH, 5:1). ν_{max} 3400 (OH), 2960, 1667 (C=O), 1630, 1600, 1530, 1400, 1325, 1300, 1200, 1175, 1100, 1050, 985, 900, 820 cm^{-1}. NMR (DMSO-d_6 ppm): 3·8(3H), 6·1(1H), 6·9(1H), 7·1, 8·0, 8·16 (1H each). Mass (*m/e*): 316 (M^+), 301, 298, 286, 273, 135, 121, 120. Found: C, 61·1; H, 4·4. $C_{16}H_{12}O_7$ requires: C, 60·76; H, 4·05%. The aqueous fraction of the hydrolysate after neutralization with $BaCO_3$ and paper co-chromatography showed the presence of rhamnose as the only sugar. Colorimetric estimation showed the aglycone:rhamnose ratio to be 1:2.

Methylation of **2** with $Me_2SO_4/K_2CO_3/Me_2CO$ gave the pentamethyl ether, mp 152–3, ex MeOH. ν_{max}: 2960, 2840, 1654 (C=O), 1620, 1540, 1500, 1480, 1375, 1310, 1280, 1225, 1190, 1140, 1000, 1030, 1015, 980, 920, 855, 830, 792 cm^{-1}. NMR ($CDCl_3$, ppm): 3·9–4·1 (15H, 5 × $OC\underline{H}_3$), 6·80 (1H), 7·08

(*d*,*J* 9 Hz), 8·15 (*d*,*J* 9 Hz) Mass (*m/e*): 372(M^+), 358, 357 (base peak), 341, 314, 299, 271, 243, 195, 172, 167, 157, 135. Found: C, 64·29; H, 5·31. $C_{20}H_{20}O_7$ requires: C, 64·51; H, 5·37%.

Alkaline degradation of **2**. **2** (60 mg) was refluxed in 50% KOH (10 ml) and EtOH (10 ml) for 6 hr under N_2, cooled, acidified (10% HCl), extracted with Et_2O (4 × 20 ml) and the Et_2O soln extracted with 10% $NaHCO_3$. The $NaHCO_3$ extract acidified and extracted with Et_2O. Chromatography over Si gel gave a colourless substance (12 mg), mp 182–4°; mmp with anisic acid 182–4°. Superimpossible IR spectrum with that of anisic acid.

Methylation of **1**. **1** (60 mg) in MeOH (5 ml) was treated with an excess of ethereal CH_2N_2 at 5° and left overnight at room temp. The residue was hydrolyzed with 2 N HCl (5 ml) in MeOH (2 ml) at 100° for 3 hr, and processed as usual to give **4**, which was crystallized from Et_2O as yellow crystals (15 mg) mp 186–9°; TLC: R_f 0·2 (EtOAc–MeOH, 5:1). It gave an olive-green colour with $FeCl_3$ and magenta colour with Mg–HCl. Mass (*m/e*): 330 (M^-), 315, 312, 301, 287, 135, 121, 120. Found: C, 61·90; H, 4·40. $C_{17}H_{14}O_7$ requires: C, 61·82; H, 4·24%.

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6a,12a-DEHYDRO-α-TOXICAROL, EIN NEUES ROTENOID AUS *AMORPHA FRUTICOSA**

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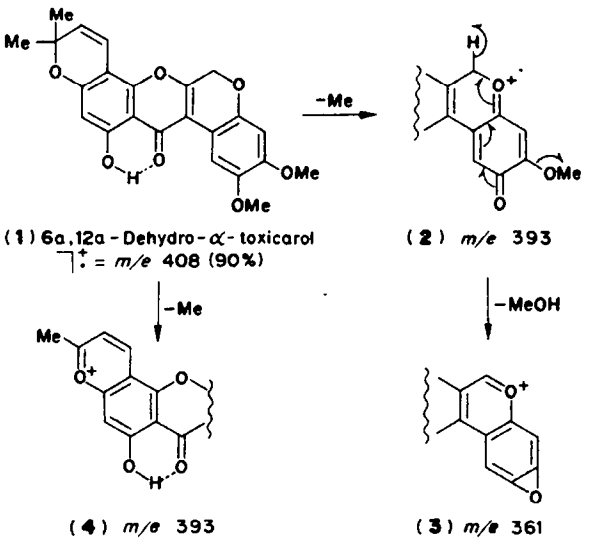
Key Word Index—*Amorpha fruticosa*; Leguminosae; 6a,12a-dehydro-α-toxicarol; rotenoid.

Aus *Amorpha fruticosa* konnten verschiedene Arbeitsgruppen Rotenoide isolieren, in denen eine isoprenoide Seitenkette in Form eines Dihydrofuranringes gebunden vorliegt [1–5]. Aufgrund allgemeiner biogenetischer Beziehungen [6,7] zwischen isoprenoiden Seitenketten und benachbarten phenolischen OH-Gruppen sollte die Pflanze auch Rotenoid-Moleküle mit anellierten Dihydropyranringen synthetisieren können. Wie sich jetzt herausstellte sind derartige Überlegungen berechtigt.

Bei der Auftrennung der Lipidfraktion der Samen von *Amorpha fruticosa* [8] fiel ein schwer trennbares Gemisch von Substanzen an, von denen die meisten mit Eisenchlorid-Lösung eine positive Phenolreaktion gaben. Der Hauptbestandteil dieses Gemisches erwies sich als 6a,12a-Dehydro-α-toxicarol (**1**).

Beim massenspektrometrischen Abbau bestehen zwischen den einzelnen Rotenoiden markante Unterschiede, je nach dem ob im Skelett die 6a,12a-Binding gesättigt oder ungesättigt ist [9,10]. Während bei den gesättigten Grundtypen in der Regel das aus der RDA-



Spaltung resultierende Radikal-Ion des Chromen-Bruchstücks in höchster Ausbeute entsteht, wird durch die Einführung der Doppelbindung die Stabilität des

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