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BIOLOGICALLY-ACTIVE FLAVONOIDS FROM *GOSSYPIUM ARBOREUM*

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Key Word Index—*Gossypium arboreum*, Malvaceae, flavonol, glycoside, quercetin, gossypetin 8-rhamnoside, antibacterial, *Pseudomonas maltophilia*, *Enterobacter cloacae*, *Heliothis zea*, *Heliothis virescens*

Abstract—A new flavonol glycoside, gossypetin 8-*O*-rhamnoside, was isolated from flower petals of *Gossypium arboreum* along with quercetin 7-*O*-glucoside, quercetin 3-*O*-glucoside and quercetin 3′-*O*-glucoside These compounds showed antibacterial activity against *Pseudomonas maltophilia* and *Enterobacter cloacae*

INTRODUCTION

Cotton flower bud flavones and aglycones have been reported to deter the development of the tobacco budworm, *Heliothis virescens* Fabricius, the corn earworm, *Heliothis zea* Boddie, and the pink bollworm, *Pectinophora gossypiella* Saunders [1–3] Other flavonoids from cotton buds are feeding stimulants for the boll weevil, *Anthonomus grandis* Boheman [4]

The chemistry of flavonoids from several species of *Gossypium* has been investigated [5–9] Parks [10] found 24 flavonoids in *Gossypium arboreum* L flower petals He was able to identify gossypetin 7-*O*-glucoside, gossypetin 8-*O*-glucoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoglucoside, quercetin 7-*O*-glucoside, kaempferol 3-*O*-glucoside, and kaempferol 3-*O*-rhamnoglucoside

In a preliminary study [unpublished data] to identify plants having biological activity for insects, flavonoid-rich extracts of *G arboreum* (var Sanguineum) flower petals were found to inhibit the growth of two bacteria, *Pseudomonas maltophilia* Hugh et Ryschenkow and *Enterobacter cloacae* (Jordan) Hormaeche et Edwards, isolated from the gut of *H zea* and *H virescens* [11] In another study [unpublished data], over 60 flavonoids were tested for inhibition of growth of these two bacteria, and most were found to be active to some degree A group of 40 flavonoids was tested for antigrowth activity toward the cotton insect *H zea*, and a number were found to be active [3] Thus, flavonoids have been shown to be antibiotic to diverse species, and antibacterial activity might therefore be expected to be a facile predictor of insect resistance factors in plants Since *G arboreum* though lower in gossypol, is known to possess resistance to various pests [12–16], it was desirable to determine whether the resistance could be attributed to the flavonoids

RESULTS AND DISCUSSION

Methanolic extracts of *G arboreum* which showed antibacterial activity yielded three known flavonoids, quercimeritrin (**1**), isoquercitrin (**2**), and quercetin 3′-*O*-glucoside (**3**), and a new flavonoid, gossypetin 8-*O*-rhamnoside (**4**) These compounds showed slight antibacterial activity when tested alone

Compounds **1**–**3** were identified by direct comparison of their TLC and UV spectral data with those of authentic samples The glycosides were hydrolysed, and UV-visible and mass spectral data of the aglycones were identical to those of quercetin The UV-visible spectra and cellulose TLC of **4** indicated that it was an 8-*O*-glycoside of gossypetin [17] Upon acid hydrolysis of **4**, the TLC and spectral data (UV-visible and MS) of the isolated aglycone were identical to those of gossypetin Cellulose TLC and CIMS of the acid hydrolysate gave rhamnose Thus, **4** is gossypetin 8-*O*-rhamnoside Gossypetin glycosides that have been previously isolated have been glucosides [5–10], therefore, this is the first report of a gossypetin rhamnoside

Compounds **1** and **2** had been previously isolated from *G arboreum* and identified by Parks [10], along with gossypetin 8-*O*-glucoside, gossypetin 7-*O*-glucoside, quercetin 3-*O*-rhamnoglucoside, kaempferol 3-*O*-glucoside, and kaempferol 3-*O*-rhamnoglucoside Parks did not find gossypetin 8-*O*-rhamnoside or quercetin 3′-*O*-glucoside in *G arboreum*

When tested for antimicrobial activity with *Pseudomonas maltophilia* and *Enterobacter cloacae*, **1**–**4** slightly inhibited growth of the two bacteria Several related flavonoids were also tested The test results are given in Table 1

The following observations relate chemical structure to biological activity against *P maltophilia* (1) All of the glycosides with substitution at the 3-position had slight activity, (2) When the sugar at the 3-position was rhamnose, higher activity was observed, however, the 8-rhamnoside showed slight activity, (3) There was no measurable difference in activity between a glucoside and a galactoside, (4) There was no apparent difference between glycosidation at the 3-, 7- and 8-positions, (5) When glycosidation was at the 3′-position in the B-ring, there was no activity, and (6) The aglycone gossypetin showed no activity

Structure–activity relationships for *E cloacae* were different from those for *P maltophilia*, and the following observations are made Gossypetin, quercetin

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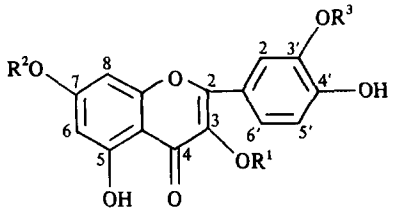
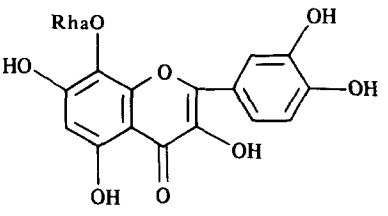


1 R^2 = Glc, R^1 = R^3 = H
2 R^1 = Glc, R^2 = R^3 = H
3 R^3 = Glc, R^1 = R^2 = H



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3-*O*-glucoside, quercetin 3-*O*-galactoside and quercetin 3-*O*-rhamnoglucoside were not active Quercetin 3′-*O*-glucoside, quercetin 7-*O*-glucoside and gossypetin 8-*O*-rhamnoside were marginally active The highest activity was from quercetin 3-*O*-rhamnoside

These findings are of importance because compounds toxic to bacteria are often toxic to insects, hence, antibacterial activity can be a facile predictor of resistance factors in cotton This information can be an aid to the plant breeder in selecting resistant varieties

EXPERIMENTAL*

The mass spectra were recorded on an HP-5985B GC/MS/DS in the CI mode The source was operated at 230 eV and 200° Methane was used as the ionizing gas at a source pressure of 0 4–0 8 torr

Extraction of plant material G arboreum flower petals (80 g of freeze-dried powder) were extracted exhaustively with boiling MeOH–H_2O (2 1), yield = 29 6 g (37 02%)

Fractionation of the MeOH–H_2O *extract* The extract was fractionated by chromatography on a Sephadex LH-20 column (l = 75 cm, i d = 5 cm, solvent = MeOH–H_2O) Nine fractions were collected, each was re-chromatographed on Sephadex LH-20 using the same column conditions All subfractions were compared by TLC on polyamide (solvent = EtOH–H_2O, 3 1) Subfractions which were similar were combined to give six fractions These fractions were chromatographed on polyamide (l = 60 cm, i d = 2 5 cm, solvent = MeOH–H_2O, 7 3) Comparison of all subfractions by polyamide TLC and recombination of similar subfractions gave eight fractions, A–H

Isolation and identification of **1** *and* **2** Prep TLC of fraction E on cellulose (solvent = *t*-BuOH–HOAc–H_2O, 3 1 1) gave two flavonol glycosides, **1** and **2** [3 92 g (4 9%) **1** and 0 48 g (0 6%) **2**] These were identified as quercetin 7-*O*-glucoside and quercetin 3-*O*-glucoside, respectively, by comparing their UV and mass spectra with those of standard compounds

Isolation and identification of **3** Fraction C was evaporated to dryness and extracted with EtOAc The insoluble portion was recrystallized from boiling water to give **3** [1 52 g (1 9%)] Compound **3** was identified as quercetin 3′-*O*-glucoside by comparison of its UV-visible and mass spectra with those of the standard compound

Isolation of **4** Fraction D was chromatographed on polyamide (l = 30 cm, i d = 2 5 cm) with an elution gradient of MeOH, Me_2CO and DMF, to give three subfractions, D_1, D_2 and D_3 Subfraction D_3 was evaporated under a fume hood to give a yellow solid, **4** [4 88 g (6 1%)]

Identification of **4** TLC was performed on cellulose in the solvent systems 15% HOAc and TBA (*t*-BuOH–HOAc–H_2O, 3 1 1) The chromatograms were viewed under UV light alone and after being exposed to NH_3 fumes The R_fs of **4** were 0 07 in 15% HOAc and 0 14 in TBA The spot appeared yellow under UV light and deep yellow under UV light after exposure to NH_3

Compound **4** had the following UV-visible spectral data, λ_{max}^{MeOH} nm 256, 374, NaOMe 240 sh, 328, 427 sh (dec), $AlCl_3$ 270, 448, $AlCl_3$/HCl 266, 440, NaOAc 277, 323, 390 (dec), NaOAc–H_3BO_3 265, 322, 396 The hypsochromic shift of 8 mm on addition of HCl to the $AlCl_3$ reagent, though less than for gossypetin or gossypitrin, is similar to that reported by Mabry *et al* [17] for gossypin (11 mm) This and the other shifts indicate that compound **4** is an 8-*O*-glycoside of gossypetin [17]

Acid hydrolysis of **4** Compound **4** (0 5 mg) was dissolved in 1 N HCl (5 ml) The soln was heated for 2 hr in a hot water bath (95°) The cooled soln was extracted with 5 ml of EtOAc to separate the aglycone, the water layer was allowed to evaporate to dryness, and the residue was dissolved in 0 5 ml of pyridine Evaporation of the EtOAc gave a yellow solid which had TLC, UV-visible and mass spectral data identical to those of gossypetin [17] TLC of the pyridine solution was performed on cellulose in the solvent system pyridine isoamyl alcohol–H_2O (7 7 2) The chromatogram was developed by spraying with anisidine phthalate (1 23 g *p*-anisidine and 1 66 g phthalic acid dissolved in

Table 1 Antimicrobial test results for flavonoid glycosides and aglycones

Test compounds	Zone width (mm)	
	P maltophilia	*E cloacae*
Quercetin 7-*O*-glucoside (**1**)	⩽ 7 0	⩽ 7 0
Quercetin 3-*O*-glucoside (**2**)	⩽ 7 0	NA*
Quercetin 3′-*O*-glucoside (**3**)	NA	⩽ 7 0
Gossypetin 8-*O*-rhamnoside (**4**)	⩽ 7 0	⩽ 7 0
Gossypetin	NA	NA
Quercetin 3-*O*-rhamnosyl-(1 → 6)-glucoside(rutin)	⩽ 7 0	NA
Quercetin 3-*O*-rhamnoside	8 5	8 5
Quercetin 3-*O*-galactoside	⩽ 7 0	NA
Quercetin 3-*O*-galactosyl-(1 → 6)-glucoside	⩽ 7 0	⩽ 7 0

*NA, No activity

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100 ml EtOH), and then heating at 120° for 5 min The sugar was identified as rhamnose by comparison with an authentic sample The mass spectrum (CI) of the sugar showed ions at *m/z* 147, 129 and 120, as compared with glucose (163, 145, 127 and 115) and rhamnose (147, 129 and 111)

Bioassay Fractions were screened for antibacterial activity against the pathogens *P maltophilia* and *E cloacae*, with bactosensitivity discs (BBL) Each fraction to be tested was dissolved in an appropriate solvent (1 mg/20 μl) A 20 μl aliquot of the soln was applied to a blank sensi-disc, and the solvent was allowed to evaporate overnight Three replications were employed for each sample A suspension of each bacterium in a 0 85% saline soln was used to streak petri plates (15 mm × 100 mm diameter) containing solidified tryptic-soy agar The sensi-discs were placed on the plates, no closer than 10–15 mm from each other (no more than seven discs per plate), and the plates were incubated overnight at 38° Inhibition was determined by measuring the diameter of the clear zone (if present) around the disc

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