$CHCl_3$–MeOH (4:1) on Si gel G. Fractions containing more than one compound were further separated by prep. TLC in $CHCl_3$–MeOH (4:1) on Si gel G and eluted with MeOH.

Compound **2** was obtained in small quantity (20 mg) and resisted crystallization from the usual solvents. R_f values for **2** were 0.8 (TLC in $CHCl_3$–MeOH, 4:1, on Si gel G) and 0.94 (PC in BAW). UV λ_{max}^{MeOH} nm (log ε): 255 (4.36), 264 (sh), 345 (4.10); λ_{max}^{NaOMe} nm: 270, 380; $\lambda_{max}^{AlCl_3}$ nm: 265, 271 (sh), 298 (sh), 360, 390; $\lambda_{max}^{AlCl_3-HCl}$ nm: 265, 271 (sh), 298 (sh), 360, 391; λ_{max}^{NaOAc} nm: 273, 365; $\lambda_{max}^{NaOAc-H_3BO_3}$ nm: 268, 350, IR ν_{max}^{KBr} cm^{-1}: 3300 (*br*), 1640. Acid hydrolysis of **2** (10 mg) with 6% HCl (10 ml) at 100° for 45 min afforded tamarixetin and rhamnose in a 1:1 ratio. The sugar residue was identified by co-PC with an authentic sample (R_f 0.41 in BAW). The aglycone crystallized from EtOAc–*n*-C_6H_{14} as pale yellow crystals mp 258–260° (lit. 259–260° [10]) TLC, R_f 0.8 ($CHCl_3$–MeOH, 9:1) on Si gel G. UV λ_{max}^{MeOH} nm (log ε): 256 (3.99), 366 (3.94). NMR (Me_2CO-d_6, TMS, δ-scale): 3.93 (*s*, –OMe), 6.24 (1H, *d*, J = 2.5 Hz), 6.51 (1H, *d*, J = 2.5 Hz), 7.07 (1H, *d*, J = 9.0 Hz), 7.71 (1H, *d*, J = 2.5 Hz) and 7.86 (1H, *dd*, J = 9, 2.5 Hz).

Methylation of **2** (5 mg) followed by acid hydrolysis of the product gave quercetin 5,7,3',4'-tetramethyl ether, mp 194–195° (lit. 195–198° [11]). When demethylated with excess pyridinium chloride at 140° for 2 hr tamarixetin (2 mg) gave quercetin which was identified by direct comparison with an authentic sample (mmp and co-TLC).

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TRISUBSTITUTED FLAVONOL GLYCOSIDES IN *CORONILLA EMERUS* FLOWERS

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Key Word Index—*Coronilla emerus*; Leguminosae; flowers; leaves; kaempferol 3-glucoside-7,4'-dirhamnoside; flavonol glycosides; UV patterning.

Abstract—A novel trisubstituted kaempferol glycoside has been isolated from leaves and flowers of *Coronilla emerus* and identified as the 3-glucoside-7,4'-dirhamnoside. It co-occurs in the flowers with the 3-glucosides and 3-glucoside-7-rhamnosides of kaempferol and quercetin. A second kaempferol triglycoside based on glucose and xylose is also present. All six glycosides contribute to the UV patterning present in the wings of the flowers. This is the first report of kaempferol triglycosides with monosaccharide units substituting hydroxyl groups at the 3-, 7- and 4'-positions.

INTRODUCTION

While studying the flavonol glycosides of yellow flowered legume species in relation to their role as UV patterning guides to insect pollinators [1], the presence of a novel flavonol glycoside with unusual colour reactions and UV spectrum was discovered in the wings of *Coronilla emerus*. This plant is pollinated by bumble bees, who are attracted to the flowers solely by the pollen, since there are no nectaries [2]. The UV absorbing flavonols, present mainly in the wings, presumably guide the insect in its approach

Table 1. Spectral and R_f properties of flavonol glycosides of *Coronilla emerus*

Glycoside	λ max (nm) in MeOH					R_f (× 100) in				Colours in UV	
	Alone	+NaOAc	$+H_3BO_3$	$+AlCl_3$	+NaOH	BAW	H_2O	15% HOAc	PhOH	Alone	$+NH_3$
Kaempferol											
3-glucoside-7,4′-dirhamnoside (**1**)	267, 320, 340	267	340	396	378	37	59	76	68	dark brown	dull brownish yellow
3,7,4′-(xylosylglucoside) (**2**)	267, 319, 340	267	340	396	378	26	58	76	61	dark brown	dull brownish yellow
3-glucoside-7-rhamnoside	267, 345	267	350	398	392	55	39	65	76	dark	bright yellow-green
3-glucoside	267, 347	274	350	396	—	73	18	49	73	dark	yellow-green
Quercetin											
3-glucoside-7-rhamnoside	257, 360	259	370	430	410	47	32	63	61	dark	bright yellow
3-glucoside	257, 358	267	378	428	—	63	14	42	59	dark	yellow

BAW, *n*-BuOH–HOAc–H_2O (4:1:5, top layer); PhOH, PhOH–H_2O (4:1).

to collect the pollen. Larger amounts of the glycoside were found to be present in the leaves of the same plant and, thus, it was obtainable in pure form. The present report describes the identification of this new kaempferol derivative and of the co-occurring glycosides in the flowers.

RESULTS

The new glycoside (**1**), isolated by PC in crystalline form, from leaves and flowers of *Coronilla emerus*, gave only glucose, rhamnose and kaempferol on acid hydrolysis and thus appeared to be a simple glycoside of this flavonol. However, in its colour reactions on paper and in its spectral properties (see Table 1), it differed from any of the usual kaempferol glycosides. The large hypsochromic shift in the neutral spectrum compared to kaempferol of 35 nm and the alkaline spectrum with a low *A* band at 378 nm were quite distinctive. The source of the novelty in its structure became apparent when complete methylation, followed by acid hydrolysis, gave kaempferol 5-methyl ether, showing that in the original glycoside all three reactive hydroxyl groups at the 3-, 7- and 4′-positions were glycosylated.

Quantitative analysis of the sugars after acid hydrolysis showed a glucose–rhamnose ratio of 1:1.98, indicating that one of the hydroxyls was linked to glucose and two to rhamnose. The only structural problem remaining was the location of these three sugars variously around the kaempferol molecule. Hydrogen peroxide oxidation, which is normally specific for the 3-substituted sugar [3], failed to work in this instance since both glucose and rhamnose were obtained. Standard enzymic hydrolysis was also not successful in locating the sugar substituents since β-glucosidase, at pH 5.0, had no effect on **1** while α-rhamnosidase (containing some β-glucosidase activity), at pH 4.4, completely hydrolysed **1** to its component parts without any intermediates being apparent.

The problem was simply solved by short incubation of **1** with the α-rhamnosidase preparation at a pH (3.8) unfavourable to β-glucosidase activity. This gave a good yield of kaempferol 3-glucoside, identified by co-chromatography with an authentic specimen. This reaction immediately established the complete structure of **1** as kaempferol 3-β-glucoside-7,4′-di-α-rhamnoside. This structure was further confirmed by carrying out partial acid hydrolysis for a very short time period (2 min) when it was possible to isolate and identify four of the five expected intermediates: the 7-rhamnoside, the 3-glucoside, the 7,4′-dirhamnoside and the 3-glucoside-4′-rhamnoside.

In the leaves of *Coronilla emerus*, this novel glycoside is the major component, whereas in the flowers it is accompanied by five other flavonol glycosides. Four of these were readily identified by standard techniques as the 3-glucosides and 3-glucoside-7-rhamnosides of kaempferol and quercetin. The sixth glycoside (**2**) was similar in colour reactions and spectrum to **1** but had a lower R_f value. On hydrolysis, **2** gave kaempferol, glucose and xylose and was clearly analogous in structure to **1**. Enzymic hydrolysis failed to give any intermediates, while partial acid hydrolysis gave insufficient of the products for identification purposes. Lack of further material prevented the complete elucidation of its structure.

Since the trisubstituted flavonols **1** and **2** have distinctive mobilities and colour reactions on 2D chromatograms, it was a relatively simple matter to survey dried leaves of other taxa of the genus *Coronilla* to see if they were present elsewhere. Compound **1** was found in each of the two subspecies of *C. emerus*, namely subsp. *emerus* and subsp. *emeroides*, but was not detected in any samples of nine other European species. Those surveyed included *C. coronata* L., *C. cretica* L., *C. juncea* L., *C. minima* L., *C. repanda* (Poiret) Guss., *C. scorpioides* (L.) Koch, *C. valentina* L., *C. vaginalis* Lam. and *C. varia* L.

DISCUSSION

All six flavonol glycosides identified (see Table 1) in flowers of *C. emerus* contribute to the UV patterning but not to the visible yellow colour which is due to carotenoids. The flavonols are located mainly in the wings, which absorb strongly in the UV and there are only trace amounts in keel and standard. This pattern in *C. emerus* is quite distinctive from that in *C. valentina* where UV absorbing yellow flavonols based on gossypetin [1] are known to contribute to both visible and UV patterning. In *C. emerus* it is not known whether the unusual glycosylation pattern of **1** and **2** has a special contribution to its pollination ecology. It is conceivable that small variations in the long wavelength maxima of the UV absorbing pigments may be picked up by insect pollinators so that the compounds could, in the long term, contribute to the maintenance of speciation in these legumes.

Although a wide range of flavonol triglycosides are known in nature, the sugars present are either located at one hydroxyl (giving a linear or branched trisaccharide) or at two positions (giving monosaccharide and disaccharide substituents) [3]. Thus kaempferol 3-glucoside-7,4′-dirhamnoside appears to be one of the first representatives of a class of glycoside with monosaccharide units attached to three separate phenolic hydroxyl groups in the flavonol nucleus.

Such compounds as **1** and **2** appear to be characteristic of *C. emerus* within the genus *Coronilla* since the leaves, at least, of several related species and the flowers of one, *C. valentina*, failed to show any derivatives of this type. They may well be present in other legume genera and a survey in this direction is in progress. They may also occur in other unrelated plants and indeed, in retrospect, it appears that one such compound is almost certainly present in a fern of the Appalachian *Asplenium* complex. Previous studies of *A. rhizophyllum* indicated the presence of an acylated complex of kaempferol glycosides, which on alkaline treatment gave, among other components, what appeared to be kaempferol 7,4′-diglucoside [4]. A reinterpretation of the spectral and other data for this compound indicates that it must have a similar structure to **1** in that three of its four hydroxyls must be substituted by glucose. It is, therefore, probably kaempferol 3,7,4′-triglucoside although this structural assignment needs confirming by measuring the aglycone–sugar ratio, or by other means. Some of the many other partly characterized kaempferol glycosides recorded in the earlier literature may well turn out to have a similar trisubstitution of their sugars.

EXPERIMENTAL

Plant sources. Flowers and leaves were collected in season from plants of *C. emerus* grown in the Botanical Garden of the University from authenticated spontaneous seed. Other *Coronilla* species were surveyed from leaf samples of herbarium specimens deposited at this University, the sheets so sampled being appropriately labelled.

Flavonoid identifications. The spectral and R_f properties of **1**, **2** and other glycosides of *C. emerus* are shown in Table 1. The four

known glycosides were identified by co-chromatography with authentic markers and by hydrolytic studies, followed by identification of intermediates in the case of diglycosides. The glycoside **1**, a cream powder, mp 248–250°, from aq. EtOH, on acid hydrolysis gave kaempferol, glucose and rhamnose. The percentage of kaempferol in **1** was determined spectrophotometrically as 42% (39% required for a triglycoside). The glucose–rhamnose ratio was determined as 1:1.98 by GC of the sugar mixture, after trimethylsilylation. After complete methylation (Me_2SO_4, Me_2CO, K_2CO_3, 8 hr) of **1**, and acid hydrolysis, kaempferol 5-methyl ether was produced. This was identified by spectral and chromatographic comparison with a synthetic sample.

Enzymic hydrolyses were carried out in acetate buffers with β-glycosidase (emulsin) or with Sigma naringinase as a source of α-rhamnosidase. Partial acid hydrolysis was conducted on **1** with 1 M HCl at 100° for 2 min. The products were separated by PC in 15% HOAc and purified. They were analysed by co-chromatography, R_f determination, spectral measurements and by sugar analysis using both PC and GC. 2D-PC chromatograms were run in BAW followed by 15% HOAc.

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FLAVONOIDS IN THE BLACK RHIZOMES OF *BOESENBERGIA PANDURATA*

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Key Word Index—*Boesenbergia pandurata*; Zingiberaceae; black rhizomes flavonoids; flavonoid methyl ethers; flavanones.

Abstract—5-Hydroxy-7-methoxyflavanone, 5,7-dimethoxyflavanone, 5-hydroxy-7-methoxyflavone 5-hydroxy-7,4′-dimethoxyflavone, 5,7-dimethoxyflavone, 5,7,4′-trimethoxyflavone, 5,7,3′,4′-tetramethoxyflavone, 5-hydroxy-3,7-dimethoxyflavone, 5-hydroxy-3,7,4′-trimethoxyflavone, 3,5,7-trimethoxyflavone and 5-hydroxy-3,7,3′,4′-tetramethoxyflavone have been isolated from the black rhizomes of *Boesenbergia pandurata*.

INTRODUCTION

Following our previous work on constituents of the Zingiberaceae of Thailand [1, 2], the present report deals with the chemical constituents of the rhizomes of *Boesenbergia pandurata* (Roxb.) Schltr. (black rhizomes) (local name: krachai-dum) which is used in folk medicine for the treatment of colic disorders.

RESULTS AND DISCUSSION

The milled rhizomes of *B. pandurata* were extracted exhaustively with hexane in a Soxhlet apparatus. The crude extract was chromatographed on a column of Si gel using hexane–ether as eluants. Further purification by prep. TLC gave 5-hydroxy-7-methoxyflavanone (**1**), 5,7-dimethoxyflavone (**2**), 5-hydroxy-7-methoxyflavone (**3**), 5-hydroxy-7,4′-dimethoxyflavone (**4**), 5,7-dimethoxyflavone (**5**), 5,7,4′-trimethoxyflavone (**6**), 5,7,3′,4′-tetramethoxyflavone (**7**), 5-hydroxy-3,7-dimethoxyflavone (**8**), 5-hydroxy-3,7,4′-trimethoxyflavone (**9**), 3,5,7-trimethoxyflavone (**10**) and 5-hydroxy-3,7,3′,4′-tetramethoxyflavone (**11**). Compounds **1**–**11** were identified on the basis of their spectroscopic data and elemental analyses. Compound **5** has been isolated in pure form from a natural source and been fully characterized for the first time. Compounds **6** and **8** do not appear to have been found previously in nature.

It is interesting to note that even though the *B. pandurata* (black rhizomes) is a variation of *B. pandurata* (yellow rhizomes) [2, 3], their chemical constituents differ substantially. Three known flavonoids, **1**, 7-hydroxy-5-methoxyflavone and 5,7-dihydroxyflavone; two known chalcones, 2′,6′-dihydroxy-4′-methoxychalcone and 2′,4′-dihydroxy-6-methoxychalcone; and a new chromenoid chalcone derivative, boesenbergin A, have been isolated from the latter plant.

EXPERIMENTAL

A voucher specimen (BKF No. 73995) of the plant material has been lodged at the Forest Herbarium, Royal Forest Department,