Table 2. ^{13}C chemical shifts of **1** and **2***

	1	2
C-4	178.8	178.6
$\underline{C}H_3\underline{C}OOR$		172.4
C-1″	102.7	99.8
C-6	99.5†	99.6
C-8	94.6†	94.6
$\underline{C}H_3COOR$		20.6‡
$\underline{C}H_3$-C-5″	17.1‡	17.1‡

* Values in ppm downfield from TMS; solvent: MeOD-D_2O (3:1); noise-decoupled, 15 MHz Fourier transform spectra; 8K data points; 4 kHz spectral width. † Signal disappears on deuteration. ‡ Changes to quartet on single-frequency, off-resonance decoupling.

Corroborative evidence for the structure of **2** as 2″-*O*-acetylquercitrin is provided by ^{13}C NMR data (Table 2). The noise-decoupled spectrum of **2** contains an ester carbonyl carbon peak and an additional Me group peak, both of which are absent in the spectrum of **1**. Moreover, the large (2.9 ppm) upfield shift of the C-1″ (rhamnose) signal in **2** would be expected only if the adjacent (C-2″) OH group was acetylated [4]. The signal for C-1″ was distinguished from those of the neighboring C-6 and C-8 signals by exchanging H-6 and H-8 in **1** with deuterium [5].

When **2** is warmed in dry deuteriopyridine the acetyl group migrates to the 3″-position and eventually an equilibrium is established between the 2″- and 3″-isomers. This finding will be reported elsewhere.

EXPERIMENTAL

Isolation. Fresh azalea flowers (Rhododendron × cv Red Wing) were extracted with boiling MeOH. **2** was purified by TLC on 2-mm layers of microcrystalline cellulose as previously described [1]. It was shown by chromatography that **2** was present in the MeOH extract before purification and was not an artifact.

Base hydrolysis. **2** (3 mg) in 0.5 N NaOH (1 ml) was heated for 15 min at 100°. The soln was acidified and quercitrin extracted with EtOAc.

Deuterium exchange. **1** (200 mg) in D_2O (2 ml) containing pyr (1 ml) was heated for 2 hr at 100°. The chilled soln was acidified with conc HCl and kept 18 hr at 4°. 6,8-d_2-*quercitrin* was obtained as bright yellow crystals.

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PHENOLIC COMPOUNDS FROM THE HEARTWOOD OF *GARCINIA INDICA**

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Key Word Index—*Garcinia indica*; Guttiferae; euxanthone; biflavanoids; volkensiflavone; morelloflavone.

No previous work has been reported on the heartwood pigments of *G. indica*, but D-leucine has been extracted from the leaves [1], (−) hydroxycitric acid from the fruit [2] and glycerides and fatty acids from the seeds [3]. Phenolic metabolites isolated from other Guttiferae heartwoods include xanthones [4], biflavanoids {5–7} coumarins [8] and biphenyls [9].

The heartwood of *Garcinia indica* was reduced to shavings and extracted with hot $CHCl_3$. Evaporation of the solvent from the extract gave a residue which separated into a yellow oil and a semi-solid black tar. The yellow oil was a mixture of aliphatic compounds (NMR and IR evidence) and was not investigated further. The black tar was chromatographed on silica. Elution with EtOAc–PhMe led to the isolation of 1,7-dihydroxyxanthone (euxanthone) [10] and the biflavanoids volkensiflavone [11] and morelloflavone [12, 11] which were separated by preparative TLC and identified by spectral comparison with authentic specimen, and by permethylations to give the chalcone-flavones by opening of ring I–C.

Dimethyl terephthalate was also isolated by chromatography but we assume this product is a plasticiser leeched out from the plastic bottle during transit from India.

These results support previous conclusions from work on *Garcinia* species which show that 3,8-linked biflavanoids and xanthones are present in the heartwood.

* Part 32 in the series "Extractives from Guttiferae". For Part 31 see ref. [6].

EXPERIMENTAL

Plant material of *G. indica* Chois was obtained from Sirsi, Karnatak, South India. All UV spectra were determined in MeOH, IR spectra as Nujol mulls. NMR spectra were measured on a Varian HA 100 instrument and MS with A.E.I. MS 12 and MS 9 spectrometers. TLC and PLC were carried out with Si gel (Merck Kieselgel G).

Extraction of G. indica. Heartwood was reduced to shavings (5 kg) and continuously extracted with hot $CHCl_3$ to give, after removal of solvent, 80 g crude extract. On standing the extract separated into 2 layers. The upper pale yellow oil was decanted off to leave a semi-solid black tar. A 16 g portion of the tar was chromatographed on Si gel and the column eluted with increasingly polar mixtures of PhMe–EtOAc and finally with MeOH. The early combined fractions gave more of the aliphatic oils previously decanted from the crude extract. Further elution gave a yellow oil R_f 0.7 (EtOAc–PhMe, 15:85) which was purified by PLC to give 1,7-dihydroxyxanthone (4 mg) mp 225–230° (lit [10], 239°) identical with an authentic sample. (Found: M^+, 228. Calc. for $C_{13}H_8O_4$ M, 228). Further elution gave dimethyl terephthalate (52 mg) as colourless needles mp 130–132° lit [13] 142°. UV λ_{max} nm ($\varepsilon \times 10^{-3}$) 275 (24.0), 289 (26.9), 321 (17.8). IR ν_{max} 1727, 1288, 1121, 1114, 1023, 961, 820, 740 and 734 cm^{-1}. NMR τ (CCl_4) 2.00 (*s*, 4H) 6.13 (*s*, 6H). (Found: M (mass spectrum), 194.0589. Calc. for $C_{10}H_{10}O_4$ M, 194.0579).

Concentration of the remaining fractions from the chromatography gave a mixture of biflavanoids (6.3 g). PLC of a portion of this mixture (300 mg) gave volkensiflavone (52 mg), and morelloflavone (190 mg) as amorphous yellow powders identical with authentic specimen. Methylation of the biflavanoids with dimethyl sulphate gave volkensiflavone hexamethyl ether M^+, 624 and morelloflavone heptamethyl ether M^+, 654.

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BIOSYNTHESIS OF GRAMINE IN *PHALARIS ARUNDINACEA*

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Key Word Index—*Phalaris arundinacea*; Gramineae; gramine; tryptophan; alkaloid biosynthesis.

Abstract—The administration of L-tryptophan-[3-^{14}C] to *Phalaris arundinacea* L. (Vantage strain) for 9 days resulted in the formation of radioactive gramine (8.2% absolute incorporation). A systematic degradation of the alkaloid indicated that essentially all its activity was located on the methylene group, indicating that its biosynthesis is the same as that occurring in *Hordeum* species and *Lupinus hartwegii*.

INTRODUCTION

Gramine (2) is found in reed canarygrass (*Phalaris arundinacea*, Gramineae) [1, 2]; several tryptamines and β-carbolines have also been isolated from this species [3, 4]. It has been established that different reed canary grass cultivars differ with respect to alkaloid concentration and the distribution patterns of specific alkaloids [4, 5]. The 'Vantage' cultivar contains only gramine, and this strain has been shown to be genetically recessive with respect to the tryptamine alkaloids [6].

It has been previously established that tryptophan (1) is a precursor of gramine in barley (*Hordeum* species) [7–9] and the lupin (Leguminosae): *Lupinus harwegii*

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