TLC ($AgNO_3$–Si gel; C_6H_6–Et_2O 7:3 as the eluent; band positions located by strip-spraying with 1% soln of $Ce(SO_4)_2$ in 2 N H_2SO_4 followed by heating) to give pachydictyol A (R_f 0.20; 5 mg) and the alcohol **11** (R_f 0.25; 8 mg), identified by comparison of their physical properties ($[\alpha]_D$, MS, NMR) with those of authentic specimens.

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DIBENZOYLMETHANES AND FLAVONES OF *MALUS*

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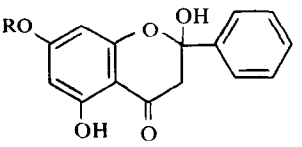
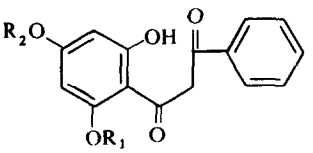
Key Word Index—*Malus*; Rosaceae; dibenzoylmethanes; flavones; dihydrochalcones; 2,4,6-trihydroxydibenzoylmethane 2-glucoside; toringin; chrysin biosynthesis.

The occurrence of an unusual glucoside in the leaf of a *Malus* cultivar (*Malus H*) has already been reported [1], together with details of its conversion to chrysin 7-glucoside both in the leaf and in extracts. The compound **1a** is known normally as a 2-hydroxyflavanone [2] but from its chemical reactions it can be regarded alternatively as a dibenzoylmethane (**2a**) or a β-hydroxychalcone (**3a**), when its 4′-glucosylation pattern then corresponds to that of the dihydrochalcone glucoside sieboldin (which also occurs in the leaf of *Malus H*) and not to the 2′-pattern of the common *Malus* dihydrochalcone phloridzin.

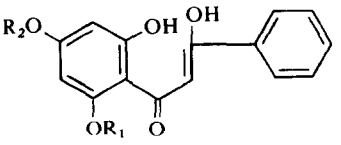
In an examination of the phenolics of *Malus* barks by PC, a substance was found in *M. fusca* bark with similar R_fs to that found in *Malus H* leaf. Sufficient of the new substance for examination was separated by chromatography on thick paper sheets. Acid hydrolysis gave only glucose and an aglucone indistinguishable in chromatographic and UV spectral properties from chrysin; this result is the same as was found on acid hydrolysis of the glucoside from *Malus H*, suggesting that the two substances might be isomeric glucosides. After storage a solution of the new compound gave a second slow-moving compound on PC in 2% acetic acid, which showed the distinctive fluorescence of the flavone glucoside toringin, chrysin 5-glucoside, already known as a bark constituent of *M. tschonoskii* [3]. Comparison of chromatographic and UV spectral properties with authentic toringin confirmed its identity.



1a R = Glc
1b R = H



2a R_1 = H; R_2 = Glc
2b R_1 = Glc; R_2 = H



3a R_1 = H; R_2 = Glc
3b R_1 = Glc; R_2 = H

Table 1. R_f values of flavonoids from *Malus*

Compound	R_f (×100) SBA	HOAc
2,4,6-Trihydroxydibenzoylmethane		
2-glucoside	70	50
4-glucoside	70	66
Chrysin 5-glucoside	65	16
2′,4′,6′-Trihydroxydihydrochalcone		
2′-glucoside	72	35
4′-glucoside	72	35
Phloridzin (reference)	70	33

The parent compound must thus be the 2-glucoside of 2,4,6-trihydroxydibenzoylmethane (**2b**), which ring closes with loss of water to give toringin, as does the 4-glucoside to give chrysin 7-glucoside.

The 4-glucoside (**1a**) gives a single strong peak λ_{max}^{EtOH} 287 nm while the 2-glucoside gives two peaks λ_{max}^{EtOH} 291, 379 nm and must be therefore largely in the enolic β-hydroxychalcone form (**3b**) [4]. In NaOEt both glucosides showed main peaks around 380 nm, (**1a**, λ_{max}^{NaOEt} 380 nm, **3b**, λ_{max}^{NaOEt} 397 nm) typical of the β-hydroxychalcone form under these conditions. The aglycone of both glucosides obtained by enzymic hydrolysis exists in the 2-hydroxyflavanone form (**1b**), λ_{max}^{EtOH} 292; the free 4-hydroxyl stabilizes this structure even in alkali, λ_{max}^{NaOEt} 326 nm with only a weak shoulder at 390 nm.

The *Malus* species in whose bark toringin and 2,4,6-trihydroxydibenzoylmethane 2-glucoside have been found include *M. fusca*, *M. kansuensis*, *M. transitoria*, *M. tschonoskii*, *M. florentina* and *M. formosana*, which all have the dihydrochalcone phloridzin in their leaf, but no sieboldin. There is thus a similarity between the glucosylation patterns of the hydroxydibenzoylmethane glucosides and the co-occurring dihydrochalcone glucosides; the 4-glucoside was originally found in the leaf of *Malus H* which produces sieboldin and not phloridzin. However, in the bark of *Malus H* both phloridzin and sieboldin are present and the 2-glucoside is found together with the 4-glucoside. Again, in the leaf of some hybrid seedlings of *Malus H* both the 2- and 4-glucosides occur together with phloridzin and sieboldin.

While the dihydrochalcone glucosides mentioned so far have 4-hydroxylation (phloridzin) or 3,4-dihydroxylation (sieboldin) in the B-ring, both the hydroxydibenzoylmethane glucosides have unhydroxylated B-rings. In the tautomeric β-hydroxychalcone, these compounds could be derived from a chalcone with unhydroxylated B-ring; chalcones are generally regarded as the initial flavonoid compounds and their conversion to dihydrochalcones has been shown [5]. Any link between the biosynthetic pathways of the dibenzoylmethanes and the common dihydrochalcones must be at an early stage preceding hydroxylation of the B-ring. It is therefore of interest that the dihydrochalcone corresponding in hydroxylation and glucosylation pattern with the 4-glucoside of 2,4,6-trihydroxydibenzoylmethane has been found to accompany it in small amounts in the leaf of *Malus H*; the substance is 2′,4′,6′-trihydroxydihydrochalcone 4′-glucoside. Similarly 2′,4′,6′-trihydroxydihydrochalcone 2′-glucoside has been found in small amounts in *M. fusca* bark with the corresponding dibenzoylmethane 2-glucoside.

The usual route to flavonoids is thought to be through a chalcone with 4-hydroxylation in the B-ring formed from *p*-coumaryl CoA, but the occurrence of those with unsubstituted B-rings in some *Malus* species suggests a parallel route from cinnamyl CoA leading to glucosides of the dibenzoylmethanes, chrysin and dihydrochalcones but not apparently to the corresponding flavonols. The usual route from *p*-coumaryl CoA leads in *Malus* to phloridzin and flavonol glycosides but not to flavones with the same hydroxylation pattern, i.e. apigenin and luteolin, which have not been found. In the closely related genus *Pyrus* the only flavones are of the apigenin and luteolin pattern and no dibenzoylmethanes have been found. In *Malus* the dibenzoylmethanes are intermediates in the formation of chrysin glucosides (as also with tectochrysin in *Populus nigra*). However, failure to detect these substances in *Pyrus* does not necessarily mean that flavones are formed by a different pathway for it has been shown that dibenzoylmethanes with B-ring hydroxylation are much less stable [6], passing very easily into flavones and so may well fail to accumulate at a detectable level. Only one natural occurrence of such a compound has so far been reported [7], the 2-glucoside of 2,4,6,3′,4′-pentahydroxydibenzoylmethane in the seed of *Galega officinalis* from which the corresponding luteolin 5-glucoside was first isolated.

EXPERIMENTAL

Bark was taken from 1-yr-old growth of trees in the Long Ashton collection of *Malus* species and extracts made with 80% EtOH–H_2O. *M. fusca*, *kansuensis* and *tschonoskii* came originally from Hilliers, *M. transitoria* and *florentina* from Kew. *M. formosana* was raised from seed of the wild species collected in Taiwan.

PC separations and 2D chromatograms were made with the solvents *sec*-BuOH–HOAc–H_2O, 70:2:28 (SBA) and 2% HOAc–H_2O (HOAc) alone or in combination on Whatman 3MM or No. 1 paper. Phenolics were detected by UV inspection at 366 and 254 nm before and after NH_3 fuming and dipping with 1% $AlCl_3$ in EtOH, and by the colour produced with *p*-nitrobenzene diazonium fluoroborate.

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