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FLAVONOIDS AND TANNINS OF *ACACIA* SPECIES

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Key Word Index—*Acacia* spp; Leguminosae; flavonoid substitution and tannin formation; nucleophilic and electrophilic properties of flavan-3,4-diols.

Abstract—The heartwoods of *Acacia giraffae* and *A. galpinii* were selected from South African *Acacias* as representative of those with abnormally high and minimal tannin contents respectively. *A. galpinii* contains amongst other analogues, the first natural (+)-2,3-*trans*-3,4-*trans*-teracacidin (7,8,4′-trihydroxyflavan-3,4-diol) and novel 3-*O*-methyl-, 7,8-di-*O*-methyl- and 7,8,4′-tri-*O*-methylflavonol analogues. (−)-2,3-*cis*-3,4-*cis*-Melacacidin (7,8,3′,4′-tetrahydroxyflavan-3,4-diol) is also present, but tannins are absent. By contrast, from the large excess of leucofisetinidin tannins which characterizes the wood of *A. giraffae*, only (+)-catechin, (+)-2,3-*trans*-3,4-*trans*-leucofisetinidin (7,3′,4′,trihydroxyflavan-3,4-diol) and all-*trans*-(+)-leucofisetinidin-(+)-catechin could be isolated.

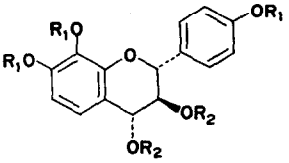
INTRODUCTION

Acacia giraffae Willd. [camel-thorn or kameeldoring (Afrikaans)] is the conspicuous great tree of the desert regions of Southern Africa, whereas *A. galpinii* Burtt Davy [monkey-thorn or apiesdoring (Afr.)] with its bright green luxuriant foliage is found along the rivers of the Transvaal bushveld [1].

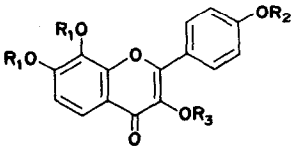
The woods of both species are hard and heavy. The dark red-brown of the heartwood of *A. giraffae* is associated with an excess of polyflavonoids with 7,3′,4′- and 5,7,3′,4′-phenolic substitution patterns. These fall within the category of condensed tannins and exhibit a high incidence of rotational isomerism [2]. By comparison the dark brown heartwood of *A. galpinii* is almost free of tannins (other than oxidation products) and contains, in common with many Australian *Acacias* [3], 7,8,4′-trihydroxyflavan-3,4-diols (teracacidins), a full range of related 7,8,4′-trisubstituted flavonoids and also (−)-melacacidin (7,8,3′,4′,tetrahydroxyflavan-3,4-diol). In this paper the basis for the striking difference in tannin content between the two species is investigated by complete analysis.

RESULTS AND DISCUSSION

A. galpinii represents the first South African *Acacia* known to contain flavonoid analogues of the 7,8,4′-trihydroxyphenolic substitution pattern, but these analogues were subsequently demonstrated in the closely-related *A. burkei* Benth. (black monkey-thorn) [4]. (−)-7,8,4′-Trihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol [(−)-teracacidin [5]] predominates in the wood extract and is accompanied by three diastereoisomers, (−)-2,3-*cis*-3,4-*trans*, (+)-2,3-*trans*-3,4-*cis* and (+)-2,3-*trans*-3,4-*trans* (**1a**) as well as by (−)-melacacidin [6] [(−)-7,8,3′,4′-tetrahydroxy-2,3-*cis*-flavan-3,4-*cis*-diol]. The (+)-*trans*-*trans* isomer (**1a**) was isolated from a natural source for the first time.



(**1a**) $R_1 = R_2 = H$
(**1b**) $R_1 = Me$; $R_2 = H$
(**1c**) $R_1 = Me$; $R_2 = Ac$



(**2a**) $R_1 = R_2 = H$; $R_3 = Me$
(**2b**) $R_1 = Me$; $R_2 = R_3 = H$
(**2c**) $R_1 = R_2 = Me$; $R_3 = H$

Attempts to purify the free phenolic form of (+)-2,3-*trans*-3,4-*trans*-diol by preparative PC were not successful due to overlap with the (−)-2,3-*cis*-3,4-*trans* diastereoisomer. However, after methylation with CH_2N_2 their methyl ethers could be separated by TLC. The PMR spectrum of the (+)-7,8,4′-trimethoxy-2,3-*trans*-flavan-3,4-*trans*-diol (**1b**) and that of its diacetate (**1c**) were

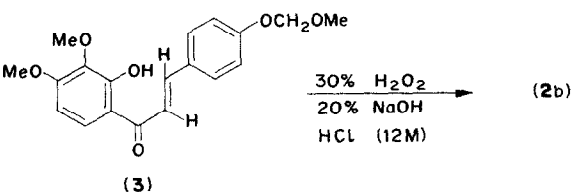
in agreement ($J_{2,3}$ 9·0, $J_{3,4}$ 6·3 Hz) with results of previous work on flavan-3,4-diols [7]. Similarly, their optical rotations {$[\alpha]_D$ −10·8°, −17·1° resp.} were in agreement with those of the corresponding derivatives of (2*R*:3*S*:4*R*)-flavan-3,4,7,3′,4′-pentaol [(+)-mollisacacidin, −9·4°, −19·6°] [8] and the same absolute configuration for (+)-7,8,4′-trihydroxy-2,3-*trans*-flavan-3,4-*trans*-diol (**1**a) follows.

The four diastereoisomeric flavan-3,4-diols are associated with the usual pattern of (±)-2,3-*trans*-dihydroflavonol, (±)-flavanone, flavonol and chalcone analogues [9], new derivatives of the first two being formed. Small quantities of natural partially methylated 7,8,4′-trihydroxyflavonols were isolated from the wood extract, namely 7,8,4′-trihydroxy-3-methoxyflavone (**2**a), 3,4′-dihydroxy-7,8-dimethoxyflavone (**2**b) and 3-hydroxy-7,8,4′-trimethoxyflavone (**2**c).

The presence of 7,8,4′-trihydroxy-3-methoxyflavone (**2**a) was indicated by the characteristic blue colour [10] under UV on PC. The PMR spectrum of **2**a indicated only one methoxy group. An additional three acetyl groups were shown after preparation of the tri-acetate, which confirmed the presence of three hydroxyls in the parent compound. Degradation with anhydrous alkali [11] resulted in fragments which were identified as pyrogallol and *p*-hydroxybenzoic acid representing the A- and B-rings respectively.

The substitution pattern of 3-hydroxy-7,8,4′-trimethoxyflavone (**2**c) was established by NMR and MS. Chemical shifts (Table 1) after acetylation indicate a 3-OH function (Δτ +0·54 for 2′ and 6′-H) whereas the MS exhibited the anticipated *m/e* 181 (20%), *m/e* 180 (3%) fragments for ring A and *m/e* 135 (15%) for the B-ring. The structure was confirmed by synthesis using (±)-7,8,4′-trimethoxy-2,3-*trans*-dihydroflavonol as starting material. Oxidation of the dihydroflavonol in hot 20% NaOH solution by bubbling air produced the desired 3-hydroxy-7,8,4′-trimethoxyflavone (**2**c) due to *trans*-elimination of hydrogens at positions 2 and 3 to give the more stable conjugated flavone molecule.

Comparison of the PMR spectrum (Table 1) of the acetylated 3,4′-dihydroxy-7,8-dimethoxyflavone (**2**b) with that of the original compound indicates chemical shifts (Δτ −0·61 for 3′ and 5′-H and +0·13 for 2′ and 6′-H) which indicate free hydroxyls on both the 3- and 4′-positions in the parent compound. The MS with fragments *m/e* 181 (2·5%) and *m/e* 127 (16%) for the A- and B-rings respectively, supported this structure, which was confirmed by synthesis. Starting materials were 4-methoxymethoxybenzaldehyde and 2-hydroxy-3,4-dimethoxyacetophenone. In the presence of alkali these undergo crossed aldol condensation to form 2′-hydroxy-4-methoxymethoxy-3′,4′-dimethoxychalcone **3**. Cyclization of the chalcone (**3**) *via* the Algar-Flynn-Oyamada (AFO) reaction and subsequent hydrolysis of the 4′-ether link resulted in the desired flavonol (**2**b).



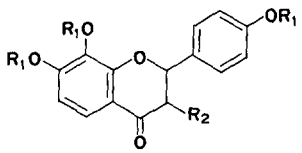
(3)

Chemical shifts [12, 13] in Table 1 indicate the anticipated deshielding effect on protons *ortho* and *para* to the hydroxyl of acetylation. For the A-ring Δτ is −0·60 (*para*) and −0·43 (*ortho*), while for the B-ring Δτ is −0·40 to −0·60 (*ortho*). Acetylation of the 3-OH resulted in a shielding effect on 2′- and 6′-protons (+Δτ) which might be diagnostic for this position.

Table 1. Chemical shifts in the aromatic region of the derivatives of 3,7,8,4′-tetrahydroxyflavone

	τ-Values (DMSO-d_6) of protons			
	2′ + 6′	3′ + 5′	5	6
7,8,4′-Trihydroxy-3-methoxyflavone	1·87	2·97	2·47	2·97
7,8,4′-Tri-*O*-acetyl-3-methoxyflavone	1·93	2·57	1·87	2·50
3-Hydroxy-7,8,4′-trimethoxyflavone	1·76	2·80	2·10	2·70
3-*O*-acetyl-7,8,4′-trimethoxyflavone	2·30	2·76	2·10	2·63
3,4′-Dihydroxy-7,8-dimethoxyflavone	1·83	2·96	2·10	2·70
3,4′-Di-*O*-acetyl-7,8-dimethoxyflavone	1·96	2·35	2·10	2·60
3,7,8,4′-Tetramethoxyflavone	1·90	2·80	2·13	2·69

Two new derivatives of the natural (±)-7,8,4′-trihydroxy-2,3-*trans*-dihydroflavonol (**4a**) were prepared, namely (±)-7,8,4′-trimethoxy derivative (**4b**) and its 3-acetate (**4c**). (±)-7,8,4′-Trimethoxyflavone (**4d**) is also a new derivative of the natural parent compound (**4** $R_1 = R_2 = H$).

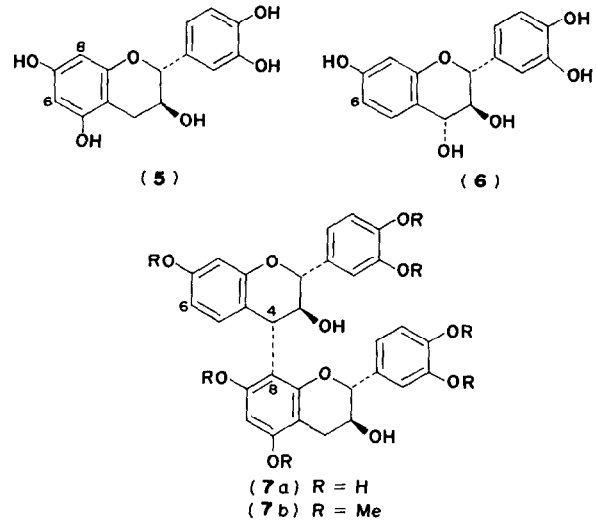


(**4a**) $R_1 = H$; $R_2 = OH$
(**4b**) $R_1 = Me$; $R_2 = OH$
(**4c**) $R_1 = Me$; $R_2 = OAc$
(**4d**) $R_1 = Me$; $R_2 = H$

The reddish colour of the heartwood of *A. giraffae* should be linked with the presence of leucofisetinidin tannins [14]. Alkali fusion of the various tannin fractions obtained by partition separation gave the fragments resorcinol, phloroglucinol and protocatechuic acid only. All fractions also gave fisetinidin chloride (3,7,3′,4′-tetrahydroxyflavylium chloride) when treated with 3 N HCl-*iso*PrOH under pressure. The isolated (+)-catechin (**5**) [(+)-5,7,3′,4′-tetrahydroxy-2,3-*trans*-flavan-3-ol] and (+)-leucofisetinidin (**6**) [(+)-7,3′,4′,trihydroxy-2,3-*trans*-flavan-3,4-*trans*-diol] accordingly represent biogenetic precursors and all-*trans*-(+)-leucofisetinidin-(+)-catechin (**7a**), the most likely prototype of the remainder of the tannins. The link in the biflavanoid is most likely 4,8, since all methoxy resonances of its heptamethyl ether (**7b**) show chemical shifts [15] during progressive addition of C_6D_6 to $CDCl_3$ solutions of the compound during PMR spectrometry.

The biflavanoid (**7a**) was first isolated from the bark of *A. mearnsii* [16] and later from the wood of *Colophospermum mopane* [15].

The wood material selected for the present study represent the extremes of tannin content among the heartwoods of some 450 species of the genus *Acacia* examined hitherto [3]. Ignoring any enzymic contribution to tannin formation and presuming that the mechanism of formation is ionic, one might examine flavonoid constituents for their potential properties as strong electrophiles and/or nucleophiles in the equivalent of benzene substitution reactions between units. Thus, the wood of *A. giraffae* is unique amongst



(**5**) (**6**)

(**7a**) R = H
(**7b**) R = Me

Scheme 1. Flavonoids and their predisposition towards condensation in tannin formation.

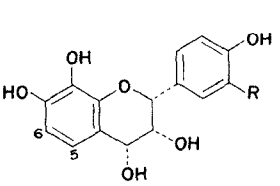
the *Acacia* heartwoods for its exceptional tannin content and presence of (+)-catechin. The latter (**5**) provides exceptionally strong nucleophilic centres at position 6 and 8, due to its *meta*-oxygenated substitution pattern. Of these the 8-position, being more accessible, provides the most favoured site for electrophilic substitution by a resonance-stabilized 4-carbonium ion arising from the flavan-3,4-diol, (+)-leucofisetinidin (**6**). A parallel situation of low residual (+)-catechin and (+)-leucofisetinidin content associated with an excess of polyflavonoids (tannins) comprised of these units exists, for example, in the woods of *Schinopsis* spp. (quebracho) [17], *Rhus lancea* (karee) [18] and in the bark of *A. mearnsii* (black wattle) [16, 19], where (+)-leucorobinetinidin [(+)-7,3′,4′,5′-tetrahydroxyflavan-3,4-diol] accompanies the above pair in the presence of an excess of leucorobinetinidin and leucofisetinidin tannins of type **7** with a (+)-catechin "terminal" group. Other examples where tannins of this type exist are *A. luederitzii* [20], *C. mopane* [15] and in the barks of a variety of Australian *Acacia* [21] related to *A. mearnsii*.

The tetraflavonoid tannin from *R. lancea* [18] and a triflavonoid from *C. mopane* [2, 15] show that extension of this presumed principle of condensation involves electrophilic attack at the sterically most available strong nucleophilic centre, namely the 6-position in the upper resorcinol-type unit of the biflavanoid (cf. 7a).

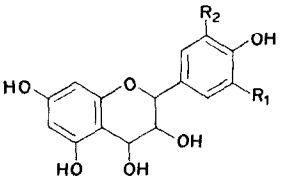
Where (+)-catechin or related strongly nucleophilic flavanoids of the phloroglucinol type are absent as, for example, in the heartwood of the black wattle (*A. mearnsii*), but where the predominant flavan-3,4-diol, (+)-leucofisetinidin (**6**) can act as both electrophile (at C-4) and nucleophile (at C-6), self-condensation of the flavan-3,4-diol occurs to form a variety of 4,6-linked biflavanoids [22], triflavanoids [23] and higher condensates. Since phloroglucinol itself is a stronger nucleophile than resorcinol [24], the tendency for self-condensation of the resorcinol-type flavan-3,4-diol in wattle wood is conceivably somewhat less. This is in line with the observation that the percentage of polyflavanoid units formed is low relative to the (+)-leucofisetinidin (**6**) content. This situation exists throughout the section Botryocephaleae and the section Uninerves subsection Racemosae of the genus *Acacia* [3] and also in the heartwood of *Robinia pseudacacia* [(+)-leucorobinetinidin] which has been studied in detail [25].

Finally, where flavan-3,4-diols of the (−)-teracacidin (**8**a) and (−)-melacacidin (**8**b) type predominate as in *A. galpinii*, 7,8-dihydroxy substitution leads to a general distribution of electron density of the unsubstituted 5- and 6-positions on the A-ring, in contrast to the strong nucleophilic sites in resorcinol- (at 6; to a lesser extent at 8) and phloroglucinol-based (at 8; to a lesser extent at 6) flavonoids.* This implies that structures of type **8** are poorer nucleophiles, and for the same reason the 4-carbonium ions which could presumably also originate from them, will be less adequately stabilized by delocalization of the charge (through resonance). This explains their lack of self-condensation and consequent absence of (4,6- or 4,5-linked) condensed tannins, other than phenol oxidation (autoxidation) products. This phenomenon is observed in all of the many *Acacia* spp. containing (−)-melacacidins and (−)-teracacidins and their analogues, particularly amongst the section Juliflorae [3].

The validity of these assumptions may be tested by random examination of those plant sources which contain phloroglucinol-type flavan-3,4-diols as precursors. From the bark of the red mangrove (*Rhizophora mucronata* Lam.), the barks of many *Acacia* spp. [21] to the testa of the giant sword-bean *Entada purseatha* DC, traces of leucocyanidin (**9**b) accompany highly condensed polyleucocyanidin tannins. The same applies to leucopelargonidins (**9**a) and leucodelphinidins (**9**c) in *Acacia* barks [21].



(**8**a) R = H
(**8**b) R = OH



(**9**a) $R_1 = R_2 = H$
(**9**b) $R_1 = OH$; $R_2 = H$
(**9**c) $R_1 = R_2 = OH$

It is of interest that the carbonyl containing flavanones, dihydroflavonols or 2-hydroxy-2-benzylcoumaranones do not participate in tannin formation of this type, presumably because the electron withdrawing effect of the 4-carbonyl function (or its equivalent) renders these compounds poor nucleophiles for attack by 4-carbonium ions. This leaves flavan-3,4-diols and flavan-3-ols to participate in tannin formation, with their B-rings nonfunctional, due to poor nucleophilic properties resulting from monohydroxylation (4'-hydroxyl); a general distribution of ring reactivity due to *ortho*-dihydroxylation (3',4'-dihydroxyls), or predominant steric factors due to high substitution (3',4',5'-trihydroxyls).

The accuracy of these chemical predictions suggest that enzymic condensations, if operative, follow the most likely electrophilic substitution paths.

EXPERIMENTAL

PMR spectra were recorded at 60 MHz in the solvents indicated with TMS as internal standard; optical rotations were in EtOH and IR spectra in $CHCl_3$. All mps are uncorr. 2D PC was run by ascent on Whatman No. 1 (28 × 46 cm) sheets in *sec*-BuOH satd with H_2O and in 2% HOAc; R_f values are indicated in this sequence. Preparative PC (PPC) was run by ascent in 2% HOAc or by descent in *sec*-BuOH satd with

* In the absence for obvious reasons (*ortho*-substitution) of the relative Hammett-Brown σ^+ values for specific sites on phloroglucinol, resorcinol and pyrogallol nuclei, the above notions are supported by the observation that in phenol-formaldehyde cold-set adhesive applications under "neutral" conditions (pH 7·3–7·8) and at ambient temperatures, resorcinol is the phenol of choice, phloroglucinol being too reactive and pyrogallol insufficiently so. The sequence of reactivity based on both nucleophilic substitution and on the stability of *p*-hydroxycarbonium ions formed under these conditions is as follows: phloroglucinol > resorcinol > pyrogallol ≫ catechol.

H_2O on Whatman No. 3 (46 × 57 cm). Bands cut from these chromatograms were eluted with 70% EtOH. TLC was on Si gel PF_{254} (0·25 mm) and PLC on the same adsorbent (1 mm). Plates were air-dried and unactivated, and sprayed with H_2SO_4–40% HCHO (40:1). Evaporations of all eluates from PC were under red pres at 60°.

CC was carried out on 2·5 × 90 cm columns using Si gel (120–230 mesh) and the flow rate was 10–12 ml per 30 min. Fractions were collected with an automatic fraction collector.

Methylations were in MeOH–Et_2O soln with an excess of CH_2N_2 at −15° for 48 hr. Hydrolysis of acetates were carried out by dissolving the compound in $CHCl_3$ (*ca* 16 mg/ml) and adding conc HCl dropwise (*ca* 0·5 ml/100 mg compound) while the mixture was refluxed at 75°. After refluxing for 45 min the mixture was neutralized with $NaHCO_3$, extracted with Et_2O, the solvent removed and the work-up continued.

Cross-sections of the trunk of the *Acacia giraffae* were cut from trees in the vicinity of Hoopstad, O.F.S., while the *A. galpinii* specimens were collected by the Government Forester in the vicinity of Louis Trichardt, Northern Transvaal.

Extraction and separation of A. giraffae. Drillings (1·22 kg) from the heartwood [after dewaxing with *n*-hexane (3 × 2·5 l.) at room temp] were extracted over 2 × 48 hr periods with Me_2CO (165 g). The extract (52 g) was partitioned using a Steady State Distribution Apparatus and H_2O–*sec*. BuOH–hexane (5:4:1). After 124 transfers the contents of every fifth tube were examined by 2D chromatography. The dimeric and higher MW flavonoids were distributed between tubes 7–25, and the monomeric phenolic compounds between tubes 77–103.

(a) *All*-trans-(+)-*leucofisetinidin*-(+)-*catechin-heptamethyl ether* (4,8-*linked*) *and its diacetate*. The material (10 g) in tubes 7–25 was separated by PPC using 2% HOAc and the band R_f 0·6 was eluted and then methylated. The product was purified by TLC in C_6H_6–Me_2CO (7:3). The band, R_f 0·41, gave a light yellow amorphous solid (117 mg), mp 105–108° (lit [16] 110°), M^+ 660 (70%). The PMR spectrum was identical with that in the literature [16]. Acetylation of the heptamethyl ether (50 mg) with $(Ac)_2O$–C_5H_5N gave an amorphous white solid (41 mg), mp 101–102° (lit [16] 103°); M^+ 744 (5%) and the PMR spectrum was the same as given in the lit [16].

(b) (+)-7,3′,4′,*Trimethoxy*-2,3-trans-*flavan*-3,4-trans-*diol*. The material (6 g) in tubes 77–103 was purified by PPC using 2% HOAc. The band R_f 0·65 was eluted and methylated. Purification of the product by TLC (R_f 0·37) and crystallization from C_6H_6–EtOH gave white needles (27 mg), mp 128° (lit [7] 129–130°); $[\alpha]_D^{24}$ −7·5° (*c* 0·6 in sym. $C_2H_2Cl_4$) {lit [7] $[\alpha]_D$ −9·5°}. The PMR spectra supported the suggested structure with $J_{2,3}$ 9·5 and $J_{3,4}$ 8·0 Hz.

(c) (+)-5,7,3′,4′-*Tetramethoxy*-2,3-trans-*flavan*-3-*ol*. From the same PC from which (b) was obtained, the band R_f 0·41 was eluted and methylated. The product was purified by TLC in C_6H_6–Me_2CO (8:2) and from the band R_f 0·39, white needles (32 mg) were obtained from Me_2CO–MeOH, mp 145° (lit [26] 143–144°); $[\alpha]_D^{25}$ −12·5° (c 1·1 in Me_2CO) {lit [26] $[\alpha]_D$ −13·4° in Me_2CO}. The PMR spectrum supported the suggested structure.

Numerous tannin fractions from the countercurrent separation were worked up by methods used for the biflavanoid leucofisetinidin under (a), but all exhibited rotational isomerism (or diastereoisomerism), thus precluding spectral analysis. All fractions gave resorcinol, phloroglucinol and protocatechuic acid on fusion with alkali under anhydrous conditions [11], and fisetinidin chloride with 3 N HCl–*iso*PrOH (1:4) under pressure at 97°.

Extraction and separation of A. galpinii. Heartwood material (2·05 kg drillings) [after dewaxing with *n*-hexane (3 × 3 l.) at room temp] was extracted over 2 × 48 hr with Me_2CO (142 g). By countercurrent separation 103 g of dry extract was dissolved and separated in a H_2O–*sec* BuOH–hexane (5:4:2). After evaluation of the distribution of components by 2D PC, the following combination of tubes were made: 6-13, 15-31, 49-74 and 80-94.

(a) (−)-7,8,3′,4′-*Tetramethoxy*-2,3-cis-*flavan*-3,4-cis-*diol* [(−)-cis-cis-*melacacidin*] *and* (−)-7,8,4′-*trimethoxy*-2,3-cis-*flavan*-3,4-cis-*diol* [(−)-cis-cis-*teracacidin*]. Material from tubes 6-13 was separated by descending PPC in H_2O satd *sec* BuOH solvent. Bands with R_f 0·38 and 0·57 were eluted and methylated separately. The product from the lower R_f band gave white needles (45 mg) from C_6H_6–MeOH. This compound was identified as the tetramethyl ether of (−)-*cis-cis*-melacacidin, mp 142° (lit [5] 144–145°); $[\alpha]_D^{23}$ −76·0 (c 0·61 in EtOH) {lit [5] $[\alpha]_D^{25}$ −83·5°, c 1·0 in EtOH}. The PMR spectrum was the same as in the lit [27]. From the higher R_f band (0·57) a product was obtained, giving fine white needles (910 mg) from Me_2CO–MeOH. This was identified as the methyl ether of (−)-*cis-cis*-teracacidin, mp 161° (lit [6] 159°); $[\alpha]_D^{23}$ −68·0° (c 0·65 in EtOH) {lit [27] $[\alpha]_D^{20}$ −71·0°, c 0·8 in EtOH}. The PMR spectrum corresponded to that in the lit [6, 27].

(b) (−)-7,8,4′-*Trimethoxy*-2,3,-cis-*flavan*-3,4-trans-*diol*, (+)-7,8,4′-*trimethoxy*-2,3-trans-*flavan*-3,4-trans-*diol* (*and its diacetate*) *and* (+)-7,8,4′-*trimethoxy*-2,3-trans-*flavan*-3,4-cis-*diol*. Material from tubes 15-31 was separated by PPC in the same way as described in (a). Two bands R_f 0·50 and 0·61 were obtained and both were cut in half, eluted and methylated. The lower half of band 0·5 gave a white amorphous solid (27 mg) which was identified as (−)-7,8,4′-trimethoxy-2,3-*cis*-flavan-3,4-*trans*-diol, mp 65–68° (lit [27] 68–78°); $[\alpha]_D^{25}$ −34·5° (c 0·4 in EtOH) {lit [27] $[\alpha]_D^{20}$ −40·3° c 0·7 in EtOH} and with a PMR spectrum the same as the lit [27]. The methylated product from the lower half of the R_f 0·5 band was purified by PLC in C_6H_6–EtOH (95:5). The band R_f 0·21 proved to be the (−)-*cis-trans*-diastereoisomer. The band R_f 0·26 was identified as (+)-7,8,4′-trimethoxy-2,3-*trans*-flavan-3,4-*trans*-diol, crystallizing as white needles (52 mg) from EtOH–H_2O, mp 95–96° (with H_2O of crystallization) and 154–155° after drying for 24 hr over P_2O_5 (lit [28] 157°); $[\alpha]_D^{24}$ − 10·8° (*c* 0·5 in EtOH). The diacetate (Ac_2O-C_5H_5N) crystallized from EtOH as white needles (41 mg), mp 142–143° (lit [28] 143°); $[\alpha]_D^{25}$ −19·6° (*c* 0·52 in EtOH); τ ($CDCl_3$) 2·60 (*d*, 2′ and 6′-H), 3·07 (*d*, 3′ and 5′-H), 3·37 (*d*, 6-H), 3·74 (*d*, 4-H), 4·47 (*ss*, 3-H), 4·82 (*d*, 2-H), 6·13, 6·20 (*s*, 3 × OMe), 8·02, 8·13 (*s*, 2 × OAc), $J_{2,3}$ 9·0, $J_{3,4}$ 6·3 Hz.

The upper part of the R_f 0·61 band from PPC was treated the same way as described above and the methylated product was identified as (+)-7,8,4′-trimethoxy-2,3-*trans*-flavan-3,4-*cis*-diol. It was a white amorphous solid (36 mg), mp 164–166° (lit [27] 168–170°); $[\alpha]_D^{25}$ +4·5° (*c* 0·47 in EtOH) {lit [27] $[\alpha]_D^{20}$ +7·0°, c 0·3 in EtOH}. The PMR spectrum corresponded to that in the lit [27].

(c) (±)-7,8,4′-*Trimethoxy*-2,3-trans-*dihydroflavonol* (*its monoacetate*) *and* (±)-7,8,4′-*trimethoxyflavanone*. The material from tubes 49-74 was separated by ascending PPC in 2% HOAc. Two bands R_f 0·19 and 0·35 were removed. From the band R_f 0·35, after elution and methylation, white needles (163 mg) were obtained from Me_2CO–MeOH. The compound was identified as (±)-7,8,4′-trimethoxy-2,3-*trans*-dihydroflavonol with mp 177°; MS M^+ 330 (26%), *m/e* 301 (11%), 181 (100), 121 (22); ν_{max} $CHCl_3$ 1686 cm^{-1} (CO-stretching); τ ($CDCl_3$) 2·29 (*d*, 5-H), 2·43 (*d*, 2′ and 6′-H), 3·00 (*d*, 3′ and 5′-H), 3·27 (*d*, 6-H), 4·90 (*d*, 3-H), 5·46 (*d*, 2-H), 6·04, 6·16 (*s*, 3 × OMe), $J_{2,3}$ 12 Hz. Acetylation produced (±)-3-*O*-acetyl-7,8,4′-trimethoxy-2,3-*trans*-dihydroflavonol as a white amorphous solid (44 mg), mp 45–47°; MS M^+ 372 (43%); τ ($CDCl_3$) 2·27 (*d*,

5-H), 2·51 (*d*, 2′ and 6′-H), 3·03 (*d*, 3′ and 5′-H), 3·27 (*d*, 6-H), 4·17 (*d*, 3-H), 4·61 (*d*, 2-H), 6·04, 6·14 (*s*, 3 × OMe), 7·97 (*s*, 3-OAc), $J_{2,3}$ 12 Hz.

The band R_f 0·19 from PPC was treated as before and yellow needles (32 mg) were obtained from Me_2CO–EtOH. The compound was identified as (±)-7,8,4′-trimethoxyflavanone mp 110–111°; MS M$^+$ 314 (43%); ν_{max} $CHCl_3$ 1683 cm^{-1} (CO-stretching); τ ($CDCl_3$) 2·17 (*d*, 2′ and 6′-H), 2·23 (*d*, 5-H), 3·01 (*d*, 3′ and 5′-H), 3·29 (*d*, 6-H), 4·47 (*q*, 2-H), 6·03, 6·10, 6·13 (*s*, 3 × OMe), 7·00 (*m*, CH_2), $J_{2,3}$ 8 Hz. The heterocyclic ABX couplings were typical of flavanones.

(d) *Derivatives of 7,8,4′-trihydroxyflavonol* (**2**. $R_1 = R_2 = R_3 = H$). The contents of tubes 80-94 were dried and acetylated and separated by column chromatography. Four related compounds were isolated and when examined on TLC their respective R_f values were 0·56, 0·50, 0·44 and 0·42 in C_6H_6–Me_2CO–EtOAc (7:1:2), *3,7,8,4′-Tetra-O-acetylflavone* (**2**. $R_1 = R_2 = R_3 = Ac$) (R_f 0·56) crystallized in light yellow needles from C_6H_6–Me_2CO–EtOH (510 mg), mp 170–173° (lit [29] 170–175°), *7,8,4′-Tri-O-acetyl-3-methoxyflavone* (**2**. $R_1 = R_2 = Ac$; $R_3 = Me$) (R_f 0·50) crystallized as colourless needles (111 mg) from EtOAc–EtOH, mp 165–166°; MS M$^+$ 426 (95%), *m/e* 384 (48), 342 (75), 341 (76), 300 (78), 299 (100), 152 (38), 121 (72); ν_{max} $CHCl_3$ 1650 (CO-stretching), 1775 cm^{-1} (acetyl); τ (DMSO-d_6) 1·87 (*d*, 5-H), 1·93 (*d*, 2′ and 6′-H), 2·50 (*d*, 6-H), 2·57 (*d*, 3′ and 5′-H), 6·10 (*s*, OMe), 7·50, 7·57, 7·63 (*s*, 3 × OAc); Found: C, 62·1; H, 4·4; Calc. for $C_{22}H_{18}O_9$: C, 61·9; H, 4·2%. *7,8,4′-Trihydroxy-3-methoxyflavone* (**2a**) was prepared from the tri-acetate by acid hydrolysis. Brown dendritic crystals (63 mg, 82% yield) were formed from MeOH–C_6H_6, mp 245–252° (decomp.); MS M$^+$ 300 (76%), *m/e* 299 (100), 272 (5), 271 (14), 257 (24), 153 (35), 152 (66), 121 (20); τ (DMSO-d_6) 1·87 (*d*, 2′ and 6′-H), 2·47 (*d*, 5-H), 2·97 (*d*, 6-H), 2·97 (*d*, 3′ and 5′-H), 3·97 (OH-resonance), 6·16 (*s*, OMe); λ_{max} MeOH 339, 315, 270, 216 (ε × 10^4 1·7, 1·9, 2·3, 2·6). λ_{max} (MeOH + NaOAc + H_3BO_3) 360, 310, 270 nm. *3-O-Acetyl-7,8,4′-trimethoxyflavone* (**2**. $R_1 = R_2 = Me$; $R_3 = Ac$) (R_f 0·42), crystallized as white needles (41 mg) from EtOAc–EtOH, mp 151–152°; MS M$^+$ 370 (4%), *m/e* 328 (100), 299 (4), 285 (3), 279 (6), 181 (10), 180 (12), 152 (8), 135 (10); ν_{max} $CHCl_3$ 1605 (CO-stretching), 1775 cm^{-1} (acetyl); τ (DMSO-d_6) 2·10 (*d*, 5-H), 2·30 (*d*, 2′ and 6′-H), 2·63 (*d*, 6-H), 2·76 (*d*, 3′ and 5′-H), 5·97, 6·00, 6·10 (*s*, 3 × OMe), 7·63 (*s*, 3-OAc). *3-Hydroxy-7,8,4′-trimethoxyflavone* (**2c**) was prepared from the acetate by acid hydrolysis and yellow needles (31 mg) were obtained from C_6H_6–EtOH, mp 199–200° (lit [30] 198°, synthetic); MS M$^+$ 328 (100%), *m/e* 329 (20), 327 (8), 313 (20), 299 (4·5), 285 (12), 181 (38), 165 (10), 152 (8), 151 (5), 148 (11), 137 (4·5), 135 (18); τ (DMSO-d_6) 1·76 (*d*, 2′ and 6′-H), 2·10 (*d*, 5-H), 2·70 (*d*, 6-H), 2·80 (*d*, 3′ and 5′-H), 6·00, 6·03, 6·13 (*s*, 3 × OMe); λ_{max} MeOH 358, 318, 265, 219 nm. (ε × 10^4 2·8, 1·5, 2·0, 2·8); Accurate mass: 328·100; Calc for $C_{18}H_{16}O_6$: 328·093. *3,4′-Di-O-acetyl-7,8-dimethoxyflavone*. (**2**. $R_1 = Me$; $R_2 = R_3 = Ac$) (R_f 0·44), white crystals (32 mg) were obtained after crystallization from EtOAc–EtOH, mp 170–171°; MS M$^+$ 398 (16%), *m/e* 356 (56), 314 (100), 299 (7), 285 (6), 279 (33), 236 (12), 181 (38), 180 (90), 152 (31), 121 (20); ν_{max} $CHCl_3$ 1625, 1645 (CO-stretching), 1775 cm^{-1} (acetyl); τ (DMSO-d_6) 1·96 (*d*, 2′ and 6′-H), 2·10 (*d*, 5-H), 2·35 (*d*, 3′ and 5′-H), 2·60 (*d*, 6-H), 5·96, 6·00 (*s*, 2 × OMe), 7·63 (*s*, 2 × OAc). *3,4′-Dihydroxy-7,8-dimethoxyflavone* (**2b**). Acid hydrolysis of the diacetate and subsequent crystallization from C_6H_6–MeOH gave yellow crystals (23 mg), mp 226–227°; MS M$^+$ 314 (100%), *m/e* 315 (22), 299 (4), 285 (4), 271 (8), 181 (2·5), 165 (8), 137 (6), 134 (4), 121 (16); τ (DMSO-d_6) 1·83 (*d*, 2′ and 6′-H), 2·10 (*d*, 5-H), 2·70 (*d*, 6-H), 2·96 (*d*, 3′ and 5′-H), 6·00 (*s*, 2 × OMe), 7·63 (*s*, 2 × OAc); λ_{max} MeOH 358, 287, 270 sh, 221 nm (ε × 10^4 0·9, 1·3, 1·1, 3·0); Found: C, 64·8; H, 4·6; Calc for $C_{17}H_{14}O_6$: C, 64·9; H, 4·5%. *3,7,8,4′-Tetramethoxyflavone* (**2**. $R_1 = R_2 = R_3 = Me$). Separation of the original crude extract by ascending PPC (2% HOAc) gave a band R_f 0·02. After elution and methylation, the product was purified by TLC using C_6H_6–EtOAc–Me_2CO (7:2:1). The band R_f 0·38 gave yellow needles (71 mg) from C_6H_6–Me_2CO, mp 145° (lit [31] 143–144°); MS M$^+$ 342 (78%); τ (DMSO-d_6) 1·90 (*d*, 2′ and 6′-H), 2·13 (*d*, 5-H), 2·69 (*d*, 6-H), 2·80 (*d*, 3′ and 5′-H), 6·00, 6·03, 6·13, 6·17 (*s*, 4 × OMe).

Synthesis of 3-hydroxy-7,8,4′-trimethoxyflavone (**2c**). Oxidation of 7,8,4′-trimethoxydihydroflavonol (**4b**) in strong alkaline soln gave the desired flavonol. The dihydroflavonol (50 mg) was heated in 3 ml 20% NaOH soln for 10 min at 100° with the passage of air. The soln was neutralized with 20% HCl to pH 3, extracted with Et_2O and the solvent removed under vacuum. The material was purified by TLC in C_6H_6–Me_2CO–EtOAc (7:1:1) and from the band R_f 0·38, yellow crystals (22 mg, 44%) were obtained from C_6H_6–EtOH, mp and mmp with the corresponding compound derived from *A. galpinii*, 198–199°. The PMR spectra of these compounds were identical.

Synthesis of 3,4′-dihydroxy-7,8-dimethoxyflavone (**2b**). This was carried in 4 stages. i) *4-(Methoxymethoxy)benzaldehyde* [32]. To a stirred suspension of finely-divided Na (0·6 g) in toluene (20 ml), a soln of 4-hydroxybenzaldehyde (3 g) in EtOH (4 ml) was added. The mixture was refluxed with stirring to complete the salt-formation. After removal of EtOH, the suspension of the Na salt in toluene was treated with monochlorodimethyl ether (20 ml) and the mixture cooled in ice. Et_2O (50 ml) was added to the mixture and the soln washed with 2% NaOH, dried and evaporated to yield 500 mg (17%) of a colourless liquid, bp 149–150° (20 mm Hg) (lit [32] bp 152–153°, 21 mm Hg); τ (acetone-d_6) −0·07 (*s*, −CHO), 2·05 (*d*, 2 and 6-H), 2·73 (*d*, 3 and 5-H), 4·66 (*s*, CH_2), 6·20 (*s*, OMe). ii) *2-Hydroxy-3,4-dimethoxyacetophenone*. 2,3,4-Trihydroxyacetophenone (500 mg) in dry Et_2O was treated with CH_2N_2 in Et_2O. After 12 hr the solvent was removed under vacuum and white crystals (406 mg) of 2-hydroxy-3,4-dimethoxyacetophenone were obtained from Me_2CO–EtOH, mp 82° (lit [33] 83°); τ ($CDCl_3$) −2·70 (*s*, 2-OH), 2·43 (*d*, 5-H), 3·46 (*d*, 6-H), 6·03, 6·07 (*s*, 2 × OMe), 7·40 (*s*, Me). iii) *2′-Hydroxy-4-(methoxymethoxy)-3′,4′-dimethoxychalcone* (**3**). To a soln of 4-(methoxymethoxy)benzaldehyde (420 mg) and 2-hydroxy-3,4-dimethoxyacetophenone (400 mg) in EtOH (20 ml), was slowly added a 40% KOH soln (5 ml). The mixture was stirred for 24 hr at 23° and poured into ice/H_2O. The ppt was collected and purified by TLC using C_6H_6–EtOAc–Me_2CO (20:2:1). The band R_f 0·43 gave a yellow amorphous solid (182 mg). From the PMR spectrum it was shown to be a mixture of the *cis*- and *trans*-chalcones. MS M$^+$ 344; ν_{max} $CHCl_3$ 1645 cm^{-1} (CO-stretching); τ (acetone-d_6) −3·45 (*s*, 2′-OH), 1·93 (*d*, 5-H), 2·10 (*s*, α-H), 2·10 (*s*, β-H), 2·13 (*d*, 2 and 6-H), 2·83 (*d*, 3 and 5-H), 3·27 (*d*, 6-H), 4·70 (*s*, CH_2), 6·16, 6·03 (*s*, 2 × OMe), 6·53 (*s*, OMe). iv) *3,4′-Dihydroxy-7,8-dimethoxyflavone* (**2b**). To soln of 2′-hydroxy-4-(methoxymethoxy)-3′,4′-dimethoxychalcone (100 mg) in MeOH (3 ml) was added 30% NaOH soln followed by dropwise addition of 40% H_2O_2 [34] (2 ml). The mixture was heated for 20 min at 60° acidified with 12 M HCl (4 ml) and heated for a further 5 min at 95°. The reaction mixture was poured into cold H_2O and extracted with Et_2O. After removal of the solvent yellow crystals (22 mg) were obtained from C_6H_6–MeOH, mp and mmp with the corresponding compound isolated from *A. galpinii*, 225–227°. The PMR spectra of these compounds was identical.

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