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Tyrosinase catalysed biphenyl construction from flavan-3-ol substrates

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Abstract

Mushroom tyrosinase catalysed oxidation of three flavan-3-ols, viz. catechin, fisetinidol and mesquitol, was conducted to construct biphenyl bonds. Exposure of the flavan-3-ols to tyrosinase and subsequent trapping of the *o*-quinone intermediates resulted in the formation of novel flavan-3-ol derivatives, the structures of which were elucidated by mono- and two-dimensional ^{1}H-NMR experiments. Application of the methodology resulted in the improved synthesis of the natural flavan-3-ol dimer, mesquitol-[5 → 8]-catechin, previously isolated from *Prosopis glandulosa*.

Keywords: Oxidative coupling; Polyphenol oxidase; Tyrosinase; Biphenyl; Flavan-3-ol dimer

1. Introduction

The browning of injured tissues of plant organs is a major cause of quality degradation in fruits and fruit-derived foods and beverages, e.g. in the wine industry during early stages of processing (Mathew & Parpia, 1971; Rouet-Mayer, Ralambosa & Philippon, 1990). The fundamental step in browning is the polyphenol oxidase (PPO, EC 1.14.18.1), also referred to as tyrosinase or phenol oxidase, catalysed transformation of *o*-diphenols to the corresponding richly coloured *o*-quinones via the interaction of molecular oxygen (Burton, 1994). The reactive *o*-quinones undergo a complex series of non-enzymatic chemical changes involved in several biosynthetic pathways including lignin, tannin and melanin formation (Brown, 1967).

The oxidation of catechin (**1**), present in numerous fruits, was reported by using PPO (Ahn & Gstirner, 1970; Goodenough, Kessell, Lea & Loeffler, 1983; Oszmianski & Lee, 1990; Rouet-Mayer et al., 1990; Guyot, Cheynier, Souquet & Moutounet, 1995) and also by chemical means (Young, Young, Roux, Brandt & Ferreira, 1987; Oszmianski, Cheynier & Moutounet, 1996). In many studies, complex mixtures of yellow pigments and colourless C–C and C–O–C linked dimers were obtained. Several derivatives obtained from the chemical oxidation of catechin (Young et al., 1987) were structurally elucidated, but the complete identification of condensation products from PPO interaction is limited only to the elegant paper by Guyot, Vercauteren and Cheynier (1996).

Hence, a study was launched to construct the biphenyl moiety in three flavan-3-ol substrates, viz. catechin (**1**), fisetinidol (**2**) and mesquitol (**3**), utilising mushroom tyrosinase (Sigma) as PPO source at pH 7. The novel flavan-3-ol derivatives were characterised by spectrometric techniques (NMR and MS) and an improved synthesis of the two atropisomers of mesquitol-(5 → 8)-catechin, isolated from *Prosopis glandulosa*, was accomplished.

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Table 1
^{1}H-NMR (300 MHz, 20°C) data of compounds **4**, **6**, **11**, **14**, **16**, **19** and **24**

Ring	H	**4** [$(CD_3)_2CO$]	**6** ($CDCl_3$)	**11** ($CDCl_3$)	**14** ($CDCl_3$)	**16** [$(CD_3)_2CO$]	**19** [$(CD_3)_2CO$]	**24** ($CDCl_3$)
A	5	–	–	6.92 (*d*, 8.8)	–	–	–	–
	6	5.84 (*d*, 2.5)[a]	6.18 (*d*, 2.2)[a]	6.52 (*dd*, 8.8, 2.5)	6.44 (*s*)	6.43 (*s*)	6.33 (*s*)	6.72 (*s*)
	8	6.00 (*d*, 2.5)[a]	6.08 (*d*, 2.2)[a]	6.48 (*d*, 8.8)	–	–	–	–
B	2′	7.00 (*br.s*)	6.94 (*br.s*)	6.92 (*br.s*)	6.92 (*d*, 2.0)	6.87–6.64 (*m*)	6.96–6.70 (*m*)	6.98 (*d*, 2.0)
	5′	6.63 (*s*)	6.64 (*s*)	6.64 (*s*)	6.84 (*d*, 8.2)	6.87–6.64 (*m*)	6.96–6.70 (*m*)	6.82 (*d*, 9.0)
	6′	–	–	–	6.95 (*dd*, 8.2, 2.0)	6.87–6.64 (*m*)	6.96–6.70(m)	6.97 (*dd*, 9.0, 2.0)
C	2	4.59 (*d*, 8.2)	4.85 (*d*, 6.3)	4.92 (*d*, 6.0)	5.22 (*d*, 5.6)	4.64 (*d*, 6.9)[a]	4.59 (*d*, 8.0)[a]	5.42 (*dd*, 5.1, 0.8)
	3	4.09–4.00 (*m*)	5.35–5.25 (*m*)	5.29–5.23 (*m*)	5.30–5.23 (*m*)	4.12–3.80 (*m*)	4.11–3.96 (*m*)	5.50 (*ddd*, 5.1, 5.1, 5.1)
	4_{eq}	2.95 (*dd*, 15.9, 5.5)	2.83 (*dd*, 16.7, 5.2)	2.95 (*dd*, 11.5, 4.8)	2.63 (*dd*, 16.5, 4.7)	2.85 (*dd*, 16.2, 5.2)[b]	2.97 (*dd*, 16.2, 5.1)[b]	2.89 (*ddd*, 17.0, 5.1, 0.8)
	4_{ax}	2.41 (*dd*, 15.9, 9.0)	2.56 (*dd*, 16.7, 6.4)	2.68 (*dd*, 11.5, 6.3)	2.45 (*dd*, 16.5, 5.9)	2.53 (*dd*, 16.2, 9.0)[c]	2.62 (*dd*, 16.2, 8.0)[c]	2.89 (*ddd*, 17.0, 5.1)
D	3″	6.01 (*dd*, 2.5)[a]	6.25 (*d*, 2.2)[a]	6.25 (*d*, 2.2)[a]	6.23 (*d*, 2.2)[a]	–	–	–
	5″	6.02 (*dd*, 2.5)[a]	6.20 (*d*, 2.2)[a]	6.21 (*d*, 2.2)[a]	6.18 (*d*, 2.2)[a]	–	–	–
	6	–	–	–	–	6.17 (*s*)	6.15 (*s*)	–
	8	–	–	–	–	–	–	7.12 (*s*)
	11	–	–	–	–	–	–	7.50 (*s*)
E	2	–	–	–	–	6.87–6.64 (*m*)	6.96–6.70 (*m*)	–
	5	–	–	–	–	6.87–6.64 (*m*)	6.96–6.70 (*m*)	–
	6	–	–	–	–	6.87–6.64 (*m*)	6.96–6.70 (m)	–
F	2	–	–	–	–	4.53 (*d*, 7.7)[a]	4.58 (*d*, 7.3)[a]	–
	3	–	–	–	–	4.12–3.80 (*m*)	4.11–3.96 (*m*)	–
	4_{eq}	–	–	–	–	2.77 (*dd*, 16.1, 5.3)[b]	2.75 (*dd*, 16.3, 6.0)[b]	–
	4_{ax}	–	–	–	–	2.64 (*dd*, 16.1, 7.2)[c]	2.84 (*dd*, 16.3, 8.6)[c]	–
	OMe		3.88, 3.84, 3.77, 3.75, 3.73 (5 × *s*), 3.76 (2 × *s*)	3.88, 3.84, 3.78, 3.76 (4 × *s*), 3.74 (2 × *s*)	3.95, 3.89, 3.87, 3.85, 3.84, 3.76, 3.62 (7 × *s*)			3.98 (MeO-9), 3.92 (MeO-10), 3.88 (MeO-5), 3.87 (MeO-4′), 3.79 (MeO-3′) (5 × *s*)
	OAc		1.86 (*s*)	1.86 (*s*)	1.95 (*s*)			2.05 (*s*)

[a,b,c] Allocations interchangeable.

2. Results and discussion

The selected flavan-3-ols (**1**, **2** and **3**) were separately exposed to catalytic quantities of mushroom tyrosinase and excess phloroglucinol in an aqueous phosphate buffer (0.05 M, pH 7, 30°C). The reactions were followed by TLC and work–up started after the first detection of polymers to ensure the recovery of any possible Michael type addition products.

Interaction of catechin and phloroglucinol with tyrosinase, afforded (2*R*,3*S*)-2,3-*trans*-6′-(2″,4″,6″-trihydroxyphenyl)-3′,4′,5,7-tetrahydroxyflavan-3-ol[1] (**4**) as the only product in 46% yield after 24h together with unreacted substrates. The mechanism for the formation of **4** presumably involves the tyrosinase catalysed oxidation of the B-ring of catechin to yield the corresponding reactive *o*-quinone intermediate (**7**). 1,4-Michael addition of nucleophilic phloroglucinol at C-6′ of the electrophilic *o*-quinone moiety yields **4** as the final product. This mechanistic rationale is corroborated by the work of Guyot et al. (1996) in which a two-electron process is favoured over a one-electron process at relative high pH values. Enhanced catechin nucleophilicity, due to a higher proportion of phenolate ions, is evident from a relative high pH of the reaction medium.

^{1}H-NMR data (Table 1) of the free phenolic, **4**, showed an ABMX-spin pattern reminiscent of the protons of the heterocyclic C-ring of catechin (Young Cronjé, Botes, Ferreira & Roux, 1985). From the remaining spin systems two AX-spin patterns for aromatic protons, one reminiscent for the A-ring of a catechin unit and the other corresponding to a phloroglucinol unit functionalised at an aromatic carbon, were evident. Two aromatic singlets at δ 7.00 and δ 6.63, for H-2′ and H-5′, respectively, confirmed the catechin B-ring functionalisation at C-6′. The *m*-doublet resonances for H-3″ and H-5″, despite their chemical equivalence, propounded the barrier to rotation of the phloroglucinol D-ring of **4**. The ^{1}H-NMR data of the heptamethyl ether acetate, **6**, (Table 1) confirmed the structure. The EIMS data of **6** indicated a molecular ion at *m*/*z* 554 (24%) in agreement with a molecular formula of $C_{30}H_{34}O_{10}$. A RDA fragment of *m*/*z* 388 (21%), representing the BD-moiety, established the B-ring substitution.

Fisetinidol (**2**) obtained from *Colophospermum mopane* (Drewes & Roux, 1966) was exposed to catalytic quantities of tyrosinase and an excess of phloroglucinol. It afforded the novel B-ring functionalised fisetinidol derivative, (2*R*,3*S*)-2,3-*trans*-6′-(2″,4″,6″-trihydroxyphenyl)-3′,4′,7-trihydroxyflavan-3-ol (**9**) and

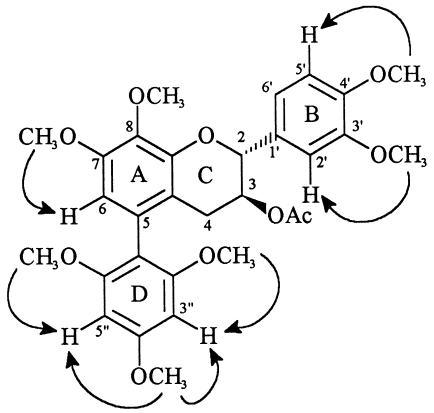


Fig. 1. NOESY associations of **14**.

was characterised after methylation and acetylation of the free phenolic product in 14% yield. A similar 1,4-Michael addition mechanism, via intermediate **8**, as proposed for catechin accounted for the product formation. ^{1}H-NMR data of the hexamethyl ether acetate **11** (Table 1) showed the ABMX-spin pattern reminiscent of the heterocyclic protons of the C-ring of fisetinidol (Drewes & Roux, 1966). The ABX-spin pattern for the aromatic protons of the fisetinidol A-ring established the unfunctionalised nature of the ring. From the remaining aromatic resonances two one-proton singlets at δ 6.92 (H-2′) and δ 6.64 (H-5′), indicating a C-6′ functionalised B-ring, and an AX-spin pattern of the phloroglucinol D-ring at δ 6.25 and δ 6.21 for H-3″ and H-5″, established the structure. The limited rotational ability of the D-ring is augmented by the AX-spin pattern. The EIMS data of **11** showed a molecular ion at *m*/*z* 524 (26%) in agreement with a molecular formula of $C_{29}H_{32}O_9$, and a RDA fragment of *m*/*z* 388 (22%) representing the BD-moiety and confirming the B-ring substitution.

The existence of rival catechol and pyrogallol moieties in mesquitol (**3**), which is a predominant metabolite in the heartwood of *P. glandulosa* (Young, Brandt, Young, Ferreira & Roux, 1986) afforded an ideal opportunity to compare a possible preference for biocatalytic oxidation of either the A-ring or the B-ring. The interaction between mesquitol and an excess of phloroglucinol, in the presence of catalytic quantities of tyrosinase, resulted in regioselective functionalisation at the mesquitol A-ring to yield the novel product, (2*R*,3*S*)-2,3-*trans*-5-(2″,4″,6″-trihydroxyphenyl-3′,4′,7,8-tetrahydroxyflavan-3-ol (**12**), in 11% yield. The structure was elucidated after methylation, acetylation and separation of the free phenolic product mixture. The preference for A-ring coupling via a 1,4-Michael addition by phloroglucinol, may be attributed to a regioselective oxidation of the A-ring by tyrosinase, because of a higher susceptibility of the pyrogal-

[1] In order to retain the 3,4-dihydroxylation numbering for the B-ring, the introduced substituent is placed at C-6′.

lol ring towards oxidation, to give intermediate **15**. ^{1}H-NMR data of the heptamethyl ether acetate (**14**) (Table 1) showed an ABMX-spin pattern in agreement with the intact heterocyclic protons of the C-ring of mesquitol (Young et al., 1986). The ABX-spin pattern of the aromatic protons (Table 1) of the B-ring was typical of the mesquitol unit. The one-proton aromatic singlet at δ 6.44 (H-6) and an AX-spin pattern at δ 6.23 and δ 6.18 for H-3″ and H-5″, represent the respective A- and D-ring substitution for compound **14**. Incorporation of ^{1}H-NMR NOESY difference spectroscopy data (Fig. 1) showed association between the methoxy protons of MeO-7 and H-6, confirming the coupling position of the phloroglucinol unit at C-5. The EIMS data of **14** indicated a molecular ion at *m*/*z* 554 (100%) suggesting a molecular formula of $C_{30}H_{34}O_{10}$. Two RDA fragments of *m*/*z* 332 (52%) and *m*/*z* 222 (7%), for the respective AD- and B-moieties, confirmed the A-ring substitution.

The natural occurrence of bi- and terphenyl type flavan-3-ols in the heartwood of *P. glandulosa* (Jacobs, Ferreira & Roux, 1983; Brandt, Young, Young & Ferreira, 1987), presumably originates from the oxidative dimerisation of the predominant metabolite, mesquitol (**3**) (Young et al., 1987). Our promising preliminary results and the reported condensation of mesquitol (**3**) and catechin (**1**) via chemical means (Young et al., 1986), prompted us to investigate the PPO catalysed construction of biphenyl type flavan-3-ols. The interaction of catechin (**1**) and mesquitol (**3**) with tyrosinase resulted in the construction of the atropisomers (*R*)- and (*S*)-mesquitol-[5 → 8]-catechin [**16** (13%) and **19** (6%), respectively] as the only identified products. ^{1}H-NMR, MS and circular dichroism data of the octamethyl ether diacetates (**18** and **21**) was in accordance with the authentic data (Young et al., 1986). The isolation of **16** and **19** as only products differ from the chemical synthesis of Young et al. (1986) during which the construction of [5,6]-bi-mesquitol and mesquitol-[5 → 6]-catechin was reported in addition to **16** and **19**. Our relative specific reaction enabled the compilation of ^{1}H-NMR data of the free phenolic atropisomers **16** and **19** for the first time (Table 1). This regioselective tyrosinase catalysed construction is believed to be the present synthesis of choice compared to the only other reported synthesis of Young et al. (1986) in which lower yields and a mixture of products were obtained.

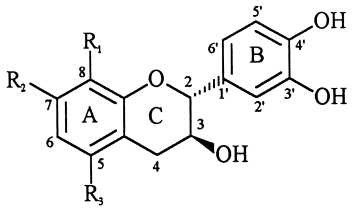
As an extension of this protocol, the tyrosinase catalysed interaction between catechin and fisetinidol was investigated. Most of the starting material was recovered and only a small quantity of a free phenolic product mixture was obtained which after methylation, acetylation and purification yielded the novel flavan-3-ol derivative, (2*R*,3*S*)-3-acetoxy-2-(3′,4′-dimethoxyphenyl)-5,9,10-trimethoxy-2,3-*trans*-3,4-dihydro-2*H*-benzofurano[2,3-*h*]chromene (**24**). ^{1}H-NMR analysis of **24** showed an ABX-spin pattern in the aromatic proton region (Table 1) which confirmed the unfunctionalised nature of the B-ring. ^{1}H-NMR NOESY difference spectroscopy data of **24** (Fig. 2) showed associations of H-2 (δ 5.42, *dd*, *J* 5.1 and 0.8, C-ring) with the two protons of the B-ring, viz. H-2′ (δ 6.98, *d*, *J* 2.0) and H-6′ (δ 6.97, *dd*, *J* 9.0 and 2.0). Due to the association between H-2 and the singlet at δ 7.50 its position was assigned as H-11 on the D-ring (Dreiding model). A sequence of associations between H-11 and MeO-10 (δ 3.92), between MeO-10 and MeO-9 (δ 3.98) and from the latter to H-8 (δ 7.12) established the positions of the two D-ring protons as well as the carbon–carbon bond and ether linkage coupling positions. The remaining singlet at δ 6.72 showed an association with MeO-5 (δ 3.88) of the A-ring. Couplings between H-2 (δ 5.42, *dd*, *J* 5.1 and 0.8) and H-3 (δ 5.50, *ddd*, *J* 5.1, 5.1 and 5.1) and between H-3 and both H-4$_{eq}$ (δ 2.89, *ddd*, *J* 17.0, 5.1 and 0.8) and H-4$_{ax}$ (δ 2.93, *dd*, *J* 17.0 and 5.1) were different from the 'normal' catechin couplings of H-2 (7.0 Hz), H-4$_{eq}$ (6.0 Hz) and H-4$_{ax}$ (8.0 Hz) to H-3 (Young et al., 1985). These *J*-value deviations suggested an abnormal C-ring conformation. From the two possible sofa conformations of the C-ring of **24** (Scheme 1), one for the B-ring in an axial orientation (*A*-conformer) and the other for the B-ring in an equatorial orientation (*E*-conformer), the observed couplings between the C-ring protons are only possible for the *A*-conformer due to their respective small dihedral angles ($\theta_{2,3} = \theta_{3,4eq} = \theta_{3,4ax} = 60^\circ$). ^{1}H- NMR data of **24** (Table 1) showed a coupling constant of 5.1 Hz between H-2 and H-3, between H-3 and H-4$_{eq}$ and between H-3 and H-4$_{ax}$, typical for protons with dihedral angles of 60°, as well as W-coupling between H-2 and H-4$_{eq}$ ($^4J_{2,4eq} = 0.8$ Hz), only possible for the *A*-conformer (Dreiding modelling). Observed NOESY associations between H-2 and H-3 and from H-3 to H-4 confirmed the *A*-conformation. The EIMS analysis of **24** indicated a molecular ion at *m*/*z* 508 (35%) in agreement with a molecular formula of $C_{28}H_{28}O_9$, and the AED-moiety was confirmed with a RDA fragment of *m*/*z* 286 (100%).

The postulated mechanism for the formation of **22** could be explained in terms of electrophilic substitution at C-8 of catechin on the B-ring of either another catechin or fisetinidol unit oxidised to a quinone. A subsequent Michael-type addition yielded the intermediate **25** after aromatisation (Scheme 2). Again a two-electron mechanism is probably favoured at the relative high pH of the reaction medium (Guyot et al., 1996). A second oxidative interaction by tyrosinase (**26**) followed by spontaneous intramolecular oxygen cyclisation, loss of a benzopyranyl unit and aromatisation of **27** yielded chromene **22**. This mechanism is

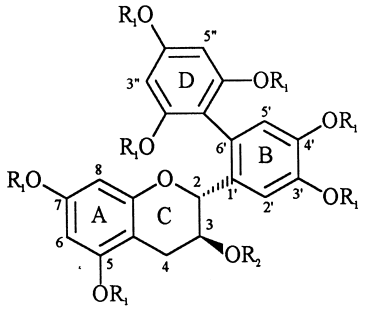
strongly supported by Guyot et al. (1996) who characterised a product similar to intermediate **27**, which was isolated as a product from the catechin condensation catalysed by grape PPO. Although we could not substantiate the nature of the benzopyranyl unit lost during the mechanistic route, we are confident that the mechanism satisfactorily accounts for formation of **22**.

Efforts towards the construction and characterisation of self-condensation products by separate exposure of catechin (**1**), fisetinidol (**2**) and mesquitol (**3**) to catalytic quantities of tyrosinase was unsuccessful. The formation of distinct yellow colours in the reaction mixture and polymerisation products were the only results evident from TLC analysis.

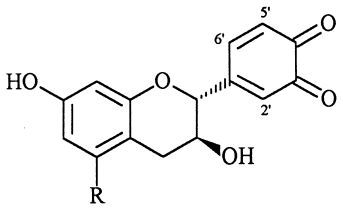
In conclusion, our results have shown that successful biphenyl constructions from flavan-3-ol substrates and phloroglucinol may be accomplished by application of commercially available mushroom tyrosinase (Sigma). To our knowledge we also for the first time demonstrated the PPO catalysed condensation of two *different* flavan-3-ol substrates.



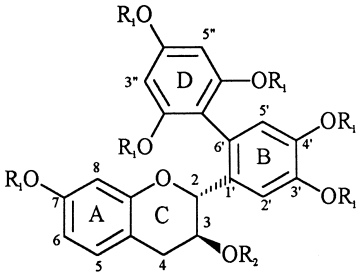
1: $R_1 = H$; $R_2 = R_3 = OH$
2: $R_1 = R_3 = H$; $R_2 = OH$
3: $R_1 = R_2 = OH$; $R_3 = H$



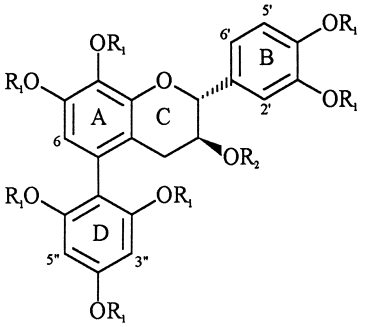
4: $R_1 = R_2 = H$
5: $R_1 = Me$; $R_2 = H$
6: $R_1 = Me$; $R_2 = Ac$



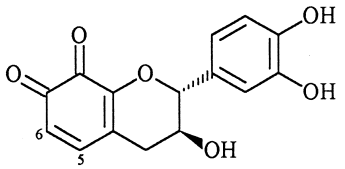
7: R = OH
8: R = H



9: $R_1 = R_2 = H$
10: $R_1 = Me$; $R_2 = H$
11: $R_1 = Me$; $R_2 = Ac$



12: $R_1 = R_2 = H$
13: $R_1 = Me$; $R_2 = H$
14: $R_1 = Me$; $R_2 = Ac$



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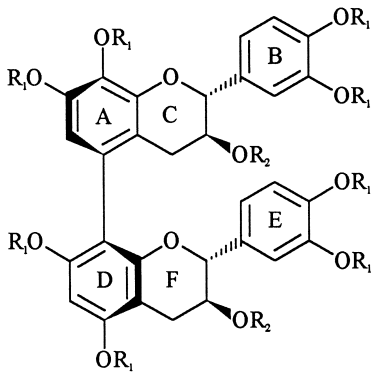
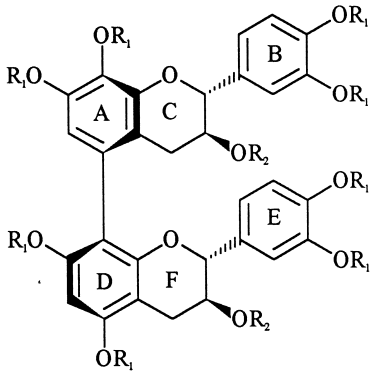
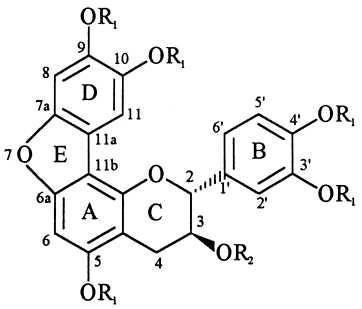


16: $R_1 = R_2 = H$
17: $R_1 = Me$; $R_2 = H$
18: $R_1 = Me$; $R_2 = Ac$



19: $R_1 = R_2 = H$
20: $R_1 = Me$; $R_2 = H$
21: $R_1 = Me$; $R_2 = Ac$



22: $R_1 = R_2 = H$
23: $R_1 = Me$; $R_2 = H$
24: $R_1 = Me$; $R_2 = Ac$

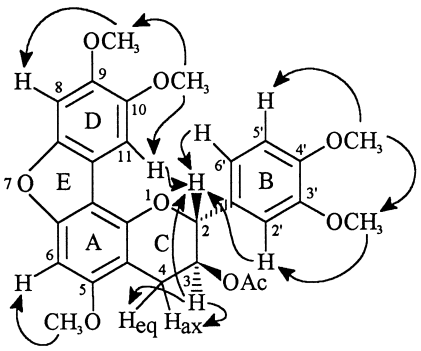
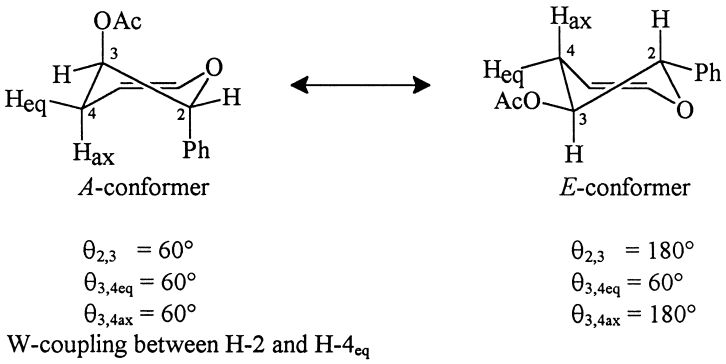


Fig. 2. NOESEY associations of **24**.

3. Experimental

^{1}H-NMR spectra were recorded at 300 MHz for solutions in $CDCl_3$ or $(CD_3)_2CO$, with TMS as int. standard. EIMS were recorded on a VG 70–70 instrument at 70 eV. TLC was performed on precoated Merck plastic sheets (silica gel 60 PF_{254} 0.25 mm) and the plates were sprayed with H_2SO_4–HCHO (40:1, v/v) after development. Prep. TLC plates, Kieselgel PF_{254} (1.0 mm) were air dried and used without prior activation. Compounds were recovered from the absorbent with Me_2CO. CC was on Sephadex LH-20 in EtOH. Methylations were performed with an excess of CH_2N_2 in MeOH–Et_2O over a period of 48 h at −15°C, while acetylations were in Ac_2O–pyridine at ambient temperatures. Evaporations were done under reduced pressure at ambient temperatures in a rotary evaporator.



Scheme 1.

3.1. Materials

Catechin, phloroglucinol and mushroom tyrosinase were purchased from Sigma. Fisetinidol from *C. mopane* (Drewes & Roux, 1966) and mesquitol from *P. glandulosa* (Jacobs et al., 1983) were used from our private collection of flavan-3-ols.

3.2. Oxidation procedure

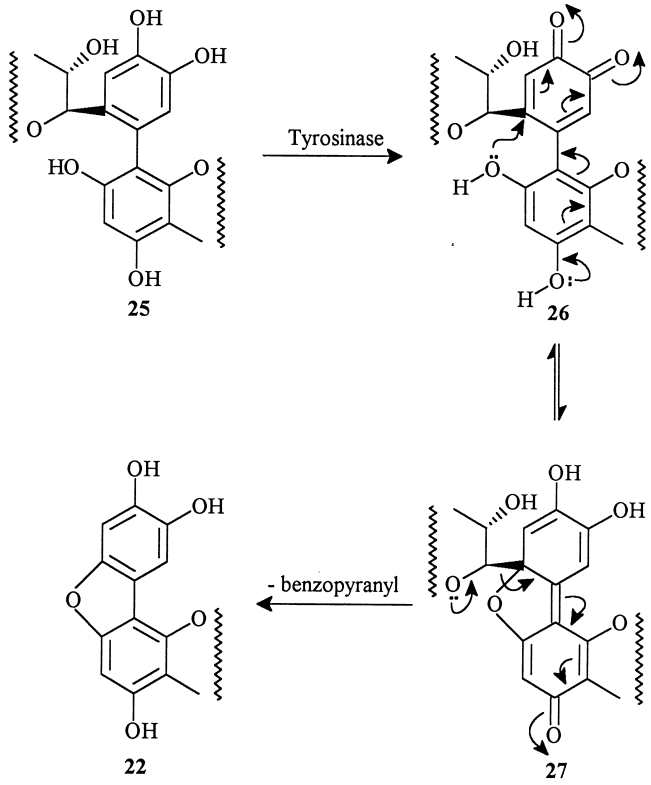
Flavan-3-ol (100 mg) and phloroglucinol (1 g) were dissolved in 0.05 M phosphate buffer (30 ml, pH 7) and after temperature stabilisation (30°C) in a water bath, tyrosinase (10 mg), dissolved in phosphate buffer (2 ml), was added and the reaction mixture gently shaken. After the first detection of polymeric material by TLC, an EtOAc extraction (3 × 30 ml) was performed, the organic layers combined, washed with brine (3 × 30 ml), dried ($NaSO_4$) and EtOAc removed under vacuum. Sephadex LH-20 afforded the separation of free phenolic product(s) from the starting material.

3.3. (2R,3S)-2,3-trans-6′-(2″,4″,6″-Trihydroxyphenyl)-3′,4′,5,7-tetrahydroxyflavan-3-ol **4**

Application of the oxidation procedure on catechin afforded unreacted catechin (33.9 mg), unreacted phloroglucinol (632.7 mg) and compound **4** (60.8 mg, 46%) as a brown amorphous solid (Ahn & Gstirner, 1970). R_f 0.31 C_6H_6–Me_2CO–MeOH (5:4:1); ^{1}H-NMR (Table 1); methylation and acetylation of **4** (60.8 mg) afforded the heptamethyl ether acetate (**6**) as a *light yellow amorphous solid* (43.2 mg, 52%). R_f 0.82 C_6H_6–Me_2CO–MeOH (18:1:1); ^{1}H-NMR (Table 1); EIMS *m/z* (rel. int.): 554 $[M]^+$ (24), 512 $[M\text{-}CH_2CO]^+$ (6), 494 $[M\text{-}HOAc]^+$ (64), 463 $[M\text{-}HOAc\text{-}OMe]^+$ (100), 388 (21), 346 (43), 328 (12), 167 (54) (found M^+, 554.2152, $C_{30}H_{34}O_{10}$ requires M^+, 554.2152).

3.4. (2R,3S)-2,3-trans-6′-(2″,4″,6″-Trihydroxyphenyl)-3′,4′,7-trihydroxyflavan-3-ol **9**

Application of the oxidation procedure on fisetinidol afforded unreacted fisetinidol (42.1 mg), unreacted phloroglucinol (712.3 mg) and an inseparable fraction of products (36.7 mg). Methylation, acetylation and TLC separation afforded the hexamethyl ether acetate (**11**) as a *light yellow amorphous solid* (29.6 mg, 14%). R_f 0.83 C_6H_6–Me_2CO–MeOH (38:1:1); ^{1}H-NMR (Table 1); EIMS *m/z* (rel. int.) 524 $[M]^+$ (26), 482 $[M\text{-}CH_2CO]^+$ (10), 464 $[M\text{-}HOAc]^+$ (23), 433 $[M\text{-}HOAc\text{-}OMe]^+$ (100), 388 (22), 346 (29), 328 (23) (found M^+, 524.2044. $C_{29}H_{32}O_9$ requires M^+, 524.2046).



Scheme 2.

3.5. (2R,3S)-2,3-trans-5′-(2″,4″,6″-Trihydroxyphenyl)-3′,4′,7,8-tetrahydroxyflavan-3-ol **12**

Application of the oxidation procedure on mesquitol afforded unreacted mesquitol (40.2 mg), unreacted phloroglucinol (640.7 mg) and an inseparable mixture of products (31.1 mg). Methylation and acetylation of the mixture followed by TLC separation afforded the heptamethyl ether acetate (**14**) as a light yellow amorphous solid (20.4 mg, 11%). R_f 0.81 C_6H_6–Me_2CO–MeOH (18:1:1); ^{1}H-NMR (Table 1); EIMS *m/z* (rel. int.) 554 $[M]^+$ (100), 512 $[M\text{-}CH_2CO]^+$ (11), 494 $[M\text{-}HOAc]^+$ (88), 479 (21), 463 $[M\text{-}HOAc\text{-}OMe]^+$ (95), 333 (90), 332 (52), 317 (21), 302 (60), 222 (7), 180 (45), 165 (21), 151 (32) (found M^+, 554.2152. $C_{30}H_{34}O_{10}$ requires 554.2152).

3.6. (R)- and (S)-Mesquitol-[5 → 8]-catechin **16** and **19**

Application of the oxidation procedure with mesquitol (100 mg) and catechin (150 mg) as nucleophile afforded unreacted catechin (43.6 mg) and the dimers **16** (26.2 mg, 13%) and **19** (11.5 mg, 6%) both as brown amorphous solids (Young et al., 1986). For ^{1}H-NMR data of **16** and **19** see Table 1.

3.7. (2R,3S)-2-(3′,4′-Dihydroxyphenyl)-3,5,9,10-tetrahydroxy-2,3-trans-3,4-dihydro-2H-benzofurano[2,3-h]chromene **22**

Application of the oxidative procedure to fisetinidol (100 mg) and catechin (150 mg) as nucleophile afforded unreacted fisetinidol (92.1 mg), unreacted catechin (101.7 mg) and an inseparable mixture of products (34.9 mg). Methylation, acetylation and subsequent TLC separation of the mixture afforded the pentamethyl ether acetate (**24**) as *a brown amorphous solid* (4.4 mg, 2%). R_f 0.50 C_6H_6–Me_2CO–MeOH (16:3:1); ^{1}H-NMR (Table 1); EIMS *m/z* (rel. int.) 508 $[M]^+$ (35), 466 $[M\text{-}CH_2CO]^+$ (11), 448 $[M\text{-}HOAc]^+$ (10), 286 (100), 271 (36), 222 (6), 180 (19) (found M^+, 508.1734. $C_{28}H_{28}O_9$ requires 508.1733).

Acknowledgements

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