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Oligomeric flavanoids. Part 27*. Interflavanyl bond formation in procyanidins under neutral conditions

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Abstract: Dimethyl(methylthio)sulfonium tetrafluoroborate (DMTSF) and silver tetrafluoroborate ($AgBF_4$) activate the C_4-S bond in the 4-thioethers of flavan-3-ols toward carbon nucleophiles to permit formation of the interflavanyl bond in procyanidins under neutral conditions.
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The structure elucidation of natural procyanidin oligomers is hampered by the absence of methodology to facilitate the progressive construction of the interflavanyl bonds. The existing semi-synthetic methods involve coupling of electrophilic C_4-substituted flavan-3-ols under either acidic or basic conditions.[1-7] Under these conditions, however, the interflavanyl bond is labile which invariably leads to an equilibrium between substrates and products. Such a labile bond and an apparent preference of the electrophile for the di- and trimeric products once condensation is initiated, furthermore give poor control regarding the level of oligomerization. The effectiveness of the thiophilic Lewis acids, dimethyl(methylthio)-sulfonium tetrafluoroborate (DMTSF)[8,9] and silver tetrafluoroborate ($AgBF_4$),[10] to activate the C_4-S bond in the 4-thioethers of flavan-3-ols towards carbon nucleophiles, and hence to generate the interflavanyl bond of procyanidins under neutral conditions, is discussed here.

In order to assess the feasibility of a procedure to establish the $C_{(sp3)}$ - $C_{(sp2)}$ interflavanyl bond in procyanidin oligomers under neutral conditions, the model reaction between the 4-thiobenzylether of a flavan-3-ol and phloroglucinol as a potent capture nucleophile was attempted (Scheme 1). Thus, treatment of a mixture of 4β-benzylsulfanylepicatechin **1**, available *via* acid-catalyzed thiolysis of Loblolly Pine purified tannin polymer[11] (see Experimental), representing the (2*R*,3*R*)-2,3-*cis*-flavan-3-ol chain extender unit of the procyanidins, and phloroglucinol in THF with a fresh batch of DMTSF (1:4:1 molar ratio) for 1h at 0^0C gave

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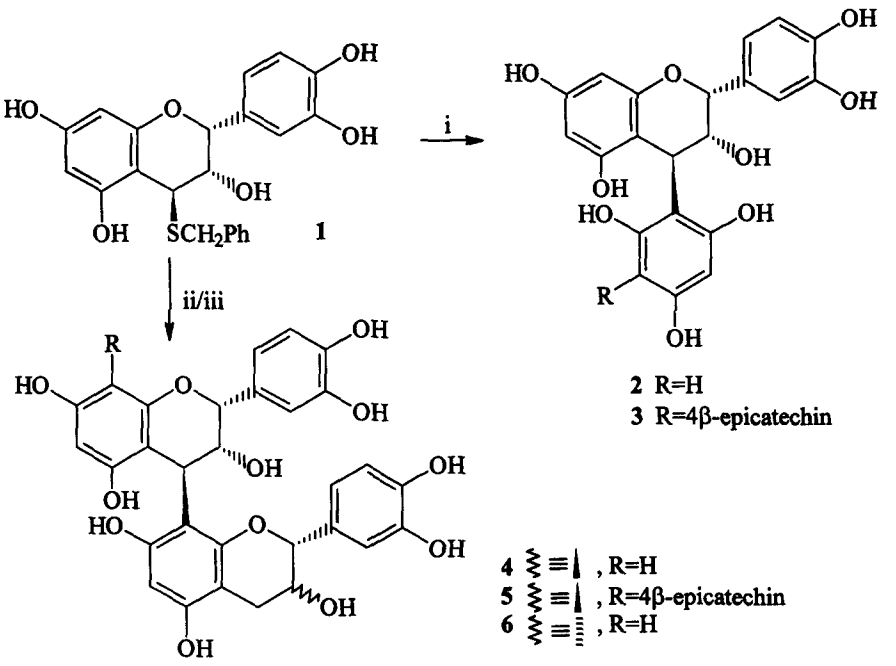
epicatechin-(4β→2)-phloroglucinol **2**[12] (28% yield) and the analogous bis-epicatechin-(4β→2: 4β→4)-phloroglucinol **3**[12] (19%).

The aqueous work-up afforded an acidic reaction mixture (pH ~ 2.5 by Merck ACILIT® indicator, pH 0-6), hence indicating that the coupling process might have occurred under acid catalysis. Repetition of the reaction in a pH 6.85 buffer solution, however, gave an identical product distribution. The stability of the C_4-S bond in the 4β-benzylsulfanylepicatechin **1** in acidic medium was additionally ascertained by treatment of a mixture of thioether **1** and phloroglucinol in 0.1M HCl solution for 3.5h at 20°C which afforded only the starting materials. The $C_{(sp3)}$ - $C_{(sp2)}$ bond formation to give the 4-arylflavan-3-ol **2** and the trimeric analogue **3** thus occurs under neutral conditions *via* activation of the C_4-S bond in the thioether **1** by DMTSF.

The potential of DMTSF to facilitate the aforementioned bond formation, prompted an attempt at generating the interflavanyl bond of the ubiquitous procyanidin B-1 **4**.[13] A mixture of 4β-benzylsulfanylepicatechin **1** and catechin in THF treated with DMTSF (1:10:1 molar ratio) at -15°C for 2h indeed



Scheme 1. Procyanidin synthesis using Lewis acid activation of 4β-benzylsulfanylepicatechin **1**; *Reagents and conditions*: i, Phloroglucinol/DMTSF in THF, 0°C, 1.25h; ii, catechin/DMTSF in THF, -15°C, 2h or catechin/$AgBF_4$ in THF, 0°C, 1h; iii, epi-catechin/$AgBF_4$ in THF, 0°C, 1h.

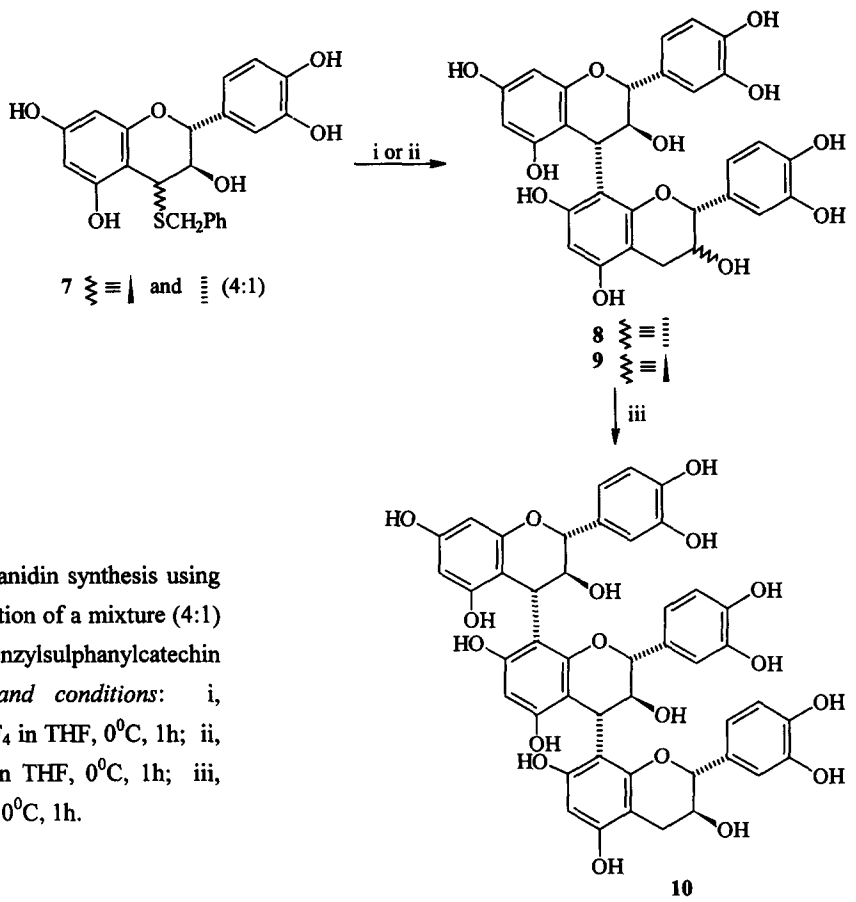
afforded procyanidin B-1[1] **4** (22%) and the analogous trimeric procyanidin **5**[14] (19%) (Scheme 1). In spite of the persistent formation of the triflavanoid **5**, formation of regiomeric dimers or of higher oligomers could not

be detected in several subsequent runs. This protocol thus compares favourably with the classical acid-catalyzed condensation of catechin-4α-ol and catechin[3,6] which gave a mixture of procyanidins B-3 **9** and B-6, the trimeric procyanidin C-2 **10** and its 4,6-regioisomer, and the presumed all-*trans*-[4,8]-linked tetraflavanoid analogue in the proportions 10:1:12:1:3 and in *ca* 45% overall yield.

Owing to problems experienced with the moisture sensitivity of DMTSF and in view of our successful activation of the benzylic carbon-sulphur bond of α-hydroxy-β-benzylsulfanyldihydrochalcones towards the intramolecular formation of a carbon-oxygen bond in the stereoselective synthesis of dihydroflavonols using AgBF4,[15] the potential of this thiophilic Lewis acid to effect interflavanyl bond formation in procyanidins was next assessed. Treatment of a solution of 4β-benzylsulfanylepicatechin **1** and catechin (10 *eq.*) in THF with $AgBF_4$ (2.5 *eq.*) for 1h at 0^0C (Scheme 1) indeed afforded procyanidin B-1[1] **4** in improved yield (38 *vs.* 22% for DMTSF as activator) as the only product formed in meaningful quantities. When this procedure was repeated with epicatechin as the capture nucleophile, procyanidin B-2[1] **6** was formed in 37% yield.

In order to broaden the scope of this thiophilic Lewis acid catalyzed interflavanyl bond formation under neutral conditions, 4-benzylsulfanylcatechin **7**, representing the (2*R*,3*S*)-2,3-*trans* chain extender unit of the procyanidins, was next used as the source of the flavan-3-ol C-4 electrophilic moiety (Scheme 2). Compound **7** was synthesized as a mixture of the 4β- and 4α-epimers (4:1) *via* reduction of (2*R*,3*R*)-2,3-*trans*-dihydroquercetin with sodiumborohydride in ethanol[3] and acid catalyzed thiolysis of the unstable catechin-4-ol intermediate using phenylmethanethiol-acetic acid. Both the 4β- and 4α-benzylsulfanylcatechin epimers **7** will be converted to the same transient C-4 carbocationic intermediate by $AgBF_4$, hence both will display the same stereochemical course of interflavanyl coupling. Separate treatment of a mixture of the epimeric 4-benzylsulfanylcatechins **7** and epicatechin and catechin in THF with $AgBF_4$ (2.5 *eq.*) for 1h at 0^0C, afforded procyanidin B-4[1] **8** (35%) and B-3[1] **9** (51%), respectively. The preferences for the formation of 4β- and 4α-interflavanyl bonds using the epicatechin- and catechin-4-thiobenzyl ethers **1** and **7**, respectively and for the (4→8)-interflavanyl linkages were anticipated,[3,6] *i.e.* the thiobenzyl ethers are converted by the Lewis acids into relatively stable intermediates permitting both the regioselective attack of the nucleophile *via* C-8 where the HOMO displays maximum amplitude,[16] and the stereoselectivity by approach from the sterically least hindered side.

Finally, we coupled procyanidin B-3 **9** and the 4-benzylsulfanylcatechin epimers **7** using $AgBF_4$ in THF to afford the trimeric procyanidin C-2[6] **10** (26%) as the only isolable product (Scheme 2). The sequences **1** → procyanidin B-1 **4** and B-2 **6**, and **7** → procyanidin B-4 **8**, B-3 **9** and procyanidin C-2 **10** using $AgBF_4$ as the thiophilic Lewis acid, no doubt, offers advantages as far as control over the level of oligomerization, reversibility and 'scattering' of the interflavanyl bond(s) are concerned in comparison with the formation of these products *via* catalysis of the coupling of catechin-4-ol and catechin by protic acids[3,6] (*vide supra*).



Scheme 2. Procyanidin synthesis using Lewis acid activation of a mixture (4:1) of 4β- and α-benzylsulphanylcatechin 7; *Reagents and conditions*: i, epicatechin/$AgBF_4$ in THF, 0^0C, 1h; ii, catechin/$AgBF_4$ in THF, 0^0C, 1h; iii, 7/$AgBF_4$ in THF, 0^0C, 1h.

EXPERIMENTAL

^{1}H NMR spectra were recorded at 298 K on a Bruker AM-300 spectrometer for solutions in $(CD_3)_2CO$ with solvent as internal standard. Column chromatography (CC) was performed on Sephadex LH-20 in EtOH or EtOH-hexane. Preparative plates (PLC) [20x20 cm, Kieselgel PF_{254} (1.0 mm)] were air dried and used without prior activation.

4β-*Benzylsulfanylepicatechin* **1**. Loblolly Pine purified tannin (5 g) and phenylmethanethiol (5.0 ml) were dissolved in EtOH (80 ml), the solution was flushed with N_2 for 15 min, HOAc (10 ml) was added, the vial was 'capped' and left at 100^0C for 16h. The mixture was cooled to room temperature, water (200 ml) was added and the excess of phenylmethanethiol was washed out with hexane (2x50 ml). The aqueous layer was then extracted with EtOAc (5x50 ml), the combined organic phases were dried (Na_2SO_4), evaporated to dryness and separated by CC in EtOH-hexane (3x85 cm column, 0.4 ml/min flow rate, 32 min fractions, first 100 ml of eluent discarded) to give the title compound **1**[11] (850 mg, tubes 206-280).

4-*Benzylsulfanylcatechins* **7**. A solution of (2*R*,3*R*)-2,3-*trans*-dihydroquercetin (500 mg, 1.64 mmol) in EtOH (20 ml) was purged with N_2 for 1h, phenylmethanethiol (1 ml, 8.5 mmol) was added and the mixture

was cooled to 0°C. $NaBH_4$ (400 mg, 10.6 mmol) was added in portions over 30 min followed by 50% HOAc (80 ml) and the mixture was stirred at room temperature for 12h. After the addition of water (200 ml) the excess phenylmethanethiol was washed out with hexane (2x30 ml), the aqueous layer was extracted with EtOAc (5x30 ml), the organic layers combined, dried (Na_2SO_4), evaporated to dryness and separated by CC in EtOH (3x65 cm column, 1.1 ml/min flow rate, 16 min fractions, first 100 ml of eluant discarded) to give 4-benzylsulfanylcatechin **7** (590 mg, tubes 38-52) as a 4:1 mixture of 4β- and α-epimers ($J_{3,4}$ = 4 and 9 Hz, resp.).

General procedure for the synthesis of procyanidins using $AgBF_4$.

The flavan-3-ol thioether (**1** or **7**) (200 mg) and catechin or epicatechin (10 *eq.*) in THF (50 ml) was cooled to 0°C, solid $AgBF_4$ (2.5 *eq.*) was added in one batch and the reaction was stirred for 1h. After the addition of H_2O (400 ml) the mixture was extracted with EtOAc (4x100 ml), the combined EtOAc layers dried (Na_2SO_4), evaporated to dryness and separated by CC in EtOH on a 3x86 cm column, 1.0 ml/min flow rate, 16 min fractions.

Procyanidin B-1[1] **4** [*epicatechin*-(4β→8)-*catechin*] 108 mg (tubes 168-204, first 400 ml of eluant discarded

Procyanidin B-2[1] **6** [*epicatechin*-(4β→8)-*epicatechin*] 102 mg (tubes 157-198, first 200 ml of eluant discarded)

Procyanidin B-3[11] **9** [*catechin*-(4α→8)-*catechin*] 141.3 mg (tubes 156-220, first 270 ml of eluant discarded]

Procyanidin B-4[1] **8** [*catechin*-(4α→8)-*epicatechin*] 97.2 mg (tubes 221-260, first 220 ml of eluant discarded)

Procyanidin C-2[6] **10** [*catechin*-(4α→8)-*catechin*-(4α→8)-*catechin*] Procyanidin C-2 **10** was prepared from thioether **7** (128 mg, 0.31 mmol) and procyanidin B-3 **9** (355.2 mg, 0.61 mmol) according to the above procedure. Separation as above (first 950 ml of eluant discarded) afforded **10** (71 mg, tubes 221-290).

These known procyanidins were identified by comparison of ^1H NMR data with those in the literature.

Epicatechin-(4β→2)-*phloroglucinol* **2**. A mixture of 4β-benzylsulfanylepicatechin **1** (50 mg), phloroglucinol (61 mg, 4*eq.*) and DMTSF (24 mg, 1 *eq.*) in THF (20 ml) was stirred at 0°C for 1.25h. Water (80 ml) was added and the mixture was extracted with EtOAc (3x50 ml), the combined organic layers were dried (Na_2SO_4) and evaporated to dryness. Separation by PLC in benzene-acetone-methanol (6:3:1, v/v) gave five bands at R_F 0.66 (4 mg), 0.51 (43 mg), 0.42 (6 mg), 0.35 (14 mg) and 0.27 (15.4 mg). The R_F 0.51 band comprised of phloroglucinol, the R_F 0.35 band gave the title compound **2**[12] and the R_F 0.27 band the bis-epicatechin-phloroglucinol adduct **3**[12]. The R_F 0.66 and 0.42 bands still comprised of mixtures and were not further investigated.

Procyanidin B-1 **4**. A mixture of 4β-benzylsulfanylepicatechin **1** (200 mg) and catechin (1.4 g, 10 *eq.*) in THF (400 ml) was stirred at -50°C and DMTSF (95 mg, 1 *eq.*) added. Stirring was then continued for 1h at -35°C and for a further 2h at -15°C. Work-up comprised the addition of a KH_2PO_4/Na_2HPO_4 buffer solution (pH ~ 6.8, 500 ml), extraction with EtOAc (4x200 ml), drying (Na_2SO_4) of organic layers and concentration to *ca* 50 ml. Water (300 ml) was added, the mixture was freeze dried and the residue (1.48 g) separated by

CC in EtOH (3x120 cm column, 1 ml/min flow rate, 16 min fractions, first 800 ml of eluant discarded) to give three fractions: A (tubes 102-124, 1.25 g), B (195-216, 60 mg) and C (290-314, 80 mg). Fraction A comprised of catechin, fraction B gave procyanidin B-1[14] **4** and fraction C, epicatechin-(4β→8)-epicatechin-(4β→8)-catechin[6] **6.**

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