Plant Proanthocyanidins. Part 7.[1] Prodelphinidins from *Pinus sylvestris*

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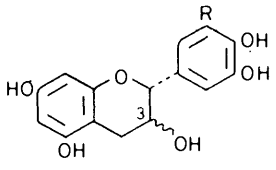
A dimeric prodelphinidin and a polymeric proanthocyanidin have been isolated from male flowers of *Pinus sylvestris*. Structural analyses of the dimer and the polymer are reported and a biomimetic synthesis of the dimer is noted.

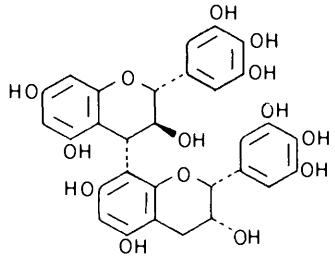
In contrast to the ubiquitous procyanidins,[2-4] comparatively little is yet known concerning the chemistry of the analogous prodelphinidins, although Bate-Smith [5] in his earlier surveys showed that a number of 'woody' plants yielded delphinidin (invariably with cyanidin) when fruit or leaf extracts were heated with hydrochloric acid in butanol. The evidence reported to date [6,7] does, however, suggest a very similar chemistry to the procyanidins [2-4] and a pattern of distribution which parallels the phylogenetic pattern of occurrence of (+)-gallocatechin (1a) and (−)-epigallocatechin (2) in plants. As Bate-Smith's observations imply, prodelphinidins are almost always found alongside procyanidins in plant tissues and the initial work in this field supports this conclusion.[6,7] Thus Porter and Foo [6] have reported the isolation of (+)-gallocatechin (1a), (−)-epigallocatechin (2), and the prodelphinidin (3) as polyacetates from *Ribes sanguineum* and the prodelphinidin (4) as its undeca-acetate from *Salix caprea*; both plants also metabolise procyanidins. Subsequently Porter and his co-workers [7] discussed the chemistry of proanthocyanidin polymers from several sources which were composed of procyanidin and prodelphinidin units.

The needles and cones of the Scot's pine (*Pinus sylvestris*) are rich sources of procyanidins, but the male flowers yield both procyanidins and prodelphinidins. In the methanol extract three prodelphinidin oligomers were identified and the prodelphinidin dimer (5a) was obtained in sufficient amount to permit characterisation. Structural analysis was achieved by methods analogous to those established in the procyanidin series.[2-4] Restricted rotation around the interflavan bond of proanthocyanidin dimers [4] has limited the use of ^{1}H n.m.r. as a structural aid in this field, but high resolution ^{1}H n.m.r. (220 and 360 MHz) analysis of the undeca-acetate of (5a) permitted a direct assignment of structure from the signals of the major rotamer which are clearly resolved at these operating frequencies and at ambient temperature. Solvolysis of the prodelphinidin (5a) in presence of toluene-α-thiol [3] gave (+)-catechin (1b) and the thioether (6) in approximately equimolar quantities. The ^{13}C chemical shift of 74.0 p.p.m. (from tetramethylsilane) for C-2 in the undeca-acetate of (5a) indicates, on the basis of earlier arguments,[3,4] the structure (5a) and the stereochemistry 4*R*.

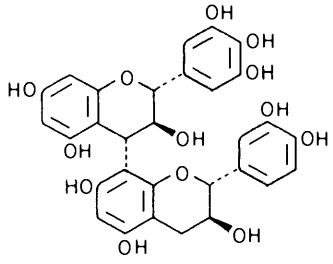
The proanthocyanidin polymer, isolated concurrently from the male flowers of *Pinus sylvestris*, gave both cyanidin and delphinidin when heated with acid in butanol (ratio *ca.* 2 : 1 by visual inspection after chromatography in Forestal solvent [8]), and was degraded by acid in the presence of toluene-α-thiol to give the thioethers (6) and (7), (+)-catechin (1b), and (+)-gallocatechin (1a).



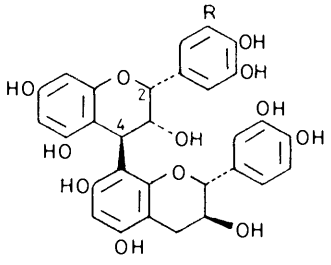
(1a) 3*S*, R = OH
(1b) 3*S*, R = H
(2) 3*R*



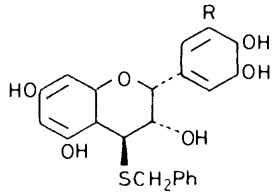
(3)



(4)



(5a) R = OH
(5b) R = H



(6) R = OH
(7) R = H

The polymer is thus composed of molecular species, the polymer chains of which contain flavan-3-ol units with predominantly 2,3-*cis*-stereochemistry and hydroxylation patterns in the B-ring of the 3,4- and 3,4,5-type. Solvolysis of the proanthocyanidin polymer in the presence of an excess of (+)-catechin (1b) gave both procyanidin B-1 (5b) and the prodelphinidin (5a).[9]

These observations lend further support to the suggestion of a relationship between the metabolism of the prodelphinidins and the gallocatechins.[6] However, the isolation of an additional prodelphinidin dimer (5a) with a mixed B-ring hydroxylation pattern and a polymer of mixed procyanidin–prodelphinidin composition has important biosynthetic implications and indicates that the biosynthesis of procyanidins and prodelphinidins is inextricably linked in some plant tissues.

EXPERIMENTAL

General procedures of analysis were as previously described.[3,4]

Isolation of Prodelphinidins.—Freshly picked (May–June) male flowers of *Pinus sylvestris* (5 kg) were macerated in methanol (*ca.* 3 × 2 000 ml) in a high speed blender to give residual plant debris and a methanol extract. Evaporation of the methanol (*ca.* 40 °C) until chlorophyll precipitation was complete gave a solution (*ca.* 500 ml) which was filtered, extracted with chloroform (2 × 300 ml) and then ethyl acetate (6 × 300 ml). Evaporation of the ethyl acetate gave the phenolic extract (*ca.* 15 g) which was dissolved in ethanol (40 ml) and separated by chromatography on Sephadex LH-20 in the same solvent. Fractions (12 ml) were collected and analysed by paper chromatography.[3,4] Appropriate fractions were grouped. Group 1 (fractions 25—50) gave (+)-catechin (1b) (3.2 g) as needles from methanol–water, m.p. and mixed m.p. 175—177 °C (Found: C, 62.0; H, 5.2. Calc. for $C_{15}H_{14}O_6$: C, 62.1; H, 4.8%); $[\alpha]_{578}$ +16.4° (*c* 1.5 in EtOH); R_F(A) 0.47 and R_F(B) 0.51. Group 2 (58—72) gave (+)-gallocatechin (1a) which crystallised from water as needles (1.2 g), m.p. 185—186 °C (Found, after drying for 24 h at 100 °C and 0.5 mmHg: C, 58.4; H, 4.9. Calc. for $C_{15}H_{14}O_7$: C, 58.8; H, 4.6%); $[\alpha]_{578}$ +14.6° (*c* 0.9 in EtOH); R_F(A) 0.43 and R_F(B) 0.52. Hexa-acetyl-(+)-gallocatechin crystallised from methanol as needles, m.p. 141—142 °C (lit.,[10] m.p. 141—143 °C); $[\alpha]_{578}$ +33.0° (*c* 0.8 in Me_2CO) and penta-*O*-methyl-(+)-gallocatechin, prepared with diazomethane, crystallised as needles from methanol, m.p. 158—160 °C (lit.,[10] m.p. 160—162 °C). Group 3 (131—180) gave procyanidin B-1 (5b) as an off-white powder (0.28 g) [R_F(A) 0.52, R_F(B) 0.33] which formed a deca-acetate, m.p. 225—227 °C (lit.,[2] m.p. 231—232 °C); $[\alpha]_{578}$ +114° (*c* 1.0 in MeCN), R_F 0.55 (acetone–benzene, 8 : 2, v/v, silica). Group 4 (215—250) gave the *prodelphinidin* (5a) which was further purified by chromatography on Sephadex LH-20 in EtOH–$CHCl_3$ (1 : 1, v/v) to give a buff-coloured powder (0.17 g) (Found, after drying for 48 h at 100 °C and 0.5 mmHg: C, 58.6; H, 5.0. $C_{30}H_{26}O_{13}\cdot H_2O$ requires C, 58.8; H, 4.6%); R_F(A) 0.50, R_F(B) 0.25; $[\alpha]_{578}$ +22° (*c* 0.4 in EtOH). The *prodelphinidin undeca-acetate* crystallised as small rosettes from methanol, m.p. 236—240 °C, with softening to an opaque glass at 130—140 °C (Found: C, 59.2; H, 4.7. $C_{52}H_{48}O_{24}$ requires C, 59.1; H, 4.5%); $[\alpha]_{578}$ +96° (*c* 0.5 in MeCN).

The insoluble plant-debris, after methanol extraction, was suspended in acetone–water (1 : 1, v/v, 2 × 1 000 ml) for 7 d at room temperature. Removal of the plant-debris and concentration of the filtrate to *ca.* 300 ml gave a suspension from which the polymeric proanthocyanidin separated as a pale brown powder (4.2 g).

(2*R*,3*S*,4*S*)-4-*Benzylthioflavan*-3,3′,4′,5,5′,7-*hexol* (6).—The prodelphinidin (5a) (0.32 g) was dissolved in ethanol (4 ml) containing toluene-α-thiol (2 ml) and acetic acid (1 ml) and the solution refluxed under N_2 for 36 h. Removal of the solvents at 30 °C and chromatography of the oily residue in $CHCl_3$–propan-1-ol (4 : 1, v/v) on Sephadex LH-20 gave (+)-catechin (0.08 g), m.p. and mixed m.p. 175—177 °C, from fractions 136—168, and the *thioether* (6) as a white solid (0.07 g) [R_F(A) 0.39, R_F(B) 0.75] from fractions 104—128. The thioether was characterised as its *hexa-acetate* which crystallised as white prisms from methanol, m.p. 153—154 °C (Found: C, 59.8; H, 4.8; S, 4.7. $C_{34}H_{32}O_{13}S$ requires C, 60.0; H, 4.7; S, 4.7%); $[\alpha]_{578}$ −28° (*c* 0.4 in $CHCl_3$); ^{1}H n.m.r. (220 MHz in $CDCl_3$) δ 2.5—2.75 (m, 5 H, Ph), 2.83 (s, 2 H, ArH ring B), 3.33 (1 H, d, *J* 2.5 Hz, 8-H), 3.45 (1 H, d, *J* 2.5 Hz, 6-H), 4.30 (1 H, s, 2-H), 4.72 (1 H, bs, 3-H), 5.85 (1 H, bs, 4-H), 5.80 and 6.15 (2 H, dd, *J* 14.0 Hz, SCH_2), 7.70 (9 H, COMe), 7.75 (3 H, COMe), 8.03 (3 H, COMe), and 8.19 (3 H, COMe).

Degradation of the Polymeric Proanthocyanidin.—The polymer (2.8 g) was suspended in ethanol (30 ml) containing toluene-α-thiol (12 ml) and acetic acid (6 ml) and refluxed for 48 h under N_2. Removal of the solvent and chromatography as above gave the thioether (7) (0.43 g) characterised as its penta-acetate, m.p. and mixed m.p. 123—125 °C, and the thioether (6) (0.26 g) characterised as its hexa-acetate, m.p. and mixed m.p. 152—154 °C. After 24 hours (+)-catechin and (+)-gallocatechin were detected by paper chromatographic analysis.

Biomimetic Synthesis of the Prodelphinidin (5a) *and Procyanidin B-1* (5b).—The polymer (3.9 g) was suspended with (+)-catechin (1.5 g) in 0.5M HCl (30 ml in dioxan–water; 1 : 1, v/v). The solution was continuously stirred at room temperature for 48 h, concentrated at 30 °C, diluted with water (75 ml), and extracted with ethyl acetate (6 × 100 ml). Evaporation of the ethyl acetate gave a gum (*ca.* 3.0 g) which was chromatographed on Sephadex LH-20 in ethanol as above. Appropriate fractions were grouped and the products acetylated to give procyanidin B-1 (5b) deca-acetate (0.32 g) and the prodelphinidin (5a) undeca-acetate (0.12 g) which were identified by t.l.c. with authentic specimens.

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