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PROCYANIDINS AND POLYPHENOLS OF *LARIX GMELINI* BARK*

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Key Word Index—*Larix gmelini*; Pinaceae; bark; catechins; procyanidins; biflavonoids; larixinol.

Abstract—Methanol extracts of *Larix gmelini* bark yielded (−)-epiafzelechin, (+)-catechin and (−)-epicatechin, dimeric procyanidins B-1, B-2, B-3 and B-4 and oligomeric procyanidins. Chemical degradation of the oligomers using toluene-α-thiol and acetic acid showed the oligomers to have flavan-3-ol terminal units possessing either the 2,3-*trans* (catechin) or 2,3-*cis* (epicatechin) stereochemistry, with the former predominating. Likewise the extension units were shown to consist of flavan-3-ol units with 2,3-*trans* or 2,3-*cis* stereochemistry in roughly equal proportion. The average degree of oligomerization was calculated as 6–7 and the number average molecular weight as 1700–2000. The structure of the residual procyanidins remaining in the bark after methanol extraction is also briefly commented upon. Larixinol is shown to have an unusual spirobiflavonoid structure and a pathway of biogenesis is proposed.

INTRODUCTION

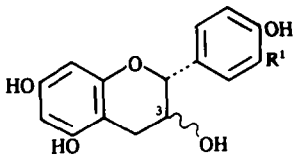
Larix gmelini (Rupr.) Rupr is an important conifer of Northern China. Its bark has been used for many years to produce tannin extracts for China's indigenous leather industry. Earlier brief reports mention the presence of flavan-3-ols and proanthocyanidins in the bark of larch species. Chumbalov and his colleagues [1] have reported the isolation of (+)-catechin (**2**), (−)-epicatechin (**3**) and (−)-epiafzalechin (**1**) from the bark of *Larix sibirica* and they proposed the structure **4** for an unusual biflavonoid, larixinol, from the same source. A preliminary communication of the revised structure (**5**) for larixinol has been made [2].

RESULTS AND DISCUSSION

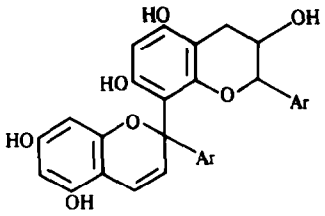
Methanol extraction of the powdered bark gave an extract which was further fractionated by liquid–liquid partition in water–chloroform then ethyl acetate. The ethyl acetate extract was chromatographed [3] on Sephadex LH-20 using ethanol as eluant to give a flavan-3-ol fraction (I), the dimeric procyanidin fraction (II) and finally using methanol as eluant the polymeric procyanidin fraction (III). Rechromatography of (I) yielded (+)-catechin (**2**), (−)-epicatechin (**3**) and (−)-epiafzelechin (**1**) and a crystalline compound (~ 0.15% of the original methanol extract) identical with the substance larixinol described by Chumbalov from *Larix sibirica* [1]. (−)-Epiafzelechin was identified by ^{1}H NMR and chromatographic comparison. (+)-Catechin and (−)-epicatechin were characterized by ^{1}H NMR and as their penta-acetates.

Larixinol (**5**, $C_{30}H_{22}O_{10}$) formed a hexa-acetate (**6**, Ac_2O–pyridine), a pentamethyl ether (**7**, Me_2SO_4–K_2CO_3) and a hexamethyl ether (**8**, NaH–DMSO–MeI). Acid treatment (5 N HCl–EtOH at 60°) did not lead to the production of any anthocyanidins and the compound was likewise stable to toluene-α-thiol and acetic acid [3]. The presence of the (−)-epiafzelechin (**1**) part structure in larixinol (**5**) was confirmed by direct comparison of the ^{1}H and ^{13}C NMR spectra. Of the remaining 15 carbon atoms in larixinol six were similarly shown to be associated with a monosubstituted phloroglucinol ring and a further six to a second *para*-(hydroxyl) substituted phenyl ring. Larixinol and its derivatives all displayed a strong absorption at ν_{max} ~ 1785–1810 cm^{-1} indicative of a γ-lactone and with this information in mind the remaining three carbon atoms in larixinol were attributed to that of a lactone carbonyl group (δ179.1, TMS), a methine carbon benzylic and attached to oxygen (δ91.0), and a quaternary carbon atom (δ61.1). Support for the lactone structure was derived by reduction of the pentamethyl ether (**7**) with lithium aluminium hydride which gave a triol (triacetate). During this transformation the lactone carbonyl group (^{13}C: δ178.38) was replaced by a new signal in the ^{13}C NMR spectrum (triplet at δ69.94) consonant with the conversion to a methylene carbon attached to oxygen. Catalytic hydrogenation of larixinol pentamethyl ether (Pt–H_2) gave at least four products which retained the parent carbon skeleton. The ^{1}H and ^{13}C NMR spectra of these compounds showed that they differed from **7** solely in the nature of the substituents which replaced the two *p*-methoxyphenyl rings (and hence the two characteristic A_2B_2 systems in the ^{1}H NMR spectra). In three of the compounds (**9**, **10**, **11**) one or both of the *p*-methoxyphenyl rings was fully saturated and in two compounds the methoxyl group had been eliminated to give a cyclohexyl substituents (**11**, **12**). The disposition of these saturated rings in compounds **9**–**12** was deduced

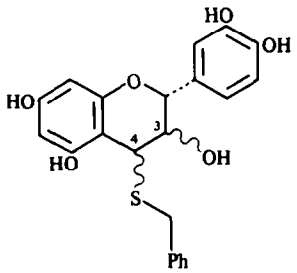
* Part 8 in the series "Plant Proanthocyanidins". For Part 7 see Gupta, R. K. and Haslam, E. (1981) *J. Chem. Soc. Perkin Trans.* 1, 1148.



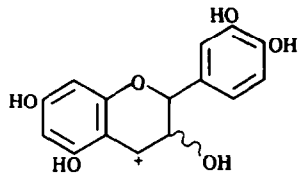
1 R^1 = H (3*R*)
2 R^1 = OH (3*S*)
3 R^1 = OH (3*R*)



4 Ar = 4'-C_6H_4-OH



16 (3*R*,4*S*)
17 (3*R*,4*R*)
18 (3*S*,4*S*)



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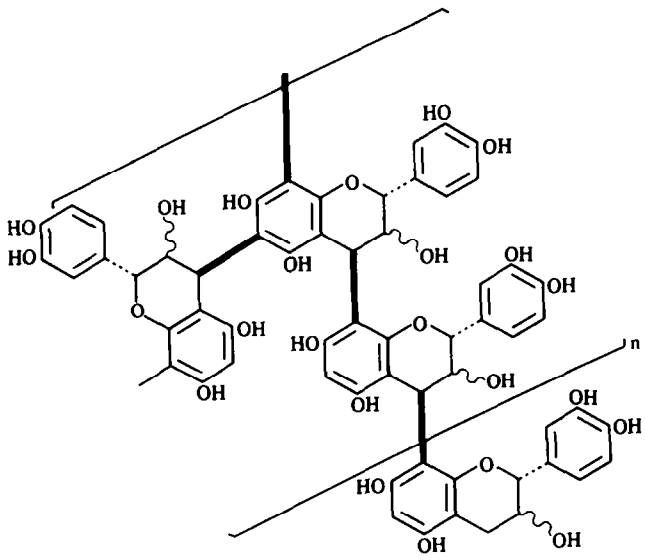

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from the multiplicity of the signals due to H-2 and H-2^1 in the ^{1}H NMR spectra.

The manner of the attachment of the (−)-epiafzelechin unit to the residual C_{15} substituent in larixinol could be deduced (from ^{1}H and ^{13}C NMR data) to involve either the 6 or 8 position and the 5- or 7-hydroxyl of the 'A' ring of the (−)-epiafzelechin fragment. In addition the relative downfield shift of the 6 or 8 proton singlet in the 'A' ring of the (−)-epiafzelechin unit at δ6.14 (TMS) indicated that the 5- or 7-hydroxyl group was acylated. NOE and solvent induced proton shifts [4, 5, $CDCl_3$ and C_6D_6] showed that each of the methoxyl groups in the pentamethyl ether (7) has one or two protons in the *ortho* positions. Based on this evidence just two structures for larixinol are permissible. The final structure of larixinol as **5** was deduced by X-ray crystallographic analysis of the metha-

nol solvate.* Figure 1 shows a computer generated representation of the spirobiflavonoid structure drawn from the final atomic co-ordinates. No attempt was made to define the absolute stereochemistry but if the epiafzelechin sub-unit has the same absolute stereochemistry as the co-occurring flavan-3-ol then the structure shown in Fig. 1 represents the absolute configuration of larixinol.

Larixinol (**5**), with its unique spirobiflavonoid structure, is representative of an entirely new class of biflavonoid. Its biogenesis is presumed (Fig. 2) to be associated with that of the C-3–C-8′ linked garcinia group of biflavonoids, e.g. saharanflavone (**13**) [6–8]. A suggested biogenetic pathway is outlined in Fig. 2 and incorporates the hypothesis that both larixinol (**5**) and the garcinia biflavonoids are formed by interception of the putative intermediate (**15**, or its biogenetic equivalent) in the oxidative flavanone–flavonol conversion from **14**.

Two dimensional paper chromatography [3] of fraction II of the original bark extract (see above) revealed that its principal components were the dimeric procyanidins B-1 [epicatechin-(4$\beta \rightarrow$ 8)-catechin], B-2 [epicatechin-(4$\beta \rightarrow$ 8)-epicatechin], B-3 [catechin-(4$\alpha \rightarrow$ 8)-catechin] and B-4 [catechin-(4$\alpha \rightarrow$ 8)-epicatechin]. HPLC analysis confirmed their identity and showed that the relative concentrations were in the comparative order B-3 > B-1 > B-2 and B-4—consonant with the greater concentration of (+)-catechin over (−)-epicatechin in the original extract and suggesting that flavan-3-ol extension units with both the (+)-catechin and (−)-epicatechin stereochemistry were abundant in the biosynthetic process [9]. The total yield of procyanidins isolated from a 60–200 year old bark was 0.5%. Each of the procyanidin dimers was isolated as its deca-acetate by silica gel flash-chromatography. The ^{1}H NMR of these deca-acetates, although they all displayed the phenomenon of atropisomerism at 30°, were identical with published spectra [10, 11]. Fraction III of the original *Larix gmelini* extract eluted from the Sephadex LH-20 column with methanol was a complex mixture of oligomeric procyanidins [12]. This fraction was characterized by chemical degradation with toluene-α-thiol and acetic acid [3] and the products isolated by chromatography on Sephadex LH-20 using chloroform–ethanol (4:1) as eluant. HPLC analysis showed the ratio of (+)-catechin and (−)-epicatechin was 4:1 (see above) and the ratio of 2,3-*trans* to 2,3-*cis* flavan-3-ol chain extension units in the oligomers—by determination of the relative amounts of **16**, **17** and **18**—was ~ 9:11. Assuming overall structures such as **19** for the procyanidin oligomers the observations also showed that the number average molecular weight was ~ 1700–2000 and the mean degree of polymerization 6–7.

From the bark residue after extraction with methanol further polymeric procyanidins could be extracted by stirring with acetone–water (1:1). The product was insoluble in ethanol. From *Larix gmelini* bark the two forms of polymeric procyanidin constituted some 4% of the plant material. There have been several reports [13–15] concerning the determination of the structure and molecular weights of polymeric proanthocyanidins by ^{13}C NMR and gel-permeation chromatography. However after repeated chromatography neither of the soluble polymeric procyanidin forms from *Larix gmelini* was directly amenable to this type of analysis, although the broad general pattern of signals was consistent with a polymeric procyanidin structure. Signals at δ 102.5–104.5 (CH) and δ 60.0–63.0 (CH_2) suggested the presence of pyranose carbohydrate residues within the heterogeneous polymeric structure and D-glucose, along with other unidentified sugars, was detected after acid hydrolysis. These observations are entirely analogous to earlier ones made with polymers from various sources including *Crataegus* sp. [16, 17].

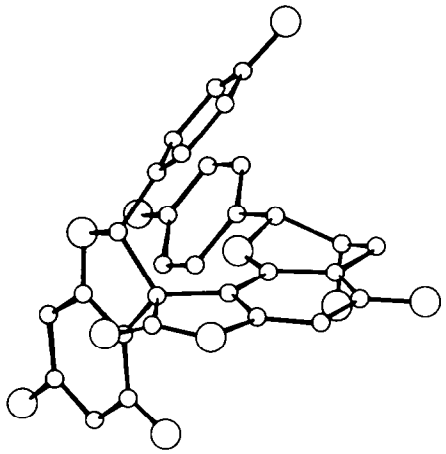


Fig. 1. Crystal structure of larixinol.

It is therefore both appropriate and significant to recall in this context the original observations of Sir Robert and Lady Robinson on the occurrence of proanthocyanidins in plant tissues [18]. These pioneers in this field drew attention to various categories of proanthocyanidins and in particular to a group (a)—"those which are insoluble in water and the usual organic solvents". In our studies in this area proanthocyanidins of this type have been regularly encountered and they are, in our experience, characteristic of all proanthocyanidin containing tissues such as stem, twig, leaf and fruit where they invariably predominate over the more freely soluble oligomeric forms, typically by as much as 5 or 20:1—based on the release of anthocyanidin with acid. Indeed in the tissues of some plants, e.g. ferns and the persimmon fruit, they overwhelmingly preponderate and they are of frequent occurrence in plant gums and exudates, e.g. *Butea frondosa* [19]. Moreover they remain in plant tissues after repeated hot solvent extraction with dimethylformamide (DMF). Similarly they have been shown to occur, alongside the soluble oligomeric procyanidins, in *Larix gmelini* bark.

Dimeric proanthocyanidins almost invariably occur free although some examples are now recorded of the occurrence of the parent flavan-3-ols and proanthocyanidins in glycosylated forms [20]. Whilst non-covalent

*Crystal data for **5**: $C_{30}H_{22}O_{10}\cdot CH_3OH$, M = 574.5, orthorhombic, space group $P2_12_12_1$, a = 11.6426(11), b = 12.7589(16), c = 18.3476(17)Å, U = 2725.5Å^3 D_c = 1.40 g cm^{-3}, Z = 4, R = 3.93, R_w = 5.42 for 2877 reflections $\theta < 76°$, $I > 3\sigma(I)$, crystal size 1.25 × 0.25 × 0.15 mm. Cu-K_α radiation λ = 1.5418Å. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this paper [2].

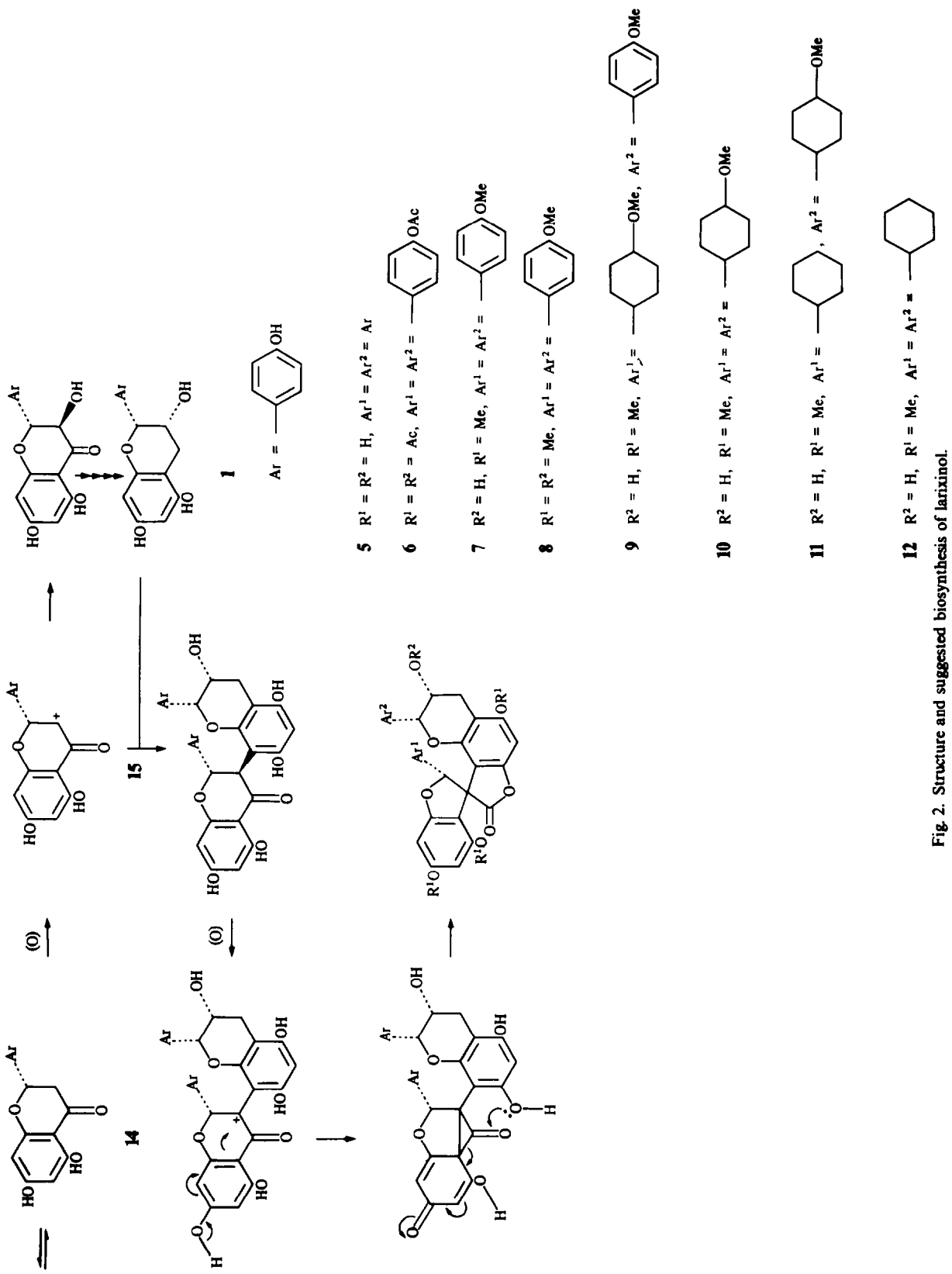
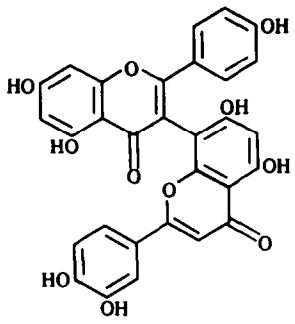


Fig. 2. Structure and suggested biosynthesis of larixinol.



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forces may be involved in the complexation of polyphenols with polysaccharides [16, 17], *one* interpretation of the present evidence is that the polymeric proanthocyanidins which fall within the category (a) defined by the Robinsons in 1935 are covalently bound to a carbohydrate matrix within the plant cell. In the light of earlier observations [9] on their biosynthesis it seems justifiable to consider that such proanthocyanidins may well result from the capture of the putative carbocation intermediate (**20**), or its quinone–methide equivalent, during biosynthesis by hydroxy-groups of saccharide structures in the plant cell (Fig. 3). In the case of plant gums such processes may occur post-mortally by processes analogous to acid-catalysed degradation in which species such as **20** are believed to be similarly released [16, 17].

Bate-Smith in his classical botanical and taxonomic surveys [21] consistently drew attention to the occurrence of proanthocyanidins in plants with a woody habit of growth and to the possibility of a relationship to lignification and a structural role for these substances. The proanthocyanidins bear many analogies to the plant lignins. Patterns of polymerization are dictated principally by the inherent chemical reactivity of the monomer precursors—intermediates such as **20** in the case of the proanthocyanidins. Soluble forms of the simple dimers (e.g. lignans and procyanidins) are found in many plants. Lignin is found not only in intimate physical contact with hemicelluloses in the plant cell wall but is directly linked by covalent linkages to such saccharides. Freudenberg suggested that this occurred during polymerization by addition of aliphatic hydroxyl groups of the carbohydrate to quinone–methide intermediates. Entirely analogous processes may be envisaged (Fig. 3) to lead directly to the 'insoluble' polymeric forms of proanthocyanidins.

EXPERIMENTAL

All mps were corrected. PC was performed at 20° using Whatman No 2 (27.5 cm^2) paper in the solvent systems: (A) 6% HOAc and (B) butan-2-ol–HOAc–H_2O (14:1:5) and sprayed as described earlier [3, 18]. Bark from 60–200 year old trees of *Larix gmelini* in the spring of 1983 was collected at the Yakeshi Tannin Extraction Plant (China).

Isolation of larixinol. Finely powdered bark (1100 g) was macerated with Me_2CO (3 × 2 l.) in a Waring blendor. After removal of the plant debris the Me_2CO extract was diluted with H_2O (1 l.) and evaporated at 30° to remove the Me_2CO. The aq. soln was extracted with $CHCl_3$ (3 × 800 ml) and then EtOAc (6 × 800 ml). The combined EtOAc extracts were evaporated at 30° to give the crude phenolic extract as a dark-red gum. The latter was chromatographed in EtOH on Sephadex LH-20 (5 × 50 cm). Rechromatography on LH-20 and then silica gel ($CHCl_3$–MeOH, 4:1) of the early fractions gave a product which when crystallized from MeOH–H_2O gave larixinol (1.55 g), mp 208–210°. Found: C, 64.6; H, 4.7. Calc. for $C_{30}H_{22}O_{10}\cdot CH_3OH$ C, 64.9; H, 4.7%. $[\alpha]_D^{20}$ − 151° (Me_2CO; *c* 1.0), R_f (A) 0.52, R_f (B) 0.83, ^1H NMR (Me_2CO-d_6): δ2.69 (2H, *m*), 4.18 (1H, *m*), 4.81 (1H, *s*), 5.89 (1H, *s*), 6.01 (1H, *d*, *J* = 2 Hz), 6.06 (1H, *d*, *J* = 2 Hz), 6.14 (1H, *s*), 6.63, 6.79 (4H, 2 × *d*, *J* = 9 Hz), 7.05 (4H, *d*, *J* = 9 Hz). ^{13}C NMR (Me_2CO-d_6): δ28.76, 61.12, 66.37, 78.74, 90.96, 91.05, 94.07, 96.81, 103.92, 105.80, 106.41, 115.28, 115.48, 127.69, 128.47, 128.00, 130.47, 157.30, 157.37, 152.20, 152.77,

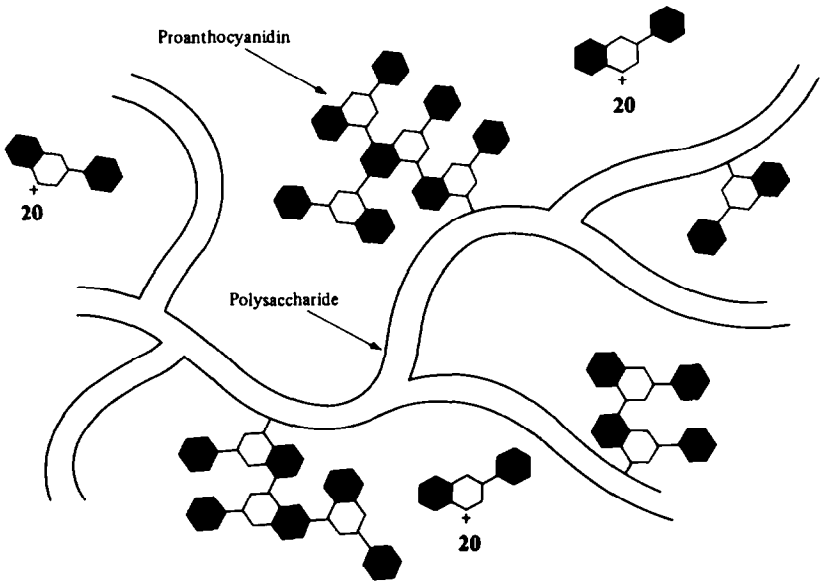

Fig. 3. Biosynthesis of 'insoluble' proanthocyanidins.

154.86, 157.82, 161.03, 163.42, 179.09. IR ν_{max}^{KBr} cm^{-1}: 3350, 1785 (γ-lactone), 1625, 1510.

Larixinol hexa-acetate (Ac_2O–pyridine) was isolated after chromatography (SiO_2; $CHCl_3$–MeOH, 40:1), and crystallized from $CHCl_3$–EtOH, mp 255–257°. Found: C, 63.2; H, 4.2. Calc. for $C_{42}H_{34}O_{16}$ C, 63.5; H, 4.28%. $[\alpha]_D^{20}$ +33.3° ($CHCl_3$; 0.9); ^{1}H NMR ($CDCl_3$): δ 1.73, 1.93, 2.27, 2.28, 2.29, 2.34 (18H, 6 × *s*), 2.96 (2H, *d*, *J* = 4 Hz), 5.12 (1H, *s*), 5.63 (1H, *m*), 6.42 (1H, *s*), 6.45 (1H, *d*, *J* = 2 Hz), 6.55 (1H, *s*), 6.78 (1H, *d*, *J* = 2 Hz), 7.05, 7.08 (4H, 2 × *d*, *J* = 9 Hz), 7.10, 7.26 (4H, 2 × *d*, *J* = 9 Hz). ^{13}C NMR ($CDCl_3$): δ 19.49, 20.68, 20.89, 21.14, 26.36, 59.65, 65.17, 76.90, 89.27, 99.04, 102.98, 109.75, 109.23, 116.88, 121.65, 121.78, 126.90, 126.95, 131.48, 133.54, 146.58, 146.71, 150.43, 151.09, 151.14, 152.47, 152.88, 162.01, 168.21, 170.24, 171.15, 171.22, 180.76. IR ν_{max}^{KBr} cm^{-1} 1810, 1765, 1620.

Larixinol pentamethyl ether (Me_2SO_4, Me_2CO, anhydr. K_2CO_3) was isolated after chromatography (SiO_2; $CHCl_3$–MeOH, 50:1) and crystallized from EtOAc, mp 183–186°, $[\alpha]_D^{20}$ −113.5° ($CHCl_3$; *c* 0.6). Found: C, 68.9; H, 5.4. Calc. for $C_{35}H_{32}O_{10}$ C, 68.85; H, 5.44%. IR ν_{max}^{KBr} cm^{-1}: 3410, 1790, 1615. ^{1}H NMR ($CDCl_3$): δ 2.4–2.95 (2H, *m*), 3.61, 3.70, 3.74, 3.84, 3.86; (15H, 5 × s), 4.08 (1H, *m*), 4.58 (1H, *s*), 6.05 (1H, *d*, *J* = 2 Hz), 6.06 (1H, *s*), 6.21 (1H, *s*), 6.23 (1H, *d*, *J* = 2 Hz), 6.62, 6.86 (4H, 2 × *d*), 7.01, 7.06 (4H, 2 × *d*, *J* = 9 Hz) (C_6D_6): δ 2.41 (1H, *dd*), 2.80 (1H, *dd*), 2.97 (3H, *s*), 3.03 (3H, *s*), 3.07 (3H, *s*), 3.38 (3H, *s*), 3.42 (3H, *s*), 3.75 (1H, *m*), 4.66 (1H, *s*), 5.98 (1H, *d*, *J* = 2 Hz), 6.06 (1H, *s*), 6.36 (1H, *d*, *J* = 2 Hz), 6.55 (1H, *s*), 6.56 (2H, *d*, *J* = 9 Hz), 6.89 (2H, *d*, *J* = 9 Hz), 7.05 (2H, *d*, *J* = 9 Hz), 7.35 (2H, *d*, *J* = 9 Hz). ^{13}C NMR ($CDCl_3$): δ 27.59, 55.17, 55.26, 55.65, 55.78, 60.21, 65.95, 77.44, 87.35, 88.95, 92.12, 92.80, 103.16, 105.72, 107.20, 112.99, 113.85, 126.47, 126.94, 128.07, 129.46, 150.03, 150.68, 151.81, 156.34, 159.29, 162.09, 163.10, 178.38. MS (probe) *m/z* (rel. int.): 612 $[M]^+$ (18), 584 (20), 434 (35), 284 (15), 269 (12), 231 (12), 163 (38), 150 (35), 135 (40), 121 (100).

Larixinol hexamethyl ether (MeI, NaH, DMSO) was isolated after chromatography on SiO_2 as a colourless gum MS (probe) *m/z*: 626 $[M]^+$, $[\alpha]_D^{20}$ −146.8° ($CHCl_3$; *c* 1.4). ^{1}H NMR ($CDCl_3$): δ 2.4–2.95 (2H, *m*), 3.03 (34, *s*), 3.62, 3.72, 3.73, 3.82, 3.83 (15H, 5 × *s*), 3.60 (1H, *m*), 4.59 (1H, *s*), 6.04 (1H, *s*), 6.05 (1H, *d*, *J* = 2 Hz), 6.18 (1H, *s*), 6.19 (1H, *d*, *J* = 2 Hz), 6.64, 6.78 (4H, 2 × *d*, *J* = 9 Hz), 6.96, 7.09 (4H, 2 × *d*, *J* = 9 Hz).

Hydrogenation of larixinol pentamethyl ether. Larixinol pentamethyl ether (70 mg) was hydrogenated (H_2, 1 atm, PtO_2) in EtOAc for 2.5 hr. The products were isolated by TLC (SiO_2 plates, EtOAc–light petrol, 1:1) to give compounds **9**–**12**. Each product was identified by mass, ^{1}H and ^{13}C NMR spectroscopy.

Compound **9** crystallized from $CHCl_3$–EtOAc, mp 202–204°. Found: C, 68.1; H, 6.0. Calc. for $C_{35}H_{38}O_{10}$: C, 67.9; H, 6.14%. ^{1}H NMR ($CDCl_3$): δ 1.4–2.2 (9H, *m*), 2.2–2.9 (2H, *m*), 3.35 (3H, *s*), 3.45 (2H, *m*), 3.58, 3.71, 3.73, 3.80 (12H, 4 × *s*), 4.11 (1H, *m*), 5.99 (1H, *d*, *J* = 2 Hz), 6.00 (1H, *s*), 6.14 (1H, *s*), 6.16 (1H, *d*, *J* = 2 Hz), 6.70 (2H, *d*, *J* = 9 Hz), 7.22 (2H, *d*, *J* = 9 Hz). ^{13}C NMR ($CDCl_3$): δ 22.35, 23.67, 28.47, 29.04, 29.74, 38.14, 55.29, 55.71, 60.65, 61.52, 75.06, 81.15, 87.27, 89.06, 92.47, 93.69, 102.87, 105.66, 107.04, 113.55, 127.04, 127.80, 151.22, 152.14, 156.40, 159.46, 159.63, 162.44, 163.25, 178.15. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2960, 2920, 1790, 1625, 1610. MS (probe) *m/z*: 618.

Compound **10**. ^{1}H NMR ($CDCl_3$): δ 1.4–2.3 (18 H, *m*), 2.6–2.9 (2H, *m*), 3.25, 3.38, 3.55, 3.74, 3.83 (15H, 5 × *s*), 3.3–3.6 (3H, *m*), 4.16 (1H, *m*), 4.62 (1H, *d*, *J* = 10.4 Hz), 5.88 (1H, *d*, *J* = 2 Hz), 6.01 (1H, *d*, *J* = 2 Hz), 6.34 (1H, *s*). MS (probe) *m/z*: 624.

$LiAlH_4$ reduction of larixinol pentamethyl ether. Larixinol pentamethyl ether (100 mg) was reduced for 24 hr in Et_2O (20 ml) with $LiAlH_4$ (0.2 g). The product was isolated as an oil (60 mg) after work up and TLC (SiO_2, light petrol–EtOAc, 1:2). Acetylation (Ac_2O–pyridine) gave a triacetate. ^{1}H NMR ($CDCl_3$): δ 1.65, 1.70, 1.76, 1.92, 1.98, 2.42 (OAc); 2.5–2.9 (2H, *m*); 3.57, 3.61, 3.65, 3.70–3.84 (OMe); 4.6–5.3 (3H, *m*), 5.07 (1H, *s*), 5.6–6.2 (4H, *m*), 6.44–6.75 (8H, *m*).

Isolation of procyanidin fractions. Bark powder (diameter 1–2 mm, 300 g) was macerated with MeOH (3 × 1000 ml) in a Waring Blendor. The methanolic soln, after removal of plant debris, and concn to small vol. (100 ml) was diluted with 500 ml distilled H_2O and then extracted with $CHCl_3$ (3 × 500 ml) and EtOAc (9 × 500 ml). The EtOAc extract was evaporated *in vacuo* to yield the crude phenolic extract (*ca* 33 g), which was dissolved in EtOH (75 ml) and chromatographed on a Sephadex LH-20 column (5 × 50 cm) in EtOH. Fractions (250 × 20 ml) were collected and analysed by one dimensional paper chromatography in solvents (A) and (B). Fractions were combined to give I (5.22 g) and II (1.634 g) for further separation. The column was finally eluted with MeOH (2000 ml). After evaporation *in vacuo*, the MeOH soluble polymeric proanthocyanidins were dried at 60° and 1 mmHg pressure for 48 hr and 6.6 g of product was obtained. After the MeOH extraction the bark residue was extracted with Me_2CO–H_2O (1:1, 2 × 2000 ml) at room temp. (2 × 48 hr). The extract, after removal of the residue, was slowly evaporated and the polymeric proanthocyanidins precipitated. The ppt was collected by high speed centrifugation and dried under the same conditions as described above.

Isolation and identification of flavan-3-ols in fraction I. Fraction I was rechromatographed on Sephadex LH-20 (5 × 30 cm) in EtOH. Fractions were collected and combined to I-1 and I-2. The fraction I-1 after chromatography on a SiO_2 column using 2-butanone–H_2O as eluant gave a compound with R_f (A) 0.43 and (B) 0.65. Its ^{1}H NMR was identical with the literature data [22] and it was identified as (−)-epiafzelechin (1).

The fraction I-2 was further separated on a SiO_2 column in $CHCl_3$–EtOH (4:1). Fractions (120 × 18 ml) were collected and 54–70 were combined to yield a mixture of (+)-catechin and (−)-epicatechin, as shown by 2D-PC. Acetylation (pyridine–Ac_2O) and chromatography on SiO_2 column in $CHCl_3$–MeOH (40:1) gave (+)-catechin penta-acetate as needles after crystallization from MeOH, mp 131–133°, $[\alpha]_{578}$ +40.1° ($CHCl_3$; *c* 1.0). Its ^{1}H NMR was identical with literature data. (−)-Epicatechin-penta-acetate was similarly obtained as needles, mp 151–152°, $[\alpha]_{578}$ −17.0° ($CHCl_3$; *c* 1.5). Its ^{1}H NMR was identical with literature data. Larixinol was crystallized from a MeOH–H_2O soln of I-2-2 fraction.

Isolation and identification of dimeric procyanidins. HPLC analysis of fraction II (5 mg in 1 ml MeOH) was performed on a Zorbax (Dupont) C_8 column (25 cm × 4.5 cm), mobile phase (A) MeOH (B) 5% HOAc. Gradient conditions: 100% B to 50% B/50% A in 20 min, 50% B/50% A to 100% A in 10 min, UV detection at 280 nm, flow rate 2 ml/min. The retention time of procyanidins B1, B2, B3 and B4 were 4'35", 6'32", 3'32" and 5'14" respectively. Fraction II (500 mg) was dissolved in a small amount of Me_2CO and chromatographed on a Sephadex LH-20 column in Me_2CO. Three fractions, which contained principally B2, B1 and B3, B4 were obtained. These three fractions were acetylated respectively with pyridine–Ac_2O. The acetylation products were purified on TLC with $CHCl_3$–EtOAc–Me_2CO (7:2:1). B2-deca-acetate (11 mg, R_f 0.44), B1-deca-acetate (16 mg, R_f 0.47), B3-deca-acetate (28 mg, R_f 0.34) and B4-deca-acetate (6 mg, R_f 0.32) were obtained. The ^{1}H NMR spectra of B1–B4 peracetates were identical with the literature data [10, 11, 19].

Chemical degradation of polymeric procyanidins. MeOH-soluble polymeric procyanidins (1.5 g) were dissolved in EtOH (30 ml) containing toluene-α-thiol (6 ml) and HOAc (4 ml). The mixture was refluxed in N_2 for 50 hr. The solvent was evaporated under vacuum and the residue was chromatographed on a

Sephadex LH-20 column (5 × 30 cm) in $CHCl_3$–EtOH (4:1). Fractions (400 × 20 ml) were collected and analysed by PC. Recombination and rechromatography on Sephadex LH-20 finally gave the thio ethers **16–18** identical with compounds previously described in the literature [3]. Compound **18** gave a tetramethyl ether (CH_2N_2), mp and mmp 57–58°, and a penta-acetate (Ac_2O–pyridine), mp and mmp 125–126°; **16** similarly gave a tetramethyl ether, mp and mmp 127–128°, and penta-acetate, mp and mmp 69°. In like manner (+)-catechin and (−)-epicatechin were similarly isolated and identified.

Determination of mean polymerization degree and number average molecular weight of polymeric procyanidins. MeOH-soluble polymeric procyanidins (100 mg) were dissolved in EtOH (8 ml) containing toluene-α-thiol (2 ml) and HOAc (1 ml). The mixture was refluxed in N_2 for 50 hr. The remaining toluene-α-thiol was removed from the reaction products by chromatography on a SiO_2 column using *n*-hexane as eluant and the mixture of degradation products were eluted with MeOH. They were then separated on a Sephadex LH20 column in EtOH and the thiol product fractions were combined. After evaporation, the residue was dissolved in 5 ml MeOH and used for HPLC analysis. The used conditions were as follows: column μ Bondapok ODS C_{18} (250 mm × 4 mm), mobile phase, A MeOH, B 5% HOAc, gradient conditions, 100%B to 50%B/50%A in 20 min, 50% B/50%A to 100%A in 5 min, detection, UV 280 nm, flow rate 2 ml/min. The retention time for (+)-catechin, (−)-epicatechin and their thio-ethers (**15**, **16**, **17**) were respectively 5'24", 8'15", 15'8", 16'15" and 18'40". The mean polymerization degree and number average molecular weight of MeOH-soluble polymeric procyanidins were calculated on the basis of quantitative determination of its degradation products by HPLC.

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