*The Chemistry of Extractives from Hardwoods. Part XXV.** (−)-epi*Afzelechin, a New Member of the Catechin Series.*

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Ether-extracts of the heartwood of *Afzelia* species contain kaempferol, dihydrokaempferol, and a previously unknown 3 : 5 : 7 : 4′-tetrahydroxyflavan. The new flavan closely resembles the 3 : 5 : 7 : 3′ : 4′-pentahydroxy-compound *epi*catechin and has thus been named *epi*afzelechin. The configurations of catechin and of *epi*catechin, concerning which there has hitherto been some ambiguity, have been re-examined in the light of current stereochemical theory, whereby the *cis*-conformation has been established for *epi*catechin and hence for its analogue *epi*afzelechin.

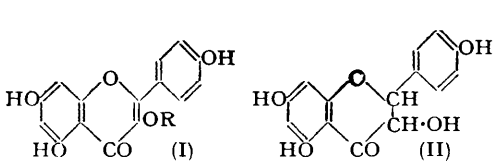
The investigation of a powdery material occluded in a sample of the commercial timber derived from *Afzelia* species revealed the occurrence of a kaempferol 3-rhamnoside, afzelin (I; R = $C_6H_{11}O_4$) (King and Acheson, *J.*, 1950, 168). The glycoside was accompanied by a small amount of a colourless compound $C_{15}H_{12}O_6$ provisionally described as a dihydrotetrahydroxyflavone, which at the time of its isolation had not been listed in abstract indexes. This substance has since been identified as a partly racemised specimen of (+)-dihydrokaempferol (II), previously found in the Japanese Judas tree (*Cercidiphyllum japonicum*) and named katuranin by Uoda, Fukushima, and Kondo (*J. Agric. Chem. Soc. Japan*, 1943, **19**, 467). We thank Professor S. Hattori for drawing attention in 1950 (personal communication) to the resemblance of the compound $C_{15}H_{12}O_6$ to katuranin, and for a specimen of the latter obtained from *C. japonicum* by Dr. M. Hasegawa (see *Chem. Abstr.*, 1953, **47**, 858) by which we have been able to confirm the identity of the product from afzelia wood. (+)-Dihydrokaempferol has also been found in *Nothofagus dombeyi* by Pew (*J. Amer. Chem. Soc.*, 1948, **70**, 3031) and with dihydroquercetin in the heartwood of *Larix decidua* by Gripenberg (*Acta Chem. Scand.*, 1952, **6**, 1152). The compound aromadendrin, long known as a constituent of *Eucalyptus kino*, has also been recognised as (+)-dihydrokaempferol (Hillis, *Austral. J. Sci. Res.*, 1952, **5**, 379).

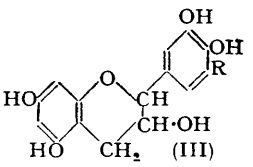
The extractives of the heartwood of *Afzelia* species have now been examined; specimens from various commercial sources were used and their derivation from members of the genus *Afzelia* was confirmed by Mr. B. J. Rendle, D.S.I.R. Forest Products Laboratory. Anatomical examination of the wood specimens alone is insufficient, however, to differentiate the individual species, and the precise botanical origin of the material could not therefore be ascertained.

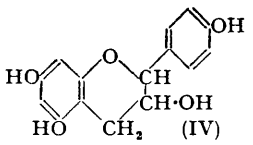
* Part XXIV, *J.*, 1955, 1338.

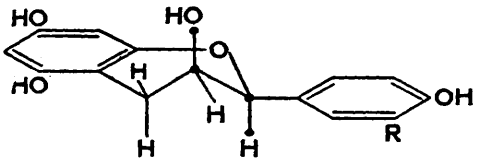
A colourless waxy material was first removed from the shredded wood with boiling light petroleum. Further extraction with boiling ether gave kaempferol (I; R = H), impure (+)-dihydrokaempferol (II), and a lævorotatory compound $C_{15}H_{14}O_5$, which with acids showed a marked inclination to polymerise. From its resemblance to the catechins (III; R = H), and in particular to *epi*catechin, this new product was later termed (−)-*epi*afzelechin and was ultimately identified as (IV). The three compounds were separated by treatment with hot water in which kaempferol is virtually insoluble; dihydrokaempferol—sometimes appreciably racemised—crystallises from the aqueous filtrate, and *epi*afzelechin is found in the residual solution. The rhamnoside afzelin (I; R = $C_6H_{11}O_4$) has not so far been encountered as a normal heartwood constituent, although it was again present among the contents (amounting to 25 g.) of a fissure in a further sample of the wood, the water-soluble glycoside being apparently the form in which the phenolic and presumably fungicidal component kaempferol is readily transported to the damaged tissues. As with the heartwood extractive, small amounts of dihydrokaempferol and of *epi*afzelechin were also found in the shake (fissure) contents, but in neither material was detected any compound of the intermediate flavan-3 : 4-diol series similar to the *leuco*anthocyanidin melacacidin (King and Bottomley, *J.*, 1954, 1399).

*epi*Afzelechin yields a tetra-acetate and thus contains four hydroxyl groups of which three were shown to be phenolic by the formation of an alkali-insoluble trimethyl ether affording a monoacetate and toluene-*p*-sulphonate. The latter ester develops an orange colour on exposure to the atmosphere, a property later ascribed to the formation of 5 : 7 : 4′-trimethoxyflavylium toluene-*p*-sulphonate. The new flavan-3-ol possesses the typical catechin reactions (see experimental section), and the positive vanillin–hydrochloric acid test indicated the presence of a phloroglucinol nucleus.

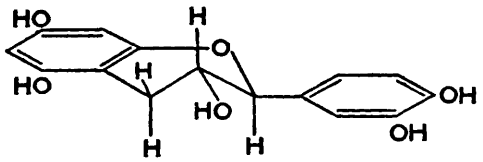




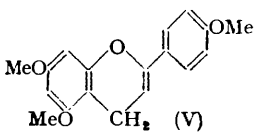


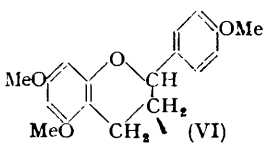


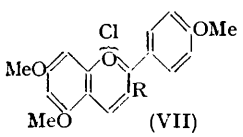
(IV*A*) *epi*Catechin (R = OH). *epi*Afzelechin (R = H)

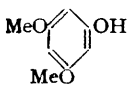


(IV*B*) Catechin

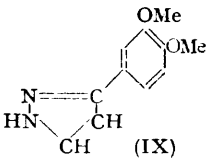


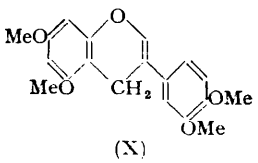






(VIII)





Oxidation of the trimethyl ether with potassium permanganate in acetone produced *p*-anisic acid and in conjunction with the data already outlined this led to the tentative adoption of structure (IV).

When the 5 : 7 : 4′-trimethyl ether 3-toluene-*p*-sulphonate was treated with anhydrous hydrazine it was smoothly converted into the flaven (V), thus resembling the corresponding

*epi*catechin derivative (Freudenberg, Fikentscher, and Harder, *Annalen*, 1925, **441**, 157) and in contrast to the analogous catechin ester which undergoes disintegration (*vide infra* and Freudenberg, Orthner, and Fikentscher, *Annalen*, 1924, **436**, 286). The flaven (V) exhibited the expected light absorption and was catalytically reduced by one molecular equivalent of hydrogen to the flavan (VI) indistinguishable from (±)-5 : 7 : 4′-trimethoxyflavan prepared by a similar reduction of 5 : 7 : 4′-trimethylapigenidin chloride (VII; R = H). Conversely, the flaven (V) was converted into the flavylium chloride (VII; R = H) by atmospheric oxidation in presence of hydrogen chloride (cf. similar oxidations, Baker, *J.*, 1929, 1593).

Corroborative evidence for the structure (IV) was obtained by catalytic hydrogenation of 5 : 7 : 4′-trimethylpelargonidin chloride (VII; R = OH) to (±)-*epi*afzelechin trimethyl ether, m. p. 105°, the latter having an ultraviolet absorption identical with that of the natural compound, m. p. 110° (mixed m. p. 105°). Similarly, natural trimethyl*epi*afzelechin acetate, m. p. 133°, did not depress the m. p. of the corresponding racemic acetate, m. p. 123°. Finally, comparison of the infrared absorption of the two methyl ethers and of their acetates in carbon tetrachloride solution was carried out by Dr. F. B. Strauss (through the kindness of Sir Robert Robinson, O.M., F.R.S.) who reports that, despite certain restrictions due to the solvent, sufficient absorption bands are present in the spectra to establish the relationship of the respective derivatives. Thus afzelechin is related to the flavonol kaempferol as the catechins are to quercetin. The gallocatechins (III; R = OH), an analogous group based on the flavonol myricetin, contain a pyrogallol residue as 2-substituent. These comprise a racemate (Bradfield and Penney, *J.*, 1948, 2249), a dextrorotatory isomer casuarin (Oshima, *J. Agric. Chem. Soc. Japan*, 1939, **15**, 636), and the gallocatechin isolated from tea by Tsujimura (*Sci. Papers Inst. Phys. Chem. Res., Tokyo*, 1925, **10**, 252). The new catechin with its 2-*p*-hydroxyphenyl group is therefore the simplest naturally occurring example of the series so far known.

The derivation of formula (IV) now leaves only stereochemical features to be discussed, although the fact has already been established by comparison of the physical and chemical properties of *epi*afzelechin with those of catechin and of *epi*catechin that the new compound, as its name implies, is a member of the *epi*-series (Table). However, an examination of

(−)-*epi*Afzelechin	(−)-*epi*Catechin	(+)-Catechin
Small needles, m. p. 240—243° (decomp.), $[\alpha]_D^{19}$ −59° (5%, EtOH)	Stout prisms, m. p. 237—239° (decomp.), $[\alpha]_{Hg}$ −69° (7%, EtOH)	Hydrate, fine needles, m. p. 93—95°; anhydrous, m. p. 174—175° (decomp.), $[\alpha]_{Hg}^{0}$ 0° (EtOH), +17° (H_2O–acetone; 1 : 1)
Tetra-acetate, m. p. 126—127°	Penta-acetate, m. p. 151—152°	Penta-acetate, m. p. 131—132°
5 : 7 : 4′-Trimethyl ether, m. p. 110°, $[\alpha]_D^{20}$ −67° (2%, EtOH)	5 : 7 : 3′ : 4′-Tetramethyl ether, m. p. 153—154°, $[\alpha]_{Hg}$ −61·5° (4%, $C_2H_2Cl_4$)	5 : 7 : 3′ : 4′-Tetramethyl ether, m. p. 143—144°, $[\alpha]_{Hg}$ −13·4° (3%, $C_2H_2Cl_4$)
Trimethyl ether acetate, m. p. 133°, $[\alpha]_D$ −73·8° (2%, $CHCl_3$)	Tetramethyl ether acetate, m. p. 91—92°, $[\alpha]_{Hg}$ −71·2° (5%, $C_2H_2Cl_4$)	Tetramethyl ether acetate, m. p. 95—96°, $[\alpha]_{Hg}$ +6·8° (10%, $C_2H_2Cl_4$)
Trimethyl ether toluene-*p*-sulphonate, m. p. 165°, $[\alpha]_D^{20}$ −9° (3%, $CHCl_3$), with anhyd. hydrazine yields 5 : 7 : 4′-trimethoxyflav-2-en, m. p. 129—130°	Tetramethyl ether toluene-*p*-sulphonate, m. p. *ca.* 165°, $[\alpha]_{Hg}^{17}$ −16·9°, with anhyd. hydrazine yields 5 : 7 : 3′ : 4′-tetramethoxyflav-2-en, m. p. 119°	Tetramethyl ether toluene-*p*-sulphonate, m. p. 86—87°, $[\alpha]_D^{14}$ +22·7° ($C_2H_2Cl_4$), with anhyd. hydrazine yields mainly fission products (phloroglucinol dimethyl ether and 3-3′ : 4′-dimethoxyphenylpyrazoline)
(±)-5 : 7 : 4′-Trimethyl ether, m. p. 105° (acetate, m. p. 123°)	(±)-5 : 7 : 3′ : 4′-Tetramethyl ether, m. p. 141—142° (acetate, m. p. 160—161°)	(±)-5 : 7 : 3′ : 4′-Tetramethyl ether, m. p. 142° (acetate, m. p. 134—135°)

the literature reveals that doubt still exists as to the true configurations of the diastereoisomeric catechins. Freudenberg in his earlier memoirs (*Annalen*, 1925, **441**, 157; **443**, 309; **446**, 87) supposed catechin to have the *cis*-structure ($H_{(2)}$ and $H_{(3)}$ *cis*) and that in the isomeric *epi*catechin the configuration was *trans*, but later this assertion was withdrawn and the question was left undecided (*Annalen*, 1927, **451**, 214; *J. Amer. Chem. Soc.*, 1932, **54**, 1917). Others, *e.g.*, Geissman and Lischner (*J. Amer. Chem. Soc.*, 1952, **74**, 3001) and

Hergert and Kurth (*J. Org. Chem.*, 1953, **18**, 521), later adopted the *cis*-structure for *epi*-catechin, the evidence on which this configuration had originally been attributed to catechin having been described by Hückel, Gerke, and Frank (*Annalen*, 1929, **477**, 159) as equivocal. On the other hand, Warburton in a recent review of *iso*flavones (*Quart. Rev.*, 1954, **8**, 67) represents catechin as the *cis*-isomer, which is in agreement with the convention adopted in " A Dictionary of Applied Chemistry " (Thorpe, 4th Ed., Vol. II, p. 43). In view of these conflicting conclusions we have re-examined the evidence concerning the stereochemistry of the catechins.

Molecular models of the conventional kind show that the heterocyclic nucleus present in the flavans is puckered and, if the concepts of the conformational theory of cyclic structures are applied to this group of compounds, the isomeric catechins can be represented by formulæ of the type (IV*A*; R = OH) and (IV*B*). With the aid of these conformational diagrams chemical differences between the two diastereoisomerides can readily be interpreted and it has thus been possible to deduce with certainty their respective stereochemical configurations. The action of hydrazine on the tetramethyl ether 3-toluene-*p*-sulphonates, for example, which as already stated affords a high yield of the related Δ^2-flaven from the *epi*catechin derivative, occurs less readily with the corresponding catechin ester and the main products are *OO*-dimethylphloroglucinol (VIII) and the pyrazoline (IX) (Freudenberg, Fikentscher, and Harder, *Annalen*, 1925, **441**, 157; Freudenberg, Orthner, and Fikentscher, *ibid.*, 1924, **436**, 286). Formation of the flaven (V) is attributed to the stereospecific *trans*-elimination (E_2) of toluene-*p*-sulphonic acid from the *epi*catechin ester, in which the four participating centres (indicated by black dots in IV*A*; R = OH) are co-planar. The configuration of groups in the catechin ester (cf. IV*B*) is unfavourable for the formation of (V), and it has been suggested that the observed products (VIII) and (IX) arise *via* the intermediate flav-3-en (Freudenberg, Carrara, and Cohn, *Annalen*, 1925, **446**, 87). The view that these eliminations proceed by an E_2 mechanism rather than E_1 is supported by the fact that fission of the carbon skeleton leading to (VIII) and (IX) occurs without molecular rearrangement, whereas when tetramethylcatechin toluene-*p*-sulphonate is heated with quinoline it yields the *iso*flaven (X) (Freudenberg, Carrara, and Cohn, *loc. cit.*). This may be interpreted as a slow heterolysis of the $C_{(3)}$–O bond and rearrangement of the resulting carbonium ion. A similar rearrangement occurs in the formation of 2-chloro-5 : 7 : 3′ : 4′-tetramethoxy*iso*flavan from tetramethylcatechin and phosphorus pentachloride (Drumm, MacMahon, and Ryan, *Proc. Roy. Irish Acad.*, 1923—24, **36**, *B*, 41; 149; 1929, **39**, *B*, 114; Freudenberg, Carrara, and Cohn, *loc. cit.*) which, in terms of current stereochemical theory, proceeds by a synchronous carbonium-ion mechanism, as with other 1 : 2-shifts. It is therefore clear that catechin is the *trans*-isomer (IV*B*), and that *epi*catechin (IV*A*; R = OH) and *epi*afzelechin (IV*A*; R = H) have *cis*-configurations. Further corroboration is to be found in the formation of the *epi*-series of compounds by catalytic reduction of anthocyanidin derivatives, a consequence of the expected *cis*-addition of hydrogen to the planar flavylium nucleus.

The probable stereochemical structures of the gallocatechins also may now be inferred from recorded data, the isomer obtained by Tsujimura (*loc. cit.*) belonging evidently to the *epi*-series from its close analogy in m. p. (218°) and specific rotation (−67·5°) to *epi*catechin (m. p. 237—239°; $[\alpha]_D$ −69°) and to *epi*afzelechin (m. p. 240—243°; $[\alpha]_D$ −59°). Similarly it can be argued that casuarin (m. p. 181—183°; $[\alpha]_D$ +19·7°) is the (+)-gallocatechin from its resemblance to (+)-catechin (m. p. 174—175°; $[\alpha]_D$ +17°). The isomers of catechin occur predominantly in the forms (+)-catechin and (−)-*epi*catechin; the isolation of (−)-*epi*afzelechin, and the existence of the gallocatechins as (−)-*epi*gallocatechin and (+)-gallocatechin, indicate an interesting uniformity in the naturally occurring geometrical species of this group of flavans.

Experimental

Except where otherwise indicated, alcoholic solutions were used for ultraviolet absorption measurements.

Isolation of Kaempferol (I; R = H) *and* (+)-*Dihydrokaempferol* (II).—Afzelia heartwood (2400 g.) was extracted in a Soxhlet apparatus with boiling light petroleum (b. p. 60—80°) for

12 hr. and then with ether for 16 hr.; evaporation of the ether left a residue (88 g., 3·7%). Repeated extraction of this residue with boiling water left an insoluble fraction of crude flavonol (*ca.* 10 g., 0·4%) which, by repeated crystallisation from acetic acid and from aqueous ethanol (50%), afforded kaempferol in small yellow prisms, m. p. 279—280° (decomp.) alone and when mixed with an authentic specimen (Found, in material dried at 110° *in vacuo*: C, 63·4; H, 3·5. Calc. for $C_{15}H_{10}O_6$: C, 62·9; H, 3·5%). Light absorption: $\lambda_{max.}$ 266 (ε 22,000) and 366 mμ (ε 17,000). Kaempferol tetra-acetate crystallised from water in colourless needles, m. p. 183°; Kostanecki, Lampe, and Tambor (*Ber.*, 1904, **37**, 2096) give m. p. 181°. Kaempferol tetramethyl ether crystallised from methanol in needles, m. p. 161—162° (Found: C, 66·7; H, 5·6; OMe, 35·1. Calc. for $C_{19}H_{18}O_6$: C, 66·7; H, 5·3; OMe, 36·3%). Ramchandra, Rao, and Seshadri record m. p. 165—166° (*Proc. Indian Acad. Sci.*, 1946, **24**, *A*, 456).

Dihydrokaempferol (II).—Crystallisation of the combined hot water extracts of the ether residue, after filtration from undissolved kaempferol, afforded partially racemised dihydrokaempferol dihydrate (20 g., 0·8%) in fine needles, m. p. 222—224° (Found: C, 56·1; H, 5·3. Calc. for $C_{15}H_{12}O_6,2H_2O$: C, 55·6; H, 5·0. Found, in material dried at 110° *in vacuo*: C, 62·8; H, 4·5; loss, 11·6. Calc. for $C_{15}H_{12}O_6$: C, 62·5; H, 4·2; loss, 11·1%), $[\alpha]_D$ +2·5° (1% in EtOH), $[\alpha]_D$ +9° (0·9% in acetone–H_2O, 1 : 1); literature values: $[\alpha]_D^{20}$ +46° (4% in acetone–H_2O, 1 : 1), $[\alpha]_D^{20}$ +13° (4% in EtOH) (Pew, *J. Amer. Chem. Soc.*, 1948, 70, 3031). Light absorption: $\lambda_{max.}$ 214 (ε 28,000) and 292 mμ (ε 17,800). Dihydrokaempferol dihydrate became yellow when heated above 200° in soda-glass capillaries and melted at 222—224° (decomp.) after shrinking at 218°; in Pyrex capillaries, it had m. p. 246° (decomp., rapid rate of heating). The dependence of the m. p. on the type of glass capillary has been noted by Hillis (*Austral. J. Sci. Res.*, 1952, **5**, 379). Dihydrokaempferol from *Afzelia* heartwood proved indistinguishable from the compound $C_{15}H_{12}O_6,2H_2O$ obtained by King and Acheson (*J.*, 1950, 168) and also from katuranin, m. p. 224—225° (Uoda, Fukushima, and Kondo, *J. Agric. Chem. Soc. Japan*, 1943, **19**, 467—477), when the three specimens were examined by mixed m. p.s, paper chromatography (see below), and by colour reactions. Dihydrokaempferol gives a brownish-purple colour with aqueous ferric chloride and a pink coloration in the magnesium– and zinc–hydrochloric acid reactions.

Kaempferol tetra-acetate (1 g.), needles, m. p. and mixed m. p. 183—184°, was obtained by addition of acetic anhydride (5 g.) to dihydrokaempferol (3·6 g.) dissolved in a slight excess of aqueous sodium hydroxide (10%) at 0° (Found, in material dried at 110° *in vacuo*: C, 60·6; H, 4·5. Calc. for $C_{23}H_{18}O_{10}$: C, 60·8; H, 4·0%).

Isolation of (−)-epi*Afzelechin* (IV).—A sample of afzelia (2800 g.), of a somewhat lighter colour than that which had yielded kaempferol and dihydrokaempferol, was extracted with light petroleum (b. p. 60—80°; 24 hr.) and then with ether (24 hr.). The residue (*ca.* 20 g., 0·7%) obtained by evaporation of the ether-extract dissolved almost completely in hot water (1·5 l.), and insoluble material was discarded. After concentration under reduced pressure the aqueous solution (200 c.c.; charcoal) deposited a yellowish solid, m. p. 243—246° (decomp.) (15 g., 0·55%). Recrystallisation of this solid from aqueous ethanol (charcoal) afforded (−)-epi*afzelechin* [(−)-cis-**3** : **5** : **7** : **4′**-*tetrahydroxyflavan*] in colourless needles (9·1 g.), m. p. 240—243° (decomp.), $[\alpha]_D^{19}$ −58·9° (3% in EtOH) [Found: C, 65·5; H, 5·6%; *M* (Rast), 269. $C_{15}H_{14}O_5$ requires C, 65·7; H, 5·2%; *M*, 274]. Light absorption: $\lambda_{max.}$ 207 (ε 43,600) and 276 mμ (ε 2200). Concentration of the mother-liquors after collection of the tannin (9·1 g.) yielded a further 3·9 g., m. p. 239° (decomp.), and 1·2 g., m. p. 241° (decomp.), which shows that the original 15 g. consisted almost entirely of (−)-*epi*afzelechin. Isolation of the new tannin was greatly facilitated by the comparative freedom of this sample of wood from kaempferol and dihydrokaempferol. Subsequent extraction of other samples gave kaempferol (0·15—0·3%) and mixtures (0·15—0·6%) shown by paper chromatography to consist of dihydrokaempferol and (−)-*epi*afzelechin; a convenient separation of such mixtures has not yet been achieved.

Colour Reactions of (−)-epi*Afzelechin*.—(−)-*epi*Afzelechin gave no colour in the magnesium– or zinc–hydrochloric acid test, or when treated with sodium amalgam and subsequently acidified. A solution of vanillin in concentrated hydrochloric acid gave an immediate red colour with (−)-*epi*afzelechin. A methanolic solution gave a faint green colour with aqueous ferric chloride, but no colour was visible on paper chromatograms sprayed with the reagent. When warmed with concentrated sulphuric acid (−)-*epi*afzelechin gave an intense red colour which became more intense as gentle heating was continued, until finally obscured by charring; (+)-catechin behaved similarly in this respect. The red colour with sulphuric acid developed slowly in the cold, but rapidly in the presence of acetic anhydride. The catechin nature of (−)-*epi*afzelechin was revealed by the phlobaphene reaction: a solution of the tannin quickly formed a reddish

precipitate (phlobaphene) when boiled with concentrated hydrochloric acid; when *epi*afzelechin was boiled with dilute hydrochloric acid a yellowish-white precipitate was formed more slowly; with boiling dilute aqueous sodium hydroxide the tannin gave a yellow solution which yielded a pink precipitate after acidification. (−)-*epi*Afzelechin gave a dense white precipitate when boiled with aqueous formaldehyde; a similar precipitate developed more slowly by the acid-catalysed reaction in the cold.

3 : 5 : 7 : 4′-*Tetra-acetoxyflavan*.—A mixture of (−)-*epi*afzelechin (0·5 g.), sodium acetate (0·5 g.), and acetic anhydride (5 c.c.) was boiled for 2 hr. The residue obtained by evaporation of the solution was insoluble in water but freely soluble in aqueous methanol; the *tetra-acetate* crystallised from aqueous acetic acid in prisms, m. p. 121—122° raised to m. p. 126—127° by recrystallisation (Found: C, 62·0; H, 4·9; Ac, 39·5. $C_{23}H_{22}O_9$ requires C, 62·4; H, 5·0; Ac, 38·9%). The acetate gave an immediate red colour with cold sulphuric acid.

(−)-cis-5 : 7 : 4′-*Trimethoxyflavan*-3-*ol*.—A mixture of (−)-*epi*afzelechin (3·3 g.), anhydrous potassium carbonate (5·5 g., 3·3 mol.), methyl sulphate (1·7 c.c., 1·5 mol.), and dry acetone (50 c.c.) was boiled gently for 3 hr. before the addition of further methyl sulphate (1·7 c.c.), and gentle boiling was maintained until the following day; then aqueous ammonia and ether were added to the suspension. Concentration of the ethereal extracts, after washing with N-sodium hydroxide and water, afforded a residue (2·9 g., 76%) of crude (−)-cis-5 : 7 : 4′-*trimethoxyflavan*-3-*ol*, m. p. 105—106° raised by recrystallisation from methanol to m. p. 110° (prisms), $[\alpha]_D^{20}$ −67·4° (2% in EtOH) [Found: C, 68·5; H, 6·6; OMe, 28·3. $C_{18}H_{20}O_5$ requires C, 68·3; H, 6·4; OMe, 29·4%; *M*, by cryoscopic method in camphor (discoloration), 298, in exaltone, 332. Required: *M*, 316]: Light absorption: $\lambda_{max.}$ 208 (ε 53,400) and 274 mμ (ε 2190). The *acetate*, prepared by boiling the trimethyl ether with acetic anhydride, crystallised readily from ethanol in needles, m. p. 133°, $[\alpha]_D^{21}$ −73·8° (2% in $CHCl_3$) (Found: C, 66·8; H, 6·25. $C_{20}H_{22}O_6$ requires C, 67·0; H, 6·2%). In carbon tetrachloride solution the carbonyl absorption band occurred at 5·75 μ.

The trimethyl ether and its acetate when warmed gently with concentrated sulphuric acid gave a red colour similar to that of (−)-*epi*afzelechin.

Potassium permanganate oxidation. A solution of (−)-*epi*afzelechin trimethyl ether (0·1512 g.) in acetone (50 c.c.) was heated on a boiling-water bath and treated with powdered potassium permanganate (1·8 g.) in portions until a faint pink colour persisted. A suspension in dilute sulphuric acid (20 c.c.) of the solid, collected by filtration, was treated with sulphur dioxide, and the resultant solution was boiled and filtered. *p*-Anisic acid (0·0153 g., 21%) crystallised from the cold solution in needles, m. p. and mixed m. p. 183—184°.

(−)-cis-5 : 7 : 4′-*Trimethoxyflavan*-3-*ol Toluene*-p-*sulphonate*.—An anhydrous pyridine (3 c.c.) solution of (−)-*epi*afzelechin trimethyl ether (1·5 g.) and toluene-*p*-sulphonyl chloride (1·2 g.) was heated on a steam-bath for 40 min. and then diluted with ethanol. The precipitated solid (1·1 g.), m. p. 162—163° (decomp.), was collected and washed with ethanol and with ether; the filtrate and washings yielded a further 0·3 g., m. p. 155° (total 1·4 g., 65%). The *toluene*-p-*sulphonate* crystallised from ethanol in needles, m. p. 165° (to an orange melt), $[\alpha]_D^{20}$ −8·7° (3% in $CHCl_3$) (Found, in material dried at 100° for ½ hr.: C, 63·4; H, 5·6. $C_{15}H_{26}O_7S$ requires C, 63·8; H, 5·6%). The formation and properties of this compound correspond closely with those of the (−)-*epi*catechin analogue (Freudenberg, Fikentscher, and Harder, *Annalen*, 1925, **441**, 157—180) including the fact that the yield is seriously diminished by extending the reaction time.

When exposed to air and light the toluene-*p*-sulphonate developed an orange colour on the surface resembling the colour of apigenidin trimethyl ether (which had $\lambda_{max.}$ 476 μ and $E_{1\,cm.}^{1\%}$ 1240 in 0·001N-hydrogen chloride in 95% ethanol). The nature of the colouring matter was confirmed by examination of a solution of the ester (0·08144 g./100 c.c. of 0·001N-HCl in ethanol) in the visible region, which revealed a single peak ($\lambda_{max.}$ 476 mμ, $E_{1\,cm.}^{1\%}$ 1·19) of the same shape and at the same position as that of the anthocyanidin; this observation is compatible with the presence in the ester examined of the equivalent of *ca.* 0·1% of apigenidin trimethyl ether. The orange coloration in the melt of the *epi*catechin analogue (Freudenberg *et al.*, *loc. cit.*) is doubtless caused by the formation of traces of luteolidin tetramethyl ether.

5 : 7 : 4′-*Trimethoxyflav*-2-*en* (V).—The foregoing ester (0·5154 g.) was heated with anhydrous hydrazine (20 c.c.) in a sealed tube at 132° (chlorobenzene vapour) for 40 min. Next day the needle crystals of 5 : 7 : 4′-*trimethoxyflav*-2-*en* (0·1812 g., 55%), m. p. 129—130° unchanged by recrystallisation from ethanol (needles), were collected on a sintered-glass Gooch crucible (Found: C, 72·4; H, 6·3. $C_{18}H_{18}O_4$ requires C, 72·5; H, 6·1%). Light absorption: $\lambda_{max.}$ 205 (ε 45,000), 247 (ε 23,800), and 272 mμ (ε 7600). Karrer and Seyhan (*Helv. Chim.*

Acta, 1950, **33**, 2209—2210) record m. p. 118—119° for the isomeric flav-3-en prepared by lithium aluminium hydride reduction of apigenidin trimethyl ether.

The experimental conditions described above for the preparation of the flav-2-en were adopted after trial experiments with aqueous and anhydrous hydrazine at the b. p. had proved unsatisfactory, although some of the flaven was undoubtedly formed. The modified conditions have the advantage over those of Freudenberg, Fikentscher, and Harder (*loc. cit.*) that a homogeneous solution is soon formed and the product separates from the cold solution in an analytically pure form.

Formation of Apigenidin Trimethyl Ether.—A solution of the flav-2-en (*ca.* 0·02 g.) and dry hydrogen chloride in chloroform (10 c.c.) and benzene (10 c.c.) developed an orange colour slowly and after seven days at room temperature it was filtered from dark brown needles. Recrystallisation from 4N-hydrochloric acid afforded apigenidin trimethyl ether pentahydrate in orange needles, m. p. and mixed m. p. 159—160° (decomp.); the light absorption curve was indistinguishable from that recorded below for an authentic specimen. For analogous conversions see Baker (*J.*, 1929, 1593).

(±)-5 : 7 : 4′-*Trimethoxyflavan* (VI).—(*a*) A solution of 5 : 7 : 4′-trimethoxyflav-2-en (0·0577 g.) in glacial acetic acid (5 c.c.) was shaken with hydrogen at room temperature and pressure over platinum black (*ca.* 0·05 g.). Hydrogen was absorbed rapidly at first (1 c.c. in 2·5 min., 2 c.c. in 7·5, 3 c.c. in 14, 3·5 c.c. in 20 min., 4 c.c. in 1 hour) and the hydrogenation was discontinued after 97 min. (4·1 c.c.; theory 4·6 c.c. at 20°/760 mm.). After filtration from catalyst the solvent was removed by freeze-drying, and crystallisation of the residue from light petroleum (b. p. 40—60°) afforded (±)-5 : 7 : 4′-*trimethoxyflavan* in small prisms, m. p. 105—106° alone and when mixed with the authentic specimen described below (Found: C, 71·4; H, 6·7. $C_{18}H_{18}O_4$ requires C, 72·0; H, 6·7%). Light absorption: $\lambda_{max.}$ 208 (ε 55,200) and 274 mμ (ε 2400). Hydrogenation of the flav-2-en in ethanol was inconveniently slow.

(*b*) An alcoholic solution of apigenidin trimethyl ether pentahydrate (1 g.) (prepared as described below) when shaken with hydrogen at room temperature and pressure over platinum black (*ca.* 0·5 g.) absorbed 56 c.c. (1 mol.) in 3 min. After absorption of the second molecular equivalent of hydrogen, which requires 3 hr., the catalyst was removed and the filtrate was concentrated under reduced pressure. An ethereal solution of the residual oil was washed with aqueous sodium hydroxide and with water, and after removal of the ether the residue separated during spontaneous evaporation of its aqueous-ethanol solution as a buff coloured powder (0·36 g., 50%), m. p. 99—100°. (±)-5 : 7 : 4′-Trimethoxyflavan crystallised slowly from light petroleum (b. p. 40—60°) in small prisms, m. p. 107—108°, and from aqueous methanol in rosettes of larger prisms, m. p. 107—108° (Found: C, 72·0; H, 7·0. Calc. for $C_{18}H_{20}O_4$: C, 72·0; H, 6·7%). The flavan is readily soluble in benzene and in alcohols, moderately soluble in hot light petroleum, and insoluble in water and in aqueous sodium hydroxide. It has a pronounced tendency to separate as an oil from aqueous-alcoholic solutions.

Preparation of Apigenidin Trimethyl ether (VII; R = H).—5 : 7 : 4′-*Trimethoxyflavylium chloride pentahydrate* was obtained in the form of orange needles (70%), m. p. 159—160° (decomp.), by the method of Pratt, Robinson, and Williams (*J.*, 1924, 205) who do not record m. p., yield, or analysis (Found: C, 51·7; H, 6·2. $C_{18}H_{17}O_4Cl,5H_2O$ requires C, 51·1; H, 6·4. Found, after drying *in vacuo* at 110°: C, 64·5; H, 5·3; loss 20·3. $C_{18}H_{17}O_4Cl$ requires C, 65·0; H, 5·2; loss 21·3%). Light absorption: $\lambda_{max.}$ 207 (ε 30,900), 278 (ε 19,850), 326 (ε 4840), and 476 mμ (ε 35,400); in 0·001N-HCl in EtOH: $\lambda_{max.}$ 208 (ε 28,200), 242 (ε 10,600), 278 (ε 21,600), 324 (ε 6300), and 476 mμ (ε 41,300).

(±)-cis-5 : 7 : 4′-*Trimethoxyflavan*-3-*ol*.—3-Hydroxy-5 : 7 : 4′-trimethoxyflavylium chloride trihydrate (1 g.) (prepared as described below) in ethanol (50 c.c.) was hydrogenated at room temperature and pressure over platinum black (*ca.* 0·5 g.). When hydrogenation was complete (4 hr.) the catalyst was removed and the filtrate was concentrated under reduced pressure, to leave a residue which dissolved in ether (charcoal). Removal of the ether, after the solution had been washed with aqueous sodium hydroxide and water, left a colourless viscous residue (A) (*ca.* 0·8 g.) which crystallised slowly at 0° from a mixture of benzene (4 c.c.) and light petroleum (6 c.c.). Recrystallisation from aqueous methanol of this crude material (0·1570 g., 17%), m. p. 97—98° to a turbid liquid clearing at 103°, afforded (±)-cis-5 : 7 : 4′-*trimethoxyflavan*-3-*ol* as a microcrystalline solid which softened at *ca.* 99° and melted to a clear liquid at 105° (Found: C, 68·4; H, 6·4. $C_{18}H_{20}O_5$ requires C, 68·3; H, 6·4%). The m. p. of the racemic compound was unaltered by admixture with (−)-*epi*afzelechin trimethyl ether (m. p. 110°) described above. The infrared absorption curves of the (−)- and the (±)-compound dissolved in carbon tetrachloride showed an exact correspondence. The light absorption of

the (±)-compound was indistinguishable from that of (−)-*epi*afzelechin trimethyl ether: $\lambda_{max.}$ 208 (ε 53,100) and 274 mμ (ε 2200).

The *acetate* of (±)-*cis*-5 : 7 : 4′-trimethoxyflavan-3-ol which separated from aqueous methanol in prisms (0·023 g.), m. p. 123° (Found: C, 67·0; H, 6·2. $C_{20}H_{22}O_6$ requires C, 67·0; H, 6·2%), was obtained by acetylation of part of the material (A). The m. p. of the (±)-acetate was unaltered by admixture with the (−)-*cis*-3-acetoxy-5 : 7 : 4′-trimethoxyflavan, of m. p. 133°, previously described. The infrared absorption curves of the (−)- and the (±)-acetate dissolved in carbon tetrachloride showed an exact correspondence; the carbonyl absorption band occurred at 5·75 μ.

Preparation of Pelargonidin Trimethyl Ether (VII; R = OH).—2-Hydroxy-4 : 6-dimethoxybenzaldehyde (30%), needles, m. p. 70°, was prepared essentially as described by Freudenberg, Fikentscher, and Wermer (*Annalen*, 1925, **442**, 309—322) except for the use of Adams's modification of the Gattermann reaction (Adams and Levine, *J. Amer. Chem. Soc.*, 1923, **45**, 2373), which may account for the lower yield. A solution of the aldehyde (4 g.) and ω-acetoxy-4-methoxyacetophenone (4 g., m. p. 59°) (Tiffeneau, *Compt. rend.*, 1910, **150**, 1182) in ethyl acetate (50 c.c.) and ethanol (10 c.c.) was saturated at 0° with dry hydrogen chloride; after 7 days at room temperature the solution was diluted with dry ether (60 c.c.) and stored at 0° after saturation at this temperature with dry hydrogen chloride. Ten days later the solution was filtered from a *dihydrate* of 3-hydroxy-5 : 7 : 4′-trimethoxyflavylium chloride (3·4 g., 38%), lustrous black prisms, decomp. 213—215° (Found: C, 54·3; H, 5·8; Cl, 13·2; acetyl, 0. $C_{18}H_{17}O_5Cl,2H_2O,0{\cdot}5HCl$ requires C, 53·6; H, 5·4; Cl, 13·2. Found, in a specimen dried to constant weight *in vacuo* at 110°: C, 62·9; H, 5·0; loss 13·3. Calc. for $C_{18}H_{17}O_5Cl$: C, 62·0; H, 4·9; loss, 13·4%). Dilution of the filtrate with dry ether precipitated further crude flavylium salt (*ca.* 4·0 g.). Recrystallisation of the salt (1·0 g.) from 1 : 1 ethanol–concentrated hydrochloric acid (100 c.c.) afforded reddish-orange needles (1·0 g.) consisting of a *hydrate* of pelargonidin 5 : 7 : 4′-trimethyl ether, decomp. 213° (Found: C, 52·8; H, 5·5; Cl, 13·1; OMe, 20·8; loss, at 110° *in vacuo*, 13·6. $C_{18}H_{17}O_5Cl,2{\cdot}5H_2O,0{\cdot}5HCl$ requires C, 52·5; H, 5·5; Cl, 12·8; OMe, 22·6; loss, 15·3%). Light absorption in EtOH containing 0·01M-HCl: $\lambda_{max.}$ 209 (841), 245 (373), 268 (468), 332 (85), 424 (229), and 518 mμ ($E^{1\%}_{1cm.}$ 975). Recrystallisation from hydrochloric acid (5%) (Karrer, Widmer, Helfenstein, Hürlimann, Nievergelt, and Monsarrat-Thoms, *Helv. Chim. Acta*, 1927, **10**, 729) afforded a less satisfactory preparation of *pelargonidin trimethyl ether monohydrate*, decomp. 197° (Found: C, 59·1; H, 4·9; Cl, 8·5, 8·6. $C_{18}H_{17}O_5Cl,H_2O$ requires C, 58·9; H, 5·2; Cl, 9·7. Found, in material dried at 110° *in vacuo*: C, 62·2; H, 5·5. Calc. for $C_{18}H_{17}O_5Cl$: C, 62·0; H, 4·9%). Karrer *et al.* (*loc. cit.*) record decomp. 130° for the compound recrystallised in this way, but Professor Karrer remarks that his specimen melts at a much higher temperature, although shrinking markedly at 130° (personal communication). A specimen supplied by Professor Karrer decomposed at 190—192°, and the decomp. point was not lowered on admixture with our materials of decomp. 197° and 213°; the light absorption of his specimen was closely similar to that recorded above.

The *perchlorate* crystallised in dark red needles, decomp. 222—225° (Found: Cl, 8·4. $C_{18}H_{17}O_9Cl$ requires Cl, 8·6%).

Afzelin.—From a fissure about 2 ft. in length in a sample of afzelia a yellow powdery material (25 g.) was removed mechanically. Chromatographic analysis showed that afzelin (kaempferol 3-rhamnoside) (I; R = $C_6H_{11}O_4$) (King and Acheson, *J.*, 1950, 168) is the main component, and that the glycoside is not accompanied by the aglycone, but that two other compounds are present in much smaller amounts. These two compounds, which are only detectable on heavily loaded chromatograms, are dihydrokaempferol (II) and *epi*afzelechin (IV).

Paper Chromatograms.—Paper chromatograms were run by the descending technique on Whatman No. 1 paper with *n*-butanol–acetic acid–water (5 : 1 : 4) (B) and *m*-cresol–acetic acid–water (50 : 2 : 48) (C) as solvents (Bate-Smith, *Biochem. Soc. Symp.*, No. 3, Partition

Substance	Range of R_F values B	C	Colour	Fluorescence in u.v.	Colour with $FeCl_3$	Colour with vanillin–HCl
(−)-*epi*Afzelechin	0·73—0·74	0·40—0·43	—	Faint blue	—	Red
Afzelin	0·82	0·58—0·59	Greenish-yellow	—	Green	—
Kaempferol	0·83	0·54—0·55	Yellow	+	Green	—
Dihydrokaempferol	0·87	0·72—0·73	—	+	Brownish-purple	—
Apigenidin trimethyl ether	0·88 (streak)	—	Orange	—	—	—

Chromatography, 1951, p. 62). Authentic specimens were used as reference compounds on each chromatogram. The chromatograms were examined in daylight and in ultraviolet light, and then sprayed in turn with alcoholic ferric chloride (1%) and vanillin (*ca.* 0·2% in concentrated hydrochloric acid). Results are tabulated.

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