Phytochemistry, 1967, Vol. 6, pp. 59 to 67. Pergamon Press Ltd. Printed in England

THE POLYPHENOLS OF *NOTHOFAGUS* SPECIES—II.

THE HEARTWOOD OF *NOTHOFAGUS FUSCA*

W. E. HILLIS and T. INOUE*

Division of Forest Products, C.S.I.R.O., South Melbourne, Australia

(*Received* 15 *June* 1966)

Abstract—From the heartwood of *Nothofagus fusca* were isolated taxifolin, catechin, aromadendrin, quercetin, kaempferol, naringenin, ellagic acid, gallic acid, resveratrol, 3,3'-di-*O*-methyl ellagic acid and a compound "nothofagin" with properties consistent with those of a mono-C-glycoside of 4,2',4',6'-tetrahydroxy-dihydrochalcone. Another compound "konnanin" appears to be a C-glycoside of 3,4,2',4',6'-pentahydroxy-dihydrochalcone. Afzelechin, epicatechin, protocatechuic acid, *p*-hydroxybenzoic acid and phloroglucinol have been identified chromatographically. The possible taxonomic significance of the dimethyl ellagic acid is discussed. Spectral and chromatographic properties of the known dihydrochalcones are described.

INTRODUCTION

THREE of the five *Nothofagus* species (Fam. Fagaceae) endemic to New Zealand (*N. fusca*, *N. menziesii*, *N. truncata*) can be readily identified by a chromatographic examination of their heartwood extractives.[1] The extractives of the other two species (*N. solandri* and *N. cliffortioides*) are very similar to each other, but differ from the above species. Provisional identification of some of the components has been made as a result of chromatographic examination.[1] This communication reports a further examination of the polyphenols of *N. fusca* (red beech) which is an important commercial timber of New Zealand.

RESULTS AND DISCUSSION

The monomeric compounds isolated have been listed in Table 1 in the order of their concentration, and these were accompanied by faint traces of three unidentified compounds. The polymeric residue from which these compounds were obtained, contained appreciable amounts of leucocyanidin polymer (as shown by the formation of cyanidin on heating with butanol–hydrochloric acid) and traces of compounds with chromatographic properties and colour reactions consistent with those for leucocyanidin "monomers". Also present were a number of monomeric ellagi-gallo-tannins which were not studied further.

The presence of dimethyl ellagic acid and the incompletely identified compounds that we have named nothofagin and konnanin is of interest.

3,3'-*Di*-O-*Methyl Ellagic Acid*

Previously, 3,3'-di-*O*-methyl ellagic acid has been recorded in the wood (and other tissues) of three families of the Order Myrtiflorae namely *Leptospermum scoparium*[2] (Fam. Myrtaceae), *Sonneratia apetala*[3] (Lythraceae) and its 4-monoglucoside in *Terminalia*

* On leave from Hoshi College of Pharmacy, Tokyo, Japan.

[1] W. E. HILLIS and H. R. ORMAN, *J. Linn. Soc.* (*Bot.*) **58**, 175 (1962).

[2] B. F. CAIN, *New Zealand J. Sci.* **6**, 264 (1963).

[3] S. N. SRIVASTAVA, D. S. BHAKUNI, V. N. SHARMA and K. N. KAUL, *J. Sci. Ind. Res.* (*India*) **21B**, 549 (1962).

TABLE 1. COMPOUNDS ISOLATED FROM THE HEARTWOOD OF *Nothofagus fusca*

(Listed in the order of amount present.)

	R_f values (× 100) in			M.p. and mixed m.p. of	
	BAW	6HOAc	For.	Original compound	Acetate
1. Taxifolin	76	38	73	240–242	87–90
2. Catechin	52	40	—	169–172*	129–130†
3. Gallic acid	52	40	65	252–254(d)	—
4. Aromadendrin	86	39	85	242–244	134–136
5. Quercetin	65	00	40	310–316	197–198
6. Naringenin	90	20	90	254–255	76–78
7. Ellagic acid	35	00	31	—	245–246‡
8. Kaempferol	87	00	55	278–280	184–186
9. Resveratrol	78	03	64	663–264	119–120
10. 3,3′-Dimethoxy ellagic acid	72	03	82	338	301–304
11. Nothofagin	76	59	81	70–100	—
12. Konnanin	71	55	71	70–100	—
13. Epicatechin	45	30	—	—	—
14. Afzelechin	74	47	—	—	—
15. Unknown *A*	64	57	—	—	—
16. Protocatechuic acid	82	53	—	—	—
17. Phloroglucinol	71	62	—	—	—
18. *p*-Hydroxybenzoic acid	92	64	—	—	—

Compounds 13, 14, 16, 17, 18 were identified by co-chromatography and colour reactions.
*$[\alpha]_D^{20}$ + 15·5° acetone–H_2O(1:1) *c.* 1·0. † $[\alpha]_D^{20}$ + 29° acetone *c.*0·9. ‡ Tetracarbethoxy derivative.

paniculata[4] (Combretaceae). It has been found also in the Order Geraniales—in the roots of *Euphorbia formosanum*[5] (Euphorbiaceae)—and in the present case in the order Fagales. 3,3′,4-Tri-*O*-methyl ellagic acid has been also found in the above *Leptospermum* and *Sonneratia* species as well as in *Eugenia maire*[6] (Myrtaceae). These methyl ellagic acids indicate a relationship between the Myrtiflorae and the Fagales or the formation of these Orders from a common stock.

Some contradictions exist concerning the response of these acids to Griessmayer's test with nitrous-nitric acid. Consistent with Lederer's observation[7] we found that ellagic acid gives a strong red and the colour of 4,4′-di-*O*-methyl derivative is unchanged. From the colour reaction of the 3,3′-di-*O*-methyl derivative (see also Ref. 5), Lederer generalized that two free 4,4′-hydroxyls are necessary for a positive reaction. However, we obtained only a weak red colour with 3,3′-di-*O*-methyl ellagic acid and Srivastava *et al.*[3] failed to observe a red colour. Apparently the colour reaction has limited value for the diagnosis of structure of methyl ellagic acids.

Nothofagin and konnanin. Chromatographic examination of extracts of different samples of *N. fusca* heartwood showed that the relative amounts of two unknown compounds to the rest of the components varied. We have named these compounds "nothofagin" and "konnanin". Several attempts were made to obtain sufficient amounts for detailed study but losses with the methods used were too great.

[4] L. R. ROW and G. S. R. S. RAO, *Tetrahedron* **18**, 357 (1962).
[5] H. SHINODA and C. P. KUN, *J. Pharm. Soc. Japan* **51**, 50 (1931).
[6] L. H. BRIGGS, R. C. CAMBIE, J. B. LOWRY and R. N. SEELYE, *J. Chem. Soc.* 642 (1961).
[7] E. LEDERER, *J. Chem. Soc.* 2115 (1949).

The spectral properties (Table 2) and the production of phloroglucinol and *p*-hydroxybenzoic acid from nothofagin by alkali fusion, and phloroglucinol and protocatechuic acid from konnanin indicate these colourless compounds to be flavonoid types. However, neither compound responded to the magnesium-hydrochloric acid or the sodium borohydride[8] colour tests, and these and the above properties would be shown only by dihydrochalcones.

TABLE 2. PROPERTIES OF NOTHOFAGIN, KONNANIN AND DIHYDROCHALCONES

Compound	R_f values (× 100) in*						Colour reactions on paper		
	BAW	6% HOAc	Phenol	I	II	III	U.V.	U.V. + NH_3	*p*-N.A.†
Nothofagin	76	59	52	51	20	07	Faint blue	Green–yellow	Yellow
Konnanin	71	55	14	34	10	02	Opaque	Green–yellow	Light brown
Aspalathin	37	45	30	04	02	00	Faint brown	Faint brown	Light brown
Phloridzin	67	40	70	15	08	00	Faint brown	Brown	Orange
Phloretin	86	12	82	65	35	19	Faint brown	Cream	Brown–pink
Asebotin	71	46	89	21	14	02	Faint brown	Brown	Orange
Asebogenin	90	10	96	72	41	63	Faint brown	Cream	Canary yellow
Gallic acid (marker)	52	40	11	50	22	17			

Compound	Spectral properties: λ_{max} in EtOH (mμ)								
	Alone	+$AlCl_3$	Δλ	+NaOEt	Δλ	+Satd. NaOAc	Δλ**	+NaOAc, H_3BO_3	Δλ
Nothofagin	294	314, 376‡	20	323	29	299, 329	5	299, 328	5
Konnanin	293	313, 377‡	20	326§	33	299, 330	6	295, 330	2
Aspalathin	291	309, 378‡	18	332	41	295, 330	39	296, 335	5
Phloridzin	288	311, 362‡	23	330	42	288, 327	0	—	
Phloretin	288	311, 373‡	23	328	40	291, 323	3	291	3
Asebotin	285	309, 363‡	24	284, 338	0	285	0	—	
Asebogenin	288	310, 375‡	22	297, 370	9	288	0	288	0

* For composition, see Experimental.
† Diazotized *p*-nitroaniline.
‡ Weak maximum.
§ Unstable.
** Shift of peak of highest intensity.

The R_f values, particularly those in 6% acetic acid (Table 2), could be possessed only by glycosides or compounds with a highly hydroxylated substituent. However prolonged heating with aqueous hydrochloric acid failed to liberate sugar or to alter the chromatographic properties, so that the compounds appear to be C-glycosyl dihydrochalcones. The u.v. spectra of the known dihydrochalcones were found to be similar to nothofagin and konnanin (Table 2).

[8] E. EIGEN, M. BLITZ and E. GUNSBERG, *Arch. Biochem. Biophys.* **68**, 501 (1957).

The spectral studies (see below) support the view that the aromatic moiety of nothofagin is 4,2',4',6'-tetrahydroxy-dihydrochalcone. The chromatographic properties (Table 2), indicate the presence of only one sugar substituent, and in view of the constitution of the known glycoflavonoids this would be in the 3'- or 5'-position. Unlike aspalathin[9], nothofagin was not converted to the corresponding flavanone when an alcohol solution was exposed to sunlight. Attempted purification of the acetate by TLC, using silica gel and acid and neutral solvents repeatedly, gave a series of bands so that sufficient amounts for further study could not be obtained.

The evidence concerning the structure of konnanin is less conclusive. Aromadendrin (dihydrokaempferol) and nothofagin give the same yellow colour with diazotized *p*-nitroaniline whereas taxifolin and konnanin give a brown colour. These pairs of compounds also show a similar relationship to each other in respect to R_f values in BAW, 6% acetic acid and Forestal solvents. The spectral studies support the view that the aromatic moiety of konnanin is 3,4,2',4',6'-pentahydroxy-dihydrochalcone. However, aspalathin[9] is the 3'-glucopyranosyl derivative of this compound and the change of its spectral properties on the addition of sodium acetate (Table 2), and its R_f values differ significantly from those of konnanin. The differences may be due to the sugar moiety but this aspect could not be checked as there was insufficient material for the necessary ozone degradation. Attempts to prepare the acetate in sufficient amounts for further study were unsuccessful.

Spectral Properties of Dihydrochalcones

The u.v. spectra of the known dihydrochalcones were determined both before and after the addition of the usual salts.[10] These compounds were phloretin (4,2',4',6'-tetrahydroxy-dihydrochalcone) and its 2'-glucoside phloridzin, asebogenin (4,2',6'-trihydroxy-4'-methoxy dihydrochalcone) and its 2'-glucoside asebotin, and aspalathin (3'-C-β-D-glucopyranosyl-3,4,2',4',6'-pentahydroxy-dihydrochalcone)[9] (see Table 2). The spectra are very similar to those of flavanones[10, 11] which also possess aromatic (B) rings that are not conjugated with the carbonyl group. Thus, the absorption maxima in alcohol solution are about 290 mμ (Table 2), and bathochromic shifts of about 20 mμ occur on the addition of aluminium chloride (as do the 5-hydroxyflavanones), and as well weak maxima were observed at about 370 mμ. The pronounced bathochromic shift in alkaline solution (Table 2) is similar to that shown by flavanones[10, 11] except in the cases of asebotin and asebogenin. In contrast to the other compounds, the expected peak in the 330–335 mμ region with asebotin is a very weak one. In asebogenin, this peak is replaced by a stronger one at 370 mμ. An explanation for this anomalous behaviour could not be found. The lack of response of asebogenin and asebotin to the addition of sodium acetate indicates that shifts in the maxima of dihydrochalcones is due to the 4'-hydroxyl (compare behaviour of the corresponding 7-hydroxyl in flavones and flavonols[10]). The distinctive difference between the other dihydrochalcones and flavanones is the formation of double peaks with the former compounds after the addition of sodium acetate. The response of one of these peaks to increasing amounts of sodium acetate differs from the other. The higher wave-length peak of phloretin, phloridzin, nothofagin and konnanin increases in intensity with increasing amounts of acetate but phloretin is the least sensitive. Aspalathin shows a similar response but in this case the intensity of the

[9] B. H. KOEPPEN and D. G. ROUX, *Tetrahedron Letters* **39**, 3497 (1965).
[10] L. JURD, In *The Chemistry of Flavonoid Compounds* (Edited by T. A. GEISSMAN), p. 107. Pergamon Press, Oxford (1962).
[11] W. E. HILLIS and A. CARLE, *Australian J. Chem.* **16**, 147 (1963).

295 mμ band decreases markedly. The response of aspalathin to boric acid and sodium acetate[10] was anomalous in that the expected bathochromic shift did not occur; similarly no shift with konnanin was observed.

EXPERIMENTAL

The samples of *Nothofagus fusca* heartwood were kindly provided by Mr. H. R. Orman, Forest Research Institute, Rotorua, New Zealand.

Chromatographic Examination

Two-dimensional chromatograms were prepared using first, *n*-butanol–acetic acid–water (6:1:2, "BAW") then 6% acetic acid ("6HOAc"). One-dimensional chromatograms were also prepared using these solvents and also phenol–water (upper phase), HCl–acetic acid–water (3:30:10, "Forestal").

The chromatograms were examined under u.v. (254 and 365 mμ) light before and after exposure to NH_3 vapour. The chromogenic sprays used were diazotized *p*-nitroaniline in 20% sodium acetate, vanillin–HCl and ferric chloride–potassium ferricyanide for polyphenols, aniline hydrogen phthalate for sugars.

Chromatoplates of Silica Gel G.F.254 (E. Merck, A. G. Darmstadt) with a thickness of 250μ were prepared in a constant temperature room at 20°. The following solvents were used: I, chloroform–ethyl acetate–formic acid (5:4:1); II, toluene–ethyl formate–formic acid (5:4:1); III, chloroform–acetic acid (8:2); IV; ethyl acetate–ethanol (1:1).

Fractionation of N. fusca *Extract*

Air-dried shavings (2 kg) of *N. fusca* heartwood were extracted in a Soxhlet-type extractor with methanol for 24 hr. The hot extract was filtered to remove insoluble material *A* (10 g) and the liquor (containing 130 g solids) concentrated under reduced pressure, mixed with an equal volume of water, and the suspension extracted in a liquid–liquid extractor with diethyl ether and then ethyl acetate. The ethyl acetate extracts contained largely unresolved material and small amounts of components with low R_f values in BAW. The aqueous extracted solution contained almost entirely polymeric material which, like the extracted shavings, when heated with butanol–HCl (95:5) yielded compounds chromatographically identified as cyanidin and ellagic acid.

The ether extracts were washed with water, and the water washes concentrated and re-extracted with ether. The combined extracts were extracted successively with saturated sodium bicarbonate, saturated sodium carbonate and N sodium hydroxide. The extracts were acidified immediately after collection, filtered and the aqueous filtrates extracted with ether and then ethyl acetate.

Chromatographic examination showed there was an accumulation of components into different fractions although the separation was not clear-cut. The main components concentrated in the sodium bicarbonate extract were gallic, ellagic and dimethyl ellagic acids, nothofagin, konnanin, phloroglucinol, *p*-hydroxybenzoic and protocatechuic acids and unknown compounds. Acidification of the sodium carbonate extracts yielded a precipitate containing taxifolin, aromadendrin, quercetin, kaempferol and naringenin, and catechin and other components were extracted from the filtrate. Resveratrol was removed with sodium hydroxide to leave waxy material in the ether solution.

Portions of these fractions were subjected to different treatments in order to isolate

certain components, and this method of treatment, together with the incomplete separation, rendered inaccurate any estimate of yield of components. However a comparison of the spot areas of the components when a two-dimensional chromatogram of the original methanol extract was made shows the relative amounts to be in the following order: taxifolin > catechin and gallic acid > quercetin = aromadendrin = ellagic acid = naringenin = dimethyl ellagic acid > kaempferol = resveratrol = nothofagin = konnanin > protocatechuic acid = *p*-hydroxybenzoic acid = phloroglucinol > unknown compounds.

Separation of Ellagic Acid

The insoluble material *A* (see previous section) was repeatedly extracted with warm ethanol and the residue extracted with methanol in a Soxhlet extractor. The microcrystals which formed in the extract were identified as ellagic acid (Table 1).

Separation of Components in Sodium Bicarbonate Extracts

The acidified liquor was filtered to remove the ellagic acid-rich precipitate and the aqueous liquor extracted with ethyl acetate. This extract was separated on a polyamide column with aqueous alcohol of increasing alcohol concentration. Gallic acid was removed with 20% alcohol, followed by protocatechuic acid, *p*-hydroxybenzoic acid and phloroglucinol (all identified chromatographically), epicatechin, catechin, nothofagin, konnanin, afzelechin[12] (identified chromatographically) and unknown compound *A*. The ellagic acid-rich precipitate was continuously extracted with methanol to remove dimethyl ellagic acid.

3,3'-O-*Dimethylellagic Acid*

The crude compound which has a low solubility in methanol was crystallized repeatedly from dioxan as slightly yellow fine needles. In addition to the properties listed in Table 1 it has R_f 0·26 in *n*-butanol:ethanol:1·5 N aqueous ammonia (4:1:3).[13] The absorption max. (in mμ) were 252, 361 (infl.), 378 (in EtOH); 256, 361 (infl.) 381, 416 (in EtOH + small amounts NaOAc); 265, 418 (in EtOH + large amounts NaOAc); 276, 315 (shoulder), 441 (in EtOH + NaOEt) (cf. Ref. 14).

The fluorescence of the compound was light blue (365 mμ u.v. light) and very strong blue (254 mμ u.v. light) changing to dull yellow in ammonia vapour. In contrast to ellagic acid it gives a weak red colour with nitrous-nitric acid.

The acid was acetylated with acetic anhydride and pyridine at 100° for 1 hr, and the acetate twice recrystallized from dioxan as fine needles, m.p. 301–304° (Row and Rao[4] report 300–302° for the 4,4'-diacetate), which showed one spot (R_f 0·78) in Solvent I.

The mother liquors obtained from the recrystallization of the above acid contained a component with the following R_f values (with that of gallic acid in brackets) in solvents BAW, 0·45 (0·52); 6HOAc, 0·04 (0·40); Forestal, 0·55 (0·65); Phenol, 0·75 (0·10); and with thin-layer chromatography solvent I, 0·24 (0·49); II, 0·28 (0·24); III, 0·05 (0·15). The regular relationship of the chromatographic properties of this compound with those of ellagic acid, 3,3'-dimethyl and 3,3',4'-trimethyl ellagic acids, in addition to the close similarity of the colour reactions with those of 3,3'-dimethyl ellagic acid suggest that the compound is a monomethyl ellagic acid.

[12] W. E. Hillis and A. Carle, *Australian J. Chem.* **13**, 390 (1960).
[13] B. F. Cain, *New Zealand J. Sci.* **5**, 390 (1962).
[14] L. Jurd, *J. Am. Chem. Soc.* **81**, 4610 (1959).

Nothofagin

The concentrates of this compound in the 25–30 per cent eluates of the polyamide column or the crude ethyl acetate extract of the neutralized sodium bicarbonate extract were streaked on to No. 3 Whatman paper and the appropriate band which fluoresced blue under u.v. light (254 mμ) was cut out and extracted with methanol. The extracts were evaporated *in vacuo* to give a dark brown residue showing five spots on TLC plates using solvents I and II.

The compound, which was very soluble in organic solvents and soluble in water, was purified by extracting repeatedly with ether, and the evaporated extract resolved on preparative layer chromatograms using solvent I. The orange band was extracted with methanol and the nothofagin in the evaporated extract was removed from the small amount of polymer by repeatedly extracting with ethyl acetate. The extract was evaporated *in vacuo* to yield a pale-tan oil which was homogeneous on chromatographic examination, and became an amorphous solid which could not be crystallized (m.p. 70–100°). Another method of purification using the neutral solvent IV failed to remove all the polymeric material.

In sunlight the compound became brown in a few days when chromatographically immobile material formed. Nothofagin possessed a yellow fluorescence on TLC plates when neutral solvents were used and was opaque with acid solvents but became yellow in ammonia vapour. On paper chromatograms it was blue in u.v. light (weak with 365 mμ, strong with 254 mμ) and a pale green–yellow when fumed with ammonia; it became blue with ferric chloride–potassium ferricyanide and with diazotized *p*-nitroaniline a distinctive orange–yellow similar to that given by phloretin. A summary of its R_f values and spectral properties are given in Table 2.

In addition to the R_f values in Table 2, the following were also determined (R_f of gallic acid in brackets), in 30% acetic acid 0·65 (0·56) and by means of TLC with solvent IV 0·20 (0·04).

Pellets of KOH were melted in a small tube and nothofagin (3 mg) added, heated for 5–25 sec, diluted with water, neutralized with HCl, extracted with ether and the extract examined by two-dimensional paper chromatography and TLC using three different solvents. Phloroglucinol and *p*-hydroxybenzoic acid were identified by direct comparison with authentic compounds although the former compound was produced in relatively smaller amounts than it was by aromadendrin.

Nothofagin showed no colour change in response to the magnesium–HCl test or when a spot of it was sprayed with sodium borohydride and fumed with hydrochloric acid.[8]

Nothofagin (3 mg) was heated at 100° for 6 hr with 5% sulphuric acid, neutralized with barium carbonate, filtered and the residue washed with hot water and methanol. The filtrate was examined chromatographically but no change in nothofagin was observed and no sugar was detected.

Nothofagin (24 mg) was acetylated with acetic anhydride and pyridine for 18 hr at room temperature, and the liquor evaporated under high vacuum. The yellow oily residue, which could not be crystallized, was separated on TLC with solvent I and the extracts of the two main bands again separated when multiple bands appeared. Further separation of the main bands gave the same result.

Konnanin

This compound was obtained from the 20–30 per cent eluates of the polyamide column and these were resolved on a number of preparative layer chromatograms using solvent I.

Konnanin is slightly soluble in ethyl ether and ethyl acetate but more soluble in ethanol, methanol and water. When freshly prepared it is a faint tan amorphous powder (m.p. 70–100°) but polymeric material forms after storage in sunlight. Methoxyl groups were absent. Konnanin possessed a yellow fluorescence when neutral chromatographic solvents were used but opaque with acidic solvents, the latter appearance changing to yellow with ammonia. Its R_f values and spectra are given in Table 2. It gave no colour with magnesium and hydrochloric acid or with sodium borohydride, but blue with ferric chloride–potassium ferricyanide, brown with diazotized *p*-nitroaniline and orange with sodium carbonate solution. Degradation with KOH for 5–15 sec produced protocatechuic acid and relatively small amounts of phloroglucinol. After heating with 2 N H_2SO_4 for 3 and 5 hr in a sealed tube konnanin was the only material detected chromatographically. When heated with 2 N H_2SO_4 for 5 hr with air circulation (Pew's oxidation) a partial change to a yellow fluorescent spot occurred but this changed to a red–brown material which could not be removed from silica gel. The crude acetate separated into several bands when purification on TLC was attempted.

Separation of Compounds in Sodium Carbonate Extracts: Taxifolin, Aromadendrin, Quercetin, Kaempferol and Naringenin

A portion (10 g) of the insoluble material obtained on acidification of the sodium carbonate extract was dissolved in *n*-butanol (1·5 l.), extracted with borate buffer of pH 8·4 (5 × 200 ml), the extracts washed with butanol (3 × 30 ml) then acidified with HCl:water (1:1). The colourless precipitate was removed and the filtrate extracted with ethyl acetate which was then evaporated and combined with the precipitate. Portions of the evaporated butanol-soluble material (2 g) and borate-extracted material (2 g) were separately resolved on a polyamide column (2 × 25 cm). Small amounts of catechin were removed from the borate-extracted material with 25% ethanol, unknown compound *A* with 30% ethanol, taxifolin with 40% ethanol and quercetin with 95% ethanol. The butanol-soluble material yielded aromadendrin, naringenin and kaempferol with 40, 45 and 95% ethanol respectively. The components were recrystallized from aqueous methanol and characterized (Table 1).

Naringenin was also isolated by streaking the fraction on preparative layers of silica gel, resolved with solvent III and the appropriate band extracted with methanol, which on evaporation yielded fine colourless needles of naringenin.

Isolation of Catechin

The aqueous liquor after neutralization of the sodium carbonate extracts was extracted with ethyl acetate and the residue obtained after evaporation of the latter was placed on a polyamide column, which was eluted with water and then aqueous alcohol of increasing alcohol concentration. Small amounts of afzelechin and epicatechin were removed with 20% alcohol together with increasing quantities of catechin.

Isolation of Resveratrol (3,5,4′-Trihydroxystilbene)

The insoluble material obtained on acidification of the sodium hydroxide extracts was recrystallized repeatedly from boiling water as colourless plates. In addition to those listed in Table 1, it had the following properties. Analysis; C, 73·1; H, 5·3. Calc. for $C_{14}H_{12}O_3$; C, 73·7; H, 5·3%. Acetate; C, 67·5; H, 5·2; CH_3CO, 35·4%. Calc. for $C_{20}H_{18}O_6$: C, 67·8; H, 5·1; CH_3CO, 36·4%. All properties are consistent with those of 3,5,4′-trihydroxystilbene.[15]

[15] W. E. HILLIS and M. HASEGAWA, *Biochem. J.* **83**, 503 (1962).

The brilliant blue fluorescence shown by the compound under u.v. light, intensified and became lighter in the presence of ammonia vapour. A strong red–orange colour was obtained when sprayed with diazotized *p*-nitroaniline.

Acknowledgements—We thank Professor Shizuo Hattori, Drs. B. H. Koeppen, and B. F. Cain for samples of authentic compounds, Misses A. Carle and E. Gloss for experimental assistance, and Dr. K. W. Zimmerman and associates for elemental analysis.