Hydroxystilbenes of *Eucalyptus wandoo*

By D. E. HATHWAY and J. W. T. SEAKINS
The British Leather Manufacturers' Research Association, Milton Park, Egham, Surrey

(*Received* 11 *December* 1958)

The heartwood and sapwood of the Australian myrtaceous tree *Eucalyptus wandoo* is used as a commercial tanning material. In preliminary experiments with the ether-soluble extractives three substances were detected which show an intense-blue fluorescence in ultraviolet light, which are red with tetrazotized benzidine and which may be hydroxystilbenes. All seven hydroxystilbenes (Table 1) which have been isolated from the Mono- and Di-cotyledonae possess a resorcinol nucleus. In the Gymnospermae, the 3:5-dihydroxystilbenes of the genus *Pinus* serve as taxonomic tracers in the Pinaceae, they protect the heartwoods from wood-rotting fungi and insects and they prevent the acid-sulphite pulping of the logs (Hägglund, 1941) by forming insoluble resins with the lignin. Hydroxystilbenes have not previously been found in the Myrtaceae.

EXPERIMENTAL

Evaporations were carried out in N_2 under reduced pressure below 35°. Melting points were determined on a Kofler block.

Separation of the ether-soluble phenols of Eucalyptus wandoo *heartwood.* Heartwood chips (500 g.) were extracted in a Soxhlet apparatus with methanol (1·5 l.) for 10 hr. and 400 ml. of water was added to the extract. Partial evaporation left a solution (300 ml.) which was continuously extracted for 30 hr. with four changes of acetic acid-free ethyl acetate in a Schacherl apparatus. Evaporation of the solvent left a solid residue which was dried over P_2O_5 at 20° *in vacuo* for 16 hr. The ethyl acetate-soluble fraction (25·2 g.) constituted 5·6% (by wt.) of the moisture-free heartwood. An aqueous solution (300 ml.) of this fraction was continuously extracted for 24 hr. with peroxide-free ether in the Schacherl apparatus, ice–water being pumped through the condenser. Evaporation of the solvent left a solid residue (18·8 g.) which was dried over P_2O_5 at 20° *in vacuo* for 16 hr. Yellow wax was removed from this fraction (18·8 g.) by extraction with benzene in a Soxhlet apparatus, and the residual material (16·6 g.), which is known as the ether-soluble phenols, constitutes 3·7% (by wt.) of the moisture-free heartwood.

Direct extraction of the initial aqueous solution with ether caused excessive frothing, which was obviated by intermediate extraction with ethyl acetate. Ether extraction of the heartwood chips (500 g.) afforded a residue (2·2 g.) which consisted principally of yellow wax (>2·0 g.).

Ether-soluble phenols from the sapwood and different locations in the heartwood. Small samples of drillings were taken from a transverse section through a 100-year-old primary limb which was 50 cm. in diameter. Samples were removed from the sapwood, the centre of the heartwood and at two locations situated at 1 cm. and 10 cm. inside the heartwood–sapwood boundary.

Paper chromatography

Chromatograms were run at 25° and dried at room temperature unless stated otherwise. Methanolic solutions (0·5%; 5 µl.) of the ether-soluble phenols and of fractions from the columns were applied to Whatman no. 2 filter papers, 25·5 cm. square, and chromatographed by the ascending method with N-acetic acid as first-way solvent and butan-1-ol–acetic acid–water (6:1:2, by vol.) as the second. Phenols were detected by their fluorescence in ultraviolet light, by the $FeCl_3$–$K_3Fe(CN)_6$ and vanillin reagents (Hathway, 1958), and by the tetrazotized benzidine reagent (Koch & Krieg, 1938; Linstedt, 1950). Catechin and gallic acid were resolved with butan-1-ol–acetic acid–water (6:1:2, by vol.) as the first-way solvent system and *m*-cresol–acetic acid–water (50:1:49, by vol.) as the second. These chromatograms were dried at 70°.

Single-way chromatography of the hydroxybenzoic acids was carried out in N-acetic acid, butan-1-ol–acetic acid–water (6:1:2, by vol.) and benzyl alcohol–*tert.*-butanol–propan-2-ol–water (3:1:1:1, by vol.) mixture containing

Table 1. *Distribution of hydroxystilbenes in the Mono- and Di-cotyledonae*

Family	Stilbene derivative	Reference
Liliaceae	3:5:4′-Trihydroxy-	Takaoka (1940*a*, *c*)
	3:5:2′:4′-Tetrahydroxy-	
Leguminosae	3:5-Dimethoxy-4′-hydroxy-	Späth & Schläger (1940); King, Cotterill, Godson, Jurd & King (1953)
	3:5:3′:4′-Tetrahydroxy-	King, King, Godson & Manning (1956)
	3:5:3′:4′:5′-Pentahydroxy-	
Moraceae	3:5:2′:4′-Tetrahydroxy-	Barnes & Gerber (1955); Mongolsuk, Robertson & Towers (1957)
	2-Homogeranyl-3:5:2′:4′-tetrahydroxy-	King & Grundon (1949).
Polygonaceae	4′-Methoxy-3:5:3′-trihydroxy-	Kawamura (1938)

1·8% (w/v) of formic acid (Stark, Goodban & Owens, 1951) respectively. The chromatograms with the third solvent system were dried at 70°.

Sugars were chromatographed by the single-way descending method (Hathway & Seakins, 1958).

Separation of compounds B_1 and B_2 by chromatography on a cellulose column

Use was made of a column (60 cm. × 5 cm.) of Solka Floc cellulose (Hathway, 1958) which had been mixed with an equal weight of acid-washed kieselguhr (British Drug Houses Ltd.) (Grassmann, Deffner, Schuster & Pauckner, 1956). A methanolic solution (250 ml.) of ether-soluble phenols (100 g.) was made into a slurry with Solka Floc cellulose (100 g.) and the solvent evaporated. The powder obtained was made into a slurry with 0·5N-acetic acid and transferred to the top of the column, which was eluted at a pressure of 25 cm. Hg with 0·5N-acetic acid which contained 0·002% of SO_2. The flow-rate was 120 ml./hr.; 500 ml. fractions of eluate were collected. For the evaporation of large volumes of eluate, a circulatory cyclone-evaporator (no. 20EF, Quickfit and Quartz Ltd., Stone, Staffs.) was used.

Traces of chlorogenic acid and *p*-coumarylquinic acid and small quantities of catechin, gallic acid and other unknown phenols were eluted in the first 4 l. of eluate.

The succeeding 500 ml. of eluate deposited crystals (0·6 g.) of pure compound B_1 on standing. Evaporation of the next 5·5 l. of eluate, together with the mother liquor from which compound B_1 had been deposited, afforded a brown glass (20 g.) from which a further quantity of compound B_1 was obtained by polyamide-column chromatography.

Elution of the column with methanol (2 l.) and evaporation of the eluate obtained gave a coloured residue (52 g.), m.p. about 250°, of impure compound B_2. Acetylation by the acetic anhydride–pyridine method gave compound B_2 triacetate, which crystallized from benzene–light petroleum (b.p. 80–100°) (1:1, v/v) as glistening plates, m.p. 118–119° (Found: C, 68·3; H, 5·2; $CH_3\cdot CO$, 35·4. Calc. for $C_{20}H_{18}O_6$: C, 67·8; H, 5·1; $CH_3\cdot CO$, 36·4%), and from aqueous ethanol fine needles, m.p. 118–119°. Alkaline hydrolysis of the triacetate under N_2 gave compound B_2, which crystallized from 2N-acetic acid in felted needles (27 g.), m.p. 264° on a preheated Kofler block, $\lambda_{max.}$ in ethanol 305 mμ (log ϵ 4·45); 220 mμ (log ϵ 4·32) (Found: C, 73·5; H, 4·9. Calc. for $C_{14}H_{12}O_3$: C, 73·7; H, 5·3%). Crystallization of the triacetate removed contaminating methoxyl-containing impurity from compound B_2.

Purification of compound B_1 by chromatography on polyamide column. A column (30 cm. × 3·5 cm.) of Perlonpulver 'feinst' (Werk Bobingen A.-G.), prepared by the method of Grassmann, Endres, Pauckner & Mathes (1957), was used. A solution of crude compound B_1 (4 g.) in methanol–water (1:1, v/v) was adsorbed on to the column, which was then eluted with the methanol–water (1:1, v/v). The progress down the column of a sharp band of compound B_1 was observed by its fluorescence in ultraviolet light, and 500 ml. of eluate was collected, and evaporated to 50 ml., which deposited clusters of needles (0·5 g.) of *compound B_1*, m.p.'s 142° (loss of H_2O), 210°; $[\alpha]_D^{22}$ −68·5° in methanol (*c*, 2·6); light-absorption max. in ethanol 305 mμ (log ϵ 4·45); 220 mμ (log ϵ 4·32) (Found: C, 58·7; H, 5·8; loss at 150°, 4·2. $C_{20}H_{22}O_8,H_2O$ requires C, 58·8; H, 5·9; H_2O, 4·4%). The *hexa-acetate* crystallized from methanol in fine needles, m.p. 107°; $[\alpha]_D^{22}$ −21° in acetone (*c*, 4·4) (Found: C, 59·3; H, 5·3; $CH_3\cdot CO$, 40·1. $C_{32}H_{34}O_{14}$ requires C, 59·8; H, 5·3; $CH_3\cdot CO$, 39·9%).

Structure of compound B_2

Compound B_2 (350 mg.) in methanol (20 ml.) was shaken in H_2 with 10% palladinized charcoal (150 mg.): 1 mol. prop. was taken up during 30 min. Isolation by evaporation and crystallization from water yielded *dihydro-B_2* (325 mg.), as needles of the monohydrate, m.p. 107°, which give the vanillin reaction. Loss of water at 100° (Found: 7·7. Calc. for $C_{14}H_{14}O_3,H_2O$: 7·3%) from the monohydrate gave anhydrous dihydro-B_2, m.p. 160°, light-absorption max. in ethanol 280 mμ (log ϵ 3·47) (Found: C, 72·7; H, 6·0. $C_{14}H_{14}O_3$ requires: C, 73·0; H, 6·1%). Dihydro-B_2 was characterized as the *tri-acetate*, which crystallized from methanol in needles, m.p. 58° (Found: C, 67·6; H, 5·8; $CH_3\cdot CO$, 35·7. $C_{20}H_{20}O_6$ requires C, 67·4; H, 5·6; $CH_3\cdot CO$, 36·2%).

Treatment of compound B_2 with diazomethane gave the tri-*O*-methyl ether, which was purified by distillation at 180° and 1 mm. Hg and twice crystallized from methanol as plates, m.p. 55–57° (Found: C, 75·2; H, 6·5; OMe, 33·5. Calc. for $C_{17}H_{18}O_3$: C, 75·5; H, 6·7; OMe, 34·4%). The m.p. (264°) of compound B_2 was not depressed by admixture with a specimen of 3:5:4′-trihydroxystilbene, m.p. 263°, prepared by the method of Takaoka (1940*b*, *d*).

Structure of compound B_1

A methanolic solution (20 ml.) of compound B_1 (500 mg.) was shaken in H_2 with 10% palladinized charcoal (150 mg.) for 30 min., when the uptake of H_2 was 1 mol. prop. Evaporation of the solvent gave a solid which, after crystallization from toluene containing a small proportion of ethanol, yielded prisms of *dihydro-B_1*, m.p. 208°, $[\alpha]_D^{22}$ −36° in acetone (*c*, 2·5), light-absorption max, in ethanol 280 mμ (log ϵ 3·47) (Found: C, 60·6; H, 6·1. $C_{20}H_{24}O_8$ requires C, 61·2; H, 6·1%), which slowly develop a red with vanillin reagent.

Permanganate oxidation. Finely powdered $KMnO_4$ (1·8 g.) was added gradually to an acetone solution (100 ml.) of the hexa-acetate (0·6 g.) of compound B_1, and the reaction was allowed to proceed at room temperature for 1 hr., when excess of $KMnO_4$ was destroyed with SO_2. The reaction mixture was evaporated and the residue was extracted with ether in a Soxhlet apparatus. Hydrolysis of the ether extract was effected by treatment for 30 min. on the boiling-water bath with N-NaOH under N_2. A mixture of phenolic acids (200 mg.) was isolated from the acidified reaction mixture with ether. Fractional crystallization from water (with charcoal) gave an acid, m.p. 165°, which when sublimed and further crystallized afforded *p*-hydroxybenzoic acid (50 mg.), m.p. 213°, undepressed by admixture with an authentic specimen, m.p. 215°. From the original mother liquors was isolated 3:5-dihydroxybenzoic acid (50 mg.), m.p. 225°, undepressed by admixture with an authentic specimen, m.p. 225°. The identity of these two acids was confirmed by paper chromatography in three solvent systems.

Hydrolysis. Treatment of compound B_1 (194 mg.) with N-H_2SO_4 (10 ml.) on the boiling-water bath for 30 min. effected hydrolysis. Extraction with ether afforded 3:5:4′-trihydroxystilbene (96 mg.), m.p. 264°, undepressed

by admixture with an authentic specimen, m.p. 264°. The residual aqueous solution was percolated (0·5 ml./min.) through a column (bed volume 13 ml.) of De-Acidite G anion-exchange resin (1·5 mg.equiv./ml.) in the free base form, and the column was washed free from sugar with 40 ml. of water. Partial evaporation of the eluate gave a solution (2 ml.), which was treated with phenylhydrazine (180 mg.) and acetic acid (0·2 ml.). Characteristic sheaves of glucosazone (200 mg.), m.p. 208° (decomp.), were formed, which had the same shape and m.p. as an authentic specimen. Oxidation afforded glucosotriazole, m.p. 195–196°, undepressed by admixture with an authentic specimen. The identity of the sugar in the eluate with glucose was further demonstrated by paper chromatography in three solvent systems. The molar ratio of aglucone to glucoside was 1:1 (Found: 49% aglucone. Calc. 56%).

Enzymic hydrolysis. Compound B_1 (100 mg.) was hydrolysed at 25° for 16 hr. with a solution containing emulsin, prepared from almonds by the method of Mann & Saunders (1938). 3:5:4′-Trihydroxystilbene (55 mg.), m.p. 264°, was isolated in 100% yield with ether from the acidified digest. Similar enzymic hydrolysis of dihydro-B_1 gave dihydro-B_2, i.e. 1-(3:5-dihydroxyphenyl)-2-(4-hydroxyphenyl)-ethane, m.p. and mixed m.p. 160°.

Hydrolysis of the methylation product. A methanolic solution (50 ml.) of compound B_1 (1·5 g.) was treated with an excess of diazomethane for 24 hr., when the excess was destroyed. The attempted crystallization of the reaction product from water afforded an amorphous powder (1 g.), m.p. about 160°. Hydrolysis of the methanolic solution (80 ml.) of this product with 4N-H_2SO_4 on the boiling-water bath under N_2 for 4 hr. gave the aglucone, which was extracted with ether. *3:4′-Dimethoxy-5-hydroxystilbene* (0·3 g.) crystallized from light petroleum (b.p. 80–100°) in plates, m.p. 115–116° (Found: C, 75·0; H, 6·1; OMe, 23·6. $C_{16}H_{16}O_3$ requires C, 75·0; H, 6·3; OMe, 24·2%). This compound differed from authentic 3:5-dimethoxy-4′-hydroxystilbene, m.p. 87–88°, which was prepared by decarboxylation of 3:5-dimethoxy-4′-hydroxystilbene-β-carboxylic acid.

RESULTS

The ether-soluble phenols of the heartwood of *Eucalyptus wandoo* are not directly extractable with ether, since these compounds are embedded in ether-insoluble membrane substances. When the heartwood was extracted with methanol, the methanolic extract transferred to water and the resulting aqueous solution extracted with ether, nearly 4% of the wood was obtained as ether-soluble phenols. The ether-soluble pinosylvin extractives of *Pinus* spp. behave similarly, and they, too, are laid down within ether-insoluble membrane substances (Hägglund, Holmberg & Johnson, 1936).

A two-dimensional chromatogram of the ether-soluble phenols showed the presence of eight constituents (Fig. 1). Three of these were chromatographically indistinguishable from chlorogenic acid, catechin or gallic acid and *p*-coumarylquinic acid respectively. Three of the remaining substances (spots 4, 6 and 7 in Fig. 1) showed an intense-blue fluorescence in ultraviolet light and their R_F values and colour reactions were not those of known naturally occurring coumarins. The hydroxystilbenes (spots 4, 6, 7) gave a red compound with tetrazotized benzidine.

Compound B_2, which exhibited absorption bands at 220 and 305 mμ, afforded a saturated dihydro-compound, $C_{14}H_{14}O_3$, the spectrum of which showed a single absorption band at 280 mμ. The fluorescence of compound B_2 was quenched by hydrogenation. This spectral shift and the quenching of the fluorescence on hydrogenation are characteristic of the hydroxystilbenes. In Table 2, compound B_2 is compared with a hydroxystilbene

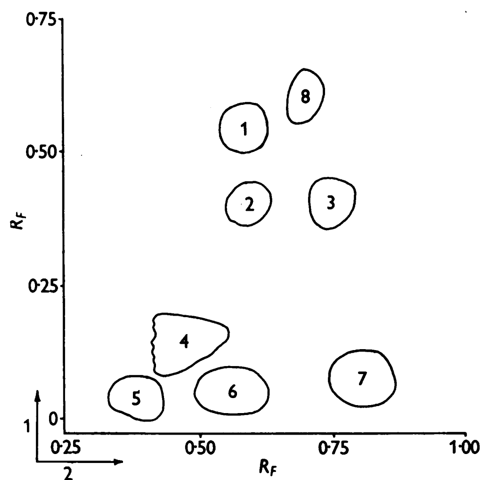

Fig. 1. Two-dimensional chromatogram of the ether-soluble phenols. The chromatogram was run first in N-acetic acid, followed by butan-1-ol–acetic acid–water (6:1:2, by vol.). Spots located by $FeCl_3$–$K_3Fe(CN)_6$: 1, chlorogenic acid; 2, catechin and gallic acid; 3, unknown; 4, compound B_1; 5, ellagic acid; 6, unknown; 7, compound B_2; 8, *p*-coumarylquinic acid.

Table 2. *Comparison of the compound of Takaoka* (1940*a*, *c*) *with compound* B_2

	Takaoka's compound	Compound B_2
Crystal form	—	Needles
M.p.	261°	264°
Colour with $FeCl_3$	Dark green	Not significant
Triacetate	Needles, m.p. 114–116°	Needles, m.p. 118–119°
Trimethyl ether	Crystals, m.p. 56–57°	Plates, m.p. 55–57°

which was isolated (Takaoka, 1940*a*, *c*) from a species of *Veratrum* (Liliaceae), and which was shown to be 3:5:4′-trihydroxystilbene (Takaoka, 1940*a*, *c*). The comparison is a close one, and the melting points and the colours with ferric chloride suggest that the material isolated in the present work is purer. The green colour described by Takaoka is incompatible with his proposed structure and probably due to slight contamination. The mixed melting point of compound B_2 and synthetic 3:5:4′-trihydroxystilbene showed that these substances were identical. 3:5:4′-Trihydroxystilbene was isolated in 1·0 % yield by wt. from a mixture of the whole heartwood from different trees.

The ultraviolet-absorption spectra of compound B_1 and its dihydro derivative, $C_{20}H_{24}O_8$, are identical with those of compound B_2 and its dihydro derivative respectively. It follows that compound B_1 is also a hydroxystilbene. Oxidative degradation of the hexa-acetate of compound B_1 yielded *p*-hydroxy- and 3:5-dihydroxy-benzoic acid. Acid hydrolysis of compound B_2 afforded 1 mol.prop. of 3:5:4′-trihydroxystilbene and 1 mol.-prop. of glucose. Compound B_1 is therefore a monoglucose derivative of 3:5:4′-trihydroxystilbene, and the lack of reducing properties of compound B_1 suggests linkage through carbon atom $C_{(1)}$ of the glucose residue. Hydrolysis of the glucoside and of the dihydroglucoside by emulsin showed that the glucoside linkage is in the β-configuration in relation to carbon atom $C_{(1)}$ of the glucose residue. The rotations of compound B_1 and of its hexa-acetate accord with those which have been recorded for phenol β-D-glucosides and their acetates (Elsner, 1935; Jermyn, 1955). Compound B_1 is therefore a β-D-glucoside of 3:5:4′-trihydroxystilbene. That the hydroxyl group in the 3-position of the aglucone may be implicated in the glucoside linkage is suggested by the slow response of the glucoside B_1 to vanillin reagent. This is confirmed by hydrolysis of the partially methylated glucoside which gave 3:4′-dimethoxy-5-hydroxystilbene, which differed in melting point from an authentic specimen of the isomeric 3:5-dimethoxy-4′-hydroxystilbene. 3:5:4′-Trihydroxystilbene-3β-D-glucoside was isolated in 0·1 % yield by wt. from a mixture of the whole heartwood from different trees.

DISCUSSION

The simultaneous occurrence of 3:5:4′-trihydroxystilbene and its 3-β-glucoside in mixed drillings from the heartwood of *Eucalyptus wandoo* Blak. (syn. *E. redunca* Schau, var. *elata*) raises the problem of the origin of these compounds and of their translocation to this tissue. Taxifolin-3′-β-glucoside has been found in the needles, cambium and sapwood of *Pseudotsuga taxifolia*, and in the wood or bark of *Larix occidentalis*, *Picea sitchensis* and *Thuja plicata* by Hergert & Goldschmid (1958). These workers suggest that taxifolin is synthesized in the needles, where it is present as the glucoside which is translocated downwards through the inner bark and transversely via the rays to the heartwood and outer bark. Taxifolin-3′-β-glucoside is hydrolysed to the aglucone by a β-glucosidase which is located at or near the sapwood–heartwood and inner–outer bark boundaries. This translocation mechanism would account for the concomitance of piceatannol (2:5:6:3′:4′-pentahydroxy-3:4-tetramethylenestilbene) (Grassmann, Endres & Pauckner, 1958) and piceatannol glucoside (2:6:4′-trihydroxy-3:4-tetramethylenestilbene-5:3′-diglucoside) (Endres, 1958) in the stembark of *Picea excelsa*. The presence of such glycolytic enzymes in the tissues of forest trees has been demonstrated. Thus Zimmermann (1958) has described an α-galactosidase which is attached to the cytoplasm of sieve tubes, and Pridham (1957 and unpublished work) has observed the presence of glycolytic enzymes, including a β-glucosidase in addition to an α-galactosidase, in the cambial and sapwood tissues of aspen (*Populus grandidentata*, *P. tacamahaca* and *P. tremuloides*). Similar considerations may apply to the hydroxystilbenes of *Eucalyptus wandoo* heartwood, but the fact that the stilbene-β-D-glucoside occurs throughout a transverse section through the heartwood (Table 3) suggests that β-glucosidases are absent from the heartwood and

Table 3. *Distribution of extractives and hydroxystilbenes in a transverse section through a 100-year-old primary limb (25 cm. radius)*

Location in the transverse section	Distance from centre (cm.)	Extractives (%)	Proximate amounts determined by comparative paper chromatography: 3:5:4′-Trihydroxystilbene (%)	3:5:4′-Trihydroxystilbene-3-β-D-glucoside (%)
Sapwood	24	6·1	Absent	Absent
Heartwood	23	25·6	0·6	1·2
Heartwood	14	21·3	1·0	0·1
Heartwood	0	16·2	1·5	0·04

sapwood of this tree, and the absence of this stilbene glucoside from the sapwood suggests that it is rapidly transported via the rays through this narrow zone (of sapwood). Alternatively, these hydroxystilbenes may be formed from non-aromatic precursors at the sapwood–heartwood boundary (Wise & Jahn, 1953).

3:5-Dihydroxy- and 3-hydroxy-5-methoxy-stilbene cause pine heartwood to resist wood-rotting fungi and insects. The activity of the stilbenes against these fungi is between 10 and 30 times greater than that of phenol (Erdtman & Rennerfelt, 1944; Rennerfelt, 1943; 1944*a–c*; 1946). Wood which had been treated with a dilute solution of 3-hydroxy-5-methoxystilbene remained uneaten by the termite *Cryptotermes brevis* Walker after 4–5 years (Wolcott, 1951). Professor H. Erdtman (personal communication) has suggested that the presence of hydroxystilbenes in dicotyledonous heartwood confers similar protection, and that the presence of such hydroxystilbenes may have contributed to the successful survival of these trees during evolution. The antifungal properties have been demonstrated of 3:5:2′:4′-tetrahydroxystilbene (Barnes & Gerber, 1955), which occurs in the heartwood of *Maclura aurantica* (Moraceae, see Table 1), and of 3:5-dimethoxy-4′-hydroxystilbene (King, Cotterill, Godson, Jurd & King, 1953), which occurs in the heartwoods of *Pterocarpus* spp. (Leguminosae, see Table 1).

The occurrence in other *Eucalyptus* spp. of such flavonoids as aromadendrin [in *E. calophylla* R.Br. and *E. corymbosa* Sm. (syn. *E. gummifera* (Gaertn.) Hochr.)] (Hillis, 1952; Maiden & Smith, 1895; Smith, 1896), aromadendrin-7-*O*-methyl ether (in *E. maculata* Hook) (Gell, Pinhey & Ritchie, 1958), kaempferol (in *E. calophylla*) (Hillis, 1952) and naringenin (in *E. maculata*) (Gell *et al.* 1958), which are related to the 3:5:4′-trihydroxystilbenes in the same way as the flavonoids of the genus *Pinus* are related to the 3:5-dihydroxystilbenes, recalls Erdtman's (1956) proposal for their ontogenesis. Since the use of ^{14}C-labelled cinnamic acid and shikimic acid and L-phenylalanine (Geissmann & Swain, 1957; Underhill, Watkin & Neish, 1957; Watkin, Underhill & Neish, 1957) has shown that the phloroglucinol ring of such flavonoids as quercetin originates direct from acetate metabolism, and that the remaining phenylpropane residue of the C-skeleton is derived from the shikimic acid metabolic pathway, it follows that the resorcinol ring of 3:5-dihydroxy- and 3:5:4′-trihydroxy-stilbene also originates from acetate and that the remaining phenylethane residue of their C-skeleton is produced from the shikimic acid metabolic pathway, if Erdtman's ontogenetic suppositions apply to the Pinaceae and Myrtaceae respectively.

SUMMARY

1. Two hydroxystilbenes have been isolated from the ether-soluble extractives of *Eucalyptus wandoo* heartwood by cellulose- and polyamide-column chromatography.

2. One of the hydroxystilbenes has been identified as 3:5:4′-trihydroxystilbene and the other as 3:5:4′-trihydroxystilbene-3-β-D-glucoside.

3. In *E. wandoo* heartwood, the 3:5:4′-trihydroxystilbenes are laid down within ether-insoluble membrane substances.

4. The translocation, function and ontogenesis of the 3:5:4′-trihydroxystilbenes is discussed.

The authors thank the Director and Council of the British Leather Manufacturers' Research Association for permission to publish this paper. We also thank Industrial Extracts Ltd., of Perth, Western Australia, for a gift of the plant material and for a grant which defrayed a part of the cost of this work, Professor W. Grassmann, München, 15, Max-Planck-Institut für Eiweiss-und Lederforschung, Schillerstrasse, 25, for a gift of Perlonpulver 'feinst', and Professor F. Wessely, Chemisches Institut der Universität Wien, Wien IX, Währinger Strasse, 38, for a gift of 3:5-dimethoxy-4′-hydroxystilbene-β-carboxylic acid.

REFERENCES

Barnes, R. A. & Gerber, N. N. (1955). *J. Amer. chem. Soc.* **77**, 3259.

Elsner, H. (1935). In *Kurzes Handbuch der Kohlenhydrate*, 4th ed., p. 271 *et seq.* Leipzig: Johann Ambrosius Barth Verlag.

Endres, H. (1958). *Chem. Ber.* **91**, 636.

Erdtman, H. (1956). *Sci. Proc. R. Dublin Soc.* **27**, 129.

Erdtman, H. & Rennerfelt, E. (1944). *Svensk Pappersmasse-Tidn.* **47**, 45.

Geissmann, T. A. & Swain, T. (1957). *Chem. & Ind.* p. 984.

Gell, R. J., Pinhey, J. T. & Ritchie, E. (1958). *Aust. J. Chem.* **11**, 372.

Grassmann, W., Deffner, G., Schuster, E. & Pauckner, W. (1956). *Chem. Ber.* **89**, 2523.

Grassmann, W., Endres, H. & Pauckner, W. (1958). *Chem. Ber.* **91**, 134.

Grassmann, W., Endres, H., Pauckner, W. & Mathes, H. (1957). *Chem. Ber.* **90**, 1125.

Hägglund, E. (1941). *Öst. Chem.Ztg.* **44**, 104.

Hägglund, E., Holmberg, J. & Johnson, T. (1936). *Svensk PappersförädlTidskr.* Special no. 37.

Hathway, D. E. (1958). *Biochem. J.* **70**, 34.

Hathway, D. E. & Seakins, J. W. T. (1958). *Biochem. J.* **70**, 155.

Hergert, H. L. & Goldschmid, O. (1958). *J. org. Chem.* **23**, 700.

Hillis, W. E. (1952). *Aust. J. sci. Res.* Ser. A, **5**, 379.

Jermyn, M. A. (1955). *Aust. J. Chem.* **8**, 403.

Kawamura, J. (1938). *J. pharm. Soc. Japan*, **58**, 405.

King, F. E., Cotterill, C. B., Godson, D. H., Jurd, L. & King, T. J. (1953). *J. chem. Soc.* p. 3693.

King, F. E. & Grundon, M. F. (1949). *J. chem. Soc.* p. 3348.

King, F. E., King, T. J., Godson, D. H. & Manning, L. C. (1956). *J. chem. Soc.* p. 4477.

Koch, J. E. & Krieg, W. (1938). *Chem.-tech. Z.* **62**, 140.
Linstedt, G. (1950). *Acta chem. scand.* **4**, 448.
Maiden, J. H. & Smith, H. G. (1895). *J. Roy. Soc. N.S.W.* **29**, 30.
Mann, F. G. & Saunders, B. C. (1938). In *Practical Organic Chemistry*, 2nd ed., p. 365. London: Longmans, Green and Co.
Mongolsuk, S., Robertson, A. & Towers, R. (1957). *J. chem. Soc.* p. 2231.
Pridham, J. B. (1957). *Analylt. Chem.* **29**, 1167.
Rennerfelt, E. (1943). *Svensk bot. Tidskr.* **37**, 83.
Rennerfelt, E. (1944*a*). *Medd. Skogsförsöksanst. Stockh.* **33**, 331.
Rennerfelt E. (1944*b*). *Skogsägaren*, **20**, 84.
Rennerfelt E. (1944*c*). *Skogsägaren*, **20**, 91.
Rennerfelt, E. (1946). *Medd. Skogsförsöksanst. Stockh.* **34**, 391.
Smith, H. G. (1896). *J. Roy. Soc. N.S.W.* **30**, 15.
Späth, E. & Schläger, J. (1940). *Ber. dtsch. chem. Ges.* **73**, 881.
Stark, J. B., Goodban, A. E. & Owens, H. S. (1951). *Analyt. Chem.* **23**, 413.
Takaoka, M. (1940*a*). *J. Fac. Sci. Hokkaido Univ.* (3), **3**, 1.
Takaoka, M. (1940*b*). *Proc. imp. Acad. Japan*, **16**, 405.
Takaoka, M. (1940*c*). *J. chem. Soc. Japan*, **61**, 30.
Takaoka, M. (1940*d*). *J. chem. Soc. Japan*, **61**, 1067.
Underhill, E. W., Watkin, J. E. & Neish, A. C. (1957). *Canad. J. Biochem. Physiol.* **35**, 219.
Watkin, J. E., Underhill, E. W. & Neish, A. C. (1957). *Canad. J. Biochem. Physiol.* **35**, 229.
Wise, L. E. & Jahn, E. C. (1953). In *Wood Chemistry*, 2nd ed., pp. 15, 16, 638 *et seq.* New York: Reinhold.
Wolcott, G. N. (1951). *J. econ. Ent.* **44**, 263.
Zimmermann, M. H. (1958). In *The Physiology of Forest Trees*, p. 381. Ed. by Thimann, K. V. New York: The Ronald Press Co.

A Study of Vitamin B_{12} Protection in Experimental Thyrotoxicosis in the Rat

By D. K. KASBEKAR, W. V. LAVATE, D. V. REGE AND A. SREENIVASAN
Department of Chemical Technology, University of Bombay, India

(*Received* 3 *March* 1958)

Occurrence of vitamin B_{12} deficiency in animals is not common. The maternal carry-over of the vitamin to the young and a substantial contribution to the host by the intestinal microflora render it difficult to induce experimental vitamin B_{12} deficiency even on strictly vitamin B_{12}-free diets. A widely practised method of producing such a deficiency is by the use of thyroid-active materials like iodinated casein. Young growing animals fed on diets supplemented with thyroprotein were therefore used by early investigators (Zucker & Zucker, 1950) for assays of liver preparations. In the presence of an ample supply of other nutrients, iodinated casein induces retardation of growth and subsequently a high mortality, which are partially counteracted by supplementation of the ration with vitamin B_{12} (Emerson, 1949; Cuthbertson, 1949; Sure & Easterling, 1950; Graham, Reichstein, Watson & Hier, 1952).

Relatively little is known, however, about the mode of action of thyroxine and the counteraction of its effects by vitamin B_{12} in intermediary metabolism. Recent work of Maley & Lardy (1955) suggests that the thyroid hormone probably acts by impairing the efficiency of oxidative phosphorylation. However, the hormone had no direct effect on the enzymes of oxidative phosphorylation, as shown by Cooper & Lehninger (1956) with digitonin preparations from mitochondria. It is possible therefore that damage to the mitochondrial morphology may be one of the reasons for the observed derangements. Fatterpaker, Marfatia & Sreenivasan (1955) studied certain model conjugation systems, such as acetylation of *p*-aminobenzoic acid and benzoylation of glycine, and observed that in the hyperthyroid animal there is a marked reduction in the efficiency of coupling of the energy-generating and -utilizing mechanisms. The counteraction of this condition by vitamin B_{12} led them to suggest that the primary manifestation of thyrotoxicosis is a deficiency of vitamin B_{12} and that vitamin B_{12} probably acts by restoration, in part at least, of oxidative phosphorylation.

The work presented here deals with the vitamin B_{12} reserves in tissues of animals fed on diets supplemented with iodinated casein. A pronounced lowering of liver and blood vitamin B_{12} is shown to be paralleled by a major derangement in the metabolism of soluble sulphydryl compounds in the liver. Observations on centrifugally separated cell fractions also indicate damage to the mitochondria. All these derangements are corrected by vitamin B_{12}, which suggests that vitamin B_{12} probably exerts protection through the maintenance of mitochondrial morphology and levels of liver sulphydryl compounds.

EXPERIMENTAL

Induction of thyrotoxicosis in rats. Weanling rats (Wistar strain) 3–4 weeks old and weighing about 40 g. were used. They were fed on a purified diet of the following composition (per cent, by wt.): ethanol-extracted casein 10, starch