POLYPHENOLS FROM THE STEM BARK OF *RHODODENDRON GRANDE* WIGHT

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ABSTRACT

Taxifolin (a dihydro flavonol) and a procyanidin have been isolated from the stem bark of *Rhododendron grande* Wight. It is of special interest that they represent neighbouring stages in the biogenetic evolution of polyphenols. The procyanidin occurs as a dimer for which probable structures are proposed based upon chemical and spectral evidence. This dimer represents a new type.

INTRODUCTION

DIHYDROFLAVONOLS and flavan-3, 4-diols represent two of the neighbouring stages in the evolution of polyphenols comprising chalkones, flavanones, flavonols, flavones and aurones besides the above two. We record here an interesting case of the co-occurrence of a dihydroflavonol and a proanthocyanidin[1] having the same hydroxylation pattern.

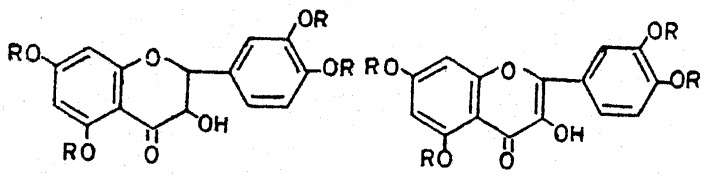
In the course of our chemical work on the stem bark of *Rhododendron grande* Wight, the acetone extract of the bark (which had previously been exhaustively extracted with petroleum ether and ether) afforded two substances, a dihydroflavonol and a proanthocyanidin. The identity of the dihydroflavonol as taxifolin[2] (5, 7, 3′, 4′-tetrahydroxydihydroflavonol) (I *a*) was proved by its properties and those of its tetramethyl ether (I *b*), by its aerial oxidation[3] to quercetin (II *a*) by boiling with aqueous sulphuric acid, and by iodine oxidation[4] of the methyl ether (I*b*) to quercetin tetramethyl ether (II *b*). All the substances were identical with authentic specimens available in the collection of Professor Seshadri.

The proanthocyanidin component yielded cyanidin on boiling with alcoholic hydrochloric acid. The identity of the flavylium salt was proved by its colour reactions, chromatographic behaviour and spectral properties. The proanthocyanidin gave a methyl ether whose methoxyl content indicated the presence of four methoxyl groups per C_{15} unit as expected. But the quantitative oxidation of the methyl ether with periodate indicated that it

consumed only half a mole of the reagent per C_{15} unit. This pointed to the existence of a dimer. The acetoxyl content of the acetate of the methyl ether agreed with that of the triacetate of a dimer made up of two leucocyanidin units. The dimeric molecule was hydrolysed[5] to the flavylium salt with ease. Using 0·6 N hydrochloric acid in alcohol flavylium salt colour appeared in 2–3 minutes. In a quantitative experiment employing the octamethyl ether, the flavylium salt colour developed almost as fast as with the tetramethyl ether of synthetic leucocyanidin treated under identical conditions.

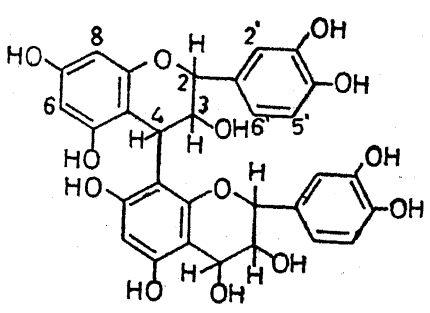
During acid treatment it was found that the proanthocyanidin yielded only cyanidin chloride and no catechin or *epi*catechin or any other flavylium salt as has been reported recently in certain cases of dimers.[6] This shows that the dimer is derived from two procyanidin units only. These facts are corroborated by the analytical data for elements and functional groups for the derivatives mentioned above. So far there is only one recorded instance of a flavandiol having condensed with itself to form a dimer;[7] but in it the resulting leucocyanidin dimer has been shown to lack a free glycol grouping and the linking involves two alcoholic hydroxyl groups, one derived from each monomer.

A number of possibilities exist for the condensation between two leucocyanidin units in which an alcoholic hydroxyl of one unit is utilised while the other unit still retains its glycol system. Either the 3 or 4 hydroxyl of the first one can be used up; the latter has been generally preferred whenever it is available. As the point of linkage in the second unit, position 8 has generally been preferred in the past, but recently the hydroxyl at position 2 of a hypothetical 2, 3, 4-flavantriol has been suggested in one case.- The observed behaviour on treatment with alcoholic HCl could be explained on the basis of either formula III or IV. NMR study which has provided valuable clues in cases of this nature enabling a clear choice, has in the present instance somehow failed to provide unequivocal evidence for the one or the other structure (*see* under experimental). It may however be mentioned that according to elemental analysis structure III seems to be the more probable.

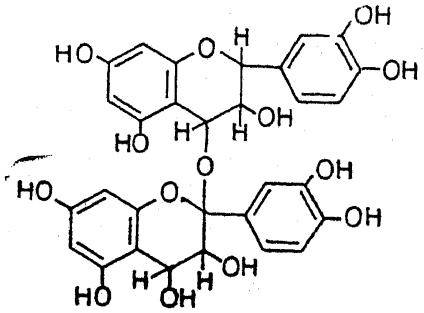


I a. R=H
b. R=CH_3

II a. R=H
b. R=CH_3



III



IV

The co-occurrence of the proanthocyanidin and dihydroflavonol supports the theory of biogenesis proposed for the flavonoid group of compounds by Seshadri *et al.*[3]

Experimental

Isolation of dihydroflavonol and proanthocyanidin

The powdered stem bark of *Rhododendron grande* was extracted successively with petroleum ether, ether and acetone. From the acetone extract the solvent was removed under reduced pressure and the semi-solid residue was repeatedly extracted with cold ethyl acetate by maceration. The combined extract after drying over $MgSO_4$ was concentrated to a small volume and excess of petroleum ether was added when a buff coloured powder was deposited. Paper chromatography indicated the presence of two components. The colour reactions and spectral properties of the components obtained pure by preparative paper-chromatography showed that they were of the nature of a proanthocyanidin and a dihydro-flavonol respectively. For obtaining sufficient quantity for chemical study separation was effected by extracting the mixture repeatedly with moist ether which removed the dihydroflavonol.

Dihydroflavonol (*Taxifolin*).—This crystallised from ethyl acetate-petroleum ether as colourless needles, m.p. 232–34° (d). It gave a bright red colour with Mg-HCl and deep violet colour with Zn-HCl and no colour with alcoholic HCl. The U.V. absorption in methanol showed max. at 228 and 288–9 mμ and with NaOEt the bands shifted to 246 and 325 mμ. These properties showed that the compound might be taxifolin. The identity was confirmed by mixed m.p. with authentic taxifolin and by paper chromatographic comparison using butanol-acetic acid-water (4 : 1 : 5) upper phase. (Found: C, 59·3; H, 4·5. $C_{15}H_{12}O_7$ requires: C, 59·2; H, 4·0%). $[\alpha]_D^{25}$ = 0° (alcohol).

Aerial oxidation of taxifolin.—Taxifolin was boiled for 30 hours with 2 N sulphuric acid. The suspension was extracted with ethyl acetate, the extract washed with water, dried, concentrated to small volume and diluted with excess of dry petroleum ether. The precipitated solid was crystallised twice from alcohol to give yellow solid, m.p. 304–8° (d). Its colour reactions and spectral properties in U.V. and visible region including shifts with standard reagents showed it to be quercetin and this was confirmed by paper chromatographic comparison with authentic quercetin and mixed m.p.

Taxifolin 5, 7, 3′, 4′*-tetra-O-methyl ether* prepared by the action of dimethyl sulphate and anhydrous potassium carbonate in acetone solution was crystallised from 50% alcohol as fine needles, m.p. 168–71°. It gave no colouration with alcoholic ferric chloride but an intense blue colour with nitric acid as described in the literature. Mixed m.p. with authentic sample was undepressed.

Oxidation of taxifolin 5, 7, 3′, 4′*-tetra-O-methyl ether to quercetin* 5, 7, 3′, 4′*-tetra-O-methyl ether by iodine.*—The tetramethyl ether mentioned above was oxidised with iodine as described by Goel[4] *et al.* The product crystallised from ethanol as pale yellow needles, m.p. 194–9° (*d*). Its properties agreed with those described in the literature for 5, 7, 3′, 4′-tetra-O-methyl quercetin.

Proanthocyanidin

The proanthocyanidin freed from the dihydroflavonol as described earlier was further purified by repeated precipitations from ethyl acetate solution by the addition of excess of petroleum ether. The almost colourless amorphous powder decomposed indefinitely at about 265°. It was highly unstable. $\lambda_{max.}$ 280 mμ (ethanol). It gave a green colour with alcoholic ferric chloride and a pinkish-red colour in 2–3 minutes on boiling with alcoholic hydrochloric acid.

Acid hydrolysis and identification of fission products. The proanthocyanidin (0·2 g.) in alcoholic hydrochloric acid (3 ml. of concentrated HCl made up to 50 ml. with alcohol) was refluxed for 2 hours and the solvent distilled under reduced pressure. After diluting the residual solution, it was extracted with ethyl acetate. The remaining aqueous solution which was coloured deep red was extracted with butanol. A drop of this butanol solution spotted on a paper chromatogram and developed in Forestal solvent (acetic acid-concentrated HCl-water, 30 : 3 : 10) gave only one spot, R_f 0·50, and in Hayashi and Abe solvent (acetic acid-concentrated

HCl-water, 5 : 1 : 5), R_f 0·32. $\lambda_{max.}$ 540 mμ; with $AlCl_3$ the value was 558 mμ. Co-chromatography confirmed its identity as cyanidin.

The ethyl acetate extract mentioned in the above paragraph was washed with a saturated solution of sodium bicarbonate followed by water. The residue obtained after removing the solvent was examined for the presence of catechin or epicatechin employing paper chromatography using the following solvent system (80 ml. of *n*-butanol shaken with 20 ml. of water and 1 ml. of ethylene glycol added to 50 ml. of organic phase[9]). No catechin or epicatechin could be detected in the test solution.

Proanthocyanidin octamethyl ether.—The methylation was carried out using dimethyl sulphate and anhydrous potassium carbonate in acetone solution. The product was purified by crystallisation from ethyl acetate-petroleum ether. A white powder sintering at about 183° and decomposing at 245° was obtained. [Found: C, 64·6; H, 6·0; OCH_3 32·3, $C_{38}H_{42}O_{13}$ (octamethyl ether of III) requires C, 64·6; H, 6·0; OCH_3 (8) 35·1%; $C_{38}H_{42}O_{14}$ (octamethyl ether of IV) requires C, 63·2; H, 5·8; OCH_3 (8) 34·4%]. $[\alpha]_D^{26}$ + 163·2° (*c*, 0·245 in methanol). The compound was insoluble in dilute aqueous sodium hydroxide and gave no colour with ferric chloride in alcohol.

The N.M.R. spectrum taken in $CDCl_3$ with tetramethyl silane as internal standard shows a very conspicuous, simple, strong and broad signal centred at 6·15 τ (methoxyl protons), but the other signals are neither conspicuous nor simple. The integration curve falls into three distinct regions: 8·33 τ to 9·47 τ integrating to 3-protons (hydroxylic protons at position 3 of one half and 3, 4 of the other half of the dimer), 4·7 τ to 7·5 τ integrating to 30 (or 29) protons [24 of 8 methoxy groups and protons at positions 2, 3, 4 (amounting to 6 according to structure III and 5 according to structure IV)] and 2·2 τ to 4·25 τ integrating to 9 (or 10) protons [aromatic protons at positions 6, 8, 2′, 5′ and 6′ (amounting to 9 according to structure III and 10 according to structure IV)].

Proanthocyanidin octamethyl ether triacetate.—The octamethyl ether described above was acetylated with acetic anhydride and pyridine (48 hour at 38°). The crude acetate was recrystallised twice from aqueous methanol, m.p. 201–05°. [Found: C, 64·0; H, 6·2, $-COCH_3$, 12·6. $C_{44}H_{48}O_{16}$ (corresponding to structure III) requires C, 63·5; H, 5·8; $-COCH_3$ (3) 15·5%. $C_{44}H_{48}O_{17}$ (corresponding to structure IV) requires C, 62·3; H, 5·7; $-COCH_3$ (3) 15·2%]. $[\alpha)]_D^{25}$ + 62·2° (*c*, 0·45 in pyridine).

Periodate oxidation of the proanthocyanidin octamethyl ether.—An aqueous solution of sodium meta periodate (25 ml.; 12·407 g./litre) was added to (*a*) proanthocyanidin octamethyl ether (93 mg. in 40 ml. ethanol) (*b*) leucocyanidin tetramethyl ether (25 mg. in 40 ml. ethanol) (*c*) analar glucose [10 mg. in water (3ml.) and 40 ml. ethanol] and (*d*) a blank (containing 40 ml. ethanol). The homogeneous solutions were kept for 48 hours. Each was then treated with 20 ml. of solution of arsenious oxide (*ca* 5·28 g./litre) and after ½ hour titrated with *ca.* N/10 iodine solution. The values for periodate consumed as calculated from these experiments are given below.

Compound	Dimeric pro-antho-cyanidin octamethyl ether	Blank	Glucose	Monomeric leucoantho-cyanidin tetra-methyl ether
Moles of Periodate consumed	1·085	Nil	5·3	1·1

Acid hydrolysis of proanthocyanidin octamethyl ether.—The octamethyl ether was hydrolysed like the parent proanthocyanidin dimer. The resulting flavylium salt isolated in the usual manner had an R_f value 0·95 (acetic acid : concentrated HCl : water, 30 : 3 : 10); $\lambda_{max.}$ 525 mμ. These values correspond to cyanidin chloride tetramethyl ether.

To assess the degree of conversion of the proanthocyanidin into flavylium salt, graded quantities of synthetic cyanidin tetramethyl ether and of the octamethyl ether of the natural proanthocyanidin dimer were boiled with 0·6 N alcoholic HCl under identical conditions. The visible absorption curves of the resulting flavylium colours were recorded and a graph of the amount of tetramethyl ether *vs.* transmittance was drawn. The plot obtained with the natural product was found to fall on the same curve showing that the rate of flavylium salt formation is nearly the same in both cases.

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Note added in proof.—In a communication dated March 7th, 1966, Drewes *et al.* (*Chem. Comm.*, 1966, 368) have described the occurrence of

a dimeric form of leucofisetinidin in the tannins of *Acacia mearnsii*. On the basis of spectral evidence they have adduced a 4, 6 linkage between the monomeric units. This paper came to the notice of the authors only by the last week of August 1966, almost 7 months after they had communicated the present paper.

REFERENCES

1. Freudenberg, K. and Weinges, K. — *Tetrahedron*, 1960, **8**, 336.
2. Hergert, H. L. .. *The Chemistry of Flavonoid Compounds*, Ed. T. A. Geissman, Pergamon Press, 1962, p. 577.
3. Krishnamurthy, H. G., Krishnamoorthy, V. and Seshadri, T. R. — *Phytochemistry*, 1963, **2**, 47.
4. Goel, R. N. and Seshadri, T. R. — *Tetrahedron*, 1959, **5**, 91.
5. Freudenberg, K. .. *Chemistry of Natural and Synthetic Colouring Matters and Related Fields*, Ed. T. S. Gore, B. S. Joshi, S. V. Sunthankar and B. D. Tilak, Academic Press (New York), 1962, p. 224.
6. Weinges, K. .. *Chem. Ber.*, 1961, **94**, 3032.
7. Lewak, S. .. *Roezniki Chemii Ann. Soc. Chim. Polonorum*, 1964, **38**, 1773; *C.A.*, 1965, 14615*c*.
8. Freudenberg, K. and Weinges, K. — *Angew. Chem. internat. Edit.*, 1962, **1**, 158.
9. ———— .. *The Chemistry of Flavonoid Compounds*, Ed. T. A. Geissman, Pergamon Press, 1962, p. 201.