Tetrahedron, 1966, Vol. 22, pp. 2367 to 2371. Pergamon Press Ltd. Printed in Northern Ireland

A NEW PROANTHOCYANIDIN FROM THE STEM BARK OF *MYRICA NAGI* THUMB.

V. KRISHNAMOORTHY and T. R. SESHADRI
Department of Chemistry, University of Delhi

(*Received* 30 *December* 1965)

Abstract—From the acetone extract of the stem-bark of *Myrica nagi* a new proanthocyanidin has been isolated. On boiling with alcoholic hydrochloric acid it gave delphinidin chloride and (+)-catechin and other complex products. The methyl ether does not consume periodic acid under conditions in which synthetic leucocyanidin methyl ether consumes one mole. From this and the analytical and spectral data (IR, NMR and mass spectra) it is concluded that in the new proanthocyanidin the 4-position of the flavan-3,4-diol is involved in the C-C linkage with the 8-position of the catechin part.

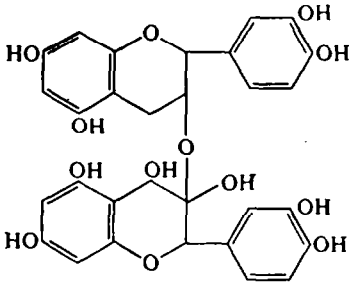
AMONG the naturally occurring polyphenolic compounds, the name proanthocyanidins, a special class of compounds which on acid hydrolysis give rise to anthocyanidin chlorides, has been proposed by Freudenberg[1] to include compounds of different hydroxylation patterns and different molecular sizes. The monomeric flavan-3,4-diols are the simplest in this class and have been extensively investigated.[2-5] The chemistry of the dimeric compounds including combinations of a flavan diol with a catechin has been studied and several possible structures have been assigned to them.

The Structure I, proposed by Forsyth and Roberts[6] for the proanthocyanidin isolated from fresh cacao beans, has been rejected by Freudenberg[9] on the basis that he isolated a proanthocyanidin identical in chromatographic behaviour and degradative studies from hawthorn berries for which he proposed structure II.[7]

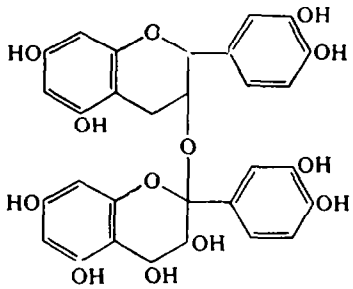
A proanthocyanidin[8] (III) which gives rise to (+) catechin and cyanidin on acid hydrolysis has been isolated from the fruits of *Gleditschia triancanthos*. Whether this exists in the keto or enol form is not clear. Recently Geissman and Dittmar, and Freudenberg and Weinges have isolated proanthocyanidins from avocado seeds[10] and cola nuts.[11] The same structure (IV) arrived at for both the proanthocyanidins differ from others in that the two C-15 units are linked through a C—C linkage. This structure has been confirmed by the NMR spectral data; the earlier structures are not supported by unequivocal evidence.

[1] K. Freudenberg and K. Weinges, *Tetrahedron* **8**, 336 (1960).
[2] A. K. Ganguly, T. R. Seshadri and P. Subramaniyan, *Tetrahedron* **3**, 225 (1958).
[3] K. R. Laumas and T. R. Seshadri, *J. Sci. Ind. Res. India* **17B**, 44, 167 (1958).
[4] G. R. Nagarajan and T. R. Seshadri, *J. Sci. Ind Res. India* **20B**, 615 (1961).
[5] A. K. Ganguly and T. R. Seshadri, *J. Sci. Ind. Res. India* **17B**, 168 (1958).
[6] W. G. C. Forsyth and J. B. Roberts, *Biochem. J.* **74**, 374 (1960).
[7] K. Freudenberg and K. Weinges, *Tetrahedron letters* 267 (1961).
[8] K. Freudenberg and K. Weinges, *Angew. Chem.* (Internat. Edit) **I**, 158 (1962).
[9] K. Freudenberg, *The Chemistry of Natural and Synthetic Colouring Matters and Related Fields* (Edited by T. S. Gore, B. S. Joshi, S. V. Sunthankar and B. D. Tilak) p. 224. Academic Press (1962).
[10] T. A. Geissmann and H. F. K. Dittmar, *Phytochem.* **4**, 359 (1965).
[11] K. Freudenberg and K. Weinges, *Chem. Communications* **11**, 220 (1965).

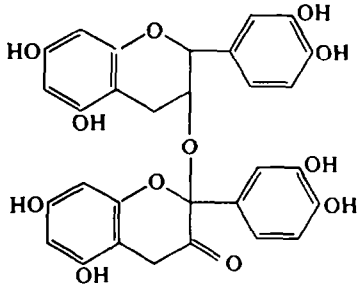
Chart I



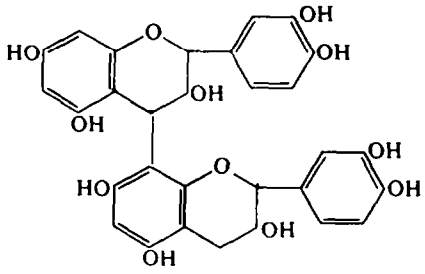
I



II



III



IV

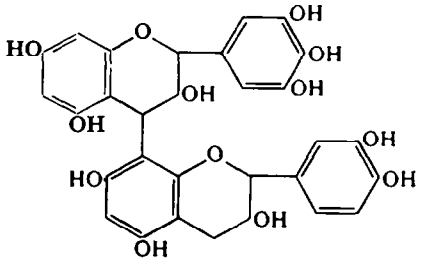
During the investigation of the stem-bark of *Myrica nagi* Thumb (f. Myricaceae), used as a tanning and dyeing agent and also as a fish poison, myriconol[12] was recently isolated from the ether extract and myricetin and myricitrin were earlier reported.[13,14] From the acetone extract by using the procedure of Ganguly and Seshadri for isolating leucoanthocyanidins,[15] an almost colourless amorphous powder was obtained. It had no definite m.p. decomposing from 200° onwards. It gave a bluish green colour with alcoholic ferric chloride and on treatment with hot acids a deep red colour developed indicating it to be a proanthocyanidin. The presence of a phloroglucinol system was indicated by the vanillin-hydrochloric acid test. The molecular formula, $C_{30}H_{26}O_{13}$, was deduced from an analytical study of its derivatives. The homogenity of the proanthocyanidin was confirmed by paper chromatography. Acetylation of the proanthocyanidin yielded a colourless product which was found to contain eleven acetoxyls showing that only two oxygens are involved in ether linkages. The methyl ether, obtained by the methylation of the proanthocyanidin further underwent acetylation to yield a diacetate, $C_{43}H_{48}O_{15}$, showing the presence of two alcoholic hydroxyls and nine phenolic hydroxyl groups. The absence of a 1,2-glycolic system was indicated by the failure of the methyl ether to react with sodium metaperiodate.

Mild acid hydrolysis of the proanthocyanidin or its acetate gave besides delphinidin

[12] V. Krishnamoorthy, N. R. Krishnaswamy and T. R. Seshadri, *Curr. Sci. India* **32**, 16 (1963).
[13] A. G. Perkin and J. J. Hummel, *J. Chem. Soc.* 1287 (1896).
[14] A. G. Perkin, *J. Chem. Soc.* 204 (1902).
[15] A. K. Ganguly and T. R. Seshadri, *Tetrahedron* **6**, 21 (1959).

chloride, (+)-catechin and unidentifiable products having lower R_f values. Vigorous acid hydrolysis using higher acid concentrations gave a good yield of delphinidin chloride identical with an authentic sample in its colour reactions, chromatography, and spectral properties. The production of delphinidin was in considerably better yields by using the method adopted by Robinson[16] in the case of crude butea gum. Based on the above degradative and analytical studies the following structure (V) has been assigned.

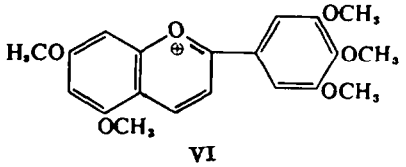
Chart II



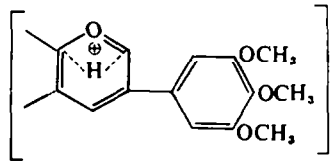
V

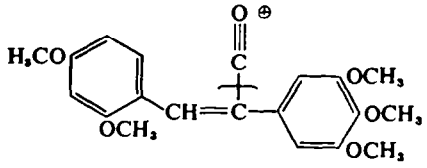
In the mass spectral data of the dimer methyl ether the molecular ion of the dimer is not recorded but there is no doubt that a dimer is involved because of signals above 400 found in the spectrum and the monomer can have mass value only below 400. There is considerable resemblance between this spectral pattern and that of the proanthocyanidin recorded by Freudenberg and Weinges.[11] The latter consisted of a unit of leucocyanidin and a unit of catechin. Since in the present compound leucodelphinidin is involved the peaks are 30 units higher than the corresponding ones of Freudenberg and Weinges. Examples are given below together with the possible structure to which they could be attributed; those given in brackets are from the spectrum of Freudenberg. (VI) 357 (327); (VII) 329 (299); (VIII) 192 (162). A number of peaks identical with those of previous workers and corresponding to the catechin part were obtained.

Chart III

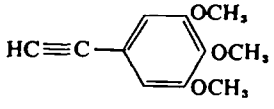


VI





VII



VIII

[16] G. M. Robinson, *J. Chem. Soc.* 1157 (1937).

In the NMR spectrum of the proanthocyanidin methyl ether acetate* were the following signals: 4·35 τ could be assigned to the proton at the 6 position of the catechin part, 3·60 τ, 4·00 τ (J = 2·5 c/s) to the metacoupled 6, 8 protons of the leucodelphinidin part, 4·80 τ to the proton at position 4 of the leucodelphinidin part, 5·50 τ to 6·70 τ (integrating to 31 protons) to nine methoxyls, two methine protons and two protons α to the acetoxyls, and 7·80 τ to 9·00 τ (integrating to 8 protons) to two acetoxyls and two benzylic protons of the catechin part. The NMR spectrum thus confirms the structure proposed for the dimer.

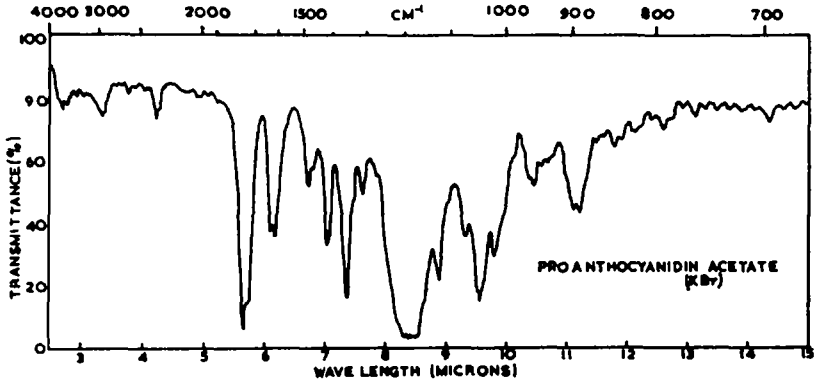


FIG. 1

EXPERIMENTAL

Isolation of the Proanthocyanidin. The finely powdered bark was extracted successively with pet. ether, ether and acetone. Acetone was removed under red. press. and the semi-solid residue thoroughly macerated and extracted with AcOEt. The extract was dried over anhydrous $MgSO_4$ and concentrated. First the coloured impurities were precipitated and removed and finally the proanthocyanidin separated. This process was repeated till an almost colourless, amorphous powder was obtained but it developed colour on exposure to air. It had no definite m.p. and decomposed over a wide range starting from 200° $[\alpha]_D^{EtOH}$ −87·9°. The analytical sample was dried over P_2O_5 at room temp. (Found: C, 54·8; H, 5·2; $C_{30}H_{26}O_{12}\cdot 3H_2O$ requires: C, 55·5; H, 4·9%.) It gave a bluish green colour with alcoholic $FeCl_3$ and a deep red colour with aqueous HCl on heating.

Proanthocyanidin acetate. A clear solution of proanthocyanidin (0·1 g) in Ac_2O (5 ml) and pyridine (1 ml) was left for 48 hr at room temp. On dilution an almost colourless solid separated which crystallized from aqueous EtOH or AcOEt–light petrol (40–60°) as a colourless powder, m.p. 130–135° with previous sintering at 115°. (Found for a sample dried over P_2O_5 at room temp: C, 56·2; H, 5·0; $COCH_3$, 41·1; $C_{52}H_{48}O_{24}$, $2H_2O$ requires C, 57·1; H, 4·8; $COCH_3$ (eleven), 42·3%), $[\alpha]_D^{EtOH}$ −88·9°. The IR spectrum of this compound is reported in Fig. 1. It gave a deep red colour on treatment with alcoholic HCl.

Proanthocyanidin methylether. To a solution of the proanthocyanidin (1 g) in acetone (100 ml), anhydrous K_2CO_3 (15 g) and dimethyl sulphate (2 ml) were added. The solution was refluxed for 6 hr, filtered free from K_2CO_3 and the acetone distilled off. The residue was treated with distilled water and the separated solid filtered off and washed with 1% NaOHaq and finally with water. The methyl ether crystallized from aqueous MeOH m.p. 160–165° with previous sintering at 156°. (Found: C, 62·8; H, 6·5; $C_{39}H_{44}O_{12}$, H_2O requires: C, 63·4; H, 6·2%.) It was insoluble in 1% aqueous alkali and optically inactive. It gave a deep red colour with alcoholic HCl.

Methylether diacetate. The foregoing methyl ether (0·1 g) in Ac_2O (5 ml) and pyridine (1 ml) was kept for 24 hr at room temp and the clear solution diluted with water. The product crystallized from

* Taken in $CHCl_3$ using tetramethylsilane as the internal standard on a varian 60 MC spectrophotometer.

MeOH as tiny prisms, m.p. 140–145° with previous sintering at 130°. (Found: C, 63·8; H, 6·5; $C_{42}H_{48}O_{18}$ requires: C, 64·2; H, 6·0%.)

Periodate oxidation. An aqueous solution of $NaIO_4$ (20 ml each; 2·036 g/250 ml) was added to (a) proanthocyanidin methyl ether (45·38 mg in aldehyde free EtOH (30 ml) (b) leucocyanidin tetramethylether (25 mg in EtOH 30 ml), (c) analar glucose (25 mg in EtOH 30 ml and water 3 ml), and a blank (containing 30 ml EtOH). The homogeneous solutions were kept for 48 hr, and then treated with a standard solution of As_2O_3 (25 ml each). After ½ hr the excess of AS_2O_3 was titrated iodimetrically and the amount of periodate calculated. The values are given below:

Compound	Proanthocyanidin methyl ether	Blank	Glucose	Leucodyanidin tetramethyl ether
Moles of periodate consumed	Nil	Nil	5·3	1·1

Delphinidin chloride. The proanthocyanidin (0·5 g) was suspended in water containing AcONa (1 g) and boiled for 8 min. After the addition of fused $ZnCl_2$ (1 g), the boiling was continued for another hr. The mixture was cooled, treated with a saturated solution of picric acid (10 ml) and then refluxed for 10 min. The refluxing was continued for 2 more hr after the addition of alcoholic HCl (110 ml; 8% v/v). Most of the alcohol was removed under red. press. and the solution diluted with distilled water. After extracting with AcOEt once, the solution was extracted repeatedly with isoamyl alcohol. The extract was washed with 1% HCl aq. The anthocyanidin was transferred into 1% HCl aq by adding excess petrol to the amyl alcohol layer. The aqueous HCl extract was thoroughly washed with benzene and petrol to remove isoamyl alcohol and concentrated to dryness in a vacuum desiccator over potash. An 1% HCl aq of the pigment gave a blue colour in amyl alcohol saturated with AcONa and this was completely destroyed in the oxidation test. R_f values of the anthocyanidin chloride 0·53 (phenol saturated with water) and 0·42 (lower phase of butanol:acetic acid:water; 4:1:5 v/v): λ_{max} 555 mμ in ethanolic 0·1% HCl.

(+)-Catechin and delphinidin chloride. The proanthocyanidin or its acetate (0·2 g) in alcoholic HCl (50 ml; 3 ml conc. HCl made up to 50 ml by alcohol) was refluxed for 2–3 hr and the solvent distilled under red. press. After diluting the residual brown solution it was extracted twice with AcOEt. The aqueous solution (deep bluish red) on working up gave delphinidin chloride. The AcOEt extract was washed thoroughly with a sat. $NaHCO_3$ aq and water. The residue obtained after removing the solvent was subjected to paper chromatography along with proanthocyanidin, catechin and epicatechin. (Solvent system: 80 ml n-butanol was shaken with 20 ml water; 1 ml ethylene glycol was added to 50 ml of the organic phase.)[17] Besides other products of low R_f values (+)-catechin was found to be present; no proanthocyanidin or epi-catechin could be detected.

[17] K. Freudenberg, *The Chemistry of Flavonoid Compounds* (Edited by T. A. Geissmann) p. 201. Pergamon, New York (1962).