Tetrahedron, 1958, Vol. 3, pp. 225–229. Pergamon Press Ltd., London

A STUDY OF LEUCOANTHOCYANIDINS OF PLANTS—I

ISOMERS OF LEUCODELPHINIDIN FROM KARADA BARK AND EUCALYPTUS GUM

A. K. GANGULY, T. R. SESHADRI and P. SUBRAMANIAN
Department of Chemistry, Delhi University, Delhi

(*Received* 30 *December* 1957)

Abstract—The leucoanthocyanidin of Karada bark (*Cleistanthus collinus*) has been isolated, and its acetate and methyl ether have been prepared and their properties and reactions studied. It is found to be a (−)-leucodelphinidin (5:7:3′:4′:5′-pentahydroxyflavan-3:4-diol). A *dextro*-rotatory leucodelphinidin is found in the kino (gum) obtained from *Eucalyptus pilularis* and it gives a different series of derivatives.

ALTHOUGH it is more than 30 years since the first study[1] of leucoanthocyanidins appeared and considerable interest has been shown in regard to their occurrence and function, the more common of the naturally occurring leucoanthocyanidins have not yet been isolated in a pure condition. This has been considered to be due to difficulties arising from their high instability and their discouraging properties. The only successful cases so far recorded are melacacidin[2] (which belongs to a special type and whose definite solubility in boiling ether helped the separation from the accompanying substances) and mollisacacidin[3] (leucofisetinidin). Consequently, efforts to elucidate precisely the part played by them in tanning, in dyeing, in regard to the taste and function of foodstuffs, in drugs and as antioxidants could not be made. A programme of exploration was therefore undertaken for suitable sources and suitable methods by which leucoanthocyanidins could be obtained pure in adequate quantity for experiments on their function. A good source has now been found in Karada bark, from which a pure sample of leucodelphinidin has been obtained.

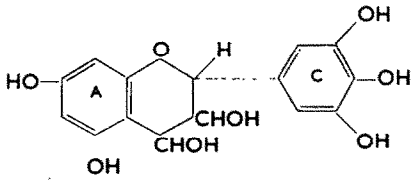
Karada bark is a popular tanning material. It is obtained from *Cleistanthus collinus*, a small tree found all over India, and it has been estimated that 10,000–12,000 tons of the bark can be collected every year. It has been reputed to contain a very high percentage of tannin and to yield good leather. Exploratory experiments indicated that it contains a single leucoanthocyanidin in good concentration. In actual isolation the crude sample amounted to 8–10 per cent of the air-dry bark, although after drastic purification only a 3 per cent yield of leucoanthocyanidin was obtained. For securing good yields it is necessary to use the bark as fresh as possible and the extraction has to be carried out at low temperatures, preferably with non-hydroxylic solvents, in order to avoid decomposition and oxidation. In our experiments extraction with low-boiling light petroleum (boiling range 40–60°) removed fatty matter, and subsequent extraction with acetone successfully removed all the leucoanthocyanidin. For purification the crude sample is taken up in ethyl acetate

[1] O. Rosenheim, *Biochem. J.* **14**, 178 (1920).
[2] F. E. King and W. Bottomley, *Chem. & Ind.* 1368 (1953); *J. Chem. Soc.* 1399 (1954).
[3] H. H. Keppler, *J. Chem. Soc.* 2721 (1957).

and subjected to fractional precipitation with light petroleum, whereby impurities are removed first and the leucoanthocyanidin is eventually obtained as a crystalline powder. Repetition of this process yields an almost colourless sample.

It has the molecular formula $C_{15}H_{14}O_8$, is *laevo*rotatory and gives the reactions typical of leucoanthocyanidins. A convenient crystalline derivative is the pentamethyl ether, obtained by means of diazomethane, and this subsequently forms a diacetate. By boiling with alcoholic hydrochloric acid the corresponding anthocyanidin and its pentamethyl ether have been obtained. The former has been identified by its colour reactions, chromatography and absorption spectra as delphinidin. Delphinidin chloride has also been obtained in a crystalline form from the leucoanthocyanidin by adopting a modification of the method used by Robinson[4] for butea gum.

Degradations were carried out with the methyl ether. Oxidation with neutral permanganate yielded trimethylgallic acid. The results of analysis and also the behaviour of the leucoanthocyanidin and the methyl ether with periodic acid conclusively showed that the compound is a flavandiol. The diol structure was further confirmed by the study of the infra-red spectrum of the methyl ether, which contained no carbonyl frequency. Hence the leucodelphinidin of Karada bark should be represented as 5:7:3′:4′:5′-pentahydroxy flavan 3:4-diol (I).



Eucalyptus gum (kino) has been used as a drug for a long time. A number of species of *Eucalyptus* are grown in India and *Eucalyptus pilularis* is one that is common in the Nilgiris. Leucodelphinidin has been extracted from this gum by adopting the same process as described above. It agreed with the leucodelphinidin obtained from Karada bark in its chemical properties. However, it is found to be *dextro*rotatory and the melting points of the derivatives are also different. It is therefore clear that the leucoanthocyanidin occurs in more than one form in nature. A detailed study of the stereochemistry involved is in progress.

EXPERIMENTAL

Isolation of the leucoanthocyanidin from Karada bark

The fresh bark of *Cleistanthus collinus* was obtained from the Central Leather Research Institute, Madras. The powdered bark (1 kg) was extracted repeatedly with low-boiling light petroleum. Concentration of the extracts yielded a colourless substance, which on crystallisation from ethanol had m.p. 141–142°. It did not give any characteristic colour with ethanolic ferric chloride, magnesium and hydrochloric acid, and zinc and hydrochloric acid, but it developed a red colour with acetic anhydride and concentrated sulphuric acid. The residual bark was first extracted with ether and subsequently with cold acetone. The ether extract did not yield any crystalline substance. The first acetone extract was deep red in colour, and the intensity of

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

the colour diminished with further extractions. The combined acetone extract was evaporated under reduced pressure to remove the acetone completely. The red sticky residue was extracted repeatedly with cold ethyl acetate. The ethyl acetate extract was dried over freshly ignited magnesium sulphate and then concentrated under reduced pressure. By gradual addition of light petroleum (boiling range 40–60°) coloured impurities were precipitated first. Further addition of light petroleum and cooling in a refrigerator yielded the leucoanthocyanidin as a crystalline mass. The process was repeated several times in order to obtain an almost colourless sample (30 g). It darkens and shrinks at 220° and does not melt up to 350° (Found: C, 52·1, 51·8; H, 5·3, 5·0. $C_{15}H_{14}O_8,1{\cdot}5H_2O$ requires C, 51·6; H, 4·9 per cent). The product was insoluble in light petroleum, ether, chloroform and benzene, but freely soluble in water, ethanol and acetone. With 10% ethanolic hydrochloric acid it developed immediate purple colour even in the cold; this deepened on keeping or on heating. With aqueous hydrochloric acid no colour was developed in the cold, but on heating a purple colour was produced along with the separation of a reddish brown amorphous material. With ethanolic ferric chloride the substance gave a blue colour. The ultra-violet spectrum of the leucoanthocyanidin showed a distinct maximum at 282 mμ and minimum at 250 mμ.

Conversion of the leucoanthocyanidin into delphinidin chloride

The leucoanthocyanidin (1 g) was suspended in water (15 ml) containing sodium acetate (2 g) and boiled for 8 min. The boiling was continued for another minute more, after the addition of fused zinc chloride (2 g). The reaction mixture was cooled thoroughly and then treated with a saturated aqueous solution of picric acid (18 ml) and the mixture was heated under reflux for 10 min. Ethanolic hydrochloric acid (200 ml, 8 per cent) was added to the reaction mixture and the heating under reflux was continued for 2 hr. The mixture was diluted with water, kept in the refrigerator for 2–3 hr, and filtered to remove the phlobaphene that separated out, and then the filtrate was extracted with *iso*amyl alcohol. The colouring matter was transferred to 1 per cent hydrochloric acid solution by the addition of light petroleum. The acid solution was washed thoroughly with benzene and concentrated at ordinary temperature over potassium hydroxide in a vacuum desiccator. The delphinidin chloride that separated was filtered off (yield 100 mg).

The following observations were made with the 1 per cent hydrochloric acid extract of the anthocyanidin chloride: (*a*) ferric chloride reaction, positive; (*b*) extraction with cyanidin reagent, no extraction; and (*c*) R_F (circular) with phenol–water, lower layer at 30°, 0·54. The ethanolic hydrochloric acid solution of the anthocyanidin chloride had an absorption maximum at 552 mμ.

Acetylation of the leucoanthocyanidin

Treatment of the leucoanthocyanidin (1 g) with acetic anhydride (10 ml) and pyridine (4 ml) in the cold for 48 hr yielded a solid (0·7 g), which crystallised from ethyl acetate and light petroleum as colourless short needles and rectangular prisms, m.p. 260° (dec. with sintering from 250°) (Found: C, 56·0; H, 4·7. $C_{29}H_{28}O_{15}$ requires C, 56·5; H, 4·5 per cent). It gave no colour with ethanolic ferric chloride. When the ethanolic solution of the acetate was boiled with concentrated hydrochloric acid, a pink colour developed.

Methylation of the leucoanthocyanidin

This was carried out with both diazomethane and dimethyl sulphate, the former reagent yielding a purer product.

Method (a). A solution of the leucoanthocyanidin (1 g) in methanol (70 ml) was treated at 0° with an excess of an ethereal solution of diazomethane until the mixture acquired a yellow colour. After the mixture had been kept for 24 hr at 0°, most of the solvent was removed and a small amount of water was added to precipitate the leucoanthocyanidin methyl ether (0·8 g). It crystallised from methanol as colourless tiny prisms, m.p. 160–164°, $[\alpha]_D^{36}$ −53·8° (ca. 0·4 per cent) (Found: C, 61·2; H, 6·2. $C_{20}H_{24}O_8$ requires C, 61·2; H, 6·2 per cent). On being heated with ethanolic hydrochloric acid, it developed a reddish colour. The methyl ether was insoluble in aqueous alkali and gave no colour with ethanolic ferric chloride.

Method (b). A solution of the leucoanthocyanidin (1 g) in dry acetone (100 ml) was heated under reflux with dimethyl sulphate (1·5 ml) and ignited potassium carbonate (5 g) for 6 hr. The potassium salts were removed by filtration, the acetone was evaporated from the filtrate and the residue was treated with cold water. The light coloured solid (0·5 g) did not yield good crystals from methanol and it had m.p. 161° (with sintering at 140°). It did not give any colour with ethanolic ferric chloride, but it developed a reddish colour on being boiled in ethanolic solution with concentrated hydrochloric acid. It was insoluble in aqueous alkali.

Infra-red spectrum. The spectrum of the leucoanthocyanidin methyl ether has been recorded in chloroform solution and the following are the main bands: 6·26 (s), 6·86 (s), 7·20 (m), 7·53 (m), 8·26 (m) and 8·79 μ (s).

Acetylation of the leucoanthocyanidin methyl ether. The methyl ether (0·2 g) was acetylated with acetic anhydride (2 ml) and pyridine (0·5 ml) in the cold for 24 hr. The acetate (0·1 g) crystallised from methanol as small cubes, m.p. 218–220° (Found: C, 60·7; H, 4·8. $C_{24}H_{28}O_{10}$ requires C, 60·5; H, 4·7 per cent).

Potassium permanganate oxidation of the methyl ether. A boiling solution of the leucoanthocyanidin methyl ether (1 g) in acetone (100 ml) was treated during 5 hr with powdered potassium permanganate (5 g) in small lots. After addition of water (10 ml) most of the acetone was removed by distillation and the mixture was decolorised by sulphur dioxide. The solution was extracted with ether and the extract was washed with aqueous saturated sodium bicarbonate. The bicarbonate solution was acidified with hydrochloric acid and then repeatedly extracted with ether. On removal of the ether, a brown sticky residue was obtained; on vacuum sublimation it yielded an acid, m.p. 165–166°, undepressed by an authentic sample of trimethylgallic acid. Circular paper chromatography was carried out, butanol saturated with ammonia being used as the irrigating solvent and bromophenol blue as the developer; a sharp ring was obtained having R_F 0·62. Trimethylgallic acid under similar condition gave R_F 0·60.

Eucalyptus pilularis gum (kino)

The sample of the gum was collected from the trees in Sims Park in Coonoor (Nilgiris).

Extraction. The finely powdered gum (600 g) was extracted successively with light petroleum, ether and acetone. The light petroleum extract contained waxy matter and did not yield any crystalline product. A small quantity of a light brown coloured

solid was obtained from the ether extract; its colour reactions indicated it to be triterpenoid. It could not be studied further owing to insufficiency of the material. Acetone extracted the leucoanthocyanidin successfully. It was purified by using a mixture of ethyl acetate and light petroleum as mentioned in the case of Karada bark (yield 12 g) (Found: C, 51·5, 51·4; H, 5·3, 4·9. $C_{15}H_{14}O_8$,1·5H_2O requires C, 51·6; H, 4·9 per cent). It darkens and shrinks at about 230° and does not melt up to 350°. It gave delphinidin chloride with ethanolic hydrochloric acid.

Acetate. The leucoanthocyanidin formed a colourless acetate with acetic anhydride and pyridine. It crystallised from a mixture of ethyl acetate and light petroleum as tiny prisms. It started melting at about 228° and finally decomposed at 240° (Found: C, 57·1; H, 4·0. $C_{29}H_{28}O_{15}$ requires C, 56·5; H, 4·5 per cent).

Methyl ether. The crystalline pentamethyl ether was prepared with diazomethane. It crystallised from methanol as colourless small prisms melting at 180–184° and the mixed m.p. with (—)-leucodelphinidin methyl ether was depressed, $[\alpha]_D^{32}$, + 72·9 (Found: C, 60·1, 60·1; H, 5·5, 6·1. $C_{20}H_{24}O_8$,0·5H_2O requires C, 59·9; H, 6·2 per cent).

Methyl ether diacetate. The methyl ether was acetylated with acetic anhydride and pyridine. The diacetate crystallised from methanol as short rectangular prisms and melted at 225–230°. The mixed m.p. with the methyl ether diacetate obtained from Karada bark was depressed (Found: C, 60·7; H, 4·8. $C_{24}H_{28}O_{10}$ requires C, 60·5; H, 4·7 per cent.).

Oxidation. Oxidation of the methyl ether with potassium permanganate yielded trimethylgallic acid, m.p. 166–167°, undepressed by admixture with an authentic sample.