LEUCOANTHOCYANIDIN-(FLAV-3-ENE-3-OL) ACETATES

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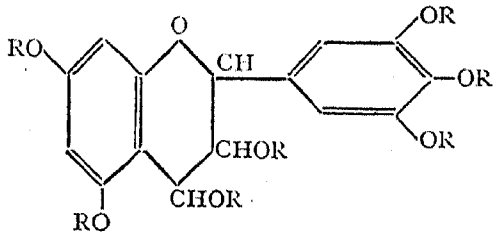
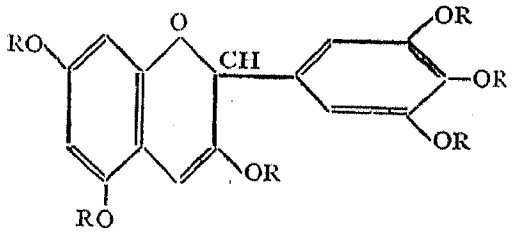
Received September 18, 1958

The conversion of leucoanthocyanidins of the flavan-3:4-diol type into the corresponding anthocyanidins gives very poor yields[1] (ca. 10%). In the course of our studies on leucoanthocyanidins obtained from various natural sources and by synthetic means, we have obtained certain results which are significant in that they offer a method of obtaining better yields in the above-mentioned conversion. For example a sample of leucodelphinidin (flavan-diol, I A) from *Phyllanthus emblica* bark[2] gave a normal heptaacetate (II) when acetylated in the cold with acetic anhydride and pyridine. On the other hand, an isomeric leucodelphinidin (flavan-diol, IB) isolated from *Myrica nagi* bark[3] when acetylated in the same way yielded a different compound which was a hexaacetate agreeing with the requirements of a flav-3-ene-3-ol acetate (III). On boiling with alcoholic hydrochloric acid it gave a comparatively high yield of delphinidin (IV). The leucodelphinidin (I A) could also be converted into its flav-3-ene-3-ol acetate by two stage acetylation, *i.e.*, first in the cold followed by heating the reaction mixture. This was found to be the same as the one obtained from (I B), and also gave a good yield of delphinidin chloride (IV). The two leucodelphinidins mentioned above represent two different stereo-isomers, (I B) being capable of losing the elements of water even in the cold and (I A) doing so only on heating. These experiments also show that flav-3-ene-3-ol acetates are good intermediates for the conversion of flavan-3:4-diols into anthocyanidins.

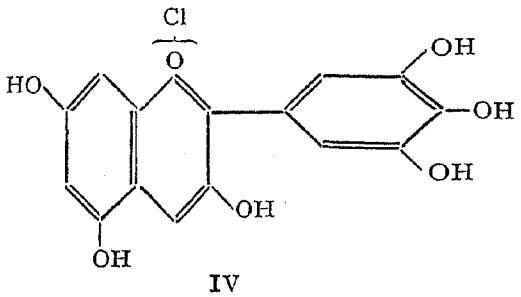


I, R=H

II, R=$COCH_3$

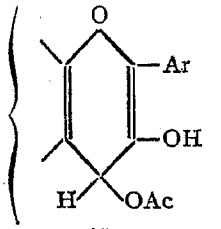


III, R=$COCH_3$

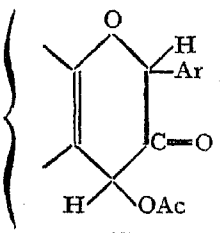


IV

In this connection should be mentioned certain observations which are of significance for the present subject. Robertson and Robinson[4] earlier carried out the reduction of rhamnetin by short boiling (7 to 8 minutes) with acetic anhydride, potassium acetate and zinc dust. The crude product was boiled with alcoholic hydrochloric acid giving a small yield of rhamnetinidin chloride besides polymerised products. Malkin and Nierenstein[5] repeated the process using only zinc and acetic anhydride and reported that they obtained only polymeric products from quercetin and rhamnetin. Recently King and White[6] described their results on the reductive acetylation of quercetin and other flavonols using acetic anhydride, sodium acetate and zinc dust and boiling for two hours. Though in each case the nature of the product was not clear and it did not have a sharp melting point, it underwent conversion into the corresponding anthocyanidin in good yields. The important point that they noted was that the intermediate compound had the same number of acetoxyl groups as the acetate of the original flavonol and that the reduction had not resulted in the increase of a hydroxyl or acetoxyl group. For example the product from quercetin had only five acetoxyl groups. King and White[6] gave (V) and (VI) as the probable structures for the reductively acetylated intermediate. Their suggestion seems to be that the carbonyl group of the flavonol alone underwent reduction yielding a derivative of anthocyanidin pseudo-base, and that one hydroxyl group escaped acetylation. But the analytical results do not agree with the proposed formula.



V



VI

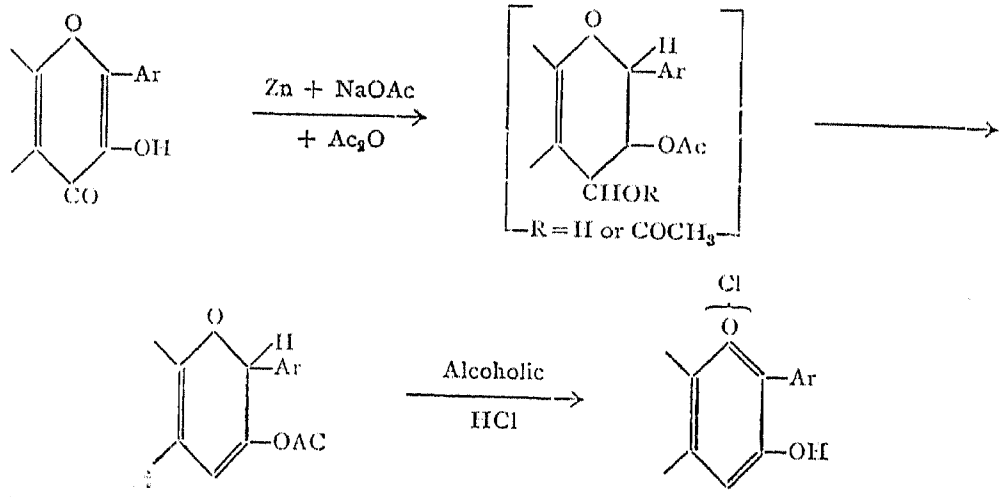
It appeared to us that the reductive acetylation procedure gave rise to flave-3-ne-3-ol acetates that have been mentioned in the earlier paras. Not

only is there close resemblance in physical properties, but the idea is fully supported by the analytical results reported by King and White.[6] The comparative data in the case of fisetin and quercetin (King and White[6]) and myricetin (present work) are given in Table I.

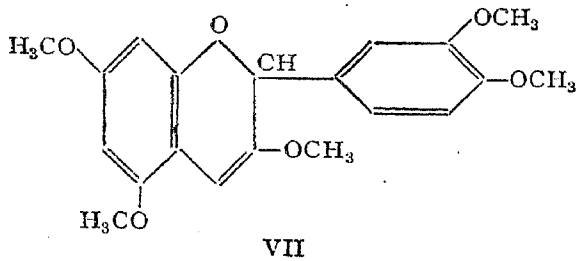
TABLE I

1	2	3	4	5	6
Original flavonol	King and White's formula for the reductive acetylation product	% Required for (2)	% Found	% Required for (6)	Present flav-3-ene-3-ol acetate formula
Fisetin	$C_{23}H_{20}O_{10}$	C, 60·5; H, 4·4	C, 62·6; H, 4·5	C, 62·6; H, 4·5	$C_{23}H_{20}O_9$
Quercetin	$C_{25}H_{22}O_{12}$	C, 58·2; H, 4·3	C, 59·8; H, 4·6	C, 60·2; H, 4·5	$C_{25}H_{22}O_{11}$
Myricetin	$C_{27}H_{24}O_{14}$	C, 56·6; H, 4·2	C, 58·0; H, 4·8	C, 58·3; H, 4·3	$C_{27}H_{24}O_{13}$

We now suggest the following explanation of the course of the reaction. The reduction could be visualised as affecting not only the carbonyl group but also the pyrone double bond. That the latter is actually more easily reduced is indicated by the earlier[7,8] finding that the reduction of chalkone with zinc dust and acetic acid yields dihydro-chalkone. The formation of the flavan-diol derivative is followed by the elimination of the elements of water giving rise to the proposed flav-3-ene-3-ol acetate. In the presence of alcoholic hydrochloric acid, deacetylation is accompanied by oxidation yielding the flavylium salt.



In support of the above may be mentioned the following points which do not agree with the formulation of King and White[6]: (i) Dihydroquercetin (taxifolin) in which the pyrone double bond is non-existent underwent reductive acetylation smoothly to yield the same acetate as was obtained from quercetin. The two products agreed in composition, melting point and infra-red spectrum. (ii) The reductive acetylation of myricetin gave a product which agreed with the leucodelphinidin-(flav-3-ene-3-ol) acetate obtained from the natural samples of leucodelphinidin isolated from *Phyllanthus emblica* and *Myrica nagi* in respect of infra-red spectrum and ready conversion into delphinidin chloride. As in other cases of reductive acetylation, the product had a lower and indefinite melting point and this may be due to the simultaneous formation of more than one isomer. (iii) Quercetin pentamethyl ether which does not contain any free hydroxyl group in 3-position underwent smooth reduction to give 3:5:7:3′:4′-pentamethoxy flav-3-ene (VII). This flavene had no acetoxyl group (as shown by the infra-red spectrum), contained all the five methoxyl groups intact and could be converted into pentamethyl cyanidin chloride.



VII

Earlier several methods were adopted for the reduction of flavonols leading to the formation of anthocyanidins. It seems to be possible that in all these cases the conjugated $O=\overset{|}{C}-C(OH)=\overset{|}{C}-$ system was hydrogenated to yield flavan-diols which under the conditions employed gave varying yields of anthocyanidin and phlobaphene.

EXPERIMENTAL

Leucodelphinidin—(flav-3-ene-3-ol) acetate

(i) The leucodelphinidin (I B) (0·3 g.) was suspended in acetic anhydride (10 c.c.) and then treated with dry pyridine (3 c.c.) to obtain a clear solution. After allowing it to stand at room temperature for 48 hours, it was poured over crushed ice with stirring. The colourless solid thus obtained was

filtered, dried and then crystallised from a mixture of ethyl acetate and light petroleum (40–60°) yielding tiny prisms (0·25 g.), m.p. 170–75° d (Found: C, 58·3; H, 4·6; $C_{27}H_{24}O_{13}$ requires C, 58·3; H, 4·3%).

(ii) As reported in a previous publication,[2] the cold acetylation of leucodelphinidin (I A) from *Phyllanthus emblica* bark yielded a leucodelphinidin heptaacetate, m.p. 240–45°. The two stage acetylation of this isomer has now been carried out as follows: The leucodelphinidin (I A) (0·2 g.) was dissolved in acetic anhydride (10 c.c.) and pyridine (2 c.c.), the solution left at the room temperature for 48 hours and then heated under reflux for 2 hours. There was some resinification during the heating. The mixture was poured over crushed ice and the dark solid that separated was collected and dried in a vacuum desiccator. It was dissolved in a small amount of ethyl acetate and the dark resinous impurities precipitated by the addition of a few drops of light petroleum (40–60°). Finally the almost colourless acetate was precipitated by the addition of excess of light petroleum. Crystallisation from a mixture of ethyl acetate and light petroleum gave rhombohedral plates (0·1 g.), m.p. 170–74° d (Found: C, 58·0; H, 4·8; $C_{27}H_{24}O_{13}$ requires C, 58·3; H, 4·3%). It agreed with the acetate form (i) in every respect.

Conversion into delphinidin chloride

The crude flav-3-ene-3-ol-acetate obtained in experiment (ii) from leucodelphinidin (I A) (0·4 g.) was converted into delphinidin chloride by heating with alcoholic hydrochloric acid as given below under (iii). The product (0·12 g.) was identical with delphinidin chloride.

(iii) *Reductive acetylation of myricetin.*—Myricetin (0·2 g.) was mixed with zinc dust (0·3 g.), fused sodium acetate (0·1 g.) and acetic anhydride (6 c.c.) and refluxed for 1 hour. The solution developed a red colour; more zinc dust (0·3 g.) was added and the refluxing continued for another hour. The mixture was filtered and the residue washed with small quantities of glacial acetic acid. The filtrate was poured over crushed ice and the separated solid filtered off. It was dissolved in a small quantity of glacial acetic acid and reprecipitated by the addition of water. It was then dried and finally purified from ethyl acetate and light petroleum (40–60°) yielding a colourless crystalline product (0·22 g.), m.p. 97–108° (Found: C, 58·4; H, 4·4; $C_{27}H_{24}O_{13}$ requires C, 58·3; H, 4·3%).

Conversion into delphinidin chloride

The above acetate (100 mg.) was refluxed with 10% alcoholic hydrochloric acid (25 c.c.) for 2 hours. Within 5 minutes the solution became

deep red. It was cooled, diluted with twice its volume of water and left in the refrigerator. The small quantity of dark red phlobaphene which had separated was filtered off and the filtrate extracted with isoamyl alcohol, which completely extracted the anthocyanidin chloride. The alcohol layer was washed with 1% aqueous hydrochloric acid. Finally more of 1% hydrochloric acid was added and the mixture shaken with a large excess of light petroleum (6 volumes). The aqueous acid solution was thoroughly washed with light petroleum and benzene to remove traces of isoamyl alcohol and then concentrated in a vacuum desiccator over potassium hydroxide. The crystalline anthocyanidin chloride (40 mg.) obtained agreed with delphinidin chloride in every respect. A drop of the 1% hydrochloric acid extract was subjected to circular paper chromatography using phenol-water (lower layer) as the irrigating solvent at 30°; a ring was observed with an Rf 0·55. A 0·1% methanolic hydrochloric acid extract of the anthocyanidin chloride showed a maximum at 540 mμ in the visible region. Its colour reactions agreed with those of delphinidin chloride fully.

Leucocyanidin-(flav-3-ene-3-ol) acetate (reductive acetylation of dihydroquercetin)

The reductive acetylation of dihydro-quercetin (0·2 g.) was carried out and the product purified in the same way as that from myricetin. The colourless tiny prisms (0·22 g.) melted at 90–100° (Found: C, 60·4; H, 5·0; $C_{25}H_{22}O_{11}$ requires C, 60·2; H, 4·5%). It agreed in analytical values and infra-red spectrum with the leucocyanidin—(flav-3-ene-3-ol) acetate obtained by the reductive acetylation of quercetin.

Conversion into cyanidin chloride

The above acetate (100 mg.) was converted into cyanidin chloride (45 mg.) in the same way as described for delphinidin chloride above. A drop of the acid solution of the anthocyanidin chloride when subjected to horizontal paper chromatography (phenol-water lower layer), gave Rf 0·72 at 30°. The anthocyanidin chloride showed an absorption maximum at 523 mμ in the visible region.

3:5:7:3′: 4′-*Pentamethoxy flav-3-ene (reduction of quercetin pentamethyl ether)*

Quercetin pentamethyl ether (0·2 g.) was subjected to reductive acetylation as in the case of myricetin. The product came out from ethyl acetate-light petroleum as colourless tiny prisms, m.p. 80–90° (Found: C, 63·9; 64·2; H, 6·1; 6·0; OCH_3 41·9; $C_{20}H_{22}O_6$, $1H_2O$ requires C, 63·8; H, 6·4; OCH_3, 43·1%). The flavene pentamethyl ether (100 mg.) was treated

with alcoholic hydrochloric acid in the same way as with leucocyanidin (flav-3-ene-3-ol) acetate. The product (25 mg.) melted at 148–50°. Pratt and Robinson[9] reported m.p. 152° for pentamethyl cyanidin chloride.

Summary

Leucodelphinidin (flavan-diol, I B) under mild conditions of acetylation and another leucodelphinidin (flavan-diol, I A) under more drastic conditions yield the same flav-3-ene-3-ol acetate which produces delphinidin chloride in much better yields than the original diols. Products of reductive acetylation of flavonols agree with flav-3-ene-3-ol acetates in important respects. Reductive acetylation of dihydro-quercetin yields the same product as that of quercetin. Quercetin pentamethyl ether suffers similar reduction under the same conditions. It is therefore concluded that reductive acetylation reduces both the ethylenic and carbonyl groups and is followed by loss of the elements of water.

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2-59 Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent,
and Published by The Indian Academy of Sciences, Bangalore.