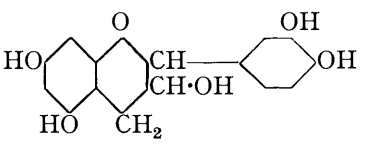
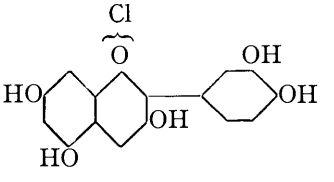
93. *The Transformation of* d-*Catechin into Cyanidin Chloride.*

By Herbert Appel and Robert Robinson.

In Freudenberg's experiments leading to the full elucidation of the relations existing between the isomeric catechins, no single observation was more significant than the formation of *dl*-epicatechin (I) by the catalytic hydrogenation of cyanidin chloride (II) (Freudenberg, Fikentscher, Harder, and Schmidt, *Annalen*, **1925**, **444**, 135). Nevertheless the reaction is difficult to bring to a successful conclusion, owing partly to the nature of the product and partly to the necessity for close attention to the activity of the catalyst and other conditions. It occurred to us that the converse transformation of catechin into anthocyanidin, if feasible, would have considerable advantages in that a method would be

(I.) 



(II.)

provided for the rapid preliminary study of various naturally occurring catechins, which could be related to anthocyanidins of known and characteristic properties. This could

be carried out even though the specimens were mixtures of stereoisomerides and the results with crude materials would be more decisive than those obtained by alkali fusion.

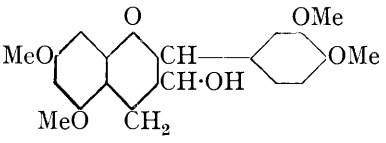
Such a conversion of catechin into cyanidin has not hitherto been realised, although several investigations on record deal with the formation of other oxonium salts from derivatives of catechin by dehydration and oxidation.

In exploratory work we found that *d*-catechin is a source of a series of coloured products when oxidised by various agents; these were usually unlike cyanidin in properties, being bluish-mauve or orange-red in acid aqueous solution. For example, the action of hydrogen peroxide on a hot alkaline solution of catechin, followed by acidification, furnishes a small yield of an orange oxonium salt, and the oxidation of tetramethylcatechin by means of chloranil in acetic acid solution gives the much bluer type of product.

The first indication of success in the anticipated direction was obtained by the use of brominated ethyl acetoacetate, but bromine in dioxan solution proved to be the key to the solution of the problem.

Judging from the colour changes, the method can be applied to *d*-catechin itself, but a smoother reaction ensues when the tetramethyl ether (III) is employed. Penta-acetylcatechin does not undergo a similar reaction.

(III.)



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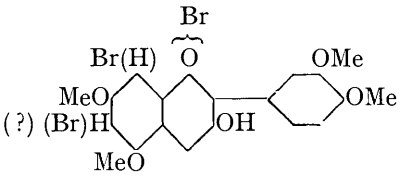
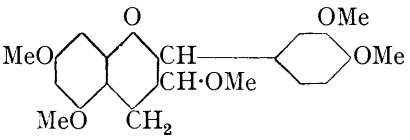


(IV.)



(V.)

The product of the action of bromine on either the tetramethyl ether or the pentamethyl ether (V) in hot technical dioxan solution is a *bromocyanidin tetramethyl ether bromide* (IV). The demethylation of (V) is strong evidence in favour of the view that the bromine first attacks the secondary alcoholic group.

In regard to the constitution of (IV) we have taken into consideration the fact that Kostanecki and Lampe (*Ber.*, **1906**, **39**, 4011) proved that bromocatechin tetramethyl ether is unsubstituted in the pyrocatechol nucleus; the most probable formulation is perhaps as an 8-bromocyanidin tetramethyl ether.

Contrary to previous experience in somewhat analogous cases (compare Willstätter and Robinson, *Ber.*, 1928, **61**, 2505), the demethylation of (IV) by means of hot hydriodic acid and phenol was accomplished satisfactorily; it was accompanied by debromination and the product was cyanidin iodide, convertible into cyanidin chloride of exceptional purity.

It is uncertain whether the addition of phosphorus, not previously employed, is responsible for this improved result or whether it is due to the presence of the bromine atom in the ring.

The cyanidin chloride thus obtained was carefully compared with specimens derived from cyanin (from blue cornflowers) and from synthetic benzoylcyanidin, with the result that no divergences of behaviour could be detected. The outcome is a further proof of the correctness of Freudenberg's catechin formula. If the alcoholic hydroxyl were situated at position 4, it is probable that the product would be a flavone or halogenated flavanone and not a benzopyrylium salt. It may be pointed out, however, that this new process is not of theoretical interest alone, for it also constitutes the most ready method of preparation of cyanidin chloride, especially on a relatively small scale.

Dioxan, recovered and distilled over sodium, could not be utilised for the reaction; this is considered to be due to the removal of the peroxides present in commercial dioxan. These peroxides evidently play an important rôle in the attack on the pyran ring and the question

of the detailed mechanism of this collaboration with the bromine is a very interesting one (compare Hannon and Kenner, J., 1934, 138). Activity is only partly restored to pure dioxan by the addition of benzoyl peroxide; thus, with "good" technical dioxan the yield averages 50%, with pure dioxan a coloration only is produced, and pure dioxan and benzoyl peroxide under the usual conditions afford about 10% of the theoretical yield. We hope to apply this procedure to the tea catechin II or gallocatechin of Tsujimura (*Sci. Papers, Inst. Phys. Chem. Research, Tokyo*, 1934, **24**, 149) and thus to confirm the conclusions of the Japanese chemist by the formation and characterisation of delphinidin.

Experimental.

Bromocyanidin Tetramethyl Ether Bromide (IV).—(A) A solution of bromine (1·85 g., 8 atoms) in technical dioxan (20 c.c.) was added to one of *d*-catechin tetramethyl ether (IV) (1·0 g., 1 mol.; m. p. 142—144°) in dioxan (100 c.c.) at 90°; a deep reddish-violet coloration developed rapidly. After about 20 seconds the mixture was quickly cooled, and crystallisation of the oxonium bromide was facilitated by alternate freezing and fusion of the solvent. The brownish reddish black crystals possessing a weak green glance were collected (0·8 g.) and washed with dioxan and ether (Found in material dried over phosphoric oxide and potassium hydroxide: C, 44·8; H, 4·3; Br, 29·1; MeO, 23·5. $C_{19}H_{18}O_6Br_2,0{\cdot}5H_2O$ requires C, 44·6; H, 3·8; Br, 31·3; 4MeO, 24·3%). The *bromide* could not be satisfactorily recrystallised, but the analyses of the crude product (which suggest the replacement of a small proportion of HBr by H_2O) are a sufficient indication of the composition.

The salt dissolves in 0·2% hydrochloric acid to a deep reddish-brown solution and the addition of sodium acetate or carbonate produces a nearly colourless, opalescent precipitate of the pseudo-base. Sodium hydroxide, especially on heating, produces a bright yellow solution when added to the salt or pseudo-base in aqueous or alcoholic media. The alcoholic solution of the salt has the intense bluish-red colour typical of cyanidin and on the addition of much water the pseudo-base is produced. The salt is not extracted from 0·2% hydrochloric acid solution by means of mixtures of toluene and *cyclo*hexanol, but the organic layer contains the pseudo-base.

(B) Under the above conditions, but using dioxan distilled over sodium (b. p. 100—101°), the salt was not isolated. Under the same conditions (pure dioxan) with the addition of benzoyl peroxide (2·0 g.), the yield of the bromide was 0·15 g.

(C) Under the conditions described under (A), but using *d*-catechin pentamethyl ether (V) (1·0 g. containing 7% of crystal-solvent) (Found in material dried in a high vacuum at 56°: MeO, 42·2. Calc. for 5MeO, 43·1%), the yield of bromide amounted to 0·55 g. (Found in material dried over phosphoric oxide and potassium hydroxide: C, 46·3; H, 4·1; Br, 33·3; MeO, 23·8. $C_{19}H_{18}O_6Br_2$ requires C, 45·4; H, 3·6; Br, 31·9; 4MeO, 24·7%). The reactions of this product are the same as those of the salt obtained from the tetramethyl ether. Neither specimen is quite homogeneous, so the question of identity does not arise. The main point, however, is that the salt under discussion contains no phenolic hydroxyl (as shown by the colour reactions) and, as it is a tetramethyl ether, the methoxyl in position 3 of the starting material has been eliminated.

Cyanidin Chloride (II).—A mixture of the crude bromocyanidin tetramethyl ether bromide (1 g.), red phosphorus (1 g.), phenol (6 g.) and hydriodic acid (50 c.c., *d* 1·7) was gently boiled in an atmosphere of carbon dioxide for 2 hours. After cooling, alcohol (10 c.c.), water (90 c.c.), ether (50 c.c.), and light petroleum (50 c.c.) were added and the solution was filtered from phosphorus. The aqueous layer was separated and concentrated under diminished pressure to about 80 c.c.; thereupon cyanidin iodide separated in its characteristic form (yield, 0·25 g.). The iodide was converted into the chloride by means of precipitated silver chloride in an alcoholic solution containing a few drops of concentrated hydrochloric acid. On evaporation of the filtrate on the steam-bath the cyanidin chloride separated in well-formed, straight, chocolate-coloured needles (Found in material dried over phosphoric oxide and potassium hydroxide: C, 53·0; H, 3·9; Cl, 10·8. Calc. for $C_{15}H_{11}O_6Cl,H_2O$: C, 52·9; H, 3·9; Cl, 10·4%). By using the bromide (1 g.) derived from catechin pentamethyl ether, cyanidin iodide (0·15 g.) was isolated and later converted into cyanidin chloride having the usual properties. The cyanidin chloride (*a*) prepared from catechin tetramethyl ether was carefully compared with a specimen (*b*) obtained by the hydrolysis of cyanin chloride and also with a synthetic specimen (*c*). The colours of acid, aqueous, and alcoholic solutions were identical and the known reactions with

sodium acetate, sodium carbonate, sodium hydroxide,* ferric chloride (both colour reaction and rate of oxidative destruction of pigment), and with buffered solutions of graded p_H (conditions of Robertson and Robinson, *Biochem. J.*, **1929**, **23**, 35) were observed in all three cases with the same results. Specimen (*a*) was light chocolate-brown in colour, (*b*) was much darker brown, and (*c*) had an intermediate colour, being more like (*a*) than (*b*); nevertheless the violet smears on paper were identical and on recrystallisation from aqueous alcoholic hydrochloric acid, by boiling off the alcohol from a test-tube, all three specimens gave prismatic needles having the same appearance under the microscope.

Advantage was taken of the opportunity to make more precise the use of the "cyanidin reagent" introduced by Robinson and Robinson (*Biochem. J.*, **1931**, **25**, **1693**). This is a mixture of *cyclo*hexanol (1 vol.) and toluene (5 vols.) and we have also used mixtures of *cyclo*hexanol (1 vol.) with toluene (3, 4, 6, and 7 vols.) which may be designated CT3, CT4, etc., according to the number of vols. of toluene.

The comparison was carried out as follows. Specimens (*a*), (*b*), and (*c*) (25·00, 25·04, and 25·03 mg. respectively) were treated in the same way; each was dissolved in 0·5% hydrochloric acid (300 c.c.) (cold solutions were deeper, browner, and bluer-red than the hot solutions), and 25 c.c. of this solution, mixed with an equal volume of 0·5% hydrochloric acid, were shaken with 50 c.c. of CT3; the experiment was repeated with CT4, CT5, CT6, and CT7. The organic layers were separated and formed a graded series of diminishing tinctorial intensity. The CT7 solutions from (*a*), (*b*), and (*c*) were directly compared in the colorimeter and found to be identical; the same was true for the CT6 solutions; and the CT5, CT4, and CT3 solutions gave the same information, but were diluted with an equal, double, and treble volume of 0·1% alcoholic hydrogen chloride, respectively, before colorimetric examination.

Under the above conditions the ratios of pigment concentration in the organic layers were found to be: CT7/6/5/4/3 = 1/1·74/3·32/5·18/9·95. Only adjacent members of the series were directly compared.

Determinations of the distribution number were made with CT3, CT4, and CT5. 10 C.c. of the organic layer with 10 c.c. of 0·5% hydrochloric acid were made up to 60 c.c. with 0·1% alcoholic hydrogen chloride. This solution was compared with 10 c.c. of the aqueous layer along with 10 c.c. of CT similarly made up to 60 c.c. The results were: CT3, D.N. 59·6 (*b*), 58·4 (*c*); CT4, 30·1 (*a*), 30·5 (*b*); CT5, 19·5 (*a*), 19·9 (*c*). We are unable to avoid errors of this order in a colorimetric method and the direct comparison described above is much more reliable than the absolute determination of the distribution number. The figures show, however, the striking effect of *cyclo*hexanol in these distribution experiments. It should be added that the compositions of the CT solvent mixtures giving similar ranges for the various anthocyanidins are widely divergent.

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