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THE OCCURRENCE OF CATECHINS IN FRUITS

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Abstract—(+)-Catechin and (−)-epicatechin have been isolated from the fruit of nine plant species using perlon powder columns. During the course of this study it was found that fresh pods of *Gleditschia triacanthos* contain not only (+)-catechin and a condensed procyanidin[1] but also a diglucoside of quercetin (m.p. 204–206°) and an unknown monoglucoside (m.p. 213–215°) of formula $C_{21}H_{22-24}O_{12}$. Neither 3-*O*-glucosyl-(−)-epicatechin nor the other products described by Gakhokidze[2] could be isolated or detected by chromatography. The physical properties of (+)-epirobidanol[3] (7,3′,4′,5′-tetrahydroxyflavan-3-ol) and of its tetramethyl ether are also described.

In connexion with investigations of condensed (or compound) proanthocyanidins,[1, 3–5] it was observed that fruits, pods, rinds, etc. formed the best sources for their isolation from plants. Condensed proanthocyanidins have, in fact, been isolated as crystalline acetates from the fruits of various species, but the elucidation of their structures is not yet complete.

There are generally only minute amounts of proanthocyanidins in the various fruits; the main phenolic products which we found and isolated were (+)-catechin or (−)-epicatechin or mixtures of the two. So far, we have not encountered any other catechins in the fruits we have examined. The catechins which occur in the various fruits are responsible for the red-brown coloration which occurs when the fruits are crushed or cut in the presence of air. Such treatment causes the catechins to come into contact with phenol oxidases which are present in the fruits and to form so-called dehydrogenation polymers. The catechins apparently do not come into contact with the oxidases during the normal metabolism in the living plant. This dehydrogenation of the catechins to give polymers observed in fruit and leaves is in contrast to the acid-catalysed intermolecular condensation of catechins to give flavonoid tannins observed in tanning woods.[6, 7] These are two completely different processes; there is nothing in common in the course of the reactions. Although the mode of formation of flavonoid tannins is well known,[6, 7] the dehydrogenation of catechins has not yet been successfully studied.

The fruit or rinds from which catechins have so far been isolated are listed in Table 1. It should be emphasized that various plants whose fruits are reported to contain catechins on

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[1] K. Freudenberg and K. Weinges, *Angew. Chem.* **74**, 182 (1962); *Angew. Chem. Internat. Edit.* **1**, 158 (1962).

[2] A. M. Gakhokidze, (a) *J. Appl. Chem.* (*U.S.S.R.*) **19**, 1197 (1946); *C. A.* **42**, 559 (1948); (b) *J. Appl. Chem.* (*U.S.S.R.*) **20**, 899 (1947); *C. A.* **42**, 4173 (1948); (c) *J. Appl. Chem.* (*U.S.S.R.*) **23**, 789 (1950); *C. A.* **46**, 9098 (1952).

[3] For the nomenclature of these compounds, see K. Freudenberg and K. Weinges, *Tetrahedron* **8**, 336 (1960).

[4] K. Freudenberg and K. Weinges, *Tetrahedron Letters 1962*, 267; *ibid. 1962*, 1073.

[5] K. Weinges, *Chem. Ber.* **94**, 3032 (1961).

[6] For reviews, see K. Freudenberg and K. Weinges, *Advances in the Chemistry of Organic Natural Products*, (L. Zechmeister), Vol. XVI, p. 1 (1958), Springer Verlag, Vienna, and in *The Chemistry of Flavonoid Compounds*, (Edited by T. A. Geissman) pp. 230 ff., Pergamon Press, London (1962).

[7] K. Freudenberg and K. Weinges, *Liebigs Ann. Chem.* **668**, 92, (1963); K. Weinges, Y. Naya and F. Toribio, *Chem. Ber.* **96**, 2870 (1963).

the basis of their detection by paper chromatography and others whose wood and bark have actually been shown to contain isolable amounts of catechins have been omitted.

It has long been known that the only naturally occurring flavanols are those with the (+)-catechin or (−)-epicatechin configurations, e.g. (+)-gallocatechin and (−)-epigallocatechin (5,7,3′,4′,5′-pentahydroxyflavan-3-ol); (+)-afzelechin and (−)-epiafzelechin (5,7,4′-trihydroxyflavan-3-ol). (−)-Robidanol,[3] (7,3′,4′,5′-tetrahydroxyflavan-3-ol), which was originally synthesized by us[12] from the corresponding hydroxyflavanonol, robidanolone,[3] and which has a configuration corresponding to that of (+)-catechin,[13] was found in nature by Roux and Maihs.[14] This is the first naturally occurring catechin without a hydroxyl group on C-5, and we have now converted it into (+)-epirobidanol. It appears extremely likely that its antipode, (−)-epirobidanol, will also be found occurring naturally.

TABLE 1. PLANTS FROM WHOSE FRUITS CATECHINS HAVE BEEN ISOLATED

Species*		(+)-Catechin	(−)-Epicatechin
Crataegus oxyacantha	F	−	+
C. monogyna	F	−	+
C. lavallei (carrierei)	F	−	+
Pyracantha coccinea	F	−	+
Theobroma cacao[8]	F	−	+
Malus various species[9]	F	−	+
Gleditschia triacanthos	F+R	+	−
Castanea vesca[10]	R	+	−
Vaccinium vitis-idaea	F	+	−
Rosa various species	F	+	−
Prunus amygdalus	R	+	+
Cola acuminata[11]	F	+	+
Wisteria sinensis	F+R	+	+

*F = Fruit-flesh, R = Rinds. Where no reference shown, this work.

During the course of this study we had already isolated (+)-catechin and a condensed proanthocyanidin[1] from the fresh fruit pods of *Gleditschia triacanthos*; on hydrolysis, the second compound yielded catechin and cyanidin. Now we have obtained from the same source two crystalline compounds, one a diglucoside of quercetin (m.p. 204–206°) and the other an unknown glucoside of formula $C_{21}H_{22-24}O_{12}$ (m.p. 213–215°). Gakhokidze[2] earlier described the isolation of 3-*O*-glucosyl-(−)-epicatechin, olmelin (4′-methoxy-5,7-dihydroxy-isoflavenone),† and acrammerin (8-methoxy-5,7,3′,4′,5′-pentahydroxyflavenone) from this source. The structure allocated by Gakhokidze[2] to olmelin (cherry-red crystals, m.p. 289–291°) is the same as that given by King *et al.* to biochanin A isolated[15] from the heartwood of *Ferreirea spectabilis*, and described as a pale yellow crystalline substance of m.p. 212–216°.

† Flavenone = flavone, cf. ref. 3.

[8] K. FREUDENBERG, R. F. B. COX, and E. BRAUN, *J. Am. Chem. Soc.* **54**, 1913 (1932).

[9] T. NAKABAYASHI, *J. Agr. Chem. Soc. Japan* **26**, 813 (1952); *C. A.* **48**, 5943 (1954).

[10] O. TH. SCHMIDT and G. HÜLL, *Chem. Ber.* **80**, 509 (1947).

[11] K. FREUDENBERG and L. OEHLER, *Liebigs Ann. Chem.* **483**, 140 (1930).

[12] K. WEINGES, *Liebigs Ann. Chem.* **615**, 203 (1958).

[13] K. WEINGES, *Liebigs Ann. Chem.* **627**, 299 (1959).

[14] D. G. ROUX and E. A. MAIHS, *Nature* **182**, 1798 (1958).

[15] F. E. KING, M. F. GRUNDON, and K. G. NEILL, *J. Chem. Soc.* *1952*, 4580.

Baker and Ollis[16] synthesized 4′-methoxy-5,7-dihydroxyisoflavenone and found it to be identical with biochanin A. 8-Methoxy-5,7,3′,4′,5′-pentahydroxyflavenone was prepared by Seshadri *et al.*[17] but was not identical with acrammerin. We were unable to isolate any of the three products described by Gakhokidze from the *G. triacanthos* pods available to us or even to detect them by chromatography. Hence although it is still an open question whether catechin glycosides occur in nature or not, Gakhokidze's observations appear to be somewhat doubtful.

EXPERIMENTAL

Isolation of (+)*-Catechin and* (−)*-Epicatechin from Fruits*

The fresh fruits were homogenized in a blendor under ethanol, and the alcoholic extract filtered off from the solid residue through cheese-cloth, and concentrated at 40° *in vacuo* under nitrogen. The resulting aqueous solution was centrifuged, saturated with NaCl and extracted repeatedly with ethyl acetate. The combined extracts were dried with Na_2SO_4, filtered, and carefully evaporated to dryness *in vacuo* under nitrogen. The residue was dissolved in a little ethanol and chromatographed on a column of perlon powder (150 × 5 cm) with ethanol as eluant; 25-ml fractions were collected automatically, and each fraction examined for catechins by paper chromatography. Water was used as the solvent, the spots being detected with ethanolic ferric chloride or methanolic vanillin/HCl. The fractions containing pure catechins were combined, taken to dryness, and the residues crystallized from a little water. The catechins were identified by m.p., specific rotation, and i.r. spectra of the free compounds and of their penta-*O*-acetyl derivatives. This general procedure was applied to all the species of fruits investigated by us (Table 1).

Phenolic Constituents of Fresh Gleditschia *Pods*

Fresh pods of *Gleditschia triacanthos* were treated according to the above general procedure. The ethanolic extract obtained was chromatographed on a perlon column and contained (+)-catechin and the condensed proanthocyanidin previously described[1] and also a quercetin diglucoside, yellow crystals from water, m.p. 204–206°. (Found: C, 52·00; H, 4·96. Calc. for $C_{27}H_{30}O_{17}$: C, 51·83; H, 4·83%.) The latter compound on hydrolysis by boiling in 2 N sulfuric acid yielded crystalline quercetin and the mother liquor contained glucose, identified by paper chromatography. The diglucoside was not investigated further.

A large quantity of phenolic substances remained dissolved in the aqueous salt solution following extraction with ethyl acetate. Re-extraction of this solution with ethyl acetate for 14 days in a liquid–liquid extractor, and subsequent purification of the extract by countercurrent distribution between ethyl acetate and water gave a fraction after 800 transfers which yielded colorless crystals on concentration. After recrystallization from water, this product gave colorless needles m.p. 213–215° of formula $C_{21}H_{22-24}O_{12}$. (Found: C, 54·14; H, 5·21. Calc. for $C_{21}H_{22}O_{12}$: C, 54·12; H, 4·76. Calc. for $C_{21}H_{24}O_{12}$: C, 53·89; H, 5·17%.) It gives a green–black color with $FeCl_3$ but no vanillin/HCl reaction. Its structure is being investigated.

(+)*-Epirobidanol from* (−)*-Robidanol* (*7,3′,4′,5′-Tetrahydroxyflavan-3-ol*)

Five grams of (−)-robidanol, which was prepared from robidan-3,4-diol,[10] and 0·05 g of sodium carbonate or acetate were dissolved in 150 ml of water under nitrogen. The flask was

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[16] W. Baker and W. D. Ollis, *Nature* **169**, 706 (1952).

[17] S. K. Balsubramanian, S. Nellakantan and T. R. Seshadri, *J. Sci. Ind. Research India* **14B**, 6 (1955); *C. A.* **50**, 2558 (1956).

closed and warmed at 80° for 48 hr. On standing for further 24 hr at room temperature, 2·2 g of unchanged (−)-robidanol precipitated out. The pale yellow supernatant contained mainly (+)-epirobidanol plus a little (−)-robidanol. The isomers were separated by counter-current distribution between ether and water. After 300 transfers, tubes 40–60 contained pure (+)-epirobidanol. After concentration of the solution *in vacuo* under nitrogen, the residue obtained was dissolved in a little water. On standing, (+)-epirobidanol precipitated out as colorless crystals. After one more recrystallization, the product was pure, m.p. 219–221°, $[\alpha]^{25}_{578} = +86{\cdot}4°$ ($c = 2$ in acetone/water 1:1). (Found: C, 62·30; H, 5·11. Calc. for $C_{15}H_{14}O_6$: C, 62·12; H, 4·87%.)

Tetra-O-methyl-(+)-epirobidanol

Methylation of (+)-epirobidanol with dimethyl sulfate and 50% aqueous potassium hydroxide and subsequent purification gave colorless crystals from aqueous methanol, m.p. 133–135°, $[\alpha]^{25}_{578} = +74°$ ($c = 2$ in *sym*-tetracloroethane). (Found: C, 65·76; H, 6·43; OCH_3, 35·55. Calc. for $C_{19}H_{22}O_6$: C, 65·95; H, 6·41; OCH_3, 35·88%.)