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Behavior of Certain 3-Hydroxyflavanones toward Bases and Basic Salts of the Alkali Metals and Ammonia

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Treatment of 3-hydroxyflavanones with bases or basic salts of the alkali metals and ammonia at a *p*H of 6.0 to 7.5 leads to the formation of monomolecular salts. Refluxing solutions of these salts causes racemization and/or conversion to the corresponding flavonol. A procedure for purification and separation of 3-hydroxyflavanones was developed on the basis of salt formation.

The preparation of naturally occurring flavonoids in pure form is often difficult and lengthy because of their instability and because of accompanying impurities. Several years ago Erdtman[1] and Lindstedt[2] reported that pinobanksin, 3,5,7-trihydroxyflavanone, precipitated out of sodium carbonate solution as an insoluble sodium salt. It could thus be separated from accompanying pinostrobin, 5,7-dihydroxyflavanone, which remained in solution. The nature of the salt was not reported. Investigation in this Laboratory indicates that treatment of *d*-dihydroquercetin and other 3-hydroxyflavanones with bases or basic salts of the alkali metals or ammonia at *p*H below 7.5 may give rise to three reactions depending upon the conditions of the treatment: monobasic salt formation, racemization and/or conversion to the corresponding flavonol.

The addition of basic salts or base to an aqueous solution of crude dihydroquercetin markedly increased the rate of its precipitation and the purity of the precipitate. Treatment of aqueous solutions of various flavonoids with basic salts and bases of sodium, potassium or ammonia increased the solubility of all except dihydroquercetin and several other 3-hydroxyflavanones. The solubility of the dihydroquercetin was decreased because of the formation of relatively insoluble "salts" which were found to contain one Na, K or NH_4 per molecule of dihydroquercetin. These salts are insoluble in dioxane and the aliphatic alcohols or ketones.

Thus, crude 3-hydroxyflavanones were purified by precipitating as the monoammonium, sodium or potassium salt, washing with acetone, acidifying the salt in an aqueous suspension and recrystallizing once from hot water. Dihydroquercetin was found to displace sodium from aqueous solutions of sodium salts derived from acids of pK_A 4 or higher. Inorganic salts of sodium or potassium derived from acids of pK_A 10 or higher could not be used over *p*H 7.0 because of the formation of soluble polybasic salts. Other flavanones or flavones were soluble in the aqueous solution or precipitated out unchanged and were soluble in the acetone wash. This procedure made possible a good separation of 3-hydroxyflavanones from other flavanones, flavones and tannins.

The 3-hydroxy group was established as being necessary, for 3′,4′,5,7-tetrahydroxyflavanone did not displace sodium from any sodium salts except the more strongly basic salts, such as sodium tribasic phosphate or sodium carbonate; *i.e.*, sodium salts of acids with pK_A 10 or higher. The flavanone-type structure also was established as necessary, for α-hydroxyacetophenone, which contains a hydroxy group adjacent to the carbonyl group as in the 3-hydroxyflavanones, and *d*-catechin, which lacks a carbonyl group, were non-reactive.

The 3-hydroxyflavanones can exist in two racemic modifications, one *cis*, the other *trans* with respect to the 2,3-hydrogens. If racemization should proceed at different rates on each of the two carbon atoms, as has been observed with epicatechin, which differs from *d*-dihydroquercetin only in the lack of a carbonyl group, a mixture of active, *dl-cis* and *trans* forms would result. Racemization of catechin with neutral or basic salts is accompanied by epimerization.[3] The resulting isomers have dissimilar physical properties and may be separated by fractional crystallization.

Careful fractionation of *d*-dihydroquercetin racemized with basic salts indicated complete homogeneity. Comparison of the X-ray diffraction patterns, derivatives, etc., of acid- or base-racemized dihydroquercetin, with *dl*-dihydroquercetin obtained by sodium hydrosulfite reduction of quercetin, showed complete identity.

Long reflux of aqueous solutions of dihydroquercetin with basic salts of Na, K or NH_3 or its Na, K or NH_3 salt alone causes conversion to quercetin. The reaction may be essentially an air oxidation. The findings support the belief that bisulfite solutions may not lose their ability to convert successive batches of dihydroquercetin to quercetin[4] and are in disagreement with the theory that dihydroquercetin is a strong promoter of bisulfite decomposition to thiosulfate.[5]

Experimental

Crude *d*-Dihydroquercetin.—Ground Douglas fir bark or cork were extracted with hot water.[6] The aqueous extract was evaporated *in vacuo* to 15% total solids content and then countercurrently extracted with ether in a column packed with glass Raschig rings. The ether extract was evaporated to dryness. Dihydroquercetin has been isolated by this procedure in this Laboratory from the barks of *Pinus ponderosa*, *Pinus jeffreyi* and *Pinus pinaster*.

Purification of Crude Dihydroquercetin with Ammonium Hydroxide.—The purification of crude dihydroquercetin was accomplished by dissolving 100 g. in hot water and treating with 30 ml. of 28% ammonium hydroxide. The mixture, *p*H 6.9, was cooled to 35°, filtered, washed with warm water and then suspended in hot water. Hydrochloric acid was added to a *p*H of 3.0. Upon cooling, a crystalline precipitate of *d*-dihydroquercetin and a trace of *dl*-dihydro-

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quercetin were obtained, m.p. 238–240°, $[\alpha]^{25}D$ +38° (*c* 4.0, acetone–water 1:1), yield (based on crude extract), 80%. *d*-Dihydroquercetin pentacetate, prepared by the reaction of acetic anhydride and pyridine at room temperature for 24 hours and recrystallization of the crude acetate derivative from methanol, had m.p. 129–130°.

Approximately similar maximum recovery of pure dihydroquercetin was obtained with sodium and potassium hydroxides or their basic salts at *p*H 6.9.

Ammonium Salt of Dihydroquercetin.—Four grams of dihydroquercetin was dissolved in 100 ml. of distilled water. The mixture was brought to boiling and 160 ml. of 14% ammonium hydroxide (1.0 molar equivalent) added. The precipitate obtained upon cooling was washed with 50 ml. of acetone to remove any unchanged dihydroquercetin. (It was found that 0.25 g. of the ammonium salt was soluble in 50 ml. of acetone, while unreacted dihydroquercetin was extremely soluble in acetone.) Recrystallization from hot water yielded white needles, m.p. 227–228° dec.

Anal. Calcd. for $NH_4C_{15}H_{11}O_{12}$: NH_4, 5.6. Found: NH_4, 5.5.

Solubility of the salt in 100 g. of distilled water at 100° was approximately 3.0 g.

An alternate procedure was developed as follows: dihydroquercetin, 10 g., was dissolved in 100 ml. of acetone. Four ml. of 14% ammonium hydroxide (1.0 molar equivalent) was added and the mixture allowed to stand for several hours. The crystalline precipitate was filtered off and recrystallized once from water; m.p. 227–228° dec. The analyses were the same as above. The addition of smaller or larger than molar amounts of ammonium hydroxide affected the yield of the ammonium salt obtained but did not change the composition.

Two-gram samples of dihydroquercetin were dissolved in 50 ml. of hot water and 5 g. of various ammonium salts added. Ammonium monobasic phosphate, ammonium sulfate, ammonium chloride or ammonium nitrate had no effect; unchanged dihydroquercetin was precipitated. When ammonium dibasic phosphate, ammonium tribasic phosphate, ammonium acetate, ammonium oxalate, ammonium carbonate or ammonium sulfite were added the ammonium salt was obtained.

Ammonium Salt of Other Flavanones.—Aromadendrin (3,4′,5,7-tetrahydroxyflavanone) and pinobanksin gave salts which contained one mole NH_4 per mole of flavanone moiety.

Insoluble ammonium salts could not be obtained under the same conditions from the following compounds: *d*-catechin (3,3′,4′,5,7-pentahydroxyflavone), eriodictyol (3′,4′,5,7-tetrahydroxyflavanone), 7,3′4′-trihydroxyflavanone, 7-hydroxyflavanone or α-hydroxyacetophenone.

Sodium Salt of Dihydroquercetin.—Four grams of dihydroquercetin and 5 g. of sodium acetate were dissolved in 100 ml. of hot water. Upon cooling, white needles of the sodium salt of dihydroquercetin were obtained, which were insoluble in dioxane, methanol or acetone. The precipitate was recrystallized once from hot water and washed with 25 ml. of acetone; yield 87%, dec. pt. 245–250°. The *p*H of a 0.0113 *M* aqueous solution (25°) was 7.6.

Anal. Calcd. for $NaC_{15}H_{11}O_7$: Na, 7.06. Found: Na, 7.21.

Dihydroquercetin, m.p. 239–241°, $[\alpha]^{25}D$ +38°, was regenerated by acidifying an aqueous solution of the Na salt with hydrochloric acid to a *p*H of 3.0.

Hot aqueous solutions of dihydroquercetin (4 g. in 100 ml.) were treated with various amounts of basic sodium salts or hydroxide. The precipitate obtained at the end of 12 hours was filtered off and dried. It was then washed with 25 ml. of acetone to remove unreacted dihydroquercetin and the insoluble residue was weighed. Maximum yields were obtained with sodium tribasic phosphate, sodium bicarbonate or sodium hydroxide at *p*H 6.5 to 7.0. Acidic or neutral salts of sodium, such as sodium chloride, sodium nitrate, sodium sulfate, sodium hydrogen sulfate or sodium monobasic phosphate did not give the sodium salt of dihydroquercetin.

Potassium Salt of Dihydroquercetin.—Equal parts of dihydroquercetin and potassium dibasic phosphate in boiling water gave the potassium salt of dihydroquercetin upon cooling, dec. without melting, 240–245°, yield 93%.

Anal. Calcd for $KC_{15}H_{11}O_7$: K, 11.40. Found: K, 11.46.

Potassium carbonate, or potassium hydroxide, and dihydroquercetin in water, also gave a potassium salt of the same composition in maximum yield at *p*H 6.5 to 7.0. Potassium chloride, potassium monobasic phosphate and potassium sulfate were ineffective.

Barium Salt of Dihydroquercetin.—Dihydroquercetin (10 g.), $Ba(OH)_2 \cdot 8H_2O$ (6.0 g., 0.57 molar equivalent) and 250 ml. water were brought to boiling. The precipitate obtained upon cooling was recrystallized once from hot water and washed with 50 ml. of acetone, dec. pt. 245–250°, yield 86%.

Anal. Calcd. for $Ba(C_{15}H_{11}O_7)_2$: Ba, 18.5. Found: Ba, 19.1.

Conversion to Flavonol.—Ten grams of the ammonium salt of dihydroquercetin were suspended in 100 ml. of water and refluxed for 16 hours. A bright yellow precipitate of quercetin was filtered off and washed with warm dilute hydrochloric acid to remove dihydroquercetin, yield 64%, m.p. 312–314°. No depression in melting point was observed upon admixture with an authentic sample of quercetin.

Aromadendrin (1.10 g.) and sodium hydrogen sulfite (0.75 g.) were dissolved in 5 ml. of water and refluxed for 2 hours; the yield was 70% of yellow crystals of kaempferol (3,4′,5,7-tetrahydroxyflavone), m.p. 278–279° (lit. 280°[7]). Similar treatment of pinobanksin (0.1 g.) with sodium hydrogen sulfite (2.0 g.) in 40 ml. of distilled water gave in 2 hours a 35% yield of the corresponding flavonol, galangin, m.p. 214–215° (lit. 215–216°[8]).

Flavanones unsubstituted in the 3-position, *i.e.*, 3′,4′,7-trihydroxyflavanone, 4′,5,7-trihydroxyflavanone, 3′,4′-dihydroxyflavanone, 7-hydroxyflavanone, eriodictyol and catechin (3,3′,4′,5,7-pentahydroxyflavane) did not react with aqueous sodium hydrogen sulfite or sodium sulfite to give the corresponding flavone or flavene. These compounds are very sparingly soluble in hot or cold water, with the exception of catechin. The solubility in hot water is greatly increased by the addition of sodium sulfite. Thus a 10% sodium sulfite solution was found to be a very good recrystallizing medium for these compounds when they were contaminated with tannins, etc. No degradation, salt formation or conversion to the corresponding chalcone was noted.

Basic Racemization of Dihydroquercetin.—Ten grams of *d*-dihydroquercetin and 15.0 g. of sodium dibasic phosphate were dissolved in 150 ml. of hot water. After refluxing for 1.5 hours, the solution was acidified to a *p*H of 3.2 with hydrochloric acid. The precipitate obtained upon cooling was filtered off and recrystallized several times from water; m.p. (after vacuum drying over P_2O_5 at 105°) 237–239°, $[\alpha]^{25}D$ +0.8 ± 0.2° (acetone–water, 1:1, *c* 4.0). Fractional crystallization indicated the material to be homogeneous. The yield was 65%. Other bases substituted for sodium dibasic phosphate were sodium carbonate, ammonium hydroxide, sodium hydroxide or trisodium phosphate. These were added in sufficient quantity to bring the solution to a *p*H of 7.0. At *p*H 5.0 to 6.0, racemization proceeded very slowly. When *d*-dihydroquercetin was refluxed with sodium hydroxide at *p*H 9.0 for 2.5 hours, only 15% could be recovered.

The acetate derivative was prepared with acetic anhydride and pyridine, m.p. 150–151°.

Comparison of the infrared spectra, X-ray powder diffraction patterns and solubilities of the *dl*-dihydroquercetin obtained by basic or acid racemization,[9] and the sodium hydrosulfite reduction of quercetin[10] indicated that they were identical.

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