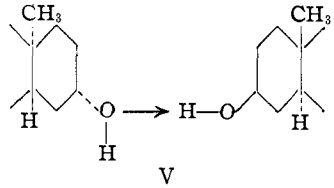
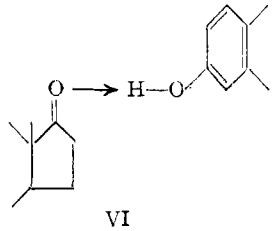
of two contiguous molecules (*cf.* V). Substances, which, like estrone, possess hydroxylic and ketonic groups at opposite ends of the molecule are ordinarily propagated in the crystal by head to tail association as in VI. Disruption of these forces in solution by intervention of solvent molecules re-



V



VI

sults in the expected shifts of carbonyl absorption in the direction of higher frequencies (Table I, column 3). The suggestion may be made that analogous frequency displacements in the digitonides are attributable to insulation, by the indifferent hydrocarbon nucleus of the digitonin molecule, of centers normally engaged in hydrogen bonding, with the further possibility of coördination of the 3β-hydroxyl groups with the glycosidic portion of the saponin.

Ultraviolet absorption of 7-ketocholesterol (no. 8) and of the corresponding digitonide in alcohol solution has also been investigated. Both substances show maximum absorption at 237.5 mμ with molar extinction coefficients of 14,000 and 12,600, respectively. Interpretation of these results is unfortunately complicated by the possibility of dissociation of the digitonide in the dilute solution employed.

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Isolation and Identification of Quercetin and Some Quercetin Glycosides from Plums (*Prunus Salicina*)

BY BYRON L. WILLIAMS AND SIMON H. WENDER

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This paper reports an extension of a series of studies of the flavonols and their glycosides present in fruits. Williams, *et al.*, have previously isolated and identified quercetin (3,3′,4′,5,7-pentahydroxyflavone) and isoquercitrin (quercetin-3-glucoside) from grapes,[1] black currants[2] and apricots.[3] This paper reports the isolation and identification of quercetin, isoquercitrin, quercitrin (quercetin-3-rhamnoside) and a quercetin-3-arabinoside from yellow plums.

(1) B. L. Williams and S. H. Wender, THIS JOURNAL, **74**, 4372 (1952).
(2) B. L. Williams, C. H. Ice and S. H. Wender, *ibid.*, **74**, 4566 (1952).
(3) B. L. Williams and S. H. Wender, *Arch. Biochem. Biophys.*, **43**, 319 (1953).

Experimental

Fifty pounds of ripe California yellow plums, *Prunus Salicina*, of the 1952 season were processed through a wet grinder with the whole fruit including the seeds and skins being ground. After 2 hours extraction with 20 gal. of distilled water at boiling temperature, the extract was filtered, then treated on an ion exchange column and later on a Magnesol column by the procedures previously used by Williams and Wender[3] for apricots. Flavonoid material was adsorbed at the top of the Magnesol column giving a zone about 40 mm. wide. The chromatogram was developed with ethyl acetate saturated with water. A zone about 30 mm. wide, yellow under ultraviolet light but not detectable in the visible, moved off the column first. The contents of this zone were found to be non-flavonoid and so were not studied further. The next zone, which will be called flavonoid fraction I, was yellow in the visible and also yellow under ultraviolet light, and its major component was later identified as quercetin. The next zone eluted, flavonoid fraction II, moved very close to the first. It was brown under ultraviolet light and yellow in the visible, and its flavonoid compound was later identified as quercetin-3-arabinoside.

The next zone eluted off the column (flavonoid fraction III) was brown in ultraviolet light and yellow in the visible, and was later shown to be a mixture of quercitrin and of a quercetin glucoside not identical with either isoquercitrin or quercimeritrin (quercetin-7-glucoside). The glucoside of fraction III has not been definitely identified as yet. The next eluted fraction (IV) was yellow in the visible and red-brown under ultraviolet light and its flavonoid was later identified as isoquercitrin. Each of the four fractions was recovered from the ethyl acetate solution by concentrating the eluate *in vacuo* to about 3 ml. and adding 10 ml. of pentane, followed by centrifugation and decantation.

Identification of Quercetin.—The quercetin zone eluted from the column was further purified as follows: The impure solid was dissolved in 10 ml. of ethyl alcohol, put on paper sheets (20 × 60 cm.) of Whatman No. 1 paper, chromatographed in 60% acetic acid–water and then air-dried for 4 hr. The resulting yellow zone on each paper at an R_f of about 0.40 was cut out and the pigment eluted with 95% ethyl alcohol in an air-tight chamber. The combined eluate from 25 sheets was concentrated to 0.5 ml. and then 10 ml. of boiling water was added. The yellow solid was separated, crystallized and identified as quercetin by the methods described in previous studies by Ice and Wender[4]; yield approximately 50 mg.

Identification of Quercetin-3-arabinoside.—The solid from fraction II was purified and then identified as quercetin-3-arabinoside by procedures similar to those reported for the isolation of this compound from the leaves of *Vaccinium myrtillus* by Ice and Wender.[4] The highly purified crystals had a m.p. 214–216°; approximate yield 16 mg.

Identification of Quercitrin.—The solid from flavonoid fraction III from the column was dissolved in 15 ml. of ethyl alcohol and put on paper strips in the manner described in previous paragraphs. The strips were first chromatographed using 15% acetic acid–water. The yellow zone at R_f about 0.6 (approximately 5 cm. wide) was cut out and eluted. Inasmuch as this zone was more dispersed than usual for the quantity recovered, a sugar analysis was made. Three mg. of the solid was hydrolyzed by refluxing for 2 hr. with 2% sulfuric acid, then the filtrate was neutralized and chromatographed as described under fraction II. By this procedure, both rhamnose and glucose were found to be present. Since the known quercetin disaccharides do not readily move on a Magnesol adsorption column with wet ethyl acetate as the chromatographing agent, and zone III did, this would indicate that zone III was most likely a mixture of a quercetin glucoside and a quercetin rhamnoside. This solid was, therefore, dissolved in ethyl alcohol once more and again put on fresh paper strips and chromatographed in 5% acetic acid–water. Each strip was loaded with 0.5 mg. of solid as before, but this time the strips were allowed to run for 14 hours. This brought the forward end of the zone to the lower end of the strip. The zone was about 10 cm. wide. The strips were allowed to air dry 3 hours, then a strip 1 cm. wide was cut out of the center of this zone and discarded. The forward part of the zone was eluted with ethyl alcohol in a leaching chamber, the alcohol evaporated, and the solid recrystallized as described under

(4) C. H. Ice and S. H. Wender, THIS JOURNAL, **75**, 50 (1953).

fraction II. Hydrolysis and subsequent sugar analysis by paper chromatography now showed rhamnose to be the only sugar present. The processing of the back part of the zone will be discussed in the next section. The yellow solid obtained from the combined eluates from the first (forward) part of the zone was recrystallized three times, then dried at 100° for 1 hr. It had a m.p. 183–184°, as did authentic quercitrin. No lowering of the melting point occurred on admixture of the two, and the isolated quercitrin showed no separation from authentic quercitrin by paper chromatography. One of the components (the forward portion) of fraction III from the column has, therefore, been identified as quercitrin; yield approximately 12 mg.

Properties of a Quercetin Glucoside.—The back part of the zone from the paper strips mentioned above was eluted, placed back again on fresh paper strips as before, and the strips chromatographed in 5% acetic acid. After 14 hours the new zone was about 5 cm. wide. The forward 2 cm. was cut off and discarded. The back 3 cm. was eluted and the ethyl alcohol evaporated and the solid hydrolyzed by refluxing for 2 hr. with 2% sulfuric acid. A sugar analysis made as described above showed glucose to be present as the predominant sugar with only a trace of rhamnose left from the quercitrin. The solid formed in the hydrolysis was identified as quercetin in the same manner as described for fraction I. Lack of sufficient material prevented further meaningful characterization of this compound. It may be identical with the quercetin glucoside reported, but not positively identified, from apricots.[3] It does show separation from both isoquercitrin and quercimeritrin on paper chromatograms processed in 15% acetic acid. Final yield of slightly impure product was about 5 mg.

Identification of Isoquercitrin.—The solid from flavonoid fraction IV was dissolved in 10 ml. of ethyl alcohol and put on strips by the procedure already described. The strips were processed in 15% acetic acid, air-dried for 3 hr., and the zone at R_f 0.45 was cut out and eluted with ethyl alcohol in an air-tight chamber. A total of 40 strips was processed, and after evaporation of the alcohol the solid was recrystallized eight times from hot water by cooling. The solid was dried at 110° for 1 hr. yielding a m.p. of 225–227°. It was identified as isoquercitrin by the methods previously described for this compound in *Vaccinium myrtillus*[4]; yield approximately 20 mg.

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The Preparation of Nicotinyl Chloride

BY H. N. WINGFIELD, JR., W. R. HARLAN AND H. R. HANMER

RECEIVED APRIL 18, 1953

Procedures for the preparation of nicotinyl chloride now in use seem to be modifications of the method of Meyer and Graf.[1] Salts of nicotinic acid are treated in various solvents and under varying conditions with thionyl chloride, and the product is vacuum distilled from pyridine or quinoline. The processes are tedious and often unsatisfactory.

We have now developed a more convenient method of preparation based upon the work of Adams and Ulich.[2] Potassium nicotinate suspended in benzene is treated with oxalyl chloride. The by-products, potassium chloride, carbon dioxide and carbon monoxide, are insoluble in benzene or are gases. No hydrogen chloride is formed and hence no hydrochloride. The nicotinyl chloride may be used immediately in the reaction flask in benzene solution, or siphoned off under anhydrous conditions, or distilled under vacuum after removal of the solvent.

Experimental

Sixteen and one-tenth grams of potassium nicotinate, which had been ground to pass a 100-mesh sieve and dried in an oven at 135°, and 75 ml. of anhydrous benzene was placed in a three-necked flask which was equipped with a stirrer and reflux condenser closed with a drying tube. The flask and contents were chilled in an ice-bath, and then 12.5 g. of oxalyl chloride in 25 ml. of anhydrous benzene was added at such a rate that the temperature remained low. Stirring was continued and 15 to 20 minutes after all the oxalyl chloride had been added, the unmelted ice in the bath was removed. The bath was allowed to come to room temperature, and heating was begun at a rate such that the bath began to simmer in about 30 minutes. After an additional 30 minutes heating at this temperature, the flask was allowed to cool. The cooled solution was siphoned off under anhydrous conditions and fractionated. The oily residue distilled at 75–90° (10–12 mm.). The yield was 85% or better.

Anal. Calcd. for C_6H_4ClNO: Cl, 24.70. Found: Cl, 24.82. On exposure to the air the oily chloride soon solidified to nicotinic acid hydrochloride.

Anal. Calcd. for $C_6H_5ClNO_2$: Cl, 22.01. Found: Cl, 22.09. A sample of the oily acyl chloride treated with absolute ethanol formed ethyl nicotinate, b.p. 222–224°.

The preparation was successful using 0.5 molar proportions. Sodium nicotinate did not give as good results as the potassium salt.

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The Neutron Irradiation of Crystalline Vitamin B_{12}

BY D. T. WOODBURY AND CHARLES ROSENBLUM

RECEIVED MAY 4, 1953

Vitamin B_{12} is a hexacovalent coördination complex of cobalt with the empirical formula[1,2] $C_{61-64}H_{86-92}N_{14}O_{14}PCo$. It is labeled[3,4] with radioactive cobalt by adding cobalt 60 to the fermentation medium in which it is produced. Direct activation of cobalt in crystalline vitamin B_{12} by neutron irradiation in a pile was thought to be improbable because of the relatively high temperatures of such a nuclear reactor, and because the high γ-ray recoil energy[5] involved in the formation of a cobalt 60 atom is far in excess of normal bond energies and should result in ejection of the radioactive cobalt atom. Radioactivation by exchange with the free Co^{60} atom is also unlikely because of the exchange stability[6] of the central cobalt atom.

In view of a report[7] that such direct activation was possible, two 21-mg. samples of crystalline vitamin were irradiated in evacuated, sealed quartz ampules in the Brookhaven National Laboratories pile for $\simeq$ 48 hours and 138

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