[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF OKLAHOMA]

The Isolation and Identification of Kaempferol and Quercetin from Strawberries (*Fragaria chiloensis*)

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This paper reports the isolation and identification of kaempferol (3,4′,5,7-tetrahydroxyflavone) and quercetin (3,3′,4′,5,7-pentahydroxyflavone) from ripe, cultivated strawberries (*Fragaria chiloensis*).

Introduction

The need for a systematic study by valid chemical methods of a limited number of fruits for the purpose of acquiring knowledge of the flavonoid compounds present therein has been emphasized by Scarborough and Bacharach.[1] Following the isolation and identification of isoquercetin and quercetin from grapes (*Vitis vinifera*)[2] and from black currants (*Ribes nigrum*),[3] a study of flavonoids present in common, edible strawberries, (*Fragaria chiloensis*), has been undertaken.

Nair and Robinson[4] reported the presence of the anthocyanin pelargonidin-3-galactoside in the wild strawberry (*Fragaria vesca*). Sondheimer and Kertesz[5] isolated the pigment which is responsible for all or most of the red color of *Fragaria chiloensis* and report the belief that it is pelargonidin-3-monoglucoside. No previous report, however, to our knowledge, has been made on flavones present in the strawberry.

Experimental

Forty pounds of ripe strawberries, grown near Tahlequah, Oklahoma, for the 1952 season and purchased from a local grocery store, was processed through a wet grinder and then extracted with 10 gal. of distilled water at boiling temperature for one hour. The extract was filtered, and the cooled filtrate was then passed over an ion exchange column containing Amberlite IRC-50(H) (Rohm and Haas, Philadelphia, Pa.). The column consisted of a glass tube 8 × 120 cm. drawn to an outlet at one end. The column containing the material adsorbed from the extract was washed with 2 gal. of distilled water to remove any sugar still present. The effluent and washings were discarded. The adsorbed material was then removed from the column by elution with 1 l. of 95% ethanol. The alcoholic eluate was taken to dryness *in vacuo* using a resin pot immersed in a hot water-bath. The pulverized residue was then extracted with three 50-ml. portions of hot, anhydrous acetone. These extracts were combined, and, after cooling, were passed through a chromatographic column 6 × 100 cm. packed to a depth of 20 cm. with Magnesol (Food Machinery and Chemical Corp., Westvaco Chemical Division, New York). Flavonoid material in the extract was adsorbed at the top of the column, giving a band about 10 mm. wide. The chromatogram was developed with ethyl acetate saturated with water. A yellow band 5 mm. wide moved off the column first, but it was still mixed with a brown band immediately behind it. The yellow band was yellow in both visible and ultraviolet light, but the brown band was evident only in the ultraviolet. The ethyl acetate eluate was reduced to 5 ml. *in vacuo* and then 20 ml. of commercial grade pentane was added. The resulting brown precipitate was separated by centrifugation. This solid became oily on exposure to the atmosphere, and was next dissolved in 25 ml. of anhydrous acetone. Paper chromatographic analysis in each of three solvent systems indicated two flavonoid zones plus a third zone (probably non-flavonoid) on the paper. The R_f values for the flavonoid spots at this stage were 15% acetic acid–water, 0.10 and 0.10; 60% acetic acid–water, 0.49 and 0.39; and butanol–acetic acid–water (40–10–50, volume) 0.87 and 0.83. On comparison with recorded R_f values of known flavonoids,[6] these R_f values indicated that one of the flavonoids was probably kaempferol (3,4′,5,7-tetrahydroxyflavone), and the other was probably quercetin (3,3′,4′,5,7-pentahydroxyflavone). To separate, purify and characterize the two flavonoids, the following procedure was used.

The two pigments were separated by descending paper chromatography making use of paper strips, size 20 × 60 cm. of S and S No. 597 paper and 60% acetic acid–water as the solvent system. The solution was put on the paper along the length of a line marked 10 cm. from one end of the strip. A margin of 3 cm. on one side and 1 cm. on the other was left in order to facilitate elution of the bands once they had been separated. After chromatographing, the strips were allowed to dry for 3 hours. Under these conditions, one effectively achieves on each paper the separation of the contents of 0.5 ml. of the mixture into three distinct zones in 10 hours. On each of these papers, the apparent non-flavonoid material had a R_f value of 0.70, with one flavonoid (kaempferol) at 0.50 and the other (quercetin) at 0.40. The kaempferol and quercetin bands were then separately cut out as strips 20 × approx. 4 cm. A strip of the paper approximately 2 cm. wide between the bands was discarded. After cutting the other end of the strip (the 1 cm. margin), to a point, the end of the strip with no adsorbed material (the 3 cm. margin) was immersed in 95% alcohol as for descending elution. The immersed strips were then placed in air-tight chambers for two hours with containers in place to catch the alcohol solution dripping from each pointed end. A total of 50 strips was thus processed. The alcohol eluates containing the material from the kaempferol bands were combined, as were separately those from the quercetin bands. The combined kaempferol fractions and the combined quercetin fractions were each separately taken to a volume of 0.5 ml. *in vacuo*, then 5 ml. of water was added to each, and the solids allowed to crystallize. Each yielded a yellow solid, which was removed by centrifugation, recrystallized three times from alcohol–water, and dried at 80° over P_2O_5 *in vacuo* for 1 hour. The pigment having R_f values corresponding to quercetin melted at 314°, and the one corresponding to kaempferol melted at 275°, both uncor. Corresponding recorded values for quercetin and kaempferol are 313–314°[7] and 272–274°,[8] respectively.

Each of these compounds was separately acetylated with acetic anhydride and sulfuric acid. The acetates were recrystallized twice from alcohol by adding water. The acetates were dried at 80° *in vacuo* over P_2O_5 for 1 hr. Melting points were: quercetin pentaacetate 194° and kaempferol tetraacetate 176–178°. These correspond to recorded values of 193–194°[7] and 179–180°,[8] respectively. The approximate yields obtained were 20 mg. of kaempferol and 30 mg. of quercetin.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY, HARVARD UNIVERSITY]

Diene Addition between *trans*-Piperylene and Citraconic Anhydride

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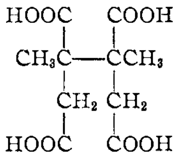
Diels–Alder reaction between *trans*-piperylene and citraconic anhydride yielded 82% of Δ^4-2,3-dimethylcyclohexene-1,2-dicarboxylic acid anhydride (VI); the structure of the adduct was proved by dehydrogenation. Oxidative cleavage of the adduct gave α,β-dimethyl-β,β'-dicarboxyadipic acid (VIII).

An optically active hexanetetracarboxylic acid, $C_{10}H_{14}O_8$, has been obtained by chromic acid oxidation of the two veratrum alkaloids, cevine[3] and germine[4]; and was shown[5] to be different from synthetic β,β'-dimethyl-β,β'-dicarboxyadipic acid (I). Fieser and Fieser[6] proposed an alternative structure, namely, α,β'-dimethyl-β,β'-dicarboxyadipic acid (II), and the present investigation was an attempt to synthesize this acid.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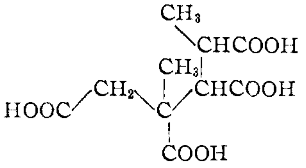
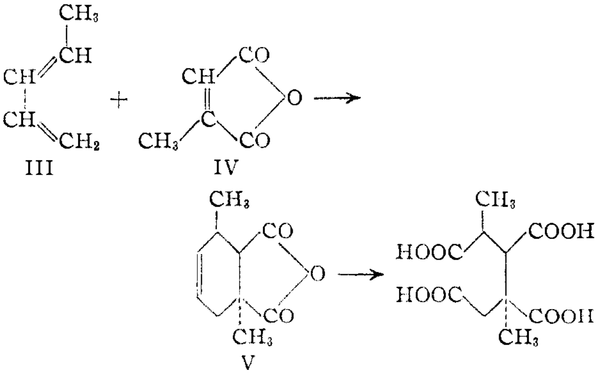
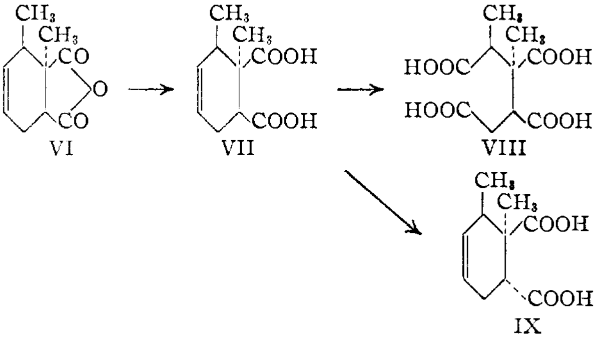

II

The particular scheme consists of a Diels–Alder addition between *trans*-piperylene (III) and citraconic anhydride (IV) followed by oxidative cleavage of the double bond of the adduct. The assumption was that the diene addition would follow the steric course illustrated. However, palladium dehydrogenation of the dihydro derivative of the dicarboxylic acid resulting from the hydrolysis of the adduct gave *o*-xylene and an acid, probably 2,3-dimethylbenzoic acid; the former was characterized by infrared spectrum as well as by permanganate oxidation to *o*-phthalic acid, identified as phthalanil. These results indicate that the isomeric adduct, Δ^4-2,3-dimethylcyclohexene-1,2-dicarboxylic acid anhydride[7] (VI), was formed in spite of the apparent steric hindrance of the two neighboring methyl groups. Consequently the synthetic tetracarboxylic ester, which was obtained by ozonolysis of the dimethyl ester of the diacid, Δ^4-2,3-dimethylcyclohexene-1,2-dicarboxylic acid (VII), followed by further oxidation with alkaline silver oxide and re-esterification, is the tetramethyl ester of α,β-dimethyl β,β'-dicarboxyadipic acid (VIII). Alkaline isomerization of the *cis*-diacid (VII) afforded a high melting acid, presumably the *trans*-diacid IX.





Experimental

Δ^4-2,3-Dimethylcyclohexene-1,2-dicarboxylic Acid Anhydride (VI).—Citraconic anhydride was prepared from citric acid in the usual way[8]; and *trans*-piperylene was obtained by dehydration of 3-pentene-2-ol[9] over alumina at 420 to 470° under 40 mm. pressure.[10]

The Diels–Alder addition was carried out as follows. A mixture of 150 g. of freshly distilled citraconic anhydride and 97 g. of *trans*-piperylene and 50 cc. of sodium-wire dried toluene was sealed in five Pyrex Carius tubes and allowed to stand at 25° for 24 hours and then heated in a steel bomb for 24 hours at 100°. After cooling to room temperature, the pale yellow viscous product was fractionated under reduced pressure and the main fraction (221 g.) distilled from 140 to 210° at 17–18 mm. Refractionation under reduced pressure gave 197 g. (82%) of the adduct, Δ^4-2,3-dimethylcyclohexene-1,2-dicarboxylic acid anhydride, b.p. 137° at 10 mm., $n^{20}\text{D}$ 1.4870, sp. gr. 28°/28° 1.158, λ^{chf} 5.46, 5.63 μ.

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