The simple chemical relationship between the flavonol and anthocyanin series of plant pigments, suggested by Combes and Everest, but not proved until Willstätter and Mallison actually produced cyanidin from quercetin by reduction in acid solution, has led to considerable speculation as to the genetical and physiological interrelations of these compounds. There is some evidence that the anthocyanins are produced in the plant from the corresponding flavonols, and not by direct synthesis. Everest, for example, has shown that glucosides of the chemically related pair myricetin and delphinidin occur side by side in purple-black forms of Viola. Before far reaching conclusions are drawn, however, it will be necessary to isolate, or otherwise identify, the pigments of a large number of species. It goes without saying that the best material for this purpose will be afforded by species whose color varieties are capable of genetic analysis or by species in which the relations between flavone and anthocyanin are capable of experimental modification.

The genus Escholtzia, abundantly distributed in California, and common in cultivation, contains garden forms with yellow, golden yellow, pale yellow, white, carmine, and rose flowers. It

*Published by permission of the Secretary of Agriculture.

1 Combes, R., Compt. rend. Acad., 1913, clvii, 1002, 1454.

495
would provide ideal material for combined genetical and biochemical investigation providing the pigments concerned were present in large quantity and easily isolable. With this idea in mind, the writers obtained for preliminary work a large quantity of petals of wild *Escholtzia*, gathered by Mr. W. W. Wagener in the vicinity of Palo Alto, California. We have called the material *Escholtzia californica* Cham., using this specific name in the broad sense, for it was of course out of the question to observe close specific or varietal differences in gathering wild material of this polymorphic genus.

**Preparation and Properties of Rutin.**

The air-dried petals afforded an abundant yield of the glucoside rutin, quercetin glucosyl-rhamnoside. They were first extracted for several days with ether, to remove fats, carotinoids, etc., and then with ethyl alcohol. The alcoholic solution was evaporated to small bulk, poured into water, and the remaining alcohol boiled off. The crude rutin, which came down as a copious crystalline precipitate, was collected on a Buchner funnel, washed with water, dried, extracted with ether until no colored impurities were removed, and finally purified by recrystallization from a large volume of hot water. By this method 7.13 gm. of rutin (dried at 140°C.) were obtained from 150 gm. of air-dried petals.

According to Perkin and Everest, rutin "is said to melt above 190°." Schmidt gives 188–190°. Our preparation began to sinter at 186° and melted at 190–192°C. (uncorrected). The color of the anhydrous compound (dried at 150–160°), as ascertained by comparison with Ridgway’s standards, was primrose-yellow; the streak was pale green-yellow. As obtained by crystallization from hot water *Escholtzia* rutin formed microscopically fine, very dense, acute-based tufts of silky crystals (Fig. 1).

Water of crystallization was determined by exposing a sample of the glucoside to a moist atmosphere under a bell jar until it

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7 Ridgway, R., Color standards and color nomenclature, Washington, 1912.
came to constant weight. It was then dried at 160° for 12 hours. The loss of water from 2.2548 gm. of glucoside was 0.1868 gm., or 8.28 per cent, according satisfactorily with the 8.13 per cent calculated from C_{27}H_{30}O_{16}·3H_{2}O, the accepted formula of rutin.

**Identification of Quercetin.**

Hydrolysis resolves rutin into one molecule each of quercetin, rhamnose, and glucose. Our anhydrous preparation, boiled with approximately 5 per cent sulfuric acid, gave quercetin yields of 0.5354 gm. and 0.6177 gm. from samples weighing 1.0812 gm. and 1.2460 gm., respectively. These figures correspond to 49.51 and 49.57 per cent. Theory requires 49.51 per cent. The

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<th>TABLE I.</th>
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<td>Rutin</td>
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<td>Quercetin</td>
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<td>Penta-acetylquercetin</td>
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crystalline quercetin (Fig. 2) was washed with cold water and dried at 140°C. It conformed in physical characteristics with quercetin from other sources. The crystals were citron-yellow; the streak light greenish yellow. In order to prevent decomposition of the material below the melting point, the bath (melted acid potassium sulfate) was heated to 300° before the sample for determination of melting point was introduced. Rosenthaler states that quercetin melts with partial decomposition at 310°. Our material darkened, but did not melt, between 300 and 305°. The melting point was not so sharp as might have been wished, but melting was complete at 310°. Wunderlich gives 305–310°.

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8 Rosenthaler, L., Der Nachweis organischer Verbindungen, Stuttgart, 813.

9 Wunderlich, A., Arch. Pharm., 1908, cxcix, 224, 241, 256.
A satisfactory identification of the compound was secured, however, by acetylation. It gave penta-acetylquercetin, melting at 189–191°. Perkin and Hummel\textsuperscript{10} found 190–191°. Samples of the latter, weighing 0.7638 and 0.5704 gm., hydrolyzed by hydrochloric acid in glacial acetic acid, gave quercetin yields of 0.4512 and 0.3376 gm., corresponding to 59.07 and 59.18 per cent. Theory requires 58.98 per cent. Combustions were made of rutin and of the quercetin and acetylquercetin derived from it. The results, concordant and agreeing well with expectation, are given in Tables I and II.

When treated with sulfuric acid in boiling glacial acetic acid, quercetin forms a finely crystalline orange-vermilion acid addition product, $C_{16}H_{10}O_7\cdot H_2SO_4$, from which the quercetin is easily regenerated by simple suspension in water. A sample of the sulfate weighing 0.2714 gm., dried at 100°, gave 0.2034 gm. of recovered quercetin, or 74.94 per cent. Theory requires 75.5 per cent.

### The Sugars.

In view of the perfect agreement of the analytical results with the figures for rutin, complete identification required only the determination of the sugars resulting from hydrolysis. The literature of rutin and its synonyms, violaquercetrin, osyritrin, myrticolorin, \textit{etc.}, shows that for many years the occurrence of glucose in the presence of rhamnose was overlooked, or vice versa. Following a modification of Perkin's procedure,\textsuperscript{11} we separated pure glucosazone and rhamnosazone from the mixed osazones,

\begin{table}
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\begin{tabular}{|c|c|c|c|c|c|}
\hline
 & Rutin & & Quercetin & & Penta-acetylquercetin \\
 & Found. & Expected. & Found. & Expected. & Found. & Expected. \\
\hline
C & 53.14 & 53.10 & 59.73 & 59.59 & 58.88 & 58.59 \\
H & 5.21 & 4.95 & 3.32 & 3.34 & 3.90 & 3.90 \\
O & 41.65 & 41.95 & 36.95 & 37.07 & 37.22 & 37.51 \\
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\end{tabular}
\end{table}


C. E. Sando and H. H. Bartlett

using the differential solubility of the compounds in acetone for the primary separation, following with recrystallization from 5 per cent pyridine in water and then from 20 per cent alcohol for final purification. In this manner rhamnosazone, melting at 181–182°, and glucosazone, melting at 205–207°, were obtained. Recrystallized from 20 per cent alcohol, under identical conditions, the crystal forms were characteristically different, as shown in Figs. 3 and 4. We were unable to distinguish two types of crystals in the mixture of osazones before fractionation by acetone, but after purification the glucosazone formed typical radiate groups of short, unbranched needles, and the rhamnosazone bifasciculate clusters of longer, more slender, branching needles.

Distribution of Rutin.

All the plants which have been reported to contain rutin have been critically examined in recent years either by Schmidt and Wunderlich or Perkin. Adding Escholtzia to the list, the known distribution of rutin is now as follows:

Santalaceae; leaves of Osyris compressa,
Polygonaceae; entire herb, but chiefly the flowers, of Fagopyrum esculentum,
Tapaveraceae; petals of Escholtzia californica,
Capparidaceae; flower buds of Capparis spinosa,
Leguminosae; flower buds of Sophora japonica,
Rutaceae; leaves of Ruta graveolens,
Violaceae; flowers of Viola tricolor,
Myrtaceae; leaves of Eucalyptus macrorhyncha,
Globulariaceae; leaves of Globularia alypum.

Only Capparis is doubtful, the rutin from this source differing from that of Ruta, etc. in sintering 10° below the usual temperature, regardless of every effort to purify the material completely. With the exception of Globularia, all the plants known to contain rutin fall within the subclass Archichlamideae of the Dicotyledones.
The petals of *Escholtzia californica* contain nearly 5 per cent of rutin (quercetin glucosorhamnoside). In view of the great range of flower colors in *Escholtzia*, from golden yellow to white, and from white to rose, this genus would appear to afford especially suitable material for study of the physiological and genetic relationships of the flavonol and anthocyanin pigments. It is hoped that the problems will interest workers who are advantageously located for carrying out both garden and laboratory studies.

Notwithstanding the brilliant work of Willstätter in showing the chemical relation of the anthocyanins and the flavonol pigments, it is quite true, as Wheldale\(^2\) has said, that in order to prove their relation in nature it is necessary to know which flavone accompanies which anthocyanin in a considerable number of plants. It would conserve effort in solving the problem if the flavones were isolated and identified in all the plants in which Willstätter determined the anthocyanins, and, conversely, if those plants in which the yellow pigments are well known were studied with respect to the anthocyanins. That it will be difficult to work out the relation, and that it cannot be done except by collaboration between chemists and geneticists, is shown by the fact that Sutton's "Black Knight" pansy, a variety of *Viola tricolor*, a species well known for its great range of flower colors, has been shown by Everest to contain glucosides of the pair myricetin-delfphinidin, whereas one would have expected from the well established occurrence of rutin in this species that the pair quercetin-cyanidin would have been the first to be detected. The share of the geneticist in the final elucidation of the pigment situation must be to provide the chemist with material of known factorial composition.

EXPLANATION OF PLATES.

PLATE 6.

Fig. 1. *Escholtzia* rutin, crystallized from hot water (X 90).

Fig. 2. Quercetin from *Escholtzia* rutin, as obtained by hydrolysis of the rutin with boiling 5 per cent sulfuric acid (X 90).

PLATE 7.

Fig. 3. Phenylglucosazone, separated from phenylrhamnosazone by acetone and purified by recrystallization. The crystals were obtained by cooling of a hot solution in 20 per cent alcohol. Under exactly the same conditions phenylrhamnosazone crystallized as in Fig. 4 (X 90).

Fig. 4. Phenylrhamnosazone, crystallized from hot 20 per cent alcohol (X 90).
Fig. 1.

Fig. 2.

(Sando and Bartlett: Rutin.)
Fig. 3.

(Sando and Bartlett: Rutin.)

Fig. 4.
RUTIN, THE FLAVONE PIGMENT OF ESCHOLTZIA CALIFORNICA CHAM
Charles E. Sando and H. H. Bartlett


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