A METHOD FOR THE SEPARATION OF CERTAIN FLAVONES PRESENT IN TOBACCO

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Flavones have been reported to be present in various tobaccos, although rutin is the only one of these flavones studied in any detail in tobacco. Hasegawa (1931) obtained crude preparations of rutin from fresh tobacco leaves. Neuberg and Kobel (1936) also isolated rutin from green tobacco leaves. Couch and Krewson (1944) obtained a patent for the extraction of rutin from high-quality flue-cured tobacco.

A second flavone, isoquercitrin, has been isolated from tobacco leaves by Kurilo (1936). However, in all these studies, other flavones, closely related in structure, and, no doubt, present in the same tobacco leaves, were not studied alongside the rutin or isoquercitrin. In the investigation reported here, a method has been devised whereby several different flavones present in tobacco may be separated from the same sample for further study. Chemical and physical analyses, including spectrophotometric analyses, have been conducted on the separated compounds to indicate that they are flavones.

EXPERIMENTAL

The sample of lug Burley tobacco, *Nicotiana tabacum*, that was used was grown and aircured on the Kentucky Agricultural Experiment Station Farm during the 1942 season. This tobacco was then stored in heavy cardboard boxes in a cool dry basement until the fall of 1945. At this time, the tobacco was finely divided in a hammer mill, and the ground tobacco powder kept in heavy-cloth bags until used.

A 100-gram sample of the powdered tobacco was boiled for sixty minutes with 1500 ml of distilled water. The mixture was filtered while hot. The re-

sulting filtrate was concentrated *in vacuo* to 400 ml, and the concentrate filtered. The precipitate was discarded. Eight such separations, as described above, were brought to this concentrated point, and then these concentrates from eight 100-gram samples were combined. To the resulting solution, 10 liters of acetone were added, with mixing. After an hour, the resulting resin-like solid was separated from the supernatant liquid by filtration with suction. The solid material, containing the particular flavones studied, was washed with cold acetone until the washings were free from color. The washings were discarded after recovery of most of the acetone.

Next, the solid which had precipitated on the addition of acetone to the water concentrate, was extracted with 200-ml portions of boiling 95-percent ethyl alcohol, and each alcohol extract filtered while hot. The resulting filtrate, containing the flavones under study, was next treated with powdered talc (c.p. Fisher). The talc had been previously washed thoroughly with ethyl alcohol.

Each alcohol solution of the colored substances which had been extracted from the acetone precipitates was thoroughly shaken with the talc and kept over night. After filtering, both the talc and the filtrate were saved. The filtrate was treated with four new, different portions of talc. The alcohol solution remaining after these four additional treatments with talc was set aside for further purification. This alcohol solution of substances not adsorbed on the talc was called Fraction A. Meanwhile, the talc fractions from the first two treatments of alcohol solution were combined, and then washed with 95-percent ethyl alcohol until the washings were colorless. In order to elute the colored matter adsorbed on the talc, the talc was washed with 300-ml portions of distilled water; the mixture was filtered, and the water elution of the talc repeated until no further color could be removed. The water eluates were combined, and then concentrated *in vacuo* to one-tenth of their original volume. This concentrate was filtered, and then centrifuged; the supernatant liquid was decanted; the precipitate discarded; and the decantate again filtered to remove any talc or other suspended matter.

Acetone was now added to the filtered decantate until the solution became cloudy in appearance, and precipitation gradually followed. After two days, the mixture was centrifuged to collect the flocculent yellow precipitate, and the acetone-water solution was removed by decantation. The precipitated pigment was redissolved in water, filtered, and reprecipitated by the addition of acetone again. This solid substance, after decantation of the supernatant liquid, was designated as Pigment W.

Fraction A, the alcohol filtrate containing pigments not adsorbed on talc, was further fractionated by means of chromatographic adsorption technique. A glass column, 17.5 cm long and 1.9 cm in diameter, constricted at one end, was used. The adsorbent consisted of 10 parts by weight of activated Fisher grade-A alumina (ground so that one-half passed through a 200-mesh sieve and the second half through a 100-mesh sieve) mixed with 2 parts by weight of Johns-Manville *Celite No. 501*. The adsorbent was packed tightly in the column, and then thoroughly washed with 95-percent ethyl alcohol. The washings were discarded. The solution, Fraction A, was next passed through the adsorbing column, with pressure. At least two pigments were adsorbed on the column, whereas one was not adsorbed at all on the alumina. The latter, in the filtrate, was lemon yellow in color, and was designated Pigment 1.

After separating the nonadsorbed Pigment 1 fraction from the colored matter adsorbed on the alumina, the adsorption column was washed thoroughly with 95-percent ethyl alcohol, until the alcohol passing into the filtrate was colorless. These washings were used later only for recovery of the alcohol. Distilled water was next passed through the column. A colored substance moved down the column and was collected. This fraction was called

Pigment 2. Some colored material still remained adsorbed on the alumina. The adsorption column was washed thoroughly with distilled water. These washings were discarded. A third fraction was obtained by elution with a 1-percent hydrochloric-acid solution. The yellow color in the hydrochloric-acid percolate was much more intense than the color of the previous two fractions. This third substance was designated as Pigment 3.

Various analyses, detailed results of which are to be reported elsewhere, have been run on the Pigments W, 1, 2, and 3. They indicate that these substances are all flavones; further investigations on these are in progress.

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