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

The Isolation of Isoquercitrin from the Seed Pods of *Cercis canadensis*

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In the course of a search for vitamin-P-active flavonoid pigments occurring naturally in plants, the drying seed pods of the red-bud tree, *Cercis canadensis,* were considered as a possible source. This paper describes the experimental procedure whereby a flavonoid pigment was isolated from the pods. By means of paper partition chromatography, as well as classical methods, this pigment has been identified as isoquercitrin, the 3-glucoside of quercetin. Isoquercitrin has been obtained from a number of natural sources, such as maize,[2] Trifolium blossoms[3] and tobacco.[4]

Experimental

Isolation.—Thirteen hundred fifty grams of the seed pods, obtained from red-bud trees on the campus of the University of Oklahoma in August, 1948, were exhausted with 95% ethanol (covering the whole pods with alcohol, boiling, and straining the alcohol through a cloth). This was repeated until the filtrate gave only weak magnesium-hydrochloric acid tests (acidifying an alcoholic solution of the pigment with concentrated hydrochloric acid and adding magnesium turnings) which is considered positive for flavonoid pigments when some shade of red develops. The alcoholic filtrate was concentrated *in vacuo.* A precipitate soon separated. This precipitate was washed with 50% alcohol until the above test on the washings was negative. The washings were combined with the original filtrate, and the remaining alcohol was removed by continuing the distillation *in vacuo.* Water was added from time to time in order to maintain a convenient volume. The solution was cooled and then extracted with re-distilled diethyl ether until the ether layer was colorless.

Several volumes of alcohol were then added to the aqueous phase. The precipitated potassium nitrate was filtered off and the filtrate concentrated *in vacuo* to a viscous sirup. This sirup was repeatedly extracted with hot alcohol, with much shaking and stirring, until the extracts gave negative magnesium-hydrochloric acid tests. All the extracts were then combined and concentrated *in vacuo* to 100 to 200 ml. Five volumes of ether were added to the dark-red concentrate, and the solution was allowed to stand overnight in the cold. A brown resinous mass containing some white crystals separated. The alcohol-ether solution was decanted and the solvents evaporated *in vacuo,* with water being added to bring the volume to 75 ml. On standing overnight in the cold, a precipitate formed which was discarded.

One-third of the aqueous solution was concentrated to a small volume. An equal volume of water was added and the hot solution was treated with 10 ml. of a solution containing 0.05 g. of lead acetate per ml. A tan precipitate separated. Tests indicated that the major portion of the flavonoid pigment had not been precipitated. The filtrate was, therefore, heated on the water-bath and treated with basic lead acetate solution, until precipitation was complete.

This yellow precipitate was suspended in water and heated. Hydrogen sulfide was bubbled in. After the formation of the lead sulfide was complete, more water was added and the resulting suspension heated until the sulfide had flocculated. The precipitate was filtered off through coarse paper. The lead sulfide was boiled repeatedly with alcohol with intermittent filtering in order to elute all adsorbed flavone. The filtrates were all combined, again treated with hydrogen sulfide, and filtered. The alcohol was removed *in vacuo,* and water was added to bring the volume to 25 ml. A yellow precipitate separated overnight. The supernatant liquid was allowed to remain in contact with the precipitate for several days. The precipitate was then centrifuged and the supernatant liquid decanted. The precipitate was washed twice with cold water and twice with hot water. It was dissolved in hot alcohol (a small amount of insoluble material was discarded). One volume of water was added; this solution was allowed to evaporate slowly until crystallization was complete. The precipitate was recrystallized four times by dissolving in boiling alcohol and adding water.

Thirty-four mg. of the dried, bright yellow crystals were obtained (yield, 0.0076%), m. p. 215° uncor.; Sando and Bartlett[2] record 216° for isoquercitrin.

Color Tests.[5]—(1) Magnesium-hydrochloric acid: pinkish-red; (2) alcoholic ferric chloride: greenish-brown; (3) aqueous lead acetate: bright yellow precipitate; (4) ammonia: bright yellow solution; and (5) concd. sulfuric acid: bright yellow solution.

Spectrum.—A solution containing 1.4 mg. per 100 ml. was used with 95% alcohol as the solvent in the Beckman Model DU spectrophotometer. The corresponding spectrum of pure isoquercitrin was determined. Both gave curves with maxima at 2550 and 3550-3600 Å. and minima at 2400 and 2850 Å.

Hydrolysis.—A small sample of the isolated yellow crystals was dissolved in 0.5 ml. of warm 0.6% sulfuric acid and refluxed for one and one-half hours. The yellow precipitate was collected on a sintered glass funnel and washed with cold water.

The Aglycone.—This yellow precipitate was recrystallized twice by dissolving in boiling alcohol, adding water to incipient cloudiness and setting aside in the cold for recrystallization; m. p. 310-315° uncor. This corresponds closely with the value for quercetin.

Color Tests (see above).—(1) Red; (2) greenish-brown; (3) orange-red; (4) bright yellow solution; and (5) bright yellow solution. These results check with those obtained on a sample of quercetin.

The spectrum of the aglycone was identical with that of quercetin (maxima at 2550 and 3750 Å.; minima at 2400 and 2850 Å.).

Pentaacetate.—This derivative of the unknown aglyclone was prepared as follows: A small amount of the aglycone was dissolved in 0.5 ml. of acetic anhydride and a drop of pyridine was added. This mixture was refluxed for two hours, then 2 ml. of water was added and the solution allowed to stand overnight in the cold. The resulting white crystals were recrystallized by dissolving in hot alcohol, adding water to cloudiness, and allowing to stand overnight in the cold; m. p. 195° uncor. Perkin and Everest[6] record 191-195° for the pentaacetate of quercetin.

The Sugar.—The filtrate from the hydrolysis mixture was used for the preparation of an osazone.[7]

The characteristic crystals of glucosazone were observed. No other osazones were formed.

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[2] Sando and Bartlett, *J. Biol. Chem.*, **54**, 629 (1922).

[3] Hattori, Hasegawa and Hagashi, *Acta Phytochim.* (*Japan*), **10**, 147 (1937).

[4] Yamafugi, *J. Agr. Chem. Soc. Japan*, **8**, 404 (1932).

[5] Klein, "Handbuch der Pflanzanalyse," Springer, Vienna, 1932, Vol. II, p. 910.

[6] Perkin and Everest, "The Natural Organic Colouring Matters," Longmans, Green and Co., London, 1918, p. 188.

[7] Bodansky and Fay, "Laboratory Manual of Physiologica Chemistry," 4th ed., John Wiley and Sons, Inc., New York, N. Y., 1938, p. 28.

Paper Partition Chromatography.[8]—The technique of one-dimensional paper partition chromatography was helpful in the identity of the isolated pigment. Ethyl acetate, 40% butanol–50% water–10% acetic acid and *m*-cresol were each used as the flowing solvent. The isolated pigment was chromatographed alone and in a mixture with a known pure sample of isoquercitrin. The R_f values for the isolated pigment are as follows: ethyl acetate: 0.65; butanol–acetic acid–water: 0.72; and *m*-cresol: 0.30. The corresponding values with pure isoquercitrin were: 0.63–0.65, 0.72, and 0.29. This method was also used in the identification of the aglycone obtained after hydrolysis. The R_f values are as follows: ethyl acetate 0.93; butanol–acetic acid–water 0.81; and *m*-cresol, 0.45. The corresponding values for quercetin were 0.93, 0.83 and 0.44.

Chromogenic Reagents.—The fluorescence in ultraviolet light of a dried spot of a solution of the unknown pigment on filter paper, after being sprayed with various chromogenic reagents,[8] was also of assistance in the identification of the isolated flavonoid pigment: alcoholic aluminum chloride, yellow; aqueous lead acetate, orange yellow; aqueous basic lead acetate, orange; aqueous sodium carbonate, yellowish-brown; boric acid–citric acid,[9] yellow. The same colors were obtained with pure isoquercitrin.

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Summary

A flavonoid pigment has been isolated from the seed pods of *Cercis canadensis* and identified as isoquercitrin. The identity was also confirmed by paper partition chromatography.

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[8] Wender and Gage, *Science*, **109**, 287 (1949).

[9] Wilson, THIS JOURNAL, **61**, 2303 (1939).

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF DUKE UNIVERSITY]

Trifluoromethyl Biphenyls[1]

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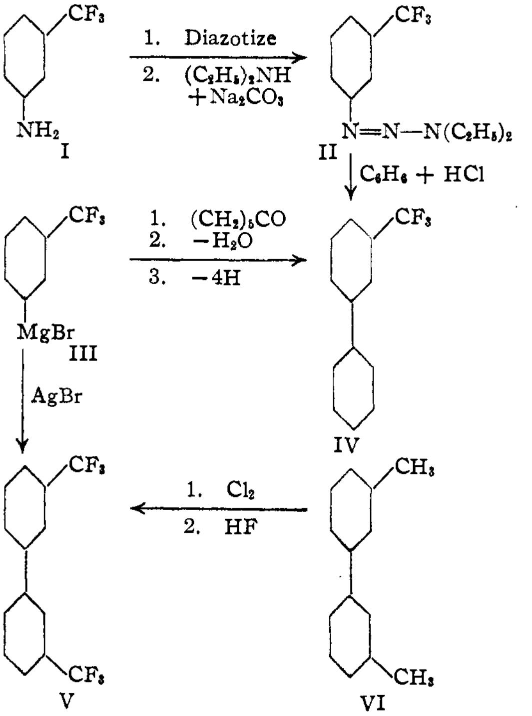
The literature affords no example of a biphenyl compound containing one or more trifluoromethyl groups. It has been found that such compounds may be prepared from trifluoromethyl benzene derivatives by application of the classical biaryl syntheses, or, in at least one case, from methylbiphenyls by an indirect fluorination process.

The first method studied was the recently developed triazene modification of the diazo reaction.[3,4] It was found that the triazene (II) produced by addition of diethylamine to diazotized *m*-(trifluoromethyl)-aniline,[5] could be decomposed in benzene solution to give a 24% yield of 3-trifluoromethylbiphenyl (IV). When benzotrifluoride or bis-trifluoromethylbenzene was used instead of benzene, no coupling was observed.[6]

The action of *m*-trifluoromethylphenylmagnesium bromide[7] (III) on cyclohexanone followed by dehydration and dehydrogenation[8] with chloranil[9] gave *m*-trifluoromethylbiphenyl (IV). When the same Grignard reagent (III) was treated with anhydrous silver bromide, 3,3′-bis-(trifluoromethyl)-biphenyl (V) was obtained in 60% yield. The same product was prepared from *m,m*′-bitolyl (VI) by complete chlorination of the methyl groups followed by halogen interchange with liquid hydrogen fluoride under pressure.



Ullmann reactions, carried out using 3-nitro-4-chlorobenzotrifluoride and 4-chloro-5-nitro-1,3-bis-(trifluoromethyl)-benzene, yielded 2,2′-dinitro-4,4′-bis-(trifluoromethyl)-biphenyl (VII)

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[1] The work reported here was carried out under contract N6ori-107 with the Office of Naval Research.

[2] Office of Naval Research Fellow.

[3] U. S. Patent 2,280,504; *C. A.*, **36**, 5658 (1942).

[4] Elks and Hey, *J. Chem. Soc.*, 441 (1943).

[5] Swarts, *Bull. Acad. roy. Belg.*, [3] **35**, 388 (1898).

[6] The triazenes from *meta*- and *para*-nitroaniline were likewise without effect on the trifluoromethylbenzenes.

[7] We were unable to prepare the related 2-chloro-5-trifluoromethyl-phenylmagnesium bromide from what is believed to be 3-bromo-4-chlorobenzotrifluoride.

[8] *Cf.* Weiss and Woidick, *Monatsh.*, **46**, 453 (1925).

[9] Arnold and Collins, THIS JOURNAL, **61**, 1407 (1939).