g. of manganese dioxide. The reaction mixture was stirred for 16 hr. after which it was filtered and the precipitate washed with chloroform. The filtrate was concentrated to dryness and the residue weighed 452 mg. This residue was chromatographed over acid-washed alumina (10 g.); elution with a gradient system consisted of benzene-chloroform afforded 162 mg. of 9α-hydroxytestosterone (VII). Recrystallization from acetone–petroleum ether (b.p. 60–80°) gave 128 mg. of a sample, m.p. 210–211°; $[\alpha]_D^{25} = +104$ (*c*, 1.0 chloroform); λ_{max}^{alc} 242 mμ (ε 15,200); $\lambda_{max}^{CHCl_3}$ 2.92, 6.03, 6.20 μ.

Anal. Calcd. for $C_{19}H_{28}O_3$ (304.41): C, 74.96; H, 9.27. Found: C, 75.32; H, 9.46.

9α-Hydroxyandrostene-3,17-dione (VIII). 9α-Hydroxytestosterone (100 mg.) was dissolved in 10.0 ml. of acetone and treated dropwise with stirring with a solution prepared by dissolving 30 mg. of chromic acid and an equivalent amount of sulfuric acid in 3.0 ml. of acetone. When the reaction was complete, the chromic sulfate was removed by centrifugation and washed with acetone. The combined acetone washings were evaporated to dryness, the residue taken up in chloroform, washed with water, dried over sodium sulfate, and the chloroform solution concentrated. The residue crystallized from acetone-hexane yielding 67 mg. of 9α-hydroxyandrostene-3,17-dione (VIII), m.p. 220–222°; $[\alpha]_D^{25} = +182$ (*c*, 0.9 chloroform); λ_{max}^{alc} 242 mμ (ε 16,000); $\lambda_{max}^{CHCl_3}$ 2.90, 5.75, 6.01, and 6.18 μ.

Anal. Calcd. for $C_{19}H_{26}O_3$ (302.40): C, 75.46; H, 8.67. Found: C, 75.18; H, 8.72.

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DEPARTMENT OF PHARMACOGNOSY
UNIVERSITY OF WISCONSIN
MADISON, WIS.

Flavonols in Spinach Leaves

ALEXIS ZANE AND S. H. WENDER

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There has been no previous report of the identification of individual flavonol compounds in spinach leaves, although a colorimetric, quantitative determination of the gross, flavonoid-like compound content of spinach (*Spinacia oleracea*) has been reported by Weatherby and Cheng.[1] Williams[2] has isolated a flavonoid compound in pure form from spinach leaves. Its exact identity, however, was not determined. This note describes the extraction from spinach and identification of the flavonol, patuletin, and also of a quercetagetin dimethyl ether. The latter compound has not been found previously in nature, and the name "spinacetin" is proposed for it. Spinacetin has been tentatively identified as quercetagetin-3′,6-dimethyl ether. Quercetagetin is 3,3′,4′,5,6,7-hexahydroxyflavone. Patuletin was first isolated by Rao and Seshadri from the flowers of *Tagetes patula*,[3] and later was proved to be quercetagetin-6-monomethyl ether.[4]

EXPERIMENTAL

Extraction of spinach leaves. Fifty pounds of packaged, frozen fresh spinach (Safeway Stores, Inc., "Belair" brand) was ground in an ice crusher, then loaded into 4-l. containers with 1.5 l. of water, and pressure-cooked at 115° for 45 min. The juice was squeezed out, and filtered through cotton cloth and then through a bed of Super Cel (Fisher Scientific). The clear, yellow-brown filtrate was adsorbed under pressure on wet Magnesol (Food Machinery and Chemical Corp., New York, N.Y.) packed on three glass funnels (22 cm. diam., 18 cm. deep). A yellow zone, 6 cm. deep, was formed on the Magnesol in each funnel. The adsorbent was washed with 1 l. of water, and the yellow zone was eluted with 70% ethyl alcohol–water. The dark brown eluate (4.5 l.) was concentrated *in vacuo* to 200 ml. and then "freeze-dried" to yield a black-appearing solid. Pulverization and then extraction with hot methyl alcohol (12 × 100 ml.) gave a reddish brown extract which was poured onto an 8-cm. column previously filled to a depth of 50 cm. with Magnesol in methyl alcohol. Development under 5 lb. pressure, with ethyl acetate saturated with water, readily moved a broad, bright yellow zone. The yellow eluate (1600 ml.) was taken to dryness *in vacuo*. Crystallization from acetone-water gave 0.205 g. of a yellow powder. Paper chromatography revealed the presence of at least two compounds. Separation into individual compounds was achieved by silicic acid chromatography. A column (6 cm. diam.) was packed to a depth of 38 cm. with silicic acid (Mallinckrodt No. 2847) in benzene-acetone (84:16 v./v.), under 5 lb. pressure. The yellow powder (0.205 g.) was dissolved in acetone (18 ml.) and diluted with benzene (102 ml.), and then chromatographed. On development with the benzene-acetone, two major zones formed, and they were eluted separately. After removal of the solvent *in vacuo*, the eluate from the faster-moving zone yielded 75 mg. of a yellow powder, called "compound A-2." From the eluate of the slower moving zone, 76 mg. of fine yellow needles called "compound A-1," were obtained.

Identification of compound A-1. The yellow product containing compound A-1 was chromatographed on a silicic acid column using benzene-acetone (84:16 v./v.), and then crystallized from ethyl alcohol–water to give yellow needles. These were dried at 110° *in vacuo*, yield 59 mg., m.p. 261–263° (all melting points are uncorrected). R_f values in 60% acetic acid, *n*-butyl alcohol–acetic acid–water (6:1:2 v./v./v.), and phenol-water (3:1 w./w.), using Whatman No. 1 chromatography paper and descending chromatography, were 0.47, 0.75, and 0.63, respectively. The ultraviolet absorption spectrum showed maxima at 257 and 375 mμ and minima at 240 and 285 mμ.

Anal. Calcd. for $C_{16}H_{12}O_8$: C, 57.83; H, 3.64; OCH_3, 9.34. Found: C, 58.05; H, 3.98; OCH_3, 9.28.

Compound A-1 (10 mg.) was refluxed for 6 hr. with 1 ml. of dimethyl sulfate in 8 ml. of anhydrous acetone and potassium carbonate (2.5 g.) to give colorless needles, m.p. 143–144°. No depression occurred on mixed melting point determination with synthetic quercetagetin hexamethyl ether. The melting point of authentic patuletin was not depressed by the addition of compound A-1. The ultraviolet and infrared spectra of compound A-1 and the reference patuletin were identical, respectively. With spectral measurements by the

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method of Jurd and Horowitz,[6] both the reference patuletin and compound A-1 behaved exactly alike. Each exhibited a 4-mμ shift of the short wave-length band, after 5 min., from 257 mμ to 261 mμ in the presence of sodium acetate. With boric acid-sodium acetate mixture,[6] there was a shift of 18 mμ for each in the higher wave-length peak, indicating the presence of the *o*-dihydroxy group in both. In sodium ethoxide, however, the higher wave-length peak disappeared in each case after 5 min. Thus, patuletin and compound A-1 are identical by every test used.

Identification of compound A-2. The yellow powder (75 mg.), containing compound A-2 obtained from the faster-moving eluate of the silicic acid column, was crystallized twice from benzene–methyl alcohol to yield fine yellow needles. These were dried at 110°, *in vacuo;* m.p. 235–236°. R_f values in 60% acetic acid; the *n*-butyl alcohol–acetic acid–water; and the phenol-water solvent systems were 0.56, 0.85, and 0.88, respectively. The ultraviolet absorption spectrum showed maxima at 257 and 373 mμ and minima at 241 and 287 mμ.

Anal., Calcd. for $C_{17}H_{14}O_8$ (346.28): C, 58.96; H, 4.08; OCH_3, 17.92. Found: C, 59.12; H, 4.03; OCH_3, 18.57.

Compound A-2 (10 mg.) was methylated with 1 ml. of dimethyl sulfate in 8 ml. of anhydrous acetone and 2.5 g. of potassium carbonate to give colorless needles, m.p. 143–144°, not depressed by authentic quercetagetin hexamethyl ether. Demethylation of compound A-2 with hydriodic acid, sp. gr. 1.7, and acetic anhydride yielded quercetagetin. The latter had R_f values of 0.27, 0.45, and 0.20, respectively, in the 60% acetic acid, *n*-butyl alcohol–acetic acid–water, and phenol-water systems.

Degradation with potassium hydroxide by a procedure used previously by Yang *et al.*[7] produced vanillic acid. Identification of vanillic acid was achieved by the method of Hergert and Goldschmid.[8] Thus, compound A-2 contained the 3′-methoxy-4′-hydroxy grouping. Compound A-2 on paper chromatograms gave a greenish-yellow color under long wavelength ultraviolet light, indicating that its 3-hydroxy group is not substituted. Spectral shift measurements[5,6] indicated that a free 7-hydroxy group is present, as the short wave length band shifts from 257 mμ to 269 mμ with sodium acetate. Boric acid–sodium acetate addition produced no shift for the higher wave length peak, indicating the absence of an *o*-dihydroxy group. In 0.002*N* sodium ethoxide, the longer wave-length peak was partially suppressed after 5 min., and disappeared after 1 hr. These data indicate that compound A-2 is the 3′,6-dimethyl ether of quercetagetin.

Both patuletin and quercetagetin-3′-6-dimethyl ether have also been isolated from fresh spinach leaves obtained from a wholesale produce company. Other flavonoid compounds have been shown by paper chromatography to be present in both the fresh spinach and frozen spinach preparations. Studies on their identification are now in progress.

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CHEMISTRY DEPARTMENT
UNIVERSITY OF OKLAHOMA
NORMAN, OKLA.

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Reaction of Indoles with Acetyl Cyanide

K. T. POTTS[1]

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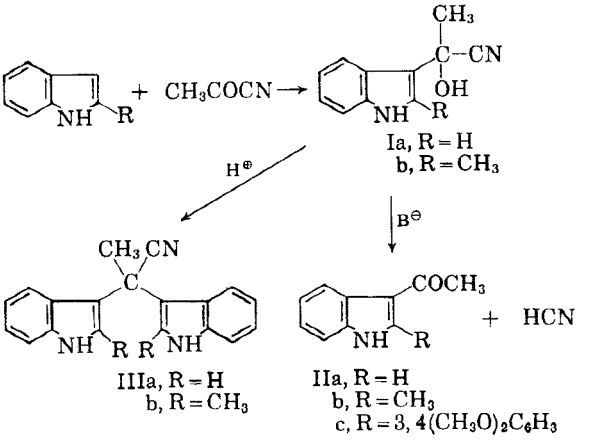
3-Acetylindole (IIa) is an important intermediate[2] in projected syntheses of several indole alkaloids and is available in moderate yield from indole and a boiling acetic acid–acetic anhydride mixture, the resultant 1,3-diacetylindole being readily converted into 3-acetylindole by base.[3] In an attempt to prepare this compound more economically, indole was treated with acetyl cyanide. Acyl cyanides are known to be effective acylating agents under a wide variety of conditions[4] and, in particular, acetyl cyanide in chloroform solution in the presence of pyridine has been reported to react with 2-methylindole forming 3-acetyl-2-methylindole (IIb) exclusively.[5]



The reaction of indole with acetyl cyanide in chloroform and in the presence of base, either at room temperature or at 62°, gave only a small amount of 3-acetylindole, the main product being a crystalline compound of empirical formula $C_{19}H_{15}N_3$. This same product was obtained almost exclusively on heating indole in an excess of acetyl cyanide. It gave a positive reaction with Ehrlich's reagent only on warming, and its infrared spectrum showed absorption bands at 3425 cm.$^{-1}$ (>NH), 2237 cm.$^{-1}$ (—CN), and those characteristic of the *o*-disubstituted benzene nucleus.[6] Its ultraviolet

(1) Present address: Department of Chemistry, University of Louisville, Louisville, Ky.

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