chloroform (35 ml.) was added dropwise to a stirred solution of 7-acetoxy-6-allyl-3,4,8-trimethylcoumarin (5.00 g., 0.0174 mole) in chloroform (50 ml.). Evaporation of solvent left 7.6 g. (98% yield) of an off-white solid, m.p. 141–145°, which was suitable for use in the next step. Crystallization from ethanol gave the material reported in Table I.

2',3,4,8-Tetramethylpsoralene (VIIa). A solution of crude 7-acetoxy-6-(2',3'-dibromopropyl)-3,4,8-trimethylcoumarin (74.5 g., 0.167 mole) in ethanolic sodium ethoxide (19.2 g. sodium in 750 ml. absolute ethanol) was heated under reflux for 1.75 hr., allowed to cool for 15 min., and poured into a mixture of ice (3 kg.) and 3.5% hydrochloric acid (3 l.). The resulting precipitate was washed three times with 5% sodium hydroxide (1-l. portions) and then with water to yield 36.2 g. (89% yield) of crude product, m.p. 190–196°. Crystallization from ethanol and then from benzene gave the material reported in Table I.

3,4-Benzo-2',8-dimethylpsoralene (VIII). A mixture of 2',8-dimethyl-3,4-cyclohexenopsoralene (3.01 g.), 5% palladium on charcoal (3.00 g.), and diphenyl ether (25 ml.) was heated under reflux for 5 hr. The catalyst was removed from the hot solution by filtration and was washed with 10 ml. of hot diphenyl ether. On cooling, the combined filtrate and wash liquor deposited a crystalline solid, which was washed with 95% ethanol and recrystallized from benzene to yield off-white prisms, (0.75 g., 25% yield), m.p. 232–233°. Analytical data are recorded in Table I.

4',5'-Dihydro-4,5'-dimethylisopsoralene (IX. R = CH_3). 7-Allyloxy-4-methylcoumarin[1] (212.5 g.) was heated at 215° (temperature of reaction mixture) for 3 hr. and the hot melt was poured into ethanol (1.5 l.). Addition of water (10 l.) gave a precipitate, which was treated with 5% aqueous sodium hydroxide (1.5 l.), in several portions, to obtain an alkali insoluble residue that crystallized from 95% ethanol in pale yellow needles (17.0 g., 8% yield), m.p. 117.6–117.8°. Acidification of the alkaline extracts gave crude 8-allyl-7-hydroxy-4-methylcoumarin which was purified in the manner described earlier.[1] Analytical results are included in Table I.

4,5'-Dimethylisopsoralene (X. R = CH_3). A mixture of 4',5'-dihydro-4,5'-dimethylisopsoralene (5.41 g.), 5% palladium on charcoal (5.0 g.), and diphenyl ether (60 ml.) was heated under reflux for 5 hr., filtered, and allowed to cool. The next day, an off-white solid (1.72 g., 32.1% yield), m.p. 179.6–182.2°, was collected by filtration. Dilution of the filtrate to 300 ml. with petroleum ether (b.p. 30–60°) gave a second crop which, when combined with the first crop, crystallized from ethanol in colorless prisms (2.33 g., 43% yield), m.p. 182–183°. A mixture of this material and a sample of 4,5'-dimethylisopsoralene from another method[1] had m.p. 182–183°. The infrared spectra of the two samples were identical.

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KALAMAZOO, MICH.

[CONTRIBUTION FROM THE UNITED STATES DEPARTMENT OF AGRICULTURE]

Spectral Studies on Flavonoid Compounds. II. Isoflavones and Flavanones[1a]

ROBERT M. HOROWITZ[1b] AND LEONARD JURD[1c]

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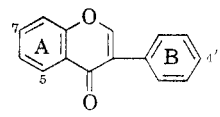
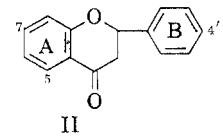
The ultraviolet spectra of isoflavones and flavanones are similar. A free 7-hydroxyl group in these compounds can be detected by spectral changes observed on the addition of sodium acetate, while a free 5-hydroxyl group can be detected by addition of aluminum chloride. Certain specifically substituted flavanones are shown to form chalcones readily in dilute alkali. A number of examples are given.

Although the ultraviolet spectra of many naturally occurring isoflavones and flavanones have been reported,[2] spectral changes in the presence of basic and complexing reagents have not been extensively employed in the structural analysis of these compounds. In view of the success with which spectral shifts produced by certain reagents have been correlated with the location of hydroxyl groups in various flavonol compounds,[1] it was of interest to determine whether similar shifts might provide useful structural information in the isoflavone and flavanone series.

Isoflavones (I) and flavanones (II) differ from flavonols in that the B-ring is not conjugated with



I



II

the carbonyl group. The spectral characteristics of isoflavones and flavanones are similar, therefore, and are determined primarily by absorption in the A-ring conjugated with the carbonyl group. These compounds usually have only one prominent ab-

(1) (a) Part I: L. Jurd and R. M. Horowitz, *J. Org. Chem.*, 22, 1618 (1957). (b) Fruit and Vegetable Chemistry Laboratory, Pasadena, Calif.[1d]; (c) Western Regional Research Laboratory, Albany 10, Calif.[1d]; (d) a laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(2) *E.g.*, W. K. Warburton, *Quart. Rev. Chem. Soc.*, 8, 67 (1954); N. L. Dutta, *J. Ind. Chem. Soc.*, **36**, 165 (1959); J. B. Harborne, *Chemistry and Industry*, 1142 (1954); D. H. Curnow, *Biochem. J.*, 58, 283 (1954); P. Crabbe, P. R. Leeming, and C. Djerassi, *J. Am. Chem. Soc.*, 80, 5262 (1958).

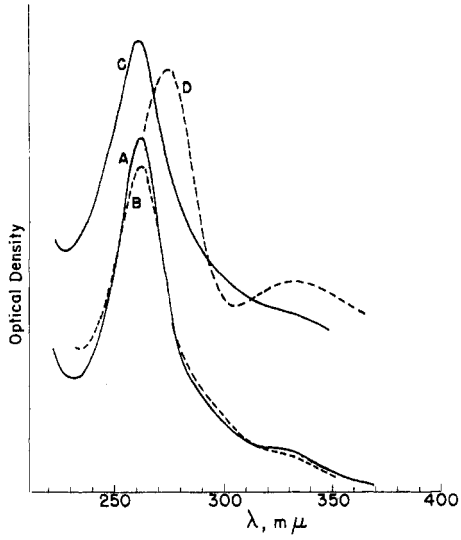


Fig. 1. Ultraviolet spectra of (A) genistin in ethanol, (B) genistin in ethanolic sodium acetate, (C) sophoricoside in ethanol, (D) sophoricoside in ethanolic sodium acetate

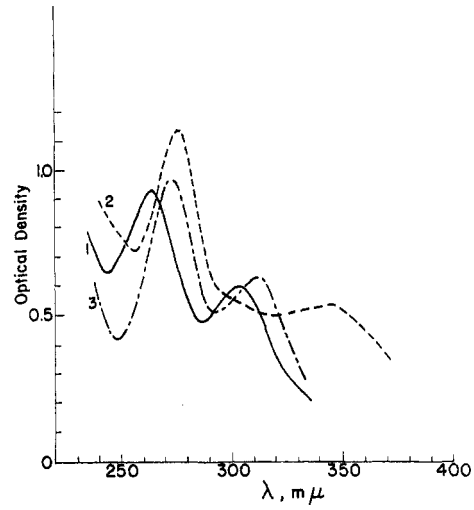
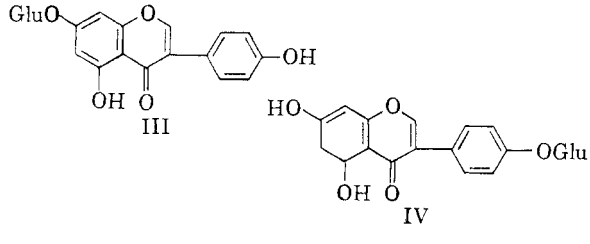


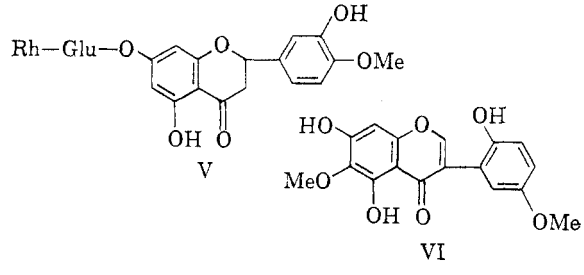
Fig. 2. Ultraviolet spectra of podospicatin in (1) ethanol, (2) sodium acetate, (3) aluminum chloride

sorption peak which occurs in the region of 250–290 mμ.

Detection of a 7-hydroxyl group. A free hydroxyl group at the 7- position of an isoflavone is sufficiently acidic to be ionized by fused sodium acetate. Ionization of a 7-hydroxyl group results in a bathochromic shift of the main absorption band of about 10 mμ for isoflavones (Table I: compounds 1, 3, 5, 7, 10, and 12) and 35–60 mμ for flavanones (Table II: compounds 1, 2, 3, 4, 5, 5, 6, 7, 8, 10, and 11). No significant changes are observed in the spectra of compounds lacking a free hydroxyl group at the 7-position (Table I: compounds 2, 4, 6, and 8; Table II: compounds 9, 13, 14, 15, 16, 17, 18, 19, and 20). These observations are particularly useful in distinguishing isomers such as genistin (III) and sophoricoside (IV), which are the 7- and 4′-glucosides of the aglycone genistein (5,7,4′-trihydroxyisoflavone), respectively. These glucosides have virtually identical spectra in ethanol, but in the presence of sodium



acetate the spectrum of genistin is unchanged while that of sophoricoside shifts 13 mμ (Fig. 1). Since the spectra of both glucosides undergo a bathochromic shift with aluminum chloride (see below), it is clear that the 5-hydroxyl group is unsubstituted. Further examples are provided by the flavanone glycoside neohesperidin (V)[3] and the new isoflavone podospicatin (VI).[4] The spectrum of neohesperidin is unaffected by sodium acetate, so that it may be inferred that sugar groups are attached through the 7-hydroxyl group. This conclusion has been confirmed by chemical degradations.[5] On the other



hand, the main absorption band of podospicatin is shifted 12 mμ on the addition of sodium acetate (Fig. 2). This would be expected on the basis of the structure assigned to podospicatin by Briggs and Cebalo.[4]

Detection of 4′-hydroxy-7-alkoxyflavanones. Sodium acetate is ordinarily the preferred reagent for ionizing the 7-hydroxyl group of flavanones. The addition of dilute sodium hydroxide (one drop of 1% sodium hydroxide in a 2.5-ml. cuvette) generally gives a spectral result similar to that obtained with sodium acetate except in the case of 4′-hydroxy-7-alkoxy- or 4′-hydroxy-7-glucosidoxyflavanones. When these structural features are present the compound is rapidly converted to its chalcone which, in the ionized form, has a broad maximum in the region of 400–450 mμ. All flavanones form chalcones eventually in concentrated alkali[6] but

(3) F. Kolle and K. Gloppe, *Pharm. Zentralhalle*, **77**, 421 (1936).

(4) L. H. Briggs and T. P. Cebalo, *Tetrahedron*, **6**, 145 (1959).

(5) Unpublished data.

(6) M. Shimokoriyama, *J. Am. Chem. Soc.*, **79**, 4199 (1957).

TABLE I

INFLUENCE OF SODIUM ACETATE AND ALUMINUM CHLORIDE ON THE SPECTRA OF ISOFLAVONES

Isoflavone	λ_{max}, mμ		
	C_2H_5OH[a]	NaOAc[b]	$AlCl_3$[c]
1. 7-Hydroxy-4′-methoxyisoflavone (Formonetin)	250	260	
2. Osajin	274	274	
3. 5,7,4′-Trihydroxyisoflavone (Genistein)	262	271	274
4. 5,4′-Dihydroxy-7-glucosidoxyisoflavone (Genistin)	262	262	273
5. 5,7-Dihydroxy-4′-methoxyisoflavone (Biochanin-A)	261	271	
6. Pomiferin	276	276	
7. 5,7-Dihydroxy-4′-glucosidoxyisoflavone (Sophoricoside)	262	275	276
8. 5,3′,4′-Trihydroxy-7-methoxyisoflavone (Santal)	263		274
9. 5,2′-Dihydroxy-6,7,5′-trimethoxyisoflavone (7-*O*-methylpodospicatin) (4)	265		277
10. 5,7,3′-Trihydroxy-6,4′,5′-trimethoxyisoflavone (Irigenin)	267	277	
11. 5,3′-Dihydroxy-6,4′,5′-trimethoxy-7-glucosidoxyisoflavone (Iridin)	268	268	
12. 5,7,2′-Trihydroxy-6,5′-dimethoxyisoflavone (Podospicatin)	263	275	273
13. 5-Hydroxy-6,7,2′,5′-tetramethoxyisoflavone (4)	262		275

[a] Absolute ethanol. [b] Absolute ethanol + fused sodium acetate. [c] Absolute ethanol + 3 drops 10% aqueous aluminum chloride.

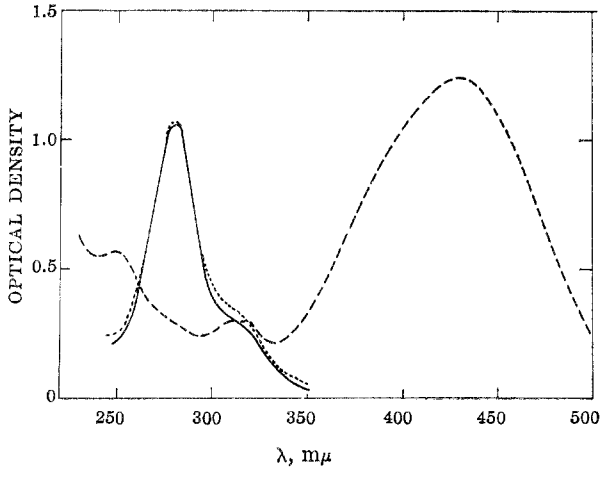


Fig. 3. Sakuranin: ——— In ethanol; _____ sodium acetate added (the curves are superimposable); ..... aluminum chloride added; - - - - 1 drop 1% sodium hydroxide added and allowed to stand ten minutes

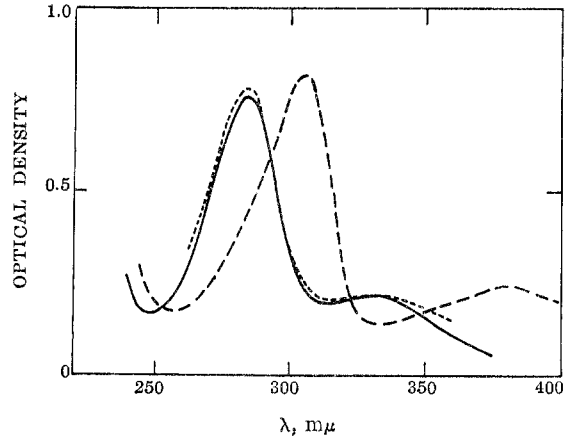
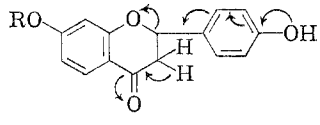


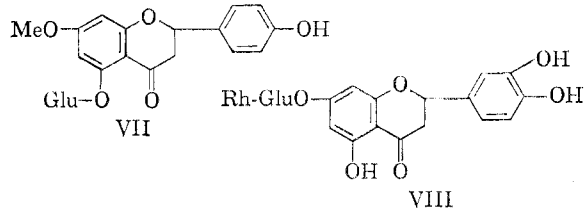
Fig. 4. Eriocitrin: ——— In ethanol; ..... sodium acetate added; - - - - aluminum chloride added

it appears that only 4′-hydroxy-7-alkoxy- or 4′-hydroxy-7-glucosidoxyflavanones form chalcones rapidly (five to ten minutes) in the very dilute alkali specified here. The susceptibility to chalcone formation in these compounds may be visualized as the result of increased acidity of the hydrogen atom *alpha* to the carbonyl group coupled with ionization of the 4′-hydroxyl group:



An example of this effect is provided by the glycoside sakuranin (VII) which is the 5-glucoside of 7-methoxy-5,4′-dihydroxyflavanone (sakuranetin). As expected, the glucoside gives no shift with sodium acetate or aluminum chloride and forms the chalcone in alkali (Fig. 3). After hydrolysis to the aglycone, a shift with aluminum chloride is observed.

Detection of a 5-hydroxyl group. It has been shown earlier that isoflavones and flavanones having a free 5-hydroxyl group form complexes with aluminum chloride and that this complex formation results in a bathochromic shift in the spectra of these compounds.[4,7,8] From the further examples reported in Tables I and II it is apparent that the principal wave length of 5-hydroxyisoflavones undergoes a remarkably constant bathochromic shift of 11–14 mμ on the addition of aluminum chloride, while that of 5-hydroxyflavanones changes by 20–30 mμ. In Fig. 4 the various



(7) T. Swain, *Chem. & Ind.*, 1480 (1954).
(8) W. J. Dunlap and S. H. Wender, *Arch. Biochem. Biophys.*, **87**, 228 (1960).

TABLE II

INFLUENCE OF SODIUM ACETATE, SODIUM HYDROXIDE, AND ALUMINUM CHLORIDE ON THE SPECTRA OF FLAVANONES

Compound	λ_{max}, mμ			
	C_2H_5OH[a]	NaOAc[b]	NaOH[c]	$AlCl_3$[f]
1. 7-Hydroxyflavanone	277	338	338	277
2. 7,4′-Dihydroxyflavanone (Liquiritigenin)	276	338	338	276
3. 7,3′,4′-Trihydroxyflavanone (Butin)	278	338	338	278
4. 5,7-Dihydroxyflavanone (Pinocembrin)	291	329	329	312
5. 5,7,4′-Trihydroxyflavanone (Naringenin)	290	328	328	311
6. 5,7,3′,4′-Tetrahydroxyflavanone (Eriodictyol)	289	328	328[d]	310
7. 3,5,7,3′,4′-Pentahydroxyflavanone (Taxifolin)	291	330	329[d]	314
8. 5,7-Dihydroxy-4′-methoxyflavanone (Isosakuranetin)	292	328	328	312
9. 5,3′,4′-Trihydroxy-7-methoxyflavanone	287	287	289[d]	309
10. 5,7,4′-Trihydroxy-3′-methoxyflavanone (Homoeriodictyol)	289	328	328	311
11. 5,7,3′-Trihydroxy-4′-methoxyflavanone (Hesperetin)	288	328	328	311
12. 5-Hydroxy-7,3′,4′-triacetoxyflavanone	274			303
13. Isosakuranetin 7-Rhamnoglucoside (Poncirin)	283	283	285	308
14. Eriodictyol 7-Rhamnoglucoside (Eriocitrin)	285	285	285[d]	306
15. Hesperetin 7-Rutinoside (Hesperidin)	285	285	287	308
16. Hesperetin 7-Neohesperidoside (Neohesperidin)	285	285	287	308
17. 5,4′-Dihydroxy-7-methoxyflavanone (Sakuranetin)	287	287	424[e]	310
18. Sakuranetin 5-glucoside (Sakuranin)	281	281	428[e]	281
19. Naringenin 7-glucoside (Prunin)	284	284	425[e]	308
20. Naringenin 7-rhamnoglucoside	284	284	428[e]	308

[a] Absolute ethanol. [b] Absolute ethanol saturated with fused sodium acetate. [c] 2.5 ml. absolute ethanol treated with 1 drop of 1% sodium hydroxide. [d] Solution decomposes rapidly. [e] Forms the chalcone. [f] Absolute ethanol saturated with aluminum chloride hexahydrate.

spectra of the new flavanone glycoside eriocitrin (VIII)[9] are shown. The bathochromic shift obtained with aluminum chloride shows the presence of a 5-hydroxyl group, while the lack of a shift with sodium acetate shows the presence of a sugar group at the 7-hydroxyl. The presence of free *o*-dihydroxyl groups is inferred from the instability of the compound in alkaline solution as well as from other evidence.[10]

Acknowledgment. We should like to thank Prof. L. H. Briggs for a sample of podospicatin and Dr. J. Naghski for a sample of sophoricoside. We should also like to thank Mr. Bruno Gentili for determining a number of spectra.

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(9) R. M. Horowitz and B. Gentili, *J. Am. Chem. Soc.*, 82, 2803 (1960).

(10) The compound decomposes irreversibly in alkali before chalcone formation can be observed.

[CONTRIBUTION FROM THE INSTITUTO DE QUÍMICA AGRÍCOLA, MINISTÉRIO DA AGRICULTURA]

Chemistry of Brazilian *Leguminosae*. II.[1] Isolation and Structure of Caviunin

OTTO RICHARD GOTTLIEB AND MAURO TAVEIRA MAGALHÃES

Received June 29, 1960

Caviunin, an extractive from *Dalbergianigria* (Fr. Allem.) is shown to be 5,7-dihydroxy-2′,4′,5′,6-tetramethoxyisoflavone.

Since early Brazilian history, the wood of *Dalbergia nigra* (Fr. Allem.), a tree belonging to the *Dalbergiae* tribe of the *Leguminosae* family, has been a much valued article of export. The species is particularly abundant in the state of Espirito Santo, but occurs also in the neighbouring states of Bahia, Minas Gerais, Rio de Janeiro, and in São Paulo, where it is called jacarandá caviuna. In other countries, however, *Dalbergia nigra*[2] is known under different names, such as Brazilian rosewood[3]

(1) Paper I: O. R. Gottlieb and M. Taveira Magalhães, *Anais assoc. brasil. quim.*, 18, 89 (1959).

(2) An anatomical and dendrometric study of *Dalbergia nigra*, as well as a list of references to the botanical literature is given by A. de Mattos Filho and A. F. Coimbra Filho, *Arquivos do Serviço Florestal* (Rio de Janeiro), 11, 157 (1957).

(3) This name is an allusion to the red color of the heartwood and the species should not be confused with the essential oil-producing trees of the genus *Aniba* (family *Lauraceae*) which we have studied in several papers entitled, "The Chemistry of Rosewood." For the most recent article, Part VI in the series, see W. B. Mors, O. R. Gottlieb, and I. de Vattimo, *Nature*, 184, 1589 (1959).