from acetic acid only one isomer, in the form of fine colorless prisms, m.p. 138°, giving a violet coloration in hot sulfuric acid. Yield: 3.5 g.

*Anal.* Calcd. for $C_{25}H_{23}N$: C, 89.0; H, 6.9; N, 4.2. Found: C, 89.2; H, 6.6; N, 4.0.

*1-(4-Bromophenyl)-2-phenyl-2-(4-isopropylphenyl)acrylonitrile* (VIII). Prepared from 9 g. of 4-isopropylbenzophenone, 9.5 g. of 4-bromobenzyl cyanide, and 5 g. of sodium amide, this product, b.p. 298–300°/13 mm., crystallized from acetic acid in fine colorless prisms, m.p. 148°.

*Anal.* Calcd. for $C_{24}H_{20}BrN$: C, 71.6; H, 5.0; N, 3.5. Found: C, 71.3; H, 5.0; N, 3.3.

*1-(4-Bromophenyl)-2-(2-chlorophenyl)-2-(4-xenyl)acrylonitrile* (IX). Prepared from 8 g. of 2-chloro-4′-phenylbenzophenone, 8 g. of 4-bromobenzyl cyanide, and 5 g. of sodium amide, this product crystallized from acetic acid in fine colorless needles, m.p. 210°.

*Anal.* Calcd. for $C_{27}H_{17}BrClN$: C, 68.9; H, 3.6; N, 3.0. Found: C, 68.6; H, 3.4; N, 2.8.

*1-(4-Chlorophenyl)-2-(2-chlorophenyl)-2-(4-xenyl)acrylonitrile* (X). This compound was prepared from 10 g. of 2-chloro-4′-phenylbenzophenone, 9 g. of 4-chlorobenzyl cyanide, and 4.2 g. of sodium amide; the portion boiling at 325–328°/13 mm. crystallized from acetic acid in colorless leaflets, m.p. 201°, giving a violet coloration in hot sulfuric acid.

*Anal.* Calcd. for $C_{27}H_{17}Cl_2N$: C, 76.1; H, 4.0; N, 3.3. Found: C, 75.8; H, 3.8; N, 3.0.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, M.R. SCIENCE INSTITUTE, GUJARAT COLLEGE]

# Chalcones and Related Compounds Derived from 2-Hydroxy-5-acetaminoacetophenone II. Flavones and Flavonols

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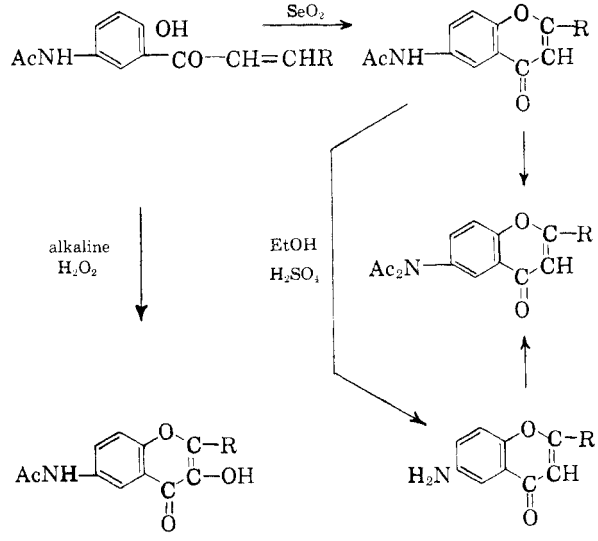
The selenium dioxide oxidation and Algar-Flynn oxidation of some acetaminochalcones derived from 2-hydroxy-5-acetaminoacetophenone have been studied. 6-Acetaminoflavones and flavonols have been synthesized. The 6-acetaminoflavones have been deacetylated by means of ethanolic sulfuric acid, the corresponding 6-aminoflavones being obtained.

In a previous paper[2], the authors have described various chalcones derived from 2-hydroxy-5-acetaminoacetophenone by condensing it with various aldehydes and the chalcones obtained have been cyclized to the corresponding 6-aminoflavanones. The work has now been extended to the synthesis of other heterocyclic compounds, and the synthesis of 6-aminoflavones and 6-acetaminoflavanols from the above chalcones is described in this paper.

When the acetaminochalcones were subjected to selenium dioxide oxidation,[3] 6-acetaminoflavones were obtained, which on deacetylation by ethanolic sulfuric acid, gave the corresponding 6-aminoflavones.

The chalcones were then subjected to Algar-Flynn oxidation[4] using alkaline hydrogen peroxide. Under these conditions, the corresponding 6-acetaminoflavonols were obtained.

Neither selenium dioxide nor Algar-Flynn oxidation of 2,2′-dihydroxy-5′-acetaminochalcone succeeded.




R = $C_6H_5$; *p*-$C_6H_4OCH_3$; 3,4-$C_6H_3(CH_2O_2)$; *m*-$C_6H_4OH$.

## EXPERIMENTAL

*6-Acetaminoflavone.* A mixture of 2′-hydroxy-5′-acetaminochalcone (0.5 g.) and selenium dioxide (0.5 g.) in dry isoamyl alcohol (15 ml.) was refluxed on an oil bath at 160–170° for 12 hr. The reaction mixture was then filtered while hot to remove precipitated selenium, and the filtrate was steam-distilled to remove isoamyl alcohol. A dark brown solid, along with some pasty mass, separated; this was filtered, dried, and extracted with benzene; the solid obtained after the removal of benzene was recrystallized twice from ethanol, producing yellowish brown needles, m.p. 174°. Yield, 0.3 g.

*Anal.* Calcd. for $C_{17}H_{13}O_3N$: C, 73.12; H, 4.66; N, 5.02. Found: C, 73.05; H, 4.47; N, 4.48.

It is soluble in ethanol, acetic acid, benzene, and chloroform. It gives greenish fluorescence with concentrated $H_2SO_4$. It does not give the $FeCl_3$ color test. It is insoluble in dilute alkali and dilute hydrochloric acid.

The *diacetyl* derivative prepared by the acetic anhydride-

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(2) A. A. Raval and N. M. Shah, *J. Org. Chem.*, **21**, 1408 (1956).

(3) K. Venkataraman *et al.*, *J. Chem. Soc.*, 866 (1935); 569 (1936).

(4) J. Algar and J. Flynn, *Proc. Roy. Irish Acad.*, **B 42**, 1 (1934).

pyridine method, crystallized from ethanol as brown granules, m.p. 256–258°.

*Anal.* Calcd. for $C_{19}H_{15}O_4N$: N, 4.36. Found: N, 4.03.

*Deacetylation: formation of 6-aminoflavone.* To a solution of 6-acetaminoflavone (0.5 g.) in ethanol (25 ml.), dilute sulfuric acid (10%; 20 ml.) was added gradually until a slight turbidity resulted. The turbidity was removed by adding more ethanol. The clear solution was refluxed on a water bath for 6 hr. The excess of ethanol was then distilled and the remaining liquor was treated with ammonia until it became alkaline. Excess of ammonia was expelled by heating the mixture on a water bath for 15 min. The brown solid was collected, washed with water, and crystallized from ethanol as brown needles, m.p. 192°. Yield, 0.2 g.

*Anal.* Calcd. for $C_{15}H_{11}O_2N$: C, 76.0; H, 4.64; N, 5.90. Found: C, 75.91; H, 4.52; N, 5.57.

It is soluble in ethanol, acetic acid, ethyl acetate, and benzene. It dissolves in concentrated $H_2SO_4$ with a blue fluorescence. It is insoluble in dilute alkali, but dissolves readily in dilute mineral acids. It does not give a $FeCl_3$ color test. The *diacetyl* derivative crystallized from ethanol as brown granules, m.p. 256–258°; a mixed melting point with the sample described earlier remained undepressed.

The following flavones were similarly prepared from different chalcones. To avoid repetition, the experimental details are omitted.

The compound *6-acetamino-4'-methoxyflavone* was prepared from 2'-hydroxy-5'-acetamino-4-methoxychalcone. It crystallized from benzene as light brown needles, m.p. 255°.

*Anal.* Calcd. for $C_{18}H_{15}NO_4$: C, 69.90; H, 4.85; N, 4.53. Found: C, 69.45; H, 4.40; N, 4.18.

It is soluble in ethanol, acetic acid, benzene, and chloroform. It is insoluble in dilute alkali as well as in dilute mineral acids. It dissolves with a yellowish brown color in concentrated $H_2SO_4$, the solution exhibiting violet fluorescence.

The *diacetyl* derivative prepared by the acetic anhydride-sodium acetate method, crystallized from ethanol as deep yellow granules, m.p. 270°.

*Anal.* Calcd. for $C_{20}H_{17}NO_5$: N, 3.98. Found: N, 3.68.

*6-Amino-4'-methoxyflavone.* The compound 6-acetamino-4'-methoxyflavone was deacetylated by dilute sulfuric acid under the same conditions as described previously. The brown solid was collected, washed with water, and crystallized from ethanol as brown needles, m.p. 147–148°.

*Anal.* Calcd. for $C_{16}H_{13}NO_3$: C, 71.91; H, 4.87; N, 5.24. Found: C, 71.25; H, 4.65; N, 4.95.

It is soluble in ethanol, acetic acid, ethyl acetate, benzene, and acetone. It dissolves in concentrated $H_2SO_4$ with greenish-blue fluorescence. It is insoluble in dilute alkali but dissolves readily in dilute mineral acids. On acetylation, it gave the *diacetyl* derivative, m.p. 270°; a mixed melting point with the sample described above remained undepressed.

*6-Acetamino-3',4'-methylenedioxyflavone* from 2'-hydroxy-5'-acetamino-3,4-methylenedioxychalcone crystallized from ethanol as brownish needles, m.p. 130°.

*Anal.* Calcd. for $C_{18}H_{13}NO_5$: C, 66.88; H, 4.02; N, 4.33. Found: C, 66.50; H, 3.91; N, 3.99.

It is soluble in ethanol, ethyl acetate, acetic acid, benzene, and chloroform. It is insoluble in dilute alkali as well as dilute mineral acids. It gives greenish fluorescence with concentrated $H_2SO_4$.

The *diacetyl* derivative, crystallized from ethanol as a brown powder, m.p. 280°.

*Anal.* Calcd. for $C_{20}H_{15}NO_6$: N, 3.83. Found: N, 3.50.

*6-Amino-3',4'-methylenedioxyflavone* obtained by deacetylating the above flavone as previously described, crystallized from ethanol as a dark brown powder, m.p. 180°.

*Anal.* Calcd. for $C_{16}H_{11}NO_4$: N, 4.98. Found: N, 4.60.

It is soluble in ethanol, acetic acid, and ethyl acetate. It gives bluish-green fluorescence with concentrated sulfuric acid. It is insoluble in dilute alkali, but it dissolves readily in dilute mineral acids.

The amino-flavone was acetylated: a *diacetyl* derivative identical with the above was obtained, m.p. 280°; a mixed melting point with the same obtained previously remained undepressed.

*6-Acetamino-3'-hydroxyflavone* from 2'-hydroxy-5'-acetamino-3-hydroxychalcone crystallized from ethanol as brownish needles, m.p. 260°.

*Anal.* Calcd. for $C_{17}H_{13}NO_4$: N, 4.74. Found: N, 4.45.

It is soluble in ethanol, acetic acid, chloroform, acetone, and benzene. It is insoluble in dilute alkali as well as in dilute mineral acids. It gives a greenish fluorescence with concentrated $H_2SO_4$.

The *acetyl* derivative, prepared by the acetic anhydride–sodium acetate method, crystallized from ethanol as brown granules, m.p. 270°.

*Anal.* Calcd. for $C_{21}H_{17}NO_6$: N, 3.69. Found: N, 3.42.

*6-Amino-3'-hydroxyflavone* obtained by deacetylating 6-acetamino-3'-hydroxyflavone as before, crystallized from ethanol as brown granules, m.p. 300°.

*Anal.* Calcd. for $C_{15}H_{11}NO_3$: N, 5.53. Found: N, 5.31.

It is soluble in ethanol, acetic acid, and benzene. It is insoluble in dilute alkali but readily dissolves in dilute mineral acids. It gives a pale greenish fluorescence with concentrated $H_2SO_4$.

The *acetyl* derivative, prepared by the acetic anhydride–sodium acetate method, crystallized from ethanol as brown granules, m.p. 270°, a mixed melting point with the same described before remaining undepressed.

*6-Acetaminoflavonol.* To an ethanolic solution of 2'-hydroxy-5'-acetaminochalcone (0.5 g. in 20 ml.), sodium hydroxide solution (5%; 20 ml.), was added. The deep red solution was cooled in an ice bath and hydrogen peroxide (16.5%; 5 ml.) was added to it. The reaction mixture was kept in an ice bath for 2 hr. and then left overnight at room temperature, whereupon the color of the mixture turned orange. It was acidified by dilution with ice-cold acidulated water; the solid that separated was filtered, washed with water, and crystallized from acetic acid as pale yellow thick plates, m.p. 278°. Yield, 0.2 g.

*Anal.* Calcd. for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.40; N, 4.74. Found: C, 69.02; H, 4.32; N, 4.63.

It is soluble in excess of dilute alkali, but insoluble in dilute hydrochloric acid. It is soluble in acetic acid, chloroform, acetone and sparingly soluble in ethanol and ethyl acetate. It gives a violet fluorescence with concentrated sulfuric acid.

The following flavonols were similarly prepared from different chalcones. To avoid repetition, the experimental details are omitted.

*6-Acetamino-4'-methoxyflavonol* was obtained from 2-hydroxy-5'-acetamino-4-methoxychalcone and crystallized from acetic acid as light yellow long needles, m.p. 257°.

*Anal.* Calcd. for $C_{18}H_{15}NO_5$: C, 66.45; H, 4.61; N, 4.30. Found: C, 66.50; H, 4.45; N, 4.03.

It is insoluble in ethanol and dilute hydrochloric acid, but dissolves in excess of dilute caustic alkali. It gives a green fluorescence when dissolved in concentrated sulfuric acid and a bluish-green fluorescence with Wilson's[5] boric acid reagent.

*6-Acetamino-3',4'-methylenedioxyflavonol* from 2'-hydroxy-5'-acetamino-3,4-methylenedioxychalcone crystallized from acetic acid as pale yellow granules, m.p. 272–273°.

*Anal.* Calcd. for $C_{18}H_{13}NO_6$: C, 63.73; H, 3.84; N, 4.13. Found: C, 63.50; H, 3.44; N, 3.78.

It is soluble in acetic acid, chloroform, and acetone, but sparingly soluble in ethanol and ethyl acetate. It gives a bluish, violet fluorescence with concentrated sulfuric acid. It is soluble in excess of dilute alkali but is insoluble in dilute mineral acids.

*6-Acetamino-3'-hydroxyflavonol* was obtained from 2'-hydroxy-5'-acetamino-3-hydroxychalcone and crystallized from acetic acid as pale yellow leaflets, m.p. >300°.

(5) C. W. Wilson, *Chem. Soc.*, **61**, 2303 (1939).

*Anal.* Calcd. for $C_{17}H_{13}NO_5$: C, 65.59; H, 4.18; N, 4.50. Found: C, 65.21; H, 4.00; N, 4.30.

It is soluble in acetic acid, chloroform, and acetone. It is insoluble in dilute mineral acids. It gives a greenish-blue fluorescence with concentrated sulfuric acid.

The deacetylation of these flavonols was not successful either with sulfuric acid or with anhydrous aluminum chloride.

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[CONTRIBUTION FROM THE LABORATORY FOR THE STUDY OF HEREDITARY AND METABOLIC DISORDERS AND THE DEPARTMENTS OF BIOLOGICAL CHEMISTRY AND MEDICINE, UNIVERSITY OF UTAH COLLEGE OF MEDICINE]

# Preparation of 5-Hydroxy-L- and D-Tryptophan[1]

ALLAN J. MORRIS AND MARVIN D. ARMSTRONG

*Received September 17, 1956*

5-Hydroxy-DL-tryptophan has been resolved by fractional crystallization of the quinine salts of N-carbobenzoxy-5-benzyloxy-DL-tryptophan. Configurations have been assigned to the resolved isomers, and 5-hydroxy-L- and D-tryptophan have been obtained by catalytic hydrogenation of N-carbobenzoxy-5-benzyloxy-L- and D-tryptophan, respectively.

Considerable interest has developed in the metabolism of 5-hydroxyindole compounds following the identification of serotonin[2] and enteramine[3] as 5-hydroxytryptamine (5HTA). DL-Tryptophan labelled with carbon-14 has been shown to be converted to 5-hydroxytryptophan (5HT) in the salivary glands of the toad *Bufo Marinus*,[4] tryptophan is converted to 5HT in *Chromobacterium violaceum*[5,6] and a specific decarboxylase which converts 5HT to 5HTA is present in the kidney tissue of dogs and guinea pigs.[7] Thus, 5-hydroxytryptophan appears to be a naturally occurring amino acid of considerable physiological importance.

Significant differences have been reported in the metabolism of the D- and the L- forms of many amino acids *in vivo*, particularly the aromatic amino acids. The administration of DL-phenylalanine leads to the excretion of phenylpyruvic acid,[8] whereas L-phenylalanine appears to be completely metabolized by infants.[9] D-Tryptophan is to some extent excreted unchanged[10] and is in part converted to indolelactic and indoleacetic acids,[11] whereas L-tryptophan is more completely metabolized by the human.[12] L-DOPA is converted in large part to homoprotocatechuic and homovanillic acids whereas D-DOPA is in part excreted unchanged and a considerable portion of the amount administered remains unaccounted for in the human.[13] Because of the natural occurrence of 5-hydroxytryptophan and because of the desirability of conducting both *in vivo* metabolic experiments and *in vitro* enzymatic studies with pure optical isomers, the resolution of this amino acid was undertaken.

Preliminary attempts to resolve 5-hydroxytryptophan as the N-formyl derivative were unsuccessful. The N-acetyl derivative was considered even less suitable because of difficulties encountered in hydrolyzing the compound in preliminary studies. N-Carbobenzoxy-5-benzyloxy-DL-tryptophan was then prepared and was resolved by fractional crystallization of the quinine salts from a benzene solution. Catalytic hydrogenation of the N-carbobenzoxy-5-benzyloxy-D- and L-tryptophans afforded 5-hydroxy-D-tryptophan and 5-hydroxy-L-tryptophan, respectively. The yield of 5-hydroxy-L-tryptophan from N-carbobenzoxy-5-benzyloxy-DL-tryptophan was 42%. Configuration of the respective antipodes was established by comparison of the rotation of the resolved isomers with those of tryptophan, by the shift in optical rotation of the L- isomer to a more positive value in acid solution[14] and by the papain-catalyzed formation of N-carbobenzoxy-5-benzyloxy-L-tryptophan anilide.[15]

## EXPERIMENTAL

*N-Carbobenzoxy-5-benzyloxy-DL-tryptophan.* 5-Benzyloxy-DL-tryptophan[16,17] (20.0 g., 0.064 mole) was suspended in a

(1) This work was supported by research grants from the National Institutes of Health, United States Public Health Service.

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(17) We wish to express our appreciation to the Upjohn Company for the gift of a generous supply of 5-benzyloxyindole.