Prototropic Reactivity in Salicylamide and Salicylanilide by Molecular Electronic Spectroscopy

STEPHEN G. SCHULMAN▲, PETER J. KOVI, and JOHN F. YOUNG

Abstract □ The acidity dependences of the electronic absorption and fluorescence spectra of salicylamide and salicylanilide were employed to demonstrate the presence of an intramolecular hydrogen bond in the ground states of the neutral molecules. Upon excitation to the lowest excited singlet state, intramolecular proton transfer from the phenolic groups to the carboxamide and phenyl carboxamide groups is observed. Hydrogen bonding and protonation of the carboxamide groups are shown to occur at the nitrogen atoms rather than at the oxygen atoms.

Keyphrases □ Salicylamide—prototropic reactivity, molecular electronic spectroscopy, presence of intramolecular hydrogen bond □ Salicylanilide—prototropic reactivity, molecular electronic spectroscopy, presence of intramolecular hydrogen bond □ Molecular electronic spectroscopy—prototropic reactivity in salicylamide and salicylanilide □ Hydrogen bonding—salicylamide and salicylanilide, prototropic reactivity, molecular electronic spectroscopy

In recent years, salicylamide and salicylanilide have become among the more notorious members of the pharmaceuticals related to salicylic acid. Nonlegend sleeping pills containing salicylamide frequently have been involved in suicide attempts, while several substituted derivatives of salicylanilide have been identified as photosensitizers. Sensitive fluorometric methods for the analysis of salicylamide are available (1–3). However, because of the similarities in the emission frequencies between the species derived from salicylamide and salicylic acid in the basic solutions on which fluorometry is carried out, there is little selectivity in fluorometric analysis and contamination by salicylate is always a problem. Little or no information about the the electronic absorption or fluorescence of salicylanilide appears to be available. As a result of our interests in the electronic structure, analytical toxicology, photochemistry, and photobiology of these compounds, the present study of the acidity dependences of the electronic spectra of salicylamide and salicylanilide was undertaken.

EXPERIMENTAL[1]

Salicylamide[2] and salicylanilide[2] were recrystallized from chloroform.

Chloroform[3] (spectroquality) and sulfuric acid[4] were used without further purification. Distilled deionized water was used to make dilutions of the sulfuric acid and to prepare acetate, phosphate, and borate buffers as well as sodium hydroxide solutions for absorption and fluorescence titrimetric studies.

[1] Absorption spectra were taken on a Beckman DB-GT spectrophotometer. Fluorescence spectra were taken on a Perkin-Elmer MPF-2A fluorescence spectrophotometer whose monochromators were calibrated against the xenon line emission spectrum and whose output was corrected for instrumental response by means of a rhodamine-B quantum counter. The pH measurements were made on an Orion model 801 pH meter with a Beckman silver–silver chloride–glass combination electrode.

[2] Eastman Organic Chemicals, Inc., Rochester, N. Y.

[3] Matheson, Coleman and Bell, Inc., Rutherford, N. J.

[4] AnalAR, Mallinckrodt Chemical Works, Inc., St. Louis, Mo.

Table I—Long Wavelength Absorption (λ_a) and Fluorescence (λ_f) Maxima[a] of Salicylamide and Salicylanilide in Aqueous Media

	Salicylamide		Salicylanilide	
	λ_a	λ_f	λ_a	λ_f
18 *M* H_2SO_4	317	383	316	364
1 *M* H_2SO_4	302	435	298	449
pH 7 phosphate buffer	302	417	298, 338	420
0.01 *M* NaOH	329	417	338	420

[a] Spectral maxima are reported in nanometers.

RESULTS

The long wavelength absorption maxima and fluoresence maxima of salicylamide and salicylanilide in representative regions of the Hammett acidity and pH scales are presented in Table I. The electronic absorption spectra of these compounds are shown in Figs. 1 and 2.

The ground-state dissociation constants for the prototropic equilibria between the protonated and neutral molecules (pKa_1) and between the neutral molecules and anions (pKa_2) derived from salicylamide and salicylanilide were estimated from the midpoints of the variations of the absorption spectra with pH and Hammett acidity (absorptiometric titrations). The excited state dissociation constants (pKa_1^* and pKa_2^*) were determined from the midpoints of the variations of relative fluorescence quantum yields with Hammett acidity and pH (fluorometric titration curves). These data are presented in Table II.

DISCUSSION

Agren (4) previously reported the pKa for the equilibrium between the neutral molecule and anion derived from salicylamide to be 8.9. However, the current spectrophotometric studies yielded a value of 8.3. Dissociation of the neutral molecule results in a shift of the long wavelength absorption maximum to longer wavelength, indicating that dissociation of the neutral salicylamide takes place from the phenolic group in the ground electronic state (5). The magnitude of the ground-state pKa for dissociation from the phenolic group of salicylamide is comparable to that of methyl salicylate (6), the only other simple derivative of salicylic acid in which the phenolic group is free to dissociate while the functional group derived from the carboxyl group of salicylic acid is uncharged.

The shift to longer wavelength of the absorption spectrum of salicylamide, accompanying protonation, is qualitatively similar to that of methyl salicylate (6) and indicates that, in the ground state, protonation of salicylamide occurs in the carboxamido group. However, while methyl salicylate can only be protonated at the carboxyl-type oxygen in concentrated acid, salicylamide can con-

Table II—Ground-State Dissociation Constants (pKa) and Lowest Excited Singlet State Dissociation Constants (pKa*) of Salicylamide and Salicylanilide.

	Salicylamide[a,b]	Salicylanilide[a,b]
pKa_1	−2.6	−4.1
pKa_1^*	−5.3	−6.6
pKa_2	8.3	6.9
pKa_2^*	2.1	—[c]

[a] The pKa values were determined by absorptiometric titrations. [b] The pKa* values were taken from the midpoints of the fluorometric titrations. [c] Equilibrium was not attained during the lifetime of the excited state of the salicylanilide zwitterion.

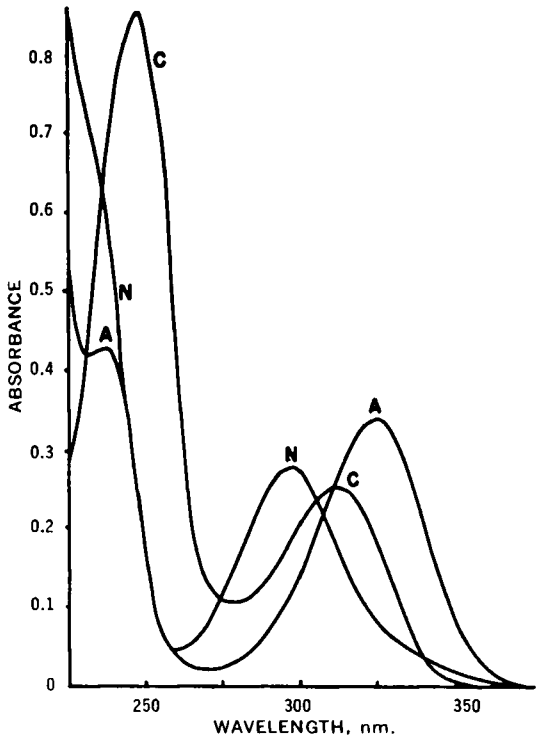


Figure 1—*Absorption spectra of the prototropic species derived from salicylamide (C) cation, $H_0 = -10$; neutral molecule (N), pH 4; anion (A), pH 12.*

ceivably be protonated at either the carbonyl oxygen atom or the amido nitrogen atom of the carbamido group. A recent study (7) showed that, in benzamide, protonation takes place in the ground state, the lowest excited singlet state, and the lowest triplet state at the amido nitrogen atom. The magnitude of the shift of the absorption spectrum of salicylamide upon protonation, which is smaller than that of methyl salicylate, and the pKa for protonation of salicylamide, which is only slightly more acidic than that of benzamide (7) and considerably less acidic than that of methyl salicylate, suggest that protonation occurs at the nitrogen atom in salicylamide. The proposed sequence of protolytic dissociations of salicylamide, beginning with the cation, is shown in Scheme I.

Upon going from concentrated acid to more dilute acid media, the fluorescence of salicylamide shifts to a longer wavelength. This indicates that the protonated molecule, present in concentrated acid,

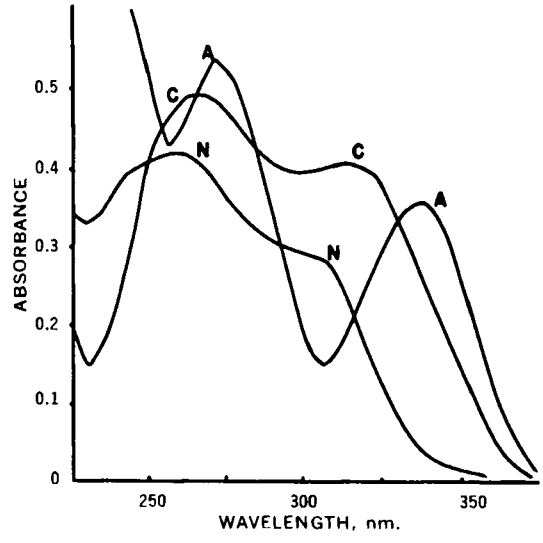
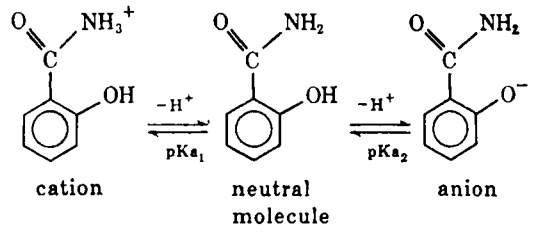


Figure 2—*Absorption spectra of the prototropic species derived from salicylanilide (C) cation, $H_0 = -10$; neutral molecule (N), pH 4; anion (A), pH 12.*



Scheme I—Protolytic reactions of salicylamide in the ground state

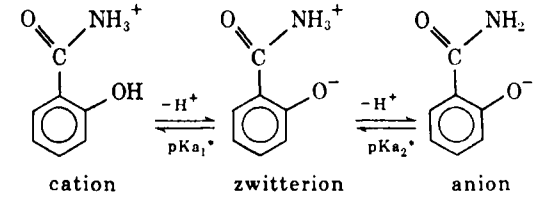
dissociates in the lowest excited singlet state from the phenolic group. In near neutral media, the fluorescence shifts to a shorter wavelength, signaling dissociation from the carboxamido group. The proposed sequence of dissociations of salicylamide in the lowest excited singlet state is represented in Scheme II.

In the lowest excited singlet state, the uncharged salicylamide is a zwitterion, while in the ground state it is a neutral molecule. As a result, no straightforward comparison between the successive ground and excited state equilibrium constants is possible.

In the region between pH 2.1 and $H_0 = -2.6$, the formation of the excited zwitterion of salicylamide must proceed entirely by phototautomerization of the neutral molecule. The pKa_1 of salicylamide is about 0.5 log unit more acidic than that of benzamide, even though the *O*-hydroxyl group might be expected to enhance the basicity of the carboxamido group. This is very much like the diminution of the basicity of the carboxyl group in salicylic acid by the presence of the hydroxyl group and suggests the presence of an intramolecular hydrogen bond between the hydroxyl group and the carboxamido group in the ground state. Presumably, the phototautomerism of salicylamide is intramolecular, consisting of readjustment of the position of the hydroxylic proton in the hydrogen bond so that the proton is essentially lost from the phenolic group and gained by the carboxamido group.

The arguments that were developed for salicylamide are all qualitatively applicable to the prototropic reactivity of salicylanilide, as seen from the similarities between the spectral shifts of the two compounds produced by protolytic dissociation. However, the presence of the phenyl group on the carboxamido nitrogen atom of salicylanilide has a substantial effect on the acid and base strengths of the various ground and excited state prototropic species derived from salicylanilide. All measurable equilibrium constants of salicylanilide are about 1.5 orders of magnitude more acidic than those of salicylamide. This observation can be rationalized in terms of the electromeric electron-withdrawing effect the phenyl group has on the lone pair of the carboxamido nitrogen atom of salicylanilide. This effect reduces the basicity of the carboxamido nitrogen, making it more difficult to protonate and thereby accounting for the lower value of pKa_1 for salicylanilide. The reduction in basicity of the carboxamido nitrogen atom also weakens the hydrogen bond between the phenolic proton and the phenyl-substituted carboxamido group. The weakening of this hydrogen bond, which stabilizes the phenolic proton in the neutral salicylamide molecule, makes the phenolic proton easier to remove. In addition, the electron-withdrawing effect of the phenyl group makes the phenyl-substituted carboxamido group more electron withdrawing than the unsubstituted carboxamido group. The combined effects of the weakening of the internal hydrogen bond and the stronger electromeric and field effects upon the hydroxyl group make pKa_2 more acidic in salicylanilide than in salicylamide.

Finally, in the lowest excited singlet state, only pKa_1^* could be measured for both compounds. Because pKa_1^* represents dissocia-



Scheme II—Protolytic reactions of salicylamide in the lowest excited singlet state

tion of the cations from the phenolic groups to form the zwitterions, hydrogen bonding is not important in this equilibrium. However, the electron-withdrawing enhancement of the carboxamido group by the phenyl group is even stronger in the lowest excited singlet state than in the ground state, as evidenced by the reduction in basicity of arylamines in the lowest excited singlet state (8). Thus, the lower pK_{a_2} of salicylanilide relative to salicylamide is attributed entirely to the greater electromeric and field effects of the phenyl-substituted carboxamido group in the lowest excited singlet state.

REFERENCES

(1) W. C. Lange, D. G. Floriddia, and F. J. Pruyn, *J. Pharm. Sci.*, **58**, 771(1969).

(2) W. H. Barr and S. Riegelman, *ibid.*, **59**, 154(1970).

(3) S. A. Veresh, F. S. Hom, and J. J. Miskel, *ibid.*, **60**, 1092 (1971).

(4) A. Agren, *Acta Chem. Scand.*, **9**, 39(1955).

(5) S. G. Schulman, *J. Pharm. Sci.*, **60**, 371(1971).

(6) P. J. Kovi, C. L. Miller, and S. G. Schulman, *Anal. Chim. Acta*, **61**, 7(1972).

(7) W. L. Paul and S. G. Schulman, *Anal. Chem.*, **45**, 415(1973).

(8) S. G. Schulman and A. C. Capomacchia, *Spectrochim. Acta*, **28A**, 1(1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 16, 1972, from the *College of Pharmacy, University of Florida, Gainesville, FL 32601*

Accepted for publication February 9, 1973.

▲ To whom inquiries should be directed.

Alkaloid Studies VIII: Isolation and Characterization of Alkaloids of *Tabernaemontana heyneana* Wall and Antifertility Properties of Coronaridine

WALTER E. MEYER, JOHN A. COPPOLA*, and LEON GOLDMAN▲

Abstract □ Extraction of the roots of *Tabernaemontana heyneana* Wall yielded the alkaloids coronaridine, voacangine, ibogamine, 19-oxocoronaridine, and the pseudoindoxyl of voacangine. Coronaridine was demonstrated to prevent pregnancies in adult female rats when administered orally.

Keyphrases □ *Tabernaemontana heyneana* Wall—isolation and characterization of alkaloids, antifertility properties of coronaridine □ Alkaloids—isolation and identification from *Tabernaemontana heyneana*, antifertility properties of coronaridine □ Antifertility agents, potential—isolation, characterization, and screening of coronaridine from *Tabernaemontana heyneana* □ Coronaridine—isolation from *Tabernaemontana heyneana*, screened for antifertility properties in rats

Investigation of the alkaloids of the Tabernaemontanoideae tribe of the Apocynaceae family has received considerable attention because of the interest aroused in the alkaloids isolated from *Tabernanthe iboga* of the same family.

The roots and bark of *Tabernaemontana heyneana* Wall were previously shown to contain the indole alkaloid coronaridine (I) (1) and its 20-hydroxy derivative heyneanine (II) (2). In this study, the roots of this botanical were examined, and the isolation and identification of additional indole alkaloids and some pharmacological properties of coronaridine are described.

DISCUSSION

An aqueous ethanolic extract of the roots of *T. heyneana* Wall was found to prevent fertilization of adult female rats when administered orally. The residue from this extract was treated with aqueous acetic acid, and the soluble components were then extracted into methylene chloride. Removal of the acidic components from the methylene chloride extract by washing with aqueous base gave, after evaporation of the solvent, a brown amorphous crude material (Fraction A), which retained all of the antifertility activity of the original extract. Chromatographic fractionation of a portion of Fraction A on silica gel yielded the alkaloid coronaridine and a closely associated alkaloid which was not obtained pure.

The alkaloids in Fraction A were precipitated as their hydrochloride salts, and the free bases were regenerated to afford a tan amorphous mixture of alkaloids (Fraction B). Sequential chromatography of Fraction B on silica gel, Grade I neutral alumina, and Grade III neutral alumina yielded additional coronaridine and the second alkaloid, which was identified as voacangine (III) by comparison with an authentic sample[1].

The syrup obtained from a methanol wash of the Grade III neutral alumina column was separated further by partition chromatography on diatomaceous earth to give a chromatographically pure, noncrystalline alkaloid. This alkaloid, which appeared on thin-layer chromatograms of solutions of coronaridine that had been exposed to air, was identical with the iodine–sodium bicarbonate oxidation [the procedure for conversion of ibogaine (IV) to 19-oxoibogaine (V) (3)] product of coronaridine. The appearance of a second carbonyl absorption band in the IR spectrum (6.06 μ) and a molecular-ion peak (m/e 352.1780) in the mass spectrum, which appeared 14 mass units higher than the molecular-ion peak for coronaridine, indicated this alkaloid to be 19-oxocoronaridine (VI), previously isolated from the bark of *Conopharyngia jollyana* Stapf. (4). [In *Reference 5*, this compound was characterized only by mass spectral analysis (m/e 352) and R_f values.]

Column chromatography of Fraction B on Grade II neutral alumina yielded a noncrystalline alkaloid fraction, which was further separated by partition chromatography on diatomaceous earth. A fraction containing 19-oxocoronaridine, contaminated with a highly fluorescent yellow material, was rechromatographed on silica gel to provide a crystalline yellow-green fluorescent alkaloid. The distinctive UV spectrum and mass spectrum indicated this material to be the pseudoindoxyl (VIII) (6) of voacangine (III).

Those fractions collected before the pseudoindoxyl (VIII) was

[1] The authors thank Dr. M. Gorman, Lilly Research Laboratories, for a sample of voacangine.