TABLE II.—PER CENT LOSS OF DYE IN BUFFERED TABLETS AFTER 60 DAYS STORAGE AT VARIOUS TEMPERATURES

Temperature, °C.	FD&C Red No. 4				FD&C Yellow No. 5				FD&C Blue No. 1			
	Unbuffered	pH 3	pH 5	pH 7	Unbuffered	pH 3	pH 5	pH 7	Unbuffered	pH 3	pH 5	pH 7
25	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	15	29	11	11	13	8
80	0	0	6	0	0	0	25	48	13	11	19	12

TABLE III.—REFLECTANCE CHANGES AT THE SURFACE OF TABLETS FOLLOWING STORAGE

	FD&C Blue No. 1				FD&C Yellow No. 5			
	Unbuffered	pH 3	pH 5	pH 7	Unbuffered	pH 3	pH 5	pH 7
Initial	1.200	1.120	1.045	1.080	0.825	0.768	0.785	0.770
25°, 60 days	1.175	1.110	1.020	1.030	0.825	0.760	0.760	0.750
80°, 60 days	1.000	0.884	0.851	0.885	0.715	0.500	0.515	0.475

explanation for this is the high concentration of dye used for the tablets.

The pH of the colored and uncolored tablets showed similar variations relative to maintenance of initial pH with storage. A slight decrease in pH was noticed in all cases except for the tablets buffered at pH 3 where essentially no change took place. Initially, it was intended to utilize the same buffer salts (phosphates) for the entire pH range of this investigation; however, it soon became apparent that the buffer capacity of the phosphate salts was not adequate at all the pH levels required. Therefore, varied buffered salt combinations were used for the different pH's to give optimum buffer capacity.

This study was designed to obtain an initial insight into the thermal stability of colorants when used in tablets. Although only limited data have been obtained, further experiments are necessary to define the complex factors contributing to dye instability. As a result of this information, additional investigations are contemplated to evaluate thoroughly the thermal stability of all FD&C colorants when employed in solid dosage forms.

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Stability of Metal Complexes of Nuclear-Substituted Salicylic Acids: Correlation with Biological Effects

By WILLIAM O. FOYE and JOSEPH G. TURCOTTE

Stability constants are reported for the chelation of ferric and aluminum ions by a series of salicylic acid derivatives having amino, alkyl, chloro, and nitro groups in the ring. The sequence of complex stabilities found is in agreement with current organic theory and shows a parallelism with the ionization constants. Three of the biological effects known for salicylates, antibacterial, analgesic-antipyretic, and fungicidal, can be related to the magnitude of complex stability or ionization constant.

A NUMBER of experimental approaches have been made in the attempt to explain the biological effects of the salicylates. One explanation for these effects has implicated the metal-binding ability of the salicylates (1–4), since the *meta*- and *para*-hydroxybenzoic acids, which are incapable of binding metal ions through complexation, exert none of the classical salicylate effects (1). The literature, however, does not provide much information regarding the relative abilities of the various salicylates to bind metal ions. The measurement of stability constants should provide a much fuller knowledge of these abilities and give us a better insight as to the importance of metal-binding in salicylate actions.

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TABLE I.—KNOWN STABILITY CONSTANTS FOR VARIOUS SALICYLATES AND METAL CATIONS (Water, 20°)

Ligand	Cation: Fe^{+++} log K'	Fe^{+++} log K''	Cu^{++} log K'	Cu^{++} log K''	Fe^{++} log K'	Fe^{++} log K''	Ref.
Salicylic acid	16.35	11.90	10.60	7.85	6.55	4.7	(6)
5-Sulfosalicylic acid	14.60	10.55	9.50	6.80	5.90	<4.0	(6)
5-Aminosalicylic acid	16.97	12.04	..	..	..	..	[a]
Methyl salicylate	..	..	5.90	..	9.74[a]	..	(6)
Salicyl aldehyde	..	..	5.75	..	..	..	(6)
Gentisic acid	10.8	8.0	7.0	4.9	..	..	(7)
Salicyluric acid	9.0	7.0	6.1	5.6	..	..	(7)
Salicylamide	10.02	6.24	..	..	..	..	[a]

[a] Ågren, A., *Acta Chem. Scand.*, **9**, 39(1955).

Stability constants for the chelation of biologically important metal ions by salicylic acid (5–7), as well as those of a few salicylic acid derivatives, have been recorded. The latter include methyl salicylate, salicylamide, 5-sulfo- and 5-aminosalicylic acids, gentisic acid, and salicyluric acid. The constants for these substances with pertinent metal ions are listed in Table I.

The literature provides ample indication that salicylic acid or its derivatives become involved in enzymatic reactions, for which temporary binding of the prosthetic metal is a likely possibility. Among the more recent reports of this nature are those of Piccinelli (8), in which sodium salicylate was found to cause marked change in sugar metabolism; Messer (9), who found that sodium salicylate and sodium acetylsalicylate inhibit the synthesis of glutamine; Adams (10), who found a correlation between anti-inflammatory activity, increase in oxygen consumption, and uncoupling of oxidative phosphorylation; Durham (11), who showed that *p*-aminosalicylic acid antagonized the oxidative dissimilation of *p*-aminobenzoic acid in flavobacteria; and Janota (12), who reported that prolonged administration of salicylates increased the activity of plasma enzymes in rabbits. Direct inhibition of a copper oxidase, tyrosinase, by salicylic acid is also known.[1]

Stability constants have now been determined for the reaction with metal ions of a series of salicylic acids, having various substituents in the 3, 4, and 5 positions of the ring, with the expectation that a correlation might be found between either strength or weakness of metal-binding and a salicylate action. Where a temporary combination with the constituent metal of an enzyme might take place, as previously postulated (13), a lowering of stability constant (from that of salicylic acid with metals) should indicate more effective compounds. That these substituents might affect other physical properties of the molecules, such as solubility characteristics, is presently under consideration.

[1] C. W. Bauer, personal communication.

The metal ions selected for this experiment were ferric and aluminum. Ferric ion was considered more appropriate than cupric, for instance, since Perrin's calculations (6) revealed that salicylic acid has a stronger affinity for ferric ion than has glycine, a representative cellular ligand, by a factor of 400:1, whereas glycine has the stronger affinity for cupric ion, by a factor of 45:1. Aluminum was selected as a suitable trivalent ion for comparative purposes. The possibility of the same chelating strength sequence for two metal ions could then be determined.

METHODS

Materials.—The metal salts used were Baker analyzed reagents $FeCl_3 \cdot 6H_2O$ and $AlCl_3 \cdot 6H_2O$. The organic ligands selected for study (listed in Table II) were commercially available with the exception of the diisopropyl and *tert*-butyl derivatives of salicylic acid which had to be prepared. The ligands obtained commercially were purified by several recrystallizations from aqueous alcohol and charcoal. Purity was confirmed by melting points and paper chromatography using butanol saturated with ammonia.

Carbonate-free 0.1000 *N* sodium hydroxide solution was prepared from a concentrated solution of sodium hydroxide U.S.P. appropriately diluted with recently boiled and cooled distilled water. It was treated with saturated barium hydroxide solution for 12 hours, filtered, and standardized.

TABLE II.—IONIZATION CONSTANTS (20°)

Compound	pKa′ ($-CO_2H$)	pKa ($-OH$)
4-Aminosalicylic acid	6.75	13.33
4-Methylsalicylic acid	5.72	13.32
3-Methylsalicylic acid	5.70	13.30
3,5-Diisopropylsalicylic acid	5.92	13.28
5-*tert*-Butylsalicylic acid	5.64	13.20
3-Isopropyl-6-methylsalicylic (*o*-thymotic) acid[a]	5.60	13.21
Salicylic acid	5.57	13.14
5-Chlorosalicylic acid	4.85	13.34
5-Nitrosalicylic acid	3.92	12.83
3-Nitrosalicylic acid	3.81	12.77

[a] Generously supplied by Dr. Paul J. Jannke, University of Connecticut, Storrs.

TABLE III.—STABILITY CONSTANTS OF THE SALICYLATE COMPLEXES (20°)

Ligand	Fe^{+++} log K'	Fe^{+++} log K''	Fe^{+++} log Ks[a]	Al^{+++} log K'	Al^{+++} log K''	Al^{+++} log Ks[b]
4-Aminosalicylic acid	16.30	13.91	30.48	15.57	12.45	28.02
4-Methylsalicylic acid	16.26	11.89	29.84	15.76	11.65	27.41
3,5-Diisopropylsalicylic acid	16.23	11.59	29.66	15.62	11.75	27.37
5-*tert*-Butylsalicylic acid	16.06	12.21	29.44	15.60	11.56	27.16
Salicylic acid	16.53	12.69	29.34	15.62	11.45	27.07
3-Isopropyl-6-methylsalicylic acid	16.03	11.34	29.08	15.45	11.50	26.95
3-Methylsalicylic acid	15.99	11.85	29.04	15.26	11.53	26.79
5-Chlorosalicylic acid	16.07	12.82	28.96	16.02	11.73	27.75
3-Nitrosalicylic acid	14.60	12.46	27.54	14.25	11.85	26.10
5-Nitrosalicylic acid	14.51	12.67	26.86	14.31	12.16	26.47

[a] Calculated from $\log Ks = -2 \log (Sc)$, when $\bar{n} = 1$. [b] Calculated from $Ks = \log K' + \log K''$.

Preparation of 3,5-Diisopropylsalicylic Acid.—The procedure of Meyer and Bernhauer (14) for 5-isopropylsalicylic acid was used with modifications. A 1,600-ml. portion of 80% sulfuric acid, 60 Gm. (0.43 mole) of salicylic acid, and 40 ml. of 2-propanol were stirred rapidly at 70° for 1 hour. The flocculant product was isolated and recrystallized from glacial acetic acid. A yield of approximately 80% of white, crystalline material was obtained which melted at 117–118°. Literature values for the melting points of both the 5-isopropyl and 3,5-diisopropyl derivatives were found to be in the range 116–118°.

Anal.[2]—Calcd. for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.90; H, 8.20.

Preparation of 5-*tert*-Butylsalicylic Acid.—Use of the method of Meyer and Bernhauer (14) was found to give a sulfonated product, so it was modified as follows. Sixty grams (0.43 mole) of salicylic acid, 75 ml. of *tert*-butyl alcohol, and 1,600 ml. of 80% sulfuric acid were stirred for 1 hour, during which time the temperature was raised to 75°. The resulting solution was allowed to cool overnight, charcoal was added, and the mixture was boiled for 5 minutes. It was filtered and allowed to cool, and the white, crystalline product was isolated and recrystallized from dilute acetic acid; m.p. 151–152°.

Anal.—Calcd. for $C_{11}H_{14}O_3$: C, 68.02; H, 7.26. Found: C, 68.00; H, 7.30.

Ionization Constants.—Values for primary and secondary ionization constants for each compound were determined by the potentiometric procedure of Osol and Kleckner (15), using 0.01 *M* solutions of the salicylates in 95% ethanol. A Beckman model E-2 electrode sensitive over the entire pH range was employed to give more accurate constants for the phenolic group. Table II lists the constants obtained.

Titrations.—The titrations were carried out under nitrogen as previously described (7) except that 0.01 *M* solutions of the ligands were made in 95% ethanol instead of in water. Aqueous base was used as before for titration, and the same volumes and concentrations were used for the determination of ionization constants as for the stability constants. The final solutions in each case contain approximately 79% alcohol. A Beckman Zeromatic pH meter with a Beckman model E-2 electrode was employed. Titrations were repeated for each system until pH values agreed to within 0.02–0.05 units for each run. A Friden fully automatic calculator, model SRW, was used for most of the calculations, which were carried out as previously described (7). The constants obtained are listed in Table III.

[2] Carbon-hydrogen analyses were done by Carol K. Fitz, Needham, Mass.

RESULTS AND DISCUSSION

In Table III are listed the values calculated for the first and second stability constants (log K' and log K'') of the metallic complexes, which correspond to the extent of formation of those having 1:1 and 2:1 ligand-metal ratios. In some cases, precipitation of the complexes prevented the determination of the log K''' values, and in other cases the values obtained were of doubtful accuracy. For purposes of comparison, "log Ks" values are listed, which indicate the overall stability of complex formation through the 2:1 complex stage. It would be unlikely that values for the third stability constant would alter the sequence of chelating abilities as shown. Therefore the log Ks values for the first and second stability constants, calculated by two different procedures (that one being listed that gave the greatest spread of values in each case) should be adequate for comparison.

The sequence of chelating abilities found was essentially the same for both the Fe^{+++} and Al^{+++} complexes. This sequence is also in agreement with that determined for copper complexes of substituted salicylaldehydes by Calvin and Wilson (16). The results reported for the salicylaldehydes in general resemble those reported here for the substituted salicylates: there was but a small variation in stability constants among the alkyl derivatives which in general were slightly higher than the value for salicylaldehyde itself, the halogen-substituted salicylaldehydes gave somewhat lower values, and the 3- and 5-nitro derivatives gave the lowest constants. No amino derivatives were included.

Comparison of the stability constants of the complexes with the ionization constants of the acids shows a rough parallelism between acidity (of both carboxyl and phenolic groups) and complex stability. One immediately questions the position of the 5-chlorosalicylic acid complexes in the stability sequence, since its phenolic ionization constant would indicate a stability comparable to that of the 4-amino- or 4-methylsalicylic acids, while its carboxyl ionization constant would place it just above the nitrosalicylic acids in the stability sequence. That the stability of the ferric complex falls between the values for the ferric complexes of the alkyl

and nitro acids indicates that the acidity of the carboxyl group contributes fully as much to the complex stability as the acidity (or basicity) of the phenolic group. However, with the aluminum complex of 5-chlorosalicylic acid, the stability of the complex is almost as great as that of the 4-amino derivative. In both cases, the value of log K' approaches that for the more stable complexes, as might be predicted from the value of the phenolic ionization constant.

The stability of the 3-methylsalicylic acid complexes is also seen to be out of position with regard to the ionization constants. In this case, a possible explanation is suggested immediately in steric hindrance which would tend to lower the stability constant to its observed value. That the 3,5-diisopropyl derivative does not produce the same demotion of stability constant may be due to the lesser numbers of α-hydrogen atoms in the 3-substituent.

A conclusion regarding the electronic effects of the substituents on complex stability can be readily seen from the constants reported here and is consistent with current organic chemical theory. Those groups capable of contributing electrons to the ring increase the basicity of the phenolic group and thus increase complex stability, whereas those groups that attract electrons decrease the phenolic basicity and complex stability as well. The 4-substituents apparently increase the basicity of the carboxyl function thus supporting the structure of the free chelating species shown in the previous paper (7). Similar observations regarding the effect of ionization constant on complex stability have been made before (7, 17).

A complete understanding of the role that complex stability plays in the biological effects of salicylates probably cannot be reached until the limiting effects of other properties, such as liposolubility, are known. However, the possibility may be advanced that the analgesic effects of salicylates do not require a high stability for possible metal complexes, whereas the antibacterial ability does. This may be concluded from the high stability noted for the complexes of 4-aminosalicylic acid, the only effective antitubercular agent among the salicylates, and the lower stability noted for the complexes of *o*-thymotic acid, reported to have a greater analgesic effect than has aspirin (18). The cresotinic acids (3-, 4-, and 5-methylsalicylic acids) have approximately the same antirheumatic efficacy as salicylic acid (19), and 4,5-dimethylacetylsalicylic acid has a similar antipyretic ability to that of aspirin (20). These observations are all in accord with the similar constants found for salicylic acid and its alkyl derivatives. It appears doubtful that the fungicidal activity of the salicylates is related to metal binding, since the most effective derivatives, the salicylanilides and alkyl ethers (21) would not be expected to bind metal ions to an appreciable extent.

SUMMARY

1. The avidities of a series of salicylic acids, having various substituents in the ring, for ferric and aluminum ions have been measured potentiometrically and recorded as stability constants.

2. The sequence of complex stabilities found with both metal ions is in agreement with that predicted from the electron-attracting and -repelling effects of the substituents, and shows a parallelism with the ionization constants of the ligands.

3. A relation between the magnitude of the complex stability constant and three of the biological effects of the salicylates has become evident. This has made possible the classification of these biological effects in terms of a physical constant (either complex stability or ionization constant). Antibacterial ability apparently requires high constants, analgesic-antirheumatic effects appear in compounds having constants similar to those of salicylic acid itself, and fungicidal ability has been found in those salicylates having no or very low metal-binding strength.

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