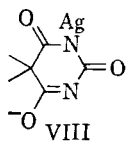
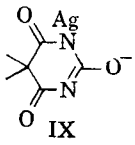
silver ion does not change the electron distribution in the enolized form.

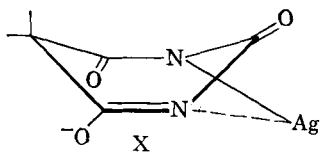
Goyan, *et al.* (11), have suggested that barbital degradation in ammonia buffer is similar to ammonolysis of an ester and that hydrolysis of the ionized species would probably be at the 4 (or 6) position. By virtue of infrared spectra of the *p*-nitrobenzyl derivatives of a series of barbiturates, Chatten and Levi (12) have proposed that the mechanism of the reaction involves enolization of the carbonyl at position 4 (or 6).

The silver-barbiturate anionic complex has been shown to be stable in alkaline solution. The silver ion, when attacking the barbiturate anion, liberates a proton, thus the nitrogen atom which is not involved in the enolization appears to be the position of the silver ion, as shown by structures VIII and IX


VIII


IX

These formulas do not appear to explain the stability of the complex to alkaline hydrolysis, especially if the 4 (or 6) carbonyl group is the position vulnerable to hydroxyl ion attack.


X

Barbiturates which have substituents on a nitrogen atom, i.e., a methyl group, are known to hydrolyze in the same way as the unsubstituted compounds. The mono silver anionic complexes are believed to be stabilized by the formation of a chelate structure involving both the 1 and 3 nitrogen atoms. This compound was previously described by Poethke and Furst (6) and is represented by structure X.

SUMMARY

1. Stability constants for the anionic silver complexes for a series of nine barbituric acid derivatives were determined potentiometrically.

2. The per cent recovery or titration efficiency was determined.

3. The molar absorptivity of the silver anionic complex of barbital was shown to be the same as that of barbital in alkaline solution.

4. The effect of silver ion on the stability of barbital in a sodium carbonate solution was described.

5. A structure for the anionic silver complex was proposed.

REFERENCES

(1) Budde, H., *Apoth. Ztg.*, **49**, 295(1934).
(2) Danielsson, B., *Svensk Farm. Tidskr.*, **55**, 125(1951); through *Chem. Abstr.*, **45**, 6798(1951).
(3) Gautier, J. A., Pellerin, F., and Pineau, J., *Ann. Pharm. franc.*, **16**, 625(1958).
(4) Perelman, Y. M., *J. Anal. Chem. U. S. S. R. English Translation*, **11**, 243(1956).
(5) Perelman, Y. M., *ibid.*, **11**, 491(1956).
(6) Poethke, W., and Furst, W., *Pharmazie*, **15**, 673 (1960).
(7) Kolthoff, I. M., and Furman, N. H., "Potentiometric Titrations," John Wiley & Sons, Inc., New York, N. Y., 1931.
(8) Kolthoff, I. M., and Elving, P. J., "Treatise on Analytical Chemistry," Vol. 1, Part 1, The Interscience Encyclopedia, Inc., New York, N. Y., 1959.
(9) Leyda, J. P., Lamb, D. J., and Harris, L. E., THIS JOURNAL, **49**, 581(1960).
(10) Werner, A. E. A., *Sci. Proc. Roy. Dublin Soc.*, **23**, 131 (1943).
(11) Goyan, J. E., Shaikh, Z. I., and Autian, J., THIS JOURNAL, **49**, 627(1960).
(12) Chatten, L. G., and Levi, L., *Appl. Spectroscopy*, **11**, 177(1957).

Synthesis of Some Derivatives of Sorbic Acid and Their Antifungal Properties

By ROBERT W. GOETTSCH†, AUGUST G. DANTI, and DALE H. CRONK

Seven amides and three amine salts of sorbic acid were prepared and screened for their antifungal activity against four pathogenic fungi. Of the 10 compounds tested, N-(2-methylpiperidyl)-sorbamide, *n*-hexylsorbamide, cyclohexylamine sorbate, phenylethylamine sorbate, and *n*-hexylamine sorbate exhibited antifungal activity.

THERE ARE many types of chemical compounds that have been studied for their potential antifungal activity. Among the diverse chemical types of antifungal agents investigated are the fatty acids, their salts, and derivatives. Hoffman, Schweitzer, and Dalby (1) have demonstrated that fatty acids and their salts are fungistatic for certain nonpathogenic fungi. Keeney and Broyles (2) reported that sodium propionate appeared effective in the treatment of certain superficial fungus infections. Keeney, Ajello, and Lankford (3) investigated the fungi-

Received October 30, 1961, from the School of Pharmacy, Northeast Louisiana State College, Monroe.
Accepted for publication December 7, 1961.
† Present address: College of Pharmacy, University of Tennessee Medical Units, Memphis 3.

static and fungicidal activity of fatty acid salts and found that fungicidal activity was acquired as the fatty acid chain was lengthened. Gooding (4) and Geigy (5) reported that certain α,β-unsaturated fatty acids and their derivatives, respectively, possessed fungistatic activity. Peck and Rosenfeld (6) found that olefinic acids possessed antifungal activity to a greater degree than their aliphatic homologs.

Sorbic acid, which is intermediate in the fatty acid series in respect to carbon chain length and is unsaturated at the α,β and γ,δ-positions, and its derivatives have been studied for their antifungal activity. Puls, Lindgren, and Cosgrove (7) reported that sorbic acid was superior to benzoic acid as a fungistatic agent. Recently some derivatives of sorbic acid were reported by Cronk, Zopf, and Jones (8) which have shown the potentialities of sorbic acid derivatives as antifungal agents. This investigation was undertaken to study the effects of some sorbic acid derivatives on certain pathogenic fungi which produce dermatoses in man.

EXPERIMENTAL PART I

Sorbyl Chloride.—To 160 Gm. (1.43 moles) of sorbic acid[1] in a 1-L. round-bottom flask was gradually added 328 Gm. (2.75 moles) of thionyl chloride. After addition of the thionyl chloride, the mixture was stirred for one and one-half hours and then heated to 80° for one-half hour. The product was purified by vacuum distillation using potassium hydroxide and a mixture of dry ice and acetone as traps. The fraction distilling at 95–99° (20 mm. Hg) was collected as a nearly colorless liquid. The yield was 135 Gm. or 72% of the theoretical.

Method I.—The substituted amides of sorbic acid were prepared by reaction of sorbyl chloride with the appropriate amine as illustrated by the following process.

N-Benzylsorbamide.—In a 500-ml. round-bottom flask was placed 6.5 Gm. (0.05 mole) of sorbyl chloride dissolved in 200 ml. of ether. A solution of 10.7 Gm. (0.1 mole) of benzylamine in 50 ml. of ether was added, dropwise. An immediate reaction took place with formation of a precipitate of the amine hydrochloride. The mixture was stirred for 30 minutes at room temperature; the resulting mixture was treated with water and the ether layer separated. The ether layer was washed with 5% hydrochloric acid, 4% sodium carbonate, and water. Evaporation of the ether layer gave the sorbamide which was purified by recrystallization from ethanol. The yield was 8.5 Gm. (84% of theoretical) of white crystals which melted at 129–131°.

Method II.—The amine salts of sorbic acid were prepared by reaction of sorbic acid with the appropriate amine as illustrated by the following process.

N-Cyclohexylamine Sorbate.—In a 500-ml. round-bottom flask was placed 5.6 Gm. (0.05 mole) of sorbic acid dissolved in 200 ml. of ether. A solution of 6.94 Gm. (0.07 mole) of cyclohexylamine, in 50 ml. of ether was added, dropwise. The mixture was stirred for one and one-half hours at room temperature and the resulting precipitate filtered and dried. The salt was purified by recrystallization from ethyl acetate. The yield was 9.6 Gm. (91% of theoretical) of white crystals which melted at 150–153°.

The recrystallization solvents, yields, melting points, and analyses are given in Table I.

EXPERIMENTAL PART II

The antifungal activity of the compounds prepared in this investigation was determined by the paper disk technique. The fungi used were: *Microsporum canis* (ATCC10214), *Microsporum gypseum* (ATCC10215), *Microsporum audouini* (ATCC10216), and *Trichophyton rubrum* (ATCC-10218). Aqueous suspensions of spores of the test organisms were prepared from 14-day agar slants which had been incubated at 28 ± 2°. The suspensions were prepared by aseptically transferring pure colony growths from the slants to a sterile 4-oz. wide-mouth glass bottle. One-half-inch sections of glass rod and 40 ml. of sterile distilled water were added to the bottle, then agitated vigorously to pulverize the colony growth into a homogeneous suspension.

The culture plates were prepared using Difco Sabouraud's dextrose agar dehydrated, 65 Gm./L., pH 5.6. Exactly 20 ml. of the dissolved agar was pipetted into test tubes and autoclaved for 15 minutes at 15 pounds pressure (121°). The media from each test tube was poured into sterile, flat bottom Petri dishes (15 × 100 mm.) and allowed to solidify. The plates were then inoculated with 0.5 ml. of the spore suspensions.

The test compounds, with the exception of cyclohexylamine sorbate, phenylethylamine sorbate, and *n*-hexylamine sorbate were dissolved in acetone in 5% w/v concentrations. The above salts were dissolved in water in the same concentration.

The testing procedure consisted of immersing the sterile paper disks[2] in the test solution of each compound and draining off the excess liquid by touching the disk to the wall of the container. One disk was placed in the center of each culture plate, running triplicate plates for each compound. The plates were then incubated at 28 ± 2° for 14 days. The zones of inhibition were recorded at the end of 7 and 14 days. The zones of inhibition were measured as the minimum distance from the periphery of the paper disk to the edge of the colony growth. The *in vitro* results of the test compounds and controls employed are given in Table II.

DISCUSSION

During the course of this investigation other derivatives were prepared in addition to those reported in Table I, namely N-piperidylsorbamide, N-(*n*-butyl)-sorbamide, N-(*sec*-butyl)-sorbamide, N-(2-aminopyridyl)-sorbamide, and 1-methyl-4-sorbyl-

[1] The sorbic acid used in this investigation was supplied through the courtesy of Union Carbide Chemicals Co., Division of Union Carbide Corp., New York 17, N. Y.

[2] Difco Bacto disks, sterile blanks, 1/2 inch (13 mm.).

TABLE I.—AMIDES AND AMINE SALTS OF SORBIC ACID

No.	Amine Component	Recrystallization Solvent	Yield, %	M.P., B.P.,[a] °C.	Formula	Nitrogen—Calcd.	Nitrogen—Found
1	Benzylamine	Ethanol	84	129–131	$C_{13}H_{15}NO$	6.95	6.72
2	Cyclohexylamine	Ethanol	88	161–162	$C_{12}H_{19}NO$	7.25	7.18
3	Phenylethylamine	Isopropanol	83	115–116	$C_{14}H_{17}NO$	6.51	6.45
4	2-Methylpiperidine	None	76	185 (20 mm.)	$C_{12}H_{19}NO$	7.25	6.97
5	*n*-Hexylamine	Ethyl ether-petroleum ether	77	74–75	$C_{12}H_{21}NO$	7.17	6.86
6	*o*-Toluidine[b]	Ethanol	86	171–173	$C_{13}H_{15}NO$	6.95	6.95
7	*p*-Phenetidine	Ethanol	81	170–172	$C_{14}H_{17}NO_2$	6.06	6.00
8	Cyclohexylamine[c]	Ethyl acetate	91	150–153	$C_{12}H_{21}NO_2$	6.64	6.80
9	Phenylethylamine[c]	Ethyl acetate	77	100–102	$C_{14}H_{19}NO_2$	6.00	6.01
10	*n*-Hexylamine[c]	Ethyl acetate	80	99–101	$C_{12}H_{23}NO_2$	6.57	6.55

[a] All melting points are uncorrected. [b] Previously reported m.p. 173° [see (11)]. [c] Amine salts of sorbic acid.

TABLE II.—ZONES OF INHIBITION, COMPOUNDS AT 5% w/v CONCENTRATIONS[a]

Compound No.	*M. canis* Days 7	*M. canis* Days 14	*M. gypseum* Days 7	*M. gypseum* Days 14	*M. audouini* Days 7	*M. audouini* Days 14	*T. rubrum* Days 7	*T. rubrum* Days 14
1	0	0	0	0	0	0	0	0
2	1	0	0	0	3	1	0	0
3	2	0	0	0	8	1	1.5	0
4	16	7	19	13.5	20	16.5	16.5	9
5	3	2.5	3	1.5	9	3	5	1
6	0	0	0	0	1	0	0	0
7	0	0	0	0	1	0	0	0
8	16	0	0	0	25.5	12	10.5	0
9	12.5	0	0	0	24.5	17	11	0
10	10.5	0	2	0	26	13	10.5	0
Sorbic acid	16	0	2.5	0	31.5	19.5	15.5	0
Acetone	0	0	0	0	0	0	0	0

[a] Zones, minimum distance from periphery of disk to edge of colony growth, average of three measurements. Measurements given in millimeters.

piperazine. Other salts prepared were benzylamine sorbate, piperidine sorbate, 1-methylpiperazine sorbate, 2-methylpiperidine sorbate, *n*-butylamine sorbate, *sec*-butylamine sorbate, and 2-aminopyridine sorbate.

It was found that many of the sorbamides and amine salts were unstable with respect to light and/or room temperature and decomposed with the formation of light tan to dark brown solids or resinous products. The unstable nature of amine salts and derivatives is well known. A technical bulletin from Union Carbide Corp. (9), in referring to amine soaps (salts), states that the color becomes somewhat darker on standing and that this color formation varies with both the amine component and the oxidizing tendencies of the fatty acid being used, but that the color change does not impair the effectiveness of the compounds. According to a treatise on sorbic acid (2,4-hexadienoic acid) (10), the acid, its esters, and salts are subject to oxidative reactions at the olefinic bonds and that autoxidation results in peroxide formation followed by a degradative and polymerization process. This ease of oxidation apparently accounts for some of the decomposition noted in the compounds prepared during this study.

In preparing the solutions for antifungal testing it was found that compounds No. 2, 3, 6, and 7 (Table I) had a maximum solubility of less than 5% w/v in acetone. This factor may have contributed to the little or no antifungal properties shown for these compounds (Table II). The fact that some solutions were made with acetone and some with water did not exhibit any discernible degree of difference in diffusion rates when the paper disks were placed on the agar media.

It should be noted that although the readings were taken on the 7th and 14th days, that in the case of *M. gypseum*, compounds No. 8, 9, and 10, (Table I), as well as the control, sorbic acid, were effective up to 5 days. After this time growth resumed, until at the end of 2 weeks, no zones of inhibition were perceived.

Compounds No. 4, 5, 8, 9, and 10 (Table I) showed the greatest degree of antifungal activity against all pathogens tested (Table II). With the exception of the test with *M. audouini*, N-(2-methylpiperidyl)-sorbamide appeared to be a more effective antifungal agent than was sorbic acid after 2 weeks of culture growth.

REFERENCES

(1) Hoffman, C., Schweitzer, T. R., and Dalby, G., *Food Research*, **4**, 539(1939).
(2) Keeney, E. L., and Broyles, E. N. *Johns Hopkins Hosp. Bull.*, **73**, 479(1943).
(3) Keeney, E. L., Ajello, L., and Lankford, E., *ibid.*, **75**, 377(1944).
(4) Gooding, C. M., "Process of Inhibiting Molds," U. S. pat. 2,379,294.
(5) Geigy, J. R., Swiss pat. 257,648, May 2, 1948; through *Chem. Abstr.*, **44**, 3524d(1950).
(6) Peck, S. M., and Rosenfeld, H., *J. Invest. Dermatol.*, **1**, 237(1938).
(7) Puls, D. D., Lindgren, L. F., and Cosgrove, F. P., THIS JOURNAL, **44**, 85(1955).
(8) Cronk, D. H., Zopf, L. C., and Jones, J. W., *ibid.*, **48**, 455(1959).
(9) "Emulsions and Detergents," 8th ed., Carbide and Carbon Corp., New York, N. Y., 1949.
(10) "Encyclopedia of Chemical Technology," 1st suppl. vol., The Interscience Encyclopedia, Inc., New York, N. Y., 1957, p. 840.
(11) Riedel, A., and Schultz, E., *Liebigs Ann. Chem.*, **367** 14(1909).