Discoloration of Tablets Containing Amines and Lactose

By ROBERT A. CASTELLO and ALBERT M. MATTOCKS

It has been noted by several workers that tablets of amine salts combined with lactose darken in color with aging, and this problem has been investigated. Results show that the darkening is due to a reaction occurring in the presence of amine base and not in the presence of the salt. The reaction in tablets can be accelerated with elevated temperature and high humidity. The release of free base from the amine salt is shown to be due to reaction of the alkaline lubricant, such as magnesium stearate, which removes hydrogen ion with the formation of stearic acid, furnishing an alkaline medium in the adsorbed moisture. The change in pH with time was recorded to illustrate the effects occurring in tablets. The use of neutral or acidic lubricants, such as glyceryl monostearate or stearic acid, prevents the discoloration from occurring. These findings are thought to be applicable to any combination of amine salt and lactose.

THOUGH lactose has long been used as a tablet excipient, the recent finding that spray-dried lactose can be compressed without granulation has greatly increased the use of this material in tablet-making. This has made more important the recognition and avoidance of incompatibilities of lactose, combinations with amines being, perhaps, the one most commonly encountered.

A number of workers have found that tablets of amine salts combined with lactose as a filler have discolored slowly on storage to become tan in color. The intensity of color generally has been dependent on the amount of amine present and the humidity and time of storage, 6 months often being required for the discoloration to be distinct. It is of concern to learn whether the reaction occurring involves decomposition of the active ingredients, the lactose, or both, and how this reaction may be prevented. For these purposes the work presented in this paper was undertaken. Amphetamine sulfate was selected as the amine salt for most of this work.

EXPERIMENTAL

Materials Used.—Spray-dried lactose,[1] amphetamine sulfate, and various lubricants and excipients used were of U.S.P., N.F., or pharmaceutical grade. All chemicals used were of reagent grade.

A series of tablets were prepared of lactose with a relatively high percentage of amphetamine sulfate and with various lubricants, the combinations being selected so as to determine the ingredients necessary for reaction. Tablets were placed on watch glasses in a humidity cabinet at 40° and 85% relative humidity, conditions previously found to cause distinct discoloration in a commercial tablet in 1–2 days. Results are given in Table I.

Received April 28, 1961 from the College of Pharmacy, University of Michigan, Ann Arbor.
Accepted for publication June 30, 1961.
Presented to the Scientific Section, A.PH.A., Chicago meeting, April 1961.

[1] Western Condensing Co., Appleton, Wis.

These results indicated several possibilities for reaction: (*a*) the free amine may be necessary for reaction, in which case the reaction may occur between the amine and lactose, or the free base may simply be undergoing alkaline oxidation, (*b*) the reaction may be a general base-catalyzed reaction of lactose.

To demonstrate clearly the alkalizing effects of the lubricants, excess amounts of lubricant (3%) were added to a 1% solution of amphetamine sulfate, the mixture was stirred continuously, and the pH was recorded over a period of several hours. Curves of pH *vs.* time are shown in Fig. 1.

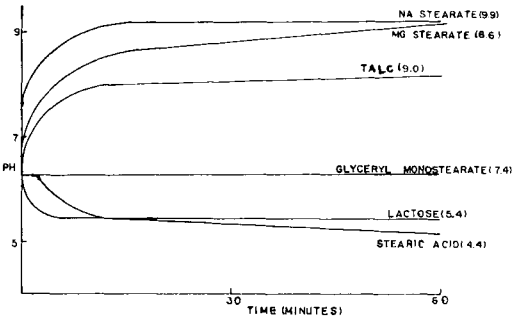

Fig. 1.—Effect of lubricants on solutions of amphetamine sulfate. Figures in parentheses are pH of suspensions of lubricants alone.

From Fig. 1 it is seen that the substances promoting discoloration, sodium stearate, magnesium stearate, and talc shifted the pH of the solution from 6.3 to values well on the alkaline side.

Results to this point indicated that the discoloration reaction required moisture, and it was concluded that the possibility of occurrence of reaction could be predicted from results with solutions. The use of solutions allowed observations to be made more quickly with discoloration being more distinct.

Results obtained with a series of solutions are shown in Table II. These data demonstrate that if an amine base or other alkali is present, the discoloration occurs. Further, it is seen that discoloration occurs only in the presence of lactose. Thus, it is clear that the discoloration is a base-catalyzed reaction of lactose.

TABLE I.—DISCOLORATION OF AMINE-LACTOSE TABLETS AFTER 48 HOURS AT 40° AND 85% RELATIVE HUMIDITY

Amine	Lubricant	Visual Observation
Amphetamine SO_4 10%	Mg Stearate 1%	Discoloration
Amphetamine SO_4 10%	Na Stearate 1%	Discoloration
Amphetamine SO_4 10%	Talc 2%	Discoloration
Amphetamine SO_4 10%	Stearic acid 1%	No discoloration
Amphetamine SO_4 10%	Glyceryl monostearate 1%	No discoloration
Amphetamine SO_4 10%	None	No discoloration
Amphetamine base 10%	None	Strong discoloration

TABLE II.—DISCOLORATION OF AQUEOUS SOLUTIONS AFTER 48 HOURS AT 60°

	Components		
Amine	Lactose, %	Other	Visual Observations
Amphetamine SO_4	10	...	No discoloration
.....	10	...	No discoloration
Amphetamine base, 1%	..	...	No discoloration
Amphetamine base, 1%	10	...	Severe discoloration
Ammonia buffer pH 9.5	10	...	Slight discoloration
.....	10	NaOH, 1%	Severe discoloration
.....	10	Mg stearate, 3%	No discoloration
Amphetamine SO_4, 1%	10	Mg stearate, 3%	Severe discoloration
Desoxyephedrine HCl, 1%	10	...	No discoloration
Desoxyephedrine base, 1%	10	...	Severe discoloration
Desoxyephedrine HCl, 1%	10	Mg stearate, 3%	Severe discoloration

The fact that the reaction product is from lactose alone and does not contain amine was substantiated by extraction and examination of the colored material. A solution of lactose, 10%, and amphetamine base, 1%, was allowed to react at 60° for several days, and the colored material was extracted with *n*-butanol. The butanol solution was washed thoroughly with water, dilute hydrochloric acid, and again with water, little or none of the color being removed in the washings. The butanol solution was then dried over anhydrous sodium sulfate and evaporated to leave a dark, tarry residue of the colored product. This material could not be recrystallized but was found to be free of nitrogen. Treatment with phenylhydrazine yielded yellowish crystals, recrystallized from methanol-water, which melted at 186–188°. This derivative has not yet been identified.

DISCUSSION

Reactions of carbohydrates with amines are well known and have been reviewed by Pigman (1). It has been found that reactions occur with aldehyde groups of open chain forms of the carbohydrates to form Schiff bases, as well as with ring forms to produce glycosylamines or N-glycosides. The reaction causing formation of a colored product, however, is more likely the browning reaction described by Maillard in 1912 (2). Beacham and Dull (3) investigated the browning of glucose, 2,3,4,6-tetramethyl-D-glucose, glyceric aldehyde, glycoaldehyde, and a number of glycoaldehyde ethers in the presence of amines. They observed that the susceptibility to browning of the D-glycosyl derivatives of the various amines was directly proportional to the basic strength of the amine. From the work of Mitts and Hixon (4) it is seen that the glucosyl derivatives of the more basic amines were most highly dissociated in aqueous solution, while those amines which formed condensation products resistant to hydrolysis did not readily cause browning. The amine derivatives of glyceric aldehyde and glycoaldehyde browned readily, indicating that furfurals are not necessary intermediates in the browning reaction. Derivatives formed from etherified glycoaldehyde and 2,3,4,6-tetramethylglucose were resistant to browning, supporting the necessity of the α-hydroxyl group in the reaction. This was in agreement with Hurd and Kelso (5) who reported that 2,3,4-tridesoxyaldopentose did not brown in the presence of glycine while 3,4-didesoxyaldopentose did. Results of these workers indicate that although a reaction of sugar with amine may be an intermediate step, it is not a necessary step in the browning reaction.

The reaction occurring between the amine salt and the basic lubricants, sodium stearate for example, may be postulated as

$$RN^+H_3 = RNH_2 + H^+ \qquad \text{(Eq. 1)}$$

$$\text{Stearate}^- + H^+ = \text{HStearate} \qquad \text{(Eq. 2)}$$

$$RN^+H_3 + \text{Stearate}^- = RNH_2 + \text{HStearate} \qquad \text{(Eq. 3)}$$

In the reaction mixture excess stearate is available; therefore, the alkali stearate is maintained at saturation. The reaction causes the solution to become saturated with stearic acid, as well, providing sufficient hydrogen ion is released from the amine salt. The equilibrium pH of the mixture may be approximated by the Henderson-Hasselbalch equation

$$\text{pH} = \text{pKa}_{(\text{HStearate})} + \log\left(\frac{\text{Stearate}^-}{\text{HStearate}}\right) \qquad \text{(Eq. 4)}$$

The theoretical value calculated by Eq. 4 was 8.49, which does not agree with the observed value of 9.2 for the sodium stearate mixture (see Fig. 1). It was felt, therefore, that either the proposed mechanism was incorrect or the pH measurements of the mixture were in error. It was noted that Jenny, *et al.* (6), reported the development of a liquid junction potential when measuring the pH of slurries and colloidal systems. This potential was said to be due

to a change in transference numbers of the potassium and chloride ions in the solution being measured. They demonstrated this effect by observing a potential drop across two calomel electrodes, one immersed in the suspension, the other in the supernatant liquid.

To eliminate this error, an equilibrium mixture of amphetamine sulfate and sodium stearate was centrifuged to remove the suspended material. The pH was again determined and found to be 8.6, which is in reasonable agreement with the calculated value, 8.49, from Eq. 4.

From these results it is proposed that Eq. 3 is descriptive of the system, and that the role of basic lubricants is the removal of hydrogen ion to promote the base-catalyzed browning of lactose. These findings coupled with later tests using inorganic alkalies indicate that the reaction is a general base-catalyzed reaction and not limited to amine bases.

It should be noted that the pH values shown in Fig. 1 are those of suspensions and contain the errors discussed above.

Results of this work furnish a practical means for predicting discoloration of tablets containing lactose with a few short-term tests. It is suggested that a new formulation be tested simply by preparing a solution or slurry of the ingredients, placing it in a bath at 60° for 2 or 3 days, and then observing the color. In this way one can determine whether the formulation is satisfactory before preparing tablets. Also, it is demonstrated that lactose should not be utilized in combination with bases, and when lactose is combined with amine salts, alkali stearates and talc should be avoided.

REFERENCES

(1) Pigman, W., "The Carbohydrates; Chemistry, Biochemistry, Physiology," Academic Press, Inc., New York, N. Y., 1957, p. 406.
(2) Maillard, L. C., *Compt. rend.*, **154**, 66(1912).
(3) Beacham, H. H., and Dull, M. F., *Food Research*, **16**, 439(1951).
(4) Mitts, E., and Hixon, R. M., *J. Am. Chem. Soc.*, **66**, 483(1944).
(5) Hurd, C. D., and Kelso, C. D., *ibid.*, **70**, 1484(1948).
(6) Jenny, H., *et al.*, *Science*, **112**, 164(1950).

Synthesis of α- and β-Amino Ketone Analogs of Amino Acids as Antibacterial Agents

By SHU-SING CHENG†, SIGURDUR JONSSON‡, and FRED T. SEMENIUK

α- and β-Amino ketone analogs of amino acids were synthesized as potential antagonists of amino acids in microbial metabolism. The hypothetical mechanism of antibacterial activity for certain analogs is proposed. The successful use of organocadmium reagents for the synthesis of the aliphatic and aromatic series of α- as well as β-amino ketones indicates a wider scope of applicability than by the method of Dakin-West or by Friedel-Crafts acylation. These series of compounds show antimicrobial activity *in vitro*.

THE ANTIBACTERIAL activity (1) reported for β-amino ketone analogs of β-alanine suggested that α-amino ketone analogs of α-amino acids might possess similar activity. Due to the structural similarity between amino ketone analogs and the natural amino acids, one may assume that these analogs function as antagonists to amino acids, thus interfering with biochemical reactions in which individual amino acids or related peptides participate.

The cell walls of most Gram-positive bacteria consist primarily of components linked together as the following sequence: 3-O-carboxyethyl-hexosamine-*dl*-alanine-*d*-glutamic acid *l*-lysine-*dl*-alanine-*dl*-alanine (2). It could be assumed that an amino ketone analog of α-alanine would interfere with the formation of this peculiar amino-sugar peptide, thus inhibiting the cell wall synthesis. Many species of bacteria are able to grow on simple media containing ammonium and other inorganic salts, plus a single organic carbon source such as glucose. Some species require the addition of certain amino acids, simple peptides, and vitamins for growth. It is obvious that many bacteria can synthesize a majority of amino acids from ammonium salt and glucose. Two ways are recognized by which a bacterium can assimilate ammonia and convert it to α-amino groups in amino acids. These are the fumarate and the α-ketoglutarate pathways (3). The fumarate pathway is dependent upon a condensation reaction between fumaric acid and ammonia to yield aspartic acid. The α-ketoglutarate pathway is

Received April 28, 1961, from the School of Pharmacy, University of North Carolina, Chapel Hill.
Accepted for publication May 8, 1961.
Presented to the Scientific Section, A.PH.A., Chicago meeting, April 1961.
Abstracted from a portion of a thesis submitted by Shu-Sing Cheng to the Graduate School of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
† Present address: Department of Bacteriology and Immunology, School of Medicine, University of North Carolina.
‡ Deceased.