

Comparison of Reactivity of Amphetamine, Methamphetamine, and Dimethylamphetamine with Lactose and Related Compounds

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The lactose browning reaction was studied in the presence of a primary amine (amphetamine), a secondary amine (methamphetamine), and a tertiary amine (dimethylamphetamine) to ascertain the relationship of amine structure to degree of browning. Reactivity of the hydrolysis products of lactose and of 5-hydroxymethylfurfural (HMF) with these amines was studied also under similar conditions. Evidence indicated that the browning of lactose in the presence of amines differed in type and degree from that observed in their absence. The lactose-amine reaction was predominantly a primary amine-carbonyl reaction and was similar in nature but distinct from the dextrose-, galactose-, and HMF-amine reactions.

IN AN ACCOMPANYING communication (1) some of the factors involved in the normal browning of lactose were reported. The term "normal browning" was used in reference to the browning of lactose occurring in the absence of added amines. It was found that 5-hydroxymethylfurfural (HMF) and/or related compounds were formed during the browning process and that pH, moisture, and the presence of common buffer components, such as phosphate, citrate, tartrate, and acetate ions, all affected the rate and degree of browning. Thus, it appears obvious that other formulation ingredients, including seemingly inert fillers and lubricants, can have an effect which could either accelerate or retard the browning process. Consequently, due consideration should be given to other components when using lactose in pharmaceutical formulations.

The discoloration of tablets containing amines and lactose was the subject of a report published by Castello and Mattocks (2). They concluded that liberation of free amine by basic lubricants in the formulation was a prerequisite to the darkening of such tablets. They reported no discoloration when neutral or acidic lubricants, like glyceryl monostearate or stearic acid, were used. However, the accelerated stability test period was only 2 days at 40° and 85% relative humidity. Also, they concluded that the brown material obtained from a lactose-amphetamine mixture was from lactose and did not contain the amine moiety. This was based on a negative nitrogen test on a tarry extract of the colored product. Observations on the use of lactose in the presence of various amines conflicted with some of the con-

clusions of the above authors and prompted this investigation.

The objective of this study was to investigate the browning reaction of lactose in the presence of added amines in an effort to differentiate between this and the browning in the absence of added amines. The three amines used in this investigation were amphetamine, methamphetamine, and dimethylamphetamine. These represent primary, secondary, and tertiary amines having otherwise identical structures and were selected in an attempt to correlate the relationship of amine structure to browning reactivity. The role of HMF in this amine reaction was investigated also since a correlation between this compound and the normal browning of spray-dried lactose was shown by Brownley and Lachman (3), and a relationship between HMF and the ultraviolet absorption spectrum of lactose was presented in the accompanying report (1). Another aspect of this work was to compare the browning reactivity of dextrose and galactose with the three amines mentioned above to ascertain the contribution by these sugars, which could be present initially as impurities, or formed subsequently by hydrolysis.

EXPERIMENTAL

The reaction of lactose with amphetamine sulfate (Miles Chemical Co.), methamphetamine hydrochloride (Abbott Laboratories), and dimethylamphetamine hydrochloride [prepared according to the procedure of Dirscherl *et al.* (4)] was studied in buffered aqueous solutions at pH 5 and 8 (citric acid-sodium hydrogen phosphate) and in water. These pH values were chosen since normal browning of lactose was minimal at pH 5 and accelerated at pH 8 (1). In view of the marked protection by borate species in the normal browning of lactose (1), the amine reaction was also studied at pH 8.0 in this buffer. Solutions containing 1% amine salt and 10% lactose were stored at 50°. The absorption spectra of suitable dilutions of these solutions were

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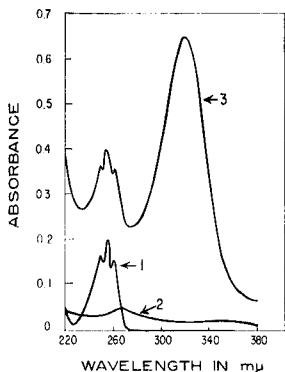


Fig. 1.—Lactose-amphetamine reaction in citrate-phosphate buffer (pH 8) heated for 7 days at 50°. Key: 1, amphetamine sulfate, 1%; 2, lactose U.S.P. spray-dried, 10%; 3, amphetamine sulfate, 1%, and lactose U.S.P. spray-dried, 10%. All solutions were diluted 2 to 100 with water.

determined at periodic intervals on a Beckman model DB spectrophotometer using 1-cm. cells and compared with the initial spectra for changes in absorption characteristics. The solutions were observed also for changes in physical appearance and pH; any resulting precipitates were isolated and studied using wet chemical tests, thin-layer chromatography, and infrared spectrophotometry.

Similar experiments were performed with amphetamine sulfate in the presence of dextrose and of galactose to determine the relative browning reactivity of the monosaccharide hydrolysis products of lactose.

A solution containing 0.05% HMF and 1% amphetamine sulfate also was studied in a similar manner to ascertain the significance of HMF in the lactose-amine reaction.

The lactose-amine reaction in tablets was investigated with amphetamine sulfate only, since the solution experiments had indicated that methamphetamine and dimethylamphetamine did not react with lactose. Tablets containing 10% amphetamine sulfate were prepared with spray-dried lactose U.S.P. and 2% of each of the following lubricants: magnesium stearate, stearic acid, glyceryl monostearate, two lots of talc U.S.P. (the aqueous slurry of one had a pH of 6.1 and the other 8.4), and sodium lauryl sulfate. These were stored at 50° and 90% relative humidity along with a lactose control and a lactose-amphetamine sulfate control containing no lubricant. The tablets were observed for changes in appearance, and the ultraviolet absorption spectra of suitable dilutions of filtered aqueous solutions were made and compared with the initial spectra of corresponding solutions. Thin-layer chromatographic experiments and solubility characteristics of the degraded tablets were used also to confirm reaction of lactose and amphetamine.

Since the solution experiments indicated that both galactose and dextrose solutions discolored more than lactose solutions in the presence of amphetamine, an experiment was devised to evaluate the contributory effect of small amounts of dextrose and/or galactose to the discoloration of amphetamine sulfate tablets. Tablets were prepared containing analytical reagent lactose and (a) 10% am-

phetamine sulfate, (b) 10% amphetamine sulfate and 0.5% dextrose, and (c) 10% amphetamine sulfate and 0.5% galactose. These tablets were observed for 1 month at 50° and 90% relative humidity.

Thin-layer chromatographic plates were made with a Brinkmann model S applicator using silica gel G (Brinkmann) of 0.2-mm. thickness as an adsorbant. The plates were allowed to dry at room temperature for 30 min. and activated at 100° for 2 hr. before application of the sample. Ascending chromatography, using the lower phase of a mixture of ethyl acetate, pyridine, and water in the ratio of 2:1:2, was employed. Reagents for detecting the chromatograms were iodine vapor, acidified 0.1 M potassium permanganate, 0.1% ninhydrin in ethyl alcohol, and aniline hydrogen phthalate.

DISCUSSION

Lactose-Amine Reaction.—Amphetamine, methamphetamine, and dimethylamphetamine have similar ultraviolet absorption spectra which could be used conveniently to study the reaction. The absorption spectra of solutions of lactose, amphetamine, and lactose-amphetamine in citrate-phosphate buffer (pH 8) stored for 1 week at 50° are presented in Fig. 1. A marked change in the absorbance curve was noted in the lactose-amphetamine solution. The intensity of absorbance at 257 mμ increased considerably, and a new peak appeared in the region of 320 mμ. Another marked difference was that the lactose-amphetamine solution became progressively darker with the appearance of a brownish-black precipitate. Different grades of lactose reacted with amphetamine sulfate in a similar manner. Reaction of lactose with amphetamine base also was similar, except that this solution discolored more rapidly. There were no changes in absorption characteristics of the three amine controls or the solutions of lactose-methamphetamine or lactose-dimethylamphetamine. No precipitation occurred with lactose-methamphetamine or lactose-

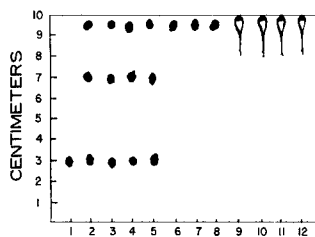


Fig. 2.—Composite thin-layer chromatogram of dextrose-, galactose-, and lactose-amphetamine reaction in citrate-phosphate buffer (pH 8) and in lactose-amphetamine tablets. Key: 1, amphetamine SO₄, 1%; 2, 3, and 4, filtrates of degraded solutions of amphetamine SO₄, 1%, with 10% dextrose, galactose, and lactose, respectively; 5, filtrate of an aqueous extract of degraded tablets of amphetamine sulfate, 1%, and lactose; 6, 7, and 8, 10% solutions of dextrose, galactose, and lactose, respectively; 9, 10, and 11, chloroform solutions of precipitates from solutions 2, 3, and 4, respectively; 12, chloroform extract of degraded tablets of amphetamine sulfate, 1%, and lactose. The spots were detected by a combination of three reagents used separately—iodine vapor, ninhydrin, and aniline hydrogen phthalate.

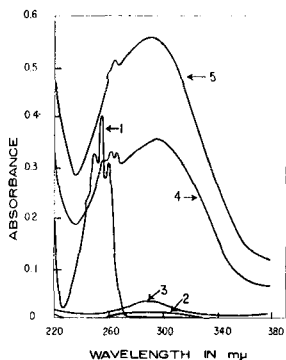


Fig. 3.—Dextrose-amphetamine and galactose-amphetamine reaction in citrate-phosphate buffer (pH 8) heated for 7 days at 50°. Key: 1, amphetamine sulfate, 1%; 2, dextrose, 10%; 3, galactose, 10%; 4, amphetamine sulfate, 1%, plus dextrose, 10%; 5, amphetamine sulfate, 1%, plus galactose, 10%. Solution No. 1 diluted 4 to 100 and all the others diluted 1 to 100 with water.

dimethylamphetamine. The lactose-methamphetamine solution did appear to have faintly more color than the control, but this was not detectable by absorption measurements. When the lactose-amphetamine solution was diluted with water, it was found that the absorbance at 320 $m\mu$ faded rapidly. This phenomenon, associated with this maximum at 320 $m\mu$, is consistent with a recent report concerning the absorption characteristics of Schiff bases (5). There was a decrease in pH from 8.0 to 7.65 in methamphetamine and dimethylamphetamine which was identical to the decrease in pH in the lactose control, whereas the pH decreased from 8.0 to 7.4 in the lactose-amphetamine sulfate solution. This liberation of hydrogen ions is characteristic of the formation of a Schiff base from an amine salt and a carbonyl compound. A solution containing 1% amphetamine sulfate and 10% sorbitol under similar conditions showed no discoloration or spectral absorbance changes. The preceding evidence supports the theory that the reaction was of a Schiff base type between the primary amine and the carbonyl group of the sugar (6, 7).

The solubility characteristics of the brown material from the lactose-amphetamine reaction were markedly different from the brown material formed in the normal browning of lactose (1). The former was soluble in chloroform, ethanol, acetone, ethyl acetate, and *n*-butanol and could be extracted easily from aqueous solutions, whereas the latter was very water soluble and could not be extracted with organic solvents.

The brownish-black precipitate isolated from the lactose-amphetamine reaction gave a positive sodium fusion test for nitrogen. This was in contradiction to the observation of Castello and Mattocks (2), who claimed that the reaction product isolated from a lactose-amphetamine solution did not contain nitrogen and that the amphetamine did not take part in the reaction. Thin-layer chromatographic data illustrated in Fig. 2 indicated the presence of two reaction products which were absent in the normal browning reaction, *i.e.*, the precipitate and a water-soluble substance. Similar experiments with solutions of lactose-methamphetamine and lactose-dimethylamphetamine indicated only one spot corresponding to the amine controls, further evidence that these two amines did not react with lactose.

From the foregoing discussion, evidence appears conclusive that amphetamine reacted with lactose, but methamphetamine and dimethylamphetamine did not. Further information indicated that the rate of this reaction and the resulting discoloration varied with various primary amines. This aspect of the lactose-amine reaction will be pursued subsequently.

Dextrose- and Galactose-Amine Reaction.—The reaction of dextrose and galactose with amphetamine in buffer (pH 8) was studied using procedures similar to those used for the lactose-amphetamine reaction. One-week data presented in Fig. 3 showed that the intensity of absorption at 257 $m\mu$ increased considerably for both dextrose and galactose and, as in the lactose-amphetamine reaction, a new peak appeared. However, the wavelength of maximum absorbance for both the monosaccharides was in the region of 290–296 $m\mu$, whereas the lactose-amphetamine peak emerged at 320 $m\mu$. In addition, fading of absorption on dilution with water was less pronounced with the monosaccharides than with lactose. Browning was apparent in 1 day with both monosaccharides and progressively darkened with the appearance of brownish-black precipitates, which had solubility characteristics similar to the lactose-amphetamine precipitate. The relative discoloration and amount of precipitation was galactose > dextrose > lactose. As in lactose-amphetamine, the precipitates from both the galactose- and dextrose-amphetamine reactions gave positive sodium fusion tests for nitrogen, an indication that the amine moiety was present in the reaction products. Similarly, thin-layer chromatographic experiments indicated the presence of a water-soluble reaction product in addition to the insoluble material (Fig. 2). The R_f values of the reaction products from the three sugars were similar in all solvent systems used, which may be due to their structural similarity.

The reaction of dextrose and galactose with meth-

TABLE I.—REACTIVITY OF METHAMPHETAMINE AND DIMETHYLAMPHETAMINE WITH DEXTROSE, GALACTOSE, AND HMF IN CITRATE-PHOSPHATE BUFFER, pH 8^a

	Dextrose, 10%			Galactose, 10%			HMF, 1%		
	Initial	A, 257 $m\mu$ ^b 4 Wk.	ΔA	Initial	A, 257 $m\mu$ ^b 4 Wk.	ΔA	Initial	A, 283 $m\mu$ ^c 4 Wk.	ΔA
Sugar and HMF controls	0.00	0.228	0.228	0.00	0.370	0.370	0.490	0.490	...
Methamphetamine HCl + controls	0.360 ^d	0.720	0.132 ^e	0.368 ^d	1.060	0.322 ^e	0.488	0.485	...
Dimethylamphetamine HCl + controls	0.353 ^d	0.595	0.014 ^e	0.358 ^d	0.836	0.108 ^e	0.500	0.490	...

^a Solutions stored at 50° for 4 weeks. ^b Diluted 2 to 50 ml. with water. ^c Diluted 1 to 2500 ml. with water. ^d There was no change in absorbance of the amine controls. ^e Corrected value after subtracting absorbance due to sugar controls.

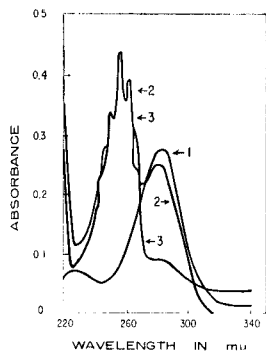


Fig. 4.—HMF-amphetamine reaction in citrate-phosphate buffer (pH 8) heated for 7 days at 50°. Key: 1, HMF, 0.05%; 2, HMF, 0.05%, plus amphetamine sulfate, 10%, initial; 3, solution No. 2 after 7 days at 50°. There was no change in the spectrum of HMF control after 1 week. All solutions were diluted 1 to 250 with water.

amphetamine and dimethylamphetamine was investigated in citrate-phosphate buffer at pH 8 in a manner similar to the reaction of these sugars with amphetamine sulfate. Four-week data, shown in Table I, indicate that galactose reacted slightly with methamphetamine and to a lesser extent with dimethylamphetamine. Dextrose reacted slightly with methamphetamine and did not react with dimethylamphetamine. It may be recalled that lactose did not react with either of these amines under similar conditions. This experiment suggests that if lactose contained appreciable amounts of the monosaccharides as impurities, it also could discolor in the presence of methamphetamine and dimethylamphetamine.

HMF-Amphetamine Reaction.—Figure 4 shows spectrophotometric data on the HMF-amphetamine sulfate reaction in citrate-phosphate buffer at pH 8. There were no changes in the absorption spectra of amphetamine sulfate and HMF controls after 1 week at 50°. In a mixture of these two, there was also no change in the absorption maximum at 257 $m\mu$ due to amphetamine; but the absorbance at 283 $m\mu$ due to HMF decreased from 0.256 to 0.090 after 1 week in solutions of comparable dilution. This could be due to the destruction of HMF by polymerization. A precipitate appeared, but the spectral absorbance curve of the solution did not resemble those obtained from the three sugar-amphetamine solutions, all of which showed the emergence of a new peak.

The infrared spectra of the precipitates from the lactose-, galactose-, dextrose-amphetamine solutions (10% sugar-1% amine) and the precipitate obtained from a solution containing 1% HMF and 1% amphetamine were determined in 5% chloroform solutions and 0.2-mm. cells on a Beckman IR-4 infrared spectrophotometer with sodium chloride optics. The three sugar reaction products yielded almost identical spectra, but differences were noted in the HMF-amphetamine spectrum (Fig. 5). The disappearance of the primary amine vibrations of amphetamine at 3335 cm^{-1} and 3400 cm^{-1} and the appearance of a band at 1666 cm^{-1} attributed to C=N stretch seemed to be further proof of the formation of a Schiff base type compound. The

bands at 1020 cm^{-1} and at about 1080 cm^{-1} found in the HMF-amphetamine spectrum were absent in the sugar-amphetamine spectrum. Both ultraviolet and infrared spectra indicated that amphetamine reacted with the sugar instead of with HMF or furfural type compounds which might be present initially as impurities or produced by degradation. No attempt was made to identify the precipitates on the basis of the infrared spectra.

From all evidence, it appeared that the reaction of amphetamine with lactose was similar in nature but could be distinguished from the reaction of amphetamine with both dextrose and galactose. Hydrolysis, therefore, is not a prerequisite to the amine browning reaction. However, since the sugar-amphetamine discoloration is in the order galactose > dextrose > lactose, the presence of these monosaccharides could make a marked contribution to browning. The presence and/or formation of HMF was shown to be an important factor in the normal browning of lactose (1, 3). However, the formation of HMF did not appear to be a prerequisite to the amine browning reaction.

Borate Protection of Sugar-Amine Reaction.—In view of the marked protection by borate ions in the normal browning of lactose, dextrose, and galactose (1), the reaction of these sugars with amphetamine was studied also in borate buffer (pH 8) at 50°. Spectral absorbance curves and visual observation indicated remarkable protection by the borate ion. No precipitation or discoloration was noticeable after 4 days; even after 7 days, only a faint tint was evident. Discoloration and precipitation became obvious after 24 hr. in the absence of borate ions. In addition, the spectral absorbance curve in the presence of borate ions showed no significant changes, like the increase in the absorbance at 257 $m\mu$ or the appearance of a new peak at 320 $m\mu$, which were observed in the absence of borate ions.

Reaction in Buffer, pH 5.—The reaction of lactose, dextrose, and galactose with amphetamine in citrate-phosphate buffer pH 5 at 50° was similar to that at pH 8, except that it was less extensive. No precipitate was formed even after 6 weeks at 50°, but the appearance of the characteristic new peaks described previously was evident after this interval. This result was expected, since the data on the nor-

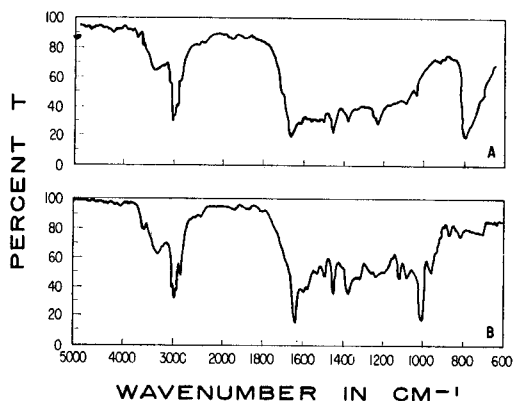


Fig. 5.—Infrared spectra of chloroform solutions of lactose-amphetamine and HMF-amphetamine reaction products. Key: A, lactose-amphetamine; B, HMF-amphetamine.

TABLE II.—DISCOLORATION OF AMPHETAMINE SULFATE-SPRAY-DRIED LACTOSE TABLETS IN THE PRESENCE OF COMMON LUBRICANTS^a

	Lubricant, 2%	Relative Degree of Browning ^d		
		1 Day	2 Days	5 Days
Lactose U.S.P. spray-dried	None	+1	+1	+2
Lactose + amphetamine SO ₄ , 10%	None	+2	+2	+5
Lactose + amphetamine SO ₄ , 10%	Talc A ^b	+2	+3	+4
Lactose + amphetamine SO ₄ , 10%	Talc B ^c	+3	+3	+4
Lactose + amphetamine SO ₄ , 10%	Stearic acid	+2	+3	+5
Lactose + amphetamine SO ₄ , 10%	Glyceryl monostearate	+2	+3	+3
Lactose + amphetamine SO ₄ , 10%	Magnesium stearate	+3	+4	+6
Lactose + amphetamine SO ₄ , 10%	Sodium lauryl sulfate	+3	+4	+5

^a Tablets stored at 50° and 90% relative humidity. ^b Acid-washed talc, aqueous slurry of which had a pH of 6.1. ^c Aqueous slurry had a pH of 8.4. ^d Browning intensity rated in comparison to room temperature controls, which were not discolored in all cases.

mal browning of lactose (1) showed a minimum reaction at pH 5.

Lactose-Amphetamine Reaction in Tablets.—Results obtained on the lactose-amphetamine reaction in tablets are summarized in Table II. All tablets were discolored more than the lactose control. Tablets containing amphetamine sulfate and lactose with no lubricant discolored more than the lactose control, indicating that liberation of free amine by basic lubricants was not a prerequisite to browning. This is further supported by the fact that discoloration took place in the presence of both acidic and neutral lubricants and also by the observation that tablets containing an acidic and a basic talc were discolored to the same extent. These results contradict the postulation of Castello and Mattocks (2), who claimed that liberation of free base was a prerequisite to discoloration.

Experiments with tablets of reagent grade lactose and amphetamine sulfate containing 0.5% each of added dextrose and galactose, respectively, and stored at 50° and 90% relative humidity indicated discoloration after 4 days. Control tablets which did not contain the monosaccharides were not discolored after 30 days. This suggests that if lactose contained monosaccharides as impurities, the browning would be more pronounced.

SUMMARY

1. The lactose-amphetamine reaction is predominantly a primary amine-carbonyl type. The reaction product, although similar in nature, was

shown to differ from that of amphetamine with dextrose, galactose, and HMF. Consequently, hydrolysis of lactose or formation of HMF does not appear to be a prerequisite to the lactose-amine reaction. However, the order of browning, galactose > dextrose > lactose, indicated that small amounts of these monosaccharides could contribute markedly to the discoloration occurring in the presence of amines.

2. Methamphetamine and dimethylamphetamine did not react with lactose or HMF. Galactose reacted slightly with both these amines, whereas dextrose reacted only with methamphetamine. Therefore, monosaccharides as impurities could contribute to browning, even with secondary and tertiary amines.

3. Accelerated stability studies with lactose-amphetamine sulfate tablets indicated that liberation of free amine by basic lubricants was not a prerequisite to browning.

4. Characteristic differences were shown to exist between the normal browning of lactose and browning occurring in the presence of amines.

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