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Stability of Tetracycline and Riboflavin

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Abstract □ The stability of tetracycline solutions in the presence of riboflavin, light, and air was investigated. Although it was found that antibiotic potency loss occurred under these conditions, the addition of ascorbic acid to the system prevented the oxidative tetracycline degradation. There does, however, appear to be a minor loss of tetracycline due to the ascorbic acid. This loss is covered by a normal product overage.

Keyphrases □ Tetracycline-riboflavin solution—stability □ Ascorbic acid effect—tetracycline-riboflavin solution stability □ Light effect—tetracycline-riboflavin solution stability □ Column chromatography—separation, analysis

In a study of the stability of antibiotic solutions containing vitamin B complex, Dony-Crotteux (1) reported that three of the tetracycline-group antibiotics (chlortetracycline, tetracycline, and oxytetracycline) lost significant potency within 2 to 4 hr. In addition, he demonstrated that the ingredient in vitamin B complex responsible for instability was riboflavin, and he proposed that degradation occurred *via* a reaction between the tetracycline and oxygen, in which riboflavin played the role of a photosensitizer. The component in light that induced antibiotic loss was reported to be UV radiation. When this work was referred to in a recent publication (2), a number of inquiries about its validity were received, since a combination of tetracycline and vitamin B complex is often administered by intravenous drip. Therefore, an investigation of this phenomenon was undertaken.

EXPERIMENTAL

The amount of tetracycline remaining in solution was evaluated by a column chromatographic technique.[1] A similar method, which is based on the authors' procedure, has been published recently (3). This method allows the simultaneous determination of tetracycline (I), anhydrotetracycline (II), and their corresponding C-4 epimers (III and IV). Studies were performed at pH 4.5 using MacIlvaine's buffer. All pH measurements were made on a meter[2] equipped with a glass and calomel electrode pair. The light source consisted of a 91.4 × 66.0 × 91.4 cm. (36 × 26 × 36 in.) light cabinet containing 12 × 30-w. and 2 × 20-w. fluorescent tubes. Lachman *et al.* (4) reported that fluorescent light produces a spectrum comparable to daylight, but somewhat higher in UV radiation. The temperature within the cabinet was 26.7°.

The aqueous solutions studied contained approximately 0.8 mg./ml. of tetracycline hydrochloride. Ascorbic acid, when present, was employed at a level of 2.5 mg./ml., and riboflavin concentrations varied from 0.01–10 mg./ml. Riboflavin was added to the solutions as the 5-phosphate ester, making necessary corrections for the difference in molecular weights. All solutions were put into 25-ml. ground-glass stoppered, clear, Pyrex glass graduates, and stored in the center of the light cabinet, equidistant from the side light sources.

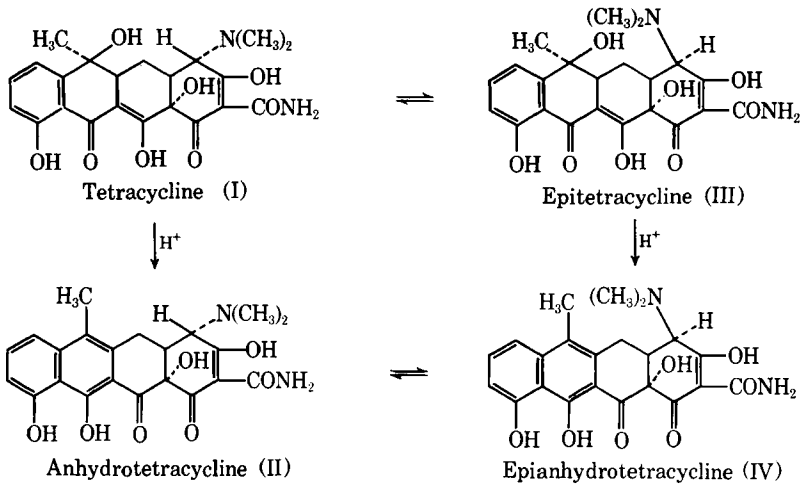
DISCUSSION

Although tetracyclines have played a prominent role in antibiotic therapy for over 15 years, information on their stability in pharmaceutical systems is somewhat meager. The degradation of tetracycline occurs by numerous pathways (5), the most common of which are shown in Scheme I.

In addition to these pathways, tetracycline also undergoes oxidation by reaction with atmospheric oxygen. This is a complex degradation scheme and most likely results in more than one product (6–8). It is this type of reaction to which Dony-Crotteux attributed the loss of antibiotic potency in the presence of riboflavin, light, and air. However, because his analytical results were only semiquantitative estimates of the remaining tetracycline content, he was unable to establish whether any of the degradation processes shown in Scheme I were also occurring. The column chromatographic technique employed in this investigation is quantitative for all of the compounds shown in Scheme I. It was found, however, that Compound III could not be determined quantitatively in the presence of riboflavin-5-phosphate due to the formation of either a riboflavin degradation product, hydrolysis of the phosphate ester linkage, or both. This unknown substance eluted on the column with Compound III, and had similar spectral absorption characteristics. Nevertheless, the possible influence of riboflavin on the conversion of I to III could be approximated by examination of the various systems investigated. In addition, prior to undertaking the study, it was established that none of the materials employed in the investigation significantly interfered with the determination of Compounds I, II, or IV.

Effect of Riboflavin—In an initial study, five aqueous solutions of tetracycline were prepared in pH 4.5 buffer, and to four of them riboflavin was added at a level of 0.01, 0.1, 1, and 10 mg./ml. The fifth solution was used as a control. All solutions were stored in the light cabinet and assayed at various times over a 24-hr. period. The results, listed in Table I, are in agreement with the observations of Dony-Crotteux on the stability of tetracycline in the

[1] The authors are indebted to Mr. Michael O'Dowd for his aid in performing the chromatographic assays reported herein.
[2] Beckman model G.



Scheme I—Some degradation pathways of tetracycline.

Table I—Stability of Tetracycline at Various Concentrations of Riboflavin in the Presence of Light and Air

Hours	Tetracycline Content (% of Initial) Riboflavin Level, mg./ml. 0	0.01	0.1	1	10
1	97.6	92.5	84.1	89.2	95.2
2	94.0	88.3	79.9	84.2	86.4
4	88.7	83.0	69.4	72.0	78.2
6	86.4	77.8	62.9	61.3	70.3
8	85.9	77.5	60.7	53.2	65.8
12	78.0	68.9	47.9	44.9	56.2
24	72.5	65.8	32.4	26.0	40.6

presence of riboflavin, air, and light. In addition, neither anhydro derivative (Compounds II or IV) was formed during the course of this nor any succeeding experiments. Therefore it appears that none of the antibiotic potency loss reported by Dony-Crotteux can be attributed to these degradation pathways (*i.e.*, I → II ⇌ IV, or I ⇌ III → IV ⇌ II). This does not exclude the reversible conversion of tetracycline to its C-4 epimer (I ⇌ III), which in fact is the reaction that accounts for antibiotic potency loss in the control solution. Evidence of this was that the sum of I and III remained constant during the course of the study. Thus, it is possible that the loss of tetracycline potency in solutions containing riboflavin represents a summation of the loss *via* epimerization and oxidation. In addition, it is uncertain whether Compound III itself undergoes oxidation. Therefore, since the degradation pathway is not simple, the authors were unable to express tetracycline loss in terms of a rate constant. An examination of the data, however, readily indicates that antibiotic loss rate increased with increasing riboflavin content to a maximum value at a riboflavin level of 0.1—1.0 mg./ml., and then decreased at the 10-mg./ml. concentration. The presence of a maximum was also reported by Dony-Crotteux. He attributed it to the fact that, at higher concentrations, the large number of riboflavin molecules at the surface of the container absorb most of the light, preventing its penetration into the bulk of the liquid.

Table II—Stability of Tetracycline and Riboflavin at 37° and pH 4.5 in the Absence of Light

Hours	Tetracycline Content (% of Initial) Riboflavin Content, mg./ml. 0	1	10
1	93.3	96.2	90.8
2	86.2	86.4	85.5
4	78.2	81.2	79.3
6	77.3	78.4	75.9
12	69.4	70.1	71.3
24	70.3	67.2	67.9

Table III — Stability of Tetracycline (TC) and Riboflavin (RF) at pH 4.5 in the Presence of Ascorbic Acid (AA)

Hr.	TC Content (% of Initial) TC Control	TC and AA	TC, RF (0.1 mg./ml.) and AA	TC, RF (1 mg./ml.) and AA	TC and RF (1 mg./ml.)
1	97.1	98.8	96.7	98.0	88.9
2	94.1	91.6	92.3	91.6	81.4
4	89.5	87.7	86.2	88.1	70.8
6	87.2	81.8	82.5	85.1	61.7
8	84.9	77.4	76.8	75.7	55.1
12	78.1	70.3	69.1	67.3	44.6
24	72.0	63.9	60.0	58.2	28.6

The importance of light on the reaction was demonstrated when samples were studied at an accelerated temperature (37°) in the absence of light. The data in Table II indicate that without light there is no difference in degradation rates between solutions with and without riboflavin.

Effect of Ascorbic Acid—In order to test the theory that antioxidants prevent tetracycline from degrading in the presence of riboflavin, light, and air, a study was performed in which ascorbic acid was incorporated into the system. Although a number of compounds are employed as antioxidants in pharmaceuticals (9), ascorbic acid was selected because it is both a solubilizer in parenteral tetracycline formulations,[3] and a commonly used antioxidant. The results of this study (Table III) make it apparent that ascorbic acid prevents the degradation of tetracycline promoted by riboflavin. An examination of the data for the tetracycline control solution, as well as all solutions containing ascorbic acid, indicates that, within experimental error, antibiotic potency loss rate is the same during the first 4 hr. Since both the tetracycline control and tetracycline plus ascorbic acid solutions degraded only *via* epimerization during this time, one may conclude that the system containing both ascorbic acid and riboflavin lost tetracycline potency because of the same reaction. This result implies that, in solutions with riboflavin where tetracycline is degrading *via* oxidation, it is also converting reversibly to the C-4 epimer. In addition, the rate constants for the forward and reverse reactions do not appear to be markedly influenced by the presence of riboflavin.

The data in Table III demonstrate that, after 4 hr., the solutions containing ascorbic acid degraded more rapidly than the control.

[3] The tetracycline HCl and ascorbic acid product is marketed by Lederle Laboratories Division, American Cyanamid Company, under the designation Achromycin tetracycline HCl intravenous.

Table IV—Material Balances of Tetracycline and its C-4 Epimer in Solution

Hours	Tetracycline Plus C-4 Epimer (% of Initial) TC Control	TC and Ascorbic Acid
1	99.6	101
2	99.3	98.2
4	99.8	99.6
6	101	98.3
8	101	94.3
12	98.2	92.0
24	97.1	87.1

Since antibiotic potency loss in the control solution proceeds *via* epimerization, it is uncertain whether the additional loss rates in ascorbic acid solutions represent an increased epimerization rate due to ascorbate, or another degradation pathway catalyzed by ascorbate. The latter hypothesis appears to be favored by the data in Table IV, which represent the summation of Compounds I and III for the tetracycline control and tetracycline with ascorbic acid solution. For the control solution, it is questionable whether even the 24-hr. data represent significant loss of total material, whereas after 6 hr., the comparable data for the ascorbic acid solution demonstrate a distinct downward trend.

Effect of Buffer—Although the data in Table IV prove that antibiotic potency loss occurs by epimerization in the control solution, the loss rate appears somewhat high for an intravenous product. It was believed, however, that this rapid rate represented catalysis by the citrate buffer (10–12). To test this hypothesis, studies were performed in nonbuffered solutions in which the pH value was simply adjusted to 4.5 with base. The results of this experiment (Table V) dramatically demonstrate the influence of citrate buffer on tetracycline stability, since the tetracycline control solution lost no potency during the 24-hr. period of the study. In addition, the loss of antibiotic potency in the system with ascorbic acid indicates that the previously suggested hypothesis of ascorbate-catalyzed degradation is probably correct.

SUMMARY AND CONCLUSIONS

The data presented in this report support the observations of Dony-Crotteux that aqueous tetracycline solutions containing riboflavin degrade in the presence of air and light. In addition, suppression of degradation by the antioxidant ascorbic acid is good evidence for his contention that the reaction is oxidative in nature. It was demonstrated, however, that although none of the degradation reported by Dony-Crotteux was due to the formation of anhydrotetracyclines, some of the potency loss he encountered resulted from epimerization catalyzed by the buffer system. This investigation demonstrates that, in the presence of light and air, riboflavin may be combined with tetracycline if ascorbic acid is included in the system. Therefore, parenteral tetracycline formulations containing ascorbic acid may be mixed with riboflavin for intravenous administration without concern for antibiotic stability. Althouth ascorbic acid itself appears to induce another degradation pathway, this loss is adequately covered by a normal product overage.

Table V—Stability of Tetracycline at pH 4.5 in Nonbuffered Systems

Hours	Tetracycline Content (% of Initial) TC in Water	TC + Ascorbic Acid in Water	TC + Riboflavin in Water
1	103	101	78.8
2	101	100	69.7
4	101	98.6	59.4
6	100	92.9	52.5
8	104	90.2	48.2
12	100	84.4	36.7
24	102	70.9	30.0

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