action of warfarin. Both of these effects seem to be due primarily to a change in the distribution of warfarin in the body.

REFERENCES

(1) G. Levy, R. A. O'Reilly, P. M. Aggeler, and G. M. Keech, *Clin. Pharmacol. Ther.*, **11**, 372(1970).

(2) R. A. O'Reilly, G. Levy, and G. M. Keech, *ibid.*, **11**, 378(1970).

(3) P. M. Aggeler and R. A. O'Reilly, *J. Lab. Clin. Med.*, **74**, 229(1969).

(4) R. A. O'Reilly and P. M. Aggeler, *Proc. Soc. Exp. Biol. Med.*, **128**, 1080(1968).

(5) R. Nagashima, R. A. O'Reilly, and G. Levy, *Clin. Pharmacol. Ther.*, **10**, 22(1969).

(6) P. M. Aggeler, R. A. O'Reilly, L. S. Leong, and P. E. Kowitz, *New Engl. J. Med.*, **276**, 496(1967).

(7) A. H. Conney, *Pharmacol. Rev.*, **19**, 317(1967).

(8) J. J. Burns, R. K. Rose, T. Chenkin, A. Goldman, A. Schulert, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **109**, 346 (1953).

(9) G. Levy and R. Nagashima, *J. Pharm. Sci.*, **58**, 1001(1969).

(10) G. Levy, *Proc. Int. Pharmacol. Congr., 4th*, **1970**, Vol. IV, 134.

(11) E. S. Vesell and J. G. Page, *J. Clin. Invest.*, **47**, 2657(1968).

(12) R. A. O'Reilly, *ibid.*, **48**, 193(1969).

(13) R. A. O'Reilly and G. Levy, to be published.

(14) M. C. Meyer and D. E. Guttman, *J. Pharm. Sci.*, **57**, 895 (1968).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 28, 1969, from the *Department of Medicine, Santa Clara Valley Medical Center and the Institute for Medical Research of Santa Clara County, and the †Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214

Accepted for publication April 30, 1970.

Supported in part by Grant HE 8058 from the U. S. Public Health Service.

The technical assistance of Geraldine M. Keech is gratefully acknowledged.

Diffuse Reflectance Studies of Solid–Solid Interactions

JOHN L. LACH and LYLE D. BIGHLEY*

Abstract □ Tetracycline and its derivatives, bishydroxycoumarin, and methantheline bromide have been studied by diffuse reflectance spectroscopy for possible solid–solid interactions with various metallic adjuvants. Examination of the spectra of some of these drug–adjuvant systems show rather small spectral changes, while other spectra show large bathochromic and hyperchromic changes, new band formation, and visual color changes which are indicative of charge–transfer interactions. Although these interactions vary in variety and intensity, they may significantly alter the availability and activity of the medicinal agent in pharmaceutical dosage forms.

Keyphrases □ Solid–solid interaction—determination □ Diffuse reflectance spectroscopy—solid–solid interaction analysis □ Tetracyclines, methantheline bromide, bishydroxycoumarin—metal-ion interactions □ Metal ions–tetracyclines, –methantheline bromide, –bishydroxycoumarin—interactions

Many articles in the pharmaceutical and medical literature deal with the problem of physiologically inactive tablet and capsule formulations. Lach and Bornstein (1), by the use of diffuse reflectance spectroscopy (DRS), have postulated that the apparent inactivity may be due in part to adsorption of the active principle onto an inert adjuvant. They have shown that solid–solid interactions do indeed exist between various chemical entities and a wide variety of adjuvants commonly found in pharmaceutical dosage forms. Although some of the interactions may be of the weak variety, they may, nevertheless, be sufficient to alter the absorption and availability of the medicinal agent.

The study of solid–solid interactions is of interest because of the effects that adjuvants may exhibit when incorporated into pharmaceutical dosage forms. These effects may include: (*a*) changes in the nature and intensity of biological activity caused by complexation with the active ingredient (2); (*b*) modifications of the physical state, particle size, and/or surface area of the drug available to the absorption sites (3); or (*c*) changes in the stability of the active principle (4–7).

The purposes of this study were to continue the investigation of solid–solid interactions of a number of drugs and to gain a better insight into the nature of these interactions.

EXPERIMENTAL

Reagents—Oxytetracycline hydrochlorde,[1] tetracycline hydrochloride,[2] chlortetracycline hydrochloride,[2] demethylchlortetracycline hydrochloride,[2] methantheline bromide,[3] bishydroxycoumarin,[4] magnesium trisilicate, ferric phosphate, aluminum hydroxide, tribasic calcium phosphate, and talc were used.

Apparatus—The following were used: Beckman model DU spectrophotometer with a diffuse reflectance attachment (1), constant-temperature water bath with rotating spindles, 150-ml. amber vials with caps, Parafilm,[5] and a glass desiccator with anhydrous calcium sulfate.[6]

Procedure—*Preparation of the Tetracyclines*—Thirty milligrams of active ingredient (tetracycline HCl, oxytetracycline HCl, chlortetracycline HCl, and demethylchlortetracycline HCl) was weighed for every 2 g. of adsorbent (pharmaceutical adjuvant) used. The powders were placed in 150-ml. amber vials, and 25 ml. of distilled water was added as the dispersion medium. The vial was then covered with Parafilm and capped. Equilibration was allowed to proceed for 2 hr. at 30 ± 0.5° to effect interaction. After equilibration, the suspension was filtered under vacuum and the powder dried

[1] Pfizer and Co.
[2] Lederle and Co.
[3] Searle and Co.
[4] K and K Laboratories.
[5] Marathon Co.
[6] Drierite, Hammond Co.

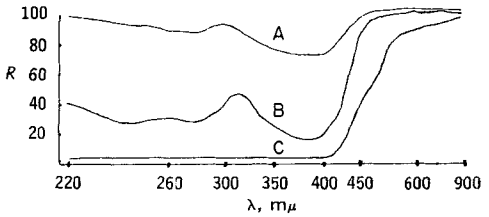

Figure 1—*DRS of chlortetracycline HCl (30 mg.) and magnesium trisilicate (2.0 g.). Key: A, control; B, equilibrated sample; and C, pure chlortetracycline HCl with no adjuvant present.*

in vacuo over anhydrous calcium sulfate. The DRS spectra of the samples were then determined, using magnesium carbonate as the standard.

Preparation of Methantheline Bromide and Bishydroxycoumarin—Sixty milligrams of active ingredient was weighed for every 2 g. of adsorbent. The powders were placed in 150-ml. vials, 10 ml. of distilled water was added to each vial, and equilibration was allowed to proceed as described previously. After equilibration, the dispersion medium was removed by drying *in vacuo* over anhydrous calcium sulfate. The DRS spectra of the samples were determined, using magnesium carbonate as the reference standard.

Preparation of the Control—The control for each experiment was prepared by physically mixing 2 g. of the adsorbent with the indicated amount of dried active ingredient, using a mortar and pestle for the trituration. The DRS of the control was then determined, using magnesium carbonate as the reference standard.

RESULTS AND DISCUSSION

Tetracycline–Adjuvant Interactions—Throughout the following discussion of the different drug–adjuvant systems, a qualitative interpretation of the results will be given because the theory and mechanism of solid–solid interactions are not fully understood at present. However, the authors feel a qualitative interpretation is of value in view of the fact that drug–adjuvant interactions do occur in the solid state. Furthermore, it is important to recognize, prior to dosage formulation, that such surface interactions may occur.

Since Lach and Bornstein (1) showed that oxytetracycline undergoes solid–solid interaction with various adjuvants, since cobalt and nickel complexes of tetracycline and two of its analogs were prepared and their spectral properties studied (8), and because the metal chelation aspects of tetracycline complexes have been investigated extensively in solution (9–13), a DRS study of the various tetracycline–metallic-containing excipients was undertaken. The objective was to determine whether significant spectral differences existed between the different derivatives and whether DRS changes observed in these antibiotic–excipient interactions could be correlated with respect to reported formation constants (9).

Figure 1, which shows the DRS of chlortetracycline hydrochloride and magnesium trisilicate, is an example of the interaction exhibited by tetracycline and its derivatives with this adjuvant. Examination of the figure shows that Spectrum B, which represents the equilibrated sample, has undergone a significant change from that of the physical mixture of the two components (A) and also from the spectrum of the pure drug (C). In addition to these spectral differences, other evidence that an interaction had taken place was noted by a change of color from white or faintly yellow in the physical mixture to a straw yellow in the equilibrated sample. The spectral and color changes occurred with all four tetracycline derivatives. It should be noted here that only in the tetracycline series were the equilibrated samples filtered first and then vacuum dried. Removal of the soluble tetracycline species from solution by the excipient under study further indicates the strength of the interaction.

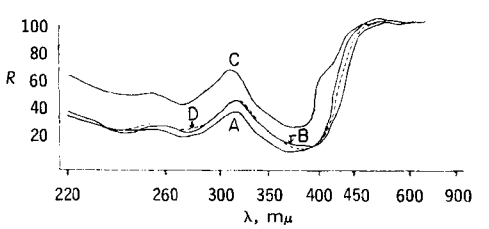

Figure 2—*DRS composite of equilibrated tetracyclines (30 mg.) and magnesium trisilicate (2.0 g.). Key: A, tetracycline HCl; B, chlortetracycline HCl; C, oxytetracycline HCl; and D, demethylchlortetracycline HCl.*

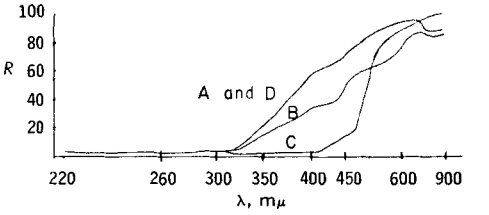

Figure 3—*DRS of tetracycline HCl (30 mg.) and ferric phosphate (2.0 g.). Key: A, control, B, equilibrated sample; C, pure tetracycline HCl with no adjuvant present; and D, pure ferric phosphate with no drug present.*

The equilibrated samples of the tetracycline–magnesium trisilicate series all show a bathochromic shift between 450 and 400 mμ and decreased reflectance throughout the visible and UV region. New band formation is seen at approximately 315 mμ in each case. This band may be considered to be the result of an interaction of the adsorbent with the tetracyclines where the adsorbent facilitates the clarification of an existing peak in this region, or it might represent the reflectance spectrum of a film of drug adsorbed onto the surface of magnesium trisilicate. The suggestion that this peak may be a clarification of an already existing peak can be seen by examining the transmittance spectra of the pure compounds in basic media (1). However, the reflectance spectra of the pure compounds (Spectrum C) do not show a maximum at this wavelength.

It appears that chemical rather than physical interactions have occurred since the spectral changes associated with physical adsorption are usually in the order of 5–10 mμ, while the maximum changes observed here approach 40–45 mμ. This, together with color formation, strongly suggests chemisorption.

Figure 2 shows the composite spectra of the various equilibrated tetracycline–magnesium trisilicate systems. With the exception of small intensity differences and small changes in the bathochromic shifts, the spectra of the equilibrated samples are very similar.

The spectra in Fig. 3 show the tetracycline hydrochloride–ferric phosphate system, which is typical of the spectra of the tetracycline derivatives with this adjuvant. Visual evidence of an interaction was again manifested by color changes. The physical mixtures were faint yellow while the equilibrated samples were tan. An examination of the spectra of the equilibrated samples in the 700–400-mμ region, as compared to the physical mixtures, shows bathochromic shifts of the order of 130 mμ and large intensity changes. Both of these changes are strongly indicative of chemisorption.

Although no differences were observed between the control and equilibrated samples over much of the UV region because of complete absorption by ferric phosphate, it is not unreasonable to assume that spectral changes would also be observed in this region if these samples were diluted with a nonabsorbing material.

Figure 4 shows the spectra of demethylchlortetracycline hydrochloride–aluminum hydroxide. These spectra are again typical of the interaction of the tetracycline series with this adjuvant. The spectra of the equilibrated samples (B) are considerably different from those of the physical mixtures (A). While the physical mixtures showed only limited absorption from approximately 450–350 mμ,

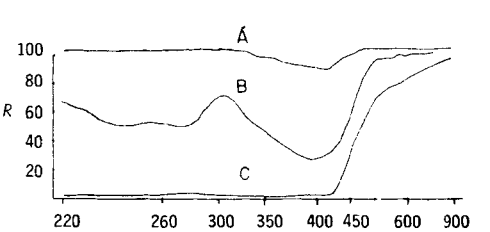

Figure 4—*DRS of demethylchlortetracycline HCl (30 mg.) and aluminum hydroxide (2.0 g.). Key: A, control; B, equilibrated sample; and C, pure demethylchlortetracycline HCl with no adjuvant present.*

Table I—Formation Constants and DRS Bathochromic Shifts of Various Tetracycline–Metal Systems

Tetracycline Derivative	log K' (1:1 Complex)	log K'' (2:1 Complex)	log Ks (Overall)	DRS Batho-chromic Shift, mμ
Aluminum Ion				
Tetracycline	7.4	6.4	19.2	98.0
Chlortetracycline	7.2	ppt.	18.0	87.5
Oxytetracycline	7.0	ppt.	18.0	98.0
Ferric Ion				
Tetracycline	9.9	8.6	25.3	130
Chlortetracycline	9.4	7.2	22.2	125
Oxytetracycline	9.6	7.2	22.5	140
Magnesium Ion				
Tetracycline	—	—	—	57
Chlortetracycline	—	—	—	44
Oxytetracycline	3.8	—	—	45

the equilibrated samples exhibited significant absorption from 500–220 mμ.

The drug concentration of 30 mg. per 2 g. of adjuvant was maintained here, since Lach and Bornstein (1) found that at this concentration the reflectance spectrum presumably represents the chemisorbed single layer. They found that when the drug–adjuvant ratio was increased, the bands in the reflectance spectrum broadened with a limit approaching the spectrum of the pure tetracyclines.

In addition to exhibiting absorption over a wider wavelength range than the physical mixtures, the equilibrated samples showed a bathochromic shift of approximately 80 mμ and a color change from white or faint yellow in the physical mixtures to an intense yellow. These spectral and visual changes are indicative of a chemical interaction of the drug molecules with the available active surface sites.

The large bathochromic shifts observed in these DRS studies of tetracycline–metallic adjuvant systems have also been reported to occur in solution spectra in the presence of metallic ions. Conover (10) has stated that there is a large bathochromic shift of the characteristic long wavelength UV absorption of solutions of tetracycline and its derivatives in an excess of many bivalent and tervalent metallic ions. He reports that these solution band displacements vary quantitatively with various metals, but qualitatively the effects are comparable. It should be pointed out that the observed bathochromic shifts and the nature of the DRS spectra also vary with various metallic adjuvants. However, the spectra of the various tetracycline derivatives for any given metal-containing adjuvant are comparable, which is reasonable and expected.

The bathochromic changes observed in the DRS spectra of these antibiotic–metallic adjuvant interactions were compared with reported formation constants (9) for the metal complexes of the tetracyclines (Table I). Since the present studies deal with surface interactions for which the stoichiometry of the interaction spectrum cannot be determined, a comparison was made between the bathochromic shifts observed in these interactions and log Ks values. Ks has been defined by Albert and Rees (9) as the product of all the partial constants. The bathochromic shifts were determined at a reflectance value between 700 and 380 mμ which exhibited the maximum change.

A comparison of the magnitude of the bathochromic shifts and the changes in the formation constants indicates some correlation,

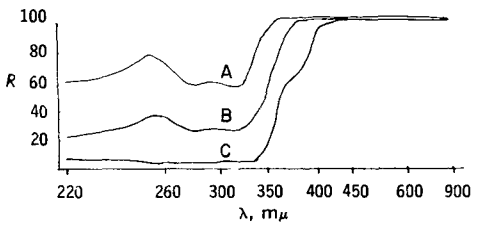

Figure 5—*DRS of bishydroxycoumarin (60 mg.) and magnesium trisilicate (2.0 g.). Key: A, control; B, equilibrated sample; and C, pure bishydroxycoumarin with no adjuvant present.*

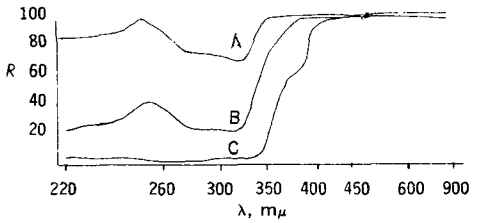

Figure 6—*DRS of bishydroxycoumarin (60 mg.) and aluminum hydroxide (2.0 g.). Key: A, control; B, equilibrated sample; and C, pure bishydroxycoumarin with no adjuvant present.*

because the ferric-containing adjuvant exhibits a larger bathochromic shift than the aluminum-containing adjuvant which, in turn, is larger than the magnesium-containing adjuvant. This is in agreement with the formation constants reported for these ions, since the value for the ferric ion is larger than the aluminum ion, which is larger than the magnesium ion. Although it is interesting that the observed DRS changes do parallel the reported formation constant values for these ions, it is difficult at this time to make a quantitative comparison.

Studies were also conducted to determine whether these drug–adjuvant interactions occurred when physical mixtures were exposed to humid conditions. Dried, physically mixed samples of the tetracyclines and magnesium trisilicate were placed on evaporating dishes in a moisture chamber, which consisted of a wax-sealed desiccator in which the desiccant had been replaced by water. The samples were allowed to stand in this moisture chamber for 4 hr., after which they were removed and dried *in vacuo* over anhydrous calcium sulfate. The spectra of these compounds were determined and found to resemble those shown in Fig. 1. The primary differences observed were that the bathochromic and intensity changes were not as pronounced, nor was the peak at 315 mμ as clearly defined. Possibly the spectral differences between water-equilibrated samples and samples exposed to humid conditions reflect an incomplete interaction such as an interaction of only the surface molecules. It is reasonable to assume that with the equilibration technique, a greater degree of interaction may occur with more pronounced spectral changes, because more surface area is exposed during the procedure.

Interaction of Methantheline Bromide and Bishydroxycoumarin with Various Metallic Adjuvants—The study of bishydroxycoumarin and methantheline bromide for possible drug–adjuvant interactions was prompted by reported therapeutic discrepancies of solid dosage forms of bishydroxycoumarin and certain quaternary compounds (14, 15).

The adjuvants chosen for this study were selected on the basis of the metal fraction which they contain and/or because of their usage in pharmaceutical formulations. Sixty milligrams of active principle was mixed with 2 g. of adjuvant, since studies with 30 mg. of active compound showed no absorbance in either the physical mixtures or the equilibrated samples.

Spectral data presented in Fig. 5 show the bishydroxycoumarin–magnesium trisilicate system. These spectra, as well as the interaction of bishydroxycoumarin with aluminum hydroxide (Fig. 6) and talc (Fig. 7), are representative of a chemisorption interaction. The spectrum of the equilibrated bishydroxycoumarin–tribasic calcium phosphate system, however, is unchanged from the spectrum of the physical mixture.

The equilibrated magnesium trisilicate (Fig. 5), aluminum hydroxide (Fig. 6), and talc (Fig. 7) systems all show a rather large bathochromic shift between 375 and 310 mμ, as well as a hyper-

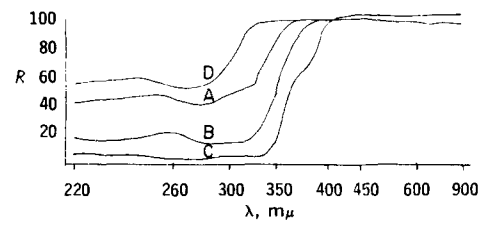

Figure 7—*DRS of bishydroxycoumarin (60 mg.) and talc (2.0 g.). Key: A, control; B, equilibrated sample; C, pure bishydroxycoumarin with no adjuvant present; and D, pure talc with no drug present.*

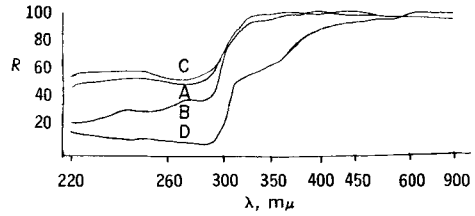

Figure 8—*DRS of methantheline bromide (60 mg.) and talc (2.0 g.). Key: A, control; B, equilibrated sample; C, pure talc with no drug present; and D, pure methantheline bromide with no adjuvant present.*

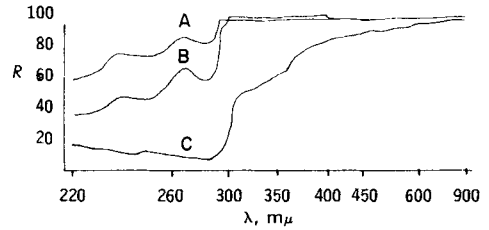

Figure 9—*DRS of methantheline bromide (60 mg.) and tribasic calcium phosphate (2.0 g.). Key: A, control; B, equilibrated sample; and C, pure methantheline bromide with no adjuvant present.*

chromic change throughout the UV region. In the magnesium trisilicate and talc systems, there is a smaller bathochromic change involving a band at 250 mμ, which has shifted to 255 mμ in the equilibrated samples. Since this particular band shift in the bishydroxycoumarin systems occurs only with the magnesium-containing adjuvants, it may indicate chelation of bishydroxycoumarin with magnesium.

Studies involving methantheline bromide and talc (Fig. 8), tribasic calcium phosphate (Fig. 9), and aluminum hydroxide (Fig. 10) showed small bathochromic shifts but rather large intensity changes. However, the interaction with magnesium trisilicate (Fig. 11) appears to be indicative of chemisorption. As in the bishydroxycoumarin–adjuvant systems, no color changes were observed when the samples were equilibrated.

Although it is evident from the spectral data presented that these interactions will vary in intensity, the spectral differences have not been correlated with bioavailability. Desorption and bioavailability experiments of these drug–excipient interactions are currently underway to gain a better understanding of the significance of such interactions.

The chemical properties of the various adjuvants may also influence the spectral changes observed with the methantheline bromide and bishydroxycoumarin systems. The availability of the metal portion in various adjuvants may differ since some surfaces are thought to be more saturated than others. The valency requirements of surface atoms may be more fully satisfied by bonding with nearby atoms, thus making them less available for chemisorption. Nor can hydration effects be overlooked, because hydration may also satisfy the valency requirements of the metallic portion and thereby decrease the number of active sites available for interaction.

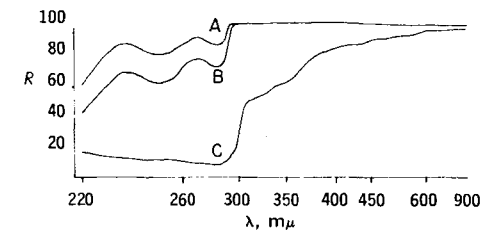

Figure 10—*DRS of methantheline bromide (60 mg.) and aluminum hydroxide (2.0 g.). Key: A, control; B, equilibrated sample; and C, pure methantheline bromide with no adjuvant present.*

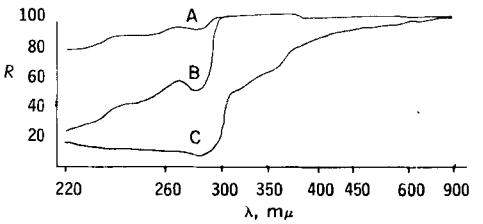

Figure 11—*DRS of methantheline bromide (60 mg.) and magnesium trisilicate (2.0 g.). Key: A, control; B, equilibrated sample; and C, pure methantheline bromide with no adjuvant present.*

A possible explanation for the bishydroxycoumarin–adjuvant interactions may be one of chelation. Further studies are in progress with respect to this hypothesis, and subsequent publications will deal with the mechanism of these interactions.

The therapeutic differences observed in presumably equivalent dosage forms may be due, in part, to surface interactions of the type described in this study. These interactions may exert an effect on the dissolution rate, as preliminary studies currently underway have indicated, and subsequently reduce the gastrointestinal absorption rates.

While it is recognized that drug release from pharmaceutical dosage forms is a complex phenomenon, the fact that surface interactions occur with various inactive adjuvants makes it imperative that the effect of these interactions on drug release be studied. Perhaps a more scientific approach to formulation through a knowledge of drug–adjuvant interactions could prevent many of the discrepancies observed in the therapeutic efficacy of pharmaceutical dosage forms.

REFERENCES

(1) J. L. Lach and M. Bornstein, *J. Pharm. Sci.*, **54,** 1730(1965).

(2) G. Levy and R. H. Reuning, *ibid.*, **53,** 1471(1964).

(3) E. H. Buckwalter and H. L. Dickison, *J. Amer. Pharm. Ass., Sci. Ed.*, **47,** 661(1958).

(4) E. G. Remmers, W. C. Barringer, G. M. Sieger, and A. P. Doerschuk, *J. Pharm. Sci.*, **53,** 1534(1964).

(5) E. G. Remmers, G. M. Sieger, A. P. Doerschuk, L. Ritter, and J. Weidenheimer, *ibid.*, **51,** 86(1962).

(6) E. G. Remmers, W. C. Barringer, G. M. Sieger, N. Anagnostakos, J. J. Corbett, and A. P. Doerschuk, *ibid.*, **54,** 49(1965).

(7) E. G. Remmers, W. C. Barringer, G. M. Sieger, and A. P. Doerschuk, *ibid.*, **53,** 1452(1964).

(8) W. A. Baker, Jr., and Patrick M. Brown, *J. Amer. Chem. Soc.*, **88,** 1314(1966).

(9) A. Albert and C. W. Rees, *Nature*, **177,** 433(1956).

(10) L. H. Conover, "Symposium on Antibiotics and Mould Metabolites," The Chemical Society, Burlington House, London, England, 1956.

(11) A. Albert, *Nature*, **172,** 201(1953).

(12) J. T. Doluisio and A. N. Martin, *J. Med. Chem.*, **6,** 16 (1963).

(13) *Ibid.*, **6,** 20(1963).

(14) E. Lozinski, *Can. Med. Ass. J.*, **83,** 177(1960).

(15) N. A. Batuyios and E. A. Brecht, *J. Amer. Pharm. Ass., Sci. Ed.*, **46,** 524(1957).

ACKNOWLEDGMENTS AND ADDRESSES

Received January 21, 1970, from the *College of Pharmacy, University of Iowa, Iowa City, IA 52240*

Accepted for publication April 6, 1970.

Abstracted in part from a dissertation presented by Lyle D. Bighley to the Graduate College, University of Iowa, in partial fulfillment of Doctor of Philosophy degree requirements.

* Fellow, American Foundation for Pharmaceutical Education. Henry S. Wellcome Memorial Fellow, 1965–1966.