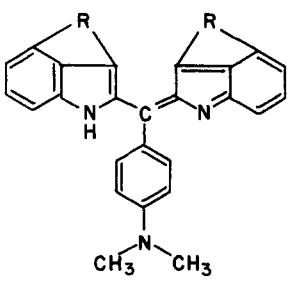
Nature of the Interference of Benzhydrol in the Colorimetric Assay of Ergot Alkaloids

By W. N. FRENCH

The reaction of skatole (3-methylindole) with *p*-dimethylaminobenzaldehyde under acidic conditions leads to the formation of a violet 1:1 condensation product (III) and a colorless 2:1 condensation product (II). Mild oxidation of the latter product gives an intensely absorbing blue compound (IV). Skatole reacts with benzhydrol to give the *N*-substituted and α-substituted condensation products (V and VI). Analogous reactions occur during the colorimetric assay of ergot alkaloids. The interference of benzhydrol in the colorimetric assay of the indole-type compounds appears to be the result of irreversible reaction of the indole nucleus with benzhydrol taking place simultaneously and in competition with the reaction of the indole nucleus with *p*-dimethylaminobenzaldehyde, thus preventing complete conversion of the indole compound to the color-producing species.

THE PRESENCE of benzhydrol in preparations of ergotamine has been observed to interfere in the colorimetric assay of this ergot alkaloid, and the extent of suppression of the color has been suggested as a direct measure of the amount of benzhydrol present (1, 2). This study was undertaken to gain insight into the nature of this interference and further knowledge of the reactions occurring in the colorimetric assay of ergot alkaloids.

The commonly used colorimetric assay procedure involves reaction of the ergot alkaloid with *p*-dimethylaminobenzaldehyde (*p*-DMAB) in the presence of sulfuric acid and a mild oxidizing agent (ferric chloride). This reaction was first described by Van Urk (3) as a means of detecting indole compounds, and later modified and improved for use as a quantitative reagent (4–6). The colored (blue) product formed in this



I

reaction has been postulated as I, which is produced by acid-catalyzed condensation of 2 moles of the lysergic acid moiety with 1 of *p*-DMAB, followed by oxidation (7). The overall reaction resembles that of benzaldehyde with methylindoles to form the bis-methylindole condensation products which are oxidized to the corresponding colored species by ferric chloride (8–11).

Since the ergot alkaloids are not readily available in large quantities and are difficult to work with because of their tendency to isomerize and solvate, the study of the effect of benzhydrol on these indole-type compounds was carried out using skatole (3-methylindole) as a model compound, and analogies made between the reactions of skatole and those of the ergot alkaloids.

EXPERIMENTAL

Reactions of Skatole and *p*-DMAB

Formation of the 2:1 Condensation Product (II).—A solution of skatole (2.0 Gm., 0.0152 mole) and *p*-DMAB (1.139 Gm., 0.0076 mole) in ethanol (10 ml.) was treated with 50% ethanolic hydrochloric acid (5 ml.). The exothermic reaction immediately produced a violet solution which was cooled to room temperature, allowed to stand for 2 hr., and poured into water (50 ml.). The mixture was extracted with chloroform (2 × 50 ml.), the chloroform extract washed with 5% sodium bicarbonate and evaporated to dryness under reduced pressure. The crystalline residue, after being washed by suspension in methanol, weighed 2.75 Gm. (92%) and melted at 217–218° (sinters 210°). Treatment with charcoal in chloroform followed by crystallization from chloroform–petroleum ether raised the melting point to 224.5–225.5°. $\lambda_{max.}^{CHCl_3}$ 270 mμ (ϵ 28,000). The product was found to be chromatographically pure by thin-layer chromatography on silica gel with chloroform–hexane (9:1) as solvent.

Anal.—Calcd. for $C_{27}H_{27}N_3$: N, 10.68. Found: N, 10.48.

Formation of the 1:1 Condensation Product (III).—A solution of skatole (200 mg., 1.52 mmoles) in ethanol (2 ml.) was added dropwise to a solution of 60% perchloric acid (0.2 ml.) and *p*-DMAB (300 mg., 2.0 mmoles) in ethanol (5 ml.). The solution was warmed on the steam bath, during which time the initial red-violet color changed to an intense blue-violet along with the formation of blue-black crystals. The solid was collected by filtration, washed with ethanol, and dried to afford 135 mg. (25%) of material, m.p. 238° dec. $\lambda_{max.}^{EtOH}$ 575 mμ (ϵ 6400) (sh 540 mμ).

Received August 8, 1965, from the Pharmaceutical Chemistry Division, Food and Drug Directorate, Ottawa, Ontario, Canada.

Accepted for publication September 22, 1965.

The author thanks Dr. Leo Levi for many valuable discussions during the course of this work.

Anal.—Calcd. for $C_{18}H_{18}N_2.HClO_4$: C, 59.59; H, 5.28; N, 7.72. Found: C, 59.34; H, 5.61; N 7.64.

Oxidation of Compound II.—An aqueous solution of sodium nitrite (18.8 mg. in 10 ml.) was added dropwise over 15 min. to a stirred solution of compound II (100 mg.) in acetone (50 ml.) containing water (5 ml.) and hydrochloric acid (10 drops). The resulting deep blue solution was stirred 5 min., then 60% perchloric acid (10 drops) was added, and the acetone removed with a rotary film evaporator. As the water content of the solution increased, dark blue (almost black) crystals began to separate. Following removal of the acetone, the mixture was filtered and the crystalline product washed with water. Yield: 117 mg. (94%). As recrystallization from organic solvents proved unsatisfactory because of solubility factors, purification was accomplished by washing the product (compound IV) several times with hot ethanol. $\lambda_{max.}^{EtOH}$ 610 mμ (ε 61,000) (sh 560, 460 mμ).

Anal.—Calcd. for $C_{27}H_{25}N_3.HClO_4$: C, 65.91; H, 5.33; N, 8.54. Found: C, 65.77; H, 5.34; N, 8.18.

Hydrogenation of Compound IV.—A mixture of calcium carbonate (100 mg.), platinum oxide (6 mg.), and compound IV perchlorate (35 mg.) in methanol (8 ml.) was stirred under an atmosphere of hydrogen for 30 min. The solution became colorless after 15 min. The inorganic solids were removed by filtration and the filtrate poured into aqueous sodium bicarbonate. Concentration of the aqueous solution under reduced pressure gave 26 mg. of crystalline product, m.p. 205–210°. Recrystallization from methanol–water raised the m.p. to 218–220°. A mixed melting point of this product and compound II was not depressed, and their infrared curves were identical.

Quantitative Reaction of Skatole with p-DMAB.—Three-milliliter aliquots of a skatole solution (30 mg. of skatole in 50 ml. of ethanol), a *p*-DMAB solution (136.5 mg. of *p*-DMAB in 25 ml. of ethanol), and a 50% ethanolic hydrochloric acid solution were mixed to give a molar ratio of 8:1 for *p*-DMAB to skatole. Similar reaction mixtures were prepared from further aliquots of *p*-DMAB solutions which had been suitably diluted with ethanol so that molar ratios of *p*-DMAB to skatole in the reaction mixtures were 4:1, 2:1, 1:1, and 0.5:1. In each case, a violet color developed rapidly, reaching a maximum after 30 min., and remaining unchanged thereafter. The absorption curves were identical to that of compound III perchlorate in ethanol. Absorptions determined at $\lambda_{max.}$ (575 mμ) are shown in Fig. 1.

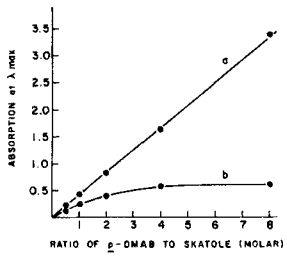


Fig. 1.—Color developed by the reaction of skatole with *p*-dimethylaminobenzaldehyde. Key: a, reaction solution initially containing 0.2 mg. of skatole per ml.; $\lambda_{max.}$ at 575 mμ; b, reaction solution diluted 62.5 times, then treated with sodium nitrite; $\lambda_{max.}$ at 610 mμ.

Solutions too concentrated for accurate measurement were diluted with ethanol–hydrochloric acid (7.5–1.5 by volume). For development of the blue color from compound II, 2-ml. aliquots from each violet solution obtained after a reaction time of 1 hr. were placed in volumetric flasks (25 ml.), diluted with ethanol (20 ml.), treated with 0.1% aqueous sodium nitrite (1 ml.), and made up to 25 ml. with ethanol. The blue color which developed within a few minutes was stable for several hours. After 15 min., the solutions were diluted fivefold with ethanol and the absorptions measured at $\lambda_{max.}$ (610 mμ) to give the results shown in Fig. 1. The absorption curves were identical to that of compound IV perchlorate in ethanol.

Reaction of Ergot Alkaloids with p-DMAB in Ethanol.—Two milliliters of a solution of ergonovine maleate (10 mg.) in ethanol (25 ml.) was treated with 2 ml. of a solution of *p*-DMAB (2.1 mg./ml.) in ethanol and 2 ml. of 50% ethanolic hydrochloric acid (molar ratio of *p*-DMAB to ergonovine of 16 to 1). A violet color developed rapidly—$\lambda_{max.}$ 540 and 575 mμ. Dilution of this solution with ethanol and addition of a few crystals of sodium nitrite produced a blue solution, $\lambda_{max.}$ 575 and 620 mμ. Similar treatment of ergotamine tartrate produced absorption curves identical to those observed for ergonovine maleate. Treatment of dihydroergotamine methanesulfonate in a similar manner, except that the initial condensation mixture was heated on a steam bath for 10 min., gave solutions displaying absorption curves with $\lambda_{max.}$ at 540 and 575 mμ before oxidation, and $\lambda_{max.}$ at 575 and 620 mμ after oxidation.

Reaction of Skatole with Benzhydrol.—Skatole (100 mg., 0.763 mmole) and benzhydrol (141 mg., 0.763 mmole) were dissolved in ethanol (5 ml.) and treated with gaseous hydrogen chloride; the solution became warm and turned pale yellow. It was allowed to stand at room temperature for 1 hr., then evaporated to dryness under reduced pressure. The residue was chromatographed on neutral alumina with benzene–petroleum ether (1:1) as solvent. The first fraction yielded 75 mg. of compound V, m.p. 140.5–141.0°.

Anal.—Calcd. for $C_{22}H_{19}N$: C, 88.85; H, 6.44; N, 4.71. Found: C, 88.87; H, 6.50; N, 4.76.

The second fraction afforded 146 mg. of compound VI, m.p. 147–148°. (A mixture of compounds V and VI exhibited a melting range of 117–132°.) Infrared: 3400 cm.$^{-1}$ ($\diagdown$N—H$\diagup$).

Anal.—Calcd. for $C_{22}H_{19}N$: C, 88.85; H, 6.44; N, 4.71. Found: C, 88.59; H, 6.41; N, 4.43.

Interference of Benzhydrol in the Reaction of Skatole with p-DMAB.—The following standard solutions were prepared: skatole [0.343 m*M*, prepared by dissolving skatole (90 mg.) in ethanol (100 ml.) and diluting 5 to 100 ml. with water], benzhydrol (0.686, 1.37, 2.06, 2.74, 3.43 m*M*, prepared by dissolving 63.2 mg. benzhydrol in 5 ml. of ethanol and diluting to 100 ml. with water and further diluting with 5% ethanol), and *p*-DMAB (0.686 m*M*, prepared by dissolving 10.25 mg. of *p*-DMAB in 100 ml. of hydrochloric acid). To 3-ml. aliquots of each benzhydrol solution (or 5% ethanol for blank) were added 3 ml. of the skatole solution followed by 6 ml. of *p*-DMAB solution. The reaction mixtures were noted to become warm (50°), and a

violet color developed quickly but faded after 5–10 min. After exactly 20 min. in the dark at room temperature, aliquots (2 ml.) of each reaction solution were diluted with ethanol (7 ml.) and treated with 0.1% aqueous sodium nitrite solution (0.5 ml.). After an additional 15 min., the deep blue solutions were made up to 10 ml. with ethanol and the absorbances determined at $\lambda_{max.}$ (610 mμ). Final solutions having molar ratios of skatole–benzhydrol–*p*-DMAB of 1:0:4, 1:2:4, 1:4:4, 1:6:4, 1:8:4, and 1:10:4, respectively, in the initial reaction medium displayed absorbances of 0.475, 0.450, 0.392, 0.347, 0.310, and 0.290, respectively, representing 5.3, 17.5, 27.0, 34.6, and 39.0% decrease in color formation due to the presence of benzhydrol.

When a similar reaction was carried out using ethanol alone as solvent in place of 5% ethanol in the initial reaction, and a molar ratio of skatole–benzhydrol–*p*-DMAB of 1:10:4, a decrease in color formation of 4% compared to that of standard solution without benzhydrol was observed.

Interference of Benzhydrol in U.S.P. Assay of Ergot Alkaloids.—Standard solutions containing 0.10 meq. of ergot alkaloid in 50 ml. of solution were prepared by dissolving ergonovine maleate (4.42 mg.) in water (50 ml.) and ergotamine tartrate (6.57 mg.) or dihydroergotamine methanesulfonate (6.80 mg.) in 1% tartaric acid (50 ml.). A benzhydrol solution containing 0.40 mmole/50 ml. was prepared by dissolving benzhydrol (7.36 mg.) in ethanol (2 ml.) and diluting to 50 ml. with water. To each of 5-ml. aliquots of the ergot alkaloid solutions were added 0, 1, 2, 3, 4, and 5 ml., respectively, of benzhydrol solution, and each sample was diluted to 10 ml. with 4% ethanol. Color development was carried out by addition of 4 ml. of *p*-DMAB reagent (125 mg. of *p*-DMAB in 100 ml. of 65% sulfuric acid with 0.05 ml. of 9% ferric chloride) to an aliquot (2 ml.) of the ergot solution with cooling in ice water. After color development in the dark at room temperature for 1 hr., the absorbance of each solution was measured at the appropriate wavelengths—540 and 610–615 mμ for ergotamine or ergonovine and at 585–590 mμ for dihydroergotamine. The results are shown in Fig. 2.

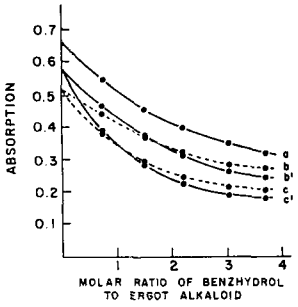
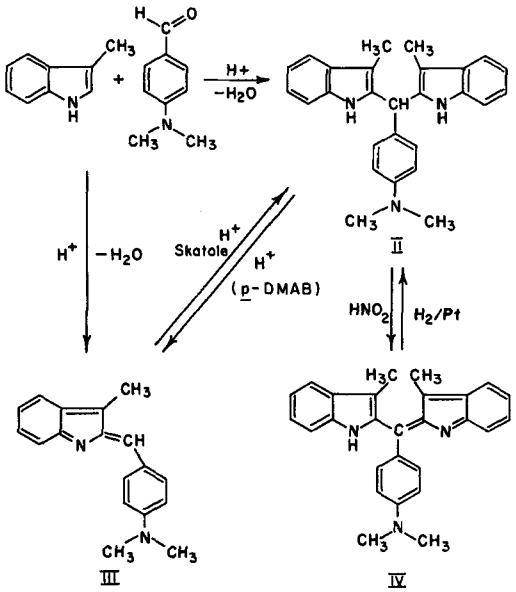


Fig. 2.—Interference of benzhydrol in U.S.P. assay of ergot alkaloids. Key: a, dihydroergotamine at 585–595 mμ; b, ergonovine at 610 mμ, and b′, at 540 mμ; c, ergotamine at 610 mμ, and c,′ at 540 mμ.

Reaction of Ergot Alkaloids with Benzhydrol.—Approximately 1 mg. of ergot alkaloid (ergotamine tartrate, dihydroergotamine methanesulfonate, ergonovine maleate, methylergonovine maleate, or methysergide bimaleate) and 1 mg. of benzhydrol were dissolved in ethanol (2 ml.) and treated with hydrochloric acid (1 ml.). The solutions were heated at 45–55° under nitrogen for 1 hr.—a violet color developed in all cases. The solutions were then poured into water, made alkaline with ammonia, and extracted with chloroform (3 ml.). Aliquots (5 μl.) of the chloroform solution were spotted on an alkaline silica gel plate (3 Gm. silica gel in 10 ml. of 0.1 *N* sodium hydroxide), along with solutions of pure alkaloids and alkaloids, processed as above but without benzhydrol, at separate locations, and the plate developed with chloroform–methanol (92:8). The compounds were detected by spraying with a 1% solution of *p*-DMAB in hydrochloric acid. Each alkaloid gave rise to three main orange spots when thus processed, while alkaloids similarly treated but without benzhydrol each gave rise to three major blue spots. The pure alkaloid without treatment gave only one spot on chromatography.

DISCUSSION

The reactions of skatole with *p*-DMAB appeared to be analogous to those of benzaldehyde with indoles (8–11) in that both 1:1 and 2:1 condensation products were formed, with the latter being readily oxidized to a highly colored product. (Scheme I.)



Reaction of Skatole with *p*-Dimethylaminobenzaldehyde. Scheme I.

The colorless product (II) was formed in the greatest amount in the condensation reaction (isolated in 92% yield from preparation in ethanolic hydrochloric acid), but some of the 1:1 condensation product (III) undoubtedly formed at the same time, as indicated by the violet color of the reaction solution. The formation of compound III was enhanced by carrying out the condensation in the presence of perchloric acid in ethanol at 100°, as described previously (12).

Compound II was sensitive to mild oxidizing agents, and crystals as well as solutions developed a blue color when exposed to air. The oxidative conversion of compound II to IV was readily carried out with nitrous acid in the presence of a small amount of hydrochloric acid using acetone as solvent while only a green color was produced in aqueous solution, and a black gummy product was obtained in the presence of perchloric acid. Oxidation failed to occur when acetic acid was used with sodium nitrite, but proceeded readily in the presence of hydrochloric

acid. Hydrogen peroxide also promoted the oxidation, but at a much slower rate than nitrous acid. Hydrogenation of compound IV to the colorless compound (II) showed that no rearrangement or cleavage had occurred during oxidation.

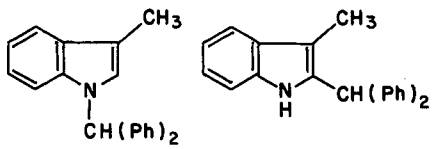
Quantitative examination of these reactions in ethanolic hydrochloric acid indicated that the formation of the violet 1:1 condensation product (III) was directly proportional to the molar ratio of *p*-DMAB to skatole (Fig. 1). The violet color was found to develop rapidly, reaching a maximum in 30 min., and remaining steady thereafter, but the amount of compound III producing the color represented only a small fraction of the reactants. The relative amount of the main product (compound II) was determined by dilution of the reaction solution and oxidation with nitrous acid. As indicated in Fig. 1, the intensity of the resulting blue solution and hence the extent of formation of compound II in the reaction solution was partially dependent on the molar ratio of *p*-DMAB to skatole, reaching a limiting value with a molar ratio of about 5:1.

As with pyrrole derivatives (12), compounds II and III could be readily interconverted, and the reactions followed by either visual observation of the color of the solutions and determination of the visible absorption spectra or by examination of the products by thin-layer chromatography. The colored compounds (III and IV) could not be chromatographed as the salts, but separations were readily achieved by using the corresponding yellow bases and chromatographing on silica gel plates with chloroform–hexane (9:1) saturated with ammonia as solvent. The colors were regenerated simply by exposing the plate to hydrogen chloride vapor. When a solution of compound II was treated with 25% sulfuric acid, a violet color formed and the solution displayed an absorption spectrum identical to that of compound III, and the presence of skatole was detected by chromatography. Similar treatment of compound III resulted in the formation of some *p*-DMAB along with apparent destruction of the compound. In weaker acid (dilute hydrochloric acid), neither compound (II nor III) was altered. In the presence of skatole and dilute hydrochloric acid, however, compound III reacted readily to form only compound II (demonstrated by chromatography), and subsequent oxidation by nitrous acid gave an overall quantitative yield of compound IV.

Observation of the absorption spectra of compounds II, III, and IV dissolved in strong acid gave further insight into the degree of stability of these compounds in strong acids. Dissolution of the violet compound (III), which displayed $\lambda_{max.}$ 575 mμ in dilute ethanolic hydrochloric acid, in 43% sulfuric acid (equivalent to that used in the U.S.P. assay procedure for ergot alkaloids) caused a decrease in the absorption at 575 mμ and an increase at 420 mμ. The violet color was not restored upon dilution of the solution with water or ethanol, and the solution further decomposed on standing. Similar treatment of compound IV, on the other hand, caused a broadening of the absorption band at 610 mμ in 43% sulfuric acid as compared with ethanolic hydrochloric acid. This absorption characteristic remained unchanged for several hours, and the original spectrum (in dilute acid) was regenerated on dilution of the sulfuric acid solution with water. The blue color which was stable in strong acid could not be produced directly from skatole and *p*-DMAB using the U.S.P. assay procedure for ergot alkaloids. Since only a green color was produced, it is possible that the strongly acidic conditions favor the formation of compound III which is subsequently destroyed by the acid.

The results of the examination of three ergot alkaloids (ergotamine, ergonovine, and dihydroergotamine) showed that their sequence of reaction was similar to that described for skatole with *p*-DMAB. Each ergot alkaloid on initial condensation with *p*-DMAB gave a violet solution whose spectrum ($\lambda_{max.}$ 575 mμ) was similar to that of compound III. Oxidation of the initial reaction mixture with nitrous acid gave a blue solution whose absorption ($\lambda_{max.}$ 610 mμ) was similar to that of compound IV. When the blue color of the ergot alkaloid was developed according to the U.S.P. assay procedure, the resulting spectra were similar to that of compound IV measured in strong acid. As expected, ergonovine and ergotamine gave identical spectra since both have the same basic substituted indole nucleus. Although these data indicated that the color reactions for ergot alkaloids are analogous to those of skatole (Fig. 1), other factors such as purity of *p*-DMAB and sulfuric acid, amounts of oxidant, and temperature of reaction may also have an effect on the over-all color development (13, 14).

The formation of compounds V and VI from the condensation of skatole with benzhydrol was found



V VI

to be affected by reaction conditions. With anhydrous hydrogen chloride in absolute ethanol, the molar ratio of the α-substituted to the *N*-substituted compound was about 2:1, whereas in aqueous acid, mainly the α-substituted compound was formed. These products were readily separated by column chromatography and differentiated by infrared spectroscopy since compound VI demonstrated a strong absorption at 3400 cm.$^{-1}$ characteristic of an

N—H group, whereas compound V did not display this absorption. The isomers were also separated by thin-layer chromatography and detected by spraying with *p*-DMAB in concentrated hydrochloric acid. The main product (VI) gave an orange spot, while the minor product (V) gave a reddish spot. In comparison, skatole and its condensation products gave deep blue spots when treated with the spray reagent. It would appear that the interference of benzhydrol in the colorimetric assay of indole-type compounds was the result of irreversible condensation of the reagents, which excluded the color-producing reaction of the indole nucleus with *p*-DMAB. The extent of interference by benzhydrol would be dependent on the difference in rate of reactions of the indole nucleus with *p*-DMAB and benzhydrol. This rate was found to be affected by the nature of the solvent system since less interference was noted when skatole and *p*-DMAB were reacted in aqueous solutions than when in alcoholic solutions.

The effect of the presence of benzhydrol on the U.S.P. method for the assay of ergotamine, ergonovine, and dihydroergotamine is depicted in Fig. 2. In the absence of benzhydrol, ergonovine and ergotamine gave the same degree of color (and exhibited identical visible spectra). However, ergotamine was more reactive with benzhydrol than ergonovine since less color was formed with any given amount of benzhydrol. Thus, using a molar ratio of benzhydrol to ergot alkaloid of 3.5:1, only 30% of the usual color was developed with ergotamine, 44% with ergonovine, and 51% with dihydroergotamine. The interference was less in ethanolic hydrochloric acid, with 62, 69, and 79%, respectively, of the color being developed. As previously noted by Alexander (2), the relative intensity of the peaks at 610 and 575 mμ for ergonovine and ergotamine changes with increasing amounts of benzhydrol, and this feature may be utilized for estimating the amount of benzhydrol present.

Thin-layer chromatographic examination of the products of the reaction of five ergot alkaloids with benzhydrol showed that two reactions occurred. On treatment with acid, each ergot alkaloid through isomerization had given rise to three main compounds, each of which developed a blue color when sprayed with *p*-DMAB reagent. In the presence of benzhydrol, however, each of these isomerized alkaloids gave rise to a new product which no longer gave a blue color with the *p*-DMAB spray. The color observed was orange, of the same hue observed for the skatole–benzhydrol condensation product (VI). These results would therefore indicate that the interference shown by benzhydrol with ergot alkaloids is the same as that with skatole.

Although benzhydrol may arise from hydrolysis of cyclizine (1-diphenylmethyl-4-methylpiperazine) which is often formulated with ergot alkaloids, the more likely source in pharmaceutical dosage forms is the hydrolysis of benzhydryl chloride used in its manufacture. Formation of benzhydrol from cyclizine appears to be negligible under normal conditions, but increased temperature and acidity caused an increase in the rate of hydrolysis, as expected. The drastic conditions required for breakdown would therefore indicate that hydrolysis of cyclizine is not likely to occur within the tablet during storage and that benzhydrol may have been present as an impurity during synthesis of the drug. Indeed, Alexander (2) has examined several preparations of ergotamine with cyclizine and found no benzhydrol. Similarly, Caws and Lawrence (1) found benzhydrol in only certain instances. However, some benzhydrol could be formed during the assay procedure under strongly acidic conditions if the reaction medium is not kept at a low temperature, and thus affect the accuracy of the determinations.

REFERENCES

(1) Caws, A. C., and Lawrence, B. E., *J. Pharm. Pharmacol.*, **14**, 59T(1962).
(2) Alexander, T. G., *J. Assoc. Offic. Agr. Chemists*, **46**, 902(1963).
(3) Van Urk, H. W., *Pharm. Weekblad.*, **66**, 473(1929).
(4) Smith, M. J., *Public Health Rept.*, **45**, 1466(1930).
(5) Allport, N. L., and Cocking, T. T., *Quart. J. Pharm. Pharmacol.*, **5**, 341(1932).
(6) Michelon, L. E., and Kelleher, W. J., *Lloydia*, **26**, 192 (1963).
(7) Pöhm, M., *Arch. Pharm.*, **286**, 509(1953).
(8) Fischer, E., and Wagner, P., *Ber.*, **20**, 815(1887).
(9) Wenzing, M., *Ann.*, **239**, 239(1887).
(10) Fearon, W. R., *Biochem. J.*, **14**, 548(1920).
(11) Dostal, V., *Chem. Listy*, **32**, 13(1938).
(12) Treibs, A., and Herrmann, E., *Z. Physiol. Chem.*, **299**, 168(1955).
(13) McGillivray, W. A., and Metcalf, W. S., *New Zealand J. Sci. Technol.*, **25**, 123(1943).
(14) Gyenes, I., and Bayer, J., *Pharmazie*, **16**, 211(1961).

Diffuse Reflectance Studies of Solid–Solid Interactions

Interactions of Oxytetracycline, Phenothiazine, Anthracene, and Salicylic Acid with Various Adjuvants

By JOHN L. LACH and MICHAEL BORNSTEIN

Data are presented for oxytetracycline-, anthracene-, phenothiazine-, and salicylic acid-adjuvant systems indicating significant interactions in equilibrated samples and those prepared by compression techniques studied by diffuse reflectance spectroscopy. Spectral changes, both in the visible and ultraviolet regions, along with color changes, substantiate these interactions. Although the mechanism of these interactions is not fully understood, data presented indicate that these complexes are of the donor-acceptor variety.

ALTHOUGH relatively little work has been done in the field of diffuse reflectance, it is, nevertheless, a useful tool for the investigation of optical properties of adsorbed molecules. Here a beam of light penetrating into the sample, which is usually a finely divided powdered solid material, is scattered in many directions, is partially absorbed, and finally re-emerges to the surface. The light emerging from the sample is then

Received July 22, 1965, from the College of Pharmacy, University of Iowa, Iowa City.
Accepted for publication September 24, 1965.
The authors thank Dr. W. B. Person, Associate Professor of Physical Chemistry, University of Iowa, Iowa City, or his assistance in preparing this article.