100 cps., and HPMC 4,000 cps. at approximately 6, 10, and 15 Kg. hardness (Figs. 1, 2, and 4). Average apparent tablet densities and corresponding hardness values are shown in Table I.

HPMC 15,000 cps. and NaCMC tablets demonstrated a slightly reduced rate of dye release during the first hour of *in vitro* dissolution as a result of increased hardness and density. The rates of dye release for the remaining dissolution period remained approximately the same, regardless of tablet hardness (Figs. 3 and 5 and Table I).

DISCUSSION

The data indicate that one can expect little or no change in dissolution rate pattern as a result of alteration in tablet density and porosity for some hydrophilic gum formulations. If changes occur, they probably will appear during the initial phase of the dissolution period and the shape of the dissolution profile will not be markedly altered. Apparently, the affinity of some gums for water will overcome any deterring influence which an increased density or decreased porosity may tend to exert on the initial rate of water penetration into the tablet surface. In those cases where the gum and/or formulation exhibit a decreased affinity for water, an increase in density will deter water penetration and reduce the rate of drug substance dissolution until a gel barrier is formed. After the gel barrier is formed, the rate of drug substance release is further reduced and becomes dependent on the rate at which drug substance diffuses through the gel and the rate at which the gel barrier is mechanically removed by agitation.

SUMMARY AND CONCLUSIONS

In an investigation of *in vitro* dissolution behavior for tablets compressed to different degrees of hardness, no changes in dissolution rate pattern were observed for three formulations. In two formulations, a slightly reduced rate of dissolution was noted during the first hour; however, no marked change in the total dissolution profile was observed. Different release rate profiles were observed with tablets prepared from different gums.

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Keyphrases

Tablet formulation dissolution
Hydrophylic resin tablet formulations
Tablet hardness
Tablet density
Simulated gastric fluid
Simulated intestinal fluid
Colorimetric analysis

Tetracyclic Phenothiazines I. Reinvestigation of the Brominations of Some Pyrido[3,2,1-*kl*]phenothiazines

By ARNOLD R. MARTIN, GERALD G. BRIGGS, and TIMOTHY J. YALE

Brominations of both 1,2-dihydro-3-keto-3*H*-pyrido[3,2,1-*kl*]phenothiazine (I) and its sulfoxide (VII) gave 3-hydroxypyrido[3,2,1-*kl*]phenothiazinium bromide (VI). Treatment of 3-keto-3*H*-pyrido[3,2,1-*kl*]phenothiazine (II) with bromine gave 2-bromo-3-hydroxypyrido[3,2,1-*kl*]phenothiazinium bromide (XII). Structural assignments of VI and XII have been made on the basis of their spectral properties, correcting those previously reported.

As a result of an interest in tetracyclic phenothiazine derivatives as intermediates for the synthesis of pharmacologically interesting agents, the reactions of 1,2-dihydro-3-keto-3*H*-pyrido-[3,2,1-*kl*] phenothiazine (I) and 3-keto-3*H*-pyrido-[3,2,1-*kl*]phenothiazine (II) with bromine (Scheme I) were reinvestigated. Harfenist (1) reported the bromination of I to give a "bromo derivative," which he assumed to be the bromoketone (III), on the basis of its elemental analysis. Heating of the "bromo derivative" in aqueous methanol caused its conversion to the unsaturated ketone (II). The reaction of II with bromine gave an unstable "dibromide," assumed to be the addition product (IV). Attempts to recrystallize IV reportedly gave the unsaturated bromoketone (V). The interpretation of the infrared and ultraviolet spectra of the two bromination products originally prepared by Harfenist requires reassignment of their structures.

RESULTS AND DISCUSSION

The infrared spectrum of the "bromo derivative" obtained from I lacks a carbonyl band, but shows the characteristics of an ionized amine salt, *i.e.*, a nearly continuous series of bands in the region of about 2300–2700 cm.$^{-1}$(2). The high melting point of the "bromo derivative" and its solubility behavior (it is insoluble in nonpolar solvents) further militate against the bromoketone structure III. The aromatized salt structure VI, on the other hand, is consistent with the analytical, spectral, and

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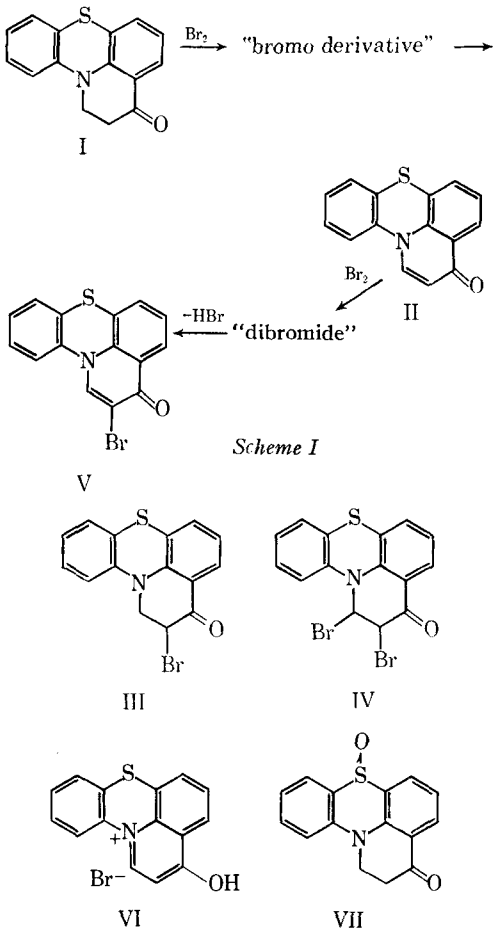


Scheme I

physical data. The salt would be expected to lose a proton in polar solvents readily to give the weak conjugate base II.

The ultraviolet spectra of I, II, and VI are shown in Fig. 1. The spectra of I and II in acetonitrile have been reported previously (3). Comparison of the spectra of I and VI reveal differences which appear unreconcilable on the basis of anticipated relationships between the spectra of an aromatic ketone and its corresponding α-bromo derivative. The relationship of VI as the conjugate acid of II was confirmed by investigating the spectral behavior of II in 50% ethanol containing varying concentrations of hydrochloric acid. The behavior of the long wavelength band is shown in Fig. 2. An isosbestic point is evident at 413 mμ. The absorption maxima of II in the presence of ethanolic hydrochloric acid are essentially the same as those of VI in acetonitrile, except for minor wavelength shifts due to differences in solvent and differences in absorptivity, probably resulting from differences in the amount of dissociation in the two solvents.

Hromatka *et al.* (4), in an effort to obtain a derivative of I chlorinated in one or both of the benzene rings, reacted the sulfoxide VII in hot aqueous ethanolic hydrochloric acid. A good yield of the unsaturated ketone II was obtained. This result prompted us to react VII with bromine, giving an excellent yield of VI. It would seem likely that the synthesis of VI (or II, depending on the workup) by bromination of I or VII, or from the action of hydrochloric acid on VII, might proceed through the common intermediate VIII. This intermediate was originally suggested by Harfenist (1) for Hromatka's preparation of II. A doubly charged intermediate like VIII has been proposed by Schmalz and Burger (5) to explain the reductive nuclear chlorination and nitration of *N*-alkylphenothiazine sulfoxides. Loss of a proton from VIII, followed by enolization, would give VI (Scheme II). The intermediate VIII could possibly rise from either of the perbromides, IX or X, or the protonated form of the sulfoxide XI, depending on the reactants.

It is noteworthy that, if the bromination of I is carried out at ice bath temperature, a brown solid containing roughly two equivalents of bromine and exhibiting a carbonyl band in the infrared can be isolated. Heating the solid causes evolution of hydrogen bromide, giving VI. Efforts to obtain a

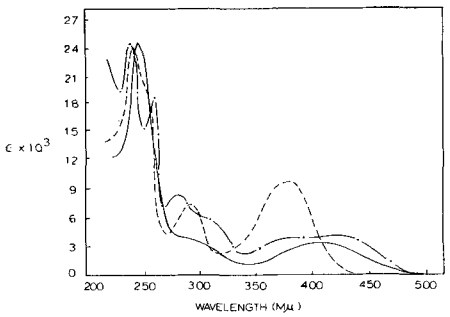


Fig. 1—1,2-Dihydro-3-keto-3H-pyrido[3,2,1-kl]-phenothiazine (I) (——) in 95% ethanol; 3-keto-3H-pyrido[3,2,1-kl]phenothiazine (II) (- - -) in 95% ethanol; 3-hydroxypyrido[3,2,1-kl]phenothiazinium bromide (VI) (— · —) in acetonitrile.

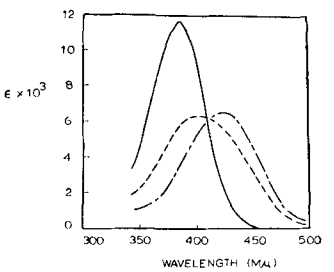
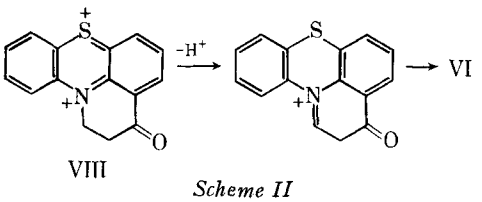
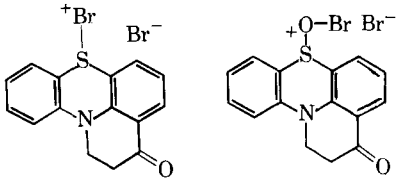


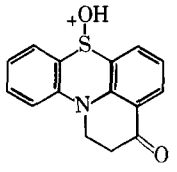
Fig. 2—3-Keto-3H-pyrido[3,2,1-kl]phenothiazine (II) (——); II in 1.6 N HCl (- - -); II in 3.8 N HCl (— - —) (all in 50% ethanol).



Scheme II



IX X

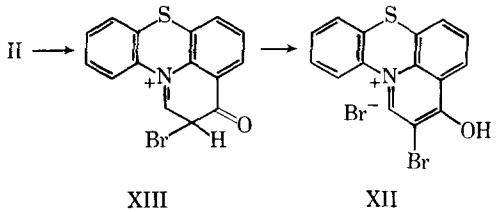


XI

satisfactory analysis of the intermediate, thought to be the S-perbromide (IX), have not been successful. The possibility that the brown solid is the bromoketone (III), which eliminates hydrogen bromide when heated, cannot be entirely ruled out.

It occurred to the authors that Harfenist's "dibromide," obtained from the reaction of II with bromine, is probably the hydrobromide salt XII. The infrared spectrum of this substance, consistent with expectations, lacks a carbonyl band and shows a continuous series of absorptions in the 2300–2700 cm.$^{-1}$ region. Efforts to study the ultraviolet spectrum of XII were frustrated by its lack of solubility in nonpolar solvents and by its conversion to the unsaturated bromoketone (V) in polar solvents. A study of the spectral behavior of V in the presence of acid demonstrated that it is a much weaker base than II. Concentrations of hydrochloric acid causing significant wavelength shifts in the spectrum of II did not effect comparable changes in the spectrum of V. Evidently, the base weakening inductive effect of the bromine atom is more important than possible stabilization of the conjugate acid of II by intramolecular hydrogen bonding of the proton with the nonbonding electrons of the bromine atom. The aromatized salt XII can be visualized as being formed *via* enolization of an intermediate ion XIII as shown in Scheme III.

The fact that II undergoes electrophilic substitution rather than addition is a further indication of its aromatic character. Infrared data also suggest a high degree of aromaticity exists in this and related systems (3). Comparable substitution reactions of *N*-substituted-2- and 4-pyridones appear in the literature (6–8). For example, bromination of 1-methyl-2-pyridone is reported to give 3,5-dibromo-2-pyridone (6). The reaction of 3,5-dicarboxy-1,2,6-trimethyl-4-pyridone with iodine in acetic acid reportedly gave the corresponding 3,5-diiodo derivative in which loss of the carboxy groups occurred (8). The behavior of II toward other electrophilic reagents is under investigation in this laboratory.



XIII XII

Scheme III

EXPERIMENTAL

Melting points were taken in a Thomas-Hoover capillary apparatus and those below 240° are corrected. Infrared spectra were determined in KBr pellets with a Beckman IR-5 spectrophotometer. Ultraviolet spectra were determined with a Cary model 15 spectrophotometer.

3-Hydroxypyrido[3,2,1-kl]phenothiazinium Bromide (VI)—The procedure employed by Harfenist (1) for the bromination of 1,2-dihydro-3-keto-3*H*-pyrido[3,2,1-*kl*]phenothiazine (I) was employed. Concentration of the mother liquid improved the yield to 85–90%. $\nu_{max.}$ 3058, 3012 (aromatic C—H); about 2300–2700 continuous band series (ionized OH); 1616 (aromatic C=C); no ketone band in the region 1680–1690 or aliphatic C—H band near 2900. When the addition of bromine was carried out in an ice bath, a brown solid was formed almost immediately. It was collected on a filter, washed with carbon tetrachloride, and allowed to dry at 25°. When heated on a melting block the substance evolved hydrogen bromide. The infrared spectrum of the brown solid showed it to contain some VI. A ketone band at 1682 cm.$^{-1}$ was also evident in the spectrum.

Bromination of 1,2-Dihydro-3-keto-3H-pyrido-[3,2,1-kl]phenothiazine-7-oxide (VII)—A solution of 3.25 Gm. (12 mmoles) of VII in hot anhydrous dioxane was cooled to 50° and 2.1 Gm. (13.2 mmoles) of bromine in 25 ml. of dioxane was added with stirring over a 3-min. period. The mixture was then heated for 10 min. on a steam bath causing copious evolution of hydrogen bromide and the precipitation of an orange solid, m.p. 255–266°, following recrystallizations from nitromethane. Concentration of the reaction mixture gave additional material, totaling 3.4 Gm. (85%).

3-Keto-3H-pyrido[3,2,1-kl]phenothiazine (II)—A mixture of 3.32 Gm. (10 mmoles) of VI and 10 ml. of triethylamine was allowed to stand 15 min. The triethylamine was removed and the resulting solid was collected on a filter and washed with water. A nearly quantitative yield of II was obtained, m.p. 206°·[Lit. (1) 207°]. $\nu_{max.}$ 3064, 3014 (aromatic C—H); 1621 (C=O).

2 - Bromo - 3 - hydroxypyrido[3,2,1 - kl]phenothiazinium Bromide (XII)—Harfenist's procedure for the reaction of II with bromine was employed (1). Recrystallization of the material thus obtained from anhydrous ethyl acetate gave orange needles, m.p. 149–155°. [Lit. (1) 146–about 159°.] $\nu_{max.}$ 3060, 3015 (aromatic C—H); about 2300–2700 continuous band series (ionized O—H); 1628 (aromatic C=C); no keto band in the region of 1680–1690 or aliphatic C—H band near 2900. Attempts to determine the ultraviolet spectrum of XII in dioxane or acetonitrile gave a spectrum identical with that of V (see below).

2 - Bromo - 3 - keto - 3H - pyrido[3,2,1 - kl]phenothiazine (V)—A mixture of 4.11 Gm. (10 mmoles) of XII and 15 ml. of triethylamine was allowed to stand 15 min. The triethylamine was removed; the residue was collected on a filter and washed with water. A nearly quantitative yield of V was

obtained, m.p. 146–147° from 95% ethanol. [Lit. (1) 145–147.3°.] $\nu_{max.}$ 3048, 3022 (aromatic C—H); 1620 (C=O). $\lambda_{max.}$ 248 (ϵ 32,000); 301 (ϵ 6,800); 387 (ϵ 11,700). The ultraviolet absorption maxima did not undergo appreciable wavelength shifts in 4 *N* hydrochloric acid in 50% ethanol.

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Keyphrases

Tetracyclic phenothiazines
Bromination of pyrido[3,2,1-*kl*] phenothiazines
IR Spectrophotometry–structure
UV Spectrophotometry–structure

Quantitative Separation and Estimation of Steroid Mixtures by Thin-Layer Chromatography II. Determination of Progesterone and Estradiol Benzoate and of Progesterone, Testosterone Propionate, and Estradiol Benzoate in Mixtures

By TATJANA BIĆAN-FIŠTER

Rapid and simple procedures were developed for the TLC separation and subsequent quantitative determination of progesterone and estradiol benzoate as well as progesterone, testosterone propionate, and estradiol benzoate in vegetable oil solutions and microcrystalline water suspensions. The solvent system proposed permits not only a rapid quantitative separation of the steroids from one another but at the same time enables the separation of the steroids from the vegetable oil. No special extraction of steroids from the oil is thus necessary. The separated steroids after extraction from the adsorbent are each determined by the colorimetric and fluorimetric method, respectively.

In a recent communication (1) concerning the quantification on a microscale of steroid mixtures after thin-layer chromatographic (TLC) separation a procedure was proposed for the determination of progesterone and testosterone propionate in oil solution.

Because of the lack of rapid methods for the routine control of preparations containing steroid mixtures, the present communication describes rapid and simple procedures for the determination of mixtures of progesterone and estradiol benzoate as well as progesterone, testosterone propionate or butyrate, and estradiol benzoate.

The procedures described have been adapted to the quantification of steroid mixtures in vegetable oil solutions and crystalline water suspensions commercially available in Yugoslovia.

EXPERIMENTAL

Materials and Apparatus

Reagents—Dissolve 0.4 Gm. of isonicotinhydrazide in methanol, add 0.5 ml. hydrochloric acid (37%), and fill up to 100 ml. with methanol. Set aside the reagent for 24 hr. before use.

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Adsorbent—Fluorescent Kieselgel HF_{254} (E. Merck, Darmstadt).

Reference Standards—Progesterone, testosterone propionate, estradiol benzoate [Organon, Oss (Holland)].

Apparatus—Thin-layer chromatography outfit with regulation thickness spreader (Desaga, Heidelberg), Agla micrometer syringe (Burroughs Wellcome & Co., London), ultraviolet lamp, 254 mμ (Hanau), fluorimeter [Kipp (Delft)].

Solvent System—Cyclohexane–ether–ammonia (8:2:0.5 v/v).

Preparation of Plates

Chromatoplates were prepared following the technique described by Stahl. Plates 20 × 20 cm. were coated (0.5 mm. layer thickness) with a slurry prepared by mixing with a pestle 35 Gm. Kieselgel HF_{254} with 85 ml. of water in a mortar. A batch suffices for six plates. The plates were air-dried for 10 min. at room temperature and thereafter activated by heating at 130° for 4 hr. and stored in a desiccator until used.

Procedures

A—TLC Separation of Progesterone and Estradiol Benzoate in Oil Injection Solutions—A 3.0-ml.