Ketonitrophenols from Mestranol and Related Compounds

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Abstract □ Mestranol (17α-ethynylestradiol 3-methyl ether), when placed on a carrier such as powdered silica gel and exposed to the atmosphere, is converted to a yellow product. The compound formed was shown to be 1α-ethynyltetrahydro-1β-hydroxy-4 - (2 - hydroxy - 5 - methoxy - 3 - nitrophenethyl) - 7a - methyl-5(4*H*)-indanone. The 3-methyl ethers of three other steroids having aromatic A rings yielded products of a similar type. Identical compounds were prepared from the respective steroids by treatment with nitrating agents in acetic acid. This reaction in acetic acid is light catalyzed. An independent synthesis of a model compound, 6-(2-hydroxy-5-methoxy-3-nitrophenyl)-3-hexanone, established the position of the constituents on the aromatic ring as well as the location of the carbonyl. The mechanism proposed for the formation of these products is an initial oxidation of the 1-substituted tetralin to form a hydroperoxide, which is ionically decomposed to form a ketophenol. The phenol is then nitrated in the *ortho*-position.

Keyphrases □ Mestranol—ketonitrophenols as decomposition products, mechanism □ Ketonitrophenols—isolated and prepared from mestranol on silica gel, mechanism □ Decomposition—preparation, isolation, and characterization of colored decomposition product from mestranol and similar products from related compounds

Tablets and dilute powder triturations of mestranol (I), when adequately protected from the atmosphere, show no loss in potency following long periods of storage[1]. However, when I was spotted on a TLC plate and exposed to the air in the laboratory for several days, it developed a cream color[2]. When the exposed TLC plate was then placed in an ammonia chamber or treated with aqueous alkali, the cream color changed to red-orange. The preparation, isolation, and characterization of this colored decomposition product from I and similar products from other related compounds are the subjects of this paper.

RESULTS AND DISCUSSION

Isolation and Characterization of Mestranol Decomposition Product—The decomposition product produced on the TLC plate was prepared on a larger scale by exposing a dried slurry of silica gel in a chloroform solution of mestranol to the air for several weeks. The organic compounds were washed off the silica gel with chloroform, and the phenolic products that had formed were extracted into aqueous alkali. The aqueous solution was acidified, the phenols were extracted back into chloroform, and the products were separated by preparative TLC.

The material that gave the yellow (407 nm) to red-orange (475 nm) color shift was extracted off the silica gel with chloroform. Evaporation of this solution yielded a bright-yellow oil, which crystallized upon scratching. The yellow crystals were subjected to high-resolution mass spectrometry[3]. The molecular weight of the compound was found to be 387.1656, corresponding within experimental error to the empirical formula $C_{21}H_{25}NO_6$.

The ions $C_8H_8NO_4$ and $C_9H_9NO_4$, two of the more abundant fragments in the spectrum, contain five and six elements of unsaturation, respectively, and probably arise from the chemically altered aromatic ring A of mestranol (I). The high degree of unsaturation as well as the three oxygen atoms and a nitrogen atom added to this portion of the molecule suggest that the ring contains a nitro group and a hydroxy. The concept that the added nitrogen atom has this character was substantiated by absorption bands in the IR spectrum at 1530 and 1315 cm^{-1}. A carbonyl absorption at 1700 cm^{-1} identified the remaining additional oxygen atom as a ketone (or aldehyde) functionality, located at some position other than ring A.

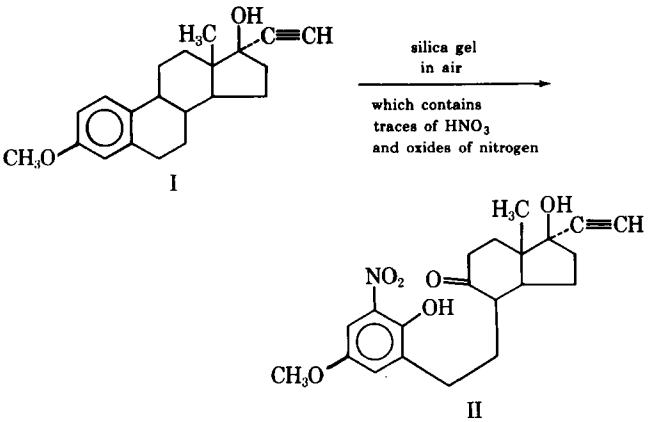
A moderately abundant fragment ion having the formula C_5H_5O is characteristic of ring D in ethynylestradiols and was taken to mean that there had been no alteration of that portion of the molecule.

The NMR spectrum indicated the presence of two aromatic protons instead of one, which would be the case if nitration and hydroxylation had occurred without any modification of the basic ring structure. Furthermore, the empirical formula found by mass spectroscopy contains 10 elements of unsaturation (rings and/or double bonds), but any derivative obtained by simply adding the list of functionalities to I would require 11, thus substantiating the NMR evidence that ring B has been opened.

The structural similarity of I to that of cumene (1) and tetralin (2) suggests that the yellow product resulted from oxidation followed by the nitration of the resulting phenol. This was borne out by the coupling constants of the two aromatic protons, which were seen to be *meta*, and the chemical shift of H-2, which indicated it to be adjacent to a nitro group rather than a hydroxy. Structure II [1α-ethynyltetrahydro-1β-hydroxy-4-(2-hydroxy-5-methoxy-3-nitrophenethyl)-7a-methyl-5(4*H*)-indanone] fits available data (Scheme I).

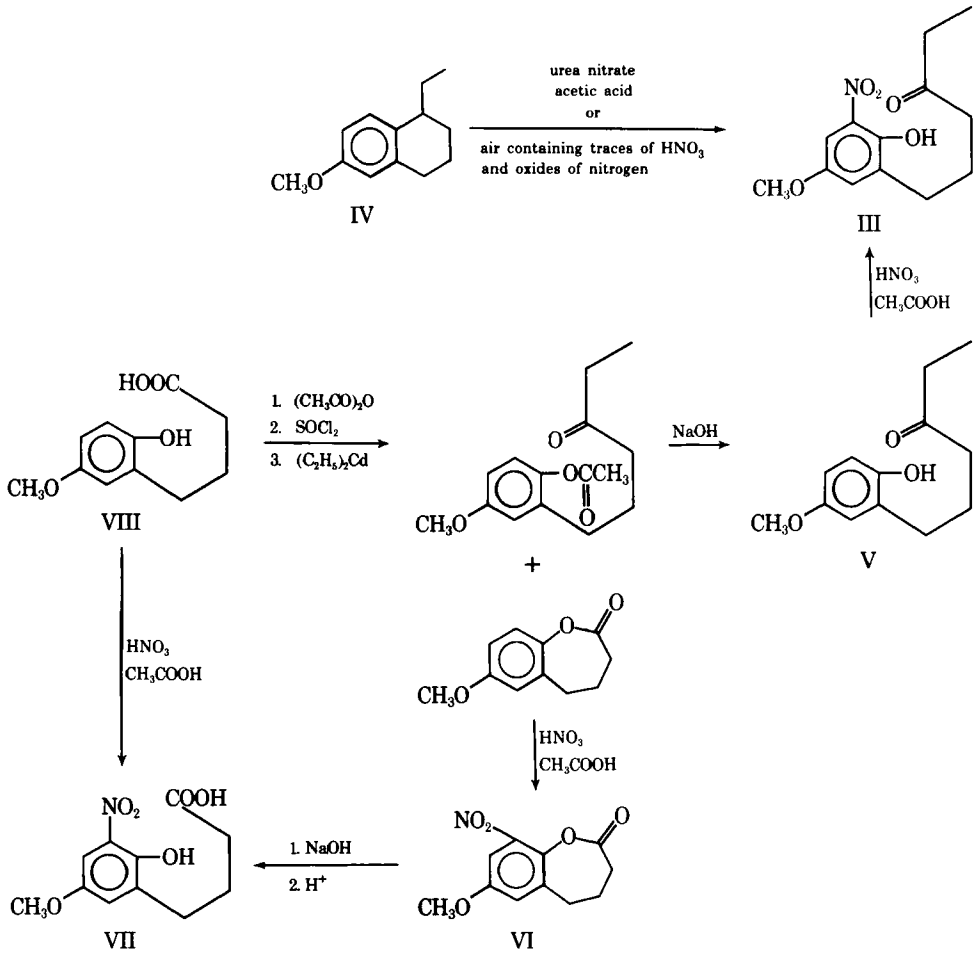
Preparation of Related Compounds—A further verification of the positions of the substituents on the aromatic ring, as well as confirmation of the location of the carbonyl, was made by the independent synthesis of 6-(2-hydroxy-5-methoxy-3-nitrophenyl)-3-hexanone (III), which was obtained from 1-ethyl-1,2,3,4-tetrahydro-6-methoxynaphthalene (IV) (Scheme II). Compound III was obtained by the exposure of IV to the air on silica and was identical to the product obtained by the nitration of the alkaline hydrolysis product (V) from the reaction of 4-(2-hydroxy-5-methoxyphenyl)butyryl chloride acetate (ester) and diethyl cadmium. In addition to III, a neutral compound, the lactone VI, was isolated following nitration of the unhydrolyzed fraction. Alkaline hydrolysis of VI yielded 4-(2-hydroxy-5-methoxy-3-nitrophenyl)butyric acid (VII), which was also obtained by direct nitration of VIII.

Several compounds closely related in structure to mestranol were also studied. Estrone 3-methyl ether, estradiol 3-methyl ether, and 3-methoxy-1,3,5-estratriene all yielded products similar



Scheme I

[1] Eli Lilly and Co., unpublished data.
[2] Paul E. Hartsaw, Eli Lilly and Co., personal communication.
[3] High-resolution mass spectrometer model 21-110, Consolidated Electrodynamics Corp.



Scheme II

to II. Estrone, which has a 3-hydroxy, was exposed to air on silica gel and yielded direct nitration products, 2-nitroestrone and 4-nitroestrone. These compounds were identified by comparison of IR spectra with those of authentic samples (3).

The nitration of estrone in acetic acid occurred instantaneously, resulting in products similar to those obtained on silica gel. These products had UV absorption peaks at 365 nm, which shifted to 400–410 nm in alkali.

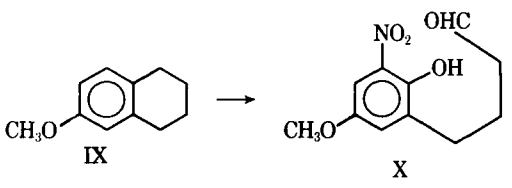
The minimum necessary structure for this reaction appears to be 1,2,3,4-tetrahydro-6-methoxynaphthalene (IX). Compound IX yielded two products in relatively small amounts, both of which had UV spectra similar to II. One of these compounds had a strong carbonyl absorption, while the other was much weaker. Complete separation of these two compounds was not accomplished. Mass spectra on a crude sample indicated a molecular weight of 239, which is in agreement with Structure X (Scheme III).

Mechanism of Reaction—The pathway to the formation of II and related compounds is most probably through a hydroperoxide intermediate. Extensive oxidation studies have been performed on cumene (4), tetralin (5–8), and closely related compounds (9). Both the conditions of the formation and the structures of the products obtained from mestranol and related compounds are similar to those produced from the simpler structures.

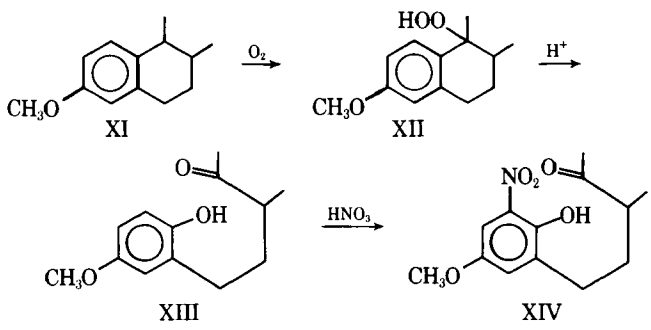
The 3-methoxyestratrienes can be considered to be 1,2-disubstituted 1,2,3,4-tetrahydro-6-methoxynaphthalenes (XI) (Scheme IV). Oxidation of XI in the air produced a hydroperoxide (XII). Ionic decomposition of XII either by acid or base yielded a (2-hydroxy-5-methoxyphenyl)alkanone (XIII), which was then readily nitrated to the *o*-nitrophenol (XIV). Nitration occurring on the silica gel carrier is facilitated because silica gel is an excellent scavenger for nitric acid and the oxides of nitrogen which are absorbed from the air (10).

The hindered position of the hydroxy group between the nitro and alkyl groups is responsible for the absorption in the visible spectrum at longer wavelengths than that for *o*-nitrophenol. The intense characteristic color of the 2-alkyl-4-methoxy-6-nitrophenols aids in their isolation. Although these compounds represent only a fraction of the starting compounds that have undergone a reaction on silica gel, they give strong evidence that the 3-methyl ethers of the estrogens are easily oxidized.

The addition of metal salts to the silica gel accelerated the formation of II. The rate of formation was about four times as fast in the presence of cupric ion as on the untreated carrier; it was also faster, but to a lesser extent, with ferric, manganic, nickelic, and co-



Scheme III



Scheme IV

Table I—Rate of Formation of II on Tricalcium Phosphate

Milligrams of I per Gram of Tricalcium Phosphate	Micrograms of II per Gram of Tricalcium Phosphate/100 hr	Micrograms of II per Milligram of I/100 hr
1.0	12.5[a]	12.5[a]
2.5	15.0[a]	6.0[a]
5.0	16.0	3.2
10.0	18.0	1.8
20.0	20.5	1.0

[a] Initial rate for first 50 hr.

Table II—Formation of II in Acetic Acid at 85° with Urea Nitrate

Reaction Time, min	Concentration of I, mg/ml	Concentration of II, μg/ml
0	9.5	0
15	9.8	70
30	8.75	115
60	—	350
90	8.12	500
180	6.5	310
240	5.9	65

baltic ions. The pH of the silica also had an effect on the formation rate and the type of product formed. Although the amount of II was initially the greatest at pH 9, the amount of II recovered from acidic silica gel was greater after several hundred hours of exposure. A large amount of unidentified dark material was found at the point of application of the material from alkaline silica gel, but only a small amount was found in this location with the acidic samples.

Other powders were tested as carriers. Next to silica gel, tricalcium phosphate was the most effective in promoting the formation of II. Due to its alkalinity, the tricalcium phosphate became pink instead of cream colored. The reaction occurred on or near the surface, so that an undisturbed sample became pink to a depth of 2–3 mm and remained colorless below that depth. In kinetic studies, the powder was mixed frequently and spread thinly to ensure that the reaction proceeded as rapidly as possible.

The effect of concentration of I on tricalcium phosphate on the rate of formation of II was studied (Table I). The reaction rate was not first order with respect to I, indicating that some other limiting factor such as surface phenomenon is involved. The rate of formation of II per milligram of I is the fastest at the lowest concentration of I.

Preparation of II in Solution—Compound II was also prepared by irradiating an acetic acid solution of I containing a small amount of nitric acid under fluorescent lamps at room temperature. Compound II formed rapidly and reached a peak concentration in less than 2 days. Exposure for a longer time brought about a rapid decrease in the concentration of II with a change in the solution color from bright yellow to brown. In the dark or in subdued light, the reaction proceeded at a much slower rate. A corresponding reaction mixture of the same composition, when heated on a steam bath for a few minutes, yielded no II even though the solution darkened.

When the oxides of nitrogen were destroyed by the addition of urea to the reaction mixture (11), the reaction proceeded more slowly both in the light and in the dark. After irradiation under a fluorescent lamp for 7 days, II was still present in appreciable amounts. By using urea nitrate as the nitrating agent, it was possible to prepare II by warming the reaction mixture in a water bath at 85°. The concentration of II reached a peak in about 90 min (Table II). The amount of II in the reaction was never large; at its maximum concentration, it was about 5% of the initial amount of I.

EXPERIMENTAL[4]

Compound II—*Preparation on Silica Gel*—One hundred grams of powdered silica gel[5] was added to a solution of 1.0 g of I in 110 ml of chloroform to make a slurry. The slurry was dried under a current of air, and the resulting powder was spread on a large filter paper. The powder was mixed and respread weekly to expose new surface to the atmosphere. After 2 months, the powder was mixed with 200 ml of chloroform and poured onto a buchner funnel. The silica gel was washed with chloroform until the washings were colorless.

The solution was then reduced in volume to 100–150 ml on a rotary evaporator. The chloroform solution was extracted with a 3% sodium hydroxide solution until the aqueous extracts were almost colorless. The red-brown aqueous layer was then washed once with a small amount of chloroform, acidified, and extracted with chloroform. The combined chloroform layers were washed once with water, dried over sodium sulfate, and evaporated to dryness on a rotary evaporator. The residue was dissolved in 2 ml of acetone and placed on a 1-mm silica gel G preparative TLC plate, which was developed with chloroform–ethyl acetate (9:1).

The orange-yellow layer, which moved about one-third of the way up the plate, was removed and extracted from the silica gel with chloroform, and the residue was rechromatographed again in the same system. The compound was identified by placing the TLC plate in an ammonia chamber and observing the shift in color from orange-yellow to deep red-orange. The product was eluted from the silica gel with chloroform. Evaporation of the solvent left a small amount of orange oil, which crystallized upon scratching. The yield was 44 mg of material, mp 130–133° with slight softening at 118°.

Light-Catalyzed Preparation in Solution—Mestranol (I) (0.4 g) was added to 50 ml of acetic acid containing 2 ml of concentrated nitric acid. The solution was placed in a 50-ml Pyrex flask and stored in a light cabinet 25.4 cm (10 in.) from two 20-w fluorescent lamps. A similar sample was stored in the dark. The sample in the light cabinet became yellow within 16 hr and changed to a brown in about 2 days. No trace of II was observed by TLC of the sample exposed for 2 days, although it was detected at 30 hr. It was still present in a sample after storage in the dark for 18 days.

The acetic acid solution was poured into five times its volume of water and extracted with chloroform until no color was apparent in the organic layer. The combined chloroform extracts were washed with water, and the isolation of the product was carried out in the same way as was described for the product obtained on silica gel. When the reaction mixture was diluted by increasing the amount of acetic acid to 200 ml, the product both formed and decomposed at a slower rate. When the amount of acetic acid was decreased to 12 ml, the sample became brown within 5 hr in the light and no II was detectable on TLC.

Preparation in Acetic Acid with Urea Nitrate—Mestranol (I) (1.27 g) was dissolved in 160 ml of acetic acid, and 3.2 g of urea nitrate was added. The solution was brought to 83–85° in a water bath and held there for 2 hr. The flask was removed from the bath and cooled. The acetic acid solution was worked up as previously described to yield 73.9 mg of II; 0.97 g of mestranol was recovered. No II was obtained when an identical reaction mixture was refluxed for 10 min, even though the solution developed an orange color.

Estrone 3-methyl ether (12), estradiol 3-methyl ether, and 1-ethyl-1,2,3,4-tetrahydro-6-methoxynaphthalene (13) were treated in the same manner as I and yielded products that were similar to II. 3-Methoxyestratriene prepared from 3-hydroxyestratriene (14) also yielded a similar product; but since the sodium and lithium salts of the ketonitrophenol were very soluble in chloroform, it could not be isolated by the method that was satisfactory for the other compounds. Removing the chloroform and replacing it with *n*-hexane[6] made possible the separation of the phenolic product from the starting material. The elemental analyses and melting points for these products are given in Table III.

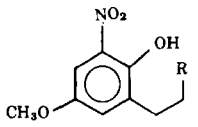
Compound VII—Two grams of 4-(2-hydroxy-5-methoxyphenyl)butyric acid (VIII) (15) was dissolved in 400 ml of acetic acid and 2 ml of concentrated nitric acid was added. After 1 min, an equal volume of water was added and the mixture was poured into

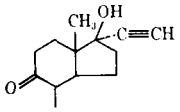
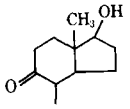
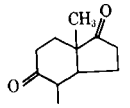
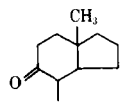
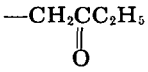
[4] Melting points were obtained on a Mel-Temp melting-point apparatus and are uncorrected.

[5] Both Syloid 63 (W. R. Grace and Co., Davison Chemical Division, Baltimore, Md.) and silica gel G (according to Stahl, E. Merck A.G., Darmstadt, Germany) were used with similar results.

[6] Skellysolve B.

Table III—Ketonitrophenols



Reactant	R	Empirical Formula	Molecular Weight	Melting Point	Analysis, % Calc.	Found
Mestranol		$C_{21}H_{25}NO_6$	387.42	130–133°	C 65.10 H 6.50 N 3.62	65.35 6.47 3.60
Estradiol 3-methyl ether		$C_{19}H_{25}NO_6$	363.40	99–101°	C 62.70 H 6.93 N 3.55	62.96 7.13 3.78
Estrone 3-methyl ether		$C_{19}H_{23}NO_6$	361.38	120–123°	C 63.14 H 6.42 N 3.88	63.33 6.67 4.09
3-Methoxy-1,3,5-estratriene		$C_{19}H_{25}NO_5$	347.40	107–110°	C 65.69 H 7.25 N 4.03	65.91 7.55 4.01
1-Ethyl-1,2,3,4-tetrahydro-6-methoxynaphthalene		$C_{13}H_{17}NO_5$	267.27	62–65°	C 58.42 H 6.41 N 5.24	58.13 6.66 5.24

1600 ml of water and extracted with chloroform. The chloroform solution was washed once with water and extracted with sodium bicarbonate solution. The alkaline solution was slowly acidified with dilute hydrochloric acid and then extracted with chloroform. The chloroform layer was washed with water and dried over sodium sulfate. The residue remaining after evaporation was recrystallized from acetone; 0.5 g, mp 126–129°, was obtained.

Anal.—Calc. for $C_{11}H_{13}NO_6$: C, 51.76; H, 5.13; N, 5.49. Found: C, 51.47; H, 5.03; N, 5.51.

Compound III—Two drops of concentrated sulfuric acid were added to a solution of 2 g of VIII in 10 ml of acetic anhydride. This solution was warmed to 40–45° and then was allowed to stand at room temperature for 30 min. The solution was poured into water and extracted with chloroform. After drying over sodium sulfate, the solvent and excess acetic anhydride were removed under reduced pressure. The residue was dissolved in thionyl chloride and warmed to start the reaction. When the reaction had subsided, the excess thionyl chloride was removed under reduced pressure. The residue was dissolved in ether and was added to a solution of diethyl cadmium (16). When the addition was completed, the mixture was refluxed for 1 hr. After cooling, cracked ice was added to the reaction mixture. Enough diluted sulfuric acid was then added to dissolve the white precipitate which had formed.

The ether layer was separated and extracted with sodium bicarbonate solution to remove any unreacted starting material. The ether solution was next extracted with sodium hydroxide solution. The sodium hydroxide solution was acidified and extracted with chloroform. The chloroform layer was washed with water, dried, and evaporated to dryness. The residue was dissolved in 10 ml of acetic acid, and the resulting solution was treated with a few drops of concentrated nitric acid. After 1 min, the solution was worked up as previously described. Five milligrams of a compound identified as III was obtained.

The ether layer was washed with water, dried over sodium sulfate, and evaporated to dryness. The oily residue was dissolved in 15 ml of methanol, and 5 ml of 3% sodium hydroxide in water was added. After 20 min, the solution was poured into water and extracted with chloroform. The chloroform solution was then extracted with sodium bicarbonate solution, washed with water, and dried. After removal of the solvent on a rotary evaporator, the residue was dissolved in acetic acid and treated with concentrated nitric acid. The reaction mixture yielded 27 mg of a material melting at 56–62°; the IR and NMR spectra of this material were identical to those of III prepared from IV.

When the neutral fraction was nitrated in acetic acid, one product was a material that moved at the solvent front on TLC. This product was isolated as an oil and was extracted from chloroform into sodium hydroxide solution. After acidification of the alkaline solution, the product was isolated as a yellow crystalline compound, mp 127–128°. The IR spectrum for this compound was identical with that of VII prepared by direct nitration of VIII.

Kinetic Studies—*Acetic Acid Solution*—Ten 5-ml glass-stoppered flasks containing 4 ml of acetic acid, 40 mg of I, and 200 mg of urea nitrate were placed in a water bath maintained at 83–85°. The flasks were removed at intervals between 15 min and 4 hr and immediately placed in an ice bath. The solutions were assayed for I using the colorimetric method described by Templeton *et al.* (17). Compound II was separated by TLC and eluted from the silica gel with chloroform. The concentration of II was determined by measuring the solution at 407 nm, using a solution of previously prepared II as a standard.

Dispersed on a Dry Powder Carrier—Mestranol was placed on tricalcium phosphate or silica gel at concentrations of 1–20 mg of I/g of carrier by making a chloroform slurry. The resulting dried powders were spread to a depth of about 0.5 cm in an open half of a petri dish and placed on a benchtop in the laboratory. The samples were mixed and respread frequently to ensure an even exposure to the atmosphere. From 0.5 to 2 g of the powder was removed periodically and placed in a tight screw-capped amber glass bottle. When all samples had been collected, II was isolated and the concentration was determined as previously described.

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Lidocaine Hydrochloride Absorption from a Subcutaneous Site

BERTON E. BALLARD

Abstract □ Subcutaneous disappearance of lidocaine hydrochloride was followed as a function of time using a specially designed "closed" subcutaneous absorption cell affixed to anesthetized rats. Unbuffered, stirred lidocaine hydrochloride solutions in cells open to the atmosphere were previously shown to increase in pH with time because of carbon dioxide loss. The closed cell was designed to prevent this loss, but pH shifts still occurred, making the derivation of a simple pharmacokinetic absorption model impossible. Because the pH of the solution shifted to higher pH values, the data suggest that precipitation of lidocaine base may have occurred in some experiments.

Keyphrases □ Lidocaine hydrochloride—absorption from subcutaneous site, design of closed subcutaneous absorption cell □ Absorption—lidocaine hydrochloride from subcutaneous site, design of closed subcutaneous absorption cell, effect of pH changes □ Parenteral dosage forms—lidocaine hydrochloride absorption from subcutaneous site, design of closed subcutaneous absorption cell

Since the publication of Schou's (1) review of drug absorption from subcutaneous connective tissue, there has been an increasing interest in quantitatively measuring the absorption rates of drugs in aqueous solution from the subcutaneous site (2–8). Quantitative measurements of drug absorption rates should result in a better understanding of which of the many possible pharmacokinetic models are appropriate for describing subcutaneous drug absorption of various drugs and of what mechanisms are involved in subcutaneous drug absorption. To date, there has not been a study where different commercial products containing the same drug have been compared in their absorption behavior from the subcutaneous region under conditions where the drug was sampled periodically at the subcutaneous absorption site, where the solution was continuously stirred, and where the surface area for absorption was held constant.

The purposes of this report are to develop and discuss the strengths and weaknesses of experimental methods that might be useful in comparing the subcutaneous absorption behavior of commercially prepared parenteral dosage forms of lidocaine hydrochloride. This drug might be considered a model compound for this purpose, because many parenteral drugs are water-soluble salts of weak organic bases and the unionized base often has limited water solubility.

EXPERIMENTAL

Animals—Female Sprague–Dawley rats were used. The anesthesia and the method used for surgically exposing the subcutaneous tissue were described previously (3).

Reagents—Lidocaine hydrochloride was obtained from two manufacturers[1,2]. According to the label claims, each preparation contained (per milliliter) 10 mg of lidocaine hydrochloride, 7 mg of sodium chloride, 1 mg of methylparaben, and sodium hydroxide to adjust the pH. Both preparations conformed to the standards for lidocaine hydrochloride injection USP without epinephrine (9). Cyclizine hydrochloride[3] (10, 11) was used as an internal standard for the GC analysis at a concentration equivalent to 243 mg of cyclizine base/liter in 0.1 *N* HCl. The *n*-hexane used as the extraction solvent in the procedure was of spectrographic grade[4]. All water was double-distilled from dissolved potassium permanganate. All glassware except the microsyringe[5] was initially cleaned in concentrated nitric acid.

Subcutaneous Absorption Cell—The design of the absorption cell used in this study differed somewhat from that used in previous work (3). The glass cell was nearly hemispheric in shape, where the maximum distance from one inside point of the open end to another inside point was 19 mm and the distance between the top inside point and the plane across the open end of the cell was 10 mm. Two holes were made in the top region of the cell. One hole, used for the stirrer, was at the top center of the cell when the cell was placed flat on a horizontal surface. A glass tube was fused to the glass surrounding this hole, and the tube extended 3 mm up

[1] Lot L 107770, Astra Pharmaceutical Products, Worcester, Mass.
[2] Lot 2016923C, Invenex Pharmaceuticals, Grand Island, N.Y.
[3] Marezine, Burroughs Wellcome & Co., Research Triangle Park, N.C.
[4] Spectroquality reagent, Matheson, Coleman and Bell, East Rutherford, N.J.
[5] Hamilton Co., Whittier, Calif.