ability study. These fluorescent characteristics by a sulfuric acid spray on the TLC plate also were observed for other 1,4-benzodiazepines and were applied for the determination of chlordiazepoxide and its metabolites in serum (17). When chloroform–2-propanol–ammonium hydroxide (95:5:0.5) is used for separation, some 1,4-benzodiazepines have different fluorescence colors under long wavelength UV light after a sulfuric acid spray with R_f values of 0.32 (chlordiazepoxide, blue fluorescence), 0.19 (demoxepam, yellowish green), 0.38 (nitrazepam, blue), 0.73 (medazepam, blue), 0.74 (prazepam, blue), and 0.06 (*p*-hydroxydesmethyldiazepam, yellow). If chloroform–methanol–acetic acid (85:15:1) is used, these compounds have similar fluorescence characteristics after a sulfuric acid spray and their R_f values are 0.79 (diazepam), 0.73 (desmethyldiazepam), 0.65 (oxazepam), 0.68 (chlordiazepoxide), 0.66 (demoxepam), 0.72 (nitrazepam), 0.77 (major spot of medazepam which has a very small spot at R_f 0.14), 0.79 (prazepam), and 0.56 (*p*-hydroxydesmethyldiazepam). By the combined use of these solvent systems and the characteristic fluorescence colors, this procedure should be applicable for the general screening test of some 1,4-benzodiazepines in both serum and urine samples.

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Facile Separation of Sulfonamides from Their Degradates by Liquid–Liquid Extraction

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Abstract □ Regulation of acidity for protonation of the free N^4-amine can provide for the selective liquid–liquid extraction isolation of a sulfonamide from its degradation products. This principle is applied for the stability-indicating determination of sulfacetamide in the presence of sulfanilamide, sulfaquinoxaline in feed, and sulfabromomethazine in dosage forms. In solution, sulfabromomethazine can exhibit photodecomposition to sulfamethazine. The mean relative errors of the these methods and the precision, represented by relative standard deviations, are each typically <2%.

Keyphrases □ Sulfonamides, various—separated from degradation products by liquid–liquid extraction □ Degradation products of various sulfonamides—separated from parent compounds by liquid–liquid extraction □ Liquid–liquid extraction—separation of various sulfonamides from degradation products □ Antibacterials—various sulfonamides, separation from degradation products by liquid–liquid extraction

Although most sulfonamides are highly stable, they can degrade (almost exclusively) by acid-catalyzed hydrolysis of the sulfur–nitrogen bond (1–7) or, occasionally, by cleavage of the nitrogen–carbon bond (1). Hence, any stability-indicating analysis for these drugs in formulations should discriminate between the intact sulfonamide and both sulfanilic acid and sulfanilamide as potential hydrolytic degradates.

BACKGROUND

Applications of separation techniques to the sulfonamides have long been of interest because of the similar chemical and physical properties of these drugs and their use in combinations. Typically, sulfonamides have been separated by TLC, and many very exacting quantitative procedures for determining mixtures of these drugs using this technique have been published. Among those studies that present data for sulfanilamide (*e.g.*, 8, 9) are evaluations of new spray reagents (10, 11) and various stationary phases (12).

Of the GLC methods, those procedures that measure the relevant amine generated from acidic sulfonamide hydrolysis (4–7) are not directly applicable for stability studies; only the methods that derivatize the intact drug (13–18) are appropriate. Some recently published high-pressure liquid chromatographic (HPLC) sulfonamide separations make use of adsorption (19, 20), ion-exchange (21, 22), ion-pair partition (23, 24), and reversed-phase (25) modes.

For stability studies of sulfonamides, simple liquid–liquid extraction is an attractive alternative to some of those tedious or time-consuming approaches. In particular, quantitative TLC methods are lengthy and require careful attention to technique to obtain good precision. The extraction is also convenient because it neither requires the derivatization essential for GLC nor relies on instrumentation, and it can be combined with standard (*e.g.*, Bratton–Marshall) analytical measurements for the sulfonamides. Although other solvents also may be satisfactory, a mixed chloroform–1% dimethylformamide solvent was selected as the organic phase to demonstrate the feasibility of this approach. The aqueous phase

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is regulated in the pH 0–4 range to give selectivity to the extraction. The primary application is for the stability-indicating determination of sulfonamides in formulations.

The relatively rapid hydrolysis of sulfacetamide can be a problem, especially with some commercial ophthalmic and dermatological solutions and tablets. The methods used for the stability-indicating determination of this drug include the thin-layer technique (26), a selective colorimetric procedure (27), adsorption column chromatographic separation (28), and HPLC (20). Unlike most other sulfonamides, sulfacetamide undergoes hydrolysis at its more labile nitrogen–carbonyl bond. Nevertheless, the amino-bearing product of this degradation, in common with conventional sulfonamide hydrolysis, is sulfanilamide. Accordingly, sulfacetamide provides one example of the application of the liquid–liquid partitioning system as a convenient alternative to other methods. The determination of sulfaquinoxaline in feed and of sulfabromomethazine in a paste and a powder formulation is also presented. In addition, a photodegradation study of sulfabromomethazine is described.

EXPERIMENTAL

Reagents—Chloroform[1], hydrochloric acid[1], and dimethylformamide[2] were used as received. The sulfonamides[3] also were used without further purification.

Determination of Distribution Ratios—Each sulfonamide was dissolved in water and diluted to 5.00×10^{-5} *M*. The pH was regulated by addition of hydrochloric acid and measured in some solutions on a pH meter[4]. A 20-ml aliquot of each solution was shaken at room temperature with an equal volume of a mixed solvent of 1% (v/v) dimethylformamide in chloroform for 0.5 hr. Sulfonamide concentration in the aqueous phase was then determined colorimetrically with the Bratton–Marshall reaction; the concentration in the organic phase also was determined similarly after evaporation of the organic solvent.

Sulfabromomethazine Photolysis—The photolytic degradation of sulfabromomethazine in ethanol (0.33 mg/ml) was studied under UV irradiation in 10-cm cells equipped with quartz windows[5] in a test chamber[6] for 24 days. Liquid chromatography of the solution was performed[7] according to a procedure described by Kram (22). A 50-cm × 2.1-mm i.d. anion-exchange[8] column was used with a flow rate of ~1 ml/min obtained with 700 psig. A 25-ml aliquot of the ethanol solution also was concentrated to 2 ml, 20 μl was spotted on a 20 × 20-cm fluorescent silica gel TLC plate[9], and the chromatogram was developed to 10 cm with a mobile phase of 50% ethyl acetate–50% chloroform.

Sulfabromomethazine Determinations—*Powder Formulation*—The following procedure was applied to a powder formulation containing sodium citrate, sodium carbonate, and edetate disodium in addition to the drug. A sample taken to provide 800 mg of sulfabromomethazine sodium was dissolved in 500 ml of 0.01 *N* NaOH and diluted to 1 liter with water. A 5.00-ml aliquot of this 5×10^{-3} *N* NaOH solution was diluted to 500.0 ml with water for analysis. A sulfabromomethazine sodium analytical standard of known concentration (8.00×10^{-3} mg/ml) was prepared in the same solvent and processed similarly.

To 20.0 ml of the sample and working standard was added 5.0 ml of 3 *N* HCl for pH regulation (pH 0.22), and the sample was partitioned in three successive equilibrations of 15, 15, and 10 ml with fresh chloroform–1% dimethylformamide. The solvents were equilibrated in 50-ml centrifuge tubes on a mechanical shaker for 10 min. The organic extracts were collected, combined, and diluted to 50.0 ml with the same solvent. A 20.0-ml aliquot of this solution was back-extracted into 15.0 ml of 0.1 *N* NaOH, 2 ml of 10% fluoroboric acid was added to 10.0 ml of this aqueous solution, and the sulfonamide was determined by the conventional Bratton–Marshall colorimetric procedure (29).

Paste Formulation—This procedure was applied to a sulfabromomethazine paste formulation that also contained mineral oil, benzyl alcohol, and a thickener[10]. The immiscibility of this formulation with water and its viscosity decreased the extraction efficiency of the drug into aqueous 0.1 *N* NaOH; this problem was overcome by the use of petroleum ether in the extraction.

[1] J. T. Baker "analyzed" reagent.
[2] Mallinckrodt analytical reagent.
[3] Merck Reference Standard, American Cyanamid (USP), Matheson, Coleman & Bell (USP), and Eastman Kodak Co.
[4] Orion model 801.
[5] Coleman.
[6] Envira-Lite model TRLC 2001.
[7] DuPont model 830 liquid chromatograph.
[8] DuPont Zipax SAX.
[9] E. Merck silica gel 60 F-254.
[10] Bentone 38, an organically modified montmorillonite clay, NL Industries.

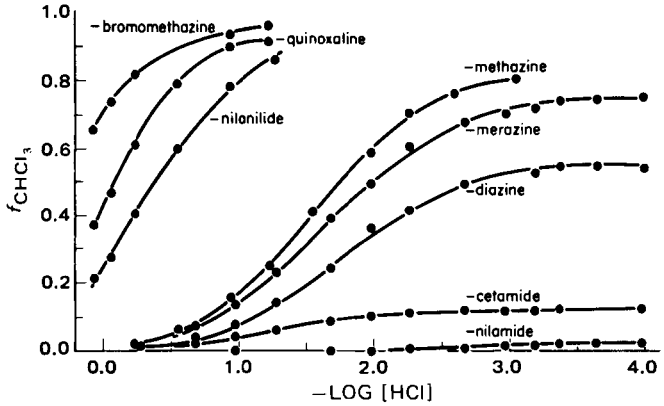

Figure 1—*Distribution ratios of selected sulfonamides into chloroform–1% dimethylformamide as a function of the acidity of the aqueous phase. The fraction in the organic phase, f_{CHCl_3}, is plotted* versus *log [HCl].*

A sample of paste taken to contain 300 mg of sulfabromomethazine sodium was treated with 15 ml of petroleum ether and 15 ml of 0.1 *N* NaOH, and the basic aqueous extracts from three successive extractions with 15.0 ml of fresh solvent were combined and diluted to 1000 ml with water. A 5.00-ml aliquot of this solution was diluted to 250.0 ml with water for analysis. To 20.0 ml of the sample was added 5.0 ml of 3 *N* HCl for pH regulation, and the sample and standard were treated as described for the powder formulation.

Determination of Sulfaquinoxaline in Feed—Sulfaquinoxaline was extracted from the feed sample into aqueous sodium hydroxide solution as prescribed in standard feed procedures (30, 31). To a 50-ml aliquot of the aqueous extract was added 15 ml of 1.2 *N* HCl, and the sample was diluted to 100 ml with water. After centrifugation, 3 g of sodium chloride was added to a portion of the sample, which was equilibrated in three successive extractions of 15, 15, and 10 ml with fresh organic mixed solvent. The combined extracts were diluted to 50.0 ml with the solvent, and a 25.0-ml aliquot was back-extracted into 15.0 ml of 0.1 *N* NaOH; then 10 ml was acidified with 2 ml of 10% fluoroboric acid prior to colorimetric measurement.

Determination of Sulfacetamide in Presence of Sulfanilamide—To a 10.0-ml aliquot of a solution containing ~0.25 mg of sulfacetamide/ml was added 10.0 ml of 0.06 *M* HCl. The sample was diluted to 100.0 ml with water, and a 10.0-ml aliquot was partitioned once with 15.0 ml of the chloroform–1% dimethylformamide mixed solvent. A 10.0-ml portion of the organic phase was evaporated under nitrogen, and the residue was dissolved in water for colorimetric measurement. The analytical reference standard sulfacetamide was treated similarly. In this procedure, the chloroform extract was taken to dryness in lieu of back-extraction into 0.1 *N* NaOH as in the sulfabromomethazine and sulfaquinoxaline procedures because of the rapid hydrolysis of the amide nitrogen–carbon bond at high pH.

RESULTS

Distribution Ratios—The distribution ratios of eight different sulfonamides between chloroform–1% dimethylformamide and water at room temperature at a concentration of 5.00×10^{-5} *M* are presented in Fig. 1 as a function of the acidity of the aqueous phase. Total recovery from both phases was typically 100 ± 3% for each drug over the pH range studied. As the pH was decreased, a greater portion of each sulfonamide remained in the aqueous phase.

Sulfabromomethazine Photolysis—The retention times of sulfabromomethazine and sulfamethazine were 9.2 and 2.2 min, respectively, using a mobile phase containing 0.1 *M* sodium nitrate and 0.01 *M* sodium borate. They increased to 64 and 5.3 min, respectively, when the nitrate concentration was reduced to 0.01 *M*. The chromatogram of the irradiated sample revealed a degradate that eluted with a retention time equal to that of sulfamethazine.

This identification was confirmed by TLC. In the described system, sulfamethazine appeared at R_f 0.56 and sulfabromomethazine appeared at 0.71. The irradiated sample exhibited two spots with the indicated R_f values corresponding to the presence of both compounds, while a control solution stored in the dark yielded the sulfabromomethazine spot only.

Sulfabromomethazine Determinations—Accuracy was measured

Table I—Sulfabromomethazine Determinations in Powder and Paste

Sample Taken			Amount Found		
Decomposition Product		Sulfabromo-		Sulfabromo-	Relative
Compound	Grams	methazine, g	$A_{547\,nm}$	methazine, g	Error, %
		Powder Formulation			
None	—	0.794	0.498	—[a]	—
	—	0.794	0.498	—	—
Sulfanilamide	0.0822	0.794	0.492	0.784	−1.26
	0.0822	0.794	0.505	0.805	1.39
Sulfanilic acid	0.0790	0.794	0.498	0.794	0.00
	0.0790	0.794	0.497	0.792	−0.25
Sulfamethazine	0.0834	0.794	0.499	0.796	0.25
	0.0834	0.794	0.498	0.794	0.00
		Paste Formulation			
None	—	None	0.008	<0.007	—
	—	None	0.002	<0.007	—
	—	None	0.002	<0.007	—
Sulfanilamide	0.0370	None	0.002	<0.007	—
	0.0407	None	0.002	<0.007	—
	0.0336	0.3067	0.366	0.304	−0.98
	0.0320	0.2956	0.359	0.298	0.68
Sulfanilic acid	0.0390	None	0.002	<0.007	—
	0.0504	None	0.002	<0.007	—
	0.0283	0.3000	0.366	0.304	1.33
	0.0281	0.2843	0.342	0.284	0.00
Sulfamethazine	0.0376	None	0.006	<0.007	—
	0.0392	None	0.008	<0.007	—
	0.0347	0.3202	0.392	0.325	1.56
	0.0350	0.2952	0.362	0.300	1.70

[a] Used as the analytical reference standard for the powder determinations.

with unmedicated powder spiked at 71.0% (w/w) sulfabromomethazine sodium by the addition of an aqueous solution of the drug to the sample during extraction. The average of four analyses was 71.0% sulfabromomethazine sodium, with a relative standard deviation of 0.2%. For the paste, the accuracy of this method was estimated by analyses for unmedicated paste spiked at 65.0 ± 1.0% sulfabromomethazine sodium by the addition of a weighed amount of the analate to individual samples of the unmedicated paste. The mean relative error from six measurements was 1.2%.

For estimates of precision, the range of four analyses of a complete water-soluble powder formulated to contain 71.4% sulfabromomethazine sodium was 1.3%. Similarly, the relative standard deviation from six measurements of a paste prepared to contain 65.0% (w/w) drug was 1.4%.

Sulfanilic acid, sulfanilamide, or sulfamethazine (the probable decomposition products of sulfabromomethazine sodium that respond to the Bratton–Marshall reaction) was added to the unmedicated powder and paste formulations at a concentration level corresponding to ~10% (w/w) of the nominal sulfabromomethazine sodium concentration in a complete formulation. Analyses by this procedure (Table I) demonstrate that the presence of these possible decomposition products neither significantly increases the background absorbance contributed by unmedicated paste nor affects the analysis of medicated formulations, confirming the efficiency of the isolation of the parent drug from its decomposition products by this procedure.

Sulfaquinoxaline in Feed—Four unmedicated feeds (a broiler feed and a turkey feed, each in both mash and pelleted forms) were spiked with a combination representing both 0.0100% sulfanilic acid and 0.0100% sulfanilamide by the addition of an aqueous solution of these sulfonamides to the feed during extraction. The absorbances from these feeds analyzed by the present procedure were equal to the background absorbance from unmedicated feed analyzed similarly (0.007–0.009 absorbance unit). These concentrations correspond to the quantitative hydrolysis of all sulfaquinoxaline in a feed containing ~0.035% drug; a typical drug concentration in feed for coccidiosis is 0.0125%. These results confirm that the procedure is not affected by the presence of sulfanilic acid or sulfanilamide in the feed matrix.

Unmedicated feeds were spiked at three concentration levels (0.0100, 0.0125, and 0.0150%) with sulfaquinoxaline. To each of these concentrations was also added either sulfanilic acid or sulfanilamide equal to 10% (w/w) of the sulfaquinoxaline concentration. The resulting analyses (Table II) demonstrate that the determination of sulfaquinoxaline is independent of the presence of these added compounds.

The accuracy and range of linearity of this procedure are represented by the mean relative error of 1.1% from 28 measurements of four feeds spiked with 0.0100–0.0150% sulfaquinoxaline. The precision of the method is estimated by the relative standard deviations of 1.2 and 1.5% from 12 determinations on each of two complete feeds formulated with sulfaquinoxaline at the 0.01 and 0.015% levels, respectively.

Sulfacetamide Determinations—Analyses of eight synthetic aqueous mixtures of sulfacetamide and sulfanilamide, covering the range of 2–8% (w/w) of the decomposition product, averaged 100.5% of the known sulfacetamide concentration with a relative standard deviation of <1% (Table III).

DISCUSSION

The separations are based on the effect of protonation of the free amino group at pH < 3 on the distribution ratios (32–35). The low basicity of

Table II—Analyses of Feeds Spiked with Sulfaquinoxaline, Sulfanilic Acid, and Sulfanilamide

Feed[a]	Sulfaquinoxaline Added, %	Sulfanilamide Added, %	Sulfanilic Acid Added, %	$A_{550\,nm}$	Sulfaquinoxaline Found, %	Relative Error, %
Broiler mash	0.0100	—	—	0.221	0.0103	3.0
Turkey mash	0.0100	0.00100	—	0.221	0.0103	3.0
Broiler pellets	0.0100	—	0.00100	0.219	0.0102	2.0
Turkey pellets	0.0125	—	—	0.270	0.0124	−0.8
Broiler mash	0.0125	0.00125	—	0.275	0.0126	0.8
Turkey mash	0.0125	—	0.00125	0.279	0.0127	1.6
Broiler pellets	0.0150	—	—	0.328	0.0149	−0.7
Turkey pellets	0.0150	0.00150	—	0.333	0.0151	0.7
Broiler mash	0.0150	—	0.00150	0.335	0.0152	1.3
Standard 0.0100% SQ				0.214		
Standard 0.0125% SQ				0.271		
Standard 0.0150% SQ				0.331		

[a] Each sample contained 5.00 g of feed.

Table III—Determination of Sulfacetamide [a] in the Presence of Sulfanilamide

Sulfanilamide Added [b]	$A_{540\ nm}$ [c]	Sulfacetamide Found, mg/ml	Relative Error, %
1.71	0.543	0.237	−0.4
1.71	0.549	0.240	0.8
3.41	0.543	0.237	−0.4
3.41	0.550	0.240	0.8
5.12	0.543	0.237	−0.4
5.12	0.548	0.239	0.4
8.53	0.557	0.243	2.1
8.53	0.552	0.241	1.3

[a] Each solution contained 0.238 mg of sulfacetamide/ml. [b] Percent of sulfacetamide (w/w). [c] Sulfacetamide standard $A_{540} = 0.545$.

this group results from its *para*-orientation to the SO_2 function. At a pH above the pKa of the free N^4-amine, the sulfonamide is uncharged and, therefore, more lipophilic than at a pH below this pKa where the sulfonamide is cationic (Fig. 1). Sulfanilic acid does not partition into chloroform–1% dimethylformamide in the pH range presented in Fig. 1 because of the strong acidity of the SO_3H substituent. In addition, because the organic phase in this system is a binary solvent mixture and each component can form hydrogen bonds, the partition properties of these sulfonamides also depend on charge, structure, and the ability to hydrogen bond with the solvents.

Photolytic loss of halogen from the aromatic ring (36) is demonstrated by the two uncorrelated chromatographic systems in this study of the degradation of sulfabromomethazine to sulfamethazine. Not only are the systems based on different chromatographic properties (ion exchange and adsorption), but the relative mobilities of the two sulfonamides are inverted between the systems as well. These results underscore the need for distinguishing sulfamethazine from sulfabromomethazine in a stability-indicating determination of this halogen-containing sulfonamide.

This separation was easily effected with the present liquid–liquid partitioning system, which provides remarkable selectivity between sulfabromomethazine and sulfamethazine despite their structural similarity. Figure 1 illustrates that the halogenated sulfonamide is much more soluble in the organic solvent than its photolytic degradate. In this procedure, pH 0.22 ($[H+] = 0.6\ M$) is selected for the separation of sulfabromomethazine from its possible interfering decomposition products. After this separation, the analate is back-extracted into 0.1 *N* NaOH for measurement. The low sensitivity of this procedure to sulfanilic acid, sulfanilamide, and sulfamethazine is demonstrated by the results of Table I.

In the pH range of Fig. 1, the maximum fraction of sulfacetamide to partition into the organic phase was 11% (between pH 2 and 4). At higher pH, this fraction decreased in a sigmoid fashion to $f = 0$ at pH 7 with an inflection at 5.5%, occurring at pH 5.40, the pKa of the sulfonamido proton on this molecule (37). At pH 2.2 ($[H+] = 6 \times 10^{-3}\ M$), sulfanilamide remained quantitatively in the aqueous phase, providing adequate conditions for determining the parent drug by comparison to a reference standard which was similarly processed through the partitioning operation (Table III).

The two official AOAC methods for the determination of sulfaquinoxaline in feed (30, 31) are not stability indicating because they rely on a Bratton–Marshall reaction without prior separation. Depending on conditions, hydrolysis of sulfaquinoxaline can yield either sulfanilic acid or sulfanilamide. The other amine that can result from hydrolysis, 2-aminoquinoxaline, is a heterocycle that does not respond to the Bratton–Marshall reaction. In the present method, the liquid–liquid partitioning step separates sulfanilic acid and sulfanilamide from sulfaquinoxaline. The pH of the aqueous phase is adjusted to ~1 for this separation (Fig. 1), and sodium chloride is added to inhibit the emulsion formation. In addition, the extension of this general technique to parts-per-million concentration level feed preparations is demonstrated by this application.

The trisulfapyrimidines in Fig. 1 reflect the effect of methyl substitution on these partitioning properties. The fractions in the organic phase where these curves level off (at pH 3–4) increase in the order sulfadiazine (0.53) < sulfamerazine (0.73) < sulfamethazine (0.80). Schumacher and Nagwekar (35), in a study to correlate partition coefficients with the transfer of drugs across biological membranes, found the same trend with increasing methyl substitution in a pH 4.3 acetate buffer–1-octanol system.

Sulfonamide transport across biological membranes has been studied extentively *in vitro via* partition coefficient measurements relative to the acidity of the sulfonamido group. In the original study (33), Bell and Roblin were unable to discern a relationship between bacteriostatic activity and the pKb of the free amino group because of the small variation of this constant among the sulfonamides. They did not consider the combined effect of this protonation and molecular structure on partitioning properties (Fig. 1). Fujita and Hansch (38) emphasized the importance of hydrophobic character on correlations with biological activity for these drugs. Because it is probable that the free amino group is the reactive center in the biochemical mechanism (32–34, 39, 40), it would be interesting to consider possible structure–activity correlations with reference to the equilibrium at this weakly basic site (Fig. 1).

It should also be feasible to develop meaningful HPLC separations based on distribution ratios in this pH range. Henry *et al.* (41) recently used this approach to correlate reversed-phase HPLC retention volumes of sulfonamides at pH 4.0 with log *P*, pKa, and biological activity. However, even if not applied to other studies, the present simple separation procedure is decidedly more convenient for analytical purposes than the many variations of more cumbersome chromatographic techniques reported previously.

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Simultaneous Solubilization of Steroid Hormones I: Estrogens and C_{21} Steroids

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Abstract □ The simultaneous solubilization of some estrogens and C_{21} steroids in aqueous polysorbate 40, tetradecyltrimethylammonium bromide, and sodium lauryl sulfate was studied. The less soluble estrogen estradiol was solubilized independently of the C_{21} steroids. The micellar solubilities of ethinyl estradiol and both corticosterone and hydrocortisone were independent of the presence of each other while the solubility of 11α-hydroxyprogesterone was enhanced by ethinyl estradiol. The solubilizations of ethinyl estradiol and the two C_{21} steroids, progesterone and 21-hydroxyprogesterone, were dependent on each other so that a varying amount of the steroid solubilized first was precipitated by an excess of the second steroid. If saturated solutions of the two steroids were mixed, no precipitation occurred. A possible mechanism for the simultaneous solubilization of steroids and its relation to structure are discussed.

Keyphrases □ Steroid hormones, various—simultaneous solubilization in aqueous surfactants □ Estrogens, various—simultaneous solubilization in aqueous surfactants □ Solubilization, simultaneous—various steroid hormones in aqueous surfactants

Micellar solubilization of drugs in aqueous solutions is well documented, and pharmaceutical systems have utilized surfactants for many years (1–3). Steroid hormones often have low aqueous solubility (3), and surfactants have been used to increase it. As early as 1944, it was noted that bile salts enhance the water solubility of steroid hormones (4). Since that time, the effect of steroid structure on solubilization and the maximum solubilization of steroids in solutions of surfactants have been investigated (5–9). Later reports described the micellar solubilization of testosterone (10–17) and the solubilization of steroids by lysophosphatidylcholine (18).

This study investigated the simultaneous solubilization of estrogens and C_{21} steroids in aqueous solutions of sodium lauryl sulfate, tetradecyltrimethylammonium bromide, and polysorbate 40. The study was undertaken to determine whether the steroids can be incorporated independently in the micelles as if separate loci for solubilization are involved or if an interaction occurs between the steroids that influences their solubility and can be related to chemical structure.

EXPERIMENTAL

Materials—The steroid hormones[1] were used as received after their melting points were found to be in good agreement with published values. Sodium lauryl sulfate[2] was purified by recrystallization from alcohol. Tetradecyltrimethylammonium bromide[3] and polysorbate 40[4] were used as received.

Solubilization Experiments—Solubilities were determined by equilibration of several concentrations of the aqueous surfactant with the steroids, followed by spectrophotometric analyses of suitably diluted aliquots as described previously (6). To two series of 5-ml ampuls, each containing the surfactant of known concentration, a sufficient amount of estrogen, *e.g.*, estradiol, or C_{21} steroid, *e.g.*, progesterone, was added to ensure an excess at equilibrium. The ampuls were closed and shaken mechanically in a thermostat at 20° (40° for sodium lauryl sulfate) for 72 hr until equilibrium was reached.

The contents of the ampuls then were filtered[5] or centrifuged to remove the undissolved steroid. The UV absorbance of the steroids was used to calculate the amount solubilized. To the estrogen-saturated surfactant solutions, an excess of C_{21} steroid was added; to the C_{21} steroid-saturated solutions, an excess of estrogen was added. The ampuls again were closed and shaken mechanically for 72 hr until equilibrium was reached. The undissolved steroid was removed, and the UV absorbance of the solutions was used to calculate steroid concentrations.

The UV absorbance of the solutions was recorded at around 280 nm for the estrogens and 240 nm for the C_{21} steroids with a spectrophotometer[6], using silica cells of 10- and 1.0-mm path length. Reference solutions containing known amounts of steroid were prepared in all surfactant solutions investigated to ascertain the possible influence of the solvent on the absorbance and for calculation of the molar absorptivity of the steroids. The simultaneous solubilization of two steroids in the same surfactant solution did not affect the molar absorptivity of each steroid.

RESULTS AND DISCUSSION

When various concentrations of polysorbate 40, tetradecyltrimethylammonium bromide, and sodium lauryl sulfate were saturated first with progesterone and then with estradiol and *vice versa*, the results were the same as if the solubilization had been done independently. The micelles of the colloids can solubilize the two steroids simultaneously without affecting their micellar solubility. In all cases, the amount of steroids solubilized increased linearly with the surfactant concentration. The amount of solubilized steroid can be calculated from the solubilization capacities measured previously (2, 3).

The micellar solubility of ethinyl estradiol is within the same range as that of progesterone in ionic surfactants and is considerably larger in nonionic surfactants (2, 3, 9). With progesterone and ethinyl estradiol as the estrogen component, the solubilization no longer occurred independently. The steroid added first to saturate the colloid solution pre-

[1] Fluka AG, Switzerland.

[2] Koch-Light Laboratories.
[3] K & K Laboratories.
[4] Tween 40, Atlas Chemical Industries.
[5] Schleicher & Schüll.
[6] Beckman DU-2.

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