TABLE V—COMPOSITION[a] AND POTENCY LOSS OF VITAMINS FROM B_1-B_{12}-NIACINAMIDE TABLETS EXPOSED TO METHANOL VAPOR FOR 1 MONTH AT ROOM TEMPERATURE

Batch No.	Potency of Each Tablet			% Loss		
	B_1, mg.	B_{12}, mcg.	Niacinamide, mg.	B_1	B_{12}	Niacinamide
12	1.4	4.0[b]	—	12.6	2.4	—
13	1.4	4.0[c]	—	14.2	4.9	—
14	1.4	—	22.0	0.0	—	0.0
15	1.4	4.0[b]	22.0	1.1	4.9	3.8
16	1.4	4.0[c]	22.0	1.1	14.6	4.0

[a] In addition to the vitamin, each tablet contained the same quantities of excipients as in the multivitamin tablet (Table I) and sufficient quantity of spray-dried lactose to adjust the weight to that of the multivitamin tablet. [b] As 1% cyanocobalamin dispersed in gelatin. [c] As 1% cyanocobalamin adsorbed on resin.

acid and vitamin B_1. No quantitative relationship between loss of vitamin B_{12} and loss of other vitamins could be observed. Nevertheless, it is quite apparent that methanol vapor when allowed to penetrate multivitamin tablets can cause loss of potency of individual vitamins, especially vitamins B_1 and B_{12}. It can be postulated that methanol vapor causes degradation of either ascorbic acid or vitamin B_1, or both, the degradation products of which in turn influence stability of vitamin B_{12}. This fact should be taken into consideration when methanol is used as a solvent in film coating of multivitamin tablets.

REFERENCES

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Keyphrases

Cyanocobalamin (vitamin B_{12})—stability

Film-coated multivitamin tablets—vitamin B_{12} stability

Methanol vapor effect—vitamins B_1, B_{12} stability

Ascorbic acid effect—vitamins B_1, B_{12} stability

Fluorometric Determination of Norgestrel and Structurally Related Steroids

By L. F. CULLEN, J. G. RUTGERS, P. A. LUCCHESI, and G. J. PAPARIELLO

A sensitive procedure, based on sulfuric acid-induced fluorescence, has been developed for the analysis of norgestrel (*dl*-13-ethyl-17α-ethinyl-17-hydroxygon-4-en-3-one) in tablets of low dosage, *i.e.*, 15–75 mcg. Optimum conditions for fluorescence have been established and the fluorescent properties of structurally related steroids studied to determine the selectivity of the reaction and mechanism of fluorescence formation. The reaction is specific for Δ^4-3-ketosteroids which have both a 17β-hydroxyl and 17α-alkyl or alkyne substitution and $\Delta^{1,3,5(10)}$-triene-3-ol steroids. A two-step mechanism is tentatively explained on the basis of the effects of temperature, time, initial acid concentration, and subsequent dilution with water on fluorogen development. Specificity of the method with respect to the analysis of intact norgestrel in the presence of its photochemical and thermal degradation products was demonstrated by comparison to quantitative thin-layer chromatography values. This procedure has been automated to permit unit dose analysis. This automated procedure is capable of analyzing 15 samples per hour with a relative standard deviation of ±1.4 percent at the 50-mcg. level.

THE SYNTHESIS of a new, extremely potent progestational steroid, norgestrel (*dl*-13-ethyl-17α-ethinyl-17-hydroxygon-4-en-3-one), and subsequent formulation of this steroid in tablets of low dosage, *i.e.*, 15–75 mcg. per tablet, had presented a challenging analytical problem. A sensitive, accurate, and rapid procedure was desired for content uniformity testing of the dosage form.

Steroids structurally related to norgestrel, having a characteristic Δ^4-3-keto group in the

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A-ring, have been quantitatively assayed utilizing the well-known 2,4-dinitrophenylhydrazine (1) and isonicotinic acid hydrazide (2) colorimetric methods, but these procedures lack the necessary sensitivity for single-tablet analyses at the dosage levels considered. A direct UV spectrophotometric procedure as well as the UV measurement of the salicyloyl hydrazone (3) or thiosemicarbazone (4) derivatives of the α,β-unsaturated carbonyl group did not exhibit the sensitivity or specificity required in the presence of tablet excipients for an accurate analytical method. Quantitative paper chromatographic (5) and thin-layer chromatographic (6) methods have been applied with some success but are limited, due to the lack of precision and accuracy characteristic of such procedures. Gas chromatographic (7) and polarographic (8, 9) procedures are available for several Δ^4-3-ketosteroids, but these procedures did not lend themselves to the analysis of large numbers of samples.

A fluorometric approach was considered to have the greatest potential and was studied further. Since the appearance of fluorescence in acid or base solution is a property of almost all steroids, there have appeared countless papers relating it to the identification, characterization, and analysis of steroids (10–13). However, there have been relatively few reports of fluorometric methods devised for Δ^4-3-ketosteroids at the sensitivity level required for unit-to-unit variation of formulations.

Bush and Sandberg (14) noted that paper chromatograms sprayed with sodium hydroxide developed an orange-yellow fluorescence specific for Δ^4-3-ketosteroids under UV irradiation. Subsequently, Abelson and Bondy (15) found that potassium *tert*-butoxide could produce the alkaline fluorescence reaction in solution in a satisfactory manner. However, the procedure requires meticulous care for satisfactory results, and it is insufficiently rapid and precise for the analysis of single tablets. Smith and Foell (5) demonstrated that Δ^4-3-ketones and $\Delta^{1,4}$-3-ketones, on paper chromatograms, exhibit a fluorescence under UV light when sprayed with isonicotinic acid hydrazide in acid alcohol. In attempting to apply this reaction to the problem at hand it was found that the procedure lacked the necessary sensitivity.

A sulfuric acid-induced fluorescence procedure used in the USP as an identification reaction for hydrocortisone (16) suggested a promising approach. A study of this reaction led to the development of a sulfuric acid fluorogenic reagent of definite and critical proportions which provided a stable and intense fluorescence suitable for quantitative measurement of microgram amounts of norgestrel in tablets of low dosage.

Although this procedure can be carried out by conventional techniques, adaptation of the method to an automated system offered advantages of accuracy, precision, and economy. Optimum conditions for the acid-induced fluorescence were established prior to the development of the automatic analyzer system. The results of a systematic study of the excitation and emission properties of norgestrel after treatment with varying concentrations of sulfuric acid and the influence of time and temperature are described. Fluorescent properties of structurally related steroids were studied to determine the selectivity of this reaction, possible applications of the procedure, and the mechanism of fluorescence formation.

EXPERIMENTAL

Apparatus—Spectral and optimum condition measurements were made on a Farrand spectrofluorometer with a fused quartz cell (10 × 10 × 45 mm. internal dimensions), using four 20-mμ slits and an RCA 1-P-28 photomultiplier tube. The excitation and fluorescent spectra are obtained on a Honeywell Electronik 15-strip-chart recorder.

A standard Technicon automatic analyzer (Auto-Analyzer) system consisting of the following modules: (*a*) Solid-prep sampler, programmed at 15 samples/hr. (*b*) Proportioning pump. (*c*) Continuous filter; speed 2. (*d*) Fluorometer; equipped with fluorescent lamp (Turner 110-853) and Corning filters: primary, No. 7-59 and secondary, No. 3-68. (*e*) Linear recorder. (*f*) Transmission lines and proportioning tubes carrying alcohol, sulfuric acid, and water consist of Acidflex tubing, while those carrying air are Tygon.

Reagents and Solutions—Eighty-five percent SDA No. 30 alcohol (ethanol–methanol, 10:1) in water (v/v); 85% sulfuric acid in water (v/v); all steroids used were of the purest grade available commercially.

Standard Norgestrel Solutions—Prepare a 0.5 mg./ml. solution of norgestrel in 85% denatured alcohol. The standard calibration curve is derived from a series of diluted solutions ranging in concentration from 15–85 mcg./ml.

Thin-Layer Chromatographic System—MN Silica Gel G-HR/UV precoated glass plates (10 × 20 cm. with a 250-μ adsorbent layer), obtainable from Analtech, Inc., were activated by heating at 105° for 0.5 hr. before use. The chromatogram was initially developed with a chloroform system. When the solvent front ascended approximately 15 cm. from the origin, the plate was removed, air-dried, and redeveloped with a benzene–chloroform (8:2 v/v) solvent system. Visualization was effected by: (*a*) inspecting the plate under short-wave (253.7 mμ) and long-wave (366.0 mμ) UV light; (*b*) spraying the plate with 85% ethanolic sulfuric acid and heating at approximately 105° for 5 min.

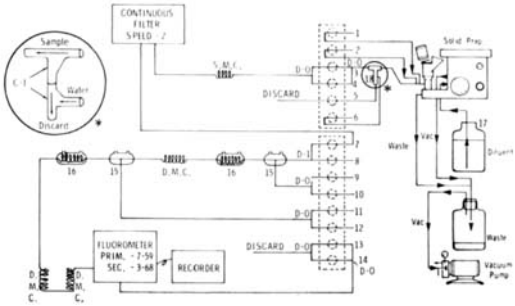


Fig. 1—Flow diagram for norgestrel. Key: 1, 0.8 ml./min. air; 2, 5.52 ml./min. 85% SDA No. 30 alcohol; 3,4, *2.03 ml./min. sample;* 5, *0.6 ml./min.;* 6, *0.42 ml./min. water;* 7, *2.76 ml./min. sample;* 8, *2.90 ml./min. air;* 9,10, *2.76 ml./min. 85% H_2SO_4;* 11,12, *2.03 ml./min. water;* 13,14, *2.03 ml./min. debubbler;* 15, *A-7 water-cooled TEE;* 16, *water-cooled mixing coil (No. 114–209);* 17, *reservoir 85% SDA No. 30 alcohol;* 18, *decantation trap.*

Automated Methodology—Figure 1 is the flow diagram indicating the automated equipment arrangement for this analytical procedure. In operation, four standards of the appropriate levels of norgestrel are placed on the sample plate, followed by samples of the intact or disintegrated tablets. Standardization is repeated at the end of a series of 15 samples in order to minimize the effects of reagent changes and instrumental variations. Samples are introduced into the Solid-prep sampler, programmed at 15 samples/hr., and homogenized in the 85% denatured alcohol. During the period of homogenization the sample is diluted and dissolution of norgestrel occurs. A small portion of the the mixture is aspirated into the flow system and is automatically filtered to remove insolubles. To prevent mechanical obstructions from the insoluble excipient materials at the tubing connections, a decantation trap between the Solid-prep unit and the proportioning pump removes the majority of the solid matter. The residual insolubles are removed by the continuous filter module. After passage through the filter module, the filtrate is segmented with air and then combined with a stream of the 85% sulfuric acid. The sample stream is immediately cooled in a water-jacketed coil and passed through a double mixing coil (D.M.C.) to permit a thorough mixing. After the stream is diluted with water, again cooled, and passed through double mixing coils, the resultant fluorometric development is measured. Calculations are made using corresponding fluorescent intensities of standards and solid dosage formulation samples.

RESULTS AND DISCUSSION

Optimum Conditions—Denatured alcohol was considered the ideal solvent for dissolution of the norgestrel during the automated extraction procedure because of the steroid's solubility in this solvent and the relatively low cost of large volumes of this solvent. In investigating the influence of alcohol concentration on acid-induced fluorescence, it was demonstrated that a significant level of alcohol could be tolerated without a fluorescent quenching effect. With a final sulfuric acid to alcohol volume ratio of 2:1 and a reaction time of 5 min., a series of acid concentrations from 25 to 100% (v/v) was evaluated. Each sample was measured against an acid–alcohol reagent blank of the appropriate composition. As illustrated in Fig. 2, maximum response is produced with 85% sulfuric acid.

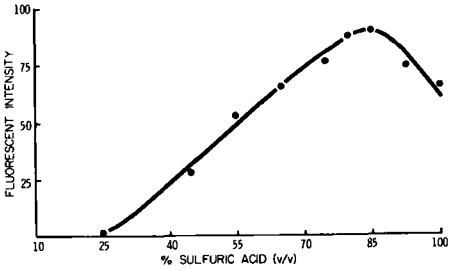


Fig. 2—Effect of initial sulfuric acid concentration on fluorescent intensity.

Considering the maximum volume of alcohol that could be aspirated into the automated flow system from the Solid-prep unit and the volume of 85% sulfuric acid required for sufficient fluorescence, it was realized that the final solution could not satisfy the essential flow dynamics of the fluorometer flow cell. As a result a series of experiments was performed to determine the effect of dilutions with water, after initial sulfuric acid-induced fluorescence. Diluting with water did not quench fluorescence, while the blank fluorescence produced by the reagents was significantly reduced. Thus, the flow difficulties could be overcome without a critical loss in sensitivity by the addition of water into the system, after initial fluorogen development with 85% sulfuric acid.

The excitation and fluorescence spectra for norgestrel after the addition of 85% sulfuric acid and on subsequent dilution with water appear in Fig. 3. Dilution with 3 ml. of water after fluorogen development corresponds to a final sulfuric acid concentration of 38%. This represents the optimum water dilution level with respect to both flow characteristics of the automated procedure and sensitivity requirements. Any further addition of water simply produced a proportional decrease in intensity. Furthermore, the addition of water to the system produced an unexpected shift in the excitation

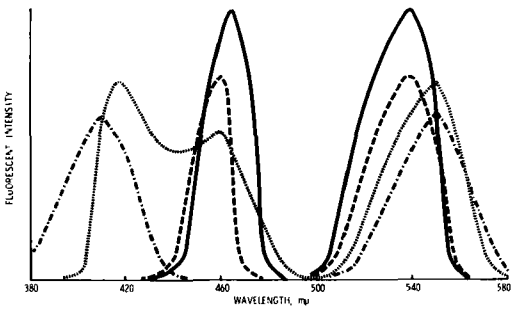


Fig. 3—Excitation and emission spectra of norgestrel. Key: ——, initial addition of 85% H_2SO_4; ---, dilution with 1 ml. H_2O; · · ·, dilution with 3 ml. H_2O; -·-, dilution with 5 ml. H_2O.

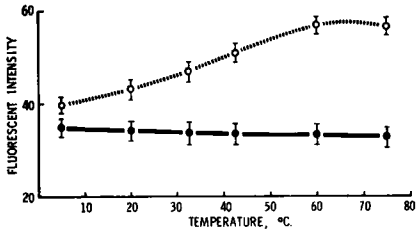


Fig. 4—Relationship between fluorescence development and temperature. Key: ---, initial addition of 85% H_2SO_4; ——, dilution with 3 ml. H_2O.

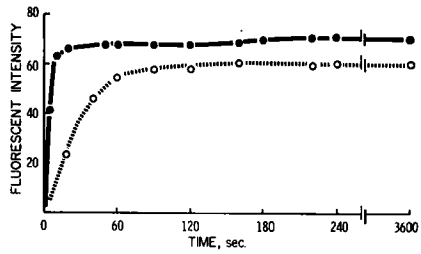


Fig. 5—Relationship between fluorescence development and time. Key: ---, initial addition of 85% H_2SO_4; ——, dilution with 3 ml. H_2O.

maxima. This unique spectral characteristic of norgestrel at the 38% sulfuric acid level suggested a basis for a more selective assay in the presence of other structurally related 3-keto-17-hydroxyl or 3,17-diketo-type steroids which have reported excitation maxima in sulfuric acid in the 460–470-mμ range (17). This will be discussed in greater detail in the section on specificity.

The fluorescence after the addition of 85% sulfuric acid and the fluorescence of the water-diluted final solution as a function of temperature and time were measured. Fluorometric measurements were made against reagent blanks treated in a similar manner. In the temperature study the intensities were recorded after exactly 5 min. The time study was run at 25°. From Figs. 4 and 5 it is evident that the fluorescence development with 85% sulfuric acid is more sensitive to time and temperature than the fluorescence of the water-diluted, final solution.

Data obtained under the above experimental conditions indicate that the maximum fluorescent yield of the initial fluorogen formation occurs above 60°. However, with respect to automated analysis adaptation, the small loss in sensitivity by maintaining the entire system at ambient room temperature overbalances the physical difficulties introduced by proportioning 85% sulfuric acid at 60°.

Specificity—In order to determine the selectivity of the reaction and the effect of group substitution on the molecule, a number of structurally related steroids were studied. The fluorescent intensities with respect to norgestrel were obtained under the exact analytical conditions described under *Automated Methodology*. The remarkable selectivity of the fluorogenic reaction is illustrated in Table I. Of the various steroids examined, only the particular group of Δ^4-3-ketosteroids which have both a 17β-hydroxyl and 17α-alkyl or alkyne substitution and $\Delta^{1,3,5(10)}$-triene-3-ol steroids produced a significant level of fluorescence. Δ^4-Steroids, $\Delta^{1,4}$-diene-3-ketosteroids, and $\Delta^{2,5(10)}$-diene-3-methoxy-steroids did not fluoresce under the prescribed conditions.

From these experiments it would appear that fluorescent intensity is strongly affected by the alkyl group at the 13-position. The replacement of a methyl group for an ethyl group as illustrated by pairs 19-norethisterone and norgestrel, nortestosterone and 13-ethyl-17β-hydroxygon-4-ene-3-one produced a decrease in the fluorescence to approximately one-half the ethyl substituted compounds.

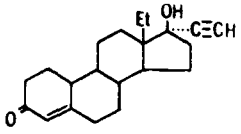
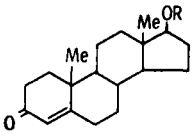
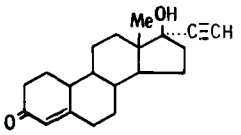
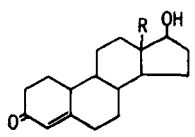
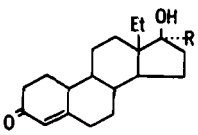
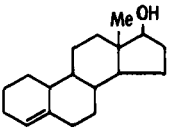
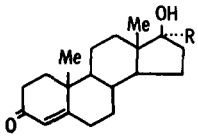
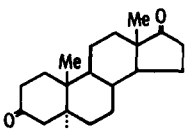
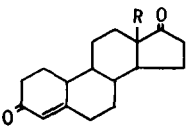
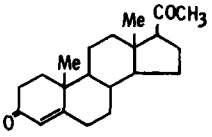
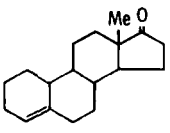
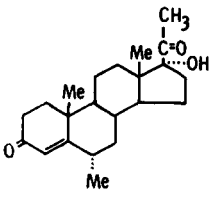
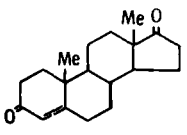
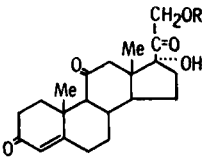
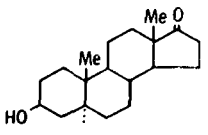
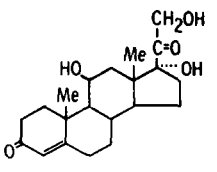
The replacement of a hydroxyl group by a ketone group at the 17-position in steroids which differ only in this single structural characteristic almost completely removes its capability to develop fluorescence.

In order to study the chemistry of the fluorogen formation and the influence of the 13-position alkyl group on the spectral characteristics, the effect of dilution with water upon the excitation spectra of all steroids which produced a fluorescent yield in the automated system were observed. As illustrated in Table II, progressive dilution produced unique sequences of change in the excitation spectra of Δ^4-3-ketosteroids with ethinyl and hydroxyl groups at the 17-position, quite comparable with those observed for norgestrel. It should be noted that further dilution beyond 31% sulfuric acid did not cause a further shift in the excitation spectra. However, Δ^4-3-ketosteroids which have a hydroxyl, keto, acetyl, ketol or combination ketol and hydroxyl functional groups at the 17-position demonstrated excitation wavelength maxima at the characteristic 460–470-mμ range and are not affected by the final acid concentration. The absence of an excitation maximum shift for steroids IV, X, and XI emphasizes the necessity for the presence of both the ethinyl group at the 17-position and α,β-unsaturated ketone in the A-ring. Furthermore, emission spectra and the data presented in Table II demonstrate that the increased intensity produced by an ethyl substitution at the 13-position is not a result of an alteration in the characteristic spectral curves of compounds containing this group, for example, compare steroid pairs I and II.

Chemistry of Reaction—Evaluation of the data suggested that under the experimental conditions used in this study the sulfuric acid-induced fluorescence of norgestrel is a two-step reaction. Eighty-five percent sulfuric acid developed a fluorescent intensity fourfold greater than 38% sulfuric acid (Fig. 2). However, after initial fluorogen formation with 85% sulfuric acid and subsequent dilution to a final sulfuric acid concentration of 38%, the relative fluorescent intensities were essentially equivalent and the unique hypsochromic shift in excitation maxima from 463 to 418 mμ was introduced (Fig. 3). The time and temperature effects (see Figs. 4 and 5) on the two-step fluorogen formation, *i.e.*, (*a*) addition of 85% sulfuric acid, (*b*) dilution with water, further support a two-stage reaction mechanism.

Fluorescence spectral data and thin-layer chromatography (TLC) data were collected to determine whether or not denatured alcohol participated in the fluorogen formation. Samples with and without alcohol showed similar excitation and fluorescence spectra with equivalent responses when treated with

TABLE I—FLUORESCENCE OF NORGESTREL AND STRUCTURALLY RELATED STEROIDS

Structure	Compound	Relative Fluorescent Intensity	Structure	Compound	Relative Fluorescent Intensity
	*Norgestrel*	100		Testosterone (R = H) Testosterone acetate (R = acetyl)	Nil Nil
	19-Norethisterone	58		13-Ethyl-17β-hydroxygon-4-ene-3-one (R = ethyl) Nortestosterone (R = methyl)	13 6
	17α-Chloroethinyl-13-ethyl-17-hydroxygon-4-ene-3-one (R = chloroethinyl) 13, 17α-Diethyl-17-hydroxygon-4-ene-3-one (R = ethyl)	23 21		Estrenol	Nil
	Ethisterone (R = ethinyl) Methyltestosterone (R = methyl)	28 166		5α-Androstane-3, 17-dione	Nil
	13-Ethylgon-4-ene-3, 17-dione (R = ethyl) Norandrostenedione (R = methyl)	3 Nil		Pogesterone	Nil
	Estrenone	Nil		Medroxyprogesterone	Nil
	Δ⁴-Androstene-3,-17-dione	Nil		Cortisone (R = H) Cortisone Acetate (R = acetyl)	Nil Nil
	Epiandrosterone	Nil		Hydrocortisone	5

(*Continued on next page.*)

TABLE I—(*Continued.*)

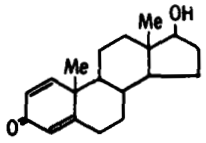
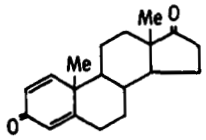
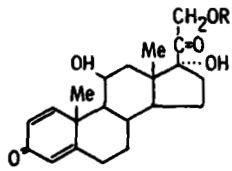
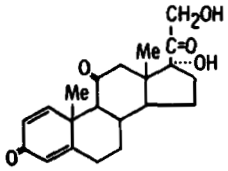
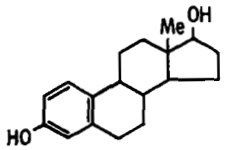
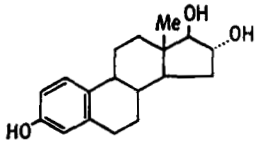
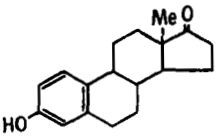
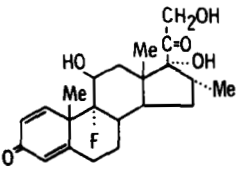
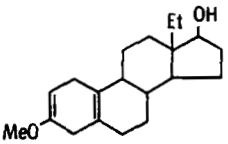
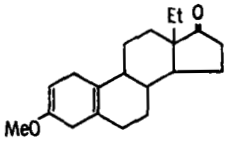
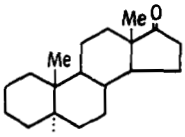
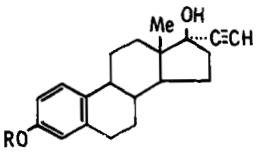
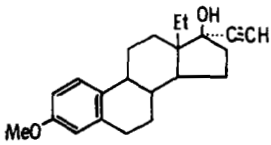
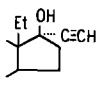
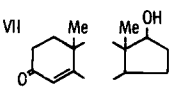
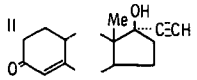
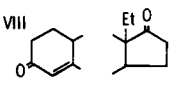
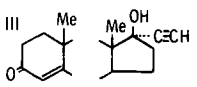
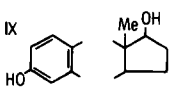
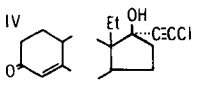
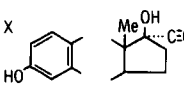
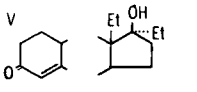
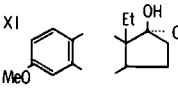
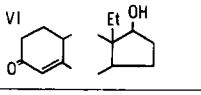
Structure	Compound	Relative Fluorescent Intensity
	$\Delta^{1,4}$-Androstadiene-17β-ol-3-one	Nil
	$\Delta^{1,4}$-Androstadiene-3,17-dione	Nil
	Prednisolone (R = H) Prednisolone acetate (R = acetyl)	Nil Nil
	Prednisone	Nil
	β-Estradiol	46
	Estriol	Nil
	Estrone	8
	Dexamethasone	Nil
	13-Ethyl-3-methoxygona-2,5(10)-diene-17β-ol	Nil
	13-Ethyl-3-methoxygona-2,5(10)-diene-17-one	Nil
	5α-Androstane-17-one	Nil
	Ethinyl estradiol (R = H) *mestranol* (R = methyl)	660 880
	13-Ethyl-17-ethinyl-3-methoxygona-1,3,5(10)-triene-17β-ol	1,020

TABLE II—EFFECT OF FINAL SULFURIC ACID CONCENTRATION ON EXCITATION PROPERTIES

Structure		Final Sulfuric Acid Concn.			Structure		Final Sulfuric Acid Concn.		
A-Ring	D-Ring	49%	38%	31%	A-Ring	D-Ring	49%	38%	31%
I		460	418	408	VII		465	465	465
II		460	412	406	VIII		462	462	462
III		465	425	415	IX		460	460	460
IV		461	461	460	X		535	535	535
V		473	472	465	XI		545	543	540
VI		470	470	465					

concentrated or diluted sulfuric acid, indicating that alcohol did not enter into the reactions. TLC confirmed these data by finding the same number of fluorophors with identical R_f values in the alcohol and alcohol-free systems.

Any hypothesis concerning the reaction mechanism must consider the reversibility or irreversibility of the individual reaction step in the fluorogen formation. TLC demonstrated that the products formed by the action of 85% sulfuric acid on norgestrel did not revert back to the intact steroid on progressive dilution of the reaction mixture with water, thereby indicating induced irreversible changes. The multiplicity of products obtained chromatographically from extracts of the water-diluted reaction mixtures was additional evidence of this irreversible reaction.

Spectral data demonstrated that in the second stage of fluorogen formation the intensities of the principal excitation maxima of norgestrel at 408 and 460 mμ are dependent on acid concentration and are reversible.

From these findings it would appear that the initial steroid-acid interaction is an extremely complex phenomenon, involving the possibility of dehydration, sulfonation, or isomerization. The second part of the mechanism is an immediate reversible reaction which suggests an ionic species interaction.

With the aid of preparative TLC, studies are now being carried out to isolate the products formed during the reaction. Based upon the identification of these fluorogens, a definitive mechanism will be presented.

Suitability as Stability Method—Specificity of the method for analysis of intact norgestrel in the presence of its photochemical and thermal degradation products was demonstrated by comparing analytical values of intentionally degraded samples to those obtained by quantitative TLC. In the TLC procedure, the norgestrel was separated from its degradation products on a TLC plate. The intact norgestrel was removed from the plate, eluted, then assayed to obtain a quantitative value. Examples of the data obtained are summarized in Table III. Since there is good agreement between the values by the two techniques it is concluded that the fluorometric procedure is stability indicating.

Linearity—An actual working curve which was obtained by analyzing standards of known concentration is demonstrated in Fig. 6. A linear relationship exists for fluorescent intensity and norgestrel concentration in the range which was studied. A recording of the steady state, during which time an 85-mcg. standard was continuously sampled, is also shown.

Precision—Repeatability of the procedure was demonstrated by assaying equal aliquots of norgestrel standard solutions. Relative standard deviations of 0.9, 1.4, 2.2, and 3.1% were determined at the 75, 50, 25, and 15-mcg. levels, respectively,

TABLE III—COMPARISON OF FLUOROMETRIC AND QUANTITATIVE TLC ANALYSIS OF INTENTIONALLY DEGRADED NORGESTREL SAMPLES

Sample Treatment	% Initial	
	Fluorometric Method	TLC Method
Suspension of the norgestrel heated in 0.1 *N* NaOH for 2 hr. at 70°	68	60
Norgestrel stored at 210° for 30 min.	90	87
Norgestrel exposed to UV light for 6 months	79	80
Synthetic mixture containing 0.5 mg. of norgestrel in 100-mg. tablet excipients stored at 100° for 49 days	42	50

Table IV—Precision of Fluorometric Method on Analysis of Composite Tablet Sample

Norgestrel, mcg. per Equivalent Tablet Weight	
49.4	50.3
50.9	50.8
48.7	50.6
49.7	49.2
50.3	50.9
51.3	49.1
50.6	49.6
50.3	50.6
49.1	49.4
50.6	49.4
Average: 50.0 mcg.	
Relative standard deviation: ±1.5%	

by performing 30 replicate assays. A series of 20 replicate assays was run on a composite sample of a tablet formulation at the 50-mcg. norgestrel per tablet level. The resulting precision data in Table IV indicate a relative standard deviation of 1.5%.

Sensitivity—The flow diagram of Fig. 1 is applicable to the analysis of samples which initially contain 15 to 200 mcg. of norgestrel. At the 15-mcg. level, the final concentration of norgestrel is approximately 0.06 mcg./ml. However, the method has been shown to be sensitive enough so that it can easily detect less than 0.02 mcg. of norgestrel/ml. Consequently, the method offers greater sensitivity than previously reported methods for the Δ^4-3-ketosteroids.

Accuracy—The accuracy of the method was determined by adding 25.0 mcg. of norgestrel to a tablet excipient mixture and measuring the percentage recovery. The data collected indicate recoveries of 97 to 103% of the theoretical amount present with a relative standard deviation of 2.1%.

A further evaluation of the accuracy of the procedure was ascertained by studying the effect of common tablet excipients and lubricants on fluorescent development. The inactive components evaluated were lactose, sucrose, magnesium stearate, stearic acid, microcrystalline cellulose (Avicel), alginic acid, an ion-exchange resin (Amberlite), methylcellulose (Methocel), and starch. No interference was experienced from these materials.

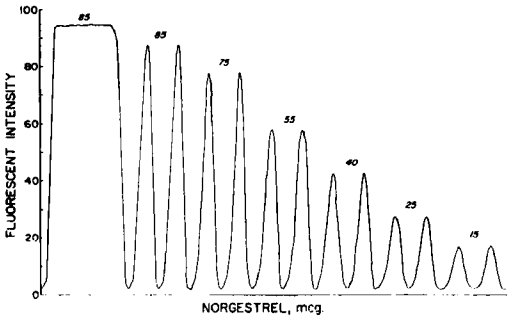


Fig. 6—Typical recording of automated system for norgestrel standards at the rate of 15 samples/hr.

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Keyphrases

Norgestrel tablets—analysis
Fluorometry—analysis
TLC—separation
UV light—spot visualization
Automated procedure—norgestrel analysis
Diagram—automated system