# Nature of Acid-Induced Fluorescence of 17α-Methyltestosterone

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**Abstract** □ The fluorescence of the 17α-methyltestosterone–trichloroacetic acid reaction product, 1,2,10,15,16,17-hexahydro-10,17,17-trimethylcyclopenta[a]phenanthren-3-one, in strong acid was investigated. Structural requirements for fluorescence were derived from absorption and fluorescence studies of related phenanthrenones and cinnamylidene compounds possessing a similar chromophore. All compounds showed fluorescence intensity that was structure and pH dependent. Fluorescence is attributed to both enol and protonated species.

**Keyphrases** □ 17α-Methyltestosterone—fluorescence of reaction product with trichloroacetic acid □ Fluorescence—study of reaction product of 17α-methyltestosterone with trichloroacetic acid □ Phenanthrenones, various—structural requirements for fluorescence in strong acid □ Cinnamylidenes, various—structural requirements for fluorescence in strong acid □ Steroids—17α-methyltestosterone, fluorescence of reaction product with trichloroacetic acid

Several fluorometric methods for the determination of steroids and steroidal glycosides devoid of native fluorescence are based on the generation of fluorophores by the action of dehydrating agents on the steroid ring (1–5). The nature and complexity of the reactions remain largely unknown, and the numerous reaction products formed are difficult to isolate (6, 7).

## BACKGROUND

Successful development of a fluorometric method with total selectivity for the determination of a particular steroid ideally depends on a reaction taking place on the ring system to form a product with unique photophysical properties. Since this reaction is difficult to achieve, steroid fluorescence analysis has depended largely on chemical transformation of the steroid skeleton using concentrated mineral acids. The use of moderately strong organic acids in steroid analysis has, however, been barely explored (5).

The use of organic acids may reduce the number of reaction products and possibly lead to more specific fluorophores from individual steroids. In an investigation to develop a new fluorometric method for digoxin analysis (8), trichloroacetic acid was highly effective in locating steroidal substances as fluorescent and strongly UV-absorbing products on TLC plates. Of more than 20 steroids subjected to this reagent, the cardiac glycosides and aglycones, 17α-methylandrost-5-ene-3,17-diol (I) and 17α-methyltestosterone (II), reacted most readily (9).

Examination of the trichloroacetic acid reaction with II under controlled conditions (10) showed the major product formed to be 1,2,10,15,16,17 - hexahydro - 10,17,17 - trimethylcyclopenta[a]phenanthren-3-one (III). Other phenanthrenones (IV and V) and cinnamylidene compounds (VI–VIII) with an approximately similar chromophore were synthesized (10–12) and studied to provide information on the structural requirements needed for the fluorescence of III.

## EXPERIMENTAL

**Apparatus**—Absorption measurements were obtained on a recording spectrophotometer[1]. Fluorescence spectra were measured with a spectrophotofluorometer[2] equipped with a 200-w mercury–xenon lamp and an x–y recorder[3]. The following instrumental settings were used for all fluorescence measurements: slit arrangement, 3; photomultiplier shutter, 5 mm; and sensitivity control, 45.

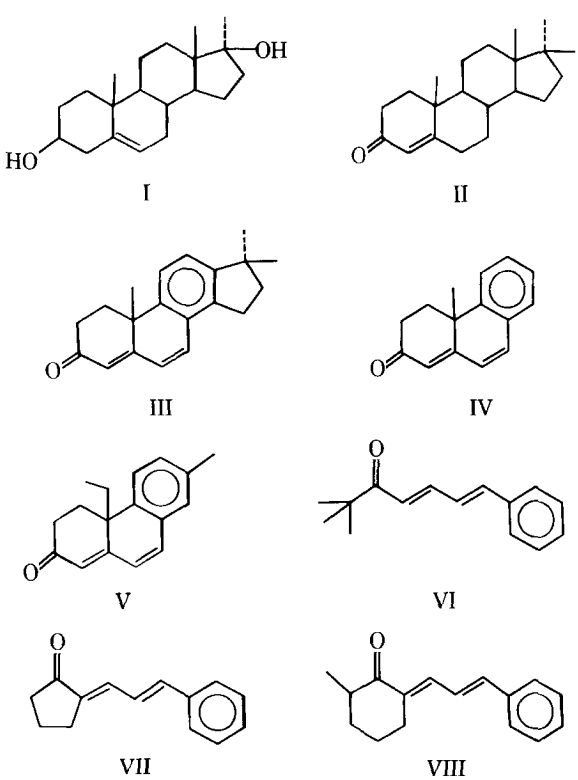
**Table I—Absorption and Fluorescence Characteristics of Phenanthrenones and Cinnamylidene Compounds in 80% Phosphoric Acid**

| Compound | Absorption $\lambda_{max}$ | Log ε | Excitation $\lambda_{max}$ | Emission $\lambda_{max}$ | Quantum Yield |
|---|---|---|---|---|---|
| III | 480 | 4.35 | 465 | 535 | 0.0015[a] |
| IV | 463 | 4.23 | 465 | 530 | 0.33[a] |
| V | 480 | 4.19 | 470 | 550 | 0.017[a] |
| VI | 430 | 4.49 | | | |
| | 320 | 4.07 | 370 | 420 | 0.032[b] |
| VII | 430 | 4.23 | 380 | 430 | 0.041[b] |
| VIII | 390 | 4.42 | 370 | 420 | 0.041[b] |

[a] Quantum yields ($\phi_f$) are relative to fluorescein ($\phi_f$ = 0.87) in 0.1 *N* NaOH (13). [b] Quantum yields ($\phi_f$) are relative to quinine sulfate ($\phi_f$ = 0.55) with excitation at 366 nm (14).

**Reagents**—Phenanthrenones (III–V) and cinnamylidene compounds (VI–VIII) were used without further purification. Analytical grade 88% phosphoric acid was diluted with double-distilled absolute ethanol BP to provide solutions of varying acidity for absorptiometric and fluorometric measurements. Analytical grade 60% perchloric acid was prepared similarly. Spectrograde hexane was used for UV studies.

**Solutions for Fluorescence Measurements**—Fluorescence efficiencies were evaluated by comparing the intensities at the wavelength of maximum emission with respect to the emission intensity of the reference compound (Table I). The sample concentration was adjusted carefully to give the same absorbance as the reference compound at the excitation wavelength chosen. Care was taken to ensure that the concentrations of the samples were well below $10^{-5}$ *M* to avoid reabsorption




[1] Beckman Acta V.
[2] Aminco-Bowman, American Instrument Co., Silver Spring, Md.
[3] Hewlett-Packard.

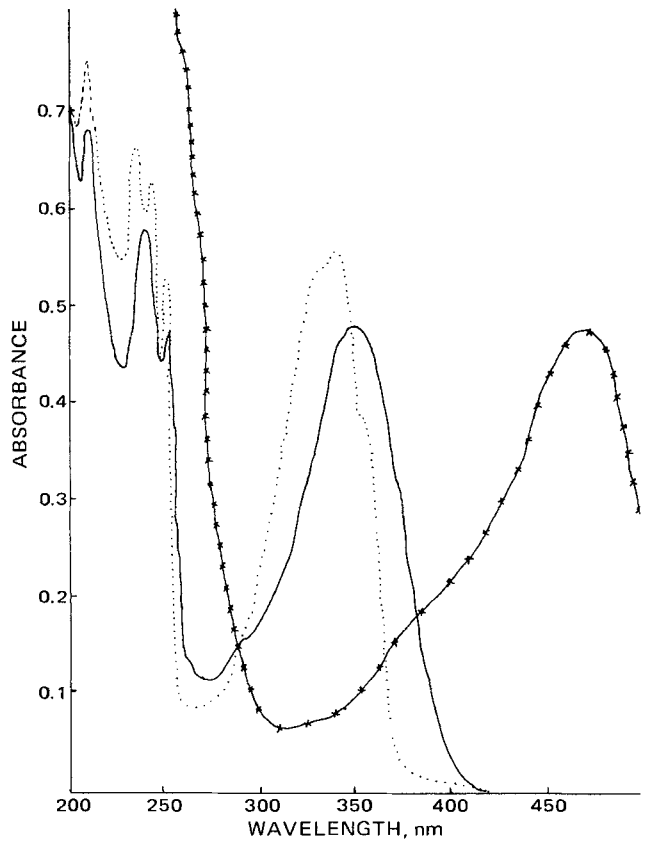



**Figure 1**—*Absorption characteristics of phenanthrenone IV in ethanol* (——), n-*hexane* (· · ·), *and 88% $H_3PO_4$* (×—).

of the fluorescence emission (15) in the 1-cm cell at the right-angle illumination viewing geometry.

No differences were observed in intensity on degassing the solutions. No corrections were made for the refractive index of the solutions. The reproducibility of duplicate determinations of the fluorescence quantum yields of the compounds was about ±5%, and the emission spectra were corrected.

## RESULTS AND DISCUSSION

The basic phenanthrenone (IV) is essentially identical in structure to III without the cyclopentane side chain and differs slightly from V in substitution. These phenanthrenones have a very similar chromophore to the cinnamylidene compounds (VI–VIII), which are, comparatively, floppy molecules. The compounds studied were chosen to elucidate the

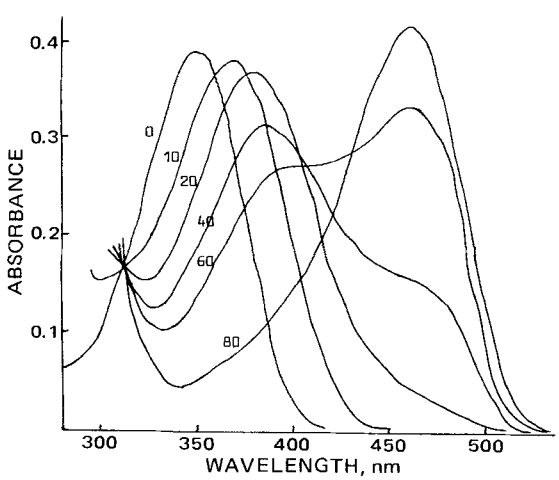



**Figure 2**—*Enolization of the chromophore of phenanthrenone IV shown as the change in absorption on increasing the phosphoric acid concentration from 0 to 80% in ethanol.*

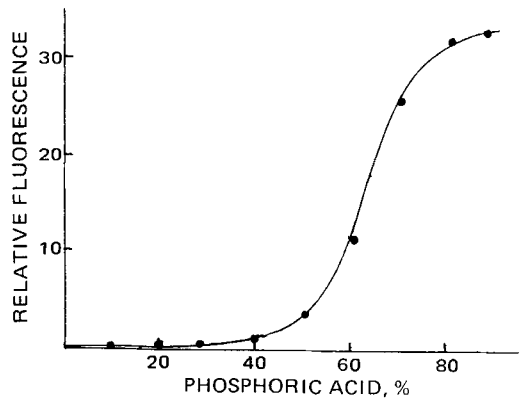



**Figure 3**—*Relative fluorescence of a $10^{-6}$ M solution of phenanthrenone IV as a function of phosphoric acid concentration in ethanol.*

structural requirements, if any, and conditions necessary to provide fluorescence emission of the 17α-methyltestosterone–trichloroacetic acid reaction product (III).

The phenanthrenone (IV) in ethanol showed three absorption bands in the UV region (Fig. 1). The intense band at about 350 nm was probably due to $\pi\pi^*$ and $n\pi^*$ absorption of the conjugated chromophore. Of similar intensity was the band at 240 nm, which was interpreted as a $\pi\pi^*$ transition with the character of an intramolecular charge transfer band (16). The most intense band, at about 200 nm, was probably a perturbed local excitation band corresponding to the benzene $^1L_a$ band.

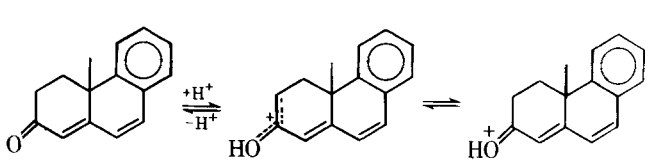
The $n\pi^*$ transition of this compound was not an isolated band. Its conjugated system was extended enough for the $n\pi^*$ band to be swamped under the more intense $\pi\pi^*$ band (17). On changing to hexane, both the charge transfer and long wavelength absorption bands showed fine structure and were accompanied by solvent shifts of 8 and 13 nm, respectively, toward shorter wavelengths. Phosphoric acid, however, caused a marked change in the chromophore, resulting in the formation of a new absorption band in the visible region (Fig. 1).

TLC evaluation of these ketones revealed that only the phenanthrenones showed appreciable fluorescence; the cinnamylidene compounds were nonfluorescent. In both hexane and ethanol, all compounds (III–VIII) were nonfluorescent. In both perchloric and phosphoric acids, however, they all exhibited intense fluorescence, which was structure and pH dependent.

The effects of pH on the absorption and emission characteristics of IV are summarized in Figs. 2 and 3. It is suggested that in strongly acidic solvents, enolization of the chromophore occurs (Scheme I) and it is the enol form that exhibits fluorescence. This suggestion is consistent with the observed disappearance of fluorescence on neutralization of an acidic solution of the compound followed by ether extraction and subsequent quantitative recovery of the ketonic species. Hence, the fluorescence intensity observed correlated closely with polarity (Fig. 3).

The quantum yields of fluorescence of both phenanthrenones and cinnamylidene compounds (Table I) correlated closely with structure. Compound IV was the most intensely fluorescent in the series since it had the high quantum yield of 0.33. It is likely that the decrease in quantum efficiency observed for both III and V was due to substitution on the aromatic ring. The floppy cinnamylidene compounds fluoresced at shorter wavelengths than the phenanthrenones, and their quantum yields were lower than that of IV by a factor of about 10. Their fluorescence may similarly be attributed to possible enolization of the chromophore under acid conditions.

The *tert*-butyl derivative (VI) is unlikely to enolize in acid. Its fluorescence may be attributed to the $\pi\pi^*$ protonated species since the ketone was recovered on neutralization. Since both excitation and emission of the *tert*-butyl derivative (VI) and the cinnamylidene compound (VIII) occurred at the same wavelengths (Table I), the fluorescence of the ketones (III–VIII) may be attributed, at least in part, to the $\pi\pi^*$ protonated species.




*Scheme I*

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# Contact Angles of Pharmaceutical Powders


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**Abstract** □ Contact angles of pharmaceutical powders were determined by measuring the maximum height of a drop of a saturated solution on a presaturated compact of the material. The results for a series of drugs are presented.



**Keyphrases** □ Powders, various pharmaceutical—contact angles, densities, and surface tensions □ Contact angles—various pharmaceutical powders □ Densities—various pharmaceutical powders □ Surface tensions—various pharmaceutical powders


The wetting of solid dosage forms is an important initial step in the process of drug dissolution both *in vitro* and *in vivo*. Determination of contact angles of solids gives a measure of their wettability, but problems arise when the solid is finely divided as a powder. Recently (1), a method that consists essentially of measuring the maximum height of a drop of a saturated solution formed on a presaturated compact of the material was used successfully. This report presents the results of contact angle determinations for an additional series of pharmaceutical powders.

## EXPERIMENTAL

**Materials**—The materials and standards are listed in Table I.

**Methods**—The contact angles were determined using a technique described earlier (1). The densities and surface tensions of the saturated solutions and the densities of the solids were determined with a balance[1], a tensiometer[2], and an air comparison pycnometer[3], respectively. The liquid measurements were carried out at 23°. Saturated solutions were prepared by allowing excess solid to equilibrate with distilled water at a constant temperature of 23°.

## RESULTS AND DISCUSSION

Table I lists the contact angles of more than 50 drugs and excipients. The values vary between 21° (ampicillin trihydrate) and 124° (aceto-

[1] Mohr, G. Kern, Ebingen, West Germany.
[2] Du Noüy K 8600 Krüss, Hamburg, West Germany.
[3] Model 930, Beckman Instruments Ned. N.V., Amsterdam, The Netherlands.

**Table I—Measured Values of Contact Angles of Pharmaceutical Powders** [a]

| Material | Standard | Surface Tension, dynes/cm | Density Solid, g/cm³ | Contact Angle, θ° |
|---|---|---|---|---|
| Acetohexamide[b] | — | 70.7 | 1.25 | 124 |
| Adipic acid[c] | Laboratory grade | 58.3 | 1.38 | 72 |
| Allobarbital[c] | Ph. Ned. VI | 69.4 | 1.28 | 61 |
| Aluminum stearate[d] | — | 69.4 | 1.05 | 120 |
| Aminophylline[e] | — | 71.5 | 1.44 | 47 |
| Aminophylline (anhydrous)[c] | Ph. Ned. VII | 65.5 | 1.44 | 40 |
| Aminopyrine[c] | Ph. Ned. VI | 57.5 | 1.19 | 60 |
| Amobarbital[f] | USP XVII, BP 1973 | 55.7 | 1.17 | 102 |
| Ampicillin (anhydrous)[g] | — | 47.9 | 1.37 | 35 |
| Ampicillin trihydrate[g] | — | 38.6 | 1.37 | 21 |
| Aprobarbital[c] | NF XII | 57.8 | 1.28 | 75 |
| Barbital[c] | Ph. Ned. VI | 63.5 | 1.24 | 70 |
| Boric acid[c] | Ph. Ned. VI | 68.0 | 1.51 | 74 |
| Butabarbital[f] | BP 1973 | 62.0 | 1.26 | 82 |
| Butalbital[f] | NF XIII | 58.8 | 1.25 | 87 |
| Butethal[c] | BP 1968 | 51.0 | 1.18 | 78 |
| Calcium carbonate[c] | Ph. Ned. VI | 72.4 | 2.68 | 58 |
| Calcium stearate[d] | — | 70.7 | 1.03 | 115 |
| Calcium sulfate dihydrate[c] | Laboratory grade | 44.5 | 2.32 | 64 |
| Cyclopentobarbital[f] | — | 60.6 | 1.29 | 76 |
| Diazepam[h] | BP 1973 | 64.0 | 1.37 | 83 |
| Digoxin[i] | Ph. Eur. | 68.1 | 1.28 | 49 |
| Ephedrine hydrochloride[c] | Ph. Ned. VI | 48.3 | 1.20 | 51 |
| Heptobarbital[c] | Ph. Ned. VI | 71.3 | 1.47 | 74 |
| Hydrochlorothiazide[j] | USP XVIII | 72.4 | 1.69 | 51 |
| Indomethacin[j] | NF | 71.5 | 1.39 | 90 |
| Isoniazid[h] | Ph. Ned. VI | 61.6 | 1.42 | 49 |
| Isoxsuprine hydrochloride[k] | — | 66.0 | 1.28 | 50 |
| Lithium carbonate[c] | Laboratory grade | 71.2 | 2.08 | 50 |
| Lithium chloride[d] | Analytical grade | 94.7 | 2.08 | 51 |
| Mephobarbital[c] | Ph. Ned. VI | 68.3 | 1.38 | 74 |
| Meprobamate[c] | Ph. Ned. VI | 51.9 | 1.24 | 83 |
| Nitrofurantoin[h] | USP XVIII | 70.6 | 1.69 | 69 |
| Oxalic acid[l] | Laboratory grade | 70.7 | 1.66 | 31 |