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A STUDY ON STABILITY OF MESTRANOL

TAUQIR AHMAD, SHAMIM AKHTAR and SAFEER HAIDER
Department of Pharmaceutical Chemistry, Faculty of Pharmacy,
University of Karachi, Karachi-75270

ABSTRACT

Mestranol, a synthetic oestrogen, is an oral contraceptive given alongwith progestogens. It is light, heat and air sensitive and on uv irradiation new polymeric species of glucosiduronate and sulphate conjugate are formed. The new species fractions of mestranol are separatable over sephadex in column chromatography and characterized by analytical techniques employed in this investigation. This;nvestigation is aimed to study formation of these species quantitatively for stability induced formulations.

Introduction

Mestranol (17a-ethynyl-3-methoxyestra-1,3,5 (10)-trien-17b-ol) is a steroidal oestrogen first synthesized by Schulz and Pfeiffer (1961) and Rosenkranz and Kind (1962), possesses an aromatic A-ring of steroidal nature related biologically to the natural human oestrogens having significant effect on various female organs and sex characteristics mannerisms (Maraud and Lyall, 1968).

The importance of mestranol lies in the use, as oral contraceptive pills more commonly employed by women throughout the world for birth control. Some of the pharmaceutical preparations in combination mainly with progestin Norethindrone are Envoid, Ovulen (Searle), Lyndiol (Organon), Norinyl, Norquen (Syntex), Ortho-novum (Ortho). The stability of oestrogens has been studied by many workers, e.g. oestrone (Fieser and Fieser, 1959), mestranol and other oestrogens (Aulesa *et al.,* 1981; Fuerst *et al.,* 1984). The study under uv irradiation of mestranol was not exhaustively investigated and still requ[i]re clear cut outl[i]ning of isolation and characterization techniques of some of the photodegradation products of polymeric origin.

Materials and Methods

Mestranol (Ethynylestradiol-3-methylether) obtained from Sigma Chemical Co., St. Louis, U.S.A. was found to be chromatographically pure, Rf 0.86 (Dichloromethane-Ether-Methanol-Water. 77:15:8:1.2 v/v) on Silica Gel G Plates. (Clarke, 1986; Rf 0.86). Suitable concentration of 100 ml 10^{-3}M Mestranol/absolute ethanol was prepared, placed in 250 ml quartz round bottom flask half immersed in water and deoxygenated by bubbling nitrogen gas into the solution. The solution was irradiated by 30 W UV Philip Tube emitting 88.74% of its radiation energy at 254nm which nearly corresponds the

absorbance maxima range of mestranol. 2.5 nil samples of irradiated solutions of mestranol taken out at 5 to 10 minute intervals were diluted to 5 ml with absolute methanol. The pH of the solutions was noted. UV spectra of irradiated solutions were recorded on Shimadzu digital double beam spectrophotometer and the solutions then subjected to TLC and column chromatography for identification and separation of irradiation products. The Rf values of different fractions were recorded.

Results and Discussion

(a) *Choice of solvent:* The experimentation conducted for mestranol solubility leads to the conclusion that mestranol solubility increases in the following order: H_2O cn-hexane <cyclohexane < ethyl alcohol. Halogenated and hydrocarbon solvents are poor and less suitable for mestranol while ethyl and methyl alcohol solvents are having excellent solvent properties.

(b) *Validity of Beer's law* Synthetic mixtures of mestranol when subjected to spectrophotometric assay, Beer-Lambert Law is satisfied over the range of concentrations used and there is no real apparent cause of chemical or instrumental deviation for the Beer-Lambert Law under controlled conditions. (Figure 1).

(c) *Correction absorbance for photolysis of mestranol*: It has been observed that 10^{4}M mestranol solutions on uv irradiation absorb significantly in the region of 200 to 290 mn (Figure 2) and other degradation products of mestranol do not absorb significantly at longer wavelengths. Therefore, a base line correction is necessary before a corrected mestranol absorbance at 287.7nm is measured for kinetic purposes. According to Shroff and Grodsky (1967), a straight line is drawn through the points given as the absorptions at 316nm and 302nm. The peak height at 287.7ern was measured from the base line. Corrected A287.7 = observed A287.7- observed 2A302 + observed Ants. The corrected absorbance of mestranol at 287.7nm during photolysis is calculated (Table 1) and the data were plotted for zero, first and 2nd order reactions (Figure 3).

(d) *Determination of molar extinction coefficients:* After confirming the validity of Beer-Lambert Law for mestranol of appropriate p11, solvents and wavelengths, the molar extinction coefficients *were* calculated by the method of least square equation E = e MC_l / s Ci, where A_l is the absorbance of a solution having concentration of CI. Extinction coefficients of mestranol in methanol at pH 6.0 is calculated as 2.071×10^3 at 279nm (A 1% 1cm = 67) (e = 1.893×10^3; Clarke, 1986), while at 287.7nm the mestranol gave 1.87×10^3 as extinction coefficient. These extinction coefficients are helpful for determination of the various unknown degradation products quantitatively in the degraded solutions.

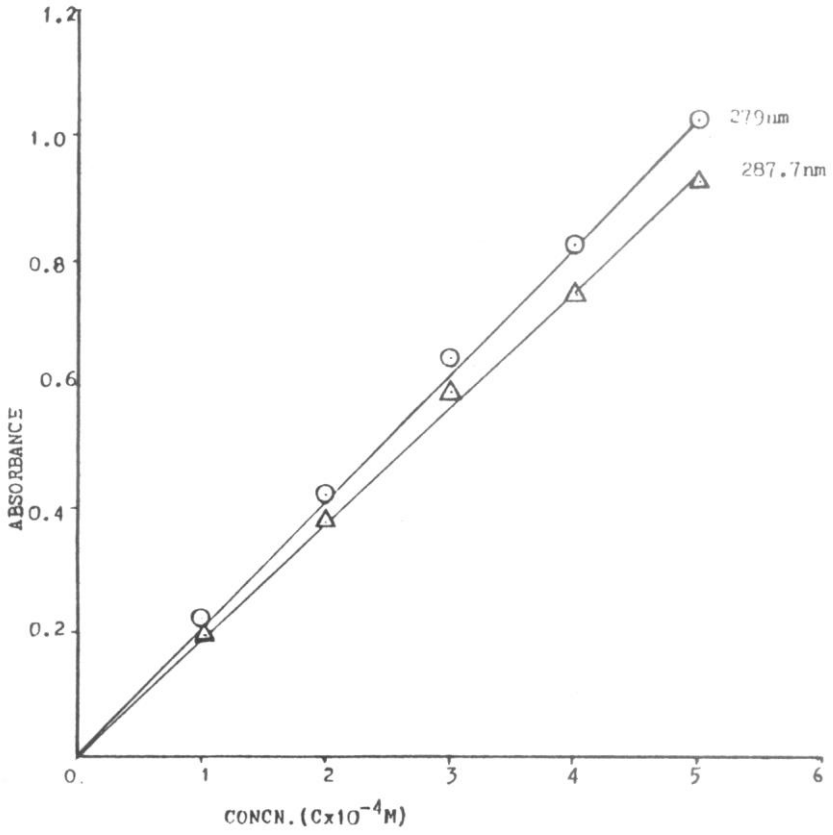

1.2
1.0
0.8
0.6
0.4
0.2
0
ABSORBANCE
1
2
3
4
5
6
CONCN. (Cx10⁻⁴M)
279nm
287.7nm

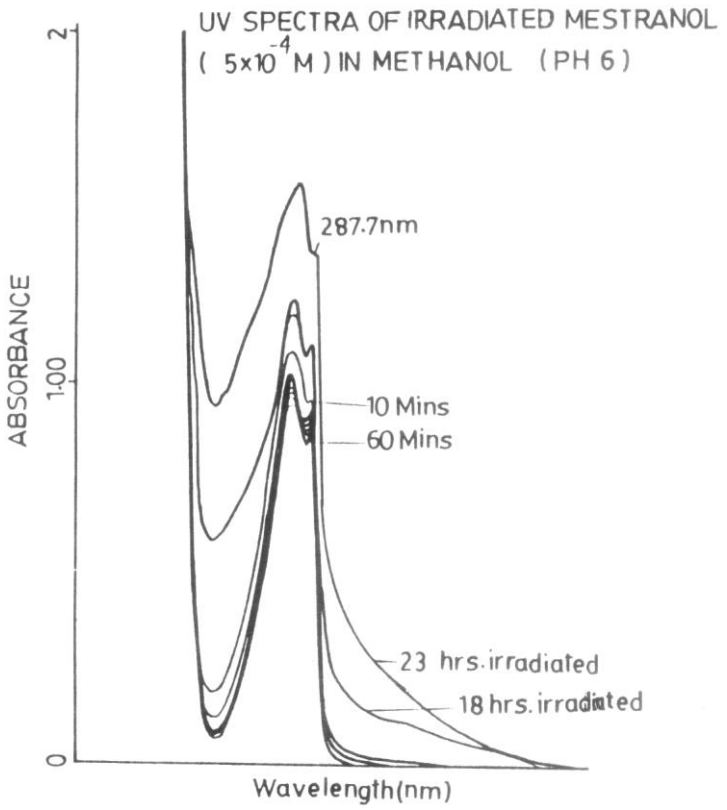
Table 1

Photolysis of 10^{-3}M Mestranol in abslute methanol at pH 6.0

Time	Observed absorbancies			corrected absorbance	corrected concentration
Mins.	316nm	302nm	287.7nm	287.7nm	x10^{-4}M/L
0	-0.016	-0.020	0.898	0.922	5.000
10	-0.002	0.002	0.881	0.875	4.745
20	-0.003	0.002	0.855	0.848	4.598
30	-0.002	0.003	0.839	0.831	4.507
40	0.002	0.003	0.817	0.813	4.409
50	0.001	0.008	0.820	0.820	4.366
60	0.002	0.012	0.806	0.786	4.252

(e) TLC of irradiated mestranol solutions: Several solvent systems of various combinations of acetone, benzene, chloroform, ether, methyl chloride and ethyl acetate (Bishara et al, 1969; Simard and Lodge, 1970; Florey, 1986 and Clarke, 1986) have been applied for determination of Rf values of mestranol authentic samples for purity checkup. But these solvent systems did not give separation of photochemical decomposition products of mestranol. Methanolic solutions of irradiated mestranol were applied on silica gel HF254 TLC plates and these spots were developed in a solvent mixture of chloroform; acetone, 86:4. The developed spots were distinguished by spraying with 10% phosphomolybdic acid in methanol. The developed spots were distinct only of mestranol (Rf 0.92) and no decomposition products *were* visible. While adopting the procedure further, the TLC plates were heated at 105°C for initially two minutes, followed by 30% H2SO4 ethanolic spraying solution and reheated for 10 minutes at 105°C. It was of great interest to note that blue coloured spots of decomposition products *were* visible in green background. With a system of CHCI3: EtOH, 29:1, the decomposition products of mestranol with streak were visible. The first spot corresponds to mestranol (Rf 0.96) while the second elongated spot (Rf 0.36 with streaking) suggests the presence of more than one substance. The solvent system chloroform: acetone, 86:4, was poor for Rf value detection. Thus the various decomposition products of mestranol were later separated on columns of sephadex LH20 using ethanol: acetone (60:40) as solvent system. Partial separation of products leads to five major groups. Each group was subjected to preparatory silica gel layer HF_{254} plates activated at 110°C for 2 hours. The chromatogram was developed, spots were made detectable by phosphomolybdic add-

H_2SO_4 spraying agent. The Rf values determined were 0.6, 0.55, 0.47, 0.34, 0.00 and correspond to fraction 1 to 5 respectively.

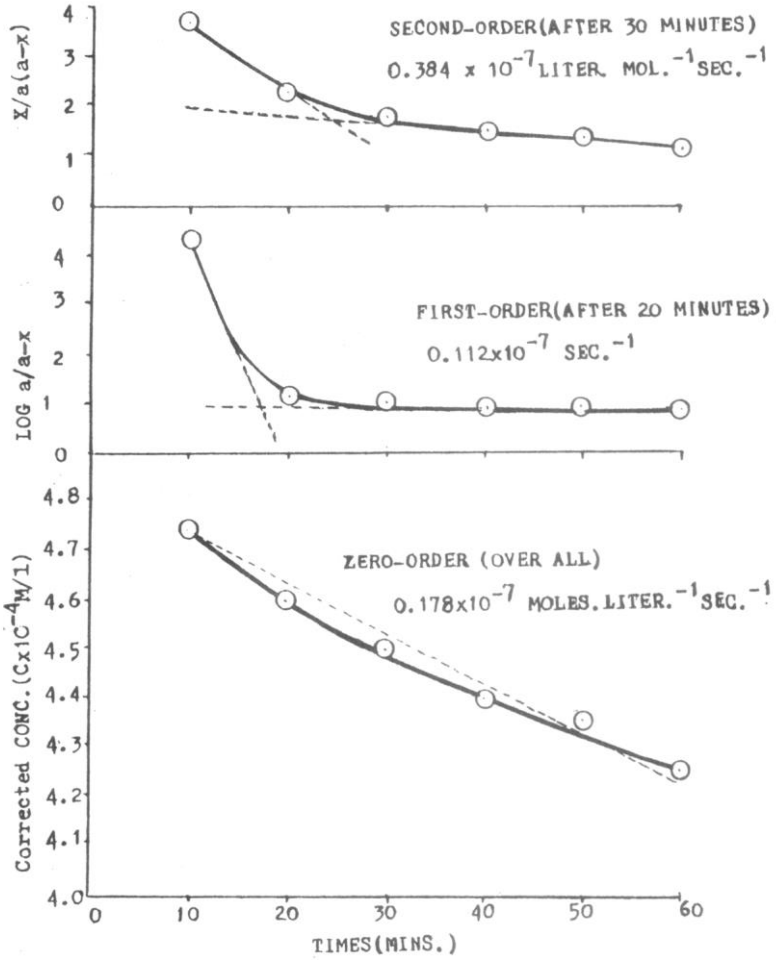


(f) *UV and IR spectra of the irradiated solutions:* The absorption bands for 10^3M mestranol/methanol during irradiation lie in the range of 260-400nm, indicating greater change in mestranol molecule. Irradiation of mestranol after 18 to 23 hours, causes shift of bands towards far uv region, showing formation and presence of some stable degradation products of mestranol. UV spectroscopic study of the above fraction in alcohol shows that these fractions (2 to 5) do not absorb in the region of 270-280nm. There is no fluorescence visible in a0 fractions, indicating the disappearance of benzene ring in mestranol after irradiation, whereas presence of absorbance at 215, 230, 260nm in fraction 1, indicate the presence of some unsaturation in the molecule. The absorption at 215nm is present in all the fractions. The fractions 1 and 2 are relatively more pure. The 1R assignments for mestranol on comparison with fraction 1 and 2 exhibited disappearance of benzene ring, acetylene group, saturated structures, rings or chains. The groups present in fractions 1 and 2 are esters, ethers, acidic, ketonic unsaturated structures.

(g) Reaction rates of mestranol: In the photolysis of 10^{-3} solutions of mestranol in alcohol, the characteristic maximally at 287.7nm (Figure 2). The corrected concentration of irradiated mestranol in methanol solution, when plotted against time in minutes for one hour of irradiation showed a linear behaviour. The zero-order rate constant calculated is $0.178x10^{-7}$ moles. $lit.^{-1}$ $sec.^{-1}$. By plotting log a/a-x and x/a(a-x) against time (minute) (Figure 3), 1st and 2nd-order plots were obtained at least to the level of initial 20 minutes of irradiation after 20 minutes of irradiation in the reaction, interferences occurs due to the newly unsatable species of mestranol. The rate of photolysis later follows quite uniformly. Thus the photolysis of mestranol exhibits a complicated trend of degradation due to unsatable form of radical polymeric producing species of glucosiduronate and sulphate conjugate like compounds. The electronic transitions due to irradiation absorption is π—π^* if extinction coefficient vary from 10^3 to 10^5, this confirms mestranol too of having π—π^* as mestranol in methanol has ε max. at 280nm = $1.94x\ 10^3$.

(h) *Molecular weights and probable structures of decomposition products:* The molecular weights determined by viscosity methods for each mestranol irradiated decomposition fractions separated by TLC are as follows.

Fraction l	(M.W.375)	Fraction 2	(M.W.368)
Fraction 3	M.W.353	Fraction 4	(M.W. 370)
Fraction 5	M.W. 364.		


SECOND-ORDER(AFTER 30 MINUTES)
0.384 x 10⁻⁷ LITER. MOL.⁻¹SEC.⁻¹
X/a(a-x)
FIRST-ORDER(AFTER 20 MINUTES)
0.112x10⁻⁷ SEC.⁻¹
LOG a/a-x
ZERO-ORDER (OVER ALL)
0.178x10⁻⁷ MOLES.LITER.⁻¹SEC.⁻¹
Corrected CONC.(Cx1C⁻⁴M/1)
TIMES(MINS.)

These molecular weights determined in this investigation are in agreement with those of Djerassic (1962) who studied mass spectroscopy of several fragmentations of mestranol produced during such type of irradiation experiments.

Thus it could be concluded that at least five decomposition products which have been separated and characterised, belong to stable species of mestranol. Moreover, several unstable species of polymeric degradation products could also be present which needs further investigation.

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