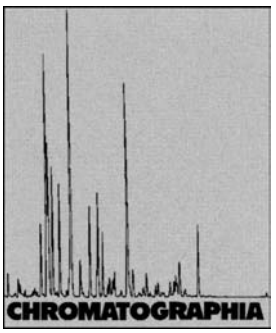
Preparation and Chromatographic Characterization of Tetrahydrogestrinone, a New ''Designer'' Anabolic Steroid



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Abstract

Tetrahydrogestrinone (THG, (17α)-13β-ethyl-17β-hydroxy-18,19-dinorpregna-4,9,11-trien-3-one, $C_{21}H_{28}O_2$, 312 m.w.) is a synthetic 19-norsteroid closely related to gestrinone ((17α)-13β-ethyl-17β-hydroxy-18,19-dinorpregna-4,9,11-trien-20-yn-3-one, $C_{21}H_{24}O_2$, 308 m.w.) which was originally developed as an oral contraceptive for women. Recent press reports detail the probable use of THG by athletes to enhance athletic performance, and the FDA has banned THG, declaring it a ''designer drug.'' THG has been difficult to analyze because of its instability, lack of commercially available analytical standards, and the fact that no published synthetic methods for the compound exist. We now report a method for the preparation of THG via the carefully controlled hydrogenation of gestrinone.

Keywords

Gas chromatography-mass spectrometry
Anabolic steroid, synthesis
Designer drug
Tetrahydrogestrinone

Introduction

Gestrinone ((17α)-13β-ethyl-17β-hydroxy-18,19-dinorpregna-4,9,11-trien-20-yn-3-one, $C_{21}H_{24}O_2$, 308 m.w.) (Fig. 1) is a synthetic 19-norsteroid originally developed as an oral contraceptive for women [1], and which has been found to have efficacy in treatment of endometriosis by suppression of ovulation [2,3]. Gestrinone has anti-estrogenic, anti-progestagenic and androgenic properties [4], and it is the latter property that has attracted athletes interested in performance-enhancing substances with anabolic properties [5], a major public health problem in the U.S. [6]. Chromatographic methods involving mass spectrometric detection have existed for anabolic steroids [7] and are in use in laboratories commissioned by the International Olympic Committee, which has banned anabolic androgens since 1976 [8]. Gestrinone is no exception, and LC-MS and GC-MS methods exist for determination of its metabolites in human samples [9–11]. Recent reports of the use of a new, closely related ''designer'' drug, an anabolic steroid identified as tetrahydrogestrinone (THG) by the IOC drug-testing laboratory at the University of California, Los Angeles [12, 13] has aroused international concern. The director of a ''nutritional supplements'' company currently faces trial in the U.S. on the basis that his company has allegedly supplied banned substances such as THG to world-class athletes in sports ranging from baseball, football, and boxing to the Olympics [14].

THG is not commercially available and, to our knowledge, there are no published articles concerning synthesis of THG in the scientific literature. To assist in the regulation of the use of THG in horse racing we have investigated the synthesis of the compound. The most direct preparation of THG is via the hydrogenation of gestrinone, a reaction which has proven difficult to limit to the terminal acetylenic group of gestrinone (Fig. 1). To avoid the formation of a mixture of products and to direct the reaction towards the reduction of only the terminal acetylenic group to an ethyl group, different palladium catalysts were tested under varying conditions. We report here the preparation of THG by carefully controlled hydrogenation of gestrinone with the Lindlar catalyst

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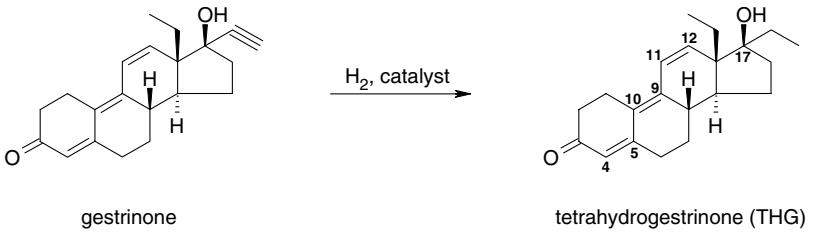

Fig. 1. THG preparation from gestrinone

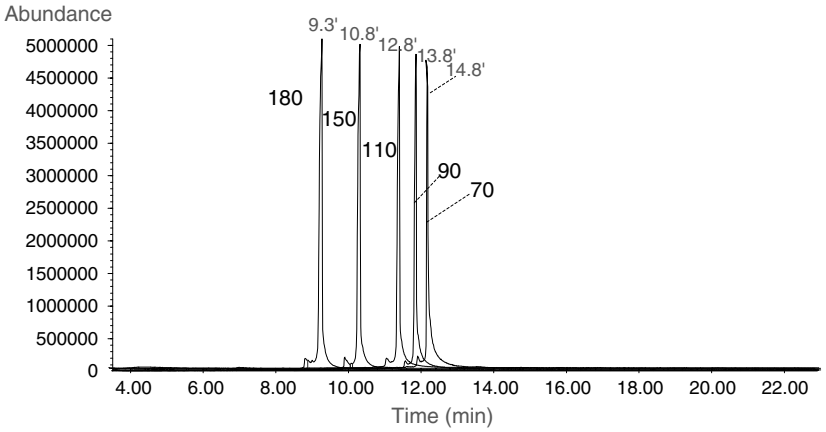

Fig. 2. Establishment of GC conditions for gestrinone. Each of the superimposed runs was run through an oven ramping procedure that began at the labeled temperature, held 2 min, then increased at 20 °C min^{-1} to a final temperature of 280 °C. The small peak preceding the prominent gestrinone peak is a 282 m.w. contaminant and likely represents loss of the acetylene side chain

(Aldrich®), followed by spectrometric characterization of the product. To our knowledge, this is the first reported synthesis of THG in the scientific literature. An in-depth report on the development of this synthetic approach is forthcoming.

Experimental

Tetrahydrogestrinone was produced by catalytic hydrogenation of gestrinone. Hydrogenation of gestrinone must be precisely controlled to prevent unwanted hydrogenation at the three other unsaturated carbon-carbon bonds, C-4, C-9, and C-11 of gestrinone. We investigated various palladium catalysts and hydrogenation reaction conditions. Identification and monitoring of reaction products was accomplished by gas chromatography-mass spectrometry (GC-MS) of the underivatized reaction mixture. At various times during the hydrogenation, a small amount (~0.5 mL) of reaction mixture was placed in a labeled vial, dried with a stream of argon and dissolved in 1 mL ethyl acetate for GC-MS analysis. The best hydrogenation conditions were achieved using the Lindlar catalyst in toluene at atmospheric pressure. Under these conditions a reaction mixture containing more than 96% of THG together with less then 1.5% unreacted gestrinone and about 2% of hexahydrogestrinone (M^+, m/z 314) as estimated on GC-MS, was obtained. Column chromatography on silica gel yielded 98% chemically pure THG as estimated by GC-MS.

Preparation of Tetrahydrogestrinone (THG, (17α)-13β-ethyl-17β-hydroxy-18,19-dinor-pregna-4,9,11-trien-3-one, $C_{21}H_{28}O_2$, 312.45 m.w.)

Gestrinone (100 mg, 0.32 mMol, Sequoia Research Products, Ltd.) was dissolved in 15 mL of toluene and 3 mg of Lindlar catalyst (palladium, 5% on calcium carbonate poisoned with lead, Aldrich®) was added. Air was removed under reduced pressure from the reaction flask, and the flask filled with hydrogen (this process was repeated three times). The reaction mixture was stirred for 36 h under hydrogen at atmospheric pressure, filtered through celite®, after which the toluene was evaporated under reduced pressure. Column chromatographic purification on silica gel, with elution by hexane – *tert*-butyl methyl ether (2:1), yielded 82 mg of THG as a yellowish oil of 98% purity (GC-MS). ^{1}H-n.m.r. (200 MHz, $CDCl_3$): δ (p.p.m.) 0.97 (t, 3 H, $CH_3C\underline{H}_2$, J 7.33 Hz), 1.03 (t, 3 H, $CH_3C\underline{H}2$, J 7.7 Hz), 1.1–2.2 (m, 12 H), 2.4-2.7 (m, 5 H), 2.7–2.9 (m, 2 H), 5.78 (s, 1 H, H-4), 6.37 (d, 1 H, J 10.25 Hz), 6.57 (d, 1 H, J 9.89 Hz).

Characterization of THG by GC-MS Analysis

Injection of gestrinone reaction products dissolved in ethyl acetate was made through the 250 °C injector port of an Agilent 6890/5972 GC-MSD (Atlanta, GA, USA), with separation on a 30 mm x 0.25 mm i.d. x 0.25 μ film thickness DB-5MS (5% phenyl-95% methylpolysiloxane) column (J&W Scientific, Folsom, CA, USA) with oven programming beginning at 180 °C (held for 2 min), then ramping at 20 °C min^{-1} to 280 °C (held for 12 min). THG eluted with adequate separation from other gestrinone hydrogenation products between 9–10 min retention time. The mass spectrometer was set up to acquire from m/z 50–550 at 1.53 scans sec^{-1} with a threshold of 150. Mass Spec Calculator Pro, Version 4.03 (Quadtech Associates, Inc., published by ChemSW, Fairfield, CA, USA, 1998) was used in the interpretation of full scan mass spectra where appropriate.

Due to the polar nature of steroid molecules, trace analysis by GC-MS typically involves derivatization to non-polar trimethylsilyl and/or methoxime derivatives, which enables gas chromatographic analysis of low concentrations of the compounds without loss due to on-column absorption. The GC analysis of steroids in the concentrations found in the synthetic reaction mixtures did not require such derivatization. Anecdotal reports and personal communications have indicated difficulties with the gas chromatographic analysis of THG. We therefore verified the satisfactory behavior of gestrinone during gas chromatography. Temperature programmed gas chromatograpic analyses with step-wise increases in initial column temperatures in the range of 70–180 °C yielded the series of chromatograms for gestrinone

(shown as an overlay) in Fig. 2. Excellent peak shape was observed at all temperatures indicating insignificant on-column adsorption or degradation of the nonderivatized steroid. The absence of derivatives avoided the creation of side-products and made the mass spectra of the reaction mixture compounds more easily interpreted.

Results

Gestrinone eluted at over 14 min retention time under our laboratory's standard GC temperature program (initial temp 70 °C programed to 280 °C at 20 °C min^{-1}, as seen at peak marked "70" in Fig. 2). We desired a more rapid assay without sacrifice of chromatographic peak shape or response, and so opted to employ the 180 degree program (Fig. 2). The resultant GC-MS method was designated THG180.M.

Gestrinone was run by GC-EI-MS, yielding the mass spectrum shown in Fig. 3 which includes assignment of peaks as indicated in the figure legend.

In order to identify optimal hydrogenation conditions, small aliquots (~0.5 mg) of reaction mixtures were placed in labeled vials, dried and resuspended in 1 mL ethyl acetate for analysis. A typical GC-MS analysis for products by the THG180 method is shown in Fig. 4. Selected ion chromatograms reveal the desired product at 10.08 min retention time (R_T), along with undesired side-products of single (310 m.w.), double (312 m.w.), triple (314 m.w.), and even quadruple (316 m.w.) double bond hydrogenations. Repeated assay of reaction products at various times enabled prediction of the ideal reaction endpoint by following the time course for appearance of the desired product, and disappearances of gestrinone (308 m.w.) and dihydrogestrinone (310 m.w.), as shown in Fig. 5. The appearance of undesired side-products such as hexahydrogestrinone (314 m.w.) could also be followed with the intention of avoiding its accumulation, either by modification of total reaction time or modification of conditions. One reaction side-product was tentatively identified as norbolethone (316 m.w.), another illicit anabolic steroid which has been previously reported in athletes' urines [15].

Selected ion chromatograms (*m/z* 308, 310, 312, 314, 316, and 318) from the final THG product are shown in Fig. 6.

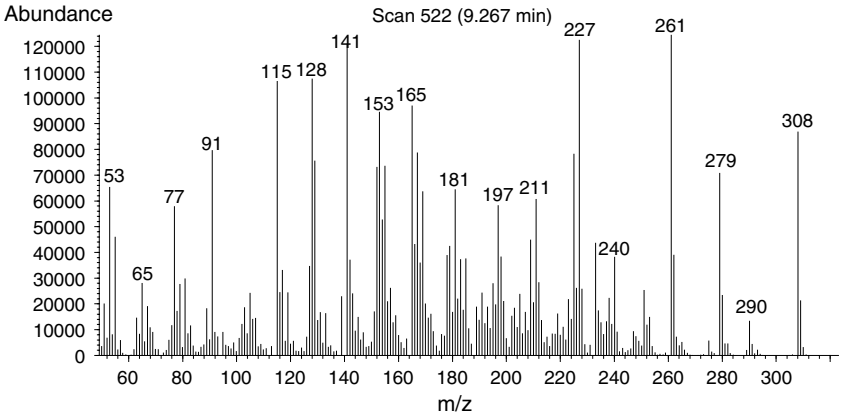

Fig. 3. Gestrinone EI-MS. Assignments: M^+, 308; *m/z* 290, loss of water; 279, loss of CH_2CH_3; 261 = 290 minus CH_2CH_3; 227, partial loss of D ring [-C_6H_{10}] ; 211, loss of D ring [-C_7H_{13}]; 197, loss of D ring [-$C_7H_{12}O$]; 181, loss of B and D rings [-$C_8H_{15}O$]; 91, C_7H_7; 77, C_6H_5; 65, C_5H_5; 53, C_4H_5

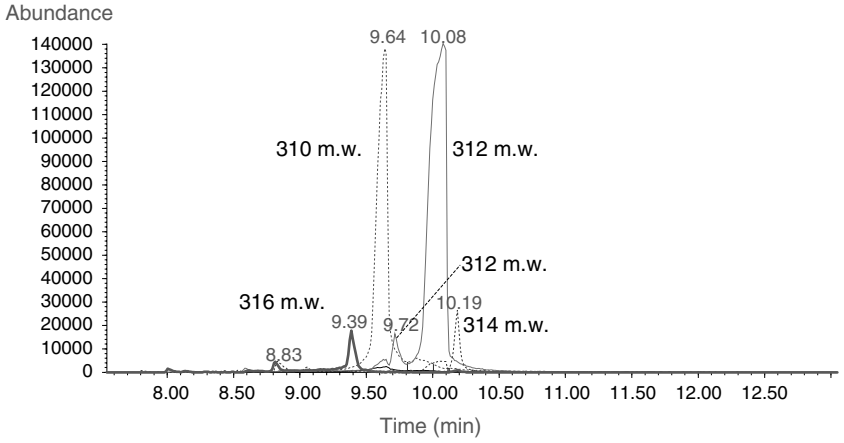

Fig. 4. Example of GC-MS of reaction products from a typical gestrinone hydrogenation experiment: $Pd/SrCO_3$ exposed to hydrogen for 10 min. A 100 µL aliquot of the reaction in toluene was dried, resuspended in 1 mL ethyl acetate, and 1 µL injected for GC-MS by the THG180 method. Selected ion chromatography revealed no 308 m.w. starting material, but candidates for single (310), double (312), triple (314), and even quadruple (316) double bond hydrogenations

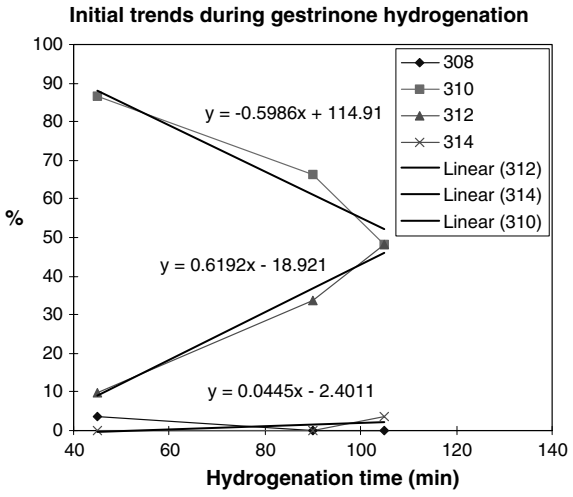

Fig. 5. An example of $Pd/SrCO_3$ hydrogenation, indicating the initial trends in % product formation and % reactant disappearance over time. Based on least squares approximation of linear trends, corresponding equations enabled estimate of 192 min for conversion to m.w. 312 [y = 0.6192x − 18.921; thus, x = 192 min total reaction time under these conditions. For other compounds of concern, m.w. 308 goes to 0 at 90 min; m.w. 310, goes to 0 well before 192 [y = − 0.5986x + 114.91 therefore, y = −0.02%], and m.w. 314, y = 0.0445 x −2.4011, therefore at 192 min, y = 6.1%

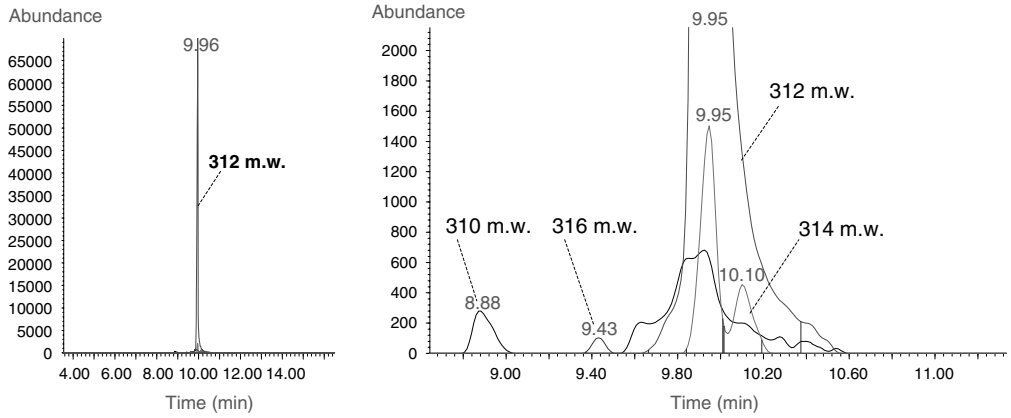

Fig. 6. Final THG product. Selected ion chromatography for starting material, final product, and side products revealed almost exclusively the 312 m.w. THG species [left panel]. Enhancement of the region around 10 min retention time [right panel] revealed minute amounts of side-products 310, 314 and 316 m.w., on the order of 2% or less combined area total. No traces of gestrinone (308 m.w.) starting material were found. Changes in chromatographic procedure increased the separation between 312 and 314 m.w. species without any corresponding shoulder or side-peak of the 312 m.w. trace, indicating lack of any secondary 312 m.w. species for which the 10.10' *m/z* 314 could be an isotopic peak

Table 1. Estimate of THG contaminants based on areas of the corresponding molecular ions (THG180.M method)

m.w.	R_T (min)	Purity (%)
310	8.86	0.7%
312	9.96	98.1%
314	10.09	1.0%
316	9.42	0.16%
318	–	0

Chromatographic analysis for the 308 m.w. starting material, and the 318 m.w. fully hydrogenated gestrinone revealed no peaks, nor were alternate 312 m.w. products found. Despite extensive gravity flow column purification, analogs of different levels of hydrogenation remained difficult to separate as might be expected. Scale expansions of the ion chromatograms disclose minute amounts of 310, 314 and 316 m.w. peaks (Fig. 6, right panel). The *m/z* 314 peak (9.95' R_T) nested directly under the large *m/z* 312 peak is appropriate to the THG isotopic peak (M + 2) abundance, but the one to the right at 10.10' R_T represents a 314 m.w. peak and not the isotopic peak to a separate overlapping 312 m.w. peak. This was verified by adjusting GC column oven ramping to increase the separation distance between the two *m/z* 314 peaks, which yielded no coincident widening or shoulder formation on the principal *m/z* 312 peak. The purity of THG final product, based on integrated area counts of molecular ion chromatograms, was 98.1% (Table 1).

In the final THG EI-mass spectrum presented in Fig. 7 only four principal high m.w. peaks differ from corresponding peaks in the gestrinone mass spectrum (Fig. 3). These are *m/z* 312, 294, 283 and 265. This is appropriate, since the corresponding peaks in gestrinone (308, 290, 279 and 261, respectively) differed by exactly 4 amu each and have been assigned structures that retained the acetylenic side-group in each case.

The mass spectrum of THG showed a distinct *m/z* 312 molecular ion 4 amu greater then the corresponding ion of gestrinone. However, this identification was considered tentative until the addition of the four hydrogen atoms was confirmed by proton nuclear magnetic resonance spectroscopy (^{1}H-n.m.r.) as occurring on the ethynyl group of C-17. The ^{1}H-n.m.r. spectrum shows two different ethyl group signals. Additionally, signals for protons H-4 (singlet), H-11 (doublet) and H-12 (doublet) are identical with the corresponding signals registered for gestrinone, a strong indication that the three conjugated carbon-carbon double bonds presented in gestrinone remained intact.

Discussion

Recent reports of the use by some prominent athletes of a new "designer" drug, an anabolic steroid identified as tetrahydrogestrinone (THG), has aroused international concern. Interest in the drug has intensified in the athletic and drug-testing communities as well as among the general public following news articles of the prosecution of persons allegedly responsible for the manufacture and distribution of the drug.

THG is not commercially available from legitimate sources. Therefore, in order to obtain an analytical standard for use in the regulation of this drug in horse racing, we have investigated the synthesis of the compound. The most direct preparation of THG is via the hydrogenation of gestrinone. To avoid the formation of a mixture of products and to direct the reaction towards the reduction of only the terminal acetylenic group of gestrinone, different palladium catalysts were tested under varying conditions.

As mentioned earlier, the final THG EI-mass spectrum (Fig. 7) revealed four principal high molecular weight ions that differed from corresponding peaks in the gestrinone spectrum. These were m/z 312 (308), 294 (290), 283 (279) and 265 (261), with parentheses showing gestrinone values. This was appropriate, since corresponding peaks differed by exactly 4 amu each and have been assigned structures that retain the acetylenic side-group in each case. Fig. 8 displays the fragmentation of high m.w. peaks beginning with the molecular ion. The smaller *m/z* 233 peak in gestrinone arising from loss of a neutral C_2H_4 molecule would be expected to give a 237 fragment in THG, and this value appears in a cluster of fragments already present in gestrinone. Lower yield of the deethylation fragment *m/z* 283 in THG may be due to competition with loss of the entire D-ring ($C_5H_{10}O$) to give the significantly larger *m/z* 227.

Hydrogenation of gestrinone produced a number of products including dihydrogestrinone, hexahydrogestrinone, and a compound tentatively identified as

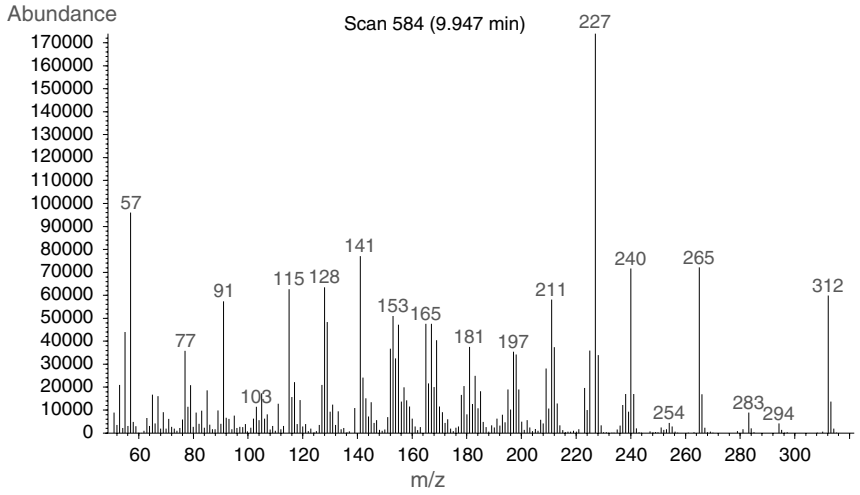

Fig. 7. Tetrahydrogestrinone final EI-mass spectrum. Notice that *m/z* values of only several high mass peaks changed significantly from those recorded for gestrinone (Fig. 3); gestrinone values listed in [brackets], including *m/z* 312 [308], 294 [290], 283 [279], and 265 [261]. In addition, *m/z* 233 was absent in contrast to gestrinone. The *m/z* values of the peaks in the remainder of the spectrum appear virtually identical to those of gestrinone

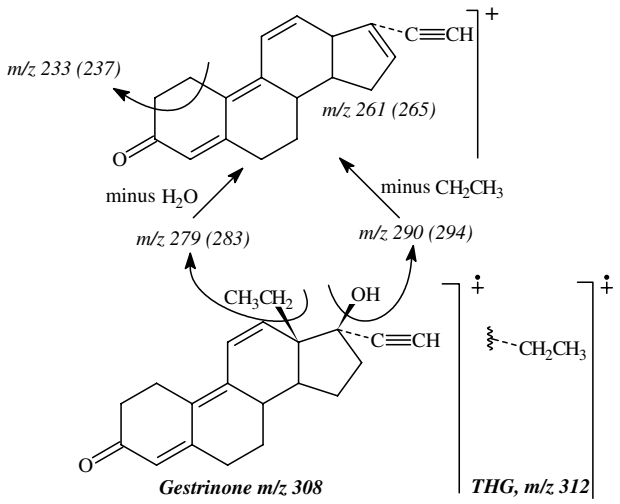

Fig. 8. Electron-impact induced fragmentations of gestrinone and THG. Gestrinone [bottom] produces an *m/z* 308 $M^{+\bullet}$; since THG substitutes the 17α-acetylenic group with ethyl, the value is *m/z* 312. Dehydration at the 17β-OH produces *m/z* 290, whereas loss of ethyl gives *m/z* 279. *M/z* 261 represents an intermediate species in which both these actions have occurred. Loss of C_2H_4 from *m/z* 261 gives *m/z* 233. Values shown represent fragment sizes arising from gestrinone, those in parentheses represent fragments from THG

norbolethone. Carefully controlled hydrogenation of gestrinone using Lindlar catalyst (Aldrich ®) produced THG in high yield. The product was purified by column chromatography to give a final product of 98.1% purity.

Acknowledgments

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