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Estimation of impurity profiles of drugs and related materials Part 16: Identification of the side-products of the ethinylation step in the synthesis of contraceptive gestogens[1,2]

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Abstract

A new apolar impurity (3,17α-diethinyl-13-ethyl-3,5-gonadiene-17-ol, IIb) was detected and identified in norgestrel with the aid of thin-layer and high-performance chromatography and spectroscopic techniques. IIb is the product of the acid-catalysed dehydration of an overethinylated side product (Ib) of the ethinylation step in the synthesis of norgestrel. IIb can be determined by thin-layer densitometry and high-performance liquid chromatography. Another impurity (17α-ethinyl-13-ethyl-4-gonene-17-ol, IV), originating from a side product of the Birch reduction step in the synthesis of norgestrel was also detected and identified. The spot of IV overlaps with that of IIb in the TLC system of USP XXIII but can be separated and quantificated by more selective TLC systems and by gas chromatograpy.

Keywords: Norgestrel; Impurities in norgestrel; Impurity profiling; Chromatography (TLC, GLC, HPLC); Spectroscopy (UV, NMR, MS, GC/MS); 3,17α-Diethinyl-13-ethyl-3,5-gonadiene-17-ol; 17α-Ethinyl-13-ethyl-4-gonen-17-ol

1. Introduction

Norethisterone (17α-ethinyl-17-hydroxy-4-oestren-3-one) and its 13-ethyl analogue, norgestrel (13-ethyl-17α-ethinyl-17-hydroxy-4-gonen-3-one) are prepared on the industrial level via total synthesis of the respective 3-methoxy-1,3,5(10)-trienes followed by their Birch reduction and finally the ethinylation of the 17-keto group in the last intermediates. The complicated, multi-step syntheses are the source of several impurities in these important contraceptive gestogens. Of these impurities 8(14)-dehydro derivatives originate from the total synthetic steps [3], 3-deoxo, 4,5-dihydro, isomeric 1-ene-3-keto and 5(10)-ene-

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3-keto as well as unreacted 3-methoxy-1,3,5(10)-triene derivatives from the Birch reduction step and the subsequent acid-catalysed hydrolysis [4–6], whereas the reason for the formation of 6α-, 6β- and 10β-hydroxy [6] as well as 4β,5β-epoxy [7] impurities is the oxidizibility of the final products and their intermediates.

The ethinylation step is also an abundant source of impurities. From among these, unreacted 17-keto derivatives [3], acetylene-bridged dimeric derivatives [3,9] and epimeric 17α-hydroxy-17β-ethinyl derivative [3,8,9] have been identified by the present authors; (see Fig. 1). It is important to note that the formation of the latter is restricted to norethisterone: in the case of norgestrel (presumably due to the steric hindrance caused by the bulkier 13-ethyl group) the epimeric side-product could not be detected.

The aim of the present paper is the description of the detection, isolation and identification of a further impurity in norgestrel, 3,17α-diethinyl-13-ethyl-3,5-gonadiene-17-ol (IIb, also included in Fig. 1). The reason for the formation of IIb is the not entirely regioselective ethinylation of the intermediate 3,17-dione. The paper contains also the identification of another impurity, interfering with the detection of IIb (17α-ethinyl-13-ethyl-4-gonen-17-ol, IV).

The present study is an example to demonstrate the necessity of the complex use of a variety of chromatographic (analytical and preparative TLC and HPLC as well as GLC), spectroscopic (UV, NMR, MS) and hyphenated techniques such as HPLC/diode-array UV and GC/MS to solve delicate problems in the impurity profiling of drugs [10].

2. Experimental

2.1. Instruments and methods

2.1.1. Thin-layer chromatography and densitometry

TLC aluminium sheets Kieselgel 60 F254 (Merck, Darmstadt, Order No. 5554) were used as the stationary phase. In addition to the use of the system of USP XXIII [10] the following two systems were used as mobile phases. System A: 95:5 v/v mixture of chloroform and acetone; system B: 60:40 v/v mixture of *n*-hexane and chloroform.

A Desaga CD-60 densitometer was used in reflection mode for measuring the densitograms and scan the reflection spectra. The densitograms after the phosphomolybdate spray [11] were scanned at 550 nm.

2.1.2. High-performance liquid chromatoraphy (analytical)

A Hewlett-Packard 1090A Series II chromatograph with built-in HP 1040 diode-array UV detector was used. Column: 250 × 4.6 mm packed with LiChrosorb 10 C-18 (Macherey Nagel). Eluent: 85:15 v/v mixture of acetonitrile and water at a flow rate of 1 ml min^{-1}. Temperature: ambient. Injected sample: 20 μl of 0.1 w/v solution in the eluent.

2.1.3. High-performance liquid chromatography (preparative)

An ISCO Model 2350 pump was used equipped with a 7125 Rheodyne injector and an ISCO Model V^4 UV detector.

2.1.4. Gas chromatography

A Hewlett-Packard 5890 gas chromatograph was used with flame-ionisation detector. Column: Ultra 1 (Hewlett-Packard) 12 m × 0.2 mm × 0.33

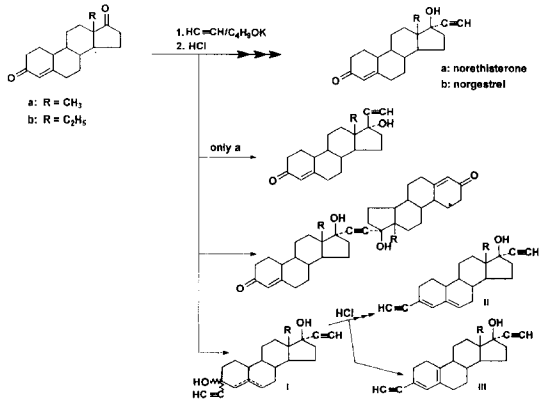


Fig. 1. Reaction scheme of the ethinylation of steroid 19-nor-4-ene-3,17-diones with side reactions.

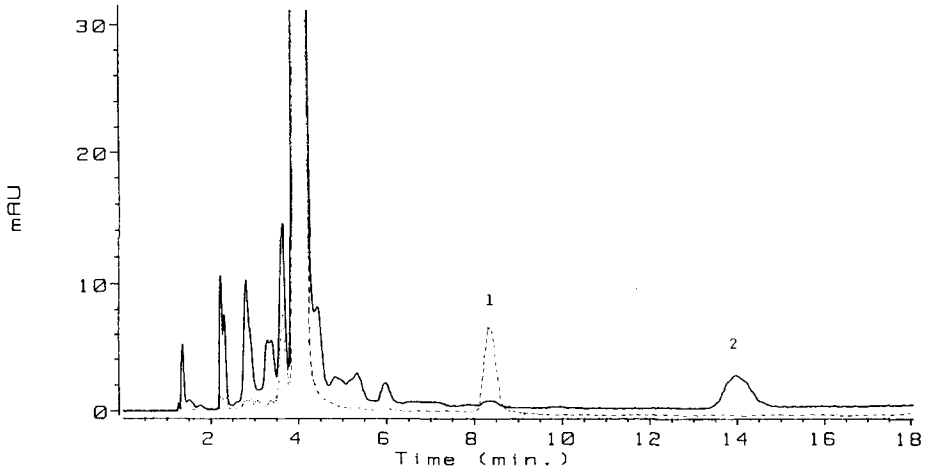


Fig. 2. Chromatogram of a crude norgestrel sample. For HPLC system see Section 2.1. Peak 1: 3,17α-diethinyl-13-ethyl-3,5-gonadien-17-ol, IIb 0.08%. Peak 2: 17α-ethinyl-13-ethyl-4-gonen-17-ol, IV, 0.22%. Monitoring at 210 nm (solid line) and at 268 nm (dotted line).

μm fused silica capillary. Carrier gas: nitrogen. Temperatures: oven 210°C, flash heater 250°C, FID 300°C. Split ratio: 1:50.

2.1.5. UV spectroscopy

A Varian Cary 3 double beam instrument was used. Solvent: 95% v/v ethanol.

2.1.6. NMR spectroscopy

The spectra were recorded on a Varian UNITY*plus* 500 NMR spectrometer at 30°C in $CDCl_3$. Chemical shifts are given relative to $\delta_{TMS} = 0.00$ ppm. The assignments were confirmed by 2D correlation experiments (DQF-COSY, HSQC, HMBC).

2.1.7. Mass spectrometry

Mass spectra were recorded on a VG TRIO-2 instrument at an electron energy of 70 eV. Temperatures: direct inlet probe 30–250°C, ion source 250°C.

2.1.8. Gas chromatography–Mass spectrometry (GC/MS)

Fisons MSD 800 instrument was used. Column and chromatographic conditions were as under 'Gas chromatography'. Ionisation mode: EI, 70 eV. Ion source temperature: 200°C.

2.2. Materials and reagents

All samples investigated were industrial or laboratory products of the Chemical Works of Gedeon Richter, Budapest. 3,17α-diethinyl-13-ethyl-3,5-gonadiene-17-ol (IIb) was synthesized by Dr. Z. Tuba, Ms. V. Abrók and Mr. J. Csörgei. HPLC grade solvents and analytical grade reagents were used.

3. Results and discussion

3.1. Detection of 3,17α-diethinyl-13-ethyl-3,5-gonadiene-17-ol (IIb) impurity in norgestrel

The continously increasing demands concerning the purity of bulk drug substances make it necessary to deal with the identification of minor, hitherto neglected impurities being present at a level below 0.1%. In the case of norgestrel (d,l-norgestrel and levonorgestrel) in addition to the impurities listed in Section 1 a minor impurity was detected at R_f 0.83 in the TLC system of the USP XXIII [11] (R_f of the main component is at 0.56). Although two of the potential impurities of norgestrel (3-methoxy-17α-ethinyl-13-ethyl-1,3,5

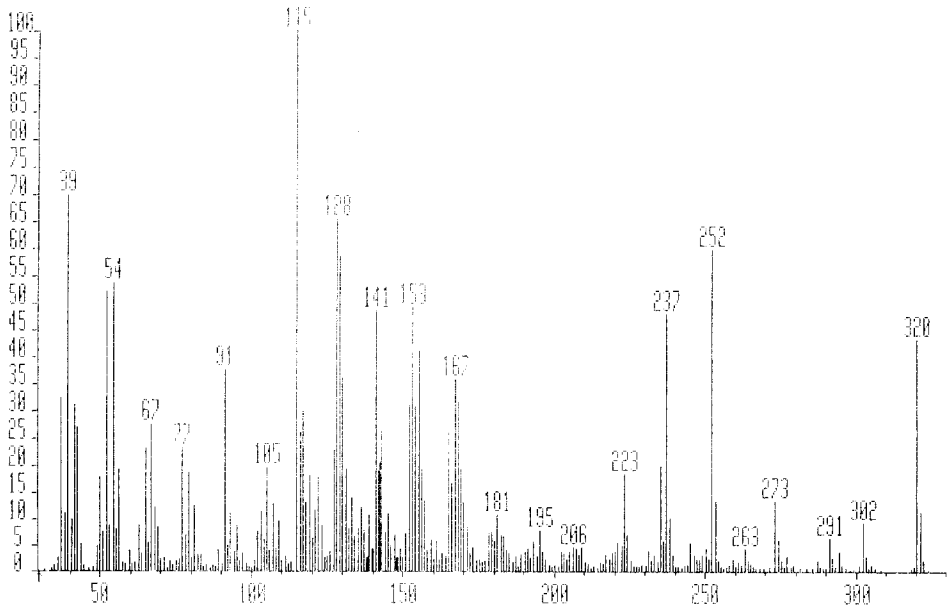


Fig. 3. Mass spectrum of 3,17α-diethinyl-13-ethyl-3,5-gonadien-17-ol (IIb) containing 18% of the 3,5(10) isomer (IIIb). See Section 2.1.

(10)-gonatrien-17-ol and 13-ethyl-5(10)-gonene-3,17-dione) appear at the same R_f value, the reflection spectrum of the material in the spot at R_f 0.83 with its maximum at 268 nm clearly indicated that the bulk of the impurity was an unknown material which was not separated from the above potential impurities in the system of the USP XXIII. For this reason we developed a more selective TLC system for the separation of the apolar impurities (System A in Section 2.1) in which the following R_f values were obtained: norgestrel: 0.55, 3-methoxy-17α-ethinyl-13-ethyl-1,3,5(10)-gonatrien-17-ol: 0.79, 13-ethyl-5(10)-gonen-3,17-dione: 0.83, 4β,5β-epoxy-norgestrel [7]: 0.74, 13-ethyl-17α-ethinyl-17-hydroxy-5(10)-gonen-3-one: 0.62, unknown: 0.80. (Of the listed five impurities the first three were absent or present below the level of 0.02% in the investigated samples.) Using this TLC system the quantity of the unknown impurity could be determined and expressed as norgestrel by using densitometry after visualization with phosphomolybdate [11].

The unknown impurity was detected also by HPLC. The eluent used for impurity profiling of norgestrel in earlier studies in this laboratory (methanol–water 70:30 v/v, [3]) is not suitable for the detection of this highly apolar impurity. As seen in Fig. 2, using an eluent with a much higher organic solvent concentration, described in Section 2.1, a peak appears at 8.4 min with the same spectral characteristics (absorption maximum at 268 with side maximum at 279 nm) as the unknown impurity; (the other peak at 14.0 min will be discussed in Section 3.3).

3.2. Identification of 3,17α-diethinyl-13-ethyl-3,5-gonadiene-17-ol (IIb)

The apolar nature of the impurity as well as the above mentioned reflection UV spectrum and the same obtained after spot elution and by the HPLC diode-array UV detector with some fine structure (maxima at 268 and 279 nm) reveal the absence of oxygen containing functional group at the 3-position and the presence of a conjugated double bond system in rings A/B. The mass spectrum obtained after TLC spot elution contains molecule peak at m/z 320 which corresponds to a 3-deoxo-A/B-diene-3,17-diethinyl structure. (Another peak at m/z 298 in the same mass spectrum will be discussed in Section 3.3).

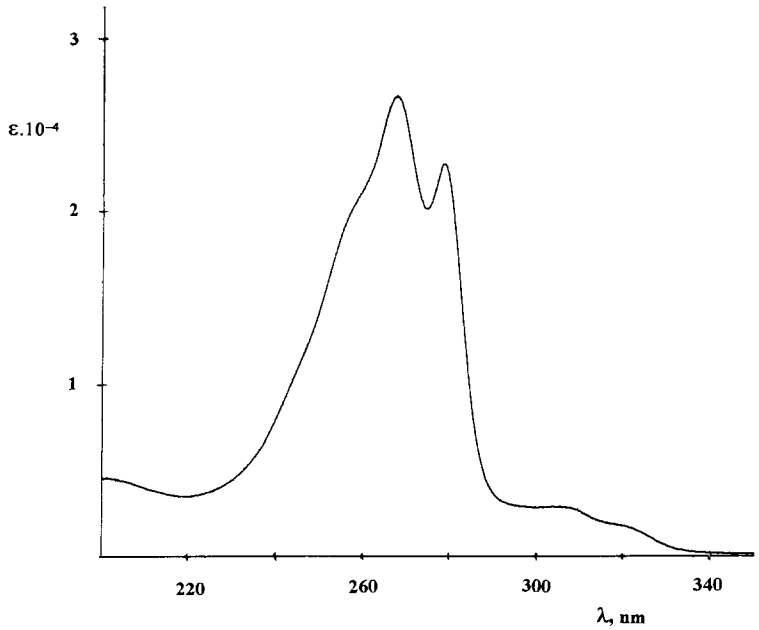


Fig. 4. Ultraviolet spectrum of 3,17α-diethinyl-13-ethyl-3,5-gonadien-17-ol (IIb) containing 18% of the 3,5(10) isomer (IIIb). See Section 2.1.

The chemical basis of the formation of this impurity is obvious: the not entirely regioselective ethinylation of the 3,17-dione-type intermediate at position 17. As is seen in Fig. 1, the configuration of the 3-hydroxy and 3-ethinyl groups and the postion of the double bond in the overethinylated side product (Ib) are uncertain. However, the acid-catalysed dehydration of Ib taking place in the course of the workup of the reaction mixture of the ethinylation leading to IIb simplifies the situation leaving uncertainity in the position of the double bonds only the possible positions being 3,5 or 3,5(10) or 2,4. On the basis of the UV spectrum the 3-ethinyl-3,5-diene structure seemed to be the most likely. The wavelength of the absorption maximum of this derivative calculated on the basis of the Woodward rule [12] is 264 nm; measured 268 nm. (In this calculation the increment of a linearly conjugated ethinyl group was taken as 30 nm, similarly to the contribution of the double bonds).

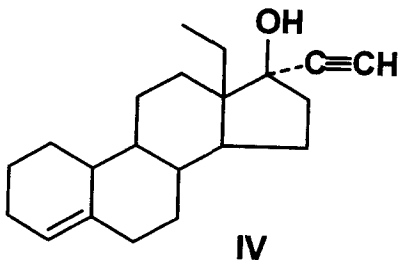
At this point of the study IIb was synthesized by diethinylating 13-ethyl-4-gonene-3,17-dione to form Ib which was transformed to IIb by acid catalysed dehydration. The details of the synthesis together with the mechanism of the dehydration reaction (involving the formation of 3,17α-diethinyl-13-ethyl-3-gonene-5β,17-diol as the intermediate) and the role of preparative HPLC in the fractionation of crude Ib and purification of IIb will be subject to a separate publication. (Unlike Ib, its 13-methyl analogue Ia is mentioned in the literature [13] as the side product of the ethinylation step in the synthesis of norethisterone-[9,11-^{3}H].)

The NMR spectra of the synthesized IIb furnished final evidence for its structure presented in

Table 1
Relative retention times (4-ene-3-keto steroids/3-deoxo analogues)[a]

4-ene-3-keto steroid/4-ene-3-deoxo steroid	Relative retention time
Norethisterone/lynestrenol	3.1
3-Ketodesogestrel/Desogestrel	3.3
Norgestrel/IV	3.5

[a] The HPLC system is described in Section 2.1.



Scheme 1.

Scheme 1: singulets of the acetylenic protons of the 3- and 17-ethinyl groups at 2.97 and 2.58 ppm, ^{13}C signals of the same at 85.9 + 77.2 and 88.0 + 74.0 ppm, respectively; multiplets of vinylic protons (H-4 at 6.40 ppm and H-6 at 5.59 ppm). Its mass spectrum (Fig. 3) contains the same molecule peak as the sample obtained by TLC spot elution (m/z 320) with identical fragmentation pattern. The UV spectrum, characteristic of the conjugated *trans*-dienyne system (see Fig. 4) is also identical with those obtained after TLC spot elution and by the HPLC diode-array detector. The retention matching of synthesized IIb with the unknown impurity of norgestrel in the described TLC and HPLC systems was also successful.

It is interesting to note that the sample of synthesized IIb contained as an impurity the isomeric 3,17α-diethinyl-13-ethyl-3,5(10)-gonadiene-17-ol (IIIb) formed as a side-product of the diethinylation/dehydration reactions. Its quantity was 18% as determined on the basis of the integrals of its characteristic ^1H-NMR signals: acetylenic proton of the 3-ethinyl group at 3,03 ppm and vinylic H-4 proton at 6.13 ppm. This isomeric impurity is poorly resolved from IIb in the HPLC system described in Section 2.2 (retention time 8.0 min). The maximum of the diode-array UV spectrum of this *cis*-dienyne derivative is at 305 nm (calculated on the basis of Woodward role 303 nm.) The two maxima/shoulders above 300 nm in Fig. 4 are due to this impurity in IIb. IIIb is present in the investigated norgestrel samples at the trace level only (below 0.02%).

3.3. Detection and identification of 17α-ethinyl-13-ethyl-4-gonene-17-ol (3-deoxo-norgestrel, IV) in norgestrel

In possession of a selective HPLC method for the determination of IIb in norgestrel (see Section 3.4) it was possible to make comparison between the results thus obtained and those obtained by TLC densitometry after visualization with phosphomolybdate. This has shown small but not negligible differences indicating that even TLC system A is not sufficiently selective: there is another impurity, too, under the spot of IIb. After having developed the even more selective system B the unknown impurity could be separated from IIb (R_f values of the unknown impurity, IIb and norgestrel in system B are 0.52, 0.48 and 0.12, respectively).

On the basis of its in situ TLC reflectance spectrum the unknown impurity was found to be UV spectrophotometrically inactive. The molecule peak in its mass spectrum obtained after TLC spot elution was at m/z 298 (see Section 3.2). On the basis of these data it was postulated that this impurity was the 3-deoxo derivative of norgestrel (17α-ethinyl-13-ethyl-4-gonene-17-ol, IV). 3-deoxo type impurities in 19-nor-4-ene-3-ketosteroids are well known in the literature as the side-products of the Birch reduction step in their syntheses [4,5]. The peak of IV appears at 14.0 min using the HPLC system described in Section 2.2 (monitoring at 210 nm). The comparison of this retention time with those of norgestrel and other pairs of 3-deoxo/3-oxo steroid drugs is summarized in Table 1. The good agreement of the relative retention times affords further evidence for the identification of IV.

Due to the low molecular weight and the apolar nature of IV its gas chromatographic properties are excellent: retention time at 11.6 min (main component at 31.2 min). The mass spectrum of IV obtained from a GC/MS scan confirmed the identification of IV.

3.4. Quantitative determination of IIb and IV in norgestrel

Preliminary experiments have shown that TLC densitometry using system B in Section 2.1 after visualization with phosphomolybdate reagent is suitable for the quantification of both impurities separately while their sum is measured when the system of USP XXIII is adopted. The selective determination of IIb can be carried out by HPLC at 268 nm using the external standard method while gas chromatography is eminently suitable for the quntitative determination of IV. Work is underway to validate these methods. The preliminary investigation of several batches of bulk norgestrel has shown that in the overwhelming majority of cases the quantity of both IIb and IV is below 0.1%.

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