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Short Communication

Preparative separation and properties of (*E*)- and (Z)-steroidal a&unsaturated ketoximes

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ABSTRACT

A procedure for the preparative resolution of(E)- and (*Z*)-norgestimate (17β-acetyloxy-13-ethyl-18,19-dinorpregn-4-en-20-yne 3-oxime) using flash chromatography is described. A longer column and less polar solvents were applied to overcome the small ΔR_F(0.05) for the isomers of norgestimate. The optical rotations of the *E* and Z isomers were +4.7° and +94.7° (in $CHCl_3$), respectively. The *E/Z* isomer ratio in the mixture was found to be 1.5 both by ^1H NMR spectrometry (4-olefinic H peaks) and by measurement of optical rotations. The validity of the above two methods was confirmed by the calibration graphs obtained with the resolved isomers. The properties of the resolved isomers are discussed with a comparison with those for testosterone oxime.

INTRODUCTION

Since the early 1970s, many α,β-unsaturated ketoximes of steroids have emerged as better drugs than their ketosteroids [1–3]. Some of them, such as norgestimate (17β-acetyloxy-13-ethyl-18,19-dinorpregn-4-en-20-yne 3-oxime) (**1**), are widely in use as oral contraceptives. These compounds are the *E* and *Z* isomers of 3-ketoximes, as two peaks were observed by HPLC with tablets [4] and in pharmacokinetic studies [5]. Norgestimate is used as a contraceptive. However, the physical properties and biological activities of *(E)*- and (*Z*)-norgestimate have never been characterized, so it was considered necessary to study the different properties and to elucidate the different biological effects of these different isomers.

As all the Δ^4-3-one oximes are very similar with respect of the differences in their isomers, the clinically important norgestimate was chosen as an example of α,β-unsaturated 3-ketoximes to study the *E* and Z isomers. These isomers have been separated as cyclodextrin inclusion complexes by HPLC on a cyanopropylsilica stationary phase [6]. Subsequently, the same group reported the separation of these isomers by conventional and overpressure TLC [7]. Only a few micrograms of isomers, which is insufficient to characterize their spectral and physical properties, can be obtained by these methods. Flash chromatography [8] was employed tentatively to separate the *E* and Z isomers of **1** on a preparative scale.

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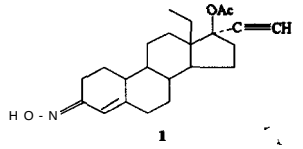

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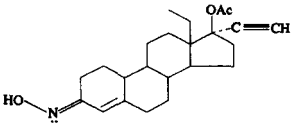

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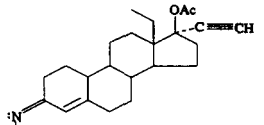

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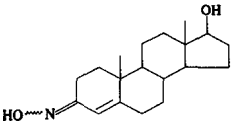

EXPERIMENTAL

Melting points were determined with the capillary method and are uncorrected. ^1H NMR spectra were recorded on a JEOL FX-90Q or Variant VXR300 Fourier transform NMR spectrometer. C^2HCl_3 was used as the solvent, while and tetramethylsilane (TMS) served as the internal standard. Optical rotations were measured in a 1-dm cell on a Perkin-Elmer Model 271 polarimeter at 589 nm (Na D line). UV spectra were obtained with a Shimazu UV-260 spectrophotometer. Mass spectra (electron impact mode, 100 eV) were measured on a VG-25-255 spectrometer. Microanalyses were carried out using a Perkin-Elmer Model 240-C elemental analyser. Microspherical silica gel [40–63 μm (230-400 mesh)] (similar to Merck silica gel 60, according to the manufacturer) used for flash chromatography and silica gel GF_{254} for TLC were purchased from Shandong Jime Chemical Factory.

17β-Acetyloxy-13-ethyl-18,19-dinorpregn-4-en-20-yne 3-oxime (1)

One-pot synthesis of **1** from 17β-hydroxy-13-ethyl-18,19-dinorpregn-4-en-20-yn 3-one gave a total yield of 80% [9]. Compound **1** (white solid): m.p. 214-216°C (lit. [10] m.p. 214-218°C); $[\alpha]_D^{15}(CHCl_3) = +40°$ (lit. [10] +41°); ^1H NMR (300 and 90 MI-Ix), δ 8.35 (s, broad, disappeared/^2H_2O, 1 H, =NOH), 6.58 [s, 0.4 H, (*Z*)-4-olefinic H], 5.92 [s, 0.6 H, (*E*)-4-olefinic H], 2.60 (s, 1 H, –C≡CH), 2.04 (s, *3* H, $-OCOCH_3$), 1.00 (t, *3* H, 18-CH_3).

Separation of la and lb

A column with an I.D. of 2.5 cm was used and 50–100-meshs and was added to cover the bottom of the column (*ca.* 1 cm depth). A slurry of silica gel in light petroleum (b.p. 30–60°C) was poured into the column, giving a height of silica in the column of 25 cm. The column was washed with light petroleum (b.p. 30–60°C)–diethyl ether (2:1) (less polar than the developing solvent used for TLC; see Table I) for 20 min at atmospheric pressure. A 300-mg amount of **1** was dissolved in CH_2Cl_2 and mixed with 1 g of silica gel. After the mixtures had dried completely, they were placed on the top of the column. Eluent [light petroleum (b.p. 30–60°C)–diethyl ether (2:1)] was added to the column and the pressure of nitrogen applied to the column was adjusted so as to make the surface of the solvent in the column fall at *ca.* 5 cm/min [8]. The eluate was collected in 10-ml fractions using an automatic fraction collector. TLC was used to monitor the appearance of the compounds. The elution was terminated after all the isomers in the mixture had been washed off the column according to the TLC examination. Fractions with the same components were combined and concentrated by rotary evaporation under vacuum at ambient temperature. Concentration nearly to dryness afforded **la, lb** or a **la-lb** mixture as while solids.

Fraction 1: (E)-1,-1a. Yield 120 mg (40%), m.p. 214–216°C, $[\alpha]_D^{15} = +4.7°$; ^1H NMR (90 MHz), δ 8.20 (s, broad, disappeared/^2H_2O, 1 H, =NOH), 5.88 (s, 1 H, 4-olefinic H), 2.59 (s, 1 H, –C≡CH), 2.04 (s, 3 H, $-OCOCH_3$), 1 .OO (t, 3 H, 18-CH_3); MS, *m/z* (relative intensity, %) 369 (M+, 23), 340 (M+ + 1 -NO, 100), 310 (M+ – OCOCH,, 42). Analysis: calculated for $C_{23}H_{31}NO_3$, C 74.70, H 8.46, N 3.79; found C 75.02, H 8.55, N 3.74%.

Fraction 2: (Z)-1,-1b. Yield 106 mg (35%), m.p. 219–221°C, $[\alpha]_D^{15} = +94.7°$; ^1H NMR (90

MHz), δ 7.64 (s, broad, disappeared/^2H_2O, 1 H, =NOH), 6.55 (s, 1 H, Colefinic H), 2.59 (s, 1 H, $-C\equiv CH$), 2.04 (s, 3 H, $-OCOCH_3$), 1.00 (t, 3 H, 18-CH_3); MS, *m/z* (relative intensity, %) 369 (M+, 22), 340 (M+ + 1 -NO, 100), 310 (M+ – OCOCH,, 36). Analysis: calculated for $C_{23}H_{31}NO_3$, C 74.70, H 8.46, N 3.79; found, C 74.66, H 8.72, N 3.78%.

In addition to **la** and **lb, 55** mg of **1** were recovered as a mixture of **la** and **lb** (yield 18%). The total recovery yield of the separation was 93%.

RESULTS

In flash chromatography, eluent(s) giving R_F = 0.35 (in TLC with the same solvent) for the centre of the mixtures and a 17-cm length of the column have been suggested [8]. An R_F value of less than 0.19 was chosen in our work in order to obtain a better resolution for the isomer mixture with small AR, (cu. 0.05). When a 25-cm column (length increased from the commonly used 17 cm) and a less polar eluent were used, the isomers of **1** were successfully resolved on a preparative scale with nearly a quantitative recovery of **1. The** purities of the isomers were determined by ^1H NMR spectrometry because studies with some analogues of **1** showed that the 4-H chemical shifts are different for different isomers [11,12]. Some properties of the *E* and *Z* isomers (**la** and **lb**), together with that of the isomers of testosterone oxime (**2**), are given in Table I.

TABLE I

SOME PROPERTIES OF ISOMERS OF α,β-UNSATURATED 3-KETOXIMES 1 AND 2

Parameter	Norgestimate (1)		Testosterone oxime (2)	
	E	*Z*	*E*	*Z*
R_F in TLC	0.22" 0.27[7,b]	0.17" 0.24[7,b]	0.33[11,c]	0.20""
M.p. ("C)	214-216	219-221	204–206	228-229
λ_{max} (nm)[d]	241.0	243.0	241.5	243.0
^1H NMR, δ_{4-H} (ppm)	5.88	6.55	5.76	6.49
$[\alpha]_D^{15}(CHCl_3)$	+4.7	+94.7		

[a] Eluent: light petroleum (b.p. 30–60°C)–diethyl ether (3:2).
[b] Eluent: n-hexane-methyl ethyl ketone-diethylamine (8:1:1).
[c] Eluent: benzene-ethyl acetate (3:1).
[d] In ethanol.

DISCUSSION

The differences in λ_{max} in the UV spectra and δ_{4-H} in the ^1H NMR spectra are consistent with the results reported for some analogues [11,12], *viz.*, $h_{,,,}(Z) - h_{,,,}(E) \approx 2$ nm and $\delta_{4-H}(Z) - \delta_{4-H}(E) \approx 0.7$ ppm. It was also found that the absorption band is broader for the *Z* isomer than the *E* isomer for **1,** which is consistent with the results reported for 2 [11]. This indicates an accuracy in the determination of the *E/Z* isomers ratio by HPLC [7,13] with detection at a certain wavelength, as it is very likely that different isomers could have different molar absorptivities at the wavelength used.

The optical rotations of these isomers, which have not previously been reported, were found to differ (see Table I). Derivatization of the keto group into an oxime does not introduce any chiral centre. However, the optical rotations for the *E* and *Z* isomers are different. First, as their parent compound is optically active, it is not surprising that the oximes showed optical activity. Second, the differing values of the optical rotation for the two isomers might be explained by the magnetic dipoles and electric dipoles being different for *E* and *Z* isomers. From the optical rotations of **1, la** and **lb, we can calcu**late that the *E/Z* isomer ratio (**la/lb**) is 1.5. This value is consistent with the result derived from the ratio of the peak areas for the 4-olefinic-H signals in the ^1H NMR spectra of the different isomers. This *E/Z* ratio of 1.5 is similar to the values reported for other analogues [7,11]. The ^1H NMR and optical rotation methods for the determination of the *E/Z* ratio are reliable, because the calibration graphs for both methods obtained with the resolved isomers confirmed their validity.

It was found that **la** and **lb are** stable in the solid state and in non-polar solvents, such as ethyl acetate and chloroform. No isomerization

was detected over at least several weeks. However, rapid isomerixation was observed in polar solvents, such as methanol and acetone. The equilibrium point (E/Z = 1.5) can be achieved in few days at ambient temperature. This is also the reason why we chose a low polarity and low boiling points of the eluents solvents in flash chromatography for resolution. The E/Z isomer ratio in the mixture or solution might be controlled by the internal energies of the different isomers. This may be understood in the future by calculations of the energies of *E* and Z isomers using appropriate molecular modelling software.

The binding of 1 to the progesterone and androgen receptors **(PR** and **AR)** was found to be different to that of its parent ketosteroid **[5,14].** This indicates that the **=NOH** group plays an important role in the binding of **1** to **PR** and **AR. As the** spatial arrangements of **(E)-** and **(Z)-1** for 0 and H atoms in the **3-oxime sub**stituent are very different, one can speculate that the binding of **la** and **lb** to the receptors could not be the same. However, it is not feasible to measure the relative binding affinity to receptors of an individual isomer, as the species would isomerize into each other in aqueous solution, which cannot be avoided in the receptor binding assay. The only way to study the binding to the receptors is through molecular modelling, which is under investigation.

In conclusion, a convenient and preparative resolution of norgestimate **(1)** (or other α,β-steroidal oximes) using flash chromatography has been developed. The studies of the characteristics of the resolved isomers indicated that the E/Z isomer ratio determined by HPLC may be inaccurate owing to the different **absorbances** of the two isomers at a certain wavelength. An accurate and convenient method for determining the *E/Z* isomer ratio is to use the ^1H NMR signals of the **4-olefinic** hydrogen, which are different for the *E* and Z isomers.

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