The photochemical decomposition of the progestogenic 19-norsteroid, norethisterone, in aqueous medium

A.G.J. SEDEE,*§ G.M.J. BEIJERSBERGEN VAN HENEGOUWEN,* H. DE VRIES,* W. GUIJT** AND C.A.G. HAASNOOT**

Introduction

In the pharmacopoeias the sensitivity to light of the (synthetic) sex hormones is mentioned. However, apart from norethynodrel[1] nothing is known from literature about the photodecomposition of these compounds. In view of the light transmittance of the human skin,[2] the bare prescription in the pharmacopoeias 'protect from light' is somewhat conflicting with the recent development of injectable and subdermal long-acting hormonal contraceptives. Furthermore oral contraceptive ingestion has been associated with light-induced side effects.[3-6]

Regarding these effects and also the possible biological activity of the steroidal photoproducts, the light-induced reactions of the contraceptive agents have not received the attention they merit, especially when the extensive daily consumption of these steroids by about 80 million women,[7] often for long periods of their lives, is taken into account. For these reasons, we initiated an investigation into photoreactions of the sex hormones.

The photochemical decomposition of norethisterone (NE, Fig. 1), a commonly applied progestogenic component in oral contraceptives and in 'the minipill', was studied. Knowledge of its photochemistry is also important because it is the main metabolite of several other progestogens.[8] Moreover, the light absorbing conjugated ketone moiety is present in other important sex hormones like norgestrel and 3-ketodesogestrel, the main metabolite of desogestrel.[9]

The photochemistry of steroidal α,β-unsaturated ketones has been the subject of extensive research for years.[10-13] However, the photochemistry of 4-en-3-oxo-19-norsteroids, *i.e.* steroids without the C10-methyl group, has received little attention, whereas it is known that slight changes in the molecular structure may influence photochemical behaviour drastically.

Not only on account of the slight but important difference in the steroidal skeleton our study is different but also because NE was irradiated in aqueous medium of pH 7.4. Irradiations of steroids were mostly performed in aprotic solvents.[10-13] Being aware of the influence of the solvent on the photoreaction of 4-en-3-oxosteroids,[10-13] we have used water with 10% ethanol as the reaction medium. In addition, lamps emitting UV-B light (280-300 nm, a part of the sunlight spectrum at sea level) and ambient temperature were applied in order to have reaction conditions as close as possible to those *in vivo*.

In this paper the photochemical decomposition of NE in aqueous medium is presented. The possible consequences for the application of NE as a contraceptive agent are discussed.

Methods

MATERIALS

Norethisterone (NE) was purchased from Sigma (St. Louis, USA) and was used without further purification. The stereoisomers 17β-hydroxy-19-nor-5α,17α-pregn-20-yn-3-one (IV; Fig. 1) and 17β-hydroxy-19-nor-5α, 17α-pregn-20-yn-3-one (V; Fig. 1) were kindly provided by Schering AG (Berlin, FRG). All solvents were dried and distilled before chromatographical application. Hexane consisted of mixed isomers ('Bakergrade', Baker,

Sedee AGJ, Beijersbergen van Henegouwen GMJ, De Vries H, Guijt W, Haasnoot CAG. The photochemical decomposition of the progestogenic 19-norsteroid, norethisterone, in aqueous medium. Pharm Weekbl [Sci] 1985;7:194-201.

*Department of Pharmacochemistry, Center for Bio-Pharmaceutical Sciences, State University of Leiden, P.O. Box 9502, 2300 RA Leiden, The Netherlands.
**Laboratory of Biophysical Chemistry, Faculty of Science, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands.
§Correspondence and reprints.

Abstract

Norethisterone, a contraceptive 19-norsteroid, was decomposed in aqueous medium (pH 7.4) by UV-B radiation (280-320 nm). This 4-en-3-oxo-19-norsteroid was not prone to the skeletal rearrangement reactions usually observed in steroids possessing a C10-methyl group. Under the reaction conditions applied, products were formed by addition of molecules, such as solvent molecules or a second steroid molecule, and by reduction of the double bond. The prevalence of addition type reactions may have consequences for the application of norethisterone-like steroids in subdermal contraceptive devices.

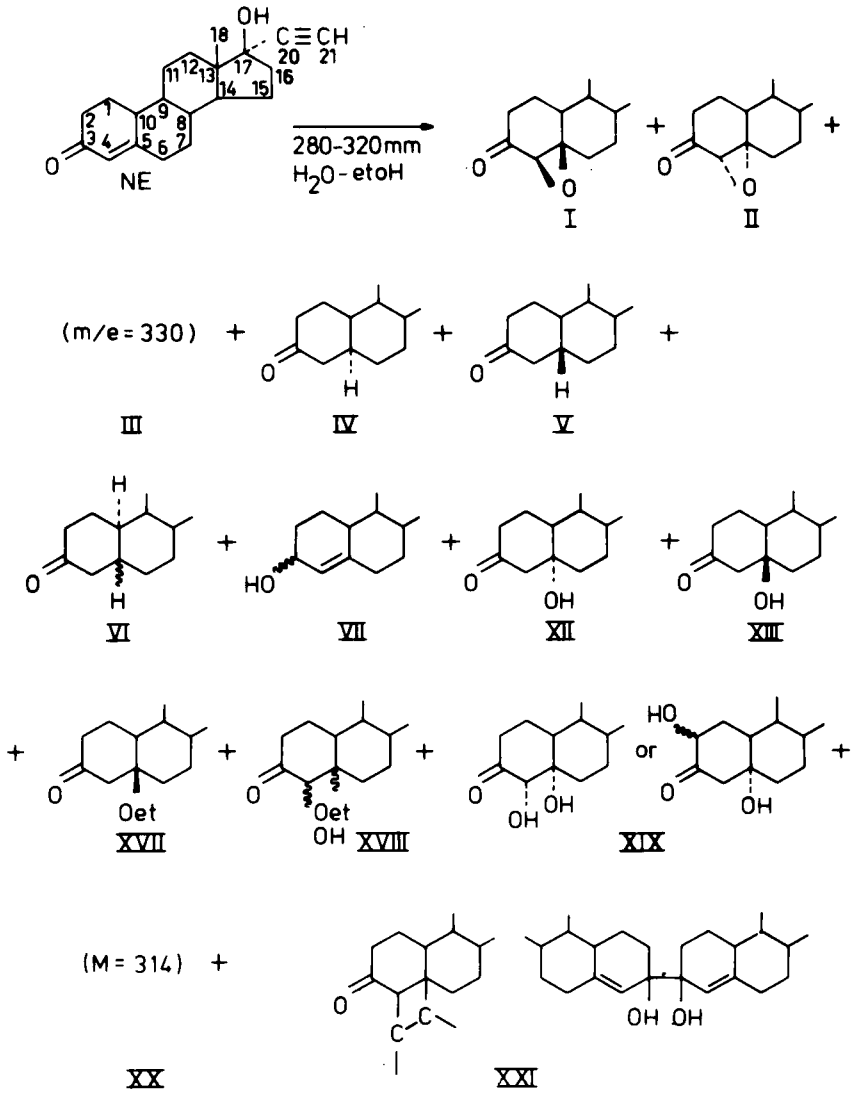


FIGURE 1
Photodecomposition by UV-B-radiation of norethisterone at ambient temperature in aqueous medium of pH 7.4 (see *Methods*). Products have been formed by addition to the 4-en-3-oxo moiety of NE and not by rearrangement

Deventer, The Netherlands). Water was demineralized and distilled.

SYNTHESIS OF STEROIDAL PRODUCTS

NE-4β,5β-epoxide (I; Fig. 1) and its reduction product 5β,17β-dihydroxy-19-nor-17α-pregn-20-yn-3-one (XIII; Fig. 1) were synthesized as described elsewhere.[14] NE-4α,5α-epoxide (II; Fig. 1) was synthesized from the 4,5-iodohydrin of NE according to the method described by Cornforth.[15] Treatment of NE-4β,5β-epoxide with sulfuric acid[16] yielded 4β,5α,17β-trihydroxy-19-nor-17α-pregn-20-yn-3-one. The orientation of its 4,5-substituents was determined by circular dichroism (CD).

PHOTOCHEMICAL DECOMPOSITION

A solution of 0.167×10^{-3} *M* NE in phosphate buffer (0.04 *M*, pH 7.4) containing 10% ethanol was divided in quantities of about 13 ml over 16 quartz tubes (15 × 1 × 1 cm). The tubes were placed in a carousel and irradiated for 40 min at ambient temperature in a Rayonet Photochemical Reactor (RPR-208, Southern New England Ultraviolet Co., Middletown, USA) equipped with two UV lamps (RUL-3000 Å, 25 watt, emitting UV-B light). The distance between the lamps and the tubes was 8 cm. The energy output of the lamps used was 1250 $\mu W \cdot cm^{-2}$ as measured near the tubes with a UVX-31 radiometer sensor (UV Products Inc., San Gabriel, USA). This is comparable with a sunny day in the summer in The Netherlands (52° North latitude). Under these conditions NE was decomposed for about 95% as determined from absorption measurements at 248 nm on a Zeiss M4Q III spectrometer (Zeiss, Jena, GDR), fitted with digital registration apparatus Optilab Multilog 311 and Multiblank 171 (Charles Goffin, Maastricht, The Netherlands).[17] This irradiation procedure was repeated fifteen times in order to obtain a sufficient amount of photoproducts. The irradiated solutions were combined and extracted with dichloromethane.

ISOLATION OF THE PHOTOPRODUCTS

After evaporation of dichloromethane the mixture of the products was dissolved in a small quantity of the eluent for column chromatographic separation: hexane–acetone–diethylether (2+1+2, vol/vol). A Lobar Fertigsäule Grosse C (440-37) Lichroprep Si60 (63-125 μm, Merck, Darmstadt, FRG) was used. A flow rate of

3.5 ml·min^{-1} was applied with an Eldex Labs Inc. (Menlopark, USA) B-94-2 pump. Detection was performed with a refractive index detector (Differential Refractometer R401, Waters Associates, Etten-Leur, The Netherlands). Six fractions were obtained. The first one was further separated on a Lobar Fertigsäule Grosse B (310-25) Lichroprep Si60 (40-63 μm, Merck) applying the solvent mixture hexane–acetone–diethylether (4+1+1, vol/vol; flow rate 3.5 ml·min^{-1}). The other fractions were subsequently chromatographed using the same column with hexane–ethylacetate mixtures (respectively 7+3, 6+4, 5+5, 4+6 and 3+7, vol/vol; flow rate 3.5 ml·min^{-1}).

Isolated fractions were further purified by means of TLC plates (DC-Fertigplatten Kieselgel 60 F254 mit Konzentrierungszone SD 0.25 mm, Merck) and, depending on the polarity of the product to be purified, one of the following mixtures (vol/vol) was used as the eluent: hexane–ethylacetate (8+2, for compounds I-V; 7+3, for compounds IV-VII, XVIII); cyclohexane–ethylacetate (3+2, for compounds IV and V); hexane–acetone–diethylether (4+1+1, for compounds II, III, XVIII; 2+1+1, for compound XII); hexane–acetone–ethylacetate (2+1+2, for compounds XIII, XVII, XX) and dichloromethane–methanol (39+1, for compounds XIX and XXI).

The plates were repeatedly eluted in the same eluent, until an appropriate separation was obtained and the desired product could be scratched off. Detection on TLC plates was performed using UV light (254 nm). Alternatively the compounds may be detected after spraying with H_2SO_4–methanol (8+2, vol/vol) followed by heating at 110°C for 5 min, as coloured spots or by means of UV light of 366 nm as spots with a characteristic fluorescence.[18]

In this way the products I, VI, VII, XII, XIII, XVII-XXI were isolated in pure form, whereas mixtures of II and III and of IV and V were obtained.

SPECTROSCOPIC METHODS

Circular dichroism spectra (MeOH) were recorded on a Dichrographe III (CNRS-Roussel-Jouan, Longjumeau, France), the IR spectra (KBr) on a Beckman IR-10 (Mijdrecht, The Netherlands), the UV spectra (MeOH) on a Perkin Elmer EPS-3T (Gouda, The Netherlands) and the mass spectra on a Kratos (Manchester, UK) MS·9/50 (70 eV, source 150°C, direct introduction). The NMR spectra (CD_3OD) were recorded on a Bruker WM-500 or WM-200 (Karlsruhe, FRG) NMR spectrometer interfaced to an ASPECT-2000 (Karlsruhe, FRG) computer and a real-time pulser board.[19]

Results

Most of the isolated products of the photochemical decomposition could be identified by their spectral data. The most prominent m/e values are indicated by exclamation marks.

I: 17β-hydroxy-4β,5β-oxido-19-nor-17α-pregn-20-yn-3-one.[20] Mass spectrum: m/e = 314, 299, 285, 247!, 229, 215, 201, 189, 173, 159!, 147, 131, 124!, 119, 105!, 91! . NMR spectrum ($CDCl_3$): δ 3.07 (s, 1H, 4α-H); δ 2.95 (s, 1H, C21–H); δ 0.95 (s, 3H, C18–H_3). CD spectrum (CH_3OH): λ_{max} = 308 nm, Δε positive.

II + III: 17β-hydroxy-4α,5α-oxido-19-nor-17α-pregn-20-yn-3-one and possibly 17β-hydroxy-4α,5α-peroxido-19-nor-17α-pregn-20-yn-3-one. Mass spectrum: m/e = 330, 314!, 298, 285, 247, 231, 215!, 201, 189, 173, 159, 149, 133, 124!, 107, 105. NMR spectrum ($CDCl_3$): δ 3.02 (s, 1H, 4β-H); δ 2.97 (s, 1H, C21–H); δ 0.96 (s, 3H, C18–H_3). These spectral data are identical to those of an authentic sample. NMR data of weaker intensity and probably belonging to III are δ 3.17 (s, 1H, 4–H?); δ 2.94 (s, 1H, C21–H?); δ 0.93 (s, 3H, C18–H_3). CD spectrum (CH_3OH): λ_{max} = 317 nm, Δε negative.

IV: 17β-hydroxy-19-nor-5α,17α-pregn-20-yn-3-one. NMR spectrum (CD_3OD): see ref. 20. CD spectrum (CH_3OH): λ_{max} = 290 nm, Δε = +1.38.

V: 17β-hydroxy-19-nor-5β,17α-pregn-20-yn-3-one. NMR spectrum (CD_3OD): see ref. 14; Sedee AGJ, Beijersbergen van Henegouwen GMJ, Guijt W, Haasnoot CAG, unpublished observations. CD spectrum (CH_3OH): λ_{max} = 288 nm, Δε = −0.59.

VI + VII: probably 17β-hydroxy-19-nor-5α,10α,17α-pregn-20-yn-3-one and/or its 5β-isomer and/or 3ξ,17β-dihydroxy-19-nor-17α-pregn-4-en-20-yne. Mass spectrum: m/e = 300, 288, 286, 273, 270, 255, 245, 231, 230, 217!, 203, 188!, 162!, 147!, 137!, 119, 107 and 55, 43!, 29; m/e = 300!, 285, 272, 271, 256!, 253, 243, 241, 230, 223, 217!, 215, 210, 205, 201, 173, 149, 131, 124, 105 and 55, 41!, 29.

XII: 5α,17β-dihydroxy-19-nor-17α-pregn-20-yn-3-one. NMR spectrum (CD_3OD): see Table I of ref. 14.

XIII: 5β,17β-dihydroxy-19-nor-17α-pregn-20-yn-3-one. NMR spectrum (CD_3OD): see Table I of ref. 14.

XVII: 5β-ethoxy-17β-hydroxy-19-nor-17α-pregn-20-yn-3-one. Mass spectrum: m/e = 344, 316, 298!, 283, 270, 247, 231!, 215!, 173, 159, 149, 125!, 110! . NMR spectrum (CD_3OD): δ 3.78 (q, $-OCH_2-$); δ 2.98 (s, C21–H); δ 1.25 (t, $-CH_3$); δ 0.94 (s, C18–H_3). IR spectrum: 3400 cm^{-1}, 3290 cm^{-1}, 2940 cm^{-1}, 2880 cm^{-1}, 1710 cm^{-1}, 1385 cm^{-1}, 1125 cm^{-1}, 1050 cm^{-1}. CD spectrum (CH_3OH): λ_{max} = 316 nm, Δε negative.

XVIII; 4ζ,5α-ethoxy,hydroxy-17β-hydroxy-19-nor-17α-pregn-20-yn-3-one. Mass spectrum: m/e = 360, 344, 342, 330, 326, 314!, 302!, 298!, 269, 259, 247, 231!, 219!, 213!, 173, 137, 124. CD spectrum (CH_3OH): λ_{max}= 292 nm, Δε positive. NMR spectrum (CD_3OD): δ 5.58 (s, C4–H); δ 4.49 (s, C4–H); δ 3.65 (m, $-OCH_2-$); δ 2.97 (s, C18–H); δ 2.95 (s, C18–H); δ 1.25 (t, $-CH_3$); δ 0.95 (s, C18–H_3); δ 0.92 (s, C18–H_3).[21,22]

XIX: 4α,5α,17β-trihydroxy-19-nor-17α-pregn-20-yn-3-one or 2ξ,5α,17β-trihydroxy-19-nor-17α-pregn-20-yn-3-one. Mass spectrum: m/e = 332, 316, 314!, 298!, 265!, 264!, 247!, 231!, 215, 203, 189, 175!, 159!, 135!, 124! . CD spectrum (CH_3OH): λ_{max} = 293 nm, Δε positive.

XX: Product M = 314. Mass spectrum: m/e = 314, 298!, 281, 270, 256, 231!, 215!, 213, 189, 173, 162, 160, 147!, 135!, 124!, 121!. The CD spectrum did not show an absorption maximum. IR spectrum (KBr):

3420 cm^{-1}, 2960 cm^{-1}, 2920 cm^{-1}, 2850 cm^{-1}, 1260 cm^{-1}, 1095 cm^{-1}, 1020 cm^{-1}, 800 cm^{-1}. NMR spectrum (CD_3OD): δ 5.88 (s); δ 3.75 (d); δ 2.96 (s); δ 2.81 (s); δ 0.93 (s).

Discussion

The photochemistry of cyclohexenones has been thoroughly investigated. The following types of reactions are commonly found: rearrangements, dimerizations, deconjugation to β,γ-ketones and reductions.[10-13] Under photolytic conditions the same reactions occur in 4-en-3-keto-steroids used in pharmaceutical preparations.[23-25] Also sex hormones with a cyclohexenone moiety are known to be photolabile.[3] Photolysis of norgestrel (having an ethyl group at C13 instead of a methyl group as in NE) and photodegradation of ethisterone (like NE, but with a C10 methyl function) upon irradiation with a sterilizing UV lamp or upon exposure to sunlight have been reported.[26,27] Decomposition of NE in tablets has been observed once.[28] Photosensitized decomposition by the colourant of the tablets has to be ruled out in this case also because NE was decomposed to a larger extent in white tablets than in dosage forms containing a light absorbing colourant.[28] However, no specific decomposition products of NE or norgestrel have been identified.

After spotting the complete reaction mixture of the photoproducts of NE on a TLC plate (Kieselgel 60 F254, Merck), which was subsequently eluted with hexane–acetone–diethylether (4+1+1, vol/vol; three times) in the first direction and with cyclohexane–ethylacetate (1+1, vol/vol; two times) in the second direction, about 25 products could be discerned on account of a different Rf value and distinct colour and fluorescence at 366 nm.[18]

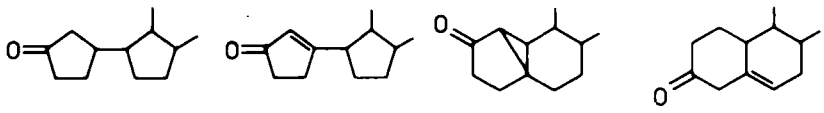
After irradiation for shorter periods than 40 min a somewhat smaller number of products, with the same Rf value, colour and fluorescence as products formed by irradiation for 40 min, were observed. To obtain a large amount of photoproducts irradiation was performed for 40 min. In view of the situation *in vivo* the types of photoreactions of NE were more significant to us than the number of products and the quantity of each product (see *Conclusion*).

An accurate quantitative determination of the products could not be performed because of losses during the laborious procedure for the isolation (column chromatography followed by TLC, as described in *Methods*) and because of the lack of knowledge of the absorption coefficient of the various compounds. A semi-quantitative approach was performed by a densitometric determination[17] after two-dimensional separation of the complete reaction mixture on pre-coated TLC plates (Sil G-25 Tenside, Machery Nagel & Co., Düren, FRG), followed by charring of the spots.[17] However, the percentage stated for each product remains an approximation, because it was assumed that all products have the same charring capacity and because some products could not be separated enough to allow proper densitometric determination.

The only exception is NE-4β,5β-epoxide (I, Fig. 1) which had been determined quantitatively before.[17] It was shown that I is a main product (12%) in the photodecomposition. A fraction (5%) containing products II and III was isolated, yielding a parent peak in its mass spectrum of m/e = 330 and the most prominent peak at m/e = 314. The NMR spectrum of the fraction showed four singlets between 2.95 and 3.20 ppm (see *Results*). One of the compounds (III) was probably decomposed, thereby yielding the α-epoxide (II), because this fraction had the same Rf value in TLC experiments as the synthesized sample of II and because its CD spectrum showed a negative maximum. Product III with m/e = 330 might be a peroxide or a hydroperoxide as observed in the base catalyzed autoxidation of 4-en-3-oxosteroids,[30] but this, probably unstable, product could not be further characterized.

In various TLC systems, a second fraction (8%, m/e = 300) showed the same Rf value as two stereoisomers IV and V. Moreover, the mass spectral fragmentation pattern of this fraction was identical with those of authentic samples of these two compounds. After recording CD spectra of the pure stereoisomers and of the isolated fraction, it was calculated that the 5α- and 5β-isomer, IV and V respectively, were formed photochemically in a 4:1 ratio. It should be noted that photoreduction of α,β-unsaturated ketones is a general process in alcoholic and aqueous media.[12,31-33] Up till now the formation of a 5β-H steroid in the photoreduction has not been reported. Several reasons may be held responsible for this fact. For instance, until now, steroids with a C10-methyl group were irradiated in these protic (alcoholic and aqueous) media, and the presence of the C19-methyl group may have prevented the formation of a 5β-H steroid. Another possibility is that a 5β-H steroid was indeed present but not isolated from the complex reaction mixture. Lastly, it could be that the presence of some 5β-H was masked by co-chromatography with its 5α-H isomer.

Two other, minor products (VI and VII; m/e = 300) were isolated of which, apart from a mass spectral fragmentation pattern, no other spectral data could be obtained because of the small quantity (2%) in which these products were formed. The precise identity of the products VI and VII is unknown; the products might be 17β-hydroxy-19-nor-5α,10α,17α-pregn-20-yn-3-one (VI) and/or its 5β-isomer, and/or 3ξ,17β-dihydroxy-19-nor-17α-pregn-4-en-20-yne (VII). According to previously reported reactions, apart from IV and V several products with m/e = 300 may be expected (Fig. 1 and 2).[31-33] However, concomitantly with VIII other rearrangement products, such as IX and X, have to be expected on the basis of photochemistry of analogues of NE



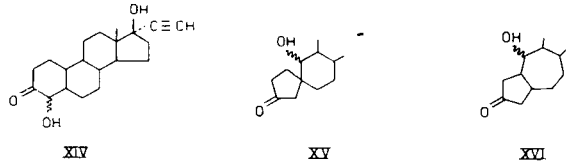
VIII IX (M=298) X (M=298) XI (M=298)

FIGURE 2
Rearrangement products of norethisterone expected to be formed according to the photochemistry of analogues of norethisterone, but not isolated

(Fig. 2).[10-12 21 31] The absence of photorearrangement products like IX and X among the isolated photodecomposition products makes it unlikely that VIII is one of the products with m/e = 300.

A rearrangement reaction of α,β-unsaturated ketones is markedly sensitive to solvent effects[12] and does not proceed smoothly in aprotic media.[10] This indicates that proton donation by the solvent, a feasible process in aqueous medium, is important. Although photorearrangement of cyclohexenones to cyclopentenones (IX), to lumiketones (cyclopropyl ketones; X) or to unconjugated β,γ-unsaturated ketones (XI)[12] are major reactions of these α,β-unsaturated ketones, such products (m/e = 298; Fig. 2) were not identified after irradiation of NE (the remaining, not identified, compounds are minor photoproducts of NE). Neither another possible photoproduct, norethynodrel, nor one of its photoproducts were found.[1] The absence of rearrangement reactions upon irradiation of a 19-norsteroid, 19-nortestosterone, in *t*-butanol (a solvent very suited to observe that type of reactions) was reported previously,[11] thus indicating the great influence of a slight change in the molecular structure of steroidal cyclohexenones on their photochemical behaviour.[34] Absence of rearrangement reactions upon irradiations of 4-en-3-keto-19-norsteroids may well be a common feature of this type of compound.

Under appropriate conditions, addition of a solvent molecule or dimerization appeared to predominate over the rearrangement processes in C19-steroids.[10 12 13] The absence of a methyl group at C10 did not prevent the addition of (parts from) solvent molecules. One of the main photoproducts of NE (m/e = 316; XII; 15%) appeared to be formed by the net addition of a water molecule and could be identified as 5α,17β-dihydroxy-19-nor-17α-pregn-20-yn-3-one.[14] Moreover, the corresponding 5β-isomer (XIII; 5%) was also isolated. It could be demonstrated that neither a C4-hydroxylated compound (XIV) nor the spirocompound XV and the cyclopentanone XVI fitted in with the 2D NMR data (Fig. 3). Compounds like XIV, XV and XVI were previously identified as photoproducts of steroid type molecules with a C10-methyl group.[35-37]



XIV XV XVI

FIGURE 3
Products with m/e = 316 expected to be formed according to the photochemistry of compounds with a 2-cyclohexenone moiety, but not isolated

An increase in molecular weight with 46 mass units in one of the products (XVII; 3%) and a negative Δε in its CD spectrum indicated the presence of an ethoxy group in a 5β position.[38] Similar nucleophilic attacks at the β-carbon of an enone system resulting in the addition of an alcoholic moiety have been reported previously.[13 39-41] Additional proof for the assigned structure was obtained from the NMR spectrum of XVII: a signal due to H4 in a C4-substituted steroid could not be detected. A product formed by addition of a molecule of the alcoholic solvent in the photoreaction of 4-en-3-ketosteroids in alcoholic medium[33 42] has not been reported previously, although such Michael-type adducts are the main products in the photoreaction of 2-cycloalkenones in alcoholic medium. In photoreactions of 4-en-3-ketosteroids with a methyl group at C10 a product formed by reduction of the double bond (as in IV) was isolated as main product in these media.[33 42]

The literature concerning the influence of carbon substituents at the β-carbon of enone systems in cyclohexenones (like C6 and C10 at C5 in 4-en-3-ketosteroids) on this alcohol addition at the double bond is not of the same tenor. For instance, it was reported by Williams that an alcohol addition did not take place in a β-dialkyl substituted enone (as in NE),[39] instead the reduction product (analogous to IV) was obtained almost quantitatively. However, photoinduced methanol addition at C3 of 3-methyl-2-cycloalkenones has also been reported:[41] the same type of reaction has been observed for NE.

Another product (XVIII; m/e = 360) was isolated in a small quantity (5%). The m/e value points to the presence of an ethoxy and a hydroxy function. This type of product was a main product in the photochemical solvent addition to 1-acetylcyclohexene in *t*-butanol.[40] From the positive maximum in its CD spectrum[38] it is concluded that product XVIII has a 5α substituent. The NMR spectrum of XVIII showed two strong singlets at 0.92 and 0.95 ppm (assigned to the hydrogens at C18) and two singlets at 4.49 ppm and 5.58 ppm (assigned to a proton at C4).[21 22] From these findings it is concluded that two isomeric compounds are present, each of which has one substituent (hydroxy or ethoxy) at C4 and one at C5 (ethoxy or hydroxy). The exact position and orien-

tation of the hydroxy and ethoxy substituents in these isomers are indistinct and XVIII is therefore best described as 4ξ,5α-ethoxy,hydroxy-17β-hydroxy-19-nor-17α-pregn-20-yn-3-one.

A product (XIX; 6%) with m/e = 332 and a prominent (M^+–18) at m/e = 314 could be rationalized by assuming an addition of two hydroxyl groups to the NE skeleton. Such an apparently unstable product has been described formerly as a metabolite of I and was named NE-4,5-dihydrodiol.[43] Acid catalyzed water addition to I (*Methods*) yielded a more stable diol with another Rf value than XIX, namely 4β,5α,17β-trihydroxy-19-nor-17α-pregn-20-yn-3-one. From the positive maximum of the CD spectrum of the photoproduct XIX it could be concluded that the substituent at C_5 has an α-orientation, identical to the synthesized product.[38] Therefore, compound XIX is either 4α,5α,17β-trihydroxy-19-nor-17α-pregn-20-yn-3-one or 2ξ,5α,17β-trihydroxy-19-nor-17α-pregn-20-yn-3-one (Fig. 1 and 4).

A minor product (XX; 4%) with a parent peak of m/e = 314, a prominent peak of M^+–16 at m/e = 298 and a, not in any other product observed, prominent fragment of m/e = 44 could not be identified by us on the basis of the data at hand. Moreover, the identification is hampered by the instability of this photoproduct. According to the absence of a maximum in its CD spectrum the ketone function has apparently disappeared. The absence of an absorption at about 1700 cm^{-1} also points to this conclusion. The prominent fragment of m/e = 44 (instead of, for instance, m/e = 43 in the mass spectra of XII and XIII) and the strong absorptions at 1260 cm^{-1} and 1095 cm^{-1} indicated the presence of a secondary alcohol function. The strong absorption at 800 cm^{-1} suggests the presence of an alkene moiety. The latter finding is corroborated by the occurrence of a resonance at 5.88 ppm in the 1D NMR spectrum. Unfortunately, further 2D NMR experiments could not be performed due to the small quantity isolated of this unstable product.

In solvent of high polarity high yields of dimer were obtained upon irradiation of 4-en-3-oxo-steroids.[10] According to the literature several fractions consisting of compounds with a high molecular weight were isolated. These fractions contain probably dimers, structure XXI represents possible dimerization products. No attempts were made to separate and identify these, but the formation of a pinacol and also of 'head to tail' or 'head to head' dimers and even addition to the ethinyl function is easily envisaged.[10,12,13] From a photobiological point of view the formation of dimers is not relevant, since the concentration of NE in the skin will be very low.

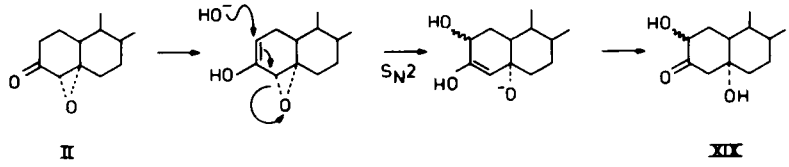
Conclusion

The main photoproducts in the decomposition of NE have been described. Some of these products are in turn photoreactive and/or thermally unstable. A few of the unidentified products amongst the about 25 that were discerned in the TLC experiment, may originate from such primary products. For example, irradiation of I is supposed to yield the enolizable β-diketone, 3,5-dioxo-10(5→4)-abeo-derivative.[11] Another example is that irradiation of C_4 hydroxy or ethoxy substituted products may result in the formation of a lactone or its ester analogue.[44] α,β-Dihydroxy-ketones are readily isomerized to the corresponding hydroxylactones.[11] For the time being no information is available to identify the remaining (not further isolated) minor products.

Nevertheless, it can be concluded that products in the photodecomposition of the 19-norsteroid, NE, in aqueous solution are mainly formed by addition of solvent molecules to the 4=5 double bond, by oxidation and by dimerization. It is remarkable that skeletal rearrangements are not observed. The latter finding is important from a photobiological point of view.

Since *in vivo* steroids are reversibly bound to protein and the present study demonstrates that in aqueous medium addition of environmental molecules predominates over rearrangement reactions, irreversible binding of NE to protein upon irradiation (sunlight) seems to be a feasible process *in vivo*. This might in turn result in an allergic response. This possibility is supported by our research into UV-B radiation induced irreversible binding of ^{14}C labelled NE to albumin. In the latter experiment 20% of the radiolabel was associated to the protein upon irradiation for 40 min with two lamps (RUL-3000 Å).[45] Apart from a direct light-induced addition reaction irreversible binding of steroidal products to albumin was observed.[20] It is known that photoproduct I is able to bind irreversibly to proteins thermochemically, the more so after enzymatic activation.[46]

The biological activity of the products of NE is unknown. Interestingly, a patient suffering from allergic-like side effects caused by a subdermal contraceptive device has been encountered (Van



II XIX

FIGURE 4
Mechanism for the formation of one of the proposed products (XIX)

Weelden H, personal communication). The question arises if the application of NE and norgestrel in especially subdermal formulations might increase the incidence of light-induced side effects.[3-6]

Acknowledgements

We wish to thank Mr. B. van Vliet for recording the IR spectra and Mr. J.J. van Houte and Dr. W. Onkenhout for recording the mass spectra. The suggestions of Dr. M. Groen (Organon International BV, Oss, The Netherlands) are gratefully acknowledged. This work was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO). NMR spectra were recorded at the Dutch National 500/200 hf-NMR facility at Nijmegen. We thank Mr. P.A.W. van Dael for keeping the NMR instruments in excellent condition.

References

[1] Williams JR, Abdel-Magid A. Photolysis of 3-oxo-$\Delta^{5(10)}$-steroids in alcoholic solvents and in the solid phase. Tetrahedron 1981;37:1675-7.

[2] Anderson RR, Parish JA. Optical properties of human skin. In: Regan JD, Parrish JA, eds. The Science of Photo-medicine. New York: Plenum Press, 1982:147-74.

[3] Wade A, ed. Martindale. The Extra Pharmacopoeia. 27th ed. London: The Pharmaceutical Press, 1977:1388.

[4] Horkay I, Tamási P, Prékopa A, Dalmy L. Photodermatoses induced by oral contraceptives. Arch Dermatol Res 1975;253:53-61.

[5] Mathison IW, Haas KL. Drug Photosensitivity. I. Light- and photosensitivities observed during oral contraceptive therapy: a review. Obstet Gynecol Surv 1970;25: 389-401.

[6] Zaun H. Hautveränderungen unter der Einnahme hormonaler Kontrazeptiva. Med Monatsschr Pharm 1981;4:161-5.

[7] Lebech PE, Ottesen B. Sex hormones and related compounds, including oral contraceptives. In: Dukes MNG, ed. Side Effects of Drugs Annual 7. Amsterdam: Elsevier Science Publishers BV, 1984:382-96.

[8] Ranney RE. Comparative metabolism of 17α-ethynyl-steroids used in oral contraceptives. J Toxicol Environm Health 1977;3:139-66.

[9] Viinikka L. Radioimmunoassay of a new progestagen Org 2969 and its metabolite. J Steroid Biochem 1978;9:979-82.

[10] Kropp PJ. Photochemical transformations of cyclohexadienones and related compounds. In: Chapman OL, ed. Organic Photochemistry. Vol 1. New York: Marcel Dekker, Inc., 1967:1-90.

[11] Jeger O, Schaffner K. On photochemical transformations of steroids. Pure Appl Chem 1970;21:247-62.

[12] Schuster DI. Photochemical rearrangements of enones. In: De Mayo P, ed. Rearrangements in ground and exited states. New York: Academic Press, 1980:167-279. (Organic Chemistry. A Series of Monographs. Vol. 42-3.)

[13] Chapman OL, Weiss DS. Photochemistry of cyclic enones. In: Chapman OL, ed. Organic Photochemistry. Vol. 3. New York: Marcel Dekker Inc., 1973:197-288.

[14] Sedee AGJ, Beijersbergen van Henegouwen GMJ, Guijt W, Haasnoot CAG. Structure elucidation of two isomeric steroids: photolytical and thermal reaction products from norethisterone studied by two-dimensional NMR. Pharm Weekbl [Sci] 1985;7:202-7.

[15] Cornforth JW, Green DT. Iodohydrins and epoxides from olefins. J Chem Soc (C) 1970;846-9.

[16] De Jongh DC, Hribar JD. The identification of some human metabolites of norgestrel, a new progestational agent. Steroids 1968;11:649-66.

[17] Sedee AGJ, Beijersbergen van Henegouwen GMJ, Blaauwgeers HJA. Isolation, identification and densitometric determination of norethisterone-4β,5β-epoxide after photochemical decomposition of norethisterone. Int J Pharm 1983;15:149-58.

[18] Sadée W, Riegelman S, Johnson LF. On the mechanism of steroid fluorescence in sulfuric acid. Part 1. The formation of trienones. Steroids 1971;17:595-606.

[19] Sedee AGJ, Beijersbergen van Henegouwen GMJ, Guijt W, Haasnoot CAG. Assignment of the ^{1}H and ^{13}C nuclear magnetic resonance spectra of norethisterone using two-dimensional nuclear magnetic resonance spectroscopy. J Chem Soc [Perkin II] 1984:1755-9.

[20] Sedee AGJ, Beijersbergen van Henegouwen GMJ, Guijt W, Haasnoot CAG. Conformational analysis of steroids in solution: 17β-hydroxy-19-nor-5α,17α-pregn-20-yn-3-one and its 5β-isomer studied by nuclear magnetic resonance. J Org Chem (in press).

[21] Caus MJ, Cánovas A, Bonet J-J. Photochemical rearrangement of a steroidal α,β-epoxylactone. Helv Chim Acta 1980;63:473-7.

[22] Huppi G, Eggart G, Iwasaki S, Wehrli H, Schaffner K, Jeger O. Die Beeinflussung der photochemischen Umsetzungen von gesättigten Ketonen durch α-Substituenten. Helv Chim Acta 1966;49:1986-2001.

[23] Savard K, Wotiz HW, Marcus Ph, Lemon HM. Effect of ultraviolet light on steroids during paper chromatography. J Am Chem Soc 1953;75:6327-9.

[24] Hamlin WE, Chulski T, Johnson RH, Wagner JG. A note on the photolytic degradation of anti-inflammatory steroids. J Am Pharm Ass 1960;49:253-5.

[25] Graham RE, Williams PA, Kenner CT. Detection of decomposition and analytical interferences in pharmaceutical preparations containing corticosteroids. J Pharm Sci 1970;59:1152-6.

[26] Cullen LF, Rutgers JG, Lucchesi PA, Papariello GJ. Fluorometric determination of norgestrel and structurally related steroids. J Pharm Sci 1968;57:1857-64.

[27] Yoshida I, Asahina H, Oyabara I. Photostability of some steroid hormones. Horumon To Rinsho 1974;22:881-5 (C.A. 1974;81:147242m).

[28] Kaminski EE, Cohn RM, McQuire JL, Carstensen JT. Light stability of norethindrone and ethinylestradiol formulated with FD & C colorants. J Pharm Sci 1979;68:368-70.

[29] Sedee AGJ, Beijersbergen van Henegouwen GMJ. Photosensitized decomposition of contraceptive steroids: a possible explanation for the observed (photo-) allergy of the oral contraceptive pill. Arch Pharm (Weinheim) 1985;318:111-9.

[30] Frimer AA, Gilinsky-Sharon P, Hameiri J, Aljadeff G. Superoxide-, *tert*-butoxide-, and hydroxide-mediated autoxidation of 3-oxo-Δ^{4}-steroids in aprotic media. J Org Chem 1982;47:2818-19.

[31] Bellus D, Kearns DR, Schaffner K. Zur Photochemie von α,β-ungesättigten cyclischen Ketonen: Spezifische Reaktionen der n,π*- und π,π*-Triplett-zustande von *O*-Acetyl-testosteron und 10-Methyl-$\Delta^{1,9}$-octalon-(2). Helv Chim Acta 1969;52:971-1009.
[32] Schuster DI, Nunez IM, Chan CB. The mechanism of photoreduction of cyclohexanones in isopropyl alcohol. Tetrahedron Lett 1981;22:1187-90.
[33] Cornell DG, Avram E, Filipescu N. Photohydration of testosterone and 4-androstene-3,17-dione in aqueous solution. Steroids 1979;33:485-94.
[34] Dauben WG, Shaffer GW, Vietmeyer ND. Alkylsubstitution effects in the photochemistry of 2-cyclohexenones. J Org Chem 1968;33:4061-9.
[35] Ferrer V, Gomez J, Bonet J-J. A type-A rearrangement in the photolysis of a steroidal α,β-unsaturated lactone. Helv Chim Acta 1977;60:1357-60.
[36] Chapman OL, Sieja JB, Welstead Jr WJ. Stereospecific rearrangement of an excited triplet ketone. J Am Chem Soc 1966;88:161-2.
[37] Williams JR, Moore RH, Li R, Weeks CM. Photochemistry of 11α- and 11β-hydroxy steroidal 1,4-dien-3-ones and 11α- and 11β-hydroxy steroidal bicyclo-[3.1.0]hex-3-en-2-ones in neutral and acidic media. J Org Chem 1980;45:2324-31.
[38] Jacobs HJC, Havinga E. Cotton effects and conformations of skeletally isomeric saturated 3-oxo-steroids. Tetrahedron 1972;28:135-53.
[39] Williams IA, Bladon P. A novel photochemical addition reaction of 3-acetoxypregna-5,16-dien-20-one. Tetrahedron Lett 1964;5:257-62.
[40] Ramey BJ, Gardner PD. Mechanism of photochemical alcohol addition to α,β-unsaturated ketones. J Am Chem Soc 1967;89:3949-50.
[41] Hart H, Chen B, Jeffares M. Stereochemistry of the photoinduced and Michael addition of methanol to seven- and eight-membered 2-cycloalkenones. The effect of methyl substituents. J Org Chem 1979;44:2722-6.
[42] Nann B, Gravel D, Schorta R, Wehrli H, Schaffner K, Jeger O. Die UV-Bestrahlung von Testosteron und 3-Oxo-17β-acetoxy-$\Delta^{1,5}$-androstadien. Helv Chim Acta 1963;46:2473-82.
[43] White INH. Chemical reactivity and metabolism of norethindrone-4β,5β-epoxide by rat liver microsomes *in vitro*. Chem Biol Interact 1980;29:103-15.
[44] Stiver S, Yates P. Stereospecificity in the photoisomerization of steroidal α-ketols to lactones. Tetrahedron Lett 1984;25:3289-92.
[45] Sedee AGJ, Beijersbergen van Henegouwen GMJ, Lusthof KJ, Lodder G. Irreversible binding of norethisterone to human serum protein induced by UV-B light. Pharm Res 1984;1267-9.
[46] Kappus H, Bolt HM. Irreversible protein binding of norethisterone (norethindrone) epoxide. Steroids 1976;27:29-45.

Received March 1985.
Accepted for publication August 1985.