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Photosensitized Decomposition of Contraceptive Steroids: A Possible Explanation for the Observed (Photo)allergy of the Oral Contraceptive Pill

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The photo-sensitized decomposition of the main steroids used in the oral contraceptive pill has been studied. Under the circumstances applied, **1** and **8** decomposed rapidly ($t_{1/2} = 11.0 \pm 0.2$ min for **1** and 5.7 ± 0.4 min for **8**). The decomposition product of **1** has been identified as 17α-ethynyl-10β-hydroperoxy-17β-hydroxyestra-1,4-dien-3-one (**2**). The decomposition products of **1** and **8** were previously found in experiments in which the irreversible binding of these steroids to protein was studied. Photosensitized decomposition of the steroids may be the cause of (photo)allergic side-effects of "the pill".

Photosensibilisierte Zersetzung der kontrazeptiven Steroide: Eine mögliche Erklärung für die beobachtete (Photo)allergie der oralen Kontrazeptiva

Die photosensibilisierte Zersetzung der wichtigsten Steroide, die in den hormonalen Kontrazeptiva angewendet werden, wurden studiert. Unter den gegebenen Umständen zersetzten sich **1** und **8** schnell ($t_{1/2} = 11.0 \pm 0.2$ min für **1** und $t_{1/2} = 5.7 \pm 0.4$ min für **8**). Das Produkt der Zersetzung von **1** wurde identifiziert als 17α-Ethinyl-10β-hydroperoxy-1,4-östradien-17β-ol-3-on (**2**). Die Produkte der Zersetzung von **1** und **8** wurden früher gefunden in Experimenten, in denen die nicht reversible Bindung dieser Steroide an Protein studiert wurde. Die photosensibilisierte Zersetzung der Steroide ist möglicherweise die Ursache (photo)allergischer Nebenwirkungen der hormonellen Kontrazeptiva.

Although side effects, induced by light, of the contraceptive pill are mentioned in literature[1,2] the responsible component is not specified in most cases. Recently we showed, that the progestogenic component norethisterone (**7**) may be responsible for photo-induced side effects on the skin but possibly as well on the inner organs[3].

In our experiments the estrogenic components ethinylestradiol(**1**) and its 3-methylether mestranol (**9**), were fairly photo-stable to sunlight (280 ~ 780 nm) in spite of their absorption maximum at 280 nm. The fluorescence of these compounds evidently compete with photochemical decomposition. Apart from *direct* photo-decomposition a photosensitizer may also be a cause of a light induced reaction. Energy, absorbed by the photosensitizer, is then transferred to the molecule provoking its reaction. If the photosensitization proceeds especially in the presence of oxygen it is called a photodynamic process. This is the case with a number of dyes and with some endogenous porphyrins[4].

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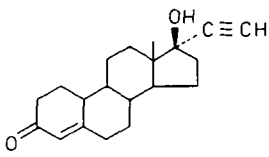
So if an estrogenic component is the cause of a light induced side-effect no direct decomposition but but decomposition by means of a photosensitizer possibly underlies this effect. There is one report[5] of decomposition of ethinylestradiol **(1)** by photosensitizing dyes in tablets, but the identity and pharmacological effects of the product(s) were not mentioned. In the body there are not only exogenous photosensitizers e.g. food dyes[6], but also endogenous ones including porphyrins. So photo-sensitized decomposition of compounds in the body is possible.

In a group of apparently normal women taking oral contraceptives 64 % had an increased urinary excretion of delta-amino laevulic acid, a precursor of the porphyrins[7]. Oral contraceptive steroids have been suggested to induce porphyria by destruction of hepatic microsomal cytochrome P-450 and induction of delta-amino laevulinate synthase and porphyrin synthesis[8,9]. In women taking oral contraceptives the chance of photosensitized decomposition of these steroids is thus enhanced: the consequences are unknown. Of two progestogenic steroids, lynestrenol **(4)** and norethynodrel **(8)** (but without ethynyl group) the products from photosensitized decomposition have already been described[10,11].

In this study we present the identity of the photosensitized decomposition product of **1**. The rate constants of the photosensitized decomposition of those steroids **1, 4, 7, 8** and **9**, that are most frequently used in the oral contraceptive pill, are given. The possible consequences of these qualitative and quantitative results are discussed in relation to the situation *in vivo*.



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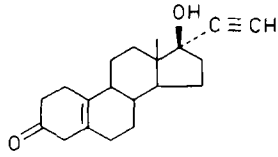

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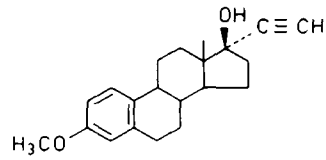

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Results and Discussion

All the steroids used have their absorption maximum beneath 300 nm. To study the photosensitized decomposition a K_2CrO_4 filter was necessary to prevent direct photodecomposition of the steroids and products[12,13,14]. With a 5RP-2 column a good separation between hematoporphyrin, decomposition products of this sensitizer, a steroid and its product(s) has been achieved (fig. 1a–d). Because of its low extinction coefficient the initial concentration of **8** is higher to allow HPLC determination with UV detection. A densitometric determination[14] was applied to **4**, because this steroid lacks an appropriate UV-absorption at those wavelengths that can be used for a HPLC detection. As internal standard **7** could be used: it was not decomposed by photosensitization (table) nor did it influence in combination with **1** the reaction rate constant of the latter (by quenching the excited state of the sensitizer for instance). So **7** was added to the solution of **4** before the reaction was started. After proving the linearity of a calibration curve in the range 1.0–20.0 µg (LR = 0.9997, with 6 samples) the conversion of **4** has been determined. With this densitometric method it is possible to determine the ration between the products **5** and **6** (fig. 1e) of the photooxidation[10].

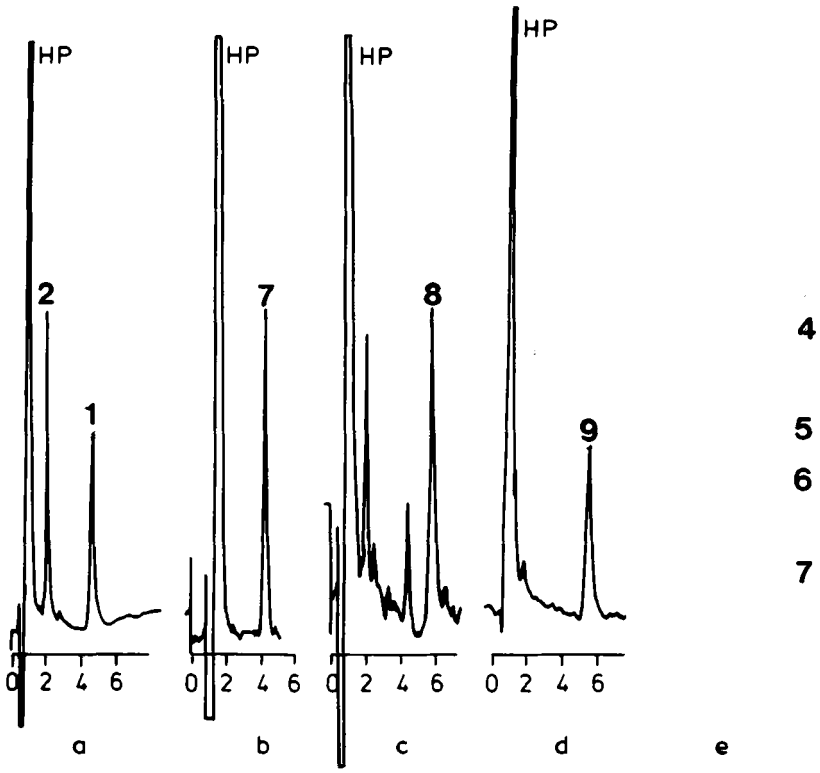
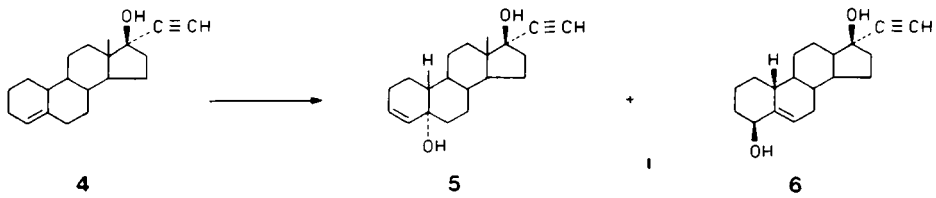


Fig. 1: Chromatograms of reaction mixtures of the steroids used (HP = hematoporphyrin)



Throughout the reaction this ratio was 62 : 38 (± 1). (This reflects the molar ratio, if the two almost identical products **5** and **6** have the same charring capacity). *Peters*[10], who performed the reaction under other circumstances, mentioned a third product in a very low quantity (< 2 %). With the exception of **7**, which did not react at all, for all steroids investigated a linear relation has been found, when $ln\ C_t/C_o$ has been plotted against time (table 1).

Taking into account constant light intensity and constant concentration of hematoporphyrin and oxygen (10^{-3} mol l^{-1}) a reaction first order in the concentration of the starting

Table 1: *Reaction rate constants*

compound	conc. x 10^{-5}M	k x 10^{-3} min^{-1}	n*	LR**
norethisterone	2.5	0.0 ± 0.3	3	0.999 (m = 5)
mestranol	2.5	9.2 ± 0.4	4	0.999 (m = 6)
lynestrenol	2.5	22 ± 1	4	0.999 (m = 6)
ethinylestradiol	2.5	63 ± 1	3	0.999 (m = 6)
ethinylestradiol	10	65 ± 2	3	0.999 (m = 6)
norethynodrel	10	122 ± 8	4	0,999 (m = 5)

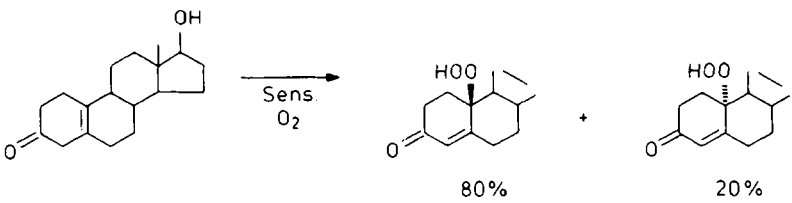
* n = the number of independent experiments

** LR = linear regression, found at least in each experiment in which m is the number of samples per experiment

material has to be expected indeed. (Obviously the slight decomposition of hematoporphyrin has no influence on the reaction rate constants under these circumstances).

7 was not decomposed by the action of a photosensitizer. A slight decomposition was observed with compounds with a comparable molecular structure[15,16]; in those cases the reaction took place at the vinylgroup. With **7** a reaction has not been found, probably because the vinylgroup is too deactivated by the ketone function. The same has to be expected for norgestrel, another frequently used progestogenic component with merely an ethyl group instead of a C18-methyl group.

Concerning the identity of the products of the other progestogenics **8** and **4**: the products **11** and **12** from 5(10-estrene-17β-ol-3-one **(10)** are described by *Maumy*[11].



10 **11** **12**

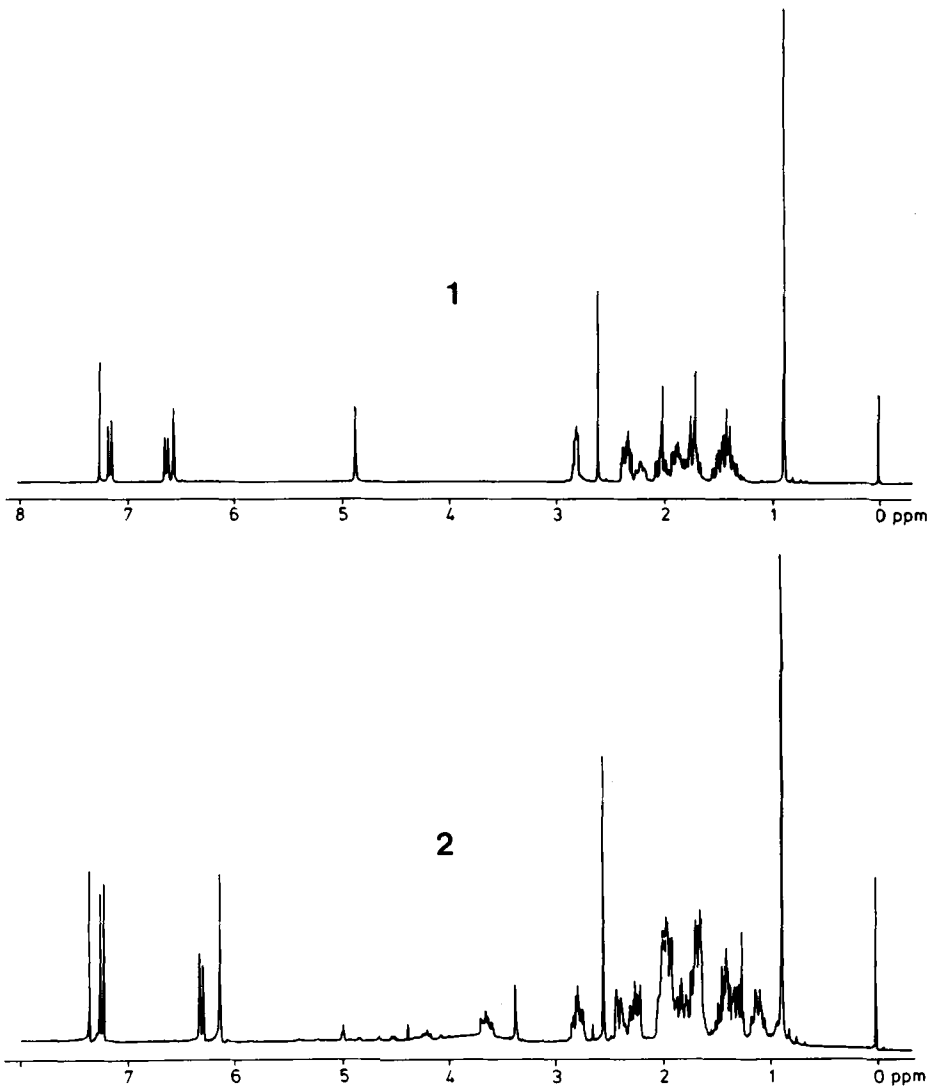
So the same kind of products have to be expected, when a 17α-ethynyl group is present. The photo-oxygenation of **4** has only been performed qualitatively[10].

The estrogenic steroids **1** and **9** showed unexpected reactivity. For only highly substituted methoxy-benzenes with at least four electron donating methoxy-groups are susceptible to photo-oxygenation[17]. We are intending to report on the product(s) of the slightly substituted methoxy-benzene **9** and their reactivity in a subsequent paper.

As far as we know photosensitized reactions of polycyclic compounds with a phenolic ring such as **1** have never been reported. Usually reactions are described of phenols subsituted with tert.-butylgroups, which have the possibility to form stable phenolate

radicals by steric hindrance[18]. When a para-substitutent is present in these "one ring" phenols a hydroperoxide is formed as primary product, that undergoes secondary reactions. The mass spectrum of **2** gave a parent peak of m/e = 328 indicating the addition of two oxygen atoms without loosing hydrogen. A parent peak of m/e = 312, which was found after reduction of **2** to **3** with KI, also points to the formation of a hydroperoxide.

From the shift of λmax from **2** and **3** with respect to **1** and the introduction of a long range coupling between C4-H and C6-H_{ax} we concluded that the aromatic A-ring has been disappeared in **2** and **3** (fig. 2). The IR data confirmed this conclusion. From the CD data it



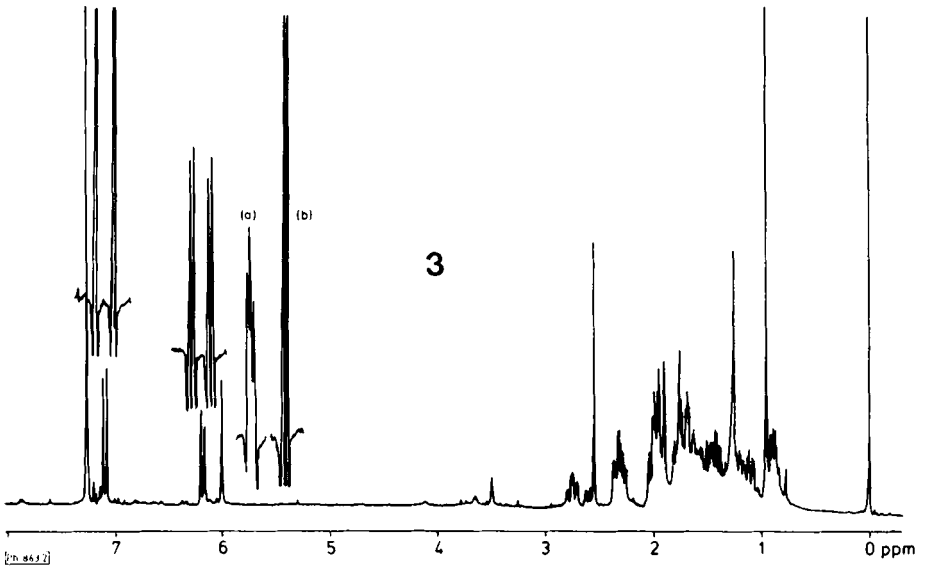
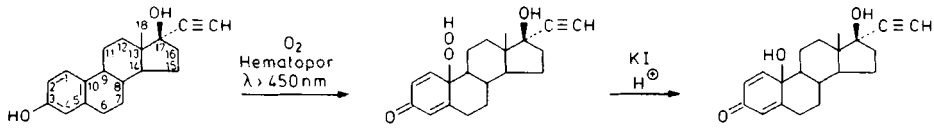


Fig. 2: ^{1}H-NMR spectra of **1**, **2** and **3**
From the spectrum of **3** the aromatic region is extended
(a) δ = 6.01 signal without decoupling on 2.8 ppm
(b) δ = 6.01 signal with decouplung on 2.8 ppm

appeared, that only the β-isomer has been formed[19]. The hydroperoxide **2** has to be 17α-ethynyl-10-hydroperoxy-1,4-estradiene-17β-ol-3-one and **3** 17α-ethynyl-1,4-estradiene-10,17β-diol-3-one (The NMR, UV and IR data of **3** are in accordance with those of *Maumy*[11] given for 19-norpregna-1,4diene-10β,14β-diol-3-one).



1 **2** **3**

The decomposition of steroids in tablets formulated with dyes, that have photosensitizing properties, will be highest when **8** and **1** are present. Fortunately from this point of view most of the organic dyes in tablets are replaced by ferrum-oxides.

In chemical literature much attention is paid to photoreactions dealing with the synthesis of new or otherwise difficultly obtainable compounds. In medical literature effects on the skin are reported, that are supposed to be caused by light. Normally these reports do not deal with the photoreactions, that underly the observed symptoms. Damage to tissues

other than the skin is seldom associated with light[6,20]. As pharmacists working on the photochemical induced side-effects of drugs we try to combine knowledge of photochemistry and of side-effects described in literature by performing experiments in vitro and ultimately in vivo to verify our hypotheses[21,22]. Also in the photosensitized oxygenation of contraceptive steroids interesting possibilities should be researched closer. *Dubertret*[23] recently showed that singlet oxygen production in human skin can be expected, when photosensitizers are incorporated into the epidermis. Singlet oxygen, a very reactive excited state of oxygen, is formed by energy transfer from the photosensitizer after the absorption of light to ground state oxygen.

By the contraceptive pill the quantity of *endogenous* photosensitizers such as protoporphyrins is raised (see introduction). Also methyldopa changes the metabolism of endogenous compounds, which leads to photobiological activity[20]. The intake of *exogenous* chemicals with photosensitizing properties themselves such as nitrazepam[24], furocoumarins[25] and other drugs and last but not least food dyes[6] potentiates further the photosensitized oxygenation of compounds such as steroids. Supposing a relation between the reaction rate constant and the feasibility of a photo-oxygenation in vivo one has to expect side-effects in the body from **1** and **8** in the first place (table 1).

In a research at the active steroid intermediate, involved in the irreversible binding of **8** to proteins and glutathione photo-oxygenation-induced binding to protein was once found[27]. *Chen*[27] argued that the 17α-ethynyl-10β-hydroperoxy-19-nortestosterone could be the reactive metabolite, that is responsible for this binding. Because **8** easily yields 10-hydroperoxides this steroid can be the cause of photo-allerby by irreversible binding of its products to protein after photosensitized decomposition. The literature does not mention anything about the products of **4** as far as we know.

Phenols can be oxidized and covalently bound in the presence of pigments serving as photosensitizers as is found for instance in the biosynthesis of alkaloids[28]. So as a consequence of photo-oxidation unwanted binding of phenolic compounds to endogenous molecules is possible, also because steroidal quinols are quite reactive[29]. They can form epoxides on basic treatment[30].

Hecker and co-workers[31] reported irreversible binding of estradiol and estron to protein, when these estrogens were incubated with rat liver microsomes. They identified the p-quinol as a metabolite. Although different enzymes might be involved, both oxygen and NADPH were required. Addition of peroxidase increased both the binding and the p-quinol formation. Besides the estrogen receptor can be labeled with tritiated estradiol on irradiation in the presence of rose bengal as photosensitizer[32]. These facts lead us to the hypothesis that upon photo-oxidation of phenolic steroids irreversible binding to protein or other endogenous molecules have to be envisaged *in vivo*. It may be an explanation for the photoallergy of estrogens[1,2]. To know to what extent the hypotheses stated above are valid *in vivo*, we have been started research with the rat as experimental animal.

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Experimental Part

Hematoporphyrin (free base), **1, 7, 8** and **9** were purchased from Sigma, **4** from Serva: all chemicals were used as such. Immediately before irradiation solutions with a final concentration of 8×10^{-5}M hematoporphyrin and $2.5 \times$ or 10×10^{-5}M steroid in phosphate buffer (pH 7.4) methanol (7 : 3) were flushed with oxygen [33,34]. The light source was a 1000 Watt Hg-Xe lamp model 6295 (Oriel Co.). Light of wavelength below 450 nm was eliminated by a 1.0 cm cuvet with a 1 % K_2CrO_4 filter. The irradiation was performed at 15°C in a 1.0 cm cuvette supplied with a magnetic stirrer at a distance of 45 cm from the lamp.

For preparative purposes 150 ml of a solution of **1** was irradiated in a tube (∅ 34 mm), while oxygen was led through the solution. The tube was surrounded by a vessel (∅ 95 mm) with the 1 % K_2CrO_4 filter. After total conversion the product was extracted with ethylacetate and after evaporation of the ethylacetate carefully washed with diethylether.

With a Spectra Physics Model 740 solvent delivery system supplied with a chrompack® 5 RP-2 column (150 × 4.6 mm ID), a LKB 2138 Uvicord-S UV detector fixed at 206 nm and a Kipp recorder separation of the concerning steroid, hematoporphyrin and the products were visualised. The eluents were methanol water mixtures: 54 : 46 for **1, 7** and **8**, 65 : 35 for **9**. The decomposition of **4** was determined densitometricly by the method described previously for **7** [1]. The plates (20 × 20 cm, Machary-Nagel & Co) were developed with hexane/ethylacetate (70 : 30); **7** (1.25×10^{-5}M) was used as int. stand. The reduction of the hydroperoxide **2** with KI and a few drops glacial acetic acid in ethylacetate was checked with TLC (Merck Kieselgel 60 F_{254}; dichlormethane/methanol (23 : 1)).

CD spectra (etOH): Dichrographe III CNRS-Ruossel-Jouan; IR-spectra (KBr): Beckman IR-10; UV-spectra (etOH): Perkin Elmer EPS-3T; Mass spectra: Kratos M5 9/50; 70 eV, source 150°C; direct introduction; NMR spectra ($CDCl_3$): chemical shift; (δ) ppm downfield from TMS; Bruker 300 Mhz.

^{1}H-NMR data of **1**: δ (ppm) = 7.16 (d, 1H, J_{1-2} = 9Hz, C-1-H); 6.63 (dd, 1H, J_{1-2} = 9Hz, J_{2-4} = 3Hz, C-2-H); 6.57 (d, 1H, J_{2-4} = 3Hz, C-4-H); 2.82 (m, C-6-H_{ax}); 0.88 (s, 3H, C-18-H_3).

^{1}H-NMR data of **2**: δ (ppm) = 7.26 (d, 1H, J_{1-2} = 10Hz, C-1-H); 6.32 (d.d. 1H, J_{1-2} = 10Hz, J_{2-4} = 2Hz, C-2-H); 6.14 (t, 1H, J_{2-4} = 2Hz, J_{4-6ax} = 2Hz, C-4-H); 2.80 (m, 1H, $J_{6ax-6eq}$ = 13.5Hz, $J_{6ax-7ax}$ = 13.5 Hz, $J_{6ax-7eq}$ = 5Hz, J_{4-6ax} = 1Hz, C-6-H_{ax}); 0.89 (s, 3H, C-18-H_3).

^{1}H-NMR data of **3**: δ (ppm) = 7.18 (d, 1H, J_{1-2} = 10Hz, C-1-H); 6.16 (d.d., 1H, J_{1-2} = 10Hz, J_{2-4} = 2Hz, C-2-H); 6.01 (t, 1H, J_{2-4} = 2Hz, J_{4-6ax} = 2Hz, C-4-H); 2.80 (m, 1H, $J_{6ax-6eq}$ = 13.5 Hz, $J_{6ax-7ax}$ = 13.5 Hz, $J_{6ax-7eq}$ = 5Hz, J_{4-6ax} = 2Hz, C-6-H_{ax}); 0.93 (s, 3H, C-18-H_3).

Decoupling on 2.8 ppm gives for δ = 6.91 ppm a doublet (1H, J_{2-4} = 2Hz). So we concluded that a long range coupling has beein introduced between C-4-H and C-6-H_{ax}.

The IR data of **3**: 1665 (c = 0 in a 6 membered α.β unsaturated ring); 1620 and 1605 (two vinyl groups); 1060 (vinyl group); 880 (vinyl group with a tertiair C-atom), 3390, 3250, 1390 and 1120 cm^{-1} (tert. alcohol).

The UV data: for **1** λmax = 283 nm (sh. at 288 nm); for **2** = 239 nm; for **3** = 240 nm.

The CD spectrum of **3** showed a $\Delta\varepsilon$max = − 0.67 at λmax = 340 nm.

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