these metals undergo reversible oxidation-reduction reactions under physiological conditions, and are capable of catalyzing autoxidations. Our observations with lead indicate that this capability is not necessary for the enhancement of oxygen toxicity. In addition, they raise the possibility that lead may interact with other oxidative agents in ways that are harmful to living organisms.

Zusammenfassung. Nachweis, dass i.v. Injektion von Bleiacetat die Überlebenszeit von Ratten, die reinen Sauerstoff atmen, dosisabhängig um 50% verkürzt, was sowohl bei 1 als auch bei 4 atm Sauerstoffpartialdruck beobachtet wird.

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1β-Hydroxylation of D-Norgestrel and Norethisterone by *Botryodiplodia malorum*

In the course of metabolic studies of the progestational agents norgestrel[1] (DL-13β-ethyl-17α-ethynyl-17β-hydroxygon-4-en-3-one, DL-I) and norethisterone (D-19-nor-17α-ethynyl-17β-hydroxyandrost-4-en-3-one, II) it was suggested that the 1β-hydroxy derivatives might be possible metabolites[2,3]. This led us to consider the preparation of D-1β-hydroxynorgestrel (D-1β, 17β-dihydroxy-13β-ethyl-17α-ethynylgon-4-en-3-one, III) and 1β-hydroxynorethisterone (D-19-nor-17α-ethynyl-1β,17β-dihydroxyandrost-4-en-3-one, IV).

Microbiological 1β-hydroxylation of 19-norsteroids has been reported with *Aspergillus ochraceus*[4] and *Botryodiplodia malorum*[5–7]; we felt, therefore, that this microbial transformation might give ready access to the desired compounds III and IV. Since 1-hydroxylated Δ^4-3-one-19-norsteroids are easily converted to their ring A aromatic congeners by base[4,5], we found through analysis of base treated solvent extracts by thin layer chromatography that *B. malorum* was the culture of choice. The experiments were designed primarily for the isolation of III and IV; other products were investigated only if they were isolated in the course of achieving this objective.

Incubation of D-norgestrel (D-I) with *B. malorum* CBS 134.50 gave the 1β-hydroxy analog III as the major product. With norethisterone (II) as steroid substrate, the 1β-hydroxy derivative IV was a very minor product, while the major product was hydroxylated at C-11β (D-19-nor-17α-ethynyl-11β,17β-dihydroxyandrost-4-en-3-one, V).

For the conversion of D-I, the inoculum was grown in shaken flasks containing a corn steep liquor: peptone: dextrose medium for 72 h at 28°. Mycelial transfers were made to a 14 l fermentor with 8 l of growth medium. After 24 h incubation the cells were filtered off and suspended in distilled water. The steroid D-I, 1.4 g added in ethanolic solution, was incubated for 71 h before harvest. The mycelium was filtered off, and the filtrate was extracted with ethyl acetate. The dried solvent extracts, dissolved in acetone, afforded 1.08 g of a crude mixture containing 5 products.

This material was chromatographed on preparative silica gel thin layer plates in chloroform: ethanol: acetone (8:1:1). The area containing the desired product was eluted and yielded, after charcoal treatment and recrystallization from acetone, 601 mg of product III, m.p.

[1] Norgestrel is a racemate (DL-I); D-norgestrel (D-I) corresponds in absolute configuration to the natural form of steroids.
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NMR assignments and optical properties of transformation products

	$[\alpha]_D$	$OH\Delta M_D^{OH}$ or $OAc\Delta M_D^{OAc}$	C-13 or C-18 Me Signal[a]	C-2 Protons	C≡CH Proton	1α or 11α Proton	C-4 Proton	Acetoxy Me Signal
D-1β-Hydroxynorgestrel (III)	−145.1[b]	−344[c]	0.95 *t* (7)	2.50 d (4.8)	2.52 *s*	4.40 *m*	5.85 *s*	
D-1β-Acetoxynorgestrel (III a)	−144.0[d]	−400[c]	1.02 *t* (7)	2.60 d (4)	2.62 *s*	5.61 *m*	5.98 *s*	2.03 *s*
1β-Hydroxynorethisterone (IV)	−153.7[b]	−397[e]	0.93 *s*	2.50 d (4)	2.52 *s*	4.45 *m*	5.88 *s*	
1β-Acetoxynorethisterone (IVa)	−135.8[d]	−399[e]	0.91 *s*	2.55 d (4)	2.57 *s*	5.53 *m*	5.88 *s*	2.01 *s*
11β-Hydroxynorethisterone (V)	− 5.8[b]	+ 76[e]	1.00 *s*		3.33 *s*	4.18 *m*	5.90 *s*	
11β-Acetoxynorethisterone (Va)	0.0[b]	+ 94[e]	1.05 *s*		2.62 *s*	5.32 *m*	5.89 *s*	2.07 *s*

[a] All NMR spectra were recorded in deuterated chloroform with the exception of V, which was recorded in deuterated dimethyl sulfoxide. All signals are given in ppm downfield from tetramethylsilane; the letters *s*, t, *m*, designate singlet, triplet, and multiplet, respectively. Numbers in parenthesis are J values; [b] Solvent was chloroform: methanol (1:1); [c] Parent compound: D-norgestrel[8]. [d] Solvent was chloroform. [e] Parent compound: norethisterone[9].

246–247°. Anal. Calcd. for $C_{21}H_{23}O_3$: C, 76.79; H, 8.59. Found: C, 76.81; H, 8.77. UV λ_{max}^{EtOH} 243 nm (ε = 15,010). $\lambda_{max}^{0.066\ N\ KOH\ in\ EtOH}$ 241 nm (ε = 9,320) and 298 nm (ε = 3,400). The latter phenoxide solution was acidified, after which it displayed a typical aromatic absorption spectrum: λ_{max} 281 nm (ε = 2,860) and 287 nm (ε = 2,550). The rapid conversion of III to its aromatic congener by mild base treatment was further confirmed by thin layer chromatography, which showed the aromatic product formed to be identical with 17α-ethynyl-13β-ethylgona-1,3,5(10)-trien-3,17-diol. The highly negative rotational increment of III (see Table) is typical for 1β-hydroxylation in 19-norsteroids, rather than of 1α-hydroxylation[4,5,10]. IR in KBr: 3.05, 3.15, 3.50, 3.58, 6.05, 6.15 μ. Mass spectrum: *m/e* 328 ($M^{+\cdot}$), *m/e* 310 ($M-H_2O$).

The D-1β-hydroxynorgestrel compound formed a monoacetate (IIIa) under mild acylating conditions, m.p. 186–187°. Anal. Calcd. for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.43; H, 8.43. IR in $CHCl_3$: 3.02, 3.50, 5.75, 5.98, 6.05 μ. The ΔM_D^{OAc} (Table) supports the 1β-assignment. NMR analysis followed by double irradiation experiments showed the C-2 protons at 2.60 ppm to be coupled to the multiplet of the C-1α-proton at 5.61 ppm and vice versa, much in the same fashion as described earlier[4], further supporting the 1β-hydroxy assignment.

The modification of norethisterone (II) to the 1β-hydroxy analog by *B. malorum* CBS 134.50 was accomplished in 1 l shaken flasks. Growth in the secondary stage was continued for 3 days prior to the addition of II. Incubation was carried out for a period of 3 to 5 days in order to effect partial elimination of the major 11β-hydroxy derivative.

A total of 15.9 g of II was fermented. The dried solvent extracts obtained from the fermentations were chromatographed on preparative thin layer plates. The area containing the 1β-hydroxy product, overlapped by the 11β-hydroxy analog, was excised and rechromatographed on thin layer plates, 0.25 mm in thickness, in chloroform: ethanol: acetone (8:1:1), with separation still incomplete. However, chromatography on thin layer plates in the solvent system cyclohexane: ethyl acetate (1:1) separated the desired IV from the 11β-hydroxy analog V and other contaminants. Elution of the desired zone and crystallization from ether gave 403 mg of IV, m.p. 191–193°. Anal. Calcd. for $C_{20}H_{26}O_3$: C, 76.40; H, 8.34. Found: C, 76.11; H, 8.38. UV λ_{max}^{EtOH} 242 nm (ε = 15,556), $\lambda_{max}^{0.066\ N\ NaOH\ in\ EtOH}$ 242 nm (ε = 9,485) and 298 nm (ε = 2,961); acidified: 280 nm (ε = 2,074), 288 nm (ε = 1,778). The proton spectrum displayed the expected signals as shown in the Table, and the rotational increment was in accordance with 1β-hydroxy assignment. IR in KBr: 3.00, 3.44, 6.01, 6.10 μ. Mass spectrum: *m/e* 314 ($M^{+\cdot}$), *m/e* 296 ($M-H_2O$). Monoacetate IVa: m.p. 177–180°. Anal. Calcd. for $C_{22}H_{28}O_4 \cdot {}^1/_4\ H_2O$: C, 73.20; H, 7.96. Found: C, 73.05; H, 7.90. IR in KBr: 2.90, 3.02, 3.40, 5.71, 6.00, 6.10 μ. Mass spectrum: *m/e* 356 ($M^{+\cdot}$), *m/e* 296 ($M-CH_3COOH$). The rotational increment and NMR spectrum of IVa are in agreement with the proposed structure.

In the course of isolating 1β-hydroxynorethisterone, we accumulated sufficient 11β-hydroxy analog V for analytical purposes, m.p. 223–224°. Anal. Calcd. for $C_{20}H_{26}O_3$: C, 76.40; H, 8.34. Found: C, 76.47; H, 8.38. UV λ_{max}^{EtOH} 242 nm (ε = 15,635). IR in KBr: 3.00, 3.45, 3.50, 6.05, 6.15 μ. Mass spectrum: *m/e* 314 ($M^{+\cdot}$), *m/e* 296 ($M-H_2O$). Monoacetate Va: m.p. 215–220°. Mass spectrum: *m/e* 356 ($M^{+\cdot}$), m/e 296 ($M-CH_3COOH$). IR in KBr: 3.00, 3.15, 3.45, 5.78, 6.06 and 6.16 μ. The 11β-hydroxyl function of the diol V could be oxidized to a ketone affording D-19-nor-17α-ethynyl-17β-hydroxyandrost-4-en-3,11-dione (VI): UV λ_{max}^{EtOH} 240 nm. IR in KBr: 2.96, 3.08, 3.50, 5.82, 6.01 and 6.16 μ. Mass spectrum: *m/e* 312 ($M^{+\cdot}$), *m/e* 176, ($C_{11}H_{12}O_2$), *m/e* 148 ($C_{10}H_{12}O$). NMR: 0.86 ppm (C-13 methyl singlet), 5.85 ppm (C-4 proton singlet). The assignment of structure is as follows: Base treatment of V gave no significant alteration of the UV-spectrum over a 24 h period, thus excluding hydroxylation at carbons 1, 2, 6 and 7. The carbonyl absorption at 5.82 μ in the IR-spectrum of compound VI excluded hydroxylation in ring D. Monoacetate formation (Va) under mild conditions indicated a secondary alcohol. The NMR-spectrum excluded hydroxylation at C-18, the ethynyl portion and C-4, leaving only C-11 and C-12 as possible alternatives. The C-12 position was excluded by the position of the C-13 methyl singlet in VI which, in the case of 12 substitution, should receive a large downfield shift compared to its parent norethisterone[11]. Actually, a small upfield shift (0.06 ppm) was observed. The mass spectral fragmentation of VI, with peaks at $C_{11}H_{12}O_2$ (176) and $C_{10}H_{12}O$ (148), gave further evidence for the hydroxylation to have occurred at C-11 rather than C-12. The molecular rotational increments of the diol V and the monoacetate Va are in agreement both in sign and magnitude with data reported by DE FLINES et al.[12], who put the range of 11β-hydroxylation in 19-norsteroids from +17° to +121°. The ΔM_D for 11α-hydroxylation is reported to be negative[12]. In addition, the shape of the 11α-proton signal in Va was in close agreement with the published pattern[13].

The two 1β-hydroxy compounds were compared for estrogenicity in mice by the method of EDGREN and CALHOUN[14]. The animals were injected s.c. with a total of 100 μg of steroid dissolved in 0.4 ml of sodium carboxymethylcellulose, 0.5% solution, over a 4-day period. None of the test animals injected with the norgestrel derivative (III) showed vaginal cornification, while 19 of 20 mice tested with the norethisterone analog (IV) gave a positive response. Neither of the parent compounds had any estrogenic effect.

Zusammenfassung. Die mikrobiologische 1β-Hydroxylierung von D-Norgestrel und Norethisteron durch den Mikroorganismus *Botryodiplodia malorum* wird beschrieben. Ausserdem wurde 11β-Hydroxynorethisteron isoliert und charakterisiert.

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