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Crystal structure of doisynolic acid and the structure of other products formed during its synthesis

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The crystal structure of the D-seco-estrogen doisynolic acid shows it to have the natural S *configuration at the position derived from C-14 in estrone. Two major by-products during the synthesis of doisynolic acid from estrone are shown to be dimeric steroids. One is an aldol condensation product, and the other appears to arise from an alkaline cleavage of the aldol product.* (*Steroids* **60**:261–264, 1995)

Keywords: doisynolic acid; estrogens; X-ray crystallography; stereochemistry

Introduction

Doisynolic acid (**1**), an estrogen D-ring seco steroid formed by alkaline fusion of estrone (**2**), and its various cogeners have been endocrine curiosities since their first preparation in the 1930s and 1940s (Figure 1).[1–3] Some isomers are exceedingly potent and long-acting stimulants of uterine growth when administered to rats orally (exceeding by a wide margin the potency of estradiol); however, the most potent isomers appear to have the unnatural *R* configuration at the carbon derived from C-14 in estrone,[3] and as a whole the members of the doisynolic acid class have affinities for the estrogen receptor that are far below that of estradiol.[4]

The stereochemistry of the doisynolic acid isomers and various B-ring unsaturated analogs has been elucidated by chemical correlations studies.[3,5] We present here the X-ray structure of (+)-*trans*-doisynolic acid derived from estrone, showing definitively that this less active isomer has the natural *S* configuration, which is consistent with the earlier assignments.[3,5] Furthermore, we have characterized by ^{1}H and ^{13}C NMR and mass spectroscopy the structure of two dimeric steroids that are prominent byproducts in the low-yield alkaline fusion by which doisynolic acid is prepared.

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Received July 3, 1994; accepted October 3, 1994

Experimental

General methods

Proton and ^{13}C magnetic resonance spectra were obtained on a Varian Unity 400 spectrometer at 400 MHz and 101 MHz, respectively. ^{1}H NMR and ^{13}C NMR chemical shifts are reported in ppm downfield from internal tetramethylsilane (δ scale). The data are reported in form (if applicable): chemical shift (multiplicity, coupling constant, number of protons, assignment). All *J* values are in Hertz (Hz). Purification was by flash chromatography.[6]

Alkaline Cleavage of Estrone

We followed the procedure of MacCorquodale[2] and Iviark. Estrone (1.0 g, 3.7 mmol) was heated with solid potassium hydroxide (20 g, 356 mmol) in a glass flask at 280–290°C for 1.25 h. After having been cooled to room temperature, the brown reaction mixture was dissolved in water, filtered, and the filtrate neutralized with dry ice, yielding a solid (950 mg) that was collected by filtration. TLC analysis (40% EtOAc/hexanes) showed three spots (staining by phosphomolybdic acid) at R_f 0.5 (estrone), 0.4 (ultraviolet [UV] active), and 0.3 (very weakly UV active). The spot at R_f 0.4 corresponds to two products—doisynolic acid (**1**, very weakly UV active) and aldol addition dimer **3a** (UV active); the spot at R_f 0.3 was identified as the novel dimeric condensation product **4**. Doisynolic acid is reported to be UV active,[7] but is only visible when applied in large quantities on a TLC plate. The spots at R_f 0.4 and 0.3 stained yellow with bromocresol purple, indicating that they are acids. The flash column chromatography provided crude doisynolic acid, which was purified by extraction into aqueous saturated $NaHCO_3$ and after reiosolation, recrystallization from hexane/actone: m.p. 194–196°C (lit.[2] 195°C).

Steroids 60:261–264, 1995

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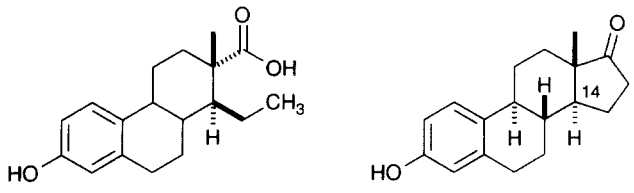


Figure 1 Structures of doisynolic acid **(1)** and estrone **(2)**.

Spectroscopic data

1. ^{1}H NMR (300 MHz, $CDCl_3$ + acetone-d_6) δ 0.91 (t, 3, J = 7.5 Hz, CH_2CH–3), 1.06 (s, 3, CH_3), 1.17–1.38 (m, 6), 1.58–1.76 (m, 2), 1.92 (td, 1, J = 10.0, 3.6 Hz), 2.00–2.08 (m, 1), 2.18–2.35 (m, 2), 2.68–2.76 (m, 2, C6-H_2), 6.46 (d, 1, J = 2.6 Hz, C4-H), 6.53 (dd, 1, J = 8.4, 2.6 Hz, C2-H), 7.02 (d, 1, J = 8.41, C1-H); ^{13}C NMR (75 MHz, $CDCl_3$ + acetone-d_6) δ 13.9 (CH_3), 23.1 (CH_2), 25.1 (CH_2), 26.2 (CH_2), 29.1 (CH_2), 36.1 (CH_2), 40.3 (CH), 42.2 (CH), 46.2 (C), 46.9 (CH), 111.9 (CH), 113.8 (CH), 125.3 (CH), 129.8 (C), 136.4 (C), 154.0 (C), 178.7 (CO); MS (EI, 70 eV) 288 (M^+, 68), 243 (10), 213 (30), 160 (100), 145 (19), 133 (28).

3a. ^{1}H NMR (300 MHz, acetone-d_6) δ 0.85 (s, 3, CH_3), 0.95 (s, 3, CH_3), 1.30–2.48 (m, 22), 2.75–3.05 (m, 8), 6.50–6.52 (m, 2, 2C4-H), 6.53–6.60 (m, 2, 2C1-H), 7.10 (d, 2, J = 8.4 Hz, 2C2-H); MS (EI, 70 eV) 522 (M^+, 74), 507 (100), 350 (12), 280 (4), 261 (6), 252 (28), 237 (11), 225 (5), 213 (63), 199 (13), 185 (13), 172 (24) 159 (72), 145 (24), 133 (54). Analysis calculated for $C_{36}H_{42}O_3$: 522.3134. Found: 522.3130.

3b. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (s, 3, CH_3), 0.92 (s, 3, CH_3), 1.30–2.25 (m, 22), 2.80–3.02 (m, 8), 3.79 (s, 6, OCH_3), 6.63–6.67 (m, 2, 2C4-H), 6.70–6.75 (m, 2, 2C1-H), 7.20–7.24 (m, 2, 2C2-H); MS (EI, 70 eV) 550 (M^+, 60), 535 (100), 375 (3), 364 (12), 349 (4), 324 (4), 275 (12), 266 (30), 251 (16), 227 (86). Analysis Calculated for $C_{38}H_{46}O_3$: 550.3447. Found: 550.3442.

4. ^{1}H NMR (300 MHz, acetone-d_6) δ 0.50 (s, 3, CH_3), 0.98–1.34 (m, 7), 1.07 (s, 3, CH_3), 1.38–1.50 (m, 1), 1.58–1.80 (m, 5), 1.80–2.02 (m, 4), 2.03–2.23 (m, 3), 2.36 (t, 1, J = 10.2 Hz), 2.55–2.75 (m, 4, 2C5-H), 5.10 (dd, 1, J = 15.2, 9.5 Hz, = CH), 5.31 (dd, 1, J = 15.2, 8.4 Hz, = CH), 6.38–6.41 (m, 2, 2C2-H), 6.41–6.55 (m, 2, 2C2-H), 6.95 (d, 1, J = 8.0 Hz, C1-H), 7.00 (d, 1, J = 8.0 Hz, C1-H); ^{13}C NMR (75 MHz, acetone-d_6) δ 13.0 (CH_3), 15.6 (CH_3), 25.0, 26.3, 26.9, 28.5, 28.62 (2), 28.62, 30.4, 37.0, 37.8, 39.4, 39.7, 43.4, 44.73 (2), 48.1, 52.1, 55.1, 55.5, 113.28 (C2 or 4 CH), 113.43 (C2 or 4 CH), 115.59 (C2 or 4 CH), 115.65 (C2 or 4 CH), 126.75 (2, 2C1-CH), 131.26 (=CH), 131.41 (C), 131.97 (C), 135.0 (=CH), 138.15 (C), 138.35 (C), 155.58 (C), 155.74 (C), 178.9 (COOH); MS (EI, 70 eV) 540 (M^+, 60), 525 (2), 494 (3), 454 (3), 439 (2), 312 (6), 267 (9), 257 (23), 254 (23), 228 (26), 213 (1000, 199 (12), 185 (21), 173 (21), 159 (56), 145 (23), 133 (64). Analysis Calculated for $C_{36}H_{44}O_4$: 540.3240. Found: 540.3226.

X-ray Crystallography

Crystal data for $C_{18}H_{24}O_3 \cdot$ ½ H_2O *1*. Transparent, colorless, and prismatic single crystal of **1** was monoclinic at −75°C, space group $P2_1$ (C_2^2; No. 4) with a = 11.194(2) Å, b = 7.298(2) Å, c = 20.042(7) Å, β = 103.47(2), V = 1592(9) Å^3 and d_{calcd} = 1.241 g cm^{-3} for Z = 4. A total of 2758 intensities were measured (Mo Kα $2\theta < 47°$, 2564 unique, R_i = 0.013) on an Enraf-Nonius CAD4 automated κ-axis diffractometer using ω/θ scans with angles of 1.50[1.00 + 0.35tan(θ)]. These data were corrected for Lorentz-polarization effects, anomalous dispersion, and absorption [μ_a(Mo Kα) = 0.79 cm^{-1}]. The structure was solved by direct methods (SHELXS-86),[8] and refined by least-squares (SHELX-76)[9] to convergence [R(F) = 0.035 and ωR (F) = 0.036 for 2133 observed reflections ($I > 2.58\sigma(I)$)]. In the final cycles of least-squares refinement, positions and anisotropic thermal coefficients were refined for non-H atoms; positions for six O bound H atoms were refined; a common isotropic H atom thermal parameter was refined; and an empirical isotropic extinction parameter was varied. Successful convertence was indicated by the maximum shift/error for the last refinement cycle. The final map had no significant features and a final analysis of variance showed no systematic errors. The absolute configuration could not be crystallographically determined using Mo radiation; however, the geometry at atoms C-8 and C-9 was established by synthesis, so the relative geometry at C-14 was determined by this experiment.

Results and discussion

We performed the KOH fusion reaction of estrone according to a previously reported procedure.[2,6,7] A crystal suitable for X-ray crystallography was grown from an acetone/hexane solution of **1.** The crystal structure of doisynolic acid (**1,** Figure 1) clearly shows that it retains the natural *S* configuration at the carbon derived from C-14 in estrone.

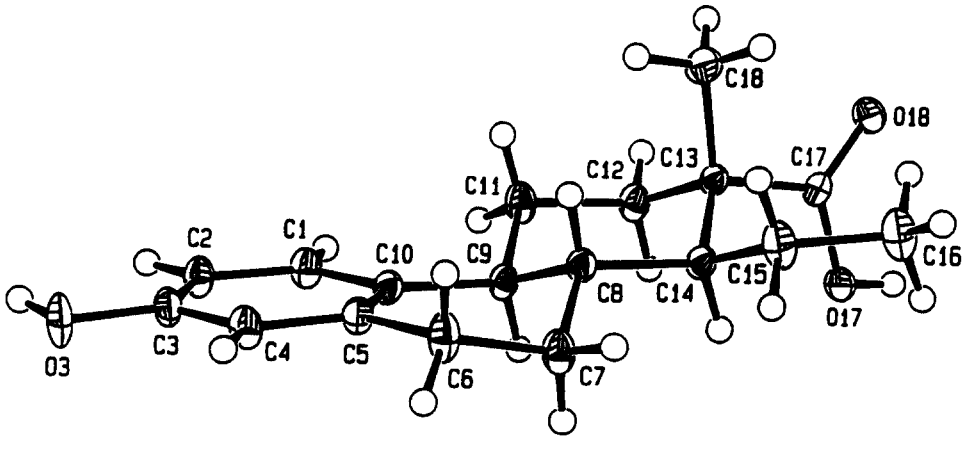
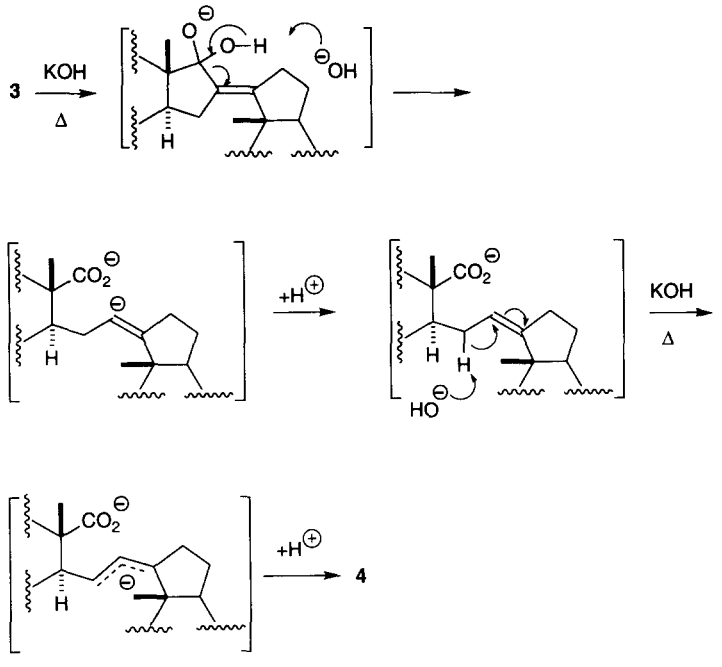


Figure 2 X-ray crystallographic structure of the doisynolic acid **1.**



Scheme 1 Proposed mechanism to form compound **4**.

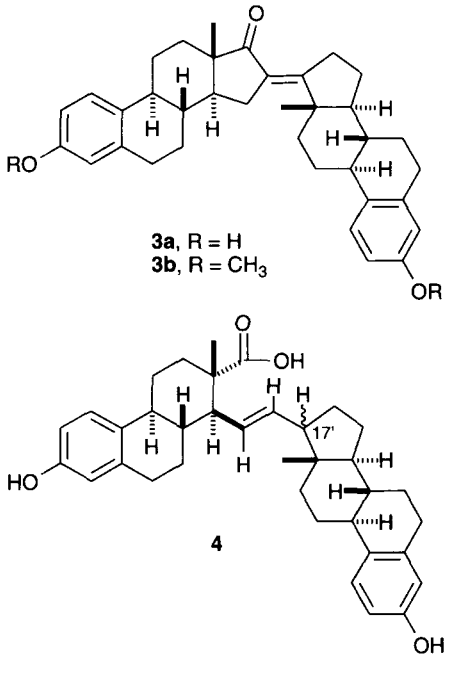


Figure 3 Structures 3 and 4.

As it is the less potent doisynolic acid isomer that is derived from estrone, this confirms that the more potent epimer has the unnatural *R* configuration at this center.[3,5]

All literature preparations of doisynolic acid from estrone by alkaline fusion report low yields. Despite considerable effort, we have been unable to improve our yield of 20–30%. However, in the course of our investigations, we have isolated two dimeric condensation products **3a** (<5%) and **4** (20–30%) that are formed during the reaction. Their structures have been established by ^{1}H and ^{13}C NMR and MS. While compound **3** results simply from an aldol addition-dehydration sequence, the more unusual structure **4** could be formed by an alkaline induced ring scission-rearrangement sequence from the aldol product as shown in Scheme 1. The distinguishing features from the ^{1}H NMR spectrum of **4** are two olefinic proton resonances (δ 5.10, J = 15.2, 9.5 Hz and δ 5.31, J = 15.2, 8.4 Hz) and two methyl singlets (δ 0.50, 1.07). In addition, ^{13}C NMR shows two sets of steroid peaks, including two new olefinic carbons (δ 131.3, 135.0; CH peaks by proton attached test experiment) and one carbonyl carbon (δ 178.9). EI mass spectrometry identified the molecular ion as m/z 540. The stereochemistry of olefin is *trans*, based on the 15.2 Hz coupling constant, and each olefinic carbon is attached to a CH group. The stereochemistry of C-17 could not be identified. The structure assigned to compound **4** is constant with all of these data (Figure 3; Scheme 1).

We also performed the KOH fusion reaction of estrone 3-methyl ether. Because of its poor solubility, starting material reacted inefficiently. The aldol addition-dehydration product **3b** was the only isolated compound (50%, based on consumed starting material). Compounds **3a** and **3b** are both UV active and have ^{1}H NMR spectra showing two C-18 methyl peaks. EI mass spectrometry of **3a** and **3b** showed molecular ions as 522 and 550, respectively.

In conclusion, we have obtained a crystal structure of doisynolic acid, derived from estrone, and shown that it has the natural 14*S* configuration. The structures of two persistent byproducts that of form during the strongly alkaline cleavage conditions have been elucidated.

Acknowledgments

We are grateful for support of this research through grants from the National Institutes of Health (PHS 5R37 DK15556) and the Eli Lilly Corporation, and for the help of Andrew Scribner.

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