Efficient Synthesis of a Novel Estrone-Talaromycin Hybrid Natural Product**

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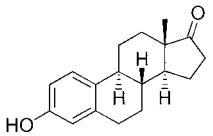
Dedicated to Professor Wolfgang Steglich on the occasion of his 65th birthday

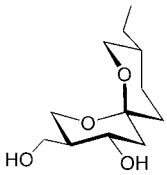
An important aim of organic chemistry is the development of new types of biologically active substances. Of particular importance are natural products that can be used in many ways, either directly or indirectly, as lead structures. Examples of this are strobilurins[1] described by Steglich, Anke et al., and epothilones[2] described by Höfle et al.

However, it should be remembered that it is not easy to find new, interesting natural products. Nevertheless an almost inexhaustible reservoir of new types of diverse structures is available from the coupling of two or more natural products to make hybrids. This route, at least in terms of the approach, is also adopted in nature, for example in the combined biosynthesis of vitamin E, which forms from a terpene and a shikimic acid.[3] In addition, vincristin is worthy of mention, which is formed from an aspidoderma and an iboga alkaloid and which is one of the most important substances in the treatment of leukemia in children.[4]

Although the enzymatic formation of hybrid natural products by gene manipulation is currently under intensive study,[5] to date there have been few attempts at the chemical synthesis of such compounds.[6] We describe here a new type of pharmacologically interesting hybrid natural product made from estrone (**1**) and the highly biologically active mycotoxin talaromycin B (**2**).[7] The combination of a toxin with a steroid was chosen because steroids are able to penetrate the cell membrane and bind to the cell nucleus.[8]

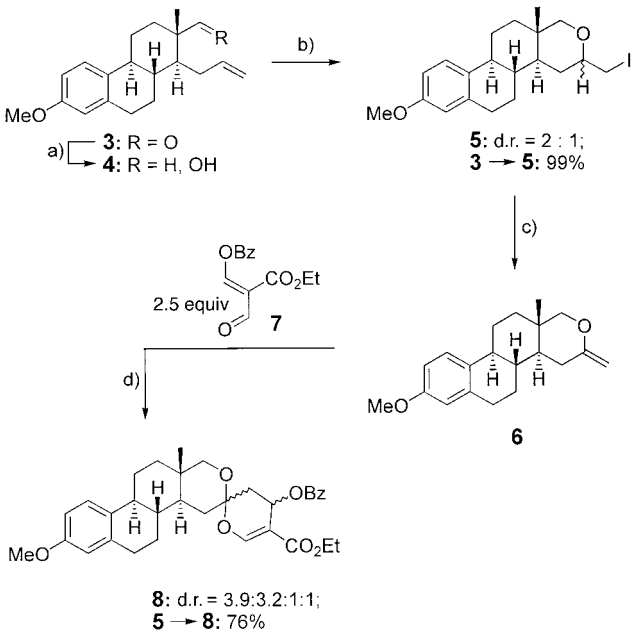


1
estrone



2
(–)-talaromycin B

For the synthesis the secoestrone derivative **3** was used which is available in four steps from **1**.[9] Reduction of **3** with sodium borohydride in methanol at 20 °C led to the alcohol **4** from which, by reaction with iodine via an intermediate iodonium ion, iodomethyltetrahydropyran **5** was obtained as a 2:1 mixture of diastereomers (Scheme 1). The low diastereoselectivity of the reaction is insignificant since the newly formed stereogenic center is destroyed in the next step. Thus, elimination with 1,8-diazabicyclo[5.4.1]undec-7-ene (DBU) as the base yielded the exocyclic enol ether **6**. The reaction was carried out at 90 °C without solvent; in this manner isomerization of the double bond could be avoided. The enol ether is, however, not stable and was therefore converted directly in a Diels–Alder[10] reaction with ethyl *O*-benzoyldiformylacetate (**7**) to the spiroacetal **8** which was obtained as a mixture of four diastereomers in the ratio 3.9:3.2:1:1 in 76% yield.



Scheme 1. a) 10 Equiv $NaBH_4$, MeOH, RT, 30 min; b) I_2, $NaHCO_3$, Et_2O/H_2O, RT, 4 h; c) DBU, 90 °C, 30 min; d) toluene, CH_2Cl_2, RT, 14 h. Bz = benzoyl.

The main diastereomer **8a** was obtained in crystalline form after chromatography and its structure was elucidated by crystal structure analysis.[11] The relative configuration of the newly formed stereogenic centers in **8a** resulted from an *exo* attack of the heterodiene **7** *anti* to the angular methyl group in **6**.

Reduction of the two ester groups in **8a** with diisobutylaluminum hydride (DIBAH) led to the diol **9** (Scheme 2) in 70% yield. The subsequent hydrogenation of the double bond in **9** at 50 bar hydrogen pressure in methanol/ethyl acetate with platinum dioxide as catalyst afforded the desired estrone–talaromycin hybrid natural product **10** in quantitative yield.[12] The newly formed stereogenic center in **10** resulted from β-addition of hydrogen to the olefinic double bond. The high stereoselectivity of the hydrogenation can be

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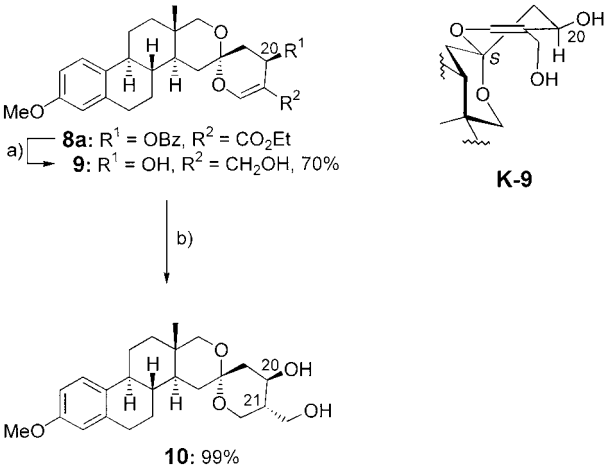

Scheme 2. a) 12 Equiv DIBAH, THF/CH_2Cl_2, $-78\,^{\circ}C$, 21 h; b) 10 mol% PtO_2, 50 bar H_2, MeOH/EtOAc, 12 h. Bz = Benzoyl.

ascribed to both stereoelectronic control and the directive effect of the β-hydroxyl group at C-21.[13] Thus, assuming that **K-9** is the most stable reactive conformation of the transition state, hydrogenation from the β-side via an energetically favorable chair form should lead to the product obtained.[14]

The configuration at C-20 was determined by analysis of the ^{1}H NMR coupling constants of the 20-H signal at $\delta = 3.81$. The coupling constants $J_1 = J_2 = 10.9$ Hz and $J_3 = 4.9$ Hz result from two axial–axial and one axial–equatorial coupling. This coupling pattern is only possible for the given diastereomer **10**.

To test the biological activities of **9** and **10** their in vitro effects on human cancer cells from the cell line A549 were determined. For this 10^2 to 10^5 cells, in each case, were placed in 6-well multiplates and incubated with different concentrations of each substance in the culture medium DMEM, which contains 10% fetal calf serum. After 11 d cultivation at $37\,^{\circ}C$ in air with a CO_2 content of 7.5% the clones were dyed with Löffler methylene blue and the relative clone formation rate was determined.[15] The effective dosage (ED_{50}) was about 30 μM, and thus lies only slightly below the cytotoxicity of aldophosphamide, the active metabolite of the well known cytostatic agent cyclophosphamide.[16]

The efficient synthesis of **10** described, in which a hetero Diels–Alder reaction is the key step, opens the way to new types of hybrid natural products which are built up from a steroid and a mycotoxin and which show interesting biological activities. For instance, in vitro tests with **9** and **10** showed strong cytotoxic activity against human cancer cells.

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[11] Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-101429. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[12] **10**: M.p. 150 °C (decomp); $[\alpha] = +109.7$ ($c = 0.3$, $CHCl_3$); ^{1}H NMR ([D$_4$]MeOH, 500 MHz): $\delta = 0.98$ (s, 3H, 18-H), 1.15–1.35 (m, 2H, 12-H_2), 1.38–1.50 (m, 3H, 14-H, 11-H_2), 1.55–1.70 (m, 3H, 19-H_b, 9-H, 8-H), 1.73 (dd, $J = 13.1$, 3.8 Hz, 1H, 7-H_b), 1.92–1.96 (m_c, 1H, 7-H_a), 2.00 (dd, $J = 12.6$, 5.0 Hz, 1H, 19-H_a), 2.23–2.33 (m, 2H, 15-H_2), 2.77–2.80 (m_c, 2H, 6-H_2), 3.08 (d, $J = 10.6$ Hz, 1H, 17-H_a), 3.29–3.31 (m_c, 3H, 21-H, 20-OH, 21-CH_2OH), 3.33 (d, $J = 10.6$ Hz, 1H, 17-H_b), 3.46 (t, $J = 11.3$ Hz, 1H, 22-H_b), 3.49 (dd, $J = 11.3$, 7.5 Hz, 1H, 22-H_a), 3.72 (s, 3H, 3-OCH_3), 3.76 (dd, $J = 11.2$, 4.8 Hz, 1H, 21-CH_aOH), 3.81 (td, $J = 10.9$, 4.9 Hz, 1H, 20-H), 3.83 (dd, $J = 11.2$, 3.8 Hz, 1H, 21-CH_bOH), 6.58 (d, $J = 2.6$ Hz, 1H, 4-H), 6.66 (dd, $J = 8.5$, 2.6 Hz, 1H, 2-H), 7.14 (d, $J = 8.5$ Hz, 1H, 1-H); ^{13}C NMR ([D$_4$]MeOH): $\delta = 16.34$ (C-18), 26.71 (C-11), 26.85 (C-12), 30.91 (C-13), 34.51 (C-6), 35.36 (C-7), 36.37 (C-15), 39.93 (C-14), 42.95 (C-9), 45.07 (3-OCH_3), 45.73 (C-19), 47.51 (C-8), 55.54 (C-21), 61.73 (C-22), 62.61 (21-CH_2OH), 66.29 (C-20), 73.16 (C-17), 99.84 (C-16), 112.6 (C-2), 114.4 (C-4), 127.0 (C-1), 133.8 (C-10), 138.8 (C-5), 159.0 (C-3).

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[14] Force field calculations on **9** indicate that **K-9** is the most stable conformation in the ground state.

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