Structure−Activity Relationships of the Estrogenic Sesquiterpene Ester Ferutinin. Modification of the Terpenoid Core

Giovanni Appendino,*[,†] Paola Spagliardi,[†,‡] Olov Sterner,[§] and Stuart Milligan[⊥]

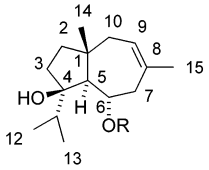
Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Università del Piemonte Orientale, Via Bovio 6, 28100 Novara, Italy, Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Via Giuria 9, 10125 Torino, Italy, Department of Organic and Bioorganic Chemistry, Lund University, P.O. Box 124, 221 00 Lund, Sweden, and Endocrinology and Reproductive Research Group, Kings College, SE1 1 UL, London, U.K.

Received June 18, 2004

Esterification of *p*-hydroxybenzoic acid, a very weak estrogenic compound, with the daucane alcohol jaeschkeanadiol (**1b**) leads to a spectacular magnification of the estrogenic activity. To identify the structural elements responsible for this effect, the terpenoid core of jaeschkeanadiol *p*-hydroxybenzoate (ferutinin, **1a**) was modified, capitalizing on the presence of two functionalities, the monoacylated, hydrogen-bonded 1,3-diol system and the double bond. The hydrogen bonding, while possibly useful, was not critical for activity, while hydrogenation and cyclopropanation of the double bond were tolerated. Conversely, oxidative modifications of the double bond that placed a hydroxyl on the α-face of the molecule proved detrimental. Taken together, these observations identified the substitution at C-8/C-9 as critical for activity.

Estrogen receptors (ERs) are relatively indiscriminate in their binding properties, to the point that "no single chemical has yet been internationally agreed to be devoid of estrogenicity",[1] but very few potent nonsteroid scaffolds are known. Most of them are relatively flat molecules that retain a certain degree of topological similarity with estradiol (E2) and the capacity to position critical phenolic hydroxyls into the hydrogen-bonding elements of ERs (the diad Arg 291/Glu 324 and His 524 for the ER-α).[2] The discovery of potent estrogenic activity in the sesquiterpene ester ferutinin (**1a**)[3] and the occurrence of large amounts of this compound in a Sardinian chemotype of giant fennel (*Ferula communis* L.)[4] gave us the opportunity to investigate the structure−activity relationships of this novel estrogenic prototype. Ferutinin is also the alleged active principle of various preparations available in the health food market as libido enhancers. These products have recently featured prominently in the media and the popular press, despite concerns about their safety.[5]

Ferutinin is a *p*-hydroxybenzoyl ester, and in a previous study, the aromatic moiety was found essential for ER binding.[6] Esters of *p*-hydroxybenzoic acid are widely used as preservatives in pharmaceutical formulations,[7] while the free acid is produced in the metabolic degradation of dietary flavonoids such as apigenin and quercetin[8] and is a common component of human urine.[9] Jaeschkeanadiol (**1b**), the parent diol of ferutinin, lacks any recognizable hormonal activity,[6] while *p*-hydroxybenzoic acid and its simple alkyl esters show only marginal estrogenic activity.[10] Therefore, the daucane core contributes critically to the activity of ferutinin, and this observation provided a rationale for undertaking a study aimed at identifying which functionalities of the terpenoid core are required, and presumably involved, in ER binding.



	R
1a	*p*-HO-C_6H_4-CO-
1b	H
1c	*p*-HO-C_6H_4-CH_2-
1d	*p*-PivO-C_6H_4-CO-
1e	*p*-AcO-C_6H_4-CO-

Results and Discussion

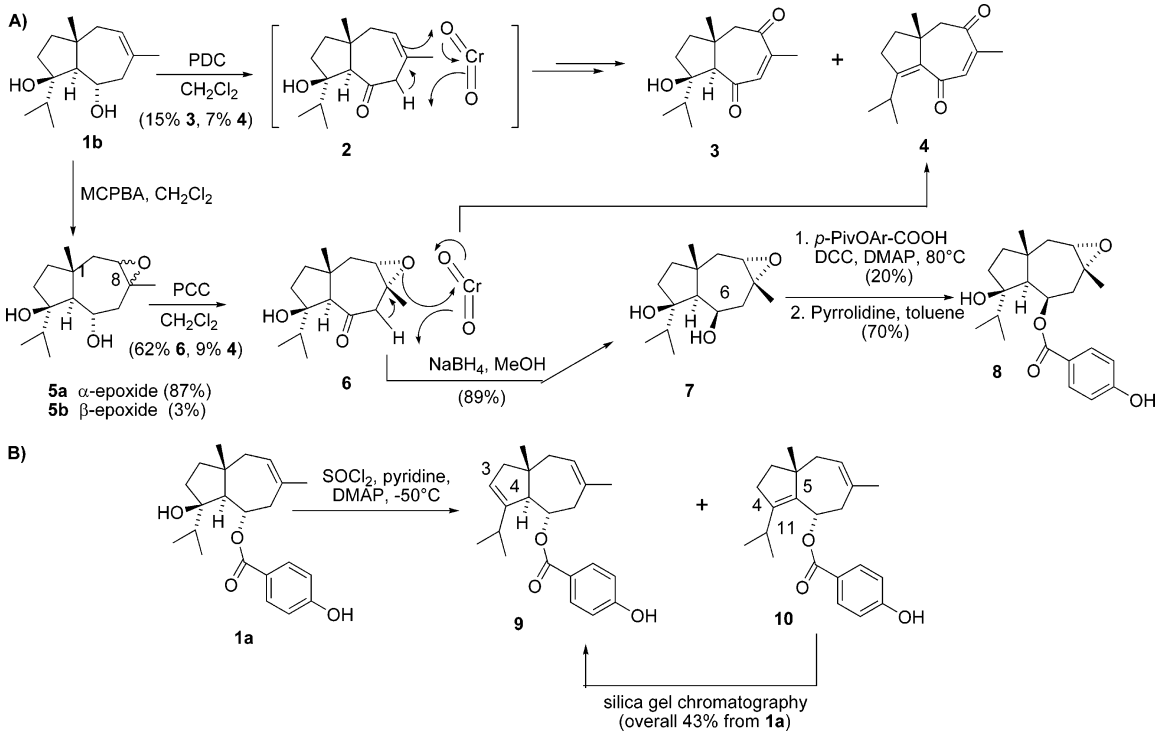
Intramolecular hydrogen bonding is possible between the tertiary hydroxyl and the ester carbonyl of jaeschkeanadiol esters.[11] This adds to the tendency of secondary esters to maximize $\sigma\rightarrow\pi^*$ conjugation by assuming a *Z*-conformation around the ester C−O single bond,[12] overall translating into a geometry where the carbonyl oxygen is oriented toward the β-face of the daucane skeleton and the aromatic ring bisects its mean plane. The perturbation of this conformational constraint seemed a good starting point to explore the magnifying effect of the daucane moiety on the hormonal activity of *p*-hydroxybenzoic acid. To this end, the replacement of the ester link with an ether bond, the epimerization of the secondary 6-hydroxyl, and the dehydration of the 4-hydroxyl were pursued.

The ether analogue **1c** was prepared by etherification of jaeschkeanadiol (**1b**) with *p*-pivaloyloxybenzyl bromide, in turn prepared from *p*-hydroxybenzaldehyde by reduction, Schotten−Baumann pivaloylation, and NBS-mediated bromine-to-oxygen exchange. The etherification reaction occurred in modest yield, delivering, after deprotection, **1c** as a foam. The secondary hydroxyl of jaeschkeanadiol proved too hindered to act as a substrate for a Mitsunobu reaction. To invert the configuration at C-6, we therefore investigated an alternative strategy, based on an oxidation−reduction protocol. The underlying rationale is that the angular β-methyl at C-1 shields the upper face of the daucane skeleton, directing attack from the opposite α-face. In the event, the oxidation of jaeschkeanadiol under a variety of conditions (PCC, PDC, TPAP, activated DMSO,

* To whom correspondence should be addressed. Tel: +39 0321 375744 (G.A.); +34 957 218267+44 20 7848 6271 (S.M.). Fax: +39 0321 375821 (G.A.); +44 7848 6280 (S.M.). E-mail: Giovanni.Appendino@pharm.unipmn.it (G.A.); Stuart.Milligan@kcl.ac.uk (S.M.).
† Università del Piemonte Orientale.
‡ Università di Torino.
§ Lund University.
⊥ Kings College.

10.1021/np049796w CCC: $27.50
Published on Web 08/25/2004

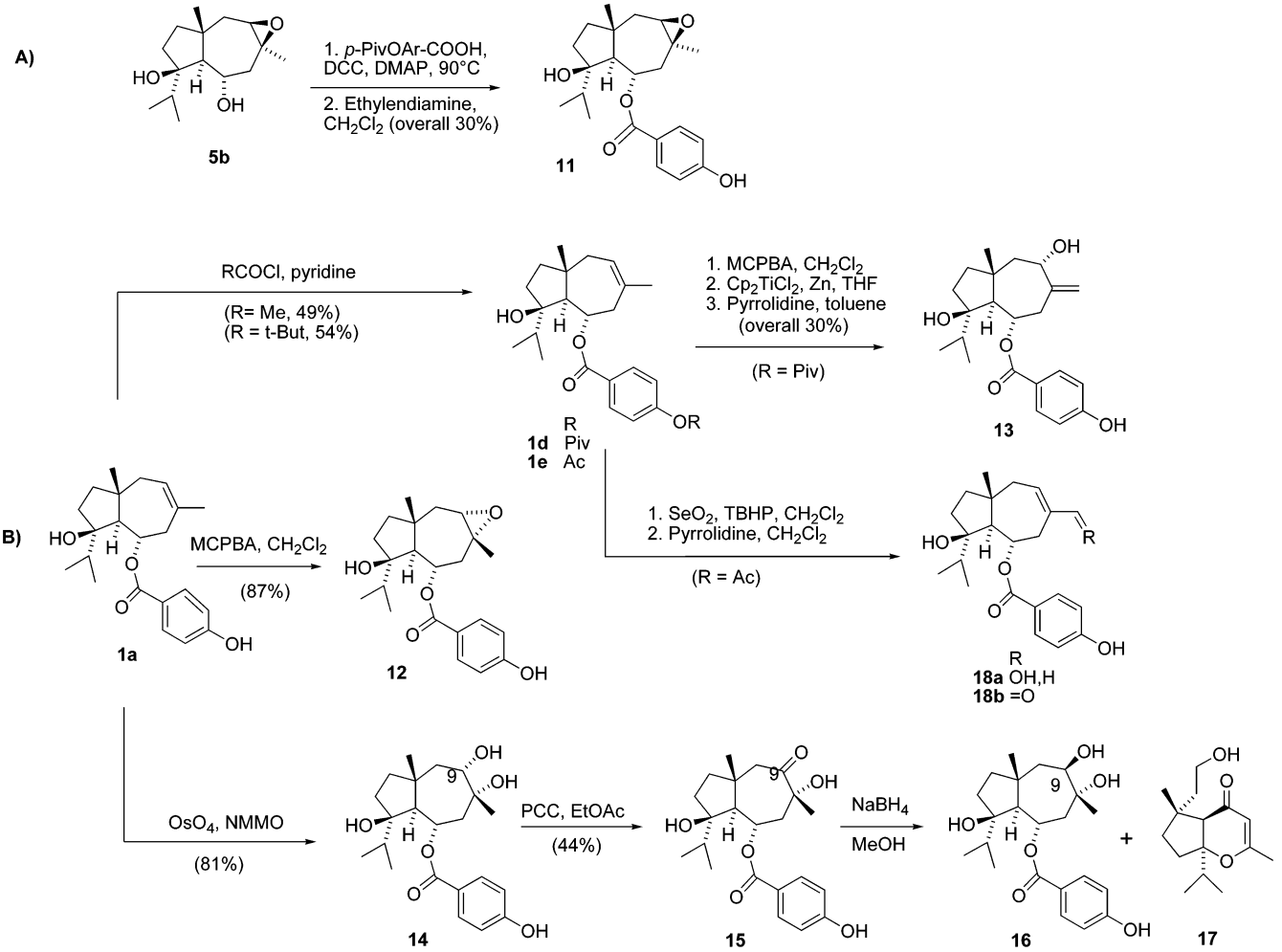
Scheme 1. Synthesis of 6-Epiferutinin-α-epoxide (**8**, A) and Dehydroferutinin (**9**, B)



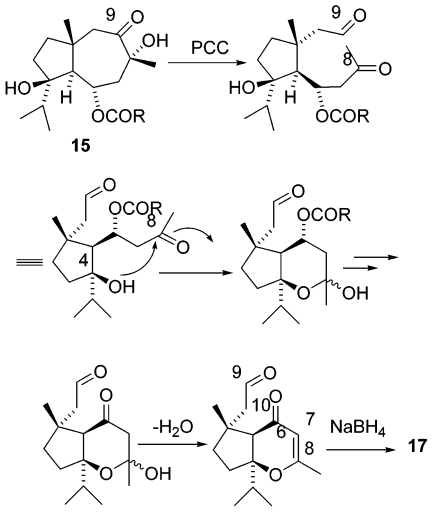
TEMPO) failed to afford the ketone **2**, rather giving a complex reaction mixture, from which, with Cr^{6+}-based reagents, a mixture of the enone **3** and its dehydration product **4** could be characterized. Formation of a conjugated enone is a foreseeable problem in the oxidation of homoallylic alcohols, but overoxidation to an ene-dione, presumably via a concerted cyclic mechanism (Scheme 1, A), was surprising. To overcome this problem, we "protected" the double bond as an epoxide. Treatment of jaeschkeanadiol with MCPBA occurred with remarkable diastereoselectivity, affording a separable 30:1 mixture of epoxides (**5a**,**b**). The oxidation of the major α-epoxide (NOE correlation between the C-1 and the C-8 methyls) was next screened with the same set of oxidants assayed for **1b**. Transition metal oxidants (Cr^{6+}, TPAP) afforded the expected epoxyketone **6**, but a certain degree of overoxidation to **4**, presumably through a variant of the mechanism proposed for its formation from **1b**, could not be avoided. On the other hand, no reaction occurred with milder oxidizers, like the couple TEMPO/trichloroisocyanuric acid.[13] The epoxyketone **6** was then treated with $NaBH_4$ to afford the 6β-alcohol **7** as the only reaction product. Esterification of **7** with *p*-pivaloyloxybenzoic acid required harsh conditions and gave a low yield (20%), but, after deprotection, 6-epiferutinin epoxide (**8**) could eventually be obtained. Since attempts to deoxygenate **8** failed (see infra), the effect of C-6 epimerization could not be assessed by point mutation, but only in an indirect way, that is, by comparing the activity of the corresponding epoxides (**8** and ferutinin epoxide (**12**), available as the only reaction product from the epoxidation of ferutinin). These unforeseen difficulties were somewhat compensated by the observation that ferutinin could be dehydrated in a highly regioselective way. Thus, treatment with thionyl chloride afforded a ca. 3:1 mixture of $\Delta^{3,4}$- and $\Delta^{4,5}$-isomers (**9** and **10**, respectively), which equilibrated to the pure $\Delta^{3,4}$-olefin **9** during chromatography on silica gel or by standing in $CDCl_3$ (Scheme 1, B). The $\Delta^{4,11}$-isomer could not be detected, either in the reaction mixture or after its acidic equilibration.

The endocyclic double bond of ferutinin served as a basis for a second set of analogues, where C-8 and C-9 are modified in an oxidative or reductive way (Scheme 2A, B). The minor epoxide from the epoxidation of jaeschkeanadiol (**5b**) was converted to its *p*-hydroxybenzoate (**11**) in the usual way, complementing the stereoselectivity observed during the epoxidation of ferutinin to **12**. In a model reaction for the synthesis of 6-epiferutinin from its epoxide **8**, protected (pivalate) ferutinin epoxide was subjected to various deoxygenation protocols. The couple zinc/titanocene dichloride,[14] while failing to materialize the expected olefin, nevertheless led to a smooth isomerization to the allylic alcohol **13**, providing access to an analogue with an exocyclic double bond. Dihydroxylation of ferutinin by the Upjohn protocol afforded the α-glycol **14** as the only reaction product, further oxidized with PCC to the ketol **15**. Examination of the crude reaction mixture indicated the formation of an aldehyde (singlet at δ 9.8, ca. 25% of ketol **15**). This compound proved too unstable on silica gel for direct isolation, but could be characterized after reduction of the crude reaction mixture with $NaBH_4$. The major reaction product was the *trans*-diol **16**, accompanied by the pyrone **17**, which could not be completely purified from other minor reaction products even by HPLC. The structure of **17** was unambiguously assigned by 1D and 2D NMR measurements. A possible mechanism for the formation of this compound is depicted in Scheme 3. Thus, overoxidation of the ketol system could afford a 8,9-*seco*-daucane, featuring two carbonyl-bearing groups bound to the cyclopentane ring. The 8-ketone is located on the β-side of the molecule, and formation of an acetal with the tertiary 4-hydroxyl could take place, next followed by the hydrolytic loss of the aromatic ester group and the oxidation of the 6-hydroxyl. This could trigger the elimination of the semiacetal hydroxyl and the overall formation of a cyclopenta[*b*]pyrone aldehyde, whose chemoselective reduction would afford the primary alcohol **17**. The characterization of a 8,9-cleaved jaeschkeanadiol ester is interesting, since the cleavage of

Scheme 2. Synthesis of Ferutinin-β-epoxide (**11**, A) and Oxidative Modification of the Double Bond of Ferutinin (B)

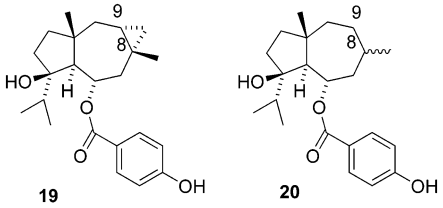


Scheme 3. Possible Mechanism for the Oxidative Fragmentation of the Daucane Core of **15** and the Formation of **17**



the 8,9-bond of jaeschkeanadiol affords compounds of relevance for the synthesis of prostanoids,[15] but has been reported to give complex mixtures with a variety of reagents.[15] Taken together, these observations suggest that the 8,9-double bond of jaeschkeanadiol esters can indeed be oxidatively cleaved, but that the cleavage products are unstable and undergo further reactions, one of which is the one leading to bicyclic pyrones.

To complete the oxidative modifications, the double bond of ferutinin acetate (**1e**) was allylically oxidized in a regioselective way, affording a mixture of a 14-hydroxy and a 14-oxo derivative, next converted by deprotection to the analogues **18a** and **18b**, respectively. As for the nonoxidative modifications, the double bond of ferutinin pivalate (**1d**) was cyclopropanated with a modified Simmons−Smith protocol[16] to a single cyclopropyl homologue, next deprotected to **19**, while catalytic hydrogenation of ferutinin gave an almost equimolecular mixture of epimeric dihydro derivatives (**20**). The lack of stereoselectivity in the catalytic hydrogenation is remarkable, since all the other additions to the double bond of ferutinin occurred with remarkable stereoselectivity.



The estrogenic activity of ferutinin (**1a**) and its analogues was investigated in a yeast screen containing the human estrogen receptor alpha (ERa). This screen provides a rapid evaluation of estrogenic agonist activity,[17] and its results are shown in Figure 1. Ferutinin (**1a**) and its dihydro derivatives (**20**) showed high estrogenic potency, ca. 2 orders of magnitude lower than estradiol. Significant

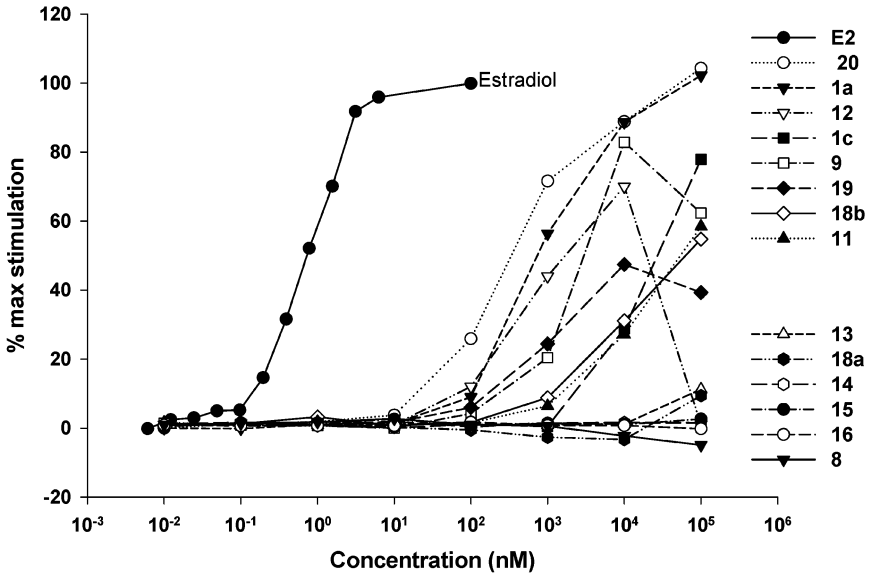


Figure 1. Relative estrogenic activity of estradiol, ferutinin (**1a**), and its analogues **1c**, **8**, **9**, **11**, **12**, **13**, **14**-**16**, **18a**, **18b**, **19**, and **20**. Estrogenic activity was assessed in a yeast screen bearing the human estrogen receptor (ERα). Results are expressed as a percent of maximal stimulation achieved with estradiol.

estrogenic activity was also detected in compounds **1c**, **9**, **11**, **12**, **18b**, and **19**, although **9**, **12**, and **19** showed clear signs of cytotoxicity at the highest concentrations assayed. Conversely, no significant activity was detected in all the remaining compounds. Ferutinin and its analogues were also assayed for antiestrogenic, androgenic, and antiandrogenic activity, but no activity could be evidenced.

The estrogenic activity of the *p*-hydroxybenzyl analogue **1c** and of the dehydro derivative **9** is somewhat unexpected and shows that the conformational constraint of the acyl and the sesquiterpene moieties via hydrogen bonding, while possibly useful, is not critical for the estrogenic activity of ferutinin. The modification of the double bond provided several interesting insights. Thus, both diastereomeric epoxides (**11** and **12**) maintained a significant share of the activity of the natural product. Conversely, all oxidative modifications that replaced the C-8/C-9 double bond with oxygenated functions (glycol, ketol) having a hydroxyl on the α-face of the molecule gave inactive analogues (compounds **13**−**16**). Taken together, these data suggest that the α-face of the eastern part of ferutinin interacts with the lipophilic domain of ER and that this critical interaction is disrupted by the presence of a hydroxyl group. The substantial retention of activity observed upon cyclopropanation and hydrogenation of the double bond supports this view, since the lipophilic pocket of the ERs can bind a wide variety of lipophilic substrates[1] and should easily accommodate a protruding methylene (as in **19)** or methyl (as in **20**). The effect of epimerization at C-6 could not be assessed by a point-mutation, since the synthesis of 6-epiferutinin proved elusive. Nevertheless, the marked decrease of activity between α-epoxyferutinin (**12**) and its 6-epimer (**8**) suggests that the configuration at C-6 is critical. The effect of the oxidation of the allylic methyl was also interesting, since the introduction of a hydroxyl was detrimental for activity, while methyl to formyl oxidation was somewhat tolerated. A possible rationalization is that, while rotation around the C-8/C-14 bond is possible for the 14-hydroxy derivative, electronic factors strongly favor a coplanar orientation for the formyl and the endocyclic double bond. Thus, while the hydroxymethyl derivative **18a** can attain inactive conformations having the hydroxyl oriented toward the α-face of the molecule, resonance effects maintain the formyl oxygen of **18b** substantially coplanar with the mean plane of the daucane skeleton, preventing the perturbation of the critical lipophilic interaction.

In conclusion, capitalizing on the presence of two functionalities, the monoacylated, hydrogen-bonded 1,3-diol system and the double bond, a series of analogues of ferutinin were synthesized, disclosing some interesting trends in its structure−activity relationships. The 4-hydroxyl, the ester carbonyl, and their intramolecular hydrogen bonding were noncritical, while lipophilic interactions involving the right-handed moiety of the molecule were relevant. Further modifications of the terpenoid core are conceivable, but could not be addressed starting from the natural product. This limitation might be circumvented by a total synthesis that incorporates the possibility to generate focused structural diversity in the target, as seminally outlined by Danishefsky in his manifesto of "diverted" organic synthesis.[18] The relevance of the aliphatic moiety is a unique feature of ferutinin that sets it apart from all other phytoestrogens and justifies efforts directed at its total synthesis.

Experimental Section

General Experimental Procedures. IR spectra were obtained on a Shimadzu DR 8001 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded at room temperature on a Bruker DRX-300 spectrometer (300 and 75 MHz, respectively) and on a Bruker DRX-500 (500 and 125 MHz). Chemical shifts were referenced to the residual solvent signal ($CDCl_3$, δ_H 7.27, δ_C 76.9; CD_3COCD_3, δ_H 2.05, δ_C 206.26). HRMS were recorded on a VG Prospect (Fisons) mass spectrometer. Silica gel 60 (70−230 mesh) was used for gravity column chromatography. HPLC separations were performed using a Microporasil Waters (0.8 × 30 cm), equipped with a Waters 6000 A pump and a Waters R 401 refraction index detector. CH_2Cl_2, benzene, and toluene were dried by distillation from P_4O_{10}, and THF by distillation from Na-benzophenone. Ferutinin esters (acetate, pivalate) were prepared by overnight treatment of crude ferutinin (**1a**) with an excess (5 molar equiv) of the corresponding chloride in pyridine. Pivaloyl and acetyl deprotection of the *p*-hydroxybenzoyl (benzyl) moiety was affected by overnight treatment with an excess (20 molar equiv) of pyrrolidine (procedure A) or ethylenediamine (procedure B)

in dry CH_2Cl_2 or toluene (ca. 1.5 mL/mmol of substrate) and workup by dilution with CH_2Cl_2 and washing with 1 N HCl and brine. Organic solutions were dried with Na_2SO_4 prior to evaporation. Zinc was activated by treatment with 1 N HCl, filtration, and sequential washing with water, acetone, and ether.

Determination of Hormonal Activity in Vitro. Estrogenic activity was determined using an estrogen-inducible yeast screen (*Saccharomyces cerevisiae*) expressing the human estrogen receptor-α and containing expression plasmids carrying estrogen-responsive sequences controlling the reporter gene lac-Z (encoding the enzyme β-galactosidase). Estrogenic activity was determined from the metabolism of chlorophenol red β-D-galactopyranoside by monitoring the absorbance at 540 nm.[17a] Antiestrogenic activity was investigated by determining whether the compounds could inhibit the stimulatory response to a submaximal concentration (1.56 nM) of estradiol.[19] Androgenic and antiandrogenic activity were assessed using a yeast screen expressing the human androgen receptor.[19]

Jaeschkeanadiol *p*-Hydroxybenzyl Ether (1c). Under a nitrogen atmosphere, to a stirred solution of dimsyl anion (obtained by reacting NaH (60% dispersion in mineral oil, 151 mg, 3.8 mmol, 2.2 molar equiv) with 5 mL of DMSO) was added jaeschkeanadiol (**1b**, 208 mg, 0.87 mmol). After stirring 5 min at room temperature, *p*-pivaloyloxybenzyl bromide[20] (358 mg, 1,32 mmol, 1.5 molar equiv) was added. After stirring a further 60 h, the reaction was worked up by dilution with petroleum ether−ether (3:1 v/v) and sequential washing with saturated NH_4Cl and brine. After drying and evaporation, the residue was purified by column chromatography (15 g silica gel) eluted with a petroleum ether−EtOAc gradient (from 98:2 to 95:5) to afford the pure product (86 mg, 23%), which was deprotected in the usual way (procedure B). After purification by column chromatography (2.5 g silica gel) eluted with petroleum ether−EtOAc gradient (from 9:1 to:8:2), 53 mg of **1c** was obtained (77%) as an amorphous foam: IR (KBr) ν_{max} 3484, 1616, 1597, 1520, 1277, 1225, 1150, 1067, 1049 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.17 (2H, AA′, acyl), 6.74 (2H, BB′, acyl), 5.50 (1H, br s, H-9), 4.65 (1H, d, J = 12 Hz, H-1′), 4.20 (1H, d, J = 12 Hz, H-1′), 3.55 (1H, br t, J = 6.7 Hz, H-6), 1.88 (3H, br s, H-14), 0.94 (3H, br s, H-15), 0.87−0.85 (each 3H, d, J = 6.7 Hz, H-12, H-13); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.1 (s, C-5′), 133.3 (s, C-8), 130.1 (d, C-3′), 128.1 (s, C-2′), 124.9 (d, C-9), 115.9 (d, C-4′), 87.4 (s, C-4), 73.7 (d, C-6), 69.9 (t, C-1′), 61.5 (d, C-5), 43.4 (s, C-1), 41.3, 40.8, 39.6 (t, C-2, C-7, C-10), 37.4 (d, C-11), 30.6 (t, C-3), 20.7 (q, C-14), 19.7, 18.3, 17.1 (q, C-15, C-12, C-13; HREIMS m/z 326.2271 $[M - H_2O]^+$ (calcd for $C_{22}H_{32}O_3 - H_2O$, 326.2246).

Oxidation of Jaeschkeanadiol (1b). To a solution of **1b** (1.0 g, 4.20 mmol) in dry CH_2Cl_2 (10 mL) was added an excess of PDC (6.32 g, 16.8 mmol, 4.0 molar equiv). After stirring at room temperature for 24 h, the reaction was worked up by dilution with ether and filtration through Celite. The filtrate was washed with 2 N H_2SO_4 and brine, dried, and evaporated. The residue was purified by column chromatography on silica gel (25 g, petroleum ether−EtOAc, 95:5, as eluent) to afford 160 mg (15%) of **3** and 67 mg (7%) of **4**.

1*R*,4*R*-4-Hydroxydauca-7-ene-6,9-dione (3): yellow oil; IR (liquid film) ν_{max} 3497, 1651, 1616, 1468, 1445, 1379, 1196, 1171 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.31 (1H, br s, H-7), 4.41 (1H, br s, OH), 2.85 (2H, br s, H-10), 2.79 (1H, s, H-5), 2.02 (3H, br s, H-14), 1.22 (3H, br s, H-15), 0.88−0.85 (each 3H, d, J = 6.7 Hz, H-12, H-13, overlapped); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.6, 200.7 (s, C-6 and C-9), 148.1 (s, C-8), 134.8 (d, C-7), 86.3 (s, C-4), 63.4 (d, C-5), 58.4 (t, C-10), 42.5 (s, C-1), 40.5 (t, C-2), 37.6 (d, C-11), 34.0 (t, C-3), 22.5, 21.2 (q, C-14 and C-15), 18.0, 17.6 (q, C-12 and C-13); CIMS (isobutanol) m/z 251 $[M + 1]^+$ $[C_{15}H_{22}O_3 + H]^+$, 233 $[M - H_2O + H]^+$ $[C_{15}H_{22}O_3 - H_2O + H]^+$.

1*R*-Dauca-4,7-diene-6,9-dione (4): yellow oil; IR (liquid film) ν_{max} 1726, 1676, 1632, 1615, 1582, 1462, 1377, 1337, 1265 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.65 (1H, s, H-7), 3.46 (1H, hept, J = 6.7 Hz, C-11), 2.91 (1H, d, J = 15.1 Hz, H-10α), 2.77 (1H, d, J = 15.1 Hz, H-10β), 2.02 (3H, br s, H-14), 1.19 (3H, br s, H-15), 1.05 (each 3H, d, J = 6.8 Hz, H-12, H-13, overlapped); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.7, 189.5 (s, C-9 and C-6), 167.0 (s, C-4), 144.0 (s, C-8), 139.8 (s, C-5), 138.2 (d, C-7), 55.1 (t, C-10), 45.1 (s, C-1), 39.2 (t, C-3), 29.6 (t, C-2), 28.3 (d, C-11), 23.1 (q, C-14), 20.7, 20.6, 20.4 (q, C-12, C-13, C-15); CIMS (isobutanol) m/z 233 $[M + 1]^+$ $[C_{15}H_{20}O_2 + H]^+$.

Epoxidation of Jaeschkeanadiol. To a solution of **1b** (2.02 g, 8.48 mmol) in dry CH_2Cl_2 (8 mL) was added MCPBA (77%, 2.93 g, 13.0 mmol, 1.5 molar equiv), resulting in the formation of a milky suspension. After stirring 40 min at room temperature, the reaction was worked up by dilution with CH_2Cl_2 and washing with 5% $Na_2S_2O_3$, 5% NaOH, and brine. After removal of the solvent, the residue was purified by column chromatography on silica gel (65 g, packing with petroleum ether−EtOAc, 9:1, elution with petroleum ether−EtOAc, 9:1 to 6:4). Fractions eluted with petroleum ether−EtOAc, 7:3, afforded 65 mg (3%) of **5b**, and fractions eluted with petroleum ether−EtOAc, 6:4, afforded 1.87 g (87%) of **5a**.

Jaeschkeanadiol α-epoxide (5a): amorphous foam; IR (KBr) ν_{max} 3416, 2959, 2863, 2361, 1379, 1051, 1030, 970, 878 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.09 (1H, dt, J = 10, 2 Hz, H-6), 2.85 (1H, t, J = 7 Hz, H-9), 1.41 (3H, br s, H-14), 1.15 (3H, br s, H-15), 0.95−0.89 (each 3H, d, J = 6.7 Hz, H-12, H-13); ^{13}C NMR (75 MHz, $CDCl_3$) δ 86.2 (s, C-4), 67.2 (d, C-6), 63.2, 61.0 (d, C-5 and C-9), 56.5 (s, C-8), 47.0 (s, C-1), 43.2, 41.0, 40.9 (t, C-2, C-7, C-10), 37.6 (d, C-11), 31.9 (t, C-3), 24.2 (q, C-14), 19.0, 18.1, 16.8 (q, C-15, C-12, C-13); HREIMS m/z 236.1713 $[M - H_2O]^+$ (calcd for $C_{15}H_{26}O_3 - H_2O$, 236.1776).

Jaeschkeanadiol β-epoxide (5b): colorless gum; IR (KBr) ν_{max} 3119, 3083, 2965, 2934, 1375, 1167, 1096, 1051, 1026 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.51 (1H, br s, OH), 4.01 (1H, dt, J = 10, 1 Hz, H-6), 2.91 (1H, d, J = 5 Hz, H-9), 1.32 (3H, br s, H-14), 1.24 (3H, br s, H-15), 0.93−0.89 (each 3H, d, J = 6.8 Hz, H-12, H-13); ^{13}C NMR (75 MHz, $CDCl_3$) δ 87.6 (s, C-4), 67.4 (d, C-6), 63.2, 57.9 (d, C-5 and C-9), 58.0 (s, C-8), 44.2 (s, C-1), 43.9, 41.9, 40.2 (t, C-2, C-7, C-10), 37.5 (d, C-11), 32.9 (t, C-3), 26.1 (q, C-14), 21.2 (q, C-15), 18.1, 16.9 (q, C-12, C-13); HREIMS m/z 236.1719 $[M - H_2O]^+$ (calcd for $C_{15}H_{26}O_3 - H_2O$, 236.1776).

6-Dehydrojaeschkeanadiol α-epoxide (6). To a solution of **5a** (1.60 g, 6.30 mmol) in dry CH_2Cl_2 (12 mL) was added PCC (2.71 g, 12.6 mmol, 2.0 molar equiv). After stirring at room temperature for 8 h, the reaction was worked up by dilution with ether and filtration on Celite. The filtrate was washed with 1 N HCl and brine, dried, and evaporated. The residue was purified by column chromatography on silica gel (40 g, petroleum ether−EtOAc, 95:5, as eluent) to afford 134 mg (9%) of **4** and 990 mg (62%) of **6**. The latter was obtained as a colorless powder: IR (KBr) ν_{max} 3428, 2961, 2361, 1676, 1383, 1287, 1125, 1015, 884 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.83 (1H, br s, OH), 2.93 (1H, t, J = 6.9 Hz, C-9), 2.76 (1H, d, J = 12 Hz, H-7α), 2.66 (1H, d, J = 12 Hz, H-7β), 2.39 (1H, br s, H-5), 1.35 (3H, br s, H-14), 1.17 (3H, br s, H-15), 0.86−0.78 (each 3H, d, J = 6.7 Hz, H-12, H-13); ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.8 (s, C-6), 85.7 (s, C-4), 67.4, 60.9 (d, C-5 and C-9), 54.9 (s, C-8), 51.4 (t, C-7), 48.1 (s, C-1), 41.1, 39.2, 36.3 (t, C-2, C-3, C-10), 39.0 (q, C-11), 23.4, 20.1 (q, C-14 and C-15), 18.1, 17.6 (q, C-12, C-13); CIMS (isobutanol) m/z 253 $[M + 1]^+$ $[C_{15}H_{24}O_3 + H]^+$, 235 $[M - H_2O + H]^+$ $[C_{15}H_{24}O_3 - H_2O + H]^+$.

6-Epijaeschkeanadiol α-epoxide (7). To a solution of **6** (990 mg, 3.93 mmol) in MeOH (13 mL) was added an excess of $NaBH_4$ (223 mg, 5.89 mmol, 1.5 molar equiv) in ca. 1 min. The reaction was then immediately worked up by dilution with EtOAc, washing with saturated NH_4Cl and brine, drying, and evaporation. The residue was purified by column chromatography on silica gel (25 g, petroleum ether−EtOAc, 9:1 to 8:2, as eluent) to afford 890 mg (89%) of **7** as an amorphous foam, which was used directly for the esterification step.

6-Epiferutinin α-epoxide (8). To a solution of **7** (830 mg, 3.26 mmol) in dry toluene were added *p*-pivaloyloxybenxoic acid[21] (2.17 g, 9.78 mmol, 3.0 molar equiv), DCC (2.02 g, 9.78 mmol, 3.0 molar equiv), and DMAP (400 mg, 3.26 mmol, 1.0 molar equiv). The mixture was stirred at 80 °C overnight and then worked up by dilution with EtOAc and washing with

saturated Na_2CO_3 and brine. After drying and evaporation, the residue was purified by column chromatography on silica gel (30 g, petroleum ether−EtOAc, 9:1, as eluent) to afford 300 mg (20%) of the *p*-pivaloyloxybenzoic ester, a part of which (100 mg) was deprotected according to procedure A. After purification by column chromatography on silica gel (3 g, petroleum ether−EtOAc, 9:1, as eluent), 56 mg of **8** was obtained as a colorless gum (70%): IR (liquid film) ν_{max} 3445, 1682, 1611, 1591, 1516, 1312, 1281, 1161, 1113, 1098 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.86 (2H, AA′, acyl), 6.88 (2H, BB′, acyl), 5.42 (1H, br t, J = 10 Hz, H-6), 2.92 (1H, t, J = 7 Hz, H-9), 1.49 (3H, br s, H-14), 1.26 (3H, br s, H-15), 0.92−0.84 (each 3H, d, J = 6.7 Hz, H-12, H-13); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.9 (s, C-1′), 161.2 (s, C-5′), 131.7 (d, C-3′), 121.1 (s, C-2′), 115.5 (d, C-4′), 86.7 (s, C-4), 70.7 (d, C-6), 61.0, 59.3 (d, C-5 and C-9), 58.6 (s, C-8), 44.8 (s, C-1), 43.7, 40.1, 39.5 (t, C-2, C-7, C-10), 35.7 (d, C-11), 31.7 (t, C-3), 27.4, 25.9 (q, C-14 and C-15), 18.4, 17.1 (q, C-12, C-13); HREIMS *m/z* 356.205 $[M - H_2O]^+$ (calcd for $C_{22}H_{30}O_5 - H_2O$, 356.1988).

3,4-Dehydroferutinin (9). Under a nitrogen atmosphere, a stirred and cooled (−50 °C) solution of ferutinin (**1a**) (294 mg, 0.820 mmol) and DMAP (96 mg, 0.860 mmol, 1 molar equiv) in dry pyridine (2 mL) was treated with thionyl chloride (0.18 mL, 0.29 g, 2.5 mmol, 3.0 molar equiv). After stirring at −50 °C for 2 h, the reaction was worked up by dilution with CH_2Cl_2 and sequential washing with 2 N H_2SO_4, saturated $NaHCO_3$, and brine. After drying and evaporation, the residue was purified by column chromatography on silica gel (8 g, petroleum ether−EtOAc, 98:2 to 9:1, as eluent) to afford 253 mg (43%) of **9** as a yellow oil: IR (liquid film) ν_{max} 3441, 1707, 1609, 1593, 1514, 1445, 1275, 1246, 1165, 1111 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.99 (2H, AA′, acyl), 7.53 (1H, br s, OH), 6.93 (2H, BB′, acyl), 5.55 (1H, br s, H-9), 5.40 (1H, br s, H-3), 5.30 (1H, m, H-6), 2.92 (1H, d, J = 11 Hz, H-5), 1.93 (1H, dd, J = 15, 3 Hz, H-7β), 1.79 (3H, br s, H-14), 0.95 (3H, br s, H-15), 0.97−0.85 (each 3H, d, J = 6.7 Hz, H-12, H-13); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.6 (s, C-1′), 160.7 (s, C-5′), 152.9 (s, C-4), 132.4 (s, C-8), 131.9 (d, C-3′), 125.0 (d, C-9), 122.1 (s, C-2′), 120,7 (d, C-3), 115.2 (d, C-4′), 71.8 (d, C-6), 60.7 (d, C-5), 45.8 (s, C-1), 46.9, 41.2, 39.2 (t, C-2, C-7, C-10), 28.1 (d, C-11), 26.7 (q, C-14), 22.7, 21.8, 20.8 (q, C-12, C-13, C-15); HREIMS *m/z* 340.2098 $[M]^+$ (calcd for $C_{22}H_{28}O_3$ 340.2038).

Ferutinin β-epoxide (11). To a solution of **5b** (55 mg, 0.216 mmol) in dry toluene (2 mL) were added *p*-pivaloyloxybenzoic acid (147 mg, 0.663 mmol, 3.0 molar equiv), DCC (134 mg, 0.648 mmol, 3.0 molar equiv), and catalytic DMAP (7.4 mg, 0.061 mmol, 0.28 molar equiv). After stirring overnight at 90 °C, the reaction was worked up by dilution with EtOAc and washing with saturated $NaHCO_3$ and brine. After drying and evaporation, the residue was purified by column chromatography on silica gel (2.5 g, petroleum ether−EtOAc, 9:1, as eluent) to afford a *p*-pivaloyloxybenzoate (57 mg, 57%), deprotected following procedure B. The residue was purified by column chromatography on silica gel (2 g, petroleum ether−EtOAc, 8:2, as eluent) to afford 24 mg (52%) of **11** as an amorphous foam: IR (KBr) ν_{max} 3546, 3281, 2361, 1709, 1676, 1611, 1595, 1516, 1314, 1281, 1238, 1159, 1098 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.93 (2H, AA′, acyl), 7.09 (1H, s, OH), 6.91 (2H, BB′, acyl), 5.57 (1H, m, H-6), 2.99 (1H, t, J = 6 Hz, H-9), 2.48 (1H, s, OH), 1.52 (3H, br s, H-14), 1.24 (3H, br s, H-15), 0.93−0.87 (each 3H, d, J = 6.7 Hz, H-12, H-13); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7 (s, C-1′), 160.8 (s, C-5′), 131.9 (d, C-3′), 121.8 (s, C-2′), 115.4 (d, C-4′), 85.2 (s, C-4), 71.4 (d, C-6), 59.9, 58.0 (d, C-5 and C-9), 52.7 (s, C-8), 42.7 (s, C-1), 42.2, 40.7, 40.0 (t, C-2, C-7, C-10), 36.8 (d, C-11), 31.9 (t, C-3), 24.2, 23.3 (q, C-14 and C-15), 18.4, 17.3 (q, C-12 and C-13); HREIMS *m/z* 356.1999 $[M - H_2O]^+$ (calcd for $C_{22}H_{30}O_5 - H_2O$, 356.1988).

Ferutinin α-epoxide (12). To a solution of **1a** (200 mg, 0.559 mmol) in dry CH_2Cl_2 (2 mL) was added MCPBA (77%, 275 mg, 1.23 mmol, 2.2 molar equiv). After stirring 3 h at room temperature, the reaction was worked up by dilution with CH_2Cl_2 and washing with 5% $Na_2S_2O_3$, 5% NaOH, and brine. After removal of the solvent, the residue was purified by column chromatography on silica gel (3 g, packing with petroleum ether−EtOAc, 8:2, elution with the same eluent) to afford 183 mg (87%) of **12** as an amorphous foam.[22]

8,9-Dihydro-8,14-dehydro-9-hydroxyferutinin (13). To a solution of ferutinin pivalate (250 mg, 0.566 mmol) in dry CH_2Cl_2 was added MCPBA (77%, 190 mg, 0.848 mmol, 1.5 molar equiv). After stirring at room temperature for 2 h, the reaction was worked up by dilution with CH_2Cl_2 and sequential washing with 5% $Na_2S_2O_3$, 5% NaOH, and brine. After drying and removal of the solvent, the crude reaction mixture was added to a solution of Cp_2TiCl_2 in THF (348 mg, 1.4 mmol, 2.5 equiv) previously treated with activated zinc (183 mg, 2.80 mmol, 5.0 molar equiv) for 45 min, until its color turned from red to deep green. After stirring 15 min at room temperature, the reaction was worked up by dilution with EtOAc and sequential washing with 2 N H_2SO_4 and brine. After drying and evaporation of the solvent, the residue was purified by column chromatography on silica gel (8 g, elution with petroleum ether−EtOAc, 9:1) to afford a pivaloyl derivative, deprotected as usual (procedure A) to afford 60 mg of **13** as an amorphous foam (overall 30%): IR (liquid film) ν_{max} 3513, 1684, 1609, 1588, 1516, 1281, 1240, 1163, 1121, 1044 cm^{-1}; ^1H NMR (300 MHz, CD_3COCD_3) δ 9.19 (1H, s, OH), 7.88 (2H, AA′, acyl), 6.91 (2H, BB′, acyl), 5.46 (1H, m, H-6), 5.25−4.90 (each 1H, d, J = 1.8 Hz, H-14, H-14′), 4,20 (1H, m, H-9), 3.50 (1H, s, OH), 1.22 (3H, br s, H-15), 0.92−0.84 (each 3H, d, J = 6.7 Hz, H-12, H-13); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 165.8 (s, C-1′), 162.1 (s, C-5′), 149.6 (s, C-8), 131.9 (d, C-3′), 122.8 (s, C-2′), 115.6 (d, C-4′), 112.2 (t, C-14), 85.7 (s, C-4), 72.7, 72.2 (d, C-6 and C-9), 55.3 (d, C-5), 52.8 (s, C-1), 43.4, 42.5, 39.3 (t, C-2, C-7, C-10), 36.5 (d, C-11), 32.1 (t, C-3), 20.8, 18.4, 17.5 (q, C-15, C-12, C-13); HREIMS *m/z* 374.2112 $[M]^+$ (calcd for $C_{22}H_{30}O_5$, 374.2093).

8*R*,9*S*-8,9-Dihydro-8,9-dihydroxyferutinin (14). To a solution of ferutinin **1a** (234 mg, 0.654 mmol) in CH_3COCH_3−H_2O, 4:1, were added OsO_4 (0.2 M in toluene, 0.030 mL, 0.0060 mmol, 0.01 molar equiv) and NMMO (227 mg, 1.94 mmol; 3.0 molar equiv). After stirring at room temperature for 36 h, the reaction was worked up by stirring with saturated Na_2SO_3 for 45 min; it was extracted with EtOAc and washed with brine. After drying and removal of the solvent, the residue was purified by column chromatography on silica gel (7 g, elution with petroleum ether−EtOAc, 8:2 to 5:5) to afford 207 mg (81%) of **14** as an amorphous foam: IR (KBr) ν_{max} 3402, 1684, 1609, 1593, 1516, 1314, 1285, 1165, 1123, 1101 cm^{-1}; ^1H NMR (300 MHz, CD_3COCD_3) δ 9.11 (1H, s, OH), 7.91 (2H, AA′, acyl), 6.91 (2H, BB′, acyl), 5.59 (1H, dt, J = 10, 3 Hz, H-6), 3.45 (1H, m, H-9), 1.27 (3H, br s, H-14), 1.19 (3H, br s, H-15), 0.94−0.84 (each 3H, d, J = 6.7 Hz, H-12, H-13); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 166.6 (s, C-1′), 162.4 (s, C-5′), 132.6 (d, C-3′), 123.7 (s, C-2′), 115.9 (d, C-4′), 86.3 (s, C-4), 75.1 (d, C-9), 75.0 (s, C-8), 71.3 (d, C-6), 53.5 (d, C-5), 50.1 (s, C-1), 44.2, 44.0, 42.8 (t, C-2, C-7, C-10), 37.0 (d, C-11), 32.9 (t, C-3), 30.4 (q, C-14), 20.5, 19.0, 17.9 (q, C-15, C-12, C-13); HREIMS *m/z* 374.2066 $[M - H_2O]^+$ (calcd for $C_{22}H_{32}O_6 - H_2O$ 374.2093).

8*R*,9*H*-8,9-Dihydro-8-hydroxy-9-oxoferutinin (15). To a solution of **14** (105 mg, 0.268 mmol) in EtOAc (3 mL) were added PCC (373 mg, 1.73 mmol, 6.5 molar equiv) and Celite (373 mg). After stirring at room temperature for 12 h, the reaction was worked up by filtration over Celite and evaporation. The residue was purified by column chromatography on silica gel (3 g, petroleum ether−EtOAc, 8:2 to 6:4, as eluent) to afford 46 mg (44%) of **15** as a yellow oil: IR (KBr) ν_{max} 3402, 1705, 1609, 1593, 1514, 1312, 1283, 1165, 1121, 1100 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.87 (2H, AA′, acyl), 6.79 (2H, BB′, acyl), 5.60 (1H, dt, J = 10, 3 Hz, H-6), 1.35 (3H, br s, H-14), 1.30 (3H, br s, H-15), 0.83−0.80 (each 3H, d, J = 6.7 Hz, H-12, H-13); ^{13}C NMR (75 MHz, $CDCOCD_3$) δ 213.4 (s, C-9), 167.0 (s, C-1′), 160.8 (s, C-5′), 132.1 (d, C-3′), 121.7 (s, C-2′), 115.3 (d, C-4′), 84.4 (s, C-4), 77.4 (s, C-8), 69.7 (d, C-6), 50.9 (d, C-5), 42.0 (s, C-1), 48.7, 40.7, 40.2 (t, C-2, C-7, C-10), 36.6 (d, C-11), 31.7 (t, C-3), 27.9, 23.1 (q, C-14 and C-15), 18.1, 17.2 (q, C-12 and C-13); HREIMS *m/z* 390.2011 $[M]^+$ (calcd for $C_{22}H_{30}O_6$, 390.2042).

8*R*,9*R*-8,9-Dihydro-8,9-dihydroxyferutinin (16). To a solution of **15** (166 mg, 0.423 mmol) in MeOH (2 mL) was

added portionwise an excess of $NaBH_4$ (160 mg, 4.23 mmol, 10 molar equiv). After 2 h the reaction was worked up by dilution with EtOAc, washing with saturated NH_4Cl and brine, drying, and evaporation. The residue was purified by column chromatography on silica gel (3 g, petroleum ether−EtOAc, 7:3, as eluent) to afford a mixture. **16** (50%) was isolated only after HPLC (hexane−EtOAc, 5:5) as an amorphous foam: IR (KBr) ν_{max} 3441, 1682, 1599, 1516, 1316, 1294, 1246, 1167, 1003 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.89 (2H, AA′, acyl), 6.87 (2H, BB′, acyl), 5.73 (1H, br t, J = 10 Hz, H-6), 3.85 (1H, br s, H-9), 1.31, 1.28 (each 3H, br s, H-14 and H-15), 0.86−0.84 (each 3H, d, J = 6.7 Hz, H-12, H-13, overlapped); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.6 (s, C-1′), 161.1 (s, C-5′), 131.8 (d, C-3′), 121.8 (s, C-2′), 115.4 (d, C-4′), 86.0 (s, C-4), 76.7 (d, C-9), 75.9 (s, C-8), 72.3 (d, C-6), 53.6 (d, C-5), 43.3 (s, C-1), 46.3, 43.1, 39.2 (t, C-2, C-7, C-10), 36.5 (d, C-11), 31.4 (t, C-3), 30.8 (q, C-14), 21.5 (q, C-15), 18.4, 17.3 (q, C-12 and C-13); HREIMS m/z 374.213 $[M - H_2O]^+$ (calcd for $C_{22}H_{32}O_6 - H_2O$, 374.2093).

When the reduction was carried out on crude **15**, the *seco*-daucane **17** could also be obtained as a minor reaction product. This compound could not be completely purified from unfragmented daucane impurities, but its structure elucidation could nevertheless be clarified by 1D and 2D NMR experiments: ^1H NMR (500 MHz, $CDCl_3$) δ 5.35 (1H, s, H-7), 4.57 (1H, dd, J = 3.8 and 7.4 Hz, 9-OH), 3.86 (1H, m, H-9α), 3.72 (1H, m, H-9β), 2.89 (1H, s, H-5), 2.06 (1H, ddd, J = 5.3, 7.7, and 14.0 Hz, H-3α), 2.04 (1H, m, H-11), 2.01 (3H, s, H-14), 1.96 (1H, ddd, J = 8.4, 8.6, and 14.0 Hz, H-3β), 1.93 (1H, ddd, J = 4.2, 9.0, and 14.7 Hz, H-10α), 1.70 (1H, ddd, J = 7.7, 8.6, and 12.8 Hz, H-2α), 1.66 (1H, ddd, J = 5.3, 8.4, and 12.8 Hz, H-2β), 1.55 (1H, ddd, J = 3.3, 5.8, and 14.7 Hz, H-10β), 0.97 (3H, s, H-15), 0.95 (3H, d, J = 6.8, H-13), 0.94 (3H, d, J = 6.8, H-12); ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.9 (s, C-6), 174.4 (s, C-8), 103.7 (s, C-7), 98.2 (s, C-4), 59.1 (t, C-9), 56.1 (d, C-5), 46.0 (t, C-10), 45.8 (s, C-1), 40.0 (t, C-2), 34.9 (d, C-11), 33.5 (t, C-3), 22.6 (q, C-15), 21.7 (q, C-14), 18.1 (q, C-13), 17.6 (q, C-12); HREIMS m/z 252.1734 $[M]^+$ (calcd for $C_{15}H_{24}O_3$, 252.1725).

Allylic Oxidation of Ferutinin Acetate (1e). To a suspension of SeO_2 (60.1 mg, 0.542 mmol, 0.51 molar equiv) in 2 mL of dry CH_2Cl_2 was added TBHP (3 M in toluene, 0.70 mL, 191 mg, 2.11 mmol, 2.0 molar equiv). After stirring at room temperature for 20 min, a solution of ferutinin acetate (422 mg, 1.06 mmol) in dry CH_2Cl_2 (3 mL) was added. Stirring was continued at room temperature for 75 min, and then the reaction was worked up by dilution with CH_2Cl_2 and washing with 10% Na_2SO_3 and brine. After drying and evaporation, the residue was purified by column chromatography on silica gel (3 g, petroleum ether−EtOAc, 9:1 to 8:2, as eluent) to afford 15 mg (13%) of the 14-oxo derivative and 27 mg (22%) of the 14-hydroxy derivative, deprotected in the usual way (procedure A) to afford, respectively, **18b** and **18a**.

14-Hydroxyferutinin (18a): amorphous foam; IR (KBr) ν_{max} 3402, 1686, 1609, 1593, 1516, 1312, 1277, 1165, 1119, 1099 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.02 (2H, AA′, acyl), 7.17 (2H, BB′, acyl), 5.82 (1H, m, H-9), 5.26 (1H, dt, J = 10, 3 Hz, H-6), 4.09 (2H, br s, H-14), 1.11 (3H, br s, H-15), 0.99−0.88 (each 3H, d, J = 6.7 Hz, H-12, H-13); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.8 (s, C-1′), 160.7 (s, C-5′), 136.4 (s, C-8), 131.8 (d, C-3′), 127.8 (d, C-9), 121.9 (s, C-2′), 115.3 (d, C-4′), 86.6 (s, C-4), 71.4 (d, C-6), 68.6 (t, C-14), 60.0 (d, C-5), 43.9 (s, C-1), 41.1, 40.5, 37.0 (t, C-2, C-7, C-10), 36.8 (d, C-11), 31.4 (t, C-3), 20.2, 18.5, 17.4 (q, C-15, C-12, C-13); HREIMS m/z 374.2074 $[M]^+$ (calcd for $C_{22}H_{30}O_5$, 374.2093).

14-Oxoferutinin (18b): amorphous foam; IR (KBr) ν_{max} 3398, 1699, 1682, 1611, 1593, 1516, 1312, 1277, 1242, 1167, 1098 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.41 (1H, s, H-14), 7.87 (2H, AA′, acyl), 6.93 (1H, m, H-9), 6.87 (2H, BB′, acyl), 5.26 (1H, dt, J = 10, 3 Hz, H-6), 1.12 (3H, br s, H-15), 0.93−0.85 (each 3H, d, J = 6.7 Hz, H-12, H-13); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.2 (d, C-14), 167.5 (s, C-1′), 161.9 (s, C-5′), 156.8 (d, C-9), 140.8 (s, C-8), 132.5 (d, C-3′), 121.8 (s, C-2′), 116.0 (d, C-4′), 87.4 (s, C-4), 70.7 (d, C-6), 60.1 (d, C-5), 44.3 (s, C-1), 43.0, 41.8 (t, C-2 and C-10), 37.4 (d, C-11), 31.7, 31.3 (t, C-3 and C-7), 21.0 (q, C-15), 18.9, 17.9 (q, C-12 and C-13); HREIMS m/z 372.1950 $[M]^+$ (calcd for $C_{22}H_{28}O_5$, 372.1937).

8,9-Methlenferutinin (19). Under a nitrogen atmosphere, a solution of ferutinin pivalate (250 mg, 0.565 mmol) in dry benzene (20 mL) was treated with diethylzinc (1 N in hexanes, 9 mL, 6.53 g, 9.0 mmol, 16 molar equiv) and diiodomethane (0.73 mL, 2.43 g, 9.0 mmol, 16 molar equiv). The mixture was stirred at 65 °C for 12 h and then worked up by cooling and sequential washing with 1 N HCl, saturated $NaHCO_3$, and finally brine. After drying and evaporation, the residue was washed with petroleum ether and then deprotected (protocol B). The final product was purified by column chromatography over silica gel (5 g, petroleum ether−EtOAc, 8:2, as eluent) to afford 74 mg of **19** as an amorphous foam (overall 35%): IR (KBr) ν_{max} 3349, 1703, 1669, 1609, 1595, 1512, 1314, 1277, 1248, 1167, 1100 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.06 (1H, s, OH), 7.91 (2H, AA′, acyl), 6.91 (2H, BB′, acyl), 5.51 (1H, dt, J = 10, 1 Hz, H-6), 2.68 (1H, s, OH), 1.30−1.21 (each 3H, br s, H-14 and H-15), 0.90−0.86 (each 3H, d, J = 6.7 Hz, H-12, H-13), 0,70 (1H, m, H-9), 0,58 (1H, dd, J = 8, 4 Hz, H-16), 0.21 (1H, t, J = 4.4 Hz, H-16); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.8 (s, C-1′), 161.7 (s, C-5′), 132.4 (d, C-3′), 122.3 (s, C-2′), 116.0 (d, C-4′), 87.4 (s,C-4), 72.2 (d, C-6), 61.8 (d, C-5), 45.7 (s, C-1), 45.7, 43.5, 41.0 (t, C-2, C-7, C-10), 37.4 (d, C-11), 32.3 (t, C-3), 25.6, 19.9 (q, C-14 and 1C-5), 24.0 (q, C 16), 21.9 (d, C-9), 18.9, 18.0 (q, C-1 and C-13), 15.3 (s, C-8); HREIMS m/z 354.2119 $[M - H_2O]^+$ (calcd for $C_{23}H_{32}O_4 - H_2O$, 354.2195).

Dihydroferutinin (20). A methanol solution of ferutinin (205 mg, 0.573 mmol) was hydrogenated (balloon) in the presence of Pd(C) (20 mg). After 24 h, the reaction was worked up by filtration over Celite to afford 179 mg (87%) of **20** as an amorphous foam: IR (liquid film) ν_{max} 2926, 2359, 1682, 1609, 1591, 1312, 1283, 1163, 1123, 1100 cm^{-1}. Major isomer: ^1H NMR (500 MHz, $CDCl_3$) δ 7.94 (2H, AA′, acyl), 6.89 (2H, BB′, acyl), 5.52 (1H, ddd, J = 4.1, 7.3 and 10.6 Hz, H-6), 2.09 (1H, m, H-8), 2.07 (1H, d, J = 10.5 Hz, H-5), 1.95 (1H, m, H-9α), 1.95 (1H, m, H-3α), 1.94 (1H, m, H-7α), 1.82 (1H, m, H-7β), 1.65 (1H, m, H-3β), 1.65 (1H, m, H-11), 1.64 (1H, m, H-2α), 1.61 (1H, m, H-9β), 1.57 (1H, m, H-2β), 1.47 (1H, m, H-10α), 1.22 (1H, m, H-10β), 1.19 (3H, s, H-15), 1.07 (3H, d, J = 7.0, H-14), 0.88 (3H, d, J = 6.8 Hz, H-12), 0.85 (3H, d, J = 6.8 Hz, H-13); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.0 (s, C-1′), 160.4 (s, C-5′), 132.0 (d, C-3′), 128.8 (s, C-2′), 115.4 (d, C-4′), 86.3 (s, C-4), 74.6 (d, C-6), 57.7 (d, C-5), 44.0 (s, C-1), 42.7 (t, C-10), 41.5 (t, C-7), 38.8 (t, C-2), 36.7 (d, C-11), 31.9 (t, C-3), 31.7 (t, C-9), 29.1 (d, C-8), 22.5 (q, C-14), 19.9 (q, C-15), 18.5 (q, C-12), 17.5 (q, C-13). Minor isomer: ^1H NMR (500 MHz, $CDCl_3$) δ 7.94 (2H, AA′, acyl), 6.89 (2H, BB′, acyl), 5.65 (1H, ddd, J = 1.3, 4.7 and 9.7 Hz, H-6), 2.23 (1H, d, J = 9.7 Hz, H-5), 1.98 (1H, m, H-7α), 1.95 (1H, m, H-8), 1.94 (1H, m, H-2α), 1.82 (1H, m, H-3α), 1.74 (1H, m, H-7β), 1.62 (1H, m, H-2β), 1.61 (2H, m, H-9), 1.58 (1H, m, H-11), 1.52 (1H, m, H-10α), 1.34 (1H, m, H-3β), 1.23 (1H, m, H-10β), 1.13 (3H, s, H-15), 0.94 (3H, d, J = 7.0 Hz, H-14), 0.88 (3H, d, J = 6.8 Hz, H-12), 0.86 (3H, d, J = 6.8 Hz, H-13); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.8 (s, C-1′), 160.4 (s, C-5′), 132.0 (d, C-3′), 128.9 (s, C-2′), 115.3 (d, C-4′), 86.7 (s, C-4), 73.3 (d, C-6), 54.8 (d, C-5), 44.5 (s, C-1), 43.1 (t, C-10), 44.1 (t, C-7), 43.1 (t, C-2), 36.6 (d, C-11), 31.7 (t, C-3), 36.1 (t, C-9), 29.6 (d, C-8), 25.2 (q, C-14), 20.2 (q, C-15), 18.5 (q, C-12), 17.5 (q, C-13); HREIMS m/z 360.2289 $[M]^+$ (calcd for $C_{22}H_{32}O_4$, 360.2301).

Acknowledgment. We are grateful to Prof. M. Ballero (Università di Cagliari, Italy) for a generous gift of the nonpoisonous chemotype of giant fennel from Sardinia, and to Prof. J. Sumpter (Brunel University, Uxbridge, UK) for providing the estrogen-responsive yeast screen. Financial support from MIUR (Fondi ex-40%, progetto Sostanze naturali ed analoghi sintetici con attività antitumorale) is gratefully acknowledged.

References and Notes

(1) Elsby, R.; Ashby, J.; Sumpter, J. P.; Brooks, A. N.; Pennie, W. D.; Maggs, J. L.; Lefevre, P. A.; Odum, J.; Beresford, N.; Paton, D.; Park, B. K. *Biochem. Pharmacol.* **2000**, *60*, 1519−1530.

(2) Brzozowski, A. M.; Pike, A. C.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J.-A.; Carlquist, M. *Nature* **1997**, *389*, 753−758.

(3) (a) Ignatkov, V. I.; Ahmedhodzjaeva, H. T.; Babichev, V. *Farmakol. Toksikol.* **1990**, *53*, 37−38. (b) Ikeda, K.; Arao, Y.; Otsuka, H.; Nomato, S.; Horiguchi, H.; Kato, S.; Kayama, F. *Biochem. Biophys. Res. Commun.* **2002**, *29*, 354−360.
(4) Appendino, G.; Cravotto, G.; Sterner, O.; Ballero, M. *J. Nat. Prod.* **2001**, *64*, 393−395.
(5) (a) Hadidi, K. A.; Aburjai, T.; Battah, A. K. *Fitoterapia* **2003**, *74*, 242−246. (b) El-Thaher, T. S.; Matalka, K. Z.; Taha, H. A.; Badwan, A. A. *Int. J. Impot. Res.* **2001**, *13*, 247−251.
(6) Appendino, G.; Spagliardi, P.; Cravotto, G.; Pocock, V.; Milligan, S. *J. Nat. Prod.* **2002**, *65*, 1612−1615.
(7) Nimmagudda, R. R.; Ramanathan, R.; Putcha, L. *Biochem. Arch.* **1997**, *13*, 171−178.
(8) Griffiths, L. A.; Smith, G. E. *Biochem. J.* **1972**, *128*, 901−911.
(9) Liebich, H. M.; Forst, C. *J. Chromatogr.* **1990**, *525*, 1−14.
(10) Routledge, E. J.; Parker, J.; Odum, J.; Ashby, J.; Sumpter, J. P. *Toxicol. Appl. Pharmacol.* **1998**, *153*, 12−19. Alkyl *p*-hydroxybenzoates (parabens) from antiperspirants and deodorants were detected in breast tumors (Darbre, P. D. *J. Appl. Toxicol.* **2003**, *23*, 89−95), and frequent underarm shaving combined with deodorant use has been linked to breast cancer (McGrath, K. G. *Eur. J. Cancer Prev.* **2003**, *23*, 479−485), though the matter is controversial (Whelan, J. *New Sci.* **2004**, Jan 24, p 11).
(11) The presence of this eight-membered intramolecular hydrogen bonding is in accordance with the sharp hydroxyl absorption (3300−3500 cm^{-1}, unaffected by dilution) in the IR spectra of jaeschkeanadiol esters and the low frequency of the ester carbonyl absorption (1730−1710 cm^{-1} for aliphatic esters, <1700 cm^{-1} for the aromatic ones) (Skiraman, M. C.; Nagasampagi, B. A.; Pandey, R. C.; Dev, S. *Tetrahedron* **1973**, *29*, 985−991, and ref 6).
(12) Mathieson, A. L. *Tetrahedron Lett.* **1965**, 4137−4140.
(13) De Luca, L.; Giacomelli, G.; Porcheddu, A. *Org. Lett.* **2001**, *3*, 3041−3043.
(14) Schobert, R. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 2693−2703.
(15) Tolstikov, G. A.; Akbutina, F. A.; Dembitskii, A. D.; Valeev, F. A.; Miftakhov, M. S. *Zh. Org. Chim.* **1992**, *28*, 2081−2090.
(16) Furukawa, J.; Kawabata, N.; Nishimura, J. *Tetrahedron* **1968**, *24*, 53−58.
(17) (a) Routledge, E. J.; Sumpter, J. P. *Environ. Toxicol. Chem.* **1996**, *15*, 241−258. (b) Milligan, S. R.; Kalita, J. C.; Heyerick, A.; Rong, H.; De Cooman, L.; De Keukeleire, D. *J. Clin. Endocrinol. Med.* **1999**, *83*, 2249−2252.
(18) Njardarson, J. T.; Gaul, C.; Shah, B.; Hjang, X.-Y.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 1038−1040.
(19) Sohoni, P.; Sumpter, J. P. *J. Endocrinol.* **1998**, *158*, 327−339.
(20) Prepared in overall 24% yield from 4-hydroxybenzaldehyde by reduction (4 molar equiv of $NaBH_4$, MeOH), pivaloylation (1.6 molar equiv of pivaloyl chloride, 1.6 molar equiv of KOH, dioxane), and treatment with NBS (1.5 molar equiv, 1.2 molar equiv of TPP, CH_2Cl_2).
(21) Prepared in 85% yield by pivaloylation of *p*-hydroxybenzoic acid (4 molar equiv of pivaloyl chloride, 0.3 molar equiv of DMAP, pyridine).
(22) Diab, Y.; Dolmazon, R.; Bessière, J.-M. *Flavour Fragr. J.* **2001**, *16*, 120−122.

NP049796W