Arzanol, an Anti-inflammatory and Anti-HIV-1 Phloroglucinol α-Pyrone from *Helichrysum italicum* ssp. *microphyllum*

Giovanni Appendino,*[,†,‡] Michela Ottino,[†,‡] Nieves Marquez,[§] Federica Bianchi,[†,‡] Anna Giana,[†,‡] Mauro Ballero,[‡] Olov Sterner,*[,⊥] Bernd L. Fiebich,[∥] and Eduardo Munoz*[,§]

Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Università del Piemonte Orientale, Via Bovio 6, 28100 Novara, Italy, Consorzio per lo Studio dei Metaboliti Secondari (CSMS), Viale S. Ignazio 13, 09123 Cagliari, Italy, Department of Organic Chemistry, Lund University, P.O. Box 124, 221 00 Lund, Sweden, Department of Psychiatry, University of Freiburg Medical School, Germany, and Departamento de Biología Celular, Fisiología e Inmunología, Facultad de Medicina, Universidad de Córdoba, Avenida de Menendez Pidal s/n, 14004 Córdoba, Spain

Received November 17, 2006

An acetone extract of *Helichrysum italicum* ssp. *microphyllum* afforded the phloroglucinol α-pyrone arzanol (**1a)** as a potent NF-κB inhibitor. Arzanol is identical with homoarenol (**2a**), whose structure should be revised. The phloroglucinol-type structure of arzanol and the 1,2,4-trihydroxyphenyl-type structure of the base-induced fragmentation product of homoarenol could be reconciled in light of a retro-Fries-type fragmentation that triggers a change of the hydroxylation pattern of the aromatic moiety. On the basis of these findings, the structure of arenol, the major constituent of the clinically useful antibiotic arenarin, should be revised from **2b** to **1b**, solving a long-standing puzzle over its biogenetic derivation. An α-pyrone (micropyrone, **7**), the monoterpene *rac-E-ω*-oleoyloxylinalol (**10**), four known tremetones (**9a**−**d**), and the dimeric pyrone helipyrone (**8**) were also obtained. Arzanol inhibited HIV-1 replication in T cells and the release of pro-inflammatory cytokines in LPS-stimulated primary monocytes, qualifying as a novel plant-derived anti-inflammatory and antiviral chemotype worth further investigation.

Helichrysum italicum G. Don. is one of the best known medicinal plants from the Mediterranean area.[1] The strong and persistent smell of *H. italicum* is reminiscent of that of curry and combines with the long-lasting bright yellow color of its flower heads to make the plant a veritable icon of the Mediterranean environment. In the Greek-Roman system of medicine, *H. italicum* was used as an anti-inflammatory and anti-infective plant,[2] and both uses are still well rooted in folk medicine today.[3] Systematic clinical studies on the anti-inflammatory properties of *H. italicum* were carried out by Santini, an Italian physician, in the 1940s.[4] Despite the promising results obtained, these investigations were largely overlooked, but the demise of COX-2 inhibitors and the current interest in new anti-inflammatory agents have led to a belated recognition of their seminal relevance.[5]

Plants from the genus *Helicrysum* are prolific producers of secondary metabolites,[6] and anti-inflammatory properties have been described for some constituents of *H. italicum*.[7] However, the low isolation yield and the overall modest activity of these compounds make it likely that more potent, specific, and still unidentified anti-inflammatory agents occur in this plant. Since the ethnobotanical use and the clinical efficacy of *H. italicum* as an anti-inflammatory agent are clearly documented, we have started a systematic study of this plant as a source of new pharmaceuticals. Inhibition of the transcription nuclear factor-κB (NF-κB) served as the end-point, and the subspecies *microphyllum* (Willd.) Nyman, a plant endemic to Sardinia,[1] was used as starting material. NF-κB is a validated target for inflammation,[8] and there is also growing awareness that its malfunctioning or disregulation is involved in several other pathologies, including cancer and AIDS.[9] Therefore, leads identified in this investigation might have broad pharmaceutical application.

* To whom correspondence should be addressed. Tel: +39 0321 375744 (G.A.); +34 957 218267 (E.M.); +46 70 5306649 (O.S.). Fax: +39 0321 375621 (G.A.); +34-957 218229 (E.M.); +46.46 2228209 (O.S.). E-mail: appendino@pharm.unipmn.it (G.A.); fi1muble@uco.es (E.M.); Olov.Sterner@organic.lu.se (O.S.).

† Università del Piemonte Orientale.

‡ Consorzio per lo Studio dei Metaboliti Secondari.

⊥ Lund University.

§ University of Cordoba.

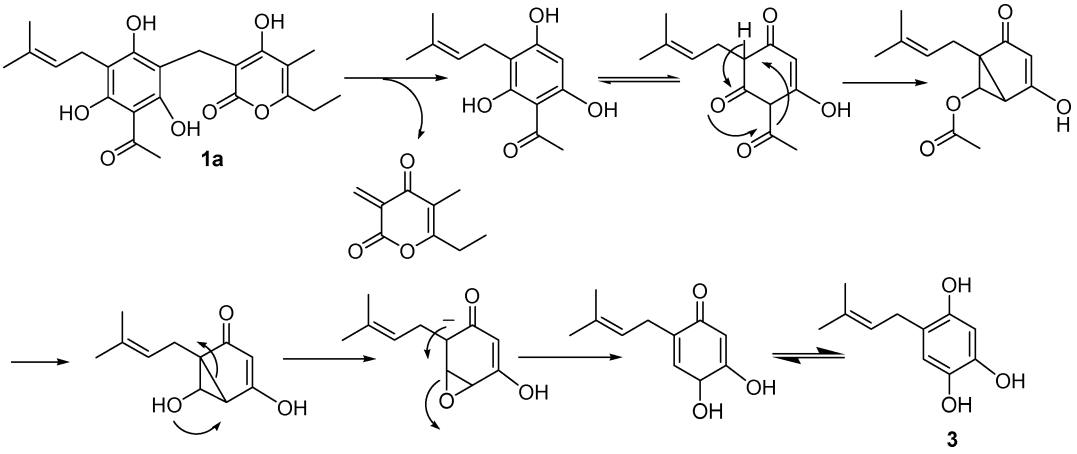
∥ University of Freiburg.

Results and Discussion

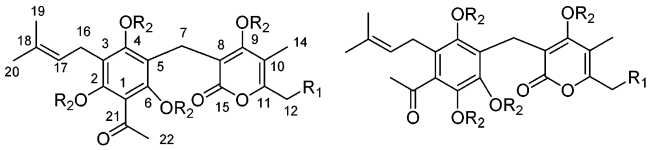
An acetone extract of *H. italicum* ssp. *microphyllum* showed potent NF-kB-inhibiting activity ($IC_{50} \approx 25\ \mu g/mL$) in a luciferase gene reporter assay, where the amount of the luciferase gene product is related to the extent of NF-κB transcriptional activation.[8] The acetone extract was separated by solid-phase extraction into three fractions (petroleum ether, EtOAc, and acetone). The active EtOAc eluate deposited a pale yellow precipitate when dissolved in petroleum ether−EtOAc (1:1) (ca. 0.05% from the dried plant material). This compound, arzanol **1a**,[10] showed powerful NF-κB-inhibiting activity ($IC_{50} \approx 5\ \mu g/mL$), superior to that of all the other products obtained by bioassay-directed fractionation of the mother liquors by gravity column chromatography. HR-MS established the molecular formula $C_{22}H_{26}O_7$, while the IR spectrum showed evidence of hydroxy (3329, 3300 cm^{-1}) and conjugated carbonyl (1671, 1626 cm^{-1}) groups. The ^1H NMR spectrum (C_6D_6) showed a series of simple spin systems corresponding to a *C*-prenyl group (δ 5.12, br t, $J = 7.1$ Hz, 1H; 3.39, br d, $J = 7.1$ Hz, 2H; 1.54 and 1.50, br s, 2 × 3H), an ethyl group (δ 2.04, q, $J = 7.6$ Hz, 2H; 0.85, t, $J = 6.7$ Hz, 3H), a methylene singlet (δ 3.62, s), and two deshielded methyls (δ 2.55 and 1.62), one of which (δ 2.55) was sharp and presumably bound to a carbonyl. The ^{13}C NMR spectrum (C_6D_6) showed two carbonyl singlets, of the ketone and ester type (δ 204.4 and 169.6, respectively), as well as five deshielded aromatic singlets typical of polyhydroxylated phenolic or enolic carbons (δ 167.5, 162.1, 161.2, 161.0, 159.1). These spectroscopic features are typical of phloroglucinol α-pyrones, characteristic compounds from plants of the genus *Helicrysum*.[11] HMBC experiments established the location of the various substituents on the aromatic ring and the pyrone moiety. Especially revealing were the HMBC correlations between the protons of the methylene bridge (δ 2.58) and the *ipso* (C-5 and C-8, δ 106.9 and 103.1, respectively) and *ortho* aromatic (C-4 and C-6, δ 162.1 and 161.0, respectively) and pyrone (C-9 and C-15, δ 167.5 and 169.6, respectively) carbons. These served as a starting point to elucidate the substitution pattern of the phloroglucinol and the pyrone moieties, completed by inspection of the HMBC correlations of the prenyl methylene and the methyl and ethyl substituents, respectively. A compound isomeric to **1a** (homoarenol, **2a**) was isolated from *H. arenarium*

10.1021/np060581r CCC: $37.00
Published on Web 02/22/2007

Scheme 1. Proposed Mechanism for the Formation of the 1,2,4-Trihydroxybenzene **3** from the Treatment of **1a** with Base (KOH)

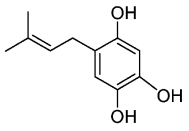


L. as an inseparable mixture with its homologue (arenol, **2b**).[12] The two compounds could be separated after acetylation, and the reported physical (mp) and spectroscopic (^{1}H NMR, IR) data for the tetraacetate of homoarenol are identical to those of arzanol tetraacetate. The two compounds are, therefore, the same, and homoarenol, a long-standing biogenetic puzzle due to the unnatural 1,2,4-trioxygenation pattern of its aromatic moiety,[13] should be revised to a mainstream phloroglucinol derivative. The original substitution pattern of arenol was established by František Šorm, one of the founding fathers of modern terpenoid chemistry,[14] on sound data. Thus, the spectroscopic analysis of the fragmentation product obtained, in unreported yield, by treatment of homoarenol tetraacetate with bases was correctly formulated as **3** on the basis of the nonequivalence and the lack of coupling between its two aromatic protons. While the correctness of this assignment is beyond doubt, it should nevertheless be noted that it is in principle possible to write a mechanism that couples the formal migration of the oxygen atom adjacent to the prenyl to the loss of the acetyl residue (Scheme 1), reconciling the HMBC data of the natural product with the structure assigned by Šorm to the fragmentation product of homoarenol. Thus, retro-Michael fragmentation of the alkylidene-bis-heteroaryl system[15] splits the natural product into its pyrone and phloroglucinol moieties, generating an acetylphloroglucinol derivative that might then undergo a cyclopropylogous retro-Fries rearrangement. Overall, carbon-to-oxygen acetyl migration takes place, a reaction reminiscent of some transformations observed in the prenylated phloroglucinol hyperforin.[16] The resulting cyclopropanol can then rearrange to an epoxide, eventually turning into the catechol derivative **3** by ring-opening and tautomerization (Scheme 1).[17]

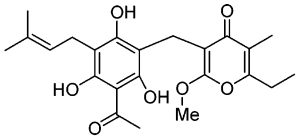


	R_1	R_2
1a	Me	H
1b	H	H
1c	Me	Ac

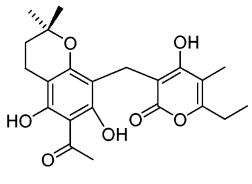
	R_1	R_2
2a	Me	H
2b	H	H
2c	Me	Ac



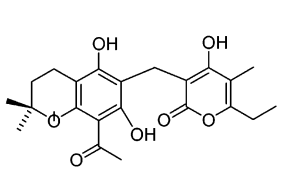
3



4



5



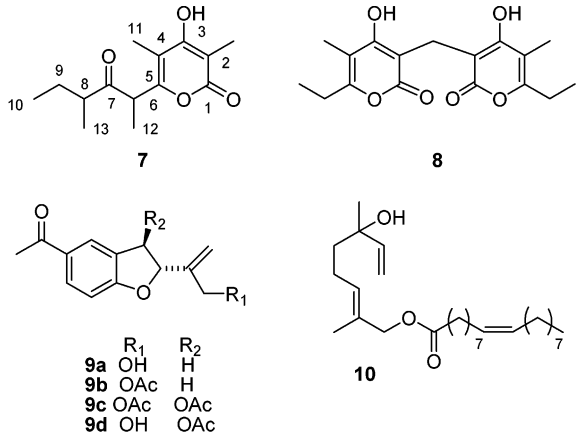
6

On the basis of these findings, the structure of arenol, the major constituent of the clinically useful antibiotic arenarin,[18] should be revised from **2b** to **1b**. Arenarin is a mixture of phenolic antibiotics from *H. arenarium* L. and was used as an antiseptic in the former USSR.[18] The long-standing uncertainties about the structure of arenol are remarkable, since the first references to arenarin date back to the 1950s,[18] with over half a century elapsing before the structure of its major constituent could finally be fully elucidated. In arzanol (**1a**) and its peracetate (**1c**), the NMR resonances of the central methylene protons and their corresponding carbon (C-7) were rather broad, suggesting slow rotation around the carbon bridging the two ring systems.[19]

Methylation of arzanol with trimethylsilyldiazomethane afforded the *O*-methyl derivative **4**, while treatment with acids afforded a separable mixture of the benzopyrans **5** and **6**, the result of the intramolecular cyclization of the prenyl group on its *ortho*-phenolic hydroxy groups. These observations showed that, unlike other phloroglucinols,[20] arzanol is amenable to chemical modification and can therefore act as a starting template for structure−activity studies. Interestingly, a benzofuran isomeric to **5** (italipyrone) was reported from *H. italicum* by Bohlmann.[21]

The major constituent of the mother liquors from the crystallization of **1a** was a new pyrone, micropyrone (**7**). This compound ($C_{14}H_{20}O_4$, HRMS) showed no NF-κB-inhibing properties (*vide infra*) and was obtained as a colorless powder. Its gross structure was deduced from the presence of two well-defined aliphatic spin systems of protons (a *sec*-butyl and an ethylidene), which were combined by HMBC correlations to a ketone carbonyl (δ 210.5, C-7) and a 3,5-dimethyl-4-hydroxypyrone moiety. Micropyrone is related to a series of β-diketones isolated from the essential oil of *H. italicum*, which might well result from its hydrolytic thermal degradation during steam distillation of the plant material.[22]

The known bis-pyrone helipyrone (**8**)[23] and four acylbenzofurans, tremetones **9a**−**d**,[6,24] were also obtained from the mother liquors of the crystallization of **1a**, as well as the optically inactive oleyl ester of *E*-ω-hydroxylinalol (**10**). The structure of **10** was confirmed by synthesis from linalol, one major constituent of the essential oil of *H. italicum*.[25] Thus, commercial linalol was stereoseletively ω-hydroxylated[26] and then acylated with oleic acid to afford a compound identical, apart from the optical rotation, with the natural product. Only the dimeric derivative helipyrone (**8**) showed significant NF-κB-inhibing properties ($IC_{50} \approx 8.7$ μM), while micropyrone and all tremetones (**9a**−**d**) were essentially inactive (data not shown).



Since the HIV-1 promoter is highly responsive to TNFα-induced NF-κB pathway, the crude acetone extracts of *H. italicum* subps. *mycrophyllum* and arzanol were also investigated for their capacity to inhibit TNFα-induced HIV-1-LTR transactivation in a T cell line stably transfected with a plasmid containing the luciferase gene driven by the HIV-1 LTR promoter. Both the extract and **1a** inhibited, in a concentration-dependent manner, HIV-LTR transactivation ($IC_{50} \approx 25$ μg/mL and 5 μM, respectively, Figure 1). In order to rule out a nonspecific mechanism of action, arzanol was further evaluated in a HeLa Tet-On-Luc assay, a model where the luciferase gene is under the control of an artificial promoter regulated by doxycycline.[27] Arzanol (**1a**) failed to inhibit luciferase activity driven by the tetracycline-sensitive artificial promoter, ruling out a possible interference with the transcriptional machinery, or with the *in vitro* activity of the luciferase enzyme (data not shown).

To further investigate the anti-HIV activity of arzanol (**1a**), Jurkat cells were infected with the pNL4-3 HIV-1 clone pseudotyped with the VSV envelope, which can bypass the natural mode of HIV-1 entry into these cells and supports a robust HIV-1 replication.[28] Upon integration into host chromosomes, this recombinant virus expresses the firefly luciferase gene, and therefore, luciferase activity in infected cells correlates with the rate of viral replication. In this assay, high luciferase activity levels were detected 24 h after cellular infection with the VSV-pseudotyped HIV-1 clone, and

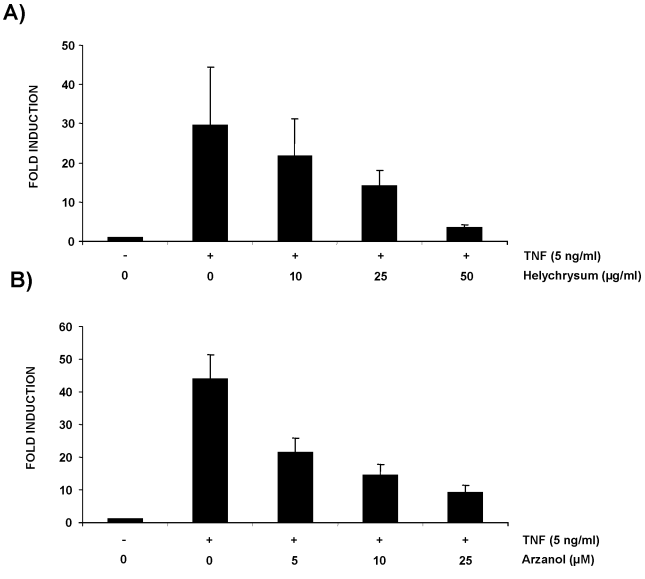


Figure 1. Effects of the acetone extract of *Helychrysum italicum* subsp. *microphyllum* extract and arzanol (**1a**) on NF-κB activation. 5.1 cells were pretreated with increasing concentrations of the isolated compounds and next treated with TNFα for 6 h, after which luciferase activity was measured and expressed as an incremental induction.

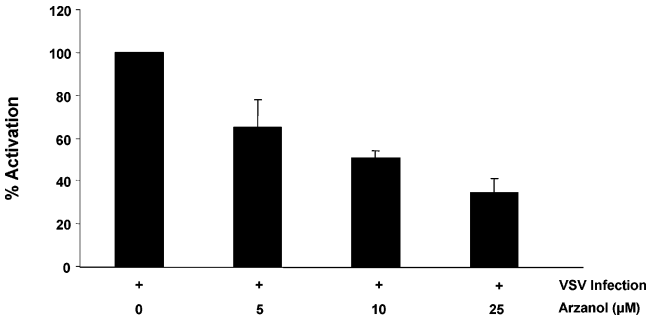


Figure 2. Effects of arzanol (**1a**) on recombinant virus replication. Jurkat T cells (10^6/mL) were pretreated with arzanol at the indicated doses and then infected with the VSV-pseudotyped-pNL4-3.Luc.R^-E^- for 24 h. Results are represented as RLU ± SD of three different experiments.

pretreatment of Jurkat cells 30 min prior to infection with increasing doses of arzanol resulted in a dose-dependent inhibition of luciferase activity (Figure 2).

NF-κB is one of the key regulators of genes involved in the immune/inflammatory response and is implicated in the regulation of several factors (cytokines, chemokines, adhesion molecules, acute phase proteins, inducible effectors enzymes) involved in inflammatory conditions.[8,9] To support the idea that arzanol acts as an anti-inflammatory and as an anti-HIV-1 agent, we therefore measured the levels of pro-inflammatory mediators in primary human monocytes. The levels of PGE2, IL-1β, IL-6, IL-8, and TNFα in LPS-stimulated human peripheral monocytes were determined by ELISA. Arzanol showed potent inhibition of the production of IL-1β and TNFα (IC_{50} = 5.6 and 9.2 μM, respectively) and, to a lesser extent, also of IL-6, IL-8, and PGE_2 (IC_{50} = 13.3, 21.8, and 18.7 μM, respectively).

Taken together, the results of this study validate *H. italicum* as a source of anti-inflammatory and anti-HIV compounds and suggest that NF-κB is one of the major targets of its activity. Although less active toward this end-point, the numerous compounds accompanying arzanol might synergize its activity by acting at other anti-inflammatory targets. Thus, dual inhibition of arachidonate metabolism via COX and LO was demonstrated for some acetophenones from *H. italicum*,[7c] and we are currently pursuing this attractive hypothesis as a mechanistic rationale for the promising clinical data reported for the anti-inflammatory activity of *H. italicum*.[4,5]

Experimental Section

General Experimental Procedures. IR spectra were obtained on a Shimadzu DR 8001 spectrophotometer. Optical rotations were determined at 22 °C on a Perkin-Elmer 141 polarimeter equipped with a sodium lamp (589 nm) and 10 cm microcell. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker DRX500 spectrometer with an inverse multinuclear 5 mm probehead equipped with a shielded gradient coil. The spectra were recorded in $CDCl_3$, and the solvent signals (δ 7.26 and 77.0, respectively) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constants (*J*) in Hz. COSY, HMQC, and HMBC experiments were recorded with gradient enhancements using sine-shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH}$ = 145 Hz and $^nJ_{CH}$ = 10 Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker XWIN-NMR software (rev. 010101). Mass spectra (HRESI) were recorded with a Micromass Q-TOF MICRO instrument and on a Finnigan MAT LCQ ion trap mass spectrometer. Silica gel 60 (70−230 mesh) was used for gravity column chromatography and vacuum chromatography.

Plant Material. *H. italicum* was collected near Arzana (NU, Sardinia) on June 30, 2001, and was identified by M.B. A voucher specimen (#729) is deposited at the Herbarium, Dipartimento di Scienze Botaniche, Università di Cagliari (CAG).

Extraction and Isolation. Dried, nonwoody aerial parts (leaves and flowerheads, 1.0 kg) were extracted with acetone at room temperature (3 × 4 L) to give 51 g of a brownish residue. The latter was adsorbed on silica gel (50 g) and fractionated by vacuum chromatography (200 g of silica gel) into three fractions, petroleum ether (7 g), EtOAc (31 g), and acetone (11 g). The EtOAc fraction was evaporated, redissolved in warm EtOAc (250 mL), and added to an equal volume of petroleum ether. After standing overnight at room temperature, a voluminous yellowish precipitate was formed. This was collected and washed with ether, to afford 780 mg of **1a** as a pale yellow powder (0.078%). The filtrate and the ether washings were pooled, evaporated, and fractionated by gravity column chromatography on silica gel (300 g, petroleum ether−EtOAc as eluant). The following compounds were obtained, in order of elution: oleyl *ω*-hydroxylinalol (**10**, 19 mg; petroleum ether−EtOAc, 95:5), helipyrone (**8**, 245 mg; petroleum ether−EtOAc, 8:2), a second crop of arzanol (**1a**, 185 mg; petroleum ether−EtOAc, 6:4), the tremetones **9b** (56 mg; petroleum ether−EtOAc 5:5), **9c** (47 mg; petroleum ether−EtOAc, 5:5), **9d** and **9a** (120 and 53 mg, respectively, petroleum ether−EtOAc, 4:6), and finally mycropyrone (**7**, 482 mg; petroleum ether−EtOAc, 3:7).

Arzanol (1a): pale yellow powder; mp 146 °C ($CHCl_3$ ether); IR (KBr) ν_{max} 3229, 1671, 1626, 1597, 1568, 1443, 1368, 1319, 1175, 1082 cm^{-1}; ^1H NMR (C_6D_6): δ 10.75 (br s, 1H, OH), 9.77 (br s, 1H, OH), 6.70 (br s, 1H, OH), 5.12 (br t, 1H, J = 7.1 Hz, H-17), 3.62 (br s, 2H, H-7), 3.39 (d, 2H, J = 7.1 Hz, H-16), 2.55 (s, 3H, H-22), 2.04 (q, 2H, J = 7.6 Hz, H-12), 1.62 (br s, 3H, H-14), 1.54 (br s, 3H, H-19), 1.50 (br s, 3H, H-20), 0.85 (t, 3H, J = 7.6 Hs, H-13); ^{13}C NMR (C_6D_6) δ 204.4 (s, C-21), 169.6 (s, C-15), 167.5 (s, C-9), 162.1 (s, C-4), 161.2 (s, C-11), 161.0 (s, C-6), 159.1 (s, C-2), 136.4 (s, C-18), 122.6 (d, C-17), 108.3 (s, C-10), 107.5 (s, C-3), 106.9 (s, C-5), 105.9 (s, C-1), 103.1 (s, C-8), 32.2 (q, C-22), 25.8 (q, C-20), 24.6 (t, C-12), 23.0 (t, C-16), 18.6 (t, C-7), 17.9 (q, C-19), 11.3 (q, C-13), 9.4 (q, C-14); HREIMS *m/z* 402.1687 $[M]^+$ (calcd for $C_{22}H_{26}O_7$,402.1679).

Acetylation of Arzanol. To a solution of arzanol (200 mg, 0.50 mmol) in dry pyridine (2 mL) was added an excess of Ac_2O (2 mL). After stirring overnight at room temperature, the reaction was quenched by the addition of MeOH (0.5 mL) and worked up by dilution with H_2O and extraction with ether. The organic phase was sequentially washed with 2 N H_2SO_4, $NaHCO_3$, and brine and evaporated, to give a residue, which was washed with ether to afford 198 mg (70%) of the tetraacetate **1c** as a white powder: mp 126−129 °C (ether−hexane) (lit. 130−132 °C);[12] IR (KBr) ν_{max} 1770, 1749, 1643, 1579, 1430, 1372, 1190, 1165, 1075 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.93 (br t, J = 6.8 Hz, H-17), 3.60 (br s, H-7), 3.10 (d, J = 6.8 Hz, H-16), 2.57 (q, J = 6.7 Hz, H-12), 2.41 (s, H-22), 2.25 (s, OAc), 2.22 (s, OAc), 2.22 (s, OAc), 2.18 (s, OAc), 1.74 (br s, H-20), 1.65 (br s, H-14), 1.64 (br s, H-19), 1.21 (t, J = 6.7 Hs, H-13); HREIMS *m/z* 570.2111 $[M]^+$ (calcd for $C_{30}H_{34}O_{11}$,570.2101).

Methylation of Arzanol. To a suspension of arzanol (100 mg, 0.25 mmol) in dry CH_2Cl_2 (2 mL) was added 2.0 M trimethylsilyldiazomethane in hexane (1.24 mL, 2.48 mmol, 10 molar equiv). The reaction was stirred at room temperature for 1 h and then worked up by washing with 2 N H_2SO_4 and brine. After evaporation, the residue was purified by gravity column chromatography on silica gel (2.5 g, petroleum ether−EtOAc, 7:3, as eluant) to afford 84 mg (78%) of **4** as a yellowish foam: IR (KBr) ν_{max} 1749, 1706, 1649, 1526, 1370, 1352, 1180, 1105, 1015 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.54 (br s, OH), 11.06 (br s, OH), 8.42 (br s, OH), 5.23 (br t, 1H, J = 6.9 Hz, H-17), 4.17 (s, 3H, OMe), 3.59 (br s, 2H, H-7), 3.34 (d, 2H, J = 6.9 Hz, H-16), 2.72 (s, 3H, H-22), 2.68 (q, 2H, J = 7.5 Hz, H-12), 1.98 (br s, 3H, H-14), 1.80 (br s, 3H, H-19), 1.70 (br s, 3H, H-20), 1.26 (t, 3H, J = 7.5, H-13); ^{13}C NMR ($CDCl_3$) δ 204.1 (s, C-21), 182.5 (s, C-9), 162.4 (s, C-15), 161.7 (s, C-4), 161.2 (s, C-11), 159.9 (s, C-2), 159.0 (s, C-6), 132.6 (s, C-18), 122.9 (d, C-17), 118.1 (s, C-10), 107.2 (s, C-3), 106.0 (s, C-1), 103.9 (s, C-5), 103.2 (s, C-8), 56.5 (q, OMe), 33.2 (t, C-22), 25.8 (q, C-20), 24.3 (t, C-12), 21.9 (t, C-16), 17.8 (q, C-19), 16.8 (t, C-7), 11.8 (q, C-13), 9.7 (q, C-14); HREIMS *m/z* 416.1830 $[M]^+$ (calcd for $C_{23}H_{28}O_7$, 416.1835).

Cyclization of Arzanol. (a) With $CeCl_3$: To a solution of arzanol (100 mg, 0.25 mol) in dry THF (1 mL) was added $CeCl_3 \cdot 7H_2O$ (100 mg), and the reaction mixture was stirred at room temperature for one week. The reaction was then worked up by dilution with EtOAc and extraction with saturated $NaHCO_3$. After washing with brine, the organic phase was evaporated and the residue purified by gravity column chromatography on silica gel (2.5 g, petroleum ether−EtOAc, 95:5, as eluant) to afford, along with starting material (35 mg), 28 mg of **5** (28%) and 18 mg of **6** (18%). (b) With HCl: Arzanol (100 mg, 0.25 mmol) was added to a cooled (0 °C) solution of HCl in MeOH (obtained by treatment of 5 mL of MeOH with 100 μL of $SOCl_2$). After stirring at 0 °C for 1 h, the reaction was left at room temperature overnight and worked up as described above. After column chromatography, 29 mg of **5** (29%) and 6 mg of **6** (6%) were obtained.

Cycloarzanol A (5): amorphous foam; IR (KBr) ν_{max} 3290, 1670, 1626, 1599, 1567, 1445, 1378, 1320, 1115, 1062 cm^{-1}; ^1H NMR ($CDCl_3$) δ 14.11 (s, 1H, 2-OH), 10.50 (s, 1H, 6-OH), 9.20 (s, 1H, 9-OH), 3.62 (s, 2H, H-7), 2.74 (s, 3H, H-22), 2.65 (br t, 2H, J = 6.8 Hz, H-16), 2.57 (q, 2H, J = 7.6 Hz, H-12), 1.96 (br s, 3H, H-14), 1.88 (t, 2H, J = 6.8 Hz, H-17), 1.48 (s, 6H, H-19 and H-20), 1.21 (t, 3H, J = 7.6 Hs, H-13); ^{13}C NMR ($CDCl_3$) δ 205.0 (s, C-21), 169.2 (s, C-15), 167.1 (s, C-9), 162.5 (s, C-2), 161.4 (s, C-11), 158.1 (s, C-6), 155.5 (s, C-4), 107.7 (s, C-10), 106.4 (s, C-1), 104.0 (s, C-5), 102.1 (s, C-8), 101.1 (s, C-3), 79.1 (s, C-18), 33.4 (q, C-22), 32.2 (7, C-17), 24.3 (t, C-12), 17.6 (t, C-7), 16.1 (t, C-16), 11.5 (q, C-13), 9.4 (q, C-14); HREIMS *m/z* 402.1687 $[M]^+$ (calcd for $C_{22}H_{26}O_7$, 402.1679).

Cycloarzanol B (6): amorphous foam; IR (KBr) ν_{max} 3290, 1671, 1626, 1600, 1568, 1445, 1379, 1330, 1123, 1062 cm^{-1}; ^1H NMR ($CDCl_3$) δ 16.03 (s, 1H, 6-OH), 10.50 (s, 1H, 4-OH), 10.04 (s, 1H, 9-OH), 3.61 (br s, 2H, H-7), 2.65 (m, 2H, 16), 2.64 (s, 3H, H-22), 2.55 (q, 2H, J = 7.6 Hz, H-12), 1.95 (br s, 3H, H-14), 1.78 (m, 2H, H-17), 1.37 (s, 6H, H-19 and H-20), 1.20 (t, 3H, J = 7.6 Hz, H-13); ^{13}C NMR ($CDCl_3$) δ 203.8 (s, C-21), 169.3 (s, C-15), 167.5 (s, C-9), 161.7 (s, C-4), 161.1 (s, C-11), 160.5 (s, C-6), 156.4 (s, C-2), 108.2 (s, C-10), 105.0 (s, C-1), 104.8 (s, C-5), 102.3 (s, C-3), 102.2 (s, C-8), 76.2 (s, C-18), 32.7 (q, C-22), 31.7 (t, C-17), 24.3 (t, C-12), 17.3 (t, C-7), 16.8 (t, C-16), 11.6 (q, C-13), 9.4 (q, C-14); HREIMS *m/z* 402.1687 $[M]^+$ (calcd for $C_{22}H_{26}O_7$, 402.1679).

Micropyrone (7): white powder; mp 97 °C (ether); $[\alpha]_D^{25}$ −21 (*c* 1.0, MeOH); IR (KBr) ν_{max} 1721, 1689, 1660, 1635, 1576, 1217, 1177, 1128, 1084, 1041 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.84 (q, J = 6.9 Hz, H-6), 2.58 (m, H-8), 2.02 (s, H-12), 2.00 (s, H-11), 1.60 (m, H.9a), 1.36 (d, J = 6.7 Hz, H-13), 1.34 (m, H-9b), 0.99 (d, J = 6.7 Hz, H-14), 0.81 (t, J = 6.7 Hz, H-10); ^{13}C NMR ($CDCl_3$) δ 210.5 (s, C-7), 166.2 (s, C-1), 165.6 (s, C-5), 155.7 (d, C-3), 109.5 (s, C-4), 99.5 (s, C-2), 48.2 (d, C-6), 45.3 (d, C-8), 26.9 (t, C-9), 16.1 (q, C-14), 13.3 (q, C-13), 11.5 (q, C-10), 9.9 (q, C-12), 8.7 (q, C-11); HREIMS *m/z* 252.1357 $[M]^+$ (calcd for $C_{14}H_{20}O_{74}$, 252.1362).

(±)-*ω*-Oleoyloxylinalol (10): colorless oil; $[\alpha]_D^{25}$ ca. 0 (*c* 1.0, MeOH); IR (KBr) ν_{max} 1736, 1458, 1383, 1265, 1240, 1163, 1119 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.93 (dd, J = 17.3, 10.1, 1H), 5.49 (br t, J = 6.9 Hz, 1H), 5.35 (br s, 2H), 5.24 (d, J = 17.3 Hz, 1H), 5.09 (d, J = 10.1 Hz, 1H), 4.45 (br s, 2H), 2.25 (m, 2H), 1.65 (br s, 3H), 0.88 (t, J = 6.8 Hz, 3H); HREIMS *m/z* 434.3769 $[M]^+$ (calcd for $C_{28}H_{50}O_3$, 434.3760).

Synthesis of (*R*)-*ω*-oleoyloxylinalol (10). To a solution of *ω*-hydroxylinalol ($[\alpha]_D^{25}$ −10 (*c* 1.10, MeOH); lit. −12.8 (*c* 1.08, MeOH))[25] (320 mg, 2.0 mmol) in dry toluene (5 mL) were added oleic acid (625 mg, 2.25 mmol, 1.12 molar equiv), DCC (463 mg, 2.25 mmol, 1.25 molar equiv), and DMAP (10 mg). After stirring 30 min at room temperature, the reaction was worked up by filtration over Celite and evaporation. The residue was purified by gravity column chromatography on neutral alumina, to afford 332 mg (34%) of (*R*)-**10**, identical, except for the optical rotation, with the natural product ($[\alpha]_D^{25}$ −6.7 (*c* 1.30, MeOH)).

Biological Assays. Cell Lines and Plasmids. Jurkat cells (American Type Culture Collection, Manassas, VA) were cultured in RPMI 1640 medium (Invitrogen, Barcelona, Spain), containing 10% heat-inactivated fetal bovine serum, 2 mM glutamine, penicillin (50 U/mL), and streptomycin (50 μg/mL), and were maintained at 37 °C in a 5% CO_2 humidified atmosphere and split twice a week. The 5.1 cell line is a Jurkat-derived clone stably transfected with a plasmid containing the luciferase gene driven by the HIV-LTR promoter and was maintained in complete media supplemented with G418 (200 μg/mL). The vector pNL4-3.Luc.R^-E^- from N. Landau was obtained from the AIDS Research and Reference Reagent Program (National Institute of Allergy and Infectious Disease, National Institutes of Health, USA) and contains the firefly luciferase gene inserted into the pNL4-3 nef gene. Two frameshifts (5′ Env and Vpr aa 26) render this clone Env^- and Vpr^-.

Luciferase Assays. To determine NF-*κ*B-dependent transcription of the HIV-LTR-luc, cells were preincubated for 30 min with increasing concentrations of either arzanol (**1a**) or the *H. italicum* acetone extract for 30 min and next stimulated with TNFα (2 ng/mL) for 6 h. Cells

were lysed in 25 mM Tris-phosphate pH 7.8, 8 mM $MgCl_2$, 1 mM DTT, 1% Triton X-100, and 7% glycerol. Luciferase activity was measured using an Autolumat LB 953 (EG&G Berthold, Bad Wildbad, Germany) following the instructions of the luciferase assay kit (Promega), and protein concentration was measured by the Bradford method. The background obtained with the lysis buffer was subtracted from each experimental value, the RLU/μg of protein was calculated, and the specific transactivation was expressed as fold induction over untreated cells. All experiments were repeated at least three times.

Production of VSV-Pseudotyped Recombinant Viruses and Infection Assays. High-titer VSV-pseudotyped recombinant virus stocks were produced in 293T cells as previously described.[29] Briefly, the cells were cotransfected with the pNL4-3.Luc.R^-E^- plasmid along with the $pcDNA_3$-VSV plasmid by the calcium phosphate transfection method. Supernatants, containing virus stocks, were harvested 48 h post-transfection, centrifuged for 5 min at 500*g* to remove cell debris, and stored at −80 °C until use. Cell-free viral stock was tested using an enzyme-linked immunoassay for antigen HIV-1 p24, and cultures were infected with 200 ng of HIV-1 gag p24 protein as follows: Jurkat cells (10^6/mL in 24-well plates) were pretreated with increasing concentrations of arzanol for 30 min and then inoculated with the virus stocks. Twenty four hours later, the cells were washed twice in PBS and the luciferase activity was measured in the cell lysates. The results are represented as the percentage of activation (considering the infected and untreated cells 100% activation). Results represent mean ± standard deviation (SD) of three independent experiments.

Isolation of Human Peripheral Monocytes and Determination of IL-6, TNF-α, IL-1-β, and PGE_2. Primary monocytes from healthy human donors were seeded in 24-well plates ($(1-2) \times 10^6$ cells/well) for ELISA analysis ($(1-2) \times 10^6$ cells/well) and incubated at 37 °C/5% CO_2. The medium and the nonadherent cells (lymphocytes) were removed, and fresh RPMI-1640 medium containing 1% human serum was added. The analysis of IL-6, TNF-α, IL-1-β, and PGE2 was done as follows: human peripheral monocytes were preincubated with increasing concentrations of arzanol (**1a**) for 30 min before stimulation with LPS (10 ng/mL) for an additional 24 h. Then, the culture supernatants were harvested and centrifuged for 10 min at 10000*g*, and the levels of the cytokines or PGE_2 in the supernatant were measured by ELISA (IL-6, IL-1, TNF-α: Pelikine, distributed by Immunotool, Frisoythe, Germany) or EIA (AssayDesign, distributed by Biotrend, Köln, Germany) according to the manufacturer's instructions. All experiments were carried out with using three buffy coats from different blood donors in triplicate.

Acknowledgment. This research was supported by the EU (Contract QLK3-CT-2000-00463, Anti-inflammatory Natural Products). We are very grateful to Dr. R. Sestu and the Pro Loco Arzana for their hospitality during the collection of the plant material.

References and Notes

(1) Pignatti, S. *Flora d'Italia*; Edagricole: Bologna, 1982; Vol. 3, p 42.
(2) *Heliochrysum ... duritias and inflammations discutit, ambustis cum melle inponitur. Contra ... lumborum vitia bibitur* (*Helichrysum* heals hardenings and inflammation, and mixed with honey soothes burnings. It is used as a potion against rheumatisms): Pliny the Elder. *Naturalis Historia*, *XXI*; pp 168−169.
(3) For a review, see: Ballero, M.; Maxia, A. *Erboristeria Domani* **2006**, *300*, 52−56.
(4) (a) Santini, L. *Considerazioni sugli Effetti Terapeutici dell'Elicriso*; Salvetti: Castelnuovo, 1948. (b) Santini, L. *Atti Soc. Lomb. Sci. Med. Biol.* **1949**, *5*, 18.
(5) A synopsis of Santini's studies on *Helicrysum* was recently published (Santini, L. *Erboristeria Domani* **2006**, *300*, 65−71).
(6) Most investigations on the chemistry of plants from the genus *Helichrysum* were carried out by Bohlmann and Jakupovic. In an impressive publication, 26 phloroglucinols, nine diterpenoids, one sesquiterpene, and one pyrone were described, besides known compounds, from 11 South African species (Jakupovic, J.; Kuhnke, J.; Schuster, A.; Metwally, M. A.; Bohlmann, F. *Phytochemistry* **1986**, *25*, 1133−1142).
(7) (a) Araceli, S.; Recio, M. C.; Giner, R. M.; Máñez, S.; Rios, J. L. *J. Nat. Prod.* **2001**, *64*, 1360−1362. (b) Araceli, S.; Recio, M. C.; Schinella, L. G. R.; Máñez, G.; Giner, R. M.; Rios, J.-L. *J. Pharmacol.* **2003**, *460*, 219−226. (c) Sala, A.; Recio, M. C.; Schinella, G.; Máñez.; Giner, R. M.; Cereda-Nicolas, M.; Rios, J.-L. *Eur. J. Pharmacol.* **2003**, *461*, 53−61.
(8) Hume, D. A.; Fairlie, D. P. *Curr. Med. Chem.* **2005**, *25*, 2925−2929.
(9) (a) Karin, M. *Nature* **2006**, *414*, 431−436. (b) Karin, M.; Ben-Neriah, Y. *Annu. Rev. Immunol.* **2000**, *18*, 621−663.
(10) The name arzanol derives from the Sardinian village (Arzana) where the plant was collected. Owing to the confusion surrounding the structure of homoarenol, we propose to replace this name with arzanol. The carbon atoms of arzanol have been numbered according to the system proposed by Bohlmann for phloroglucinol α-pyrones.[6]
(11) (a) Singh, I. P.; Bharate, S. B. *Nat. Prod. Rep.* **2006**, *23*, 558−591. (b) Ciochina, R.; Grossman, R. B. *Chem. Rev.* **2006**, *106*, 3963−3986.
(12) Vrkoč, J.; Dolejš, L.; Sedmera, P.; Vašíčková, S.; Šorm, F. *Tetrahedron Lett.* **1971**, 247−250.
(13) Bohlmann, F.; Zdero, C. *Phytochemistry* **1980**, *19*, 153−155.
(14) For a short biography of F. Šorm, see: http://www.garfield.library.upenn.edu/essays/v15p051y1992-93.pdf
(15) Appendino, G.; Cravotto, G.; Nano, G. M.; Palmisano, G.; Annunziata, R. *Helv. Chim. Acta* **1993**, *76*, 1194−1201.
(16) (a) Verotta, L.; Lovaglio, E.; Sterner, O.; Appendino, G.; Bombardelli, E. *Eur. J. Org. Chem.* **2004**, 1193−1197. (b) Verotta, L.; Lovaglio, E.; Sterner, O.; Appendino, G.; Bombardelli, E. *J. Org. Chem.* **2004**, *69*, 7869−7864.
(17) Owing to the lack of details on the conditions of the rearrangement of homoarenol tetraacetate to **3**,[12] we were unable to reproduce this reaction. In a typical experiment, treatment of the tetraacetate of **1b** with 1 M KOH in MeOH at room temperature led to the complete consumption of the starting material in less than 6 h, affording a complex mixture from which no pure product could be characterized.
(18) Rashba, O. Y. *Acad. Nauk. Ukr. R.S.P.* **1957**, *19*, 25−28; *Chem. Abstr.* **1958**, *51*, 87680. Arenarin was successfully employed as an eye antibiotic.
(19) Atropisomerism was detected in flavan−acylphloroglucinol dimers around the bond connecting these two moieties (Sawadjoon, S.; Kittakoop, P.; Kirtikara, K.; Vichai, V.; Tanticharoen, M.; Thebtaranonth, Y. *J. Org. Chem.* **2002**, *67*, 5470−5475).
(20) Appendino, G.; Bianchi, F.; Minassi, A.; Sterner, O.; Ballero, M.; Gibbons, S. *J. Nat. Prod.* **2002**, *65*, 334−338.
(21) Hänsel, R.; Cybulski, E.-M.; Çubukçu, B.; Meriçli, A. H.; Bohlmann, F.; and Zdero, C. *Phytochemistry* **1980**, *19*, 639−644.
(22) (a) Tira, S.; Di Modica, G.; Casinovi, C. G.; Galeffi, C.; Pela, A. *Tetrahedron Lett.* **1967**, 143−148. (b) Manritto, P.; Monti, D.; Colombo, E. *Phytochemistry* **1972**, *6*, 2122−2124.
(23) Opiz, L.; Hänsel, R. *Tetrahedron Lett.* **1970**, 3369−3370.
(24) The tremetone bitalin A (12-hydroxytremetone, **9a**) had already been isolated from *H. italicum* (Zapesochnaya, G. G.; Dzyadevich, T. V.; Karasartov, B. S. *Khim Prir. Soedin.* **1990**, 409−410; *Chem. Abstr.* **1991**, *114*, 98154). Tremetone is the (in)famous white snakeroot (*Eupatorium rugosum*) toxicant excreted in cows' milk and responsible for the so-called "milk sickness" of humans (Panter, K. E.; James, L. F. *J. Anim. Sci.* **1990**, *68*, 892−904). Some tremetones show anti-inflammatory activity (Favier, L.; Tonn, C.; Guerriero, E.; Rotell, A.; Pelzer, L. *Planta Med.* **1998**, 64, 657−659).
(25) (a) Satta, M.; Tuberoso, C. I. G.; Angioni, A.; Pirisi, F. M.; Cabras, P. *J. Essent. Oil Res.* **1999**, *11*, 711−715. (b) Angioni, A.; Barra, A.; Arlorio, M.; Coisson, J. D.; Russo, M. T.; Pirisi, F. M.; Satta, M.; Cabras, P. *J. Agric. Food Chem.* **2003**, *51*, 1030−1034.
(26) Behr, D.; Wahlberg, I.; Nishida, T.; Enzell, C. R. *Acta Chem. Scand. B* **1978**, *32*, 228−234.
(27) Marquez, N.; Sancho, R.; Macho, A.; Calzado, M: A.; Fiebich, B. L.; Muñoz, E. *J. Pharmacol. Exp Ther.* **2004**, *308*, 993−1001.
(28) Canki, M.; Thai, J. N.; Chao, W.; Ghorpade, A.; Potash, M. J.; Volsky, D. J. *J. Virol.* **2001**, *75*, 7925−7933.
(29) Sancho, R.; Medarde, M.; Sanchez-Palomino, S.; Madrigal, B. M.; Alcami, J.; Muñoz, E.; San, Feliciano, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4483−4486.

NP060581R