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Short Route to the Total Synthesis of Natural Muricadienin and Investigation of Its Cytotoxic Properties

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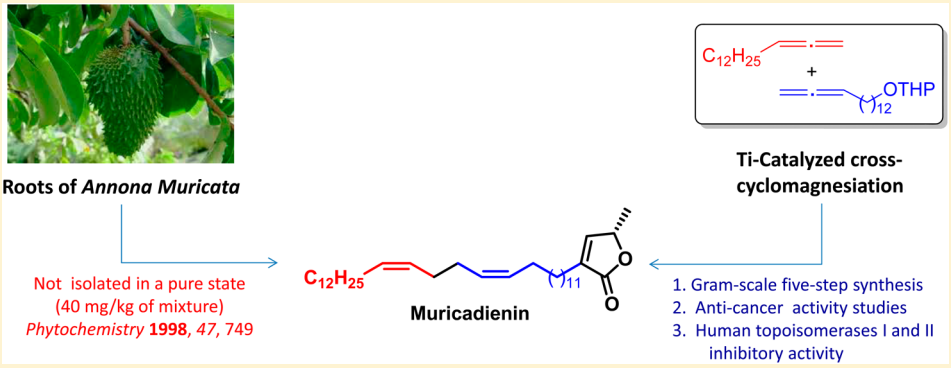
Supporting Information



ABSTRACT: An original synthesis of the acetogenin muricadienin, the bioprecursor of solamin, has been developed. The key step in the five-step 41% overall yield synthesis is the catalytic cross-cyclomagnesiation reaction of functionally substituted 1,2-dienes with EtMgBr in the presence of Cp_2TiCl_2 and magnesium metal. It has been demonstrated for the first time that muricadienin exhibits a moderate in vitro inhibitory activity against topoisomerases I and IIα, key cell cycle enzymes. Using flow cytometry, muricadienin was shown to have high cytotoxicity toward the HEK293 kidney cancer cells (IC_{50} 0.39 μM).

Acetogenins represent a group of naturally occurring, unbranched fatty acids (C_{32}–C_{34}) containing a γ-lactone moiety, which have been isolated from the leaves, roots, seeds, and bark of Annonaceae plants.[1] In most cases, acetogenin molecules contain additional hydroxy, keto, epoxy, tetrahydrofuran, and tetrahydropyran groups as well as double and triple bonds (Figure 1).

The enhanced interest in this class of compounds is caused by their broad spectrum of biological activities, for example, insecticidal, antifeedant, antiprotozoal, immunosuppressive, and antitumor activities.[2] Acetogenins were shown to exhibit cytotoxicity against multiple-drug-resistant tumors[3] and are the most active currently known mitochondrial complex I inhibitors.[4]

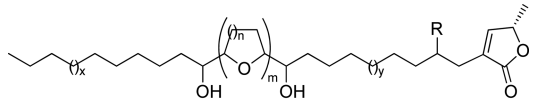


Figure 1. General skeleton of annonaceous acetogenins.

It is postulated that the bis-hydroxytetrahydrofuran moiety of acetogenins is formed via mono- and bis-epoxy derivatives of long-chain 1*Z*,5*Z*-dienoic acids. For example, the biosynthesis of solamin, the prototype of most monotetrahydrofuran-containing acetogenins, starts from muricadienin, which had been isolated in minor quantities from *Annona muricata* roots, and involves the epoxymurin and diepoxymuricanin intermediates.[5,6]

Despite the large number of studies dealing with the antitumor activity of solamin and its derivatives,[7] data on the biological activity of its bioprecursor, muricadienin, are still

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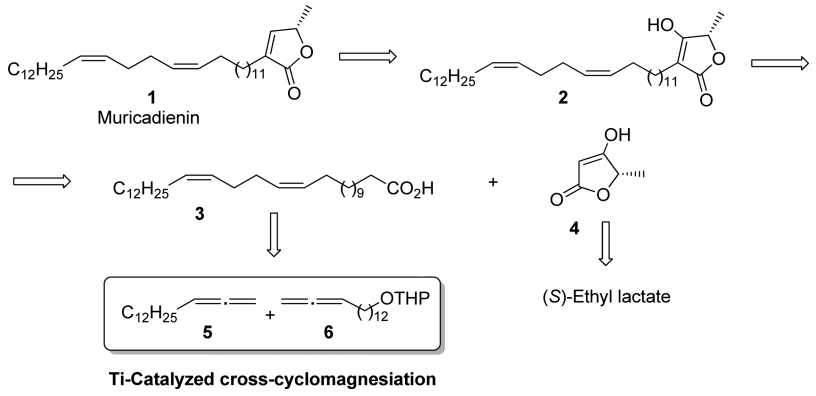
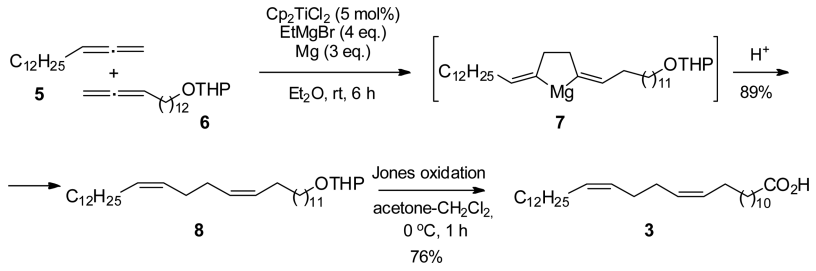
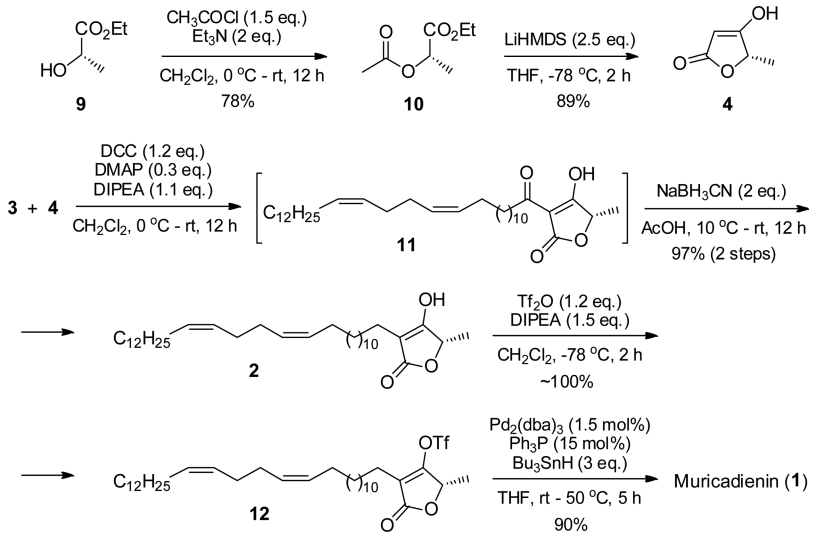
Scheme 1. Retrosynthetic Analysis of Muricadienin



Scheme 2. (*Z*,*Z*)-Stereoselective Synthesis of (13*Z*,17*Z*)-Triacontanoic Acid



Scheme 3. Installation of the Terminal α-Substituted Butenolide Moiety and Completion of the Total Synthesis of Muricadienin (1)



missing. It is noteworthy that muricadienin had not been isolated in a pure state, and the only total synthesis reported in 2014 by Stark and co-workers involves 11 steps with 42% yield[6] and is thus, in our opinion, of limited practical use.

In view of the significance of an efficient access to acetogenins and their analogue as well as research into their antitumor activities, a short synthesis was devised for muricadienin resorting to the newly developed, Cp_2TiCl_2-catalyzed cross-cyclomagnesiation of O-containing and terminal aliphatic 1,2-dienes with EtMgBr in the presence of magnesium metal as a key step.[8]

RESULTS AND DISCUSSION

Chemistry. The retrosynthetic analysis implied (13*Z*,17*Z*)-triaconta-13,17-dienoic acid (**3**), stereoselectively prepared by catalytic cross-cyclomagnesiation of dienes **5** and **6** with subsequent construction and attachment of the terminal α-substituted butenolide in muricadienin (**1**), including a Fries rearrangement in the final step (Scheme 1).

According to the developed scheme, (13*Z*,17*Z*)-triaconta-13,17-dienoic acid (**3**), the key building block in the synthesis of muricadienin, was prepared in two steps (68% overall yield) using the previously developed[8] cross-cyclomagnesiation of 1,2-pentadecadiene (**5**) and 13,14-pentadecadienol pyranyl ether (**6**) with EtMgBr (**5**:**6**:EtMgBr:Mg:[Ti] = 10:11:40:30:0.5;

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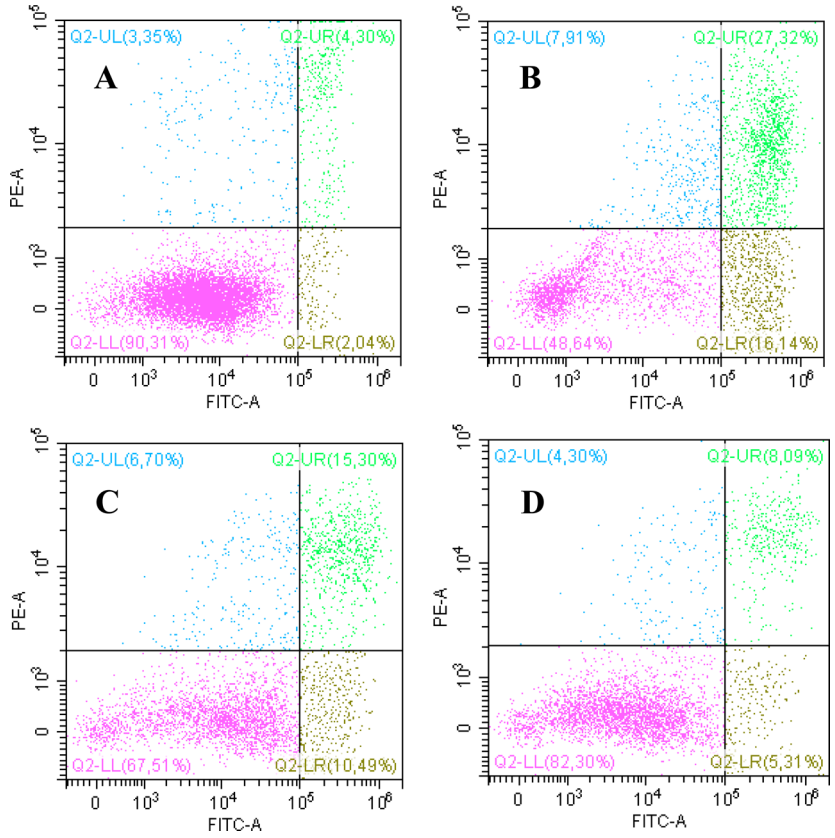

Figure 2. HEK293 cells treated with different concentrations of muricadienin (**1**) were double-stained with annexin V/PI and analyzed by flow cytometry. (A) Control; (B) **1** (0.48 μM); (C) **1** (0.19 μM); (D) **1** (0.1 μM).

Et_2O, 6 h, rt) catalyzed by Cp_2TiCl_2 (5 mol %) followed by Jones oxidation of the (13*Z*,17*Z*)-triaconta-13,17-dienol pyranyl ether (**8**), formed upon hydrolysis of the magnesacyclopentane **7** (Scheme 2).

The terminal butenolide moiety was installed via a published method[9] involving a 4-(dimethylamino)pyridine (DMAP)-catalyzed Fries rearrangement (Scheme 3). In situ O-acylation of (*S*)-5-methyltetronic acid **4**, prepared in two steps from (*S*)-ethyl lactate **9** according to a known procedure,[10] with acid **3** followed by a DMAP-initiated Fries rearrangement afforded intermediate **11**, which was directly reduced with $NaBH_3CN$ in HOAc to give the α-alkylated butenolide **2** in high yield (~97%).

The final stage included the transformation of **2** to the butenolide triflate **12**[6] and its $Pd_2(dba)_3$-catalyzed reduction with Bu_3SnH, to afford the target (+)-muricadienin (**1**) in ~41% overall yield.

Flow Cytometry Studies. The muricadienin-induced apoptosis in the HEK293 cell culture was detected by flow cytometry using an Alexa Fluor 488 annexin V/dead cell apoptosis kit. Phosphatidylserine externalization on the outer surface of the plasma membrane is an exact and reliable indication of cell apoptosis or necrosis. The difference between these two forms of cell death is that in the early stages of apoptosis the cell membrane remains relatively undamaged, whereas upon necrosis, the cell membrane loses integrity. In the living, normally functioning cells, phosphatidylserine is present on the cell surface membrane in minor quantity; hence, its interaction with the V Alexa Fluor 488 annexin is insignificant. Furthermore, the undamaged cell membrane is impermeable to propidium iodide (PI). At the early stage of apoptosis, phosphatidylserine molecules appear on the cell surface, but the membrane is still impermeable to DNA-binding dyes such as PI. The membrane integrity is lost at later stages of cell death. Thus, while detecting apoptosis, one can distinguish four types of cells: living cell (annexin V−/PI−), early apoptosis (annexin V+/PI−), late apoptosis (annexin V+/PI+), and necrosis (annexin V−/PI+). As can be seen from Figure 2, the action of muricadienin on the tumor cell culture results in a substantial dose-dependent increase in the total number of apoptotic cells. The highest percentage of apoptosis of 43.4% (16.1% early + 27.3% late) was observed at a 0.48 μM concentration of the compound. In the samples with 0.19 μM and 0.1 μM muricadienin, the proportions of apoptotic cells were 25.8% (10.5% early + 15.3% late) and 13.4% (5.3% early + 8.1% late), respectively.

MTT Assay. MTT is a colorimetric assay for evaluation of cell metabolic activity. The NADPH-dependent cellular oxidoreductases present in the living cell can reflect, under certain conditions, the cell viability. These enzymes can reduce tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) or yellow tetrazole, to give insoluble formazan, which develops a purple color, particularly in living cells. Thus, the color gradient facilitates determination of the degree of cytostatic activity (shift from proliferation to dormancy) of drug candidates and toxic compounds. The antiproliferative effect of muricadienin was measured on a human tumor cell line (HEK293) employing the MTT assay. Etoposide served as a positive control. The IC_{50} value found in the experiment was 0.39 μM. Thus, muricadienin shows higher cytotoxicity toward HEK293 cells than etoposide (IC_{50} 0.6 μM), analogues of which are widely used in clinical practice.

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Topoisomerase Inhibition Assay. The cytotoxic action of biologically active compounds is attributed to their ability to affect molecular targets, most often enzymes that play key roles in the cell cycle. Previously, it was shown that higher (5*Z*,9*Z*)-dienoic acids, which exhibit antitumor and antibacterial activities, are efficient inhibitors of topoisomerases I and II.[8d−f,11] Considering the facts that long-chain unsaturated acids are the biological precursors of acetogenins[12] and that muricadienin (**1**) contains a (1*Z*,5*Z*)-diene moiety, it was assumed that the ability to inhibit the DNA-dependent topoisomerase enzyme is a possible factor responsible for its high cytotoxicity. The studies demonstrated for the first time that muricadienin (**1**) at concentrations of >50 μM inhibits hTop I and at concentrations of >1 μM inhibits hTop II in vitro. However, (13*Z*,17*Z*)-triaconta-13,17-dienoic acid (**3**), which is the precursor of muricadienin, did not inhibit topoisomerases even at concentrations above 400 μM.

As a continuation of studies dealing with the development of stereoselective syntheses of natural biologically active compounds based on the new Ti-catalyzed cross-cyclomagnesiation of 1,2-dienes with Grignard reagents, a five-step synthesis of muricadienin, the bioprecursor of acetogenins, was developed. It was demonstrated for the first time that muricadienin in moderate concentrations (>50 μM) inhibits human topoisomerase I and in even smaller concentrations (>1 μM) inhibits human topoisomerase II and shows high cytotoxicity toward the HEK293 kidney cancer cell line (IC_{50} 0.39 μM). This new method will facilitate the synthesis of a wide range of muricadienin analogues from various 1,2-dienes and thus the investigation of structure−activity relationships in a series of analogues.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were recorded on a Stuart SMP3. IR spectra were recorded on a Bruker VERTEX 70 V using KBr discs over the range of 400−4000 cm^{-1}. ^1H and ^{13}C NMR spectra were obtained using a Bruker Ascend 500 spectrometer in $CDCl_3$ operating at 500 MHz for ^1H and 125 MHz for ^{13}C and a Bruker AVANCE 400 spectrometer in $CDCl_3$ operating at 400 MHz for ^1H and 100 MHz for ^{13}C. Mass spectra were obtained with a MALDI TOF/TOF spectrometer in a α-cyano-4-hydroxycinnamic acid matrix. High-resolution mass spectra (HRMS) were measured on a Bruker maXis instrument using electrospray ionization (ESI).[13] In experiments on selective collisional activation activation energy was set at maximum abundance of fragment peaks (see figure legends). A syringe injection was used for solutions in MeCN−H_2O, 50/50 vol % (flow rate 3 mL/min). Nitrogen was applied as a dry gas; interface temperature was set at 180 °C. Elemental analyses were measured on a 1106 Carlo Erba apparatus. The purity of the synthesized compounds was controlled using TLC on Sorbfil plates; anisic aldehyde in HOAc was used for color development. Column chromatography was carried out on Acrus silica gel (0.060−0.200 mM). All solvents were dried (1,4-dioxane, THF, Et_2O over Na) and freshly distilled before use. (*S*)-5-Methyltetronic acid (**4**) was synthesized according to a literature procedure.[10]

Synthesis of 2-(Pentadeca-13,14-dien-1-yloxy)tetrahydro-2*H*-pyran (6). Paraformaldehyde (2.6 g), copper iodide (3.4 g, 17.8 mmol), and dicyclohexylamine (14.0 mL, 70.4 mmol) were sequentially added to a solution of 2-(tetradec-13-yn-1-yloxy)-tetrahydro-2*H*-pyran (10.0 g, 34.0 mmol) in anhydrous dioxane (120 mL). The resulting mixture was heated under reflux for 24 h. The addition of 2 M HCl (50 mL) and extraction with Et_2O (2 × 100 mL) was followed by an extraction of the organic layer with $NaHCO_3$, water, and brine and drying with anhydrous $MgSO_4$. The solvent was evaporated in vacuo, and the residue was purified by silica gel column chromatography (*n*-hexane−EtOAc, 30:1) to afford 2-(pentadeca-13,14-dien-1-yloxy)tetrahydro-2*H*-pyran (**6**) (8.1 g, 78%) as a colorless liquid.

*2-(Pentadeca-13,14-dien-1-yloxy)tetrahydro-2H-pyran (**6**):* colorless liquid; IR (film) ν_{max} 2928, 2851, 1463, 1379, 1354, 1301, 1248, 1108, 1068, 815, 722 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.24−1.42 (18H, m), 1.49−1.62 (6H, m), 1.71 (1H, m), 1.83 (1H, m), 1.99 (2H, m), 3.38 (1H, m), 3.49 (1H, m), 3.73 (1H, m), 3.87 (1H, m), 4.58 (1H, m), 4.64 (2H, m), 5.08 (1H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 208.5 (C), 98.8 (CH), 90.0 (CH), 74.4 (CH_2), 67.6 (CH_2), 62.2 (CH_2), 30.8 (CH_2), 29.8 (CH_2), 29.6 (signals of 4C, CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 28.3 (CH_2), 26.2 (CH_2), 25.5 (CH_2), 19.7 (CH_2); anal. C 77.69, H 11.73%, calcd for $C_{20}H_{36}O_2$, C 77.87, H 11.76%.

Cross-Cyclomagnesiation of Pentadeca-1.2-diene (5) and 2-(Pentadeca-13,14-dien-1-yloxy)tetrahydro-2*H*-pyran (6) with EtMgBr in the Presence of Mg Metal and Cp_2TiCl_2 Catalyst (General Procedure). Diethyl ether (30 mL), pentadeca-1,2-diene (**5**) (1.0 g, 4.8 mmol), 2-(pentadeca-13,14-dien-1-yloxy)tetrahydro-2*H*-pyran (**6**) (1.1 g, 4.0 mmol), EtMgBr (40.0 mmol) (as 1.5 M solution in Et_2O), Mg powder (0.7 g, 30.0 mmol), and Cp_2TiCl_2 (0.1 g, 0.4 mmol) were placed in a glass reactor with stirring under argon (~0 °C). The reaction mixture was warmed to room temperature (20−22 °C) and stirred for 6 h. The reaction mixture was treated with a 5% solution of NH_4Cl in H_2O (20 mL) and extracted with Et_2O (2 × 100 mL). The combined organic phases were dried over $MgSO_4$ and filtered, and the solvents were removed under reduced pressure. Silica gel column chromatography (*n*-hexane−EtOAc, 35:1) of the residue gave compound **8** (1.98 g, 89%) as a pale yellow, oily liquid.

*2-[(13Z,17Z)-Triaconta-13,17-dien-1-yloxy]tetrahydro-2H-pyran (**8**):* pale yellow oily liquid; IR (film) ν_{max} 2929, 2853, 1465, 1384, 1360, 1303, 1256, 1110, 1075, 815, 722 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.90 (3H, m), 1.26−1.39 (38H, m), 1.52−1.63 (6H, m), 1.73 (1H, m), 1.85 (1H, m), 2.03 (4H, m), 2.09 (4H, m), 3.40 (1H, m), 3.51 (1H, m), 3.75 (1H, m), 3.89 (1H, m), 4.59 (1H, m), 5.35−5.43 (4H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 130.4 (CH), 130.4 (CH), 129.1 (CH), 129.1 (CH), 98.8 (CH), 67.7 (CH_2), 62.3 (CH_2), 31.9 (CH_2), 30.8 (CH_2), 29.8−29.3 (signals of 18C, CH_2), 27.4 (CH_2), 27.4 (CH_2), 27.3 (CH_2), 26.3 (CH_2), 25.5 (CH_2), 22.7 (CH_2), 19.7 (CH_2), 14.1 (CH_3); anal. C 81.01, H 12.82%, calcd for $C_{35}H_{66}O_2$, C 80.89, H 12.79%.

Oxidation of 2-[(13*Z*,17*Z*)-Triaconta-13,17-dien-1-yloxy]-tetrahydro-2*H*-pyran (8) with the Jones Reagent. To a solution of 2-[(13*Z*,17*Z*)-triaconta-13,17-dien-1-yloxy]tetrahydro-2*H*-pyran (**8**) (1.2 g, 2.3 mmol) in acetone (20 mL) and CH_2Cl_2 (5 mL) at rt was added dropwise Jones reagent (3.0 mL). The reaction mixture was stirred at rt for 1 h, the reaction was quenched with H_2O (5 mL), the solution was concentrated under reduced pressure to remove the excess of acetone and CH_2Cl_2, and the aqueous layer was extracted with Et_2O (3 × 30 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography eluting with *n*-hexane−EtOAc (30:1) to afford (13*Z*,17*Z*)-triaconta-13,17-dienoic acid (**3**) (0.82 g, 76%).

*(13Z,17Z)-Triaconta-13,17-dienoic acid (**3**):* white crystals; mp 34−36 °C; IR (film) ν_{max} 2921, 2853, 1711, 1465, 1377, 1309, 1286, 1260, 1233, 1208, 1185, 965, 935, 725, 721 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.91 (3H, t, J = 6.4 Hz), 1.27−1.38 (36H, m), 1.66 (2H, m), 2.05 (4H, m), 2.10 (4H, m), 2.37 (2H, t, J = 7.2 Hz), 5.37−5.42 (4H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 180.3 (C), 130.4 (CH), 130.4 (CH), 129.2 (CH), 129.2 (CH), 34.1 (CH_2), 31.9 (CH_2), 29.8−29.1 (signals of 16C, CH_2), 27.4 (CH_2), 27.4 (CH_2), 27.3 (CH_2), 27.3 (CH_2), 24.7 (CH_2), 22.7 (CH_2), 14.1 (CH_3); anal. C 80.12, H 12.56%, calcd for $C_{30}H_{56}O_2$, C 80.29, H 12.58%.

Synthesis of (5*S*)-4-Hydroxy-5-methyl-3-[(13*Z*,17*Z*)-triaconta-13,17-dien-1-yl]furan-2(5*H*)-one (2). N,N-diisopropylethylamine (0.3 mL, 1.9 mmol) was added to a suspension of butenolide **4** (0.2 g, 1.9 mmol), fatty acid **3** (0.8 g, 1.8 mmol), 4-DMAP (66.0 mg, 0.5 mmol), and N,N'-Dicyclohexylcarbodiimide (0.4 g, 2.2 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was stirred overnight with warming to rt. The yellow solution was filtered, and the solid was washed with Et_2O. The filtrate was concentrated, and the residue was

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dissolved in EtOAc (5 mL). The organic phase was washed with a solution of 1 N HCl (10 mL) and brine (10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. In order to remove residual urea derivative, the mixture was dissolved in Et_2O (10 mL), filtered, and concentrated in vacuo to yield a brownish solid, which was directly used in the subsequent reduction step. Thus, the crude product was dissolved in HOAc (10 mL), and $NaBH_3CN$ (0.2 g, 3.6 mmol) was slowly added at 10 °C. The reaction mixture was stirred overnight with warming to rt and then poured into a solution of 1 N HCl (5 mL). The aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with H_2O (10 mL) and brine (10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo (3 × codistillation with toluene to remove HOAc). The title compound (**2**) (0.93 g, 97%) was obtained in analytically pure form.

*(5S)-4-Hydroxy-5-methyl-3-[(13Z,17Z)-triaconta-13,17-dien-1-yl]-furan-2(5H)-one (**2**):* colorless solid; $[\alpha]^{19}_D$ −18 (*c* 0.4, $CHCl_3$); IR (film) ν_{max} 3011, 2917, 2848, 1764, 1707, 1626, 1466, 1344, 1313, 1264, 1246, 1224, 1110, 1082, 1054, 777, 722 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.89 (3H, t, *J* = 6.5 Hz), 1.24−1.32 (38H, m), 1.48 (2H, m), 1.52 (3H, d, *J* = 6.5 Hz), 2.03 (4H, m), 2.09 (4H, m), 2.21 (2H, t, *J* = 7.5 Hz), 4.84 (1H, q, *J* = 6.5 Hz), 5.36−5.43 (4H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.5 (C), 177.2 (C), 130.4 (CH), 130.4 (CH), 129.1 (CH), 129.1 (CH), 100.9 (C), 75.2 (CH), 31.9 (CH_2), 29.8−29.3 (signals of 17C, CH_2), 28.1 (CH_2), 27.4 (CH_2), 27.4 (CH_2), 27.3 (CH_2), 27.3 (CH_2), 22.7 (CH_2), 21.1 (CH_2), 17.8 (CH_3), 14.1 (CH_3); HRMS *m*/*z* 531.4772 $[M + H]^+$ (calcd for $C_{35}H_{63}O_3$, 531.4777), 553.4591 $[M + Na]^+$ (calcd for $C_{35}H_{62}NaO_3$, 553.4597).

Synthesis of (2S)-2-Methyl-5-oxo-4-((13Z,17Z)-triaconta-13,17-dien-1-yl)-2,5-dihydrofuran-3-yl Trifluoromethanesulfonate (12). DIPEA (0.5 mL, 2.6 mmol) was added to a stirred solution of compound **2** (0.9 g, 1.7 mmol) in CH_2Cl_2 (10 mL) at rt. The solution was cooled to −78 °C, and Tf_2O (0.6 g, 0.3 mL, 2.0 mmol) was slowly added. The mixture was stirred at −78 °C for 2 h. After complete conversion, CH_2Cl_2 (10 mL) was added, and the reaction mixture was extracted with a solution of 1 N HCl (10 mL). The combined organic phases were washed with H_2O (10 mL) and brine (10 mL), dried over $MgSO_4$, and filtered. The solvents were removed under reduced pressure. Flash chromatography (5% ethyl acetate in hexanes) of the residue gave triflate **12** (1.1 g, >99%) as a pale yellow oil.

*(2S)-2-Methyl-5-oxo-4-[(13Z,17Z)-triaconta-13,17-dien-1-yl]-2,5-dihydrofuran-3-yl trifluoromethanesulfonate (**12**):* pale yellow oil; $[\alpha]^{19}_D$ +29 (*c* 0.4, $CHCl_3$); IR (film) ν_{max} 3006, 2925, 2854, 1783, 1700, 1436, 1341, 1223, 1139, 1068, 808, 764, 722, 605, 509 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.90 (3H, t, *J* = 6.4 Hz), 1.26−1.38 (38H, m), 1.56 (3H, d, *J* = 6.8 Hz), 1.60 (2H, m), 2.04 (4H, m), 2.09 (4H, m), 2.34 (2H, m), 5.13 (1H, q, *J* = 7.6 Hz), 5.37−5.42 (4H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.1 (C), 163.4 (C), 130.3 (CH), 130.3 (CH), 129.1 (CH), 129.1 (CH), 121.9 (C), 118.3 (q, J_{C-F} = 320.9 Hz), 74.4 (CH), 31.9 (CH_2), 29.8−29.1 (signals of 18C, CH_2), 27.4 (CH_2), 27.4 (CH_2), 27.3 (CH_2), 27.3 (CH_2), 26.7 (CH_2), 22.7 (CH_2), 17.7 (CH_3), 14.1 (CH_3); HRMS *m*/*z* 680.4530 $[M + NH_4]^+$ (calcd for $C_{36}H_{65}F_3NO_5S + NH_4$, 680.4536), 685.4084 $[M + Na]^+$ (calcd for $C_{36}H_{61}F_3NaO_5S + Na$, 685.4089), 701.3823 $[M + K]^+$ (calcd for $C_{36}H_{61}F_3KO_5S + K$, 701.3829).

Synthesis of (5S)-5-Methyl-3-[(13Z,17Z)-triaconta-13,17-dien-1-yl]furan-2(5H)-one (Muricadienin, 1). $Pd_2(dba)_3$ (20.6 mg, 0.022 mmol, 1.5 mol %) and PPh_3 (59.0 mg, 0.23 mmol, 15.0 mol %) were dissolved in anhydrous THF (10 mL). After stirring for 5 min at rt, triflate **12** (1.0 g, 1.5 mmol) and Bu_3SnH (1.2 mL, 4.5 mmol) were added to the orange solution. The mixture was heated and stirred at 50 °C for 5 h. After complete conversion of the starting material the reaction mixture was cooled to rt, diluted with H_2O (5 mL), and extracted with Et_2O (3 × 15 mL). The combined organic phases were dried over $MgSO_4$ and filtered, and the solvents were removed under reduced pressure. Flash chromatography (2% EtOAc in *n*-hexane to 5% EtOAc in *n*-hexane) of the residue gave (+)-muricadienin (**1**) (0.68 g, 90%).

*(5S)-5-Methyl-3-[(13Z,17Z)-triaconta-13,17-dien-1-yl]furan-2(5H)-one (muricadienin, **1**):* colorless, waxy solid; $[\alpha]^{19}_D$ +13 (*c* 0.7, $CHCl_3$); IR (film) ν_{max} 3006, 2924, 2853, 1761, 1655, 1465, 1374, 1318, 1198, 1118, 1077, 1027, 967, 858, 805, 722, 637, 610 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.89 (3H, t, *J* = 7.0 Hz), 1.25−1.38 (38H, m), 1.41 (3H, d, *J* = 6.5 Hz), 1.56 (2H, m), 2.03 (4H, m), 2.09 (4H, m), 2.28 (2H, t, *J* = 7.5 Hz), 4.99 (1H, qd, *J* = 6.5, 1.5 Hz), 5.35−5.43 (4H, m), 6.99 (1H, d, *J* = 1.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.9 (C), 148.8 (CH), 134.3 (C), 130.4 (CH), 130.4 (CH), 129.1 (CH), 129.1 (CH), 77.4 (CH), 31.9 (CH_2), 29.8−29.2 (signals of 17C, CH_2), 27.4 (CH_2), 27.4 (CH_2), 27.4 (CH_2), 27.3 (CH_2), 27.3 (CH_2), 25.2 (CH_2), 22.7 (CH_2), 19.2 (CH_3), 14.1 (CH_3); HRMS *m*/*z* 515.4826 $[M + H]^+$ (calcd for $C_{35}H_{63}O_2$, 515.4823), 537.4648 $[M + Na]^+$ (calcd for $C_{35}H_{62}NaO_2$, 537.4642), 532.5093 $[M + NH_4]^+$ (calcd for $[C_{35}H_{66}NO_2]^+$, 532.5088).

Cell Culture. Human embryonic kidney cells HEK293 were obtained from the HPA Culture Collections (UK). The cell line was cultured as monolayers and maintained in DMEM (Gibco BRL) supplemented with 10% fetal bovine serum and 1% penicillin−streptomycin solution at 37 °C in a humidified incubator under a 5% CO_2 atmosphere. Muricadienin was dissolved in DMSO and diluted with the tissue culture medium before use.

Annexin V-FITC/PI Assay. Apoptosis was quantified by the detection of phosphatidylserine surface exposure on apoptotic cells using an Alexa Fluor 488 annexin V/dead cell apoptosis kit. HEK293 cells were incubated with or without muricadienin for 24 h. The adherent and floating cells were collected and washed with phosphate-buffered saline. Then, the cells were resuspended in 90 μL of annexin V binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$; pH 7.4). Annexin V (5 μL) and 1 μL of PI were added to the reaction mixture and incubated for 15 min at room temperature in the dark. After the addition of 300 μL of binding buffer, the stained cells were analyzed with a CytoFLEX flow cytometry system (Beckman Coulter, Inc., USA).

MTT Assay. MTT tests were carried out following the known procedure.[14] For further information see the Supporting Information.

DNA Topoisomerase I and II Assays. The inhibitory activity of muricadienin (**1**) was determined using the topoisomerase I drug screening kit TG-1018-2 and the topoisomerase IIα drug screening kit TG1009-2 protocols by Topogen (USA) (for further information see the Supporting Information).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00335.

NMR data, NMR spectra, and general procedure (PDF)

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Notes

The authors declare no competing financial interest.

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