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SYNTHETIC TRANSFORMATIONS OF SESQUITERPENE LACTONES. V.* SYNTHESIS AND CYTOTOXICITY OF 13-ARYL-SUBSTITUTED TOURNEFORIN DERIVATIVES

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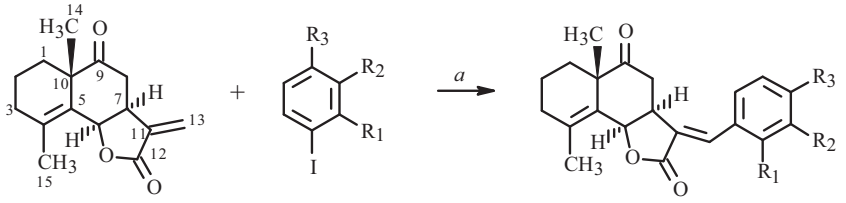
*13-Aryl-substituted tourneforin derivatives were synthesized via the Heck reaction with aryliodides. The structure of (*E*)-13-(3,4-dimethoxybenzyl)-eudesma-4(5),11(13)-dien-6α,12-olide was confirmed by an XSA. A study of the cytotoxicity of the synthesized derivatives for CEM-13, MT-4, and U-937 tumor models showed promise for the modification.*

Keywords: tourneforin, Heck reaction, 13-aryleudesmanolides, tumor cells, XSA.

Compounds containing an α-methylene-γ-lactone, in particular sesquiterpene lactones, are interesting because of a variety of physiological activity, including antitumor properties [2]. It was found during a study of the structure–activity relationship of a series of sesquiterpene lactones that high cytotoxicity correlated with the presence of an exocyclic double bond in the lactone ring [3]. It was proposed that the antitumor activity of these compounds was due to induction of apoptosis of tumor cells by activating caspase-3 [4]. The antitumor drug Arglabin was developed based on a sesquiterpene methylenelactone [5]. We showed earlier that the structure of eudesmane-type methylenelactones can be modified using the Heck reaction [6]. Herein C^{13} arylation of tourneforin (**1**), an available sesquiterpene lactone of *Artemisia tournefortiana* Rchb. [7], is reported.



1 **2a - c** **3a - c**

2a, 3a: $R_1 = H$, $R_2 = R_3 = OCH_3$
2b, 3b: $R_1 = R_3 = OCH_3$, $R_2 = H$
2c, 3c: $R_1 = R_2 = H$, $R_3 = F$

a. $Pd(OAc)_2$, $P(o\text{-Tol})_3$, Et_3N, DMF, 120°C, 16 h

The reaction of **1** with 4-iodoveratrol (**2a**), 2,4-dimethoxyiodobenzene (**2b**), and 4-iodo-1-fluorobenzene (**2c**) was carried out in the catalytic system $Pd(OAc)_2$–$(o\text{-Tol})_3P$ (4/16 mol%) in DMF solution in the presence of Et_3N. Heating the mixture of components for 16 h resulted in complete conversion of the starting compound. Column and analytical chromatography over silica gel isolated the corresponding (*E*)-13-aryleudesma-4(5),11(13)-dien-6α,12-olides (**3a–c**) (27–45% yields).

*For No. IV, see Ref. [1].

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TABLE 1. Cytotoxicity of Tourneforin (**1**) and 13-Aryl-Substituted Derivatives **3a**–**c**

Compound	CEM-13 tumor cells, $CCID_{50}$, μM	U-937 tumor cells, $CCID_{50}$, μM	MT-4 tumor cells, $CCID_{50}$, μM
1	> 200	> 200	> 200
3a	25.7	54.5	5.5
3b	18.6	56.1	12.3
3c	11.8	41.2	8.9

$\overline{CCID_{50}}$ is the dose inhibiting tumor cell viability by 50%.

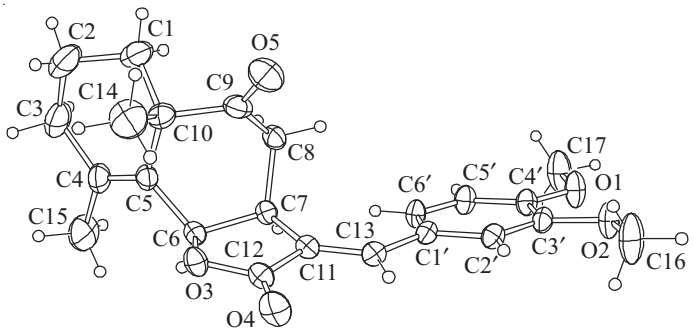

Fig. 1. Molecular structure of **3a** from an XSA.

The structures of the synthesized compounds were established based on elemental analyses and spectral characteristics. The structure of **3a** was confirmed by an x-ray crystal structure analysis (XSA) (Fig. 1). The bond lengths of the tricyclic moiety were similar to analogous ones in starting **1** [7]. The lactone ring had the half-chair conformation with a folding angle of 15.8(6)° [atoms C7, C11, C12, O3 were planar within ±0.009(8) Å]. The angle between the benzene ring (C1′÷C6′) and the plane of C7C11C12O3 was 16.4(6)°.

The methoxyls $O2C16H_3$ and $O1C17H_3$ were practically coplanar with the benzene ring. The torsion angles C3′-C4′-O1-C17 and C4′-C3′-O2-C16 were 178.3(2) and 179.3(2)°, respectively. The six-membered ring (C5÷C10) adopted a distorted twist conformation. The cyclohexene moiety had a conformation similar to a boat with C1 deviating by 0.664(5) Å from the plane of C2C3C4C5C10 [planar within ±0.040(3) Å]. The crystal structure had molecular sheets extended along the *b* axis that were formed by H-bonds C6′–H…O4 and C7–H7…O4 (with parameters C–H 0.93 and 0.98, H…O 2.50 and 2.41, C…O 3.383(2) and 3.175(2) Å, C–H…O 158 and 135°, respectively) and H-bond C5′–H…O3 [parameters C–H 0.93, H…O 2.58, C…O 3.330(2) Å, C–H…O 138°].

PMR spectra of **3a–c** contained resonances for H-13 in the range 7.42–7.50 ppm. The (*E*)-configuration of the C^{11}–C^{13} double bond of **3a–c** was inferred from the presence in the ^{13}C NMR spectra (single-resonance mode) of C–H ^3J-*cis* coupling constants between the olefinic proton and the lactone carbonyl C atom (^3J = 7.2 Hz). A characteristic feature of the PMR spectra of **3a–c** was a weak-field shift of H^7 (δ 3.98–4.03 ppm) compared with the position of the corresponding proton in the spectrum of **1** (δ 3.65 ppm). According to PMR spectra and GC–MS of the reaction mixture, **3a**–**c** were the only reaction products of cross-conjugation of **1** with aryliodides **2a**–**c**.

The course of the Heck reaction of the methylenelactones depended significantly on the structure of the lactone. Thus, the reaction of α-methylene-γ-butyrolactone with the aryliodides occurred with formation of products with the (*Z*)-configuration [8]. The reaction of isoalantolactone with arylhalides produced (*E*)-13-aryleudesma-4(15),11(13)-dien-8α,12-olides and products of isomerization of the double bond and configuration inversion at C(8), i.e., 13-aryleudesma-4(15),7(11)-dien-8β,12-olides [6]. The reaction of 11,13-dehydrosantonin with the aryliodides gave exclusively the corresponding 13-arylidenelactones with the (*E*)-configuration of the double bond. Products of a double-bond shift were not observed [9]. We obtained an analogous result for the Heck reaction of **1**.

Table 1 presents the cytotoxicity of **1** and its arylidene-substituted derivatives **3a**–**c**. It can be seen that introducing the aromatic substituent into the C(13) position of **1** increased the cytotoxicity against lymphoid tumor cells.

Thus, a method for preparing 13-aryl-derivatives using the Heck reaction was proposed based on the available *A. tournefortiana* metabolite tourneforin. Significant cytotoxicity was found for the new derivatives of eudesmane-type methylenelactones against MT-4 and CEM-13 tumor cell lines.

EXPERIMENTAL

NMR spectra were recorded in $CDCl_3$ or CD_3OD solutions on Bruker AV-400 (operating frequency 400.13 for ^1H and 100.78 MHz for ^{13}C) and AV-600 (operating frequency 600.30 for ^1H and 150.96 MHz for ^{13}C) spectrometers. Resonances in NMR spectra were assigned using various types of H–H and C–H shift correlation spectroscopy (COSY, COXH, COLOC, NOESY). Multiplicities of resonances in ^{13}C NMR spectra were determined by recording spectra in J-mode. A DFS Thermo Scientific high-resolution mass spectrometer (ionizing electron energy 70 eV, vaporizer temperature 230–280°C) was used to record mass spectra and to determine molecular weights and elemental compositions. Melting points were determined on a Stuart SMF-38 heating stage.

IR spectra were recorded in KBr pellets on a Vector-22 instrument. UV absorption spectra were recorded in EtOH on an HP 8453 UV–Vis spectrometer. Specific rotations $[\alpha]_D^{20}$ were measured on a PolAAr3005 polarimeter. X-ray diffraction experiments for **3a** were performed at room temperature on a Bruker Kappa APEX II diffractometer (Mo Kα-radiation, graphite monochromator, CCD-detector, maximum angle $2\theta = 54.2°$).

Reaction products were separated by column chromatography over silica gel (Acros, 0.035–0.070 mm) and additionally by preparative TLC on a loose layer of silica gel containing K-35 luminophore (1%) on plates (20 × 20 cm) with a thin layer of sorbent (1 mm) using benzene:EtOAc and $CHCl_3$:EtOH as eluents. $Pd(OAc)_2$ was synthesized by the literature method [10]; 2,4-dimethoxyiodobenzene (**2b**), as before [11].

(3a*S*,5a*S*,9b*R*,*E*)-3-(3,4-Dimethoxybenzylidene)-5a,9-dimethyl-3a,4,5a,6,7,8-hexahydronaphtho[1,2-*b*]furan-2,5(3*H*,9b*H*)-dione (3a). A two-necked glass ampul was filled with Ar, in a stream of which the ampul was charged successively with **1** (247 mg, 1 mmol), 4-iodoveratrol (**2a**, 290 mg, 1.1 mmol), $Pd(OAc)_2$ (9 mg, 0.04 mmol), *tris*-(*o*-tolyl)phosphine (49 mg, 0.16 mmol), DMF (5 mL), and Et_3N (142 mg, 1.4 mmol). Molecular sieves (3 Å) were added. The ampul was sealed (slight excess of Ar pressure). The mixture was heated for 16 h at 120°C and cooled. The ampul was opened. The contents were poured into a Petri dish. The solid precipitate was dissolved in the minimal amount of $CHCl_3$ and chromatographed over silica gel ($CHCl_3$:EtOH eluent, 100:0→10:1). *tris*-(*o*-Tolyl)phosphine, starting lactone, and a mixture of lactone and reaction products ($CHCl_3$:EtOH eluent) were eluted sequentially. Repeated chromatography on a plate with a loose layer of silica gel and recrystallization from EtOH afforded **3a** (164 mg, 45%), mp 196–198°C (EtOH), $[\alpha]_{589}$ –78.5° (*c*, $CHCl_3$). IR spectrum (ν, cm^{-1}): 825, 849, 926, 964, 1021, 1105, 1140, 1160, 1193, 1218, 1250, 1266, 1319, 1334, 1423, 1443, 1463, 1518, 1597, 1644, 1713, 1734, 2836, 2937, 2970. UV spectrum (EtOH, λ_{max}, nm, log ε): 203 (4.37), 242 (4.12), 330 (4.35).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.33 (3H, s, H-14), 1.53 (1H, ddd, J = 12.8, 5.8, 3.6, H-1), 1.59 (1H, ddd, J = 13.0, 12.8, 3.4, H-1), 1.63–1.79 (2H, m, H-2), 1.85 (3H, s, H-15), 2.14 (1H, m, H-3), 2.49 (1H, dd, J = 13.2, 3.1, H-8), 2.82 (1H, dd, J = 13.2, 8.7, H-8), 3.88 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.98 (1H, dddd, J = 8.7, 7.3, 3.1, 1.5, H-7), 5.65 (1H, d, J = 7.3, H-6), 6.88 (1H, d, J = 8.3, H-5′), 6.95 (1H, d, J = 1.5, H-2′), 7.09 (1H, dd, J = 8.3, 1.5, H-6′), 7.49 (1H, d, J = 1.5, H-13).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 17.69 (C-2), 19.39 (C-14), 24.42 (C-15), 32.13 (C-3), 33.83 (C-1), 37.60 (C-8), 38.83 (C-7), 47.83 (C-10), 55.87 (2×OCH_3), 75.05 (C-6), 111.17 (C-2′), 112.99 (C-5′), 123.99 (C-6′), 125.35 (C-5), 126.25 (C-1′), 128.76 (C-11), 137.98 (C-4), 138.03 (C-13), 149.01 (C-4′), 150.86 (C-3′), 171.32 (C-12), 213.35 (C-9).

Mass spectrum (*m*/*z*, I_{rel}, %): 382 (100), 245 (15), 217 (24), 151 (11), 91 (6). Found: MW 382.1773. $C_{23}H_{26}O_5$. Calcd: 382.1775.

(3a*S*,5a*S*,9b*R*,*E*)-3-(2,4-Dimethoxybenzylidene)-5a,9-dimethyl-3a,4,5a,6,7,8-hexahydronaphtho[1,2-*b*]furan-2,5(3*H*,9b*H*)-dione (3b). The method described above was used with **1** (247 mg, 1 mmol) and 2,4-dimethoxyiodobenzene (**2b**, 290 mg, 1.1 mmol) to afford **3b** (172 mg, 45%), mp 221–222°C (EtOH), $[\alpha]_{589}$ –48° (*c* 1.23, $CHCl_3$). IR spectrum (ν, cm^{-1}): 837, 928, 970, 1032, 1120, 1159, 1180, 1225, 1275, 1302, 1335, 1423, 1464, 1500, 1603, 1637, 1736, 2931, 2976. UV spectrum (EtOH, λ_{max}, nm, log ε): 201 (3.79), 213 (3.80), 221 (3.93), 245 (3.97), 300 (4.09), 341 (4.36).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.31 (3H, s, H-14), 1.51 (1H, ddd, J = 12.8, 3.8, 3.6, H-1), 1.57 (1H, ddd, J = 13.0, 12.8, 3.4, H-1), 1.71 (1H, m, H-2), 1.80 (1H, m, H-2), 1.83 (3H, s, H-15), 2.11 (2H, m, H-3), 2.43 (1H, dd, J = 13.5, 3.5, H-8), 2.73 (1H, dd, J = 13.5, 8.5, H-8), 3.82 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.98 (1H, dddd, J = 8.6, 8.5, 7.5, 2.0, H-7), 5.61 (1H, d, J = 7.5, H-6), 6.44 (1H, d, J = 2.4, H-3′), 6.47 (1H, dd, J = 8.5, 2.4, H-5′), 7.31 (1H, d, J = 8.5, H-6′), 7.42 (1H, d, J = 2.0, H-13).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 17.76 (C-2), 19.39 (C-14), 24.60 (C-15), 32.21 (C-3), 33.76 (C-1), 37.65 (C-8), 38.78 (C-7), 47.63 (C-10), 55.37 (OCH_3), 55.49 (OCH_3), 74.86 (C-6), 98.28 (C-32), 105.25 (C-52), 115.50 (C-1′), 124.61 (C-5), 128.92 (C-11), 130.12 (C-6′), 132.60 (C-13), 137.97 (C-4), 160.21 (C-2′), 162.81 (C-4′), 171.68 (C-12), 213.51 (C-9).

Mass spectrum (*m*/*z*, I_{rel}, %): 382 (100), 367 (6), 351 (7), 339 (6), 323 (9), 309 (11), 245 (28), 217 (35), 151 (27), 91 (10). Found: MW 382.1769. $C_{23}H_{26}O_5$. Calcd: 382.1775.

(3a*S*,5a*S*,9b*R*,*E*)-3-(4-Fluorobenzylidene)-5a,9-dimethyl-3a,4,5a,6,7,8-hexahydronaphtho[1,2-*b*]furan-2,5(3*H*,9b*H*)-dione (3c). The method described above was used with **1** (247 mg, 1 mmol) and 4-iodo-1-fluorobenzene (**2c**, 245 mg, 1.1 mmol) to afford **3c**. The compound was purified by column chromatography over silica gel and Al_2O_3 and by TLC. Yield 92 mg (27%) of an oil. IR spectrum (ν, cm^{-1}): 2979, 1746, 1713, 1650, 1600, 1510, 1372, 1326, 1313, 1294, 1192, 1176, 1153, 1132, 1104, 970, 927, 837, 756. UV spectrum (EtOH, λ_{max}, nm, log ε): 194 (3.52), 206 (3.96), 220 (3.84), 286 (4.02), 294 (4.01), 302 (3.93).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.31 (3H, s, H-14), 1.50 (1H, ddd, J = 12.6, 3.7, 3.5, H-1_a), 1.57 (1H, ddd, J = 12.9, 12.8, 3.4, H-1_b), 1.73 (1H, m, H-2_a), 1.81 (1H, m, H-2_b), 1.83 (3H, s, H-15), 2.13 (2H, m, H-3), 2.38 (1H, dd, J = 13.3, 3.2, H-8_a), 2.82 (1H, dd, J = 13.3, 8.7, H-8_b), 4.03 (1H, dddd, J = 8.7, 7.2, 3.2, 1.7, H-7), 5.65 (1H, d, J = 7.2, H-6), 7.09 (2H, dd, J = 8.6, 8.5, H-3′, H-5′), 7.44 (2H, dd, J = 8.5, 5.4, H-2′, H-6′), 7.50 (1H, d, J = 1.7, H-13).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 17.67 (C-2), 19.32 (C-14), 24.37 (C-15), 32.12 (C-3), 33.78 (C-1), 37.56 (C-8), 38.56 (C-7), 47.72 (C-10), 75.18 (C-6), 116.18 (C-3′, C-5′), 127.76 (C-5), 128.58 (C-11), 129.65 (C-1′), 132.02 (C-2′, C-6′), 136.64 (C-13), 138.22 (C-4), 163.42 (C-4′), 170.86 (C-12), 213.06 (C-9). $C_{21}H_{21}FO_3$.

X-ray Crystal Structure Analysis of 3a. Crystals of **3a** were orthorhombic, $a = 9.3112(4)$, $b = 13.1142(6)$, $c = 17.233(1)$ Å, V = 2104.4(2) $Å^3$, space group $P2_12_12_1$, $Z = 4$, $C_{23}H_{26}O_5$, $d_{calcd} = 1.207$ g/cm^3, $\mu = 0.084$ mm^{-1}, crystal size 0.43 × 0.25 × 0.15 mm. Intensities of 4462 independent reflections were measured. Absorption corrections were applied using the SADABS program (transmission 0.9647–0.9875). The structure was solved by direct methods using the SHELXS-97 program and refined by anisotropic and isotropic (for H) methods using the SHELXL-97 program. Positions of H atoms were calculated geometrically. Parameters of H atoms were refined isotropically using a rider model. The final refinement parameters were $wR_2 = 0.1106$, S = 1.046. A total of 258 parameters were refined (R = 0.0382 for 3709 $F > 4\sigma$). Atomic coordinates and bond lengths and angles were deposited in the Cambridge Crystallographic Data Centre (CCDC-825956).

Cell Culture. We used human tumor cell lines MT-4, CEM (human T-cell leucosis cells), and U-937 (human monocytes). Cells were cultured in RPMI-1640 medium containing fetal calf serum (10%), L-glutamine (2 mmol/L), gentamicin (80 μg/mL), and lincomycin (30 mg/mL) at 37°C in a CO_2 incubator. Test compounds were dissolved in DMSO and added to cell culture at the required concentrations. We used three wells for each concentration. Cells incubated without added test compounds were used as the control.

MTT Test. The standard MTT test was used to determine $CCID_{50}$ (dose inhibiting cell viability by 50%) values [12, 13].

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