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Chemical and biotransformation of Gelomulide F: a rare diterpene lactone

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Chemical and biotransformations of a rare diterpene lactone, Gelomulide F (**1**), from the cytotoxic extract of leaves of *Suregada multiflora* were studied. Fermentation of compound **1** with *Sachromyces cerevisiae* transformed it to Gelomulide D (**2**) and E (**3**), whereas its treatment with 2N KOH yielded Gelomulide D (**2**). In addition, a novel compound (**4**) was obtained by its diastereo and chemoselective reduction with $NaBH_4$. All of these compounds were characterized on the basis of extensive ^{1}H-NMR, ^{13}C-NMR, 2D NMR, and mass spectral analyses.

Key Words: Diterpene lactone, Gelomulide, *Suregada multiflora*, *Sachromyces cerevisiae*, diastereo and chemoselective reduction

Introduction

A series of rare diterpene lactones were isolated from the leaves of *Suregada multiflora*.[1] In continuation of our previous work to accomplish structural modifications in this rare class of diterpene lactones,[2] the present investigation was carried out on Gelomulide F (**1**). Both chemical and biotransformation methods were employed and structurally interesting analogs (**2-4**) were obtained.

Experimental

Column chromatography (CC): Merck silica gel 60 (70-230 mesh) TLC: Merck silica gel GF 254 (0.25 mm); detection by vanillin reagent. All reagents used were of analytical grades. Supermarket baker's yeast NATU

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(China) was used. M. pts.: a micromelting point apparatus (Yanaco MP-S3). $[\alpha]_D$: JASCO DIP-360 digital polarimeter. UV spectra: a Hitachi U 3200 spectrophotometer; λ_{max} (log ε) in nm. IR spectra: JASCO A-302 IR spectrophotometer; in $CHCl_3$ on KBr disks; in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: on Bruker Avance: at 400/100 MHz; resp., δ in ppm referenced with respect to the residual solvent signal of $CDCl_3$, coupling constants J in Hz. Mass spectra: a double-focusing mass spectrometer (Varian MAT 311 A); high-resolution electron impact mass spectra (HR/EI-MS) with a Jeol HX 110 mass spectrometer; in m/z (rel. %).

Microbial transformation of Gelomulide F (1) by *Saccharomyces cerevisiae*

Saccharomyces cerevisiae (baker's yeast, 40 g) was added in portions to a stirred solution of sugar (100 g) in water (300 mL) in a conical flask at 35 °C. Gelomulide F (200 mg in 2 mL of DMF) was added after 4 h. The reaction was monitored by TLC. After 4 days, the incubation mixture was filtered and extracted with chloroform. The combined extracts were dried over anhydrous Na_2SO_4, and the solvent was evaporated to give 321 mg of brown residue. The crude residue was chromatographed on a silica gel column and eluted with EtOAc/hexane to obtain the transformed metabolites **2** (38%) and **3** (22%).

β-Elimination of Gelomulide F (1) by KOH

Compound **1** (10 mg, 0.25 mmol) in THF (2 mL) was put in a 25-mL round-bottomed flask and 2 mL of 2 N KOH was added. The solution was stirred at room temperature. After 2 h, the reaction mixture was neutralized with aq. HCl and extracted with CH_2Cl_2. The organic layer was washed with ammonium chloride, dried over magnesium sulfate, and evaporated under vacuum. The residue was recrystallized by acetone-hexane to obtain compound **2** (85%).

2-Ene-8β,14β-epoxy-1-oxo-13,15-abiatene-16,12-olide (2)

Appearance: Colorless prism; R_f: 0.34 (20% acetone-hexane); mp: 229 °C; $[\alpha]_D$: +85° ($CHCl_3$; c 0.003); UV: (MeOH) λ_{max} (log ε) 223 nm (5.6).); IR: ν_{max}: 2954, 1746, 1665, 1438, 1363, 1249, 1090 cm^{-1}; EI-MS: m/z (rel. int. %.): 328 (M^+, 52), 310 (16), 295 (12), 267 (32), 232 (27), 214 (27) 186 (33), 160 (20), 150 (89), 137 (70), 96 (49), 53 (100).; HREI-MS: 328.1648 (for $C_{20}H_{24}O_4$, 328.1674); ^{1}H- and ^{13}C-NMR: Table.

6β-Acetoxy-2-ene-1-oxo-8β,14β-epoxy-13,15-abiatene-16,12-olide (3)

Appearance: Colorless needles; R_f: 0.27 (20% acetone-hexane); mp: 267 °C; $[\alpha]_D$: –13.3° ($CHCl_3$; c 0.003); UV: (MeOH) λ_{max} (log ε) 227 nm (6.3); IR: ν_{max} cm^{-1}: 2960, 1758, 1730, 1240, 1668, 1460, 1378 and 1025; EI-MS: m/z (rel. int.%): 386 (M^+, 17), 326 (54), 308 (43), 293 (18), 283 (9), 265 (37), 230 (8), 212 (10), 163 (17), 137 (100), 105 (37); HREI-MS: m/z= 386.1542 (for $C_{25}H_{22}O_4$, 386.1518); ^{1}H- and ^{13}C-NMR: Table.

Reduction of Gelomulide F (1) by $NaBH_4$

Compound **1** (20 mg, 0.5 mmol) was treated with $NaBH_4$ (3 mg, 0.8 mmol) in MeOH under stirring at room temperature for 1 h. The reaction was quenched by addition of aq. HCl. The reaction mixture was extracted

with diethylether, and the organic layer was washed with NH_4Cl solution, dried over $MgSO_4$, and concentrated over a rotary evaporator. The white solid obtained showed only one product (**4**) on TLC.

Table. ^{1}H- and ^{13}C-NMR data of compounds **1-4** in $CDCl_3$; δ in ppm (J in Hz).

	1		**2**		**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1		210.2		204.5		202.6	3.64 *t* (2.6)	72.0
2	2.38 *dd* (13.5, 3.4) 3.17 *dd* (13.5, 3.6)	39.5	5.8 *d* (10)	124.1	5.82 *d* (10)	122.6	1.92-2.2 *m*	30.3
3	5.02 *t* (3.5)	80.6	6.4 *d* (10)	155.0	6.31*d* (10)	156.5	4.94 *t* (2.8)	78.5
6		20.1		21.0	5.13 *ddd* (11,4.9)	70.1		
9	2.68 *d* (7.3)	40.8	2.9 *d* (7.2)	39.8	2.66 *d* (7.1)	38.9	2.60 *d* (7.6)	41.6
12	4.84 *ddd* (13, 5.4, 2)	75.7	4.83 *ddd* (12.1, 5.4, 2.1)	74.8	4.79 *m* ($w_{1/2}$ = 19)	76.1	4.56 *m* ($w_{1/2}$ = 16)	73.2
13	-	154.6	-	156.4	-	153.1	2.90 *m*	37.2
14	3.8 *s*	56.0	3.7 *s*	56.4	3.8 *s*	55.6	3.00 *s*	59.0
15	-	128.1	-	128.7	-	129.4	2.90 *m*	37.2
16	-	173.8	-	171.2	-	173.2	-	
17	1.94 *d (1.8)*	8.7	1.95 *d* (2.0)	9.0	1.96 *d* (1.7)	8.81	1.40 *d* (6.6)	11.5
18	0.96 *s*	27.7	1.10 *s*	31.3	1.16 *s*	33.7	0.91(*s*)	28.1
19	1.20 *s*	22.1	1.11 *s*	22.7	1.30 *s*	22.5	0.94 (*s*)	22.3
20	1.30 *s*	16.8	1.31 *s*	18.8	1.37 *s*	19.2	0.83(*s*)	20.6
OCO<u>CH$_3$</u>	2.03 *s*	20.9			2.05 *s*	21.4	2.1 s	21.6
O<u>CO</u>CH$_3$	-	170.0	-		-	170.1	-	169.9

3β-acetoxy-1β-hydroxy-8β,14β-epoxy-abiatane-16,12-olide (4)

Appearance: White powder; Yield: (73%); mp: 264 °C; $[\alpha]_D$: –18.3° ($CHCl_3$; c 0.003); UV: (MeOH) λ_{max} (log ε) 220 nm (6.1); IR: ν_{max} cm^{-1}: 3464, 2927, 1752, 1449, and 1028; EI-MS: m/z (rel. int. %): 392 (13), 336 (2), 319 (6), 285 (19), 241 (70), 135 (20), 55 (100); FAB-MS (+ve): 393; ^{1}H- and ^{13}C-NMR: Table.

Results and discussion

Saccharomyces cerevisiae (baker's yeast) was selected due to its well known ability to reduce carbonyl groups. However, interestingly, Gelomulide F (**1**) was transformed into compound **2** via β-elimination and **3** through subsequent acetylation at C-6 (Figure 1). Comparison of all the physical and spectral data identified the transformed product as Gelomulide D (**2**) and E (**3**), which have already been isolated from the same plant.[1] Treatment of substrate **1** with ethanolic KOH also afforded Gelomulide D (**2**) through β-elimination of the

3-OAc group. Hence, based on these transformations, it can be proposed that Gelomulide D (**2**) and E (**3**) are biogenetically derived from Gelomulide F (**1**), which is a major constituent of the leaf extract of *S. multiflora.*[1]

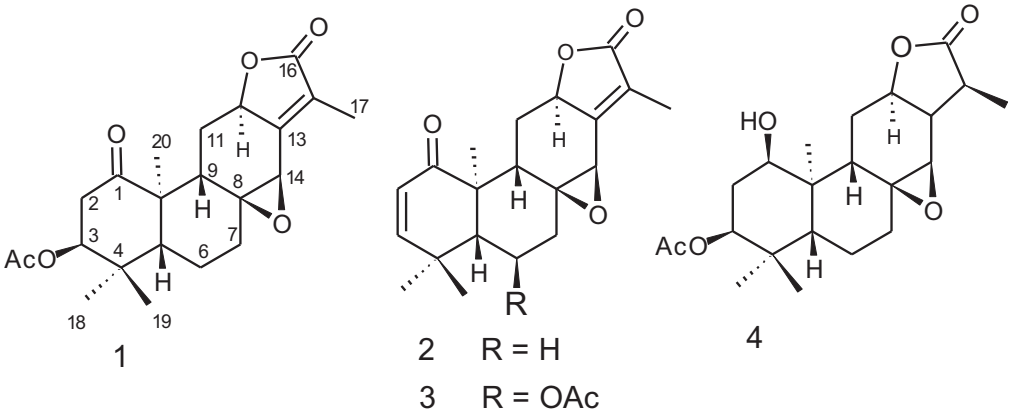

Figure 1. Compounds **1-4**.

Moreover, a very interesting compound (**4**) was obtained by chemo- and diastereoselective reduction of Gelomulide F (**1**) with $NaBH_4$ at room temperature (Figure 1). The EIMS spectrum of compound **4** exhibited the M^+ at m/z 392 and FAB-MS (+ve) at m/z 393. In the IR spectrum, the presence of –OH (3578 cm^{-1}) and the absence of ketonic absorption indicated that the keto group at C-1 of Gelomulide F (**1**) was reduced be to a hydroxyl group. The ^{1}H-NMR spectrum of compound **4** (Table) showed an extra methine signal at δ 3.64 attributed to H-1. The ^{13}C-NMR spectrum further confirmed this modification as a new methine carbon that appeared at δ 72.0 instead of carbonyl carbon resonating at δ 210.2 in Gelomulide F (**1**). The stereochemistry of the hydroxyl group at C-1 was deduced to be β-oriented based on the NOE interactions between H-1 (δ_H 3.64) and α-oriented Me-20 (δ_H 0.83) in the NOESY experiment (Figure 2). It is well documented that prediction of the predominant product of a metal hydride reduction depends on the space requirement.[3] We envisioned, as the axial approach of the hydride is strongly inhibited by an axial 3-OAc group in Gelomulide F (**1**), that the hydride approaches from the less hindered equatorial side (Figure 3), and as a result the predominant product is kinetically controlled axial alcohol in compound **4**.

Another interesting selectivity was introduced by $NaBH_4$ as the result of conjugate reduction of the lactone ring. The singlet of C-14 proton, geminal to the epoxy group, was shifted upfield at δ 3.00, while Me-17 appeared at δ 1.4 (d, J= 6.6 Hz), suggesting that the double bond in the lactone ring was also reduced. NOE interactions between H-12 and H-15 indicated that Me-17 is β-oriented. A 2-proton multiplet at δ 2.90 was attributed to H-13 and H-15, which also showed HMBC interaction with C-14 and C-16 (Figure 2). All this spectral evidence supported the structure of the reduced product (**4**) as 3β-acetoxy-1β-hydroxy-8β,14β-epoxy-abiatane-16,12-olide. The product is plausibly formed by initial conjugate addition of hydride anion to β-carbon (C-13), followed by ketonization of the less stable enol product (Figure 3). Selective conjugate reduction of α,β-unsaturated carbonyl compounds is already reported, usually requiring efficient catalysts like rhodium amido complexes, indium(III) chloride, or $Rh(PPh)_3Cl$.[4–6] α,β-Unsaturated esters can reduced to saturated analogs by $NaBH_4$ only if an additional electron withdrawing group is present at the α-position or under drastic conditions,[7–12] whereas reduction of α,β-unsaturated lactones is rarely reported.[13] The current

report is, therefore, a significant addition for the purpose of achieving conjugate addition of α,β-unsaturated lactone in very mild conditions.

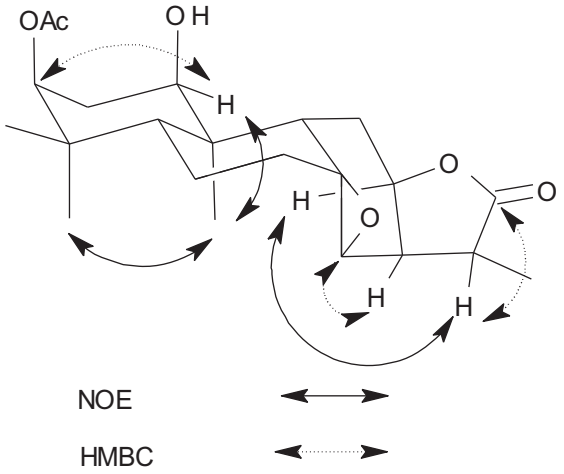

Figure 2. Key NOE and HMBC interactions in compound **4**.

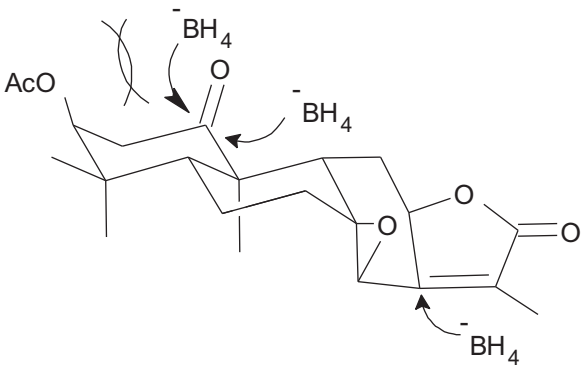

Figure 3. Plausible selectivity in reduction of compounds **1** to **4**.

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