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TRITERPENOID SAPONINS FROM *VERBASCUM SONGARICUM*

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Key Word Index—*Verbascum songaricum*; Scrophulariaceae; triterpenoid saponins; songarosaponin E and F.

Abstract—From the aerial parts of *Verbascum songaricum* the two new triterpenoid saponins songarosaponin E, F and the known buddlejasaponin I have been isolated and identified as 3-*O*-{[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranosyl}-olea-11,13-diene-3β,23,28-triol, 3-*O*-{[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranosyl}-olea-11,13-diene-3β,16β,23,28-tetrol and 3-*O*-{[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranosyl}-13β,28-epoxyolea-11-ene-3β,16β,23-triol.

INTRODUCTION

Plants belonging to the genus *Verbascum* are well-known drugs in European folk medicine, mainly used due to their expectorant and mucolytic activity [1]. *Verbascum songaricum* Schrenk, a perennial plant of Central Asia, is also used in traditional medicine in Russia. In the course of our investigations on triterpenoid saponins of *V. songaricum*, we isolated songarosaponin A–D [2, 3]. In this report, we present the isolation and structure elucidation of two new triterpenoid saponins, besides the known buddlejasaponin I [4], which has been isolated for the first time from this plant.

RESULTS AND DISCUSSION

The methanolic extract of the dried aerial parts of *V. songaricum* was subjected to CC and TLC on silica gel, followed by HPLC separation to afford songarosaponin E (**1**), F (**2**) and buddlejasaponin I (**3**). The negative liquid SIMS of **1** exhibited the $[M-1]^-$ ion at *m/z* 1087. The fragment ions at *m/z* 925, 763, 601 and 455 showed the sequential loss of three hexose moieties and one 6-deoxyhexose moiety. The attachment of the 6-deoxyhexose directly to the aglycone was indicated by the fragment ion at *m/z* 601, formed due to the loss of three hexoses and further by the fragment ion at *m/z* 455, which was consistent with the loss of 6-deoxyhexose from the ion at *m/z* 601.

In the positive ion mass spectrum of peracetylsongarosaponin E (**4**) was found not only the tetrasaccharide unit (*m/z* 1137) and the aglycone moiety (*m/z* 523) $[C_{34}H_{51}O_5-16]^+$, but also the molecular ion (*m/z* 1676). Further fragment ions were observed at *m/z* 331 and at *m/z* 1329 $[M-16-331]^+$ corresponding to the terminal position of a hexose in the glycosidic part of **4**. The ion at *m/z* 998 $[M-(16+2\times331)]^+$ was in accord with two terminal hexoses. The disaccharide fragment ion at *m/z* 619 confirmed the linkage of two hexoses. Based on this fragmentation pattern, a terminal hexose and a disaccharide unit must be linked at the inner 6-deoxyhexose.

In the ^{1}H NMR spectrum of **4** (500 MHz, $CDCl_3$), the presence of three β-D-glucopyranosyl units and a β-D-fucopyranosyl unit was established by the doublets of four anomeric proton signals at δ 4.66 (J = 8.0 Hz), 4.60 (J = 8.0 Hz), 4.53 (J = 8.0 Hz), 4.24 (J = 7.7 Hz) and the doublet at δ 1.14 (J = 6.4 Hz) due to a methyl group. A detailed analysis of the ^{1}H NMR data of the carbohydrate chain of **4** (in $CDCl_3$) revealed that H-4′ fucose at δ 5.18 showed a remarkable downfield shift in comparison with H-2′ (δ 3.80) and H-3′ (δ 3.71). Therefore, fucose must be acetylated in position C-4′ and linked to two glucoses at position C-2′ and C-3′ fucose. The highfield shift of H-4″ of the 1,4 linked β-D-glucose compared to H-4‴ and H-4⁗ of the terminal glucoses (δ 5.06, 5.09) was due to the glycosylation at C-4″ glucose. Within the range of measuring accuracy, the chemical shifts and coupling constants (in $CDCl_3$) of the sugar protons of **4** and **5** coincide with the sugar protons of peracetylsongarosaponin C [2] and D [3]. 2D ROESY experiments of the sugar chain sequence of peracetylsongarosaponin C confirmed the above described glycosidic linkages and furthermore revealed that the cellobiose unit was attached to C-3′ fucose.

Concerning the most representative signals, the ^{1}H NMR spectrum (500 MHz, pyridine-d_5) of **1** showed six tertiary methyl groups characterized by the singlets at δ 0.79, 0.86, 0.94, 0.94, 1.01 and 1.06. The assignments of the protons were performed by means of ^{1}H–^{1}H COSY 45° experiments of the glycoside (**1**) in pyridine-d_5 and of its peracetate (**4**) in $CDCl_3$ (Table 1). The signals at δ 6.37

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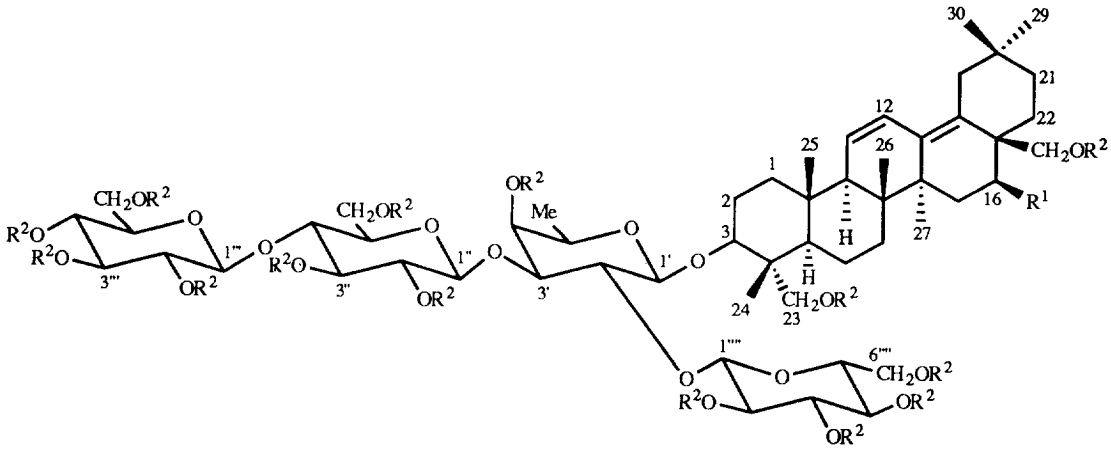

	R^1	R^2
1	H	H
2	OH	H
4	H	Ac
5	OAc	Ac

Table 1. ^{1}H NMR spectral data of the triterpenoid moieties of **4** and **5**

H	**4**($CDCl_3$)	**5**($CDCl_3$)
$2_{ax}/2_{eq}$	1.82/1.92	1.82/1.94
3_{ax}	3.48 (*dd*, $J_{2eq,3ax}$ = 4.7 Hz, $J_{2ax,3ax}$ = 11.2 Hz)	3.48 (*dd*, $J_{2eq,3ax}$ = 4.8 Hz, $J_{2ax,3ax}$ = 11.4 Hz)
9_{ax}	1.92	1.93
11	6.37 (*dd*, $J_{11,12}$ = 10.7 Hz, $J_{9ax,11}$ = 3.0 Hz)	6.38 (*dd*, $J_{11,12}$ = 10.7 Hz, $J_{9ax,11}$ = 2.9 Hz)
12	5.55 (*brd*, $J_{11,12}$ = 10.7 Hz)	5.60 (*brd*, $J_{11,12}$ = 10.7 Hz)
15_{ax}		1.88 (*dd*, $J_{15ax,15eq}$ = 12.3 Hz, $J_{15ax,16ax}$ = 12.8 Hz)
15_{eq}		1.27 (*dd*, $J_{15eq,15ax}$ = 12.3 Hz, $J_{15eq,16ax}$ = 4.4 Hz)
16_{ax}		5.06 (*dd*, $J_{15ax,16ax}$ = 12.8 Hz, $J_{15eq,16ax}$ = 4.4 Hz)
$22_{ax}/22_{eq}$	2.40/1.67	2.42/1.71
23a	4.05 (*d*, $J_{23a,23b}$ = 11.2 Hz)	4.03 (*d*, $J_{23a,23b}$ = 11.5 Hz)
23b	4.24 (*d*, $J_{23a,23b}$ = 11.2 Hz)	4.26 (*d*, $J_{23a,23b}$ = 11.5 Hz)
24–27	0.68 (*s*, 3H)	0.76 (*s*, 3H)
	0.76 (*s*, 6H)	0.769 (*s*, 3H)
	0.89 (*s*, 3H)	0.773 (*s*, 3H)
		0.89 (*s*, 3H)
28a	3.95 (*d*, $J_{28a,28b}$ = 11.3 Hz)	4.07 (*d*, $J_{28a,28b}$ = 11.7 Hz)
28b	4.16 (*d*, $J_{28a,28b}$ = 11.3 Hz)	4.49 (*d*, $J_{28a,28b}$ = 11.7 Hz)
29–30	0.93 (*s*, 6H)	0.95 (*s*, 3H)
		1.08 (*s*, 3H)

and 5.55 of **4** were assigned to the olefinic protons H-11 and H-12. The remarkable downfield shift of H-11 in comparison with that of peracetylsongarosaponin D indicated a conjugated diene, established by the four olefinic ^{13}C NMR signals (125 MHz, $CDCl_3$) at δ125.4, 126.5, 133.2 and 137.0 (Table 2). Based on these observations, **1** was suggested to possess a diene moiety at C-11, 13 (18). The chemical shifts of the protons at the oxygen bearing carbon atoms C-3, C-23 and C-28 of **1**, which were superimposed by the sugar protons in the ^{1}H NMR, were located by the cross-peaks in the ^{1}H–^{1}H COSY 45° experiment. The existence of two acylable hydroxyl functions in the aglycone was confirmed by acetylation of **1** to tetradecaacetylsongarosaponin E (**4**). The significant downfield shift of the C-3 signal (δ83.8) in the ^{13}C NMR spectrum of **4** in comparison with triterpene A confirmed the glycosylation in position 3 [5]. The ^{13}C NMR data of the aglycone of **4** were in good agreement with those of peracetylsongarosaponin A [2]. Consequently, the structure of songarosaponin E (**1**) was determined to be 3-*O*-{[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranosyl}-olea-11,13-diene-3β,23,28-triol.

Songarosaponin F (**2**) showed a very similar ^{1}H NMR spectrum to **1**. The ^{1}H NMR and the ^{1}H–^{1}H COSY 45° experiments revealed that both saponins possess the same

Table 2. ^{13}C NMR spectral data of the triterpenoid moieties of **4** and **5** in $CDCl_3$

C			4		5
1	CH_2		38.1[a]		37.7
2	CH_2		25.1[a]		25.1
3	CH		83.8		83.7
4	C		41.8[c]		41.8
5	CH		47.5		47.5
6	CH_2		17.9		17.8[c]
7	CH_2		32.2		32.3
8	C		40.3		40.3
9	CH		54.0		54.0
10	C		36.2		36.2
11	CH=		126.5		127.2
12	CH=		125.4		125.1
13	C		137.0		136.9
14	C		37.9		42.1[a]
15	CH_2		33.0		30.9
16		CH_2	24.3[b]	CH	77.6
17	C		42.1[c]		43.9[a]
18	C		133.2		131.0
19	CH_2		37.6[a]		37.7
20	C		32.9		32.0
21	CH_2		34.9		34.8
22	CH_2		30.1		29.7
23	CH_2		65.4[d]		63.7[b]
24	Me		12.5		12.5
25	Me		18.3		18.3[c]
26	Me		*		16.8
27	Me		16.6		20.5
28	CH_2		65.7[d]		65.3[b]
29	Me		32.2		32.8
30	Me		24.1		24.2

[a-d]Assignments may be reversed.
*Superimposed with acetyl moieties.

sugar chain sequence. An additional acetyl group in the ^1H NMR spectrum of peracetylsongarosaponin F (**5**) in contrast to **4** indicated a further hydroxyl function in the aglycone moiety of **2**. Indeed, in the ^{13}C spectrum of **5**, the signals belonging to the carbon atoms of ring A–C and E, and of their substituents were coincident with those obtained for **4** (Table 2), while the signals of the ring D carbons and of its substituents showed significant downfield shifts at C-14, C-16, C-17 and C-27 due to a hydroxyl group at C-16. In the ^1H–^1H COSY 45° experiment of **5** in $CDCl_3$, the double doublet of H-16 at δ5.06 ($J_{15ax,\,16ax}$ = 12.8 Hz, $J_{15eq,\,16ax}$ = 4.4 Hz) was coupled with H-15_{ax} at δ1.88 and with H-15_{eq} at δ1.27, indicating the axial configuration of H-16 (Table 1).

A comparison of the ^{13}C NMR data of **5** and those of saikogenin A (olea-11,13-diene-3β,16β,23,28-tetrol) showed the presence of saikogenin A as an aglycone in **2** [6]. The ^{13}C NMR chemical shifts of the aglycone atoms of **5** in $CDCl_3$ were assigned according to those of saikogenin A in pyridine-d_5, because up to now no ^{13}C NMR data of peracetylsaikogenin A ($CDCl_3$) exist. In order to consider the shifts originated by acetylation and solvent change (peracetylsaikogenin A in $CDCl_3$ in comparison with saikogenin A in pyridine-d_5), the ^{13}C NMR data of the aglycone atoms of peracetylsongarosaponin A in $CDCl_3$ and songarosaponin A (aglycone; olea-11,13-diene-3β,23,28-triol) in pyridine-d_5 were compared as well [2].

Since no acidic conditions were employed during extraction and isolation, we can reasonably assume that **1** and **2** are primary constituents of the plant, and not artifacts derived from the 13,28-ether precursor [7].

Recently, Yamamoto *et al.* [4] isolated from *Buddleja japonica* (Buddlejaceae), buddlejasoponin I, whose oligosaccharide chain was identical with that of songarosaponin A and B.

Verbascum songaricum yields saikosaponin-like compounds, containing oleanane derivatives as aglycone and different combinations of glucose, fucose and rhamnose. Saikosaponins are considered to be the major pharmacological active components in *Bupleurum* species (Umbelliferae) [6–8], *Corchorus acutangulus* (Tiliaceae) [9] and *Polycarpone loeflingiae* (Caryophyllaceae) [10]. Besides their haemolytic effect [11], which is generally known from monodesmosidic saponins, some of them exhibit antiviral [12], cytotoxic [8], antiinflammatory [13], antihepatotoxic [14] and plasma-cholesterol lowering activities [15]. Therefore, the pharmacological activity profile of songarosaponins is of interest in view of their structural similarity to the saikosaponins.

EXPERIMENTAL

General. ^1H NMR spectra were recorded at 500 MHz. ^{13}C NMR were obtained at 125 MHz. The negative ion mass spectra were recorded on an electron attachment mass spectrograph (Finnigan MAT 8500). The matrix for the liquid SIMS was glycerine. CC was carried out on silica gel 60 (0.063–0.2 mm), TLC on silica sheets (0.3 mm, HF_{254}) and prep. TLC on silica gel sheets (1 mm, PF_{254}). The spots were detected with 'triterpene reagent' (1% soln of vanillin in 50% H_3PO_4), which indicated the aglycone moiety, and with 'sugar reagent' (4% ethanolic aniline–4% ethanolic diphenylamine–H_3PO_4, 5:5:1), which indicated the carbohydrate moiety.

Isolation. The plants were cultivated from seeds in the gardens of the Institute for Plant Biochemistry, Halle (a voucher specimen of *V. songaricum* is deposited in the herbarium of the Institute). The dried and powdered aerial parts (160 g) of *V. songaricum* were extracted with MeOH at 22°. The solvent was concd under red. pres. at 50°. In the crude extract **1** and **2** could be detected by HPLC on reversed phase silica gel (RP-8) eluted with MeCN–H_2O (1:5). The crude saponin fr. (900 mg) was obtained by repeated CC on silica gel by eluting with $CHCl_3$–MeOH-mixts. This fr. was first chromatographed on prep. TLC with $CHCl_3$–MeOH–H_2O (74:23:3) and then subjected to HPLC on reversed phase silica gel (RP-8) eluted with MeCN–H_2O (1:5) to yield 3 mg **1**, 2 mg **2** and 1 mg **3**.

Songarosaponin E (**1**). ($C_{54}H_{88}O_{22}$, M_r 1089.29); $[\alpha]_D^{24}$ +24° (pyridine; *c* 0.04); liquid SIMS negative ion mode *m/z* (rel. int.): 1087 $[M-1]^-$ (100), 925 $[M-1-Glc]^-$

(43), 763 [M−1−(2×Glc)]⁻ (51), 601 [M−1−(3×Glc)]⁻ (22), 455 [M−1−(3×Glc+Fuc)]⁻ (13), 453 (14); ^{1}H NMR (500 MHz, pyridine-d_5): δ6.55 (1H, *dd*, *J* = 10.6, 3.0 Hz, H-11), 5.66 (1H, *br d*, *J* = 10.6 Hz, H-12), 5.59 (1H, *d*, *J* = 8.0 Hz, G''''-1), 5.27 (1H, *d*, *J* = 8.0 Hz, G''-1), 5.19 (1H, *d*, *J* = 8.0 Hz, G'''-1), 4.92 (1H, *d*, *J* = 7.8 Hz, F'-1), 4.42 (1H, H-23b), 4.19 (1H, H-3_{ax}), 4.08 (1H, H-28b), 3.73 (1H, H-23a), 3.72 (1H, H-28a). 2.50 (1H, *d*, *J* = 13.9 Hz, H-22_{ax}), 2.28 (1H, H-2_{eq}), 2.08 (1H, H-9_{ax}), 2.02 (1H, *dd*, *J* = 11.7, 3.3 Hz, H-2_{ax}), 1.84 (1H, H-1_{eq}), 1.80 (1H, H-22_{eq}), 1.40 (3H, *d*, *J* = 6.4 Hz, F'-6), 1.08 (1H, H-1_{ax}), 1.06 (3H, *s*),1.01 (3H, *s*), 0.94 (6H, *s*), 0.86 (3H, *s*) 0.79 (3H, *s*).

Peracetylsongarosaponin E (**4**). EIMS 70 eV *m/z* (rel. int.): 1676 [M]⁺ (14), 1617 (23), 1515 (3), 1329 (8), 1269 (4), 1137 (16), 998 (3), 619 (19), 559 (17), 523 (18), 463 (87), 403 (18), 331 (100), 271 (43), 229 (19), 211 (19), 169 (72), 127 (14), 109 (36), 43 (17); ^{1}H NMR (500 MHz) of the aglycone moiety: Table 1; ^{1}H NMR (500 MHz, $CDCl_3$) of the sugar sequence: δ 1.14 (3H, *d*, *J* = 6.4 Hz, F'-6), 3.44 (1H, *m*, G'''-5), 3.59 (1H, *m*, G''''-5), 3.60 (1H, *m*, F'-5), 3.66 (1H, *m*, G'''-5), 3.71 (1H, *dd*, *J* = 9.8, 3.4 Hz, F'-3), 3.80 (1H, *dd*, *J* = 7.7, 9.8 Hz, F'-2), 3.84 (1H, *dd*, *J* = 9.4, 9.7 Hz, G''-4), 3.97 (1H, *m*, G''-6a), 4.01 (1H, *m*, G''''-6a), 4.03 (1H, *m*, G'''-6a), 4.24 (1H, *d*, *J* = 7.7 Hz, F'-1), 4.31 (1H, *m*, G''''-6b), 4.39 (1H, *m*, G'''-6b), 4.53 (1H, *d*, *J* = 8.0 Hz, G'''-1), 4.60 (1H, *d*, *J* = 8.0 Hz, G''''-1), 4.66 (1H, *d*, *J* = 8.0 Hz, G''-1), 4.69 (1H, *m*, G''-6b), 4.87 (1H, *dd*, *J* = 8.0, 9.3 Hz, G''-2), 4.88 (1H, *dd*, *J* = 8.0, 9.3 Hz, G''''-2), 4.90 (1H, *dd*, *J* = 8.0, 9.3 Hz, G'''-2), 5.03 (1H, *dd*, *J* = 9.3, 9.4 Hz, G''-3), 5.06 (1H, *dd*, *J* = 9.4, 9.7 Hz, G'''-4), 5.09 (2H, G''''-3, G''''-4), 5.12 (1H, *dd*, *J* = 9.3, 9.4 Hz, G'''-3), 5.18 (1H, *dd*, *J* = 3.4, 1.0 Hz, F'-4); ^{13}C NMR (125 MHz): Table 2.

Songarosaponin F (**2**). ($C_{54}H_{88}O_{23}$, M_r 1105.29); $[\alpha]_D^{24}$ +26° (pyridine; *c* 0.04); liquid SIMS negative ion mode *m/z* (rel. int.): 1103 [M−1]⁻ (19), 941 [M−1−Glc]⁻ (11), 779 [M−1−(2×Glc)]⁻ (11), 617 [M−1−(3×Glc)]⁻ (4), 471 [M−1−(3×Glc+Fuc)]⁻ (3), 469 (3); ^{1}H NMR (500 MHz, pyridine-d_5): δ6.50 (1H, *dd*, *J* = 11.3, 3.2 Hz, H-11), 5.66 (1H, *d*, *J* = 11.3 Hz, H-12), 5.59 (1H, *d*, *J* = 8.0 Hz, G''''-1), 5.27 (1H, *d*, *J* = 8.0 Hz, G''-1), 5.19 (1H, *d*, *J* = 8.0 Hz, G'''-1), 4.92 (1H, *d*, *J* = 7.8 Hz, F'-1), 1.40 (3H, *d*, *J* = 6.4 Hz, F'-6), 1.05 (6H, *s*), 0.95 (3H, *s*), 0.93 (3H, *s*), 0.85 (3H, *s*), 0.83 (3H, *s*).

Peracetylsongarosaponin F (**5**). EIMS 70 eV *m/z* (rel. int.): 1734 [M]⁺ (2), 1674 (7), 1615 (12), 1614 (11), 1387 (7), 1137 (6), 996 (2), 619 (15), 581 (12), 559 (13), 521 (15), 461 (67), 401 (15), 331 (100), 271 (34), 229 (14), 211 (11), 169 (70), 127 (7), 109 (28), 43 (15); ^{1}H NMR (500 MHz) of the aglycone moiety: Table 1; ^{13}C NMR (125 MHz): Table 2.

Buddlejasaponin I (**3**). ($C_{54}H_{88}O_{22}$, M_r 1089.29); liquid SIMS negative ion mode *m/z* (rel. int.): 1087 [M−1]⁻ (19), 941 [M−1−Rha]⁻ (7), 925 [M−1−Glc]⁻ (6), 779 [M−1−(Glc+Rha)]⁻ (11), 617 [M−1−(2×Glc+Rha)]⁻ (7), 471 [M−1−(2×Glc+Rha+Fuc)]⁻ (4), 439 (6); ^{1}H NMR (500 MHz, pyridine-d_5): δ5.96 (1H, *d*, *J* = 10.7 Hz, H-11), 5.86 (1H, *d*, *J* = 1.1 Hz, R'''-1), 5.63 (1H, *d*, *J* = 10.7 Hz, H-12), 5.59 (1H, *d*, *J* = 7.9 Hz, G''''-1), 5.27 (1H, *d*, *J* = 7.9 Hz, G''-1), 4.92 (1H, *d*, *J* = 7.8 Hz, F'-1), 4.50 (1H, H-16_{ax}), 4.39 (1H, H-23a), 4.38 (1H, H-28a), 4.17 (1H, H-3_{ax}), 3.71 (1H, H-23b), 3.33 (1H, H-28b), 2.49 (1H, *d*, *J* = 13.0 Hz, H-22_{ax}), 1.74 (3H, *d*, *J* = 6.2 Hz, R'''-6), 1.38 (3H, *d*, *J* = 6.4 Hz, F'-6), 1.37 (3H, *s*, H-26), 1.32 (1H, H-22_{eq}), 1.08 (3H, *s*, H-27), 1.05 (3H, *s*, H-24), 0.95 (3H, *s*, H-25), 0.91 (3H, *s*, H-29), 0.88 (3H, *s*, H-30).

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