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# STRUCTURE OF HISTAMINE RELEASING GUAIANOLIDES FROM *THAPSIA* SPECIES

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**Key Word Index**—*Thapsia garganica*; *T. villosa*; *T. maxima*; Apiaceae; thapsigargin; thapsigargicin; thapsitranstagin; thapsivillosin A–J; hexanoic acid; octanoic acid; 6-methylheptanoic acid; 6-methyloctanoic acid; sesquiterpene lactone; guaianolide; histamine.

**Abstract**—The structures of six hexaoxygenated guaianolides esterified with four non isomeric carboxylic acids have been established by $^1$H NMR and $^{13}$C NMR spectroscopy. The acyl residues have been located by chemical ionisation mass spectrometry. Some of the acyl residues are novel for sesquiterpene lactones. The acyl residues in two additional guaianolides esterified with isomeric acids have been located by partial hydrolysis.

## INTRODUCTION

Species of the umbelliferous genus *Thapsia*, which according to Flora Europoea consists of the three species *T. garganica*, *T. maxima* and *T. villosa*, have been utilized in medicine since ancient time [1]. Phytochemical investigations have led to the isolation of eight hexaoxygenated and two pentaoxygenated guaianolides which are very potent non-cytotoxic histamine releasing compounds [2, 3]. The biological properties make these compounds interesting tools for mediator release studies [3]. Except for thapsigargin (**1**) and thapsigargicin (**2**) [4, 5] no attempts have been made to elucidate the stereochemistry of the sesquiterpene nucleus or to locate the acyl residues in any of the isolated guaianolides. This paper reports assignment of stereochemistry to the hexaoxygenated guaianolide nuclei as evidenced by NMR spectroscopy and localizations of the acyl residues on the nucleus as evidenced by chemical ionisation mass spectrometry or partial hydrolysis.

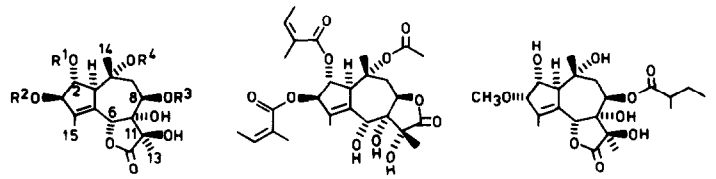
In addition, the structures of minor constituents isolated from extracts of *T. garganica*, *T. villosa* and *T. maxima* are described.

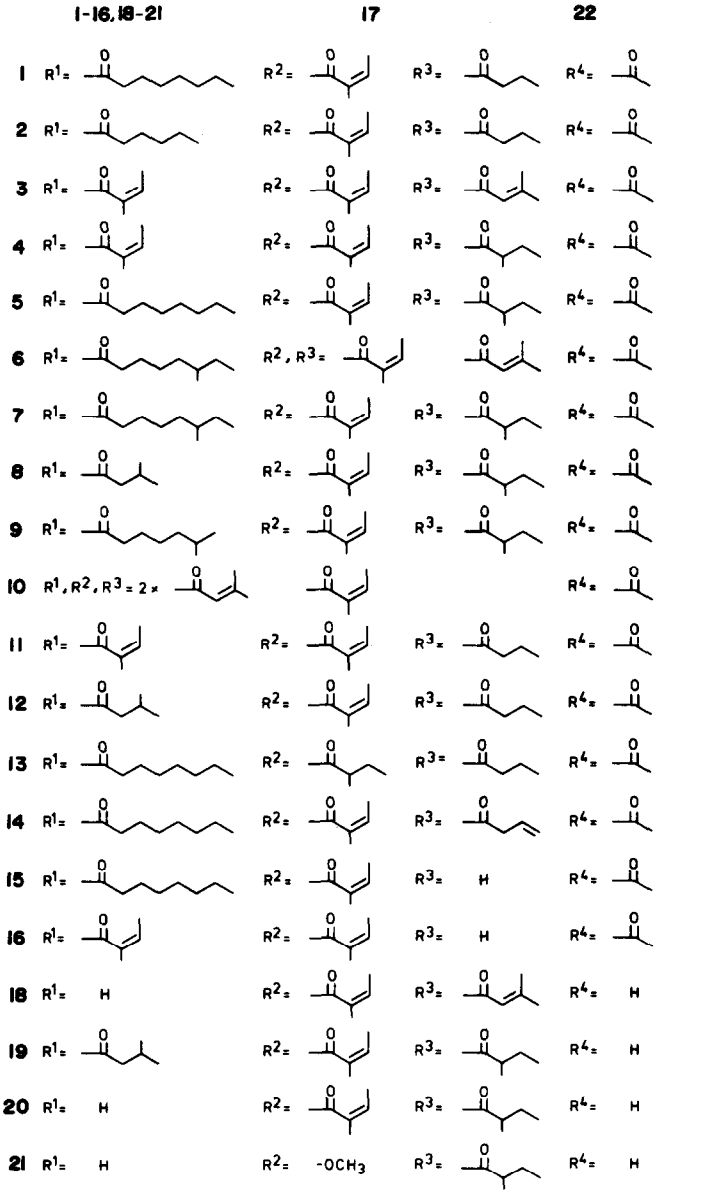
## RESULTS AND DISCUSSION

The sesquiterpene lactones were purified by chromatography over silica gel, followed by chromatography over silanized silica gel and by preparative HPLC. Besides the previously isolated but not structurally elucidated six hexaoxygenated guaianolides, thapsivillosin A, B, C, D and E (**3**, **4**, **5**, **6** and **7**, respectively), and thapsitranstagin (**8**) [2], further minor constituents were isolated, *viz.* thapsivillosin G (**9**) from *T. villosa*, thapsivillosin H (**10**) from *T. maxima*, and thapsivillosin I and J (**11** and **12**) from *T. garganica*. The total amount of skin irritating compounds was found to vary between 0.1 and 0.5 per cent in all of the investigated fresh plant materials.

In the $^{13}$C NMR spectra of thapsigargin (**1**), thapsigargicin (**2**), and the compounds **3**–**12**, fifteen signals were found at the same chemical shift values, within the limits of uncertainty. Based on this finding it was concluded that all the compounds possess the same hexaoxygenated guaianolide nucleus and carry free hydroxy groups at C-7 and C-11. Likewise, in the $^1$H NMR spectra, the invariant chemical shift values and coupling constants of the signals assignable to the protons attached to the sesquiterpene nucleus also support the presence of a common guaianolide moiety. The identities, but not the positions, of the acyl residues of the natural products were ascertained by interpretation of the $^1$H NMR and $^{13}$C NMR spectra. The calculated molecular weights were confirmed by chemical ionization mass spectrometry using *iso*-butane as reagent gas.

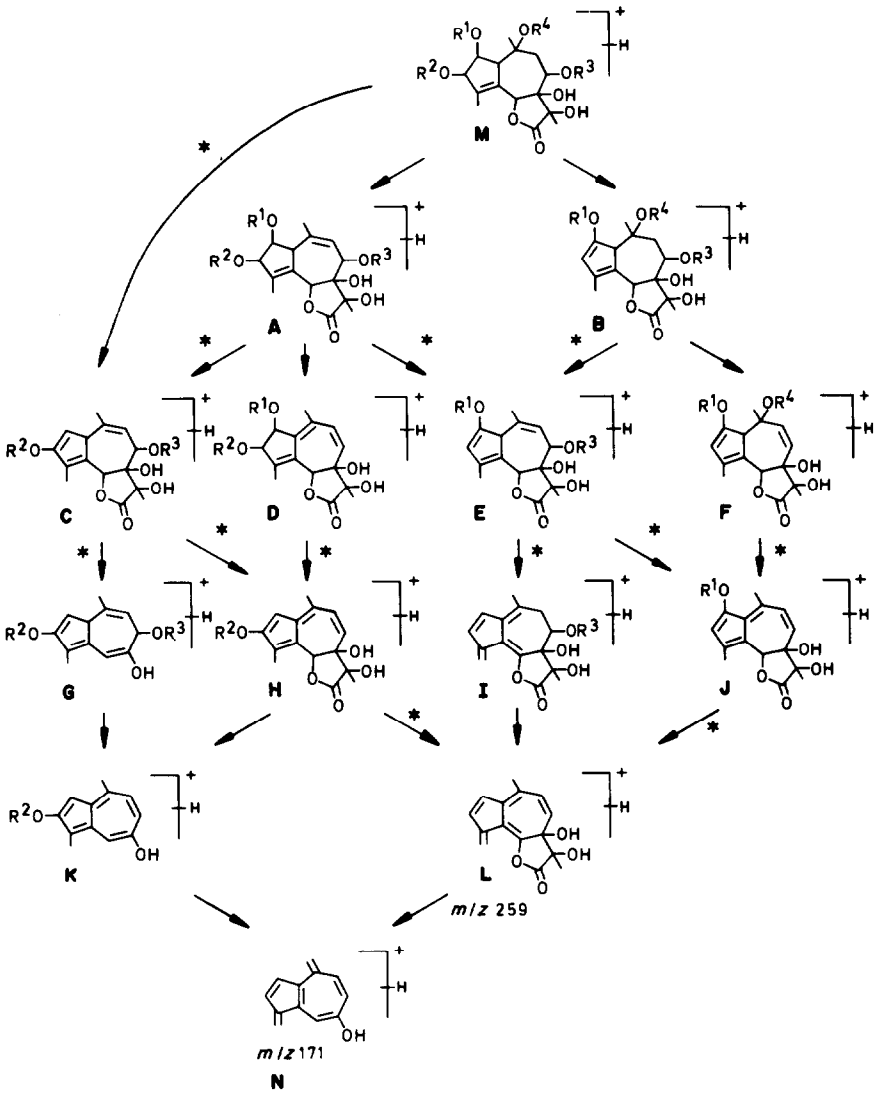
Scheme 1 shows the predominant part of the fragmentation pattern of compound **1**, involving successive eliminations of carboxylic acid moieties and, at later stages, loss of a $C_3H_4O_3$ unit from the lactone ring. The same pattern was found in the spectra of other compounds of known structure: **2**, **13** (prepared by catalytic hydrogenation of **1**) and **14** (obtained by treatment of **15** with vinylacetic anhydride). Assuming that this scheme holds for all the isolated guaianolides, we are able to locate non-isomeric acyl groups, as shown in the following analysis. The first acids to be lost from the molecular ion are those esterified with the tertiary and allylic hydroxy groups at C-10 and C-3, producing ions **A** and **B**, respectively. Subsequent losses of carboxylic acids ultimately give ion **L**, which, being devoid of acyl groups, is common to the spectra of all the compounds. Loss of a $C_3H_4O_3$ fragment from ion **L** can then lead to ion **N**, likewise common to all the spectra. As indicated in Scheme 1 some of the processes are supported by metastable ions. If these were the only fragmentation routes it would not be possible to locate the four acyl residues. Fortunately, unique ions are found, namely ions **G** and **K**. The ion **G**, formally of azulenic structure, is formed, as supported by a metastable ion, by loss of the $C_3H_4O_3$ fragment from ion **C**. Ion **K** may be formed from both **G** and **H**. The differences in mass between ions **K** and **N** and between ions **G** and **K**






equal the molecular weights of the acids esterified with O-3 and O-8, respectively. Since, as noted above, formation of ions **A** and **B** is due to losses of acyl residues attached to O-10 and O-3 it is now possible to calculate the molecular weight of the acid esterified with O-10. The fourth acid, then, must be located at C-2.

Four of the isolated lactones, thapsitranstagin (**8**), thapsivillosin A (**3**), D (**6**) and H (**10**), contain isomeric acyl groups. Shortage of material prevented further characterization of **6** and **10**, whereas partial saponification of **3** and **8** enabled localization of the acyl residues. Treatment of **3** with dilute potassium hydroxide afforded three products **16**, **17** and **18**, which were identified by comparison of their NMR spectra with those of the analogous products obtained from **1** [5]. The difference in chemical shifts of H-8 in the spectra of **3** and **16** proves the location of the senecioyl group. The locations of one of the angeloyl moieties and the acetyl moiety at O-2 and O-10, respectively, appeared from the chemical ionisation mass spectrum.




Scheme 1. Dominating fragmentation pattern in the chemical ionisation mass spectra of compounds **1–14** using *iso*-butane as reagent gas. Processes supported by metastable ions are indicated with an asterisk. No single spectrum exhibits all metastable ions.

Acid hydrolysis of **8** in methanol yielded a mixture of four products (**19–22**), all of which contain an α-methylbutanoyl group. Comparison of the chemical shift values of the protons attached to the sesquiterpene nuclei of **8** and **19–22** established the locations of the acyl residues. NOE experiments proved the positions of the methoxy groups in **21** and **22**. The relative configurations at C-3 of these compounds follow from the magnitudes of the H-2 and H-3 couplings. In the *trans*-isomer (**21**) the value (3.5 Hz) equals the value observed in the natural product (**8**), compared to 6.1 Hz for the *cis*-epimer (**22**). A similar relationship has been observed in five membered rings, which cannot deviate appreciably from planarity [6]. X-ray crystallography revealed deviations of less than 0.08A from least-square of the carbons of the cyclopentene ring of the 7,11-epoxide of **1**. According to NMR-studies the same conformations of the cyclopentene and cycloheptane rings of **1** and its 7,11-epoxide are preferred [4, 5]. The formation of the two epimeric methyl ethers, **21** and **22**, suggest that an $A_{AL}$ 1 mechanism [7] operates in the cleavage of the angeloyl ester of **8**.

Although all the acids present in the natural products **1–12** have been found in higher plants no oxygenated sesquiterpene lactone esterified with hexanoic, octanoic, 6-methylheptanoic, or 6-methyloctanoic acid has previously been described [8]. 6-Methylheptanoic and 6-methyloctanoic acids are derived from isoleucine and valine, respectively, in *Bacillus colistinus* [9].

Further investigations are necessary to evaluate the significance of the observed invariant locations of the acyl residues bound to two or more of the investigated hexaoxygenated guaianolides.

## EXPERIMENTAL

Chemical ionisation mass spectra were obtained on a VG 70-70

Table 1. Dominating ions in the chemical ionisation mass spectra of compounds **1–14** using *iso*-butane as reagent gas [*m/z* values (rel. int.)]

| | $[M]^+$ | A | B | C | D | E | F | G | H | I | J | K | L | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 651 (17) | 591 (36) | 551 (78) | 447 (68) | 503 (12) | 491 (100) | 463 (27) | 359 (57)† | 359 (57)† | 347 (17) | 403 (39) | 271 (52) | 259 (34) | 171 (56) |
| **2** | 623 (6) | 563 (21) | 523 (49) | 447 (66) | 475 (11) | 463 (100) | 435 (29) | 359 (78)† | 359 (78)† | 347 (22) | 375 (40) | 271 (57) | 259 (26) | 171 (20) |
| **3** | 619 (7) | 559 (28) | 519 (51) | 459 (100)* | 459 (100)* | 459 (100)* | 419 (13) | 371 (49) | 359 (47)† | 359 (47)† | 359 (47)† | 271 (64) | 259 (26) | 171 (50) |
| **4** | 621 (6) | 561 (14) | 521 (49) | 461 (100)* | 459 (8) | 461 (100)* | 419 (12) | 373 (33) | 359 (30)† | 361 (15) | 359 (30)† | 271 (57) | 259 (16) | 171 (40) |
| **5** | 665 (18) | 605 (40) | 565 (80) | 461 (62) | 503 (10) | 505 (100) | 463 (26) | 373 (34) | 359 (22) | 361 (17) | 403 (23) | 271 (59) | 259 (20) | 171 (62) |
| **6** | 677 (6) | 617 (21) | 577 (34) | 459 (25) | 517 (71)* | 517 (71)* | 477 (62) | 317 (17) | 359 (49)† | 359 (49)† | 417 (69) | 271 (49) | 259 (27) | 171 (44) |
| **7** | 679 (14) | 619 (36) | 579 (76) | 461 (55) | 517 (12) | 519 (100) | 477 (22) | 373 (42) | 359 (26) | 361 (21) | 417 (29) | 271 (71) | 259 (26) | 171 (68) |
| **8** | 623 (16) | 563 (33) | 523 (71) | 461 (65)* | 461 (65)* | 463 (100) | 421 (12) | 373 (36) | 359 (17) | 361 (32) | 361 (32) | 271 (53) | 259 (17) | 171 (39) |
| **9** | 665 (3) | 605 (8) | 565 (15) | 461 (17) | 503 (41) | 505 (30) | 463 (100) | 373 (13) | 359 (70) | 361 (11) | 403 (96) | 271 (28) | 259 (29) | 171 (18) |
| **10** | 619 (7) | 559 (15) | 519 (46) | 459 (77)* | 459 (77)* | 459 (77)* | 419 (89) | 371 (18) | 359 (100)† | 359 (100)† | 359 (100)† | 271 (36) | 259 (24) | 171 (30) |
| **11** | 607 (5) | 547 (16) | 507 (65) | 447 (100)* | 459 (7) | 447 (100)* | 419 (9) | 359 (41)† | 359 (41)† | 347 (11) | 359 (41)† | 271 (29) | 259 (15) | 171 (26) |
| **12** | 609 (4) | 549 (15) | 509 (33) | 447 (34) | 461 (51) | 449 (56) | 421 (100) | 359 (88)* | 359 (88)* | 347 (11) | 361 (97) | 271 (53) | 259 (31) | 171 (24) |
| **13** | 653 (0.3) | 593 (5) | 551 (18) | 449 (17) | 505 (51) | 491 (25) | 463 (56) | 361 (77)* | 361 (77)* | 347 (4) | 403 (100) | 273 (19) | 259 (15) | 171 (7) |
| **14** | 649 (1) | 589 (3) | 549 (5) | 445 (6) | 503 (40) | 489 (8) | 463 (100) | 357 (3) | 359 (55) | 345 (2) | 403 (80) | 271 (9) | 259 (13) | 171 (3) |

In all spectra a prominent peak appeared at *m/z* 199. In the spectrum of **6** this peak was base peak.

*†In the same horizontal line: isomeric ions.

instrument equipped with a dual EI/CI ion source, using the direct inlet system. Reagent gas *iso*-butane; ion source temperature 220°. The NMR spectra were obtained on a Bruker HX-270S spectrometer in the FT mode by using quadrature detection.

After concn of an ethanolic extract of powdered dried root material the residue was distributed between $H_2O$ and EtOAc. The upper phase was concd and the residue chromatographed over silica gel using $CH_2Cl_2$–EtOAc as an eluent. The fractions showing skin irritating properties were rechromatographed on silanized silica gel RP2 (Merck 0.063–0.200 mm) using aq. MeOH as an eluent. Final purification was performed by preparative HPLC over LiChrosorb RP18 (Knauer Pre-Packed 16 × 250 mm) using aq. MeOH as an eluent and detecting the peaks by monitoring UV absorption at 254 nm and by refractive index. All the compounds were isolated as colourless, amorphous powders.

$^1$H *NMR data for compounds* **1–14** ($CDCl_3$, TMS): the chemical shift values of the protons of the sesquiterpene nucleus correspond to the values given for **1** and **2** [5]. Acyl residues acetyl $\delta$1.9 (*s*); angeloyl 6.1 (*q*), 2.0, 1.9; butyryl 2.3, 1.6, 0.9 (*t*); hexanoyl and octanoyl 2.3, 1.6, 1.3, 0.9 (*t*); 2-methylbutyryl 2.3, 1.7, 1.5, 1.1 (*d*), 0.9 (*t*); 3-methylbutyryl 2.3, 0.9 (*d*); 6-methylheptanoyl 2.3, 1.6–1.1, 0.8 (*d*); 6-methyloctanoyl 2.3, 1.6, 1.3, 1.1, 0.9; senecioyl 5.8, 2.2 (*s*), 2.0.

$^{13}$C *NMR data for compounds* **1–14** ($CD_3OD$, TMS): sesquiterpene nucleus $\delta$59.1 (C-1), 79.5 (C-2), 85.8 (C-3), 141.4 (C-4), 133.1 (C-5), 78.4 (C-6), 79.7 (C-7), 67.7 (C-8), 39.5 (C-9), 86.3 (C-10), 79.7 (C-11), 178.7 (C-12), 16.2 (C-13), 23.4 (C-14), 13.2 (C-15); acetyl 172.7 (C-1), 23.0 (C-2); angeloyl 169.2, 128.8, 140,0, 16.4, 20.9; butyryl 174.4, 37.7, 19.2, 14.7; hexanoyl 174.5, 35.4, 25.9, 32.6, 23.6, 14.6; 2-methylbutyryl 176.3, 42.7, 27.3, 12.0, 16.7; 3-methylbutyryl 173.4, 44.2, 27.2, 22.8; 6-methyloctanoic 174.1, 35.6, 26.3, 27.7, 35.2, 37.5, 30.6, 11.8, 19.6; 6-methylheptanoic 174.3, 35.2, 27.2, 27.9, 39.8, 29.0, 23.0; octanoyl 174.4, 35.3, 26.1, 30.1, 30.1, 32.9, 23.7, 14.3; senecioyl 166.2, 117.1, 158.8, 20.4, 27.4.

*Hydrolysis of thapsitranstagin* (**8**). To a soln of **8** (48 mg) in MeOH (1.6 ml) was added $H_2O$ (0.2 ml) and $CF_3COOH$ (40 $\mu$l) and the mixture was left in a sealed vessel for 72 hr at 92°. After concn the residue was chromatographed over Lichrosorb RP 18 (Knauer Pre-Packed 8 × 250 mm) using MeOH–0.5% AcOH (5:1) as an eluent to yield: starting material (12.3 mg). Compound **19** (2.4 mg); $^1$H NMR ($CDCl_3$, TMS): $\delta$5.76, 5.66 (H-3 or H-6), 5.55, 5.26 (*t*, *J* = 3.5 Hz, H-2 or H-6) 3.53 (H-1), 2.34 (*dd*, *J* = 3 and 14 Hz, H-9$\alpha$), 1.91 (H-15), 1.50 (H-13), 1.19 (H-14). Compound **20** (7.7 mg); $^1$H NMR (DMSO-$d_6$, TMS): $\delta$5.56, 5.49 (H-3 or H-6), 5.38 (*t*, *J* = 3.5 Hz, H-8), 4.18 (*t*, *J* = 3.5 Hz, H-2), 3.29 (H-1), 2.33 (*dd*, *J* = 3 and 14 Hz, H-9$\alpha$), 1.69 (H-15), 1.24 (H-13), 0.98 (H-14). In **19** and **20** the signal of H-9$\beta$ is hidden under the signals of the acyl residues. Compound **21** (7.81 mg); $^1$H NMR (DMSO-$d_6$, TMS): $\delta$5.52 (H-6), 5.35 (*t*, *J* = 3.5 Hz, H-8), 4.07 (*t*, *J* = 3.5 Hz, H-2), 3.82 (H-3), 3.38 (MeO), 3.15 (H-1), 2.27 (*dd*, *J* = 3 and 14 Hz, H-9$\alpha$), 1.74 (H-15), 1.62 (*dd*, *J* = 3 and 14 Hz, H-9$\beta$), 1.22 (H-13), 0.95 (H-14). Irridation of the methoxy-signal affords an 11 per cent increment of the H-3 signal. Compound **22** (1.5 mg); $^1$H NMR (DMSO-$d_6$, TMS): $\delta$5.46 (H-6), 5.35 (*t*, *J* = 3.5 Hz, H-8), 4.29 (*dd*, *J* = 3.5 and 6.1 Hz, H-2), 3.93 (*d*, *J* = 6.1 Hz, H-3), 3.37 (MeO and H-1), 2.39 (*dd*, *J* = 3 and 14 Hz, H-9$\alpha$), 1.78 (H-15), 1.60 (*dd*, *J* = 3 and 14 Hz, H-9$\beta$), 1.23 (H-13), 0.93 (H-14). Irridation of the methoxy signal affords a 6 per cent increment of the H-3 signal.

*Hydrolysis of thapsivillosin A* (**3**). To a soln of **3** (22.6 mg) in MeOH (1.13 ml) was added aq. 2 M NaOH (1.13 ml) and the mixture was left for 15 min at 20°. The reaction was stopped by addition of 4 M HCl (1.13 ml). $H_2O$ (10 ml) was added and the soln extracted with $Et_2O$ (3 × 10 ml). The combined $Et_2O$ extracts were concd and the residue was chromatographed over Lichrosorb RP 18 (Knauer Pre-Packed 8 × 250 mm) using MeOH–0.5% AcOH (5:2) as an eluent to yield: starting material (5.4 mg). Compound **18** (2.9 mg); $^1$H NMR ($CDCl_3$, TMS): $\delta$5.69, 5.32 (H-6 or H-3), 5.50 (*t*, *J* = 3.5 Hz, H-8), 4.26 (*t*, *J* = 3.5 Hz, H-2), 3.40 (H-1), 2.35 (*dd*, *J* = 3.5 and 14 Hz, H-9$\alpha$), 1.90 (H-15), 1.48 (H-14), 1.15 (H-13). The signal of H-9$\beta$ was hidden under the signal of the acyl moieties. Compound **17** (3.6 mg); $^1$H NMR ($CDCl_3$, TMS): $\delta$5.70 (H-3), 5.47 (*t*, *J* = 3 Hz, H-2), 5.13 (*dd*, *J* = 3 and 14 Hz, H-8), 4.65 (H-6), 3.59 (H-1), 2.84 (*dd*, *J* = 3 and 16 Hz, H-9$\alpha$), 1.90 (H-15), 1.80 (*dd*, *J* = 14 and 16 Hz, H-9$\beta$), 1.65 (H-14), 1.48 (H-13). Compound **16** (8.1 mg); $^1$H NMR ($CDCl_3$, TMS): $\delta$5.81, 5.79 (H-3 or H-6), 5.8 (*t*, *J* = 3.5 Hz, H-2), 4.33 (*t*, *J* = 3.5 Hz, H-8), 4.24 (H-1), 2.87 (*dd*, *J* = 3.5 and 14 Hz, H-9$\alpha$), 2.50 (*dd*, *J* = 3.5 and 14 Hz, H-9$\beta$), 1.9 (H-15), 1.49 (H-14), 1.47 (H-13).

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