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MONO-TETRAHYDROFURAN RING ACETOGENINS FROM *GONIOTHALAMUS DONNAIENSIS*

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Key Word Index—*Goniothalamus donnaiensis*; Annonaceae; roots; acetogenins; mono-THF rings; γ-hydroxymethyl-γ-lactone; goniodonin; 34-*epi*-goniodonin; *cis*-goniodonin; 34-*epi-cis*-goniodonin.

Abstract—A new type of annonaceous acetogenin was isolated from an ethanolic extract of the roots of *Goniothalamus donnaiensis*. The structures of two epimeric pairs, goniodonin and 34-*epi*-goniodonin, *cis*-goniodonin and 34-*epi-cis*-goniodonin, characterized by the presence of a γ-hydroxymethyl-γ-lactone moiety, were elucidated by spectral data and chemical derivatization. Two known mono-THF acetogenins, 2,4-*cis*- and *trans*-gigantetrocinone, were also found. © 1997 Elsevier Science Ltd

INTRODUCTION

The annonaceous acetogenins are a new group of powerful bioactive agents and over 200 acetogenins have been isolated from several genera; three types of γ-lactone ring have been reported [1]. Our investigation on the ethanolic extract of the roots of *Goniothalamus donnaiensis* resulted in the isolation of two novel epimeric pairs belonging to a new subclass of acetogenins, characterized by the presence of a γ-hydroxymethyl-γ-lactone, *viz.* goniodonin (**1**) and 34-*epi*-goniodonin (**1′**), *cis*-goniodonin (**2**) and 34-*epi-cis*-goniodonin (**2′**).

RESULTS AND DISCUSSION

Dried pulverized roots of *G. donnaiensis* were extracted with 95% EtOH and the residue of the extract F_1, was partitioned through a standard extraction scheme (see Experimental). The extract F_5 was subjected to repeated open and flash silica gel column chromatography to yield a mixture of **1**(**1′**) and **2**(**2′**). Analytical reverse-phase HPLC of the mixture failed to resolve them; normal-phase analytical HPLC, however, gave near-baseline separation of **1**(**1′**) and **2**(**2′**).

Compounds **1** and **1′**, eluted on repeated reverse-phase and normal phase HPLC, gave a sharp single peak. Mass spectrometry and elemental analysis indicated a molecular formula of $C_{35}H_{64}O_8$ and an identical carbon skeleton. However, the ^{13}C NMR spectrum revealed duplication of several signals that appeared at δ 69.1–71.0; 105.0–105.3, 131.7–132.4, 150.7–149.6 and 172.0–172.8, with a relative intensity of 55–45 for all of them, suggesting the presence of two epimeric compounds. ^1H NMR, ^1H–^1H COSY and ^{13}C–^1H COSY indicated that the chemical shift of protons H-3 and H-4 were obviously different for **1** and **1′**. H-3a and H-3b of **1** were observed at δ 2.27 (*dq*) and 2.51 (*dd*), respectively, correlating with the carbon signal at δ 33.1; H-4 of **1** were at δ 3.89 (*m*) correlating with the carbon at δ 69.1. In the case of **1′**, H-3a and H-3b resonated at δ 2.41 (*m*) correlating with the carbon at δ 32.0; H-4 appeared at δ 3.78 (*m*) correlating with the carbon at 71.0. In the HMBC spectrum, the carbon signal that appeared at δ 69.1 correlated with the protons at δ 2.27 and 2.51, while the carbon at δ 71.0 correlated with the protons at δ 2.41. Accordingly, it is possible to assign the NMR data for **1** and **1′** (Tables 1 and 2). The spectral characteristics in the γ-lactone moiety of **1** and **1′** clearly distinguished them from previously known acetogenins. Firstly, in the ^1H NMR spectrum, the signal for H-34 (*ca* δ 5.04 or 4.50) disappeared and the H-35 protons exhibited a signal at δ 1.66 (*s*) instead of 1.42 (*d*). Secondly, in the ^{13}C NMR spectrum, the signals at δ 105.0–105.3 (C-34) replaced the signal at *ca* δ 77.9 (or 82.5). These data indicated that for **1** and **1′**, H-34 was substituted by a hydroxyl; this was confirmed by a DEPT experiment, which indicated that C-34 of **1**(**1′**) was a quaternary carbon.

In order to confirm that **1** and **1′** were epimeric at C-34, like some similar lactol compounds [2, 3],

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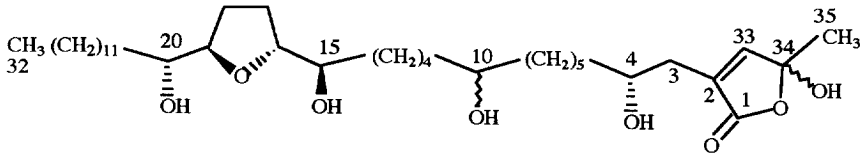
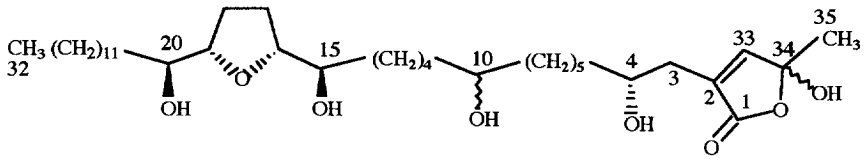


1 (1')



2 (2')

phenylhydrazone derivatives (**3**) were prepared [4]. The mechanism of formation of **3** was nucleophilic addition, the lactol ring opening with the nucleophilic reagent to form a Schiff base followed by aminolysis of ester. In the ^{13}C NMR spectrum of **3**, all the signals appeared to be singlets; in its ^{1}H NMR spectrum, the signals for H-4, H-3a, and H-3b appeared at δ 3.92 (*m*), 2.70 (*dq*) and 2.86 (*dd*), respectively, suggesting that it was one compound. The NMR spectra of the mixture of **1** and **1'** and the derivative **3** (Tables 1 and 2) indicated that both are mono-THF acetogenins with a flanking hydroxyl group on both sides. The positions of the THF ring and the hydroxyl groups were established by EI mass spectrometry (Fig. 1) and further confirmed by the analogous EI mass spectral fragmentations of **3** and its *per*-TMSi derivative (**3a**) (Fig. 2).

The relative configurations of the THF system of **1** and **1'** were assigned as *threo/trans/threo* from the ^{1}H NMR signals at δ 3.41 (*m*, 2H, H-15, 20), 3.78 (*m*, 2H, H-16, 19), 1.97 (*m*, 2H, H-17a, 18a) and 1.63 (*m*, 2H, H-17b, 18b) and ^{13}C NMR resonances at δ 74.4 (1C, C-15), 74.3 (1C, C-20), 82.5 (1C, C-16), 82.7 (1C, C-19) and 28.9 (2C, C-17, C-18) [5, 6]. The absolute stereochemistry at C-4, C-15 and C-20 was assigned by studying the *per*-Mosher ester derivatives (**3s** and **3r**) of **3** [7]. The ^{1}H NMR chemical shift data of **3s** and **3r** showed that the absolute configuration at C-4

Table 1. ^{1}H (500 MHz, *J* in Hz) NMR resonances (δ) for compounds **1**, **1'**, **3**, **3s** and **3r**

Position	**1**	**1'**	**3***	**3s**	**3r**	**Δ3s–3r**
35	1.66, *s*	1.66, *s*	2.38, *s*	2.19	2.23	negative
33	6.95, *s*	6.95, *s*	7.08, *s*	6.76	6.90	negative
3a	2.27, *dq* (14.1, 9.3)	2.41, *m*	2.70, *dq* (13.9, 8.2)	2.68	2.74	negative
3b	2.51, *dd* (14.1, 4.2)	2.41, *m*	2.86, *dd* (13.9, 2.6)	3.03	3.07	negative
4	3.89, *m*	3.78, *m*	3.92, *m*	5.53	5.50	*R*
5	1.2–1.6, *m*	1.2–1.6, *m*	1.2–1.6, *m*	1.62	1.60	positive
6–9	1.2–1.6, *m*	1.2–1.6, *m*	1.2–1.6, *m*	—	—	—
10	3.63, *m*	3.63, *m*	3.58, *m*	—	—	—
11–13	1.2–1.6, *m*	1.2–1.6, *m*	1.2–1.6, *m*	—	—	—
14	1.2–1.6, *m*	1.2–1.6, *m*	1.2–1.6, *m*	1.50	1.46	positive
15	3.41, *m*	3.41, *m*	3.40, *m*	4.89	5.02	*R*
16	3.78, *m*	3.78, *m*	3.79, *m*	3.88	4.00	negative
17a, 18a	1.97, *m*	1.97, *m*	1.97, *m*	1.64	1.92	negative
17b, 18b	1.63, *m*	1.63, *m*	1.63, *m*	1.35	1.62	negative
19	3.78, *m*	3.78, *m*	3.79, *m*	3.92	4.00	negative
20	3.41, *m*	3.41, *m*	3.40, *m*	4.95	5.02	*R*
21	1.2–1.6, *m*	1.2–1.6, *m*	1.2–1.6, *m*	1.58	1.52	positive
22–31	1.2–1.6, *m*	1.2–1.6, *m*	1.2–1.6, *m*	—	—	—
32	0.88, *t* (6.8)	0.88, *t* (6.8)	0.88, *t* (6.8)	—	—	—

* Aromatic signals: δ 7.37 (1H, *t*), 7.46 (2H, *t*), 7.56 (2H, *d*).

Table 2. ^{13}C (125 MHz) NMR resonances (δ) for compounds **1**, **1′**, **3**, **2**, **2′** and **4**

Position	1	1′	3*	2	2′	4*
1	172.0	172.8	161.5	172.0	172.8	161.5
2	131.7	132.4	132.0	131.7	132.4	132.0
3	33.1	32.0	33.4	33.1	31.9	33.4
4	69.1	71.0	70.7	69.0	70.8	70.7
5–9	22–38	22–38	22–40	22–38	22–38	22–40
10	71.5	71.5	71.7	71.4	71.4	71.7
11–14	22–38	22–38	22–40	22–38	22–38	22–40
15	74.4	74.4	74.1	74.5	74.5	74.1
16	82.5	82.5	82.7	82.6	82.6	82.7
17, 18	28.9	28.9	28.8	28.1	28.1	28.1
19	82.7	82.7	82.7	82.6	82.6	82.7
20	74.3	74.3	74.0	74.2	74.2	74.0
21–31	22–38	22–38	22–40	22–38	22–38	22–40
32	14.1	14.1	14.1	14.1	14.1	14.1
33	150.7	149.6	141.7	150.7	149.6	141.7
34	105.0	105.3	145.4	105.0	105.3	145.4
35	24.3	24.3	21.0	24.3	24.3	21.0

* Aromatic signals: δ 125.6 (2C), 128.2 (1C), 128.8 (2C), 142.0 (1C).

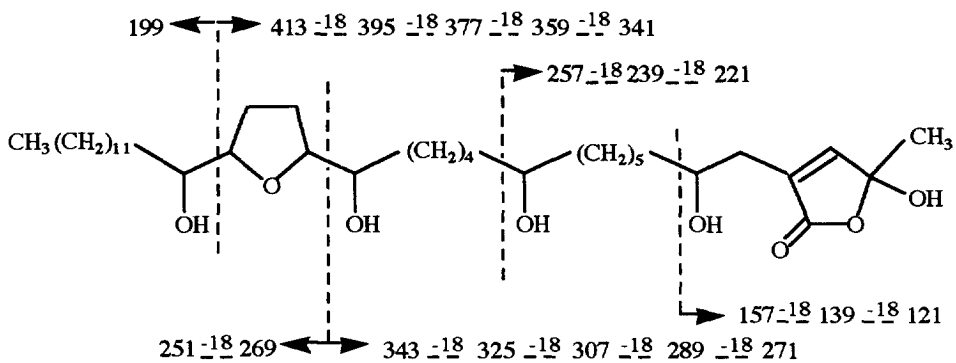


Fig. 1. Diagnostic EI mass spectral fragment ions (m/z) of compounds **1**(**1′**) and **2**(**2′**).

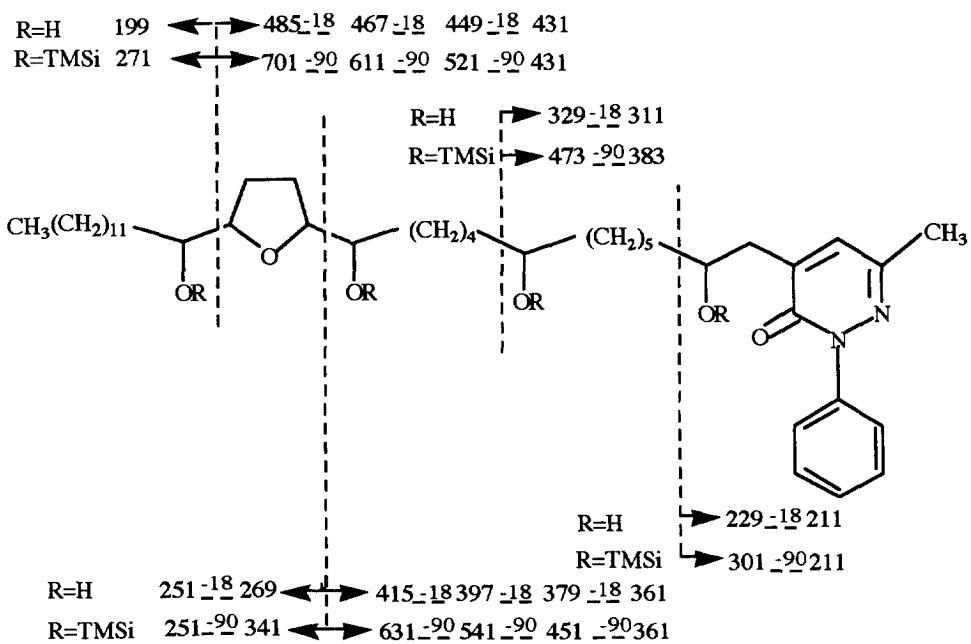


Fig. 2. Diagnostic EI mass spectral fragment ions (m/z) of compounds **3**, **4** (R = H), **3a**, and **4a** (R = TMSi).

Table 3. ^{1}H (500 MHz, J in Hz) NMR resonances (δ) for compounds **2**, **2′**, **4**, **4s** and **4r**

Position	**2**	**2′**	**4***	**4s**	**4r**	$\Delta\delta$**4s–4r**
35	1.66, *s*	1.66, *s*	2.38, *s*	2.19	2.23	negative
33	6.95, *s*	6.95, *s*	7.08, *s*	6.75	6.90	negative
3a	2.26, *dq* (14.1, 9.3)	2.41, *m*	2.70, *dq* (13.9, 8.2)	2.68	2.74	negative
3b	2.51, *dd* (14.1, 4.2)	2.41, *m*	2.86, *dd* (13.9, 2.6)	3.03	3.07	negative
4	3.89, *m*	3.78, *m*	3.92, *m*	5.53	5.50	*R*
5	1.2–1.6, *m*	1.2–1.6, *m*	1.2–1.6, *m*	1.62	1.60	positive
6–9	1.2–1.6, *m*	1.2–1.6, *m*	1.2–1.6, *m*	—	—	—
10	3.63, *m*	3.63, *m*	3.58, *m*	—	—	—
11–13	1.2–1.6, *m*	1.2–1.6, *m*	1.2–1.6, *m*	—	—	—
14	1.2–1.6, *m*	1.2–1.6, *m*	1.2–1.6, *m*	1.71	1.69	positive
15	3.41, *m*	3.41, *m*	3.40, *m*	4.86	4.92	*R*
16	3.78, *m*	3.78, *m*	3.79, *m*	4.06	4.10	negative
17a	1.93, *m*	1.93, *m*	1.93, *m*	1.79	1.80	negative
17b	1.63, *m*	1.63, *m*	1.63, *m*	1.34	1.38	negative
18a	1.93, *m*	1.93, *m*	1.93, *m*	1.42	1.46	negative
18b	1.63, *m*	1.63, *m*	1.63, *m*	0.82	0.86	negative
19	3.78, *m*	3.78, *m*	3.79, *m*	3.85	3.87	negative
20	3.41, *m*	3.41, *m*	3.40, *m*	5.06	5.04	*S*
21	1.2–1.6, *m*	1.2–1.6, *m*	1.2–1.6, *m*	1.32	1.35	positive
22–31	1.2–1.6, *m*	1.2–1.6, *m*	1.2–1.6, *m*	—	—	—
32	0.88, *t* (6.8)	0.88, *t* (6.8)	0.88, *t* (6.8)	—	—	—

* Aromatic signals: δ 7.37 (1H, *t*), 7.46 (2H, *t*), 7.56 (2H, *d*).

of **1** and **1′** is *R*. Similarly, the Mosher ester data allowed the absolute stereochemical assignment of the carbinol centres adjacent to the mono-THF ring in **1** and **1′** as C-15 *R* and C-20 *R* (Table 1). The absolute configuration at C-10 could not be ascertained by direct application of the Mosher ester method, because the chemical shifts of H-9 and H-11 were virtually indistinguishable by ^{1}H NMR.

Compounds **2** and **2′** exhibited similar properties to those of **1** and **1′**. Repeated reverse-phase and normal-phase HPLC gave a sharp peak. Mass spectral and elemental analysis indicated a molecular formula $C_{35}H_{64}O_8$ and an identical carbon skeleton. The ^{1}H and ^{13}C NMR spectra showed duplication of several signals with a ratio of 55–45, suggesting that **2** and **2′** were epimeric at C-34, like **1** and **1′**. This was confirmed by the formation of the phenylhydrazone derivative (**4**). As for **1**(**1′**), the NMR spectra of the mixture of **2** and **2′** and the derivative **4** (Tables 2 and 3) indicated that **2** and **2′** are mono-THF acetogenins with a flanking hydroxyl group on both sides. The positions of the THF ring and the hydroxyl groups were established by EI mass spectrometry (Fig. 1) and further confirmed by the analogous fragmentations of **4** and its *per*-TMSi derivative (**4a**) (Fig. 2). Differences between **1**(**1′**) and **2**(**2′**) were observed in their NMR spectra: in **2** and **2′**, the protons of H-17a, 18a resonated at δ 1.93 instead of 1.97, H-17b, 18b appeared at δ 1.74 instead of 1.63, and C-17, 18 appeared at δ 28.1 instead of 28.8. These observations indicated that the rings in **2** and **2′** had the *threo*/*cis*/*threo* configuration [6, 8].

The absolute stereochemistry of C-4 in **2** and **2′** was assigned by the preparation of the *per*-Mosher ester derivatives (**4s** and **4r**) of **4**. ^{1}H COSY analyses of these derivatives were then performed. The ^{1}H NMR chemical shifts (Table 4) indicate the C-4*R* configuration. Similarly, the absolute configuration of the THF system of **2**(**2′**) was assigned as 15*R*, 16*R*, 19*S*, 20*S* by studying the Mosher ester data (Table 3). This result was identical to all *cis*-mono-THF ring acetogenins examined so far [9].

The mixtures of **1**(**1′**) and **2**(**2′**) showed potent cytotoxicity against the HCT-8 (human colon adenocarcinoma) cells ($IC_{50} < 10\ \mu g\ ml^{-1}$).

EXPERIMENTAL

General. Mps are uncorr. ^{1}H NMR (500 MHz), COSY (500 MHz) and ^{13}C NMR (125 MHz) spectra (all in $CDCl_3$ with TMS as int. standard) were obtained on a Bruker AM-500 spectrometer. HPLC was carried out using a silica gel (7 μm) column (250 × 4.6 mm) equipped with a UV detector. Analytical TLC was performed on silica gel developed with $CHCl_3$–MeOH (9:1) and cyclohexane–EtOAc–MeOH (6:3:1), and visualized by spraying with 5% H_2SO_4 in EtOH, followed by heating.

Bioassays. Cytotoxicity of isolated compounds against human solid tumour cells was measured in 5-day MTT assays at the Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences.

Plant material. Dried roots of *G. donnaiensis* Finet et Gagnap were collected from Long Jin County, Guangxi Provience, People's Republic of China. A voucher specimen has been deposited in the herbarium of Guangxi College of Traditional Chinese Medicine.

The plant material was pulverized using an electric mill.

Extraction and isolation. Pulverized roots (9.5 kg) were extracted exhaustively with 95% EtOH to yield extract F1 (2.05 kg), which was partitioned between H_2O and $CHCl_3$ (1:1), giving the H_2O-soluble fr. F2 (448 g), the $CHCl_3$-soluble fr. F3 (820 g) and the insoluble interface fr. F4 (201 g). F3 was then partitioned between 90% aq. MeOH–petrol (1:1) to yield the petrol-soluble fr. F6 (42 g) and the aq. MeOH-soluble fr. F5 (638 g). F5 (91 g) was applied to a column of silica gel (120–180 mesh), eluting with $CHCl_3$ containing gradually increasing amounts of MeOH. Impure components were combined according to TLC analysis and these were again subjected to repeated chromatography to yield crude **1**(**1′**) and **2**)**2′**). The mixts of **1**(**1′**) and **2**(**2′**) were sepd by silica gel HPLC eluting with cyclohexane–ether–MeOH (10:3:1, flow rate 1 ml min^{-1}) to afford the two amorphous powders **1**(**1′**) and **2**(**2′**) (R_ts 30.7 and 28.6 min, respectively).

Goniodonin (**1**) *and* 34-epi-*goniodonin* (**1′**). White, amorphous powder, mp 78–80°. $[\alpha]_D^{18}$ +3.4° (MeOH, *c* 0.17). IR ν_{max}^{KBr}, 3514, 2921, 2852, 1734, 1467 cm^{-1}. FABMS *m/z*: $[M+Na]^+$ 635. EIMS (70 eV, direct, inlet) *m/z* (rel. int.): $[MH-H_2O]^+$ 595 (3), 577 (3), 559 (5), 541 (9), 523 (4), 413 (1), 395 (1), 377 (5), 359 (35), 341 (18), 343 (4), 325 (2), 307 (90), 289 (65), 271 (15), 257 (2), 239 (25), 221 (5), 157 (5), 139 (15), 121 (30). Elemental analysis (%), found: C, 68.80, H, 10.40; calc. for $C_{35}H_{64}O_8$: C, 68.63, H, 10.46. ^1H NMR and ^{13}C NMR: Tables 1 and 2.

cis-*Goniodonin* (**2**) *and* 34-epi-cis-*goniodonin* (**2′**). White, amorphous powder, mp 80–82°. $[\alpha]_D^{18}$ +3.8° (MeOH, *c* 0.10). IR ν_{max}^{KBr}, 3413, 2920, 2851, 1735, 1465 cm^{-1}. FABMS *m/z*: $[M+Na]^+$ 635. EIMS (70 eV, direct, inlet) *m/z* (rel. int.): $[MH-H_2O]^+$ 595 (3), 577 (3), 559 (5), 541 (8), 523 (4), 413 (1), 395 (2), 377 (5), 359 (30), 341 (15), 343 (5), 325 (2), 307 (93), 289 (68), 271 (12), 257 (3), 239 (20), 221 (8), 157 (3), 139 (20), 121 (35). Elemental analysis (%), found: C, 68.45, H, 10.51; calcd for $C_{35}H_{64}O_8$: C, 68.63, H, 10.46. ^1H NMR and ^{13}C NMR: Tables 3 and 2.

Phenylhydrazone derivatives of **1**(**1′**) *and* **2**(**2′**). A mixt. of 4 mg sample and 1 mg phenylhydrazine in 5 ml EtOH was refluxed for 2 hr. The viscous mass (**3** or **4**) which was obtained after removal of solvent *in vacuo* was purified by prep. TLC.

Compound **3**. White powder. EIMS (70 eV, direct. inlet) *m/z* (rel. int.): $[M]^+$ 684 (3), 666 (2), 648 (2), 630 (2), 612 (1), 485 (18), 467 (16), 449 (10), 431 (4), 415 (6), 397 (65), 379 (15), 361 (3), 329 (12), 229 (23). ^1H NMR and ^{13}C NMR: Tables 1 and 2.

Compound **4**. White powder. EIMS (70 eV, direct. inlet) *m/z* (rel. int.): $[M]^+$ 684 (4), 666 (3), 648 (3), 630 (2), 612 (2), 485 (15), 467 (13), 449 (8), 431 (5), 415 (5), 397 (70), 379 (20), 361 (5), 329 (15), 229 (25). ^1H NMR and ^{13}C NMR: Tables 3 and 2.

TMSi derivatives of **3** *and* **4**. Samples of less than 1 mg were treated with 20 μl of *N,O-bis*(trimethylsilyl)-acetamide and 2 μl of pyridine and heated at 70° for 30 min to yield the respective TMSi derivatives (**3a** and **4a**).

Compound **3a**. EIMS (70 eV, direct. inlet) *m/z* (rel. int.): 701 (95), 611 (38), 521 (10), 431 (5), 631 (100), 541 (30), 451 (8), 361 (3), 473 (23), 301 (8).

Compound **4a**. EIMS (70 eV, direct. inlet) *m/z* (rel. int.): 701 (90), 611 (40), 521 (5), 431 (8), 631 (100), 541 (35), 451 (10), 361 (5), 473 (18), 301 (10).

MTPA derivatives of **3** *and* **4**. (*R*)-(+) or (*S*)-(−)-methyl α-(trifluoromethyl)phenylacetic acid (MTPA, 15 mg) and *N,N′*-dicyclohexylcarbodiimide (DCC, 10 mg) were added to a 1.5 mg sample of **3** or **4** dissolved in dry CH_2Cl_2 with a few crystals of (dimethylamino)pyridine. The mixt. was stirred at room temp. for 6 hr and the product (**3s**, **3r**, **4s** or **4r**) was purified by prep. TLC. **3s** and **3r**. Colourless oil. ^1H NMR resonances: Table 1. **4s** and **4r**. Colourless oil. ^1H NMR resonances: Table 3.

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