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# New pregnane glycosides from *Caralluma negevensis*

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Dedicated to the memory of Professor Serena Catalano

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**Abstract**—Twenty new pregnane glycosides were isolated from the whole plant of *Caralluma negevensis*. Their structures were elucidated by extensive spectroscopic methods including 1D-($^1$H, $^{13}$C, $^{13}$C DEPT, TOCSY, ROESY) and 2D-NMR experiments (DQF-COSY, HSQC, HMBC, HOHAHA) as well as ESI-MS analysis. Pregnane glycosides were tested for their cytotoxic and genotoxic activity. © 2002 Elsevier Science Ltd. All rights reserved.

## 1. Introduction

The plants of the family Asclepiadaceae are known to contain cytotoxic and tumoricidal *C/D-cis*-polyoxypregnane esters and glycosides.[1] *Caralluma negevensis* Zohary is a succulent perennial herb occurring wild on the rocky desert of East Saharo–Arabian subregion,[2] and it is used by Bedouins to treat chronic lung diseases, such as tuberculosis and cancer.

Previous phytochemical studies on plants of the genus *Caralluma* have reported the isolation of several pregnane glycosides and/or their ester,[3,4] some of which showed antitumor activity, and others were postulated as precursors of cardenolides.[1]

In the course of our screening for biologically active natural products, twenty new pregnane glycosides (compounds **1**–**20**), along with two known pregnane glycosides russelioside B and russelioside C,[4] were isolated from the whole plant of *C. negevensis*. The structures of these compounds are based on the known polyoxypregnane skeleton of boucerin,[5,6] calogenin,[4,7] or 5α-dihydrocalogenin,[8] as well as acetyl, tigloyl, and *o*-hydroxybenzoyl ester moieties linked at C-12 and/or at C-20 of the aglycons. In addition all compounds possess an oligosaccharide portion linked at C-3 and/or C-20 to the aglycon consisting of one to six sugar unit, well known to occur in Asclepiadaceae plants.

This paper deals with the isolation of these pregnane glycosides and their structural elucidation by means of one- and two-dimensional NMR techniques ($^1$H, $^{13}$C, $^{13}$C DEPT, 1D-TOCSY, 1D-ROESY, DQF-COSY, HSQC, HMBC, HOHAHA), ESI-MS, and with evaluation of cytotoxic[9] and genotoxic[10,11] activity on some of them selected on the basis of their structural differences.

## 2. Results and discussion

The chloroform and methanol extracts of the whole plant of *C. negevensis* were processed as described in the Section 3 to afford twenty new pregnane glycosides (**1**–**20**) together with the known derivatives russelioside B and russelioside C.

The compounds can be sorted into three series by the nature of the pregnane aglycons: compounds **1**–**16** are characterized by the boucerin aglycon, compounds **17**–**19** have the calogenin structure, and compound **20** presents the 5α-dihydrocalogenin skeleton. $^1$H and $^{13}$C NMR assignments for all compounds **1**–**20** (Section 3 and Tables 1–4) were based on the analysis of NMR spectral data ($^1$H, $^{13}$C, DEPT, DQF-COSY, 1D-TOCSY, HOHAHA, HSQC, HMBC).

*Compounds **1**–**16***. ESI-MS and NMR data of compounds **1**–**16** indicated that they were derivatives of the *C/D-cis*-polyoxypregnane boucerin by comparison with aglycon data previously reported in the literature.[5,6] In addition to the pregnane moiety, the $^1$H and $^{13}$C NMR spectra of compounds **1**–**3** and **5**–**16** showed signals due to tigloyl and/or acetyl and/or *o*-hydroxybenzoyl groups. The ester

*Keywords*: pregnane glycosides; *Caralluma negevensis*; cytotoxicity; genotoxicity.

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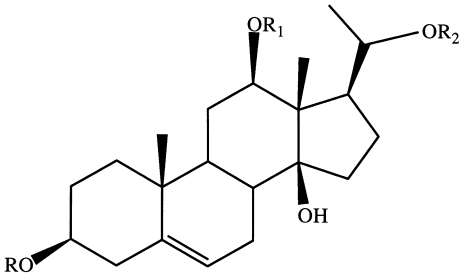
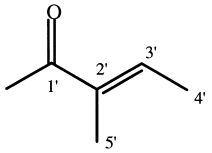
linkages were located at C-12 and/or C-20 on the basis of the chemical shifts of the double doublet of H-12 ($\delta\sim4.60$ when esterified, $\delta\sim3.65$ when free) and the quartet of H-20 ($\delta\sim4.95$ when esterified, $\delta\sim3.80$ when free) (see Experimental Section). This was confirmed by the results of HMBC experiments which showed clear long-range correlation peaks between the carbonyl carbon of the tigloyl, acetyl, and *o*-hydroxybenzoyl groups and H-12 and/or H-20 of the aglycons.

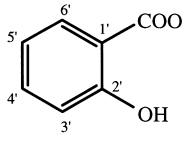



| Compounds | R | $R_1$ | $R_2$ |
|---|---|---|---|
| **1** | E | H | Tig |
| **2** | E | Tig | Tig |
| **3** | F | Tig | Ac |
| **4** | G | H | H |
| **5** | G | *o*-OH-Bz | H |
| **6** | G | Tig | Ac |
| **7** | G | Tig | Tig |
| **8** | G | Tig | H |
| **9** | W | Tig | H |
| **10** | W | Tig | Tig |
| **11** | W | *o*-OH-Bz | H |
| **12** | W | Ac | H |
| **13** | I | Tig | Tig |
| **14** | M | H | Tig |
| **15** | M | Tig | Tig |
| **16** | L | Tig | Ac |

Tig = tigloyl




*o*-OH-Bz = *o*-hydroxybenzoyl




Ac = acetyl

*Compounds **1** and **2** (chain E).* Compounds **1** and **2** had molecular formulae $C_{53}H_{86}O_{19}$ and $C_{58}H_{92}O_{20}$, respectively, deduced from ESI-MS and NMR analysis. An examination of their NMR spectra revealed signals of protons and carbons attributed to aglycon 20-*O*-tigloylboucerin for compound **1** and 12β, 20-*O*-ditigloylboucerin for compound **2**, respectively. In addition to the aglycon signals, $^{13}C$ NMR spectra of both compounds exhibited 27 signals ascribable to the saccharide portion made up of three 3-*O*-methyl-2,6-dideoxyhexopyranosyl and one hexopyranosyl units. A detailed comparison of their sugar region NMR spectra showed that the saccharide chain was identical in the two compounds. Their $^1H$ NMR spectra also supported the above results by the presence of four anomeric protons at $\delta$ 4.48, 4.65, 4.83, and 4.89, three doublet methyls at $\delta$ 1.21, 1.23, 1.40, and three *O*-methoxyl at $\delta$ 3.47, 3.48, 3.50. All proton signals due to the four sugar units (Table 2) were assigned by a DQF-COSY experiment together with HOHAHA and 1D-TOCSY experiments which allowed the sequential assignments of hydrogens from H-1 to H-6 within each sugar unit starting from the anomeric and methyl signals. The β-linkages of the four sugar moieties were shown by the large ($J\approx9.0$ Hz) coupling constants of the anomeric proton signals as well as by the resonances of C-2, C-3, and C-5 characteristic of β forms.[12,13] The assignments of all protonated carbons were accomplished by interpretation of the HSQC NMR spectrum and allowed therefore the identification of the sugars as a terminal β-D-glucopyranosyl as well as a β-D-oleandropyranosyl and two β-D-cymaropyranosyl inner units. Each sugar unit was glycosylated at C-4 as shown by the glycosidation shifts observed for $C\text{-}4_{cym\ I}$, $C\text{-}4_{cym\ II}$, and $C\text{-}4_{ole}$ (Table 2). Direct evidence for the sugar sequence and their linkage sites was derived from the results of HMBC experiment which showed unequivocal correlation peaks between $H\text{-}1_{cym\ I}$–C-3, $H\text{-}1_{cym\ II}$–$C\text{-}4_{cym\ I}$, $H\text{-}1_{ole}$–$C\text{-}4_{cym\ II}$, $H\text{-}1_{glc}$–$C\text{-}4_{ole}$. It is also interesting to note that C-1 of D-cymarose characteristically resonates upfield (97.0 ppm) when linked at C-3 of the aglycon in contrast with the resonance at 101.0 ppm when it is linked to the hydroxyl group of a sugar unit.[14] On the basis of this NMR evidence, the structure of the sugar chain of compounds **1** and **2** was determined to be β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

*Compound **3** (chain F).* The aglycon moiety of compound **3** was determined to be 12β-*O*-tigloyl-20-*O*-acetylboucerin by spectroscopic evidence (Section 3 and Table 1), while the sugar chain attached to the C-3 position was a tetrasaccharide, as revealed by four anomeric signals ($^1H$ NMR $\delta$ 4.47, 4.64, 4.81, and 4.87; $^{13}C$ NMR $\delta$ 96.9, 101.2, 102.6, and 104.1) in its spectra. The proton coupling network of each sugar residue was derived from a combination of 1D- and 2D-NMR experiments, which indicated that a β-D-quinovopyranose unit was present instead of the β-D-oleandropyranose unit observed in the saccharidic chain E (Section 3). In fact, the 1D-TOCSY subspectrum obtained when irradiating the anomeric proton signal at $\delta$ 4.47 (1H, d, $J$=7.5 Hz) showed a set of coupled proton signals at $\delta$ 3.14, 3.54, 3.47, 3.45, 1.45 assigned from H-1 to $H_3$-6 of a β-D-quinovopyranose unit. To establish the nature of the sugar sequence required analysis of the HMBC spectrum which showed correlation peaks between H-1 of quinovose and C-4 of the second unit of cymarose and between H-1 of glucose and C-4 of quinovose. Thus, the structure of compound **3** was established as 12β-*O*-tigloyl-20-*O*-acetylboucerin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

**Table 1.** $^{13}C$ NMR data for aglycon moieties of compounds **1**–**3**, **5**, **8**, **12**, **17**, **18**, **20** (600 MHz, $CD_3OD$)

| Position | **1** | **2** | **3** | **5** | **8** | **12** | **17** | **18** | **20** |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 38.2 (t) | 38.3 | 38.2 | 38.3 | 38.3 | 38.3 | 38.5 (t) | 38.5 | 38.2 (t) |
| 2 | 30.5 (t) | 30.4 | 30.6 | 30.4 | 30.6 | 30.4 | 30.6 (t) | 30.6 | 30.3 (t) |
| 3 | 80.4 (d) | 79.0 | 80.0 | 79.0 | 79.2 | 79.0 | 79.9 (d) | 80.0 | 79.3 (d) |
| 4 | 39.5 (t) | 39.7 | 39.5 | 39.8 | 39.6 | 39.8 | 39.5 (t) | 39.5 | 35.2 (t) |
| 5 | 141.0 (s) | 141.0 | 140.5 | 141.0 | 141.0 | 141.0 | 141.0 (s) | 141.0 | 45.6 (d) |
| 6 | 123.2 (d) | 123.0 | 122.8 | 123.2 | 123.1 | 123.2 | 123.8 (d) | 123.8 | 28.7 (t) |
| 7 | 28.3 (t) | 27.9 | 28.3 | 28.0 | 27.9 | 28.0 | 28.1 (t) | 28.1 | 30.6 (t) |
| 8 | 37.9 (d) | 37.8 | 38.4 | 37.2 | 37.4 | 37.2 | 37.8 (d) | 37.8 | 41.3 (d) |
| 9 | 45.0 (d) | 44.6 | 44.5 | 44.7 | 44.6 | 44.7 | 47.7 (d) | 47.7 | 50.6 (d) |
| 10 | 38.1 (s) | 38.0 | 38.0 | 37.6 | 37.6 | 37.6 | 37.8 (s) | 37.8 | 37.0 (s) |
| 11 | 26.5 (t) | 27.1 | 26.5 | 26.7 | 26.8 | 26.7 | 21.1 (t) | 21.1 | 21.2 (t) |
| 12 | 71.4 (d) | 78.2 | 78.8 | 78.8 | 79.0 | 78.8 | 41.3 (t) | 41.3 | 41.9 (t) |
| 13 | 50.4 (s) | 53.4 | 53.2 | 53.8 | 53.8 | 53.8 | 47.0 (s) | 47.0 | 47.5 (s) |
| 14 | 84.7 (s) | 87.4 | 87.0 | 87.5 | 87.5 | 87.5 | 86.0 (s) | 86.0 | 86.1 (s) |
| 15 | 32.8 (t) | 32.6 | 32.8 | 33.4 | 33.3 | 33.4 | 33.8 (t) | 33.8 | 33.2 (t) |
| 16 | 25.4 (t) | 25.0 | 25.5 | 26.0 | 26.5 | 26.0 | 20.5 (t) | 20.5 | 21.5 (t) |
| 17 | 52.0 (d) | 50.9 | 52.0 | 53.3 | 53.4 | 53.3 | 55.5 (d) | 57.9 | 57.7 (d) |
| 18 | 9.8 (q) | 9.8 | 10.2 | 11.0 | 10.0 | 11.0 | 15.0 (q) | 15.0 | 12.3 (q) |
| 19 | 19.7 (q) | 19.8 | 19.8 | 19.6 | 19.6 | 19.6 | 19.6 (q) | 19.6 | 15.5 (q) |
| 20 | 78.8 (d) | 74.8 | 75.2 | 71.3 | 71.7 | 71.3 | 75.5 (d) | 79.0 | 78.8 (d) |
| 21 | 19.6 (q) | 19.5 | 19.7 | 23.0 | 22.8 | 23.0 | 19.5 (q) | 17.3 | 16.9 (q) |
| Tig at C-12 | | | | | | | | | |
| 1′ | | 169.5 | 169.0 (s) | | 169.9 (s) | | | | |
| 2′ | | 130.1 | 130.0 (s) | | 130.9 (s) | | | | |
| 3′ | | 138.2 | 138.8 (d) | | 139.0 (d) | | | | |
| 4′ | | 14.3 | 14.0 (q) | | 14.0 (q) | | | | |
| 5′ | | 12.0 | 12.3 (q) | | 12.0 (q) | | | | |
| Tig at C-20 | | | | | | | | | |
| 1′ | 168.8 (s) | 168.9 | | | | | | | |
| 2′ | 130.1 (s) | 129.9 | | | | | | | |
| 3′ | 138.6 (d) | 138.3 | | | | | | | |
| 4′ | 14.3 (q) | 14.4 | | | | | | | |
| 5′ | 12.0 (q) | 12.0 | | | | | | | |
| Ac | | | | | | | | | |
| CO | | | 178.0 (s) | | | 178.0 | 178.0 | | |
| $CH_3$ | | | 21.3 (q) | | | 21.2 | 21.0 | | |
| *o*-OH-Bz | | | | | | | | | |
| 1′ | | | | 112.0 (s) | | | | | |
| 2′ | | | | 161.5 (s) | | | | | |
| 3′ | | | | 119.4 (d) | | | | | |
| 4′ | | | | 120.7 (d) | | | | | |
| 5′ | | | | 131.2 (d) | | | | | |
| 6′ | | | | 138.3 (d) | | | | | |
| COO | | | | 173.0 (s) | | | | | |

*Compounds **4**–**8** (chain G)*. Compounds **4**–**8** had molecular formulae $C_{48}H_{80}O_{19}$, $C_{55}H_{84}O_{21}$, $C_{55}H_{88}O_{21}$, $C_{58}H_{92}O_{21}$, and $C_{53}H_{86}O_{20}$, respectively, as deduced from ESI-MS and NMR spectra. Their aglycons were identified as boucerin,[5,6] 12β-*O*-*o*-hydroxybenzoylboucerin, 12β-*O*-tigloyl-20-*O*-acetylboucerin, 12β,20-*O*-ditigloylboucerin, and 12β-*O*-tigloylboucerin, respectively, by NMR data (Table 1). A detailed comparison of their NMR spectra showed that the sugar chain was the same. Also in this case, the proton coupling network within each sugar residue was determined using a combination of NMR experiments, which led to the identification of two β-D-cymaropyranose, one 6-deoxy-3-*O*-methyl-β-D-allopyranose, and one β-D-glucopyranose. Once again direct evidence of the sugar sequence and the linkage site was derived from HSQC and HMBC experiments. The absence of any $^{13}C$ glycosidation shift for the glucopyranose residue suggested that this sugar was the terminal unit, while the glycosidation shift on C-$4_{cym\ I}$, C-$4_{cym\ II}$, C-$4_{all}$ indicated the sequence of the saccharidic chain. Also in this case one D-cymarose unit was linked to C-3 of the aglycon as shown by HMBC experiment; key correlations were observed between H-$1_{cym\ I}$–C-3, H-$1_{cym\ II}$–C-$4_{cym\ I}$, and H-$1_{all}$–C-$4_{cym\ II}$. Thus the structure of chain G was established as β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

*Compounds **9**–**12** (chain W)*. Compounds **9**–**12** had molecular formulae $C_{53}H_{86}O_{20}$, $C_{58}H_{92}O_{21}$, $C_{55}H_{84}O_{21}$, and $C_{50}H_{82}O_{20}$, respectively, as deduced from MS and NMR analyses. The NMR spectra revealed that the aglycon moieties of **9**–**12** were 12β-*O*-tigloylboucerin, 12β,20-*O*-ditigloylboucerin, 12β-*O*-*o*-hydroxybenzoylboucerin, 12β-*O*-acetylboucerin, respectively. $^1H$ and $^{13}C$ NMR spectra revealed that **9**–**12** had an identical tetrasaccharidic sugar chain made up of two D-cymarose, one D-thevetose, and one D-glucose units. By means of NMR spectral data each carbon and hydrogen signal was assigned as shown in Table 3. Consequently it was possible to identify a terminal β-D-glucopyranose as well as two inner β-D-cymaropyranose, and a β-D-thevetopyranose, each glycosylated at C-4 as inferred by HSQC and HMBC

**Table 2.** $^{1}H$ and $^{13}C$ NMR data for sugar moieties of compounds **1**, **2**, **4**–**8**, **13** (600 MHz, $CD_3OD$)

| Position | **1**, **2** chain E | | **4**–**8** chain G | | **13** chain I | |
|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| D-Cym I | | | | | | |
| 1 | 4.89 dd (9.5, 2.0) | 97.0 | 4.89 dd (9.5, 2.0) | 97.2 | 4.87 dd (9.5, 2.0) | 97.6 |
| 2a | 1.54 br dd (16.0, 12.0) | 36.5 | 1.56 br dd (16.0, 12.0) | 36.5 | 1.61 br dd (16.0, 12.0) | 36.3 |
| 2b | 2.09 br dd (16.0, 3.0) | | 2.09 br dd (16.0, 3.0) | | 2.14 br dd (16.0, 3.0) | |
| 3 | 3.87 q (3.0) | 78.5 | 3.87 q (3.0) | 78.0 | 3.86 q (3.0) | 78.7 |
| 4 | 3.30 dd (9.5, 3.0) | 83.8 | 3.35 dd (9.5, 3.0) | 83.4 | 3.27 dd (9.5, 3.0) | 84.0 |
| 5 | 3.90 dq (9.5, 6.0) | 69.8 | 3.83 dq (9.5, 6.0) | 69.8 | 3.83 dq (9.5, 6.0) | 69.7 |
| 6 | 1.21 d (6.0) | 18.4 | 1.21 d (6.0) | 18.5 | 1.21 d (6.0) | 18.4 |
| –OMe | 3.48 s | 58.4 | 3.46 s | 58.5 | 3.45 s | 58.5 |
| D-Cym II | | | | | | |
| 1 | 4.83 dd (9.5, 2.0) | 101.0 | 4.83 dd (9.5, 2.0)) | 101.4 | | |
| 2a | 1.62 br dd (16.0, 12.0) | 36.4 | 1.60 br dd (16.0, 12.0) | 36.3 | | |
| 2b | 2.13 br dd (16.0, 3.0) | | 2.15 br dd (16.0, 3.0) | | | |
| 3 | 3.88 q (3.0) | 78.6 | 3.86 q (3.0) | 78.4 | | |
| 4 | 3.24 dd (9.5, 3.0) | 84.0 | 3.26 dd (9.5, 3.0) | 84.0 | | |
| 5 | 3.84 dq (9.5, 6.0) | 69.9 | 3.82dq (9.5, 6.0) | 70.0 | | |
| 6 | 1.23 d (6.0) | 18.0 | 1.22 d (6.0) | 18.2 | | |
| –OMe | 3.50 s | 58.2 | 3.45 s | 58.6 | | |
| D-Ole | | | | | | |
| 1 | 4.65 dd (8.5, 1.5) | 102.7 | | | 4.63 dd (8.5, 1.5) | 103.0 |
| 2a | 1.48 m | 37.6 | | | 1.46 m | 37.5 |
| 2b | 2.35 m | | | | 2.34 m | |
| 3 | 3.45 m | 80.4 | | | 3.50 m | 79.2 |
| 4 | 3.25 t (9.5) | 84.0 | | | 3.22 t (9.5) | 84.0 |
| 5 | 3.44 dq (9.5, 6.0) | 72.0 | | | 3.45 dq (9.5, 6.0) | 72.6 |
| 6 | 1.40 d (6.0) | 19.0 | | | 1.40 d (6.0) | 19.0 |
| –OMe | 3.47 s | 58.4 | | | 3.46 s | 59.0 |
| –OMe D-Allo | | | | | | |
| 1 | | | 4.61 d (8.0) | 103.5 | 4.78 d (8.0) | 102.3 |
| 2 | | | 3.38 dd (8.0, 2.5) | 72.3 | 3.40 dd (8.0, 2.5) | 72.6 |
| 3 | | | 3.98 dd (3.5, 2.5) | 83.1 | 3.97 dd (3.5, 2.5) | 83.2 |
| 4 | | | 3.35 (9.0, 3.5) | 84.0 | 3.35 (9.0, 3.5) | 84.2 |
| 5 | | | 3.39 m | 72.0 | 3.37 m | 73.0 |
| 6 | | | 1.28 d (6.0) | 18.5 | 1.28 d (6.0) | 18.4 |
| –OMe | | | 3.62 s | 61.5 | 3.62 s | 61.7 |
| D-Glc | | | | | | |
| 1 | 4.48 d (8.0) | 104.5 | 4.37 d (8.0) | 106.0 | 4.39 d (7.8) | 106.6 |
| 2 | 3.22 dd (9.5, 8.0) | 75.6 | 3.21 dd (9.5, 8.0) | 75.5 | 3.20 dd (9.5, 7.8) | 75.6 |
| 3 | 3.37 d (9.5) | 78.2 | 3.37 d (9.5) | 77.8 | 3.38 d (9.5) | 78.0 |
| 4 | 3.27 d (9.5) | 71.7 | 3.26 d (9.5) | 71.8 | 3.25 d (9.5) | 71.8 |
| 5 | 3.28 m | 78.3 | 3.32 m | 78.0 | 3.29 m | 78.3 |
| 6a | 3.67 dd (12.0, 5.0) | 63.0 | 3.68 dd (12.0, 5.0) | 62.8 | 3.68 dd (12.0, 5.0) | 62.9 |
| 6b | 3.89 dd (12.0, 3.5) | | 3.94 dd (12.0, 3.0) | | 3.81 dd (12.0, 3.0) | |

*J* values are in parentheses and reported in Hz; chemical shifts are given in ppm.

spectral data. The position of each sugar unit was confirmed by 1D-ROESY experiments that showed a cross peak between the signal at $\delta$ 4.89 (H-$1_{cym\ I}$) and the signal at $\delta$ 3.53 (H-3), and other key correlation peaks between the signal at $\delta$ 4.83 (H-$1_{cym\ II}$) and the signal at $\delta$ 3.30 (H-$4_{cym\ I}$), $\delta$ 4.37 (H-$1_{the}$) and 3.31 (H-$4_{cym\ II}$), $\delta$ 4.45 (H-$1_{glc}$) and 3.40 (H-$4_{the}$). NMR spectra also revealed that chain W is related to chain G by replacement of the unit of 6-deoxy-3-*O*-methyl-β-D-allopyranose with a β-D-thevetopyranose. On the basis of the above data, the structure of the tetrasaccharide of compounds **9**–**12** was deduced as β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

*Compound **13** (chain I).* The structure 12β,20-*O*-ditigloyl-boucerin 3-*O*-β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside was assigned to compound **13** on the basis of the following evidence. Compounds **13** and **7** were shown to be isomers with identical molecular formulae ($C_{58}H_{92}O_{21}$); the structural differences, as shown by their NMR spectra, involved a different sugar in the tetrasaccharide moiety. In particular chain I was related to chain G by replacement of the second unit of cymarose with an oleandrose, as deduced from results of DQF-COSY, 1D-TOCSY, and HSQC experiments (Table 2). Unambiguous determination of the interglycosidic linkages and sugar sequence was obtained from HMBC experiment. Key cross peaks due to long-range correlation were observed between H-1 of oleandrose and C-4 of cymarose, H-1 of 6-deoxy-3-*O*-methyl-allose and C-4 of oleandrose, and H-1 of glucose and C-4 of 6-deoxy-3-*O*-methyl-allose.

*Compounds **14** and **15** (chain M).* Compounds **14** ($C_{66}H_{108}O_{29}$) and **15** ($C_{71}H_{114}O_{30}$) were 20-*O*-tigloyl-boucerin and 12β,20-*O*-ditigloylboucerin derivatives, respectively. $^{1}H$ and $^{13}C$ NMR data suggested that the sugar chain of compounds **14** and **15** consists of six

**Table 3.** $^{1}H$ and $^{13}C$ NMR data for sugar moieties of compounds **9**–**12**, **14**–**16** (600 MHz, $CD_3OD$)

| Position | **9**–**12** chain W | | **16** chain L | | **14**, **15** chain M | |
|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| D-Cym I | | | | | | |
| 1 | 4.89 dd (9.5, 2.0) | 97.0 | 4.89 dd (9.5, 2.0) | 97.0 | 4.89 | 97.0 |
| 2a | 1.56 br dd (16.0, 12.0) | 36.2 | 1.57 br dd (16.0, 12.0) | 36.2 | 1.57 | 36.2 |
| 2b | 2.09 br dd (16.0, 3.5) | | 2.08 br dd (16.0, 3.5) | | 2.08 | |
| 3 | 3.87 q (3.5) | 78.5 | 3.87 q (3.5) | 78.6 | 3.87 | 78.6 |
| 4 | 3.30 dd (9.5, 3.5) | 83.8 | 3.26 dd (9.5, 3.5) | 83.8 | 3.26 | 83.8 |
| 5 | 3.90 dq (9.5, 6.0) | 69.8 | 3.86 dq (9.5, 6.0) | 70.0 | 3.88 | 70.0 |
| 6 | 1.22 d (6.0) | 18.4 | 1.21 d (6.0) | 18.4 | 1.21 d | 18.4 |
| –OMe | 3.46 s | 58.4 | 3.45 s | 58.5 | 3.45 s | 58.5 |
| D-Cym II | | | | | | |
| 1 | 4.83 dd (9.2, 2.0) | 101.0 | 4.83 dd (9.2, 2.0) | 100.1 | 4.83 | 100.3 |
| 2a | 1.63 br dd (16.0, 12.0) | 36.4 | 1.63 br dd (16.0, 12.0) | 36.6 | 1.63 | 36.6 |
| 2b | 2.17 br dd (16.0, 3.5) | | 2.17 br dd (16.0, 3.5) | | 2.17 | |
| 3 | 3.88 q (3.0) | 78.6 | 3.87 q (3.0) | 78.8 | 3.85 | 78.8 |
| 4 | 3.31 dd (9.5, 3.0) | 84.2 | 3.25 dd (9.5, 3.0) | 84.0 | 3.25 | 83.8 |
| 5 | 3.84 dq (9.5, 6.0) | 69.9 | 3.87 dq (9.5, 6.0) | 69.9 | 3.87 | 69.8 |
| 6 | 1.32 d (6.0) | 18.4 | 1.31 d (6.0) | 18.2 | 1.31 | 18.2 |
| –OMe | 3.45 s | 58.6 | 3.45 s | 58.6 | 3.45 | 58.6 |
| D-The | | | | | | |
| 1 | 4.37 d (8.5) | 106.0 | 4.36 d (8.5) | 106.0 | 4.36 | 106.0 |
| 2 | 3.32 t (8.7) | 74.9 | 3.32 t (9.0) | 74.9 | 3.32 | 74.8 |
| 3 | 3.21 t (8.7) | 86.1 | 3.21 t (9.0) | 86.0 | 3.21 | 86.0 |
| 4 | 3.40 t (8.7) | 82.4 | 3.38 t (9.0) | 82.7 | 3.38 | 82.8 |
| 5 | 3.49 m | 72.4 | 3.49 m | 72.4 | 3.49 | 72.4 |
| 6 | 1.40 d (6.0) | 18.4 | 1.40 d (6.0) | 18.6 | 1.40 | 18.6 |
| –OMe | 3.65 s | 61.0 | 3.66 s | 61.1 | 3.66 | 61.1 |
| –OMe D-Allo | | | | | | |
| 1 | | | 4.60 d (8.0) | 103.8 | 4.60 | 103.8 |
| 2 | | | 3.40 dd (8.0, 2.5) | 72.3 | 3.40 | 72.3 |
| 3 | | | 3.99 dd (3.5, 2.5) | 83.2 | 3.99 | 83.2 |
| 4 | | | 3.37 dd (9.0, 3.5) | 83.8 | 3.37 | 83.6 |
| 5 | | | 3.40 m | 72.4 | 3.40 | 72.4 |
| 6 | | | 1.31 d (6.0) | 18.4 | 1.31 | 18.4 |
| –OMe | | | 3.63 s | 61.9 | 3.63 | 61.9 |
| D-Glc I | | | | | | |
| 1 | 4.45 d (8.0) | 102.9 | 4.46 d (7.8) | 106.1 | 4.38 | 106.2 |
| 2 | 3.20 dd (9.5, 8.0) | 75.7 | 3.21 dd (9.5, 7.8) | 75.6 | 3.20 | 75.6 |
| 3 | 3.38 d (9.5) | 78.0 | 3.37 d (9.5) | 77.9 | 3.29 | 84.0 |
| 4 | 3.24 d (9.5) | 71.8 | 3.24 d (9.5) | 71.8 | 3.25 | 71.0 |
| 5 | 3.29 m | 78.3 | 3.28 m | 78.2 | 3.33 | 77.8 |
| 6a | 3.63 dd (12.0, 5.0) | 63.0 | 3.68 dd (12.0, 5.0) | 63.1 | 3.65 | 62.0 |
| 6b | 3.90 dd (12.0, 3.5) | | 3.92 dd (12.0, 3.5) | | 3.90 | |
| D-Glc II | | | | | | |
| 1 | | | | | 4.46 d (8.0) | 104.1 |
| 2 | | | | | 3.21 dd (9.5, 8.0) | 75.6 |
| 3 | | | | | 3.37 d (9.5) | 77.8 |
| 4 | | | | | 3.24 d (9.5) | 71.6 |
| 5 | | | | | 3.28 m | 78.1 |
| 6a | | | | | 3.68 dd (12.0, 5.0) | 63.0 |
| 6b | | | | | 3.92 dd (12.0, 3.0) | |

*J* values are in parentheses and reported in Hz; chemical shifts are given in ppm.

residues: six anomeric proton signals that resonated at $\delta$ 4.36, 4.38, 4.46, 4.60, 4.83, and 4.89, and correlated in the HSQC spectrum to carbons at 106.0, 106.2, 104.1, 103.8, 100.3, and 97.0 ppm, respectively, were easily identified. By a combination of 1D- and 2D-NMR experiments, 1D-TOCSY subspectrum of six monosaccharide units could be interpreted and, at the same time, the type of sugar and its configuration assigned (Table 3). The β-configuration at the anomeric carbons of the six sugars was confirmed by the *J* values of their anomeric signals (Table 3).[12,13] An HSQC experiment correlated all proton resonances with those of the corresponding carbons and allowed to deduce the position of interglycosidic linkages. The sugar sequence was confirmed by HMBC cross peaks that showed correlations between H-$1_{glc\ II}$–C-$3_{glc\ I}$, H-$1_{glc\ I}$–C-$4_{all}$, H-$1_{all}$–C-$4_{the}$, and H-$1_{the}$–C-$4_{cym\ II}$. Finally confirmation of the sugar sequence was derived from the chemical shift of C-$1_{cym\ I}$ (97.0 ppm). Therefore the sugar chain of compounds **14** and **15** was established to be β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

*Compound **16** (chain L).* Compound **16** possessed a pentasaccharide sugar chain attached to C-3 of the aglycon that was identified as 12β-*O*-tigloyl-20-*O*-acetylboucerin. The

**Table 4.** $^{1}H$ and $^{13}C$ NMR data for sugar moieties of compounds **17**–**20** (600 MHz, $CD_3OD$)

| Position | **17**, **20** chain B | | **18**, **19** chain C | | **18** chain D | |
|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| –OMe D-Fuc | | | | | | |
| 1 | 4.38 d (8.0) | 103.0 | 4.39 d (8.0) | 103.0 | | |
| 2 | 3.62 dd (9.5, 8.0) | 71.6 | 3.63 dd (9.5, 8.0) | 71.7 | | |
| 3 | 3.27 dd (9.5, 4.0) | 85.9 | 3.26 dd (9.5, 4.0) | 85.8 | | |
| 4 | 4.20 dd (4.0, 2.5) | 74.8 | 4.20 dd (4.0, 2.5) | 74.7 | | |
| 5 | 3.67 m | 71.0 | 3.61 m | 71.4 | | |
| 6 | 1.32 d (6.0) | 17.3 | 1.30 d (6.0) | 17.3 | | |
| –OMe | 3.53 s | 58.0 | 3.54 s | 58.3 | | |
| D-Glc I | | | | | | |
| 1 | 4.41 d (8.0) | 104.3 | 4.43 d (7.8) | 104.4 | | |
| 2 | 3.17 dd (9.5, 8.0) | 75.2 | 3.23 dd (9.5, 7.8) | 76.0 | | |
| 3 | 3.39 d (9.5) | 78.9 | 3.39 d (9.5) | 77.7 | | |
| 4 | 3.27 d (9.5) | 71.0 | 3.37 d (9.5) | 71.2 | | |
| 5 | 3.28 m | 78.2 | 3.49 m | 76.5 | | |
| 6a | 3.70 dd (12.0, 5.0) | 62.5 | 3.82 dd (12.0, 5.0) | 70.0 | | |
| 6b | 3.89 dd (12.0, 3.5) | | 4.15 dd (12.0, 3.0) | | | |
| D-Glc II | | | | | | |
| 1 | | | 4.64 d (8.0) | 104.2 | | |
| 2 | | | 3.24 dd (9.5, 8.0) | 75.3 | | |
| 3 | | | 3.37 d (9.5) | 78.2 | | |
| 4 | | | 3.29 d (9.5) | 71.6 | | |
| 5 | | | 3.30 m | 78.1 | | |
| 6a | | | 3.69 dd (12.0, 5.0) | 62.4 | | |
| 6b | | | 3.90 dd (12.0, 3.5) | | | |
| D-Glc III | | | | | | |
| 1 | | | | | 4.41 d (8.0) | 104.5 |
| 2 | | | | | 3.23 dd (9.5, 8.0) | 75.5 |
| 3 | | | | | 3.37 d (9.5) | 78.0 |
| 4 | | | | | 3.38 d (9.5) | 71.1 |
| 5 | | | | | 3.35 m | 78.2 |
| 6a | | | | | 3.61 dd (12.0, 5.0) | 68.4 |
| 6b | | | | | 3.98 dd (12.0, 3.5) | |
| D-Glc IV | | | | | | |
| 1 | | | | | 4.63 d (7.8) | 104.2 |
| 2 | | | | | 3.22 dd (9.5, 7.8) | 76.0 |
| 3 | | | | | 3.40 d (9.5) | 78.8 |
| 4 | | | | | 3.37 d (9.5) | 71.1 |
| 5 | | | | | 3.48 m | 77.3 |
| 6a | | | | | 3.81 dd (12.0, 5.0) | 68.5 |
| 6b | | | | | 3.98 dd (12.0, 3.0) | |
| L-Rha | | | | | | |
| 1 | | | | | 4.74 d (1.8) | 102.0 |
| 2 | | | | | 3.83 dd (2.5, 1.8) | 72.6 |
| 3 | | | | | 3.66 dd (9.0, 2.5) | 72.3 |
| 4 | | | | | 3.38 t (9.0) | 74.0 |
| 5 | | | | | 3.67 m | 69.8 |
| 6 | | | | | 1.29 d (6.5) | 18.0 |

*J* values are in parentheses and reported in Hz; chemical shifts are given in ppm.

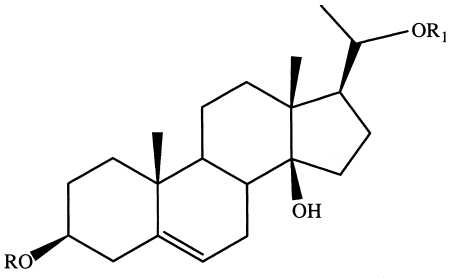
ESI-MS spectrum of compound **16** (molecular formula $C_{62}H_{100}O_{25}$) showed the $[M+Na]^+$ ion at *m/z* 1267 and a fragmentation pattern similar to that of **15**. The $^{13}C$ and $^{13}C$ DEPT NMR spectra of **16** showed 62 signals of which 34 were assigned to the saccharidic portion. Analysis of sugar chain NMR data of compound **16** and comparison with those of **15** showed that **16** differed from **15** only in the absence of the terminal glucopyranosyl unit (Table 3). Therefore, the structure 12β-*O*-tigloyl-20-*O*-acetylboucerin 3-*O*-β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside was assigned to compound **16**.

*Compound 17 (chain B).* Compound **17** was assigned $C_{36}H_{58}O_{13}$ molecular formula, as shown by its ESI-MS data (*m/z* 721 $[M+Na]^+$) in combination with the $^{13}C$ NMR spectrum. Compound **17** showed two anomeric protons and carbons at $\delta_H$ 4.38 and 4.41 and $\delta_C$ 103.0 and 104.3 (Table 4). The structure of the sugar unit was determined by NMR spectral data as 3-*O*-methyl-β-D-fucose attached through a 1→4 linkage to β-D-glucose and confirmed by comparison with literature values.[4] The genin was identified as calogenin from a careful analysis of its NMR spectra and by comparison with literature data.[4,7] Attachment of the glycoside chain at C-3 was indicated by the significant downfield shift observed for this carbon in compound **17** relative to the corresponding signal in calogenin and was subsequently confirmed from a long-range correlation between C-3 (79.9 ppm) and H-$1_{fuc}$ ($\delta$ 4.38) in the HMBC spectrum. 1D-ROESY experiment showed a correlation peak between H-$1_{glc}$ and H-$4_{fuc}$ that

established the position of each sugar unit. The ester was determined to be an acetyl group as shown by NMR data ($\delta_H$ 1.93; $\delta_C$ 21.0, 178.0). The ester linkage was located at C-20 on the basis of the chemical shift of the double doublet signal ($\delta$ 4.42) ascribable to H-20 of esterified calogenin and confirmed by HMBC peak between H-20 and signal at 178.0 ppm. From the foregoing evidence, compound **17** was deduced as 20-*O*-acetylcalogenin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-3-*O*-methylfucopyranoside or 20-*O*-acetyl russelioside C.

*Compound **18** (chains C and D)*. Compound **18** ($C_{58}H_{96}O_{31}$) was identified as a further calogenin derivative possessing two sugar moieties at C-3 and C-20. The $^{13}C$ and $^{13}C$ DEPT NMR spectra of **18** showed 58 signals, of which 21 were assigned to the steroid moiety and 37 to the saccharide portion. In compound **18**, C-20 appeared at 79.0 ppm in the $^{13}C$ NMR and H-20 at $\delta$ 4.03 in the $^{1}H$ NMR spectra, indicating that the hydroxyl group at C-20 was glycosylated. Attachment of the glycosidic chain at C-3 was confirmed by comparison with compound **17** spectral data. The identity and sequence of sugar units was deduced by a combination of 1D and 2D NMR techniques. The results of 1D-TOCSY and DQF-COSY experiments allowed the sequential assignments of all the proton resonance to the individual monosaccharide as reported in Table 4. Thus, the chemical shifts of the sugar resonance were attributable to the β-D-3-*O*-methylfucopyranosyde ($\delta_{H\text{-}1fuc}$=4.39), β-D-glucopyranoside ($\delta_{H\text{-}1glc\ I}$=4.43), β-D-glucopyranoside ($\delta_{H\text{-}1glc\ II}$=4.64), β-D-glucopyranoside ($\delta_{H\text{-}1glc\ III}$=4.41), β-D-glucopyranoside ($\delta_{H\text{-}1glc\ IV}$=4.63), and α-L-rhamnopyranoside ($\delta_{H\text{-}1rha}$=4.74). HSQC experiment led to the correlation of all the proton resonances with those of each corresponding carbon. The absence of any glycosidation shift for one β-D-glucopyranose and α-L-rhamnopyranose moieties suggested that these sugars were terminal units. Glycosidation shifts were observed for C-$4_{fuc}$ (74.7 ppm), C-$6_{glc\ I}$ (70.0 ppm), C-$6_{glc\ III}$ (68.4 ppm), and C-$6_{glc\ IV}$ (68.5 ppm). Finally the position of the sugar residues in **18** was defined by the HMBC experiment. A cross peak due to long-range correlation between C-3 (80.0 ppm) of the aglycon and H-$1_{fuc}$ indicated that 3-*O*-methylfucose was the residue linked to C-3; a cross peak between C-$4_{fuc}$ and H-$1_{glc\ I}$ indicated that glucose I was the second unit; a cross peak between C-$6_{glc\ I}$ and H-$1_{glc\ II}$ indicated that glucose II was the third unit of the trisaccharide chain at C-3. Similarly, the sequence of the trisaccharide chain at C-20 was indicated by the cross peaks between C-20 and H-$1_{glc\ III}$, and between C-$6_{glc\ III}$ and H-$1_{glc\ IV}$ and between C-$6_{glc\ IV}$ and H-$1_{rha}$. On the basis of all this evidence compound **18** was identified as calogenin 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-β-D-3-*O*-methylfucopyranoside-20-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6) β-D-glucopyranoside.

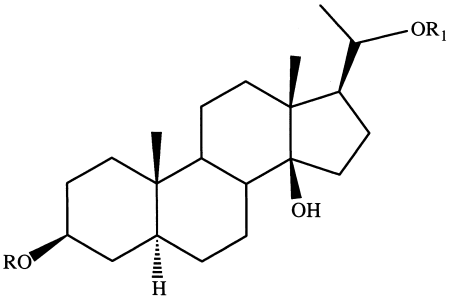
*Compound **19** (chains C and A)*. Compound **19** had molecular formula $C_{46}H_{76}O_{22}$ as obtained from NMR and ESI-MS spectral data. Its NMR spectra showed, together with signals of the aglycon calogenin, 25 signals ascribable to sugar units. ESI-MS of compound **19** showed the $[M+Na]^+$ ion at *m/z* 1003 with prominent fragments at *m/z* 841 $[(M+Na)-162]^+$ (cleavage of one hexose unit) and *m/z* 519 $[(M+Na)-(162+160+162)]^+$ due to the subsequent loss of two hexose units. Analysis of the NMR data of compound **19** and comparison with those of **18** showed they possessed the same sugar chain at C-3 (Table 4), while saccharidic moiety at C-20 was the point of difference. The sugar chain at C-20 of compound **19** was established to be one β-D-glucopyranose (Experimental section). Therefore, the structure of calogenin 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-β-D-3-*O*-methylfucopyranoside-20-*O*-β-D-glucopyranoside was assigned to **19**.



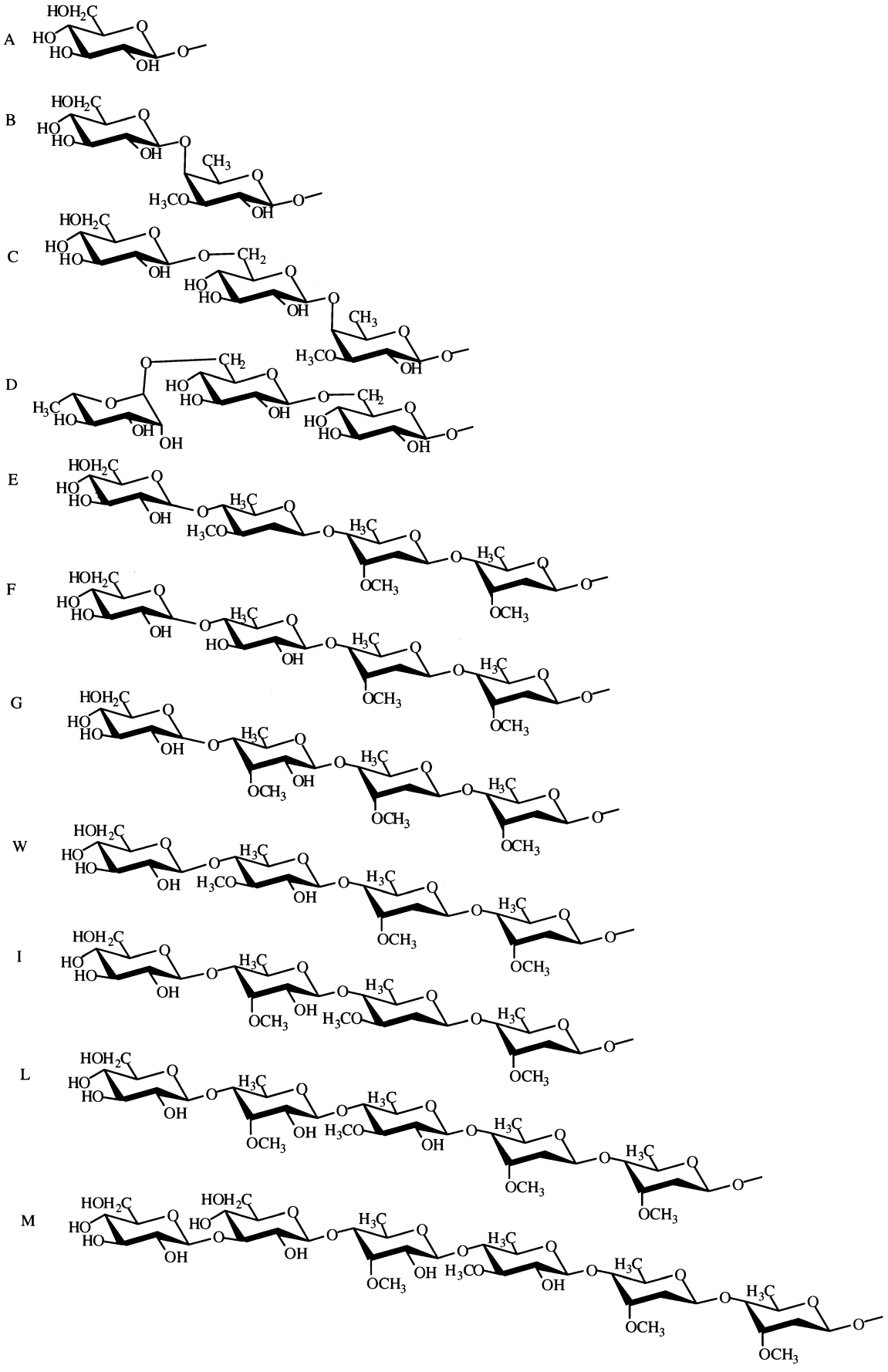

| Compounds | R | $R_1$ |
|---|---|---|
| **17** | B | Ac |
| **18** | C | D |
| **19** | C | A |

*Compound **20** (chains B and A)*. Finally, compound **20** had molecular formula $C_{40}H_{68}O_{17}$ ($^{13}C$ NMR data and ESI-MS, *m/z* 843 $[M+Na]^+$) that presented only two mass unit of difference if compared with russelioside B.[4] This evidence, together with NMR analysis, suggested the presence of the 5α-dihydrocalogenin aglycon[8] with the same sugar moiety of russelioside B. The comparison of the $^{1}H$ and $^{13}C$ NMR spectra of **20** with those of russelioside B showed the lack of the double bond between C-5 and C-6 and the presence of one methyne and one methylene at $\delta$ 1.11 and 1.09, 2.05, respectively, which correlated in the HSQC spectrum with signals at 45.6 and 28.7 ppm (Table 1). Thus the structure of compound **20** was established as 5α-dihydrocalogenin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-3-*O*-methylfucopyranoside-20-*O*-β-D-glucopyranoside or dihydrorusselioside B.

The known compounds were identified as russelioside B and russelioside C by comparison with literature NMR data.[4]




| Compound | R | $R_1$ |
|---|---|---|
| **20** | B | A |


A
B
C
D
E
F
G
W
I
L
M

### 2.1. Cytotoxic and genotoxic activity

Some pregnane glycosides from *C. negevensis*, selected on the basis of their structural differences were submitted to cytotoxic and genotoxic activity evaluation studies. The cytotoxic test[9] assesses the ability of a molecule to arrest or inhibit tumor cell growth, while the genotoxic assay evaluates the induction of micronuclei (small chromatine mass resembling the main nucleus and easily observed in the cytoplasm of cells undergoing at least one cell division). This methodology has been previously used to prescreen biological activity of newly characterised chemicals of plant origin.[10,11] None of the tested compounds inhibited tumor cell (LoVo and HT29) growth up to 25 μg/mL, except for compound **7** that is cytotoxic for both cell lines. This effect seems to be the consequence of a general toxicity rather than of a specific activity against the DNA as there is no difference in the pattern of toxicity between LoVo and HT29 cell lines. Compound **7** slightly increased micronuclei (MN) frequency only at 150 μg/mL.[10,11] These results indicated that the tested compounds did not show effective anticancer activity against the two cell lines used and were not genotoxic in the in vitro human lymphocyte micronucleus assay, especially when compared to other related molecules which are active at concentrations at least 50-fold lower.

## 3. Experimental

### 3.1. General

Melting points were determined on a Kofler apparatus. Optical rotations were measured on a Perkin–Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. UV spectra were recorded on a Perkin–Elmer-Lambda 11 spectrophotometer. A Bruker DRX-600 NMR spectrometer, operating at 599.19 MHz for $^1$H and 150.86 MHz for $^{13}$C, using the UXNMR software package, was used for NMR experiments; chemical shifts are expressed in $\delta$ (ppm) referring to the solvent peaks $\delta_H$ 3.34 and $\delta_C$ 49.0 for $CD_3OD$. $^{13}$C DEPT, 1D-TOCSY, 1D-ROESY, $^1$H–$^1$H DQF-COSY, HOHAHA, $^1$H–$^{13}$C HSQC, and HMBC experiments were carried out using the conventional pulse sequences as described in the literature.[15] ESI-MS (positive mode) were obtained from a Finningan LC-Q Deca instrument from Termoquest (San Jose, CA, USA) equipped with Excalibur software. TLC was performed on precoated Kieselgel 60 $F_{254}$ plates (Merck); compounds were detected by spraying with $Ce(SO_4)_2/H_2SO_4$ solution followed by heating. Column chromatography was performed over Si gel (40–63 μm, Merck) and Sephadex LH-20 (Pharmacia); droplet counter-current chromatography (DCCC) was performed on an apparatus manufactured by Büchi, equipped with 300 tubes; HPLC separations were conducted on a Shimadzu LC-8A series pumping system equipped with a Waters R401 refractive index detector and with a Waters μ-Bondapak $C_{18}$ column and Shimadzu injector.

### 3.2. Plant material

The whole plant of *C. negevensis* Zohary was collected in Dabbet Hanoot, Qa' al Naqab, Jordan, in April, 1999 and identified by Professor Sawsan Al Oran, Department of Biology, University of Jordan, Amman, Jordan. A voucher specimen is deposited at the Orto Botanico, Università di Pisa, Pisa, Italy (No. 2000-0051/1).

### 3.3. Extraction and isolation

The dried whole plant (80 g) of *C. negevensis* was defatted with *n*-hexane and then extracted successively at room temperature with $CHCl_3$ and MeOH to give 4.9 and 9.5 g of residues, respectively. The chloroform extract was dissolved in a mixture MeOH–$H_2O$ 8:2 and part of the soluble portion (1.1 g) was submitted to flash column chromatography over Si gel with $CHCl_3$ containing an increasing amount of MeOH (99:1, 98:2, 9:1, 8:2, 1:1, V/V) to give six main fractions. Fractions 4 (190 mg), 5 (180 mg), and 6 (45 mg) were further purified on RP-HPLC on a C-18 μ-Bondapak column, (30 cm×7.8 mm, flow rate 2.0 mL/min) with MeOH–$H_2O$ 4:1 for fraction 4, MeOH–$H_2O$ 75:25 for fraction 5, and MeOH–$H_2O$ 7:3 for fraction 6, to yield compounds **16** (2.0 mg, $t_R$=17 min), **1** (2.5 mg, $t_R$=22 min), **3** (5.5 mg, $t_R$=25 min), and **2** (6.3 mg, $t_R$=45 min) from fraction 4, compounds **4** (2.3 mg, $t_R$=10 min), **5** (2.7 mg, $t_R$=30 min), **9** (5.8 mg, $t_R$=35 min), **6** (6.5 mg, $t_R$=45 min), **14** (3.8 mg, $t_R$=50 min), **13** (4.5 mg, $t_R$=82 min), **10** (3.0 mg, $t_R$=90 min), and **7** (6.5 mg, $t_R$=98 min) from fraction 5; russelioside C (3.5 mg, $t_R$=22 min), compounds **11** (2.0 mg, $t_R$=56 min), **12** (5.3 mg, $t_R$=65 min), and **8** (2.4 mg, $t_R$=70 min) from fraction 6, respectively. The methanolic residue was partitioned between *n*-BuOH–$H_2O$: the *n*-BuOH fraction (4.0 g) was chromatographed on Sephadex LH-20 with MeOH as eluent, collecting 20 major fractions. Fraction 3 was purified by DCCC ($CHCl_3$–MeOH–$H_2O$–*n*-PrOH (9:12:8:1), descending mode, flow 10 mL/h) to give three fractions A (100 mg), B (40 mg), and C (60 mg). Fraction C was submitted to final separation by RP-HPLC on a C-18 μ-Bondapak column, (30 cm×7.8 mm, flow rate 2.0 mL/min) with MeOH–$H_2O$ 83:17 as eluent to obtain pure compound **15** (5.0 mg, $t_R$=25 min). Fraction 5 was submitted to DCCC (*n*-BuOH–$Me_2CO$–$H_2O$ (33:10:50) descending mode, flow 10 mL/h), to yield pure compound **19** (4.0 mg) together with two main fractions A′ (80 mg) and B′ (50 mg). Fraction B′ was purified by RP-HPLC on a C-18 μ-Bondapak column, (30 cm×7.8 mm, flow rate 2.0 mL/min) with MeOH–$H_2O$ 1:1 as eluent to yield pure compound **17** (6.2 mg, $t_R$=35 min). Fraction 6 was submitted to DCCC (*n*-BuOH–$Me_2CO$–$H_2O$ (33:10:50) descending mode, flow 10 mL/h), to yield pure compound **18** (6.5 mg) together with one fraction B″ (70 mg) that was finally purified by RP-HPLC on a C-18 μ-Bondapak column, (30 cm×7.8 mm, flow rate 2.0 mL/min) with MeOH–$H_2O$ 3:2 as eluent to yield pure russelioside B (50 mg, $t_R$=21 min) and compound **20** (5.8 mg, $t_R$=27 min).

**3.3.1. Compound 1.** Amorphous powder, mp 135–138°C; $[\alpha]_D^{25}$=+10.3 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 218 sh (2.56) nm; $^1$H NMR of the aglycon: $\delta$ 1.05 (3H, s, Me-19), 1.06 (3H, s, Me-18), 1.29 (3H, d, *J*=6.5 Hz, Me-21), 1.87 (3H, d, *J*=6.5 Hz, H-4′), 1.89 (1H, m, H-7a), 1.94 (3H, s, H-5′), 2.05 (1H, m, H-17), 2.20 (1H, dd, *J*=10.2, 2.5 Hz, H-4a), 2.27 (1H, m, H-7b), 2.38 (1H, br

t, $J$=9.5 Hz, H-4b), 3.45 (1H, m, H-3), 3.66 (1H, dd, $J$=12.0, 4.0 Hz, H-12), 4.62 (1H, m, H-20), 5.48 (1H, br d, $J$=4.0 Hz, H-6), 6.96 (1H, br q, $J$=6.5 Hz, H-3′); $^{13}$C NMR data of the aglycon: see Table 1, $^{1}$H and $^{13}$C NMR data of the sugar moiety: see Table 2; ESI-MS $m/z$: 1049 $[M+Na]^+$; anal. C 61.90% H 8.46%, O 29.64%, calcd for $C_{53}H_{86}O_{19}$, C 61.95%, H 8.44%, O 29.60%.

**3.3.2. Compound 2.** White crystalline powder, mp 133–135°C; $[\alpha]_D^{25}$=+19.7 ($c$ 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 215 sh (3.31), 272 (2.90) nm; $^{1}$H NMR of the aglycon: $\delta$ 1.01 (3H, s, Me-18), 1.03 (3H, s, Me-19), 1.15 (3H, d, $J$=6.5 Hz, Me-21), 1.82 (3H, d, $J$=6.5 Hz, H-4′ at C-12), 1.83 (3H, s, H-5′ at C-20), 1.84 (1H, m, H-7a), 1.88 (3H, d, $J$=6.5 Hz, H-4′ at C-20), 1.90 (3H, s, H-5′ at C-12), 2.06 (1H, m, H-17), 2.16 (1H, dd, $J$=10.0, 2.5 Hz, H-4a), 2.22 (1H, m, H-7b), 2.36 (1H, br t, $J$=9.5 Hz, H-4b), 3.51 (1H, m, H-3), 4.72 (1H, dd, $J$=12.0, 4.3 Hz, H-12), 5.11 (1H, m, H-20), 5.47 (1H, br d, $J$=4.0 Hz, H-6), 6.79 (1H, br q, $J$=6.5 Hz, H-3′ at C-12), 6.93 (1H, br q, $J$=6.5 Hz, H-3′ at C-20); $^{13}$C NMR data of the aglycon: see Table 1; $^{1}$H and $^{13}$C NMR data of the sugar moiety: see Table 2; ESI-MS $m/z$: 1131 $[M+Na]^+$; anal. C 62.75%, H 8.37%, O 28.88%, calcd for $C_{58}H_{92}O_{20}$, C 62.78%, H 8.36%, O 28.86%.

**3.3.3. Compound 3.** Amorphous powder, mp 130°C; $[\alpha]_D^{25}$=+19.0 ($c$ 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 219 sh (4.12) nm; $^{1}$H NMR of the aglycon: $\delta$ 1.05 (3H, s, Me-19), 1.08 (3H, s, Me-18), 1.14 (3H, d, $J$=6.5 Hz, Me-21), 1.82 (3H, d, $J$=6.5 Hz, H-4′), 1.90 (1H, m, H-7a), 1.91 (3H, s, H-5′), 2.00 (3H, s, CO*Me*), 2.08 (1H, m, H-17), 2.18 (1H, dd, $J$=10.0, 2.5 Hz, H-4a), 2.26 (1H, m, H-7b), 2.38 (1H, br t, $J$=9.0 Hz, H-4b), 3.52 (1H, m, H-3), 4.64 (1H, dd, $J$=12.0, 4.0 Hz, H-12), 4.95 (1H, m, H-20), 5.47 (1H, br d, $J$=4.3 Hz, H-6), 7.05 (1H, br q, $J$=6.5 Hz, H-3′); $^{13}$C NMR data of the aglycon: see Table 1; $^{1}$H NMR of the sugar moiety: $\delta$ 1.20 (3H, d, $J$=6.4 Hz, H-6$_{cym\ I}$), 1.25 (3H, d, $J$=6.0 Hz, H-6$_{cym\ II}$), 1.45 (3H, d, $J$=6.0 Hz, H-6$_{qui}$), 1.53 (1H, br dd, $J$=16.0, 12.0 Hz, H-2b$_{cym\ I}$), 1.63 (1H, br dd, $J$=16.0, 12.0 Hz, H-2b$_{cym\ II}$), 2.09 (1H, br dd, $J$=16.0, 3.0 Hz, H-2a$_{cym\ I}$), 2.15 (1H, br dd, $J$=16.0, 3.0 Hz, H-2a$_{cym\ II}$), 3.14 (1H, dd, $J$=10.0, 7.0 Hz, H-2$_{qui}$), 3.20 (1H, dd, $J$=9.5, 7.5 Hz, H-2$_{glc}$), 3.23 (1H, d, $J$=9.5 Hz, H-4$_{glc}$), 3.25 (1H, dd, $J$=9.5, 3.0 Hz, H-4$_{cym\ I}$), 3.26 (1H, m, H-5$_{glc}$), 3.30 (1H, dd, $J$=9.5, 3.0 Hz, H-4$_{cym\ II}$), 3.37 (1H, d, $J$=9.5 Hz, H-3$_{glc}$), 3.45 (1H, m, H-5$_{qui}$), 3.47 (1H, t, $J$=10.0 Hz, H-4$_{qui}$), 3.51 (3H, s, OMe$_{cym\ I}$), 3.53 (3H, s, OMe$_{cym\ II}$), 3.54 (1H, t, $J$=10.0 Hz, H-3$_{qui}$), 3.64 (1H, dd, $J$=12.0, 3.5 Hz, H-6b$_{glc}$), 3.82 (1H, dq, $J$=9.5, 6.0 Hz, H-5$_{cym\ II}$), 3.85 (1H, dq, $J$=9.5, 6.4 Hz, H-5$_{cym\ I}$), 3.87 (1H, q, $J$=3.0 Hz, H-3$_{cym\ I}$), 3.88 (1H, q, $J$=3.0 Hz, H-3$_{cym\ II}$), 3.94 (1H, dd, $J$=12.0, 5.0 Hz, H-6a$_{glc}$), 4.47 (1H, d, $J$=7.5 Hz, H-1$_{qui}$), 4.64 (1H, d, $J$=7.5 Hz, H-1$_{glc}$), 4.81 (1H, dd, $J$=9.0, 2.0 Hz, H-1$_{cym\ II}$), 4.87 (1H, dd, $J$=9.5, 2.0 Hz, H-1$_{cym\ I}$); $^{13}$C NMR of the sugar moiety: $\delta$ 18.0 (q, C-6$_{cym\ I}$), 18.5 (q, C-6$_{cym\ II}$), 18.6 (q, C-6$_{qui}$), 36.5 (t, C-2$_{cym\ I}$), 36.8 (t, C-2$_{cym\ II}$), 58.0 (q, OMe$_{cym\ I}$), 58.1 (q, OMe$_{cym\ II}$), 62.9 (t, C-6$_{glc}$), 69.7 (d, C-5$_{cym\ I}$), 69.9 (d, C-5$_{cym\ II}$), 71.8 (d, C-4$_{glc}$), 72.6 (d, C-5$_{qui}$), 74.4 (d, C-2$_{qui}$), 75.3 (d, C-2$_{glc}$), 78.1 (d, C-5$_{glc}$), 78.0 (d, C-3$_{qui}$), 78.2 (d, C-3$_{glc}$), 78.6 (d, C-3$_{cym\ I}$), 78.7 (d, C-3$_{cym\ II}$), 83.6 (d, C-4$_{qui}$), 83.7 (d, C-4$_{cym\ II}$), 84.0 (d, C-4$_{cym\ I}$), 96.9 (d, C-1$_{cym\ I}$), 101.2 (d, C-1$_{cym\ II}$), 102.6 (d, C-1$_{glc}$), 104.1 (d, C-1$_{qui}$); ESI-MS $m/z$: 1093 $[M+Na]^+$; anal. C 60.49%, H 8.11%, O 31.40%, calcd for $C_{54}H_{86}O_{21}$, C 60.54%, H 8.09%, O 31.36%.

**3.3.4. Compound 4.** Amorphous powder, mp 165–170°C; $[\alpha]_D^{25}$=+29.0 ($c$ 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 225 sh (2.24), 280 (3.38) nm; NMR data for the aglycon moiety are superimposable on those for boucerin;[5,6] $^{1}$H and $^{13}$C NMR data of the sugar moiety: see Table 2; ESI-MS $m/z$: 983 $[M+Na]^+$; anal. C 59.90%, H 8.42%, O 31.68%, calcd for $C_{48}H_{80}O_{19}$, C 59.98%, H 8.39%, O 31.63%.

**3.3.5. Compound 5.** Amorphous powder, mp 118–120°C; $[\alpha]_D^{25}$=+20.3 ($c$ 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 222 sh (4.20), 250 sh (3.76), 278 (5.05) nm; $^{1}$H NMR of the aglycon: $\delta$ 1.05 (3H, s, Me-19), 1.23 (3H, d, $J$=6.5 Hz, Me-21), 1.29 (3H, s, Me-18), 1.85 (1H, m, H-17), 1.88 (1H, m, H-7a), 2.19 (1H, dd, $J$=9.5, 2.5 Hz, H-4a), 2.26 (1H, m, H-7b), 2.40 (1H, br t, $J$=9.5 Hz, H-4b), 3.53 (1H, m, H-3), 3.83 (1H, m, H-20), 4.63 (1H, dd, $J$=12.0, 4.0 Hz, H-12), 5.48 (1H, br d, $J$=4.5 Hz, H-6), 6.60 (1H, t, $J$=8.0 Hz, H-4′), 7.20 (1H, t, $J$=8.0 Hz, H-5′), 7.77 (1H, d, $J$=8.0 Hz, H-3′), 7.91 (1H, d, $J$=8.0 Hz, H-6′); $^{13}$C NMR data of the aglycon: see Table 1, $^{1}$H and $^{13}$C NMR data of the sugar moiety: see Table 2; ESI-MS $m/z$: 1103 $[M+Na]^+$; anal. C 61.06%, H 7.84%, O 31.10%, calcd for $C_{55}H_{84}O_{21}$, C 61.10%, H 7.83%, O 31.07%.

**3.3.6. Compound 6.** Amorphous powder, mp 133–139°C; $[\alpha]_D^{25}$=+35.6 ($c$ 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 220 sh (3.86), 282 (4.76) nm; NMR data for the aglycon moiety are identical to those for compound **3**; $^{1}$H and $^{13}$C NMR data of the sugar moiety: see Table 2; ESI-MS $m/z$: 1107 $[M+Na]^+$; anal. C 60.80%, H 8.20%, O 31.00%, calcd for $C_{55}H_{88}O_{21}$, C 60.87%, H 8.17%, O 30.96%.

**3.3.7. Compound 7.** Amorphous powder, mp 152–157°C; $[\alpha]_D^{25}$=+34.7 ($c$ 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 280 (3.99) nm; NMR data for the aglycon moiety are superimposable on those of compound **2**; $^{1}$H and $^{13}$C NMR data of the sugar moiety: see Table 2; ESI-MS $m/z$: 1147 $[M+Na]^+$, 1047, 947, 489; anal. C 61.88%, H 8.27%, O 29.85%, calcd for $C_{58}H_{92}O_{21}$, C 61.90%, H 8.24%, O 29.86%.

**3.3.8. Compound 8.** Amorphous powder, mp 133–135°C; $[\alpha]_D^{25}$=+25.0 ($c$ 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 220 sh (2.40) nm; $^{1}$H NMR of the aglycon: $\delta$ 1.05 (3H, s, Me-19), 1.23 (3H, d, $J$=6.5 Hz, Me-21), 1.29 (3H, s, Me-18), 1.84 (3H, d, $J$=6.5 Hz, H-4′), 1.85 (1H, m, H-17), 1.88 (1H, m, H-7a), 1.89 (3H, s, H-5′), 2.19 (1H, dd, $J$=9.5, 2.0 Hz, H-4a), 2.26 (1H, m, H-7b), 2.40 (1H, br t, $J$=9.5 Hz, H-4b), 3.53 (1H, m, H-3), 3.83 (1H, m, H-20), 4.63 (1H, dd, $J$=12.0, 4.0 Hz, H-12), 5.48 (1H, br d, $J$=4.0 Hz, H-6), 6.98 (1H, br q, $J$=6.5 Hz, H-3′); $^{13}$C NMR data of the aglycon: see Table 1; $^{1}$H and $^{13}$C NMR data of the sugar moiety: see Table 2; ESI-MS $m/z$: 1105 $[M+Na]^+$, 1005; anal. C 61.04%, H 8.30%, O 30.66%, calcd for $C_{53}H_{86}O_{20}$, C 61.02%, H 8.31%, O 30.67%.

**3.3.9. Compound 9.** Amorphous powder, mp 120–123°C; $[\alpha]_D^{25}$=+4.0 ($c$ 0.4, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 220

sh (2.29) nm; NMR data for the aglycon moiety are identical to those for compound **8**; $^1$H and $^{13}$C NMR data of the sugar moiety: see Table 3; ESI-MS *m/z*: 1065 $[M+Na]^+$, 965; anal. C 60.98%, H 8.33%, O 30.69%, calcd for $C_{53}H_{86}O_{20}$, C 61.02%, H 8.31%, O 30.67%.

**3.3.10. Compound 10.** Amorphous powder, mp 152°C; $[\alpha]_D^{25}$=+16.3 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 217 sh (3.50), 278 (3.88) nm; NMR data for the aglycon moiety are superimposable on those of compound **2**; $^1$H and $^{13}$C NMR data of the sugar moiety: see Table 3; ESI-MS *m/z*: 1147 $[M+Na]^+$, 1047, 585; anal. C 61.81%, H 8.27%, O 29.92%, calcd for $C_{58}H_{92}O_{21}$, C 61.90%, H 8.24%, O 29.86%.

**3.3.11. Compound 11.** Amorphous powder, mp 160°C; $[\alpha]_D^{25}$=+16.0 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 220 sh (3.85), 247 sh (2.92), 280 (3.68) nm; NMR data for the aglycon moiety are identical to those for compound **5**; $^1$H and $^{13}$C NMR data of the sugar moiety: see Table 3; ESI-MS *m/z*: 1103 $[M+Na]^+$; anal. C 61.07%, H 7.85%, O 31.08%, calcd for $C_{55}H_{84}O_{21}$, C 61.10%, H 7.83%, O 31.07%.

**3.3.12. Compound 12.** Amorphous powder, mp 140–142°C; $[\alpha]_D^{25}$=+12.0 (*c* 0.3, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 225 (3.90) nm; $^1$H NMR of the aglycon: $\delta$ 1.05 (3H, s, Me-19), 1.23 (3H, d, *J*=6.5 Hz, Me-21), 1.29 (3H, s, Me-18), 1.85 (1H, m, H-17), 1.88 (1H, m, H-7a), 2.05 (3H, s, CO*Me*), 2.19 (1H, dd, *J*=10.0, 2.5 Hz, H-4a), 2.26 (1H, m, H-7b), 2.40 (1H, br t, *J*=10.0 Hz, H-4b), 3.53 (1H, m, H-3), 3.83 (1H, m, H-20), 4.63 (1H, dd, *J*=12.0, 4.2 Hz, H-12), 5.48 (1H, br d, *J*=4.5 Hz, H-6); $^{13}$C NMR data of the aglycon: see Table 1; $^1$H and $^{13}$C NMR data of the sugar moiety: see Table 3; ESI-MS *m/z*: 1025 $[M+Na]^+$, 965; anal. C 59.79%, H 8.26%, O 31.95%, calcd for $C_{50}H_{82}O_{20}$, C 59.86%, H 8.24%, O 31.90%.

**3.3.13. Compound 13.** White crystalline powder, mp 140–145°C; $[\alpha]_D^{25}$=+49.0 (*c* 0.3, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 218 sh (3.21), 276 (3.30) nm; NMR data for the aglycon moiety are superimposable on those of compound **2**; $^1$H and $^{13}$C NMR data of the sugar moiety: see Table 2; ESI-MS *m/z*: 1147 $[M+Na]^+$; anal. C 61.91%, H 8.19%, O 29.90%, calcd for $C_{58}H_{92}O_{21}$, C 61.90%, H 8.24%, O 29.86%.

**3.3.14. Compound 14.** Amorphous powder, mp 125–128°C; $[\alpha]_D^{25}$=+19.3 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 221 sh (4.15), 279 (4.30) nm; NMR data of the aglycon moiety are identical to those for compound **1**; $^1$H and $^{13}$C NMR data of the sugar moiety: see Table 3; ESI-MS *m/z*: 1387 $[M+Na]^+$, 1287; anal. C 58.00%, H 7.98%, O 34.02%, calcd for $C_{66}H_{108}O_{29}$, C 58.05%, H 7.97%, O 33.98%.

**3.3.15. Compound 15.** Amorphous powder, mp 165–170°C; $[\alpha]_D^{25}$ +34.0 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 218 sh (4.42) nm; NMR data for the aglycon moiety are superimposable on those of compound **2**; $^1$H and $^{13}$C NMR data of the sugar moiety: see Table 3; ESI-MS *m/z*: 1469 $[M+Na]^+$; anal. C 58.84%, H 7.96%, O 33.20%, calcd for $C_{71}H_{114}O_{30}$, C 58.91%, H 7.94%, O 33.16%.

**3.3.16. Compound 16.** Amorphous powder, 185–190°C dec; $[\alpha]_D^{25}$=+24.0 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 220 sh (2.45), 275 (4.21) nm; NMR data for the aglycon moiety are identical to those for compound **3**; $^1$H and $^{13}$C NMR data of the sugar moiety: see Table 3; ESI-MS *m/z*: 1267 $[M+Na]^+$, 1207; anal. C 59.71%, H 8.10%, O 32.19%, calcd for $C_{62}H_{100}O_{25}$, C 59.79%, H 8.09%, O 32.12%.

**3.3.17. Compound 17.** Amorphous powder, mp 174°C; $[\alpha]_D^{25}$=-22 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 265 (4.25) nm; $^1$H NMR of the aglycon: $\delta$ 1.06 (3H, s, Me-19), 1.14 (3H, s, Me-18), 1.32 (3H, d, *J*=6.4 Hz, Me-21), 1.70 (1H, m, H-17), 1.90 (1H, m, H-7a), 1.93 (3H, s, CO*Me*), 2.25 (1H, m, H-7b), 2.31 (1H, dd, *J*=10.0, 2.5 Hz, H-4a), 2.47 (1H, br t, *J*=9.5 Hz, H-4b), 3.53 (1H, m, H-3), 4.42 (1H, m, H-20), 5.45 (1H, m, H-6); $^{13}$C NMR data of the aglycon: see Table 1; $^1$H and $^{13}$C NMR data of the sugar moiety: see Table 4; ESI-MS *m/z*: 721 $[M+Na]^+$, 661; anal. C 61.82%, H 8.38%, O 29.80%, calcd for $C_{36}H_{58}O_{13}$, C 61.87%, H 8.37%, O 29.76%.

**3.3.18. Compound 18.** Amorphous powder, mp 142°C; $[\alpha]_D^{25}$=−4.0 (*c* 0.6, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 277 (4.37), 320 sh (3.37) nm; $^1$H NMR of the aglycon: $\delta$ 1.08 (3H, s, Me-19), 1.15 (3H, s, Me-18), 1.31 (3H, d, *J*=6.4 Hz, Me-21), 1.70 (1H, m, H-17), 1.90 (1H, m, H-7a), 2.25 (1H, m, H-7b), 2.31 (1H, dd, *J*=9.5, 2.5 Hz, H-4a), 2.47 (1H, br t, *J*=9.5 Hz, H-4b), 3.55 (1H, m, H-3), 4.03 (1H, m, H-20), 5.45 (1H, br d, *J*=4.0 Hz, H-6); $^{13}$C NMR data of the aglycon: see Table 1; $^1$H and $^{13}$C NMR data of the sugar moiety: see Table 4; ESI-MS *m/z*: 1311 $[M+Na]^+$; anal. C 54.00%, H 7.52%, O 38.48%, calcd for $C_{58}H_{96}O_{31}$, C 54.03%, H 7.50%, O 38.47%.

**3.3.19. Compound 19.** Amorphous powder, mp 195°C; $[\alpha]_D^{25}$=−11.0 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 220 sh (4.13), 283 (3.78) nm; NMR data for the aglycon moiety are superimposable on those of compound **18**; $^1$H NMR of the sugar moiety: chain A $\delta$ 3.16 (1H, dd, *J*=9.5, 7.8 Hz, H-2$_{glc\ III}$), 3.28 (1H, d, *J*=9.5 Hz, H-4$_{glc\ III}$), 3.30 (1H, m, H-5$_{glc\ III}$), 3.38 (1H, d, *J*=9.5 Hz, H-3$_{glc\ III}$), 3.68 (1H, dd, *J*=12.0, 5.0 Hz, H-6a$_{glc\ III}$), 3.88 (1H, dd, *J*=12.0, 3.0 Hz, H-6b$_{glc\ III}$), 4.40 (1H, d, *J*=7.8 Hz, H-1$_{glc\ III}$); chain C: see Table 4; $^{13}$C NMR of the sugar moiety: chain A $\delta$ 62.4 (C-6$_{glc\ III}$), 71.7 (C-4$_{glc\ III}$), 75.3 (C-2$_{glc\ III}$), 77.6 (C-3$_{glc\ III}$), 78.2 (C-5$_{glc\ III}$), 104.5 (C-1$_{glc\ III}$); chain C: see Table 4; ESI-MS *m/z*: 1003 $[M+Na]^+$, 841, 519; anal. C 56.28%, H 7.82%, O 35.90%, calcd for $C_{46}H_{76}O_{22}$, C 56.31%, H 7.81%, O 35.88%.

**3.3.20. Compound 20.** Amorphous powder, 270–275°C dec; $[\alpha]_D^{25}$=−6.0 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 225 (4.55), 238 sh (3.78) nm; $^1$H NMR of the aglycon: $\delta$ 0.86 (3H, s, Me-18), 1.10 (3H, s, Me-19), 1.30 (3H, d, *J*=6.4 Hz, Me-21), 1.65 (1H, m, H-17), 3.67 (1H, m, H-3), 4.03 (1H, m, H-20); $^{13}$C NMR data of the aglycon: see Table 1; for $^1$H and $^{13}$C NMR data of the sugar moiety see compound **19** (chain A) and Table 4 (chain B); ESI-MS *m/z*: 843 $[M+Na]^+$; anal. C 58.45%, H 8.37%, O 33.18%, calcd for $C_{40}H_{68}O_{17}$, C 58.52%, H 8.35%, O 33.13%.

### 3.4. Cytotoxic and genotoxic activity

**3.4.1. In vitro cytotoxicity test for anticancer activity in tumor cell lines.** Cell lines: HT29, colorectal human cancer cell line, DNA mismatch repair proficient, established from non polyposic sporadic tumor; LoVo, colorectal human cancer cell line, DNA mismatch repair deficient, established from non polyposic hereditary tumor. The two cancer cell lines were treated with 10, 25, 75, and 100 μg/mL from 24 h to 48 h. All tested compounds were dissolved in DMSO not exceeding 1% final concentration. Negative controls were set up according to the maximum amount of solvent used to prepare each chemical. The ability of single cells to proliferate and form colony during 48 h grown in culture medium was measured. The results are expressed, in percent, as relative cloning efficiency that is the ratio of the number of cell grown after chemical treatment with respect to untreated cell growth.[9]

**3.4.2. In vitro genotoxicity test in human somatic cells.** Phytohemagglutinin-stimulated peripheral blood lymphocytes cultured up to 72 h were used. The addition of cytochalasin B to cultures 44 h before cell harvest blocked the cytodieresis of dividing lymphocytes which assumed a characteristic binucleated shape (a cell with two main nuclei) at the following interphase. Only in these cells was observed the presence of micronuclei. Lymphocyte cultures were treated with 0.1, 1.0, 5.0 50, 100, and 150 μg/mL from 24 to 72 h. All tested compounds were dissolved in DMSO not exceeding 1% final concentration. Negative controls were set up according to the maximum amount of solvent used to prepare each chemical. The frequency of micronuclei (MN) is expressed as the number of micronucleated lymphocytes (containing one or more MN) on 1000 cells scored.[10,11]

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