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Isolation and Structural Characterization of New Glycolipid Ester Type Dimers from the Resin of *Ipomoea tricolor* (Convolvulaceae)[1]

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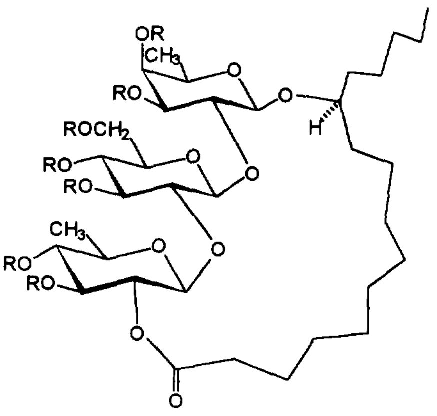
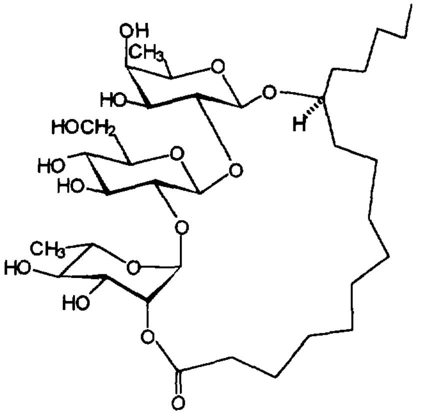
Abstract: An extensive structural investigation using high field NMR spectroscopy and FAB mass spectrometry led to the characterization of tricolorins F-J, minor phytogrowth inhibitor glycolipids purified by recycling HPLC from the resins of *Ipomoea tricolor* Cav. (Convolvulaceae). The potential of various two-dimensional NMR techniques in deducing information about the saccharide substitution and the positions of acylation in these complex oligosaccharides is presented. Tricolorins F and G were found to be trisaccharides forming a macrolactone with the aglycone, (11*S*)-hydroxyhexadecanoic acid (jalapinolic acid). Tricolorins H, I and J consisted of three ester type dimers of either tricolorins F or G and tricoloric acid C, a new hetero-trisaccharide of jalapinolic acid. © 1997 Elsevier Science Ltd.

Several biological activities of the species belonging to *Ipomoea* genus, a member of the morning-glory family (Convolvulaceae), have been attributed to their glycoresin contents.[2,3] In tropical zones of Mexico, farmers make intensive use of *Ipomoea tricolor* Cav. (*I. violacea* L.), among other members of the Convolvulaceae, as a cover crop. This plant has the useful property of suppressing the growth of other plants, including invasive weeds. In a previous investigation, we reported the isolation and structural elucidation of five novel oligosaccharides, tricolorins A-E. These complex glycolipids were isolated and purified by recycling HPLC from the less polar phytogrowth inhibitory fractions of the resin obtained from the $CHCl_3$ soluble extract of this plant material.[4,5] The isolated compounds were linear hetero-tetrasaccharides forming a macrocyclic ester with the aglycone moiety, (11*S*)-hydroxyhexadecanoic acid (jalapinolic acid), and this chemical feature is the most significant among all the individual constituents of the so-called "resin glycosides" of convolvulaceous plants. The complexity of these resins because of the similarity in the structures of some of their individual constituents, due to the fact that they mainly represent isomers[5] involving different sites of esterification and (or) different short-chain acids ester-linked at the oligosaccharide cores, has prevented the isolation of pure glycolipids with high molecular weight from these mixtures of bioactive plant constituents.



1: R = H
7: R = Ac



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Further investigation on the phytogrowth inhibitory fractions of the oligosaccharide mixture of *I. tricolor* allowed the isolation of five additional glycolipids, tricolorins F-J (**1-5**). Once again, the success for total separation of these compounds to homogeneity was achieved by HPLC using a micropore bonded-phase medium in the recycle mode. The maximal resolution of these compounds was obtained with an aminopropylmethylsilyl bonded silica gel column,[5] which was eluted isocratically with CH_3CN-H_2O (84:16). Compounds **1-5** were present in the relative amounts of 2.7% (*Rt* 8.6 min), 1.1% (*Rt* 14.3 min), 2.6% (*Rt* 16.3 min), 76.8% (*Rt* 22.2 min) and 16.5% (*Rt* 28.9 min), respectively. This paper describes the structural investigation using high field NMR spectroscopy and FAB-mass spectrometry of two new macrolactone trisaccharides of jalapinolic acid, tricolorins F (**1**) and G (**2**), and the characterization of tricolorins H (**3**), I (**4**) and J (**5**) as three novel glycolipid ester type dimers of either compounds **1** or **2** and tricoloric acid C (**6**), a new hetero-trisaccharide of jalapinolic acid. The potential of various one- and two-dimensional NMR spectroscopic methods in deducing information about the interglycosidic linkage, the sequence of monosaccharide residues and the positions of esterification in all these complex oligosaccharides is discussed in detail.

Either positive or negative-ion FAB-MS of compounds **1-5** were obtained. Data from these high resolution experiments were useful in determining the molecular formula of each compound. Positive ion mass spectra generated by tricolorins F (**1**) and G (**2**) were found to be identical, with a pseudo-molecular ion at *m/z* 731 $\{M + Na\}^+$ and therefore these constituents should represent diastereoisomeric compounds of molecular formula $C_{34}H_{60}O_{15}$ (M^+ 708). For compound **3**, the negative ion mode revealed a quasi-molecular ion peak $\{M - H\}^-$ at *m/z* 1499, so indicating a molecular formula of $C_{73}H_{128}O_{31}$. Compounds **4** and **5** showed the same $\{M - H\}^-$ ion at *m/z* 1415; consequently, these natural products represented another pair of diastereoisomers having the molecular formula of $C_{68}H_{120}O_{30}$. The negative ion FAB-mass spectra of compounds **3-5** showed the same fragmentation pathways that resulted from the cleavage of a glycolipid ester type dimer to form the corresponding high-mass fragment ions representing each of the two monomeric units.[6] For compound **3**, these peaks were observed at *m/z* 791 (monomeric unit A) and 725 (unit B). Besides the common fragment peaks[5] produced by glycosidic cleavage[7] of the sugar moieties in each of the oligosaccharide cores (Fig. 1), i.e. *m/z* 579 {725 - 146 (methylpentose)}$^-$, 417 {579 - 162 (hexose)}$^-$, and 217 {417 - 146 (methylpentose)}$^-$, compounds **4** and **5** showed a peak at *m/z* 707 (unit A) in place of that detected at *m/z* 791 in the spectrum of compound **3**. The mass difference between these peaks (84 mass units; C_5H_8O) indicated the presence of a (2*S*)-methylbutanoic acid (mba)[5] residue esterifying the oligosaccharide core of the monomeric unit A in compound **3**. This result was confirmed by GC-MS analysis[4] of the organic acid fraction liberated by alkaline hydrolysis of tricolorin H (**3**).

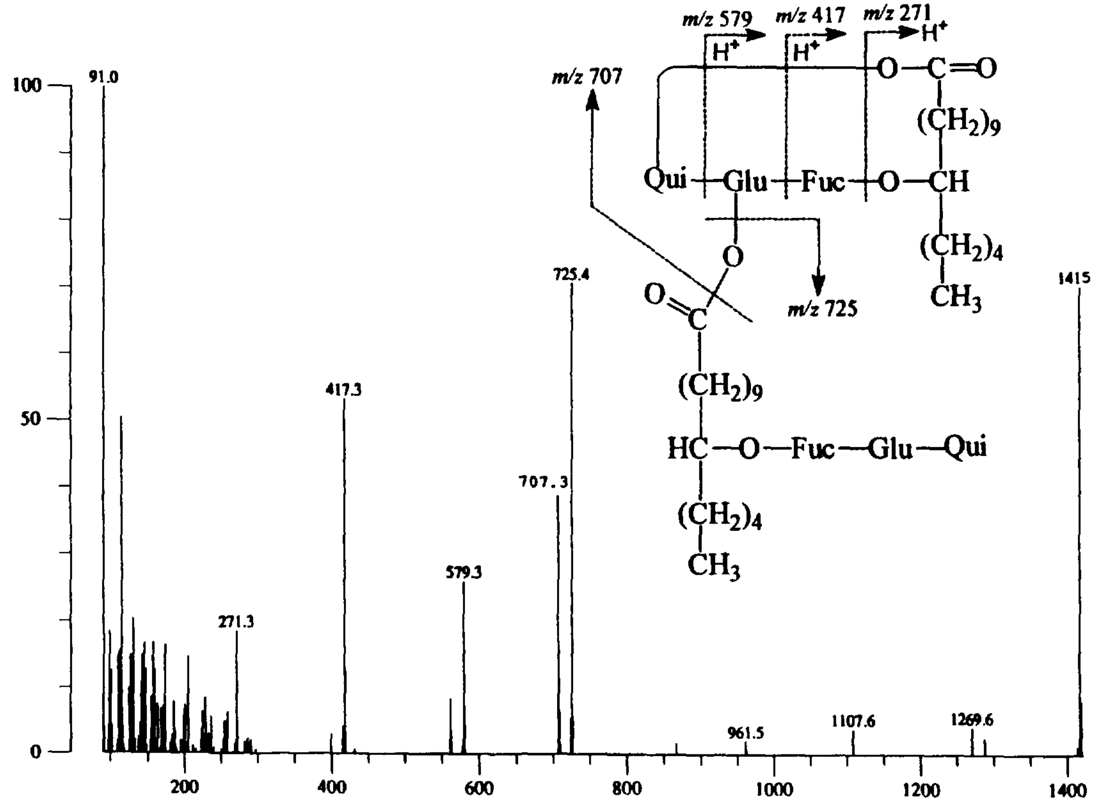


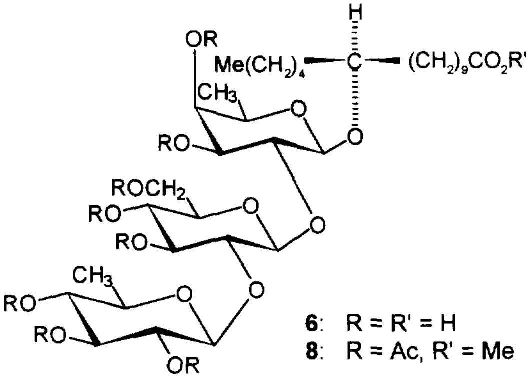
Fig. 1. Negative ion FAB-mass spectrum in a glycerol matrix of tricolorin J (**5**). This technique provides a strong [M – H]$^-$ peak (*m/z* 1415). All the characteristic ions of the sugar sequence are clearly observed. The high-mass ions [M/2 – H]$^-$ (*m/z* 707) and [M/2 – H + H_2O]$^-$ (*m/z* 725) correspond to the cleavage of the dimeric structure.

Table 1. ^{1}H-NMR Data for Tricolorins F (**1**), G (**2**) and derivatives **6**, **7** and **8**^{a}

Proton^b	**1**	**2**	**6**	**7**	**8**
Fuc-1	4.74 d (7.5)	4.80 d (8.0)	4.80 d (7.5)	4.91 d (7.5)	4.74 d (7.6)
2	4.36 dd (7.5, 8.4)	4.75 dd (8.0, 9.0)	4.33 dd (7.5, 9.0)	4.42 dd (7.5, 10.0)	4.24 dd (7.6, 9.7)
3	4.17 dd (3.0, 8.4)	4.27 dd (3.5, 9.0)	4.19 dd (3.0, 9.0)	5.36 dd (3.0, 10.0)	5.48 dd (3.4, 9.7)
4	4.06* bs	3.86 d (3.5)	4.11-4.02*	5.83 d (3.0)	5.51 d (3.4)
5	3.81*	3.77 q (6.5)	3.83 q (6.5)	4.06 q (6.0)	3.90-3.84*
6	1.55 d (6.1)	1.55 d (6.5)	1.56 d (6.5)	1.25 d (6.0)	1.22 d (6.4)
Glu-1	5.23 d (7.5)	5.80 d (8.0)	5.17 d (7.5)	5.33 d (8.0)	5.17 d (7.6)
2	4.07-4.02*	3.98 dd (8.0, 9.0)	4.06 dd (7.5, 8.5)	4.19 dd 8.0, 9.5)	3.95-3.91 m*
3	4.17*	4.12*	4.21-4.13*	5.76 dd (9.5, 9.5)	5.61 dd (9.4, 9.7)
4	4.17*	4.12*	4.15 dd (9.0, 9.0)	5.33 dd (9.5, 9.5)	5.34 dd (9.7, 9.7)
5	3.77 bd (9.0)	3.55 dt (4.2, 9.5)	3.73 m*	4.03 ddd (3.0, 5.0, 9.5)	3.98 ddd (2.4, 4.2, 9.7)
6a	4.38 m*	4.09*	4.41 dd (3.0, 11.2)	4.58 dd (5.0, 12.5)	4.59 dd (4.2, 12.1)
6b	4.38 m*	4.09*	4.29 dd (4.5, 11.2)	4.29 dd (3.0, 12.5)	4.36 dd (2.4, 12.1)
Rha-1		5.71 d (2.0)			
2		6.15 dd (2.0, 3.5)			
3		4.80 dd (3.5, 9.5)			
4		4.29 dd (9.5, 9.5)			
5		4.89 dq (6.0, 9.5)			
6		1.90 d (6.0)			
Qui-1	5.23 d (7.5)		5.25 d (7.5)	5.44 d (7.5)	5.02 d (8.0)
2	5.65 dd (7.5, 8.5)		4.07 dd (7.5, 8.5)	5.26 dd (7.5, 9.5)	5.41 dd (8.0, 9.5)
3	4.19 dd (8.5, 9.0)		4.15 dd (8.5, 9.0)	5.49 dd (9.5, 9.5)	5.68 dd (9.5, 9.5)
4	3.77 dd (9.0, 9.0)		3.71 dd (9.0, 9.0)	5.14 dd (9.5, 9.5)	5.35 dd (9.5, 9.5)
5	3.81*		3.81 dq (6.0, 9.0)	3.81 dq (6.0, 9.5)	3.95-3.91 m*
6	1.72 d (5.8)		1.69 d (6.0)	1.50 d (6.0)	1.54 d (6.1)
Jal-2a	2.98 m	2.48 ddd (3.5, 8.5, 14.0)	2.55 t (7.4)	2.66 ddd (6.0, 9.0, 15.0)	2.37 t (7.4)
2b	2.45 m	2.32 ddd (3.5, 9.0, 14.0)		2.54 ddd (6.5, 9.0, 15.0)	
11	3.94 m	3.91 m	3.91 m	3.80 m	3.90-3.84* m
16	0.85 t (7.0)	0.83 t (7.0)	0.85 t (7.0)	0.86 t (7.0)	0.84 t (6.9)

a) Data recorded at 500 MHz in C_5D_5N. Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (J) is given in parenthesis in Hz. Chemical shifts marked with an asterisk (*) indicate overlapped signals. Spin-coupled patterns are designated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad signal. All assignments are based on ^{1}H-^{1}H COSY and TOCSY data.

b) Abbreviations: Fuc = fucose, Glu = glucose, Rha = rhamnose. Qui = quinovose, Jal = 11-hydroxyhexadecanoyl.



6: R = R' = H
8: R = Ac, R' = Me

On alkaline hydrolysis, compound **1** yielded an H_2O-soluble glycosidic acid, named tricoloric acid C (**6**). The acid-catalyzed hydrolysis of **6** gave (11*S*)-hydroxyhexadecanoic acid,[4,5] as the aglycone portion and a mixture of monosaccharides. The saccharide units were identified as fucose, glucose and quinovose by using reversed-phase HPLC.[5] To complete the determination of the primary structure of compounds **1** and **2**, ^{1}H and ^{13}C NMR spectra of these compounds were recorded. In contrast to the well resolved ^{1}H NMR spectrum of compound **2**, tricolorin F (**1**) showed a severe signal overlap in the proton region between δ 3.5-4.5, and this situation initially hampered the complete ^{1}H (Table 1) and ^{13}C (Table 2) assignments for the natural glycolipid. To enhance signal dispersion, we decided to prepare the peracetyl derivative 7 from compound **1**, as well as the peracetyl methyl ester **8** from tricoloric acid C (**6**). No derivatization was needed to achieve the total structural assignment of tricolorin G (**2**) which was readily made through an extensive investigation using NMR spectroscopy. A combination of ^{1}H NMR spectra in conjunction with 2-D homonuclear techniques including DQF-COSY and TOCSY allowed all C-bonded protons to be sequentially assigned within each ring system (Table 1) and by the use of the ^{1}H-detected $\{^1H, ^{13}C\}$ one-bond correlation experiment (HMQC) all the resonances in the ^{13}C NMR spectra were assigned (Table 2). This NMR analysis led to the identification of fucose, glucose and rhamnose as the monosaccharides present in compound **2**, confirming the diastereoisomeric nature of tricolorins F (**1**) and G (**2**). The interglycosidic connectivities, as well as the site of lactonization, were established entirely on the basis of the observed ^{13}C-^{1}H long-range crosspeaks ($^{2,3}J_{CH}$) in the HMBC experiments.[8,9] For example, the following ^{13}C-^{1}H long-range cross peaks (^3J) were observed in the spectrum of tricolorin G (**2**) and unambiguously assigned as those between C-1 (δ_C 102.6) of fucose and H-11 of the aglycone (δ_H 3.91), C-1 of glucose (δ_C 101.5) and fucose H-2 (δ_H 4.75), and C-1 of rhamnose (δ_C 101.4) and glucose H-2 (δ_H 3.98). The same long-range heteronuclear coupling correlations were used to determine the linkages within the trisaccharide core of peracetyl tricolorin F (7), e.g. a connectivity was observed between H-

Table 2. ^{13}C-NMR Data for tricolorins F (**1**), G (**2**) and derivatives **6**, **7** and **8**[a]

^{13}C[b]	**1**	**2**	**6**	**7**	**8**
Fuc-1	102.4	102.6 (160.0)	102.1 (160.0)	101.9	101.6
2	81.7	76.0	82.4	75.8	76.9
3	75.4	76.4	74.9	75.8	74.6
4	72.6	73.2	72.2	71.1	71.4
5	71.3	71.3	71.1	68.6	69.5
6	17.4	17.2	17.1	16.5	16.4
Glu-1	103.6	101.5 (164.7)	103.8 (164.6)	103.5	101.7
2	85.0	84.8	85.4	78.1	80.0
3	77.3	77.7	77.6	72.9	75.2
4	72.3	72.6	71.7	69.8	69.5
5	76.1	76.9	76.2	71.9	71.3
6	63.6	62.9	63.0	62.9	62.7
Rha-1		101.4 (172.5)			
2		74.1			
3		70.4			
4		74.7			
5		70.1			
6		19.3			
Qui-1	103.9		106.4 (162.4)	100.2	101.5
2	76.1		76.3	75.2	72.3
3	76.9		77.3	73.6	73.7
4	76.9		77.2	73.4	73.8
5	74.3		73.8	71.3	70.0
6	18.4		18.2	17.4	17.9
Jal-1	173.2	173.5	176.3	172.4	174.0
2	35.6	35.0	37.4	34.7	34.1
11	81.2	79.6	79.7	81.8	80.3
16	14.4	14.2	14.2	14.2	14.3

a) Data recorded at 125.7 MHz in C_5D_5N. Chemical shifts (δ) are in ppm relative to TMS. Coupling constants ($^1J_{CH}$, in Hz) are in parnenthesis. All assignments are based on HMQC and HMBC data.

b) Abbreviations: Fuc = fucose, Glu = glucose, Rha = rhamnose, Qui = quinovose, Jal = 11-hydroxyhexadecanoyl.

2 on glucose at δ 4.19 and the anomeric carbon of the quinovose at δ 100.2, and between H-2 on fucose (δ 4.42) and C-1 of glucose (δ 103.5). Verification of the position of lactonization was also provided by the ${}^{3}J_{CH}$ connectivities between the carbonyl group of the aglycone and proton H-2 of the terminal monosaccharide unit in both natural trisaccharides, compounds **1** and **2**. To determine conclusively the anomeric configuration of the glycosyl linkages a series of gated decoupling experiments were performed on tricolorin G (**2**) and tricoloric acid C (**6**). From the completely resolved multiplets for the three anomeric ^{13}C atoms of compound **6** (Table 2), all ${}^{1}J_{CH}$ values were found to be < 165 Hz. Such coupling constants established the mode of glycosidic linkage as β in the ${}^{4}C_{1}$ conformation of the pyranose[10] type for each of the monosaccharide units.[5] These results for compound **2** showed ${}^{1}J_{CH}$ coupling values of 160.0 Hz for fucose, 164.7 Hz for glucose and 172.5 Hz for rhamnose. The latter value is typical for the equatorial configuration of the anomeric proton, confirming a ${}^{1}C_{4}$ conformation for the α-L-rhamnose residue.[11,12]

In this way, the primary structure of tricolorin F (**1**) was established as (11S)-hydroxyhexadecanoic acid 11-*O*-β-D-quinovopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1→2)-β-D-fucopyranoside-(1,2'''-lactone). A different triglycoside of jalapinolic acid was identified as the oligosaccharide core of tricolorin G (**2**) and the structure of this natural product was determined to be (11*S*)-hydroxyhexadecanoic acid 11-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-β-D-fucopyranoside-(1,2'''-lactone).

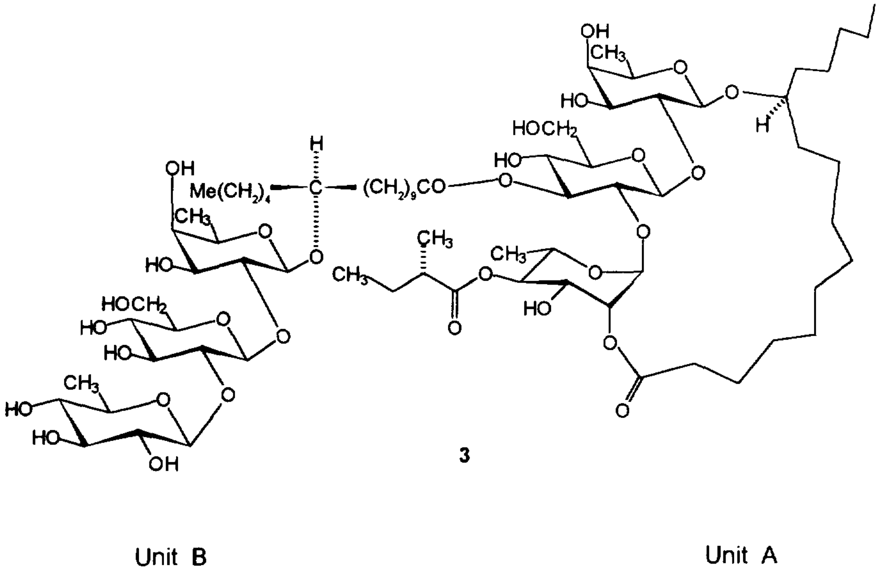


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Table 3. ^1H and ^{13}C NMR Data for Tricolorin **H** (**3**)[a]

	Unit A		Unit B	
Residue[b]	^1H	^{13}C	^1H	^{13}C
Fuc-1	4.80 d (8.0)	102.2	4.85 d (7.5)	101.6
2	4.78 dd (8.0, 9.0)	75.0	4.35 dd (7.5, 9.0)	82.8
3	4.25*	76.6	4.19*	74.9
4	3.99*	73.3	4.07*	72.1
5	3.84*	71.3	3.87*	71.1
6	1.59 d (6.5)	17.2	1.58 d (6.5)	17.3
Glu-1	5.89 d (8.0)	100.6	5.19 d (8.0)	103.8
2	4.01 dd (8.0, 9.5)	83.5	4.14*	84.9
3	5.84 dd (9.5, 9.5)	78.1	4.21*	78.1
4	4.21*	69.6	4.23*	71.5
5	3.49 dt (3.5, 9.5)	77.2	3.75*	76.4
6a	4.08 dd (3.5, 11.7)	61.6	4.44 dd (3.0, 11.4)	62.8
6b	4.01 dd (3.0, 11.7)		4.37 dd (4.6, 11.4)	
Rha-1	5.39 d (1.5)	100.4		
2	5.62 dd (1.5, 3.2)	74.6		
3	4.69 dd (3.2, 9.5)	67.3		
4	5.70 dd (9.5, 9.5)	75.2		
5	4.87 dq (6.5, 9.5)	67.3		
6	1.68 d (6.5)	19.0		
Qui-1			5.37 d (7.5)	106.2
2			4.13*	76.4
3			4.13*	77.7
4			3.75*	77.5
5			3.82*	73.8
6			1.74 d (6.0)	18.3
Jal-1		173.8		174.1
2a	2.24 m	34.8	3.03 ddd (6.4, 9.5, 16.2)	34.6
2b	2.19 m		2.66 ddd (6.5, 9.2, 16.2)	
11	3.91 m	79.5	3.95 m	78.7
16	0.86 t (7.0)**	14.3	0.85** t (7.0)	14.2
mba-1		176.4		
2	2.39 tq (7.0, 7.0)	41.4		
2-Me	1.17 d (7.0)	16.9		
3-Me	0.89** t (7.0)	11.6		

a) Data recorded in C_5D_5N at 500 MHz for ^1H and 125.7 MHz for ^{13}C. Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (*J*) is given in parenthesis in Hz. Chemical shifts marked with an asterisk (*) indicate overlapped signals and those with double asterisks (**) interchangeable resonances. Spin-coupled patterns are designated as follows: d = doublet, t = triplet, q = quartet, m = multiplet. ^1H assignments are based on ^1H-^1H COSY and TOCSY data. ^{13}C assignments are based on HMQC and HMBC data.

b) Abbreviations: Fuc = fucose, Glu = glucose, Rha = rhamnose, Qui = quinovose, Jal = 11-hydroxyhexadecanoyl, mba = 2-methylbutanoyl.

Tricolorin H (**3**) represented a heterodimer of the two previously described trisaccharides. A mild alkaline hydrolysis of this natural product afforded each of the monomeric units which were identified by co-elution in HPLC with natural tricolorin G (**2**) and tricoloric acid C (**6**). The ^{1}H NMR spectrum of compound **3** was completely assigned by the combination of DQF-COSY and TOCSY experiments. The ^{1}H chemical shifts and coupling constants are listed in Table 3. The diagnostic resonances observed in the downfield region at δ 5.89, 5.39, 5.37, 5.19, 4.85 and 4.80 were assigned to the anomeric protons by virtue of their mutiplicities as doublets. A group of three non-anomeric ring proton signals was found to be paramagnetically shifted to δ 5.84, 5.70 and 5.62 (Table 3), reflecting the presence of three sites of acylation. In general, the remaining region where the majority of the methyne resonances appear was intricate due to partial spectral overlap. However, expansion of the TOCSY spectrum for the region δ 3.4-5.9 showed that at least one proton signal for each monosaccharide unit was completely resolved. Therefore, edited ^{1}H-NMR sub-spectra[8] for each individual monosaccharide moiety were obtained to accomplish the assignment of all resonances.

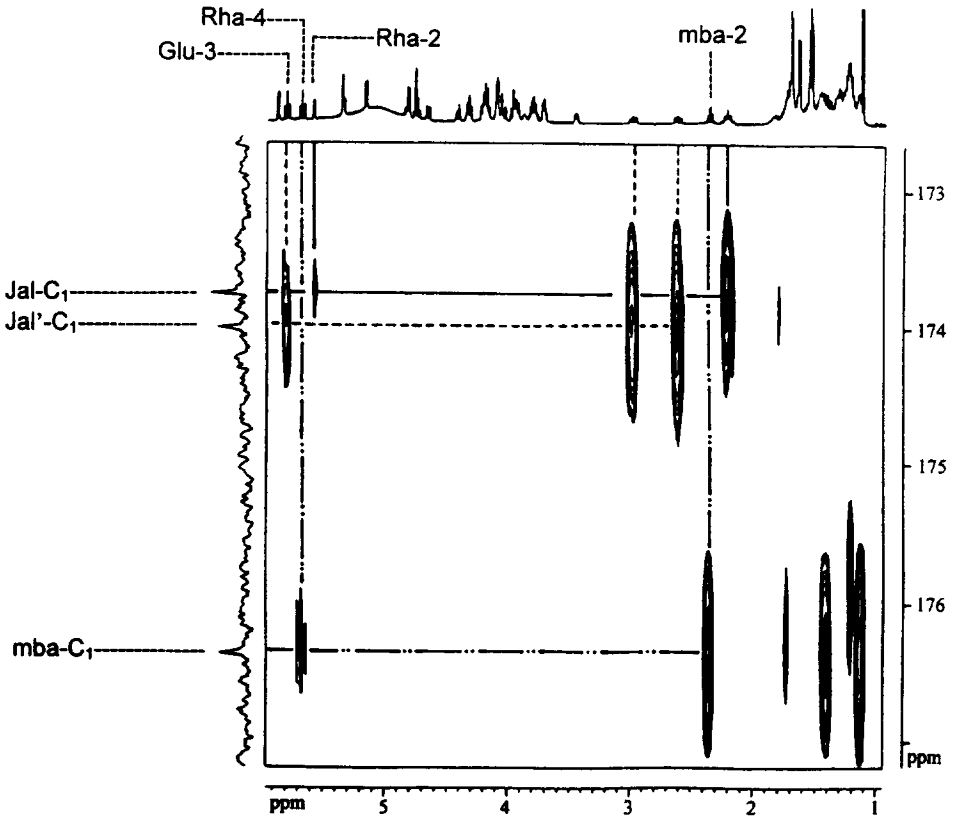
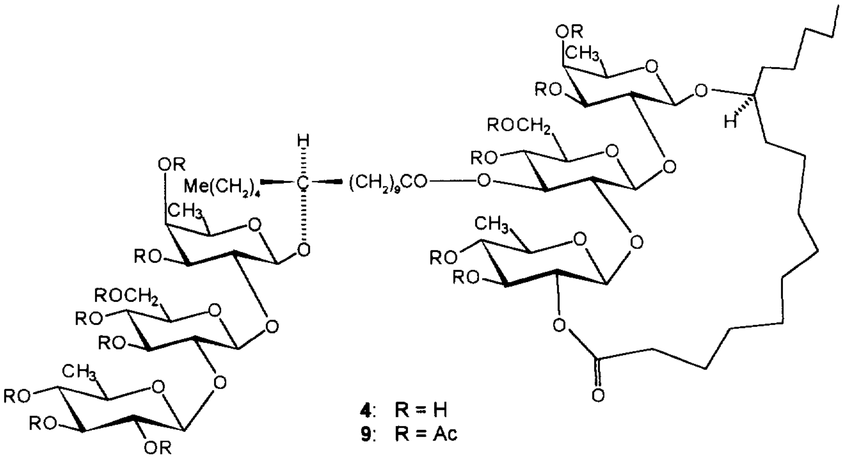


Fig. 2. ^{1}H-Detected heteronuclear multiple-bond correlation (HMBC) spectrum of tricolorin H (**3**). Expanded region showing the $^3J_{CH}$ connectivities of the ^{13}C carbonyl signals with the oligosaccharide proton resonances.

Table 4. ^1H and ^{13}C NMR spectral data for derivative **9**[a].

	Unit A		Unit B	
Residue	^1H	^{13}C	^1H	^{13}C
Fuc-1	4.89 d (8.0)	102.1	4.75 d (8.0)	101.7
2	4.40 dd (8.0, 10.0)	76.0	4.25 dd (8.0, 10.0)	77.0
3	5.36 dd (3.0, 10.0)	75.8	5.49 dd (3.5, 9.5)	74.7
4	5.80 d (3.0)	71.3	5.52 dd (1.0, 3.5)	71.4
5	4.04 q (6.5)	68.7	3.87 dq (1.0, 6.5)	69.0
6	1.23 d (6.5)	16.5	1.22 d (6.5)	16.6
Glu-1	5.34 d (8.0)	103.6	5.17 d (7.0)	101.9
2	4.21 dd ((8.0, 9.5)	78.2	3.94 dd (7.0, 9.5)	80.1
3	5.81 dd (9.5, 9.5)	72.9	5.62 dd (9.5, 9.5)	75.4
4	5.35 dd (9.5, 9.5)	70.0	5.37-5.33*	69.6
5	4.04*	72.1	3.98 ddd (2.5, 4.0, 10.0)	71.5
6a	4.58 dd (5.0, 12.2)	63.1	4.59 dd (4.0, 12.0)	62.9
6b	4.29 bd (12.2)		4.36 dd (2.5, 12.0)	
Qui-1	5.45 d (7.5)	100.3	5.03 d (8.0)	101.7
2	5.26 dd (7.5, 9.5)	75.4	5.41 dd (8.0, 9.5)	72.5
3	5.48 dd (9.5, 9.5)	73.9	5.68 dd (9.5, 9.5)	73.9
4	5.12 dd (9.5, 9.5)	73.6	5.37-5.33*	74.0
5	3.81 dq (6.0, 9.5)	71.5	3.91 dq (6.5, 10.0)	70.2
6	1.48 d (6.0)	17.6	1.54 d (6.5)	17.9
Jal-1		172.7		173.6
2a	2.69 m	34.9	2.77 ddd (7.5, 7.5, 16.4)	34.2
2b	2.59 ddd (7.5, 8.0, 15.5)		2.57 ddd (8.0, 8.0, 16.4)	
11	3.78 m	81.9	3.85 m	80.4
16	0.85 t (7.0)	14.3	0.85 t (7.0)	14.3

a) For instrumental conditions and abbreviations, see Table 3.



4: R = H
9: R = Ac

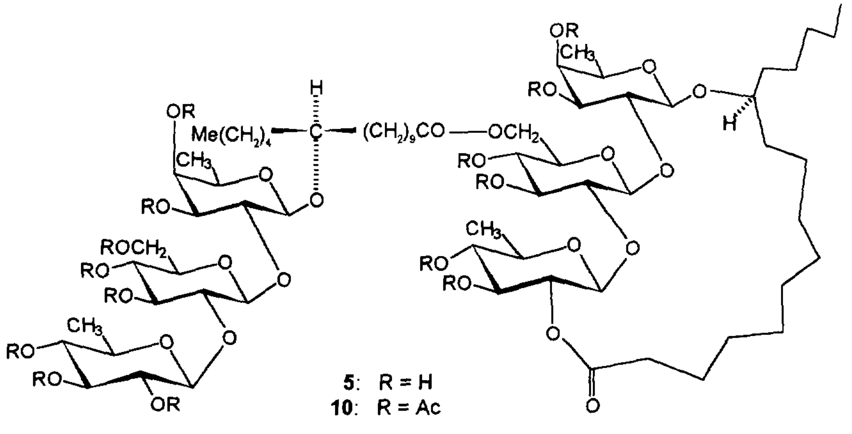
HMQC studies then allowed assignment of the corresponding carbons via one-bond ^{13}C-^{1}H (Table 3) correlations, while HMBC technique was used to locate the methylbutyric acid residue, the ester linkage with tricoloric acid C, i.e. monomeric unit B, and the position of lactonization on the oligosaccharide core of monomeric unit A. The most shielded carbonyl resonance (δ_C 173.8) was easily assigned to the lactone[5] functionality and its observed ^{2}J-coupling with each of the methylene protons at δ_H 2.19 and 2.24 allowed the assignment of the C-2 position of the aglycone moiety of monomeric unit A. The site of lactonization was corroborated as being placed at C-2 of rhamnose by the observed ^{3}J-coupling between the carbonyl carbon of the lactone and its geminal proton (δ_H 5.62) in the pryranose ring. The location of the 2*S*-methylbutanoate residue was determined by the observed correlations between the multiplet at δ 2.39 (H-2 mba; $^{2}J_{CH}$) and the triplet at δ 5.70 (H-4 Rha; $^{3}J_{CH}$) with the carbonyl resonance at δ 176.4 (C-1 mba). Similarly, verification of the position of esterification by tricoloric acid C (**6**) on the oligosaccharide core of monomeric unit A was provided by the $^{3}J_{CH}$ connectivities between the carbonyl group at δ 174.1 and H-3 (δ_H 5.84) of glucose on the macrocyclic monomer. It was also possible to link the carbonyl ester group of monomer B with their corresponding vicinal methylene protons $(^{2}J_{CH})^{12}$ at δ 3.03 and 2.66 in the 11-hydroxyhexadecanoyl moiety (Fig. 2). It is interesting to mention that all NMR spectral data for tricolorin G (**2**) and tricoloric acid C (**6**) were entirely superimposable with those registered for compound **3** except for those resonances downfield shifted on monomeric unit A of the latter compound as a consequence of acylation with the fatty acid residues, i.e. H-3 (δ 5.84) and C-3 (δ 78.1) of glucose and H-4 (δ 5.70) and C-4 (δ 75.2) of rhamnose. Consequently, the glycolipid ester-type dimeric structure of tricolorin H (**3**) was determined to be (11*S*)-hydroxyhexadecanoic acid 11-*O*-α-L-{4-*O*-(2*S*-methylbutanoyl)}-rhamnopyranosyl-(1 → 2)-*O*-β-D-{3-*O*-(11*S*-hydroxyhexadecanoyl 11-*O*-β-D-quinovopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-fucopyranoside)}-glucopyranosyl-(1→2)-β-D-fucopyranoside-(1,2'''-lactone).

The diastereoisomeric structures of tricolorins I (**4**) and J (**5**) were established as ester type dimers of the same oligosaccharide core since both compounds on alkaline hydrolysis yielded tricoloric acid C (**6**), as the single saponification product. Under mild alkaline hydrolysis, compounds **4** and **5** afforded tricolorin F (**1**) and tricoloric acid C (**6**), which represented each of the monomeric units of the natural product dimers. Therefore, the only difference should be the location of the ester linkage established between the two monomers forming tricolorins I (**4**) and J (**5**). High field NMR spectra were obtained for both natural products. Unfortunately, severe signal overlap due to the structural simmetry of the dimers prevented a detailed assignment of all resonances in the ^{1}H and ^{13}C NMR spectra. To unambiguously establish the position of esterification between each of the monomeric units of tricolorin I (**4**) and J (**5**), a derivatization process from the native oligosaccharides was conducted. On acetylation, natural compounds **4** and **5** afforded derivatives **9** and **10**, respectively. Comparison of the ^{1}H NMR spectra obtained for peracetyl tricolorin F (**7**) and compounds **9** and **10** provided support for the location of the ester linkage. After measuring the ^{1}H-^{1}H COSY, TOCSY and ^{13}C-

Table 5. ^{1}H and ^{13}C NMR spectral data for derivative **10**[a].

	Unit A		Unit B	
Residue	^{1}H	^{13}C	^{1}H	^{13}C
Fuc-1	4.89 d (7.5)	101.8	4.75 d (8.0)	101.5
2	4.39 dd (7.5, 10.0)	75.7	4.24 dd (8.0, 10.0)	76.8
3	5.35 dd (5.0, 10)	75.7	5.48 dd (3.5, 9.5)	74.5
4	5.80 d (3.0)	71.1	5.50 dd (1.0, 3.5)	71.1
5	4.04 q (6.0)	68.5	3.85 dq (1.0, 6.5)	68.8
6	1.22 d (6.0)	16.3	1.21 d (6.5)	16.4
Glu-1	5.31 d (8.0)	103.5	5.17 d (7.0)	101.7
2	4.18 dd ((8.0, 9.5)	78.0	3.92 dd (7.0, 9.5)	79.8
3	5.75 dd (9.5, 9.5)	72.9	5.61 dd (9.5, 9.5)	75.1
4	5.35 dd (9.5, 9.5)	69.7	5.37-5.32*	69.4
5	4.04*	71.9	3.98 ddd (3.0, 4.0, 10.0)	71.3
6a	4.61 dd (5.0, 12.2)	62.6	4.58 dd (4.5, 12.2)	62.8
6b	4.35 dd (2.5, 12.2)		4.35 dd (2.5, 12.2)	
Qui-1	5.42 d (8.0)	100.1	5.01 d (8.0)	101.5
2	5.23 dd (8.0, 9.5)	75.1	5.41 dd (8.0, 9.5)	72.2
3	5.47 dd (9.5, 9.5)	73.6	5.67 dd (9.5, 9.5)	73.6
4	5.11 dd (9.5, 9.5)	73.3	5.39 dd (9.5, 9.5)	73.7
5	3.79 dq (6.0, 9.5)	71.3	3.90 dq (6.0, 9.5)	69.9
6	1.47 d (6.0)	17.4	1.54 d (6.0)	17.7
Jal-1		172.4		173.3
2a	2.52-2.46 m	34.6	2.52-2.46 m	34.1
2b	2.68-2.61 m		2.52-2.46 m	
11	3.82-3.76 m	81.7	3.87-3.82 m	80.2
16	0.86 t (7.0)	14.2	0.83 t (7.0)	14.2

a) For instrumental conditions and abbreviations, see Table 3.



5: R = H
10: R = Ac

^1H HETCOR (Tables 4 and 5), the esterification for tricolorin I (**4**) was established on C-3 of the glucose in monomeric unit A, since the corresponding proton signal in compound **9** (δ_{H-3} 5.81) had been shifted downfield ($\Delta\delta$ = 0.05) relative to the same H-3 glucose resonance in compounds **7** and **10**. Therefore, the site of esterification for tricolorin J (**5**) was corroborated as being placed at C-6 of the glucose unit in the monomeric moiety carrying the lactone functionality. Additional evidences for the position of esterification were provided by the observed HMBC correlation between the carbonyl carbon of the ester group (δ_C 173) in monomeric unit B and its geminal proton in the pyranose ring of monomer A, i.e. H-3 of glucose in compound **9** and H-6 of glucose (δ_{H-6} 4.61) in compound **10**.

In conclusion, the structure of tricolorin I (**4**) was elucidated as (11*S*)-hydroxyhexadecanoic acid 11-*O*-β-D-quinovopyranosyl-(1 → 2)-*O*-β-D-{3-*O*-(11*S*-hydroxyhexadecanoyl 11-*O*-β-D-quinovopyranosyl-(1→ 2)-*O*-β-D-glucopyranosyl-(1→2)-β-D-fucopyranoside)}-glucopyranosyl-(1→2)-β-D-fucopyranoside-(1,2'''-lactone). The structure of tricolorin J (**5**) was established as (11*S*)-hydroxyhexadecanoic acid 11-*O*-β-D-quinovopyranosyl-(1 → 2)-*O*-β-D-{6-*O*-(11*S*-hydroxyhexadecanoyl 11-*O*-β-D-quinovopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1→2)-β-D-fucopyranoside)}-glucopyranosyl-(1→2)-β-D-fucopyranoside-(1,2'''-lactone).

Finally, we can infer that the resins of *Ipomoea tricolor* represent complex mixtures of glycolipids formed by several tri- and tetrasaccharide cores,[5] acylated by lower molecular weight organic acids, and the mixture of some of their dimers, e.g. tricolorins H-J (**3-5**). This investigation provides an additional support to the general structure proposed for the resins of convolvulaceous plants on the basis of their behaviour towards alkalis and acids.[13] Accordingly, the resin glycosides could represent mixtures of oligomers of high molecular weight as suggested for one of the oligosaccharide constituents of the MeOH-soluble fraction obtained from the roots of *Exogonium purga* by Mannich and Schumann.[14] Recently, this suggestion was also confimed by the isolation of merremin, the first example of an ester-type dimer of an individual resin glycoside from the roots of *Merremia hungaiensis*, a Chinese medicinal member of the Convolvulaceae family.[6]

EXPERIMENTAL

The general experimental procedures, including HPLC instrumentation and NMR techniques, along with the handling of plant material and purification of the crude resin glycosides from *Ipomoea tricolor* are described in the preceding papers of this series,[4,5] unless stated otherwise.

Isolation and purification of tricolorins F-I. The polar fractions 146-155 (4.1 g) collected from the original column used for fractionation[4] of the $CHCl_3$ extract of *I. tricolor* were submitted to a secondary purification process by flash column chromatography (Baker Si gel, 40 μm). Subfractions (2.0 g) eluted with $CHCl_3$-Me_2CO (3:2) were analysed by HPLC. 200 μg of sample (20 μl) were applied to a Waters standard column for carbohydrate analysis (300 × 3.9 mm), and five new oligosaccharides (tricolorins F-J) were detected according to their retention times (*Rt*) in CH_3CN-H_2O (88:12, flow rate = 0.5 ml/min). This analytical separation was then transferred to a preparative column using the recycle mode operation. The HPLC system equipped with a Waters aminopropylmethylsilyl amorphous silica column (150 × 19 mm; μBondapak, 10 μm, 125 Å) was used to perform the separation. Sample load and solvent consumption were determined as previously described.[5] Elution was conducted isocratically with the same solvent system used for analytical chromatography with a flow rate of 6.0 ml/min. Compounds **2** (*Rt* 14.3 min, peak area 1.1%) and **3** (*Rt* 16.3 min, 2.6%) were collected during the first pass through the column. Eight cycle passes achieved complete separation of compounds **1** (*Rt* 8.6 min, 2.7%), **4** (*Rt* 22.2 min, 76.8%) and **5** (*Rt* 28.9 min, 16.5%) to homogeneity.

***Tricolorin F* (1).** Mp 116-118°; $[\alpha]_D$ -30° (c = 0.1, MeOH); ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; positive FAB-MS *m/z* (rel. int.) $[M + Na]^+$ 731 (50.8); positive HRFAB-MS *m/z* 731.3835 $[M + Na]^+$, $C_{34}H_{60}O_{15}Na$ requires 731.3829.

***Tricolorin G* (2).** Mp 98-100°; $[\alpha]_D$ -42° (c = 0.1, MeOH); ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; positive FAB-MS *m/z* (rel. int.) $[M + Na]^+$ 731 (37.4); positive HRFAB-MS *m/z* 731.3891 $[M + Na]^+$, $C_{34}H_{60}O_{15}Na$ requires 731.3829.

***Tricolorin H* (3).** Mp 118-120°; $[\alpha]_D$ -22° (c = 0.1, MeOH); ^{1}H and ^{13}C NMR see Table 3; negative FAB-MS *m/z* (rel. int.) $[M - H]^-$ 1499 (10), 791 (23), 725 (43), 579 (20), 417 (29), 271 (76); negative HRFAB-MS *m/z* 1499.8370 $[M - H]^-$, $C_{73}H_{127}O_{31}$ requires 1499.8361.

***Tricolorin I* (4).** Mp 136-138°; $[\alpha]_D$ -70° (c = 0.1, MeOH); ^{1}H NMR (C_5D_5N): 5.76 dd (9.6, 9.6 Hz), 5.53 d (7.5 Hz), 5.33 d (7.5 Hz) 4.81 d (7.5 Hz), 4.69 d (7.5 Hz), 4.42 bd (11.5 Hz), 2.66 m, 2.56 m, 2.36-2.34 m, 1.70 d (6.0 Hz), 1.54 d (6.4 Hz), 0.84 t (7.0 Hz); ^{13}C NMR (C_5D_5N): 172.9, 172.8 (2 × C=O), 106.1, 103.7, 103.2, 103.0, 101.6, 101.0, 84.8, 82.6, 81.3, 78.8, 78.0, 77.6, 77.5, 77.2, 76.3, 75.7, 75.1, 74.9, 74.1, 73.7, 72.0, 71.5, 71.1, 70.9 (methyne signals, from DEPT), 62.7, 35.0, 34.7, 34.6, 34.5, 34.4, 32.3, 30.1, 30.0, 29.8, 29.7, 29.5, 29.3, 28.8, 25.7, 25.6, 25.4, 25.2, 25.1, 22.9, 22.8 (methylenes), 18.3, 18.1, 17.3, 17.2, 14.2 14.1 (methyls); negative FAB-MS *m/z* (rel. int.) $[M - H]^-$ 1415 (3.4), 725 (12), 707 (16.1), 579 (8), 417 (6.4), 271 (9.4); negative HRFAB-MS *m/z* 1415.8007 $[M - H]^-$, $C_{68}H_{119}O_{30}$ requires 1415.7786.

***Tricolorin J* (5).** Mp 131-133°; $[\alpha]_D$ -110° (c = 0.1, MeOH); ^{1}H NMR (C_5D_5N): 5.63 dd (8.0, 8.0 Hz), 5.35 d (7.0 Hz), 5.24 m, 5.16 d (7.6 Hz), 4.83 d (7.6 Hz), 4.73 d (7.3 Hz), 4.43 dd (2.7, 11.3 Hz), 1.71 d (6.0 Hz), 1.55 d (6.3 Hz), 0.89 t (6.5 Hz), 0.84 t (7.0 Hz); ^{13}C NMR (C_5D_5N): 173.7, 172.9 (2 × C=O), 106.2 (2 × C), 103.9 (2 × C), 103.3, 101.6, 84.9, 82.8, 81.4, 78.7, 78.1, 77.7, 77.5, 76.6, 76.4, 74.9, 74.0, 73.8, 72.0, 71.6, 71.4, 71.1 (methyne signals, from DEPT), 64.6, 62.8, 35.4, 35.1, 34.4, 32.5, 32.3, 30.4, 30.1, 29.9, 29.7, 29.5, 28.9, 25.8, 25.6, 25.3, 25.1, 23.0, 22.9 (methylenes), 18.3, 17.3, 17.2, 14.3, 13.7 (methyls); negative FAB-MS *m/z* (rel. int.) $[M -H]^-$ 1415 (70.2), 725 (70.7), 707 (38.9), 579 (26.0), 417 (53.3), 271 (18.5); negative HRFAB-MS *m/z* 1415.7867 $[M - H]^-$, $C_{68}H_{119}O_{30}$ requires 1415.7786.

***Mild alkaline hydrolysis of tricolorin H*.** A solution of compound **3** (14.6 mg) in 25% NH_4OH and *p*-dioxane-H_2O (1:2) (10 ml)[6] was heated for 14 h at 40°. The reaction mixture was made acidic to pH 4.0 with 2N HCl and extracted with ether. The aqueous layer was extracted with *n*-BuOH (4 × 3 ml) and the organic solvent removed to dryness. The crude product was submitted to semi-preparative reversed phase HPLC. An ISCO C-18 column (250 × 10 mm, 10 μm) was used. The elution was isocratical with 70% of MeOH-H_2O and a flow rate of 1.5 ml/min to afford tricolorin G (**2**) (2.6 mg, peak area 16%, *Rt* 24.8 min) and tricoloric acid C (**6**) (5.1 mg, 32%, *Rt* 16.6 min), besides unreacted starting material **3** (4.3 mg, 29%, *Rt* 24.2 min) and several unidentified minor derivatives. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was directly analysed by GC-EIMS[4] with only one peak detected (*Rt* 3.57 min), which was identified as (2*S*)-methylbutanoic acid.

Mild alkaline hydrolysis of tricolorin I and J. Compounds 4 and 5 were independently deacylated and worked up in the same way as for tricolorin H (**3**). Deacylation of compound **4** (10 mg) afforded tricolorin F (**1**) (2.1 mg, *Rt* 8.6 min) and tricoloric acid C (**6**) (3.0 mg, *Rt* 16.6 min), as well as unreacted starting material **4** (4.0 mg, *Rt* 22.2 min). The same products were obtained from the hydrolysis of tricolorin J (**5**) (10 mg): compounds **1** (2.0 mg) and **6** (3.3 mg), besides unreacted starting material **5** (3.8 mg, *Rt* 28.9 min).

Saponification of tricolorins F, I and J. Compounds **1** (15.4 mg), **4** (30.4 mg) and **5** (21.8 mg) afforded the same glycosidic acid under treatment with 5% KOH-H_2O. This derivative was purified by reversed phase HPLC (C-18) using the same conditions as indicated above to yield 12.1 mg, 24.0 mg and 16.2 mg of tricoloric acid C (**6**), respectively: colorless powder; mp 110-112°; ^1H NMR see Table 1; ^{13}C NMR see Table 2; negative FAB-MS *m/z* (rel. int) $[M + Na - 2H]^-$ 747 (55.7), $[M - H]^-$ 725 (100.0), $[M - H - 146 (C_6H_{10}O_4)]^-$ 579 (20.1), $[579 - 162 (C_6H_{10}O_5)]^-$ 417 (28.5), $[417 - 146 (C_6H_{10}O_4)]^-$ 271 (16.2), $[271 - 18,]^-$ 253 (5.4); positive FAB-MS *m/z* (rel. int.) $[M + 2Na - H]^+$ 771 (58.0), $[M + Na]^+$ 749 (17.7); positive HRFAB-MS *m/z* 749.3981 $[M + Na]^+$, $C_{34}H_{62}O_{16}Na$ requires 749.3935.

Acid hydrolysis of tricoloric acid C. A solution of derivative **6** (8 mg) in 2N HCl (1 ml) was heated at 90° for 2h. The reaction mixture was diluted with water (2 ml) and extracted with ether (3 × 3 ml). The aqueous layer was neutralized with 2M KOH and extracted with *n*-BuOH. The organic phase was washed with H_2O and evaporated to dryness to afford 2.8 mg of a mixture of monosaccharides, which was analyzed by HPLC using the Waters carbohydrate analysis column (78% CH3CN-H_2O, flow rate 0.5 ml/min)[5]: *Rt* 12.2 (D-quinovose), *Rt* 13.5 (D-fucose), and *Rt* 20.1 (D-glucose). The absolute configuration of the sugars was corroborated by their positive $[\alpha]_D$ values. An aliquot of this hydrolysis mixture (1 mg) was derivatized with Sigma Sil-A for 35 min at 70° and analyzed by GC-MS as previously described.[4] Components coeluted with the trimethylsylil ethers of standard fucose (*Rt* 16.1 min), quinovose (6-deoxy-β-D-glucose, *Rt* 17.9 min) and glucose (*Rt* 24.2 min). The combined organic layers (Et_2O) were evaporated to dryness. The residue was purified by tlc to give 1.1 mg of the aglycone which was treated with an excess of CH_2N_2 in Et_2O at room temperature to afford methyl (11*S*)-hydroxyhexadecanoate. Its physical and spectral properties were identical to those previously reported.[4]

Preparation of derivatives 7, 9 and 10. Tricolorins F (**1**) (15.0 mg), I (**4**) (52.12 mg) and J (**5**) (25.7 mg) were independently acylated with Ac_2O-pyridine (1:1). The peracetylated derivatives were purified by HPLC, using a preparative silica gel column (250 × 21.2 mm, 10 μm, ISCO). The mobile phase was 50% *n*-hexane-EtOAc (flow rate = 9 ml/min). Elution of each reaction mixture afforded compouds 7 (14.7 mg, *Rt* 22.0 min), **9** (47.8 mg, *Rt* 26.15 min) and **10** (13.5 mg, *Rt* 37.7 min).

Compound 7. Mp 68-70°; ^1H NMR see Table 1; ^{13}C NMR see Table 2.

Compound 9. Mp 74-76°; ^1H and ^{13}C NMR see Table 4.

Compound 10. Mp 72-74°; ^1H and ^{13}C NMR see Table 5.

Preparation of derivative 8. Tricoloric acid C (**6**) (14.4 mg) was acetylated with 1 ml Ac_2O-pyridine (1:1) during 24 hrs at room temperature. The peracetyl derivative was further esterified with freshly prepared CH_2N_2 in Et_2O to afford 16.4 mg of the corresponding peracetyl methyl ester (**8**): oil, ^1H NMR see Tables 1; ^{13}C NMR see Table 2.

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