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# Detailed FAB-Mass Spectrometry and High Resolution NMR Investigations of Tricolorins A-E, Individual Oligosaccharides from the Resins of *Ipomoea tricolor* (Convolvulaceae)[1]

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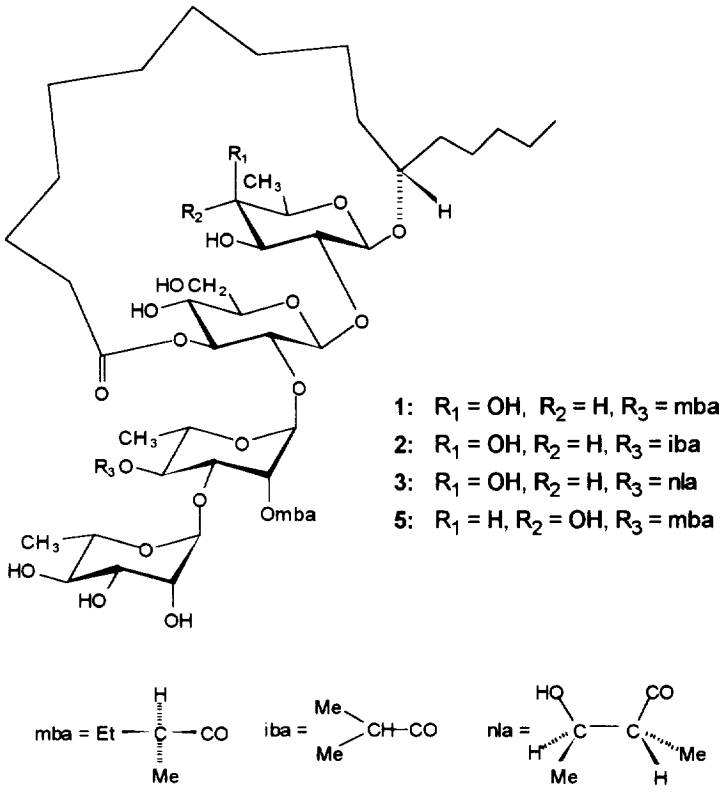
*Key words: Ipomoea tricolor*, Convolvulaceae, oligosaccharides, tricolorins A-E, tricoloric acids A and B, recycling HPLC, FAB-MS, $^{1}H$ and $^{13}C$ NMR, COSY, TOCSY, ROESY, HMQC, HMBC.

***Abstract:*** Extensive NMR and FAB-MS studies have led to the characterization of tricolorins A-E, major phytogrowth inhibitors present in the allelopathic glycoresin of *Ipomoea tricolor* Cav. (Convolvulaceae). The purification of these tetraglycosides was successfully achieved by an amino bonded-phase HPLC methodology. Tricolorins B-D differed from the previously reported macrocycle structure of tricolorin A in the site of lactonization as well as in the type and location of short-chain acids ester-linked at the oligosaccharide core (tricoloric acid A). The structure of tricolorin E was based on a new linear tetraglycoside of jalapinolic acid (tricoloric acid B).


The most conspicuous anatomical feature of species belonging to any genus of the morning-glory family (Convolvulaceae) is the occurrence of secretory cells with resinous contents in foliar tissues, roots and rhizomes. These glycoresins represent an important chemotaxonomic marker of this family[2] and are responsible for the purgative properties of some medicinal members[3,4] of the Convolvulaceae, e.g., *Ipomoea purga*,[5] *I. orizabensis*,[6] and *Convolvulus scammonia*.[7]

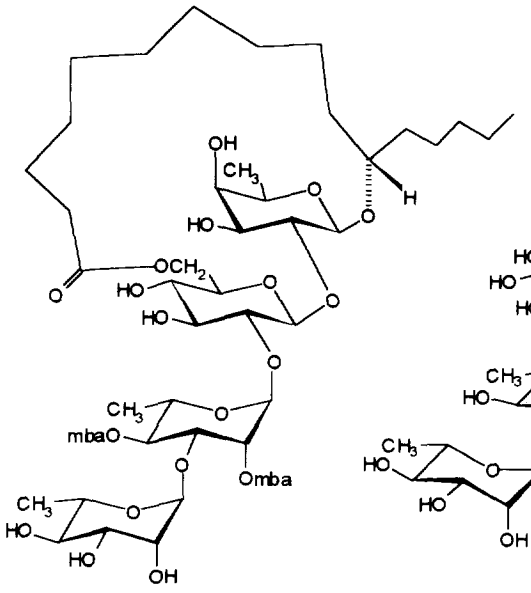
*Ipomoea tricolor* Cav. (*I. violacea* L.) is an economically important plant whose utilization goes back to prehispanic times. In Mexico, the seeds of this plant are used as hallucinogenics in magic rituals due to their content of ergot type alkaloids.[8] This species is also used in traditional agriculture as a weed controller, and its allelopathic potential has been demonstrated.[9] Bioactivity-directed fractionation of crude extracts led to the identification of the resin glycosides as the phytogrowth inhibitory principles in *Ipomoea* species.[9,10] We also became interested in this type of compounds in part because of their cytotoxic and antimicrobial potential for development of pharmacological agents.[3] This paper deals with the results of further studies on the glycoresins of *I. tricolor* involving the isolation by recycling HPLC and detailed high resolution NMR investigations of tricolorins A-E (**1-5**), major oligosaccharides isolated in pure form.




We previously reported the structure of tricolorin A (1) as a result of extensive spectroscopic studies.[9] The isolation of compound 1 was easily achieved by crystallization from the $CHCl_3$-soluble resins due to its considerable abundance in this complex mixture of oligosaccharides. However, the purification of additional glycolipids to homogeneity proved to be a very difficult task. The complexity of the so-called "resin glycosides" of convolvulaceous plants because of the close similarity in the structures of their individual constituents has posed fundamental difficulties associated with the oligosaccharides isolation.[11,12]

In the present study, the key to successful separation of compounds 1-5 was the use of a micropore bonded-phase media by HPLC in the recycle mode. In particular, analytical and preparative chromatography through an aminopropylmethylsilyl bonded silica gel column, which was eluted isocratically with $CH_3CN$-$H_2O$ (92:8), provided maximal resolution of the mixture.

Careful analysis allowed the quantitative determination of tricolorin A (1) and four additional major constituents present in the less polar fraction of the glycoresin. The integration areas obtained for each peak indicated that tricolorins A-E (1-5) were in the mixture in relative amounts of 62.83% (*Rt* 19.7 min), 11.11% (*Rt* 21.7 min), 9.40% (*Rt* 32.0 min), 0.93% (*Rt* 14.6 min), and 7.68% (*Rt* 16.0 min), respectively.



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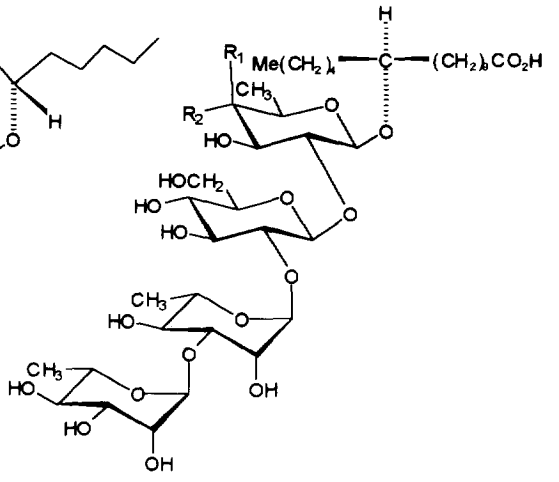

**6**: $R_1$ = OH, $R_2$ = H
**7**: $R_1$ = H, $R_2$ = OH

All purified compounds were amorphous white solids exhibiting negative specific rotation. A combination of one-dimensional (1-D) and two-dimensional (2-D) NMR spectroscopy,[13,14] including COSY, TOCSY, HMQC, HMBC and ROESY experiments, in conjunction with FAB-mass spectrometry, was used for the characterization of compounds **1-5**.

MS data have been obtained using positive and negative-ion FAB techniques. As already reported, negative-ion FAB-MS was successful in giving a base peak $\{M - H\}^-$ ion for compound **1** (*m/z* 1021; $M^+$ 1022, $C_{50}H_{86}O_{21}$).[9] Likewise, the negative ion mode afforded a weak $\{M - H\}^-$ ion at *m/z* 1007 for compound **2**, in contrast with the strong pseudomolecular ion detected at *m/z* 1009 by the positive ion mode. These experiments indicated a molecular formula of $C_{49}H_{84}O_{21}$ for glycolipid **2**, one methylene group less than that of compound **1**. For compound **3**, the negative ion mode revealed a weak $\{M - H\}^-$ ion at *m/z* 1037 ($C_{50}H_{86}O_{22}$). Accordingly, the observed difference of 16 mass units between compound **1** and **3** suggested the presence of an additional hydroxyl group. Compounds **4** and **5** showed the same $\{M - H\}^-$ ion peak at *m/z* 1021 (negative ion FAB-MS), and therefore these constituents should represent diastereoisomeric compounds of molecular formula $C_{50}H_{86}O_{21}$.

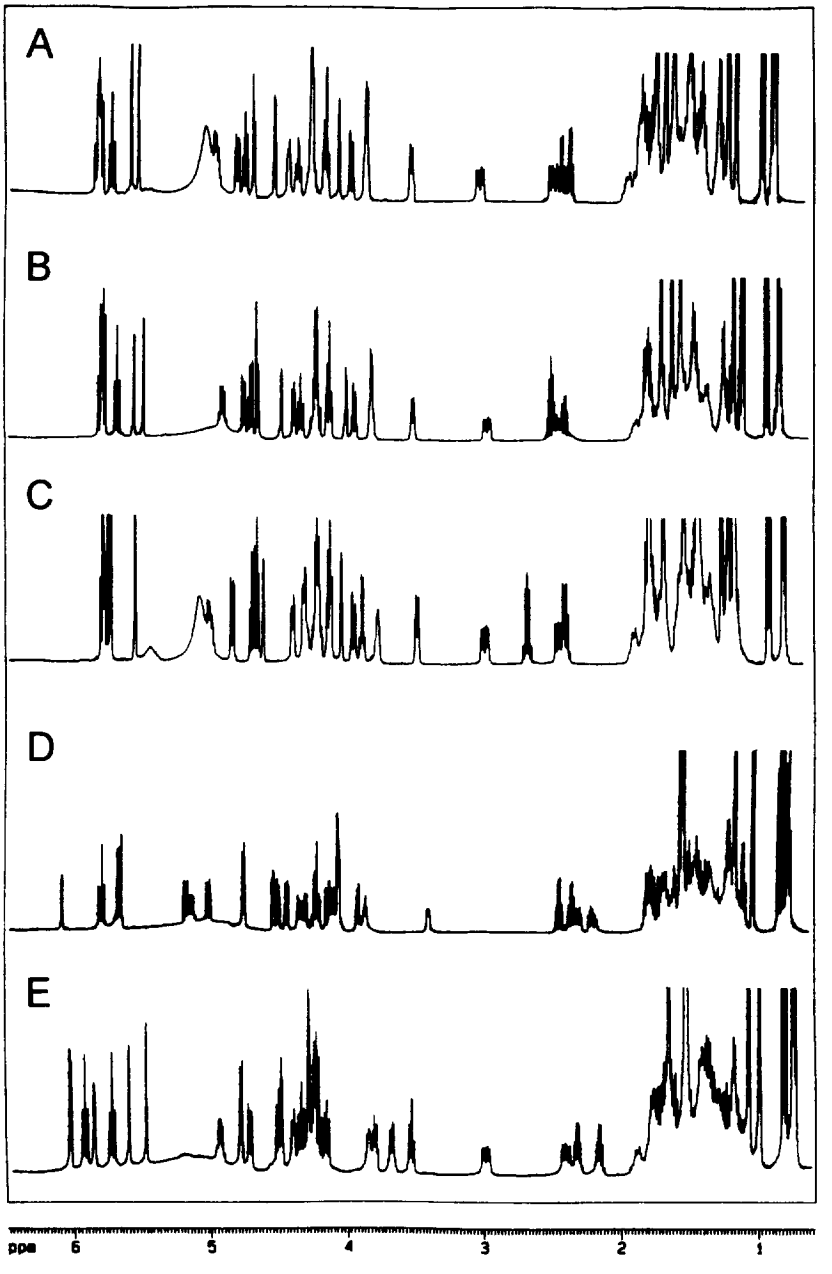


Fig. 1. 500-MHz $^1$H NMR spectra of tricolorins A-E (**1-5**) at 25 °C in pyridine-$d_5$.

Patterns of substitution on individual saccharide units in tricolorins A-E (**1-5**) were studied by $^1H$ NMR spectroscopy. 1-D spectra were initially obtained in $CD_3OD$ and $(CD_3)_2CO$ but these were difficult to interpret due to a poor spectral resolution. Signal dispersion was greatly obtained in pyridine-$d_5$.[13] Use of this solvent and 2-D homonuclear techniques[14] (COSY and TOCSY) made possible the assignment of chemical-shift values for all C-bonded protons in each moiety (Table 1). $^{13}C$ NMR were also obtained for each compound and signals were assigned by 2-D HMQC studies in the same solvent (Table 2). The $^1H$ NMR spectra of all five compounds showed the following common features (Fig. 1): (i) signals attributable to H-1 through H-6 of three methylpentoses and one hexose moieties were identified (Fig. 2); (ii) resonances assignable to the non-equivalent protons of the methylene group at C-2 in the aglycone moiety; (iii) a methyne proton for the H-11 of the aglycone; (iv) signals for two short-chain fatty acid residues esterifying the oligosaccharide core.

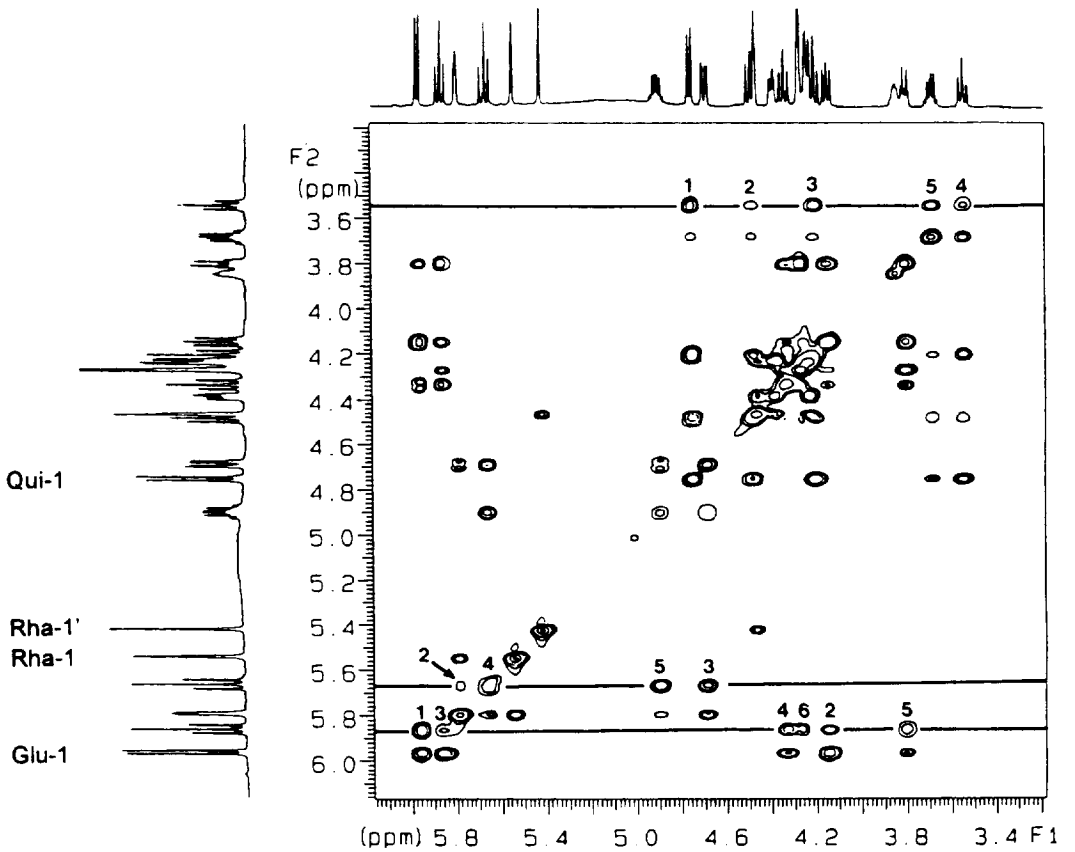


Fig. 2. Expanded region of TOCSY spectrum with high resolution 1-D projection for the oligosaccharide core of tricolorin E (**5**). Selected region shows each interacting $H_1$-$H_5$ subspectrum for all the monosaccharide units; those in the $F_2$ cross sections through quinovose H-4, glucose H-3 and rhamnose H-4 on the diagonal are indicated.

Table 1. $^1$H-NMR Data for Tricolorins A-E {1-5} and Tricoloric Acids A {6} and B {7}[a]

| Proton[b] | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Fuc-1 | 4.64 d (7.7) | 4.65 d (8.0) | 4.63 d (7.9) | 4.77 d (7.5) | | 4.80 d (7.5) | |
| 2 | 4.70 dd (7.7, 8.6) | 4.70 dd (8.0, 9.2) | 4.68 dd (7.9, 8.4) | 4.53 dd (7.5, 9.2) | | 4.51 dd (7.5, 9.5) | |
| 3 | 4.79-4.23* | 4.18-4.28* | 4.25-4.35* | 4.37 dd (3.5, 9.2) | | 4.39 dd (3.5, 9.5) | |
| 4 | 4.01 d (2.8) | 4.00 d (3.0) | 4.02 d (3.1) | 4.06-4.11* | | 3.88 d (3.5) | |
| 5 | 3.79-3.82* | 3.82 q (6.5) | 3.87 q (6.4) | 3.95 q (6.5) | | 3.76 q (6.5) | |
| 6 | 1.56 d (6.3) | 1.56 d (6.5) | 1.51 d (6.4) | 1.61 d (6.5) | | 1.48 d (6.5) | |
| Qui-1 | | | | | 4.76 d (8.0) | | 4.85 d (8.0) |
| 2 | | | | | 4.49 dd (8.0, 9.5) | | 4.21 dd (8.0, 8.5) |
| 3 | | | | | 4.21 dd (9.0, 9.5) | | 4.38 dd (8.5, 9.0) |
| 4 | | | | | 3.55 dd (9.0, 9.0) | | 4.59 dd (9.0, 9.0) |
| 5 | | | | | 3.692 dq (6.0, 9.0) | | 3.76 dq (9.0, 6.0) |
| 6 | | | | | 1.61 d (6.0) | | 1.58 d (6.0) |
| Glu-1 | 5.75 d (7.5) | 5.77 d (7.5) | 5.71 d (7.4) | 5.66 d (7.5) | 5.96 dd (7.5) | 5.57 d (7.5) | 5.63 d (7.0) |
| 2 | 4.11 dd (7.5, 9.3) | 4.13 dd (7.5, 9.0) | 4.10 dd (7.4, 9.0) | 4.13 dd (7.5, 8.5) | 4.15 dd (7.5, 9.0) | 4.23 dd (7.5, 9.0) | 4.15 dd (7.0, 8.5) |
| 3 | 5.78 dd (9.3, 9.3) | 5.80 dd (9.0, 9.0) | 5.77 dd (9.0, 9.0) | 4.06-4.11* | 5.86 dd (9.0, 9.5) | 4.14 dd (9.0, 9.0) | 4.17 dd (8.5, 9.0) |
| 4 | 4.31 dd (9.3, 9.3) | 4.34 dd (9.0, 10.0) | 4.25-4.35 * | 4.06-4.11* | 4.33 dd (9.5, 9.5) | 4.06 dd (9.0, 9.0) | 4.08 dd (9.0, 9.0) |
| 5 | 3.48 dt (2.4, 9.3) | 3.52 dt (3.2, 10.0) | 3.47 dt (2.7, 9.7) | 3.44 dt (1.5, 6.5) | 3.81 dt (3.5, 9.5) | 3.60 ddd (3.4, 5.5, 9.0) | 3.77 ddd (2.5, 5.5, 9.0) |
| 6a | 4.11* | 4.13 dd (3.2, 12.0) | 4.10* | 5.02 dd (3.0, 12.0) | 4.27-4.29* | 4.28 dd (3.4, 11.5) | 4.38 dd (2.5, 11.5) |
| 6b | 3.92 dd (2.4, 11.7) | 3.93 dd (3.2, 12.0) | 3.93 dd (2.7, 11.6) | 4.17 dd (1.5, 12.0) | 4.27-4.29* | 4.18 dd (5.5, 11.5) | 4.25 dd (5.5, 11.5) |
| Rha-1 | 5.54 d (1.0) | 5.55 d (1.5) | 5.53 s* | 6.34 d (1.7) | 5.55 d (2.0) | 6.34 d (2.0) | 6.23 d (1.0) |
| 2 | 5.77* | 5.79 dd (1.5, 3.2) | 5.77* | 6.06 dd (1.7, 3.2) | 5.79 dd (2.0, 3.5) | 4.98 dd (2.0, 2.7) | 4.93 dd ( 1.0, 3.7) |
| 3 | 4.76 dd (2.8, 9.9) | 4.75 dd (3.2, 9.7) | 4.82 dd (3.3, 9.8) | 5.18 dd (3.2, 9,7) | 4.69 dd (3.5, 9.7) | 4.89 dd (2.7, 9.5) | 4.78 dd (3.7, 9.7) |
| 4 | 5.67 dd (9.9, 9.9) | 5.68 dd (9.7, 10.0) | 5.73 dd (9.8, 9.8) | 5.78 dd (9.7, 9.7) | 5.67 dd (9.7, 9.7) | 4.47 dd (9.5, 9.5) | 4.42 dd (9.7, 9.7) |
| 5 | 4.91 dq (6.2,9.9) | 4.91 dq (6.0, 10.0) | 4.99 dc (6.2, 9.8) | 5.14 dq (6.5, 9.7) | 4.90 dq (6.0, 9.7) | 5.08 dq (6.5, 9.5) | 4.92 dq (6.0, 9.7) |
| 6 | 1.62 d (6.2) | 1.62 d (6.0) | 1.77 d (6.2) | 1.63 d (6.5) | 1.62 d (6.0) | 1.83 d (6.5) | 1.73 d (6.0) |
| Rha'-1 | 5.49 (1.0) | 5.49 d (1.5) | 5.53 s* | 5.64 d (1.5) | 5.42 d (1.5) | 6.02 d (1.5) | 5.99 d (1.0) |
| 2 | 4.49 (1.0, 2.5) | 4.48 dd (1.5, 3.5) | 4.59 dd (1.9, 3.3) | 4.56 dd (1.5, 3.5) | 4.47 dd (1.5, 3.5) | 4.71-4.76* | 4.71 dd (1.0, 3.0) |
| 3 | 4.39 (2.5, 8.2) | 4.39 dd (3.5, 9.0) | 4.37 dd (3.3, 8.5) | 4.46 dd (3.5, 9.2) | 4.39 dd (3.5, 8.7) | 4.63 dd (3.2, 9.2) | 4.67 dd (3.0, 9.5) |
| 4 | 4.19-4.23* | 4.18-4.28 * | 4.17-4.24* | 4.24 dq (9.2, 9.2) | 4.24-4.26* | 4.25 dd (9.2) | 4.23 dd (9.5, 9.5) |
| 5 | 4.19-4.23* | 4.18-4.28* | 4.17-4.24* | 4.32 dq (6.0, 9.2) | 4.24-4.26* | 4.71-4.76* | 4.70 dq (6.0, 9.5) |
| 6 | 1.69 d (3.4) | 1.70 d (6.0) | 1.67 d (5.6) | 1.62 d (6.0) | 1.73 d (5.5) | 1.57 d (6.5) | 1.57 d (6.0) |

Table 1. (continued)[a]

| Proton[b] | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Jal-2a | 2.98 ddd (1.5, 8.0, 16.8) | 2.97 ddd (2.0, 8.5, 16.4) | 2.97 ddd (1.5, 7.6, 16.5) | 2.36 ddd (2.0, 7.0, 14.5) | 3.02 ddd (2.0, 8.2, 16.0) | 2.52 t (7.5) | 2.57 t (6.5) |
| 2b | 2.44 ddd (2.0, 9.5, 16.8) | 2.46 ddd (1.5, 7.5, 16.4) | 2.43 ddd (1.8, 8.7, 16.5) | 2.26 ddd (2.0, 7.0, 14.5) | 2.46 ddd (2.0, 9.5, 16.0) | | |
| 11 | 3.79-3.82* | 3.82 * | 3.75 m | 3.89 m | 3.87 m | 3.90 m | 3.89 m |
| 16 | 0.82 t (7.0) | 0.84 t (7.0) | 0.80 d (7.0) | 0.86 t (7.0) | 0.85 t (7.2) | 0.85 t (7.0) | 0.86 t (6.7) |
| mba-2 | 2.32 tq (7.0, 7.0) | 2.41 tq (7.0,7.0) | 2.38 tq (6.8, 6.8) | 2.41 tq (7.0, 7.0) | 2.38 tq (7.0, 7.0) | | |
| 2-Me | 1.10 d (7.0) | 1.18 d (7.0) | 1.15 d (6.9) | 1.11 d (7) | 1.10 d (7.0) | | |
| 3-Me | 0.83 t (7.4) | 0.93 t (7.0) | 0.91 t (7.4) | 0.89 t (7.5) | 0.92 t (7.2) | | |
| mba'-2 | 2.38 tq (7.0, 7.0) | | | 2.50 tq 7, 7 | 2.22 tq (7.0, 7.0) | | |
| 2-Me | 1.16 d (7.0) | | | 1.24 d (7) | 1.17 d (7.0) | | |
| 3-Me | 0.91 d (7.0) | | | 0.92 t (7.5) | 0.85 t (7.2) | | |
| iba-2 | | 2.51 sept (7.0) | | | | | |
| 3 | | 1.11 d (7.0) | | | | | |
| 3' | | 1.10 d (7.0) | | | | | |
| nla-2 | | | 2.66 dq (7.2, 7.2) | | | | |
| 3 | | | 4.17-4.24* | | | | |
| 2-Me | | | 1.16 d (7.1) | | | | |
| 3-Me | | | 1.24 d (6.2) | | | | |

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 shifs marked with an asterisk (*) indicate overlapped signals. Spin-coupled patterns are designated as follows: s = singlet, d = doublet, t = triplet, m = multiplet, sept = septet. All assignments are based on $^1H$-$^1H$ COSY and TOCSY data.

b) Abbreviations: Fuc = fucose, Qui = quinovose, Glu = glucose, Rha = rhamnose, Jal = 11-hydroxyhexadecanoyl, mba = 2-methylbutanoyl, iba = 2-methylpropanoyl, nla = 3-hydroxy-2-methylbutanoyl.

On alkaline hydrolysis, compounds **1-5** liberated an organic acid fraction together with an $H_2O$-soluble linear tetraglycoside of 11*S*-hydroxyhexadecanoic acid (jalapinolic acid).[15,16] The organic acids were analyzed by gas chromatography-mass spectrometry, while the glycosidic acids were examined by NMR spectroscopy. Organic acids provided from tricolorin B (**2**) were 2-methylpropanoic (iba) and (2*S*)-methylbutanoic (mba) acids. Tricolorin C (**3**) furnished nilic, 3*R*-hydroxy-2*R*-methylbutanoic (nla), and (2*S*)-methylbutanoic acids. The short-chain acids yielded from tricolorins A (**1**), D (**4**) and E (**5**) were proved to be identical, i.e. (2*S*)-methylbutanoic acid. Compounds **2-4** yielded the same glycosidic acid, tricoloric acid A (**6**),[9] previously obtained from saponification of compound **1**. Saponification of tricolorin E (**5**) yielded a new glycosidic acid, named tricoloric acid B (**7**). Subsequent acid-catalyzed hydrolysis of **7** gave jalapinolic acid, as the aglycone portion, and a mixture of monosaccharides which were characterized as rhamnose, glucose and quinovose by using both reverse-phase HPLC[17] of the crude carbohydrates mixture and gc-ms analysis of their corresponding trimethylsilyl ethers.[18] The negative ion FAB-MS of **6** and **7** showed the same fragmentation pattern with the common fragment peaks observed at m/z 871 $\{M - H\}^-$, 725 $\{M - H - 146$ (methylpentose unit)$\}^-$, 579 $\{725 - 146$ (methylpentose unit)$\}^-$, 417 $\{579 - 162$ (hexose unit)$\}^-$, and 217 $\{417 - 146$ (methylpentose unit; jalapinolic acid $- H\}^-$, indicating that tricoloric acid B (**7**) was also a linear tetraglycoside. The $^1H$ NMR spectrum of **7** was completely assigned by the combination of double-quantum-filtered (DQF) COSY and TOCSY. These results allowed the pertinent resonances in the $^{13}C$ spectrum to be assigned. This was accomplished via $^1H$-detected $\{^1H, ^{13}C\}$ one-bond correlation experiment[13,14] (HMQC) and by comparison with those previously observed for tricoloric acid A (**6**).[9] The major differences in the $^1H$ and $^{13}C$ NMR spectra (Tables 1 and 2) of derivative **7** were the assignments for the inner methylpentose unit in the oligosaccharide core. From the information discussed above, it was apparent that the fucopyranosyl unit of tricoloric acid A was replaced by a quinovose residue in tricoloric acid B and therefore its structure was elucidated as (11*S*)-hydroxyhexadecanoic acid 11-*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1→2)-β-D-quinovopyranoside.

From the $^1H$ NMR spectra, esterified positions on the tetraglycosides were easily identified through strong deshielding effects. Thus, in all compounds, the hydroxyl groups on positions C-2 and C-4 of inner rhamnose were obviously acylated. The site of lactonization at C-3 of glucose for tricolorins A-C (**1-3**) and E (**5**) was established by the significant downfield shift observed for its geminal proton. In tricolorin D (**4**), a significant downfield shift owing to acylation was observed for the methylene protons on C-6 of glucose and suggested that the ester linkage of jalapinolic acid was placed at this position. These postulations were corroborated by FAB-mass spectrometry in the negative mode. Besides the common fragment peaks observed at *m/z* 271 and 417, compounds **1-5** showed a peak at m/z 561 in place of that detected at *m/z* 579 in the spectrum of tricoloric acids A (**6**) and B (**7**). The difference of 18 mass units suggested that the ester linkage of the aglycone moiety must be placed at any of the hydroxyl groups of the glucopyranosyl unit.[9]

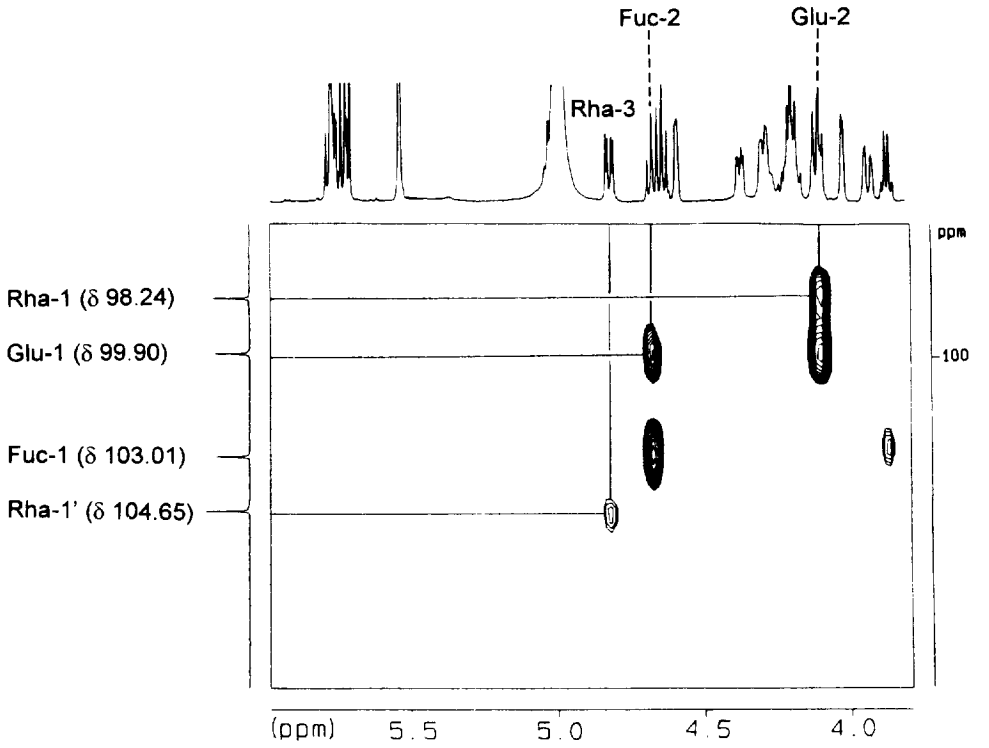


Fig. 3. $^{1}$H-Detected heteronuclear multiple-bond correlation (HMBC) spectrum of tricolorin C (**3**) with high resolution 1-D $^{1}$H and $^{13}$C projections. Expanded region showing the $^{3}J_{CH}$ connectivities of the anomeric carbons across the glycosidic linkages.

The interglycosidic connectivities were established almost entirely on the basis of detailed long-range ($^{2}J$, $^{3}J$) heteronuclear coupling correlations by HMBC studies.[13,14] These experiments also provided additional evidences for the location of the ester substituents. The following features were common to all compounds **1-5**:

(i) A $^{3}J_{CH}$-interaction between carbon C-11 of the aglycone and the anomeric proton of the inner sugar moeity of the tetraglycoside core, i.e. the fucose unit in compounds **1-4** and quinovose in compound **5**.

(ii) $^{13}$C-$^{1}$H long-range cross peaks (Fig. 3) were observed and unambiguously assigned as those between the anomeric carbon of glucose (C-1) and proton H-2 of the inner methylpentose unit of the oligossacharide, i.e. H-2 of fucose in compounds **1-4** and H-2 of quinovose in glycolipid **5**; C-1 of inner rhamnose and glucose H-2, and C-1' of terminal rhamnose and inner rhamnose H-3.

(iii) The most shielded carbonyl resonance ($\delta_C$ 172) could be easily assigned to the lactone functionality by the $^{2}J$-coupling with each of the methylene protons $\delta_H$ *ca.* 2.3-3.0 on the adjacent C-2 position of the aglycone moiety. In compounds **1-3** and **5**, the site of lactonization could be corroborated as being placed at C-3 of glucose by the observed $^{3}J$-coupling between the carbonyl carbon of the aglycone moiety and its geminal proton

Table 2. $^{13}$C-NMR Data for Tricolorins A-E {1-5} and Tricoloric Acids A {6} and B {7}[a]

| Carbon[b] | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Fuc-1 | 103.1 | 103.2 | 103.0 | 103.0 | | 103.0 (157.7) | |
| 2 | 74.7 | 74.9 | 74.8 | 78.4 | | 78.7 | |
| 3 | 76.2 | 76.2 | 76.1 | 76.7 | | 76.2 | |
| 4 | 73.2 | 73.2 | 73.2 | 73.2 | | 73.1 | |
| 5 | 71.3 | 71.3 | 71.2 | 71.3 | | 71.2 | |
| 6 | 17.4 | 17.3 | 17.3 | 17.3 | | 17.4 | |
| Qui-1 | | | | | 102.8 | | 102.4 (162.4) |
| 2 | | | | | 72.3 | | 80.8 |
| 3 | | | | | 78.9 | | 78.8 |
| 4 | | | | | 77.0 | | 76.8 |
| 5 | | | | | 72.8 | | 72.5 |
| 6 | | | | | 18.5 | | 18.5 |
| Glu-1 | 99.8 | 99.9 | 99.9 | 102.2 | 99.7 | 102.7 (164.7) | 102.6 (164.7) |
| 2 | 80.7 | 80.8 | 80.3 | 77.3 | 80.8 | 78.8 | 78.7 |
| 3 | 73.0 | 79.1 | 79.1 | 78.9 | 79.2 | 79.2 | 79.4 |
| 4 | 69.6 | 69.7 | 69.6 | 70.4 | 70.0 | 72.8 | 72.5 |
| 5 | 76.3 | 76.3 | 76.2 | 74.9 | 76.9 | 77.1 | 77.4 |
| 6 | 61.3 | 61.5 | 61.3 | 63.0 | 61.8 | 63.4 | 63.3 |
| Rha-1 | 104.6 | 98.4 | 98.2 | 97.7 | 98.6 | 102.2 (173.9) | 102.2 (173.9) |
| 2 | 72.8 | 72.8 | 72.8 | 72.9 | 72.8 | 72.1 | 71.6 |
| 3 | 75.9 | 76.0 | 75.8 | 75.7 | 75.7 | 80.6 | 79.4 |
| 4 | 73.3 | 73.5 | 73.4 | 74.0 | 73.3 | 73.2 | 72.9 |
| 5 | 67.4 | 67.3 | 67.5 | 66.9 | 67.3 | 70.0 | 70.2 |
| 6 | 18.5 | 18.4 | 18.6 | 18.5 | 18.6 | 19.1 | 18.8 |
| Rha'-1 | 98.3 | 104.6 | 104.6 | 104.5 | 104.4 | 104.3 (171.7) | 103.6 (171.6) |
| 2 | 72.3 | 72.4 | 72.2 | 72.5 | 76.5 | 72.6 | 72.2 |
| 3 | 72.5 | 72.6 | 72.5 | 72.5 | 72.5 | 72.9 | 72.5 |
| 4 | 73.4 | 73.5 | 73.5 | 73.5 | 73.4 | 74.4 | 74.2 |
| 5 | 70.5 | 70.5 | 69.2 | 70.4 | 70.6 | 70.2 | 69.9 |
| 6 | 18.3 | 18.4 | 18.3 | 18.5 | 18.3 | 18.7 | 18.4 |

Table 2. (Continued)[a]

| Carbon[b] | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Jal-1 | 172.3 | 172.4 | 172.3 | 173.5 | 172.4 | 177.4 | 180.6 |
| 2 | 34.4 | 34.5 | 34.4 | 34.4 | 34.4 | 35.9 | 38.2 |
| 11 | 80.9 | 81.0 | 80.8 | 80.8 | 81.0 | 80.8 | 80.6 |
| 16 | 14.2 | 14.2 | 14.2 | 14.2 | 14.2 | 14.4 | 14.2 |
| mba-1 | 175.6 | 175.7 | 175.8 | 175.5 | 175.8 | | |
| 2 | 41.4 | 41.5 | 41.4 | 41.8 | 41.5 | | |
| 2-Me | 17.0 | 16.9 | 17.0 | 16.7 | 17.2 | | |
| 3-Me | 11.8 | 11.8 | 11.8 | 11.8 | 11.9[c] | | |
| mba'-1 | 175.8 | | | 175.9 | 175.8 | | |
| 2 | 41.6 | | | 41.4 | 41.4 | | |
| 2-Me | 16.9 | | | 16.8 | 16.9 | | |
| 3-Me | 11.8 | | | 11.9 | 11.8[c] | | |
| iba-1 | | 176.1 | | | | | |
| 2 | | 34.4 | | | | | |
| 3 | | 19.1 | | | | | |
| 3' | | 18.1 | | | | | |
| nla-1 | | | 174.5 | | | | |
| 2 | | | 49.8 | | | | |
| 3 | | | 70.4 | | | | |
| 2-Me | | | 14.0 | | | | |
| 3-Me | | | 21.2 | | | | |

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 parenthesis. All assignments are based on HMQC and HMBC data.
b) Abbreviations: Fuc = fucose, Qui = quinovose, Glu = glucose, Rha = rhamnose, Jal = 11-hydroxyhexadecanoyl, mba = 2-methylbutanoyl, iba = 2-methylpropanoyl, nla = 3-hydroxy-2-methylbutanoyl.
c) Values may be interchanged.

($\delta_H$ *ca.* 5.8) in this piranose ring. Verification of the position of lactonization in compound **4** was provided by the $^3J_{CH}$ connectivities between the carbonyl group of the aglycone and the non-equivalent protons of the methylene group at C-6 of glucose (Fig. 4).

(iv) The remaining esterified positions are, therefore, the location of the two additional ester linkages for the short-chain acid residues esterifying the oligosaccharide core. Through HMBC experiments, it was possible in all the cases to link specific carbonyl ester groups with their corresponding vicinal methyne group resonances ($^2J_{CH}$) and also with the pyranose ring proton at the position of esterification ($^3J_{CH}$). This multiple-bond correlation NMR technique proved to be invaluable in establishing the location of the *O*-acyl groups in these complex glycolipids. In all oligosaccharides (**1-5**), a 2*S*-methylbutanoate could be placed at C-2 in the inner rhamnose unit, and accordingly the additional acyl substituent exhibited a $^3J_{CH}$ coupling with the H-4 signal of the same sugar (Fig. 4). For compound **2**, the $^2J_{CH}$ coupling between the most deshielded carbonyl resonance ($\delta_C$ 176.1) and the septet-like proton signal on the adjacent carbon ($\delta_H$ 2.5) were used to identify the isobutyrate residue and the location of this acyl group at C-4 was established by the $^3J_{CH}$ coupling with the signal at $\delta$ 5.68 (Fig. 5). By a similar analysis, the carbonyl resonance of the methylbutyrate group ($\delta_C$ 175.7) at C-2 was recognized. In tricolorin C (**3**), the same $^{2,3}J_{CH}$ interactions were used to differenciate between the carbonyl resonances for the nylate ($\delta_C$ 174.5) and the methylbutyrate ($\delta_C$ 175.8) substituents on the inner rhamnose unit.

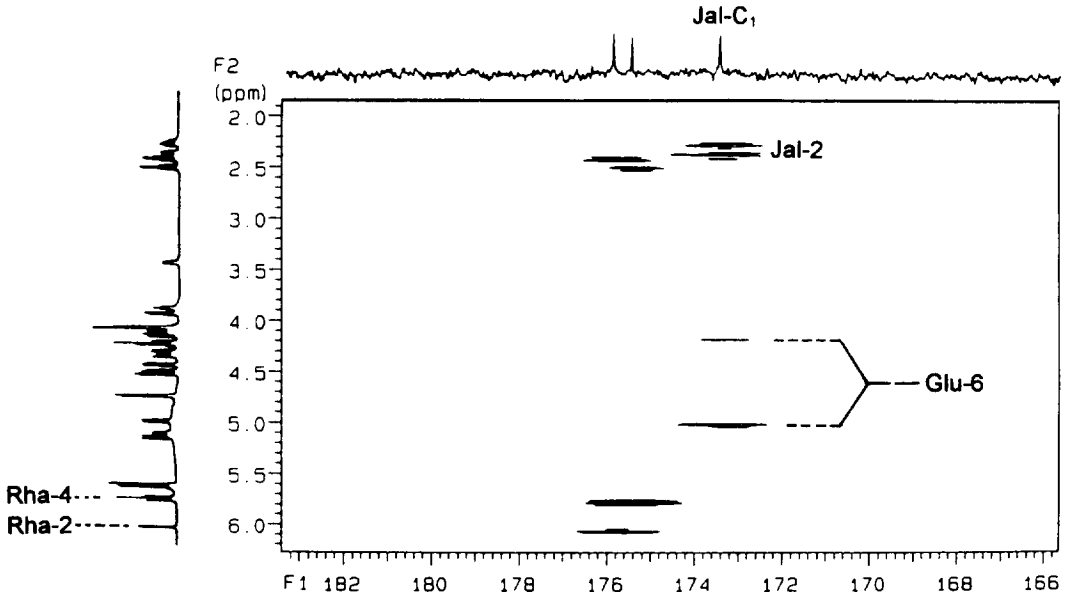


Fig. 4. $^1$H-Detected heteronuclear multiple-bond correlation (HMBC) spectrum of tricolorin D (**4**). $^{13}$C expanded region ($F_1$) showing the $^3J_{CH}$ connectivities for the carbonyl resonances of the ester groups with their corresponding geminal proton signals in the oligosaccaride core.

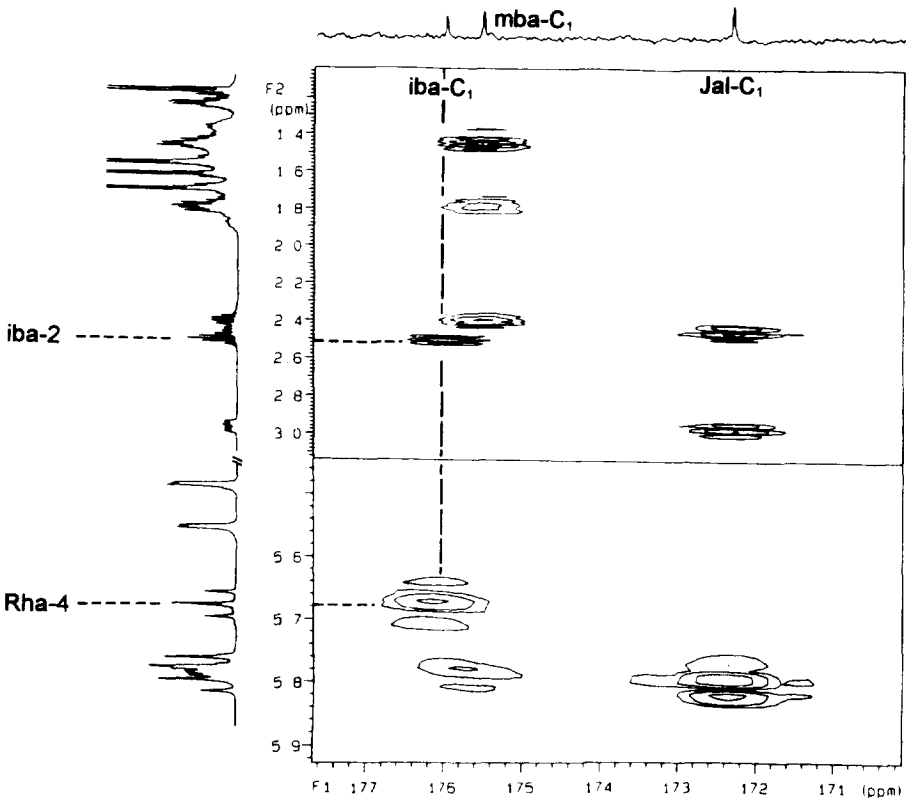


Fig. 5. $^{1}$H-Detected heteronuclear multiple-bond correlation (HMBC) spectrum of tricolorin B (**2**). Expanded region showing the $^{3}J_{CH}$ connectivities of the $^{13}$C carbonyl signals with the oligosaccharide proton resonances. The carbonyl signal at 176.1 ppm was assigned to the isobutirate group by virtue of its $^{2}J_{CH}$ correlation with the septet at 2.51 ppm (iba H-2).

ROESY spectra were also useful for determining the saccharide substitution and anomeric configuration. Crosspeaks were observed for all possible interglycosidic connectivities (Fig. 6). In the same way, observation of intraglycosidic ROESY crosspeaks also discriminated between the α- and β-anomers of rhamnopyranosyl units.[13] A strong interaction between H-1 and H-2 was observed, while on the contrary H-3 and H-5 did not present any correlation with the anomeric proton, confirming a $^{1}C_{4}$ conformation for the α-L-rhamnose residues. Alternatively, the same information was obtained by measuring anomeric $^{1}J_{CH}$ coupling constants (Table 2) from a series of gated decoupling studies undertaken with tricoloric acids A (**6**) and B (**7**). The results are exemplified by those of compound **7** which showed $J_{C1\text{-}H1}$ values of 171.6 Hz for the terminal rhamnose, 173.9 Hz for the inner rhamnose, 164.7 Hz for glucose and 162.4 Hz for quinovose. These values indicated that all the monosaccharide units are of the pyranose type,[13,14] that rhamnose is present as the α-anomer[19] and that the mode of glycosidic linkage of glucose and quinovose is β in the $^{4}C_{1}$ conformation.[20]

In conclusion, the primary structure of the oligosaccharide core of tricolorins A-D (**1-4**) is based on tricoloric acid A (**6**).[9] Tricolorins B (**2**) and C (**3**) are macrocycle structures differing from previously reported tricolorin A (**1**) in the type of residues esterifying position C-4 of the inner rhamnose unit, i.e. isobutyric acid in tricolorin B (**2**), and nilic acid as the acyl substituent for tricolorin C (**3**). The structure of tricolorin D (**4**) was elucidated as (11*S*)-hydroxyhexadecanoic acid 11-*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-α-L-{2-*O*-(2*S*-methylbutanoyl)-4-*O*-(2*S*-methylbutanoyl)}-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl)-(1→2)-β-D-fucopyranoside-(1,6'')-lactone).

A different linear tetraglycoside of jalapinolic acid, tricoloric acid B (**7**), was identified as the oligosaccharide core of tricolorin E (**5**). An epimeric structure of tricolorin A (**1**) at C-4 of the inner methylpentose unit was identified for this natural product. Therefore, the structure of tricolorin E (**5**) was elucidated as (11S)-hydroxyhexadecanoic acid 11-*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-α-L-{2-*O*-(2*S*-methylbutanoyl)-4-*O*-(2*S*-methylbutanoyl)}-rhamnopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-quinovopyranoside-(1,3'')-lactone).

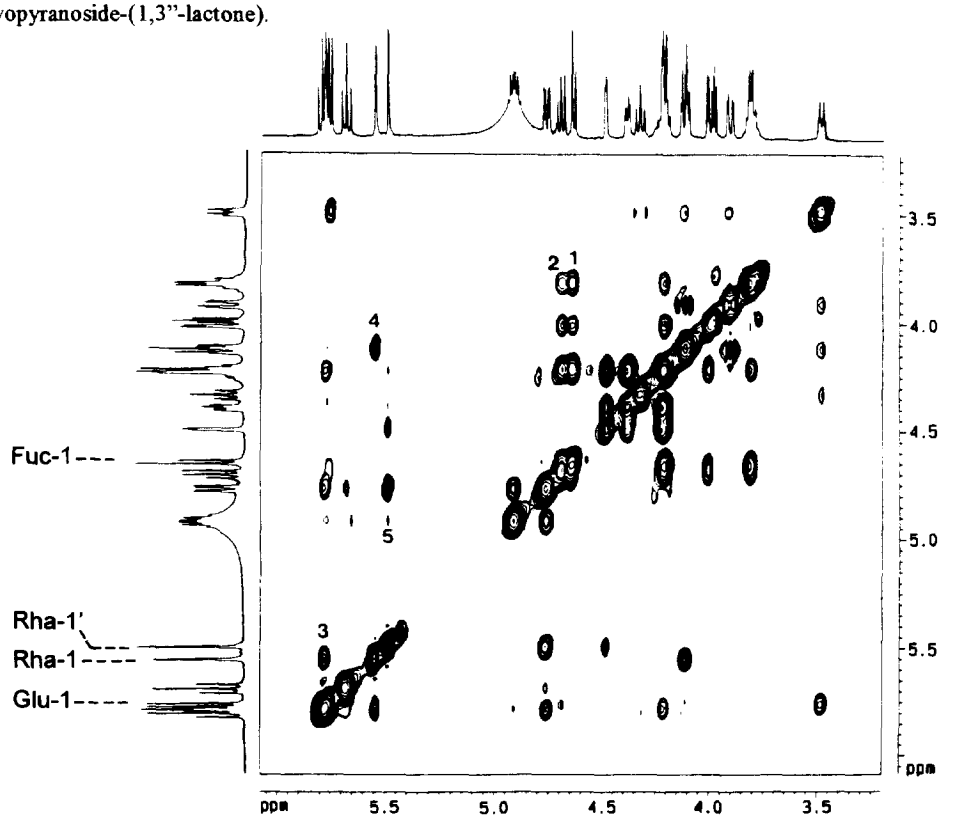


Fig. 6. The ROESY spectrum of tricolorin A (**1**) showing five through-space $^1H$-$^1H$ NOE responses corresponding to the interglycosidic connectivities: 1, Fucose $H_1$-aglycone $H_{11}$; 2, Fucose $H_2$–aglycone $H_{11}$; 3, glucose $H_1$-rhamnose $H_1$; 4, glucose $H_2$-ramnose $H_1$; 5, rhamnose $H_3$-rhamnose $H_{1'}$.

## EXPERIMENTAL

***General Experimental Procedures***. The instruments used, conditions under which measurements were made, and the source of plant material, along with the handling of the plant extracts, are given in the preceding article[9] unless otherwise specified. Positive and negative ion FAB-mass spectra were recorded using a glycerol matrix on a VG 7070E mass spectrometer. $^{1}H$ (500 MHz), $^{13}C$ (125.7 MHz), DEPT, COSY, TOCSY, HMQC, HMBC and ROESY NMR experiments were conducted either on a Bruker AMX-500 or a Varian Unity-500 spectrometers. Portions (15-40 mg) of each sample were dissolved in 0.75 ml of pyridine-$d_5$. 2-D NMR spectra were recorded by using standard Bruker pulse program: in the HMQC and HMBC experiments, $\Delta$ = 1 s and $J$ = 140, 8 Hz, respectively; the correlations maps consisted of 512 × 1K data points per spectrum, each composed of 16 to 64 transients COSY, TOCSY and ROESY experiments were carried out using standard Varian sofware. COSY spectra were acquired in absolute value mode while TOCSY, ROESY, and HMQC spectra were acquired in phase-sensitive mode and HMBC was processed using mixed-mode processing (phase sensitive along $f_1$, absolute value along $f_2$). Mixing times of 0.1 sec were used for TOCSY and ROESY experiments while HMBC experiments were optimized for 8 Hz coupling constants. A BIRD nulling delay of 0.25 sec was used for HMQC while $J$ filters, BIRD pulses, and HMQC delays were all set corresponding to $J$ = 155 Hz. Chemical shifts are in δ (ppm) and referred to TMS as internal reference.

***HPLC analysis***. Instrumentation consisted of a Waters (Millipore Corp., Waters Chromatography Division, Milford, MA, USA) 600E Multisolvent Delivery System equipped with a Waters 410 differential refractometer detector and a computer (OptiPlex 466/Le, Dell). Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium 2000 software program (Waters). Our attemps to achieve reasonable resolution of the oligosaccharide mixture with C-18 and cyano bonded reversed phase analytical columns were unsuccessful. However, the standard column for carbohydrate analysis (Waters) gave complete separation of the sample in a run time of 40 min (80% $CH_3CN$-$H_2O$). Therefore, an analytical separation was first developed on a Waters aminopropylmethylsilyl amorphous silica column (300 × 3.9 mm; μBondapak, 10 μm, 125 Å) in order to determine the optimum mobile phase composition and sample load for the isolation. The analytical HPLC *Rt* was determined for all compounds eluted isocratically with $CH_3CN$-$H_2O$ (92:8, flow rate = 0.5 ml/min). A loadling study revealed that a maximun of 500 μg of sample could be applied to the analytical column while retaining adequate resolution.

***Isolation and purification of tricolorins A-E***. Details of the crude resin glycosides isolation from the $CHCl_3$ extract of *Ipomoea tricolor* were previously described.[9] Sample preparation for HPLC analysis was performed by purification of the less polar phytogrowth inhibitory fractions (140-145; 5.9 g)[9] using a flash column chromatography over silica gel and a gradient of MeOH in $CHCl_3$. Combined fractions eluted with $CHCl_3$-MeOH (4:1) afforded a white amorphous powder (3.9 g). HPLC separations were performed employing a preparative column (150 × 19 mm) packed with the same micropore bonded-phase media used for analytical chromatography. The elution was isocratical with $CH_3CN$-$H_2O$ (92:8) and a solvent flow rate of 6 ml/min was used. The analytical sample load was scaled-up 120 times to 60 mg (500 μl) for preparative separation. Fractions across the peaks of interest were colleted by the technique of heart-cutting, and aliquots were injected on the analytical column to assess purity. Fractions containing major components **1** (*Rt* 19.7 min) and **3** (*Rt* 32.0 min) were >99% pure. The preparative HPLC system was then operated in recycle mode to further separate and guarantee maximal purity of the three remaining peaks, i.e compounds **2** (*Rt* 21.7 min), **4** (*Rt* 14.6 min) and **5** (*Rt* 16.0 min) by coupling a sample recycle valve and a solvent recycler (MiniMizer, MetaChem

Technologies Inc.) with the pump. The sensitivity setting of the refractometer was increased from 8× to 64× so that all minor impurities could be easily detected. Eight to twelve recycle passes achieved complete separation of all components to homogeneity.

***Tricolorin A* (1).**- Mp 118-120°; $[\alpha]_D$ -30° (c = 1.5, MeOH); $^1$H NMR see Table 1; $^{13}$C NMR see Table 2; positive FAB-MS *m/z* (rel. int.) $[M + H]^+$ 1023 (100), 877 (80); negative FAB-MS *m/z* (rel. int.) $[M - H]^-$ 1021 (100), $[M - H - C_5H_8O]^-$ 937 (27), 561 (70), 417 (85), 271 (18); negative HRFAB-MS *m/z* 1021.5584 $[M - H]^-$, $C_{50}H_{85}O_{21}$ requires 1021.5583.

***Tricolorin B* (2).**- Mp 119-120°; $[\alpha]_D$ -38° (c = 1.0, MeOH); $^1$H NMR see Table 1; $^{13}$C NMR see Table 2; positive FAB-MS *m/z* (rel. int.) $[M + H + glycerol]^+$ 1101 (30), $[M + H]^+$ 1009 (100), 863 (40); negative FAB-MS *m/z* (rel. int.) $[M - H]^-$ 1007 (30), $[M - H - C_4H_6O]^-$ 937 (7), 561 (15), 417 (10), 271 (100); negative HRFAB-MS *m/z* 1007.5427 $[M - H]^-$, $C_{49}H_{83}O_{21}$ requires 1007.5426.

***Tricolorin C* (3).**- Mp 125-126°; $[\alpha]_D$ -29° (c = 1.0, MeOH); $^1$H NMR see Table 1; $^{13}$C NMR see Table 2; negative FAB-MS *m/z* (rel. int.) $[M - H]^-$ 1037 (7.3), $[M - H - H_2O]^-$ 1019 (12), $[M - H - C_2H_4O]^-$ 993 (50), $[M - H - C_5H_8O_2]^-$ 937 (32), 561 (78), 417 (71), 271 (30); negative HRFAB-MS *m/z* 1037.5534 $[M - H]^-$, $C_{50}H_{85}O_{22}$ requires 1037.5532.

***Tricolorin D* (4).**- Mp 105-106°; $[\alpha]_D$ -26° (c = 1.0, MeOH); $^1$H NMR see Table 1; $^{13}$C NMR see Table 2; negative FAB-MS *m/z* (rel. int.) $[M - H]^-$ 1021 (87), $[M - H - C_5H_8O]^-$ 937 (10.9), 919 (7.5), 561 (32), 417 (25), 271 (23); negative HRFAB-MS *m/z* 1021.5583 $[M - H]^-$, $C_{50}H_{85}O_{21}$ requires 1021.5583.

***Tricolorin E* (5).**- Mp 115-116°; $[\alpha]_D$ -58° (c = 1.0, MeOH); $^1$H NMR see Table 1; $^{13}$C NMR see Table 2; negative FAB-MS *m/z* (rel. int.) $[M - H]^-$ 1021 (22), $[M - H - C_5H_8O]^-$ 937 (4), 561 (8), 417 (16), 271 (21); negative HRFAB-MS *m/z* 1021.5585 $[M - H]^-$, $C_{50}H_{85}O_{21}$ requires 1021.5583.

***Saponification of tricolorins A-E*.** Solutions of compounds **1-5** (20 mg) in 5% $KOH/H_2O$ were refluxed at 95° for 2 h. The reaction mixtures were adjusted to pH 4 with 4 N HCl, and extracted with $Et_2O$ (2 × 10 ml). The organic layer were washed with $H_2O$, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. Each residue was directly analyzed by GC-EIMS.[9] Compounds **1**, **4** and **5** afforded the same single peak (*Rt* 5.6 min), which was identified as (2*S*)-methylbutanoic acid (mba): $[\alpha]_D$ + 17° (*c* = 4.3, MeOH). Compound **2** gave two peaks, *Rt* 3.0 min (2-methylpropanoic acid) and 5.6 min (mba). Compound **3** also afforded two peaks, *Rt* 5.6 (mba) and 15.8 min: 3*R*-hydroxy-2*S*-methylbutanoic acid; $[\alpha]_D$ + 25° (*c* = 5.0, MeOH).[6] The aqueous phases were extracted with *n*-BuOH (2 × 10 ml), and the organic layers were evaporated in vacuo to give tricoloric acid A (**6**) from compounds **1**, **2**, **3** and **5**. The physical and spectral properties of derivative **6** were identical to those previously reported.[9] Compound **4** afforded 9 mg of tricoloric acid B (**7**) which was purified by reverse phase HPLC (C-18, 10.0 × 250 mm, 10 µm; MeOH-$H_2O$, 7:3; flow rate = 2.5 ml/min): colorless powder; mp 124-125°; $^1$H NMR see Table 1; $^{13}$C NMR see Table 2; negative FAB-MS *m/z* (rel. int.) $[M - H]^-$ 871 (100), 725 (20), 579 (18), 417 (23), 271 (10); negative HRFAB-MS *m/z* 871.4539 $[M - H]^-$, $C_{40}H_{71}O_{20}$ requires 871.4538.

***Acid Hydrolysis of tricoloric acid B (7): sugar analysis*.** A solution of derivative **7** (9 mg) in 2 N HCl (1 ml) was heated at 90° for 2 h. The reaction mixture was diluted with $H_2O$ (2 ml) and extracted with $Et_2O$ (2 × 1.5 ml). The aqueous layer was neutralized with KOH and extracted with *n*-BuOH (2 × 1.5 ml). This organic layer was washed with $H_2O$ and evaporated to

afford 4.2 mg of a mixture of monosaccharides, which was subjected to HPLC analysis employing Waters carbohydrate analysis column (78% $CH_3CN$-$H_2O$; flow rate = 0.5 ml/min)[15]: *Rt* 11.9 (rhamnose), 12.2 (quinovose) and 20.1 min (glucose). An aliquot of this hydrolysis mixture (1 mg) was derivatized with Sigma Sil-A for 35 min at 70°. GC-MS analysis[9] gave three peaks: *Rt* 14.8, 17.9 and 24.2 min which coeluted with those of TMSi-ethers of standard α-L-rhamnose, 6-deoxy-β-D-glucose (quinovose) and β-D-glucose, respectively. The combined organic layers ($Et_2O$) were evaporated to dryness. The residue was purified by tlc to give 1.2 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, $[\alpha]_D$ = + 1.0° (*c* = 0.9, MeOH). Its physical and spectral properties were identical to those previously reported.[9]

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tasteless purgative resin with a slight smoky odour, often given to children as an anthelminthic on account of the small dose required. It is reputed to be an excellent purge used in constipation, pain and colic in the bowels and general intestinal topor, being combined, in compound powder, with other laxatives, and with carminatives such as ginger and cloves. This drug derives its local name from the City of Xalap in the state of Veracruz in Mexico, where it is abundant. The traditional production system of the root of jalap has been recently reviewed by Linajes A.; Rico-Gray, V.; Carrion, G. *Economic Bot.* **1994**, 48, 84-89.

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