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TRICOLORIN A, MAJOR PHYTOGROWTH INHIBITOR FROM *IPOMOEA TRICOLOR*[1]

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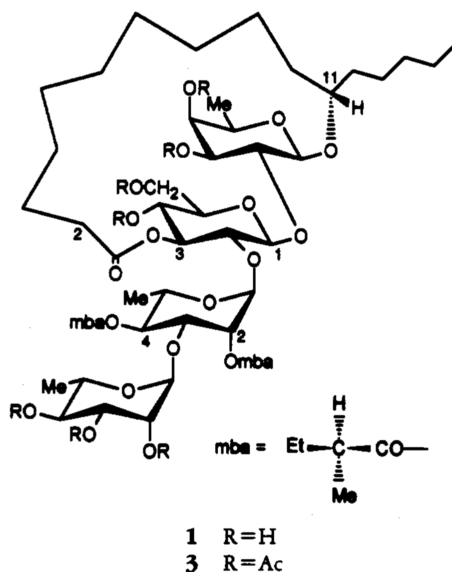
ABSTRACT.—The allelopathic potential of *Ipomoea tricolor* (Convolvulaceae), used in Mexican traditional agriculture as a weed controller, has been demonstrated by measuring the inhibitory activity of organic extracts on seedling growth of *Amaranthus leucocarpus* and *Echinochloa crus-galli*. Bioactivity-directed fractionation of the active $CHCl_3$ extract led to the isolation of the allelopathic principle, which turned out to be a mixture of the so-called "resin glycosides" of convolvulaceous plants. The structure of tricolorin A, the major phytogrowth inhibitor present in the active fraction, was elucidated as (11*S*)-hydroxyhexadecanoic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-[2-*O*-(2*S*-methylbutyryl)-4-*O*-(2*S*-methylbutyryl)] rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2')-β-D-fucopyranoside-(1,3''-lactone) [**1**], based on chemical methods and spectral analysis including ^1H-^1H COSY, ^1H-^{13}C HETCOR, long range ^1H-^{13}C COLOC, and selective INEPT experiments. Bioassays showed that radicle elongation of the two weed seedlings tested was inhibited by tricolorin A [**1**] with IC_{50} values ranging from 12 to 37 μM. *Staphylococcus aureus* was sensitive to compound **1** with an MIC value of 1.8 μg/ml. Significant cytotoxic activity against cultured P-388 and human breast cancer cells (ED_{50} 2.2 μg/ml) was demonstrated for compound **1**, and it also inhibited phorbol 12,13-dibutyrate binding using calf brain homogenate as a source of protein kinase C (IC_{50} 43 μM).

The allelopathic nature of plants in the genus *Ipomoea* (Convolvulaceae), especially the suppressive effects on weed growth of sweet potatoes, *Ipomoea batatas* (L.) Lam., has been suggested in previous studies (1,2). These species produce very aggressive competitive effects, due to their strong propagative power, complemented with a high allelopathic interference (3). In tropical an temperate zones of Mexico, polycultures represent a traditional and efficient management of resources in agroecosystems that provide a natural control of weeds. In such systems, the cultivation of some semi-domesticated legumes, as well as some *Ipomoea* species, is a common practice to minimize the growth of companion weeds (4).

Ipomoea tricolor Cav. is used extensively as a cover crop, especially during August to October, the fallow period in the sugar-cane fields of the state of Morelos, in the southeastern intertropical region of Mexico. The allelopathic potential of this species has been previously demonstrated by measuring the inhibitory activity of aqueous lixiviates and organic extracts on seed germination and seedling growth of *Amaranthus leucocarpus* Watts. and *Echinochloa crus-galli* (L.) Beauv. Bioactivity-directed fractionation of the active organic extract led to isolation of the phytogrowth-inhibitor principle, which turned out to be a mixture of resin glycosides (1).

[1]This paper was presented, in part, at the 33rd annual meeting of the American Society of Pharmacognosy, Williamsburg, Virginia, 26–31 July, 1992.

Our objective in the present investigation was to characterize the structure of the major active constituent, tricolorin A [**1**], from *I. tricolor*, and to determine its relative toxicity as an allelopathic chemical. In addition to the procedures of isolation and structure elucidation of **1**, we report the biological potential of this compound in an array of in vitro test systems.



1 R=H
3 R=Ac

RESULTS AND DISCUSSION

Screening of organic-solvent-soluble extracts (hexane, $CHCl_3$, and MeOH) of *I. tricolor* on seedling growth bioassays detected noteworthy activity in the $CHCl_3$ solution. When this $CHCl_3$ extract was subjected to cc, the phytogrowth-inhibitory activity was concentrated in the crude resin glycosides. Further chromatographic analysis of the active fraction yielded the major component in pure form, to which we have accorded the trivial name tricolorin A [**1**]. Table 1 summarizes the results of the biological screening procedures.

The allelopathic potential of compound **1** was then studied in greater detail. As shown in Figure 1, phytogrowth-inhibitory activity bioassays showed significant inhibition of seed germination in both weed plants tested. Linear regression analysis indicated a concentration value required to cause 50% inhibition (IC_{50}) of 36.9 μg/ml (36.1 μM) for *E. crus-galli*. Seed germination of *A. leucocarpus* was less sensitive to compound **1**, with an IC_{50} value of 152.7 μg/ml (149.4 μM). As shown in Figure 2, radicle growth of *E. crus-galli* and *A. leucocarpus* was inhibited by compound **1** in a dose-dependent manner. At low concentrations (1 μg/ml), both 2,4-dichlorophenoxyacetic acid (2,4-D), which was used as a positive standard, and compound **1** stimulated the growth of both plants by 4 to 10% over the untreated controls. The IC_{50} value of compound **1**, estimated by probit analysis for radicle growth of *E. crus-galli*, was 12.6 μg/ml (12.3 μM), with a 95% confidence interval of 17.4–7.8 μg/ml. For radicle elongation of *A leucocarpus*, the IC_{50} was 37.0 μg/ml (36.2 μM), with a 95% confidence interval of 46.8–29.3 μg/ml. These results clearly demonstrate the strong inhibitory effect displayed by tricolorin A [**1**], since the concentration threshold required for most of the natural phytogrowth inhibitors tested in similar experimental designs is often in the 100–1000 μg/ml range (5).

TABLE 1. Phytogrowth Inhibitory Activity of Extracts, Crude Resin Glycosides, and Pure Compound **1** from *Ipomoea tricolor* on Radicle Elongation of *Amaranthus leucocarpus* and *Echinochloa crus-galli*.

Sample[a]	Radicle growth (mm)[b]		Growth inhibition (%)[c]	
	Amaranthus	*Echinochloa*	*Amaranthus*	*Echinochloa*
Control	12.8±0.41	22.8±0.89	8.6	0.9
Hexane extract	11.7±0.85	22.6±1.44		
Control	7.9±1.08	13.4±1.83	30.4[d]	32.8[d]
MeOH extract	5.5±1.71	9.0±2.80		
Control	8.2±0.71	9.5±1.31	48.8[d]	63.2[d]
$CHCl_3$ extract	4.2±1.25	3.5±0.17		
Control	14.0±0.78	24.3±7.40	51.4[d]	72.4[d]
Crude resin glycosides	6.8±1.01	6.7±0.52		
Control	10.4±1.16	17.0±1.04	68.3[d]	78.8[d]
Tricolorin A [**1**]	3.3±0.12	3.6±0.12		

[a]Samples tested at 100 μg/ml.
[b]Each value represents the means ± SD of 4 replicates, 20 seedlings per replicate.
[c]Percentage of untreated seedlings (controls).
[d]$P \leq 0.05$, ANOVA with Duncan's multiple-range test.

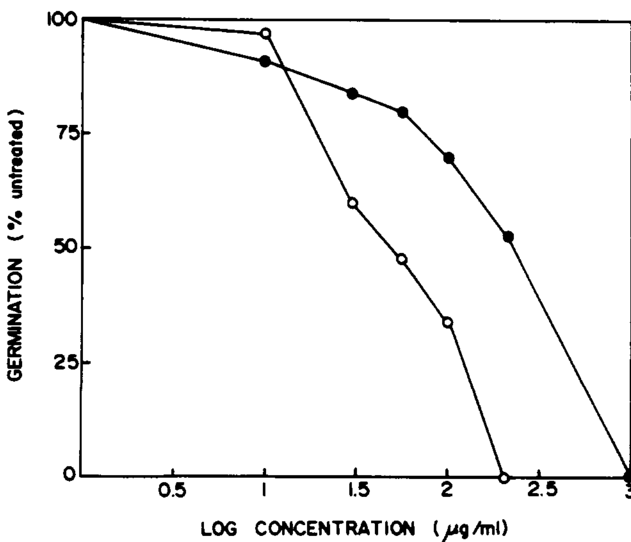


FIGURE 1. Seed germination bioassays of *Amaranthus leucocarpus* (●) and *Echinochloa crus-galli* (○) treated with different concentrations of tricolorin A [**1**]. Additional details are provided in the Experimental section.

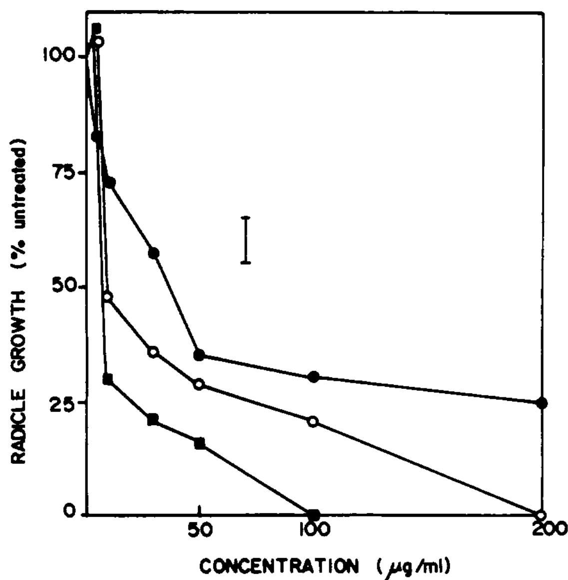


FIGURE 2. Radicle growth of *Amaranthus leucocarpus* (●) and *Echinochloa crus-galli* (○) treated with different concentrations of tricolorin A [1]. Both tested plants showed similar responses to 2,4-D (■), used as a positive control. The bar represents the maximum standard deviation (see Experimental).

The molecular formula of tricolorin A [1] was determined as $C_{50}H_{86}O_{21}$ by elemental analysis. Its negative ion fabms exhibited a quasi-molecular ion peak at *m/z* 1021 $[M-H]^-$. On alkaline hydrolysis, compound **1** liberated an organic acid fraction together with an H_2O-soluble glycosidic acid **2**. Analysis of the organic acid fraction by gc-ms afforded a single peak which was identified as (2*S*)-methylbutyric acid (6). The acid-catalyzed hydrolysis of **2** gave (11*S*)-hydroxyhexadecanoic acid (jalapinolic acid) (6,7), as the aglycone portion, and a mixture of monosaccharides. The negative ion fabms of **2** showed an $[M-H]^-$ peak at *m/z* 871 along with the fragment peaks at *m/z* 725 $[M-H-146$ (methylpentose unit)$]^-$, 579 $[725-146$ (methylpentose unit)$]^-$, 417 $[579-162$ (hexose unit)$]^-$, and 271 [417−146 (methylpentose unit); jalapinolic

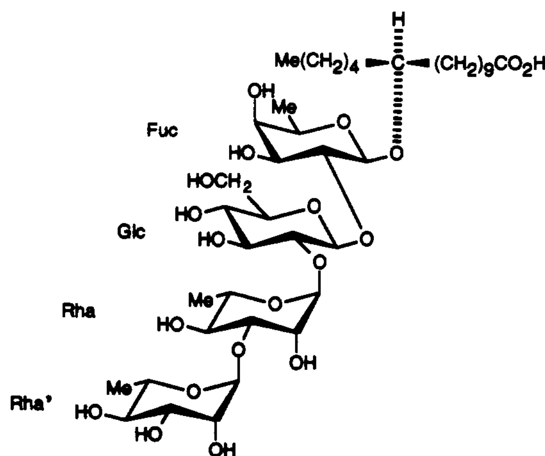


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TABLE 2. ^{1}H-nmr Data for Tricolorin A [**1**] and Derivatives **2**–**4**[a].

Unit	Compound			
	1	**2**	**3**	**4**
Fuc-1	4.68 d (7.8)	4.85 d (7.4)	4.47 d (7.8)	4.55[b]
2	4.78 dd (7.8, 8.0)	4.59 dd (7.4, 9.0)	4.32–4.37 m[b]	4.35 dd (9.0)[i]
3	4.26–4.28 m[b]	4.45 dd (3.4, 9.0)	5.13 dd (3.4, 10.2)	4.68 m[c]
4	4.06 d (3.2)	3.92 d (3.4)	5.38 d (3.4)	4.08–4.15 m[d]
5	3.84–3.86 m[c]	3.80 q (6.5)	4.30 q (6.4)	3.90[e]
6	1.60 d (6.0)	1.48 d (6.5)	1.17 d (6.4)	1.71 d (6.5)
Glc-1	5.81 d (7.2)	5.65 d (7.6)	5.05 d (7.6)	5.51 d (7.8)
2	4.15 dd (7.2, 8.0)[d]	4.30 dd (7.6, 9.0)	3.89 dd (7.6, 9.0)	4.08–4.15 m[d]
3	5.85[e]	4.19 dd (9.0)[i]	5.59 dd (9.0)[i]	5.72 dd (9.0)[i]
4	4.38 dd (9.3)[i]	4.09 dd (9.0)[i]	5.24 dd (9.0)[i]	4.08–4.15 m[d]
5	3.50 dt (1.9, 9.3)	3.66 ddd (2.7, 5.0, 9.0)	3.79 dt (2.0, 9.0)	3.55 ddd (3.0, 5.0, 9.0)
6	3.93 dd (1.9, 11.0)	4.23 dd (5.0, 12.0)	4.09 dd (2.1, 12.2)	3.75 m[f]
	4.16[d]	4.33 dd (2.7, 12.0)	4.32–4.37 m[b]	3.90[e]
Rha-1	5.58 d (1.1)	6.41 d (1.3)	5.25 d (1.5)	5.52 d (1.0)
2	5.85[e]	5.03 dd (1.3, 2.9)	5.53 dd (1.5, 3.4)	5.67 dd (1.5, 3.0)
3	4.81 dd (3.0, 9.9)	4.94 dd (2.9, 9.4)	4.28 dd (3.4, 10.0)	4.68 m[c]
4	5.74 dd (9.9)[i]	4.52 dd (9.4)[i]	5.47 dd (10.0)	5.62 dd (9.8)[i]
5	4.98 dq (6.0, 9.9)	5.16 dq (6.0, 9.4)	4.32–4.37 m[b]	4.55[b]
6	1.68 d (6.0)	1.85 d (6.0)	1.40 d (6.1)	1.54 d (6.0)
Rha′-1	5.54 d (1.0)	6.07 d (1.0)	5.18 d (1.0)	5.58 d (1.0)
2	4.55 dd (1.0, 3.0)	4.78[b]	5.29 dd (1.0, 3.2)	4.40–4.55 m[g]
3	4.44 dd (3.0, 10.0)	4.67 dd (3.4, 9.2)	5.43 dd (3.2, 9.8)	4.40–4.55 m[g]
4	4.26–4.28 m[b]	4.29 dd (9.2)[d]	5.33 dd (9.8)[i]	3.82 dd (9.8)[i]
5	4.26–4.28 m[b]	4.78[b]	3.76 dq (6.0, 9.8)	4.08–4.15 m[d]
6	1.72 d (6.0)	11.55 d (6.2)	1.04 (6.0)	1.44 d (6.0)
jla-2	2.48 ddd (3.0, 10.0, 16.0)	2.52 t (7.0)	2.26 ddd (3.0, 9.0, 16.0)	2.38–2.55 m[h]
	3.02 ddd (3.0, 8.0, 16.0)		2.68 ddd (3.0, 9.0, 16.0)	2.70 ddd
11	3.84–3.86 m[c]	3.94 m	3.58 m	3.75 m[f]
16	0.87 t (7.0)	0.84 t (7.0)	0.59 t (7.0)	0.83 t (7.0)
mba-2	2.25–2.45 m[b]	—	2.21 tq (7.0, 7.0)	2.38–2.55 m[h]
2-Me	1.22 d (7.0)	—	1.13 d (7.0)	1.23 d (7.0)
3-Me	0.95 t (7.0)	—	0.81 t (7.0)	0.96 t (7.0)
mba′-2	2.25–2.45 m[b]	—	2.57 tq (7.0, 7.0)	2.38–2.55 m[h]
2-Me	1.15 d (7.0)	—	0.99 d (7.0)	1.17 d (7.0)
3-Me	0.85 t (7.0)	—	0.80 t (7.0)	0.93 t (7.0)
MeC=O	—	—	1.74–1.97 (s×7)	—
$(Me)_2C$	—	—	—	1.43–1.56 (s×6)

[a]Data recorded at 300 MHz in pyridine-d_5. Chemical shifts are in δ (ppm). Coupling constants (J in Hz) are given in parentheses. The assignments were made by a combination of COSY and HETCOR.

[b–h]Overlapped signals.

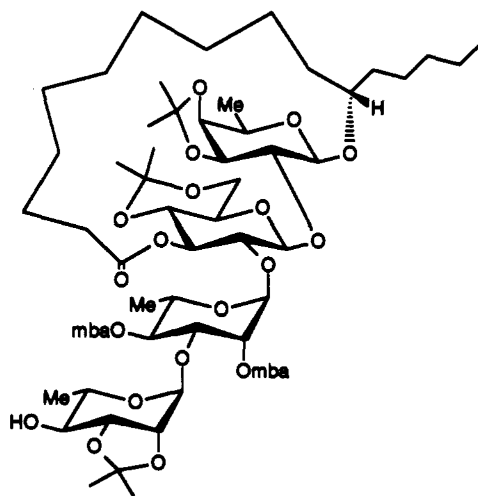
[i]Triplet-like signal.

acid−H]$^-$, indicating that tricoloric acid [**2**] is a linear tetraglycoside of jalapinolic acid composed of 1 mol of hexose and 3 mol of methylpentose (6,8). The hydrolysis mixture of sugars was characterized by gc-ms analysis of their trimethylsilyl ethers (9) as rhamnose, fucose, and glucose in a ratio of 2:1:1.

The ^{1}H-nmr spectrum of **1** showed signals at δ 2.48 and 3.02 (each 1H) due to the nonequivalent protons of the methylene group at C-2 in the aglycone moiety, as well as the signals for two residues of 2-methylbutyric acid (Table 2). This spectral evidence indicated that the active compound **1** is a resin glycoside, similar to those isolated previously from other *Ipomoea* species (8,10), having the carbonyl group of the aglycone linked to an oligosaccharide core to form a macrocyclic ester and the small fatty acid

residues esterifying the sugars. The existence of three acylated sites was confirmed by acetylation of **1** to the hepta-acetyl derivative **3**.

The ^{1}H and ^{13}C signals arising from the sugar moieties in the spectra of compounds **1**–**4** were assigned by ^{1}H-^{1}H DQ-COSY and ^{1}H-^{13}C HETCOR analyses (Tables 2 and 3). The ^{13}C-nmr spectrum of **2** showed the glycosylation shifts at fucose C-2 ($\Delta\delta$ 6.3), glucose C-2 ($\Delta\delta$ 3.6), and at C-3 ($\Delta\delta$ 8.0) of one of the rhamnose units, when compared with those of corresponding methyl pyranosides (11). The selective INEPT technique (12), applied to tricolorin A [**1**], confirmed the nature of saccharide substitution. Thus, irradiation of the anomeric proton H-1 of fucose at δ 4.68 ($^{3}J_{CH}$=6 Hz) selectively enhanced C-11 (δ 80.76) of the aglycone. When H-2 of fucose (δ 4.78) was irradiated ($^{3}J_{CH}$=6 Hz), a clear enhancement of the anomeric carbon C-1 of glucose resulted (δ 99.76). Irradiation of the anomeric proton of one of the rhamnose units at δ 5.58 ($^{3}J_{CH}$=4 Hz) enhanced the glucose C-2 (δ 80.60). Finally, irradiation of the inner rhamnose H-3 at δ 4.81 ($^{3}J_{CH}$=6 Hz) resulted in the polarization transfer to the anomeric carbon C-1′ (δ 104.49) of the terminal rhamnose. The application of a COLOC nmr experiment (Figure 3) on **1** further confirmed the sequence of the sugar moiety. ^{13}C-^{1}H long-range cross peaks were observed and unambiguously assigned as those between C-1 of fucose and H-11 of the aglycone, C-1 of glucose and fucose H-2, C-1 of inner rhamnose and glucose H-2, and C-1′ of terminal rhamnose and inner rhamnose H-3. From the information discussed above, it was apparent that the structure of tricoloric acid [**2**] corresponded to (*S*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-β-D-fucopyranoside.



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In order to establish the site of lactonization and the location of the two additional ester linkages at the oligosaccharide core, the signals for the sugar moiety in the ^{1}H-nmr spectra of **1** and **2** were compared. Significant downfield shifts owing to acylation were observed for glucose H-3 ($\Delta\delta$ 1.66) and inner rhamnose protons H-2 ($\Delta\delta$ 0.82) and H-4 ($\Delta\delta$ 1.22). Subsequently, in a selective INEPT experiment, magnetization transfer from rhamnose H-4 at δ 5.74 ($^{3}J_{CH}$=4 Hz) enhanced the carbonyl ester resonance at δ175.42. Therefore, one of the methyl butyric acid residues is concluded to be esterified at the geminal hydroxyl group at this position. In the negative fabms of **1** and **2**, besides the common fragment peaks observed at *m/z* 271 and 417, tricolorin A [**1**] showed a peak at *m/z* 561 in place of that detected at *m/z* 579 in the spectrum of **2**. The difference of

TABLE 3. ^{13}C-nmr Chemical Shift Values of Tricolorin A [**1**] and Tricoloric Acid [**2**][a].

Unit	Compound				
	1	**2**	β-D-Fuc[b]	β-D-Glc[b]	α-L-Rha[b]
Fuc-1	103.09	102.71	105.9		
2	74.62	78.32	72.0		
3	76.16	76.00	75.2		
4	73.12	72.82	72.6		
5	71.24	70.91	71.3		
6	17.24	17.18	17.2		
Glc-1	99.76	102.35		105.4	
2	80.60	78.38		74.8	
3	78.96	79.10		78.1	
4	69.62	72.59		71.4	
5	76.25	76.94		78.1	
6	61.31	63.13		62.5	
Rha-1	98.20	101.93			102.4
2	72.74	71.93			71.9
3	75.84	80.47			72.5
4	73.30	72.96			73.6
5	67.23	69.68			69.4
6	18.25	18.89			18.4
Rha′-1	104.49	104.17			
2	72.25	72.36			
3	72.46	72.66			
4	73.34	74.17			
5	70.44	69.88			
6	18.48	18.50			
jla-1	172.13	176.15			
2	34.36	34.92			
11	81.00	80.55			
16	14.15	14.20			
mba-1	175.55				
2	41.53				
3	27.92				
4	11.76				
2-Me	16.91				
mba-1	175.42				
2	41.37				
3	27.06				
4	11.76				
2-Me	16.91				

[a]Data recorded at 75.4 MHz in pyridine-d_5. Chemical shifts are in δ (ppm). The assignments were verified by long range ^{1}H-^{13}C correlation.

[b]Data for the methyl glycopyranosides were reported previously (11) and are listed here for comparison.

18 mass units suggested that the ester linkage of jalapinolic acid is placed at C-3 of glucose. Independent verification of the position of lactonization was provided by selective INEPT nmr experiments on acetonide **4**. The H-3 proton of glucose (δ 5.85), which overlapped the rhamnose H-2 signal in compound **1**, was clearly observed as a triplet at δ 5.72 in the ^{1}H-nmr spectrum of derivative **4**, which upon irradiation ($^{3}J_{CH}=6$ Hz) produced the enhancement of the resonance at δ 172.07 corresponding to the carbonyl group of the aglycone moiety. Similarly, irradiation of protons H-2 (δ 5.67) and H-4 (δ 5.62) of the inner rhamnose unit using $^{3}J_{CH}=3$ Hz gave maximal enhancements of the carbonyls in the methylbutyric acid moieties (δ 175.65 and 175.84). Therefore,

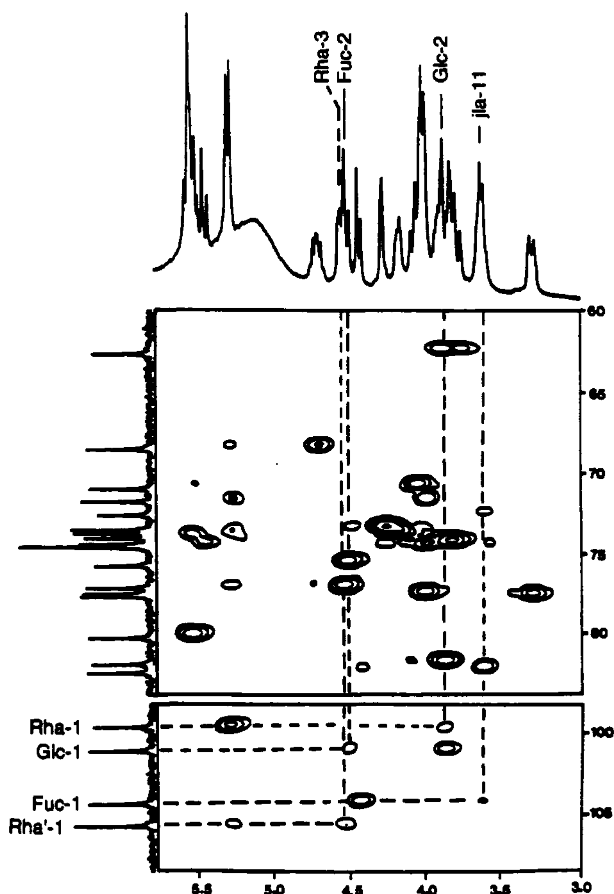

FIGURE 3. Expansion of ^{1}H-^{13}C COLOC spectrum for the tetrasaccharide unit of tricolorin A [1] in pyridine-d_5–D_2O (5:1). Long-range connectivities are indicated for anomeric carbons.

the structure of tricolorin A, major phytogrowth-inhibitor from *I. tricolor*, was elucidated as (11*S*)-hydroxyhexadecanoic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-[2-*O*-(2*S*-methylbutyryl)-4-*O*-(2*S*-methylbutyryl)]-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-β-D-fucopyranoside-(1,3″-lactone) [**1**].

Additional biological evaluation procedures for tricolorin A [**1**] were performed. Quantitative antimicrobial assays (13) against *Staphylococcus aureus* allowed the determination of an MIC of 1.8 μg/ml for compound **1**. The crude resin glycosides and pure compound **1** showed comparable lethality to brine shrimp (14). Probit analysis of data indicated that the LC_{50} for the glycosidic mixture was 36.8 μg/ml with a 95% confidence interval of 62.5–20.5 μg/ml. For pure compound **1**, the LC_{50} was 18.2 μg/ml, with a 95% confidence interval of 37.1–5.8 μg/ml. In spite of this larvicidal activity, compound **1** displayed only marginal cytotoxicity with ten human cancer cell lines and murine lymphocytic leukemia in cell culture (P-388) (15). Potential antimitotic activity was evaluated in an astrocytoma (ASK) assay (16). As summarized in Table 4, the most intense cytotoxic activity (ED_{50} 2.2 μg/ml) was observed with human breast cancer and P-388 cells. No demonstrable antimitotic activity was observed as judged by the ASK assay. Finally, competitive antagonism of phorbol 12,13-dibutyrate binding to partially purified protein kinase C was analyzed as described previously (17,18). Tricolorin A [**1**]

TABLE 4. Cytotoxic Activity of Tricolorin A [**1**].[a]

Compound	Cell line										
	A-431	BC-1	Col-2	HT-1080	KB	KB-V1	LNCaP	Lu-1	Mel-2	ZR-75-1	P-388
1	10.3	2.2	8.9	6.3	>20	>20	5.8	5.5	7.6	8.1	2.2

[a]Results are expressed as ED_{50} values (μg/ml). Abbreviations: A-431, epidermoid carcinoma; BC-1, breast; Col-2, colon; HT-1080, sarcoma; KB, nasopharyngeal carcinoma; KB-V1, drug-resistant KB; LNCaP, prostate; Lu-1, lung; Mel-2, melanoma; ZR-75-1, breast; P-388, murine lymphocytic leukemia.

was found to demonstrate a dose-dependent inhibition of the specific binding of [^{3}H]PDBu with an IC_{50} of 44.1 μg/ml (43.2 μM) (Figure 4).

In conclusion, the *Ipomoea* resin glycosides are potent inhibitors of plant growth that are primarily responsible for the allelopathic interference exhibited by these species. These active principles may also be involved in the chemical ecology of the plant family Convolvulaceae, as demonstrated by the broad range of biological activities displayed by tricolorin A [**1**].

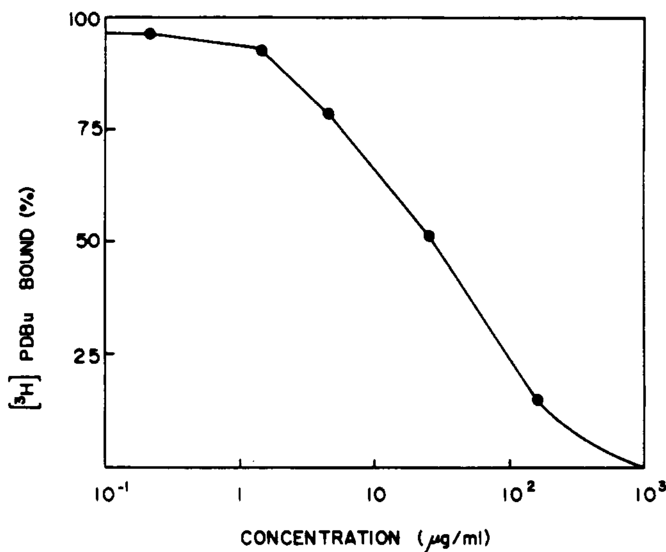

FIGURE 4. Inhibition of [^{3}H]PDBu binding to partially purified protein kinase C by tricolorin A [**1**]. [^{3}H]PDBu binding as a function of compound **1** concentration was assayed using a 60-min incubation at ambient temperature as described in the Experimental section. Points shown are mean values ± SD of 4 replicates.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's, optical rotations, ir, and low resolution ms were measured as described previously (1). All nmr spectra were obtained in C_5D_5N with TMS as an internal standard. ^{1}H- and ^{13}C-nmr spectra were obtained on a Varian XL-300 spectrometer operating at 300 MHz and 75.44 MHz, respectively. Standard Varian pulse sequences were used to obtained ^{1}H-^{1}H DQ-COSY, HETCOR, and ^{1}H-^{13}C COLOC ($^3J_{CH}$=8 Hz) nmr spectra. Selective INEPT experiments were performed on a Nicolet NMC-360 (90.8 MHz) spectrometer. Fabms was obtained using a Finnigan MAT 90 instrument. Gc-ms analyses were conducted on a Varian 112S mass spectrometer/gas chromatograph, as previously reported (9). Analytical and preparative tlc were performed on Si gel 60 F_{254} Merck plates, and the spots were visualized by spraying with a 10% solution of $Ce(SO_4)_2$ in 2 N H_2SO_4, followed by heating at 110°. Cc was carried out on Si gel 60 (70–230 mesh, Merck).

PLANT MATERIAL.—Whole plants (1.2 kg) of *I. tricolor* were collected in Cañón de Lobos, Morelos, México, in February 1990. A voucher specimen (Anaya No. 90-I) has been deposited in the National Herbarium (MEXU), Instituto de Biología, Universidad Nacional Autónoma de México. The seeds of *A. leucocarpus* and *E. crus-galli* were purchased from Valley Seed Service, Fresno, California.

BIOLOGICAL SCREENING PROCEDURES.—Phytogrowth-inhibitory activity was determined by seed germination and radicle elongation studies, according to the procedures previously described (1). The activity of extracts, chromatography fractions, and resin glycosides was routinely evaluated on seeds of *A. leucocarpus* and *E. crus-galli*. The assays were conducted in 6-cm-diameter Petri dishes with four replicates per treatment. Twenty seeds were germinated in a Petri dish on Whatman No. 1 paper moistened with 1.5 ml of distilled, deionized H_2O. Test samples dissolved in MeOH-H_2O (1:1) were added to each Petri dish, followed by solvent evaporation by air drying. Treatment solutions were prepared to final concentrations of 100 μg/ml. 2,4-Dichlorophenoxyacetic acid (2,4-D, Sigma) was used as a positive control. The same protocol was used for negative controls containing solvent, except they received no test sample. Petri dishes were placed in the dark at 27°, and percentages of germination and radicle lengths were obtained after 24 h for *A. leucocarpus* and 48 h for *E. crus-galli*.

SOLVENT EXTRACTION.—The air-dried, milled plant material (1.2 kg) was defatted exhaustively by maceration at room temperature with hexane. This solution upon evaporation under low pressure at 40° yielded 8.2 g of a yellowish resinous extract. The residual material was extracted with $CHCl_3$ three times overnight. After evaporation to dryness, the $CHCl_3$ extract gave 27.3 g of a dark-green residue. Finally, the marc was further extracted by percolation with MeOH to afford 76.3 g of a dark brown resinous extract. The $CHCl_3$ extract showed the strongest phytogrowth-inhibitory activity.

ISOLATION OF TRICOLORIN A [**1**].—The crude $CHCl_3$ extract (27.8 g) was chromatographed over 700 g of Si gel in a gravity column using a gradient of Me_2CO/MeOH in $CHCl_3$. The inhibitory effect was found to concentrate in the resin glycoside fraction (10 g), which was rechromatographed over Si gel (400 g) eluting with $CHCl_3$-Me_2CO-MeOH (5:5:1). A total of 180 fractions (100 ml each) were collected. Combined fractions 140–145 (6 g), which displayed most of the activity, were treated with activated charcoal followed by crystallization (MeOH) to afford 4.8 g (0.4% w/w) of tricolorin A [**1**].

BIOLOGICAL EVALUATION PROCEDURES FOR TRICOLORIN A [**1**].—The potential activity of tricolorin A [**1**] as an inhibitor of weed growth was performed as described above. Tests were conducted with 0, 1, 5, 10, 30, 50, 100, and 200 μg/ml of compound **1**. Statistical differences between the treatments were evaluated by analysis of variance. IC_{50} values were obtained by probit analysis (19). Quantitative antimicrobial activity against *S. aureus* (ATCC 25923) was accomplished using the agar diffusion technique (13). Critical MIC was calculated by applying the statistical method of least squares (20) to the data obtained from a doubling dilution experiment, which included concentrations, in duplicate, ranging from 100 to 0.19 μg/ml of compound **1**. The brine shrimp lethality bioassay followed the procedures reported by Anderson *et al.* (14). In vitro cytotoxic activities on several tumor cell lines were determined according to protocols described previously (15). KB, A-431, HT-1080, LNCaP, ZR-75-1, and P-388 cell lines were purchased from the American Type Culture Collection, and BC-1, Lu-1, Mel-2, and Col-2 cell lines were established from primary human tumors in the Division of Surgical Oncology, University of Illinois, College of Medicine at Chicago. In addition, antimitotic potential was evaluated using cultured ASK cells (16). The phorbol 12,13-dibutyrate ([^{3}H]PDBu) receptor binding assay was performed essentially by the method of de Vries *et al.* (17,18). In brief, assays were carried out in a final volume of 250 μl containing 50 mM Tris/HCl (pH 7.4), $CaCl_2$ (1 mM), [^{3}H]PDBu (39.2 nM), 50 μg/ml calf brain homogenate, and various concentrations of compound **1** (in DMSO). Non-specific binding was defined as the portion of total binding not displaceable by a 100-fold excess of unlabelled PDBu. After 1 h at ambient temperature, the reaction mixtures were collected and washed on glass fiber filters using a Skatron Cell Harvestor. The bound radioactivity was determined by scintillation counting. After correcting for nonspecific binding, a dose-response curve was prepared using 4 replicates with five concentrations of the test solution (five-fold serial dilutions from 200 to 0.32 μg/ml).

Tricolorin A [**1**].—Mp 118–120° (colorless needles, MeOH); $[\alpha]_D$ −30.32° (c=1.5, MeOH); ir ν max (KBr) 3420, 2930, 1740, 1470, 1390, 1240, 1130, 1060 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 3; positive fabms m/z (rel. int.) $[M+H]^+$ 1023 (100), 877 (80); negative fabms m/z (rel. int.) $[M-H]^-$ 1021 (100), 875 (15), 561 (70), 417 (85), 271 (18). *Anal.* calcd for $C_{50}H_{86}O_{21}$, C 58.70%, H 8.41%; found C 58.76%, H 8.45%.

ALKALINE HYDROLYSIS OF **1**.—A solution of compound **1** (500 mg) in 5% KOH/H_2O (10 ml) was refluxed at 95° for 1 h. The reaction mixture was adjusted to pH 4 with 4 N HCl, and extracted with Et_2O

(2×10 ml). The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was directly analyzed by gc-eims with only one peak detected (Rt 3.57 min), which was identified as (2*S*)-methylbutanoic acid (6): [α]D +17° (*c*=4.3, MeOH); eims *m/z* (rel. int.) $[M]^+$ 102 (1), 101 (1), 87 (22), 85 (1), 75 (4), 74 (97), 73 (16), 69 (7), 57 (62), 56 (18), 55 (17), 45 (25), 41 (91), 26 (15), 15 (7).

The aqueous phase was extracted with *n*-BuOH (2×10 ml), and the organic layer was evaporated in vacuo to give tricoloric acid [**2**] (325.9 mg): colorless powder; mp 104–106°; [α]D −61.92° (*c*=0.86, MeOH); ir ν max (KBr) 3400, 1710, 1130, 1060 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 3; negative fabms *m/z* (rel. int.) $[M-H]^-$ 871 (100), 725 (18), 579 (16), 417 (19), 271 (5), 268 (9), 142 (8). *Anal.* calcd for $C_{40}H_{72}O_{20}$, C 55.03%, H 8.31%; found C 55.05%, H 8.32%.

ACETYLATION OF **1**.—Compound **1** (100 mg) was acetylated with 2.5 ml of Ac_2O-C_5H_5N (3:2) for 24 h at room temperature to afford derivative **3** (85 mg) as a white powder: mp 74–76°; [α]D −31.92° (*c*=1.7, MeOH); ir ν max (KBr) 2930, 1740, 1440, 1370, 1230, 1070 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 3.

PREPARATION OF ACETONIDE **4**.—A small amount of molecular sieve (4 Å, Merck) (ca. 300 mg) was added to a solution of **1** (100 mg) and a trace of *p*-toluenesulfonic acid monohydrate in anhydrous Me_2CO (3 ml) and 2,2-dimethoxypropane (3 ml). The reaction mixture was stirred at room temperture for 36 h. After filtration, the solvent was evaporated to dryness. The residue was poured into H_2O and extracted with EtOAc (3×5 ml). The organic portions were combined and the solvent removed at reduced pressure. The major product was purified by tlc [$CHCl_3$-EtOAc-Me_2CO (8:1:1)] to yield 70.52 mg of product **4** (R_f 0.5) as a white solid: mp 73–75°; [α]D −21.2° (*c*=0.2, MeOH); ^1H nmr see Table 2, ^{13}C nmr see Table 3.

ACID HYDROLYSIS OF **1**: SUGAR ANALYSIS.—A solution of tricolorin A [**1**] (90 mg) in 2 N HCl (10 ml) was heated at 90° for 2 h. The reaction mixture was diluted with H_2O (5 ml) and extracted with Et_2O (2×10 ml). The aqueous layer was neutralized with KOH and extracted with *n*-BuOH (2×10 ml). This organic layer was washed with H_2O and evaporated to afford 42 mg of a mixture of monosaccharides, which was subjected to tlc analysis [$CHCl_3$-MeOH-H_2O (6:4:1)]: R_f 0.24 (glucose); 0.45 (fucose); 0.51 (rhamnose). An aliquot of this hydrolysis mixture (1 mg) was derivatized with Sigma Sil-A for 35 min at 70°. Gc-ms analysis gave three peaks: Rt 14.97, 16.67, and 21.86 min, in the ratio of ca. 2:1:1, which co-eluted with those of TMSi-ethers of standard rhamnose, fucose, and glucose, respectively.

ACID HYDROLYSIS OF **2**.—A solution of derivative **2** (100 mg) in 1,4-dioxane–H_2O (10:1) (5 ml) was heated with 10% H_2SO_4 (2 ml) under reflux for 2 h. The reaction mixture was cooled and extracted with Et_2O (3×2.5 ml). The combined organic layers were evaporated to dryness. The residue was purified by tlc [$CHCl_3$-MeOH (4:1)] to give 20.5 mg of the aglycone (R_f 0.62): mp 60–63°; [α] +0.7° (*c*=0.9, $CHCl_3$). This was treated with an excess of CH_2N_2 in Et_2O at room temperature followed by crystallization from Et_2O, to afford 19.8 mg of a white crystalline solid: mp 40–43°; [α]D +1.0° (*c*=0.9, MeOH); ir ν max (KBr) 3325, 2920, 2850, 1740, 1460, 1210, 1165 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.90 (3H, t, *J*=7 Hz, Me-16), 1.20–1.35 (16H, m), 1.43 (4H, m), 1.60 (4H, m) 2.32 (2H, t, *J*=7.0 Hz, H-2), 3.58 (1H, m, H-11), 3.68 (3H, s, -CO_2*Me*); eims *m/z* (rel. int.) $[M]^+$ 286 (0.4), 255 (2), 229 (10), 215 (15), 186 (18), 183 (63), 157 (10), 143 (30), 101 (56), 95 (38), 87 (87), 83 (74), 81 (40), 74 (32), 69 (22), 67 (15), 59 (36), 57 (30), 55 (100), 43 (40), 41 (50). Its physical and spectral properties were identical to those previously reported for methyl (11*S*)-hydroxyhexadecanate (6,7).

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