The Withanolides of Iochroma fuchsioides

Robert F. Raffauf, Melvin J. Shemluck, and Philip W. Le Quesne

J. Nat. Prod., **1991**, 54 (6), 1601-1606• DOI:
10.1021/np50078a017 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 6, pp. 1601-1606, Nov-Dec 1991

THE WITHANOLIDES OF *IOCHROMA FUCHSIOIDES*

ROBERT F. RAFFAUF,* MELVIN J. SHEMLUCK, and PHILIP W. LE QUESNE*

College of Pharmacy and Allied Professions and Department of Chemistry, Northeastern University, Boston, Massachusetts 02115-5096

ABSTRACT.—In addition to the known withanolide D [**1**], three new withanolides have been isolated from *Iochroma fuchsioides*, which we have named as derivatives of withanolide D: 18-acetoxywithanolide D [**3**], 18-acetoxy-4-deoxy-5,6-deoxy-5-withenolide D [**5**], and 18-acetoxy-5,6-deoxy-5-withenolide D [**6**]. Two compounds characterized in this study were shown to be artifacts of isolation resulting from addition of MeOH to **1** and **3** during extraction of the plant with that solvent: 3-methoxy-2,3-dihydrowithanolide D [**2**] and 18-acetoxy-3-methoxy-2,3-dihydrowithanolide D [**4**]. Alkaloids were not detected; eight common amino acids and NH_4^+ were identified.

Iochroma fuchsioides Miers (Solanaceae) is a shrub with orange-red flowers that grows in the Andean highlands of Colombia and Ecuador. It is a putative medicinal and hallucinogen used by the medicine men of the Kamsá Indians of the Sibundoy Valley of Colombia and is known locally as "borrachera" or "borrachera andake" (intoxicant) (1). Little botanical work has been undertaken since the genus was first described in the 1800s and no chemical studies of *Iochroma* have come to our attention.

Its position among the ca. 80 recognized genera of the Solanaceae (2) is a matter of dispute among taxonomists. Hunziker's recent classification of the South American Solanaceae into 12 tribes (3) places *Iochroma* in the subtribe Solaneae along with *Solanum, Physalis, Lycopersicon, Acnistus, Dunalia,* and others, whereas Baehni's treatment of the family (2) includes it along with *Latua, Dunalia,* and *Acnistus* in a subtribe of their own, the Iochromineae. Certainly its placement on purely morphological grounds appears difficult.

Further, Hunziker (3) and Schultes (1) disagree on the number of species within the genus. At least three names have been associated with the plants used in our study: *I. fuchsioides, Iochroma gesnerioides*, and *Iochroma umbrosa*. Schultes has merged *I. umbrosa* into *I. fuchsioides*. We have examined two collections, one from Colombia and the other from Ecuador. Superficial morphological differences between the two may be due to the state of development of the plants at the two collection sites, the former being more mature, woody, and with less glabrous leaves. By comparison with available herbarium materials we have accepted both as *I. fuchsioides*.

RESULTS AND DISCUSSION

A search for alkaloids in *I. fuchsioides* was conducted on the basis that (a) it is a member of a plant family rich in these compounds; (b) it has been reported as a hallucinogen by indigenous people in Colombia; and (c) it gives positive Dragendorff's spot tests, a classical screening method for alkaloids. In the event, no alkaloids were obtained from the plant, but a group of withanolides was found which gave strong positive tests with the Dragendorff's reagent. Two collections of plant material, each consisting of leaves and stems, were examined, one from Ecuador and the other from Colombia. They were extracted and fractionated separately.

Dried, ground plant material was defatted with hexane and then extracted with MeOH. Si gel chromatography of the solvent-partitioned MeOH extract of the Ecuadorian material gave as the major component withanolide D [**1**], identified by correspondence of its physical constants and spectral data with those reported in the literature (4) and by comparison with an authentic sample. ^{1}H- and ^{13}C-nmr and hrms spectra of

this compound (the ^{13}C-nmr spectrum appears not to have been reported previously) formed the basis of structure assignments for the congeners and artifactual compounds described below.

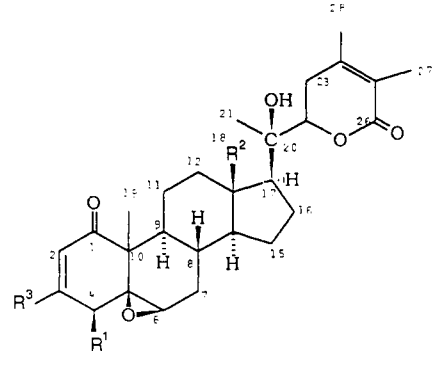
The first withanolide, withaferin A, was isolated from *Withania somnifera* (5), and since then more than 100 withanolides have been obtained from nine genera of the Solanaceae. Withanolide D [**1**] is one of the more important members of the series, and since the compounds we have obtained in this investigation are closely related to withanolide D, we have named them as its derivatives rather than by the necessarily elaborate IUPAC nomenclature.

Preparative tlc and hplc of the withanolide-D-yielding material mentioned above yielded three further compounds. The first, assigned structure **3**, on cims showed $[M+1]^+$ at *m/z* 529, corresponding to the formula $C_{30}H_{40}O_8$. Since the ^{1}H- and ^{13}C-nmr spectra were clearly analogous to those of withanolide D but showed loss of a quarternary *C*-methyl and gain of an acetoxymethyl function, we inferred that this compound is 18-acetoxywithanolide D. The argument of Kupchan *et al.* (6) for assigning to withacnistin the structure 18-acetoxywithaferin A applies equally well in the present case to support the 18-acetoxywithanolide D structure. In corroboration of this assignment, the ^{13}C-nmr spectra of withanolide D and the new compound are identical with respect to the Me-19 signal.

The next withanolide, assigned structure **4**, contained an additional CH_4O unit as compared with compound **3**. From inspection of spectra this compound was clearly a methanol adduct with the ring-A enone function, this Michael addition probably having occurred during the extraction of the plant material. The MeOH addition reaction did not change the chemical shift of the methylene carbon bearing the acetoxyl group, further supporting the location of the latter function at C-18 rather than C-19.

The final withanolide isolated from the Ecuadorian material was assigned structure **2**, the Michael adduct of MeOH and withanolide D. Support for the idea that compounds **2** and **4** are artifacts of extraction comes from our finding that withanolide and its 18-acetoxy derivative react with MeOH under the conditions of extraction to yield **2** and **4**.

The Colombian plant material, processed similarly to the Ecuadorian, gave compounds **1**, **2**, and **4**, as well as two compounds assigned the unsaturated desoxy-struc-



	R^1	R^2	R^3	Other
1	-OH	-Me	-H	
2	-OH	-Me	-OMe[31]	2,3-dihydro-
3	-OH	$-CH_2OAc^{29}_{30}$	-H	
4	-OH	$-CH_2OAc$	-OMe	2,3-dihydro-
5	-H	$-CH_2OAc$	-H	$\Delta^{5,6}$ (deoxy)
6	-OH	$-CH_2OAc$	-H	$\Delta^{5,6}$ (deoxy)

tures **5** and **6**. These structural assignments are based on characteristic and predictable changes in the ^1H- and ^{13}C-nmr spectra consequent upon the functional group changes at C-5 and C-6. The differences in withanolide profiles between the two plant samples may reflect differences in the age of the samples or else may arise from "chemical race" variations such as are well known for other withanolide-producing plants.

With the idea that psychoactive alkaloids might be found in the aqueous extracts we investigated the H_2O-soluble constituents. Positive tests were obtained for carbohydrates, tannins, and amino acids; eight of these last, along with NH_4^+, were identified by the hplc profiles of their phenylisothiocyanate derivatives compared to those of standard amino acids (7–9).

The non-polar extract obtained by defatting the plant with hexane gave evidence of the presence of sterols by the Liebermann-Burchard test; it was not examined further. A similar extract of the closely related *Acnistus breviflorus* had been found earlier to contain a wide assortment of C_{26} and C_{27} sterols, hydrocarbons, phytol, and fatty acids (10).

The discovery of withanolides in *Iochroma* is, at the moment, only suggestive of its chemical relationship to allied genera. Only one other plant, a Costa Rican collection of *Acnistus arborescens*, has been found to contain 18-acetoxy compounds (6). This may be of chemotaxonomic significance following further chemical studies of additional genera and species of this portion of the Solanaceae, which have been difficult to classify on purely morphological grounds.

The withanolides have been shown to possess biological activities including antimicrobial activity, cytotoxicity, effects on the immune system both positive and negative, and antifeedant activity against insect herbivory (11–16). D. Lavie (personal communication) doubts that they have psychoactive properties unless they are ingested in near-toxic doses. This might be presumed based on ethnobotanical reports of the use of one to three cupfuls of a strong decoction over a 3-hr period which can leave the "brujo" ill for at least a day (1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Chromatography was done on Merck SiO_2 60, 230–400 mesh; tlc and preparative tlc on Analtech plates (SiO_2) with fluorescent indicator, and general visualization by both long- and short-wave uv light. Specific groups of compounds were visualized with H_2SO_4 and heat (steroids), ninhydrin (amino acids), and Dragendorff's reagent ("alkaloids," i.e., withanolides). Depending on the nature of the mixture to be separated, hplc was conducted with a variety of commercially available instruments and columns which are described in the procedures for the individual compounds discussed below. Melting points were taken on a Gallenkamp apparatus and are uncorrected. Spectra were taken on the following instruments: ir, Perkin-Elmer 566B; uv, Perkin-Elmer 3B UV/VIS; nmr, Varian XL 300 at 75.429 MHz for ^{13}C and 299.943 MHz for ^1H with TMS or $CHCl_3$ as internal standards; ms, Finnegan 4021B using INCAS data system. Optical rotations were taken on a Perkin-Elmer Model 241 Polarimeter.

PLANT MATERIAL.—Plant material was obtained from two sites in South America. That from Colombia was collected in the environs of Sibundoy, Putumayo, where it is somewhat of a weed growing along roadsides, field edges, and house boundaries. The Ecuadorian sample was harvested from gardens in Quito where it is used as an ornamental; it appears to be more juvenile, less woody, with fewer flowers and fruit, and with more glabrous leaves. Voucher specimens of both collections have been deposited in the Economic Botany Herbarium of Harvard University, Cambridge, Massachusetts 02138.

ISOLATION AND CHARACTERIZATION.—*Ecuadorian collection.*—Milled leaf and stem (5.4 kg), defatted with hexane and extracted with MeOH (7 × 8 liters, room temperature, one month) gave, after removal of the solvent, 906 g of a syrupy residue. Assuming the presence of alkaloids based on preliminary qualitative tests, a portion of this syrup (424 g) was partitioned between $CHCl_3$ and 5% HCl. The latter extract was made alkaline with NH_4OH and extracted with $CHCl_3$ to recover these compounds. The alkaloid fraction (6.28 g) gave a strongly positive test with Dragendorff's reagent as well as a hydroxamic acid test indicating the presence of an ester functionality. It was fractionated by flash chromatography (fcc) on SiO_2 using $CHCl_3$ and increasing amounts of MeOH to yield 138 fractions of which 4–14 ($CHCl_3$, 1.05

g) were pooled on the basis of tlc profiles and strong positive reaction with the Dragendorff's reagent. This was subjected to a second fcc to give 24 fractions from which 13–18 ($CHCl_3$ 1,2,3% MeOH, 865 mg) were similarly selected for a final fcc giving 44 fractions. The addition of MeOH to 9–19 ($CHCl_3$/3% MeOH, 457 mg) resulted in the precipitation of a single (tlc) *N*-free product which reacted positively to both Dragendorff's and Mayer's reagents. It was crystallized from EtOAc: mp 253–255°; ir, uv, ^{1}H nmr, and ms entirely consistent with those reported for withanolide D [**1**] (4). Our ^{13}C-nmr data, apparently new to the literature, are recorded in Table 1.

With the realization that withanolides rather than alkaloids were the likely compounds of interest in the plant, a modified partition scheme was carried out as follows. The remainder of the MeOH syrup was partitioned between hexane and 10% aqueous MeOH to remove additional non-polar, Dragendorff-negative material, and the aqueous layer was then exhaustively extracted with Et_2O to give a crude withanolide fraction (21.5 g). Four grams, in 50-mg increments, were chromatographed on a Supelco LC-SDB C_{18} semiprep column (13 × 250 mm) using 50% MeOH as the isocratic mobile phase and a flow rate of 4.7 ml/min, and with the detector set at 254 nm to yield seven fractions. The first two and the last were Dragendorff's negative.

Fraction 3 (40 mg) was purified by normal phase preparative tlc using a mobile phase of $CHCl_3$-EtOAc (1:1) and triple development. Bands were visualized by short wave uv and recovered by extraction with $CHCl_3$, EtOAc, and iPrOH to give 27 mg of a crystalline product identified as 18-acetoxywithanolide D [**3**]: $C_{30}H_{40}O_8$; needles from EtOAc-hexane; mp 148–153°; uv max 215 nm (log ε 4.18, $CHCl_3$); $[\alpha]_D$ +71.9° (c = 5, $CHCl_3$); ir (KBr) 3600, 3450, 3030, 1700, 1200–1000 cm^{-1}; ^{1}H nmr δ 6.96 (dd, H, J = 5.6, 10.5 Hz), 6.2 (d, H, J = 10.5 Hz), 4.25 (dd, H, J = 3.5, 6.4 Hz), 4.12 (d,

TABLE 1. Withanolide ^{13}C-nmr Chemical Shifts in ppm.

Carbon	Compound					
	1	**2**	**3**	**4**	**5**	**6**
C-1	202.2	209.8	202.4	209.9	204.3	203.5
C-2	132.4	39.7	132.1	34.5	124.2	130.3
C-3	141.9	56.7	142.4	56.7	145.3	142.3
C-4	70.0	74.7	69.6	74.6	33.3	69.1
C-5	63.9	64.9	63.7	64.8	135.8	138.8
C-6	62.4	59.6	61.8	59.6	127.7	128.8
C-7	31.5	31.0	31.4	31.4	31.5	31.0
C-8	29.2	28.8	29.5	29.1	32.8	32.4
C-9	44.1	42.6	44.1	42.7	42.8	42.8
C-10	47.7	50.3	47.6	50.2	50.4	49.2
C-11	21.9	21.1	21.7	21.2	23.2	22.7
C-12	39.7	39.2	34.7	39.5	35.0	35.0
C-13	42.7	42.6	45.1	45.1	45.2	45.2
C-14	56.5	56.4	56.0	56.1	56.2	56.2
C-15	23.8	23.6	23.6	23.5	23.6	23.6
C-16	21.9	21.8	21.7	21.6	21.5	21.6
C-17	54.6	54.-	54.7	54.6	54.7	54.8
C-18	13.5	13.3	61.6	61.6	61.9	61.9
C-19	17.3	15.2	17.2	15.5	18.8	22.7
C-20	75.1	74.9	74.4	74.5	74.6	74.7
C-21	20.7	20.6	21.1	21.1	21.1	21.2
C-22	80.9	80.7	80.7	80.7	80.8	80.9
C-23	31.1	31.1	31.0	31.1	30.7	31.5
C-24	148.9	149.0	148.5	148.6	148.6	148.7
C-25	122.0	121.7	121.9	121.9	121.8	122.0
C-26	166.1	166.1	166.0	166.0	166.0	166.0
C-27	12.5	12.3	12.4	12.3	12.3	12.4
C-28	20.5	20.5	20.3	20.3	20.4	20.5
-*C*OMe			171.0	171.0	171.1	171.1
-CO*Me*			20.2	20.5	20.5	20.5
-O*Me*		56.8		56.8		

H, J = 5.6 Hz), 3.78 (d, H, J = 6.4 Hz), 3.23 (s, H), 3.08 (bs, H), 2.07 (s, 3H), 1.96 (s, 3H), 1.40 (s, 3H); ^{13}C nmr see Table 1; positive cims *m/z* (% rel. int.) $[M+1]^+$ ($C_{30}H_{41}O_8$) 529 (14), 511 (19), 469 (81), 451 (75), 433 (43), 343 (30), 327 (17), 169 (41), 125 (94), 61 (100).

Fraction 4 (single spot on tlc) proved to be 18-acetoxy-3-methoxy-2,3-dihydrowithanolide D [**4**]: $C_{31}H_{44}O_9$; needles from EtOAc; mp 242–245°; $[\alpha]_D$ +13.2° (c = 7.87, $CHCl_3$); uv max 222 nm (log ϵ 4.02, (EtOH); ir (KBr) 3600, 3450, 3030, 1700, 1200–1000 cm^{-1}; ^{1}H nmr δ 4.35 (dd, H, J = 3.5, 13.7 Hz), 4.21 (dd, H, J = 3.5, 11.4 Hz), 3.75 (s, 3H), 3.55 (m, H), 3.37 (s, 3H), 3.22 (s, 3H), 3.00 (m, H), 2.60 (dd, H, J = 5.5, 13.0 Hz), 2.39 (bm), 2.04 (s, 3H), 1.98 (s, 3H), 1.38 (s, 3H); ^{13}C nmr see Table 1; ms (cims) *m/z* (% rel. int.) 501 (70), 469 (19), 451 (15), 417 (29), 405 (40), 375 (44), 343 (100), 325 (27), 169 (48), 125 (68).

Fraction 5 was identified as withanolide D by its hplc retention time compared to that of the sample described above and by coinjection with an authentic sample.

Fraction 6 (single spot on tlc) was identified as 3-methoxy-2,3-dihydrowithanolide D [**2**], $C_{29}H_{42}O_7$, needles from EtOAc, mp 222–228°; $[\alpha]_D$ −119.3° (c = 2.8, $CHCl_3$); uv max 226 nm (log ϵ 3.98, EtOH); ir (KBr) 3600, 3450, 3030, 1700 (broad), 1200–1000 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 4.21 (dd, H, J = 4, 13 Hz), 3.70 (m, H), 3.36 (s, H), 3.23 (s, H), 2.95 (dd, H, J = 6, 13 Hz), 2.63 (dd, H, J = 3.6, 15 Hz), 2.45 broad t, H, J = 15 Hz), 1.96 (s, 3H), 1.88 (s, 3H), 1.28 (s, 3H), 1.25 (s, 3H), 0.82 (s, 3H); ^{13}C nmr see Table 1; eims *m/z* (rel. int.) 485 (8), 377 (19), 359 (59), 341 (39), 327 (26), 299 (22), 273 (33), 168 (22), 126 (100), 125 (86).

Colombian collection.—The milled leaf and stem (6.8 kg) were extracted by the second procedure described for the Ecuadorian material to give a similar crude withanolide fraction which was used for direct injection onto a Waters 6000 Multisolvent Delivery System using either a manual Waters U6K or an automated Wisp Model 712 sample injector. The detector was a Waters Lambda Max Model 481 LDC variable wavelength spectrophotometer. Data were recorded on an LDC Milton-Roy CI-10 integrator using a Dynamax Macro (21.4 × 250 mm) column and 50% aqueous MeOH as initial mobile phase at 15 ml/min that went to 100% MeOH in 39 min and held there for 9 min. Fourteen injections (125 mg each) resulted in the following products.

Fractions 1–3 were but weakly Dragendorff's positive and were not further investigated.

Fraction 4 (394 mg) showed two spots on tlc which were separated on the same hplc system using a mobile phase of 55% MeOH to give **4** and **2**, identified by direct comparison of ^{1}H-nmr, ^{13}C-nmr, and ms data as given for these compounds above.

Fraction 5 was shown to be **1** by comparison of its hplc retention time with that described above and by coinjection with an authentic sample.

Fraction 6 was separated using the same hplc system and 70% MeOH as an isocratic mobile phase and a flow rate of 15 ml/min to give 94 mg of 18-acetoxy-4-deoxy-5,6-deoxy-5-withenolide D [**5**]: $C_{30}H_{40}O_6$; needles from MeOH; mp 132–135°; uv max 223 nm (log ϵ 4.09, $CHCl_3$); ir (KBr) 3600, 3450, 3030, 1700, 1200–1000 cm^{-1}; ^{1}H nmr δ ($CDCl_3$) 6.82 (dd, H, J = 5.6, 10.5 Hz), 5.88 (dd, H, J = 6.5 Hz), 4.25 (dd, H, J = 3.5, 12.3 Hz), 4.19 (d, H, J = 5.6 Hz), 3.35 (broad s, H), 3.27 (broad s, H), 2.88 (d, H, J = 5.8 Hz), 2.18 (d, H, J = 5.8 Hz), 209 (s, 3H), 1.98 (s, 3H), 1.90 (s, 3H), 1.43 (s, 3H), 1.23 (s, 3H); ^{13}C nmr see Table 1; eims (% rel. int.) $[M]^+$ 493 (trace), major fragments at 311 (6), 169 (5), 125 (15), 43 (100).

Again in an attempt to clean up the crude withenolide fraction prior to hplc, 12.5 g were subjected to fcc on SiO_2 using hexane, hexane-CH_2Cl_2 (1:1), CH_2Cl_2 with 5, 10, 20, and 50% iPrOH, and finally MeOH. A total of 120 fractions (125 ml each) were collected. Only fractions 64–66 (265 mg) were demonstrably Dragendorff's positive and showed the presence of three compounds by tlc and analytical hplc. These were separated by preparative hplc on a 21.7 × 250 mm ODS column of 8 μ particle size (Rainin). The mobile phase was a gradient of 50–65% MeOH over 23 min followed by a step up to 80% MeOH during 2 min and held for 8 min. Injection loads varied from 20 to 60 mg.

Fractions 1 (80 mg) and 2 (150 mg) were shown to be **3** and **4**, respectively, by ^{1}H- and ^{13}C-nmr spectra identical to those established previously for these compounds.

Fraction 3 (88 mg), recrystallized from EtOAc, was identified as 18-acetoxy-5,6-deoxy-5-withenolide D [**6**]: $C_{30}H_{40}O_7$; $[\alpha]_D$ +66.3° (c = 4.39, $CHCl_3$); uv max 221 nm (log ϵ 4.29, $CHCl_3$); ir (KBr) 3600, 3450, 3030, 1695, 1200–1000 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 6.78 (dd, H, J = 5.6, 10.5 Hz), 5.90 (d, H, J = 10.5 Hz), 5.87 (s, H), 4.60 (d, H, J = 5.6 Hz), 4.23 (dd, H, J = 3.5, 13.5 Hz), 4.16 (d, 2H, J = 6.8 Hz), 2.03 (s, 3H), 1.93 (s, 3H), 1.87 (s, 3H), 1.40 (s, 3H), 1.38 (s, 3H); ^{13}C nmr see Table 1; eims (% rel. int.) $[M]^+$ 512 (4), major fragments at 494 (4), 452 (7), 434 (10), 387 (7), 327 (100), 309 (18), 169 (22), 125 (18), 43 (63).

METHANOL ADDUCTS.—While using the retention time of withanolide D as a reference for the hplc profiles of the other withanolides, we observed the MeOH solutions of the substance changed with time on standing at room temperature, giving rise to a second peak corresponding to **2**. This suggested that **2**, and

by analogy **4**, were not naturally occurring but resulted from the addition of MeOH to **1** and **3**, respectively, during extraction of the plant. This was established in two ways.

First, a sample of pure withanolide D was refluxed with MeOH for several days, fresh solvent being added from time to time to maintain constant volume. An analytical reversed-phase column, a Waters 6000 pump, a Rheodyne injector, and an LDC/Milton-Roy variable wavelength detector were used to examine the resulting solution, which exhibited two peaks corresponding to a mixture of **1** and **2**. After 36 h reflux, an estimated 65% conversion had taken place.

Second, a small sample of plant material was extracted with Et_2O, in which the withanolides are soluble. Using the same analytical hplc comparison, peaks corresponding to **2** or **4** were not observed in the withanolide fraction thus obtained.

The column used in these experiments was a Beckman C_{18}, 25 cm in length and having a particle size of 5 μ. Injections were 5 μl = 50 μg/injection using 55% MeOH, a solvent flow of 1 ml/min, and a chart speed of 0.5 cm/min.

AMINO ACIDS.—Qualitative tests on the aqueous fraction remaining after the removal of the withanolides by extraction with Et_2O from both plant samples gave evidence for the presence therein of amino acids, tannins, phenols, and carbohydrates. Only the amino acids were further investigated.

Small portions of the residues after removal of the H_2O were partially dissolved in absolute MeOH and filtered from nondescript solids, and the filtrate was evaporated to yield ninhydrin-positive material. Tlc [SiO_2, *n*-BuOH–HOAc–H_2O (2:1:1)] exhibited at least six positive spots indicative of the presence of amino acids. By preparative tlc using *n*-BuOH–HOAc–H_2O (3:1:1), these were isolated as a group, converted to their phenylisothiocyanate derivatives, and subjected to hplc by published procedures (7–9). A typical result for both Ecuadorian and Colombian samples established the following identifications: serine, threonine, alanine, proline, NH_4^+, tyrosine, valine, isoleucine, and leucine. Several minor peaks were observed; their identification was not pursued.

ACKNOWLEDGMENTS

We are indebted to Mrs. Luis W. Levy of Quito, Ecuador, for the collection of the plant material from that area and to Mr. Pedro Juajibioy of Sibundoy, Colombia, for the collection of the Colombian sample. We thank Dr. D. Lavie, Weizmann Institute, Rehovot, Israel, for an authentic sample of withanolide D, Dr. David Forsyth of the Department of Chemistry, Northeastern University, for helpful discussions on the ^{13}C-nmr aspects of the problem, and Dr. Catherine Costello, Massachusetts Institute of Technology, for the high resolution mass spectrum of withanolide D.

LITERATURE CITED

1. R.E. Schultes, *J. Psychedelic Drugs*, **9**, 45 (1977).
2. C. Baehni, *Candollea*, **10**, 882 (1946).
3. A. Hunziker, in: "Biology and Taxonomy of the Solanaceae." Ed. by J.G. Hawkes, R.N. Lester, and A.D. Skelding, Academic Press, New York, 1979, pp. 49–85.
4. D. Lavie, I. Kirson, and E. Glotter, *Isr. J. Chem.*, **6**, 671 (1969).
5. A. Yarden and D. Lavie, *J. Chem. Soc.*, 2925 (1962).
6. S.M. Kupchan, W.K. Anderson, P. Bolliger, R.W. Doskotch, R.W. Smith, J.A. Saens Renauld, N.K. Schnoes, A.L. Burlingame, and D.H. Smith, *J. Org. Chem.*, **34**, 3858 (1969).
7. S. Cohen, T. Tarvin, and B. Bidlingmeye, *Am. Lab.*, **16**, 48 (1984).
8. D.R. Koop, E.T. Morgan, G.E. Tarr, and M.J. Coon, *J. Biol. Chem.*, **25**, 8472 (1982).
9. J.A. Saunders, J.M. Saunders, S. Morris, and S.A. Wynne II, *Chromatogram*, **9**, 2 (1988).
10. G.J. Bukovits and E. Gross, *An. Asoc. Quim. Argent.*, **69**, 15 (1981).
11. J. Rosazza, *Lloydia*, **41**, 297 (1978).
12. M.D. Anton, D. Abramson, R.L. Tyson, C. Chang, J.L. McLaughlin, G. Peck, and J.M. Cassaday, *J. Nat. Prod.*, **44**, 579 (1981).
13. R.B. Bates and D.J. Eckert, *J. Am. Chem. Soc.*, **94**, 8528 (1972).
14. M.J. Begley, L. Crombie, P.J. Ham, and D.A. Whiting, *J. Chem. Soc., Perkin Trans. 1*, 296 (1976).
15. K.R.S. Ascher, M. Eliyahu, E. Glotter, I. Kerson, and A. Abraham, *Phytoparasitica*, **12**, 147 (1984).
16. K.R.S. Ascher and E. Glotter, in: "Proceedings." 6th International Congress on Pesticide Chemistry, Ottawa, 1986.

Received 22 April 1991