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IOCHROMOLIDE: A NEW ACETYLATED WITHANOLIDE FROM *IOCHROMA COCCINEUM*

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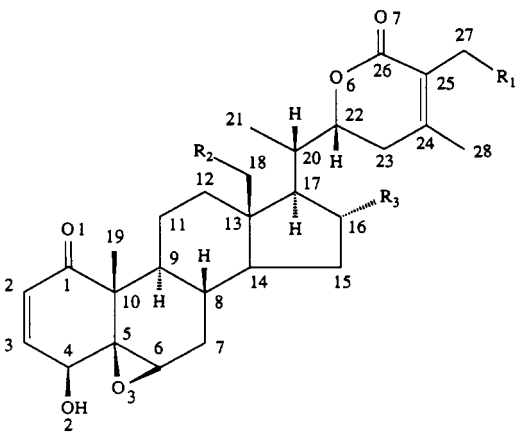
ABSTRACT.—The phytochemical investigation of *Iochroma coccineum* (Solanaceae) afforded three withanolides: withaferine A [**1**], withacnistine [**2**], and a new compound, the (17*R*,20*S*,22*R*)-4β-hydroxy-1-oxo-5β,6β-epoxy-16α-acetoxywitha-2,24-dienolide, which we called iochromolide [**3**]. The structure of **3** was deduced on the basis of nmr and mass spectral comparison with **1** and **2** and definitively established by X-ray crystallography.

Withanolides are natural steroidal lactones produced as far as known only by plants in the Solanaceae (1,2). They can be classified into four main groups (1): 20-deoxywithanolides (withaferine A and related compounds), 20-hydroxywithanolides (e.g., withanolide D), withanolides with the C-17 side chain α-oriented (like withanolide E), and withanolides whose basic framework is modified or chlorinated (physalins, withaphysalins, physalolactones, etc.). It has been observed that one chemotype or one given species generally produces withanolides of one of these types only. The biological and pharmacological properties of withanolides are of great interest. Although only a few of them have been tested, we know that they may exhibit antitumor, antifeedant, immunomodulating, and anti-inflammatory activities (1,2). During our investigation of *Iochroma coccineum* Scheidweiler (3) we isolated three substances belonging to the 20-deoxywithanolide class. The chemistry of the genus *Iochroma* Benth. (4) has, to our knowledge, not been studied before, except for the recent isolation of three hydroxycinnamic acid amides from *Iochroma cyaneum* (5).

RESULTS AND DISCUSSION

The defatted H_2O/MeOH extract of the leaves of *I. coccineum* furnished, after extrac-



1 $R_1 = OH$, $R_2 = R_3 = H$
2 $R_1 = R_3 = H$, $R_2 = OAc$
3 $R_1 = R_2 = H$, $R_3 = OAc$

tion with Et_2O and separation by various chromatographic steps, three withanolides (see Experimental).

The ^1H- and ^{13}C-nmr spectra (Tables 1 and 2), the mass spectra, the uv absorption maxima, and the melting points allowed us to identify two of them as withaferine A [**1**] and withacnistine [**2**] by comparison with earlier reported data (6–10).

The uv maximum of the third isolated compound **3**, taken in MeOH, is 216 nm with log ϵ 4.16, which is typical for withanolides, since both unsaturated lactones and enone groups (ring A) exhibit absorption maxima between 215 and 230 nm with high molar extinction coefficients (11,12).

The mass spectrum of **3** showed a molecular peak at *m/z* 512. The ion at *m/z* 452, corresponding to the loss of HOAc, indicated the presence of an acetate group. The presence of these two peaks in the mass spectrum of **2** let us assume the new compound to be an isomer of **2**. Fission between C-20 and C-22 led to the base peak at *m/z* 125 ($C_7O_2H_9$), suggesting the absence of an OH group on the side chain, since hydroxylated lactones, like **1**, give an intense ion at *m/z* 141, corresponding to $C_7O_3H_9$ (6). The ion corresponding to the loss of the side chain including two carbons of ring D (C-16 and C-17), present in the spectra of **1** (*m/z* 197) and **2** (*m/z* 181), was missing in the spectrum of **3**; this could be an indication for a change of structure occurring at ring D. The major fragmentation ions showed further fissions corresponding to the loss of H_2O and HOAc: in addition to *m/z* 452 we could observe *m/z* 494 (512 − 18), *m/z* 434 (512 − 60 − 18), *m/z* 281 (299 − 18). Other typical ions were present (*m/z* 55, 67, 79, 97, 105, 267, 285, 299), resulting from the cleavage of the steroidal skeleton, as well as the ion *m/z* 124 due to the fission of ring B with possible hydrogen transfer; all these peaks were observed in the mass spectra of the three isolated withanolides.

TABLE 1. ^1H-nmr Spectral Data of Relevant Protons of **1–3**.[a]

Proton	Compound		
	1	**2**	**3**
H-2	6.21 (d, 10.1)	6.20 (d, 10.1)	6.21 (d, 10.1)
H-3	6.94 (dd, 6.1, 10.1)	6.94 (dd, 5.8, 10.1)	6.95 (dd, 5.8, 10.1)
H-4	3.77 (d, 6.1)	3.77 (d, 5.8)	3.77 (d, 5.8)
H-6	3.24 (br s)	3.25 (br s)	3.24 (br s)
H-7b	2.16 (dt, 3.2, 5.0, 14.9)	2.16 (dt, 2.9, 4.8, 14.9)	[b]
H-16	—	—	4.90 (t, 7.2, 9.0)
H-18	0.72 (s)	3.83 (d, 11.9)[c] 4.21 (d, 11.9)	0.76 (s)
H-19	1.42 (s)	1.41 (s)	1.41 (s)
H-21	0.99 (d, 6.6)	1.12 (d, 6.5)	1.02 (d, 6.5)
H-22	4.43 (dt, 3.2, 4.3, 13.3)	4.38 (dt, 3.2, 4.3, 13.3)	4.17 (dt, 3.2, 4.3, 13.1)
H-23b	2.50 (dd, 13.3, 18.0)	2.45 (br t)	2.42 (br t)
H-27	4.35 (d, 13.0)[c] 4.39 (d)[d]	1.88 (s)	1.88 (s)
H-28	2.04 (s)	1.94 (s)[e]	1.93 (s)[e]
H-30[f]	—	2.08 (s)[e]	1.96 (s)[e]

[a]Chemical shifts are reported in ppm, signal multiplicities and coupling constants (Hz) are shown in parentheses.

[b] Signal overlapped.

[c]AB system.

[d]J not measurable, the signal being overlapped by that of H-22.

[e]Signals within a vertical column may be interchanged.

[f]Protons from -OAc.

TABLE 2. ^{13}C-nmr Spectral Data of **1–3**.[a]

Carbon	Compound		
	1	**2**	**3**
C-1	202.3 (s)	202.3 (s)	202.2 (s)
C-2	132.3 (d)	132.1 (d)	132.1 (d)
C-3	142.2 (d)	142.1 (d)	142.1 (d)
C-4	69.9 (d)	69.8 (d)	69.8 (d)
C-5	63.9 (s)	63.8 (s)	63.8 (s)
C-6	62.3 (d)	62.6 (d)	62.6 (d)
C-7	31.3 (t)	31.4 (t)	31.1 (t)
C-8	29.8 (d)	30.0 (d)	29.2 (d)
C-9	44.2 (d)	44.3 (d)	44.1 (d)
C-10	47.8 (s)	47.7 (s)	47.6 (s)
C-11	22.1 (t)	22.3 (t)	22.0 (t)
C-12	39.4 (t)	34.6 (t)	39.5 (t)
C-13	42.6 (s)	45.5 (s)	43.4 (s)
C-14	56.1 (d)	55.6 (d)	57.5 (d)
C-15	24.3 (t)	24.1 (t)	34.6 (t)
C-16	27.3 (t)	27.0 (t)	79.5 (d)
C-17	52.1 (d)	52.6 (d)	53.6 (d)
C-18	11.6 (q)	61.9 (t)	13.0 (q)
C-19	17.3 (q)	17.6 (q)	17.5 (q)
C-20	38.9 (d)	39.1 (d)	37.6 (d)
C-21	13.3 (q)	13.7 (q)	13.5 (q)
C-22	78.8 (d)	78.1 (d)	78.2 (d)
C-23	29.9 (t)	29.4 (t)	29.9 (t)
C-24	153.0 (s)	148.8 (s)	148.4 (s)
C-25	125.8 (s)	122.2 (s)	122.4 (s)
C-26	167.0 (s)	167.0 (s)	166.7 (s)
C-27	57.4 (t)	12.5 (q)	12.5 (q)
C-28	20.0 (q)	20.5 (q)[b]	20.4 (q)[b]
-O-*C*O-CH$_3$	—	171.1 (s)	170.1 (s)
-O-CO-*C*H$_3$	—	21.0 (q)[b]	21.1 (q)[b]

[a]Chemical shifts are reported in ppm; signal multiplicities are shown in parentheses.

[b]Signals within a vertical column may be interchanged.

^{13}C-nmr data of **3** (Table 2) were quite similar to those of **2**: both contained signals for 30 carbons, but the DEPT analysis indicated the presence of one CH and one Me more and two CH_2 less for **3** than for **2**. The triplet assigned to C-18 in **2** disappeared, but one additional quartet was observed at 13.0 ppm which corresponds to the signal for C-18 (Me) of **1**. On the other hand, the triplets at about δ24 and δ 27, due to C-15 and C-16 and present in the spectra of **1** and **2**, were lacking, but the spectrum of **3** showed another triplet at δ 34.6 and a further doublet at δ 79.5. The environment of C-15 and C-16, i.e., C-13, C-14, and C-17, underwent small changes in chemical shifts. The signals for all the other carbons showed similar multiplicities and shifts. Therefore, we expected the third withanolide to be isomeric to **2**, with an acetoxyl group on C-15 or C-16 and with C-18 being a methyl.

The ^{1}H-nmr spectrum of **3** (Table 1) also showed strong similarities with those of **1** and **2** but without the AB system (H-18) of **2**; six methyl groups were present, giving five singlets corresponding to Me-18 (acetoxylated in **2**), Me-19, Me-27 (hydroxylated in **1**), Me-28 and Me-30 (Me from acetoxyl), and one doublet for Me-21, as well as one hydroxyl group (δ 2.59) which proton was exchangeable with D_2O. The spectrum was

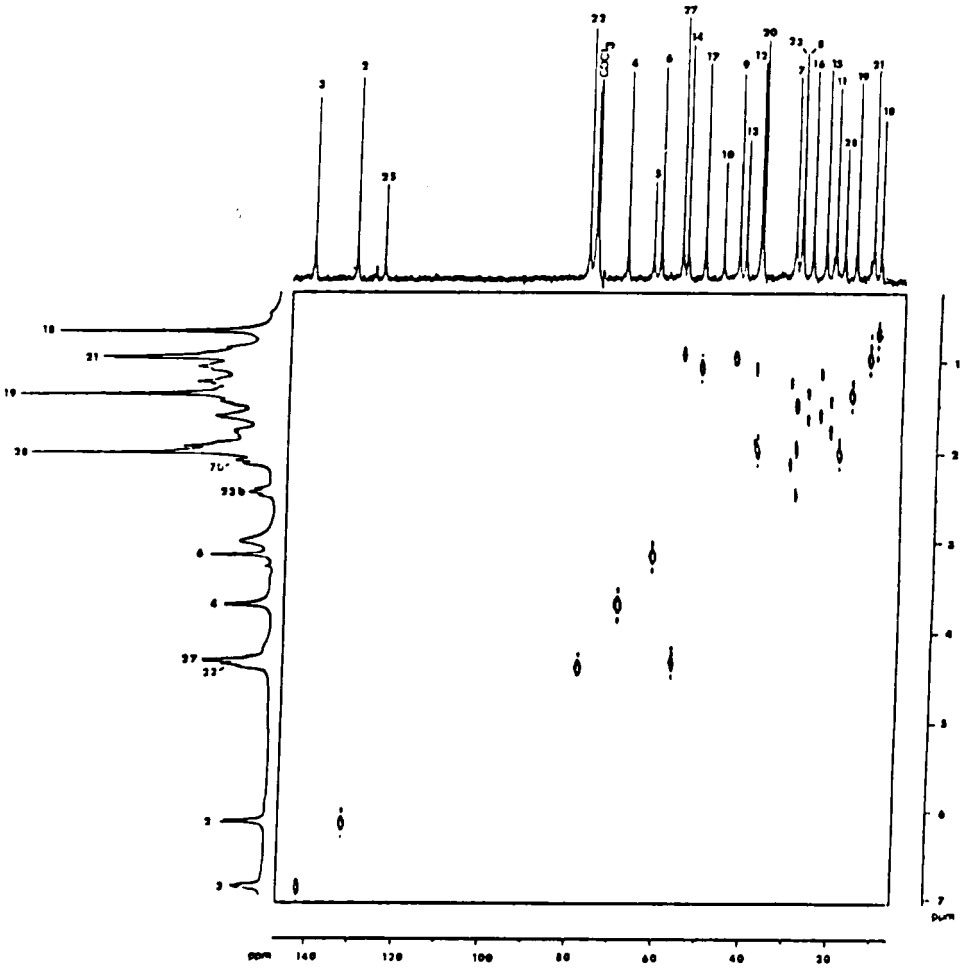


FIGURE 1. 2D ^{1}H-^{13}C heteronuclear shift correlation of iochromolide [**3**].

in good agreement with the presumed structure but did not indicate whether the acetoxyl group was on C-15 or C-16. Figure 1 shows the ^{1}H-^{13}C heternuclear shift correlation spectrum of **3**.

The definitive structure of **3** was determined by X-ray crystallography (Figure 2), which confirmed the above assumptions and clearly indicated that the acetoxyl was attached at C-16 and α-oriented. This compound constitutes the first known example of a 16-acetoxywithanolide. No essential differences were observed between the two independent molecules of the asymmetric unit. The relative stereochemistry being fixed by the X-ray structure determination, the absolute configuration of the molecule has been

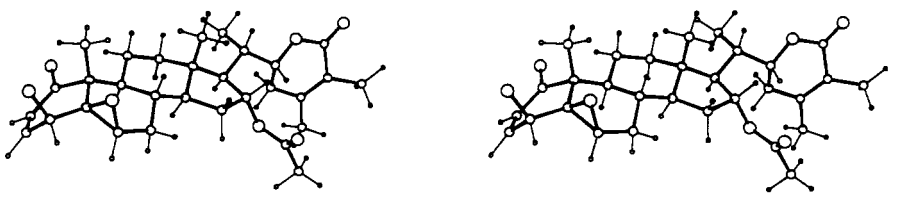


FIGURE 2. Stereoscopic view of iochromolide [**3**].

assumed as equivalent to those of withaferin A derivatives (13). An analysis of the ring conformations of the steroid is reported in Table 3. The ring fusions (A/B cis; B/C trans; C/D trans; 9,10 anti; 8,14 anti) and geometrical parameters of both molecules are close to those observed for withaferin A. The molecular packing is fixed by hydrogen bonds involving the 4β-hydroxyl and the 16α-acetoxyl groups: O(5) . . . $(02)_{x+1,y+1,z-1}$ = 2.787 (13) Å, O(2) . . . $O(05)_{x,y+1,z-1}$ = 2,800 (13) Å.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were measured on a Tottoli apparatus and are uncorrected. Uv spectra were determined on a Lambda 3 uv/vis spectrophotometer (Perkin-Elmer). ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AMX 360 at 360.13 and 90.53 MHz, respectively, in $CDCl_3$ at 23°, using TMS as internal standard (δ). Eims were determined at 70 eV on a VG 7070E spectrometer. Analytical and semi-preparative hplc were performed on a Waters 600 E apparatus, using respectively a Waters 990 Photodiode Array Detector and a Waters 484 Tunable Absorbance Detector.

PLANT MATERIAL.—Seeds of *I. coccineum* (3) were provided by the Jardim Botanico da Universidade de Lisboa, Portugal. Plants were grown at the Station Fédérale de Recherches Agronomiques, Centre des Fougères, in Conthey, Wallis, Switzerland. A voucher specimen is deposited in our laboratory. Leaves were dried at 40°.

EXTRACTION AND ISOLATION OF **1**, **2**, AND **3**.—Powdered dried leaves of *I. coccineum* (100 g) were extracted with MeOH 80% at 40° for 15 h. After filtration and evaporation, the residue was dissolved in

TABLE 3. Ring-Puckering Parameters (Q_2, Q_3, Q_T, φ_2, θ_2) according to Cremer and Pople (18) and Minimum Values of Asymmetry Parameters (ΔC_s, ΔC_2) according to Nardelli (19) for Iochromolide [**3**].

	Mol. 1	Mol. 2
Ring A	(C-1–C-2–C-3–C-4–C-5–C-10)	
Conformation	twist-boat	twist-boat
Q_2	0.4613	0.3918
Q_3	0.1671	0.1686
Q_T	0.4907	0.4265
φ_2	−148.2	−156.5
Θ_2	70.1	66.7
ΔC_2	C-1–C-2: 0.006 (7)	C-1–C-2: 0.023 (6)
Ring B	(C-5–C-6–C-7–C-8–C-9–C-10)	
Conformation	half-chair	half-chair
Q_2	0.3753	0.3690
Q_3	0.3541	0.2963
Q_T	0.5160	0.4732
φ_2	−165.8	−166.0
Θ_2	46.7	51.2
ΔC_2	C-5–C-6: 0.057 (5)	C-5–C-6: 0.057 (5)
Ring C	(C-8–C-9–C-11–C-12–C-13–C-14)	
Conformation	chair	chair
Q_2	0.0668	0.0932
Q_3	0.5641	0.5508
Q_T	0.5681	0.5587
φ_2	−108.1	−107.8
Θ_2	6.8	9.6
ΔC_2	C-9–C-11: 0.013 (5)	C-9–C-11: 0.017 (5)
Ring D	(C-13–C-14–C-15–C-16–C-17)	
Conformation	envelope	envelope
Q_2	0.4410	0.4401
φ_2	−173.5	−174.94
ΔC_s	C-13: 0.043 (6)	C-13: 0.033 (6)

MeOH-CCl_4-H_2O (57:20:18); after separation from the CCl_4 layer, the H_2O/MeOH layer was further extracted with CCl_4 and, after elimination of MeOH, with Et_2O. The Et_2O phase gave, after evaporation, 700 mg of residue which was chromatographed over a Sephadex LH-20 (Pharmacia) column (2 × 60 cm) with a gradient of MeOH in H_2O (0 to 100% of MeOH, 10% increments). The fractions corresponding to 20 to 60% of MeOH were collected, the residue obtained after evaporation of the solvent was applied on a Lobar Lichroprep Si 60 column (A size, Merck), and the elution was performed with hexane/$CHCl_3$ (gradient from 20 to 100% of $CHCl_3$, 5% increments). An impure mixture (90 mg) of **2** and **3** was obtained with 25 to 40% of $CHCl_3$, and **1** (27 mg) was eluted with 50 to 55% of $CHCl_3$. Compounds **2** and **3** were isolated by semi-preparative hplc [μ Bondapak C18, 19 × 150 mm, MeCN-H_2O (40:60), 10 ml·min^{-1}, detector at 215 nm]. Compound **3** was crystallized from EtOAc and gave white needles. Compound **1** was further purified by crystallization from MeOH/H_2O and Me_2CO/hexane.

Fractions were monitored by analytical hplc [μ Bondapak C18, 3.9 × 30 mm, MeCN-H_2O (40:60), 1.3 ml·min^{-1}, detection at 215 and 227 nm] and by tlc. The methods used for tlc were: (a) (original method) *i*-BuCOMe–HexOH–hexane-HOAc (30:30:40:1) saturated with H_2O, on Si gel 60 F_{254} plates (Merck), **1** R_f 0.27, **2** R_f 0.47, **3** R_f 0.52, and (b) MeOH-H_2O (70:30), on RP 18 F_{254}s plates (Merck), **1** R_f 0.28, **2** R_f 0.23, **3** R_f 0.23. Compounds **1**, **2**, and **3** were positive with Dragendorff's reagent and also gave positive reactions for epoxide (14).

CRYSTALLOGRAPHIC DATA AND STRUCTURE REFINEMENT OF IOCHROMOLIDE [**3**].—$C_{30}H_{40}O_7$/$C_4H_8O_2$, mol wt = 512./88, crystallized in the triclinic system, space group *P1*, with two independent molecules in the asymmetric unit and one molecule of EtOAc used as solvent of crystallization; a = 9.435 (2), b = 13.603 (3), c = 14.468 (4) Å, α = 65.26 (2), β = 70.98 (2), γ = 69.67 (2)°, Z = 2, d_c = 1.20 g·cm^{-3}, μ = 0.079 mm^{-1}, Fooo = 600. The diffracted intensities were measured at room temperature on a Nonius CAD4 diffractometer with graphite monochromated MoKα radiation. Data collection: sin θ/λ < 0.53; h = −9, 9; k = −14, 14; l = 0, 15, ω/2θ scans, no absorption correction. Of the 3844 measured reflections, 3086 were considered as observed [|Fo| > 4σ(Fo)] and used in structure refinement. The structure was solved by direct methods (MULTAN-87) (15) and refined by full-matrix least-squares analysis. Atomic scattering factors and anomalous-dispersion terms are taken from *International Tables for X-ray Crystallography* (16). All coordinates of the H atoms were calculated. The hydrogen atom of the hydroxyl group has not been observed. The EtOAc molecule is disordered, and seven atomic sites have been observed and refined with fixed values of population parameters (0.5) and isotropic displacement parameters (U = 0.05 Å^2). The maximum and minimum residual electron densities in the final ΔF synthesis were 0.59 and −0.35 e Å^{-3}, respectively. The final R factor was R = 0.087 [ωR = 0.062, w = 1/σ^2(Fo)]. All calculations were performed with the XTAL-3.0 (17) program.[1]

Withaferine A [**1**].—Mp after crystallization from MeOH/H_2O 230–235°, from Me_2CO/hexane 247–248°; eims *m/z* (%) $[M]^+$ 470 (11), $[M-H_2O]^+$ 452 (6), $[452-H_2O]^+$ 434 (4), $[434-H_2O]^+$ 416 (3), 387 (13), 347 (24), 329 (5), 311 (5), 285 (22), 267 (20), 241 (34), 197 (21), 141 (81), 124 (100), 123 (58), 105 (35), 95 (89), 79 (31), 67 (58), 55 (58); uv λ max (MeOH) 214 and 333 nm; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Withacnistine [**2**].—Mp after crystallization from C_6H_6 136–142°; eims *m/z* (%) $[M]^+$ 512 (3), $[M-H_2O]^+$ 494 (3), 466 (3), $[M-HOAc]^+$ 452 (4), 434 (6), 406 (7), 389 (11), 329 (4), 311 (5), 285 (8), 181 (10), 157 (10), 145 (12), 154 (11), 125 (100), 124 (20), 105 (12), 97 (21), 79 (10), 67 (15), 55 (12); uv λ max (EtOH 95%) 216 and 330 nm; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Iochromolide [**3**].—Mp after crystallization from EtOAc 146–154°; eims *m/z* (%) $[M]^+$ 512 (0.7), $[M-H_2O]^+$ 494 (0.6), $[M-HOAc]^+$ 452 (2), 434 (6), 419 (2), 329 (2), 299 (7), 285 (5), 281 (7), 267 (2), 175 (9), 152 (12), 125 (100), 124 (12), 105 (9), 97 (18), 79 (9), 67 (11), 55 (10); uv λ max (MeOH) 216 and 330 nm; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

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[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

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