Iodinated Indole Alkaloids From *Plakortis simplex* − New Plakohypaphorines and an Evaluation of Their Antihistamine Activity

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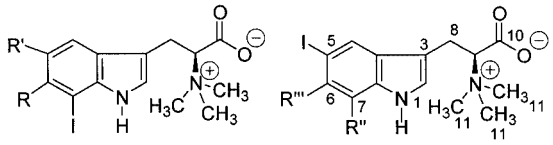
Three new iodinated tryptophan derivatives, plakohypaphorines D–F (**4**–**6**), have been isolated from the Caribbean sponge *Plakortis simplex*. Their structures have been determined by applying spectroscopic methods and microwave-assisted selective dehalogenation. Compound **5** is the first naturally occurring triiodinated indole, while compound **6** is a unique metabolite because it possesses both chlorine and iodine atoms on the indole nucleus. We have evaluated the antihistamine activity of the whole series of plakohypaphorines A–F, but only the diiodinated analogues proved to be active: they display a specific antagonism of the noncompetitive type.

Introduction

Halogen-containing secondary metabolites are particularly abundant in nature, especially in the marine environment; at least 4000 naturally occurring organohalides have been reported to date.[1] Among them, chlorinated or brominated derivatives are predominant, while fluorinated or iodinated molecules are rare.[1] The very few examples of iodine-containing natural products can be grouped into five structural classes: 1) Volatile compounds having very short carbon frameworks (e.g., iodomethane[2]); 2) nucleoside derivatives;[3] 3) tyrosine derivatives (human thyroid hormones and secondary metabolites such as the simple alkaloids dakaramine[4] and turbotoxin,[5] or the more-complex depsipeptide geodiamolide[6]); 4) fatty acid derivatives;[7] and 5) terpene derivatives (e.g., the recently reported tasihalides[8]).

During the last few years, our research group has been working on a detailed analysis of the secondary metabolite composition of *P. simplex*, and we have isolated a series of unusual cycloperoxide-containing compounds (the antimalarial plakortin[9,10] and its congeners[11]), several different oxygenated polyketides (plakortethers,[12] plakortones,[13] and furano esters[9]), alkaloids,[14] and immunomodulating glycolipids[15]. Recently, chemical investigation of the polar fractions obtained from the organic extract of this sponge afforded three simple tryptophan-based betaines, named plakohypaphorines A−C (**1**−**3**),[16] which are unique because they are the first examples of natural iodoindole molecules. To gain additional amounts of these metabolites for pharmacological testing, we conducted chemical screening of a new specimen of *P. simplex*. As expected, the polar fractions contained **1**−**3**, but, remarkably, three additional iodinated indole derivatives, plakohypaphorines D−F (**4**−**6**), also were isolated. In this paper, we describe the isolation and structural elucidation of these three new iodinated compounds and the results of an evaluation of the anti-histamine activity of the whole series of plakohypaphorines.



1 R = R' = H
2 R = I R' = H
3 R = H R' = I

4 R''' = I R'' = H
5 R''' = R''= I
6 R''' = H R'' = Cl

Results and Discussion

A specimen of *P. simplex* collected in the Caribbean Sea in the summer of 2002 was homogenized and exhaustively extracted with methanol and then with chloroform. The methanolic layer was partitioned between *n*BuOH and water and the organic phase, combined with the chloroform extract, was subjected to chromatography over a column packed with reverse-phase silica gel (RP18) and eluted with a solvent gradient from H_2O/MeOH (9:1) to MeOH. Frac-

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tions eluted with two of these eluents (H_2O/MeOH, 6:4 and 4:6) were pooled and fractionated by MPLC over silica gel using an eluent system of increasing polarity (from EtOAc to MeOH). As indicated by a preliminary spectroscopic analysis, the methanolic tails were rich in aromatic betaines; therefore, these fractions were purified further by RP18 HPLC (eluent: MeOH/H_2O, 6:4), to provide plakohypaphorines A−F (**1**−**6**) in their pure states.

The elemental composition of plakohypaphorine D (**4**) was determined by HR-electrospray mass spectrometry (ESIMS, positive mode) to be $C_{14}H_{16}I_2N_2O_2$. The ^{1}H NMR spectrum ([D_6]DMSO) of **4** (Table 1) has only a few resonances: it displays a D_2O-exchangeable signal at δ = 11.08 ppm, three singlets in the aromatic region (δ = 8.24, 7.96, and 7.20 ppm), a singlet integrating for nine protons at δ = 3.20 ppm, and three multiplets (one proton each) at δ = 3.66, 3.18 and 3.13 ppm, which a COSY spectrum allowed us to assign to a CH_2CH moiety. The ^{13}C NMR spectrum of **4** ([D_6]DMSO, Table 2) contains 12 resonances: three in the sp^3 region, eight in the aromatic region (between δ = 94.0 and 138.0 ppm), and a signal at δ = 168.7 ppm that we attribute to a carboxylate function.

Table 1. ^{1}H NMR spectroscopic data (500 MHz) of plakohypaphorines D−F (**4**−**6**)[a]

Position	**4** δ_H (mult., int.)	**5** δ_H (mult., int.)	**6** δ_H (mult., int.)
1-NH	11.08 (s, 1 H)	10.87 (s, 1 H)	11.50 (s, 1 H)
2	7.20 (s, 1 H)	7.20 (s, 1 H)	7.20 (s, 1 H)
4	8.24 (s, 1 H)	8.13 (s, 1 H)	7.98 (s, 1 H)
6			7.42 (s, 1 H)
7	7.96 (s, 1 H)		
8a	3.18 (m, 1 H)	3.18 (m, 1 H)	3.18 (m, 1 H)
8b	3.13 (m, 1 H)	3.13 (m, 1 H)	3.13 (m, 1 H)
9	3.66 (dd,[b] 1 H)	3.65 (dd,[b] 1 H)	3.68 (dd,[b] 1 H)
$N(CH_3)_3$	3.20 (s, 9 H)	3.20 (s, 9 H)	3.20 (s, 9 H)

[a] Recorded in [D_6]DMSO. [b] J = 10.3, 3.4 Hz.

Table 2. ^{13}C NMR spectroscopic data (125 MHz) of compounds **4**−**6**[a]

Position	**4** δ_C (mult.)	**5** δ_C (mult.)	**6** δ_C (mult.)
2	125.5 (CH)	127.1 (CH)	126.4 (CH)
3	109.8 (C)	110.9 (C)	110.4 (C)
3a	129.8 (C)	129.1 (C)	131.9 (C)
4	128.9 (CH)	129.2 (CH)	126.5 (CH)
5	94.8 (C)	94.6 (C)	80.7 (C)
6	101.2 (C)	111.2 (C)	127.6 (CH)
7	122.0 (CH)	91.6 (C)	116.7 (C)
7a	137.2 (C)	138.9 (C)	132.2 (C)
8	25.0 (CH_2)	23.2 (CH_2)	23.0 (CH_2)
9	78.7 (CH)	78.7 (CH)	78.4 (CH)
10	168.7 (C)	167.9 (C)	170.6 (C)
$N(CH_3)_3$	52.0 (CH_3)	51.2 (CH_3)	51.2 (CH_3)

[a] Recorded in [D_6]DMSO.

By performing an HSQC experiment, we associated the ^{1}H NMR spectroscopic resonances to the signals of the directly attached carbon atoms; consequently, six ^{13}C NMR spectroscopic signals, all resonating in the sp^2 region, were assigned to non-protonated carbon atoms (one being the carboxylate carbon atom). The pattern of the proton and carbon atom resonances we obtained resemble that reported for two diiodoindole derivatives: plakohypaphorines B (**2**) and C (**3**).[16] In particular, the eight aromatic resonances suggest an indole nucleus linked to two strongly shielding iodine atoms (δ_C = 101.2 and 94.8 ppm). Investigation of ^{1}H−^{13}C long-range correlations, through a gradient-selected HMBC experiment, confirmed the hypaphorine nature of compound **4** and contributed to the assignment of all the resonances in the NMR spectra. g-HMBC correlations of 9-H and 8-H_2 with the carboxylate carbon atom, of 9-H with the carbon atom resonances at δ = 52.0 ppm (C-11), and of 8-H_2 with C-3 established the 2-(trimethylamino)propionate nature of the aliphatic side chain and its location at C-3 of the indole nucleus. Because C-2 is a protonated carbon atom [δ_C = 125.5 ppm, δ_H = 7.20 ppm; g-HMBC cross-peaks: 2-H/C-3, 2-H/C-8; ROESY correlation: 2-H/1-H (δ = 11.08 ppm)], it is necessary for the two iodine atoms to be located on ring B. The spatial proximity (evidenced through a ROESY experiment) of the methylene protons, however, at C-8 (δ = 3.13 and 3.18 ppm) with the hydrogen atom resonating as singlet at δ = 8.24 ppm clearly identifies this latter proton as 4-H and, consequently, excludes C-4 as an iodine-substituted position. Thus, taking into account the lack of multiplicity of the aromatic signals, which excludes an *ortho* relationship for the corresponding protons, only a 5,6- or a 5,7-diiodo substitution remains possible. Since 5,7-diiodohypaphorine has been described previously, as plakohypaphorine C (**3**),[16] the structure of plakohypaphorine D (**4**) must be assigned as 5,6-diiodohypaphorine. The key g-HMBC cross-peaks — of 1-H with C-2, C-3, C-3a, C-7, and C-7a, of 4-H with both the iodine-linking C-5, C-6, and C-7a, and of 7-H with C-6, C-5, and C-3a — fully support this structure.

In plakohypaphorine E (**5**), which has the molecular formula $C_{14}H_{15}I_3N_2O_2$, which we assigned through HR-ESIMS analysis (positive mode), a third iodine atom replaces one of the hydrogen atoms of plakohypaphorines B−D (**2**−**4**). The ^{1}H NMR spectrum of **5** ([D_6]DMSO, Table 1) is almost identical to that of **4** (Table 1) except for the lack of a resonance in the aromatic region. Analogously, the ^{13}C NMR spectrum of **5** ([D_6]DMSO, Table 2) closely resembles that of **4**, but, in this case, the resonances of three (instead of two) strongly shielded sp^2-hybridized unprotonated carbon atoms (δ = 111.2, 94.6, and 91.6 ppm) are present; we attribute these signals to the iodine-linking carbon atoms. Through inspection of a ROESY spectrum, we assigned the aromatic proton singlet at δ = 7.20 ppm to 2-H (correlations with 1-H, 8-H_2, and 9-H), while spatial contacts between the proton resonating at δ = 8.13 ppm and both 8-H_2 and 9-H are only compatible with its location at C-4. Thus, plakohypaphorine E (**5**) must be 5,6,7-triiodohypaphorine. A thorough analysis of HSQC and HMBC spectra allowed us to completely assign of all the resonances in the NMR spectra (Table 1 and 2), which, thus, confirms the

proposed structure for plakohypaphorine E (**5**) as that of the first natural triiodinated indole compound.

ESIMS analysis of plakohypaphorine F (**6**) indicated pseudomolecular ion peaks at *m/z* = 407 and 409 $[M + H]^+$ (ratio 3:1), which suggests the presence of a chlorine atom. High-resolution measurement of the lower-mass peak indicated that the molecular formula for **6** is $C_{14}H_{16}ICIN_2O_2$. Analysis of spectroscopic data identified plakohypaphorine F (**6**) as a close analogue of plakohypaphorine C (**3**); a chlorine atom replaces one of the two iodine atoms. The ^1H and ^{13}C NMR spectra (Table 1 and 2, respectively) of **6** are similar to those reported for **3** and, with the assistance of COSY, HSQC, and g-HMBC experiments, we easily identified the H/C signals of the 2-(trimethylamino)propionate moiety. As far as the aromatic region is concerned, the eight signals of sp^2-hybridized carbon atoms are indicative of the presence of an indole nucleus, whose three protons resonate as singlets in the ^1H NMR spectrum. Among them, we attribute the signal at δ = 7.20 ppm to 2-H on the basis of its ROESY correlation with 1-H (δ = 11.50 ppm) and its HMBC cross-peaks with C-3, C-7a, and C-8, while we identified the proton resonating at δ = 7.98 ppm as 4-H on the basis of its spatial proximity with both 8-H_2 and 9-H (ROESY correlations). Therefore, the remaining hydrogen atom, resonating as a singlet at δ = 7.42 ppm, could be attached at either C-6 or C-7. A qualitative evaluation of heteronuclear coupling constants, which can be accomplished through inspection of phase-sensitive HMBC, can be used to discriminate between the two different possible structures.[16] In this case, 4-H shows "large" coupling constants (indicative of $^3J_{C,H}$ in planar systems) with both C-7a and the protonated carbon atom at δ = 127.6 ppm and "small" coupling constants (indicative of $^2J_{C,H}$ or $^4J_{C,H}$ in planar systems) with C-3a, C-5, and C-7. Analogously, the proton resonating at δ = 7.42 ppm shows "large" coupling constants with both C-4 and C-7a and "small" coupling constants with the remaining carbon atoms of indole ring B. As a whole, these data are in full agreement with a 5,7-disubstituted indole nucleus, but, because the two halogen atoms in **6** are different, both 7-chloro-5-iodohypaphorine and 5-chloro-7-iodohypaphorine structures could be proposed. Unfortunately, even HMBC spectra did not provide reliable evidence to allow an unambiguous choice to be made between these two possibilities.

Taking into account, however, that reduction of aromatic halides is much more effective for iodinated derivatives than for chlorinated ones, the relative position of the halogen atoms on **6** were deduced by inspecting the products of the selective dehalogenation. Several different methods have been described to accomplish this conversion, but in our case, because the substrate is a natural product available in very minute amounts (1.5 mg), we needed a reaction that occurs in high yield and has an easy workup. With these characteristics in mind, the dehalogenation reaction proposed recently by Jones et al.[17] appeared to be the best choice avaliable. In this reaction, the halogenated compound is treated with potassium formate and palladium acetate under microwave irradiation. The choice of solvent is crucial: when the reaction is carried out in ethanol, a nonselective reduction of all carbon−halogen bonds is observed, whereas deiodination and debromuration products are obtained selectively and in high yields when using DMSO as the solvent. Thus, microwave irradiation for 25 s of a DMSO solution of plakohypaphorine F (**6**), HCOOK, and $Pd(OAc)_2$ afforded compound **7** (90% yield, Figure 1). The chlorohypaphorine structure of **7** was attested by ESIMS (*m/z* = 281 and 283, ratio 3:1, $[M + H]^+$), while its ^1H NMR spectrum included, in the low-field region, a singlet at δ = 7.28 ppm (attributable to 2-H), two doublets (δ = 7.12 and 7.60 ppm) and a triplet (δ = 7.02 ppm). This multiplicity is exclusively compatible with a 7-chlorohypaphorine and, therefore, the starting molecule must possess a chlorine atom at C-7 and an iodine atom at C-5 (Figure 2). Consequently, compound **6** is defined unambiguously as 7-chloro-5-iodo-*N*,*N*,*N*-trimethyltryptophan betaine. Plakohypaphorine F (**6**) is a unique metabolite because it possesses both chlorine and iodine atoms on its indole nucleus. On the other hand, 7-chloroindole derivatives are well-known; for example, chlorination of tryptophan to 7-

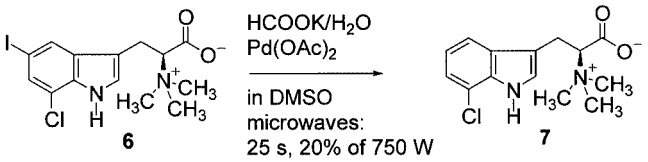

Figure 1. Microwave-assisted deiodination of plakohypaphorine F (**6**)

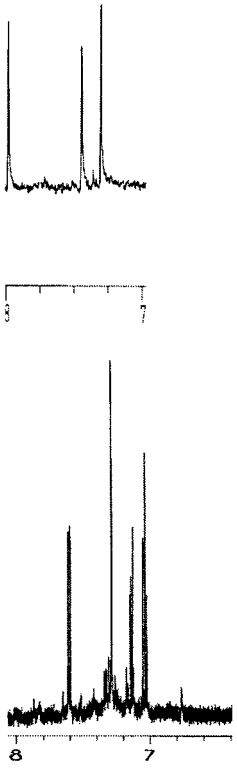

Figure 2. A comparison between the ^1H NMR spectra (low-field regions) of compound **7** (lower) and plakohypaphorine F (upper)

chlorotryptophan is the first step in the biosynthesis of the potent antifungal antibiotic pyrrolnitrin.[18]

It is known that halogenation of the benzene ring on tryptophan derivatives does not affect the sign of optical rotation.[19] Therefore, simply identifying the signs of the $[\alpha]_D$ measurements for plakohypaphorines D−F (**4**−**6**) and comparing them with those detected in the same solvent (CH_3OH/CF_3COOH, 8:1) for plakohypaphorines A−C (**1**−**3**),[16] L-hypaphorine, and L-6-bromohypaphorine[19] allowed us to propose the L (*S*) configuration at C-9 for **4**−**6**.

Recently, conicamin, a simple indole derivative isolated by our group from the tunicate *Aplidium conicum*,[20] has been shown to possess a selective histamine antagonist activity. Stimulated by this finding, we decided to test plakohypaphorines (**1**−**6**) for antihistaminic activity on isolated guinea pig ileum.[21] Plakohypaphorines B (**2**), C (**3**), and D (**4**), at the concentrations of 10^{-6}−10^{-5} M, produced a significant ($p < 0.001$; $n = 5$) concentration-dependent reduction of histamine-induced (10^{-9}−10^{-5} M) contractions (Figure 3). Under the same conditions, plakohypaphorine E (**5**) was much less active and its inhibitory effect showed no concentration dependence (Figure 3), while plakohypaphorines A (**1**) and F (**6**) were completely inactive. Although calculations of the values of pA_2 indicate a noncompetitive antagonistic effect, the histamine antagonism of **2**−**4** is specific because these molecules did not affect acetylcholine- and $BaCl_2$-induced contractions.

The antihistaminic activity of these simple iodinated indole derivatives appears to be connected to the number and nature of the halogen atoms on the aromatic nucleus. Indeed, only the diiodinated analogues proved to be consistently active, regardless the relative position of the halogen atoms. Interestingly, removal of one of the iodine atoms (in **1**), addition of a further iodine atom (in **5**), and substitution

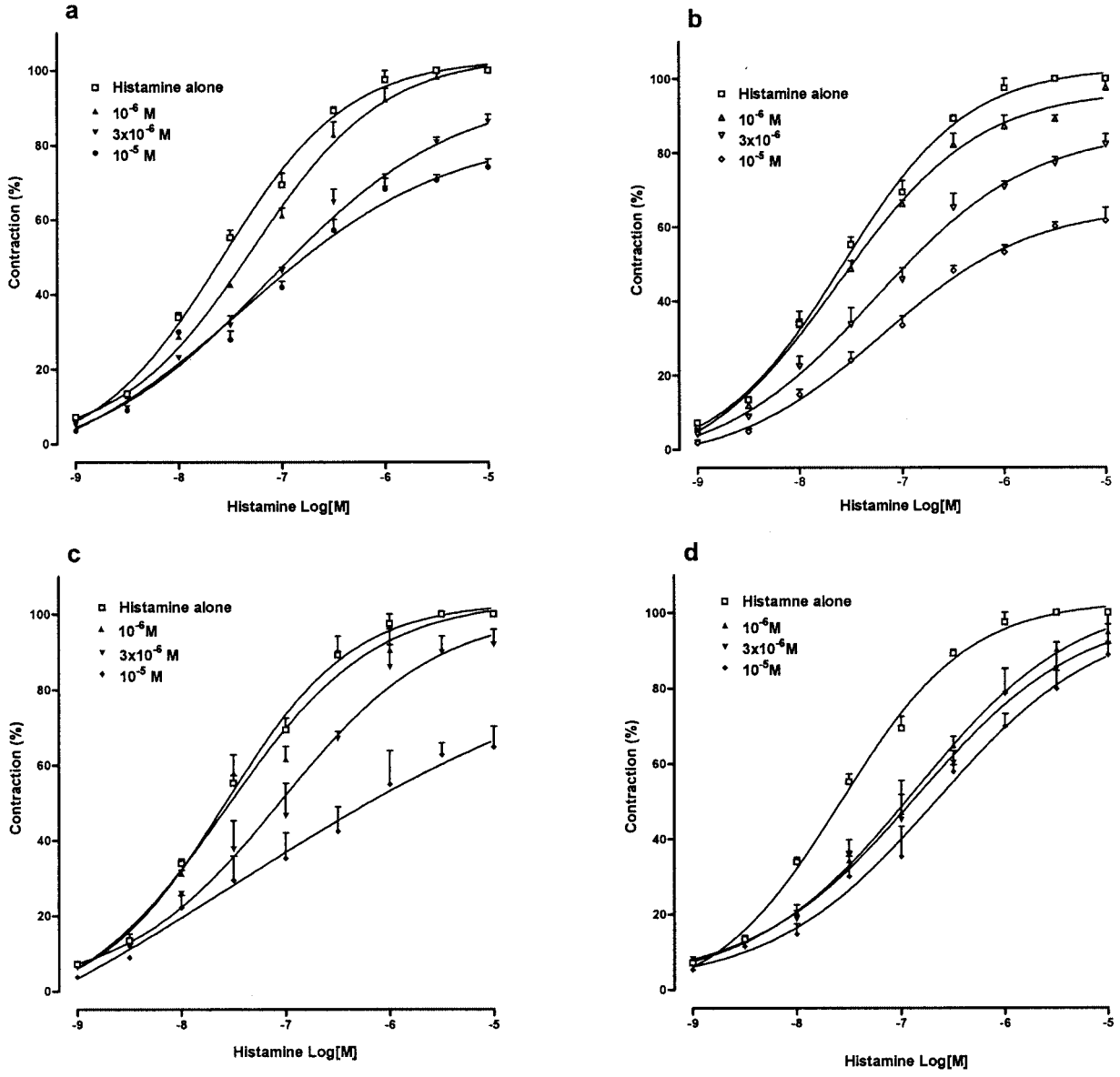

Figure 3. Effects of plakohypaphorine B (**a**), plakohypaphorine C (**b**), plakohypaphorine D (**c**), and plakohypaphorine E (**d**) (10^{-6}−10^{-5} M) on histamine-induced contraction. Each point represents the mean ± S.E.M. obtained from five experiments

of an iodine atom with a chlorine atom (in **6**) cause a dramatic decay in the antihistaminic activity. Interpretation of these results is not simple and requires further experiments.

Conclusion

The isolation of plakohypaphorines A−F (**1**−**6**) from two different specimens of *P. simplex* is particularly remarkable because these metabolites represent the only natural iodoindole derivatives isolated to date. Parallel brominated or chlorinated tryptophan betaines were not detectable in *P. simplex*, which, thus, suggests that the enzyme yielding **1**−**6**, most likely starting from tryptophan, is highly specific for iodine. The distribution of halogen atoms on the indole nucleus of the six plakohypaphorines is also worthy of note. Indeed, apparently, iodine can be attached at positions 5, 6, and 7 with no selectivity, while positions 2 and 4 appear not to be amenable for halogenation. Biosynthetic halogenation of aromatic compounds is still a debated issue and both haloperoxidases (producing hypohalogenic acid as the actual halogenating agent) and NADH-dependent halogenases (transforming the substrate so that the halide ion may be used directly as nucleophile) have been proposed to catalyze this reaction.[22] In this context, plakohypaphorine biosynthesis would be an intriguing subject of investigation for research groups working on enzymatic halogenation.

The finding of a specific antihistaminic activity for plakohypaphorines is also remarkable; questions regarding their physiological role and their real producer (the sponge and/or symbiont microorganism) still remain to be answered.

Experimental Section

General Remarks: Optical rotations were measured in MeOH/CF_3COOH (8:1) using a Perkin−Elmer 192 polarimeter equipped with a sodium lamp (λ = 589 nm) and a 10-cm microcell. IR (KBr) spectra were recorded with a Bruker model IFS-48 spectrophotometer. UV spectra were obtained in MeOH using a Beckman DU70 spectrophotometer. ESI-MS mass spectra were recorded with a Finnigan MAT LCQ mass spectrometer. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured with a Bruker AMX-500 spectrometer; chemical shifts are referenced to the residual solvent signal ([D$_6$]DMSO: δ_H = 2.49 ppm, δ_C = 39.5 ppm). Homonuclear ^1H connectivities were determined by performing COSY experiments. One-bond heteronuclear $^1H-^{13}C$ connectivities were determined using an HSQC pulse sequence (interpulse delay set for $^1J_{C,H}$ = 130 Hz). Two- and three-bond $^1H-^{13}C$ connectivities were determined by gradient-selected HMBC experiments optimized for $^{2,3}J$ = 6.0 Hz. Heteronuclear coupling constant were evaluated qualitatively by using a HMBC pulse sequence. Medium-pressure liquid chromatography (MPLC) was performed using a Büchi 861 apparatus having RP18 and SiO_2 (230−400 mesh) stationary phases. HigH-performance liquid chromatography (HPLC) separations were achieved on a Beckman apparatus equipped with an RI detector and LUNA (Phenomenex) columns (C_{18}, 250 × 4 mm).

Animal Material, Extraction, and Isolation: A specimen of *Plakortis simplex* was collected in July 2002 along the coasts of the Bahamas. A voucher specimen is deposited at the Dipartimento di Chimica delle Sostanze Naturali, Italy (ref. number: 02−10). The organism was frozen immediately after collection and kept frozen until extraction, when the sponge (43 g, dry weight after extraction) was homogenized and extracted with methanol (4 × 500 mL) and chloroform (4 × 500 mL). The methanol extract was initially partitioned between H_2O and *n*BuOH and then the organic phases were combined and concentrated in vacuo to afford a viscous brown oil (22.1 g). This oil was subjected to chromatography on a column packed with RP18 silica gel and eluted with H_2O/MeOH (9:1, A_1; 7:3, A_2; 4:6, A_3; 2:8, A_4; 1:9, A_5). Fraction A_3 (441.6 mg) was chromatographed further by MPLC (SiO_2, 230−400 mesh; solvent gradient system of increasing polarity from EtOAc to MeOH). The fractions that eluted with EtOAc/MeOH (8:2 to 0:10) were rechromatographed by HPLC (MeOH/H_2O, 6:4; flow, 0.8 mL/min) to afford plakohypaphorines A (**1**, 3.2 mg), B (**2**, 2.2 mg), and C (**3**, 1.8 mg) and the novel plakohypaphorines D (**4**, 2.1 mg), E (**5**, 4.3 mg), and F (**6**, 1.5 mg) in a pure state.

Plakohypaphorine D (4): Pale-yellow amorphous solid. $[\alpha]_D^{25}$ = +27.1 (*c* = 2.0 mg/mL in CH_3OH/CF_3COOH, 8:1). IR (KBr): $\tilde{\nu}_{max.}$ = 3385, 1610, 1550 cm^{-1}. UV (CH_3OH): $\lambda_{max.}$ (ε) = 288.0 (3700), 224.0 nm (17200). ^1H and ^{13}C NMR ([D$_6$]DMSO): see Table 1 and 2, respectively. LR-ESIMS: *m*/*z* = 499 [M + H$^+$], 521 [M + Na$^+$]. HR-ESIMS: observed *m*/*z* = 498.9388 [M + H]$^+$; calcd. for $C_{14}H_{17}I_2N_2O_2$, 498.9379; Δ = 1.8 ppm.

Plakohypaphorine E (5): Pale-yellow amorphous solid. $[\alpha]_D^{25}$ = +31.2 (*c* = 4.0 mg/mL in CH_3OH/CF_3COOH, 8:1). IR (KBr): $\tilde{\nu}_{max.}$ = 3388, 1610, 1554 cm^{-1}. UV (CH_3OH): $\lambda_{max.}$ (ε) = 288.0 (3740), 224.0 nm (17211). ^1H and ^{13}C NMR ([D$_6$]DMSO): see Table 1 and 2, respectively. LR-ESIMS: *m*/*z* = 625 [M + H$^+$], 647 [M + Na$^+$]. HR-ESIMS: observed *m*/*z* = 624.8355 [M + H]$^+$; calcd. for $C_{14}H_{17}I_3N_2O_2$, 624.8346; Δ = 1.4 ppm.

Plakohypaphorine F (6): Pale-yellow amorphous solid. $[\alpha]_D^{25}$ = +26.7 (*c* = 1.5 mg/mL in CH_3OH/CF_3COOH, 8:1). IR (KBr): $\tilde{\nu}_{max.}$ = 3390, 1609, 1562 cm^{-1}. UV (CH_3OH): $\lambda_{max.}$ (ε) = 286.0 (3740), 226.0 nm (17200). ^1H and ^{13}C NMR ([D$_6$]DMSO): see Table 1 and 2, respectively. LR-ESIMS: *m*/*z* = 407/409 (ratio 3:1) [M + H$^+$], 429/431 (ratio 3:1) [M + Na$^+$]. HR-ESIMS: observed *m*/*z* = 407.0009 [M + H]$^+$; calcd. for $C_{14}H_{17}{}^{35}ClIN_2O_2$, 407.0023; Δ = 3.4 ppm.

Microwave-Assisted Dehalogenation of Plakohypaphorine F: $Pd(OAc)_2$ (1.5 mg) and an aqueous HCOOK solution (3.5 mg/7 μL) were added to a solution of plakohypaphorine F (**6**, 1.0 mg) in DMSO (1.0 mL). This mixture was purged with nitrogen gas, placed in a beaker in an upright position, and subjected to microwave heating (25 s, 20% of 750 W power setting; Milestone Ethos 1600 oven) under atmospheric pressure. The black suspension obtained was filtered and the filtrate was purified by HPLC (RP18, MeOH/H_2O, 6:4) to afford compound **7** (0.6 mg, 90% yield) as a colorless amorphous solid. $[\alpha]_D^{25}$ = +28.9 (*c* = 0.6 mg/mL in CH_3OH/CF_3COOH, 8:1). ^1H NMR ([D$_6$]DMSO): δ = 3.13 (m, 1 H), 3.18 (m, 1 H), 3.20 (s, 9 H), 3.64 (dd, *J* = 10.3, 3.4 Hz, 1 H), 7.02 (t, *J* = 7.5 Hz, 1 H), 7.12 (d, *J* = 7.5 Hz, 1 H), 7.28 (s, 1 H), 7.60 (d, *J* = 7.5 Hz, 1 H), 11.10 (s, 1 H) ppm. LR-ESIMS: *m*/*z* = 281/283 (ratio 3:1) [M + H$^+$]. HR-ESIMS: observed *m*/*z* = 281.1070 [M + H]$^+$; calcd. for $C_{14}H_{17}{}^{35}ClN_2O_2$, 281.1057; Δ = 4.6 ppm.

Pharmacological Tests: Male guinea pigs weighing 250−350 g were used (Harlan, Italy). The animals were maintained under controlled conditions (temperature: 24 ± 2 °C; humidity: 60%) and had free access to water and food. All experiments complied with the Italian

D.L. no. 116 27/1/1992 and associated guidelines in the European Communities Council Directive of 24/11/1986 (86/609/ECC). Histamine hydrochloride, acetylcholine hydrochloride, and $BaCl_2$, purchased from Sigma (Milan, Italy) were dissolved in distilled water. The guinea pigs were killed by asphyxiation with CO_2 and segments (1−1.5 cm) of ileum were removed, flushed of luminal contents and placed in Krebs solution [concentrations (mM): NaCl, 119; KCl, 4.75; KH_2PO_4, 1.2; $NaHCO_3$, 25; $CaCl_2$, 2.5; $MgSO_4$, 1.5; and glucose, 11]. The tissues were prepared as previously reported.[21] In brief, ileum segments were set up (to record contractions mainly from the longitudinal axis) in an organ bath containing Krebs solution (20 mL) equilibrated with 95% O_2 and 5% CO_2 at 37 °C. The tissues were connected to an isotonic transducer (load: 0.5 g) connected to a "Gemini" recording apparatus (Ugo Basile, Comerio, Italy). After an equilibration period of 1 h, we recorded concentration−response curves for histamine (10^{-9}−10^{-5} M), acetylcholine (10^{-8}−10^{-5} M), and $BaCl_2$ (10^{-4}−10^{-2} M). These materials were added to the bath and left in contact with the tissue for 30 s and then washed out. After at least two stable concentration response curves were obtained, the curves were repeated in the presence of plakohypaphorines A−F (**1**−**6**). To determine statistical significance, we used one-way analysis of variance followed by a Bonferroni test. Results are expressed as means ± S.E.M ($P < 0.05$ is considered significant).

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