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The ^{13}C and ^{1}H nmr spectra of chloroindoles and chlorotryptophans in methanol-d_4 were assigned based on 1-D and 2-D nmr techniques, including COSY, inverse-detected direct (HMQC) and long-range (HMBC) correlation. Chlorine substitutent effects in chemical shifts (SCS) for chlorotryptophans and chloroindoles were calculated and compared. The correlations were linear except for 4-chlorotryptophan, which suggests structural changes on the indole ring between 4-chlorotryptophan and 4 chloroindole. The conformational analysis based on the coupling constants of the side chain also showed a change in the fractional population of the rotamers between 4-chlorotryptophan and the other chlorotryptophans.

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Introduction.

After 6-chloro-D-tryptophan was identified as a nonnutritive sweetener [2], there has been an increasing interest in chemical and biological properties of chlorotryptophans. 4-Chloro-L-tryptophan was isolated from a hydrolysate of the protein of pea seeds [3], and 5-chloro-D-tryptophan was isolated from the hydrolysate of the antibiotic longicatenamycin [4]. 7-Chlorotryptophan was found to be a substrate of L-tryptophan-2,3-dioxygenase from *Pseudomonas aureofaciens* [5]. Despite the broad spectrum of practical applications of chlorotryptophans, the ^{13}C nmr spectra have not been studied. Among the reported ^{1}H chemical shifts (CS) of 4-, 5- and 6-chlorotryptophans [3,4,6], only those of 5-chlorotryptophan were assigned [3]. In another study, nmr spectra of 5-substituted indoles have been reported, in which the ^{1}H and ^{13}C CS of 5-chloroindole were assigned [7]. The ^{13}C CS values of chlorotryptophans have not been reported, even in recent syntheses of chlorotryptophans [3,5,8,9]. In the present paper we unambiguously assign ^{1}H and ^{13}C nmr spectra of chlorotryptophans **2b-e** and chloroindoles **1a-e**, calculated the chlorine SCS, and compare the chlorine SCS of **2b-e** with those of **1b-e**.

Results and Discussion.

Table 1

^{1}H Chemical Shifts of Chloroindoles and Chlorotryptophans

Compound	H-2	H-3	H-4	H-5	H-6	H-7	α-H	β-H	β-H'
1a	7.23	6.44	7.55	7.00	7.10	7.39	-	-	-
2a [a]	7.14	-	7.70	7.01	7.10	7.33	3.57	3.29	2.91
1b	7.31	6.53	-	7.03	7.07	7.34	-	-	-
2b	7.20	-	-	6.97	7.01	7.28	3.66	3.58	3.08
1c	7.29	6.43	7.53	-	7.07	7.36	-	-	-
2c	7.20	-	7.73	-	7.05	7.31	3.54	3.26	2.90
1d	7.26	6.46	7.51	6.99	-	7.39	-	-	-
2d	7.18	-	7.67	6.99	-	7.35	3.55	3.27	2.93
1e	7.31	6.52	7.50	6.98	7.12	-	-	-	-
2e	7.24	-	7.67	7.00	7.12	-	3.56	3.29	2.95

[a] Data taken from Ref 1.

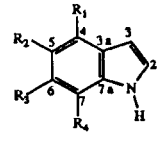
The ^{1}H and ^{13}C nmr data for chloroindoles and chlorotryptophans in methanol-d_4 are given in Tables 1 and 2, respectively. For comparision, the analogous data of tryptophan are also included. Since the solubility of most chlorotryptophans in methanol was very low (<0.1%), the spectra of **2a-e** (20-25 m*M*) were measured in 0.1N sodium deuterioxide/methanol-d_4.

Table 2

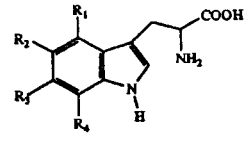
^{13}C Chemical Shifts of Chloroindoles and Chlorotryptophans [a]

Compound	C-2	C-3	C-3a	C-4	C-5	C-6	C-7	C-7a
1a	125.4	102.2	129.4	121.1	119.9	122.1	112.1	137.6
2a [b]	124.5	112.5	129.1	119.7	119.7	122.3	112.2	138.2
1b	126.4	100.7	128.0	126.5	119.6	122.8	111.0	138.4
2b [c]	126.3	113.1	125.5	126.9	120.6	122.7	111.3	139.8
1c	127.2	102.1	130.5	120.4	125.6	122.3	113.3	136.0
2c	126.2	112.4	130.2	119.2	125.5	122.4	113.3	136.6
1d	126.5	102.5	128.1	122.1	120.5	128.0	111.9	138.0
2d	125.5	112.9	127.8	120.9	120.1	128.2	111.9	138.5
1e	126.7	103.4	131.3	120.0	120.8	121.6	117.6	134.6
2e	125.7	113.8	131.0	118.7	120.5	121.7	117.6	135.0

[a] The ^{13}C CS for α-C, β-C and COO- except **2b** are 57.9-58.0, 32.5-32.7 and 182.2-182.4 ppm, respectively. [b] Data taken from Ref 1. [c] The ^{13}C CS for α-C, β-C and COO- are 59.2, 33.7 and 182.4 ppm, respectively.



1a-e



2a-e

	R_1	R_2	R_3	R_4
1a, 2a	H	H	H	H
1b, 2b	Cl	H	H	H
1c, 2c	H	Cl	H	H
1d, 2d	H	H	Cl	H
1e, 2e	H	H	H	Cl

The ^{1}H-^{1}H assignments of **1a-e** and **2b-e** were achieved by ^{1}H-^{1}H COSY [10], HMQC (heteronuclear multiple quantum coherence) [11] and HMBC (heteronuclear

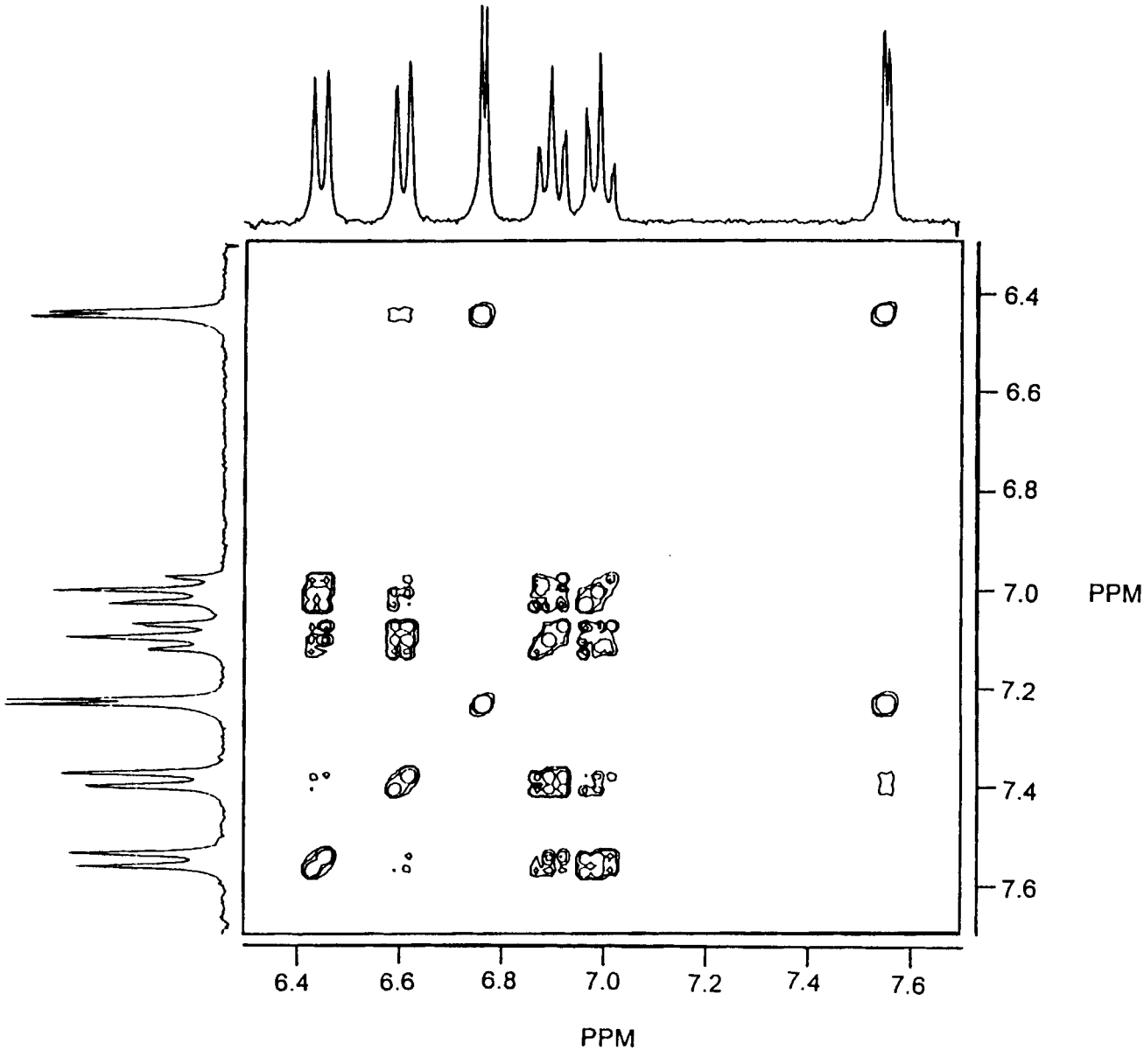


Figure 1. Contour plot of the COSY spectrum of indole.

multiple bond correlation) [12] 2D experiments, in addition to ^{1}H and ^{13}C 1D experiments. Since the ^{1}H CS of indole were solvent and concentration dependent [13], it was necessary to unambiguously assign ^{1}H spectra of **1a** in methanol-d_4 which has not been used previously as solvent. The assignments were obtained from COSY data shown in Figure 1. The results confirmed the previously reported long-range coupling between H-3 and H-7 (1.0 Hz) as well as between H-4 and H-7 (0.6 Hz) [14,15]. The correlation of these assignments with the ^{13}C signals in the HMQC spectra made it possible to assign the ^{13}C resonances, and the order of ^{13}C CS agreed with those in chloroform-d [16]. These characteristic long-rang couplings in indole have been applied to the assignments of ^{1}H spectra of chloroindoles.

The assignments of ^{13}C spectra of 5- and 7-substituted indoles **1c, 1e** and tryptophans **2c, 2e** have been accomplished from HMQC spectra (Figure 2). The ^{13}C resonance signals for the quaternary carbons, 3a and 7a, were differentiated based on the positions of the corresponding signals of indole and tryptophan. The characteristic upfield signals for C-5 and C-7 made them straightforward to assign. The signals of chlorinated carbons were always higher than C-3a and C-7a, but lower than C-3, for chlorotryptophans. The order of the assigned CS of **1c** agreed with those reported, even though the solvent was different [7].

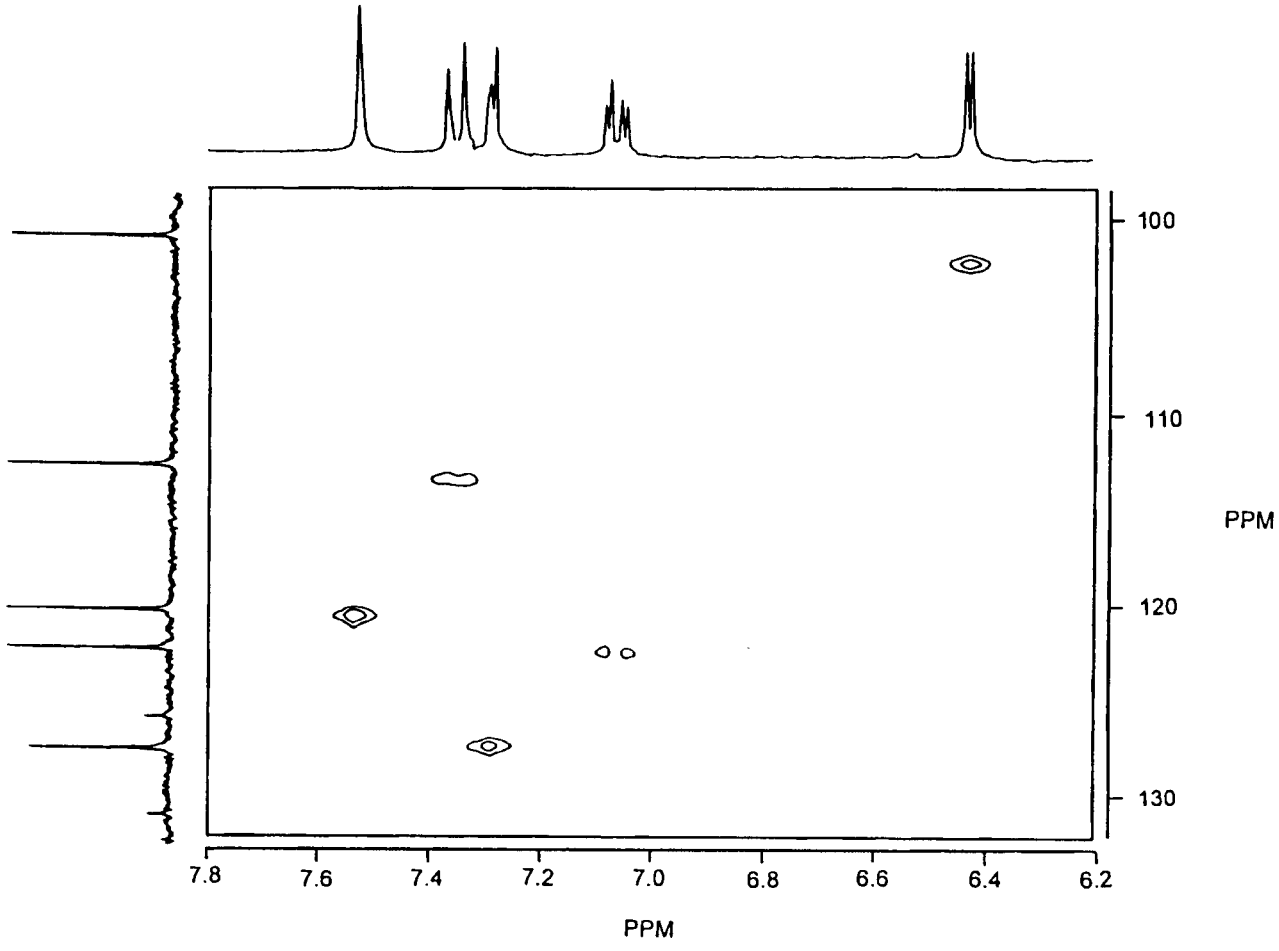
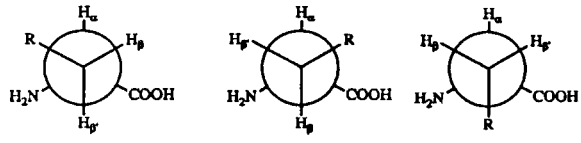


Figure 2. Contour plot of the 2D ^{1}H-^{13}C HMQC spectrum of 5 -chloroindole.

The ^{13}C assignments of 4- and 6-substituted compounds, **1b, 1d, 2b, 2d,** required HMBC as well as HMQC, because some signals appeared in a very narrow range. In the case of **2d**, there were three pairs of signals within 1 ppm of each other that were around 112, 120 and 128 ppm. Among these, the pair at 112 ppm were trivial to assign due to the difference in the chemical nature. The pair at 120 ppm was differentiated on the basis of the HMQC data, in which the crosspeaks appeared between the ^{1}H doublet at 7.67 ppm and the ^{13}C signal at 120.9 ppm, as well as between resonances at 6.99 ppm and 120.1 ppm [17].

The resonances of the remaining pair of quaternary carbons have been assigned based on the comparison of the peak height. The signal at 128.2 ppm (C-6) was about twice as high as the other at 127.8 (C-3a). These assignments were confirmed from the HMBC plot, in which the former had two crosspeaks corresponding to H-4 and H-7. On the other hand, the latter had crosspeaks with H-2 and H-5 signals, as shown in Figure 3. The same strategy was used for assigning the C-3a and C-4 resonances of **1b** and **2b**, assigning the chlorinated carbon by comparision of heights of peaks, and finally confirming the assignments by HMBC [17]. On the other hand, the C-3a and C-6 resonances of **1d** were so close (<0.1 ppm) that the assignments based on the comparison of heights were not confirmed by HMBC.

After we assigned all ^{13}C resonances of **1a-e** and **2b-e**, we calculated the chlorine SCS for indole and tryptophan by subtracting the ^{13}C CS for indole and tryptophan from those of chloroindoles and chlorotryptophans, and the results are shown in Table 3. The chlorine SCS for the benzene ring of chloroindoles and chlorotryptophans are $5 \sim 7$ for α-carbons, $-3 \sim 1$ for β-carbons, $0.3 \sim 1.9$ for γ-carbons, and $-1.7 \sim -0.9$ for δ-carbons, in terms of dif-



$P_I = (J_{\alpha\beta'} - J_g)/(J_t - J_g)$ $P_{II} = (J_{\alpha\beta'} - J_g)/(J_t - J_g)$ $P_{III} = 1 - P_I - P_{II}$

$J_t = 13.56$ $J_g = 2.60$ (1)

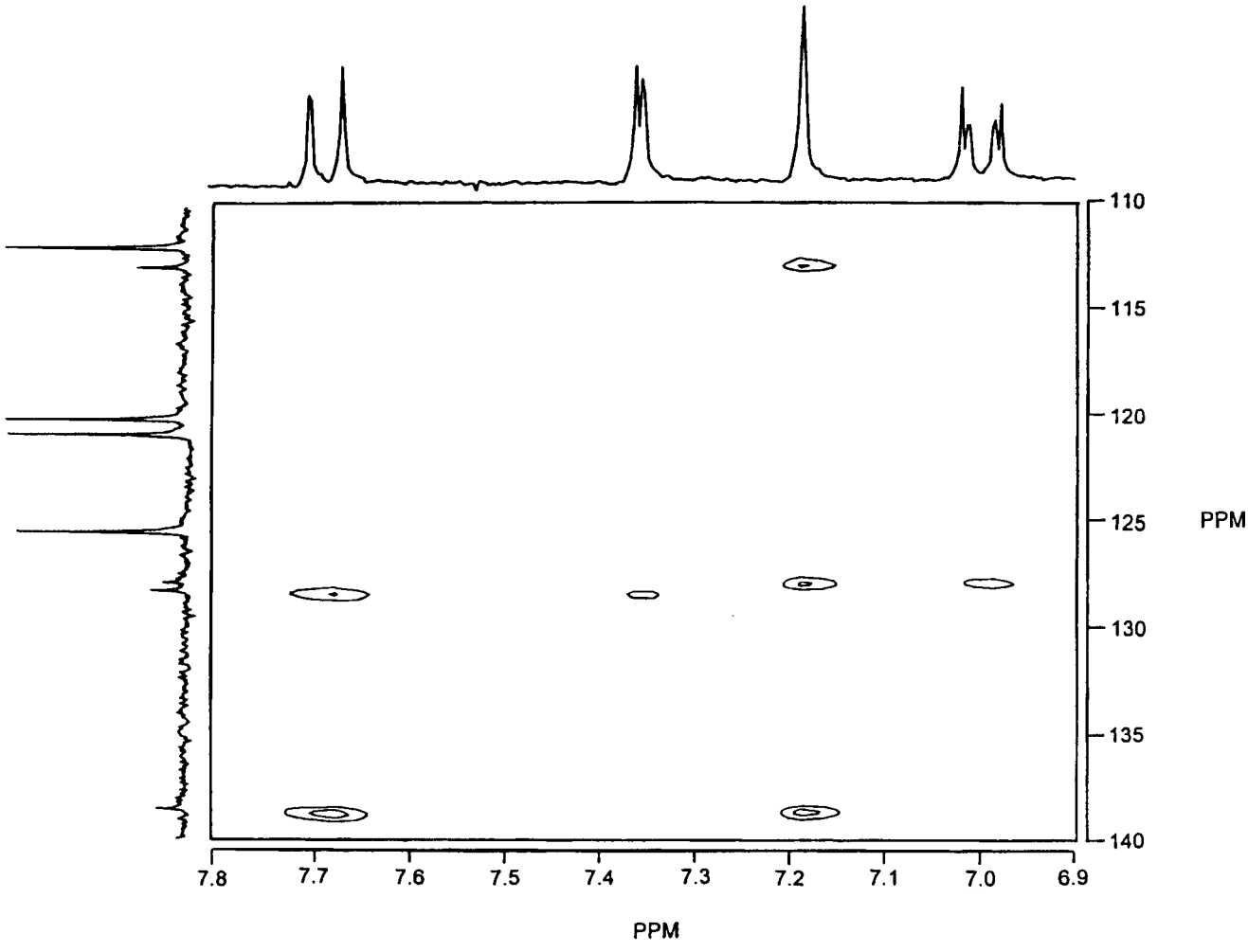


Figure 3. Portion of the 2D ^{1}H-^{13}C HMBC spectrum of 6-chlorotryptophan.

ferences from indole and tryptophan. The changes in the C-2 resonances were always 1 ~2 ppm downfield. On the other hand, C-3 varied from 1.3 ppm downfield to 1.5 ppm upfield. Like other aromatic compounds, δ-effects are stronger than γ-effects except in the case of **1e** and **2e**.

The comparison of the chlorine SCS for tryptophan with those of indole are shown in Figure 4. The correlation is linear to an excellent approximation, with correlation coefficient of 0.99, except in the case of **1b** and **2b**. The ^{1}H CS of one of the β-protons of **2b** (3.58 ppm) was different from the others (3.25-3.29 ppm), which suggests that there are

Table 3

The Chlorine SCS for Chloroindoles and Chlorotryptophans

Compound	C-2	C-3	C-3a	C-4	C-5	C-6	C-7	C-7a
1b	0.9	-1.5	-1.4	5.4	-0.3	0.7	-1.0	0.8
2b	1.9	0.7	-3.6	7.3	1.0	0.4	-0.9	1.6
1c	1.8	-0.1	1.1	-0.7	5.7	0.2	1.2	-1.6
2c	1.8	0.0	1.2	-0.4	5.9	0.1	1.2	-1.7
1d	1.1	0.3	-1.3	1.0	0.6	5.9	-0.2	0.4
2d	1.0	0.4	-1.3	1.3	0.5	5.9	-0.2	0.3
1e	1.3	1.2	1.9	-1.1	0.9	-0.5	5.5	-3.0
2e	1.2	1.3	1.9	-0.9	0.9	-0.6	5.4	-3.2

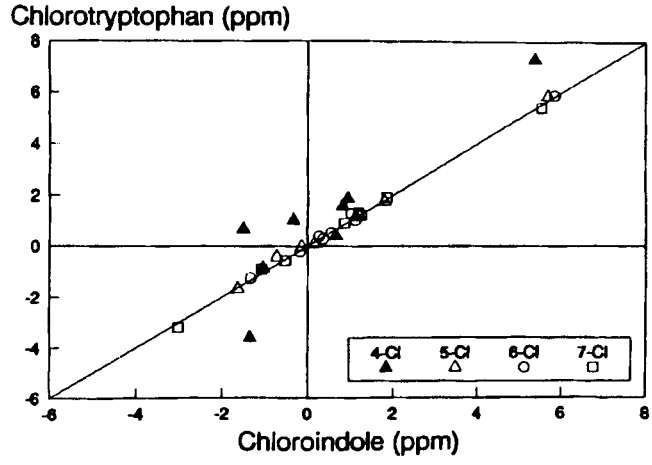


Figure 4. Linear relationship of the chlorine SCS between chlorotryptophans and chloroindoles.

structural changes between **2b** and the others. The coupling constants between the α- and β-protons of **2b** were also different from those of the others (Table 4).

Table 4

The Fractional Population of Chlorotryptophans [a]

Compound	Jαβ	Jαβ'	P_I	P_{II}	P_{III}
2a	4.25	8.64	55	15	30
2b	5.1	8.3	52	23	25
2c	4.4	8.4	53	16	31
2d	4.5	8.3	52	17	30
2e	4.46	8.24	51	17	32

[a] The assignments of two β-protons were based on Refs 19, 20.

The nature of the structural change in **2b** was clear when we compare the side chain rotamer populations. The fractional population can be calculated from the coupling constants according to Pachler's equation (equation 1) [18]. The fractional population of rotamer I (P_I) of **2b** was slightly smaller than those of tryptophan and the other chlorotryptophans, but that of rotamer II (P_{II}) was significantly larger than those of tryptophan and the chlorotryptophans (Table 4). The population of rotamer III (P_{III}) of **2b** was also smaller than those of the others.

It was expected that the effects due to the structural changes of **2b** would be concentrated on the carbons around the chlorine and side chain. Thus the largest differences in the chlorine SCS between **1b** and **2b** (CS of **2b** - CS of **1b**) were shown at C-3 (−2.2), C-3a (2.2), C-4 (−1.9) and C-5 (−1.3). These differences were smaller than those of the methyl SCS between 4-methylindole and 4-methyltryptophan (C-3, −2.5; C-3a, 1.8; C-4, −2.8; C-5, −1.9) [1]. On the other hand, the fluorine SCS of 4-fluorotryptophan did not show any unusual effects, compared with those of other fluorotryptophans [21]. An analogous steric effect has been observed for 1,8-disubstituted naphthalenes [22-24] and geometric distortions were suggested as an explanation for the effect [23]. Our results suggest the angle distortion may be accompanied by a conformational change in the case of **2a**. Thus, whenever the SCS of indoles with large substituents is applied to the corresponding tryptophans, it should be checked for steric effects in the 4-substituted derivative. These results suggest that the 4-substituted tryptophans, when incorporated into peptides, will result in structural and conformational differences compared with the natural peptides.

EXPERIMENTAL

The ^1H and ^{13}C spectra of **1a-e** and **2b-e** were recorded on Bruker AC-300 (300.135 MHz) and AC-250 spectrometers (62.896 MHz), using digital resolution of 0.20 Hz and 0.72 Hz per points, respectively. Solutions (20-25 m*M*) in methanol-d_4 or 0.1 *N* sodium deuterioxide/methanol-d_4 were placed in 5 mm o.d. sample tubes at 20°. Chemical shifts were referenced to internal TMS.

The HMQC 2D spectra were obtained on the Bruker AC-300 spectrometer using the pulse sequence developed by Bax and coworkers [11]. The fixed delays correspond to a $^1J(CH)$ coupling of 150 Hz. Other typical parameters were as follows: spectral width, 1.54 KHz (^1H-**1a-d**), 1.67 KHz (^1H-**2b-d**), 2.1 KHz (^{13}C-**1a-d**), 4.5 KHz (^{13}C-**2b-d**); data matrix, 512 x 512; recycle delay, 2 seconds; number of transients, 256; increments of the delay time, 64. Total acquisition time for the data was 11 hours.

The long range HMBC data were acquired using the pulse sequence of Bax and Summers [12]. Because of the remaining ambiguity about the correlations of the two quaternary carbons (C-3a and C-6) of **2d**, we ran the HMBC twice using different ^{13}C spectral widths. The fixed delays correspond to a single long range coupling of 10 Hz. Other typical parameters were as follows: spectral width, 1.5 KHz (^1H-**1b**), 1.67 KHz (^1H-**2b, 2d**), 2.1 KHz (^{13}C-**1b, 2d**), 5.8 KHz (^{13}C-**2b**); data matrix, 512 x 512; recycle delay, 2 seconds; number of transients, 256; increments of the delay time, 64. Total acquisition time for the data was 11 hours.

COSY spectra were acquired using a standard pulse sequence employing phase cycling of 45° instead of 90° [10]. Other typical parameters were as follows: spectral width, 1.5 KHz; data matrix, 1K x 512; recycle delay, 2 seconds; number of transients, 256; increments of the delay time, 8. Total acquisition time for the data was 90 minutes.

Compounds **1a-d** were purchased from Aldrich Chemical Co. and were used without further purification. Compound **1e** was prepared by the procedure of Rydon and Tweddle [25]. Chlorotryptophans **2b-d** were synthesized enzymatically from L-serine and corresponding chloroindoles according to our procedure [26].

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