allowed to stand overnight in the ice-box, the yellow precipitate, which consisted largely of 7-azaindole together with some product, was separated by filtration and triturated with 5% hydrochloric acid. The residue, which was only slowly soluble in this medium was recrystallized from 1:1 benzene–cyclohexane for analysis; fine yellow needles, m.p. 214.5–215.0°. It was later found that the compound, like its indole analog,[13] is soluble in dilute sodium hydroxide solution. The preparation was not repeated, but it is probable that the reaction would be more nearly complete at a higher *p*H.[13]

Anal. Calcd. for $C_{13}H_{10}N_4$: C, 70.25; H, 4.54; N, 25.21. Found: C, 70.50; H, 4.52; N, 25.0.

Ultraviolet Spectra.—Spectra were determined on a Beckman model DU quartz spectrophotometer at a concentration of 10^{-4} *M*.

AMHERST, MASSACHUSETTS

[CONTRIBUTION FROM THE COURTAULD INSTITUTE OF BIOCHEMISTRY]

Synthesis and Absorption Spectra of the Symmetrical Chloroindigos

BY PETER W. SADLER AND IN PART BY REGINALD L. WARREN

RECEIVED SEPTEMBER 12, 1955

The ultraviolet and visible absorption spectra of the symmetrical chloroindigos were determined in tetrachloroethane solution. Contrary to earlier work[1,2] the degree of halogenation was not found to be paralleled by the extent of the bathochromic shift. In fact both bathochromic and hypsochromic shifts were found to occur, depending on the position of the substituents. A simple relationship was deduced between the absorption maxima of the four dichloroindigos and the more highly substituted compounds.

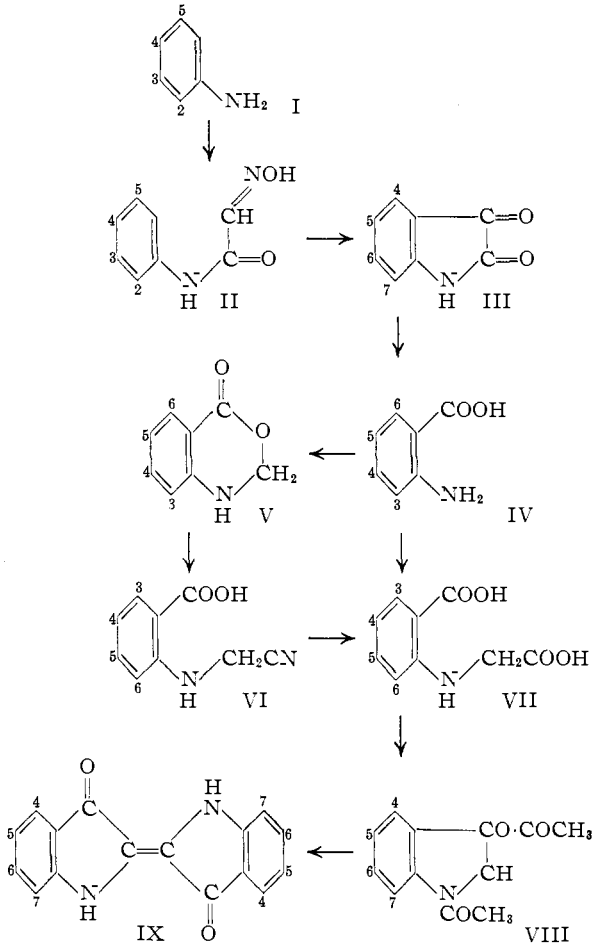
Introduction

As part of a larger program, relating absorption spectra to chemical constitution in indigoid dyes, the fifteen symmetrical chloroindigos were synthesized. All of the dyes were obtained from the corresponding diacetylindoxyl compounds by alkaline hydrolysis followed by aerial oxidation. Many of the chloroindigos previously prepared have been obtained by the direct chlorination of indigo, their configuration having been proved by degradation to known compounds; in this present work the synthetic route chosen has removed all ambiguity, as several known compounds occur as intermediates in the synthesis of each indigo, making the configuration of each dye certain.

Several of the chloroamines I which were used as starting materials were obtainable commercially,[3] with the exception of 3,5-dichloro,[4] 3,4,5-trichloro-[5] and 2,3,5-trichloroaniline[6] which were prepared and characterised by methods in the literature.

The substituted α-isonitrosoacetanilides II were prepared from the appropriate chloroamines I by the Sandmeyer synthesis,[7] using the Marvel–Hiers modifications.[8] The crude reaction products were extracted with warm 2 *N* sodium hydroxide (hot concentrated alkali decomposes the isonitrosoacetanilides) and filtered over "Hyflo-Supercel." Slow addition of 2 *N* hydrochloric acid precipitated the derivatives in a reasonably pure and often crystalline form. Aqueous ethanol was used for recrystallization. Details are listed in Table IV.

The isonitrosoacetanilides were cyclized to the isatins III in concentrated sulfuric acid,[7] the necessary modifications of temperature and duration of heating are given in Table V. In each case



the crude product was dissolved in the required amount of 2 *N* sodium hydroxide (water being added if necessary to prevent precipitation of the sodium isatinate), then filtered rapidly (charcoal) after making the hot solution faintly acid by the addition of glacial acetic acid. By this method a clear solution of the isatin was obtained, free from unchanged isonitrosoacetanilide and tarry by-

(1) J. Formanek, *Angew. Chem.*, **41**, 1133 (1928).

(2) W. R. Brode, E. G. Pearson and G. M. Wyman, THIS JOURNAL, **76**, 1034 (1954).

(3) L. Light & Co. Ltd., Poyle Trading Estate, Colnbrook, Bucks, England.

(4) G. M. Dyson, H. J. George and R. F. Hunter, *J. Chem. Soc.*, 3043 (1926).

(5) H. Hodgson and J. Walker, *ibid.*, 1620 (1933).

(6) H. Hodgson and A. Kershaw, *ibid.*, 2920 (1929).

(7) T. Sandmeyer, *Helv. Chim. Acta*, **2**, 234 (1919).

(8) C. S. Marvel and G. S. Hiers, *Org. Syntheses*, **V**, 71 (1925).

products. The isatin was isolated by further acidification of the hot filtrate with concentrated hydrochloric acid. Isomeric mixtures of isatins resulted from the cyclization of 3-chloroisonitrosoacetanilide (4- and 6-chloroisatin) and 3,4-dichloroisonitrosoacetanilide (4,5-dichloroisatin and 5,6-dichloroisatin). After preliminary purification the 4-chloroisatin was precipitated from a solution containing the two substituted sodium isatinates by the addition of excess acetic acid, the 6-chloroisatin was subsequently isolated by strong acidification with hydrochloric acid. In the case of the 4,5-dichloro- and 5,6-dichloroisatins the acetic acid separation was incomplete, but fractional crystallisation from ethanol produced each in a pure state.

Alkaline peroxide oxidation (footnote *a* to Table VI) of the chloroisatins III gave the corresponding chloroanthranilic acids IV. Both 5-chloro- and 3,5-dichloroanthranilic acids were more readily obtained by the direct chlorination of anthranilic acid. Details are listed in Table VI. Anthranilic acids containing a substituent in the 3-position will not condense with chloroacetic acid to form the corresponding phenylglycine-*o*-carboxylic acids VII, so for these an alternative route *via* the anthranilic acid formalide (V) was used. They were prepared essentially by Villiger's method,[9] *i.e.*, refluxing the anthranilic acid in the minimum quantity of methanol, formalin (2 mole, 40%) being added dropwise. Usually the formalide separated after a few minutes refluxing as well-defined crystals, often requiring no further purification. When more drastic conditions were necessary, the anthranilic acid was stirred in 20% formalin (5 mole) at 60°. In these heterogeneous reactions the anthranilic acid swells and becomes a voluminous fluffy precipitate as the reaction proceeds. The reaction products can easily be purified by extracting with sodium bicarbonate solution, then recrystallising the residue from methanol, ethanol or dioxane. Experimental details are given in Table VII.

The substituted phenylglycinenitrile-*o*-carboxylic acids VI were obtained from the corresponding anthranilic acid formalides V by shaking or stirring with aqueous sodium cyanide (2 moles) at 60°. In each case the formalide slowly dissolved and when solution was complete the reaction mixture was allowed to stand for a further half-hour. Then on careful acidification with cooling, the phenylglycinenitrile-*o*-carboxylic acid was precipitated. The product was washed well with water and dissolved in aqueous sodium bicarbonate. Removal of unchanged formalide was effected by filtration, and then acidification gave the nitrile which was crystallized (charcoal) from aqueous ethanol. Details are listed in Table VIII.

Alkaline hydrolysis of VI with boiling 2 *N* sodium hydroxide gave the corresponding chlorophenylglycine-*o*-carboxylic acids VII; the remainder, *i.e.*, those lacking a substituent in the 3-position, were prepared by the direct route, by condensing the substituted anthranilic acid with chloroacetic acid. Experimental details are given in Table IX. The chlorophenylglycine-*o*-carboxylic acids VII were converted to the corresponding diacetylindoxyl compounds VIII by heating a solution in excess acetic anhydride under reflux in the presence of excess (4 moles) anhydrous sodium acetate. After removal of excess acetic anhydride, the diacetylindoxyl compounds were hydrolyzed with hot 0.1 *N* sodium hydroxide, the free indoxyl compounds formed, being readily converted to the symmetrical indigoid dyes by aerial oxidation. The chloroindigos were purified by first washing with hot water, then hot ethanol, before crystallizing from a suitable solvent as indicated in Table X.

Owing to the considerable difference in solubility between indigoid dyes substituted in the 4- or 7-position and those with only 5- or 6-substituents, chloroform is not a suitable solvent for spectroscopic studies. However, the higher boiling tetrachloroethane enabled a direct comparison to be made of the spectra of both the soluble and sparingly soluble indigoid dyes. It has been shown, by an examination of the infrared spectra, that this enhanced solubility of indigoid dyes containing 4- or 7-substituents is a result of the absence in these compounds of intermolecular hydrogen bonding which has been shown to occur in indigo itself, and also in derivatives with substituents in only the 5- or 6-position.[10]

Discussion of Results

It has been stated by Formanek,[1] and Brode, Pearson and Wyman,[2] that the degree of halogenation of indigoid dyes is paralleled by the extent of the bathochromic shift occurring in the visible absorption maximum. This has not been sub-

TABLE I

ABSORPTION SPECTRA DATA OF THE SYMMETRICAL CHLOROINDIGOS IN TETRACHLOROETHANE AT 20°

Position of substituents	λ_1, mμ	ϵ_1	λ_2, mμ	ϵ_2
None	605	16,580	285	25,500
4,4′-	610	25,890	290	39,130
5,5′-	620	17,950	290	35,260
6,6′-	590	14,860	291	26,370
7,7′-	600	20,550	291	40,020
4,4′,5,5′-	622.5	20,830	299	34,820
4,4′,6,6′-	595	22,300	295	32,800
4,4′,7,7′-	610	24,500	295	42,530
5,5′,6,6′-	605	....	295	....
5,5′,7,7′-	617.5	19,950	299.5	37,500
6,6′,7,7′-	590	21,830	293	34,130
4,4′,5,5′,6,6′-	610	....	302.5	....
4,4′,5,5′,7,7′-	615	16,700	302.5	35,000
4,4′,6,6′,7,7′-	595	20,500	296	43,050
5,5′,6,6′,7,7′-	600	16,040	303	38,500
Octachloro compd.	610	16,030	307.5	26,010

TABLE II

EFFECT OF SUBSTITUTION ON λ_1 IN SYMMETRICAL DICHLOROINDIGOS

Position of substituents	λ_1, mμ	Shift (mμ) compared with indigo
None	605	..
4,4′-	610	+ 5
5,5′-	620	+15
6,6′-	590	−15
7,7′-	600	− 5

(9) V. Villiger, *Ber.*, **42**, 3540 (1909).

(10) S. J. Holt and P. W. Sadler, to be published.

TABLE III

λ_1 CALCULATED AND OBSERVED FOR SYMMETRICAL CHLOROINDIGOS, IN TETRACHLOROETHANE AT 20°

Substituents	λ_1 calcd., mμ	λ_1 obsd., mμ
4,4',5,5'-	625	622.5
4,4',6,6'-	595	595
4,4',7,7'-	605	610
5,5',6,6'-	605	605
5,5',7,7'-	615	617.5
6,6',7,7'-	585	590
4,4',5,5',7,7'-	620	615
4,4',6,6',7,7'-	590	595
4,4',5,5',6,6'-	610	610
5,5',6,6',7,7'-	600	600
Octachloro compd.	605	610

TABLE IV

SUBSTITUTED α-ISONITROSOACETANILIDES

Substituents	Refluxing time, min.	Yield, %	M.p., °C. Found	M.p., °C. Reptd.
2-Chloro	50	47	152	150[a]
3-Chloro	0	40	146	154[a]
2,3-Dichloro	30	13	172	[b]
3,4-Dichloro	0	50	154	158[a]
3,5-Dichloro	15	31	230	185[a]
3,4,5-Trichloro	[f]	60	210	[c]
2,3,5-Trichloro	[g]	34	173	[d]
2,4,5-Trichloro	[f]	43	171	[e]

[a] T. Sandmeyer, *Helv. Chim. Acta*, **2**, 234 (1919). [b] *Anal.* Calcd. for $C_8H_6O_2N_2Cl_2$: C, 41.2; H, 2.6. Found: C, 41.2; H, 2.8. [c] *Anal.* Calcd. for $C_8H_5O_2N_2Cl_3$: C, 35.9; H, 1.9. Found: C, 36.1; H, 2.1. [d] *Anal.* Calcd. for $C_8H_5O_2N_2Cl_3$: C, 35.9; H, 1.9. Found: C, 36.2; H, 2.2. [e] *Anal.* Calcd. for $C_8H_5O_2N_2Cl_3$: C, 35.9; H, 1.9. Found: C, 35.9; H, 1.9. [f] Reaction carried out on 0.5 mole scale using four times the usual volume of water. The amine was dissolved in the minimum quantity of hot ethanol before addition to the hot reaction mixture of chloral hydrate, hydroxylamine hydrochloride and hydrochloric acid. The mixture was boiled for 15 min., then four times the usual amount[8] of anhydrous sodium sulfate was added and the mixture allowed to cool overnight, depositing white crystals of the isonitrosoacetanilide. [g] As above, but diluted five times, and refluxed for 1 hr. before adding the sodium sulfate.

stantiated by the present work, for by a consideration of the spectral data of the chloroindigos listed in Table I, it can be seen that both 4,4'-dichloroindigo and octachloroindigo have the same absorption maximum of 610 mμ, as also have the 4,4',7,7'-tetrachloro- and 4,4',5,5',6,6'-hexachloro compounds. Indeed the dye showing the greatest bathochromic shift is only tetra-substituted, *i.e.*, 4,4',5,5'-tetrachloroindigo (λ_1 622.5 mμ). There is, however, a simple relationship between the maxima of the more highly substituted symmetrical chloroindigos, and the maxima of the four dichloroindigos. The shifts in λ_1 of the dichloroindigos, compared with that of indigo, are shown below (Table II). Substitution in positions 6,6'- and 7,7'-produces equal and opposite effects to substitution in the 5,5'- and 4,4'-positions, respectively. From a consideration of these incremental shifts, it can be seen that the value of λ_1 for the symmetrical tetra- and hexachloroindigos may be simply calculated by adding to the basic absorption maximum of indigo (605 mμ) the respective shift for each pair of symmetrical substituents. For example λ_1 for 4,4',5,5',6,6'-hexachloroindigo is equal to the sum of λ_1 for indigo plus the increments for the 4'4-, 5,5'-, and 6,6'-dichloro-substituents (λ_1 = 605 + 5 + 15 − 15 = 610 mμ, λ_1 observed = 610 mμ). This relationship has been found to hold good for every possible symmetrical tetra- and hexachloroindigo, and also for the octachloro derivative as shown in Table III. It is possible that a similar relationship may obtain for substituents other than chlorine and this is being further investigated.

Experimental

Spectra.—Concentrations of about 10 mg. per liter were used, and the spectra recorded in a Hilger "Uvispek" spectrophotometer using the solvent as reference in 1 cm. matched quartz cells at 20°. For the very sparingly soluble dyes (5,5'-, 6,6'-dichloro- and 5,5',6,6'-tetrachloroindigo) supersaturated solutions containing 1–2 mg. per liter were prepared and the spectra recorded at 20° in 4-cm. cells.

TABLE V

CHLOROISATINS

Substituents	Conditions	Yield, %	Color and solvent	M.p., °C. Found	M.p., °C. Reptd.
4-Chloro	90–95° 0.5 hr.	33[k]	Orange red needles (acetic acid)	256	256.5[a]
6-Chloro	90–95° 0.5 hr.	31[k]	Yellow plates (acetic acid)	263	259[a]
7-Chloro	90–95° 0.5 hr.	35	Orange needles (acetic acid)	175	180[b] 175[c]
4,5-Dichloro	90–95° 0.5 hr.	41[k]	Red plates (ethanol)	250	245[d]
4,6-Dichloro	90–95° 0.5 hr.	30	Yellow needles (acetic acid)	251	250[e]
4,7-Dichloro	90–95° 0.5 hr.	37	Light orange needles (acetic acid)	245	252[e]
5,6-Dichloro	90–95° 0.5 hr.	10[k]	Orange red needles (ethanol)	278	268[d] 277[f]
6,7-Dichloro	120° 10 min.		Yellow orange needles (acetic acid)	204	[g]
4,5,6-Trichloro	90–95° 1 hr.	17	Copper platelets (acetic acid)	275	[h]
4,5,7-Trichloro	100° 2 hr.	47	Red cubes (hot acetic acid) orange cubes with solvent of crystallization (cold acetic acid)	258	[i]
4,6,7-Trichloro	95–100° 1 hr.	35	Yellow needles (acetic acid)	239	[j]

[a] A. E. Senear, H. Sargent, J. F. Mead and J. B. Koepfli, THIS JOURNAL, **68**, 2695 (1946). [b] M. B. Chaudhari and K. S. Nargund, *J. Univ. Bombay*, **28**, 65 (1950). [c] T. Sandmeyer, *Helv. Chim. Acta*, **2**, 234 (1918). [d] B. R. Baker, R. E. Schaub, J. P. Joseph, F. J. McEvoy and J. H. Williams, *J. Org. Chem.*, **17**, 149 (1952). [e] E. Grandmougin and P. Seyder, *Ber.*, **47**, 2368 (1914). [f] M. M. Rapport, A. E. Senear, J. F. Mead and J. B. Koepfli, THIS JOURNAL, **68**, 2700 (1946). [g] *Anal.* Calcd. for $C_8H_3O_2NCl_2$: C, 44.4; H, 1.4; N, 6.5. Found: C, 44.7; H, 1.7; N, 6.7. [h] *Anal.* Calcd. for $C_8H_2O_2NCl_3$: C, 38.3; H, 0.8; N, 5.6. Found: C, 38.2; H, 0.8; N, 5.8. [i] *Anal.* Calcd. for $C_8H_2O_2NCl_3$: C, 38.3; H, 0.8; N, 5.6. Found: C, 38.4; H, 0.9; N, 5.8. [j] *Anal.* Calcd. for $C_8H_2O_2NCl_3$: C, 38.3; H, 0.8; N, 5.6. Found: C, 38.4; H, 0.9; N, 6.2. [k] Denotes one of a pair of isomers.

TABLE VI

CHLOROANTHRANILIC ACIDS

Substituents	Route	Yield, %	M.p., °C. Found	M.p., °C. Reptd.
6-Chloro	*a*	84	146–147	146–147[d]
5-Chloro	*b*	30	210	204[e]
4-Chloro	*a*	86	235	235–236[f]
3-Chloro	*a*	99	192	182[g]
5,6-Dichloro	*a*	85	170	165–167[h]
4,6-Dichloro	*a*	98	190	[l]
3,6-Dichloro	*a*	80	153	151–153[i]
4,5-Dichloro	*a*	92	207	208[k]
3,5-Dichloro	*b*	35	230	224[e]
3,4-Dichloro	*a*	81	238	237–238[i]
4,5,6-Trichloro	*a*	80	191	[m]
3,5,6-Trichloro	*a*	82	176	180[j]
3,4,6-Trichloro	*a*	92	210	210[k]
3,4,5,6-Tetrachloro	*c*	83	182	183

[a] Isatin (1 mole) dissolved in *N* sodium hydroxide (3 moles) by warming, hydrogen peroxide (2 mole, 100 vol.) added in parts to hot solution till oxidation was complete, mixture cooled and neutralized. After filtering (charcoal) the anthranilic acid was obtained by careful acidification of the filtrate. [b] Anthranilic acid (20 g.) was added with stirring over 10 min. to sulfuryl chloride (26 g.) in sodium-dried ether (350 ml.) at −10° (temperature important) stirred for further 5 min. and the ether removed by distillation under reduced pressure. Water (150 ml.) was added to the residue and the solid collected stirred with 8% hydrochloric acid (200 ml.) for 30 min. at 60–70° and filtered. The solid residue was 3,5-dichloroanthranilic acid. Sodium acetate was then added to the cooled filtrate, precipitating at *p*H 7 the crude 5-chloro compound. This was dissolved in the minimum quantity of cold absolute alcohol, treated with charcoal and filtered. The filtrate was then heated to boiling and water added until precipitation from the boiling solution commenced. On cooling 5-chloroanthranilic acid was obtained. [c] Prepared from tetrachlorophthalic anhydride; *cf.* W. R. Orndorff and E. H. Nichols, *Amer. Chem. J.*, **48**, 475 (1912). [d] P. Cohn, *Monatsh.*, **22**, 481 (1901). [e] W. Eller and L. Klemm, *Ber.*, **55**, 221 (1922). [f] T. S. Moore, M. T. Marrack and A. K. Proud, *J. Chem. Soc.*, 119, 1789 (1921). [g] M. B. Chaudhari and K. O. Nargund, *J. Univ. Bombay*, **28**, 65 (1950). [h] B. R. Baker, R. E. Schaub, J. P. Joseph, F. J. McEvoy and J. H. Williams, *J. Org. Chem.*, **17**, 149 (1952). [i] V. Villiger, *Ber.*, **42**, 3549 (1909). [j] C. Graebe and S. Rostowgew, *Ber.*, **34**, 2110 (1901). [k] F. Beilstein and A. Kuhlberg, *Ann.*, **152**, 240 (1869). [l] *Anal.* Calcd. for $C_7H_5O_2NCl_2$: C, 40.8; H, 2.4. Found: C, 40.6; H, 2.2. [m] *Anal.* Calcd. for $C_7H_4O_2NCl_3$: C, 34.9; H, 1.7. Found: C, 35.1; H, 2.0.

TABLE VIII

CHLOROPHENYLGLYCINENITRILE-*o*-CARBOXYLIC ACIDS

Substituents	Yield, %	M.p., °C. Found	M.p., °C. Reptd.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
6-Chloro	93	128	..	51.3	51.4	3.3	3.2
3,6-Dichloro	80	122	123[b]				
4,6-Dichloro	85	158	157[a]				
5,6-Dichloro	96	177	..	44.1	43.9	2.5	2.8
3,4,6-Trichloro	84	167	..	38.6	38.8	1.8	1.8
3,5,6-Trichloro	78	145	..	38.6	38.6	1.8	1.9
Tetrachloro	90	175	176[c]				

[a,b,c] Same as in Table VII.

TABLE VII

CHLOROANTHRANILIC ACID FORMALIDES

Substituents	Conditions	Yield, %	M.p., °C. Found	M.p., °C. Reptd.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
3-Chloro	Stirred in 20% formalin for 1 hr.	85	276	..	52.3	52.4	3.3	3.1
3,5-Dichloro		82	171	171[a]				
3,4-Dichloro		92	231	..	44.0	43.9	2.3	2.5
3,6-Dichloro	Refluxed in methanol, 40% formalin added dropwise	87	160	161[b]				
3,5,6-Trichloro		93	207		38.0	38.1	1.6	1.8
3,4,6-Trichloro		78	188		38.0	38.0	1.6	1.7
Tetrachloro		80	210	213[c]				

[a] German Patent, B. 52378. [b] V. Villiger, *Ber.*, **42**, 3540 (1909). [c] W. R. Orndorff and E. H. Nichols, *Amer. Chem. J.*, **48**, 480 (1912).

TABLE IX

CHLOROPHENYLGLYCINE-*o*-CARBOXYLIC ACIDS

Substituents	Route	Yield, %	M.p., °C. Found	M.p., °C. Reptd.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
3-Chloro	*a*	31	185	175[d]				
4-Chloro	*a*	98	210	210–215[e]				
5-Chloro	*a*	93	215	215[f]				
6-Chloro	*b*	99	167	....	47.0	46.8	3.5	3.5
3,4-Dichloro	*a*	85	158–160	....	40.9	40.6	2.6	2.5
3,5-Dichloro	*a*	70	180	....	40.9	40.5	2.6	2.6
3,6-Dichloro	*b*	96	156	156[g]				
4,5-Dichloro	*a*	94	218	....	40.9	40.8	2.6	2.7
4,6-Dichloro	*b*	82	242	240[h]				
5,6-Dichloro	*b*	99	243	....	40.9	40.9	2.6	2.6
3,4,5-Trichloro	*a*	62	190	....	36.2	36.0	2.0	1.6
3,4,6-Trichloro	*b*	92	176	....	36.2	35.9	2.0	2.0
3,5,6-Trichloro	*b*	84	202	....	36.2	36.1	2.0	1.9
4,5,6-Trichloro	*c*	15	245	....	36.2	35.9	2.0	2.1
Tetrachloro	*b*	95	198	198[i]				

[a] Equimolar proportions of the chloroanthranilic acid and chloroacetic acid heated under reflux in aqueous solution with the addition of sufficient sodium carbonate to keep the reaction mixture alkaline. [b] The chlorophenylglycinenitrile-*o*-carboxylic acid boiled in 2 *N* sodium hydroxide until evolution of ammonia had ceased. [c] Direct chlorination of 5-chloro-

phenylglycine-*o*-carboxylic acid in 15% w./v. hydrochloric acid gave the low yield recorded of 4,5,6-trichlorophenylglycine-*o*-carboxylic acid, no other isomers were isolated [d] B.A.S.F., German Patent 231962. [e] B.A.S.F., German Patent 148615. [f] G. Heller and L. Hessel, *J. prakt. Chem.*, **120**, 73 (1929). [g] V. Villiger, *Ber.*, **42**, 3541 (1909). [h] B.A.S.F., German Patent 148615. [i] W. R. Orndorff and E. H. Nichols, *Amer. Chem. J.*, **48**, 483 (1912).

TABLE X

CHLOROINDIGOS

Substituents	Solvent	Color and λ_1 (mμ) in tetrachlorethane		Lit. λ_1 (mμ) or color
4,4'-Dichloro	Chlorobenzene	Blue	610	602.5 tetralin[a,b]
5,5'-Dichloro	Nitrobenzene	Green blue	620	606.5 tetralin[a,c]
6,6'-Dichloro	Nitrobenzene	Mauve	590	561 tetralin[a] red, nitrobenzene[d]
7,7'-Dichloro	Chloroform	Blue violet	600	*j*
4,4',5,5'-Tetrachloro	Nitrobenzene	Green blue	622.5	*e*
4,4',6,6'-Tetrachloro	Tetrachlorethane	Blue mauve	595	*k*
4,4',7,7'-Tetrachloro	Nitrobenzene	Royal blue	610	*f*
5,5',6,6'-Tetrachloro	Nitrobenzene	Blue	605	Blue violet[g]
5,5',7,7'-Tetrachloro	Chlorobenzene	Green blue	617.5	613.5 Carbon tetrachloride[a,h]
6,6',7,7'-Tetrachloro	Tetrachloroethane	Mauve	590	*l*
4,4',5,5',6,6'-Hexachloro	Tetrachloroethane	Royal blue	610	*m*
4,4',5,5',7,7'-Hexachloro	Tetrachlorethane	Blue	615	615.5 tetralin[a]
5,5',6,6',7,7'-Hexachloro	Tetrachloroethane	Blue violet	600	*i*
4,4',6,6',7,7'-Hexachloro	Tetrachloroethane	Blue mauve	595	*n*
Octachloro	Tetrachloroethane	Royal blue	610	Blue violet[i]

[a] J. Formanek, *Angew. Chem.*, 41, 1133 (1928). [b] L. Gindraux, *Helv. Chim. Acta*, 12, 921 (1929). [c] C. Mettler, *Ber.*, **38**, 2809 (1905). [d] F. Sachs and E. Sicket, *Ber.*, **37**, 1861 (1904). [e] B.A.S.F. German Patents 234961, 409618. [f] E. Grandmougin and P. Seyder, *Ber.*, **47**, 2365 (1914). [g] German Patent 254467. [h] L. Kalb, *Ber.*, **42**, 3653 (1909). [i] C. Van De Bunt, *Rec. trav. chim.*, **48**, 121 (1929). [j] *Anal.* Calcd. for $C_{16}H_8O_2N_2Cl_2$: C, 58.0; H, 3.0; N, 8.5. Found: C, 58.2; H, 3.0; N, 8.7. [k] *Anal.* Calcd. for $C_{16}H_6O_2N_2Cl_4$: C, 48.0; H, 1.5; N, 7.0. Found: C, 48.0; H, 1.5; N, 6.7. [l] *Anal.* Calcd. for $C_{16}H_6O_2N_2Cl_4$: C, 48.0; H, 1.5; N, 7.0. Found: C, 48.2; H, 1.3; N, 7.0. [m] *Anal.* Calcd. for $C_{16}H_4O_2N_2Cl_6$: C, 40.9; H, 0.9; N, 6.0. Found: C, 41.0; H, 1.0; N, 6.2. [n] *Anal.* Calcd. for $C_{16}H_4O_2N_2Cl_6$: C, 40.9; H, 0.9; N, 6.0. Found: C, 40.8; H, 0.8; N, 6.0.

Acknowledgment.—The authors are indebted to Dr. S. J. Holt for several stimulating discussions and suggestions concerning this problem.

LONDON, W. 1, ENGLAND

[CONTRIBUTION FROM THE NATIONAL RESEARCH COUNCIL OF CANADA, PRAIRIE REGIONAL LABORATORY]

The 3-*o*-Nitrophenyl- and 3-(Phenyl-*p*-azo-phenyl)-2-thiohydantoins of Amino Acids[1]

BY L. K. RAMACHANDRAN[2] AND W. B. MCCONNELL

RECEIVED SEPTEMBER 6, 1955

The synthesis of the 5-alkyl-3-(*o*-nitrophenyl)-2-thiohydantoins and the 5-alkyl-3-(phenyl-*p*-azo-phenyl)-2-thiohydantoins derived from several of the naturally occurring amino acids is reported. The ultraviolet spectra of the 3-(phenyl-*p*-azo-phenyl)-thiohydantoins have been studied over the region 225–380 mμ and the molecular extinction coefficients for the peaks of absorption recorded.

2,4-Dinitro-1-fluorobenzene and phenyl isothiocyanate are widely used[3] as reagents for studies on the sequence of amino acids in peptides and proteins. The partial destruction of dinitrophenyl amino acids during the hydrolysis of dinitrophenylated proteins is a serious disadvantage in the use of the former reagent. Phenyl isothiocyanate reacts with amino acids to give N-phenylthiocarbamyl derivatives which can undergo ring closure to yield 5-substituted 3-phenyl-2-thiohydantoins.[4] With a peptide, the phenylthiocarbamyl peptide formed undergoes rearrangement to yield the phenylthiohydantoin of the amino acid occupying the N-terminal position and exposing the amino group of the adjacent residue, thus offering a means for the stepwise degradation starting from the N-terminal amino acid. The phenylthiohydantoins are ordinarily identified by paper chromatography[5,6] or by infrared spectrophotometry,[7] and quantitative determinations are made by ultraviolet spectrophotometry.[3]

It appeared that chromatography of the amino acid thiohydantoins could be facilitated if an isothiocyanate containing a chromophore were used in place of phenyl isothiocyanate. Further, the colored derivatives would be expected to have improved absorption properties. Determination of these thiohydantoins by measurement of the absorption in the visible region of the spectrum might also become possible. The feasibility of this ap-

(1) Issued as paper No. 216 in the Uses of Plant Products series and as N.R.C. No. 3896.

(2) National Research Council of Canada Postdoctorate Fellow, 1953–1955.

(3) H. Fraenkel-Conrat, J. I. Harris and A. L. Levey, in "Methods of Biochemical Analysis," Vol. II, Edited by D. Glick, Interscience Publishers, N. Y., 1955, p. 389.

(4) P. Edman, *Acta Chem. Scand.*, **4**, 277 (1950).

(5) J. Sjoquist, *ibid.*, **7**, 447 (1953).

(6) W. A. Landmann, M. P. Drake and J. Dillaha, THIS JOURNAL, **75**, 3638 (1953).

(7) L. K. Ramachandran, A. Epp and W. B. McConnell, *Anal. Chem.*, **27**, 1734 (1955).