222 ml. of water was cooled to −5°. A solution of 11.1 g. of sodium dichromate in 67.2 ml. of water was added dropwise over a 2-hour period with the temperature maintained at −10° to −5°. Stirring was continued for 3 more hours at −10° to −5°. After standing at room temperature overnight, the mixture was cooled to 0° and 22.2 g. of sodium dichromate in 134.4 ml. of water was added over a 2-hour period. Stirring was continued at 0 −2° for 1 hour and then for 2 hours at room temperature. The acidic reaction mixture was extracted with five 1-liter portions of ether. The combined ether extract was washed once with water saturated with sodium sulfate. The ether extract was dried over anhydrous magnesium sulfate and concentrated under reduced pressure to leave 7.1 g. of reddish colored oil. This oil was extracted with five 200-ml. portions of warm petroleum ether. Concentration of the extract under reduced pressure left 5.6 g. of reddish oil.

A small chromatographic column was prepared using 60 g. of alumina (Brockmann, Grade II) and petroleum ether. The reddish oil was placed on the column using petroleum ether. The column was developed using petroleum ether. A large orange-colored band moved quite rapidly down the column leaving purple, violet and yellow bands behind. The petroleum ether eluate was concentrated under reduced pressure to leave 5.17 g. of reddish oil. The oil was redissolved in petroleum ether; the solvent was slowly removed under reduced pressure to leave yellow-orange needles of 6-ethoxy - 5 - methoxy - 2 - methylbenzoquinone, m.p. 34–35° (micro-block). A small sample sublimed at 75–80° at 0.1 mm. pressure melted at 33–34° (micro-block).

Anal. Calcd. for $C_{10}H_{12}O_4$: C, 61.21; H, 6.17. Found: C, 61.11; H, 6.07.

6-Ethoxy-5-methoxy-2-methylhydroquinone Dimethyl Ether (XIX).—One gram of 6-ethoxy-5-methoxy-2-methylbenzoquinone was dissolved in a mixture of 5 ml. of ethanol and 1 ml. of water. With stirring, 0.4 g. of sodium hydrosulfite was added in portions. The benzoquinone was reduced to the hydroquinone. An additional 0.1 g. of sodium hydrosulfite was added. Then, 6.3 g. of dimethyl sulfate was added. Finally, a solution of 2 g. of sodium hydroxide in 8 ml. of water was added over a 2.5-hour period at room temperature with stirring. Some more sodium hydrosulfite was added during this time. Stirring was continued at room temperature overnight. Water was added to the reaction mixture; it was extracted three times with ether. The combined ether extract was washed three times with 2 *N* sodium hydroxide solution and four times with salt water. After drying over anhydrous magnesium sulfate, the ether extract was concentrated under reduced pressure to leave 0.7 g. of crude dimethyl ether of 6-ethoxy-5-methoxy-2-methylhydroquinone as a pale yellow oil.

4-Ethoxy-2-methyl-3,5,6-trimethoxybenzyl Chloride.—The 0.7 g. of 6-ethoxy-5-methoxy-2-methylhydroquinone dimethyl ether (XIX) was added to a mixture of 35 ml. of concentrated hydrochloric acid and 1.0 ml. of formaldehyde. The suspension was stirred while gaseous hydrogen chloride was introduced. Gentle heating was maintained for 2.5 hours. The reaction mixture was diluted with water and the mixture was extracted three times with ether. The ether extract was washed four times with water. After drying over anhydrous magnesium sulfate, the ether extract was concentrated under reduced pressure to leave 0.75 g. of crude 4-ethoxy-2-methyl-3,5,6-trimethoxybenzyl chloride as a yellow colored oil. A small sample in ethanol–water solution gave a silver chloride precipitate when treated with silver nitrate.

4-Ethoxy-2-methyl-3,5,6-trimethoxybenzyl Cyanide (XX).—To 0.3 g. of potassium cyanide dissolved in 4 ml. of water was added 0.75 g. of 4-ethoxy-2-methyl-3,5,6-trimethoxybenzyl chloride in 30 ml. of ethanol. The mixture was refluxed with stirring for 4.5 hours. The ethanol was removed under reduced pressure. Water was added and the mixture was extracted three times with ether. The combined ether extract was washed three times with water. After drying over anhydrous magnesium sulfate, the ether extract was concentrated under reduced pressure to leave 0.75 g. of 4-ethoxy-2-methyl-3,5,6-trimethoxybenzyl cyanide as a nearly colorless oil.

4-Ethoxy-2-methyl-3,5,6-trimethoxyphenylacetic Acid (XII).—A solution of 0.75 g. of 4-ethoxy-2-methyl-3,5,6-trimethoxybenzyl cyanide (XX) and 17.9 g. of potassium hydroxide in 160 ml. of 50% methanol was refluxed with stirring under nitrogen for 11.5 hours. Ammonia was slowly evolved. The reaction mixture was concentrated under reduced pressure to remove methanol. More water was added to the concentrate which was then extracted with four portions of chloroform. The dried chloroform extract yielded 0.42 g. of pale yellow oil. The alkaline liquor was cooled and acidified to *p*H 2 with concentrated hydrochloric acid; an oil separated. The mixture was extracted with four portions of chloroform. After drying over anhydrous magnesium sulfate. the combined chloroform extract was concentrated under reduced pressure to leave 0.34 g. of nearly colorless oil which rapidly crystallized. The 4-ethoxy-2-methyl-3,5,6-trimethoxyphenylacetic acid was purified by sublimation. The product was distilled at 145–150° at 0.1 mm. pressure to yield a colorless oil which readily crystallized, m.p. 87–89° (micro-block).

Anal. Calcd. for $C_{14}H_{20}O_6$: C, 59.14; H, 7.09. Found: C, 58.89; H, 7.11.

Acknowledgment.—We are indebted to Dr. Nelson R. Trenner and Mr. Byron H. Arison for n.m.r. analyses, Mr. Richard N. Boos for microanalytical data, and Mr. Robert Walker for certain infrared measurements.

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Substitution, Oxidation and Group Participation in the Bromination of Indoles

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The structure of the dibromoskatole, resulting from the action of N-bromophthalimide on skatole in benzene, has been proved to be II by acid hydrolysis to 6-bromo-3-methyloxindole (III), an isomer of the bromination product IX of 3-methyloxindole, and by oxidative degradation to 2-acetamino-4-bromobenzoic acid (V). Electrophilic substitution of indoles in the 6-position has been shown, in the case of 2-phenylskatole (VI), to proceed *via* an unstable yellow perbromide intermediate (formulated as VII), rearranging rapidly to the 6-bromo compound VIII. In aqueous media, intramolecular participation of the carboxyl group of X, possibly by displacement on a bromonium intermediate XVI, has led to (5-bromo)-dioxindolespirolactones of type XI which have been hydrogenolyzed to oxindole-3-propionic acid (XII).

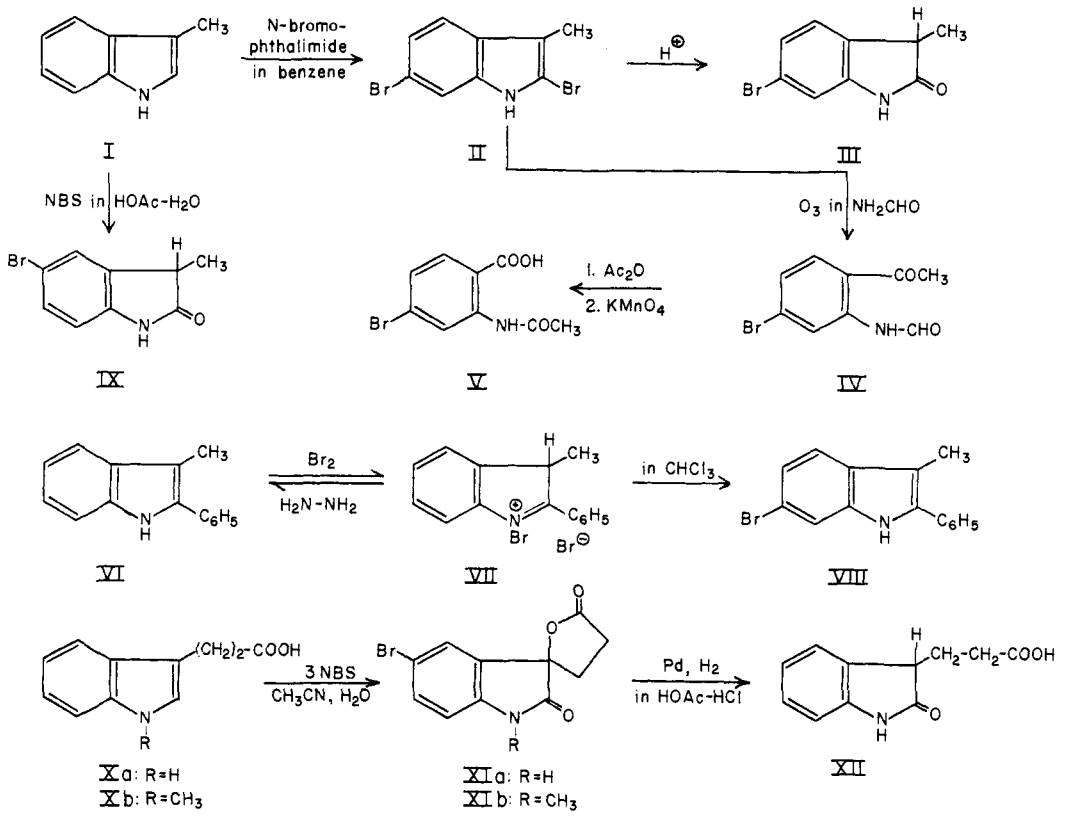
The action of brominating agents upon indoles has received comparatively little attention,[1,2] and has been studied mainly in non-aqueous media. The effect of water on the course of the reactions has not been generally recognized. In connection with the selective cleavage of tryptophyl peptide bonds by positive halogen[3-5] we had occasion to

(1) *Cf.* R. Brunck, *Ann.*, **272**, 206 (1893).

(2) W. C. Sumpter and F. M. Miller, "Heterocyclic Compounds with Indole and Carbazole Systems," Interscience Publishers, Inc., New York, N. Y., 1954, pp. 29–81.

(3) A. Patchornik, W. B. Lawson and B. Witkop, THIS JOURNAL, **80**, 4748 (1958).

CHART I



study this problem. Some observations on the reactions of indoles with halogenating agents in both aqueous and non-aqueous media are reported herein.

Bromination in Non-aqueous Media.—The recorded instances of the bromination of indoles in non-aqueous solvents show that, in general, substitution eclipses oxidation of the indole nucleus, that the first bromine atom enters position 3, if accessible, and the second bromine substitutes either position 2 or 6, depending upon the presence of other substituents. With one exception, position 2 is favored over 6. Indole and 2-methylindole are converted to 3-bromo derivatives by dioxane dibromide in dioxane solution.[6] The reaction of N-bromosuccinimide in dioxane with lysergic acid derivatives leads to 2-bromo derivatives.[7]

Also in glacial acetic acid bromosuccinimide converts simpler 3-substituted indoles such as skatole and indole-3-propionic acid to the 2-bromo derivatives.[8] By contrast, bromine in acetic acid brominates 3-carbethoxyindole in the 6-position.[9] The lack of reactivity of the 2-position in this case is probably due to the proximity of an electron-attracting group which inactivates the 2,3-double bond. Plant and Tomlinson[10] reported nuclear 4- or 6-bromination of 2,3-diphenylindole and 2-phenylskatole with bromine in acetic acid. The location of the bromine atom in the product from 2,3-diphenylindole was later proved to be the 6-position.[11]

We obtained further support for this course of bromination by determining the structure of a dibromoskatole first prepared by Putokhin.[12] The dibromide II (λ_{max} 278 mμ, ϵ 8,800), obtained by treatment of skatole (I) with 2 moles of N-bromophthalimide in boiling benzene, gave a negative Ehrlich reaction and was converted into a brominated 3-methyloxindole (III, $\lambda\lambda_{max}$ 285, ϵ 1,800; 253 mμ, ϵ 6,800) on refluxing in hydrochloric acid–dioxane. These facts, together with the observation that only one-fourth of the two bromine atoms were removed after 20 min. in boiling aniline,[13] indicate that one bromine atom is in the 2-position, that the other substitutes the benzene ring, and that the methyl group is unsubstituted. In addition, the nuclear magnetic resonance spectrum of the dibromoskatole agreed with both an α-bromoindole and an intact 3-methyl group.[14] Ozonization of II[15] furnished a bromo-*o*-aminoacetophenone (IV), which was converted by acetylation and permanganate oxidation to 4-bromo-2-acetaminobenzoic acid (V),[16] identical

(4) A. Patchornik, W. B. Lawson and B. Witkop, THIS JOURNAL, **80**, 4747 (1958).
(5) L. K. Ramachandran and B. Witkop, *ibid.*, **81**, 4028 (1959).
(6) L. A. Yanovskaya, *Doklady Akad. Nauk, U.S.S.R.*, **71**, 693 (1950).
(7) F. Troxler and A. Hofmann, *Helv. Chim. Acta*, **40**, 2160 (1957).
(8) R. L. Hinman, private communication.
(9) R. Majima and M. Kotake, *Ber.*, **63**, 2237 (1930).
(10) S. G. P. Plant and M. L. Tomlinson, *J. Chem. Soc.*, 955 (1933).
(11) C. F. Koelsch, THIS JOURNAL, **66**, 1983 (1944).
(12) N. I. Putokhin, *J. Gen. Chem. USSR*, **15**, 332 (1945).
(13) A Patchornik and S. E. Rogozinski, *Anal. Chem.*, **31**, 985 (1959).
(14) We are indebted to Dr. L. A. Cohen for his advice and interpretation.
(15) *Cf.* B. Witkop, *Ann.*, **556**, 103 (1944).

with a synthetic sample. This establishes the structure of the dibromide as 2,6-dibromoskatole (II).

Plant and Tomlinson[10] observed the formation of a yellow intermediate during the bromination in acetic acid of 2-phenylskatole (VI) to 4- or 6-bromo-2-phenylskatole (VIII), and noted that the intermediate reacted with potassium iodide. We succeeded in isolating the yellow intermediate by carrying out the bromination in a mixture of cyclohexane and acetic acid. Recrystallization was accomplished by the rapid addition of pentane to a freshly prepared chloroform solution of the intermediate. The analytical data and oxidation equivalent are in agreement with the formula $C_{15}H_{13}NBr_2$, a complex of bromine with 2-phenylskatole (VII). The role of the dibromide as an intermediate is supported by the facts that (i) in solution it is rapidly rearranged to the final product, 6-bromo-2-phenylskatole (VIII), and (ii) that it is readily reduced to the starting material VI by hydrazine. In the solid state the intermediate VII is fairly stable and may be kept for a few weeks at $-20°$. At room temperature it darkens after a day or two. In solution its stability varies with the polarity of the solvent. Its rearrangement to the 6-bromo compound is easily assayed *in situ* by following the spectral changes at 312 mμ. This rearrangement follows first-order kinetics and may well be an intramolecular process. In chloroform (0.75% EtOH) the half-life of the intermediate is 47 min. at 20°, while in acetonitrile it is only of the order of 2 min.

The spectral properties of the intermediate agree with structure VII, the "perbromide" of the indolenine tautomer of 2-phenylskatole. The ultraviolet spectrum (in chloroform) shows $\lambda\lambda_{max}$ at 258 (ϵ 9,070) and 342 mμ (ϵ 17,400). The infrared spectrum in the 6–7 μ range is strikingly similar to the spectrum of 3,3-dimethyl-2-phenylindolenine hydrochloride[17] and quite different from that of 2-phenylskatole.

If intermediates of type VII are representative of the general course of nuclear bromination of indoles in non-aqueous media, the substitution of the 6-position is easily explained: The electron-attracting indoleninium group deactivates positions 5 and 7 for electrophilic substitution, while the 6-position is activated by a *p*-alkyl substituent.[18]

Bromination in Aqueous Media.—The little information in the literature on the action of halogens on indoles in aqueous medium indicates that, in general, oxidation rather than substitution of the heterocyclic ring takes place[19a]; N-alkylindoles are oxidized to 3,3-dibromo-N-alkoxindoles by the action of sodium hypobromite.[19b] Reduction of these compounds provides a route to N-alkyloxindoles.[20] Oxidation of skatole with aqueous iodine gave an unidentified iodine-free compound, possibly 3-methyldioxindole or the hydrate of oxyskatole.[21] 2-Methylindole and indole itself were converted by aqueous iodine to 3-iodo derivatives. Under slightly different conditions indole was oxidized to indigo.[19a,21]

When skatole (I) was treated with 2.5 moles of N-bromosuccinimide in aqueous acetic acid, a bromo-3-methyloxindole[22] IX, λ_{max} 254 mμ (ϵ 12,200), was isolated. This material was different from the 6-bromo-3-methyloxindole (III), obtained by hydrolysis of 2,6-dibromoskatole, but identical with 5-bromo-3-methyloxindole, prepared by bromination of 3-methyloxindole (atroxindole) in aqueous acetic acid. The assignment of the bromine atom to the 5-position is based upon the analogy with the bromination of oxindole in water which leads to 5-bromooxindole.[23]

The reaction of N-bromosuccinimide with indole-3-propionic acid (Xa), a close model to tryptophan, in a mixture of aqueous acetic buffer, *p*H 4, and alcohol or acetone, led to the uptake of 3 moles of N-bromosuccinimide within a few minutes. Titration of bromide ion liberated in the process of addition of N-bromosuccinimide showed that in its early stages the reaction was primarily an oxidation. After 2 moles of N-bromosuccinimide had reacted with indole-3-propionic acid, about 1.9 moles of bromide ion was formed. The third mole of N-bromosuccinimide was involved in a substitution reaction, since only two moles of bromide ion were found after the consumption of three moles of N-bromosuccinimide.

When indole-3-propionic acid was treated with three moles of N-bromosuccinimide on a preparative scale, a crystalline neutral compound, $C_{11}H_8NO_3Br$, was obtained. The spectrophotometric data, $\lambda\lambda_{max}^{EtOH}$ 308 (ϵ 1,600) and 260 mμ (ϵ 10,200) and $\lambda\lambda_{max}^{KBr}$ 5.62 μ (five-membered lactone) and 5.76 μ (oxindole), suggested the structure XIa of a spirolactone of a dioxindole-3-propionic acid with a bromine atom in the 5-position in analogy to the product IX from skatole. The conversion of XIa to oxindole-3-propionic acid (XII)[24] by hydrogenolysis confirmed the presence of a benzylic oxygen function as part of the lactone ring.

The reaction between 3-substituted indoles in aqueous media and N-bromosuccinimide or bromine is immeasurably fast for ordinary technique. The indole absorption maximum in the ultraviolet decreases instantaneously, and there is a rapid release of hydrogen bromide. N-Bromoacetamide, on the other hand, reacts at a measurable rate.[5,25] A possible pathway from skatole to 3-

(16) P. Friedländer, S. Bruckner and G. Deutsch, *Ann.*, **388**, 23 (1912).

(17) B. Witkop, J. B. Patrick and H. M. Kissman, *Ber.*, **85**, 949 (1952).

(18) Enzymatic hydroxylation of indoles in the 6-position, as observed in the conversion of tryptamine to 6-hydroxytryptamine [J. B. Jepson, S. Udenfriend and P. Zaltzman, *Federation Proc.*, **18**, 254 (1959)] may be mentioned in this connection.

(19) (a) L. K. Ramachandran and P. S. Sarma, *J. Sci. Ind. Research*, **11B**, 161 (1952); *cf.* L. K. Ramachandran, *Chem. Revs.*, **56**, 205 (1956). (b) E. Fischer and O. Hess, *Ber.*, **17**, 559 (1884).

(20) H. G. Colman, *Ann.*, **248**, 116 (1888); A. Michaelis, *Ber.*, **30**, 2809 (1897).

(21) H. Pauly and K. Gundermann, *ibid.*, **41**, 3999 (1908). *Cf.* F. Troxler and A. Hofmann, *Helv. Chim. Acta*, **42**, 793 (1959)), for the conversion of lysergic acid derivatives into dioxindoles with calcium hypochlorite.

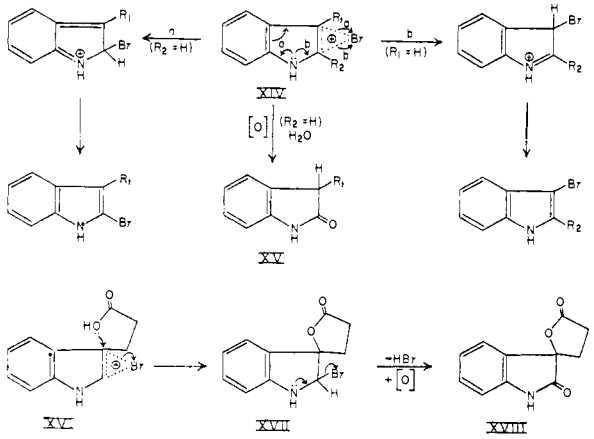
(22) This compound was first prepared by Dr. Arthur A. Patchett.

(23) W. C. Sumpter, F. M. Miller and L. N. Hendrick, THIS JOURNAL, **67**, 1656 (1945); *cf.* the analogous 5-bromination of dihydroindoles: A. P. Terent'ev, M. N. Preobaženskaja, *Ž. obšč. Chim.*, **29**, 317 (1959).

(24) P. L. Julian and H. C. Printy, THIS JOURNAL **75**, 5301 (1953).

(25) A. Patchornik, W. B. Lawson, E. Gross and B. Witkop, *ibid.*, **82**, 5923 (1960).

CHART II



methyloxindole (XV) is shown in Chart II. The conversion of the postulated bromonium intermediate XIV, tautomeric with a 3-bromo-3-methylindoleninium ion, *via* the hydrated form, into XV, is analogous to the acid-catalyzed conversion of 3-haloindoles to oxindole.[26]

In indole-3-propionic acid (Xa), 1,5-interaction of the carboxyl group with the 3-position in the hypothetical intermediate XVI gives rise to a lactonic intermediate XVII. A second mole of N-bromosuccinimide is required to oxidize the bromo- or hydroxy-indoline (equivalent to an indolenine) to the lactone XVIII of dioxindole-3-propionic acid. There is a close analogy between this reaction and the intramolecular participation of functional groups in the halogenation of unsaturated acids,[27] esters[27,28] and amides.[29] Further bromination of XV and XVIII occurs very easily, and usually the 5-bromo compounds IX and XIa are isolated as the major products. The possibility that substitution of the hydrogen atom on the indole nitrogen by bromine might give a reactive N-bromo intermediate was excluded by the smooth conversion of N-methylindole-3-propionic acid (Xb) to the N-methylated bromo-lactone XIb in high yield with three moles of N-bromosuccinimide.

At the present time the hypothetical brominium intermediate XIV rationalizes best the three possible pathways: (i) formation of 2-bromo derivatives (R_2 = H, arrows a); (ii) of 3-bromo derivatives (R_1 = H, arrows b) and (iii) hydrolysis to an oxindole in aqueous medium.

The participation of the carboxyl group of the propionic acid side chain is pictured in XVI as a concerted displacement reaction on a transient bromonium intermediate, in preference to an elimination on a more stable 3-bromoindolenine.[30] Additional factors, such as electron-attracting or -releasing substituents at the indole imino group, or in the pyrrole and benzene rings, may exercise directing influences so that more than one mechanism may obtain in different cases.

There is a similar dualism in the formation of 2- and 3-hydroxyindoles with peracids, *e.g.*, the oxidation of skatole to 3-methyloxindole with peracetic acid, and to formaminoacetophenone (probably *via* 3-hydroxy-3-methylindolenine) with perbenzoic acid. In this and other cases, β-hydroxyindolenines have been favored as intermediates in the oxidation of indoles[31] in preference to the postulation of 2,3-oxiran intermediates.[32]

This transformation of indole-3-propionic acid (Xa) into the bromolactone XIa with N-bromosuccinimide suggested the application of neighboring group effects to the selective cleavage of C-tryptophyl peptide bonds. The detailed studies on model peptides underlying the application to proteins[5] are described in the following paper.[25]

Experimental[33]

2,6-Dibromoskatole (II).—Skatole was brominated by the use of N-bromophthalimide in boiling benzene according to Putokhin.[12] The dibromo derivative was obtained as very pale tan (not green, as reported) plates from aqueous ethanol, decomposing at about 100° (reported[12] m.p. 100–102°), $\lambda_{max}^{C_2H_5OH}$ (ϵ) 288 mμ (8400), 277 mμ (8800).

Anal. Calcd. for $C_9H_7NBr_2$: C, 37.40; H, 2.44; N, 4.85; Br, 55.31. Found: C, 37.56; H, 2.52; N, 4.91; Br, 54.97.

2-Acetamino-4-bromobenzoic Acid (V). **A.** *Via* **Ozonization of 2,6-Dibromoskatole.**—A solution of 580 mg. (2 mmoles) of dibromoskatole in a mixture of 5 ml. of formamide[15] and 15 ml. of acetic acid was treated at room temperature with a stream of ozone (1 mg. per sec.) for 110 sec. The amount of ozone absorbed was 98 mg. (2.04 mmoles), as determined by iodometric titration of the gas leaving the reaction mixture. The solution was extracted with ether, and the extract evaporated to dryness with an air stream. After addition of acetic acid containing some hydrochloric acid, possible peroxides were destroyed by addition of a little sodium thiosulfate–potassium iodide solution. The solution was heated to boiling and let stand overnight. After neutralization with sodium hydroxide, the product was extracted with ether and precipitated from the dried ethereal solution as its hydrochloride by the addition of gaseous hydrogen chloride. The crude 2-amino-4-bromoacetophenone hydrochloride (137 mg., 30%) was acetylated in the cold with acetic anhydride. The resulting neutral 2-acetamino-4-bromoacetophenone without further purification was refluxed with a solution containing 460 mg. of potassium permanganate and 500 mg. of magnesium sulfate in 50 ml. of water for 6 hr. After a small amount of residual permanganate had been destroyed by the addition of oxalic acid, the manganese dioxide was removed by filtration, and the solution acidified with hydrochloric acid. Extraction with methylene chloride, followed by evaporation of the extract, gave 60.9 mg. (40%) of 2-acetamino-4-bromobenzoic acid (V), m.p. 209–214°. Recrystallization from aqueous ethanol gave material having m.p. 212–217°. A mixed melting point with an authentic sample, m.p. 214–218°, was 212–218°, and the infrared spectra of the two (Nujol) were identical.[34]

(26) R. Weissgerber, *Ber.*, **46**, 651 (1913).

(27) R. T. Arnold, M. deMoura Campos and K. L. Lindsay, THIS JOURNAL, **75**, 1044 (1953).

(28) W. P. Miller, Ph.D. Thesis, University of Minnesota, 1957.

(29) L. Goodman and S. Winstein, THIS JOURNAL, **79**, 4788 (1957).

(30) Likewise, the concerted displacement reaction of a phenolic hypobromite (path a) has been favored over an elimination of a tribromoquinol (path b) in the formation of the spirolactone from phloretic acid and three moles of N-bromosuccinimide: G. L. Schmir, L. A. Cohen and B. Witkop, *ibid.*, **81**, 2228 (1959).

(31) B. Witkop and H. Fiedler, *Ann.*, **558**, 91 (1947); *cf.* A. Ek, H. Kissman, J. B. Patrick and B. Witkop, *Experientia*, **8**, 36 (1952).

(32) There is ony one case of an indole-2,3-oxiran in the literature and that requires confirmation, namely, the "epoxide" of 6-nitro-2,3-dimethylindole; C. M. Atkinson, J. C. E. Simpson and A. Taylor, *J. Chem. Soc.*, 165 (1954).

(33) All melting points are corrected. The microanalyses were performed by Dr. W. C. Alford and associates of the Microanalytical Services Unit of this Laboratory.

(34) The melting point of this material was lowered considerably by admixture of 2-acetamino-5-bromobenzoic acid, m.p. 221–224°, reported m.p. 224°; *cf. Ber.*, **22**, 1645 (1889)], and the infrared spectra of the two isomers were quite different.

B. By Synthesis.—Nitration[35] of *p*-bromotoluene gave 2-nitro-4-bromotoluene, m.p. 43–45° (reported[35] m.p. 43°, 47° resp.), which upon oxidation with permanganate[36] gave 2-nitro-4-bromobenzoic acid, m.p. 160–164° (reported[37] m.p. 163°). Reduction of this with hydrogen in the presence of 10% palladium-on-charcoal gave 4-bromoanthranilic acid, m.p. 219–222° (reported[37] m.p. 222°). Treatment with acetic anhydride produced 2-acetamino-4-bromobenzoic acid, m.p. 217–221° (reported[16] m.p. 217°).

6-Bromo-3-methyloxindole (III).—A solution of 600 mg. (2.17 mmoles) of 2,6-dibromoskatole in a mixture of 30 ml. of dioxane and 30 ml. of 3.0 *N* sulfuric acid was refluxed for 18 hr. After dilution with 59 ml. of water, the solution was extracted twice with 50-ml. portions of ethyl acetate. The organic layers were washed with 1.0 *N* potassium bicarbonate and saturated sodium chloride and evaporated to dryness. Crystallization of the residue from benzene gave 331 mg. (71%) of yellow needles of 6-bromo-3-methyloxindole, m.p. 167–176°. Recrystallization from benzene afforded an analytical sample as very pale yellow needles, m.p. 175–176° (shrinking from 165°); $\lambda\lambda_{max}^{C_2H_5OH}$ 286 (ϵ 1,830), 252 mμ (ϵ 6,850).

Anal. Calcd. for C_9H_8NOBr: C, 47.81; H, 3.57; N, 6.20. Found: C, 47.52; H, 3.74; N, 6.27.

5-Bromo-3-methyloxindole (IX). A. By the Treatment of Skatole with N-Bromosuccinimide in Aqueous Medium.—To a suspension of 2.62 g. (0.02 mole) of skatole in a mixture of 30 ml. of acetic acid and 20 ml. of water was added 8.90 g. (0.05 mole) of N-bromosuccinimide with stirring over a period of 5 minutes. During the addition, the temperature was kept below 50°. The solution was evaporated at room temperature, and the residue taken up in chloroform. After several extractions with 1.0 *N* potassium bicarbonate, the chloroform was evaporated, and the residue recrystallized from 25 ml. of benzene to give 1.42 g. (32%), of the oxindole IX, m.p. 172–178°. Recrystallization raised the m.p. to 183–186°; λ_{max}^{EtOH} 253 (ϵ 12,500), shoulder at 280–290 mμ. There was a large depression observed on admixture with the isomeric 6-bromo-3-methyloxindole (m.p. 176°).

Anal. Calcd. for C_9H_8NOBr: C, 47.81; H, 3.57; N, 6.20. Found: C, 47.95; H, 3.57; N, 6.07.

B. By Bromination of 3-Methyloxindole.—Treatment of 500 mg. (3.35 mmoles) of 3-methyloxindole with 6.04 mg. (3.35 mg.) of N-bromosuccinimide in 50% acetic acid gave 350 mg. (42%) of 5-bromo-3-methyloxindole, m.p. 182–185°, after recrystallization from benzene. A mixture with the compound prepared by method A melted at 183–186°.

Bromine Adduct VII of 2-Phenylskatole (VI).—To an ice-cold solution of 1.0 g. (4.85 mmoles) of 2-phenylskatole (VI) in a mixture of 8 ml. of glacial acetic acid and 4 ml. of cyclohexane, 2 ml. of acetic acid containing 775 mg. (4.85 mmoles) of bromine was added. The yellow precipitate which immediately formed was filtered, washed with cyclohexane and ether and dried *in vacuo* to give 1.38 g. (78%) of the intermediate VII, m.p. 88–90° dec. Recrystallization, carried out by the addition of pentane to a freshly prepared and filtered chloroform solution of VII at 0°, gave canary-yellow microcrystals, m.p. 92–95° dec., λ_{max} 342 mμ (ϵ 9,700) and 258 mμ (ϵ 17,400) in chloroform.

Anal. Calcd. for $C_{15}H_{13}NBr_2$: C, 49.07; H, 3.57; N, 3.82; mol.wt., 367. Found: C, 48.37; H, 3.76; N, 3.92; mol.wt., 386 (iodometric titration).

Reconversion of the Adduct VII to 2-Phenylskatole (VI).—A solution of 503 mg. (1.38 mmoles) of the dibromo adduct VII in a mixture of 3 ml. of hydrazine hydrate and 10 ml. of ethanol was heated to the boiling point and let stand for 1 hour at room temperature; 100 ml. of 0.05 *N* hydrochloric acid was added, and the solution was extracted with ether. Evaporation of the ether and crystallization from ether–petroleum ether gave 130 mg. (46%) of 2-phenylskatole, m.p. 88–91° (m.m.p. with authentic material, m.p. 90–92°, was 88–91°), λ_{max} 309 mμ (ϵ 20,200) and 229 mμ (ϵ 28,000) in acetonitrile.

6-Bromo-2-phenylskatole (VIII). A. By Direct Bromination.—The bromination of 2-phenylskatole (VI) in acetic acid according to Plant and Tomlinson gave 6-bromo-2-phenylskatole (VIII), m.p. 146–147° (reported[10] m.p. 147°), λ_{max} 313 mμ (ϵ 23,700) and 240 mμ (ϵ 31,800) in CH_3CN.

B. By Rearrangement of the Perbromide VII.—A solution of 460 mg. (1.2 mmoles) of intermediate VII in 50 ml. of acetonitrile was stirred overnight and evaporated. Crystallization of the residue from cyclohexane gave 161 mg. (47%) of 6-bromo-2-phenylskatole, m.p. 135–145°. Recrystallization raised the m.p. to 144–147°.

Kinetics of the Conversion of Perbromide VII to 6-Bromo-2-phenylskatole.—Using a Nylon adder-mixer,[38] a small quantity of the perbromide VII was added to the solvent in a quartz cuvette. The optical density of the solution was measured as a function of time at 312 mμ. The initial concentration of intermediate was calculated from the optical density of the product after the reaction was essentially complete, and with this information, together with the extinction coefficients of the intermediate and product, the concentration of product at any time was calculated from the equation: $C_p = (O - O_s)/(\epsilon_p - \epsilon_i)$, where O is the optical density at time t, O_s is the optical density at the start of the reaction, ϵ_p is the extinction coefficient of the product and ϵ_p is the extinction coefficient of the intermediate. The reaction followed first-order kinetics at least up to 90% completion. The half-life of the intermediate in reagent chloroform (0.75% ethanol) was 47 min., and in acetonitrile was *ca.* 2 min. at 20°.

5-Bromodioxindole-3-propionic Acid Lactone (XIa).—A solution of 2.84 g. (16 mmoles) of N-bromosuccinimide in 25 ml. of acetonitrile was added dropwise to a well-stirred solution of 1.0 g. (5.3 mmoles) of indole-3-propionic acid in a mixture of 25 ml. of acetonitrile and 50 ml. of 0.4 *M* acetate buffer, *p*H 4. After 10 min. at room temperature, 20 ml. of 0.4 *M* ammonium formate buffer of *p*H 4 was added to destroy residual N-bromosuccinimide. Treatment with 140 ml. of 1.0 *N* potassium bicarbonate, followed by cooling to 0°, produced 815 mg. (52%) of colorless needles of the lactone, m.p. 195–199°. A further 185 mg. was obtained from the aqueous solution by ether extraction to give a total yield of 64%. An analytical sample, m.p. 199.5–200.5°, was obtained as fine needles by recrystallization from methanol–water. The ultraviolet spectrum exhibited maxima at 261 mμ (log ϵ 4.01) and 309 mμ (log ϵ 3.21).

Anal. Calcd. for $C_{11}H_8NO_3Br$: C, 46.83; H, 2.86; N, 4.97; Br, 28.33; mol. wt. (sapon. equiv. based on consumption of alkali), 282. Found: C, 46.70; H, 2.92; N, 4.92; Br, 28.46; mol. wt., 300.

Oxindole-3-propionic Acid (XII) from the Bromolactone XIa.—A solution of 282 mg. (1 mmole) of the bromolactone XIa in a mixture of 25 ml. of acetic acid and 0.5 ml. of concd. hydrochloric acid was hydrogenated in the presence of 50 mg. of 10% palladium-on-charcoal at room temperature and atmospheric pressure. Uptake of hydrogen amounted to 1 mmole during the first hour, and then slowed down considerably. After 1.5 days, when the uptake was slightly more than 2 mmoles, the solution was filtered and lyophilized. The residue was dissolved in aqueous potassium bicarbonate, extracted with ether to remove neutral components and then acidified and extracted with ethyl acetate. Evaporation gave a crystalline mass which was recrystallized from water to give 80 mg. (39%) of oxindole-3-propionic acid, m.p. 166–169°. A mixed melting point with an authentic sample of oxindole-3-propionic acid, m.p. 164–168° (prepared by the method of Julian and Printy[24]), was 164–168°, and the infrared spectra of the two samples were identical. In the ultraviolet, oxindole-3-propionic acid had a maximum at 249 mμ (log ϵ 3.73) as well as a shoulder in the range 270–280 mμ (log ϵ 3.09).

N-Methylindole-3-propionic Acid (Xb).—Five grams (0.025 mole) of methyl indole-3-propionate[39] was methylated in liquid ammonia solution with methyl iodide and sodamide by the method of Potts and Saxton.[40] The crude, oily methyl N-methylindole-3-propionate, which possessed no NH peak in the infrared, was hydrolyzed by hot aqueous methanolic sodium hydroxide to give 3.1 g.

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(57%) of N-methylindole-3-propionic acid, m.p. 122–124°. Recrystallization from methanol–water afforded an analytical sample, m.p. 124–126°.

Anal. Calcd. for $C_{12}H_{13}NO_2$: C, 70.91; H, 6.45; N, 6.89. Found: C, 70.67; H, 6.30; N, 7.03.

5-Bromo-N-methyldioxindole-3-propionic Acid Lactone (XIb).—A solution of 1.63 g. (9 mmoles) of N-bromosuccinimide in 20 ml. of acetonitrile was added to a solution of 609 mg. (3 mmoles) of N-methylindole-3-propionic acid (Xb) in a mixture of 20 ml. of acetonitrile and 40 ml. of 0.4 *M* acetate buffer, *p*H 4. After 1 hour at room temperature, the acetonitrile was evaporated *in vacuo*, 50 ml. of 1.0 *N* potassium bicarbonate added and the mixture extracted twice with 25-ml. portions of ethyl acetate. The dried (sodium sulfate) extract was evaporated, and the residue crystallized by addition of ether. The yield of crude, dry lactone was 930 mg. (78%). After recrystallization from methanol, 530 mg., m.p. 161–163°, was obtained.

Anal. Calcd. for $C_{12}H_{10}NO_3Br$: C, 48.67; H, 3.40; N, 4.63; Br, 26.99. Found: C, 48.93; H, 3.51; N, 4.79; Br, 26.62.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MD.]

The Use of N-Bromosuccinimide and N-Bromoacetamide for the Selective Cleavage of C-Tryptophyl Peptide Bonds in Model Peptides and Glucagon[1]

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Fifteen model peptides III–XVII (Table I) derived from indole-3-propionic acid or N-carbobenzyloxy- and N-benzoyltryptophan have been subjected to cleavage by N-bromosuccinimide and N-bromoacetamide in aqueous acetic–formic acid buffer systems as well as in 10.0 *M* lithium acetate solution of *p*H 4.0. Maximal yields of 60–90% of liberated amino acids have been obtained. Of the 28 peptide bonds present in the pancreas hormone glucagon, only the one following the single tryptophan present was cleaved with liberation of the known tetrapeptide Leu-Met-Asp-Thr.

The reactivity of proteins with brominating agents has been known for some time,[4] and the reaction of amino acids with hypobromite, hypochlorite[5] or with N-bromosuccinimide[6] has been used as a method for decarboxylation of amino acids[7,8] and for the analysis of terminal carboxyl groups on peptides and proteins.[6] On the other hand, the action of sodium hypobromite on simple peptides converts the N-terminal amino acid to a substituted nitrile,[9] an interesting method which so far has found little application. The prolonged action of excess hypohalite in all of these reactions causes many side reactions. For instance, after treatment of ovalbumin with sodium hypobromite, cystine, lysine, tyrosine and tryptophan were destroyed.[9c]

A novel, much milder and more selective use of positive halogen for the cleavage of tryptophyl peptides was suggested by the intramolecular participation reaction which occurs in the transformation of indole-3-propionic acid (I) to the lactone II of 5-bromodioxindole-3-propionic acid during oxidative bromination with N-bromosuccinimide.[1,10] This paper describes the conditions for the selective cleavage of C-tryptophyl peptide bonds in model compounds and in the polypeptide hormone glucagon.[1,11]

When carbobenzyloxytryptophan (Cbz-Try) was treated with successive increments of N-bromosuccinimide (NBS) the initial indole spectrum (Fig. 1A, I), after the addition of 1.53 moles of NBS, changed to an oxindole spectrum (Fig. 1A, II) similar to that of the bromospiroöxindole II ($\lambda\lambda_{max}^{EtOH}$ 261 (ϵ 10,300), 309 mμ (ϵ 1,630)). Figure 1B, I, II, III presents these same changes expressed as difference spectra, whereby the reference cell contains the starting material in the same concentration as the observation cell.

The ability of peptide bonds to participate in the lactonization reaction was demonstrated, *e.g.*, with N-carbobenzyloxy-L-tryptophylglycine III. Figure 2 shows that the decrease in optical density at 280 mμ and the liberation of glycine go parallel and reach a maximum after the addition of 1.5–2.5 moles of N-bromosuccinimide.

In an alcoholic aqueous acetate buffer of *p*H 4, glycine was liberated in 39% yield. The maximum yield of glycine was obtained between *p*H 3 and 5. The carbobenzyloxy group in III apparently adversely affects the cleavage,[12] since

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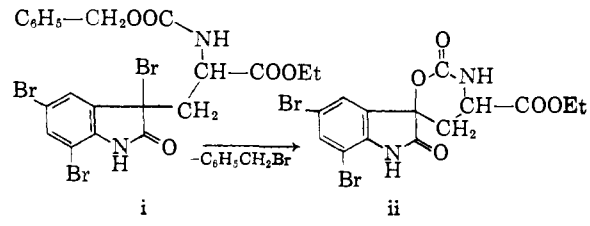
(12) Apart from the fact that benzyl ethers are oxidized by N-bromosuccinimide [D. G. Markees, *J. Org. Chem.*, **23**, 1490 (1958)], N-carbobenzyloxytryptophan ethyl ester with N-bromosuccinimide in acetic acid gave a labile bromo compound which on recrystallization from petroleum ether lost benzyl bromide (smell). The analysis of the resulting colorless needles, m.p. 235°, agreed with the formula $C_{14}H_{12}N_2O_5Br_4$, possibly the 2-ketotetrahydrooxazine ii formed *via* the labile bromoöxindole i.



i ii