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CONVERSION OF 3-ALKYLINDOLE TO 3-ALKYL-2-AMINO-3H-INDOL-3-OL VIA ALKYL 2-FORMAMIDOPHENYL KETONE

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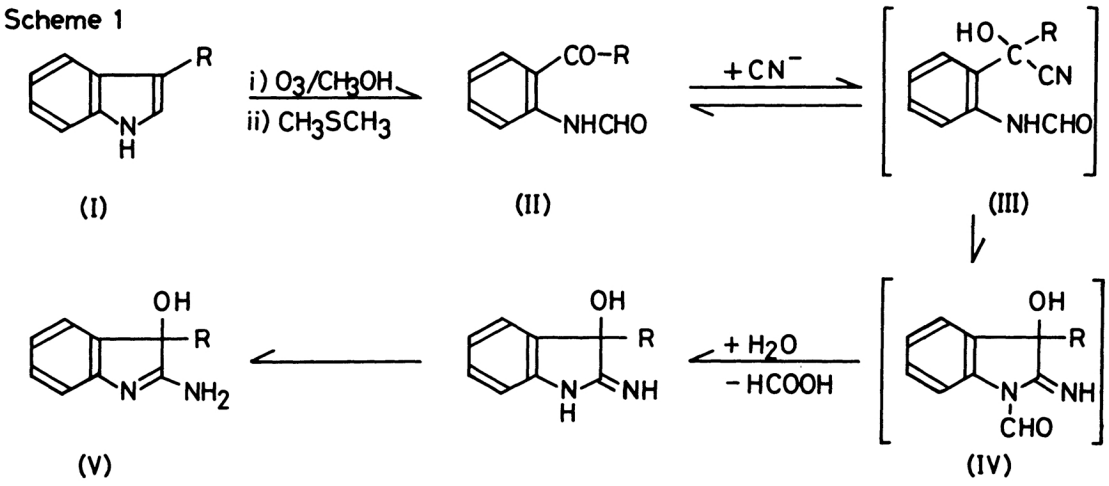
A new method for conversion of 3-alkylindoles to 3-alkyl-2-amino-3H-indol-3-ols is described. In this reaction, the indole nucleus was first oxidized with ozone to alkyl 2-formamidophenyl ketone, which was subsequently subjected to the reaction with cyanide ion in weakly alkaline medium to cyclize to the 3H-indole ring. The overall yield of the reaction was approximately 75 %. By this method an ionizable amidine function was introduced at the side chain of tryptophan.

Tryptophan is readily converted to N'-formylkynurenine either by chemical oxidation with ozone and other oxidants or by oxidation in the metabolic process of biological organisms. Conversely no attempts have been reported for the reconstitution of the indole nucleus at the side chain of kynurenine or N'-formylkynurenine. Recently, for the elucidation of microenvironment of tryptophan in proteins,[1] we have devised a method to utilize kynurenine as an environment-sensitive fluorescent probe since the former was converted to the latter by chemical modification technique.[2,3] In this connection, we have undertaken to reconstitute an indole skeleton by the introduction of an extraneous carbon atom, especially carbon-13, at the side chain of kynurenine. In the present paper we will present the results of the investigation of the conversion of 3-alkylindole to 3-alkyl-2-amino-3H-indol-3-ol via alkyl 2-formamidophenyl ketone, which was formed on ozonization of the indole derivative.

The conversion of 2-alkylindole to 3-alkyl-2-amino-3H-indol-3-ol by the present reaction is shown in Scheme 1. In this scheme, the 3-alkylindole(I) was

Scheme 1



(I) (II) (III) (IV) (V)

first oxidized to alkyl 2-formamidophenyl ketone(II) by the reduction of methoxyhydroperoxidic intermediate formed on methanolic ozonization of (I) under the conditions described previously.[4] Usually this ozone-oxidation for ring opening of (I) proceeded almost quantitatively. Following procedures were employed to convert (II) to (V). As a typical experiment the conversion of N-α-acetyl-N'-formyl-L-kynurenine(II, R=-$CH_2CH(NHCOCH_3)COOH$) to N-α-acetyl-β-(2-amino-3-hydroxy-3H-indol-3-yl)-L-alanine is shown below. N-α-Acetyl-N'-formylkynurenine(II, 558 mg, 2 mmol), which had been prepared by ozone-oxidation of N-α-acetyl-L-tryptophan,[4] was dissolved with potassium hydrogencarbonate(200 mg, 2 mmol) in water(3 ml). To this solution solid potassium cyanide(140 mg, 2.2 mmol) was added and the solution was stirred at room temperature. As the reaction proceeded, a typical UV absorption due to alkyl 2-formamidophenyl ketone disappeared gradually and, instead, new absorption maxima at 270 nm and 303 nm started to appear. At the end of the reaction a spectrum similar to an 2-amino-3H-indole chromophore[5] was recorded(Fig. 1).

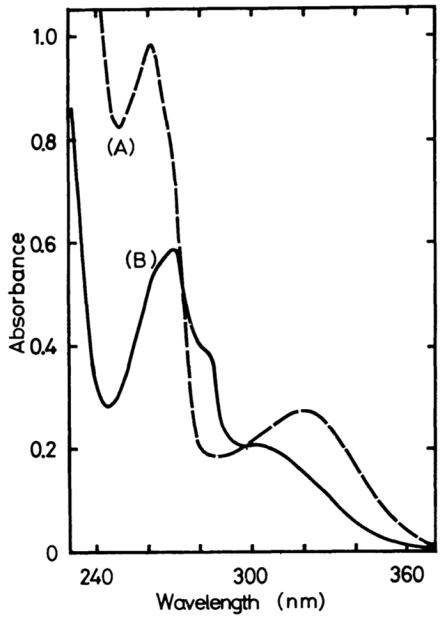


Fig. 1. Change of UV absorption spectrum in the reaction of N-α-acetyl-N'-formylkynurenine(A) with cyanide ion. Curve(B) corresponds to the final reaction product obtained after 12 hours. Reaction condition: N-α-acetyl-N'-formylkynurenine(10 mM) and potassium cyanide(40 mM) reacted each other in 0.5 M phosphate buffer at pH 7.4 at room temperature. The spectra were recorded after dilution with water.

After the reaction was continued for 4 hours, the reaction mixture was concentrated to a small volume under reduced pressure and acidified to pH 4-5 with 0.4 M hydrochloric acid. The faintly turbid solution was then allowed to stand at room temperature for a few hours until crystals were completely separated out. The crystalline products were collected by filtration, washed with water and methanol, successively, and dried. Pure N-α-acetyl-β-(2-amino-3-hydroxy-3H-indol-3-yl)-L-alanine was obtained by recrystallization from 50 % acetic acid. Yield 234 mg (42 %). M.p. 251°C(dec.). $[\alpha]_D^{18}$ -37.6°(c 0.5, 0.1 M HCl). Rf.[6] (SiO_2) 0.50. Found: C, 56.23; H, 5.39; N, 15.13%. Calcd. for $C_{13}H_{15}N_3O_4$: C, 56.31; H, 5.45; N, 15.13%. IR(KBr): 3210, 1675, 1622, 1585, 1295, 1193, 1093, and 750 cm^{-1}. ^{13}C-NMR(DCl salt, ppm relative to the internal standard(dioxane, 67.4 ppm) in D_2O): 174.2, 174.1, 173.8(acetyl CO, α-CO, and C-2), 140.6(C-7a), 132.1(C-6), 130.5(C-3a), 126.4, 125.3(C-4 and C-5), 113.7(C-7), 80.4(C-3), 48.9(α-CH), 39.3(β-CH_2), and 22.1 (acetyl CH_3). ^1H-NMR(DCl salt, ppm from TMS in DMSO-d_6 with a few drops of D_2O):

7.51-7.09(arom. 4H), 3.99(d-d, 1H, α-CH), 2.65-2.56(m, 2H, β-CH_2), and 1.45(3H, acetyl CH_3).

From mother liquor, additional crystals, highly soluble in water, were isolated by concentration and fractional crystallization from aqueous ethanol. Transparent rod-like crystals decomposed at 189°C, but, on drying for 10 hours at 80°C, the anhydrous material(77 mg, 15%) was obtained. M.p. 224-226°C(dec.). $[\alpha]_D^{18}$ -12.8°(c 0.5, 0.1 M HCl). Rf.[6] (SiO_2) 0.50. Found: C, 56.05; H, 5.42; N, 14.92%. These values of elemental analysis were in good accordance within experimental error with those calculated for N-α-acetyl-β-(2-amino-3-hydroxy-3H-indol-3-yl)-L-alanine. However, a subtle difference in the chemical shift of the protons corresponding to the α-CH(3.79 ppm), β-CH_2(2.81-2.35 ppm), and CH_3CO(1.81 ppm) groups was observed between the first and the second crops, although the UV absorption spectra of both crops were the same. Eventually we concluded that these two materials in each crop are diastereoisomers formed by the introduction of an additional asymmetric center at position 3 in the 3H-indole ring of this L-alanine derivative.

Other four 3-alkylindoles were similarly converted to the corresponding 3-alkyl-2-amino-3H-indol-3-ols in moderate yields by the ring-opening reaction of the indole with ozone and the subsequent ring closure with cyanide ion(Table 1).

Table 1. 3-Alkyl-2-amino-3H-indol-3-ols prepared by the reaction with cyanide subsequent to ozonization of the corresponding 3-alkylindole

R in (V)	Yield[a]	M.p.(°C)		Elemental analysis Calculated C	H	N	Found C	H	N
$-CH_3$[b]	76 %	198-202(dec.)	$C_9H_{10}N_2O$	66.65	6.22	17.27%	66.47	6.14	17.05%
$-CH_2CH_2COOH$[c]	78	221-227(dec.)	$C_{11}H_{12}N_2O_3$	58.78	5.60	12.46	58.67	5.59	12.21
$-CH_2CH_2NHCOCH_3$[d]	73	122-123	$C_{12}H_{15}N_3O_2$	59.48	6.65	17.34	59.32	6.88	17.16
$-CH_2\underset{CONH_2}{CHNHCOCH_3}$[e]	47	191(dec.)	$C_{13}H_{16}N_4O_3$	56.51	5.84	20.28	56.62	5.81	20.04

a) Based on the starting indole derivative. b) This compound was hydrolyzed with aqueous alkali(pH 10.5) at room temperature for two weeks and the hydrolysis product(m.p. 163-163.5°C) was identified as 1,3-dihydro-3-hydroxy-3-methyl-2H-indol-2-one(3-methyldioxindole, lit., 161.5-162.5°C[7]). c) 1/4 H_2O. d) 1/2 H_2O. e) A less water-soluble isomer of diastereoisomers with L-configuration.

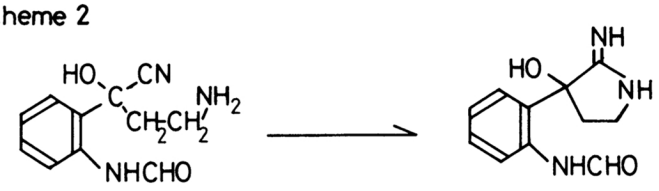
3-Alkyl-2-amino-3H-indol-3-ol is a derivative of aromatic amidines: ionization constant of N-α-acetyl-β-(2-amino-3-hydroxy-3H-indol-3-yl)-L-alanineamide was determined to be 6.92 at 25°C by UV-titration at 283 nm.

The introduction of an extraneous carbon atom of cyanide ion into the 3H-indole nucleus was confirmed by ^{13}C-NMR spectroscopy. For instance, in a ^{13}C-NMR spectrum of 2-amino-3-methyl-3H-indol-3-ol, only a resonance was detected at 175.5 ppm below pD 4 when ^{13}C-enriched cyanide ion was used in the ring closure reaction, indicating that the cyanide carbon was introduced into the position 2 of the 3H-indole ring. Accordingly the formyl carbon in alkyl 2-formamidophenyl ketone was not incorporated into the skeleton of the newly constructed hetero-ring. This means that the formyl group was eventually eliminated from the 3H-indole ring at a certain stage of the reaction, probably after the ring closure of (III) to (IV)

as shown in Scheme 1.

It is obvious that the formation of a cyanohydrin intermediate(III) is prerequisite to ring closure. Therefore, if another nucleophile is present at the position where the interaction with the cyano group is favorable, the intramolecular cyclization of (III) must take place in a different way from the case shown in Scheme 1. In fact, no 3H-indole derivative was formed from N'-formylkynurenamine, whereas 2-imino-3-(2-formamidophenyl)pyrrolidin-3-ol(VI, yield 86 %. M.p. 159-161°C(dec.). Found: C, 60.35; H, 6.03; N, 19.18%. Calcd. for $C_{11}H_{13}N_3O_2$: 60.26; H, 5.98; N, 19.15%. IR(KBr): 3430, 3300, 1635, 1580, and 1100 cm^{-1}) was obtained by the reaction of the cyano group with the unsubstituted amino group (Scheme 2). This type of cyclization was also observed for N'-formylkynurenine, an oxidation product of free tryptophan.

Scheme 2



(III) $R=CH_2CH_2NH_2$ (VI)

The role of the formyl group in the present cyclization reaction should be emphasized from the following two points. First acetylkynurenine lacking the acyl substituent at the aromatic amino group failed to react with cyanide ion and the 3H-indole nucleus was not formed. Secondly the formyl group was not replaceable with an acetyl group. This finding is reminiscent of the report of Bell and Wei,[8] who had pointed out that, in the cyclization of 2-aminobenzophenone with cyanide ion to the 3H-indole ring, the substitution of the aromatic amino function by the dichloroacetyl group was necessary for ring closure. Consequently, these facts imply that, in order to effect the ring closing reaction of alkyl or aryl 2-aminophenyl ketone, the aromatic amino function should be blocked with an acyl group such as a formyl or dichloroacetyl group, which may serve to enhance the reactivity of the aromatic carbonyl function toward cyanide ion.

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