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It was established that indole and 2-methylindole are converted in soil to stable compounds, which were isolated and identified by means of mass spectrometry (lowand high-resolution), IR, UV, and PMR spectroscopy, and by comparison with model samples. Under the influence of peroxide compounds in the soil, indole is converted to 2,2-di(3-indolyl)indoxyl and very small amounts of indoxylrot, indirubin, and indigo. Similarly, 2-methylindole is converted in soil to 2-methyl-2-(2methyl-3-indolyl)-3-oxoindoline.

The waste waters of coal-tar chemical and petroleum-refining enterprises, the proportion of indoles in which may sometimes reach 15.6 mg/liter, atmospheric air contaminated with the industrial refuse of factories, and various indole-containing products of plant and animal origin may be sources of indole and its homologs in soil [1, 2].

Our experiments have shown that indole in chernozem soil with a moisture content of 15-20% forms stable yellow I in high yields and a small amount of three other compounds (2-5%) of the weight of I) at 20°C.

According to high-resolution mass spectrometry, I, with mp 246-247°C and R<sub>f</sub> 0.30, had empirical formula  $C_{24}H_{17}N_{3}O$  [the molecular weight determined for the molecular ion (M<sup>+</sup>) was 363.13669, as compared with the calculated value of 363.13723]. Preliminary analysis of the mass spectrum showed that I consists of three indolyl fragments, of which one contains an oxygen atom. Absorption bands at 1720 (CO) and 3490 (NH) and a series of bands at 1620, 1600, 1510, 1490, 1350, 1420, and 1320 cm<sup>-1</sup>, which are ascribable to the stretching vibrations of the indole ring [3], are observed in the IR spectrum. The absorption bands in the UV spectrum at 226, 260, and 280 nm are characteristic for unsubstituted indole [4]. The absence of a long-wave absorption band indicates that there is no conjugation between the indole rings. A broad singlet at 10.90 ppm, which we assigned to the signal of NH protons, and a complex multiplet of aromatic protons at 7.06-8.12 ppm are observed in the weak-field region of the PMR spectrum. The assignment is confirmed by the integral intensity ratio (3:14).

The substance is extremely stable with respect to the action of alkalis, and we obtained indole in 10-20% yield only in the case of fusion with KOH. Mineral acids cause slow decomposition of the substance at room temperature, and gentle heating with alcohol solutions of organic acids (acetic acid, etc.) gives the same effect. Indole is formed in no higher than 20% yield in all cases.

Picric acid decomposes I to indole and a dimer, which, after decomposition of its picrate, was found to be identical to one of the products of transformation (II) that was isolated from the soil. This substance was a red compound with mp 212°C and  $R_f$  0.60. A mass of 246.07931 (as compared with a value of 246.07883 calculated for empirical formula  $C_{1.6}H_{1.0}N_{2}O$ ) was determined for the M<sup>+•</sup> peak from high-resolution mass spectrometric data.

The IR and UV spectra of II are identical to the spectra of indoxylrot [i.e., 2-(3'-indolyl)indoxyl], which we obtained for comparison by an independent method [5]. No melting-point depression was observed for a mixture of II and the synthesized sample. The PMR spectrum of this compound virtually coincided with the spectrum of I (the positions of the signals remained the same, although the integral intensity ratio became 1:9, respectively). On the basis of these data we proposed the previously described [6-8] 2,2-di(3'-indoly1)indoxyl structure for I.

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It has been reported that I is formed in the oxidation of indole with hydrogen peroxide in glacial acetic acid [6] or with hydrogen peroxide in the presence of plant peroxidase [7]. An instance of enzymatic oxidation of indole with *Claviceps purpurea* cells to give mainly I has also been described [8]. The authors [6-8] also note the formation of two modifications: a low-melting modification (Ia) with mp 202-207°C and a high-melting modification (Ib) with mp 245-247 or 250-251°C, the UV and IR spectra of which are identical. Our studies showed that the previously described picrate of I [7] is actually the picrate of II, since picric acid decomposes I.

To confirm our proposed structure of I we modeled the conditions described in [6-8]. We obtained the UV, IR, and mass spectra for the isolated reaction products and found that they were completely identical to the spectra of indole-transformation product I (Table 1).

Ejection of a neutral CO particle occurs in the first step of the fragmentation of the molecular ion under the influence of electron impact. This process is characteristic for all of the investigated indoles [9-11]. The existence of an  $(M - CO)^{+\bullet}$  ion in the form of a coplanar system is responsible in the subsequent elimination of a hydrogen atom for the existence of a stable rearranged ion with structure a [the condensation of the rings in ions a and b (and  $a - H_2$ )<sup>+</sup> is confirmed by the presence of doubly charged ions in the mass spectra]. These data were proved rigorously by recording the high-resolution mass spectra: as a result, values of 334.13681 (as against the calculated value for empirical formula  $C_{12}H_16N_3$  of 334.13449) for ion a and 217.07683 (as against the calculated value for empirical formula  $C_{15}H_9N_2$  of 217.07657) for ion b were obtained. The trend of the fragmentation of M<sup>+•</sup> is illustrated by the following scheme:



(Here and subsequently, the m/e values are given for the ion peaks.)

An additional confirmation of structure I is afforded by a comparison of the mass spectra obtained in this study with the spectrum of its isomer (III) (Table 1) [12].



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TABLE	(≈5%)	

V	V, in- digo	100,0 52,9 9,9 15,7 15,7 15,7 15,7 1,5,7 1,5,7 1,5,7 1,5,7 1,5,7 1,5,7 1,5,7 5,9,9 5,9,9 1,5,7 5,7 1,5,7 1,5,7 1,5,7 1,5,7 1,5,7 1,5,7 1,5,7 5,4,7 5
IV and	IV, in- dirubin	$\begin{array}{c} 100,0\\ 11,3\\ 21,5\\ 6,1\\ 6,1\\ 12,5\\ 8,5\\ 8,5\\ 8,5\\ 8,5\\ 8,5\\ 8,5\\ 8,5\\ 8$
lon		$ \begin{array}{l} M^+ (262) \\ (M^- CO)^+ (231) \\ (M^- CO)^- 111^+ (233) \\ al (M^- M-11NCO)^+ (219) \\ (M^- 11NCO)^+ (219) \\ (M^- C)^- (205) \\ (M^- C_7 H_5 O)^+ (205) \\ (M^- C_7 H_5 O)^+ (205) \\ (M^2 + (131) \\ (C_7 H_5 NO)^+ (119) \\ (C_7 H_5 NO)^+ (119) \\ (C_7 H_5 NO)^+ (119) \\ (C_6 H_5 N)^+ (101) \\ (C_6 H_5 N)^+ (101) \end{array} $
:	=	23,1 23,1 23,1 23,1 23,1 23,1 23,1 23,1
	HOT	$ \begin{array}{l} M^+ (246) \\ (M^-H)^+ (245) \\ (M^-H)^+ (245) \\ (M^-CO)^+ (218) \\ (M^-CO)^{-1}1]^+ (217) \\ a^-H)^+ (216) \\ (a^-H)^+ (216) \\ (a^-HCN)^+ 190 \\ (a^-HCN)^- 11]^+ (189) \\ (C_8H_5N)^+ (117) \\ (C_8H_5N)^+ (117) \end{array} $
III (isomer	of I)	81.0 14.5 100.0 6.3 6.3
	Ic, mp 270°C	5,0 5,0 5,0 5,0 5,0 5,0 5,0 5,0 5,0 5,0
	1b, mp 260°C	38.9 33.3 5.4 5.4 8.3 8.3 7.2 8.3
П	la, mp 202°C	37,7 37,7 37,7 5,7 5,7 5,0 5,0 5,0
	I (from soil) mp 246°C	32,5 32,5 5,4 7,9 5,4 7,9 5,4 7,9 5,4 7,9 5,4 7,9
	Ions	$\begin{array}{c} M^{+} \left( 363 \right) \\ (M^{-}H)^{+} \left( 363 \right) \\ (M^{-}H)^{+} \left( 362 \right) \\ (M^{-}CO)^{+} \left( 335 \right) \\ (M^{-}CO)^{-}H]^{+} \left( 334 \right) \\ al(M^{-}CO)^{-}H]^{+} \left( 334 \right) \\ (a \cdot H_{2})^{+} \left( 322 \right) \\ b \left( a \cdot H_{2} \right) + \left( 322 \right) \\ b \left( 26 H_{7} \right) \right) \\ m^{2+} \left( 167 , 5 \right) \\ (C_{6} H_{7} N)^{+} \left( 117 \right) \end{array}$

\*The values presented in parentheses indicate the mass numbers of the lons.

The IR and UV spectra of III also contain appreciable differences.

As we stated above, we isolated another two transformation products — red IV, which sublimes, has  $R_f 0.48$ , and was identified as indirubin, and dark-blue V, with mp 390°C and  $R_f 0.17$ , which was identified as indigo — from the soil. Samples of IV and V isolated from the soil did not depress the melting points of indirubin and indigo that we synthesized by independent methods. Their UV and IR spectra were qualitatively identical to the spectra of samples of I and II. However, the empirical compositions of IV and V were confirmed in both cases by high-resolution mass spectrometry: the masses of the M<sup>+•</sup> ions for IV and V were, respectively, 262.07963 and 262.07398 (the value calculated for empirical formula  $C_{16}H_{10}N_2O_2$ is 262.0742). The character of the fragmentation of M<sup>+•</sup> in both cases confirms the structures of IV and V (Table 1).

As previously demonstrated, two factors — microorganisms that completely consume the indole without the formation of stable compounds, and peroxide compounds, which are products of biochemical reactions catalyzed by soil enzymes — act upon indole in the soil. To verify this assumption we excluded the action of the microorganisms by carrying out the oxidation of indole with hydrogen peroxide with sterile soil and, for comparison, in various solvents at room temperature.

Preparative chromatographic separation of the reaction mixture showed that the principal product in the oxidation of indole in sterile soil is I (the high-melting modification with mp 260°C), and substances with structures II, IV, and V are present as impurities.

The principal reaction product was indoxylrot (II) when the oxidation was carried out in methanol at 50°C. A third modification of I, with mp 270°C, the UV, IR, and PMR spectra of which are identical to the spectra of the two remaining modifications of I, is formed under these conditions in addition to I V and V. No appreciable differences were observed in the intensities of the principal characteristic ions (with allowance for the 5 rel.% reproducibility of the mass spectra) when their mass spectra were compared (Table 1); this could constitute evidence for a change in the structure of I, although it was found that their mobilities on various supports — aluminum oxide, Silufol, and other sorbents — differ in the case of analysis by means of thin-layer chromatography (TLC).

The results of our experiments make it possible to assume that indole in both soil and in organic solvents is oxidized by peroxide compounds via the scheme



As a result of transformation of 2-methylindole in soil under the same conditions we were able to isolate only 2-methyl-2-(methyl-3-indolyl)-3-oxoindoline (VI). The same compound is also formed in the oxidation of 2-methylindole with hydrogen peroxide in methanol and in sterile soil:



Absorption bands with maxima at 1720 (CO) and 3400  $cm^{-1}$  (NH) are observed in the IR spectrum of VI. Signals of the ring protons of the benzene ring of indole (6.73-7.20) and indoline (7.37-7.73, 4H), singlets of two methyl groups at 2.40 (indole ring) and 1.90 ppm of

indoxyl (attached, respectively, to  $sp^2$  and  $sp^3$  carbon atoms), and broad signals of an NH group at 7.82 (indoxyl) and 4.98 ppm (indole) were recorded in the PMR spectrum (in CDCl<sub>3</sub>). The latter signals are found at 10.81 (indole) and 7.64 ppm (indoxyl) in DMSO solution. The character of the fragmentation of the M<sup>+•</sup> ion of this compound confirms the proposed struc-

ture. The process  $M^+ \xrightarrow{-CO} (M-CO)^+$  that occurs in the first step constitutes evidence for the presence of a carbonyl group. Steric factors promote the elimination of a methyl group

[13]  $M^{+} \xrightarrow{-CH_3} (M - CH_3)^+$  and cleavage of the interannular bond with the recording of indole (130 and 131) and indoxyl (146) fragments.



## EXPERIMENTAL

The IR spectra of solutions of the compounds in acetonitrile and chloroform were recorded with a UR-20 spectrometer. The UV spectra were recorded with a Specord UV spectrophotometer. Chromatography in a loose thin layer of aluminum oxide (activity II) and preparative separation were carried out by elution with acetone hexane systems (1:2 and 2:1). The PMR spectra of solutions of the compounds in DMSO were recorded with an XL-100 spectrometer with tetramethylsilane as the internal standard. The mass spectra were recorded with a Varian Mat-311 spectrometer with a system for direct introduction of the samples into the ion source; the ionizing-electron energy was 70 eV, the cathode emission current was 300  $\mu$ A, the accelerating voltage was 3 kV, and the ionization-chamber temperature was 180°C. The high-resolution mass spectra were obtained with the same spectrometer under the same conditions with M/AM 15000; PFK was used as the standard.

<u>Transformation of Indole in Soil (Typical Method)</u>. A 5-g sample of indole was added to 1 kg of soil with a moisture content of 20% (see [1] for information regarding the effect of the moisture content of soil on the yields of transformation products), and the mixture was maintained at 20°C until the indole vanished completely (as determined by TLC monitoring). The soil was then extracted with ether in a Soxhlet apparatus, after which the extract was dried, and the solvent was removed by distillation. The resulting crystalline substances were separated with a column filled with aluminum oxide. This procedure gave 2 g (40%) of 2,2-di(3-indoly1)indoxyl (I). Found: C 79.4; H 4.8; N 11.8%. C<sub>24</sub>H<sub>17</sub>N<sub>3</sub>O. Calculated: C 79.3; H 4.7; N 11.6%. Also obtained was 0.04 g (0.8%) of 2-(3-indoly1)indoxyl (indoxylrot) [5] (II), with mp 212°C (from acetonitrile). Found: C 78.2; H 4.2; N 11.1%. C<sub>1e</sub>H<sub>10</sub>N<sub>2</sub>O. Calculated: C 78.0; H 4.1; N 11.3%.

Indirubin (IV) and indigo (V) were isolated from the soil in up to 0.01-g amounts, which are sufficient for identification by means of the IR, UV, and mass spectra but insufficient for elementary analysis.

The mass spectra of isomers IV and V are almost identical (Table 1). The only substantial difference in the spectrum of IV is the appearance of an ion peak with m/e 219, which is due to detachment of an HNCO particle from the molecular ion because of different (as compared with V) ring fusion [13]. Mass spectrum of V (the ion peaks with intensities 5% of the maximum peak in the spectrum are presented): 41 (14.3), 43 (20.6), 50 (20.0), 51 (17.1), 52 (9.6), 53 (5.1), 55 (16.7), 57 (17.1), 63 (9.9), 64 (8.4), 67 (5.0), 69 (9.1), 71 (6.7), 74 (6.6), 75 (17.3), 76 (45.7), 77 (32.3), 78 (7.7), 83 (8.3), 89 (7.7), 90 (6.0), 91 (14.0), 102 (19.4), 103 (34.9), 104 (48.6), 105 (11.4), 119 (11.6), 129 (6.6), 130 (7.3), 131 (7.9), 157 (11.4), 158 (14.9), 178 (7.0), 179 (8.4), 205 (45.7), 206 (21.6), 233 (9.9), 234 (52.9), 235 (8.9), 262 (100.0), 263 (19.3).

The transformation of 2-methylindole in the soil was carried out similarly. The transformation products were extracted with chloroform, and 2-methyl-2-(2-methyl-3-indolyl)indoxyl (VI) was purified with a column filled with aluminum oxide. The yield of VI, with mp 204-206°C (from acetonitrile),  $R_f$  0.25, and M<sup>+</sup> 276, was 75 g (35%). Mass spectrum: 77 (11.3), 89 (5.9), 93 (6.1), 102 (10.2), 103 (6.8), 116.5 (5.0), 117 (5.0), 123 (8.0), 123.5 (5.9), 127 (7.6), 128 (13.6), 129 (14.1), 130 (22.8), 131 (12.8), 138 (17.6), 146 (5.1), 218 (5.0), 230 (8.9), 231 (21.3), 232 (26.1), 233 (98.0), 234 (22.8), 246 (5.0), 247 (67.4), 248 (43.5), 249 (7.7), 260 (6.5), 261 (100), 262 (18.5), 276 (220.0)[sic], 277 (7.4). Found: C 78.4; H 5.9; N 10.0%. Calculated: C 78.2; H 5.8; N 10.1%.

Oxidation of Indole with Hydrogen Peroxide. A) A 33% solution of hydrogen peroxide was added dropwise with stirring to a solution of 1.17 g (0.01 mole) of indole in methanol, and the mixture was maintained at room temperature for 3 days, during which the solution became reddish-brown, and black crystals precipitated. The crystals were removed by filtration and separated with a column filled with aluminum oxide with a benzene chloroform hexane system (6:30:1). This procedure gave two modifications of I, the UV, IR, PMR, and mass spectra of which were identical to the spectra of Ia with mp 202-204°, and Rf 0.31. The yield was 18%. Found: C79.3; H 4.82; N 11.9%. C<sub>24</sub>H<sub>17</sub>N<sub>3</sub>O. Calculated: C 79.3; H 4.7; N 11.6%. The second modification (Ib) had mp 260° and Rf 0.29 and was obtained in 36% yield. Found: C 79.5; H 4.8; N 11.1%. C<sub>24</sub>H<sub>17</sub>N<sub>3</sub>O. Calculated: C 79.3; H 4.7; N 11.6%.

B) Both modifications of I were similarly obtained at  $50^{\circ}$ C after 6 h, but another modification (Ic with mp 270°C and R<sub>f</sub> 0.13) was also isolated in 36% yield. The UV, IR, and PMR spectra were identical to the spectra of I isolated from the soil and both of the lower melting modifications. Found: C 79.8; H 4.8; N 11.7%.

C) Two previously described [6] modifications of I (Ia with mp  $202-204^{\circ}C$  and  $R_f 0.31$ , and Ib with mp  $246^{\circ}C$  and  $R_f 0.29$ ), which were completely identical to the above-described modifications [the UV, IR, and PMR spectra were identical, and the mass spectra contained slight differences that are difficult to explain (see Table 1)], were obtained by oxidation of indole with hydrogen peroxide in glacial acetic acid by the method in [6].

The oxidation of 2-methylindole was carried out by method A. The yield of 2-methyl-2-(2-methyl-3-indolyl)indoline (VI), with mp 204-206°C and  $R_f$  0.22, was 50%. Found: C 78.4; H 5.9; N 10.0%. C<sub>10</sub>H<sub>10</sub>N<sub>2</sub>O. Calculated: C 78.2; H 5.8; N 10.1%.

No melting-point depression was observed for a mixture of this product with a sample obtained from the soil.

## LITERATURE CITED

- 1. V. A. Medvedev, G. P. Lipnitskaya, A. K. Sheinkman, and V. D. Davydov, Pochvovedenie, No. 3, 32 (1975).
- 2. V. A. Medvedev and V. D. Davydov, Pochvovedenie, No. 11, 57 (1972).
- 3. A. R. Katritzky, Rec. Trav. Chim., 78, 995 (1959).
- 4. A. Stern and K. Timmons, Electron Absorption Spectroscopy in Organic Chemistry [Russian translation], Mir, Moscow (1974), p. 202.
- 5. O. Schmitz-DuMont, K. Hamann, and K. H. Geller, Ann., 504, 1 (1933).
- 6. B. Witkop, J. Patrik, and H. Kissmann, Ber., 85, 949 (1952).
- 7. A. G. Holmes-Siedle and B. Sannders, Chem. and Ind., No. 3, 265 (1957).
- 8. S. H. Loo and D. O. Woolf, Chem. Ind., No. 9, 1123 (1957).
- 9. I. C. Powers, J. Org. Chem., 90, 678 (1968).
- 10. R. Marchelli, W. D. Jamieson, S. Safe, O. Hutzinger, and R. A. Heacock, Can. J. Chem., 49, 1296 (1971).
- 11. R. A. Khmel'nitskii, Khim. Geterotsikl. Soedin., No. 3, 291 (1974).
- 12. G. I. Zhungietu and L. P. Sinyavskaya, Khim. Geterotsikl. Soedin., No. 2, 204 (1976).
- 13. I. M. Rice, G. O. Dudek, and M. Barber, J. Am. Chem. Soc., 89, 4569 (1965).