SECONDARY METABOLITES OF Pyricularia oryzae

I. o-NITROPHENOL DERIVATIVES

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o-Nitrophenol derivatives have been isolated from the culture liquid of the deuteromycete Pyricularia oryzae Cav.: 4-hydroxy-3-nitrophenylacetic acid, 4-hydroxy-3-nitrobenzyl alcohol, 1- and 2-(4-hydroxy-3-nitrophenyl(ethanols, N-(4-hydroxy-3-nitrophenylethyl)acetamide, and pyriculamide. This is the first time that any of these compounds has been isolated from natural sources. The o-nitrophenols obtained possess moderate growth-inhibiting activity in relation to rice shoots.

As is known, fungi are rich source of various secondary metabilites, among which there are many biologically active compounds. Pyricularia oryzae Cav., belonging to the order Deuteromyces, is the causative agent of pyriculariosis, one of the economically important diseases of rice. It is interesting as a possible source of phytotoxins, plant growth regulators, and similar compounds. α-Picolinic acid and pyricularin, which possess phytotoxic action, have been isolated from this microorganism previously [1]. Later, α-tetralone derivatives were isolated – 3,4,8-trihydroxy-, 4,6,8-trihydroxy-, and 3,4,6,8-tetrahydroxytetralones and also the pentaketide aldehydes pyriculol and pyriculariol and their reduced analogues – 6-hydroxymellein, 2,4-dihydroxy-6-(2-oxopropyl)benzoic acid, tenuazonic acid, and the diketopiperazine Pro-Leu [2]. Pyriculol, pyriculariol, and tenuazonic acid possess a high phytotoxicity. The α-tetralone derivatives, which participate in the biosynthesis of melanin in this fungus, possess a moderate growth-regulating activity. Yet another α-tetralone was isolated later: 4,6-dihydroxytetralone (isosclerone) [3]. The close species P. grisea has yielded 5-hydroxymethylfuran-2-carboxylic acid [4], which has shown moderate cytotoxic activity, and pyrichalasin H [5] – a phytotoxin of the cytochalasin group.

We have investigated the composition of the metabolites of P. oryzae VG-345. The fungus was grown by the deep-fermentation method in a medium based on molasses. The culture liquid acidified to pH 2.5, was extracted with ethyl acetate, and the extract was separated into acid and neutral fractions by the usual methods.

In the course of separating the acid fraction, a complex of α-hydroxy and α-keto acids (products of the deamination of hydrophobic aliphatic amino acids) and also artefactual compounds formed from them in the process of extraction and separation were isolated. Calculated as keto acids, their amount in the culture liquid was 30 mg/liter. In addition to them, from the acid fraction we isolated two acids of aromatic nature. One of them was identified as furan-2-carboxylic acid by comparing its physicochemical characteristics with those described in the literature [6]. The native nature of this acid, like that of 5-hydroxymethylfuran-2-carboxylic acid from P. grisea [4], requires proof, since they could be formed from pentoses and hexoses, respectively, in the process of fermentation under acid conditions.

The second compound consisted of a bright yellow crystalline substance with the composition $C_8H_7NO_5$ (I). The presence of a carboxy group was confirmed by the appearance of absorption bands at 3100-2500, 1689, 1429, and 917 cm^{-1} in the IR spectrum, of a broad signal at 6.0-6.8 ppm in the PMR spectrum, and also by the formation of the methyl ester (Ia) on treatment with CH_2N_2. With a large excess of CH_2N_2, the dimethyl derivative (Ib) was formed. Absorption at 1626 and 1575 cm^{-1} indicated the presence of an aromatic nucleus. Signals at 10.31 and 10.53 ppm in the PMR spectra of (I) and (Ia), respectively, and also absorption of moderate intensity at 3253 cm^{-1} in their IR spectra and the disappearance of these features in the spectra of (Ib) permitted the assumption of the presence of a phenolic hydroxyl forming an intramolecular hydrogen bond. The UV spectra of (I) and (Ia) were completely identical

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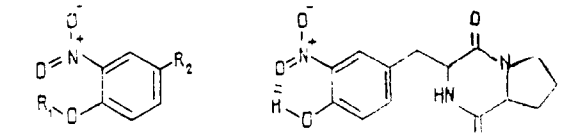
with the spectrum of o-nitrophenol [7]. The presence of a nitro group was also confirmed by absorption at 1531 and 1526 cm^{-1} for (I) and (Ia), and for the derivative (Ib) at 1520 and 1347 cm^{-1}, respectively [8]. In the aromatic region of the PMR spectrum of the acid (I) there were signals at (ppm) 7.14 (d, J = 8.5 Hz), 7.51 (dd, J = 8.5 and 2.1 Hz), and 8.03 (d, J = 2.1 Hz) relating to a 1,3,4-substituted benzene nucleus. The corresponding protons of o-nitrophenol resonate at 7.10, 7.53, and 8.03 ppm [9]. The weak-field part of the spectrum of (Ia) was practically identical with the spectrum of (I), while in the derivative (Ib) these signals had undergone an upfield shift by 0.1-0.2 ppm because of the disappearance of the hydrogen bond and a disturbance of the coplanarity of the nitro group with the aromatic nucleus. The aliphatic part of the spectrum of the spectrum of (I) was represented by a singlet at 3.6 ppm (2 H), broadened because of spin–spin coupling with the H-2 and H-6 protons of the aromatic nucleus.



I $R_1 = H$, $R_2 = CH_2COOH$
Ia $R_1 = H$, $R_2 = CH_2COOMe$
Ib $R_1 = Me$, $R_2 = CH_2COOMe$
II $R_1 = H$, $R_2 = CH(OH)Me$
III $R_1 = H$, $R_2 = CH_2CH_2OH$
IV $R_1 = H$, $R_2 = CH_2OH$
VI $R_1 = H$, $R_2 = CH_2CH_2NHAc$

These facts, taken together, permitted (I) to be identified as 4-hydroxy-3-nitrophenylacetic acid. The ^{13}C NMR spectrum also confirmed this structure, since the chemical shifts of the carbons of the nucleus coincided with the calculated shifts for 4-methyl-2-nitrophenol to within ±1.0 ppm [10].

When the neutral fraction was separated, another five o-nitrophenol derivatives were isolated in the form of bright yelow crystalline substances. Their PMR spectra contained signals relating to a 4-hydroxy-3-nitrophenyl residue and were practically identical with the corresponding signals of the acid (I). Of them, (II) and (III), isomers with the empirical formula $C_8H_9NO_4$, were alcohols, as followed from their IR spectra and the presence in their PMR spectra of signals at 2.03 and 1.93 ppm which disappeared on the addition of D_2O. The assignment of the other signals in the aliphatic region of their PMR spectra was easy and permitted the ascription to them of the structures of 2- and 1-(4-hydroxy-3-nitrophenyl)ethanols, respectively, for (II) and (III). Compound (IV), isolated in minor amount contained in its PMR spectrum, in addition to signals relating to the aromatic nucleus, a signal at 1.63 ppm of a hydrogen atom exchanging with D_2O, and a two-proton singlet at 4.69 ppm. Possessing the compositions $C_7H_7NO_4$, it was identified unambiguously as 4-hydroxy-3-nitrobenzyl alcohol.

According to the results of elementary analysis and mass spectrometry, compound (V) contained on additional nitrogen atom and had the empirical formula $C_{10}H_{12}N_2O_4$. The aliphatic part of its PMR spectrum contained the signal of a methyl group at 1.96 ppm, which was assigned to a N-acetyl group, as was also confirmed by signals in the ^{13}C NMR spectrum at 170.68 and 22.64 ppm. The presence of an amide group also followed from the IR spectrum (1650 and 1535 cm^{-1}) and from a broad signal at 5.6 ppm of a H atom slowly exchanging with D_2O. The remaining signals corresponded to a $-CH_2CH_2-$ grouping and, consequently, (V) was N-(4-hydroxy-3-nitrophenylethyl)acetamide.

Compound (VI), which we have called pyriculamide, had a somewhat more complex structure than the substances discussed above. The results of PMR (250 MHz) and ^{13}C NMR (20 MHz) spectroscopies, which are given in Table 1, permitted the structure of pyriculamide to be divided into three fragments. The first fragment corresponded to a 4-hydroxy-3-nitrophenyl group, and its signals differed little from the signals of the compounds given earlier. The second fragment was represented by a ABX system, which, on the addition of D_2O, gradually changed into a A_2X system. The X proton of this system had long-range SSCCs with the third fragment for which, on the basis of the results of double resonance on H-2', H-3'a, H-3'b, H-5'a, and H-5'b, the structure $-CHCH_2CH_2CH_2-$ was established. On the other hand, the empirical formula of pyriculamide, $C_{14}H_{15}N_3O_5$, the presence in the ^{13}C NMR spectrum of signals at 170.32 and 164.49 ppm, corresponding to carbonyl functions, and the neutral nature of the substance

TABLE 1. PMR and ^{13}C NMR Spectra of Pyriculamide (VI)

Position	^{13}C NMR, $CDCl_3$, ppm	PMR, ppm (50 MHz)			Position[6]	SSCC, Hz
		acetone-d_6	acetone-d_6 + D_2O[1]	acetone-d_6 + D_2O[2]		
1	170,32*	—	—	—	2—3a	5,1
2	59,06**	4,46 br.t	4,47 ttd	4,47 br.t	2—3b	5,1
3a	45,37	3,18 dd	3,17 dd	3,18 d	2—2′	1,8
b		3,28 dd	3,23 dd		2—5′a	0,8
4	128,58	—	—	—	2—5′b	0,8
5	139,18	8,12 d	[3]	[3]	3a—3b	15,0
6	133,33	—	—	—	5—9	2,3
7	154,12	—	—	—	8—9	8,6
8	120,19	7,11 d	[3]	[3]	2′—3′a	10,1
9	125,81	7,69 dd	[3]	[3]	2′—3′b	6,7
1′	164,49*	—	—	—	3′a—3′b	12,1
2′	56,14**	4,13 br.dd	4,13 ddd	[4]	3′a—4′a	10,1
3′a	28,26	1,74 m	1,58 dddd	1,42 m	3′a—4′b	8,5
b		2,15 m	2,13 dddd	2,14 m	3′b—4′a	5,7
4′a	22,42	1,85 m	1,82 m	1,79 m	3′b—4′b	3,7
b		(2H)	(2H)	(2H)	5′a—4′a	7,5
5′a	35,05	3,39 br.ddd	3,45[4]	3,40 m	5′a—4′b	5,1
b		3,51 br. ddd			5′b—4′a	8,2
NH		7,11 br.s	—	—	5′b—4′b	8,2
7—OH		10,35 s	—	—	5′a—5′b	12,0

[1]and [2]Amounts of D_2O added approximately 1/100 and 1/20 of the volume of the solution of (VI).
[3]The chemical shifts and SSCCs scarcely changed on the addition of D_2O, and therefore the spectrum of the aromatic region was not recorded.
[4]Overlaps with the HOD signal.
[5]For all SSCCs with the 4'a and 4'b protons the assignment to them is ambiguous because of the unresolved nature of the multiplet given by 4'a and 4'b.
[6]The SSCCs of the protons of the aromatic region and of the 5'a and 5'b protons were taken from the spectrum recorded in acetone-d_6, and the others from the spectrum taken with the addition of 1/100 of a volume of D_2O.
*,**) No explanation given in Russian original — editor.

permitted a hypothesis to be put forward of the presence of two amide groupings. The chemical shifts of the protons at 4.13 and 4.46 ppm and the carbon shifts corresponding to them at 59.06 and 56.14 ppm lay within the limits of the shifts characteristic for α-amino acid residues. The facts given permitted the assumption of the peptide nature of pyriculamide and the proposal for it os the structure of the diketopyrazine from nitrotyrosine and proline. The existence of long-range spin-spin coupling between the α-protons of the two amino acids with the considerable constant of 1.8 Hz is characteristic for diketopiperazines having the boat conformation. The nature of the second amino acid as proline was also confirmed by the presence in the mass spectrum of an ion with m/z 70, which corresponds to the ion of protonated Δ^1-pyrroline, C_4H_6N.

Very few o-nitrophenyl derivatives isolated from natural sources are known, and the closest in the structural respect to the compound that we have isoalted is an o-nitrophenol glucoside from Rhizoctonia solani Kuhn [11], so that their isolation from P. oryzae opens up a new source of natural o-nitrophenols.

It is known from the literature that 4-hydroxy-3-nitropheyllacetic acid possesses a moderate phytotoxicity according to the results of field trials on six species of cultivated plants [12]; it causes a 600% increase in the level of NH_3 in pine seedlings [13]. In tests for the inhibition of the synthesis of chlorophyll in clover and duckweed it lowered its amount from 52 to 36% at concentrations of 10^{-4} and 10^{-3} M, respectively [14]. We have carried out experiments to investigate the growth-inhibiting activity of the o-nitrophenols (I) and (III), using rice shoots as the test plant. Each compound possessed a moderate activity, inhibiting the growth of the roots by 70 ± 7%, and 45 ± 7% respectively, at a concentration of 60 μg/ml. It must also be mentioned that (III), unlike (I), causes deformation of the roots.

EXPERIMENTAL

Mass spectra were taken on a LKB 9200 instrument (Sweden), and IR spectra on a Shimadzu IR-435 instrument, of the substances in the form of films or suspensions in paraffin oil. UV spectra were recorded on a Shimadzu UV-260 spectrophotometer (Japan) and NMR spectra on Bruker WM-250 and AC80 instruments (FRG). Spectific optical rotations were determined on a Perkin-Elmer 402 spectropolarimeter.

TLC was conducted on Kieselgel 60 plate (Merck, FRG) in the following solvent systems: 1) ethyl acetate; 2) ethyl acetate–benzene (1:1); 3) benzene; 4) chloroform–methanol–acetic acid (90:10:1). The substances were detected by viewing the plates under UV illumination (254 and 366 nm, Chromato-Vue instrument) and also by spraying the chromatograms with 5% vanillin in 1% H_2SO_4 in ethanol, or by their exposure to iodine vapor.

Treatment of the Culture Liquid. A culture of P. oryzae VG-345 was grown by the deep-fermentation method in a medium based on molasses. The culture liquid after the separation of the mycelium was acidified with 30% H_3PO_4 to pH 2.5, and was extracted twice with an equal volume of ethyl acetate. The mean yield of extract after the solvent had been distilled off (rotary evaporator, 40°C) was 0.35 g/liter.

The extract (86 g) was distributed between 2 liters of 90% methanol and 3 liters of hexane. The hexane extracts were discarded. The residue after the elimination of the methanol (77 g) was dissolved in 2 liters of ethyl acetate and the solution was washed with 5% $NaHCO_3$ (5 × 40 ml). Evaporation of the organic layer, after it had been dried over Na_2SO_4, gave 6 g of a neutral fraction in the form of a brown resin. The aqueous extract was acidified to pH 3.0 and was re-extracted with 3 liters of ethyl acetate. Evaporation of the organic layer in vacuum gave 44 g of an acid fraction in the form of a red-brown oily liquid with a sharp odor.

The acid fraction was separated on silica gel (BDH, 20-120 mesh, 6.5 × 17 cm) with elution by hexane, C_6H_6, and C_6H_6–MeCN mixtures. The fractions obtained were separated further on Sephadex LH-20 (Pharmacia, Sweden, 2.5 × 80 cm) in 95% ethanol and in a $CHCl_3$ → $CHCl_3$–95% ethanol (1:1) gradient. A number of derivatives of α-hydroxy and α-keto acids, and also two crystalline substances, were isolated. Their recrystallization from hexane–acetone gave 0.21 g of furan-α-carboxylic acid and 0.19 g of (I).

Chromatography of the neutral fraction on Sephadex LH-20 in 95% ethanol led to 9 fractions. The separation of fraction 3 on the same sorbent in the $CHCl_3$–C_6H_{14}–MeOH (4:6:1) system, followed by chromatography on silica gel (Chemapol, 15/40 μm, $CHCl_3$–MeOH (0% → 2%), and filtration through activated carbon (200 mg) in MeOH led to 110 mg of (V). When fraction 4 was chromatographed on LH-20 in a $CHCl_3$ → $CHCl_3$–95% ethanol (1:1) gradient, material was obtained the further purification of which on LH-20 in $CHCl_3$–C_6H_{14}–MeOH (4:6:1) with subsequent recrystallization from acetone gave 25 mg of (VI). The same fraction yielded a mixture of three substances which were separated by HPLC (LiChrosorb 100, 10 μm, 1.0 × 25 cm) in the heptane–ethyl acetate (6:4) system. The substances obtained were purified additionally on a column of polyamide (Woelm for TLC, 0.9 × 25 cm) in hexane–$CHCl_3$ (4:1). Subseqent recrystallization from hexane–ethyl acetate gave 5 mg of (II), 33 mg of (III), and 23 mg of isosclerone. An analogous separation of fraction 5 gave additional amounts of (II) and (III), and also 5 mg of (IV).

4-Hydroxy-3-nitrophenylacetic Acid (I). Bright yellow needles from hexane–acetone with mp 145-147°C (according to the literature: 146-147°C from ethanol [7]). UV spectrum, λ_{max}^{MeOH} 216,277, 358 nm (log ε: 4.22; 3.83; 3.50). IR spectrum, λ_{max} (paraffin oil), cm^{-1}: 3260, 3100-2500 series of bands, 1689 s, 1626, 1575 w, 1531 s, 1488 w, 1429 s, 1407, 1325, 1300, 1252 s, 1212 s, 1175 s, 1126 w, 1080 w,s 917 w, 843 w, 819 w, 807 w, 760 w, 743 w, 715 w, 640 broad, 564.

PMR spectrum ($CDCl_3$, ppm): 3.66 (2H, s, CH_2COO); 6.00-6.80 (1H, br., COOH): 7.14 (1H, d J = 8.5 Hz, H-5); 7.51 (1H, dd, J = 8.5; 2.1 Hz, H-6); 8.03 (1H, d, J = 2.1 Hz, H-2); 10.31 (1H, br. s, OH-4).

^{13}C NMR spectrum (methanol-d_4, ppm): 40.19 (CH_2), 120.78 (C5), 126.50 (C2), 128.41 (C1), 135.37 (C3), 139.30 (C6), 154.42 (C4), 174.69 (COOH).

Mass spectrum m/z (%): 197 (M^+, 43) 179(2), 153(9), 152(100), 135(4), 107(3), 106(21), 105(9), 94(3), 78(5), 77(12). R_f 0.54(syst. 1) 0.34 (syst.4).

Methyl 4-Hydroxy-3-nitrophenylacetate (Ia). A solution of diazomethane in ether was added in small portions to a suspension of 15 mg of the acid (I) in 1 ml of dry ether. When the spot of the initial substance had almost disappeared (TLC, syst. 3), the reaction mixture was evaporated, and the residue was dissolved in benzene and passed through 5 g of silica gel. Recrystallization from hexane–ethyl acetate gave 13 mg of yellow plates with mp 64-66°C (according to the literature, 68-69°C [15]). UV spectrum, λ_{max}^{MeOH} 215, 274, 355 nm (log ε: 4.21; 3.80; 3.48). IR spectrum, λ_{max} (paraffin oil), cm^{-1}: 3253, 1721 s, 1627, 1574, 1526 s, 1480 w, 1415, 1370, 1349, 1322, 1300, 1258, 1210 sh., 1190 sh., 1160 s, 1117 w, 1079 w, 910, 871 w, 828 w, 820 w, 763 w, 726 w, 630, 560.

PMR spectrum ($CDCl_3$, ppm): 3.62 (2H, br.s, CH_2); 3.71 (3H, s, COOMe); 7.13 (1H, dd, J = 8.3, 0.4, H-5); 7.51 (1H, ddd, J = 8.3; 2.1; 0.6 Hz, H-6); 8.01 (1H, dd, J = 2.1; 0.4; 0.6 Hz, H-2); 10.53 (1H, s, OH-4).

Mass spectrum, m/z (%): 211 (M^+, 37), 179(1), 153(8), 152(10), 136(2), 135(8), 107(2), 106(19), 105(10), 94(2), 78(2), 79(2), 77(7), 59(10). R_f 0.82 (syst. 2); 0.27 (syst. 3).

Methyl 4-Methoxy-3-nitrophenylacetate (Ib). An excess of a solution of diazomethane in ether was added to a solution of 15 mg of the acid (I) in 1 ml of acetonitrile. After 1 h, the mixture was evaporated, and the residue was separated by preparative TLC (syst. 3; 20 × 20 cm; 0.2 mm). The upper yellow band gave 3 mg of (Ia), and the lower band 8 mg of (Ib), which formed colorless acicular crystals from hexane–ethyl acetate with mp 100-102°C. UV spectrum, λ_{max}^{MeOH} 216, 260, 330 nm (log ε: 4.21; 3.55; 3.40). IR spectrum, λ_{max} (paraffin oil), cm^{-1}: 1723 s, 1621, 1574 w, 1520 s, 1495 w, 1430, 1397 w, 1347 s., 1305, 1265 s., 1218, 1196, 1184, 1168, 1159 sh., 1083 w, 1016, 992, 919 w, 900 w, 824, 810, 757, 720, 575.

PMR spectrum ($CDCl_3$, ppm): 3.61 (2H, br. s, CH_2); 3.70 (3H, s, COOMe); 3.94 (3H, s, MeO-4); 7.05 (1H, d, J = 8.6 Hz, H-5); 7.46 (1H, dd, J = 8.6; 2.2 Hz, H-6); 7.78 (1H, d, J = 2.2 Hz, H-2).

Mass Spectrum, m/z (%): 225 (M^+ 38), 211(3), 195(8), 167(9), 166(100), 152(6), 151(4), 146(5), 136(3), 135(6), 120(4), 106(4), 105(8), 92(8), 91(10), 90(35), 89(3), 59(6).

1-(4-Hydroxy-3-nitrophenyl)ethanol (II). Pale yellow acicular crystals from hexane–ethyl acetate with mp 80-82°C. UV spectrum, λ_{max}^{MeOH} 215, 275, 355 nm (log ε: 4.20, 3.81, 3.49). IR spectrum, λ_{max} (paraffin oil). cm^{-1}: 3290, 3200, 1623, 1579, 1537 s, 1480, 1458, 1418, 1375, 1333, 1306 s., 1265 w., 1253, 1243, 1206 w, 1177 s, 1138 w, 1128 w, 1085, 1075 w, 1028, 1085, 1075 w, 1028, 930 w, 894 w, 880, 842 w, 828, 824 w, 762, 720 br., 650 br., 598, 567 w.

PMR spectrum ($CDCl_3$, ppm): 1.49 (3H, d, J = 6.4 Hz, Me); 1.93 (1H, br,s, CHOH); 4.91 (2H, d, J = 6.4 Hz, CHOH); 7.14 (1H, d, J = 8.5 Hz, H-5); 7.61 (1H, dd, J = 8.5; 2.2 Hz, H-6); 8.10 (1H, d, J = 2.2 Hz, H-2); 10.54 (1H, s, HO-4).

Mass spectrumm m/z (%): 183 (M^+ 20), 169(8), 168(100), 166(3), 152(6), 135(3), 123(9), 12(22), 121(5), 106(4), 94(10), 92(3), 91(3), 77(3), 65(9). R_f 0.64 (syst. 2).

2-(4-Hydroxy-3-nitrophenyl)ethanol (III). Bright yellow acicular crystals from hexane–ethyl acetate with mp 55-56°C. UV spectrum, λ_{max}^{MeOH} 214, 276, 358 nm (log ε: 4.23, 3.82, 3.50). IR spectrum λ_{max} (paraffin oil). cm^{-1}: 3250 br., 1626, 1575, 1531 s, 1481, 1463, 1422, 1365, 1305 br, s, 1251 s, 1245 sh., 1137 s, 1120, 1080 s, 1055, 1021, 980 w, 899, 853 w, 836, 817, 760, 753, 700 w, 670 w, 621 br, 587.

PMR spectrum $CDCl_3$, ppm): 203 (1H, br,s, CH_2OH); 2.80 (2H, t, J = 6.5 Hz, CH_2CH_2OH); 3.82 (2H, t, J = 6.5 Hz, CH_2OH); 7.06 (1H, d, J = 8.5 Hz, H-5); 7.44 (1H, dd, J = 8.5; 2.2 Hz, H-6); 7.92 (1H, d, J = 2.2 Hz, H-2); 10.42 (1H, s, OH-4).

Mass spectrum, m/z (%): 183 (M^+, 34), 165(6), 153(16), 152(100), 136(7), 135(52), 107(10), 106(55), 105(35), 95(3), 94(10), 91(7), 79(4), 78(21), 77(47), 66(10), 65(10), 55(3), 52(6), 51(5), 50(17). R_f 0.55 (syst. 2).

4-Hydroxy-3-nitrobenzyl Alcohol (IV). Bright yellow needles from hexane–ethyl acetate with mp 122-123°C.

Mass spectrum, m/z (%): 169 (M^+, 100), 168(28), 152(12), 140(4), 135(4), 123(52), 122(32), 121(10), 109(4), 106(38), 105(23), 95(31), 94(25), 93(12), 81(5), 79(9), 78(12), 77(24), 66(22), 65(51), 55(9), 53(11), 51(10).

PMR spectrum ($CDCl_3$, ppm): 1.65 (1H, br, s, OH): 4.69 (2H, s, CH_2); 7.15 (1H, d, J = 8.6 Hz, H-6); 7.60 (1H, dd, J = 8.6; 2.1 Hz, H-5); 8.11 (1H, d, J = 2.1 Hz, H-2); 10.53 (1H, s, 4-OH).

N(4-Hydroxy-3-nitrophenylethyl)acetamide (V). Bright yellow needles from benzene–hexane with mp 95-98°C. IR spectrum, ν_{max} (film), cm^{-1}: 3250 s., 3070, 2900, 16.40 br, 1650 s, 1630 s,sh, 1620 s, 1535 s, 1525 s, 1480, 1420, 1355 sh, 1310 s, 1240 s, 1175 s, 1120, 1075, 925 w, 800 w, 830 sh, 820, 750 s, 655 br, 580 br.

PMR spectrum ($CDCl_3$, ppm): 1.96 (3H, s, Ac); 2.82 (2H, br, t, J = 7.0 Hz, CH_2CH_2NH)); 3.49 (2H, br. q, J = 7.0 Hz, CH_2CH_2NH); 5.6 (1H, br, NH); 7.10 (1H, d, J = 8.6 Hz, H-6); 7.49 (1H, dd, J = 8.6; 2.1 Hz, H-5); 7.92 (1H, d, J = 2.1 Hz, H-2); 10.45 (1H, s, OH-4).

^{13}C NMR spectrum ($CDCl_3$, ppm): 22.64 (CH_3CO), 34.11 (CH_2CH_2NH), 40.17 (CH_2CH_2NH), 119.81 (C-5), 124.13 (C-6), 131.30 (C-1), 133.22 (C3), 137.98 (C-2), 153.37 (C-4), 170.68 (CH_3CO).

Mass Spectrum, m/z (%): 224 (M^+ 49), 207(11), 206(82), 166(13), 165(100), 164(7), 152(10), 148(4), 136(10), 135(40), 120(7), 119(11), 118(7), 107(11), 106(23), 105(28), 92(10), 91(24), 89(8), 79(7), 78(18), 77(32), 72(32), 71(7), 65(14), 63(9), 60(36), 51(22), 43(100).

Pyriculamide (VI). Bright yellow needles from acetone with mp 183-185°C (decomp), $[\alpha]_{\lambda}^{20}$ (nm): −84.4° (589; D), −87.5° (578), −99.6° (546), −174.9° (435) (c 0.66; MeOH. UV spectrum, λ_{max}^{MeOH}: 214, 274, 356 nm (log ε: 4.30; 3.78; 3.45); $\lambda_{max}^{0.1NKOH}$ 212, 237, 274 sh, 417 nm (log ε: 4.02; 4.25; 3.57; 3.63). IR spectrum, ν_{max} (paraffin oil), cm^{-1}: 3230 sh, 3170, 3040, 1665 s, 1635 s, 1570 w, 1520, 1440 s, 1370 w, 1305 s, 1240, 1170, 1180 br, 825, 750 w, 595 w.

The PMR and ^{13}C NMR are given in Table 1.

Mass spectrum, m/z (%): 305 (M^+ 60), 287(41), 259(4), 181(4), 154(100), 153(12), 152(15), 135(15), 125(48), 106(1), 105(3), 97(1), 70(45).

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