

METABOLIC FORMATION AND CHEMICAL SYNTHESIS OF 5,6-CYCLOPENTENO-4, 11-DIHYDRO-3H-PYRIDO[3,2-*a*]CARBAZOL-3-ONE, THE MAJOR INTESTINAL BACTERIAL METABOLITE OF THE PYROLYSIS MUTAGEN 5,6-CYCLOPENTENO-11H-PYRIDO[3,2-*a*]CARBAZOLE (LYS-P-1)

David G.I. Kingston* and Yue-Zhong Shu

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 24061-0212, U.S.A.

Roger L. Van Tassell and Tracy D. Wilkins

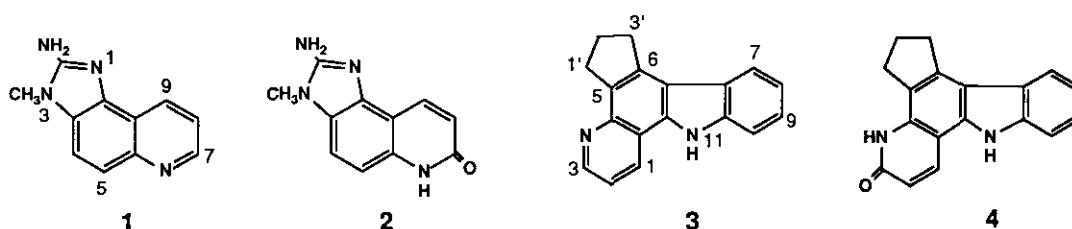
Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 24061-0305, U.S.A.

Abstract — Anaerobic incubation of the lysine pyrolysis mutagen 5,6-cyclopenteno-11H-pyrido[3,2-*a*]carbazole (Lys-P-1, **3**) with human intestinal bacterial mixtures yielded the major metabolite 5,6-cyclopenteno-4, 11-dihydro-3H-pyrido[3,2-*a*]carbazol-3-one (3-HO-Lys-P-1, **4**). The metabolite (**4**) has been synthesized from 4-aminoindane in six steps. NAD and NADP significantly enhanced the metabolism, suggesting that the transformation of **3** leading to **4** involves a hydration and subsequent dehydrogenation processes.

Our continuing studies on the anaerobic metabolism of food mutagens have shown that the potent dietary mutagen 2-amino-3-methyl-3H-imidazo[4,5-*f*]quinoline (IQ, **1**) was metabolized by human intestinal bacteria to the quinolone derivative 2-amino-3, 6-dihydro-3-methyl-7H-imidazo[4,5-*f*]quinolin-7-one (7-HO-IQ, **2**).¹ Significantly, 7-HO-IQ (**2**) is a powerful direct-acting mutagen.² These results have suggested a potential human health risk in that various mutagenic heterocyclic amines such as **1**, which are present in the pyrolysates of proteinaceous foods, may reach the human colon after ingestion and become metabolically activated by colonic

flora. The genotoxic bacterial metabolite(s), therefore, may play more of a role in colon carcinogenesis than the parent compounds.

5, 6-Cyclopenteno-11*H*-pyrido[3,2-*a*]carbazole (Lys-P-1, **3**)³ is the major mutagen isolated and identified from pyrolysis mixtures of L-lysine.⁴ In *in vitro* mammalian mutagenic assays, Lys-P-1 (**3**), similarly to Trp-P-2, induced mutagenesis of Chinese hamster V79 cells in a dose related fashion, while other heterocyclic pyrolysis mutagens were not active in this assay.⁵ The chemical structure of Lys-P-1 (**3**) is of particular interest from a metabolic viewpoint, since it shares with IQ (**1**) the structural feature of a quinoline ring system. We thus elected to investigate the metabolism of mutagenic Lys-P-1 (**3**) by human intestinal flora.



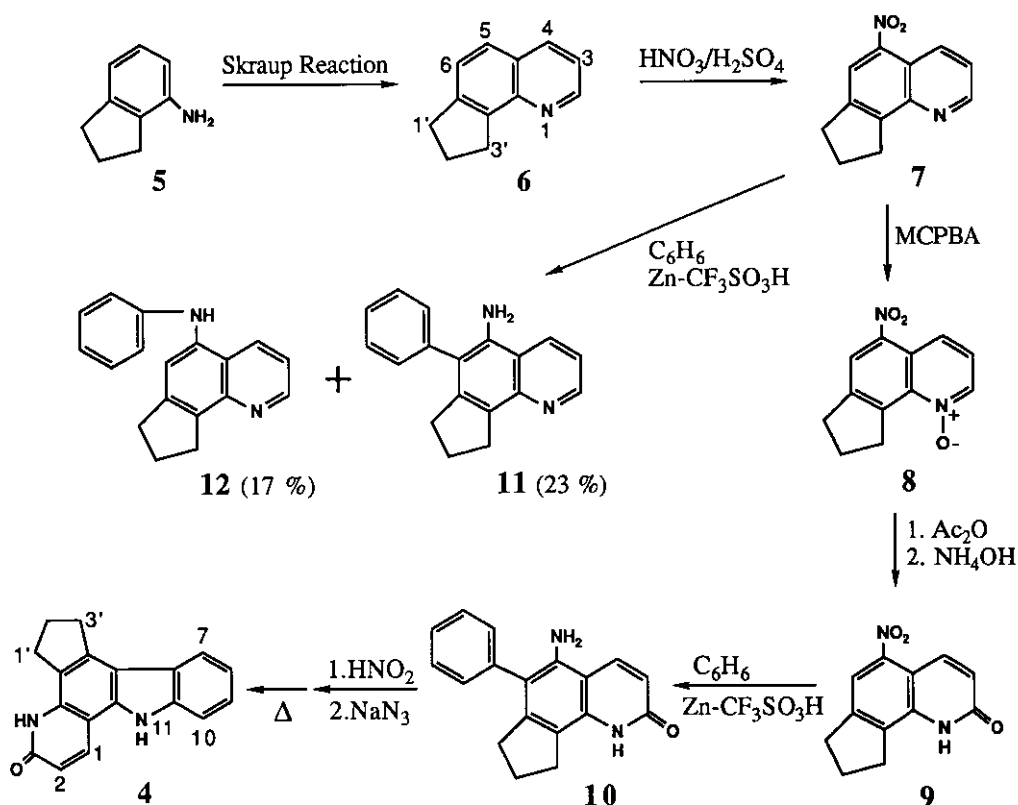
RESULTS AND DISCUSSION

The mutagen Lys-P-1 (**3**) was prepared by the method briefly reported by Sugimura and coworkers,⁴ using optimized or modified procedures as needed. The structure of **3** was confirmed by spectroscopic data, which have not previously been reported. Incubation of **3** with human feces-seeded BHI broth followed by extraction of aromatic metabolites with blue cotton,⁶ led to the detection of one major metabolite on tlc, which showed yellowish fluorescence under a long wavelength uv lamp. This metabolite was also detected by a reversed phase hplc system. The compound was not produced in a control experiment in which **3** was incubated with autoclaved bacterial broth, indicating that it is a bacterial metabolite (Table 1). Overall metabolite formation was however low, to the extent of *ca.* 3% of the substrate (Table 1); material for structural characterization was isolated from the incubated bacterial mixtures of **3** and purified by preparative tlc.

The mass spectrum of the metabolite showed a molecular ion peak at *m/z* 274, 16 mass units higher than that of Lys-P-1 (**3**), indicative of the presence of an extra hydroxyl group in the metabolite molecule. The uv absorption maxima of the metabolite were not shifted bathochromically on treatment with base (1*N* NaOH), suggesting that the extra hydroxyl group exists in the tautomeric ketone form rather than the phenol form. Since most 2- and 4-

hydroxyquinolines are known to exist essentially exclusively in the tautomeric quinolone form,⁷ and since our previous work on the anaerobic metabolism of compounds with a quinoline skeleton had shown that these compounds underwent oxidation at the 2-position of the quinoline ring,^{1a,8} we postulated that the metabolite would have the structure (4). The synthesis of 4 was thus initiated to confirm the structure of the biological sample.

The direct conversion of 3 to 4 was considered as a route to 4, but was rejected because of the poor solubility of 3 in most organic solvents. We thus elected a total synthesis route to 4. Skraup reaction of 4-aminoindane (5)⁹ in nitrobenzene yielded 7,8-cyclopentenoquinoline (6) (61 %). Nitration of 6 with mixed acid gave almost exclusively the desired 5-nitro product (7) in 78 % yield. Treatment of 7 with *m*-chloroperbenzoic acid afforded the *N*-oxide (8) (65 %). Rearrangement of 8 in the presence of acetic anhydride followed by mild hydrolysis (29% NH₄OH)^{1b,10} provided the 2(1*H*)-quinolinone derivative (9) (48 %).



The method for reductive phenylation of nitroarenes with benzene, zinc dust, and triflic acid was successfully applied to the introduction of a phenyl group at the 6-position of **9**.¹¹ The desired 6-phenyl product (**10**) (49 %) was the only isolable compound from the reaction mixture; no 5-*N*-phenylation product was isolated.¹² While in the preparation of Lys-P-1 (**3**) compound (**7**) underwent the same reaction to give substantial amounts of 5-*N*-phenylation product (**12**) (17 %) in addition to the 6-*C*-phenylation product (**11**) (23 %) (detailed data not shown). The outcome of efficient formation of **10** may have arisen because the presence of the quinolone system tended to increase the stability of the nitrenium ion derived from **9**, a claimed intermediate ion in the model reaction,^{11a} and thus render carbon-6 more reactive toward the nucleophilic attack by benzene.

Completion of the synthesis was brought about by thermolysis of the 5-azido derivative of **10**, the method previously used to make Lys-P-1, to give 5,6-cyclopenteno-4, 11-dihydro-3*H*-pyrido[3,2-*a*]carbazol-3-one (3-HO-Lys-P-1, **4**) (79%). The synthetic product had identical chromatographic (tlc, hplc) and spectroscopic (uv, eims) properties to those of the metabolite obtained from Lys-P-1 (**3**).

Our previous observations have indicated that the conversion of IQ (**1**) to 7-HO-IQ (**2**) by anaerobic bacteria involved a hydration-dehydrogenation sequence,^{1b} and the formation of **4** from **3** may thus also proceed through the same pathway. Dehydrogenases isolated from anaerobic bacteria often require NAD(P) as cosubstrate(s),¹² and hence an increase in the transformation of **3** to **4** on adding NAD or NADP would be indicative of this pathway. When NAD and NADP were added to assess their stimulative effect on the metabolism of **3** to **4**, both of the electron acceptors significantly enhanced the transformation in a dose-dependent fashion (Table 1).

Table 1
Transformation of Lys-P-1 (**3**) to 3-HO-Lys-P-1 (**4**) by human intestinal bacteria under anaerobic conditions

Addition or treatment	3-HO-Lys-P-1 (4) formed (μmol)	Transformation %
None	0.09	3
Autoclaved	0	0
NAD (4.78 μmol)	0.53	17
NAD (9.56 μmol)	0.85	27
NADP (4.78 μmol)	0.66	21
NADP (9.56 μmol)	1.04	33

Each tube (10 ml) contained 0.8 mg of Lys-P-1 (**3**, 3.10 μmol) and the incubation was carried out at 37°C for 4 days

It is noteworthy that molybdenum hydroxylases such as aldehyde oxidase and xanthine oxidase/dehydrogenase have been known to catalyze the nucleophilic hydroxylation of *N*-heterocyclic substrates; the attack is usually at the electropositive carbon adjacent to a ring *N* atom, and the oxygen atom incorporated into the product may be ultimately derived from water.¹³ However, this particular enzyme activity has rarely been reported in anaerobic bacteria.¹⁴ Thus, it is at present unknown whether the formation of **4** is catalyzed by a molybdenum hydroxylase. In conclusion, we have metabolically formed and synthetically prepared 3-HO-Lys-P-1 (**4**), a new bacterial metabolite of the pyrolysis mutagen Lys-P-1 (**3**).

EXPERIMENTAL

General

General experimental procedures were as previous described.^{1,2}

Synthesis of Lys-P-1 (**3**)

Lys-P-1 (**3**) was prepared from indan by modifications of the procedures of Wakabayashi *et al.*⁴ Compound (**3**), grey amorphous powder (MeOH/benzene), mp 283-285°C (decomp.). Uv (MeOH) λ_{max} 344 nm (log ϵ 3.97), 295 (4.95), 248 (4.75), 242 (4.76), 223 (4.59); ir (KBr) 3448, 1560, 1459, 1343, 778, 740 cm^{-1} ; ^1H -nmr (DMSO- d_6) δ 2.51 (2H, qui, $J=7.4$ Hz, $\text{H}_2\text{-2}'$), 3.55 (2H, t, $J=7.4$ Hz, $\text{H}_2\text{-3}'$), 3.72 (2H, t, $J=7.4$ Hz, $\text{H}_2\text{-1}'$), 7.42 (1H, br t, $J=7.6$ Hz, 8-H), 7.60 (1H, br t, $J=7.5$ Hz, 9-H), 7.75 (1H, dd, $J=8.3, 4.3$ Hz, 2-H), 7.83 (1H, d, $J=7.5$ Hz, 10-H), 8.26 (1H, d, $J=7.7$ Hz, 7-H), 9.06 (1H, d, $J=8.3$ Hz, 1-H), 9.07 (1H, d, $J=4.3$ Hz, 3-H); eims m/z (relative intensity) 258 (M^+ , 65), 257 (63), 243 (13), 203 (28), 199 (35), 149 (44), 137 (42), 129 (39), 128 (43), 91 (55), 71 (60). Calcd for $\text{C}_{18}\text{H}_{14}\text{N}_2$: M^+ 258.1157. Found: M 258.1156.

Incubation of Lys-P-1 (**3**) with human fecal bacterial mixtures

Human feces from five healthy donors (four males, one female) were mixed anaerobically, diluted 5-fold in distilled water and stored at -70°C prior to experimental use.^{1a} A 0.2 ml sample of this mixture was then inoculated into 1000 ml of prereduced BHI broth (Difco Lab, Detroit, MI), and the broth was cultivated anaerobically for 24 h at 37°C . To the broth was added Lys-P-1 (**3**, 70 mg) dissolved in DMSO (2 ml). The supplemented culture was incubated anaerobically for 4 days at 37°C , and then shaken twice with blue cotton (35 g each) (ICN Biochemicals, Inc., Costa Mesa, CA)⁶ for 30 min. The blue cotton was washed with water (2000

ml X 3) and dried. The compounds adsorbed to the cotton were eluted twice with MeOH/29% NH₄OH (100:3) (400 ml each). The extract was concentrated, and the residue was chromatographed on a preparative silica gel G tlc plate (CHCl₃/AcOEt/MeOH, 5:1:0.5) to afford metabolite (4) (0.8 mg, R_f 0.32) and unmetabolized 3 (34 mg, R_f 0.67). Metabolite 4: Uv (MeOH) $\lambda_{\max}$ 346 nm (log ϵ 4.12), 291 (4.58), 254 (4.56), 248 (4.55), 235 (4.53), 222 (4.45); eims m/z (relative intensity) 274 (M⁺, 100), 273 (55), 259 (13), 243 (12), 137 (11), 121 (10).

Quantitative analysis of metabolite 4 formed in bacterial mixtures

To a 24 h BHI broth (10 ml/tube) seeded with human feces 0.1ml of Lys-P-1 (3, 0.8 mg) in DMSO and 0.2 ml of an appropriate cofactor were added. Incubation was carried out anaerobically at 37°C for 4 days. The metabolic mixture was treated with blue cotton (0.6 g/tube) in the same fashion as described above. The blue cotton extract was then dissolved in MeCN (2 ml) and filtered with a membrane filter (0.2 μ m, Gelman Scientific). An aliquot (10 μ l) of the filtrate was injected into an hplc system: pump, Waters 501 solvent delivery system equipped with a Biorad 1306 uv monitor; column, Radial-Pak cartridge NV C₁₈ (Waters Assoc. Inc., Milford, MA); mobile phase, MeCN/0.1M NH₄OAc (51:49, v/v); detection at 290 nm with a range of 0.02 a.u.f.s. Under these conditions Lys-P-1 (3) had a retention time of 7.55 min, and the metabolite (4) 5.04 min. Linear calibration curves of these two compounds were prepared for quantitation by using standard samples.

7,8-Cyclopentenoquinoline (6)

4-Aminoindane (5, 5.5 g, 44.36 mmol)⁹ was heated with stirring at 135°C with glycerol (19 ml, 260 mmol), nitrobenzene (2.76 ml), FeSO₄ (1.55 g) and conc. H₂SO₄ (11 ml) for 4 h, after which time tlc showed the absence of starting material. The reaction mixture was diluted with water, basified with 25% NaOH to pH 10, and then extracted with CH₂Cl₂. The CH₂Cl₂ fractions were combined, dried over Na₂SO₄, and evaporated. Flash column chromatography (CH₂Cl₂/MeOH, 100:1) yielded 6 (oil, 4.3 g, 61.5%). Uv (MeOH) $\lambda_{\max}$ 297 nm (log ϵ 3.49), 236 (4.69); ir (neat) 2951, 1614, 1507, 1354, 1309, 832, 785 cm⁻¹; ¹H-nmr (CDCl₃) δ 2.30 (2H, qui, J=7.5 Hz, H₂-2'), 3.17 (2H, t, J=7.5 Hz, H₂-1'), 3.47 (2H, t, J=7.5 Hz, H₂-3'), 7.33 (1H, dd, J=8.3, 4.3 Hz, 3-H), 7.48 (1H, d, J=8.2 Hz, 5-H), 7.66 (1H, d, J=8.2 Hz, 6-H), 8.16 (1H, dd, J=8.3, 1.7 Hz, 4-H), 8.92 (1H, dd, J=4.3, 1.7 Hz, 2-H); eims m/z (relative intensity) 169 (M⁺, 55), 168 (100), 154 (12), 139 (12), 115 (8), 83 (18). Calcd for C₁₂H₁₁N: M⁺ 169.0891. Found: M 169.0901.

5-Nitro-7,8-cyclopentenoquinoline (7)

To a stirred mixture of **6** (1.92 g, 11.36 mmol) in conc. H_2SO_4 (5 ml) was added dropwise a mixture of conc. H_2SO_4 /conc. HNO_3 (5 ml/5 ml) at 0°C . The reaction mixture was stirred for an additional 3 h at 80°C . The solution was then poured into ice water, basified with 25% NaOH to pH 10, and extracted with AcOEt. The organic fractions were combined, dried (Na_2SO_4) and evaporated. Crystallization of the residue from hexane/benzene gave yellowish needles of **7** (1.90 g, 78.2%), mp $97-99^\circ\text{C}$. Uv (MeOH) λ_{max} 331 nm (log ϵ 3.89), 230 (4.61); ir (KBr) 2955, 1514, 1410, 1341, 838, 811, 779 cm^{-1} ; ^1H -nmr (CDCl_3) δ : 2.49 (2H, qui, $J=7.5$ Hz, H_2-2'), 3.26 (2H, t, $J=7.5$ Hz, H_2-1'), 3.55 (2H, t, $J=7.5$ Hz, H_2-3'), 7.59 (1H, dd, $J=9.4, 3.6$ Hz, 3-H), 8.33 (1H, s, 6-H), 9.01 (1H, d, $J=9.4$ Hz, 4-H), 9.02 (1H, d, $J=3.6$ Hz, 2-H); eims m/z (relative intensity) 214 (M^+ , 100), 213 (75), 184 (25), 167 (83), 139 (15), 115 (8), 83 (12). Anal. Calcd for $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_2$: C, 67.29; H, 4.67; N, 13.08. Found: C, 66.70; H, 4.56; N, 12.85.

5-Nitro-7,8-cyclopentenoquinoline-N-oxide (8)

A solution of *m*-chloroperbenzoic acid (2.9 g, 16.82 mmol) in 50 ml of CHCl_3 was added dropwise over 30 min to a stirred solution of **7** (1.8 g, 8.41 mmol) in 20 ml of CHCl_3 at 0°C . Stirring was continued overnight at room temperature. The solution was then treated with 3% Na_2CO_3 and finally with water. The organic layer was dried (Na_2SO_4) and evaporated *in vacuo*. Crystallization of the residue from EtOH yielded **8** (1.25 g, 64.6%) as yellow plates, mp $157-159^\circ\text{C}$ (decomp.). Uv (MeOH) λ_{max} 370 nm (log ϵ 3.74), 259 (sh, 3.74) 235 (4.37); ir (KBr) 3422, 1560, 1509, 1408, 1330, 1279, 804, 743 cm^{-1} ; ^1H -nmr (CDCl_3) δ 2.25 (2H, qui, $J=7.6$ Hz, H_2-2'), 3.13 (2H, t, $J=7.6$ Hz, H_2-1'), 4.07 (2H, t, $J=7.6$ Hz, $3'-\text{H}_2$), 7.37 (1H, dd, $J=9.0, 6.0$ Hz, 3-H), 8.25 (1H, s, 6-H), 8.39 (1H, d, $J=9.0$ Hz, 4-H), 8.47 (1H, d, $J=6.0$ Hz, 2-H); eims m/z (relative intensity) 230 (M^+ , 80), 214 (100), 213 (78), 184 (36), 167 (95), 139 (22), 115 (12), 83 (25). Anal. Calcd for $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_3$: C, 62.61; H, 4.35; N, 12.17. Found: C, 62.45; H, 4.20; N, 11.83.

5-Nitro-7,8-cyclopenteno-1H-quinolin-2-one (9)

A solution of **8** (770 mg, 3.35 mmol) in 25 ml of acetic anhydride was heated with stirring at 140°C for 2.5 h. Solvent was then removed *in vacuo* and the residue was dissolved in MeOH (3 ml) and basified with 29% NH_4OH (20 ml). The mixture was stirred for an additional 3 h, and then extracted with AcOEt. The organic phases were combined, dried (Na_2SO_4) and evaporated. Purification by flash column chromatography

(CH₂Cl₂/MeOH, 100:2) afforded **9** (370 mg, 48%) as an amorphous powder, mp > 300°C (AcOEt). Uv (MeOH) $\lambda_{\max}$ 341 nm (log ϵ 3.87), 238 (4.46); ir (KBr) 2967, 1662, 1511, 1340 cm⁻¹; ¹H-nmr (DMSO-d₆) δ 2.15 (2H, qui, J=7.5 Hz, H₂-2'), 3.03 (2H, t, J=7.5 Hz, H₂-1'), 3.10 (2H, t, J=7.5 Hz, H₂-3'), 6.68 (1H, d, J=10.0 Hz, 4-H), 7.80 (1H, s, 6-H), 8.22 (1H, d, J=10.0 Hz, 3-H), 9.54 (1H, br s, NH); eims m/z (relative intensity) 230 (M⁺, 100), 184 (24), 166 (12), 156 (17), 154 (15), 128 (15). Calcd for C₁₂H₁₀N₂O₃: M⁺ 230.0691. Found: M 230.0694.

5-Amino-6-phenyl-7,8-cyclopenteno-1H-quinolin-2-one (**10**)

To an ice-chilled mixture of **9** (300 mg, 1.3 mmol) and trifluoromethanesulfonic acid (3.45 ml, 39 mmol) in benzene (6 ml, 63.5 mmol) was added Zn-dust (726 mg, 11 mmol) in three portions with stirring. Stirring was continued for an additional 8 h at room temperature until the starting material was consumed (tlc), and the solution was then poured into ice water, basified with 25% NaOH to pH 9, and extracted with AcOEt. The AcOEt fractions were combined, dried (Na₂SO₄), and concentrated. The residue was purified by column chromatography (CHCl₃/MeOH, 100:1) to give compound **10** as a yellowish amorphous powder (176 mg, 48.9%), mp 232-234°C (decomp.) (toluene/AcOEt). Uv (MeOH) $\lambda_{\max}$ 332 nm (log ϵ 3.93), 319 (3.95), 278 (4.21), 239 (sh, 4.25), 228 (4.37); ir (KBr) 3460, 3344, 1635, 1552, 1498, 1416, 828, 753, 705, 684 cm⁻¹; ¹H-nmr (CDCl₃) δ 2.13 (2H, qui, J=7.3 Hz, H₂-2'), 2.72 (2H, t, J=7.3 Hz, H₂-1'), 2.97 (2H, t, J=7.3 Hz, H₂-3'), 6.55 (1H, d, J=9.8 Hz, 4-H), 7.30 (2H, dd, J=7.6, 1.4 Hz, 2", 6"-Ph-H), 7.40 (1H, tt, J=7.3, 1.4 Hz, 4"-Ph-H), 7.49 (2H, br t, J=7.5 Hz, 3", 5"-Ph-H), 7.86 (1H, d, J=9.8 Hz, 3-H), 9.11 (1H, br s, CONH); eims m/z (relative intensity) 276 (M⁺, 100), 275 (62), 247 (13), 199 (17), 138 (6), 124 (5), 115 (8). Calcd for C₁₈H₁₆N₂O: M⁺ 276.1263. Found: M 276.1273.

5,6-Cyclopenteno-4,11-dihydro-3H-pyrido[3,2-a]carbazol-3-one (3-HO-Lys-P-1, **4**)

To a suspension of **10** (164 mg, 0.6 mmol) in 14.5% H₂SO₄ (7 ml) a solution of NaNO₂ (46 mg, 0.66 mmol) in 1.5 ml of water was added dropwise in an ice-salt bath, and the mixture was stirred for 2 h. A solution of NaN₃ (49 mg, 0.75 mmol) in 3 ml of water was added, and stirring was continued for an additional 3 h at room temperature. The reaction mixture was then basified with 10% NaOH, and extracted with AcOEt. The AcOEt phases were combined, dried (Na₂SO₄), and evaporated. The residue was suspended in anhydrous decahydronaphthalene (6 ml), and the stirred mixture was heated at 155°C for 2 h, then allowed to cool, and

chilled in an ice bath for 4 h. The precipitate was filtered off, washed with hexane six times and again filtered. The crude product was further purified by flash column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 100:2) to yield **4** as a brownish amorphous powder (128 mg, 78.6 %), mp $>300^\circ\text{C}$ (MeOH/benzene). Ir (KBr) 3412, 3234, 1648, 1628, 1553, 1436, 1382, 1334, 1245, 828, 732 cm^{-1} ; ^1H -nmr (DMSO- d_6) δ : 2.28 (2H, qui, $J=7.3$ Hz, $\text{H}_2\text{-}2'$), 3.11 (2H, t, $J=7.3$ Hz, $\text{H}_2\text{-}3'$), ca. 3.44 (2H, t, $J=7.3$ Hz, $\text{H}_2\text{-}1'$, overlapped by H_2O signal), 6.54 (1H, d, $J=9.6$ Hz, 1-H), 7.21 (1H, br t, $J=7.6$ Hz, 8-H), 7.37 (1H, br t, $J=7.8$ Hz, 9-H), 7.57 (1H, d, $J=7.9$ Hz, 10-H), 7.98 (1H, d, $J=7.6$ Hz, 7-H), 8.47 (1H, d, $J=9.6$ Hz, 2-H), 11.24 (1H, br s, -CONH). Calcd for $\text{C}_{18}\text{H}_{14}\text{N}_2\text{O}$: M^+ 274.1106. Found: M 274.1103. The synthesized product was identical (uv, eims, tlc, hplc) with the metabolite isolated from fecal bacterial mixtures as described above.

ACKNOWLEDGEMENT

Financial support of this work was provided by the National Cancer Institute, National Institutes of Health, through grant number CA 40821.

REFERENCES AND NOTES

1. a) M. Bashir, D. G. I. Kingston, R. J. Carman, R. L. Van Tassell, and T. D. Wilkins, *Mutation Res.*, **1987**, *190*, 187. b) M. Bashir, D. G. I. Kingston, R. J. Carman, R. L. Van Tassell, and T. D. Wilkins, *Heterocycles*, **1987**, *26*, 2877. c) R. L. Van Tassell, R. J. Carman, D. G. I. Kingston, and T. D. Wilkins, *Microbial Ecol. Health Diseases*, **1989**, *2*, 123. d) M. Bashir, D. G. I. Kingston, R. L. Van Tassell, and T. D. Wilkins, *Heterocycles*, **1989**, *29*, 1915.
2. R. J. Carman, R. L. Van Tassell, D. G. I. Kingston, M. Bashir, and T. D. Wilkins, *Mutation Res.*, **1988**, *206*, 335.
3. The name of Lys-P-1 was first assigned as 3, 4-cyclopentenopyrido[3,2-*a*]carbazole in ref. 4. It is more reasonable to name it as 5, 6-cyclopenteno-11*H*-pyrido[3,2-*a*]carbazole. The nomenclature for similar compounds recently reported can be seen in a) Y. Kumar, A. K. Saxena, P. C. Jain, and N. Anand, *Indian J. Chem.*, **1980**, *19*, Sect. B, 996. b) C. Dionne, L. G. Humber, A. Asselin, J. McQuillan, and A. M. Treasurywala, *J. Med. Chem.*, **1986**, *29*, 1452. c) E. Lescot, G. Muzard, J. Markovits, J. Belleney, B. P. Roques, and J.-B. Le Pecq, *J. Med. Chem.*, **1986**, *29*, 1731.

4. K. Wakabayashi, K. Tsuji, T. Kosuge, K. Takeda, K. Yamaguchi, K. Shudo, Y. Iitaka, T. Okamoto, T. Yahagi, M. Nagao, and T. Sugimura, *Proc. Japan Acad.*, **1978**, *54*, Ser. B, 569.
5. S. Takayama, and M. Tanaka, *Toxicol. Lett.*, **1983**, *17*, 23.
6. H. Hayatsu, T. Oka, A. Wakata, Y. Ohara, T. Hayatsu, H. Kabayashi, and S. Arimoto, *Mutation Res.*, **1983**, *119*, 233.
7. R. M. Acheson, "An Introduction to the Chemistry of Heterocyclic Compounds", Interscience, New York, 1960, pp. 220-221.
8. M. Bashir, D. G. I. Kingston, R. J. Carman, R. L. Van Tassell, and T. D. Wilkins, *Heterocycles*, **1990**, *31*, 1333.
9. M. Pailer and H. Grunhaus, *Monatsh. Chem.*, **1974**, *105*, 1362
10. E. Ochiai and T. Okamoto, *Yakugaku Zasshi*, **1948**, *68*, 88.
11. a) T. Okamoto, K. Shudo, and T. Ohta, *J. Am. Chem. Soc.*, **1975**, *97*, 7184. b) T. Ohta, R. Machida, K. Takeda, Y. Endo, K. Shudo, and T. Okamoto, *J. Am. Chem. Soc.*, **1980**, *102*, 6385.
12. T. Akao, T. Akao, M. Hattori, T. Namba, and K. Kobashi, *J. Biochem.*, **1986**, *99*, 1425.
13. C. Beedham, "Progress of Medicinal Chemistry", Vol. 24, ed. by G. P. Ellis and G. B. West, Elsevier Science Publishers, New York, 1987, pp. 85-127.
14. C. A. Woodfolk and J. S. Downard, *J. Bacteriol.*, **1977**, *130*, 1175.

Received, 8th July, 1991