Short Communication

Synthesis of ($\pm$)-Gizzerosine, an Inducer of Gizzard Erosion in Broiler Chicks

Kenji MORI,* Tomomi OKAZAKI,
Tadashi NOGUCHI and Hiroshi NAITO

Department of Agricultural Chemistry,
The University of Tokyo, 1-1-1, Yayoi,
Bunkyo-ku, Tokyo 113, Japan

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It is known that certain kinds of fish meal induce gizzard erosion in broiler chicks.¹⁾ Very recently Okazaki *et al.* isolated about 100 μ g of a new compound named gizzerosine from 10 kg of heated fish meal.²⁾ This is an extremely potent inducer of gizzard erosion in chicks. Within a week severe gizzard erosion was observed by feeding less than 50 μ g/day (or 2.20 ppm in diets) of gizzerosine to chicks. Extensive spectral studies on the isolated gizzerosine were concluded by the proposal of its structure as 2-amino-9-(4-imidazolyl)-7-azanonanoic acid **1** with an unknown absolute configuration at C-2.²⁾ This proposal has now been confirmed by an unambiguous synthesis of ($\pm$)-**1** as shown in the Scheme.

Cyclopropyldimethylcarbinol **2** was converted to an iodide **3**, bp 85~86°C (21 mm-

Hg); n_D^{21} 1.5097, in an overall yield of 57% in 6 steps (48% hydrobromic acid; sodium cyanide; potassium hydroxide; lithium aluminum hydride; tosyl chloride; sodium iodide). Alkylation of diethyl acetaminomalonate with **3** in the presence of sodium ethoxide in refluxing ethanol gave **4**, mp 43.0~43.5°C, in an 86% yield. This was oxidized with *m*-chloroperbenzoic acid to give an oily epoxide **5**. Treatment of **5** with periodic acid dihydrate in ether-tetrahydrofuran (5:1) at 0°C for 10 min gave a crude aldehyde **6**, IR $\nu_{\max}^{\text{film}}$ cm⁻¹: 2720; NMR (60 MHz, CDCl₃) δ : 9.8 (~1H, t, J =1.5 Hz), in a quantitative yield from **4**. Reductive amination of **6** with histamine dihydrochloride and sodium cyanoborohydride (NaBH₃CN)³⁾ in methanol yielded a diester **7** as a crude oil in an 87% yield. This was dissolved in hydrochloric acid and the resultant solution was heated under reflux for 5 hr to give the trihydrochloride of ($\pm$)-**1** as a crude gum in an 82% yield. This gum was purified by chromatography over Amberlite IR CG-50 (*N*-ethylmorpholine form). After washing the column with 2 M aqueous *N*-ethylmorpholine, ($\pm$)-**1** was eluted with 3 M aqueous ammonia to give almost pure material. Its final purification by preparative TLC over acid-washed cellulose powder gave pure ($\pm$)-**1** after development with 2-propanol-28% aqueous ammonia-water (16:3.5:4). The synthetic ($\pm$)-**1** was identical with the isolated gizzerosine on the basis of chromatographic and spectral comparisons:

* Address correspondence to this author.

TLC [cellulose; 2-propanol–28% aqueous ammonia–water (16:3.5:4)]: R_f 0.58; ^1H -NMR (400 MHz, D_2O) δ : 1.47 (2H, m), 1.76 (2H, dt, $J_1=16$, $J_2=8$ Hz), 1.90 (2H, dt, $J_1=10$, $J_2=7$ Hz), 3.12 (2H, t, $J=8$ Hz), 3.16 (2H, t, $J=8$ Hz), 3.38 (2H, t, $J=8$ Hz), 3.75 (1H, t, $J=7$ Hz), 7.35 (1H, s), 8.55 (1H, s); ^{13}C -NMR (100 MHz, D_2O) δ =22.4, 23.7, 25.9, 30.7, 47.4, 48.0, 55.5, 117.2, 132.9, 136.7, 175.3; EI-MS (as methyl ester diacetamide) m/z : 338.19496 (M^+ , Calcd. for $\text{C}_{16}\text{H}_{26}\text{O}_4\text{N}_4$: 338.19540). ($\pm$)-Gizzerosine dihydrochloride crystallized from methanol–water (5:1) as fine prisms, mp 253~256°C (dec.); IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3130 (s), 2780 (s), 2450 (s), 1640 (s), 1605 (s), 1525 (s), 1495 (s), 1355 (m), 1335 (m), 1235 (m), 1095 (m), 1055 (m), 960 (m), 835 (m), 795 (m), 720 (m). *Anal.* Found: C, 41.92; H, 7.16; N, 17.20. Calcd. for $\text{C}_{11}\text{H}_{22}\text{O}_2\text{N}_4\text{Cl}_2$: C, 42.08; H, 7.08, N, 17.89%. ($\pm$)-Gizzerosine **1** was proved to be bioactive. The addition of 6.25 ppm of ($\pm$)-**1** in experimental diets for chicks caused severe erosion of their gizzards. The

activity, however, was only about 1/3 as strong as the isolated material. This probably means that only one enantiomer of **1** is bioactive.

Synthesis of gizzerosine enantiomers *via* the enzymatic resolution of ($\pm$)-**8** is in progress and will be reported elsewhere together with a full account of this work.

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