β-Carbolines from Japanese Sake and Soy Sauce: Synthesis and Biological Activity of Flazin and Yellow Substance YS (Perlolyrine)[1)]

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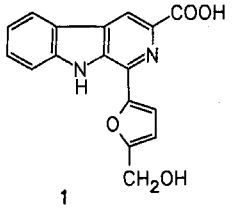
The β-carbolines flazin (**1**) and perlolyrine (**2**, substance YS) occuring in Japanese sake and soy sauce were synthesized starting with the methyl ester of L-tryptophan or tryptamine, respectively. The inhibition of the monoamine oxidases A and B by **1** and **2** were determined and compared with the inhibitory effects of harmine and harmaline.

β-Carboline aus japanischem Sake und aus Soya-Sauce: Synthese und biologische Aktivität des Flazins und der gelben Substanz YS (Perlolyrin)

Die β-Carboline Flazin (**1**) und Substanz YS = Perlolyrinis (**2**), die in japanischem Sake und japanischer Soya-Sauce vorkommen, wurden ausgehend von L-Tryptophanmethylester respektive Tryptamin synthetisiert. Die Hemmung der Monoaminoxidasen A und B durch **1** und **2** wurde im Vergleich mit Harmin und Harmalin gemessen.

The highly fluorescent β-carbolines flazin and substance YS, isolated from Japanese soy sauce, were reportet to have structures **1** and **2** respectively[1)]. Both compounds also occur in Japanese sake[2)], and **2** seems furthermore to be identical with the alkaloid perlolyrine isolated from rye-grass[3)]. Perlolyrine (**2**) was synthesized from (±)-tryptophan and its structure confirmed by an X-ray analysis of its hydrobromide[3)]. Flazin methylester (**7**) prepared by the Japanese investigators from flazin (**1**) with diazomethane[1)] is structurally related to 3-(ethoxycarbonyl)-β-carboline (CEE) which strongly binds to benzodiazepine receptors[4)] and has for this reason aroused considerable interest. Perlolyrine (**2**), on the other hand, is structurally related to the *Harmala* alkaloid harmine, a known inhibitor of monoamine oxidase A[5)]. The presence of **1** and **2** in food and their chemical relationship to well known psychomimetics provided incentive to prepare them in sufficient quantity for testing.



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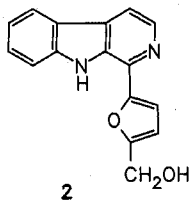

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The route chosen by us resembles in principle the reported synthesis of **2**[3)], obtained from (±)-tryptophan by *Pictet-Spengler* reaction with 5-(acetoxymethyl)furfural, and followed by oxidation of the reaction products with chromic acid[3)], but varies in details.

Direct condensation of L-tryptophan with commercially available 5-(hydroxymethyl)furfural[6)] did not readily yield the desired tetrahydrocarbolines. However, success was achieved when the methyl or ethyl ester of L-tryptophan was reacted with 5-(acetoxymethyl)furaldehyde in refluxing benzene in the presence of a catalytic amount of p-toluene-sulfonic acid to yield *Schiff* base **3**, which cyclized spontaneously to a 3:5 mixture of cis- and trans-tetrahydrocarbolines **4** and **5**, respectively. The mixture could be separated by column chromatography and configuration of the two compounds was established on the basis of C-13 NMR data[7)]. Aromatization of **4** and **5** with Pt/C catalyst in the presence of oxygen afforded the desired carboline **6**, with a faster aromatization rate noted for trans isomer **5**. Removal of the acetyl group in **6** with 0.5 % NH_4OH in methanol led to compound **7**, identical with flazin methylester[1)]. Hydrolysis of **6** in 0.1 N methanolic NaOH yielded a yellow, crystalline product, identical with flazin **1**[1)].

Attempts to convert flazin **1** into yellow substance **2** by decarboxylation failed to give positive results. YS (perlolyrine) was therefore synthesized in a way similar to that described above. Condensation of tryptamine with 5-(acetoxymethyl)-furfural in refluxing benzene led to *Schiff* base **8**. This compound, contrary to **3**, did not spontaneously cyclize under these conditions to the desired tetrahydrocarbolines **9** and **10**, but cyclization could be achieved with HCl gas in ether solution. The mixture of enantiomers **9** and **10** aromatized to compound **11** under conditions identical to those described for the conversion of **4** and **5** into **6**. Removal of the acetoxy group from **11** with 0.5 % NH_4OH in methanol gave product **2**, identical in all respects with YS reported by *Nakatsuka* et al.[1)], and with perlolyrine reported by *Jeffreys*[3)].

Flazin (**1**) und YS (**2**) do not inhibit binding of tritiated diazepam to benzodiazepine receptors nor do these compounds inhibit MAO B. Compound YS (**2**) and its acetylated derivative **11**, but not flazin, were effective inhibitors of MAO A at about 1000 fold less potency than harmaline or harmine (see Biological Testing).

[1)] Unserem Freund und Kollegen Professor Dr. Bernhard Witkop zum 70. Geburtstag gewidmet.

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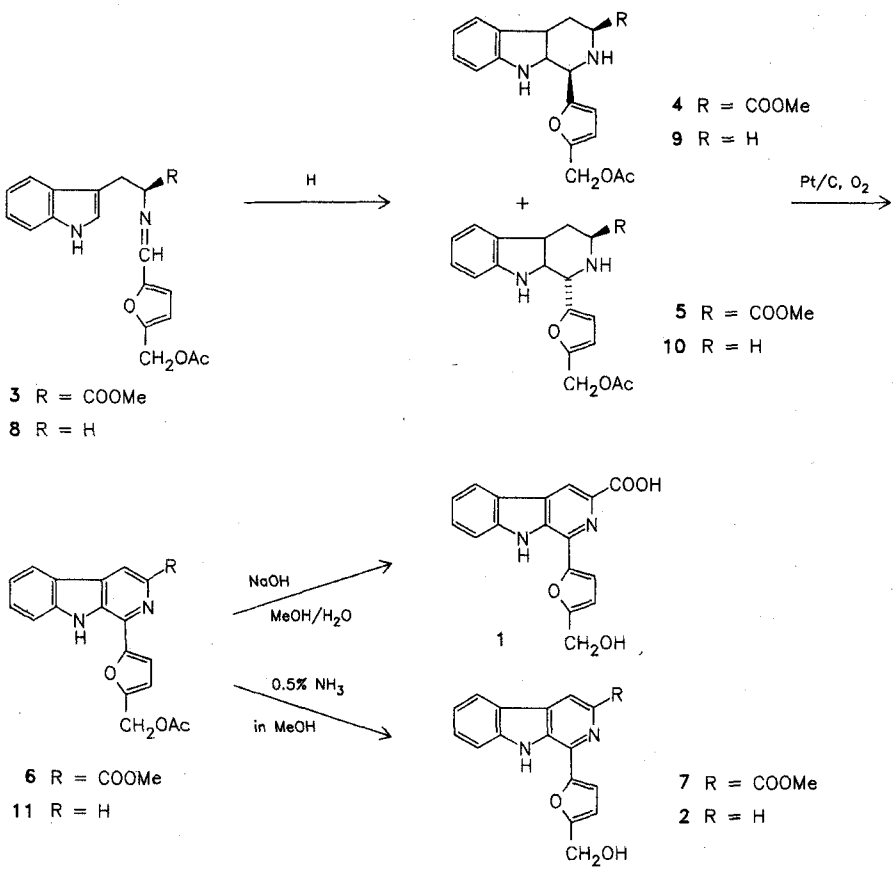

Experimental Part

Synthesis and Analysis

Melting points: Fisher-Johns apparatus, corrected. – ^{1}H-NMR spectra: Varian XL-300 spectrometer, TMS as the internal reference. – ^{13}C-NMR spectra: JEOL FX-100 spectrometer, TMS as the internal reference. – Chemical ionization mass spectra (CIMS): Finnigan 1015D spectrometer with ammonia as the reagent gas and a Model 6000 data collection system. – Ultraviolet spectra: Hewlett-Packard 8450A UV-VIS spectrometer. – IR spectra: Beckman 4230 instrument. – Elemental analyses: Atlantic Microlab Inc., Atlanta, Georgia. – Silica gel 60 for short column flask chromatography (0.015–0.040 mm) was from E. M. Reagents.

1-(5-Acetoxymethyl-2-furyl)-3-carbomethoxy-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole **(4 *and* 5)**

Solutions of 6.15 g (28 mmol) of L-tryptophane methyl ester (prepared from L-tryptophane by esterification with MeOH and $SOCl_2$) in 100 ml of benzene, and 5.00 g (30 mmol) of 5-(acetoxymethyl)-furaldehyde (prepared by acetylation of 5-(hydroxymethyl)furfural) in 100 ml of benzene were combined in 500 ml flask, a few mg of p-toluenesulfonic acid were added, and the reaction mixture was refluxed for 4 h. It was then evaporated to dryness, and the residue was chromatographed on SiO_2 column, using $CHCl_3$-MeOH-NH_4OH 990:9:1 mixture as an eluent, to yield 2.74 g of cis isomer **4** (absolute configuration 1R 3S) followed by mixture of **4** and **5** (0.70 g), and 4.62 g of trans isomer **5** (absolute configuration 1S 3S). Total yield 8.06 g (71 %).

Isomer cis **4**: mp 63–65 °C (from methanol). – $[\alpha]_D^{25} = 2.35°$ (chloroform). – CIMS: m/z 369 ($M^+ + 1$). – IR ($CHCl_3$): 3635, 3465, 2950, 2840, 1740, 1435, 1375, 1355, 1305, 1010 cm^{-1}. – UV (MeOH): 224, 274, 280 (sh), 290 nm. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 8.80 (s; 1H, NH indole), 7.90–7.55 (m; 4H, aromat.), 6.38 (d; 1H, H-4', J = 8 Hz), 6.32 (d; 1H, H-3', J = 8 Hz), 5.39 (s; 1H, H-1), 5.05 and 5.01 (2 s; 2H, CH_2OCO), 3.99 (dd; 1H, H-3, J_1 = 11 Hz, J_2 = 4.5 Hz), 3.81 (s; 3H, $COOCH_3$), 3.18 (m; 1H, H-4), 2.93 (m; 1H, H-4), 2.2 (broad s; 1H, NH), 2.06 (s; 3H, CH_3CO). – ^{13}C-NMR ($CDCl_3$): δ (ppm) = 173.1, 170.8, 154.0, 149.8, 136.1, 131.6, 126.9, 122.2, 119.7, 118.3, 111.6, 111.1, 108.6, 108.2, 58.1, 56.5 (C-3), 52.3, 51.7 (C-1), 25.5, 20.9. – $C_{20}H_{20}N_2O_5$ (368.2) Calc. C 65.2, H 5.28, N 7.6, Found C 65.6, H 5.71, N 7.3.

Isomer **5** (trans) mp 130–131 °C (methanol). – $[\alpha]_D^{25} = -1.33°$ (chloroform). – CIMS m/z 369 ($M^+ + 1$). – IR ($CHCl_3$): 3465, 2950, 2840, 1735, 1440, 1370, 1370, 1350, 1305, 1010 cm^{-1}. – UV (MeOH): 226, 275 (sh), 282, 290 nm. – ^{1}H-NMR ($CDCl_3$): δ (ppm) 8.28 (s, 1H, NH indole), 7.52–7.08 (m, 4H, aromat.), 6.29 (d; 1H, H-3', J = 3 Hz), 5.98 (d; 1H, H-4', J = 3 Hz), 5.31 (s; 1H, H-1), 5.01 and 5.00 (2s; 2H, CH_2OCO), 3.91 (dd; 1H, H-3, J_1 = 9 Hz, J_2 = 4.5 Hz), 3.75 (s; 3H, $COOCH_3$), 3.17 (dd; 1H, H-4, J_1 = 15 Hz, J_2 = 4.5 Hz), 2.95 (dd; H, H-4, J_1 = 15 Hz, J_2 = 9 Hz), 2.3 (broad s; 1H, NH), 2.04 (s; 3H, CH_3CO). – ^{13}C-NMR ($CDCl_3$): δ (ppm) 173.7, 170.7, 155.2, 149.6, 136.2, 122.2, 119.5, 118.3, 111.3, 111.1, 108.8, 108.6, 58.2, 52.3, 52.2 (C-3), 49.1 (C-1), 25.1, 20.8. – $C_{20}H_{20}N_2O_5$ (368.2) Calc. C 65.2, H 5.48, N 7.6, Found C 65.2, H 5.52, N 7.6.

1-(5-Acetoxymethyl-2-furyl)-3-carbomethoxy-9H-pyrido[3,4-b]indole **(6)**

A mixture of isomers **4** and **5** (4.65 g) was dissolved in 150 ml of toluene, 2.5 g of 5 % Pt/C was added and the reaction mixture was refluxed with stirring for 6 h, while air was passed slowly through the mixture. Then an-

other 1.0 g of Pt/C was added and the reaction mixture was refluxed for the next 6 h. It was then filtered through Celite and evaporated to dryness to yield 4.5 g of a yellow solid, which was crystallized from methanol to give 4.21 g (91 %) of yellow crystals, mp 154–156 °C. – CIMS: m/z 365 ($M^+ + 1$). – UV (MeOH): 215, 272, 298, 362, 377 nm. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 8.81 (s; 1H; NH indole), 8.20–7.26 (m, 5H, aromat.), 7.35 (d; 1H, H-3′, J = 3.5 Hz), 6.65 (d; 1H, H-4′, J = 3.5 Hz), 5.29 (s; 2H, CH_2OCO), 4.06 (s; 3H, $COOCH_3$), 2.19 (s; 3H, CH_3CO).

*1-(5-Hydroxymethyl-2-furyl)-9H-pyrido[3,4-b]indolo-3-carboxylic acid (**1** = Flazin).*

Compound **6** (70 mg) was dissolved in 30 ml of 1:1 water-methanol mixture, 1 ml of 2N NaOH was added and the reaction mixture was stirred at room temp. for 1 h. Then 1 ml of 2N HCl was added dropwise, a yellow precipitate was filtered, washed with water, and dried to give 52 mg of yellow material. The product was dissolved in 15 ml of hot methanol and left in a refrigerator overnight to yield yellow crystals mp 231–232 °C (lit.[1]: 230–232 °C). – CIMS: m/z 309 ($M^+ + 1$). – UV (MeOH): 215, 269, 295, 364, 379 nm. – ^{1}H-NMR (DMSO-d_6): δ (ppm) = 12.7 (broad s; 1H, COOH), 11.60 (s; 1H, NH), 8.86 (s; 1H, H-4), 8.43 (d; 1H, aromat., J = 8 Hz), 7.83 (d; 1H, aromat., J = 8 Hz), 7.66 ("t", 1H, aromat., J = 8 Hz), 7.44 (d; 1H, H-3, J = 3.5 Hz), 7.36 ("t", 1H aromat., J = 8 Hz), 6.64 (d; 1H, H-4, J = 3.5 Hz), 5.5 (broad s; 1H, OH), 4.70 (s; 2H, CH_2O).

1-(5-Hydroxymethyl-2-furyl)-3-carbomethoxy-9H-pyrido[3,4-b]indole **(7)**

Compound **6** (500 mg) was dissolved in 300 ml of methanol, then 0.5 ml of a concentrated aq. NH_4OH was added and the reaction mixture was kept at room temp. for 2 h. It was then evaporated to dryness and crystallized from methanol to give beige crystals mp 205–208 °C (lit.[1]: 199–200 °C). – CIMS: m/z 323 ($M^+ + 1$). – UV (MeOH): 215, 273, 299, 363, 378 nm. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 8.58 (s; 1H, NH indole), 8.07–7.25 (m; 6H, aromat.), 6.40 (d; 1H, H-4′, J = 3.5 Hz), 4.80 (s; 2H, CH_2O), 4.04 (s; 3H, $COOCH_3$).

1-(5-Acetoxymethyl-2-furyl)-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole **(9** *and* **10)**

Solutions of 5-acetoxymethylfuraldehyde (800 mg) in 50 ml of benzene and tryptamine (900 mg) in 50 ml of benzene were combined together, few mg of p-toluenesulfonic acid was added and the reaction mixture was refluxed for 3 h, while water was removed azeotropicaly with benzene. It was then evaporated to dryness, dissolved in 100 ml of ethylene glycol dimethyl ether, and few ml of Et_2O saturated with HCl gas was added (a yellow oil precipitated), and the reaction mixture was left at room temperature for 2 h. The solvent was then evaporated under reduced pressure, and a crude product was chromatographed on a silica gel column to yield 1.0 g (68 %) of the desired tetrahydro-β-carboline as a yellow oil. A small sample was crystallized from methanol to give colorless crystals mp 144–145 °C (lit.[3]: 148 °C, from ethyl acetate). – CIMS: m/z 311 ($M^+ + 1$). – UV (MeOH): 226, 275 (sh) 282, 290 nm.

1-(5-Acetoxymethyl-2-furyl)-9H-pyrido[3,4-b]indole **(11)**

1-(5-Acetoxymethyl-2-furyl)-β-tetrahydrocarboline (950 mg) was dissolved in 50 ml of toluene, 200 mg of 5 % Pt/C was added and the reaction mixture was refluxed for 8 h with stirring, while air was passed through the solution. It was then filtered through Celite, evaporated to dryness and chromatographed on a silica gel column to yield 470 mg (50 %) of the desired β-carboline, which was crystallized from ethyl acetate to give yellow crystals mp 157–159 °C (lit.[3]: 161 °C). – CIMS: m/z 307 ($M^+ + 1$). – UV (MeOH): 237, 252, 273, 292, 366, 381 nm.

*1-(5-Hydroxymethyl-2-furyl)-9H-pyrido[3,4-b]indole (**2** = Perlolyrine)*

Compound **11** (200 mg) was dissolved in 50 ml of methanol, then 0.1 ml of concentration aqueous solution of NH_3OH was added, and the reaction mixture was kept at room temp. for 3 h. It was then evaporated to dryness and crystallized from ethyl acetate to give 120 mg (70 %) of crystals mp 179–181 °C (lit.[1]: 186 °C). – CIMS: m/z 265 ($M^+ + 1$). – UV (MeOH): 237, 253, 272, 291, 366, 382 nm.

Biological Testing

Receptor Binding Assays

The ability of **6** to inhibit [^{3}H]diazepam binding to benzodiazepine receptors was evaluated in rat cerebral cortical membranes prepared as previously described[4]. In brief, incubations consisted of 0.3 ml tissue homogenate (1:100 in 50 mM Tris-HCl buffer, pH 7.4), 0.1 ml radioligand (final concentration 2 nM), 0.1 ml drug solution (final concentration 50–5000 nm), and buffer (50 nM Tris-HCl, pH 7.4) to final volume 1 ml. Nonspecific binding was defined by replacing 0.1 ml of buffer with an equal volume of flunitrazepam (final concentration 3 μM). Incubations were performed at 0–4 °C for 90 min and terminated by rapid filtration and washing over GF/B glass fiber filters. Radioactivity was determined in a Beckman LS 5801 liquid scintillation spectrometer.
No inhibition of [^{3}H]diazepam binding by compound **6** was observed at concentrations of up to 5 μM (results not shown).

Enzyme Inhibition Studies

The ability of these compounds to inhibit either highly purified human placental MAO A[8] or human liver MAO B[9] was also tested using previously established methods[10]. None of the alkaloids tested were found to inhibit MAO B, but compounds **2** (YS), and its acetylated derivative **11** were effective competitive inhibitors of MAO A, with K_i's of 7.2 μM for **2** and 1.1 μM for **11** (Table 1). These were the only compounds which produced any inhibition of MAO activity of those tested within the synthetic route beyond the acid cyclization step (results not shown). Although these compounds are very effective inhibitors of MAO A, they were about three orders of magnitude less potent than either harmine or harmaline (1.9 nM and 5.3 nM, respectively).

Table 1
Inhibition of MAO A by Selected β-Carbolines

Compound	Type of Inhibition	K_i
2	competitive	7.2 μM
11	competitive	1.1 μM
harmine	competitive	1.9 nM
harmaline	competitive	5.3 nM

Compounds were dissolved in dimethyl sulfoxide (**YS** and **11**), or water (harmine and harmaline). Control experiments were conducted by adding dimethyl sulfoxide without test compound to determine the extent of inhibition due to solvent. Kinetics of oxidation were measured versus various concentrations of kynuramine (0.8–9 K_m) in 50 mM sodium phosphate buffer, pH 7.5 in a final volume of 0.5 ml. Initial rates of reaction were measured in duplicate on a Beckman DU7HS spectrophotometer at 314 nm at 30° during the first 3 min of incubation. For inhibitor studies, increasing amounts of a particular test compound were added to each set of different concentrations of kynuramine (0.8–9 K_m). *Lineweaver-Burk* analysis was conducted on all sets of data, and graphs were analyzed to determine the type of inhibition. Since all compounds exhibited competitive inhibition, replots of the slopes of the lines versus concentration of inhibitor were performed to obtain K_i values.

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