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STRUCTURES OF FLAZIN AND YS, HIGHLY FLUORESCENT COMPOUNDS ISOLATED FROM JAPANESE SOY SAUCE

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Summary: The structures of flazin and YS were determined to be β-carboline derivatives, 1 and 2, substituted with a hydroxymethylfuran moiety at 1-position.

In 1936, Higashi[1] first found a highly fluorescent compound in sake(Japanese rice wine) lees and named it flazin. Flazin has also been found in Japanese rice vinegar[2], soy sauce[3] and miso[4](fermented soy bean paste), but its structure[5] has not been determined. YS(yellow substance) was also isolated from an old sake[6] and soy sauce[7]. Although the structural and synthetic studies of YS were reported recently[8], the spectroscopic data of the natural and synthetic YS were not consistent each other. Now, we report the structures of flazin and YS isolated from soy sauce.

Flazin and YS were isolated from Japanese soy sauce(Ichibiki brand tamari) as shown in fig. 1. Crude flazin was precipitated from ether extracts after standing for one week at 20°C. Filtration and washing with ether gave a crystalline powder, mp 220-225°C, which was further purified by recrystallization from methanol to give flazin (1), mp 230-232°C [IR ν_{max}(KBr) 3420, 1601, 1360, 1320 cm^{-1}; UV λ_{max}(MeOH) nm(ε) 263(26800), 290(22800), 355(11800), 370 (12400)]. ^{1}H-NMR spectrum of 1 in DMSO-d_6 showed only broad signals. Flazin (1) was an extremely polar compound and its very limited solubility in organic solvents as well as in water prevented further structural studies without derivatization.

Treatment of flazin (1) with diazomethane in methanol afforded monomethyl ester 3 in 90% yield [mp 199-200°C; MS m/z 322(M^+); ^{1}H-NMR δ($CDCl_3$) ppm 4.02 (3H, s), 4.79(2H, br.s), 6.43(1H, d, J=3.5 Hz), 7.32(1H, m), 7.38(1H, d, J=3.5 Hz), 7.56(2H, m), 8.08(1H, d, J=8 Hz), 8.62(1H, s)]. The ^{1}H-NMR spectrum of 3 indicated the presence of a 2,5-disubstituted furan moiety (6.43 and 7.38 ppm) and a hydroxymethyl group (4.79 ppm). The hydroxymethyl group was proved by acetylation of 3 with acetic anhydride/pyridine at 20°C to give a monoacetate 4[9] [-CH_2OAc; 5.23 ppm(2H, s)] in quantitative yield. Hydrogenolysis of 4 with H_2/5% Pd-C in methanol at 20°C gave a methylfuran derivative 5 in 95% yield [mp 158-159°C; MS m/z 306(M^+); ^{1}H-NMR δ($CDCl_3$) ppm 2.54 (3H, s), 4.04(3H, s), 6.24(1H, d, J=3.5 Hz), 7.30(1H, m), 7.32(1H, d, J=3.5 Hz, 7.58(2H, m), 8.14(1H, d, J=8 Hz), 8.70(1H, s), 9.44(1H, br.s)].

Presence of furan moiety was certified by further hydrogenation of 5 with H_2/20% Pd-C in methanol at 60°C for 2 hr to its tetrahydro derivative 6 in 85% yield [oil; MS m/z 310(M^+); UV λ_{max} (MeOH) nm(ε) 235(20700), 269(31800); ^{1}H-NMR δ($CDCl_3$) ppm 1.46(3H, d, J=7 Hz), 1.66(1H, m), 2.20(1H, m), 2.44(1H, m), 2.64(1H, m), 4.04(3H, s), 4.34(1H, m), 5.48(1H, t, J=8 Hz), 7.32(1H, m), 7.56(2H, m), 8.16(1H, d, J=8 Hz), 8.76(1H, s)]. The presence of the -CHX-CH_2-CH_2-CHX-CH_3group was confirmed by decoupling experiments on the ^{1}H-NMR spectrum of 6.

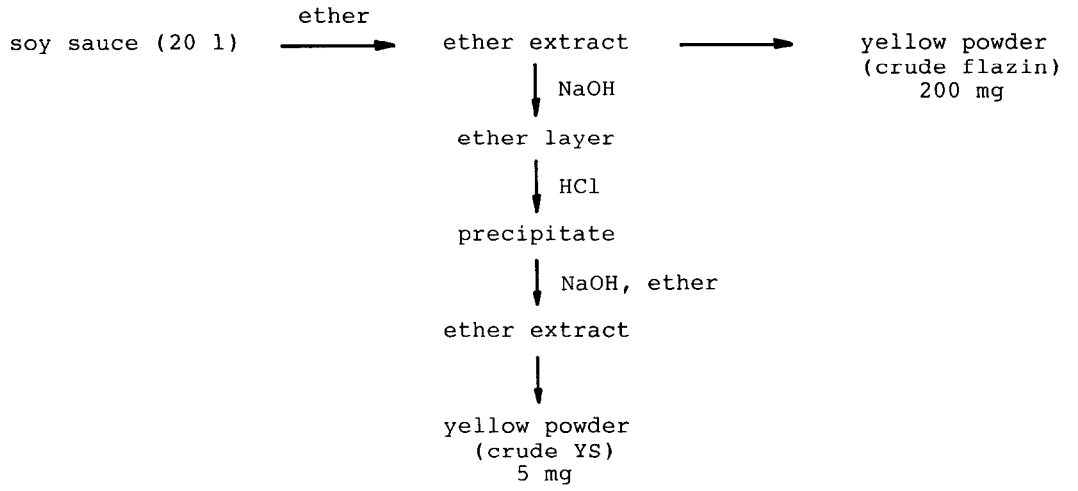
Thus, the furan moiety of 5 was transformed to the 2,5-disubstituted tetrahydrofuran moiety in 6 by hydrogenation. The UV spectrum and the aromatic proton signals in the ^{1}H-NMR spectrum of 6 were very similar to these of methyl β-carboline-3-carboxylate 7.[10] Therefore the structure of 6 was determined to be methyl 1-(5-hydroxymethyl-2-tetrahydrofuryl)-β-carboline-3-carboxylate. Consequently, the structure of flazin was concluded to be 1-(5-hydroxymethyl-2-furyl)-β-carboline-3-carboxylic acid (1).

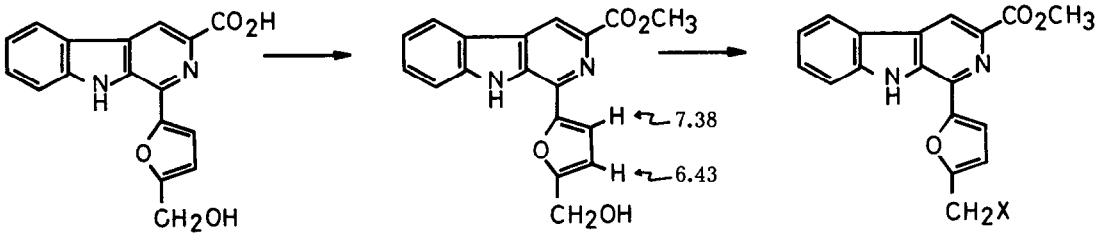
YS was isolated as follows: the ether extract was treated with 1N NaOH and the organic layer was acidified with conc. HCl to afford a small amount of precipitates(YS hydrochloride). The hydrochloride was treated with 1N NaOH and extracted with ether. After evaporation of the organic solvent, the residue was purified by thin layer chromatography on a silica gel plate to give YS (2) as yellow powder [mp 186°C; MS m/z 264(M^+); ^{1}H-NMR δ(CD_3OD) ppm 4.76(2H, s), 6.58(1H, d, J=3.5 Hz), 7.21(1H, d, J=3.5 Hz), 7.38(1H, t, J=8 Hz), 7.68(1H, t, J=8 Hz), 7.69(1H, d, J=8 Hz), 8.00(1H, d, J=5.5 Hz), 8.18(1H, d, J=8 Hz), 8.28(1H, d, J=5.5 Hz)].

^{1}H-NMR spectrum of YS was very similar to those of flazin methyl ester (3) except two proton signals appeared at 8.00 and 8.28 ppm and also similar to those of harmane. UV spectra of YS (2) and harmane were almost identical. Thus, the structure of YS was concluded to be 1-(5-hydroxymethyl-2-furyl)-β-carboline (2). The structure of YS is identical with these of perlolidin isolated from perennial rye-grass(Lolium perenn L.)[11], L. chuanxiong(a medicinal plant for treatment of angina pectoris)[12], and Korean ginseng[13]. The spectroscopic data of YS and and its acetate 8[14] were completely identical with those reported previously.[11]

The structures of flazin (1) and YS (2) are similar to those of biologically active β-carboline analogs, pyridindolol[15] (a β-galactosidase inhibitor), ethyl β-carboline-3-carboxylate[β-CEE][16] (a potent inhibitor of the specific binding of ^{3}H-diazepam to its brain receptors), etc. Synthetic studies of flazin (1) and biological tests of these β-carboline derivatives are now in progress.

Fig. 1. Isolation procedure of flazin (1) and YS (2).



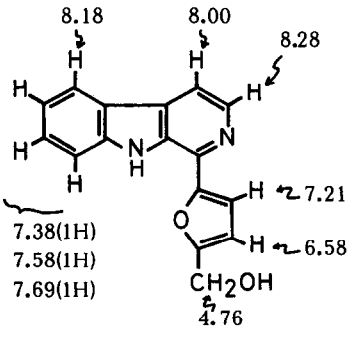


Flazin (1)

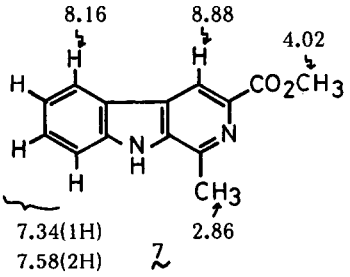
3

4 X=OAc

5 X=H



YS (2)
(Perlolidin)



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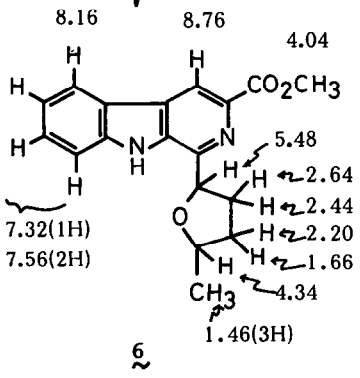

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9. **4**: oil; MS m/z 364(M^+); ^{1}H-NMR δ($CDCl_3$) ppm 2.16(3H, s), 4.03(3H, s), 5.23 (2H, s), 6.60(1H, d, J=3.5 Hz), 7.28(1H, m), 7.30(1H, d, J=3.5 Hz), 7.60 (2H, m), 8.08(1H, d, J=8 Hz), 8.72(1H, s).
10. **7** was prepared by condensation of α,β-dehydrotryptophan methyl ester with acetaldehyde [^{1}H-NMR δ($CDCl_3$) ppm 2.86(3H, s), 4.02(3H, s), 7.34(1H, m), 7.58(2H, m), 8.16(1H, d, J=8 Hz), 8.88(1H, s), 8.99(1H, br.s)]. S. Nakatsuka, K. Yamada and T. Goto, in preparation.
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14. **8**: MS m/z 306(M^+); ^{1}H-NMR δ($CDCl_3$) ppm 2.17(3H, s), 5.38(2H, s), 6.63(1H, d, J=3.5 Hz), 7.19(1H, d, J=3.5 Hz), 7.88(1H, d, J=5.5 Hz), 8.12(1H, d, J= 8 Hz), 8.42(1H, d, J=5.5 Hz).
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