REACTION OF 4-ACETOXY-1,4-BENZOXAZIN-3-ONE WITH AMINO ACID DERIVATIVES

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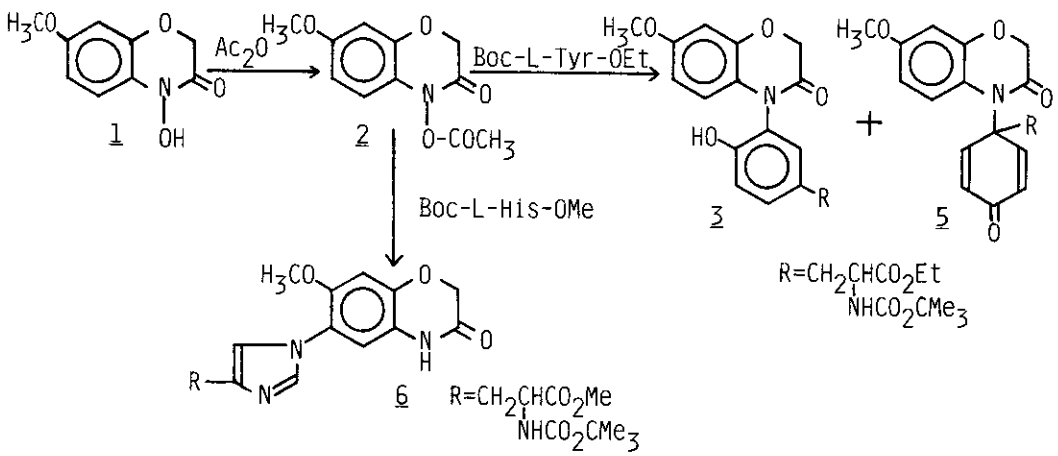
Abstract ——— A reaction of 4-acetoxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (2) with t-butoxycarbonyl-L-tyrosine ethyl ester (Boc-L-Tyr-OEt), t-butoxycarbonyl-L-histidine methyl ester (Boc-L-His-OMe) and t-butoxycarbonyl-L-tryptophan methyl ester (Boc-L-Trp-OMe) was described. The 4 and 6 positions in the benzozaxinone ring were attacked by the amino acids. The reaction with Boc-L-Trp-OMe gave hexahydropyrrolo-indoles.

A group of 1,4-benzoxazinones, which exhibits antifungal and insectistat properties, has been isolated from Job's tears, rye, wheat and corn.[1] It has two unique structural features: a benzoxazinone ring system and a cyclic hydroxamate. Recently, we found that some of them were moderately strong mutagens to bacteria.[2] We anticipated that 4-hydroxy-1,4-benzoxazinones reacted with biological macromolecules, such as proteins and nucleic acids, because some muta-carcinogenic arylhydroxylamines and arylhydroxamic acids were known to react with them after activation.[3] In fact, the acetate (2) of 4-hydroxy-7-methoxybenzoxazinone (1) reacted with DNA and guanylic acid,[4] and with nucleophiles such as phenols and indoles which are nucleophilic fragments of peptides.[5] In this paper we describe a reaction of 2 with amino acid derivatives: Boc-L-Tyr-OEt, Boc-L-His-OMe and Boc-L-Trp-OMe[6] as model compounds of peptides.

A freshly perpared 2 was treated with Boc-L-Tyr-OEt (8 equiv.) in benzene at room temperature. After the mixture was stirred for 30 min., the solvent was evaporated in vacuo and the residue was submitted to a silica gel column chromatography, eluted with ethyl acetate-methylene chloride. A tyrosine derivative (3) bound with the benzoxazinone [Anal. Calcd. for $C_{25}H_{30}N_2O_8$, C: 61.72, H: 6.22, N: 5.76, Found, C: 61.48, H: 6.28, N: 5.53] and 7-methoxy-2H-1,4-benzoxazin-3(4H)-

one (4) were obtained in 58% and 11% yields, respectively. In addition, a labile compounds (5), which decomposed on the silica gel column, was isolated by a careful work-up and with alumina column chromatography as a light yellow oil in 16% yield.

The structure of 3 was deduced from nmr (in DMSO-d_6): six aromatic protons [6.19 ppm (1H, d, J=8.9 Hz), 6.42 ppm (1H, dd, J=2.5, 8.9 Hz), 6.65 ppm (1H, d, J=2.5 Hz) and 6.80-7.20 ppm (3H, m)], a phenolic hydroxy proton (9.56 ppm) and the C^2-methylene (4.73 ppm). The structure of 5 was deduced from high resolution mass spectroscopy: obs. M^+ 486.1988 (Calcd. for $C_{25}H_{30}N_2O_8$: 486.1999), and



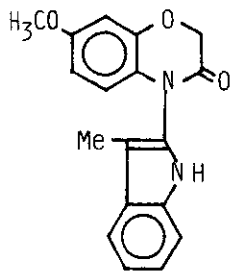
nmr (in $CDCl_3$): olefinic protons [6.39 ppm (2H, m) and 7.34 ppm (2H, m)], three aromatic protons [6.41 ppm (1H, dd, J=2.8, 9.2 Hz), 6.58 ppm (1H, d, J=2.8 Hz) and 6.71 ppm (1H, d, J=9.2 Hz)] and the C^2-methylene (4.47 ppm). The absorptions of lactam NH and phenolic OH were not found in nmr spectrum. The assigned structures were supported by the comparison with UV and nmr spectra of 3a and 5a (3 and 5: R=CH_3 prepared from 2 and p-cresol.[5]

Reaction of 2 with Boc-L-His-OMe (8 equiv.) in DMF at room temperature gave a histidine derivative (6) bound with the benzoxazinone as a light brown amorphous [Anal. Calcd. for $C_{21}H_{26}N_4O_7 \cdot \frac{1}{4}H_2O$, C: 55.93, H: 5.92, N: 12.42; Found, C: 55.93, H: 5.92, N: 12.19, Mass M^+ (obs.) 446.1833] in 61% yield. The structure of 6 was

deduced from nmr (ppm in $CDCl_3$): 6.92 (1H, br s), 7.69 (1H, br s) (those were assigned to the imidazole protons attached to the carbon atoms), and 6.68 (1H, s), 6.81 (1H, s) and 9.24 (1H, br s) (those were assigned to the aromatic protons of position 8 and 5, and NH proton of the benzoxazinone, respectively. It shows that the nitrogen atom of the imidazole ring of the histidine attacked the benzoxazinone ring. The UV spectra of 6 at neutral, acidic and basic media were very similar to those obtained for 6a (6, R=H) prepared from 2 and imidazole. 1,5-Disubstitution should shift the UV spectrum from that of 6a. Therefore, the structure of 6 is not rotationally hindered, and comprises 1,4-disubstituted imidazole rather than 1,5-disubstituted one.

Though a reaction of 2 with 3-methylindole gave 2-substituted 3-methylindole (7) in a low yield,[5] 2 reacted with Boc-L-Trp-OMe (2 equiv.) in methylene chloride at room temperature to give colorless prisms 8 (mp 185-186°C) and colorless powder 9 (mp 85-95°C) in 20% and 21% yield, respectively, after purification by the silica gel chromatography. Elemental analyses and mass spectra suggested the molecular formula, $C_{26}H_{29}N_3O_7$, for both the compounds. It appears that they were diastereoisomers, since their spectral data were very similar. 8 showed IR: ν_{max}(KBr, cm^{-1}), 3380 (NH), 1750 and 1685 (CO), UV: λ_{max}(95% EtOH, nm), 237, 286, 310 (sh), mass M^+ 495. 9 showed IR: ν_{max}(KBr, cm^{-1}), 3360 (NH), 1745 and 1688 (CO), UV: λ_{max}(95% EtOH, nm), 241, 286, 310 (sh), mass: M^+ 495. Their ^{13}C-nmr showed twenty-four absorptions including three carbonyl carbons, twelve aromatic carbons and nine aliphatic carbons. ^1H-nmr spectrum of 8 in $CDCl_3$ revealed seven aromatic proton absorptions at 6.39 ppm (1H, dd, J=2.8, 8.9 Hz), 6.57 ppm (1H, d, J=2.8 Hz), and 6.90 ppm (1H, d, J=8.9 Hz) (those were assigned to the benzoxazinone aromatic protons), at 6.60-6.82 ppm (2H, m), 7.13 ppm (1H, m) and 7.56 ppm (1H, m) (those for the indole ring protons). In addition, there appeared no signal attributable to the proton of the indole 2 position. Instead, a singlet at 5.82 ppm was found and assigned to the 8a proton of 1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole. Similarly, ^1H-nmr of 9 in $CDCl_3$ showed seven signals at 6.41 ppm (1H, dd, J=2.8, 8.9 Hz), 6.55 ppm (1H, d, J=2.8 Hz), 6.60-6.80 ppm (2H, m), 6.92-7.22 ppm (2H, m) and 7.52 ppm (1H, m), and the 8a-proton signal at 5.57 and 5.62 ppm as two singlets.[7] Therefore, it was concluded that the N^4 atom in the benzoxazinone attacked the 3 position of the indole to give an indolenine, and successive cis annulation which is more sterically favourable than trans one, led to the hexahydropyrroloindoles. The stereochemistry of 8 and 9 was deduced from their

spectra. The methyl signal of the ester group of 9 was obserbed at a higher field (3.26 ppm) than that of 8 (3.70 ppm). On the other hand, the signal of the hydrogen (position 2) of 9 was obserbed at a lower field (4.58 ppm) than that of 8 (4.13 ppm). The protons of the methyl ester of 9 and the methine hydrogen of 8



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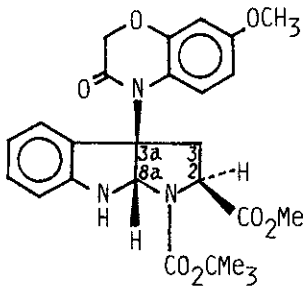

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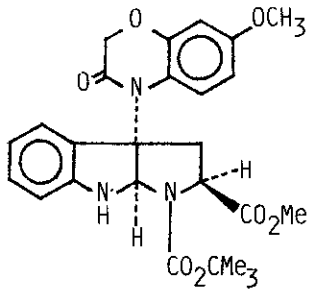

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were shielded by the benzene ring. The molecular model suggests that the methyl ester of the structure 9 (the anti isomer between 2-carbomethoxy and 3a-benzoxazinone ring) can be shielded by the benzene ring of the hexahydropyrroloindole. The spectral aspects of 8 and 9 correspond to the reported data for the syn and anti isomers of racemic 3a-hydroxy derivatives.[8]

In summary, 4-acetoxybenzoxazinone (2) reacted with tyrosine, histidine and tryptophan derivatives. Since 2 also reacted with some alkyl and aromatic thiols,[5] it probably reacts with cysteine. The result suggests that 4-hydroxybenzoxazinones, after activation such as esterification of the 4-hydroxy group, react with nucleophilic amino acid residues in proteins.

REFERENCES AND NOTES

1) O.Wahlroos and A.I.Virtanen, Acta. Chem. Scand., 1959, 13, 1906. A.I.Virtanen and E.Honkanen, ibid., 1960, 14, 1214. L.J.Coruera, M.D.Woodward, J.P.Helgeson, A.Kelman and C.D.Upper, Plant Physiol., 1978, 61, 791.

2) Y.Hashimoto, K.Shudo, T.Okamoto, M.Nagao, Y.Takahashi and T.Sugimura, Mutation Res., 1979, 66, 191.

3) J.A.Miller, Cancer Res., 1970, 30, 559.

4) T.Ishizaki, Y.Hashimoto, K.Shudo and T.Okamoto, Tetra. Lett., 1982, 23, 4055.

5) Y.Hashimoto, T.Ohta, K.Shudo and T.Okamoto, ibid., 1979, 1611.

6) Boc-L-Tyr-OEt (mp 99-104°C), Boc-L-His-OMe (mp 128-129°C) and Boc-L-Trp-OMe (mp 148-150°C) were prepared by the protection of the amino groups of L-amino acid esters with 2-(t-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON) and triethylamine in dioxane-water.

7) The nmr of 9 in $CDCl_3$ at room temperature showed two singlets at 1.14 and 1.51 ppm (4:1) for N-$COOCMe_3$ and two singlets at 5.57 and 5.62 ppm (1:4) for the 8a-proton. The split seems to be probably due to the restricted rotation, because those signals at 50°C in $CDCl_3$ changed to slightly broad singlets at 1.41 and 5.62 ppm, respectively.

8) M.Nakagawa, H.Watanabe, S.Kodato, H.Okajima, T.Hino, J.L.Flippen and B.Witkop, Proc. Natl. Acad. Sci. USA, 1977, 74, 4730.

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