STRUCTURE OF TYRIVERDIN, THE IMMEDIATE PRECURSOR OF TYRIAN PURPLE

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Stereoisomeric tyriverdins A and B were isolated from <u>Thais clavigera</u>. The former, which is identical with tyriverdin isolated by Baker, was confirmed by ¹³C-NMR spectrum to be 2,2'-bis(methylthio)-6,6'-dibromo-2,2'-diindoxyl. None of these compounds are detected in the gastropod, suggesting that they are formed during the isolation process probably from tyrindoxyl sulfate, a genuine constituent.

Although Tyrian purple, an ancient dye of molluscan origin, has been identified as 6,6'-dibromoindigotin 1 in 1909¹⁾, the dye itself is not present in the molluscs²⁾. In search of the precursor, Baker isolated from <u>Dicathais orbita</u> a pale yellow-green photosensitive substance named tyriverdin and demonstrated it to be the immediate precursor of 1. Furthermore he concluded that the substance is the quinhydrone type complex (formula 2a) between tyrindoxyl 3 and dehydrotyrindoxyl 4 on the basis of elemental analyses and ¹H-NMR and mass spectra²⁾. However, another structure, 2,2'-bis(methylthio)-6,6'-dibromo-2,2'-diindoxyl 2b, was later proposed by Christophersen from the similarity in behavior of tyriverdin and the model compound, 2,2'-bis(methylthio)-2,2'-diindoxyl^{3a)}, and also in ¹H-NMR spectra of tyriverdin and the synthetic 2,2'-bis(methylthio)-6,6'-dibromo-2,2'-diindoxyl (two stereoisomers)^{3b)}. Therefore, final proof was needed as to homogeneity and structure of tyriverdin and its genesis.



During our study on the chemical constituents of <u>Thais clavigera</u>⁴⁾ (Muricidae), a common gastropod in Japan, we have isolated a green substance from its hypobranchial glands (yellow or pale green) by extraction with ether in the absence of sunlight⁵⁾. The substance is stable in the dark in solid (argon atmosphere) but unstable in solution and yielded <u>1</u> on exposure to sunlight. The substance was identified as Baker's tyriverdin from this behavior and its spectra [λ ^{MeOH} 237 (ϵ 23350 sh), 252 (32410), 275 (12000 sh), 350 (2570), 402 (2870), 598 nm (710); v^{KBr} 3387, 2924, 1680, 1602, 1571, 1448, 1421, 1363, 1315, 1273, 1240, 1190, 1110, 1090, 1045, 1030, 959, 900, 851, 814, 771, 719, 641, 605, 565, 537 cm⁻¹; δ ^{DMSO-d6} 1.88 (s, Me), 6.97 (dd, J=8, 1.8), 7.31 (d, J=1.8), 7.48 (d, J=8), 8.22 (br.s)]^{2,6,7)}. However, when measured in very short period after isolation, the ¹H-NMR spectrum clearly exhibits a series of signals due to another compound with very similar structure in addition to those shown above [δ ^{DMSO-d6} 1.92 (s, Me), 6.89 (dd, J=8, 1.7), 7.08 (d, J=1.7), 7.33 (d, J=8), 8.03 (br.s)]. Therefore we designated them tyriverdins A and B (2A and 2B), respectively. <u>2B</u> is considerably less stable than 2A and converted to <u>1</u> on standing in DMSO, leaving 2A in pure form⁸⁾. Furthermore, the ¹H-NMR data of <u>2B</u> are identical with those of the stereoisomer reported by Christophersen^{3b)}. Thus, tyriverdin in its origin is a mixture of stereoisomers, tyriverdins A and B.

The presence of two isomers in tyriverdin implied Christophersen's formulation 2b is correct. For the final proof on the structure, 13 C-NMR spectrum of 2A was measured (DMSO-d₆ solution, proton-noise decoupled, 115,333 transients, 0.64 sec pulse interval). The spectrum clearly showed 9 signals, revealing the symmetrical dimeric structure. The signals were properly assigned as shown in comparison with those of isatin $5^{(9)}$ and 6-bromisatin $6^{(10)}$. The key signal for the structure 125.6 195.5 assignment is the weak singlet (quaternary origin) at 72.7 ppm which

undoubtedly indicates the presence of a tetragonal carbon in the ring system and verifies the structure $\frac{2b}{2b}$. 13 C-NMR spectrum of the mixture of 2A and 2B exhibited only signals shown (10,000 transients, 0.727 sec pulse interval)¹¹⁾.



In order to solve the problem that whether 2A and 2B are already present in the living cell system or the artifacts formed during isolation process, fresh hypobranchial glands from 15 bodies were placed in an NMR tube and the ¹H-NMR spectrum was measured immediately after the addition of DMSO-d₆ and mixing (343 transients, 4 sec pulse interval).

The spectrum shows signals due mainly to tyrindoxyl sulfate [8 2.44 (s), 7.08 (dd, J=8, 2), 7.44 (d, J=2), 7.57 (d, J=8)] and β,β-dimethylacrylylcholine^{2,12)} [δ 1.91 (s), 2.14 (s), 3.13 (s), 4.48 (br.m), 5.75 (br.s)]; no signal due to 2A or 2B was observed. However, the same solution exhibited signals due to 2A after being kept chilled in the dark for 24 days. These experiments demonstrate beyond doubt that the genuine constituent of the mollusc is tyrindoxyl sulfate²⁾ which hydrolyzes and undergoes the oxidative coupling to stereoisomeric tyriverdins, the immediate precursor of Tyrian purple, during the isolation $^{13)}$.

References and Notes

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- (1974) and references cited therein. See also, Idem, Pure and Appl. Chem., 48, 35 (1976).
- 3) a. C. Christophersen, F. Wätjen, O. Buchardt, and U. Anthoni, Tetrahedron Lett., 1977, 1747, b. Idem, Tetrahedron, 34, 2779 (1978).
- 4) Collected at Miyato Island, Miyagi Prefecture, during the period of April-June, in 1978 and 1979.
 5) 4, 6 and 2, 2-bis(methylthio)-6-bromindolinone²) were also isolated by preparative TLC (SiO₂ and Al₂O₃).
- 6) 1 H-NMR and 13 C-NMR chemical shifts were expressed in ppm from TMS.
- 7) J.T. Baker, personal communication (March 12, 1979).
- 8) In the absence of sunlight, pure 2A seemed stable in methanol.
- 9) V. Galasso, G. Pellizer, and G.C. Pappalardo, Org. Magn. Reson., 9, 401 (1977). The different assignment proposed [P.G. Gassman, B.W. Cue, Jr., and T.-Y. Luh, J. Org. Chem., <u>42</u>, 1344 (1977)] was found erroneous on the basis of the assignment of <u>6</u>.
- 10) <u>δ</u>: δ^{DMSO-d}6 114.9 (C-7), 116.9 (C-3a), 125.5 (C-5), 126.0 (C-4), 131.5 (C-6), 151.6 (C-7a), 159.1 (C-2), 183.1 (C-3).
- 11) It is not clear as to whether 2B gives identical signals with 2A or too unstable to be detected. After the measurement, no 2B was detected by ¹H-NMR.
- 12) J.T. Baker and C.C. Duke, Tetrahedron Lett., 1976, 1233.
- 13) We are indebted to Dr. J.T. Baker, Roche Research Institute of Marine Pharmacology, for spectra of tyriverdin, and to Mr. K. Aoki, ANELVA, Corp., for some of ¹³C-NMR measurements.

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