CHEMISTRY LETTERS, pp. 631-632, 1980. © The Chemical Society of Japan 1980

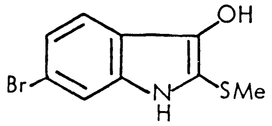
STRUCTURE OF TYRIVERDIN, THE IMMEDIATE PRECURSOR OF TYRIAN PURPLE

Yutaka FUJISE, Katsuyuki MIWA, and Shô ITÔ*

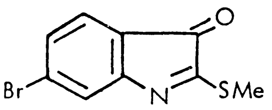
Department of Chemistry, Tohoku University, Sendai 980

Stereoisomeric tyriverdins A and B were isolated from Thais clavigera. The former, which is identical with tyriverdin isolated by Baker, was confirmed by ^{13}C-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.

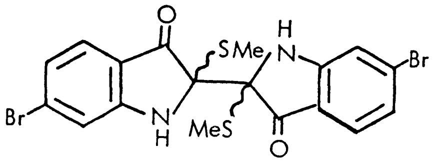
Although Tyrian purple, an ancient dye of molluscan origin, has been identified as 6,6'-dibromoindigotin **1** in 1909[1], the dye itself is not present in the molluscs[2]. In search of the precursor, Baker isolated from Dicathais orbita a pale yellow-green photosensitive substance named tyriverdin and 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 ^{1}H-NMR and mass spectra[2]. 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 ^{1}H-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.



3



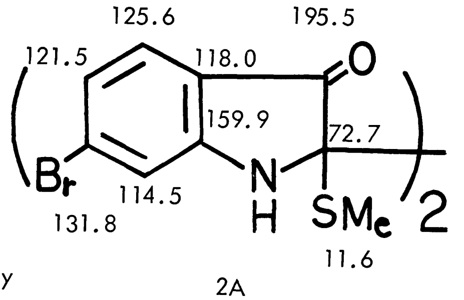
4



2b

During our study on the chemical constituents of Thais clavigera[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 sunlight[5]. The substance is stable in the dark in solid (argon atmosphere) but unstable in solution and yielded **1** on exposure to sunlight. The substance was identified as Baker's tyriverdin from this behavior and its spectra [λ_{max}^{MeOH} 237 (ϵ 23350 sh), 252 (32410), 275 (12000 sh), 350 (2570), 402 (2870), 598 nm (710); ν^{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-d_6} 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 ^{1}H-NMR spectrum clearly exhibits a series of signals due to another compound with very similar structure in addition to those shown above [δ^{DMSO-d_6} 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. **2B** is considerably less stable than **2A** and converted to **1** on standing in DMSO, leaving **2A** in pure form8). Furthermore, the ^{1}H-NMR data of **2B** are identical with those of the stereoisomer reported by Christophersen3b). 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-d_6 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 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 **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).



2A

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 ^{1}H-NMR spectrum was measured immediately after the addition of DMSO-d_6 and mixing (343 transients, 4 sec pulse interval). The spectrum shows signals due mainly to tyrindoxyl sulfate [δ 2.44 (s), 7.08 (dd, J=8, 2), 7.44 (d, J=2), 7.57 (d, J=8)] and β,β-dimethylacrylylcholine2,12) [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 isolation13).

References and Notes

1) P. Friedeländer, Ber., 42, 765 (1909). Idem, Angew. Chem., 22, 992, 2494 (1909).
2) J.T. Baker and M.D. Sutherland, Tetrahedron Lett., 1968, 43. J.T. Baker, Endeavour, 33, (118), 11 (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 (SiO_2 and Al_2O_3).
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., 42, 1344 (1977)] was found erroneous on the basis of the assignment of **6**.
10) **6**: $\delta^{\mathrm{DMSO}\text{-}d_6}$ 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 ^{1}H-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 ^{13}C-NMR measurements.

(Received March 8, 1980)