

ISOLATION OF CHOLINE AND CHOLINE ESTER SALTS OF TYRINDOXYL SULPHATE FROM THE
MARINE MOLLUSCS DICATHAIS ORBITA AND MANCINELLA KEINERI

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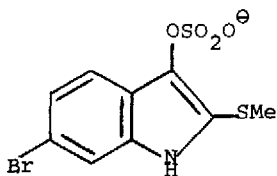
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(Received in UK 4 February 1976, accepted for publication 1 March 1976)

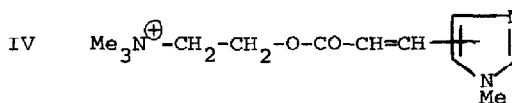
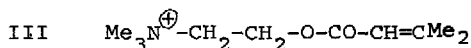
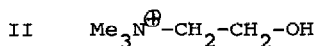
The hypobranchial glands of many dye-producing molluscs have been found to contain choline and choline esters^{1,2,3}. It has also been noted^{2,3} that the region of the glands containing the dye precursors also contain high concentrations of choline and/or choline esters. Examination of the ethanol extracts of the hypobranchial glands of the dye-producing gastropod molluscs, Dicathais orbita and Mancinella keineri has revealed an apparent association between tyrindoxyl sulphate⁴ (6-bromo-2-methylthioindoxyl sulphate) I and choline II (D. orbita) or β,β -dimethylacrylylcholine III (M. keineri). From the ethanol extract of the hypobranchial glands of D. orbita, after column chromatography on cellulose (Whatmann CC31, solvent n-butanol 9% water), were isolated colourless crystals identified as the choline II salt of tyrindoxyl sulphate I. Yield 1.49g from 500 glands, m.p. 164-166° (decomp.) analyses for $C_{14}H_{21}BrN_2O_5S_2 \cdot H$ n.m.r. (CD_3OD, TMS) δ 2.45 s (3H)SCH₃; δ 3.12 s (9H)N[⊕](CH₃)₃; δ 3.37 m (2H)CH₂N[⊕]; δ 3.90 m (2H)-CH₂O; δ 7.04 m (1H) ArH; δ 7.37 m (1H) ArH; δ 7.59 m (1H) ArH. Assignments are comparable with those of sodium tyrindoxyl sulphate and commercial choline chloride. U.V. λ_{max} (95% EtOH) 303.5, 226.5 nm, ϵ 17,300, 39,400. Similarly from the ethanol extracts of the freeze-dried glands of M. keineri were isolated colourless crystals identified as the β,β -dimethylacrylylcholine III salt of tyrindoxyl sulphate I. Yield 0.62g from 4.96g of freeze-dried glands, m.p. 69-72°, analyses for $C_{19}H_{27}BrN_2O_6S_2 \cdot H$ n.m.r. (CD_3OD, TMS) δ 1.93 d, J=2 (3H) C=C-CH₃; δ 2.18 d, J=2 (3H) C=C-CH₃; δ 2.48 s (3H) SCH₃; δ 3.13 s (9H) N[⊕](CH₃)₃; δ 3.59 m (2H) CH₂N[⊕]; δ 4.47 m (2H) CH₂OCO; δ 5.70 m (1H) HC=C; δ 7.10 m (1) ArH; δ 7.43 m (1H) ArH; δ 7.63 m (1H) ArH. Assignments are comparable with those of sodium tyrindoxyl sulphate and synthetic β,β -dimethylacrylylcholine iodide⁵. U.V. λ_{max} (95% EtOH) 303.5, 226.5 nm., 17,350, 51,750.

T.l.c. examination of fresh ethanol extracts of the molluscs using Merck 0.1mm cellulose plates with n-butanol:ethanol:acetic acid:water: 8-2:1:3 showed the presence of tyrindoxyl sulphate and of choline and choline esters. Visualisation was effected by (A) heating with dilute HCl for tyrindoxyl sulphate and (B) spraying with phosphomolybdic acid - SnCl_2 for choline and choline esters. D.orbita showed a U.V. active spot at Rf 0.67 giving positive tests (A) and (B). Another U.V. active spot at Rf 0.22 gave a negative test (A) and a positive test (B). This latter compound has been separated by paper chromatography and tentatively identified as an N-methylmurexine IV. Similar t.l.c. examination of M.keineri showed a U.V. active spot Rf 0.82 giving positive tests (A) and (B) and no other spots giving positive tests for either (A) or (B).

In D.orbita there is obviously a preference for the association of tyrindoxyl sulphate with choline II rather than association involving the N-methylmurexine IV also present. The association of tyrindoxyl sulphate with β,β -dimethylacrylylcholine III in M.keineri is very clear as both the sodium tyrindoxyl sulphate and β,β -dimethylacrylylcholine chloride have different Rf's from that of the associated compound when examined as separate salts. The presence of equimolar amounts of tyrindoxyl sulphate and choline ester is also evident in the high yield of salt isolated from the glands of M.keineri (15.5% of dry wt.).



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Acknowledgements: The work, supported by an ARGC Research Grant, was performed at the James Cook University of North Queensland.

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