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THE OCCURRENCE OF D-(+)-14/25-CYCLOHEXANETETROL IN *MONOCHRYSIS LUTHERI* DROOP*

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The only naturally occurring cyclohexanetetrol reported in the literature is betitol, weakly dextrorotatory (exact rotation not reported), m.p. 224°, which was isolated from sugar beet molasses in 1901[1]. Recently McCasland and co-workers[2] have suggested that this compound may be one of the two possible optically active diastereoisomeric 12/45-cyclohexanetetrols. The proposed structure of betitol rests mainly on elemental analyses, a negative response to Fehling's reagent, and its conversion to quinone by oxidation with potassium permanganate and sulfuric acid[1]. As the structure and configuration of betitol still require elucidation the above classification may be incorrect. In this communication we report the first unambiguous proof for the structure of a naturally occurring optically active

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cyclohexanetetrol, and on the basis of rotation data predict its absolute configuration.

The chrysophycean alga Monochrysis lutheri Droop was harvested by centrifugation after 10 days growth in an enriched seawater medium[3] under continuous light, and extracted with boiling 80% aqueous-ethanol. This extract was shaken with chloroform and separated into chloroform (discarded) and water soluble fractions. After desalting[4], the aqueous solution was reduced to a small volume and applied to sheets of Whatman No. 3 chromatography paper using the streak technique. D-(+)-14/25-Cyclohexanetetrol (I) was subsequently isolated on a paper chromatogram with n-butanol-ethanol-water (525:320:155 v/v) as the developing phase; a periodate-benzidine reagent[5] was used to detect the position of the compound on the guide strips. Compound I was obtained by eluting with water, clarifying the resulting aqueous solutions with animal charcoal, and evaporating the solutions to dryness. This procedure yielded approximately 250 mg of I from 50 l of culture.

Compound I was obtained as prisms from ethanol, $[\alpha]_D^{21}$+22.5° (c, 1.06 in water), m.p. 205-207° with decomposition (sintered at 131-132°). It was assigned structure I on the basis of the following evidence.

1) Acetylation of I with acetic anhydride in pyridine gave a viscous oil, $[\alpha]_D^{21}$+10.1° (c, 1.53 in $CHCl_3$), M.W. 314 (theory 317; osmometer method), which analysed correctly for a tetraacetate derivative (II). Found: C, 53.17; H, 6.35. Calcd. for $C_{14}H_{20}O_8$: C, 53.16; H, 6.37. Proton magnetic resonance (p.m.r.) of II in

$CDCl_3$ confirmed the presence of four equivalent acetoxyl groups (sharp singlet at τ 7.93), four methylene protons (multiplet with a width of 20 cps at average τ 7.88), and four methine protons geminal to the acetoxyl groups (multiplet with a width of 20 cps at average τ 4.92). A tetrabenzoate derivative of I, $[\alpha]_D^{21}$ -28.3° (c, 1.06 in $CHCl_3$), was also obtained as a viscous oil by reaction with benzoyl chloride in pyridine. The tetra-O-trifluoroacetyl, m.p. 104-106°, and tetra-O-trimethylsilyl, m.p. 57-59°, derivatives of I were crystalline.

2) The p.m.r. spectrum of I in pyridine at 60° gave signals for four methylene protons (multiplet at average τ 7.49), four methine protons geminal to hydroxyl groups (multiplet at average τ 5.51), and four hydroxyl protons (singlet at τ 4.54 which was temperature dependent). In dimethylsulfoxide solution four equivalent secondary hydroxyl protons gave a doublet at τ 5.58 with a spacing of 3 cps. This evidence, together with that in 1), requires that I have the molecular formula $C_6H_8(OH)_4$, and that the hydroxyl protons be magnetically equivalent even though the secondary hydroxyl groups are in an asymmetric environment (i.e. I is optically active).

3) Attempts to form isopropylidene and phenylboronate[6] derivatives of I were unsuccessful. Consequently I does not have a pair of cis hydroxyl groups in a vicinal or 1,3-diaxial relationship. Of the eight possible optically active cyclohexanetetrols[2] only structure I fulfills this requirement.

4) All p.m.r. data support the conclusion that members of each species of proton in I and II are magnetically equivalent. The narrow patterns for the methylene and methine protons in II [see 1) above] collapse to a broad singlet on double irradiation, and the acetoxyl methyl protons give a sharp singlet in a variety of solvents (pyridine, acetone-$\underline{d}_6$, benzene-$\underline{d}_6$ and carbon disulphide). Similarly, the hydroxyl protons of I are magnetically equivalent [see 2) above]. This evidence suggests a rapid interconversion between two equivalent conformations. Proof of this phenomenon was obtained by lowering the temperature of a solution of II in acetone-$\underline{d}_6$. At -98° the acetoxyl signal was present as a pair of partially resolved doublets (spacing 1.4 cps) of equal intensity with an average chemical shift between the pairs of 5.8 cps. Once again the only fully acetylated optically active cyclohexanetetrol which would be expected to behave in this manner is II.

5) At this stage the synthesis of DL-14/25-cyclohexanetetrol[2] came to our attention. The p.m.r. spectrum reported for this compound in deuterium oxide solution, was identical to that obtained with I.

6) By applying the method for calculating molecular rotations originated by Whiffen[7] and developed by Brewster[8], D-(+)-14/25-cyclohexanetetrol should have a rotation of +45 which is in good agreement with the value of +33.3 (148x22.5/100) obtained for I.

All p.m.r. spectra were recorded on a Varian A-60 spectrometer equipped with variable temperature probe and V-6058 spin-decoupler.

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