

MINOR ALKALOIDS OF TYLOPHORA ASTHMATICA

X-RAY ANALYSIS OF TYLOPHORINIDINE

V. K. WADHAWAN* and S. K. SIKKA

Nuclear Physics Division, Bhabha Atomic Research Centre

Trombay, Bombay 400 085, India

and

N. B. MULCHANDANI

Bio-Organic Division, Bhabha Atomic Research Centre

Trombay, Bombay 400 085, India

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Tylophorinidine is a minor phenolic alkaloid of Tylophora asthmatica Wight et Arn syn. T. indica (Burm) Merril. This potential antitumour compound was isolated by one of us (N. B. M.)¹ and, on the basis of the UV, IR and mass spectra of the parent compound, the UV and IR spectra of its methyl ether, and the UV, IR and NMR spectra of its diacetate, structure I¹ was assigned to it. The absence of any alcoholic group in the compound was inferred from the IR spectrum of its methyl ether, since it lacked the absorption band of the hydroxyl group in the usual range. The NMR of the diacetate, run on 60 MHz, indicated the presence of two meta and two para oriented protons. This also seemed to favour the above structure assignment to tylophorinidine. However, this could not be considered unequivocal, since varying assignments of NMR values to protons in the phenanthrene ring have appeared in recent times.²⁻⁵ An x-ray analysis was therefore undertaken for establishing the structure unambiguously and for obtaining information about the stereochemistry of the molecule. This has led to a revision of the structure of tylophorinidine, which has been found to be II. In this structure there is one alcoholic hydroxyl at C₁₄, one phenolic hydroxyl at C₆, and two methoxyls at C₃ and C₇.

For x-ray analysis purposes, a heavy-atom derivative, namely, tylophorinidine

diacetate methiodide, was prepared by refluxing tylophorinidine diacetate with methyl iodide in chloroform for one hour and working up the reaction mixture as usual. The product was crystallised from a methanol-water mixture, and the yellow, needle-shaped crystals obtained were dried under vacuum. The crystals darkened slightly on long exposure to x-rays.

Weissenberg and oscillation photographs indicated that the compound crystallises in the monoclinic system, with lattice parameters: $a = 26.89 \text{ \AA}$, $b = 12.35 \text{ \AA}$, $c = 16.62 \text{ \AA}$ and $\beta = 109^\circ$. From the observed systematic absences of reflections and the breakdown of Friedel's law, the space group was derived as C2. The density of the crystal, determined by measuring the loss of weight on immersion in petroleum ether, was 1.52 g cm^{-3} . This agreed well with the calculated value, 1.51 g cm^{-3} , obtained by assuming the presence of eight units of $\text{C}_{27} \text{H}_{30} \text{NO}_6 \text{ I}$ in the unit cell. There are, therefore, two molecules in the asymmetric unit of the cell.

Three-dimensional Weissenberg data were collected by mounting the crystal along the needle-axis (c-axis) and using Ni-filtered $\text{CuK}\alpha$ radiation. No measurable intensities could be recorded for Bragg angles beyond 45° even with very long x-ray exposures. Intensities of 1387 independent reflections were estimated by visual comparison with a standard intensity strip. After the usual data reduction, a three-dimensional Patterson synthesis was made, from which the positions of the two iodine atoms in the asymmetric unit could be readily deduced. These iodine positions were used to compute the first Fourier synthesis. Positions of 36 of the 70 non-hydrogen atoms could be located from this. The remaining non-hydrogen atoms were located by successive Fourier syntheses and least-squares refinement. Because of the large number of parameters involved, the least-squares refinement was done by varying the parameters in batches.

The absolute configuration was determined by comparing the observed differences in intensity of seven Bijvoet pairs of strong reflections with their calculated values, taking $\Delta f' = -1.2$ and $\Delta f'' = 7.0$ for the anomalous scattering of $\text{CuK}\alpha$ radiation by the iodine atoms.

At the present stage of refinement, with isotropic temperature factors for all atoms, the R value (for 934 observed reflections) is 0.13. The differences in the corresponding bond lengths and angles in the two molecules in the asymmetric unit are within the estimated standard deviations. These have, therefore, been averaged, and Fig. 1 shows a computer-plot of the average molecule. From this the structure of tylophorinidine was inferred as II.

At this stage the mass spectra of tylophorinidine monomethyl ether and tylophorinidine diacetate were also available to us. These revealed molecular ions at 379 and 449, respectively.

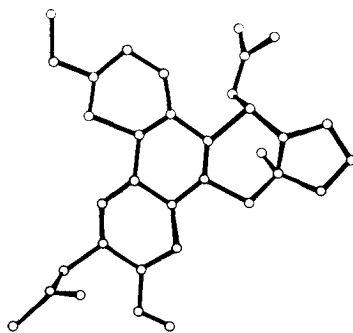
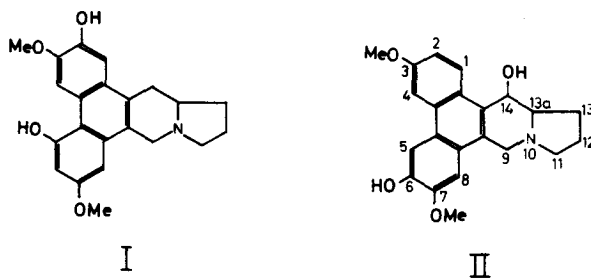


FIG. 1

vely. Also, the M-60 ion due to the loss of acetic acid in tylophorinidine diacetate could be seen at 389, thus confirming the assignment of structure II to tylophorinidine.

A re-examination of the IR spectrum of the methyl ether of tylophorinidine showed the presence of a band at 3175 cm^{-1} . This can be attributed to the $\text{C}_{14}\text{-OH}$, provided the latter is postulated as involved in hydrogen bonding. This is possible if the methyl ether exists as a dimer.

When this work was completed, Govindachari et al.⁶ reported a reinvestigation of tylophorinidine by non-X-ray methods. The gross structure proposed by them agrees with that obtained by us from X-ray diffraction. Also, their conclusion about the trans-diaxial disposition of the $\text{C}_{14}\text{-OH}$ and the $\text{C}_{13a}\text{-H}$ is confirmed by our study. But, for the orientation shown in II, the $\text{C}_{14}\text{-OH}$ is below the plane of the molecule, and $\text{C}_{13a}\text{-H}$ above it. This is just the opposite of that suggested by Govindachari et al.

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