Electronic Supplementary Material (ESI) for ChemComm.

Triple helix and rod structures of the antiseptic drug bibrocathol revealed by electron crystallography

Electronic Supplementary Information

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Powder X-Ray Diffraction Studies

In-house PXRD measurements were carried out using a Panalytical X'pert Pro diffractometer (Cu Kα1,2, λ_1 = 1.540598 Å, λ_2 = 1.544426 Å) using a Bragg−Brentano geometry, loading the samples on zero-background Si plates. Variable temperature experiments were carried out using an Anton Paar XRK 900 high-temperature chamber, connected to a rotation pump. The Pawley fit against PXRD data was carried out using TOPAS Academic V6.[1]

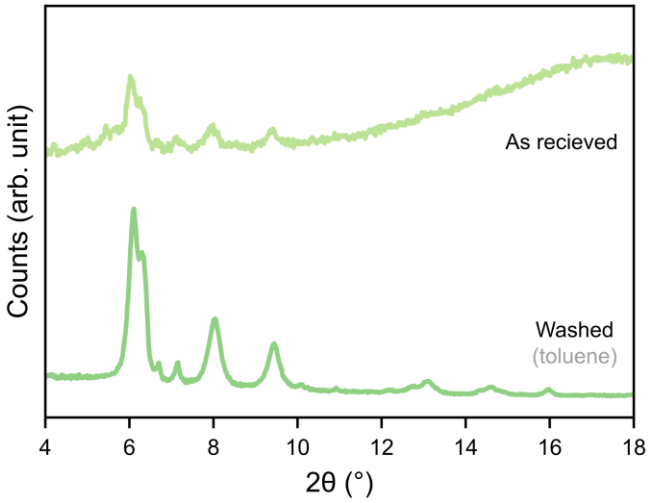

Fig. S1. Measured powder X-ray diffraction patterns of as-received bibrocathol formulation as an ointment and the solid obtained after washing the product with toluene (λ_1 = 1.540598 Å, λ_2 = 1.544426 Å).

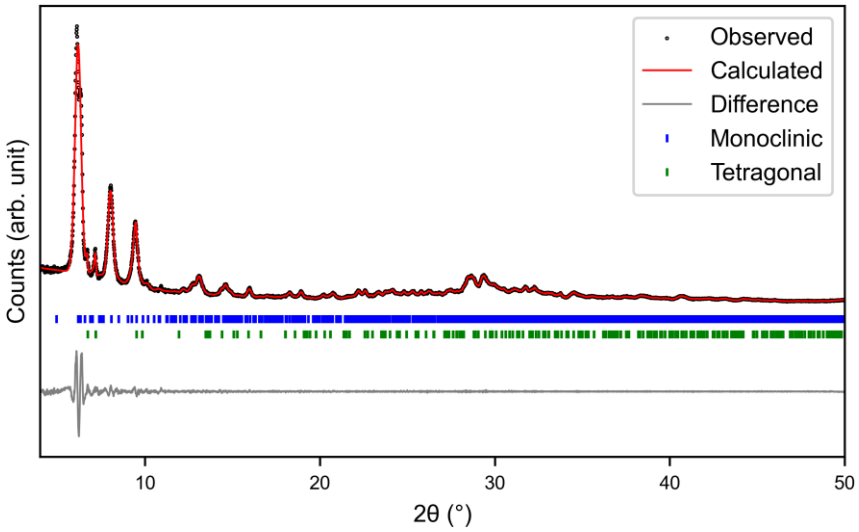

Fig. S2. Plot for the Pawley fit of both the monoclinic and tetragonal phases of bibrocathol. The data were collected using Cu Kα radiation (λ_1 = 1.540598 Å, λ_2 = 1.544426 Å).

Table S1. Crystallographic table for the Pawley fit of bibrocathol against PXRD data using the unit cells acquired from the 3DED measurements (Table S2).

Crystal system	**Monoclinic**
Space group	$P2_1/n$ (No. 14)
Unit cell dimensions	a = 16.48(7) Å
	b = 26.91(9) Å
	c = 24.08(3) Å
	β = 96.6(2) °
Volume (Å^3)	10610(62) Å^3
Crystal system	**Tetragonal**
Space group	$I4_1/a$ (No. 88)
Unit cell dimensions	a = 26.27(2) Å
	c = 13.90(2) Å
Volume (Å^3)	9591(16) Å^3
Wavelength	λ_1 = 1.540598 Å λ_2 = 1.544426 Å
Refinement method	Pawley
Refinement statistics	R_{wp} = 4.14 %
	GOF = 2.03

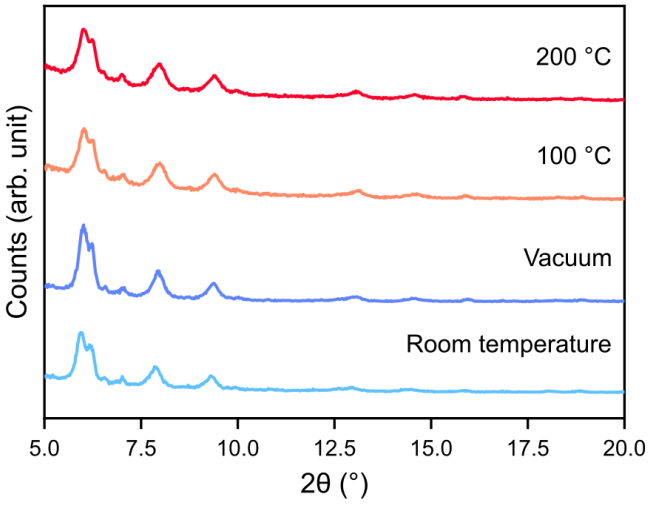

Fig. S3. Variable temperature powder X-ray diffraction patterns of bibrocathol, collected under reduced pressure (approximately 0.5 mbar, λ_1 = 1.540598 Å, λ_2 = 1.544426 Å).

3D electron diffraction (3DED)

The sample was prepared by sprinkling toluene-washed bibrocathol (obtained from a formulation of Noviform® 5%) onto holey carbon grids. Three-dimensional electron diffraction data were collected using a JEOL JEM2100 TEM, equipped with a Timepix detector from Amsterdam Scientific Instruments, while continuously rotating the crystals at 0.45° s^{-1}. The experiment was carried out using Instamatic,[2] with data reduction and merging (5 datasets for the monoclinic phase and 7 datasets for the tetragonal phase) being performed in XDS.[3] The acquired intensities were then used to solve the structure of each phase using SHELXT,[4] and refined using SHELXL,[5] with electron scattering factors as previously published by Peng.[6] From the 3DED data, all non-hydrogen atoms could be located in the initial structure solution using the program SHELXT.

Table S2. Crystallographic table of 3DED data and the refinement of the **Monoclinic** (5 crystals) and **Tetragonal** (7 crystals) phases of bibrocathol.

Compound	**Monoclinic**	**Tetragonal**
Chemical formula	$C_{60}Bi_{10}Br_{40}O_{25}$	$C_{12}BiBr_8O_5$
Formula weight	6406.59 g mol^{-1}	1072.34 g mol^{-1}
Temperature	293(2) K	293(2) K
Wavelength	0.0251 Å	0.0251 Å
Crystal system	Monoclinic	Tetragonal
Space group	$P2_1/n$ (No. 14)	$I4_1/a$ (No. 88)
Unit cell dimensions	a = 17.01(9) Å	a = 25.16(13) Å
	b = 27.26(14) Å	c = 13.98(7) Å
	c = 26.21(13) Å	
	β = 94.7(2)°	
Volume	12113(105) $Å^3$	8850(99) $Å^3$
Z	4	16
Density (calc.)	3.513 g cm^{-3}	3.219 g cm^{-3}
Index ranges	$-19 \le h \le 19$	$-27 \le h \le 30$
	$-31 \le k \le 30$	$-27 \le k \le 30$
	$-29 \le l \le 29$	$-16 \le l \le 16$
Reflections collected	120385	83501
Completeness	89.8 % (0.86 Å)	99.4 % (0.83 Å)
Independent reflections	18190	4171
	[R(int) = 0.2785]	[R(int) = 0.5154]
Data / restr. / param.	18190 / 12 / 481	4171 / 76 / 237
Goodness-of-fit on F^2	1.114	1.120
Final R index [$I > 4\sigma(I)$]	R1 = 0.2346	R1 = 0.2111

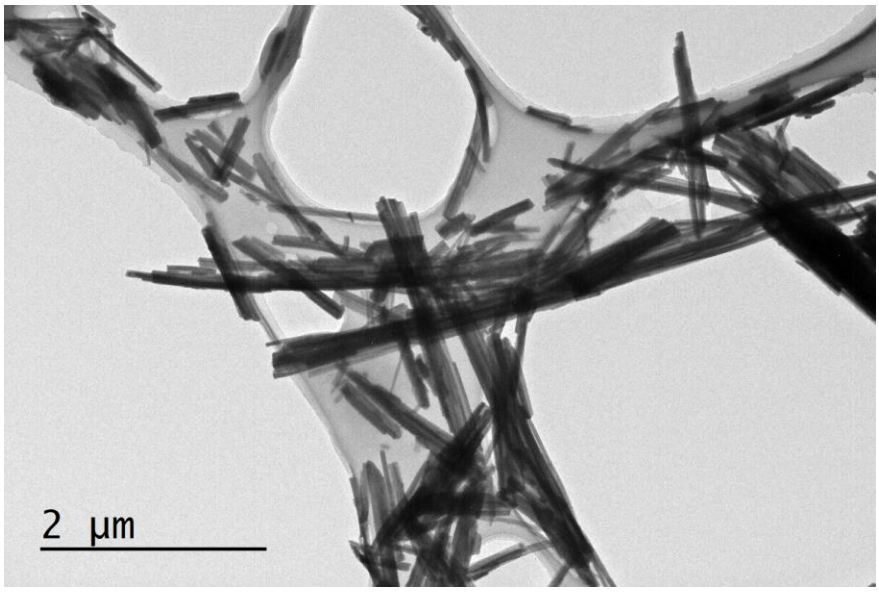

Fig. S4. Brightfield transmission electron microscopy image of aggregated bibrocathol crystals, showing a polydisperse distribution of crystals having a plank-shaped morphology.

Elemental analysis

The elemental analysis was performed by Medac Ltd (United Kingdom) on the same toluene-washed sample that was used for the 3D ED measurements. The results showed a larger than expected carbon and hydrogen content as well as a lower than expected bromine and bismuth content, likely owing to the presence of residual petroleum oil that was not fully washed away.

Table S3. Elemental analysis of toluene-washed bibrocathol.

Element	C (wt. %)	H (wt. %)	N (wt. %)	Br (wt. %)	Bi (wt. %)	Weight ratio, Br:Bi
Observed	21.47	2.34	<0.10	37.84	26.54	1.42
Expected, **monoclinic**	11.25	0	0	49.89	32.62	1.53
Expected, **tetragonal**	13.40	0.28	0	59.44	19.43	3.06

Supplemental references

(1) Coelho, A. TOPAS-Academic V6. Coelho Software. 2016.
(2) Cichocka, M. O.; Ångström, J.; Wang, B.; Zou, X.; Smeets, S. High-Throughput Continuous Rotation Electron Diffraction Data Acquisition via Software Automation. *J. Appl. Crystallogr.* **2018**, *51* (6), 1652–1661.
(3) Kabsch, W. *XDS*. *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2010**, *66* (2), 125–132.
(4) Sheldrick, G. M. SHELXT - Integrated Space-Group and Crystal-Structure Determination. *Acta Crystallogr. Sect. A Found. Crystallogr.* **2015**, *71* (1), 3–8.
(5) Sheldrick, G. M. A Short History of SHELX. *Acta Crystallogr. Sect. A Found. Crystallogr.* **2008**, *64* (1), 112–122.
(6) Peng, L. M. Electron Atomic Scattering Factors and Scattering Potentials of Crystals. *Micron* **1999**, *30* (6), 625–648.