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Synthesis and Crystal Structure of Pilloin

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Pilloin was synthesized by methylation of luteolin and its structure was characterized by element analysis, ^{1}H-NMR, IR, and single-crystal X-ray diffraction analysis. It crystallizes in the monoclinic crystal system, space group $P2_1/n$, with a = 7.336(3) Å, b = 14.257(5) Å, c = 13.298(5) Å, β = 93.516(5)°. The flavone molecules of pilloin are linked into puckering sheets via $R_3^3(14)$ and $R_2^2(9)$ graph-set ring motifs defined by the C-H... O and O-H... O hydrogen bonds. $\pi-\pi$ stacking interactions lead to the flavone skeletons of columns that are held together through centrosymmetric$R_2^2(8)$ motifs. Hydrogen bonding and aromatic $\pi-\pi$ stacking interactions assemble the title compound into a 3-dimensional network structure.

Key Words: Pilloin, crystal structure, hydrogen bonding, $\pi...\pi$ stacking, motif.

Introduction

Flavonoids (2-phenylbenzo-γ-pyrones) are a broad class of polyphenolic secondary metabolites that are abundant in plants and in various common foods such as apples, onions, tea, and red wine.[1] Apart from their important biological roles in nitrogen fixation and chemical defense, flavonoids possess a broad range of pharmacological properties including anti-oxidant, anti-cancer, anti-viral, and anti-inflammatory properties.[2] Studies have also found that hydroxyflavones and methoxyflavones displayed a significant growth inhibitory action against Jurkat, PC-3, and Colon 205 cancer cell lines.[3] Luteolin (3', 4', 5, 7-Tetralydroxyflanone), a polyphenolic compound available in foods of plant origin, belongs to the flavone subclass of flavonoids, usually occurring as glycosylated forms in celery, green pepper, perilla leaf, and camomile tea.[4,5] It has been reported to display anti-mutagenic and anti-platelet aggregation and anti-cancer effects,[6,7] and has been described as the most potent and efficient inhibitor of TNF-α, interleukin-6, and NO expression in LPS-stimulated macrophages.[8] Pilloin (3', 5-dihydroxy-4', 7-dimethoxylflavone) is a derivative of luteolin and has potential

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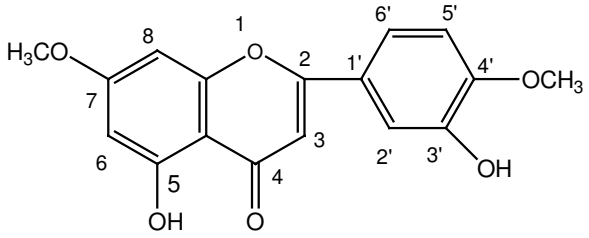
medical applications. It has been reported that pilloin was isolated from many genera of plants[9−14] and exhibited trypanocidal activity,[11] possible immunomodulatory and cytotoxic effects,[12] and the lymphocyte transformation process by exerting cytotoxic action targeted at the transformed lymphoblasts.[14] Previously, pilloin was synthesized by condensation of 2,4 -dimethoxy-6-hydroxyacetophenone with isovanillin followed by SeO_2 oxidant and $AlCl_3$ demethylation.[15] Simultaneously, Gupta et al.[16] synthesized pilloin using hesperidin as a starting compound through a complex series reaction. In this study, pilloin was synthesized easily by methylation of luteolin and its crystal structure was determined by single-crystal X-ray diffraction. The structures of pilloin can be expressed as in the Scheme.



Scheme

Experimental

General

Chemicals were of analytical reagent grade and used directly without further purification. Elemental analyses were determined using a PE-2400 elemental analyzer. Melting points were determined on a digital melting point instrument (Electro thermal model WRS-1B). The infrared spectra were recorded as KBr pellets on a Nicolet 170SX FT-IR spectrometer in the 4000-400 cm^{-1} region. The ^{1}H-NMR spectra were recorded on a Bruker AM-300 spectrometer with TMS as internal reference and DMSO-d_6 as solvent. The crystal structures were determined with a Bruker Smart-1000 CCD diffractometer.

Synthesis of Pilloin

Luteolin (1.0 g) was dissolved into acetone (30 mL) and KOH (1 mL, 37%) was added. Then, dimethyl sulfate (4 mL) was added dropwise to the solution with strong stirring. After the mixture was stirred at room temperature for 8 h, it was poured into 50 mL of water and yellow precipitation appeared. The precipitate was filtered off and put into 50 mL of NaOH (2 mol/L) with stirring. After that, the solution was filtered off and the filtrate was acidified with HCl (5%) until the pH of the filtrate was 7, and a precipitate appeared. It was filtered and recrystallized from alcohol to give pale yellow needles of pilloin in good yield (88.3%), mp 513-515 K. Crystal of pilloin suitable for X-ray analysis was recrystallized from ethyl acetates after 2 weeks at room temperature. Anal. calcd for $C_{17}H_{14}O_6$(%): C 64.97, H 4.49; found C 64.78, H 4.63. IR (KBr, cm^{-1})ν: 3298, 2933, 2843, 1658, 1603, 1506, 1448, 1270, 1200, 1148, 1037, 930, 866, 814 cm^{-1}. ^{1}H NMR(DMSO-d_6, 300 MHz): 6.82(s, 1H, H-C_3), 6.39(s, 1H, H-C_6), 6.76(s, 1H, H-C_8), 7.47(s, 1H, H-$C_{2'}$), 7.10(d, 1H, J=8.5 Hz, H-$C_{5'}$), 7.58(d, 1H, J=8.5 Hz, H-$C_{6'}$), 3.88(s, 3H, C_7-OCH_3), 3.32(s, 3H, $C_{4'}$-OCH_3), 12.94(s, 1H, C_5-OH), 9.44(s, 1H, $C_{3'}$-OH). ^{13}C NMR(DMSO-d_6, 300 MHz): 181.2(C_4), 165.1(C_7), 163.8(C_2), 160.8(C_5), 157.2(C_{8a}), 151.2($C_{4'}$), 146.6($C_{3'}$), 122.8($C_{1'}$), 118.9($C_{6'}$), 112.9($C_{5'}$), 112.2($C_{2'}$),

104.6(C_{4a}), 103.7(C_3), 97.9(C_6), 92.7(C_8), 56.0(C_7-O**C**H$_3$), 55.7($C_{4'}$ -O**C**H$_3$). The ^{1}H-NMR data of pilloin are in agreement with those in the literature.[17]

Crystallographic Data and Structure Determination

Single-crystal structure measurements were obtained on a Bruker Smart 1000 CCD area detector with graphite monochromatized Mo$K\alpha$ radiation (λ= 0.71073 Å). The data were collected in the range of 2.10 < θ <25.09° for pilloin using the ψ and ω scan technique at a temperature of 293(2) K. LP correction was applied to the data. The structure was solved by direct methods using the SHELXL[18,19] program and refinement on F^2 was performed using SHELXL-97 by full-matrix least-squares with anisotropic thermal parameters for all non-hydrogen atoms. Phenol hydroxyl H atoms were placed in calculated positions, with O—H = 0.82 Å, and refined using a riding model with Uiso(H) = 1.5 Ueq(O). H atoms bonded to C atoms were placed in calculated positions with C—H = 0.93 Å and 0.96 Å and refined as riding, allowing for free rotation of the rigid methyl groups; Uiso(H) = 1.2 Ueq(C) or 1.5 Ueq(Cmethyl). Crystallographic data and experimental details for the structural analyses are summarized in Table 1. The selected bond lengths and angles and the hydrogen bonding are given in Tables 2 and 3, respectively.

Results and Discussion

The molecular structure of pilloin is depicted in Figure 1. It is composed of a benzopyranone moiety, a phenyl moiety, 2 hydroxyl groups, and 2 methoxyl groups. The geometry of the bond lengths and angles of the flavone skeleton of pilloin are similar to those in both Luteolin[21], 5-hydroxy-4',7-dimethoxyflavone[22] and Eupatorin.[23] The atoms of the benzopyranone moiety including ring A (C4–C9) and ring C (C1–C4/C9/O1) are essentially coplanar [the mean deviation form the least-squares planes is 0.031(2) Å], the dihedral angle between the rings being 3.2(1)°. The phenyl rings B (C10–C15) and benzopyranone moieties, in turn, make an angle of 6.0(1)° [torsion C2–C1–C10–C15 175.3(2)°], which shows that the flavone skeletons deviate only slightly from planarity. On the other hand, pilloin has an additional possible intramolecular hydrogen bond of the C–H—O type, which is a relatively short intramolecular contact from C15–H15 to O1 [geometry for a C–H bond length of 0.93 Å, H—O = 2.33 Å, C15—O1 = 2.671(3) Å, angle at H = 101.3°]. This

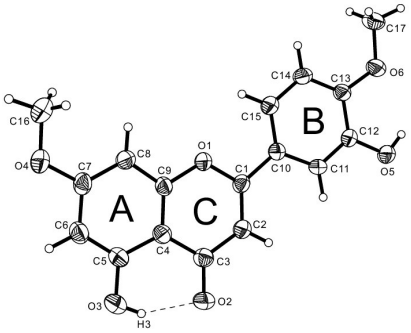


Figure 1. A view of a molecule of pilloin showing the atom-numbering scheme and with 50% probability displacement ellipsoids. Thin dashed lines represent intramolecular hydrogen bonds.

contact is inherent to the flavone molecule if the phenyl and benzopyrane are roughly coplanar. This was previously pointed out by Etter et al.[24] The marked planarity of pilloin suggests that there could be extensive conjugation between the heterocyclic ring and the exocyclic phenyl ring.

Table 1. Crystal data and structure refinement parameters for pilloin.

Formula	$C_{17}H_{14}O_6$
Formula weight	314.28
Color/shape	Yellow/needle
Crystal system	Monoclinic
Space group	P 2_1/n
Unit cell dimensions	a = 7.336(3) Å b = 14.257(5) Å c = 13.298(5) Å β = 93.516(5)°
Volume	1388.2 (9) Å^3
Z	4
Density (calculated)	1.504 mg/m^3
Absorption coefficient	0.115mm^{-1}
F(000)	656
Crystal size	0.17 × 0.14 × 0.11 mm^3
The range for data collection	2.10 to 25.09°
Index ranges	-8≤ h ≤8, -17≤ k ≤8, -15≤ l ≤15
Reflection collected	6831
Independent reflections	2470 [R(int) = 0.0383]
Completeness to theta = 25.09°	99.9%
Absorption correction[20]	Multi-scan for equivalents
Max. and min. transmission	0.9870 and 0.9810
Refinement method	Full-matrix least- squares on F^2
Data/parameters	2468/213
Goodness-of-fit on F^2	1.032
Final R indices [I>2σ(I)]	R1 = 0.0468, wR2 = 0.1051
R indices (all data)	R1 = 0.0884, wR2 = 0.1314
Largest diff. peak and hole	0.165 and -0.191 e. Å^{-3}

Table 2. Selected bond lengths (Å) and angles (°).

Bond lengths		Bond angles	
C3-O2	1.265(3)	C(2)-C(1)-O(1)	121.1(2)
C1-C2	1.346(3)	C(2)-C(1)-C(10)	127.8(2)
C1-C10	1.467(3)	C(1)-C(2)-C(3)	122.3(2)
C2-C3	1.427(3)	C(11)-C(10)-C(1)	120.7(2)
C1-O1	1.361(3)	C(15)-C(10)-C(1)	121.0(2)

Table 3. Hydrogen bonding geometry (Å,°).

D-H···A	D-H	H···A	D···A	D—H···A
O3—H3···O2	0.82	1.88	2.610(3)	148.1
O5—H5···O2^{i}	0.82	2.10	2.814(2)	144.8
C2—H2···O6^{ii}	0.93	2.53	3.332(3)	144.8
C14—H14···O4^{iii}	0.93	2.53	3.421(3)	160.5
C15—H15···O1	0.93	2.33	2.671(3)	101.3
C16—H16A···O5^{iv}	0.96	2.53	3.451(3)	160.5
C16—H16B···O5^{v}	0.96	2.59	3.323(3)	133.5

Symmetry codes: (i) 3/2-x, -1/2+y,$-z-1/2$; (ii) 3/2-x, 1/2+y,$-z-1/2$; (iii) 3/2-x, -1/2+y,$-z+1/2$; (iv) 1-x, 2-y, -z; (v)x, y, 1+z.

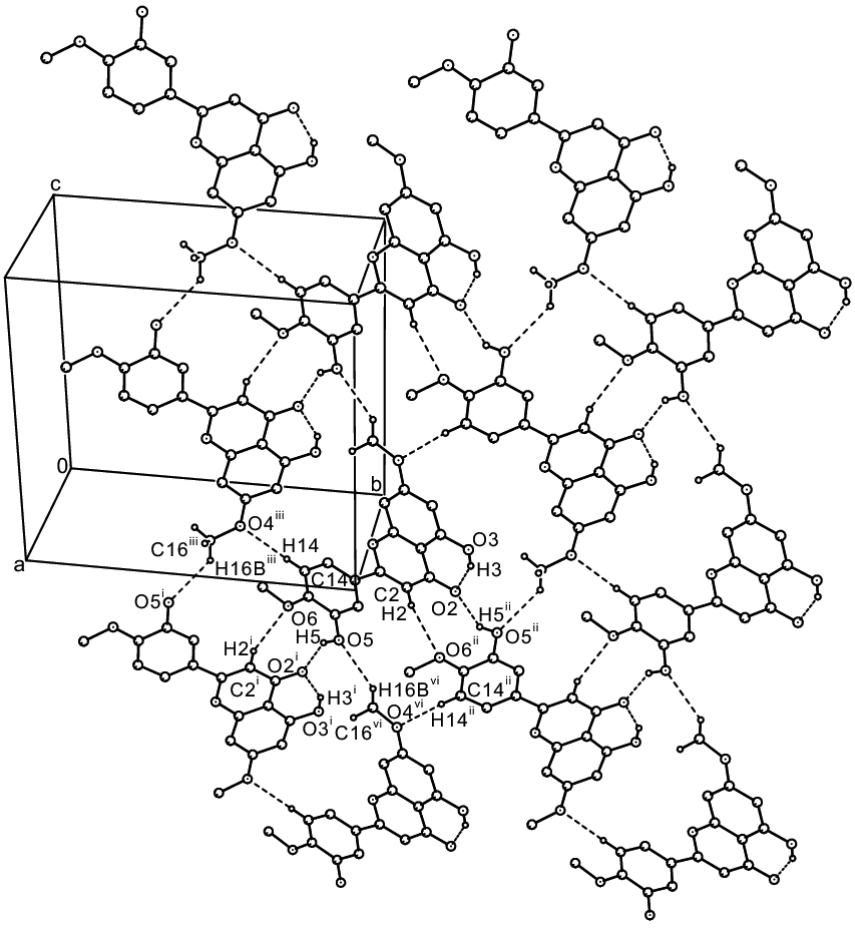


Figure 2a. Part of the crystal structure of pilloin showing the formation of sheets via the R_3^3 (14) and R_2^2 (9) motifs. For clarity, H atoms bonded to atoms not involved in the motifs shown were omitted. Thin dashed lines indicate the hydrogen bonding interactions. [Symmetry code i, ii, and iii (Table 4). In addition, (vi) x, y,z-1.]

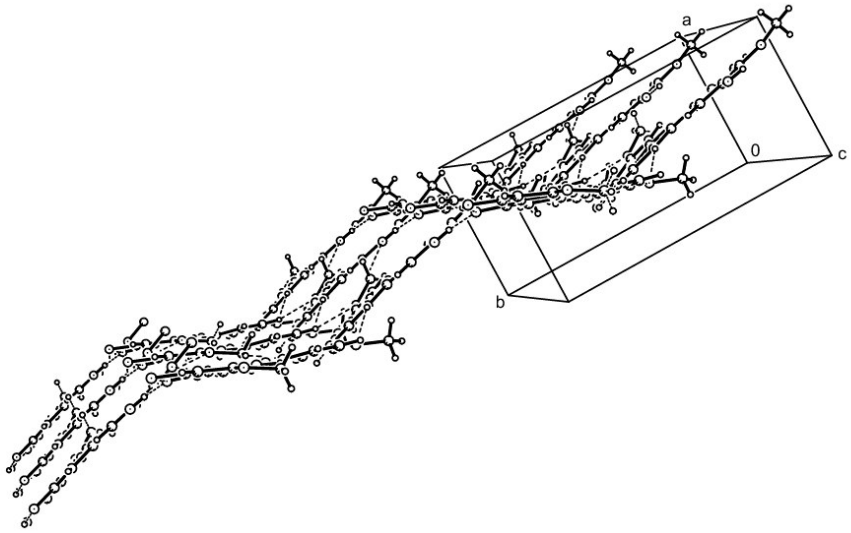


Figure 2b. A packing plot of pilloin viewed along the crystallographic *c*-axis.

The methoxyl groups at C7 and C13 are nearly coplanar with its attached rings, ring A and ring B, as revealed by the torsion angles C16–O4–C7–C6 = 171.4(2)° and C17–O6–C13–C14 = 6.5(4)°. Moreover, an independent O3–H3· · ·O2 intramolecular hydrogen bond of pilloin forms a characteristic intramolecular S(6) motif[25−27](details of the hydrogen bonding are given in Table 3).

The flavone molecules of pilloin were linked into infinite puckering sheets via $R_3^3(14)$ and $R_2^2(9)$ graph-set ring motifs defined by the C–H. . . O and O–H. . . O hydrogen bonds. As shown in Figure 2(a), an R_3^3 (14) graph-set ring motif was determined by the atoms $O4^{iii}$ [symmetry code: (iii) 3/2-x, -1/2+y,−z+1/2], $O5^i$ [symmetry code: (i) 3/2-x, -1/2+y,−z-1/2] and O6, via C—H· · ·O hydrogen bonds and linked the flavone skeletons (i) and (iii) into a plane. Simultaneously, another R_3^3 (14) motif was defined by C2—H2· · ·$O6^{ii}$, $C14^{ii}$—$H14^{ii}$· ··$O4^{vi}$ and $C16^{vi}$—$H16B^{vi}$· · ·O5 interactions [symmetry code: (ii) 3/2-x, 1/2+y,−z-1/2; (vi) x, y,z-1] and generated another plane, the flavone skeletons (x, y,z) and (x, y,z-1) lay in a second plane. These planes were further linked into infinite puckering sheets via the R_2^2 (9) motifs, which were defined by O5–H5. . . $O2^i$ and $C2^i$—$H2^i$ · ··O6 hydrogen bonds (details of the hydrogen bonding are given in Table 4). The puckering sheets extended in the crystallographic *ab* plane and looked like steps [Figure 2(b)]. The dihedral angle between the planes of steps was 143.3(3)°. For clarity, H atoms bonded to atoms not involved in the motifs shown were omitted. Thin dashed lines indicate the hydrogen bonding and $\pi - \pi$ stacking interactions. *CgA*, *CgB,* and *CgC* are the centroids of the rings *A*, *B* and *C*, respectively, as defined in Figure 1. [See Table 4 for symmetry codes. Additionally, (vii) -1+x, y, z.]

The flavone skeletons of pilloin were arranged in antiparallel fashion and $\pi - \pi$ stacking interactions existed between them, linking the flavone skeletons into the columns along the *a*-axis (Figure 3). These columns propagated via centrosymmetric R_2^2 (8) motifs that were formed by paired C16—H16A· · ·$O5^{iv}$ and C16—H16B· · ·$O5^v$ [symmetry code: (iv) 1-x, 2-y, -z; (v) x, y, 1+z] hydrogen bonds (entries 6 and 7 in Table 4). Rings B of the flavone skeletons stacked with the rings C of the adjacent flavone skeletons, with CgB. . . CgC^{iv} = CgC. . . CgB^{iv} = 3.773(2)Å, where CgB and CgC^{iv} were the centroid of the rings B and C at (x, y, z) and (1-x, 2-y, -z), respectively. Additionally, another kind of $\pi - \pi$ stacking interaction linked together these flavone molecules, which was defined by the rings A and B of the neighboring flavone skeletons, with CgA^{iv}. . . CgB^{vii} = CgB^{iv}. . . CgA^{vii} = 3.774(2)Å, where CgA^{iv} and CgB^{vii} were the centroid

of the rings A and B at (1-x, 2-y, -z) and (-1+x, y, z), respectively. The centroid-to-centroid distances lay in the normal range of 3.3-3.8Å[28], indicative of $\pi - \pi$ stacking interactions. Hydrogen-bonding and aromatic $\pi - \pi$ stacking interactions played a key role in assembling the 3-dimensional network structure (Figure 4).

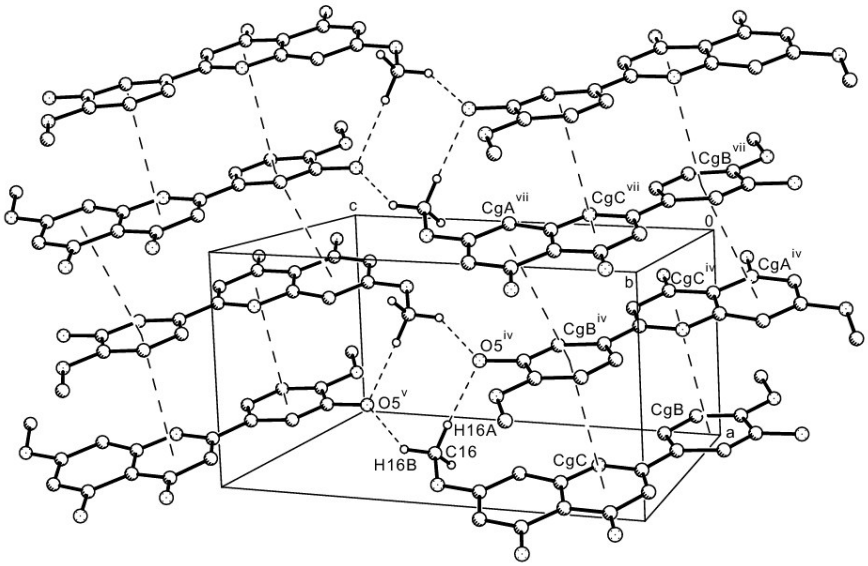


Figure 3. Part of the crystal structure of pilloin showing the $\pi - \pi$ stacking interactions and t.

In page 7, the caption of Figure 3 should be Part of the crystal structure of pilloin, showing the $\pi - \pi$ stacking interactions and the R_2^2 (8) motifs. For clarity, H atoms bonded to atoms not involved in the motifs shown have been omitted. Thin dashed lines indicate the hydrogen bonding and $\pi - \pi$ stacking interactions. *CgA, CgB* and *CgC* are the centroids of rings *A, B* and *C*, respectively, as defined in Figure 1. [See Table 3 for symmetry codes. Additionally, (vii) $-1 + x, y, z$.].

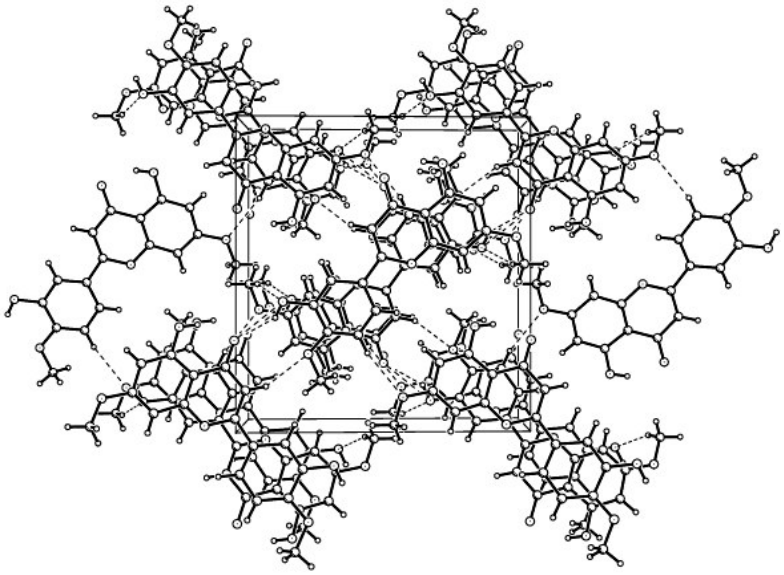


Figure 4. The packing diagram of pilloin.

Supplementary materials

CCDC-639443 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/deposit [or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033; email: deposit@ccdc.cam.ac.uk].

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